# SYNTHESIS AND APPLICATION OF THE 11-BISHOMODRIMANE -8α-OL -12-ONE

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Abstract: This review deals the methods of preparation of the 11-bishomodrimane- $8\alpha$ -ol-12-one, a convenient synton for the synthesis of drimanic and bishomodrimanic compounds, including the biologically active ones. The triterpenic tetracyclic derivatives of the onoceranic range and the fragrance compounds with ambergris odour can be also obtained from this compound.

**Keywords:** 11-Bishomodrimane-8α-ol-12-one, 11-monoacetate of drimane-8α,11-diol, 12-hydroperoxy-8α,12-epoxy-homodrimane, onocerane diol, *nor*-ambraketal, synthesis.

#### Introduction

11-Bishomodrimane- $8\alpha$ -ol-12-one (14,15,16-trinorlabdane- $8\alpha$ -ol-12-one) is a relatively accessible compound, which has been and may be further used as a starting material to prepare both sesqui – and higher terpenoids. The present review deals with the methods of obtaining of this compound as well as its usage.

#### Synthesis of 11-bishomodrimane-8a-ol-12-one

The first synthesis of the hydroxy ketone (1) was effectuated by the authors [1] from norambreinolide (2) with the aim to confirm firmly the absolute configuration of drimane sesquiterpenoids through their correlation with labdane diterpenoids, whose stereochemistry was known [2]. Norambreinolide (2) was saponified with alcoholic base to give the hydroxy acid (3), which was treated with methyllithium giving the mixture of the hydroxy ketone (1) and the bitertiary diol (4) (scheme 1), separated by column chromatogrphy on alumina. The yield of the hydroxy ketone (1), taking into account the recovered starting compound (12%), was up to ~80%. The yield of a bitertiary diol (4), obtained on further reaction of the hydroxy ketone (1) with methyllithium was ~18%. The structures of the hydroxy ketone (1) and diol (4) were proved by the data of elemental analysis and IR-spectroscopy. In such a way, the reaction of the hydroxy acid (3) with methyllithium gives not only the target compound (1), but also the by-product (4). Besides, a part of the starting hydroxy acid (3) remains unchanged. However, if the reaction is carried out up to the complete use of the hydroxy acid (3), then the yield of diol (4) increased in the detriment of the yield of the target hydroxy ketone (1).

Latter it was found out that the method of obtaining of the hydroxy ketone (1) can be simplified as it can be obtained in a good yield (65%) directly from the reaction of norambreinolide (2) with methyllithium [3], avoiding the saponification step of lactone (2). In order to obtain a high yield of the hydroxy ketone (1) the optimal molar ratio of norambreinolide and methyllithium should be 1:2. Under such conditions the yield of diol (4) constituted 18%, and the amount of the recovered starting lactone (2) was up to ~2%. If the molar ratio of lactone (2) and CH<sub>3</sub>Li is lower (1:1.5), the amount of the unchanged starting lactone raised. However if the ratio is bigger (1:4), the yield of the hydroxy ketone (1) is smaller, due to the increase of the yield of diol (4) (scheme 1).



It is necessary to note that latter an alternative, shorter route for the preparation of the hydroxy keton (1) from sclareol (5) was elaborated [4] (scheme 2). On the ozonolysis of sclareol (5) and subsequent treatment of the ozonolysis products with ammonium chloride the dimeric product (6) was formed [4], whose structure was proved by spectral data and confirmed by the X-ray analysis. Compound (6) resulted on condensation of methanal, the cleavage product of

the vinyl group of sclareol (5), with two molecules of the intermediately formed hydroxy ketone (7) or its zwitterions precursors.

This route to compound (6) was confirmed by the fact that on the simultaneous ozonolysis of (5) and hexadeuterobutadiene the reaction product (6) contained two deiterium atoms. It should be noted that the best results were obtained on treatment of the ozonolysis products with ammonium chloride. If ammonium nitrate was used instead of  $NH_4Cl$ , the yield of the dimeric product (6) dropped to 67%. In order to carry out this transformation a lot of acids, either acid and neutral salts, the cationite Ku-2,  $P_2O_{5}$ , and other reagents were tested. But in the all causes the compound (6) was obtained in low yield, or it is not formed at all.



Reagents: a) 1. O<sub>3</sub>, CH<sub>3</sub>OH, 2.NH<sub>4</sub>Cl; b) 1. O<sub>3</sub>, C<sub>6</sub>H<sub>14</sub>, 2. H<sub>2</sub>O, Δ; c) KOH, EtOH, Δ, 1h; d) Δ.

#### Scheme 2

On ozonolysis of bis- $(8\alpha, 13$ -epoxy-14, 15-bisnorlabd-12-en-12-yl) methane (6) in hexane at  $-65 \div -70^{\circ}$ C and subsequent heating of the ozonolysis products in water at  $70^{\circ}$ C, the unstable bis(13, 14, 15, 16-tetranorlabdane- $8\alpha$ , acetoxy-12-on-12-yl) methane (8) was obtained. The reaction product is not stable, readily decomposed in solution of organic solvents and during chromatography. That is why it was characterized only on the basis of spectral data and was used further without purification [5] (scheme 2).

The alkaline cleavage of the compound (8) afforded a mixture (1:1) of the hydroxy acid (3) (48%) and the hydroxy ketone (1) (46%). The hydroxy acid (3) is easily transformed into lactone (2) in a good yield, which, as was pointed out above, can be converted into the hydroxy ketone (1) in the yield of 65%. Taking into consideration this fact, the overall yield of the hydroxy ketone (1) from compond (6) constituted ~77%. Hence, hydroxy ketone (1) can be synthesized from sclareol (5) or norambreinolide (2), in a high yield. This lactone (2) can also be obtained from sclareol (5) as well as from other labdane diterpenoids [6].

#### **Application of hydroxy ketone (1)**

#### Correlation of drimane sesquiterpenoids with labdane diterpenoids

The group of drimane sesquiterpenoids includes compounds with the drimane (9) carbon skeleton. These compounds are of practical interest because many of them are biologically active [7,8]. The stereochemistry of drimane sesquiterpenoids was determined by the authors [9], who transformed drimenol (10) into the known acids (11) and (12). The saturated acid (11) was obtained on degradation of oleanolic acid (12), and drimic acid (13), was prepared on oxidation of abietic acid (14).



Latter on Wenkert E. and Strike D.P. [10] synthesized drimenol (10) and some related compounds, starting with drimic acid (13), correlating thus drimane sesquiterpenoids with tricyclic diterpenoids of known stereochemistry. However, on transformation of abietic acid (14) to the drimic one (13), the asymmetric center C-9 is distroit, and it was necessary to reconstruct it, but there were no guaranty that the reestablished configuration would be the same as it was in abietic acid (14).

More firmely correlation of drimane sesquiterpenoids with labdane diterpenoids was accomplished via the hydroxy ketone (1), in which all asymmetric atoms of the bicyclic part of sclareol (5) were kept unchanged [1]. On oxidation of the hydroxy ketone (1) by the complex of boron trifluoride etherate and the concentrated hydrogen peroxide (94%), a complex mixture of compounds which containes ketones (15)-(17), acetates (18)-(20) and a  $\gamma$ -lactone, of unknown structure was formed. The overall yield of acetates (18)-(20) constituted 31%, and that of drimenyl acetate (18) - 8%. On saponification of compound (18) with an alcoholic solution of potassium hydroxide drimenol (10) was obtained, confirming in such a way that the stereochemistry of the bicyclic systems of drimane sesquiterpenoids and labdane diterpenoids is identical. It should be mentioned that the hydroxy ketone (1) is not oxidized by either perbenzoic or monoperphtalic acids, and that ester (20) is biologically active [7,8]. Latter this compound was found in natural sourses and was called albicanyl acetate [10]. It should be note that, according to [11], the unsaturated ketone (17) has amber odour (scheme 3).



Reagents: a) BF<sub>3</sub> H<sub>2</sub>O<sub>2</sub>, Et<sub>2</sub>O, 25°C, 1,5 h; b) KOH, EtOH, Δ;

#### Scheme 3

# Synthesis and use of 11-monoacetate of drimane-8a,11-diol (21) and 12-hydroperoxy-8a,12-epoxy-homodrimane (22)

The oxidation of the hydroxy ketone (1) with an excess of trifluoroperacetic acid, prepared in situ from trifluoroacetic anhydride and 50% hydrogen peroxide, in dichloromethane solution in the presence of sodium hydrogen carbonate, with the molar ratio NaHCO<sub>3</sub>:(CF<sub>3</sub>CO)<sub>2</sub>O, ca. 1:1, afforded the 11-monoacetate of driman-8 $\alpha$ ,11-diol (21) in quantitative yield [3] (scheme 4). The yield of the hydroxy acetate (21) constituted only 30-40% if the reaction was carried out without sodium hydrogen carbonate. In this case, the dehydration products of the hydroxy acetate (21) were formed.

To assure a high yield of hydroxy ester (21), the ratio of sodium hydrogen carbonate and trifluoroacetic anhydride is of particular importance. If this ratio is more than one, the yield of hydroxy acetate (21) is lowered because of the simultaneous formation of hydroperoxide (22). The yield of compound (22) reached the maximum value (75%) if the molar ratio NaHCO<sub>2</sub>:(CF<sub>3</sub>CO)<sub>2</sub>O was 6.4:1. In this case, the yield of hydroxy acetate (21) drops to 24% (scheme 4).

Hydroperoxide (22) is formed from the cyclic semi-acetale form (23) of the hydroxy ketone (1) which are in equilibrium in solution. Structural elucidation of hydroperoxide (22) was accomplished on the basis of spectral data and its further chemical transformations. Thus, the reaction of (22) with triethyl phosphite affords a mixture of products, from which hydroxy ketone (1) and the dimeric homodrimane (24) were isolated. The structure of hydroperoxide (22) and the dimeric peroxide (24) were confirmed by X-ray analysis.

The hydroxy acetate (21) proved to be a convenient starting material for the synthesis of a range of drimane sesquiterpenoids, in particular, of their first representative drimenol (10) and its acetate (18), as well as their double bonds isomers. Barrero A.F. et al. [12, 13] synthesized drimenol acetate (18) in a low yield on dehydration of the hydroxy acetate (21) with SnCl<sub>4</sub> Authors [14] accomplished the synthesis of the albicanyl acetate (20) starting with the hydroxy acetate (21). The hydroxy acetate (21) was dehydrated with thionyl chloride into the mixture of acetates (18)-(20), which was subjected to oxidation with m-CPBA. The peracid oxidized only the the isomers (18) and (19), isomer (20) remains unchanged. Recently, the dehydration of hydroxy acetate (21) on the interaction with a wide range of dehydration reagents was investigated in detail [15, 16]. The best regioselectivity was obtained on the reaction of the hydroxy acetate (21) with Me<sub>3</sub>SiSO<sub>3</sub>Me, in acetonitrile. As a result, drimenyl acetate (18) was obtained in 65-70% yield besidesdiene (25) was formed in ~17% yield. Their mixture was separated by column chromatography [17]. In this case, the isomers of drimenyl acetate (18) were not formed. Drimenyl acetate is also the predominant component of the reaction products of the hydroxy acetate (21) with Me<sub>3</sub>SiSO<sub>3</sub>CF<sub>3</sub>. Unfortunately, the products, in which the isomers (19) and (20) considerably predominated, were not obtained.



Reagents: a) 50% H<sub>2</sub>O<sub>2</sub>, (CF<sub>3</sub>CO)<sub>2</sub>O/NaHCO<sub>3</sub> (1:1), CH<sub>2</sub>Cl<sub>2</sub>,; b) P(OEt)<sub>3</sub>;

#### Scheme 4

It is necessary to note, that the authors [18] describes a simple convenient and efficient one-step method for the transformation of the hydroxy acetate (21) into drimenol (10). It was found that on treatment of hydroxy acetate (21) with an ethanolic solution of  $H_2SO_4$  under mild conditions the dehydration and the simultaneous deacetylation took place, giving a crystalline mixture of drimenol (10) and albicanol (25) in 10:1 ratio in an 60% overall yield. Recrystallization of this mixture from hexane gave pure drimenol (10) (yield 53%). This method of the transformation of the hydroxy acetate (21) into drimenol (10) was patented [19]. It should be menthioned that drimenol (10) and its acetate (18) have been used as starting compounds for the synthesis of a large number of natural drimanic sesquiterpenoids and their analogous, a part of which possess biological activities [7,8, 20-22].

The spectral data demonstrated that hydroperoxide (22) in  $\text{CDCl}_3$  solution is in equilibrium with its C-12 epimer (27) (ratio 1:4). Obviously, in other solvents the hydroperoxide (22) (scheme 5) is also in equilibrium with its epimer at C-12, while on crystallization from petroleum ether only the less soluble epimer (22) was precipitated, gradually shifting the equilibrium towards the compound (22). The mixture of hydroperoxides (22) and (27) proved to

be readily obtained in a good yield on treatment of the alcoholic solution of the hydroxy ketone (1) at room temperature with 30% hydrogen peroxide in the presence of acetic acid as a catalyst [23] (scheme 5). The mixture of hydroperoxides (22) and (27) possess a high antifungal activity, in particular against *Candida albicans* [24].

# Efficient synthesis of triterpenoid onoceranediol (32) from the mixture of 12-hydroperoxy-8a,12-epoxy-11bihomodrimanes (22) and (27).

The mixture of hydroperoxides (22) and (27) was used as a starting compound for the synthesis of some derivatives of tetracyclic onocerane triterpenoid series.

It is known that the tertiary alkoxy radicals undergo  $\beta$ -cleavage of C-C bonds to give carbon radicals and carbonyl compounds [25,26]. The carbon radicals, in turn, are stabilized either through dimerization or by transformation into olefins with proton elimination. Hence, the alkoxy radical (28), obtained from hydroperoxides (22) and (27), can be converted via the intermediate acetoxy-substituted radical (29) into onoceranediol diacetate (30) [27] and/or drim-9(11)-en-8 $\alpha$ -ol acetate (31) [28,29].

In order to carry out the reductive cleavage of hydroperoxides (22) and (27), their reaction with FeSO<sub>4</sub>7H<sub>2</sub>O and Cu(OAc)<sub>2</sub>·H<sub>2</sub>O under various conditions was studied. When the reaction was carried out in the mixture of AcOH and H<sub>2</sub>O (ratio 9:1), the compounds (22) and (27) are almost totally converted into the hydroxy ketone (1). However in methanol solution, onoceranediol diacetate (30) was the major reaction product (yield 68%), accompanied with the unstable drim-9(11)-en-8 $\alpha$ -ol acetate (31) (yield 28%) [30]. On reduction of onoceranediol diacetate (30) by LiAlH<sub>4</sub> onoceranediol (32) was formed in 88% yield (scheme 6).

Thus, it was demonstrated that the mixture of hydroperoxides (22) and (27) is a suitable starting material for an efficient short synthesis of the derivatives of tetracyclic triterpenoid with onocerane structure.



Reagents: a) CH<sub>2</sub>SO<sub>2</sub>SiMe<sub>3</sub>,CH<sub>2</sub>CN, 20°C; b) H<sub>2</sub>SO<sub>4</sub>, EtOH, 20°C; c) 30% H<sub>2</sub>O<sub>2</sub>, EtOH, AcOH;

Scheme 5



Reagents: a) FeSO<sub>4</sub> 7H<sub>2</sub>O, Cu(OAc)<sub>2</sub>H<sub>2</sub>O, CH<sub>3</sub>OH, 0°C, 68%; b) LiAlH<sub>4</sub>, Et<sub>2</sub>O, Δ, 88%;

#### Scheme 6

#### Synthesis of the ambrox analogous

The hydroxy ketone (1) proved to be a convenient starting material for the synthesis of an ambrox analogue with a strong ambergris odour.

On reduction of hydroxy ketone (1) by lithium boron hydride, obtained in situ from potassium boron hydride and lithium chloride in isopropanol according to the method [31], the mixture of epimeric glycols (33) and (34) was formed, in the yields of 52% and 47,8%, respectively. The glycols were separated chromatographically. When glycol (33) was distilled *in vacuo*, in the presence of p-toluenesulfonic acid, according to the procedure [32], mostly one product was obtained – the oxide (35) with a strong ambergris odour in 89% yield, with traces of the epimeric oxide (36) (scheme 6). When glycol (34) was dehydrated under similar conditions the odorous oxide (36) was formed in 59% yield, alongside with the unsaturated alcohols (37) in 17% yield. It should be mentioned that the structure and stereochemistry of compounds (33)-(36) were determined earlier by the authors [33].

The selectivity of glycol (34) dehydration is lower that those of its epimer (33) and the yield of oxide (36) is also lower than of oxide (35). Obviously, this fact can be explained by the mutual repulsion of methyl groups at C-8 and C-12, which are in 1,3-*cis* position on the  $\beta$ -side of the molecule in the pre-reaction conformation of glycol (34), giving the oxide (36) (scheme 6).

Taking into consideration the results of the dehydration of glycols (33) and (34), the following scheme of obtaining of the ambrox analogue from diketone (8) was proposed: compound (8) was treated with base, the product was heated at 135-140°C to convert the hydroxy acid (3) into lactone (2) and the resulting mixture of hydroxy ketone (1) and lactone (2) was reduced by lithium boron hydride, obtained *in situ* from potassium boron hydride and lithium chloride in isopropanol. The reduction product was distilled *in* vacuo in the presence of p-toluenesulfonic acid, giving the analogue of ambrox with a strong ambergris odour. According to the GLC data, the latter contained ambrox (39) (31%), isoambrox (40) (3%), oxides (35) (18%) and (36) (16%), as well as the mixture of bicyclohomofarnesols (41) and bicyclobishomofarnesols (37) and (38) (32%). A strong ambergris odour of this analogue of ambrox (39) is due to the presence in the final product whose yield constituted 48% calculated from sclareol (5), of oxides (35), (36), (39) and (40), the content of which constituted 68% of the weight of the final product. On the ambrox analogue preparation the intermediate products were not purified but used as such.



Reagents: a) pTsOH, distilled at 135°C;

Scheme 7

#### Synthesis of diastereomeric nor-ambraketal (44) and (45).

Ambraketal (42) is known to be among the compounds with a very strong ambergris odour, while its diastereomer (43) is odourless [6, 34]. The authors [35] carried out the synthesis of *nor*-ambraketal (44) and found out that it has a strong ambergris odour as well as ambraketale (42). For the studying of the dependence of odour on structure it was of interest to synthesize also the diastereomer (45) of *nor*-ambraketal (44). A starting compound for this synthesis was the hydroxy ketone (1) [36]. On its dehydration by POCl<sub>3</sub> in pyridine or by Swern reagent the mixtures of unsaturated isomeric ketones (15)-(17) were obtained, the prevailing component of which was the target ketone (17), whose content constituted 52% and 58%, respectively [37]. That is why an indirect approach of obtaining ketone (17) from the hydroxy ketone (1) was also effectuated. The hydroxy ketone (1) was acetylated by acetyl chloride in dimethyl aniline to give the ketone (46) in 84% yield. On the pyrolysis of acetoxy ketone (46) on short-time heating at 200°C, without solvent, the mixture of ketones (15):(16):(17) in the ratio 30:18:52 was obtained in 91% yield (scheme 7). If the elimination of acetic acid from acetoxy ketone (46) was carried out on heating in DMSO, in the presence of sodium bicarbonate [38-40], the mixture of ketones (15):(16):(17) in the ratio 10:5:85 in 100% yield was obtained. The oxidation of the ketones mixture (15)-(17) by monoperphthalic acid afforded the ketone (17), the isomers (15) and (16) being selectively epoxydised. Ketone (17) can also be obtained by column chromatography of the ketones mixture on SiO<sub>2</sub>, but this procedure is less convenient.

The oxidation of ketone (17) by a catalytic amount of  $OsO_4$ , in the presence of potassium ferrocyanide as a cooxidant, led to nor-ambraketale (44) in 40% yield. The yield of ketale (44) turned out to be twice lower than on oxidation of ketone (17) by  $OsO_4$  and of tri-methylamine N-oxide [35] (scheme 8).



Reagents: a) POCI<sub>3</sub>/Py or Swern reagent; b) ACCI, C<sub>6</sub>H<sub>3</sub>NMe<sub>2</sub>; c)  $\triangle$  or DMSO, NaHCO<sub>3</sub>; d) m-CPBA; e) OSO<sub>4</sub>, K<sub>3</sub>[Fe(CN)<sub>6</sub>]; f) ZnCl<sub>2</sub>

Scheme 8

Since, on the ketone (17) oxidation by  $OsO_4$  and co-oxidants, nor-ambraketale (45) was not formed, an indirect approach was used to obtain the epoxide (47). The latter was obtained in 81% yield on ketone (17) oxidation by m-clorperbenzoic acid. Below are given the reagents which were successfully used for the isomerization of oxide (47), and in brackets are indicated the yields of nor- ambraketale (45):  $ZnCl_2$  (85%),  $SiO_2$  (81%),  $SiO_2$ , impregnated by H<sub>2</sub>BO<sub>2</sub>(80%), Al<sub>2</sub>O<sub>2</sub>(78%), Nafion H<sup>+</sup> (75%) (scheme 7).

Thus, when the epoxy ketone (47) is isomerized on treatment with various reagents, the only reaction product was nor-ambraketale (45), its diastereomer (44) is not formed. As expected, the nor-ambraketale (45) is odourless.

### Conclusion

The data mentioned above provide an additional confirmation that the hydroxy ketone (1) turned out to be an available and convenient synton to prepared a number of organic compounds, a part of which may be not only of theoretical, but also of practical interest. Obviously, this compound will be of use also on solving other synthetic problems as well.

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# APPLICATION OF CHEMICAL METHODS TO THE SOLID WASTE MANAGEMENT

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Abstract. The present article is a synthesis analysis of application of chemical methods for the development of technologies of hazardous waste management. Here are offered some technologies of neutralization of the waste containing hexacyanofferates, galvanic wastes and those with contain of vanadium, which are collected at Power Thermoelectric Plants.

Keywords: waste, hexacyanoferrates, galvanic wastes, waste from Heat and Power Plants, environmental assessment

#### Introduction

A large number of modifications has happened in the world due to the technical and scientific progress and as a result of manufacturing methods modernization. Among these changes is the creation of an enormous industrial and agricultural potential, transport development, unlimited use of natural resources, etc. A massive industrialization during almost two last centuries has contributed to resource depletion as well as favored accumulation of waste with a strong negative impact on the environment.

In the given context, in 1991, participants of the Dobrosh Castle conference, the Czech Republic[1] that represented 50 European states, clearly recognized a large number of essential factors that influence environment: climate change, ozone layer deterioration, acid rains, hazards, urban stress and chemical risk among the others. This subject was discussed by UNEP as well, that approved "Program of the United Nations for the environment at the end of the second millennium" [2]

The wastes present an important factor of environmental pollution. Many concepts, strategies, procedures and methods of environmental protection and health security had been elaborated since the negative impact of waste was found out. However, those didn't avert discords of economic development and nature, but they improved partly the enlargement of contradiction. Negative environmental effects at the regional and global level as well as the environmental conditions at the local level are keeping on deterioration.

One of the major directions of waste management and environmental protection is the reduction of the waste amount based on the improving of the technologies and management. According to data of the Global conference in Strasbourg, 1992 [3] the total amount of waste in the OECD countries in 1990 made up more than 2 billions tons, but in 1993 this exceeded up to 2, 446 billions tone from which the domestic waste amount of up to 5%, industrial waste up to 13,5%, agricultural up to 28,6%, ore waste -14 up to 7%, waste from Power Plants - up to 1,1%, silt (mud) of waste water treatment - up to 25,3%, building waste - up to 10,6%. [4]

According to the data [5], the amount of domestic solid waste (DSW) in Europe is in the keen of growing, coming up to 306 million tons or 415 kg/year per capita. Amount of DSW varies from country to country and makes up to 685 kg/year per capita in Iceland and 105 kg/year per capita in Uzbekistan. (tab.1). DSW come up to 14% of total amount in Western Europe and 5% in Eastern Europe. The basic procedure of solid waste management is neutralization by burial ground disposal. Index of waste accumulation grows annually in the most of the countries, the index of waste treatment and management keeping low. The biggest amount of waste generation (1,14 t kg/year per capita) is in the U.S. The similar tendency is observed in other countries as well. The highest environmental impact is accounted for by waste from industry (ISW). The amount of DSW and ISW in the countries of the European Union and Moldova are in the tab.1.

Table 1

# Amount of waste generated in the countries of the EU and Moldova per capita in the year 2003 [6]

Country	Total, waste	Municipal waste, %	Industrial waste,		
	kg/per capita *		%		
Belgium	1391	25,3	74,7		
Denmark	1058	51,0	49,0		
Germany	1959	28,6	71,4		
France	1070	37,5	62,5		
Ireland	1235	34,8	65,2		

Holland	1132	48,8	51,2
Great Britain	1664	35,5	64,5
Romania	2079	17,31	82,90
Republic of Moldova**	1700	3.8	96.2

\*) Badea A., Apostol T, Mărculescu C, 2003[10]; \*\*) Departament of Statistics and Sociology of Moldova. *Anuarul Statistic al Republicii Moldova anul 2003.* Chişinău, 2003, 704 p.

The industrial wastes play the most important role in the solid waste composition. In the European Union countries it comes up to (%): Belgium 74,7; Germany 49,0; France 71,4; Ireland 65,2; Holland 51,2; Great Britain 64,5 and Romania 82,9, but in the Republic of Moldova 93-95%. These data demonstrate that the ratio of industrial waste makes up from 51,2 (Holland) to 82,9% (Romania) but in Moldova the amount of solid waste is the biggest part in total amount. The dynamics of industrial waste generation in Moldova is presented in table 2.

Table 2

	Amount of waste generated in Moldova in 2001-2005*									
Type of	2001		2002		2003		2004		2005	
waste	Thous. t	%	Thous.t	%	Thous. t	%	Thous.t	%	Thous.t	%
Industrial	5963,3	98,4	5172,3	93.0	3579,2	96,2	4692,8	96,3	4140,0	95,9
Municipal	97,7	1,6	391,7	7,0	140,8	3,8	180,2	3,7	178,8	4,1
Total	6061,0	100	5564	100	3720	100	4873	100	4323	100

\*) Anuar. Calitatea factorilor de mediu și activitatea Inspectoratului Ecologic de Stat. // Chișinău, 2001.

Departamentul Statisticii și Sociologiei al Republicii Moldova. *Anuarul Statistic al Republicii Moldova anul* 2002 Chișinău: Tipografia S.A."CRIO", 2002. 525 p.; Departamentul Statisticii și Sociologiei al Republicii Moldova. *Anuarul Statistic al Republicii Moldova anul 2003.* Chișinău, 2003, 704 p.; Starea mediului în Republica Moldova în anul 2004, Ministerul Ecologiei și Resurselor Naturale al Republicii Moldova,123 p.; Starea mediului în Republica Moldova în anul 2005, Ministerul Ecologiei și Resurselor Naturale al Republicii Moldova, 116 p.

According to the data from Table 2, the quantities of industrial waste during the mentioned period of time come to 98,4-93%, that proves the necessity of their reuse.

Dynamics of waste generation and accumulation unambiguously demonstrates the reduced efficacy of waste management. This fact has strongly initiated a re-orientation in the frame of national strategy of waste management, leading towards activity of waste prevention and recycling. A diminution of DSW amount per capita is observed in Canada and Great Britain. Basically, DSW are generated and just saved in some opened areas that stipulates for the occupation of large zones and provokes the negative effect on the environment due to dangerous emissions and waste water formation. Thus waste generation remains an acute problem that seeks for a elaboration of new systems and technologies of solution. Dangerous environmental impact at regional and global scale, the deteriorated environmental situation keeps on worsening from year to year. This fact concerns Moldova, too.

There is a very critical situation in Moldova with the dangerous waste, such as: vine-making waste (6678 t), pesticides (POP-s) (more than 1678 t), vanadium contained waste (about 500 t), galvanic waste (more than 2000 t), mud from the water purification plant which provokes green house emissions (more than 600 thousands tons) [7]. This situation is an argument in the favor to investigate and to find solutions from the scientific point of view, connected with evaluation of waste chemical composition and adequate its management. The problem can be solved by non conventional way, what include both: the multivarious ecological waste investigations and the elaboration of chemical-technological methods of waste valorification.

In this context a conception of sustainable development plays an important role for natural resources conservation, because the industrial and agricultural waste are examined as a second source of row materials. The goal of this paper is to elaborate the procedures and technologies of valorification of some industrial waste generated at the factories from the R.M.

#### Waste generation

### Hexacyanoferrate wastes

In the last two decades as a result of development of vine-making branch in the RM similar with the other states from the CIS ( the Ukraine, Russia, Georgia, Armenia, Azerbaidjan and some countries from Europe and Est-Europe, such as Romania and Bulgaria) an essential quantity of dangerous waste is vine-making waste. In the period of 1994-1998 years the quantity of this waste was increased up to 7374 tones. Then, their quantity has decreased to 6622 tons in the 1999 year. The maximum quantity was registered in the period of the 2001year - 7463 t (fig.1). At present the quantity of the hexacyanofferarate waste is 6678 t [9]. The vine-making waste in the Republic was increased from 3300 up to 7463 t, in the last 12 years the annual average quantity has the values of 5800-5900 t (fig.1).

The vine-making wastes are deposited in the territories of vine factories and can include from 10 up to 30% of sediments of vine-making waste. There are metallic and betony capacities and tanks for depositing these wastes. In the upper part of tank (capacity) is a liquid phase which consists from vine and the mixture of the gelatin destruction products and the fish glue, used as the flocculants for vine purification and precipitation of the colloidal suspension. Liquid phase (pH 3-5) includes also the part of the ferrocyanide (vine-making waste) compounds and have a specific smell of the acetic acid and spoiled protein, in the bottom layers the ferrocyanide compounds are increased from 10 up to 60 %. For waste concentration **can be used cylinder which contain a special adsorption (diatomit, silicium compounds and other substances) layer.** As a result the homogeny viscose mixture is obtained. The liquid phase consists of 90% of bentonite, the vine residue and 5-10% of ferrocyanide.

There are the Cu<sup>2+</sup> ions in the vine, as a result of the vineyard treatment with CuSO<sub>4</sub> solution, as well as the Fe<sup>2+</sup> and Fe<sup>3+</sup> ions from the iron installation used for vine production. The presence of these ions has a negative influence on the vine quality. That is why hexacyanoferrate complex ion  $[Fe(CN)_6]^{4-}$  is used for its precipitation. The maximal admissible concentration of  $[Fe(CN)_6]^{4-}$  is 0,005 g/l [10].

The oxidation of Fe in Fe<sup>2+</sup> ion can be presented by chemical reaction:

$$2H^{+} + Fe^{0} = Fe^{2+} + H_{2}$$
(1)

As a result of partial oxidation of  $Fe^{2+}$  the mixture of  $Fe^{2+}$  and  $Fe^{3+}$  is formed. The process of  $Fe^{2+}$ ,  $Fe^{3+}$  and  $Cu^{2+}$  ions neutralization with  $K_4[Fe(CN)_6]$  is presented in (2) – (4).

$$2 \operatorname{Fe}^{2+} + [\operatorname{Fe}(\operatorname{CN})_{6}]^{4-} = \operatorname{Fe}_{2}[\operatorname{Fe}(\operatorname{CN})_{6}] \downarrow$$
(2)

$$4 \operatorname{Fe}^{3+} + 3 [\operatorname{Fe}(\operatorname{CN})_{6}]^{4-} = \operatorname{Fe}_{4} [\operatorname{Fe}(\operatorname{CN})_{6}]_{3} \downarrow$$
(3)

$$2 Cu^{2+} + [Fe(CN)_{6}]^{4-} = Cu_{2}[Fe(CN)_{6}] \downarrow$$
(4)

A suspension of bentonite and fish clay is supplemented, too, to facilitate the sedimentation of these hexacyanoferrates. The vine waste remains after vine decantation and filtration. There is the colloidal yeast fraction, tartaric acid, vine residues in the waste, too. The content of hexacyanoferrates in the waste is 5-10% (50-100 g/kg) from the dry mass. Early the vine waste was liquidated by landfills disposal without some treatment. After 1980 this waste was accumulated at the factories of its, generation.



Fig. 1. The vine waste quantities deposited at the vine factories in RM

There are many procedures of hexacyanoferrate waste neutralization, such as chemical treatment by sedimentation, oxidation, catalytic methods and others [11-23].

For hexacyanoferrate waste deactivation by Covalev V., Covaleva O. [18] was proposed the filtration through the sawdust of tree mixed with coal dust and following incineration. The gas emissions were purified. The advantage of this method is the use of cheap and accessible materials, but the process is time consuming, and it is necessary to purify and to verify the gas emissions for exclusion of the air pollution. That caused the supplementary cost.

Another method is the treatment of hexacyanoferrate for the transformation of waste in the soluble state by alkali using, following a treatment by catolyt and oxidation with anolyt [19]. This technology is advantageous, but because the oxidation process is difficult to be realized (respecting of strong parameters regime such as concentration of oxidizer, pH, a high temperature, etc.) and a high cost of reagent  $(H_2O_2)$  is used.

Thus, the diverse methods of vine waste treatment have been elaborated [11-23], majority of them is interesting from the applicative point of view [18,19], but its implementation for waste **detoxcation** at large scale has not been realized so far.

As a result of analysis of diverse technologies and of the possibilities of its implementation, we have drawn a conclusion that it is necessary to elaborate a new chemical procedure for hexacyanoferrate waste **detoxcation** which can be used exactly at the site of waste formation and disposal, using the installations from the vine factory and excluding the necessity of special has been modern installations with high costs.

#### Galvanic waste

The elaboration of technologies of valorification of diverse types of waste including galvanic waste with the high content of heavy metals is in the focus of great attention in the mondial practice of waste management.

The quantity of galvanic waste deposited at the "ALFA" factory the city of Chişinău at the end of the 2002 year was about 1000 tons, and these wastes were deposited in the special basin built according to the requirement.

With the dependence of the site of waste collection the Cu content was 15-35% and more or less for other metals (AL, Cr, Zn, Fe and other). The galvanic waste containing a large spectrum of metals, it could serve as rich second ores of color metals. A estimation has demonstrated that the treatment of 1000 tons of galvanic waste in order to obtain of cuprum and other metals valorification will contribute to the obtaining of 150-300 tons of metals and to the waste neutralization.

There are different methods for wastewater purification from heavy metals. Some authors proposed the waste water purification (the removal of heavy metals) by  $Na_2CO_3$  solution treatment [24]. The deficiency of this method is an unsatisfactory grade of water purification and the great consumer of  $Na_2CO_3$ . The other procedures of wastewater galvanic purification [25] consist of the use of 3,0% of Ca(OH)<sub>2</sub> for heavy metals precipitation. But this method doesn't provide the complete waste water purification either. Schegliuc and coauthors [26] proposed a procedure for the treatment of heavy metal containing wastewater with the use of iron (II) and iron (III) in the ratio of Fe(II) to Fe(III) for1:2 gram-ion/l and as well an addition of iron(II)  $\geq$  0,5 gram-equivalent up to the content the of general heavy metals - 1 gram-equivalent, after that the introduction of alkali in the mixture by intensively mixing in the continuum or alternative electromagnetic field with the current strength no more than 1A/m<sup>2</sup>.

In the case of Cr(VI) containing wastewater it is necessary to respect a ratio of Fe(II) to Cr(III). It is established that 4,5 gram-ion Fe(II) to 1 gram-ion of Cr(VI) are optimal. This fact is necessary for formation of ferrites of heavy metals: MeOoFeO; MeOoFeO; MeOoFeO;.

Budilovscii and auth. [27] proposed the galvanic wastewater treatment with hydroxide of iron (pH 8,0-8,5), but they involved the supplementary addition of Al (III) in the quantity of 2-10 mg/l for improving of nickel sedimentation, which creates the conditions of complete wastewater purification from nickel. The effective Al (III) and Ni (II) ions sedimentation is conditioned by the reaction of some chemical complex of aluminum and nickel ions and the aluminates ions formation (the mixt complexes of salts of AL and Ni with alkaline metals). The specific action of the aluminum takes place not only for Ni, but and for other metals.

For color metals extraction from sediments formed after wastewater treatment, Covalev O., Covalev V., Duca Gh. [28] used the process, including the solubilization of metal compounds with  $NH_4Cl$ ,  $(NH_4)_2P_2O_7$  and ammonium hydroxide in the presence of magnetic fluidization. The mixture from  $N_2H_4 \cdot H_2O$  (hydrate of hydrazine), HCOH (formol) and NaBH<sub>4</sub> (borohidrure of sodium) was used as a reducer. The advantage of such procedure consists in the possibility of simultaneous extraction of some metals by magnetic fluidization. The method necessitates using of some chemical substances for sediments solubilization and for metals reduction, some of them are toxic (ammonium), the others have high costs (NaBH<sub>4</sub>). Another procedure proposed by Covalev O. and auth.[29] utilizes the sulfuric acid for the heavy metals extraction and its solubilization from waste water sediments, supplementary treatment with SO<sub>2</sub> (gas) and the mixture of next reducers: hypophosfite of sodium and borohydrure of sodium in the rate of (5-10):1, as chemical agents for metal separation and application of high frequency current and magnetic fluidization. The quantity of chemical reagents in the comparison with the quantity of extracted heavy metals must be (1,5-2,0):1, that exceeds those according to the stoechiometric coefficients of chemical reactions. The advantage of this method is the possibility of simultaneously extraction of some of metals for metals and the possibility of simultaneously extraction of some of metals for metals must be (1,5-2,0):1, that exceeds those according to the stoechiometric coefficients of chemical reactions. The advantage of this method is the possibility of simultaneously extraction of some of metals for metals and the process of purification. The method is technologically

advantageous, but it is difficult, because it is necessary to use the complicated modern installation, such as magnetic fluidization, installation with the high frequency currents, the generator for the  $SO_2$  generation, the purchase of it for most laboratories from the Republic isn't possible. These procedures propose some of chemical substances for sediments solubilization and for metal reduction, some of them are very toxic (ammonium) and others have high costs (NaBH<sub>4</sub>).

Another procedure of purification of galvanic residual water [30] includes the treatment RW at pH 6...8 with reduction reagents in the following concentrations (M/l): lithium borhydride of sodium: 0,06...0,08; hypophosfite of sodium 0,26 - 0,30; formic aldehide 0,60 - 0,70; hydrasine 0,30 - 0,40. This procedure utilizes the electromagnetic field (frequency of 60 - 74 kHz) and Ni-Zn as a catalyzer in the rate of 1 : (1,5 - 2). The advantage of the present procedure in comparison with those described in [28,29] consists in the possibility of waste water purification to a high grade. Only deficiency is the necessity to use a special equipment of the laboratory with different modern installations.

Stewart and auth. [31] have elaborated the procedure for consecutive iron and zinc extraction from acid galvanic solutions. For this, it is necessary the NaOH addition till a value of pH 5,4. The sediment, which is formed, is separated by filtration at the pH 5,4. Then the new quantity of NaOH is added till the value of pH 9,4, and the sediment of  $Zn(OH)_2$  which is formed and is removed by filtration. The disadvantage of the procedures mentioned earlier is not only in the non qualitative galvanic waste water purification, but also in the impossibility of consecutive heavy metals extraction from galvanic solution, if more than 2 components are presented in solution.

The comparative analysis of procedures for galvanic waste water purification demonstrated that, the choice of one or another method depends on the chemical composition of waste water, and there doesn't exist a universal method for chemical purification of the galvanic waste. So, the galvanic waste from "Alfa» factory represents the fluid mass containing the mixture of  $Cu^{2+}$ ,  $Fe^{3+}$ ,  $Al^{3+}$ ,  $Cr^{3+}$  and  $Zn^{2+}$  ions, and for its valorification it was necessary to elaborate the method for metals separations and waste water purification.

#### Waste Containing Vanadium

As a result of the combustion of petrol products at Power Thermo electric Plant (PTP) the generation of some waste containing vanadium has happened the last decade. According to the statistical data recently received its quantity at PTP -1 is about 500 tons. It is necessary to be mentioned that these waste from the second class of toxicity present a risk for environment and people health. Its valorification will contribute to solve both important problems: waste liquidation and recuperation of about 18 tones of  $V_2O_5$ , with the total cost 17 millions lei.

There are different methods for extraction of vanadium from waste. According to Philippe G. [33] for vanadium recuperation from fly ashes (resulted after the petrol products combustion) the concentrated NaOH solution is used for vanadium selective solubilization. Vanadium separation was carried out by CaO addition in the quantity of 6-40 % in comparison with the quantity of silica (SiO<sub>2</sub>) included in ash.

For vanadium extraction from ashes of the coal combustion Kounhei Nimomya [34] has used the solubilization by NaOH at the 80-200°C. The big part of vanadium and small quantity of calcium, iron and other metals are solved in the alkaline solution from which the vanadium is extracted by trioctilamine in an organic solvent at the pH 1-4. The vanadium selective recuperation was carried out as a result of formation of a couple of vanadium ions with trioctilamine from the organic phase. Nickel from the resting solution has been precipitated with a selective oxime addition. Magnesium from resting solution was precipitated with an alkali.

The selective method of vanadium extraction from fly ashes is described by Musucava [35]. This method is proposed for the ashes solubilization in the relatively diluting alkaline solution at high temperature, with solution separation from the rest and solution treatment with the amine containing solvent for selective extraction of vanadium. Similar procedure is also proposed by Vaicutam L. and auth. [36]. According to Fassi and co-coath. [37], the vanadium waste is exposed to a thermal treatment at high temperature. The final product represents the second ores with the high  $V_2O_5$  content. For vanadium extraction Bretschneider G. and Fritz [38] proposed the addition of a melt of carbonate and chloride of alkaline metals mixture to the material with vanadium contents in the presence of oxidizer with the following water solubilization and regulation of pH value up to 6,5 and addition of ammonium salt at the temperature 90°C. After this process, solutions were cooled up to 15 - 20 °C and the metavanadate of ammonium takes place with  $V_2O_5$  obtaining.

For vanadium recuperation Goddard J[39] has proposed the method which consists in the frieding of vanadium containing waste with alkaline metal, following by the water solubilization. The vanadium extraction from the rest of combustion petrol products is carried out by the direct solubilization of the rest with  $Na_2CO_3$  and NaOH.

Other authors [40] use for the vanadium extraction a direct treatment of vanadium containing materials with alkaline reagents, sediment separation, its washing and collected soluble fractions were treated with ammonium compounds for metavanadate ammonium obtaining. The next step was effectuated similarly as in the case of Bretschneider G. and Fritz procedure.

There is another method for vanadium extraction - the sonic treatment with the frequency of 300-2000 kHz. Yoshimori Tadahico [41] used this method for vanadium and nickel extraction from the waste dispersed in water. Then the solution was filtrated for the ashes removing and obtaining of the vanadium solution. Haehn Reinhard and co-auth. [42] for facilitation of vanadium extraction process carry out the frieding of the mixture of vanadium containing the waste with additives (carbonates, chlorides, sulfates or nitrates) with the following solubilization of the mixture components. According to the work [43] the ashes are treated at pH 7-10 in the presence of the carbonates, hidrocarbonates of alkaline metals or ammonium. The rest of carbon is separated from ashes, and it has been granulated for use as solid combustible.

According to the methods elaborated by some authors [44-46] vanadium separation from waste has been carried out by frieding of waste with carbonate or sulfate of alkaline metal and solubilization of vanadates. Haehn [47] proposes a method for frieding of initial waste without alkaline additives followed by the extraction (first step) and the second frieding after supplementation of additive to the solid rest and a second solubilization.

Besides the alkaline methods, there are the methods with acid solubilization of vanadium from waste, too (Mizin [48]. Vanadium waste with a high content of calcium has been pounded, then, it was treated with  $H_2SO_4$  for  $CaSO_4$  formation, after the solution filtration  $V_2O_5$  was obtained from the solution.

The method of Woolery M. şi Rigsby L. [49] forecast the treatment of ashes bitumen in the low acid medium, followed by the oxidation to V<sup>5+</sup>, at the temperature of 80-85°C, precipitation of polyvanadates and its separation from nickel and magnesium that remain in the solution.  $H_2SO_4$  solution for vanadium extraction has been employed by Corigliand [50], too. The acid solution after oxidation was treated with agents for chelating (for example, EDTA), followed by treatment with NH<sub>4</sub>OH for selective precipitation of polyvanadates of ammonium. The acid treatment is used by other authors [51].  $H_2SO_4$  has been injected in the mixture of ashes and water then hydrazine has been added as a reducer. At the beginning pH value in the mixture was2-3, then after supernatant separation and hydrazine supplementation at permanent stirring the value of pH was increasing to 5-7 and the insoluble compound of vanadium has been separated by centrifugation. Kohlein [52] for vanadium extraction used solubilization following by gradual extraction of vanadium and nickel and its separation by ionic change chromatography. Vanadium is precipitated as a compound -  $V_2O_3$ 

The analysis of a large spectrum of the articles in the field of vanadium valorification from different types of waste (ashes, residues, industrials waste from steel melt containing vanadium) present some essential phases of vanadium containing waste treatment

- The thermal pretreatment (frieding) with the additives (carbonates, chlorides, sulfates, nitrates), sonic treatment;
- The alkaline (with NaOH, KOH) or acid treatment (with H<sub>2</sub>SO<sub>4</sub>) for vanadium solubilization. The chemical treatment can be in the combination with oxidizer (O<sub>2</sub>), or reducer (SO<sub>2</sub>);
- Extraction by organic reagents in some of the cases.
- The vanadium precipitation from solutions by ammonium salts for obtaining of the NH<sub>4</sub>VO<sub>3</sub> or polyvanadates;
- Thermal decomposition of  $NH_4VO_3$  for  $V_2O_5$  obtaining.

#### **Experimental part**

The experimental investigations have been carried out with the application of a performing apparatus. The waste probes and the substances obtained in experimental process have been analyzed with the utilization of atomic absorption spectroscopy (AAS), roentgeno fluorescent spectroscopy VRA – 30 (Carl-Zeiss, Germany), roentgeno-fluorometric BARS-3, thermo spectroanalyzer DERIVATOGRAF Q-1000, (firm MOM, Budapesta), oscilopolarograf ŢLA-03, fotocolorimeter FEK-2, potentiometer PM-10 (Germany). The analyses of hexacyanoferrate ions content in the washing water after hexacyanoferrate extraction were carried out by the Chişinău Sanitaro Epidemiologic Station

AAS was used for the determination of vanadium quantity in the residual water after the boiler washing at the TPT-1 and of the content of heavy metals in the galvanic solutions after its separation.

The vanadium content after the extraction from TPT-1 waste by different extragents has been determined by photo colorimeter KFK-2. For experiments were used: a mixture from 20 g of the waste probe and 5 g  $(NH_4)_2S_2O_8$ , 100 ml H<sub>2</sub>O and 5 g of solid NaOH in the porcelain cups was treated at 100 - 140° C with a careful agitation. After cooling to the 20°C, the mixture is solved in the bidistillate. The obtained solution was filtrated through a paper filter and the quantity was transferred in the 250 ml retort. The vanadium content in solution was determined by the photo colorimetric method.

The total vanadium content in the dry vanadium waste from TPT-1 and the vanadium content resting in the waste after vanadium(V) extraction by alkaline method (12,5 N NaOH in the presence of  $(NH_4)_2S_2O_8$  as the oxidizer) has been determined by roentgen-fluorescent spectroscopy method. The content of the metals, extracted together with vanadium by the alkaline method in the presence of oxidizer has been effectuated by the roentgen-fluoremetric method.

All experiments were carried out by the use the bidistillate water. The acids solutions ( $H_2SO_4$ ,  $HNO_3$ , HCl) were the "chemical pure" qualifications. The salts ( $ZnCl_2$ ,  $K_2Cr_2O_7$ ,  $AlCl_3$ ) used for standard solutions were "chemical pure".

For standards graphics of calibration in the case of roentgen-fluorometric (Bars-3) were used the following salts of metals with "analytic pure" and "chemical pure" qualification:  $CaCO_3$ ,  $MnSO_4$ ,  $Cr_2(SO_4)_3$ ,  $Co(NO_3)_2$ ,  $NiSO_4$ ,  $ZnSO_4$ ,  $CuSO_4$ ,  $Fe_2(SO_4)_3$ . Determination of the metals in the waste was carried out after vanadium extraction from dry sample.

#### Hexacyanoferrate waste valorization

In this work has been elaborated a new procedure for hexacyanoferrate waste neutralization (see scheme in fig. 2) [11]. The procedure is based on the hexacyanoferrate transformation in the soluble state by CaO or Ca(OH)<sub>2</sub> addition, the separation of a soluble fraction by filtration and removing of the sediment (bentonite, levure fraction). The ions of  $[Fe(CN)_{6}]_{3}^{4}$  from solution are precipitated by soluble Fe(III) salt.

There are the next steps of the procedure:

- 1. Hexacyanoferrate waste is mixed with water up to 5% (volume), then the supplementation of CaO, what interacts with H<sub>2</sub>O and (CAUTION !!!) exothermic reaction takes place with Ca(OH)<sub>2</sub> formation. The fresh Ca(OH)<sub>2</sub> is very active and contributes to solubilization of hexacyanoferrates, according to the reaction (5). The optimal hexacyanoferrates concentration is 5%.
- 2.  $\operatorname{Me}_{2}[\operatorname{Fe}(\operatorname{CN})_{6}] + 2\operatorname{Ca}(\operatorname{OH})_{2} \rightarrow 2\operatorname{Me}(\operatorname{OH})_{2} + \operatorname{Ca}_{2}[\operatorname{Fe}(\operatorname{CN})_{6}]$ a. where Me – ions Cu<sup>2+</sup> or Fe<sup>2+</sup> (5),
- 3. Separation of aqua soluble hexacyanoferrate by decantation. There are bentonite and rest of  $Ca(OH)_2$  and ferrocyanide in the solid phase. This sediment was washed with  $H_2O$  for complete soluble hexacyanoferrate removing (till the negative reaction to cyanide test). The solid rest is removed to the site of disposal. The residual washing water is used in the closed cycle for dilution of initial vine waste (to concentration 5%).
- 4. Addition of HCl solution to Ca<sub>2</sub>[Fe(CN)<sub>6</sub>] solution (till pH 3-5).
- 5. Sedimentation of ferrocyanide liquid phase by addition of soluble Fe(III) salt and formation of the insoluble  $Fe_4[Fe(CN)_6]_3$  according to the reaction (6):

$$4\text{FeCl}_{3} + 3\text{Ca}_{2}[\text{Fe}(\text{CN})_{6}] = \text{Fe}_{4}[\text{Fe}(\text{CN})_{6}]_{3}\downarrow + 6\text{CaCl}_{2}$$
(6)

6. Sediment removal by filtration and its dehidratation. The technology [11] has been used for neutralization of 1000 t of hexacyanoferrate waste at the vine factory "Vismos".

#### Galvanic waste valorization

The elaborated new procedure for heavy metal extraction from galvanic waste has importance from both aspects: ecological and economical [17]. The ecologic aspect is to liquidate this type of waste which is dangerous for environment and economic – a possibility of essential quantitative recovery of metals is necessary for our national economy (Cu, Al, Zn and other metals).

The procedure proposed in this work includes the solubilization of partial liquid galvanic waste in solution of the concentrated solution of  $H_2SO_4$  in the rate of 1:1, separation of the solution by decantation and the removing of the insoluble rest (SiO<sub>2</sub> and other solid particles).

The metallic iron (iron sawdust, generated as a waste in the process of detail fabrication) was used for Cu separation from galvanic residual solution by cementation method. This process was carried out by continued agitation at the temperature of 40 -  $60^{\circ}$ C. The reduction of Cu<sup>2+</sup> ions and Cu<sup>o</sup> formation, according to the reaction (7):

$$Cu^{2+} + Fe^{\circ} = Cu^{\circ} + Fe^{2+}$$
 (7)

Metallic copper is separated from the solution by filtration it was washed for 2-3 times with  $H_2O$ . This residual water is supplemented to the supernatant. 0.8-1.2 cm<sup>3</sup> of 6N HNO<sub>3</sub> are added to the solution after Cu separation and the solution is treated thermo for 2-3 min. The complete oxidation of iron (II) in iron (III) takes place [32], according to the reaction (8):

$$3FeCl_2 + 4HNO_3 \rightarrow Fe(NO_3)_3 + 2FeCl_3 + NO\uparrow + 2H_2O \quad (pH<1)$$
(8)

In the obtained solution is added 4-8 N NaOH solution till the pH value 4,6-5,4. As a result, the  $Fe(OH)_3$  and  $Al(OH)_3$  hydroxides are formed and their co-precipitation takes place according to the reactions (9 and 10):

$$\operatorname{Fe}^{3+} + 3\operatorname{OH}^{-} \to \operatorname{Fe}(\operatorname{OH})_{3} \downarrow \qquad (\mathrm{pH}\ 4,8-5,2)$$

$$\tag{9}$$

$$Al^{3+} + 3OH^{-} \rightarrow Al(OH)_{3} \downarrow \qquad (pH 4, 8-5, 2) \tag{10}$$

The obtained mixture is separated through filtration. The filtrate is treated with  $Na_2SO_3$  (for the Cr<sup>6+</sup> reduction in Cr<sup>3</sup> in the acid medium according to the reaction (11), and then with the NaOH to the pH value 7,8- 9,4, (is desirable 6,8- 8,8), according to the reactions (12) and (13):

$$H_2Cr_2O_7 + 3Na_2SO_3 + 6HNO_3 \rightarrow Cr_2(SO_4)_3 + 4H_2O + 6NaNO_3$$
(11)

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Sedimentation with the NaOH: 
$$Cr^{3+} + 3OH^{-} \rightarrow Cr(OH)_{3} \downarrow (pH 5,4-6,8)$$
 (12)

$$\operatorname{Zn}^{2+} + 2\operatorname{OH}^{-} \to \operatorname{Zn}(\operatorname{OH}), \downarrow$$
 (pH 6,8-8,8) (13)

As a result of the reactions in alkaline medium,  $Zn(OH)_2$ ,  $Cr(OH)_3$  and other components sedimentation takes place at these pH values. The sediments of  $Cr(OH)_3$  and  $Zn(OH)_2$  were separated by filtration. Then, the resting heavy metals were determined in the filtrate by atomic absorption spectroscopy method. The proposed technological scheme for consecutively heavy metals extraction is presented in fig. 3 [33].



Fig. 2. Technological Scheme of hexacyanoferrate vine waste treatment

Thus, the procedure elaborated for heavy metals separation from waste water includes the next steps:

• the copper reduction by "cementation" method when the metallic Fe is added in quantity of 5-10 times more than the stoechiometric quantity that is necessary for the copper substitution and its separation from waste water;

• the Fe(II) oxidation in the Fe(III) by  $HNO_3$ , the addition of the alkaline solution and the heavy metals (Fe<sup>3+</sup> and Al<sup>3+</sup>) separation in the forms of hydroxides at the pH 4,8-5,4;

• the separation of the sediments which were formed while iron and aluminum hydroxides and other components were mixed by filtration.

• the reduction of Cr (VI) in Cr(III) with  $Na_2SO_3$  according to the reaction (11) and its sedimentation together with Zn <sup>2+</sup> in form of hydroxides when NaOH is added;

• the separation of hydroxides  $Cr(OH)_3$  and  $Zn(OH)_2$ 

The data (tab. 3) demonstrate that the proposed procedure permits the heavy metals removing from the residual galvanic water up to a high degree, so its content after purification don't exceed 0,5 % from the initial quantity, excluding chromium ions. The rest of chromium content in the solution is 2,7 %.



Fig. 3. Technological Scheme of Consecutive Separation of the Metals from Waste

Table 3

Metal	Metals content in	n initial solution	Metals content in solution after metals			
			separation			
	(mg/l)	%	mg/l	% restant		
Cu <sup>2+</sup>	675	100	1,7	0,25		
Cr <sup>3+</sup>	52,5	100	1,4	2,67		
Fe <sup>3+</sup>	192,5	100	0,5	0,26		
Zn <sup>2+</sup>	112,0	100	0,5	0,41		
Al <sup>3+</sup>	96,0	100	0,4	0,42		

#### Valorization of the Waste Containing Vanadium

As a result of the comparison of methods for valorization of waste containing vanadium, we can conclude that the majority of them are used for valorization of waste with high vanadium content. Take into attention that the waste from the power thermoelectric station PTS-1(Chisinau) has a low content of vanadium ( $\sim$  3,0 %), that why it is necessary to elaborate the new chemical technology for vanadium valorization from this waste.

Vanadium in the waste is found in the insoluble state with different metals:  $FeVO_4$ ,  $FeV_2O_4$ ,  $Mn(VO_3)_2$ ,  $CaV_2O_6$ ,  $CrVO_4$ . Vanadium extraction from these wastes can be carried out by alkaline and acid solubilization. The results of determination of optimal concentrations for extragents are demonstrated in table 4.

Reagent for extraction	Content of vanadia 20 g o	um extracted from f waste	%, vanadium extraction from the total vanadium in waste				
	Mass (g)	%					
2N solution HCl	$0,23 \pm 0,012$	$1,2 \pm 0.06$	$30,0 \pm 1,5$				
9 % soluțion $H_2SO_4$	$0,24 \pm 0,012$	$1,2 \pm 0,06$	$31,3 \pm 1,6$				
2N solution NaOH	$0,55 \pm 0,028$	$2,8 \pm 0,14$	71,6 ± 3,6				
HNO <sub>3</sub> conc. + $(NH_4)_2S_2O_8$	$0,25 \pm 0,013$	$1,3 \pm 0,07$	32,8 ± 1.6				
12,5N NaOH +(NH <sub>4</sub> ),S,O <sub>8</sub>	$0,74 \pm 0,037$	3,7 ± 0,19	$96,4 \pm 4,8$				

Vanadium extraction from waste PTS-1

The data from table 4 demonstrated that if the extragent is NaOH in the presence of  $(NH_4)_2S_2O_8$  (as oxidizer) the extracted vanadium quantity from **PTS-1 waste** is of 3 times more than in the case of acid extraction [54].

As a result of experimental research of conditions and a screening of reagents for vanadium extraction from **PTS-1** waste and application of the mathematic experimental planning (factorial experiment) EFF<sup>7-3</sup> the optimal conditions were determined. These data have been used for elaboration of a technological procedure (fig. 4) of vanadium that is obtained from the waste containing vanadium.



Fig. 4. Technological Scheme for Vanadium extraction from waste

There are the next steps for vanadium obtaining, according to the alkaline method:

- Vanadium extraction from the waste in diverse state of oxidation (V<sub>2</sub>O<sub>3</sub>, V<sub>2</sub>O<sub>4</sub>) (with NaOH in the presence of oxidizer (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at the temperature about 120-140°C ) with obtaining of vanadium as: NaVO<sub>3</sub> (metavanadate), Na<sub>4</sub>V<sub>2</sub>O<sub>7</sub> (pirovanadate) or Na<sub>3</sub>VO<sub>4</sub> (ortovanadate) according to the reaction (14)
   V<sub>2</sub>O<sub>3</sub> + 6NaOH + 2(NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> = 2NaVO<sub>3</sub> + 2(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> + 2Na<sub>2</sub>SO<sub>4</sub> + 3H<sub>2</sub>O (14)
- Filtration and treatment of the solution with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> at temperature 70-90° C (during 1 hour) in the goal of transformation of the vanadium compounds in NH<sub>4</sub>VO<sub>3</sub> according to the reaction(15): 2NaVO<sub>3</sub> + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> = 2NH<sub>4</sub>VO<sub>3</sub> + Na<sub>2</sub>SO<sub>4</sub> (15)
- $NH_4VO_3$  sedimentation at the temperature 18°C.

In the case of acid extraction with  $H_2SO_4$  the vanadium is extracted as  $(VO_2)_2SO_4$ , which is transformed in  $Na_2O \cdot 5V_2O_5 \cdot 2H_2O$  and then - in  $NH_4VO_3$ , according to the reactions (16), (17). The recovery of vanadium as  $NH_4VO_3$  by this method is about 30 %.

$$Ca(VO_3)_2 + 2H_2SO_4 = (VO_2)_2SO_4 + CaSO_4 + 2H_2O$$
 (16)

$$(VO_2)_2SO_4 + 4NaOH = 2NaVO_3 + Na_2SO_4 + 2H_2O$$
 (17)

The alkaline extraction in the presence of the oxidizer is more advantageous in comparison with the acid extraction, because the first method assures a high vanadium  $(NH_4VO_3)$  recovery (about 94 % from vanadium total content in the waste).

#### Discussion

As a result of our investigations for elaboration of some procedure of waste valofication by chemical methods, the optimal conditions of extraction have been established. Such, for chemical neutralization of the hexacyanoferrate waste the maximum concentration of waste in suspension didn't exceed 5%. One of the conditions for quantitative solubilization of the hexacyanoferrate complexes is the reaction with the fresh  $Ca(OH)_2$  (that is obtained as a result of interaction CaO with  $H_2O$ ) that is very active. Another condition for the high solubilization of hexacyanoferrate complexes is a careful agitation of the mixture.

After solid sediments separation (bentonite, rest of  $Ca(OH)_2$  and yeast) its careful washing by water for ions  $[Fe(CN)_6]^{4-1}$  removing has been carried out up to the negative test at the presence of ions  $[Fe(CN)_6]^{4-1}$ . Washing residual water is used in the closed cycle for the dilution of a new portion of initial hexacyanoferrate waste.

The solution containing the  $[Fe(CN)_6]^4$  ions is acidulated by HCl to the value of pH 3-5 and then the sedimentation of ions $[Fe(CN)_6]^4$  is effectuated, according to the reaction (6). After  $Fe_4[Fe(CN)_6]_3$  sedimentation this precipitation is dehydrated and in its dry form it can be used as pigment.

Another type of waste is galvanic waste. There are the following ions in its composition:  $Cu^{2+}$ ,  $Fe^{3+}$ ,  $Al^{3+}$ ,  $Cr^{3+}$  and  $Zn^{2+}$ . Another waste is the iron sawdust that is used for copper removing from the acid solution, it is obtained after the galvanic waste solubilization. One of the conditions for this process is the careful magnetic agitation at the temperature of 40 - 60°C. Another condition for Cu precipitation is the excess of the iron sawdust. The copper reduction by the cementation method at the metallic Fe addition in the quantity of 5-10 times more than the stoechiometric quantity is necessary for copper substitution and separation from waste water.

There is a mixture of  $Fe^{2+}$  and  $Fe^{3}$  in the solution after cooper removing. For sedimentation of these ions the solution of 6N HNO<sub>3</sub> and thermic treatment for 3-4 min are used. As a result, all of iron ions are in the state of Fe<sup>3+</sup>. The qualitative removal of the Fe<sup>3+</sup> and Al<sup>3+</sup> is realized at the 6-8 N NaOH addition, the temperature of 40-45°C, at the magnetic agitation for a quantitative coagulation of Fe(OH)<sub>3</sub> and AL(OH)<sub>3</sub> mixture.

For  $Cr^{6+}$  removing it is necessary to provide a reduction of  $Cr^{6+}$  ions by  $Na_2SO_3$ , according to the reaction (11) at the constant agitation. The next step is the addition of NaOH solution up to the value of pH 5,4-6,8 for  $Cr^{3+}$  and  $Zn^{2+}$  co precipitation. Complete sedimentation of  $Zn(OH)_2$  takes place after increasing the pH value up to 8,8-9,0. The data (tab. 3) demonstrate, that the proposed procedure permits the heavy metals removing from the residual galvanic water up to a high degree, so the metals content after purification didn't exceed 0,5 % from the initial quantity except chromium ions. The resting chromium content in the solution is 2,7 %. This fact can be explained by the partial chromium solubilization at the pH value that is necessary for sedimentation of hydroxides of chromium and zinc. It is worth mentioning that the content of analyzed solution in this work is more complex than in the other investigation [31] because of the supplementation of iron and zinc, galvanic waste including cooper, chromium and aluminum in the essential quantities. Consecutive separation of the metals was possible to carry out, as a result of the combination of method of cementation and chemical methods of separation with reagents. The elaborated procedure assures the qualitative galvanic residual water purification from heavy metals, this fact was confirmed by the low residual concentrations of metals in the residual water after purification [17].

The generation of PTS waste is a serious environmental problem for the Republic of Moldova and especially for Chisinau municipal. This fact has put the beginning for investigating the vanadium content and other components of these wastes with in the goal of its valorification. For vanadium extraction the diverse reagents solutions have been tested: 2N HCl, 9 % H<sub>2</sub>SO<sub>4</sub>, 2N NaOH, HNO<sub>3</sub>conc. + (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, 12,5N NaOH +(NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>. The results of investigation demonstrate that a optimal vanadium quantity can be extracted in the latter case, that is with the NaOH solution and (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (as an oxidizer). Another important condition is the temperature of extraction. There are three essential conditions for maximal vanadium extraction: NaOH concentration, utilization of (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> as an oxidizer, the optimal temperature regime (90-140°C). The role of oxidizer is to transform all the form of vanadium in vanadium (V). The utilization of 30% of NaOH assures the solubilization of V<sub>2</sub>O<sub>5</sub> and the formation of the NaVO<sub>3</sub> [53]. After vanadium solubilization from waste, the next step is the reaction with some compounds of ammonium. This operation is realized between NaVO<sub>3</sub> and (NH<sub>4</sub>),SO<sub>4</sub> at 70-90 °C during 1 hour for NH<sub>4</sub>VO<sub>3</sub> obtaining.

# Conclusions

1. The technology of waste containing hexacyanoferrates has been elaborated and implemented -a contribution for reduction of an essential volume of such waste.

2. The technological scheme for galvanic waste treatment, that guarantees a high degree of residual water purification, has been founded theoretically and elaborated practically.

3. The chemical technology for valorification of waste from PTS and for vanadium obtaining (as  $NH_4VO_3$ ) has been elaborated. It implementation will contribute to the reduction of the impact on environment and to an economical profit of about 17 millions lei.

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# **CYCLODEXTRINS – FIELDS OF APPLICATION. PART I**

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**Abstract:** This review is dedicated to different fields of use of cyclodextrins, a family of three cyclic natural oligosaccharides and their derivatives. The first part of the review gives a brief description of the main and the most recent developed applications of cyclodextrins in food, cosmetic industry, environmental protection technologies and agriculture. Different products based on inclusion complexes with cyclodextrins and technologies with different use of cyclodextrins and inclusion complexes are described.

Keywords: cyclodextrins, food, cosmetic industry, environmental protection, agriculture

Cyclodextrins are a family of three cyclic natural oligosaccharides and their semi-synthetic derivatives. Naturally occurring  $\alpha$ -,  $\beta$ - and  $\gamma$ -cyclodextrins have torus-like macro-rings built up from 6, 7 and 8 glucopyranose units, respectively. As a consequence of the  ${}^{4}C_{1}$  conformation of the glucopyranose moieties, all the primary hydroxyl groups are situated on the one edge of the torus, while all the secondary ones are situated on the other. The internal cavity of the cyclodextrins is lined by the hydrogen atoms and the glycosidic oxygen bridges, which produce a high electron density there and lend to it somewhat hydrophobic character.

In an aqueous solution, the cyclodextrin cavity is occupied by water molecules that are energetically unfavored due to polarapolar interactions, and therefore can be readily substituted by any less polar guest-molecule added to the solution. The properties of the included molecule are very often significantly modified and the formed inclusion complexes find wide distribution in different fields of application.

The main reasons why the cyclodextrins are that popular as guests for inclusion of different substances:

• they are produced from a renewable natural material (starch) applying environmental-friendly technologies (enzymatic conversion);

• they are relatively cheap and are produced in amounts of thousands of tones per year;

• they are nontoxic in consumable concentrations and their secondary toxic effects can be easily eliminated by an appropriate chemical modification;

• they are biodegradable and do not pollute the environment. The main fields of cyclodextrins application are:

- 1. agriculture;
- 2. biotechnological applications;
- 3. chemical and biological analysis;
- 4. chemical synthesis;
- 5. cosmetic industry;
- 6. environmental protection technologies;
- 7. food industry;
- 8. pharmaceutical industry;
- 9. textile industry.



Fig. 1. Forms of natural cyclodextrins

This paper is dedicated to application of cyclodextrins in food and cosmetic industry, environmental protection technologies and agriculture.

#### **Food industry**

Food and cosmetic industries are the largest consumers of cyclodextrins and their derivatives [1]. In the food industry cyclodextrins are employed for the production of a wide variety of goods from alcohol powder to low-cholesterol cheese and milk.

Cyclodextrins are used to replace:

- a) carbohydrates with a high glycemic index such as sugar, starch or starch-derived products to reduce the energy value and glycemic load of the food;
- b) fat in table spreads;
- c) fermentable fibers, some of which may be less suitable because of their high viscosity, insufficient stability, taste or presence of by-products.

In other cases, cyclodextrins are added to foods in which they doesn't directly replace any particular component but perform a range of technical functions such as:

- a) a carrier for natural colors, flavors and vitamins;
- b) a stabilizer of oil in water emulsions, a solubilizer of lipids;
- c) a flavor and aroma modifier by suppressing undesirable characteristics.

 $\gamma$ -cyclodextrin was proved to possess the best flavor retention properties among 3 native cyclodextrins [2]. A group of flavor substances are known to be used under the form of inclusion complexes with cyclodextrin: coffee flavor [3], wasabi flavor additive [4], vanillyl alcohol derivatives [5], lemon oil [6]. Color stabilization in food is another widespread application of cyclodextrins [7-9]. Most stabilized are natural colorants like lycopene, quercetine,  $\beta$ -carotene and others.

The estimated levels of cyclodextrins used as a food ingredient are higher (up to approximately 15%) than when they are used for a technological function (approximately 1%).

Cyclodextrins can stabilize emulsions of fats and oils [10], shielding them from oxidation and thus preventing rancidity. They can also be used to debitter citrus juices through removal of bitter components rather than masking them. [11, 12]

Besides, they can be used in production of powdered products like powdered tea [13], powdered honey [14] or powdered alcohol [15]. Very often cyclodextrins are used for sweeteners stabilization and solubilization: aspartame (1), neotame (2), steviolmonoside (3,  $R=\beta Glc$ ), steviolbioside (3,  $R=\beta Glc$ ) and others. [16-18]



Fig. 2. Structures of some sweeteners for which cyclodextrin complexes have been developed: aspartame (1), neotame (2), steviolmonoside (3, R=βGlc), steviolbioside (3, R=βGlc-βGlc)

With respect to bioconversions and fermentations, cyclodextrins apparently possess an advantageous combination of properties. First, they enhance solubilization of organic compounds. Second, they reduce toxicity since complexation of organic substrates and/or products may significantly reduce their concentrations. Third, they are biocompatible. Cyclodextrins are known to cause no damage either to free enzymes or to micro-organisms.

A wide range of cyclodextrin technologies are applied to produce healthy food products with different effects:

- reduced cholesterol content: homogenized milk [19], cream [20]; cheddar cheese [21], butter [22],

- egg yolks [23]
- hypocholesterolemic effect [24]
- reduced glycemic index [25]
- antiallergic effect [26]

Other possible applications of cyclodextrins in food industry are production of gasified products [27], food cleaning products [28]. In the table 1 are presented different food products and cyclodextrin concentrations approved for them.

Food name		Cyclodextrin		Hydration factor		Level used in modeling,	
	concentration		for αCD /dilution		g/kg		
	level, %		factor for γCD				
	αCD	γCD	αCD	γCD	αCD	γCD	
Baked goods		2,0				20	
Beverage whiteners	1,0	1,0			10	10	
Biscuits	10,0	1,0			100	10	
Breads	5,0	1,0			50	10	
Breakfast foods		2,0				20	
Chewing gum	10,0	1,0			100	10	
Dairy desserts	1,0	3,0	7,8		1,3	30	
Dry mixes for beverages		1,0		10		*1,0	
Dry mixes for dressings, gravies and sauces		1,0		5		*2,0	
Dry mixes of soups		1,0		20		*0,5	
Dry mixes for puddings, desserts, jellies and		1.0		6		*1.7	
fillings		1,0		0		•1,7	
Ice cream/ice confection	2,5				25		
Fat based fillings		5,0				50	
Fermented or rennetted milk	2,5				25		
Fruit based fillings		3,0				30	
Instant coffees and teas	1,0	1,0	113	80	0,088	*0,125	
Processed cheese		3,0				30	
Salt	3,0				30		
Savory snacks	1,0	1,0			10	10	
Soy beverages	2,0	2,0			2,0	20	
Sugar confectionary (compressed)	15,0	1,0			150	10	
Oil emulsions (<80% fat)	20,0				200		
Table spreads		20,0				200	

 $\alpha$ - and  $\gamma$ -cyclodextrins in foods/levels of use [29, 30]

Table 1

\* in the 'ready to consume' version of the food

# **Cosmetic industry**

The cosmetic industry is constantly looking for new and effective products endowed with a satisfactory biological activity and an efficient delivery on the skin, hair and nails. Cyclodextrins, as cosmetic ingredients, are used to solubilize and stabilize sensitive components; stabilize emulsions; improve the absorption of active compounds; stabilize emulsions; reduce or eliminate bad smells of some cosmetic ingredients, and reduce the loss of active component through photodestruction, oxidation, volatilization. Besides, they are used in hygienic products aiming to interact with body secretions (sebum, sweat).

Below are demonstrated different examples of cyclodextrin application in cosmetic products:

1. Use as delivery systems or solubilizers for different cosmetic compounds

- delivery of fragrance materials – linalool (4) and benzyl acetate (5) [31];

- delivery of biologic active compounds coenzyme Q10 (6) [32], adapalene (7) [33],
- solubilizers for hydrophobic preservatives [34, 35].

2. Improvement of physical and chemical stability:

- protection of active ingredient(s) against oxidation – vitamin F in dermatological preparations [36];

- light-induced reactions - retinol [37], 4-methylbenzylidene camphor [38], ferulic acid (8) [39] photostabilization;

- prevention of degradation, heat-promoted decomposition storage stability [40, 41]

- reduction of loss by volatility, sublimation [42-45]
- stabilization of emulsions as emulsifiers [46]
- 3. Improvement of sensoric properties:

- elimination or reduction of undesirable tastes and odors, microbiological contamination – use in deodorants [47];

- modifying hair properties - smoothing hair combability [48]

4. Reduction of penetration of sunscreen agents in skin layers [49, 50]

5. Production of cosmetic preparations for the removal of sebum and seborrhea treatment [51]



Fig. 3. Structures of some cosmetic ingredients for which cyclodextrin complexes have been developed: linalool (4), benzyl acetate (5), coenzyme Q10 (6), adapalene (7), ferulic acid (8)

#### **Environmental protection technologies**

Cyclodextrins have been widely studied as for removal of different contaminants from waste gases, wastewater, contaminated soil. Besides, cyclodextrins are used in technologies of reduction of wastes emissions. Several technologies have been developed and approved by pilot scale experiments. Despite the obvious advantages, the environmental application of CDs is still hindered by their price.

### 1. Gaseous and volatile pollutants sorption:

- Sorption of organic solvent vapor from waste gases of chemical industry by CD solution and CD polymers [52-54];

- Sorption of radioactive iodine from the waste gases of nuclear power plants by CD solutions or CD polymers [55]

#### 2. Wastewater treatment:

- Sorption of toxic compounds by CD polymers [56-60],

- CD-assisted *in situ* oxidation (catalytic effect) – photodegradation of organophosphorus pesticides [61, 62], bisphenol [63];

- Improved biodegradation of xenobiotics by activated sludge - improved bioavailability of the contaminants reduced toxicity, use of cyclodextrin polymers as support for biofilms [64-66]

3. Contaminated soils treatment:

- Removal of organic and inorganic pollutants by soil washing with aqueous solutions of CD derivatives ("sugar flushing" or cyclodextrin-enhanced *in situ* flushing) – pentachlorophenol flushing [67], PAHs flushing [68], pesticide removal [69-72], different organic compounds and heavy metals removal [73, 74].

- Fig. 4 shows a conceptual illustration of the cyclodextrin-enhanced *in situ* flushing (CDEF) of contaminated porous media. The operation generally begins with the injection of a water-based cyclodextrin solution. There are two treatment options: using a system of designated injection and extraction wells to flush the source zone (see Figure 3) or injecting and extracting the flushing solution from one and the same wells, i.e., a push-pull operation. Because the magnitude of solubilization of organic contaminants is a linear function of the aqueous cyclodextrin concentration, the contaminant removal rate increases with the cyclodextrin concentration.

- Mineralization and detoxification of the organic and inorganic pollutants: mercury compounds [75];

- CD-assisted in situ oxidation (catalytic effect) – HPβCD-mediated iron-activated persulfate oxidation of trichloroethylene and tetrachloroethylene [76], photo-Fenton treatment of 2,4,6-trinitrotoluene contaminated soil after flushing with cyclodextrin [77], remediation of hexachlorobenzene in soil by enhanced Fenton process [78];

- CD-assisted electrokinetic remediation (improved mobility) – for 2,4-dinitrotoluene removal [79], for hexachlorobenzene removal [80], for phenanthrene removal [81], for a mixture of organic compounds and heavy metals removal [82];



Fig. 4. Conceptual scheme of the CDEF technology (CD - cyclodextrin, DNALP - dense nonaqueous phase liquid).

- CD-assisted soil bioremediation (improved bioavailability of the contaminants, enhanced biomass production, reduced toxicity) – for phenols and PAHs [83], for 2-nitrobiphenil [84], for pyrene [85], for phenonthrene [86], fluorene [87], transformed oil [88];

- CD-assisted phytoremediation of PAHs contaminated soils [89], PCBs contaminated soils [90];

- CDs for assessment of bacterial degradability of PAHs and other pollutants in soils [91, 92]

4. Reduction of emission of wastes:

- Dose reduction of agrochemicals (insecticides, pesticides, fungicides) by complexation – for 10-undecyn-1-ol [93], for iprodine [94]

- CD-containing, biodegradable plastics [95, 96].

### Agriculture

The main field of cyclodextrins' application in agriculture is pesticides complexation. Technological advantages that can be achieved applying cyclodextrins in pesticides production technologies are:

- 1. stable, standardized compositions;
- 2. improved wettability;
- 3. simple dosing and handling of dry powders;
- 4. reduced packing and storage costs;
- 5. more economical manufacturing processes
- 6. reduced labor costs.

Further are described the general advantages of pesticide-cyclodextrin complexes use with examples of such preparations (Fig. 3).

- improvement of the physicochemical characteristics of pesticides (disguised lipophilicity, improved wettability, decreased vapor pressure, enhanced aqueous solubility, etc.) for chloramidophos [97], for thiabendazole (9), carbendazim (10) and fuberidazole (11) [98], for 3-chloro-*p*-toluidine (12) [99], for 2,4-dichlorphenoxyacetic acid (13) [100];
- enhancement of bioavailability of poorly soluble and absorbable pesticides for imazalil (14) [101], for different fungicides [102];
- improvement of shelf-life (improved stability to heat, light, oxygen) for chloramine phosphate [103];
- minimizing the container/content interaction in packaged formulations;
- improved homogeneity and content uniformity in finished product;
- reduction of the applied dose, minimizing environmental pollution.





Fig. 5. Structures of some pesticides for which cyclodextrin complexes have been developed: thiabendazole (9), carbendazim (10), fuberidazole (11), 3-chloro-p-toluidine (12), 2,4-dichlorphenoxyacetic acid (13), imazalil (14)

Most of the above benefits can be attained by cyclodextrin complexation without chemical modification of the active substances. This means that no new chemical entities are introduced and just the known active compounds are developed in new, molecularly dispersed, stable, water-activated form. This saves time and money on registration process. Moreover, as enzyme-modified starch derivatives, cyclodextrins are considered non-toxic and from the environmental standpoint they are regarded as biodegradable carbohydrates.

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# DESIGN AND SYNTHESIS OF COORDINATION COMPOUNDS WITH TETRADENTATE LIGANDS DERIVED FROM ALKYLATED THIOSEMICARBAZIDE

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Abstract. This work is devoted to the investigation of the template reactions of organic compounds derived from S-alkylisothiosemicarbazide and  $\beta$ -dicarbonylic compounds (acetyl(benzoyl)acetone, 2,6-diformylpyridine, 2,6-diformyl-4-methylphenol, 1-hydroxy-2-naphthaldehyde, sodium nitromalonic dialdehyde, 1,1,3,3-tetraetoxypropane, 3-ethoxyacroleine) in the presence of 3d-metal ions, determination of the structure and properties of the prepared complexes.

There were elaborated original methods for synthesis of open-chain or macrocyclic coordination compounds of nickel(II), copper(II), oxovanadium(IV) and cobalt(II) with  $N_4$ ,  $N_3O$  and  $N_2O_2$  tetradentate ligands. Two types of hexaazamacrocyclic systems with *trans*- and *cis*- arrangement of thiosemicarbazide fragments have been obtained using template condensation of different precursors.

The biostimulative properties (biomass accumulation and increasing of enzymatic activity) of complexes have been established. Some complexes of nickel(II) have been studied as coloring agents for plastics.

Keywords: Template synthesis; coordination compounds; 3d-metal complexes; Schiff base; S-alkylisothiosemicarbazide.

#### Introduction

For some time, semi- and thiosemicarbazones have been a subject of interest to researchers of different profiles. In view of the fact that these organic compounds form with many metals complexes of diverse chemical, physical and structural characteristics, they are of special interest for the specialists in coordination chemistry [1–5]. In addition, many of these organic reagents as well as their metal complexes, have shown a wide spectrum of biological activity, so that they have also become a subject of intense research for pharmacologists [6].

A number of complexes, primarily of transition metals, involving thiosemicarbazones of different denticity have been prepared and investigated [1-8]. In contrast to thiosemicarbazide fragment, which ones are usually coordinated *via* azometinic nitrogene and sulfur atoms, the isothiosemicarbazides and their derivatives, as a rule, involve for coordination the nitrogen atom of the isothioamide group instead of the sulfur [3, 9].

A consequence of this fundamental difference in the coordination mode of thiosemicarbazones and isothiosemicarbazones is the change in the reactivity of the  $NH_2$ -group. Hence, in the case of isothiosemicarbazones, this group undergoes condensation reaction with bifunctional carbonyl compounds, yielding, among others, macrocyclic complexes [4, 5].

# **Results and discussion**

The main goal of this work is the design and synthesis by template method of new coordination compounds with diverse composition, on the matrix of different metals using S-alkylisothiosemicarbazides and different carbonylic compounds as ligsons.

As dicarbonylic compounds were used acetylacetone, benzoylacetone, nitromalonic dialdehyde, 3-ethoxyacroleine, 1,1,3,3-tetraetoxypropane, 1-hydroxy-2-naphthaldehyde, 2,6-diformylpyridine, and 2,6-diformyl-4-methylphenol.

The study of the reaction of S-methylthiosemicarbazide with acetylacetone in the presence of oxovanadium(IV) and cobalt(II) ions showed that the property to orientate the reactant species is characteristic not only for nickel(II) ions, but for other metal ions too. In both cases the formation of the compounds **1** and **2**, with open-chain ligand acetylacetone bis-(S-methylthiosemicarbazone) according to the scheme 1 was observed:

The acetylacetone bis-(S-methylthiosemicarbazone) in solutions is involved in a series of tautomeric and protolitic transformations (Scheme 2), and upon the value of pH and the nature of metal ions, different forms can be stabilized. For the nickel and cobalt(II) cases the moderate alkalinity (presence of acetate ions) gives rise to the stabilisation in the complex **2** of the uninegatively charged tetradentate  $N_4$  ligand, (form F). When the reaction occurs in the presence of

oxovanadium (IV) ions, the formation of the complex 1 requests a more alkaline medium (the presence of a strong base triethylamine), and as a result, the stabilisation of the twice deprotonated  $N_4$  ligand (form E) is observed.



Scheme 2

The nature of the initial  $\beta$ -dicarbonilic precursor il also responsible for the sense of the reactions in these systems. In the case of benzoylacetone the keto-enole tautomerism is much more directed to the formation of the enolic species and only the formation of benzoylacetone mono-S-alkylisothiosemicarbazone is possible. This organic reagent can be involved in a lot of template reactions. In the presence of nickel ions the formation of two types of complexes (**3** and **4**) has been observed (Scheme 3). After separation, purification of the products an X-ray investigation has carried out. The molecular structures of the complexes are presented in the Figs. 1 and 2.





Fig. 1. Molecular structure of the complexes 3 and 4

The appearance of NiL<sup>3</sup> (4) complex is an usual already process, which can be explained by the condensation of two molecules of benzoylacetone mono-(S-methyllthiosemicarbazone) in the coordination sphere of nickel ion [11]. The synthesis of NiL<sup>2</sup>(3) denotes a much more complicated state of the system, which is attended with a degradation of the initially compound with elimination of acetophenone. This compound may be considered as pseudo-macrocyclic, stabilized through the formation of hydrogen bond. This structure, once more, shows the possibility of acyclic arrangement of the systems around the metal ion, facilitated by the formation of the hydrogen bonds of marginal group.

An unexpected result is the coordination mode of the isothiosemicarbazidic fragment in  $[NiL^2]$ . Coordination of the second fragment via N(5) atom is dictated by the stereochemical peculiarities of the nickel ion, which one has the tendency to form compounds with square- planar configuration and by versatile character of the thiosemicarbazide fragment's coordination.

This phenomenon is emphasizing, when the number of coordination centres offered by ligand exceeds the requirements of central atom. For optimization of the structure and to satisfy the stereochemical necessity of the central ion the ligand accepts an unusual conformation. This behaviour was also observed in the case of nickel coordination compounds with 2,6-diformyl- and 2,6-diacetyl pyridine bis-(S-methylthiosemicarbazone) [10]. In general these two ligands are potentialy pentadentate and can be coordinated by the metal ions through N<sub>5</sub> set of donor atoms, including the pyridine nitrogen, two azometinic nitrogen atoms of the both isothiosemicarbazidic moieties, as well as the marginal nitrogen atoms of the same fragments. This coordination mode was confirmed for the manganese 2,6-diacetylpyridine bis-(S-methylthiosemicarbazone) compound, where the organic ligand is pentacoordinated by the central atom in the equatorial plan. In the case of nickel complexes the both ligands adopt a tetradentate coordination as is shown in the Fig. 2 for the 2,6-diformyl-derivative.



# [Ni(HL<sup>4</sup>)]I (5)

Fig. 2. Molecular structure of the complex 5

The presence of the double bonds in the organic reagent invokes an effective conjugation on whole molecule, which prompts a planar structure. These desiderates offered by ligand are not accepted by nickel ions, which dictates to the ligand an other configuration. The both isothiosemicarbazidic fragments are condensed in different configurations, vis-à-vis to azometinic bonds –RC=N-. One fragment adopts E configuration and uses the marginal nitrogen atoms for coordination, an the second one - a Z configuration, which offers the possibility to be coordinated to the nickel ion via donor atom N(2). Thus, nickel ion conducts the reaction of condensation in a certain way and hinders the formation of compound, which can contain both thiosemicarbazidic fragments in E configuration. This is an example of metal promoted reactions. For the same central atom the nature of the dicarbonylic block has an essential impact on the complex geometry. Comparing the nickel compounds prepared by template reaction of S-methylthiosemicarbazide with 2,6-diformyl- or 2,6-diacetyl-pyridine, the formation of the complexes of the same composition NiLI can be observed. The difference is manifested in the complex configuration: one of them has a plan-square structure, the second - the configuration of a tetragonal pyramid with coordination of the iodine ion in apical position.

Apparently there are no significant changes of the power of the crystalline field formed by ligand after substitution of the hydrogen in 2,6-diformylpyridinic moiety by methyl group. The observed change of complex geometry can be explained by the presence of the methyl bulk groups which ones taking off the species [NiL]<sup>+</sup> one of another, creates conditions to arrange the iodine ion in apical position, so the coordination number of nickel becomes 5. In the case of 2,6-diformilpyridine, these restrictions are far-off, the species [NiL]<sup>+</sup> are arranged more compact, so in apical position is placed the sulfur atom of the neighboring molecule. At these conditions, distance Ni-S remains less than the sum of the Van der Waals rays and can not be consider as a chemical bond. The complex configuration remains square-planar.

Combination of the benzoylacetone mono-S-alkylisothiosemicarbazone with other carbonylic components (for example 2, 6-diformyl-4-methylphenol or 2-oxynaphthaldehyde) makes possible to obtain on the matrix of nickel, cobalt, oxovanadium(IV) or copper(II) new coordination compounds containing double condensed isothiosemicarbazide fragment [12]



#### Scheme 4

The formation of the complexes 6-17 has been demonstrated using a number of spectral, magnetic, and chromatographic methods. For the complexes 10 and 13 an X-ray investigation has been undertaken. Their molecular structures are presented in the fig. 3.

The above mentioned complexes **6-17** are not the only species, which are present in the system. The partial desegregation of the initial ligson produces fragments with an increased reactivity, which ensures their fit in new combinations, with an unusual arrangement of atoms and bonds. In the presence of 2,6-diformyl-4-methylphenol or oxynaphthaldehyde the formation of the complexes **3** and **4** has been also observed. These transformations prove the complexity of the process which induces the diversity of the final products. Thus, the reaction with the higher yield can not be considered as single possible transformation. It is the reaction the more advanced, but not the only process.

The lockout of another process as frequently occurs by solvent implication. The interaction of benzoylacetone mono-S-methylthiosemicarbazone with 2,6-diformyl-4-methylphenol in methanol gives rise to the condensation in molar ratio 1:1. The acetals formation prevents the condensation with the second group and thus the generation of dinuclear compound [12].



[NiL<sup>7</sup>] (10)

 $[CoL^7]$  (13)

Fig. 3. Molecular structures of the complexes 10 and 13

The above mentioned complexes **6-17** are not the only species, which are present in the system. The partial desegregation of the initial ligson produces fragments with an increased reactivity, which ensures their fit in new combinations, with an unusual arrangement of atoms and bonds. In the presence of 2,6-diformyl-4-methylphenol or oxynaphthaldehyde the formation of the complexes **3** and **4** has been also observed. These transformations prove the complexity of the process which induces the diversity of the final products. Thus, the reaction with the higher yield can not be considered as single possible transformation. It is the reaction the more advanced, but not the only process. The lockout of another process as frequently occurs by solvent implication. The interaction of benzoylacetone mono-S-methylthiosemicarbazone with 2,6-diformyl-4-methylphenol in methanol gives rise to the condensation in molar ratio 1:1. The acetals formation prevents the condensation with the second group and thus the generation of dinuclear compound [12].

The initially prepared complexes using template methodology can be involved in the next step to build new macrocyclic edifices with a given configuration. Thus, the acetylacetone bis-(S-methylthiosemicarbazone) cobalt and nickel complexes, prepared by template reaction, were used as already assembled form, in order to produce macrocyclic systems. The macrocyclic compounds (18) and (19) [8] with *cis*- arrangement of thiosemicarbazidic fragments were prepared by tailoring the sodium salt of the nitromalonic dialdehyde or 1,1,3,3-tetraetoxypropane to the chelate precursor of the type 2, following the scheme 5. The compound (18) is paramagnetic, stable at high temperatures and can be thermally decomposed at 193 °C. The central ion is formally in oxidation state +2, confirmed by magnetic properties.

The macrocyclic cobalt(III) complex (19) with coordination number five can be synthesized coming from the open-chain compound  $[CoL^{1}I]I \cdot CH_{3}OH$  with square-pyramidal structure as matrix (or template) and 3-etoxyacroleine as tailoring agent.

The condensation of 3-etoxyacroleine with the thioamidic nitrogen atom of isothiosemicarbazidic fragment leading to the formation of a macrocyclic system was confirmed by IR spectra and mass-spectrometry. In the mass-spectra of (19), the most intense pick  $[M-I]^+$  and molecular pick  $[M]^+$ , which belongs to cobalt complex with macrocyclic equatorial ligand were recorded. The presence of the pick  $[M]^+$ , confirms the coordination of I<sup>-</sup> to the central atom. Taking into account these data square pyramidal structure with I<sup>-</sup> anion in apical position has been suggested for (19).


#### Scheme 5

Other nickel(II) and copper(II) coordination compounds (20 - 23) with hexaazamacrocyclic ligands with cis-configuration of the isothiosemicarbazide fragments can be prepared starting from nitromalonic dialdehyde bis-(S-methylthiosemicarbazone) and 1,1,3,3-tetraetoxypropane as closing agent [8] (scheme 6):



#### Scheme 6

Their purity, composition, structure and physico-chemical properties have been established using the elemental analysis and a number of physical methods such as, thermogravimetry, mass-spectrometry, IR, UV-VIS, NMR spectroscopy). For the copper(II) complex 23 an X-ray investigation was undertaken. The molecular structure of 23 is shown in fig. 4.



Fig. 4. Molecular structure of the complex 23

The complexes prepared using this technology has not only an original structure or a new arrangement of the atoms and bonds. They possess also a number of useful properties. So, the influence of NiL<sup>6</sup> (8) and CuL<sup>6</sup> (9) on the *Aspergillus niger 412* fungus production of lipolytic enzymes were studied. The obtained result proves obvious the increasing of lipolytic activity of studied fungus, biomass yield when NiL<sup>6</sup> and CuL<sup>6</sup> complexes have been added.

The influence of nickel(II), copper(II) and oxovanadium(IV) cooordination compounds:  $[ML^7]$  (10 – 12) and  $[ML^8]$  (14 - 16) on pectolytic enzymes biosynthetic process by *Penicillium viride* fungus has been studied. Tested complexes demonstrate the stimulation properties (the biomass accumulation, the increase of enzymatic activity). These properties are influenced by the presence of Ni<sup>2+</sup> and Cu<sup>2+</sup> ions and ligands nature [14].

Some complexes of nickel(II)  $[NiL^7]$  (10) and  $[NiL^8]$  (14) have been studied as dying agents for plastic [15, 16]. Varying the concentration of coloring agent and polymer type has been obtained the samples with different intensity and transparency of the color. These compounds are suitable for coloring the plastic (polyethylene and polystyrene) with high photostability.

#### Conclusions

The geometry, composition and the properties of compounds prepared by template methodology using the dicarbonylic blocks and alkylisothiosemicarbazidic fragments can be diversified by the variation of the carbonylic component, template center, and substituents. The nature of the used β-dicarbonylic blocks for assembling, as well as the structure of the precursors are responsible for the succession of the condensation of the reactant blocks. By their selection can be targeted the synthesis of coordination compounds with *trans*- and *cis*- arrangement of isothiosemicarbazidic fragments. Some compounds of this type display biologic activity and stimulate the biosynthesis process of some fungus - producers of extracellular hydrolyze (biomass accumulation, the increase of enzymatic activity). The biologic activity is influenced by the nature of metal ion and of ligands. The colouristic properties of some complexes can be used to colouring the plastics [15].

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## EMERGING CHEMISTRY ON THE OIL OF THE FUTURE. REVIEW PAPER ON DAN NOCERA'S RESEARCH

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"When you bite a green leaf, you are chewing the photons of light" /Dan Nocera/

**Abstract.** The paper is an overview of Daniel Nocera's work towards obtaining energy from the splitting of water. The review describes the challenge of storing high energy bonds and presents some of the important advances the researcher managed to accomplish.

Keywords: renewable energy, photocatalysis, porphyrin macrocycles, Rh bimetallic complexes

#### **Designing the Oil of the Future**

The main reason I became curious in Dan Nocera's work was his genuine and active interest in the "molecular chemistry of renewable energy" (1) as he himself calls it. Essentially, he is developing new ways of obtaining energy from the splitting of water, because of the large pressure between the forecast of energy usage and the energy availability for the future. He built up his research on the oil of the future on a natural model, i.e. that of plants. Plants are basically capturing the solar energy and storing it in a very interesting form. As Nocera explains in a web cast, "The Role of New Technology in a Sustainable Energy", plants take up water, that has low energy bonds, use the solar energy to break up the low energy bonds and form high energy bonds. The plant then stores these and releases the energy difference when it is needed by combining the hydrogen and oxygen back to forming the low energy bonds of water. So, "when you bite a green leaf, you are chewing the photons of light" (1).

One of the interesting questions he brought up during his talk was why plants do not make plastics and waxes or other types of oil instead of water. It must be that water is the most efficient reactant *and* product. Thus, Nocera decided that the oil of the future must be water. The challenge that Nocera come across when working with water was mainly the storage of the high-energy H-H or O-O bonds. The papers that I chose for this review concentrate on dealing with this challenge.

#### The Chemistry *Think*

One of the ways to deal with the challenge of storing the high energy bonds is by synthesizing metal complexes or macrocycles that would be stable enough to store them but sensitive enough to light in order to release them. There are a couple of issues with this such as the efficiency of the process, the impact of side products, and the reusability of the initial reactants and so on.

Nocera looked at nature one more time. He knew that water is regenerated at the end of the cycle, and can be reused in subsequent cycles. Therefore, he felt the need of designing a chain of reactions in which the complexes used in holding the high energy bonds will be regenerated at the end and could be subsequently reused. The reactions use only light energy, thus the chain takes the form of a photocycle. The advantages of photocycles include the need of less initial reactants and higher product yields, since the reactants will be used over and over again. This deals partly with the efficiency issue and the reusability of the initial reactants.

The one thing left to design are the reactants. Here, the issue of side product is taken into account. Also, the reactants or any of the chemical compounds used in the process should be stable, non-toxic, easy to handle and relatively easy to synthesize. As source of hydrogen, he considered the hydrogen halides. As source of oxygen, he considered water. As for the compounds used to store or hold the high energy bonds or to help in their formation, Nocera considered 3 types of photocatalysts with different ligands that would have 2 electrons available in their inventories. Thus, Nocera's group tried two-electron mixed valency electrons with stabilizing ligands for the M<sup>n</sup>-M<sup>n+2</sup> construct, metal complexed porphyrinogens and bimetallic macrocycles.

#### Do

In a paper published in 2004, Nocera and his collaborates describe their research on oxygen and hydrogen photocatalysis using the bimetallic complexes, porphyrinogens and macrocycles. The idea of developing the twoelectron mixed valence bimetallic complexes comes from the need of two electrons in a two electron step reaction. As previously studied, the one-electron mixed valence complexes are used in one electron steps. Hydrogen, oxygen and water activation are, however, multielectron processes and thus the need for two-electron mixed complexes.

H<sub>2</sub> Generation



Fig.1. Reactions of the photocycle in Scheme 1 (2).

#### Hydrogen

Initially, the cycle was constructed around a homo two-metal complex with ligands that would easily leave the complex as soon as it reacts with hydrogen halides (Scheme 1). Two halogens would take the place of the ligand and a mole of H2 would be produced after photolysis generating a bimetallic halide blue intermediate that readily converts to a mixed valence bimetallic halide complex. By using a halogen trap and photoactivating the metalhalogen bond, the halogens can be replaced with the initial ligands and thus the complex would be regenerated (See Figure 1). For instance, when using Rh as the metal, it is necessary to have it complexed with ligands that stabilize the Rh0-RhII rather than RhI-RhI. The bidentate ligands bis(difluorophosphino)methylamine (dfpma) and bis(difluoroethoxyphosphino)methylamine (tfepma) (Figure 2) can destabilize the RhI-RhI and thus ease the conversion to the mixed valence complex. In addition, the CO ligand was studied for its ability to open a coordination site for the hydrogen halide attack (2).



Scheme 1. Photocycle for Hydrogen production from hydrogen halides. (2)

More important however, would be complexes that are able to hold on to the hydrogen and then eliminate it as a high energy H-H bond. For that, iridium, a third-row metal, was used as the core metal of the complex, since it has a greater stability when bonded to H, than Rh, a second-row metal, does. So, except the increased stability that iridium provides to hydrogen and hydro-halides complexes, it also allows for an easy addition or elimination of the hydrogen. First, the hydrogen adds as an H-H bond to the metal in the higher oxidation state. Next, one of the hydrogens can either pass to the second iridium to give rise to a *fac* stereochemistry, or, both hydrogens end up on the same iridium metal, thus forming the *mer* stereochemistry of the two electron mixed valence complex. The elimination of the hydrogen is just the reverse of the addition (2).

The importance of the partially reduced bimetallic complex intermediate being less stable than the  $M_2^{II}$  complex is stressed. What is studied much more in detail or otherwise more clearly explained in this article is the relation between the ligands and the metal core, namely the metal-metal bond stabilization by different combinations of a bridging bidentate ligand. For instance, as shown in Figure 2, the ligand containing the electron donating nitrogen and two electron withdrawing phosphines on the sides suits best the purposes of the photocatalysts in the designed photocycle because it stabilizes the two-electron mixed valency bimetallic complex (3).

Furthermore, experiments were carried out to determine the structure of the blue intermediate (See Scheme 1). First of all, it was determined that it is produced through an intra-molecular mechanism, which could happen only if the hydrogen adds to produce the mer stereochemistry. The blue intermediate is not stable in the presence of the electron donating nitrogen of the dfpm. In order to isolate and study it, however, Nocera's team replaced the dfpma ligands by the tfepm. Spectroscopic data showed that the intermediate was actually characterized by a d<sup>8</sup>---d<sup>8</sup> transition metal pattern of peaks. Thus, they synthesized Rh and Ir d<sup>8</sup> complexes where the metals with an oxidation of I are bonded to tfepm ligands and two halogens, i.e. Cl. Rh,<sup>1</sup> was stable in solution, but Ir,<sup>1</sup> converts to the asymmetric mixed valence two electron complex  $Ir_2^{0,II}$ . Therefore, the hypothesis that predicts the tfepma and dfpma ligands to stabilize the mixed valence bimetallic complexes for their two  $\pi$ -accepting structures connected through a  $\pi$ -donating group holds. A new and more detailed cycle can thus be drawn (Scheme 2).



Scheme 2. Complete photocycle for hydrogen production from hydrogen halides using Rh complexes (3).



Fig. 2. The bimetallic Rh complexes stabilized by the bidentate ligands (3).

A crucial role in the photocycle is played by the metal halide complex. In order to close the cycle it is completely necessary to regenerate the initial compound from the metal halide complex. This is done by the elimination of the halogen. Nocera's team worked on increasing the efficiency of the halogen photo elimination by using early transition metals in high oxidation states for their larger oxidizing power(2). One option is then to use the complexes in which the early transition metals are most commonly encountered, such as porphyrins. Although porphyrin framework is favorable for halogen coupling, experiments proved activation of the M-X bond to fail as a result of the photoreduction of the porphyrin. Another option then would be to apply the design of the photocatalysts described earlier for Rh and Ir to early transition metals. The molybdenum and tungsten complexes developed in this manner did not work as well, for they were inert to oxidation (2).

The second type of complexes that Nocera's group developed are macrocycles. These are unique complexes and very different from the two electron mixed bimetallic ones because the ligands are the ones handling the electrons and not the metals as in the bimetallic complexes. Here they

used porphyrinogens (Figure 3), but modified the core metal to Zn and used "redox-inert and spectroscopically silent counterions" (2). They studied the 3 oxidation states of the Zn porphyrinogen and compared them with the Rh and Ir bimetallic complexes. The distinction between the two lies in the fact that the intermediate of the bimetallic complex is symmetrically oxidized and reduced. In the case of the Zn porphyrinogen, the redox process occurs only at the margin of the complex, which does not affect the geometry of the metal. In the case of Rh and Ir, the oxidation state of one of the metals changes, and so does the geometry of the coordination sphere (2).

A subsequent idea for research thus emerged - develop catalysts for three electron processes. Nocera's group synthesized a porphyrinogen with an iron center that is supposed to be redox-active and thus change its oxidation state contributing one more electron to the process in addition to the two from the ligands (2).

At this point in time Nocera deduced that the only way that the energy efficiency of this cycle can be improved is by concentrating the research on the photoelimination step. In the earlier paper he tried out prophyrins and porphyrinogens as ligands which failed to ease the activation of the metal-halogen bond (2). Now he turns his attention to the third-row transition metal – gold. Some of the characteristics that favor gold as the core of the bimetallic complex are its multiple oxidation states, high reduction potential, good coordination geometry, better sensitivity to light when it comes to halogen photoelimination and, in addition, Au-Au complexes have already been studied (3).

A digold complex with the standard compared to and structurally analyzed and compared to

the previously developed Rh and Ir complexes. Also, a mixed metal complex Rh---Au was synthesized and structurally analyzed. It was determined that just like the  $Au_2$  complex, there is no formal bond between Rh and Au metals and that the geometry around the rhodium metal is almost square planar. In a more recent paper a follow-up of the idea of heterobimetallic mixed-valence complexes was investigated. This time the research concentrated on synthesizing a bimetallic complex that would subsequently produce a Rh<sup>II</sup>-Au<sup>II</sup> complex. The latter could then be used in the photocycle (4).

The geometry around the two metals was determined by X-ray crystallography to be square planar for Rh<sup>I</sup> and distorted trigonal for Au<sup>I</sup>. The distance between the two metals clearly attests that there is no formal bond. However, oxidation of this complex gives rise to a Rh<sup>II</sup>-Au<sup>II</sup> complex that has formal bond with a pseudooctahedral geometry around the rhodium and a square planar geometry around the gold metal. Yet, Rh<sup>II</sup>-Au<sup>II</sup> complex is not stable at ambient temperature and the bond between the two metals breaks, thus forming two other metal complexes: one of Rh<sup>III</sup> and another one of Au<sub>2</sub><sup>II</sup>. The conclusion drawn is that the bond in the heterobimetallic complex is highly polarized towards Au, leading to a destabilization that induces one electron transfer from the rhodium's d<sup>7</sup> to gold's d<sup>9</sup> generating a d<sup>6</sup>-d<sup>10</sup> complex. This heterobimetallic complex is therefore unstable toward the disproportionation of the bond and, subsequently, it cannot be used in the photocycle. That is why there is a need of investigating other metals that would be bonded so that there is no tendency towards electron transfers. Since the Au-X bond is sensitive to light and would contribute to the increase in quantum efficiency of the cycle, it would make sense to keep this metal and investigate one that would form a stable covalent bond with gold. Nocera is proposing Pt<sup>III</sup>, which has a closer orbital energy to that of gold than rhodium (4).

#### Oxygen

When dealing with oxygen it is important to consider the complexity of making the high energy O-O bonds and again look back at "Mother Nature", specifically at Photosystem II in plants. Just like with storing the H-H, there are some challenges with storing O-O. First, the source of oxygen could be water, which has to be activated in order to obtain the oxygen. This would definitely be a multi-electron process. Second, unlike for the hydrogen, complexes that would be holding the oxygen would probably be more complex geometrically since they have to accommodate a bigger atom. It is an extremely difficult task to keep in mind these two points in designing the chain of reactions that would activate oxygen from water, generate and store it for use, since all reactions in the chain should, in addition, be easy to control with close to no side products (3).

The complexes Nocera came up with for the oxygen photocatalysis are the Pacman porphyrins, which are basically 2 porphyrin macrocycles cofacially arranged and connected by xanthene or diphenyl furan. In his research during 2004, he initiated a light design of the oxygen photocycle. Unlike the hydrogen phtocatalysis in the oxygen photocycle, oxygen is not eliminated as a molecule, but rather taken in by the bisporphyrin complexes, which can eventually oxidize phosphines, sulfides and olefins, thus releasing the sought energy (2).



Scheme 3. Photocycle for the activation of oxygen with cofacial porphyrins bridged by xanthene or diphenyl furan (2).

As shown in Scheme 3, when oxygen is introduced in the system, the two irons immediately capture it. Light is then shined on the complex and one of the irons loses the covalent bond and returns to the original oxidation state, the other iron though undergoes an increase in the oxidation state becoming Fe<sup>IV</sup> with oxygen doubly bonded to it. This species then releases its oxygen in oxidizing phosphines and sulfides to regenerate the di-iron (II) bisphosphyrin (2).

The oxygen atom transfer efficiency depends on the bridge between the two porphyrin groups. Nocera's team determined that when using the diphenyl furan the complex is "spring-loaded", or in other words it has the ability to open up the space between the two porphyrins more than when xanthene is used in bridging. Thus, the diphenyl furan allows for more room where oxygen is attacked. To increase even more the oxidizing power of porphyrins some ligands were replaced with fluorine that is strong enough to leave the complex relatively deficient in electrons and thus more sensitive to photolysis. This modified complex is able to oxidize olephines in addition to sulfides and phosphines using a longer wavelength (2).

Nocera tried to increase even more the pocket size in order to avoid the formation of the bond between the metals and the oxygen. Iron has been replaced with Co(II) and xanthene and diphenylfuran bridging ligands were used again. These complexes are perfect for the reduction of oxygen to water, which is actually a 4 electron process. Still, they did not manage to prevent the formation of the  $\mu$ -oxo metal bond. The logical solution to avoid the formation of this bond was to synthesize a monomeric porphyrin complex and that is exactly what they did (Figure 3) (3).



Fig. 3. The water hanging off of the pendant acid group on the Hangman platform (3).

This novel complex has a Hangman platform that permits the suspension of a water molecule over the metal hydroxide. From the structural point of view, the platform works in a closely similar manner to the cytochromes. It is basically an orthogonal proton coupled electron transfer process (PCET). So, while the oxygen undergoes a bond making reaction with the Fe of the complex, two electrons are taken up by the porphyrin ring, while the protons are transferred to the pendant acid group. It was determined that this group plays the same role as the water channels in natural enzymes. The reaction was monitored by stopped flow kinetics and it was found that the splitting of the  $O_2$  is clean and occurs heterolytically for the presence of the pendant acidic group (3).

The bisporhyrin complexes were developed and used in activating the oxygen through olephines, this latter mentioned monoporphyrin complex was developed to activate the oxygen from water by splitting it. As described in the paper (dampsey) the hydroxide ion is more difficult to oxidize so the research team worked on replacing the pending acid group which is unable to stabilize the ion with groups like amidine. In addition, they increased the electron withdrawing potential of the porphyrin macrocycle by fluorination, as it was also discussed in the previous paper. Further research that Nocera's group is looking into is the effects of photooxidants in the macrocycle and other macrocycles like Re(I) polypiridyl complexes (3).

#### Get (or the All-in-a-snapshot-part)

Through continuous and intense research Nocera and his team are on the way of developing the oil of the future and more specifically methods to get the energy out of it as efficiently as possible using only light as an input.

As specified throughout the paper, there are two main sides on which Nocera is concentrating: the hydrogen and the oxygen. Water contains low energy bonds and it is used by plants as a recyclable medium to create the high energy bonds of H-H and O-O. Living organisms have very efficient mechanisms of obtaining and storing this high energy bonds and eventually using the energy off of them by producing water (1). Based on this idea, Nocera developed complexes that would activate hydrogen halides, olephins or water to obtain the high energy H-H and O-O compounds. The complexes are usually metallic or bimetallic cores with stabilizing ligands (2-4).

With hydrogen, Nocera started out with a homobimetallic complex and a rough picture of the cycle that the complex would go through (2). He got as far as synthesizing a heterobimetallic complex with two electron mixed valency  $Pt^{III}$ -Au<sup>II</sup> and ligands dfpma and tfepma that stabilize the end product complex but not the symmetrically reduced and oxidized intermediate (4). The structure and properties of the intermediate where determined (3). The gold is used as one of the metals for its light sensitive Au-X bond, fact that enhances the photoelimination to regenerate the starting complex, thus increasing the photochemical quantum yield (3). Pt will substitute the Rh in the M-Au complex, which was studied in one of the papers, but was found unfavorable for the process for the high metal-metal bond disproportionation, because of the large energy difference. Pt is still a d<sup>7</sup> transition metal, but the energy gap between Pt and Au is smaller, hence the one electron transition from the d<sup>7</sup> to the d<sup>9</sup> would not be expected (4).

With oxygen, the approach is a little bit more difficult, since the final goal would be the splitting of water. Initially, Nocera sought the design of a similar photocycle as that for the hydrogen photooxidation, however the challenge was to accommodate the bigger oxygen. Therefore, he used a macrocycle made of two porphyrins bridged by xanthene or diphenyl furan instead (2). The aim was still the activation of water, but that was not possible for the formation of the bond between metal core of the porphyrin, i.e. Fe(III)-O-Fe(III) (2,3). Although the energy efficiency of the cycle using this complex was not as best as it could be, the bisporhyrin could still be used for studying purposes. For instance, when shining light on it, the Fe<sup>II</sup>Fe<sup>IV</sup>=O complex forms. It reduces olefins easily, forming epoxides that are very reactive species. The initial bimetallic complex is then regenerated (2). The research was taken one step further synthesizing

completely new monoporphyrin complexes as a Hangman platform that have the ability to hold an orthogonal PCET reaction for O-O formation and heterolytic splitting with water being able to enter the complex and hang from the pending acid-base group (3).

#### Breadth, Depth and the Rest

One of the most interesting things I found about all three papers, is the assumption that the work on this research topic is followed by the reader starting the first article of the group published in this area. In other words, the more recent the article is the less information about the general goals of the research and the discoveries or conclusions made so far is expressed. For instance, the last article analyzed provides minimal data about the photocatalytic cycle and the purpose of it, whereas the first article (and probably more in one of the first ones published by Nocera's group) contains enough relevant information on the way in which the cycle works and how it has been developed. Probably, if I had time to venture, or if I knew exactly that reading another article, for that matter one of the first ones, would decrease my time spent on understanding the purposes behind Nocera's experiments, I would have definitely read one more.

A very strong point related to the writing style in the first paper were the small summaries or important points written in bullet form at the end of each compartment that described a certain part of the experiment. They reinforced the goal of the research as well as the actual importance of the experiments discussed antecedently.

#### **My Perspective and Proposal**

So far in this paper I described the research that Dan Nocera and his group developed in the field of molecular chemistry of renewable energy. I talked about water being the perfect reactant and product in this process. I also mentioned that the problem that scientists are facing at the moment is the lack of an efficient system to produce, hold and then

release  $H_2$  and  $O_2$  to form water and give out energy. So, precisely this issue was at the core of Nocera's research. He approached it by constructing metal complexes that would hold the bonds and he designed reactions that would run in cycles and use solar energy. This system is, in essence, simulating the process of photosynthesis but outside the leaves. I will take my time in this last section, however, to propose some further investigations on Nocera's research, from my own perspective, that of an undergraduate. Hence, this section will focus on two different respects:

- improvements to Nocera's method of hydrogen production
- alternative complexes for oxygen activation.

Although the photocycle with the two-electron mixedvalence bimetallic complexes seems to be working, the questions that I posed myself from the start were related to the use of HCl as a reactant. How much hydrogen halide will be used? Where would this hydrogen halide come from? And what can be done with the halide obtained after the photoeleimination? Water is more abundant in comparison to hydrogen halides and would be perfect to use instead. I believe that the complex that Nocera developed, however, won't be able to hold the oxygen, and water might add to the metal as aqua instead of separate hydrogen and oxygen molecules. With



Fig. 4. Complete photocycle (from Ref. 2) with the incorporated water-halide cycle

the hydrogen halides the whole hypothesis of producing energy from greener sources collapses, but water in this case is more difficult to handle. Hydrogen halides suit this photocycle better because although each of the hydrogen halide adds to one metal, the network of ligands and the two-electron mixed valency of the metals allow bridging transfers to occur and, hence, facilitate the photoelimination (3).

This whole process has been proven to work only on a laboratory level. If, however, it is upscaled Nocera would have to confront the hydrogen halide issue. In his research he determined that it is the photoactivation of the M-X bond that blocks the efficiency of the cycle. So, he changed the metal from rhodium to gold, which is known for its photosensitive halide bonding (2, 4). He also changed the solvent from 2,3-dimethyl-1,3-butadiene (DMBD) to tetrahydrofuran (THF). A ten fold increase in efficiency was observed (5). However, THF probably creates a problem, because it constitutes the halide radical trap and if the process is upscaled there will be some THF residue.

To increase the efficiency of the use of hydrogen halides and to avoid any THF residue a cycle that would

regenerate the HX could be constructed (See Figure 4). For instance, if we use hydrogen chlorides we could try to regenerate Cl<sub>2</sub> at the end of the photocycle according to this simplified reaction:

2HCl  $\rightarrow$  H<sub>2</sub> + Cl<sub>2</sub>

The eliminated chlorine could be activated and let to interact with water and produce the following (7,8,10):  $Cl_2 + H_2O \Leftrightarrow HCl + HClO$ 

It was determined that the equilibrium for the chlorine hydrolysis lies much more to the right than for bromine (8). Therefore, the use of chlorine as the halogen in the photocycle is more suitable. The HCl will be used in further reactions in the photocycle. The HClO under specific conditions could dissociate to form another mole of HCl and half the molar amount of oxygen (personal assumption, not found supporting literature):

HClO  $\rightarrow$  HCl +  $\frac{1}{2}$  O<sub>2</sub>

The oxygen product from this reaction could subsequently be activated by the oxygen photocycle also described by Nocera.

The reactions above can be integrated into Nocera's photocycle as depicted in Figure 4. The main focus of the cycle would be the photoelimination of the halogen without the use of a trap. The idea is to eliminate an activated halogen that would be able to interact with water and regenerate the hydrogen halide. The radical halogens formed during photolysis are very reactive species and could be used. As described earlier, in Nocera's experiments the radicals interact with the THF solvent (2,3,5). We want them however to interact with the water.

One potential solution to this problem would be to apply a biphasic photoactivation<sup>1</sup>. In his early experiments, Nocera used DMBD as a solvent (2). DMBD is insoluble in water (6) and the bimetallic complex was reported to be stable in this solvent (2). The objective is to do a one-pot photoreaction with the complex sitting in DMBD, but have water present at the same time. As soon as light is shined, the M-X bond will be activated and halogen radicals will be formed. The photoelimination of the M-X bond is in the presence DMBD solvent as reported in Nocera's work as well, but the radicals would be able to move in the water phase. Theoretically, water could play the role of a trap for the radical, akin the THF. Since the DMBD solvent cannot serve as a trap for the halogen, as long as the surface contact with water is large enough, the halogen radical would recombine with itself and interact with water to generate HCl and HClO in a clean reaction.

Another potential solution could be redesigning the complex. Figure 5 presents a model of a theoretical complex. I used only two chelate ligands and I simplified them to the form shown in Figure 5, where they don't contain the methyl group off of the nitrogen and the fluorinated groups off of the phosphorous. The main goal here is to be able to dissolve the bimetallic complex in water and therefore achieve the photoelimination with water being the radical trap in the immediate vicinity. In addition, I simplified the structure of the complex to this point in order to be able to attack the two metal chores with 4 hydrogen halides instead of two. In this fashion, each cycle will give twice as much hydrogen, and the halide can then be recollected as hydrogen halide and reused in another cycle. The ligands used in addition to the bidentate ligands should not interfere with the solubility of the complex in water, for instance even halogens could be attached.



Fig. 5. Model bimetallic complex. M is the metal Rh,Ir or Au, and L stands for a ligand The stability of the bimetallic complex from Figure 5 in water can be predicted by computational analysis as well as by carrying out the first few reactions in the cycle. In the work done by Nocera with the rhodium complex, one of the intermediates was blue in color and it was determined to be the Rh<sup>1</sup>-Rh<sup>1</sup> complex. This intermediate would immediately disproportionate to Rh<sup>0</sup>-Rh<sup>II</sup>, the bimetallic complex that would allow for the elimination of the halogen. It is the stability of the latter that is essential. Hence, it is important to check for the color change for the newly designed complex. This will prove the stability of the two mixed electron valence rhodium complex in water. In addition, the reaction could be monitored by <sup>103</sup>Rh NMR (11) with bulkier phosphorous substituents that slow down the reactions and allow for the isolation and purification of the intermediates (2).

In the water-hydrogen halide cycle that I developed in addition to the complete photocycle for the production of hydrogen (Figure 4), one of the side products obtained was HXO. The oxohydrogen halide can dissociate into HX and oxygen upon imposing alkaline conditions. There will then be a tendency to produce H<sup>+</sup> to counteract the basic

environment. It has been reported in literature that hypobromous acid would disproportionate to produce HBrO<sub>3</sub> and HBr (9). If this issue cannot be avoided by increasing the temperature to decompose HBrO<sub>3</sub>, then chlorine again turns out to be the better candidate because it does not disproportionate (7,10). The oxygen obtained could be further activated through the porphyrin photocylcle also proposed by Nocera (2, 3).

A third possibility, and probably the easier one to be realized would be to apply the system developed for oxygen photoactivation to hydrogen. In this case the experiments will focus on characterizing the structure of the

<sup>&</sup>lt;sup>1</sup> Dr. Nataro's suggestions

metal porphyrinogen hydride complex and the reaction stoichiometry. At the same time porphyrinogen complexes of a range of metals like Zn, Fe and Co should be tested for efficiency to eliminate the hydrogen. Similar to the oxygen photoactivation, complexes containing cofacial porphyrinogens and porphyrinogen with a Hangman complex hanging from the bridging xanthenes or diphenylfuran could be tested. I hypothesize that in the case of the bisporphyrinogens the hydrogen and the halide will add to the two different metals. The xanthene will reduce the space between the two porphyrinogens allowing for bridging transfer reactions to occur that will facilitate the hydrogen and chlorine elimination. One of the challenges encountered could be that during the halogen photoelimination, the halogen radical might abstract or remove the hydrogens off of the ligands.

In summary, the improvements I proposed for the hydrogen production cycle that Nocera developed are of two kinds and stem from the issues I believe Nocera might deal with when upscaling his energy production. First of all, I suggested an additional cycle to eliminate the possibility of side products and reactants. This additional cycle involves the usage of the photoeliminated halogen to regenerate the hydrogen halide. I hypothesized that a biphasic photoactivation would eliminate the problem of the halogen interacting with the solvent and creating solvent residues. Furthermore, I designed an alternative complex that would use water as a solvent and use it right away in the regeneration of HX. Lastly, the oxygen photocycle could be tested for hydrogen with different metals and ligand arrangements. Provided an abundant and clean renewable energy source is one of the main focuses of the development in science and technology and since hydrogen is probably the most ideal carrier, this research would be important for Nocera's photocatalysis since it deals with upscaling issues of the process.

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### ADSORPTION OF AMMONIA IONS AND AMMONIUM FROM AQUEOUS SOLUTIONS ON MODIFIED ACTIVATED CARBONS

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**Abstract:** The adsorption process of ammonia ions and ammonium from aqueous solutions on the intact activated carbon obtained from nutshells through activation with phosphoric acid and oxidized with  $H_2O_2$  and later impregnated with salt containing  $Co^{2+}$ ,  $Ag^+$ ,  $Ni^{2+}$ ,  $Cu^{2+}$ , has been investigated. The efficiency of ammonia ions and ammonium elimination from various water categories, using both activated carbon (the intact CAN-7 and CAN-7 oxidized with  $H_2O_2$  and impregnated with  $Co^{2+}$ ,  $Ag^+$ ,  $Ni^{2+}$ ,  $Cu^{2+}$  ions) has been demonstrated.

Keywords: adsorption, ammonia ion, ammonia and activated carbon.

#### Introduction

Adsorption of ammonium vapors on activated carbons has been studied using adsorption technique [1-10] or those of calorimetry technique [11-14]. In literature, one can find few data regarding the adsorption of ammonia ions from aqueous solutions on modified activated carbons.

We have studied the adsorption of ammonia ions from aqueous solutions on activated carbons CAN-7 and CANO-7 obtained from nutshells, activated with phosphoric acid and oxidized with H<sub>2</sub>O<sub>2</sub>.

As the result of activated carbon CAN-7 oxidation, the content of acid functional groups has increased from 1.4 to 1.62 mg-echiv/g.

The oxidized activated carbons have been treated with aqueous solutions of Co<sup>2+</sup>, Ag<sup>+</sup>, Ni<sup>2+</sup> and Cu<sup>2+</sup> salts.

#### **Results and discussions**

The percentage of  $NH_3$  in the solutions formed from ammonium salts varies significantly depending on the solution's pH and temperature [15]. Analytical determination of  $NH_4^+$  content [15] is actually reduced to the determination of ammonia ions and ammonium total quantity.

Fig.1 shows the adsorption isotherms of  $NH_4^+$  ions and  $NH_3$  from aqueous solutions on CAN-7, CANO-7 + Co<sup>2+</sup>, CANO-7 + Cu<sup>2+</sup>, CANO-7 + Ag<sup>+</sup>, CANO-7 + Ni<sup>2+</sup> on initial pH 6,13 and temperature 20<sup>o</sup>C.



Fig.1. Adsorption isotherms of  $NH_4^+$  ions and  $NH_3^-$  from aqueous solution on intact CAN-7 (1), CANO-7 + Co<sup>2+</sup> (2), CANO-7 + Cu<sup>2+</sup> (3), CANO-7 + Ag<sup>+</sup> (4), CANO-7 + Ni<sup>2+</sup> (5) on initial pH 6,13 and temperature 20<sup>o</sup>C.

Data shown in fig.1 demonstrate that the oxidation of activated carbon CAN-7, followed by impregnation with various metal cations, modify substantially both form of  $NH_4^+$  and  $NH_3$  adsorption isotherms. Also the adsorption capacity of CAN-7 increases. It should be noted that impregnation of activated carbon CANO-7 with Co<sup>2+</sup> greatly increases (compared with other samples of investigated activated carbons) the adsorption capacity of  $NH_4^+$  ions and  $NH_3$ . It is also worth mentioning that the content of metals in the samples of modified activated carbon is of: Co – 0.2%, Ag – 1%, Ni – 0.04%, Cu – 0.16%.

The high value of ammonia ions and ammonium adsorption on CANO-7 +Co<sup>2+</sup> activated carbon samples can be explained by the increased capacity of Co<sup>2+</sup> to form coordinative compounds with NH<sub>3</sub>. In the solution, Co<sup>2+</sup> forms five coordinative compounds with NH<sub>3</sub>. For  $[Co(NH_3)_6]^{2+}$  pK<sub>1,2,3,4,5</sub> = 5,13. K<sub>1,2,3,4,5</sub> = 7,4 · 10<sup>-6</sup> [16].

Fig. 2 shows the dependence of adsorption values of ammonia ions and of ammonium on the pH of equilibrium concentrations solutions on CANO-7 + $Co^{2+}$  and CAN-7 at t = 20<sup>o</sup>C.

The data show a considerable increase of adsorption values, starting with certain values of pH. At relatively high values of pH (in the alkaline medium), the adsorption of ammonium formed in these conditions becomes significant.



Fig.2. Dependence of adsorption  $NH_3$  and  $NH_4^+$  ions upon the pH of equilibrium concentrations solutions on activated carbon CAN-7 (•) and CANO-7 + Co<sup>2+</sup>(•). t= 20°C.

It has been found [17] that ammonium is adsorbed on the carbon surface through chemosorption (fig.3).



Fig. 3. Interaction of ammonium with the surface of the adsorbent [17].

The maximum value of ammonia ions adsorption on intact CAN-7 and CANO-7 +  $Co^{2+}$  activated carbon can be calculated on the basis of Dubinin-Serpinschi equation (DS-1) inferred for the process of water vapors adsorption on the active centers from the surface of the adsorbent. Its linear form has been utilized:

$$Ce/Cs/a = 1/amC - 1/am Ce/Cs$$
,

where Ce is the equilibrium concentration of  $NH_4^+$  and  $NH_3^-$  (mmol/l); Cs is the solubility of  $NH_3^-$  (mmol/l); *a* is the adsorption value of  $NH_4^+$  ions and  $NH_3^-$  (mmol/g). Substitution, in equation DS-1, of P/Ps for Ce/Cs (to be applied in solutions) is, in our opinion, totally justified taking into consideration the applicability of Dubinin-Radushchievichi equation for solutions [18].

Fig. 4 shows the dependence Ce/Cs/a on Ce/Cs of the isotherm of ammonia ions and of the ammonium on intact CAN-7 (1) and CANO-7+ Co  $^{2+}$  activated carbon.



Fig.4. Adsorption isotherm of  $NH_4^+$  and  $NH_3^-$  from aqueous solutions on intact CAN-7 (1) and CANO-7+ Co<sup>2+</sup> (2) in the coordinates of DS-1 equation.

The adsorption value  $(a_m)$  on intact CAN-7 is about 50 mmol/g and on CANO-7+ Co<sup>2+</sup> is 66 mmol/g. Considering this supposition, the result of the present investigation must be taken into account as a possibility of purifying the water containing ammonia ions and ammonium in significant quantities.

#### Conclusions

- 1. It was established that at high pH values (10,6), the value of NH<sub>4</sub><sup>+</sup> and NH<sub>3</sub> adsorption increases considerably due to the formation, in the alkaline medium, of ammonium which exhibits a mechanism and adsorption capacity totally different from that of ammonia ions.
- 2. The possibility of water purifying from ammonia ions utilizing activated carbon, oxidized and impregnated with Co<sup>2+</sup>, Ni<sup>2+</sup> and Cu<sup>2+</sup> ions, has been found.

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## SHELF-LIFE PREDICTION OF PLUM FOODS USING ANTIOXIDANT ACTIVITY INDICES

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**Abstract:** The reducing activity and radical scavenging activity of hydrosoluble antioxidants in food compositions from plums during the storage were evaluated. The speeds of changing the reducing activity and radical scavenging activity were different. It was observed that the reducing activity diminished by 63.2% after 62...105 days of storage. The same modification of radical scavenging activity determined *in vitro* was discovered after 110...254 days of storage. Shelf-life of plum foods was calculated using Weibull distribution. The functional relationship of failure probabilities between the reducing and radical scavenging capacities have been detected ( $r^2$ = 0.94-0.98).

Keywords: shelf-life, antioxidant activity, Weibull distribution, plum, chokeberry

#### Introduction

Vegetable food is the most important source of antioxidants in human nutrition. Fruits, berries and vegetables contain different groups of antioxidants such as polyphenolics, carotenoids, anthocyans,  $\alpha$ ,  $\beta$ ,  $\gamma$ -tocopherol, ascorbic acid, etc [1-4]. Due to their activity, antioxidants play a positive role in prevention of cancer, gastrointestinal and cardiovascular diseases [5-8].

Basic characteristics of antioxidant activity are the inhibition of oxidation processes and scavenging of free radicals. The modification of antioxidant's reducing state "*in vitro*" in food compositions is the result of security effect from oxidative degradation of food. The action of antioxidants "*in vivo*" in human organism refers to deliverance from free radical R, ROO',  $O_2$ , OH excess and maintaining the equilibrium of oxido-reducing processes on cellular level.

There exists some correlation between reducing state of antioxidants and their capacities to inactivate free radicals or their radical scavenging activity [9]. This research is an attempt to identify the existence of a correlation between the described properties of antioxidants. The goal of investigation was to determine the modification of activity of hydrosoluble antioxidants in plum juice and sauce during storage.

#### Materials and methods

*The food compositions* from plums such as juice and sauce were examined. For increasing the content of antioxidants the samples of juice and sauce from plums with addition of pulp of chokeberries (*Aronia melanocarpa Elliot*) in amount of about 8.0% of total weights were prepared. Thus, juice with pulp from plums (JP), juice with pulp from plums and chokeberries (JPC), and sauce from plums and chokeberries (SPC) were pasteurized at 100°C and stored during 12 months in nonadjustable conditions.

*The reducing activity* (RA) of hydrosoluble antioxidants in tested food was determined by potentiometric method [9, 10]. The mode of this method consists in carrying out to equality, or equilibrium state of redox potential between solution of tested food and solution of L-hydroascorbic acid in two separated electrochemical cells. L-hydroascorbic acid was used as etalon of reducing activity for food antioxidants. The measurement of redox potentials was effectuated at the room temperatures  $(20\pm2^{\circ}C)$  by multi-parameter analyzer Consort C-835 with combination of silver and platinum electrodes. The differences between redox potential values of food solution and etalon solution were equalized by dosing of L- hydroascorbic acid concentration in etalon solution. Index *K* expressed the reducing activity of antioxidants in tested food in equivalent of L- hydroascorbic acid. Index K is the ratio of concentration of L- hydroascorbic acid (AA) to concentration of hydrosoluble substance in food determined by refractive index (mgAA/gRI).

Index K is calculated by equation:

$$K = \frac{C_1 \times V_1 \times m_1}{m_2 \times C_2 \times m_3}$$

where:  $C_1$  – concentration of L- hydroascorbic acid, etalon solution, mg/cm<sup>3</sup>;

 $C_{2}$  - mass fraction of hydrosoluble substance in food, g/cm<sup>3</sup>;

 $V_1 - used$  volume of L- hydroascorbic acid solution,  $cm^3$ ;

 $m_{1}$  weight of food probe after dilution, g;

 $m_2$  - weight of etalon solution, g;

 $m_3$  - weight of tested food sample, g.

The calculations of reducing activity index (K) in tested food were made with confidence factor P=0.05. The coefficient of correlation between concentration of L- hydroascorbic acid and concentration of hydrosoluble reducing substance in tested food in state of equilibrium was  $r^2 = 0.90-0.98$ .

The assay for measurement peroxyl radical scavenging activity (RSA) is based on the degree of inhibition of potassium iodide oxidation by antioxidants that scavenge peroxyl radicals, generated from thermal degradation of 2,2'-azobis(2-amidinopropane)-dihydrochloride (AAPH). Peroxyl radical scavenging activity of extracts was assayed in vitro by method Sano M. et al. [11] in our modification [12]. Namely, 0.7 ml of sample solution was added to 2 ml acetonitrile-phosphate buffer (1:1), followed by addition of 100µl of saturated KI solution. After preincubation of the mixture at 39±1°C for 2min. radical-induced oxidation was started by the addition of 200µl of 0.5M AAPH. After 60min of incubation at  $39\pm1^{\circ}$ C in the dark, the reaction vessel was chilled immediately in an ace bath to stop the radical production from AAPH and was allowed to stand for 5min in an ace bath. Subsequently, the volume of the reaction mixture was adjusted to 30ml with water. The concentration of molecular iodine in the mixture was determined by potentiometric titration with 0.25mM Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The minimum titration amount of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> was fixed at 0.01ml and the maximum potential difference was determined as the end point of the titration. The RSA of tested food is expressed as the percentage inhibition for iodine release of blank reagent without sample (control). The RSA was calculated from the following equation:

RSA (%) = 
$$\left(1 - \frac{V_s(\text{sample})}{V_c(\text{control})}\right) \times 100$$

where  $V_s$  - amount of  $Na_2S_2O_3$  expended for sample titration, ml;  $V_c$  - amount of  $Na_2S_2O_3$  expended for blank reagent titration (control), ml.

The reproducibility of RSA assay is good. The coefficients of variance were less than 4.78% and mean relative errors were within  $\pm 7.01\%$  [13].

#### **Results and discussions**

The end of shelf-life of food is ultimately assessed by activity failure of antioxidants. The curves of antioxidants activity modification in tested food are presented in fig 1, 2. The activity indices of antioxidants in food have undergone permanent changing practically during all time of storage. The modification of reducing activity (fig.1) and radical scavenging activity (fig.2) had a nonadjustable character, but in general, tended to decreasing of both the reducing and the radical scavenging activities. The most profound fluctuations of antioxidants activity were observed at the primary stage of storage, in first 50...100 days.



Fig. 1. Modification of reducing activity of antioxidants in food: • - JP;  $\blacksquare$  -JPC  $\blacktriangle$  - SPC during the storage

Perhaps the fluctuation of antioxidants activity appeared as results of different processes that were developed simultaneously in nonadjustable conditions. During the storage the diffusion processes of anthocyans and polyphenolics from solid phase (pulp of plums and chokeberries, their skins) to liquid phase took place. That has been led to increasing of concentration and activity of antioxidants in liquid phase. At the same time the speeds of oxidation and degradation reactions of antioxidants were accelerated concurrently because of symbiosis and antagonism effects between antioxidants and prooxidants in conditions when the temperature and viscosity of food were variable.



Fig. 2. Modification of radical scavenging activity of antioxidants in food: • - JP; • -JPC • -SPC during the storage

In these complicated compositions and nonadjustable conditions the decreasing degree of antioxidants activity by time can be valued by determining the probability of modification appearances [14-18]. The probability of antioxidants activity evolution in tested food was analyzed by Weibull distribution (1):

$$f(\tau) = \frac{\beta}{\eta} \left(\frac{\tau - \gamma}{\eta}\right)^{\beta - 1} e^{-\left(\frac{\tau - \gamma}{\eta}\right)^{\beta}}$$
(1)

where:  $\gamma$  - localization parameter expresses the minimal duration to time when the antioxidants activity does not manifest the modification;

 $\beta$  - shape parameter reflects the dynamics of decreasing process of antioxidants activity;

 $\eta$  - scale parameter reflects "the characteristically activity" of antioxidants, named Weibull's parameter;

 $\tau$  - time of food storage.

Experimental results were analyzed by application of Weibull distribution; the dynamics of decreasing process (parameter  $\beta$ ) and the characteristic value of antioxidants activity (parameter  $\eta$ ) were calculated (table 1). The third parameter of Weibull distribution, parameter of localization ( $\gamma$ ) was equal to 0, that means there have not observed any minimal periods of storage time when the activity of antioxidants was stable.

The decreasing probability of reducing activity degree and radical scavenging activity of antioxidants during the food storage was calculated by two parameters Weibull (Weibull++) distribution. The probability of product failure  $F(\tau)$  is related to days of storage  $(\tau)$  as follows:

$$F(\tau) = 1 - e^{-\left(\frac{\tau}{\eta}\right)^{\nu}}$$
<sup>(2)</sup>

where:  $\eta$  and  $\beta$  are the scale and shape parameters of Weibull distribution, respectively.

Whereas that the meaning of parameter  $\eta$  "characteristically activity" of antioxidants is the storage time during  $\tau = \eta$  days, from equation (2) we obtaine:

$$F(\tau) = 1 - e^{-\left(\frac{\eta}{\eta}\right)^{p}} = 1 - e^{-(1)} = 1 - 0.368 = 0.632$$
(3)

Parameter  $\eta$  characterized the duration of storage when the antioxidants activity has been decreased by 63.2% from initial activity 100%. Results presented in table 1 show that antioxidants in juice from plums and chokeberries were the most stable according to reducing activity. The loss of reducing activity by 63.2% was fixed after 105 days of storage, but the same decreasing index of radical scavenging activity was obtained just only after 184 days.

The antioxidants in sauce from plums and chokeberries demonstrated the sufficient stability of radical scavenging activity. Activity loss by 63.2% was determined after 254 storage days, at the same time the reducing activity decreased during 86 days. There was observed that the diminution of reducing activity and loss of radical scavenging activity of food antioxidants had different kinetics.

To mention, that the speed of decreasing the reducing activity was bigger than the radical scavenging activity. Using the parameters from table1 the relationship between reducing activity and radical scavenging activity was calculated.

Table 1

(4)

Food	Parameters of Weibull distribution					
	Reducing act	ivity (K) of	Radical scavenging activity (RSA)			
	antioxi	dants	of antioxidants			
	β	η	β	η		
Juice from plums (JP)	0.65	61.59	0.91	109.9		
Juice from plums and chokeberries (JPC)	0.78	105.5	1.0	184.36		
Sauce from plums and chokeberries (SPC)	0.82	86.72	2.45	254.58		

#### Weibull distribution parameters of antioxidants activity

Figures 3, 4 and 5 show the relationship between reducing activity and radical scavenging activity of antioxidants in tested food during the storage. In juice from plums the relationship between reducing activity and radical scavenging activity had the exponential correlation with coefficient equal to 0.98. The function of this correlation is (4):



$$RSA = 7,94$$
 (exp 0,027 K)

Fig. 3. Correlation ( $r^2=0.98$ ) between probabilities of reducing activity (K) and radical scavenging activity (RSA) failure of antioxidants in juice from plums



Fig. 4. Correlation ( $r^2=0.97$ ) between probabilities of reducing activity (K) and radical scavenging activity (RSA) failures of antioxidants in juice from plums and chokeberries

The decreasing of antioxidants activities in juice from plums and chokeberries and sauce from plums and chokeberries carried the character of logarithmic dependence of reducing activity on radical scavenging activity with correlation coefficients 0.94-0.97.

In composition of juice from plums and chokeberries RSA = 34.4 Ln (K) - 61.8 (5)

In composition of sauce from plums and chokeberries 
$$RSA = 30.2 Ln (K) - 27.4$$



Fig. 5. Correlation ( $r^2=0.94$ ) between probabilities of reducing activity (K) and radical scavenging activity (RSA) failures of antioxidants in sauce from plums and chokeberries

#### Conclusion

Thus, the reducing and radical scavenging capacities of antioxidants in plum food have a functional relationship with high coefficients of correlation  $r^2 = 0.94-0.98$ . The differences of speed of reducing activity and radical scavenging activity degradation reflect the diverse mechanisms of oxido-reduction processes and reactions of free radical inactivation *in vitro*, which depend on antioxidants composition in food.

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(6)

## THE STUDY OF THE BIOLOGICAL ACTIVITY OF SOME COBALT(III) DIOXIMATES WITH FLUORINE CONTAINING ANIONS

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**Abstract.** It was elaborated the methodology of synthesizing a series of new dioximates of Co(III) with the general formula  $[Co(DioxH)_2(L)_2]X \cdot nH_2O$ , where DioxH - the dioxime anione: dimethylglyoxime  $(DH_2)$ , 1,2-cyclohexanedionedioxime  $(NioxH_2)$ ; L - thiocarbamide (Thio), pyridine (Py), aniline (An), sulphanilamide (Sam); X -  $[BF_4]^2$ ,  $[ZrF_6]^2$ ;  $[TiF_6]^2$ ; whose structures have been studied with the help of contemporary physical methods: spectroscopy IR, UV-Vis, RMN <sup>1</sup>H, <sup>19</sup>F, X-ray analysis. There were elaborated optimal conditions of using cobalt dioximates with fluorine in order to intensify the biosynthesis of amylases and lipases by *Aspergillus* and *Rhizopus* strains, respectively. The tested compounds increase the lipolytic activity of *Rhizopus arrhisus* micromycetes.

**Keywords:** Co(III) dioximates, anions with fluorine, models of biological objects, biostimulating properties, fungi, enzymes.

#### Introduction

The capacity of dioximates to get complex with transition metals draws the researchers' attention not just from the perspective of synthesizing  $B_{12}$  vitamin models or haemoglobin [1-4], but also substances which manifest a wide spectrum of synthetic, analytic and structural possibilities [5-7]. The inclusion of fluorine ions or fluorine complex anions in cobalt dioximates favored the discovery of some ways of assembling complex molecules different from the case of using other halogens [8,9]. The positive action of cobalt (III) dioximates in the metabolic processes of microorganisms is well known. Modern biotechnology draws a special attention to the oriented synthesis of bioactive substances by microorganisms due to the important results obtained in the plan of fundamental studies with implications in medicine, microbiologic industry, chemistry, pharmaceutics, cosmetology etc. Due to some specific peculiarities like an increased reaction to environment changes, adaptive metabolism, short cycle of development, microorganisms are convenient for different researches. From another point of view, microorganisms are recognized as advantageous economic sources of obtaining a wide range of important bioactive substances.

A class of biologically active substances with a high importance in biology and multiple practical applications are the enzymes. Enzymatic preparations are widely used in different technological processes as well as in other fields of socio-human activities. Hydrolases – the enzymes which catalyze hydrolyze reactions, i.e. the reaction of splitting up compound substances of the type of polymers into simpler substances - have a major industrial importance, especially in medicine. The reactions of enzymatic hydrolysis are at the base of many modern technological processes. The growth of enzyme production can be obtained by improving the biosynthetical potential of microorganisms-sources. In this aspect, their use as biostimulators of the synthesizing process of coordinative compounds enzymes is of interest. Usually, these substances fulfill well determined functions of a vital importance for the organism: of transportation, accumulation, catalysts and biosynthesis stimulators of many biologic active substances. It is sufficient to mention that hemoglobin (central atom - Fe<sup>2+</sup>), chlorophyll (central atom - Mg<sup>2+</sup>) are metal complexes. The ions of many metals manifest an effect of activators of enzymatic systems [12]. The elements coordinately joined are less toxic and have a higher reagent capacity [13]. The physiological activity of metal complexes is mentioned in a series of works [13,14,15] which point out their function as regulators and growth, development and productivity biostimulators of plants, as well as their important role in increasing animals specific immunity. The specialists in coordination chemistry explain the high activity of metal complexes through the molecule energetic and conformational state, conditioned by space geometry, the unusual system of link lengths, the coordination number etc. [16].

The use of coordination compounds is justified by two moments:

- a) Considering their structure, metal complexes are close to natural biologic complexes which ensure the vital functions of the organism.
- b) In most of the metal complexes, generators of complex are biometals irreplaceable biologic catalysers, whose function is bound to proteins, specific ferments.

The analysis of different data proved that the biomass of *Spirulina platensis*, obtained through cultivation in the presence of cobalt coordinative compounds is characterized by a high content of ciancobalamine, protein, carotenoids and is balanced after other components [17]. Also, we found out that cobalt compounds with fluorine have stimulating

properties upon some microorganisms [18]. The formation of compounds with cobalt reduces the toxic action of fluorine ions present in the nutritive medium. Structural and composition complexity, the presence of metals as central atoms attest the perspective of using coordinative compounds as stimulators and regulators of biologic processes in the microbial cell.

The stimulating action of coordination compounds upon the biosynthesis of enzymes can be exercised by the metal atom from the structure of complexes, in our case by the atom of Co(III). The importance of metals in enzymology is well known. The presence or the absence of metals in the medium can influence many characteristics of the microbial cell (at AND and ARN level, protein-enzymes synthesis etc.) [19,20].

Using different proceedures, we synthesized a series of dioximates with fluorine or fluorinecontaining anions, whose composition, structure and physico-chemical properties were determined with the help of different modern methods [21-45] according to the scheme:

$$CoX_{m} \cdot nH_{2}O + 2 \underset{HO-N}{\overset{R-C-C-R}{\overset{H}{\underset{N-OH}}} + 2L}{\overset{H}{\underset{N-OH}} + 2L} \xrightarrow{CH_{3}OH/H_{2}O}_{O_{2}} + \underset{HF}{\overset{R-C-C-R}{\underset{R-O-H-O}{\overset{H}{\underset{N-C}}}}_{C=N} \underset{N-C-R}{\overset{L}{\underset{N-C}}}_{L} \times \cdot nH_{2}O \times nH_{2}O$$

Considering the fact that cobalt(III) dioximates represent models of molecules with vital functions, their properties as biostimulators upon some fungi strains were tested. It was found that the introduction of cobalt(III) dioximates in a nutritive medium of *Rhizopus* and *Aspergillus* fungi stimulates biosynthetic processes (biomass accumulation, increase of enzymatic activity, reduction of the period of cultivation) and establishes enzymogenesis processes under conditions of stress [22,23,32-34,42-44].

#### Results

The method of synthesizing Co(III) dioximates which contain as axial ligands: thiocarbamide, aniline, sulphanilamide with the anions of  $[BF_4]^-$ ,  $[ZrF_6]^{2-}$  and  $[TiF_6]^{2-}$  was elaborated. The composition of complexes was established and investigations with the help of the methods: spectroscopy in UV-Vis, IR and the crystalline structures of the complexes:  $[Co(DH)_2(Sam)_2][BF_4] \cdot H_2O$ ,  $[Co(DH)_2(Py)_2][BF_4]$ ,  $[Co(DH)_2(Sam)_2]_2[ZrF_6] \cdot 3H_2O$ ,  $[Co(DH)_2(Thio)_2]_2[ZrF_6] \cdot H_2O$ ,  $[Co(DH)_2(An)_2]_2[ZrF_6] \cdot 2H_2O$ ,  $[Co(NioxH)_2(An)_2]_2[ZrF_6] \cdot 3H_2O$ ,  $[Co(NioxH)_2(Thio)_2]_2[TiF_6] \cdot 3H_2O$  etc. were determined by X-ray method. In the IR spectra of the complexes with dimethylglyoxime there are the bands of valence oscillations absorption and those of  $Co(DH)_2$  group deforming:  $v_{as}(CH_3)=2930$  cm<sup>-1</sup>,  $v_s(CH_3)=2867$  cm<sup>-1</sup>,  $v_{as}(C=N)=1545$  cm<sup>-1</sup>,  $\delta_{as}(CH_3)=1460$  cm<sup>-1</sup>,  $\delta_s(CH_3)=1374$  cm<sup>-1</sup>,  $v_{as}(N=O)=1237$  cm<sup>-1</sup>,  $v_s(C=N)=1285$  cm<sup>-1</sup>,  $v_s(N=O)=1083$  cm<sup>-1</sup>,  $\gamma(OH)=973$  cm<sup>-1</sup>,  $\delta(CNO)=730$  cm<sup>-1</sup>,  $v_{as}(Co-N)=505$  cm<sup>-1</sup> and  $v_s(Co-N)=430$  cm<sup>-1</sup>. The complexes are a part of Co(III) *trans*-dioximates.

In  $[Co(DH)_2(Sam)_2][BF_4] \cdot H_2O$  and  $[Co(DH)_2(Sam)_2]_2[ZrF_6] \cdot 3H_2O$  two molecules of sulphanilamide are located in axial positions Lig-Co-Lig:  $v_{as}(NH_2)_{free}=3350 \text{ cm}^{-1}$ ,  $v_s(NH_2)_{free}=3250 \text{ cm}^{-1}$ ,  $v_{as}(NH_2)_{coord}=3280 \text{ cm}^{-1}$ ,  $v_s(NH_2)_{coord}=3165 \text{ cm}^{-1}$ ,  $[v_{as}(CC)+\delta_{as}(CCH)]=1595 \text{ cm}^{-1}$ ,  $[v_s(CC)+\delta_s(CCH)]=1488 \text{ cm}^{-1}$ ,  $v_{as}(SO_2)=1312 \text{ cm}^{-1}$ ,  $v_s(SO_2)=1145 \text{ cm}^{-1}$ ,  $\gamma(CCC)=672 \text{ cm}^{-1}$  and 562 cm}{-1}. The NH<sub>2</sub> amino-group is coordinated to the atom of cobalt. In the external sphere of the complexes there are the  $[ZrF_6]^{2^{\circ}}$  or  $[BF_4]^{\circ}$ , the molecules of crystallized water ( $v(OH)H_2O=3400-3600 \text{ cm}^{-1}$ ). The band of  $[ZrF_6]^{2^{\circ}}$  ion are registered in the region  $v(Zr-F)=472 \text{ cm}^{-1}$ , and those of the ion  $[BF_4]^{\circ}$ ;  $v_{as}(BF_4)=1085 \text{ cm}^{-1}$ ,  $v_s(BF_4)=760 \text{ cm}^{-1}$ ,  $\delta(F-B-F)=525 \text{ cm}^{-1}$ . In the crystals  $[Co(DH)_2(Sam)_2][BF_4]\cdot H_2O$  and  $[Co(DH)_2(Sam)_2]_2[ZrF_6]\cdot 3H_2O$  are achieved complicated cyclic fragments both with and without the participation of complex anions (Fig. 1) are achieved.

In the spectra of the complexes containing thiocarbamide there take place shifts of absorption bands resembling with some exceptions: frequency values v(NH),  $[v(CS)+v(CN) + \delta(NH_2) + \delta(HNC)]$  (around 1463 cm<sup>-1</sup>);  $[v(CN)+v(CS)+\delta(HNC)]$ ,  $\delta(NCN)$  and  $\delta(NCS)$  reduce its values at thiocarbamide coordination, but the frequency values of  $\delta(NH_2)$  and  $[v(CS)+v(CN) + \delta(NH_2)+\delta(HNC)]$  (around 1415 cm<sup>-1</sup>) increase by 10-20 cm<sup>-1</sup> [45]. In fresh solutions containing  $[Co(DH)_2(Thio)_2]^+$ , we can notice two absorption bands in regions 235 and 335 nm, the first corresponding

to  $Co(DH)_2$  grouping, which forms the cation equatorial plan, and the second – to those two thiocarbamide molecules located in a *trans* position [46].

In  $[Co(DH)_2(Thio)_2]_2[ZrF_6] \cdot H_2O$ , the coordination polyhedron of the atom of Co<sup>3+</sup> represents an octahedron N4S2, formed of four nitrogen atoms of two dioxime anions and two sulphur atoms of thiocarbamide. Thiocarbamide molecules achieve movements under different angles towards the equatorial fragment: parallel, perpendicular and intermediary (Fig. 2).



Fig.1. The process of forming cyclic fragments in [Co(DH)<sub>2</sub>(Sam)<sub>2</sub>][BF<sub>4</sub>]·H<sub>2</sub>O and [Co(DH)<sub>2</sub>(Sam)<sub>2</sub>]<sub>2</sub>[ZrF<sub>6</sub>]·3H<sub>2</sub>O



Fig. 2. The structure of [Co(DH)<sub>2</sub>(Thio)<sub>2</sub>]<sub>2</sub>[ZrF<sub>6</sub>]·H<sub>2</sub>O

In the complexes  $[Co(DH)_2(Thio)_2]_2[ZrF_6]\cdot H_2O$  and  $[Co(NioxH)_2(Thio)_2]_2[ZrF_6]\cdot 3H_2O$ , the coordination octahedron of the cobalt atom 4N2S includes 4 nitrogen atoms of dioxime radicals and two sulphur atoms of thiocarbamide molecules. Dimethylglyoxime monodeprotoned anion are located practically in the same plane, between the oximic groups of the later being achieved the O-H…O intermolecular hydrogen links. Also there take place  $\pi$ - $\pi$  and N-H…O nonvalent intermolecular interactions. In the complex with 1,2–cyclohexanedionedioxime, two complex cations (A and B), crystalographically independent, are joined by means of N-H…F hydrogen links which they form with  $[ZrF_6]^2$  complex anion (Fig. 3).

In  $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot 2H_2O$ , two complex cations  $[Co(DH)_2(An)_2]^+$ , centrosymmetrical and crystallographycally independent are united through a system of hydrogen links which occurs between the centrosymmetric anions  $[ZrF_6]^{2-}$  and the molecules of crystallized water. The coordination polyhedron of Co<sup>3+</sup> atom presents a slightly denaturized octahedron N6 (Fig. 4).

In the compound of  $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot 3H_2O$ , the basic role belongs to the complex anion  $[ZrF_6]^{2^2}$ , which forms hydrogen links with the complex cation N-H…F and C-H…F. The anions from the external sphere and the molecules of crystallizing water form a super molecular system on the base of some complicated nets of hydrogen links occurring between them (Fig. 5).



Fig. 3. Complex cation A

Complex cation B



Fig. 4. The joining fragment of molecules A and B in the compound of [Co(DH)<sub>2</sub>(An)<sub>2</sub>]<sub>2</sub>[ZrF<sub>6</sub>]·2H<sub>2</sub>O

IR spectra analysis of dioximates with Py allows us to affirm that two pyridine molecules are coordinated through nitrogen to the cobalt atom, the main proofs being the rise of frequency values of most of the absorption bands: v(CH) (3090–3125 cm<sup>-1</sup>),  $[v_{as}(CC)+\delta(CCH)]$  (1580–1610 cm<sup>-1</sup>) and  $[v_s(CC)+\delta(CCH)]$  (1480–1500 cm<sup>-1</sup>) [47]. In  $[Co(DH)_2(Py)_2][BF_4]$ , in an equatorial plane to the central atom there coordinate two dimethylgyoxime anions, joined through O-H…O intramolecular hydrogen links, but in the positions 1.6 of the octahedron, there are nitrogen atoms of pyridine molecules. In the external sphere there are  $[BF_4]$  anions (Fig. 6).

New dioximates of Co(III) with  $[TiF_6]^{2-}$  ions were obtained. In the IR spectra of synthesized compounds there are bands which indicate to their trans-configuration. The crystalline structure of  $[Co(NioxH)_2(Thio)_2]_2[TiF_6]\cdot 3H_2O$  (Fig. 7) was deciphered by means of X-rays.

The presence in the compound of a big number of  $NH_2$  donor-acceptor groups leads to the formation of a complicated system of intermolecular hydrogen links and to a special packing of crystal molecules which influence upon Thio molecules direction in relation with the equatorial plane. An important role in the process of forming crystalline structures is played by  $[BF_4]^2$ ,  $[ZrF_6]^{2^2}$  or  $[TiF_6]^{2^2}$  complex anions.



Fig. 5. The supramolecular system formed of  $[ZrF_{4}]^{2}$  anions and water molecules

Obtaining of new cobalt(III) *trans*-dioximates widens the circle of compounds with a perspective of being used as catalyzers of biosynthetic processes. The common property of metal complexes, microelements and enzymes is the capacity of provoking in live cells new biological processes, used in small concentrations - 0,5-15 mg/L. An important role in manifesting the properties of metal complexes is played by the ligands from their component. The study of biological properties of a series of cobalt(III) complexes with different ligands and fluorinated anions in the external sphere marked their beneficient influence upon the enzymogenesis process of some fungal strains producing exocellular hydrolases with different enzymatic systems.



Obtaining of new cobalt(III) *trans*-dioximates widens the circle of compounds with a perspective of being used as catalyzers of biosynthetic processes. The common property of metal complexes, microelements and enzymes is the capacity of provoking in live cells new biological processes, used in small concentrations - 0,5-15 mg/L. An important role in manifesting the properties of metal complexes is played by the ligands from their component. The study of biological properties of a series of cobalt(III) complexes with different ligands and fluorinated anions in the external sphere marked their beneficient influence upon the enzymogenesis process of some fungal strains producing exocellular hydrolases with different enzymatic systems. Thus, the introduction in the cultivation medium of *Aspergillus niger* CNMN FD 01 strain, producer of lipases, to the metal complex  $[Co(MH)_2(Py)_2][BF_4]\cdot H_2O$  increased the enzymatic activity by 30,046,2%. The coordinative compounds of cobalt(III)  $[Co(DH)_2(Thio)_2]_3F[SiF_6]\cdot1,5H_2O$ ,  $[Co(DH)_2(Thio)_2]_2[SiF_6]\cdot3H_2O$ ,  $[Co(DH)_2(Thio)_2][BF_4]\cdot3H_2O$  proved to be efficient stimulators for *Rhizopus arrhizus* F67 - producer of pectinases and *Aspergillus niger* 33 CNMN FD 06A - producer of amylases. The above mentioned compounds increased the enzymatic synthesis by 97,1-115,3% and 26.3-42,6 %, depending on the applied concentration and the strain origin [48]. The researches proved that the tested coordinative compounds modify not only the enzymatic activity but also the term of manifesting the maximum of enzymatic synthesis. It is important to establish the effect of coordination compounds depending on the composition of the internal and external sphere of metal complexes.

The present research had as object of study *Aspergillus niger* 33-19 CNMN FD 02A and *Rhizopus arrhizus Fischer* CNMN FD 03L strains. As inoculation was served the aqueous suspension of spores of 14 days culture, grown on oblique surfaces of agarized malt, at 28-30 °C in a quantity of 10 % with the concentration of 10<sup>6</sup> spores/ml was used as inoculation. In order to intensify the biosynthetical capacity of fungal strains there were used new dioxymates of cobalt(III) which contain in the internal sphere organic molecules (dimethylglyoxime, 1,2 cyclohexanedionedioxime, aniline), and in the external sphere fluorinated anions:  $[ZrF_6]^{2-}$ ;  $[TiF_6]^{2-}$ ;  $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot H_2O$  (I),  $[Co(DH)_2(An)_2]_2[TiF_6]$  (III). The metal complexes were tested in three concentrations - 1, 5, and 10 mg/L.

In the cultural liquids obtained in the experimental variants (with coordination compounds) and in the control variant (without coordination compounds) the enzymatic activity specific to the studied strains was essayed: the amylolytic activity was determined by means of the colorimetric method with iodine, using as a substrate 1% soluble starch solution in standard conditions of hydrolysis at pH-4,7 for ordinary amylases and at pH-2,5 for acid stable amylases. A unit of amylolytic activity is equivalent to the enzyme capacity in conditions determined by temperature, pH and the duration of cultivation of a gram of starch up to dextrins of a different molecular mass. The lipolytic activity was determined after the action upon olive emulsion oil in polyvinylic alcohol (Oto-Iamada method).

The analysis of data, obtained as a result of the study regarding the influence of cobalt(III) new dioximates upon the biosynthesis of ordinary (Tab. 1) and acid stable (Tab. 2) amylases by *Aspergillus niger* 33-19 CNMN FD 02A micromycete points out that the first two substances of  $[Co(NioxH)_2(An)_2]_2[ZrF_6] \cdot 3H_2O$  and  $[Co(DH)_2(An)_2]_2[ZrF_6] \cdot 2H_2O$ , characterized by identical external sphere – the anion of  $[ZrF_6]^2$  and differences in the composition of ligands – DH and NioxH<sup>-</sup>, manifest a stimulating action during the initial stage of the synthesis of amylases (during the fourth and the fifth days of cultivation) at their application in small concentrations – 1-5 mg/L. The stimulating effect was 20,0 % and 15,0 % for ordinary amylases, and 22,1 % and 23,0 % for acid stable amylases.

Table 1

#### Conc. Activity day 5 day 4 day 6 day 7 CC, Coordination compounds % towards % towards % towards % towards u/ml u/ml u/ml u/ml mg/L control control control control 175,9 159,3 117,0 183,5 106,0 142,6 80,1 104,9 1 5 163,4 120,0 173,1 100,0 132,5 74,5 167,5 100,0 [Co(NioxH)<sub>2</sub>(An)<sub>2</sub>]<sub>2</sub>[ZrF<sub>6</sub>]·3H<sub>2</sub>O (I) 10 122,7 90,0 173,1 100,0 132,5 74,5 167,5 100,0 1 178,3 94,4 138,5 101,7 103,0 167,8 167,5 100,0 5 199.1 175.9 $[Co(DH)_2(An)_2]_2[ZrF_6] \cdot 2H_2O(II)$ 160,1 110,8 115.0 112,3 63,1 104.9 10 109,0 159,3 138,5 101,7 188,7 152,7 85,8 95,1 96,5 1 178,3 130,9 208,6 120,5 171,8 184,2 109,9 96,5 5 178.3 227,4 171.8 192,4 $[Co(DH)_2(An)_2]_2[TiF_6]$ (III) 130.9 131.3 114,9 113,6 10 188,7 138,6 227,4 131,3 202,2 184,2 109,9 100 100 173,1 100 177,9 Control \_ 136,2 167,5 100,0

# Modification of ordinary amylases activity (pH 4,7) of Aspergilllus niger 33-19 CNMN FD 02A micromycete under the influence of cobalt(III) complexes with fluorinated anions (u/ml)

The stimulating effect is more powerfully expressed at the complex containing the 1,2-cyclohexanedionedioxime anion. This fact is due to the capacity of some species of micromycetes to assimilate effectively and to include methoxylate cyclic substances [49] in the metabolic processes.

#### Table 2

		Activity							
Coordination compounds	CC, mg/L	day 4		day 5		day 6		day 7	
		u/ml	% towards control	u/ml	% towards control	u/ml	% towards control	u/ml	% towards control
$[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot 3H_2O(I)$	1	262,1	122,1	335,1	128,0	227,3	76,3	251,0	108,8
	5	255,8	118,8	307,5	117,5	227,3	76,3	240,6	104,3
	10	196,1	91,1	227,6	86,9	229,9	93,9	219,8	95,3
$[\text{Co(DH)}_2(\text{An})_2]_2[\text{ZrF}_6]\cdot 2\text{H}_2\text{O} (\text{II})$	1	216,1	100,3	307,5	117,5	277,4	93,1	219,8	95,3
	5	227,6	105,7	321,9	123,0	171,1	54,4	251,0	108,8
	10	216,1	100,3	278,4	106,4	252,3	84,7	230,7	100,0
$[Co(DH)_2(An)_2]_2[TiF_6] (III)$	1	252,4	117,2	310,1	118,5	220,4	74,0	256,2	111,0
	5	276,6	128,4	335,1	128,0	249,4	83,7	261,3	113,3
	10	284,6	132,2	368,4	140,8	333,2	111,9	240,6	104,3
Control	-	215,3	100	261,7	100	297,8	100	230,7	100,0

# Modification of acid stable amylases activity (pH 2,5) of Aspergillus niger 33-19CNMN FD 02A micromycete under the influence of cobalt(III) complexes with fluorinated anions

To the end of the process of cultivation (the 5-th and the 6-th days), the difference between experiment and control in the variants I, II equilibrates. The data allow us to suppose that the tested compounds intensify microorganism development stages (lag-stage and exponential stage) characterized by processes of microorganisms adaptation and multiplication, a phenomenon which provoke a precocious biosynthesis of amylases confirmed by a considerable biosynthesis intensification of both ordinary and acid stable amylases (by 20,0 % and, respectively, 22,1 %) during the fourth day towards the control variant. Microbiological researches confirm the supposition by stating micellar precocious abundance and the active sporulation in the experimental variants.

The activity of both types of amylases is strongly modified by the III-rd substance  $[Co(DH)_2(An)_2]_2[TiF_6]$  which contains the metal of titanium in the composition of the fluorinated anion, a fact which distinguishes it from the II-nd substance which contains zirconium in the fluorinated anion. The application of this complex accelerates the process of establishing the stationary stage of the strain biologic cycle – during the 4-th and the 5-th days in the experimental variant towards the 6-th day in the control variant, reducing the technologic cycle by 48 hours, a fact which presents economic advantages, besides, it considerably increases the enzymatic biosynthesis. The maximum of standard (ordinary) amylases activity in the experimental variants (metal complex concentration was 5 and 10 mg/L) represents 227,4 u/ml in comparison with 177,9 u/ml in the control variant, and that of acid stable amylases consists of 368,4 u/ml in the experimental variant towards 297,8 u/ml in the control variant. The stimulating effect represents 38,6 % and 32,2 % for standard and acid stable amylases, respectively. The activity of amylases in this variant remains superior to control during the whole process of cultivation, a fact which confirms that this complex stimulates not only microorganism development, but also provokes "de novo" biosynthesis of enzymes (Fig. 8).

Concerning the similar composition of the II-nd and III-rd compounds mentioned above in the text -  $[Co(DH)_2(An)_2]_2[ZrF_6]$ ,  $[Co(DH)_2(An)_2]_2[TiF_6]$ , marked by the difference of complex anions -  $[ZrF_6]^2$ ,  $[TiF_6]^2$ , we can conclude that the different action of this compound upon enzymatic synthesis and development processes of *Aspergillus niger* 33-19 CNMN FD 02A fungal strain is marked by the properties of  $[TiF_6]^2$ - complex anion.

The new access of increasing enzymatic activity in some experimental variants during the 7-th day of cultivation is provoked by microorganism secondary growth. At this stage we can follow under microscope the growth of young micelle new catenae.

As a result of researches there were selected optimal conditions for the application of cobalt(III) dioximates which contain fluorine in order to intensify the biosynthesis of ordinary and acid stable amylases by *Aspergillus niger* 33-19 CNMN FD 02A fungal strain, producer of exocellular amylases: when metal complexes -  $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot 3H_2O$ ,  $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot 2H_2O$  are used as stimulators, the concentration was 1-5 mg/L, the cultivation period was 5 days; for the compound  $[Co(DH)_2(An)_2]_2[TiF_6]$  the concentration was 5-10 mg/L, the cultivation period was 4-5 days, depending on the applied concentration. The process is accomplished in standard conditions of cultivation established for *Aspergillus niger* 33-19 CNMN FD 02A fungal strain in submerse culture [24].



Fig. 8. Modification of the activity of ordinary amylases (pH 4,7) synthesized by *Aspergillus niger* 33-19 CNMN FD 02A micromycete under the influence of  $[Co(DH)_2(An)_2]_2[TiF_6]$ a. activity of ordinary amylases (pH 4,7)

b. activity acid stabile amylases (pH 2,5)

The obtained data were applied in the process of elaborating a procedure of directed synthesis of amylases by *Aspergillus niger* 33-19 CNMN FD 02A fungal strain in submerse culture with the application of  $[Co(DH)_2(An)_2]_2[TiF_6]$ .

The procedure consists in: introducing the coordinative compound of  $[Co(DH)_2(An)_2]_2[TiF_6]$ , in a concentration of 5-10 mg/L, in the standard cultivation medium of *Aspergillus niger* 33-19 CNMN FD 02A in a submerse culture. The compound was added to the medium after the medium autoclaving, namely before the inoculation of the microorganism, aimed at avoiding the destruction of the complex structure. The culture was developed on 1 L Erlenmeyer flasks containing 200 ml of nutrition medium, in conditions of continuing agitation (200 rot./min.<sup>-1</sup>), at 28-30 °C, the duration of cultivation was 4-5 days. The composition of the nutrition medium (g·L<sup>-1</sup>): starch - 3,0; been flour - 9,0, wheat bran - 18,0;  $[Co(DH)_2(An)_2]_2[TiF_6] - 0,01$ ; MgSO<sub>4</sub> - 0,5; KH<sub>2</sub>PO<sub>4</sub> - 2,0; KCl - 0,5; water - 1L; pH-3,0. The procedure ensures amylolytic activity with 23,7-28,9 % towards the control, the technological cycle reduction with 24-48 hours.

Also studied was the influence of cobalt(III) dioximates with fluorine upon the process of *Rhizopus arrhizus Fischer* CNMN FD 03 L micromycete enzymogenesis which is an active producer of lipases. Lipases are the keyenzymes in the metabolism of lipids of all live organisms. The intensive study of lipolytic enzymes is conditioned not only by the theoretic interest towards them, but also by the possibility of using them in different technological processes. The data obtained after having determined the lipolytic activity in dynamics in the cultural liquid during the 1-st, 2-nd and the 3-rd days of cultivation are given in table 3.

Concerning the I-st and the II-nd compounds, their stimulating effect manifests beginning with the first day of cultivation, the enzymatic activity being with 5,5-11,1 % superior to control in the variants with mediums of 5-10 mg/L concentration. The maximum of biosynthesis for all the compounds is marked during the second day of cultivation, representing 122,2-166,6 % towards control and corresponds to the time of manifesting the maximum of biosynthesis in the control variant. Microscopical researches don't mark any indexes of culture development acceleration, a fact confirmed by biochemical results. This can be explained by the short cycle of *Rhizopus arrhizus Fischer* CNMN FD 03 L strain developments of 2 days, unlike Aspergillus niger 33-19 CNMN FD 02A strain whose cycle of development is of 6 days. The highest activity was obtained for the II-nd complexe, in the variants with the concentration of 5 mg/L, representing respectively 93750 u/ml by comparison with 56250 u/ml in the control variant, fact which represents a rise of 66,6 %. The III-rd complex manifests a mechanism of action upon the process of strain enzymogenesis different from other complexes. The curve of the culture development cycle on the medium with such a complex repeats the curve on the witness medium, the difference consisting only in the maximum of lipases biosynthesis, more expressed during the second day of cultivation. Irrespective of the concentration in the applied diapason of this complex - 1, 5, or 10 mg/L, the enzymatic activity represents 75000 u/ml towards 56250 u/ml in the control variant or by 33,3 % superior to control. This differing behavior can be prescribed to the complex anion with titanium from the external sphere of the compound.

#### Table 3

Coordination compounds	C	Lipolytic activity, u/ml						
	Conc	day 1		d	ay 2	day 3		
	CC, mg/L	u/ml	% towards control	u/ml	% towards control	u/ml	% towards control	
$[Co(NioxH)_2(An)_2]_2[ZrF_6] \cdot 3H_2O (I)$	1	54625	105,5	68750	122,2	45000	100,0	
	5	54625	105,5	68750	122,2	45000	100,0	
	10	57500	111,1	68750	122,2	35000	77,8	
$[\text{Co(DH)}_2(\text{An})_2]_2[\text{ ZrF}_6] \cdot 2\text{H}_2\text{O} (\text{II})$	1	51750	100,0	75000	133,3	35000	77,8	
	5	57500	111,1	93750	166,6	45000	100,0	
	10	57500	111,1	81250	144,4	35000	77,8	
$[Co(DH)_2(An)_2]_2[TiF_6]  (III)$	1	46000	88,9	75000	133,3	40000	88,9	
	5	51750	100,0	75000	133,3	45000	100,0	
	10	51750	100,0	75000	133,3	45000	100,0	
Control	-	51750	100	56250	100	45000	100,0	

# Modification of Rhizopus arrhizus Fischer CNMN FD 03 L micromycete lipolytic activity under the influence of cobalt(III) with fluorinated anions

#### Conclusions

- The method of synthesizing Co(III) dioximates, which contain thiocarbamide, pyridine, aniline, sulphanilamide with anions of  $[BF_4]^2$ ,  $[ZrF_4]^2$  and  $[TiF_4]^2$  as axial ligands was elaborated.

- The composition of complexes was established and their crystalline structure by means of contemporary physical methods (IR, UV-Vis, DTGA).

As a result of the researches there were elaborated optimal conditions for using the dioximates of cobalt(III) with fluorine in order to intensify the biosynthesis of standard and acid stable amylases by *Aspergillus niger* 33-19 CNMN FD 02A strain and exocellular lipases by *Rhizopus arrhizus Fischer* CNMN FD 03 L strain:

The above-mentioned coordinative compounds are included in a sterile nutritive medium, after autoclaving in the form of a solution with well defined concentrations, directly before inoculation.

-The inclusion in the cultivation medium (in a concentration of 1-5 mg/L) of  $[Co(NioxH)_2(An)_2]_2[ZrF_6]\cdot 3H_2O$  (I),  $[Co(DH)_2(An)_2]_2[ZrF_6]\cdot H_2O$  (II) modified the duration of *Aspergillus niger* 33-19 CNMN FD 02A strain cultivation, reducing the technologic cycle by 24 hours.

- The introduction of *Aspergillus niger* 33-19 CNMN FD 02A strain in the cultivation medium (in a concentration of 5-10 mg/L) of  $[Co(DH)_2(An)_2]_2[TiF_6]$  (III) coordinative compounds, which contain the complex anion with titanium and fluorine, reduce the technologic cycle by 48 hours, ensuring the rise of standard amylases activity by 27,8 % and acid stable amylases by 23,7 %. The enzymatic activity in this variant remains superior to control during the whole period of cultivation.

- The tested dioximates of cobalt(III) with fluorine don't modify the duration of *Rhizopus arrhizus Fischer* CNMN FD 03 L micromycete – producer of exocellular lipases.

- At the introduction of  $[Co(NioxH)_2(An)_2]_2[ZrF_6]$ ·3H<sub>2</sub>O (I) and  $[Co(DH)_2(An)_2]_2[TiF_6]$  (III) compounds in the cultivation medium of *Rhizopus arrhizus Fischer* CNMN FD 03 L micromycete, the lipolytic activity superior to control, registered in the variants with the concentration of 1 mg/L for complexe (I) representing 68750 u/ml by comparison with 56250 u/ml on the control medium, which represents a rise of 22,25 %. The highest lipolytic activity was registered in the variants with coordinative compounds of  $[Co(DH)_2(An)_2]_2[ZrF_6]$ ·2H<sub>2</sub>O (II) at the application of the concentration of 5 mg/L – 93750 u/ml towards 56250 u/ml on the control medium, the increasing constituting 66,6%.

- The external sphere influence of cobalt(III) coordination compounds with fluorine experimented upon the processes of enzymatic development and synthesis at micellar fungi-producers of exocellular hydrolysis was marked.

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# CARBOXYLATE SUBSTITUTION PATTERN AS STRUCTURAL DIRECTIVE FOR THE FINAL PRODUCTS: SYNTHESIS, STRUCTURE AND PROPERTIES OF [Fe<sub>4</sub>Ca,O,(µ,-HCCl,COO)<sub>10</sub>(µ<sub>3</sub>-HCCl,COO),(THF)<sub>6</sub>]

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**Abstract:** A novel hexanuclear iron-calcium-oxo complex has been synthesized and characterized by different physicochemical methods and X-ray single crystal structural analysis:  $[Fe_4Ca_2O_2(\mu_2-HCCl_2COO)_{10}(\mu_3-HCCl_2COO)_2(THF)_6]$ . The molecular structure shows that there are two types of coordination for COO<sup>-</sup> anions: bidentate and tridentate. The corresponding variable temperature susceptibility measurement shows that in the complex there exists an antiferromagnetic interaction ( $|J_{12}| = |J_{34}| = -71.86 \text{ cm}^{-1}$ ). The iron(III) high spin state (5/2) is proved by Mössbauer spectroscopy. High magnetic EPR measurements of **1** indicates the presence of S=0 ground state with low-lying S=1 excited state centred around g = 2.0054 ±0.0001.

Keywords: Iron(III) calcium(II) heterocluster; crystal structure; antiferromagnetic interaction.

#### Introduction

Polynuclear carboxylates of 3d transition metals have been attracting renewed interest because of their intramolecular magnetic exchange interactions [1, 2] and their application as the simple models of oligonuclear active sites in metalloproteins [3-6]. The unambiguous attribution of the individual site in the trimer to one or another metal in these complexes is impeded by at least two factors: *i*) the close atomic numbers of the Fe and M metals and *ii*) the statistical distribution of the clusters in the crystal structure. The extensive investigations of other complexes with dichloroacetic acid have been reported [7-9]. However the information about  $\{M_3O\}$  core containing bivalent metal that does not belong to 3d elements, proved by X-ray crystallography is still absent.

Here we presnt a novel hexanuclear iron-calcium-oxo complex  $[Fe_4Ca_2O_2(\mu_2-HCCl_2COO)_{10}(\mu_3-HCCl_2COO)_2(THF)_6]$  (1) which is aggregated from two  $\mu_3$ -oxo fragments. The detailed crystal structure and magnetic property were discussed.

#### **Results and discussion**

X-ray diffraction revealed that crystal **1** at 293 K has a molecular structure consisted of neutral complexes, the charge balance of which is in agreement with formation of  $[Fe_4Ca_2O_2(\mu_2-HCCl_2COO)_{10}(\mu_3-HCCl_2COO)_2(THF)_6]$  species. Molecular structure of **1** at 293 K is depicted in figure 1. Two Ca<sup>2+</sup> and four Fe<sup>3+</sup> metal ions are associated into hexanuclear cluster through two  $\mu_3$ -oxo atoms (Ca1-O1 2.417(3), Fe1-O1 1.840(1), Fe2-O(1) 1.827(1) (Å) for 293 K; Ca1-O1 2.410(3), Fe1-O1 1.833(3), Fe2-O(1) 1.831(3) (Å), average values for A and B independent complexes at 130 K) and twelve dichloroacetate ligands. Ten carboxylates act as  $\eta^1:\eta^1:\mu$  bidentate bridging units while two others as bridging tridentate  $\eta^1:\eta^2:\mu$  units. The molecular structure of **1** could be described as a combination of two centrosymmetrically related trinuclear [Fe<sub>2</sub>CaO(CHCl<sub>2</sub>COO)<sub>6</sub>(THF)<sub>3</sub>] entities, which closely resemble published [Fe<sub>2</sub>CaO(CHCl<sub>2</sub>COO)<sub>6</sub>(THF)<sub>4</sub>] [10]. The metal···metal separation within the {Fe<sub>4</sub>Ca<sub>2</sub>( $\mu_3$ -O)<sub>2</sub>} core exhibits the following values: Ca1···Fe1 3.635(2), Ca1···Fe2 3.704(2), Fe1···Fe2 3.204(2), Ca1···Ca1' 3.957(2) (Å) for 293 K; Ca1···Fe1 3.632(2), Ca1···Fe2 3.676(2), Fe1···Fe2 3.203(2), Ca1···Ca1' 3.918(2) (Å) in average for A and B independent complexes at 130 K. Each Fe<sup>3+</sup> ions has a similar octahedral coordination completed by six oxygen atoms: four from bidentate bridging carboxylate groups in equatorial plane; two another from THF molecule and  $\mu_3$ -O in apical position. The coordination number of Ca<sup>2+</sup> ion reaches the value 8 due to the coordination of the oxygen atom (Ca1-O2 2.450(3) Å for 293 K; 2.457(3), Å for 130 K) from tridentate  $\eta^1:\eta^2:\mu$  carboxylate unit.

X-ray single crystal structural study at 293 and 130 K has demonstrated that compound 1 exhibits a structural

phase transition in this temperature range. In the frame of the same space group ( $P\overline{1}$ ), the lowering of the temperature from 293 to 130 K leads to the doubling of the unit cell volume with two crystallographic independent molecules. The molecular structures of two complexes at 130 K are quite similar and slightly differ only by the orientation of the THF ligands coordinated to Ca atoms.



Fig. 1. Molecular structure of  $[Fe_4Ca_2(\mu_3-O)_2(CHCl_2COO)_{12}(THF)_6]$  at 293 K. Chlorine atoms are omitted for clarity. The thermal ellipsoids are drawn at the 40% probability level.

The thermal decomposition of **1** in air is a multistage process. The elimination of THF molecules starts at 50°C. The first endothermic process is characterized by a maximum at ~70 °C and it ends at ~80 °C with ~6.5 mass loss, which corresponds to the elimination of two THF molecules per formula unit from of the crystal. The second endothermic process showed the maximum at 220 °C on the DTG curve. The total mass loss in these processes is ~68% that corresponds to removal of all THF molecules and partial decomposition of the dichloroacetate groups. An unexpected endothermal process has been observed in the range 410-455 °C. The peak registered was not accompanied by any mass change. Probably, it results from a phase transition of the reaction product. However, this statement requires additional confirmation. According to DTG data, the residual sample after decomposition consists in about ~19% of the initial sample mass and most probably corresponds to CaO·Fe<sub>2</sub>O<sub>3</sub>.

The Mossbauer spectrum (MS) of 1 was measured at 293 K. The room temperature 57Fe MS of 1 shows two symmetrical central absorption lines which were assigned to one doublet with the parameters:  $\delta_{Fe} = 0.41$  mm/s;  $\Delta E_Q = 0.53$  mm/s. They indicate the presence of Fe(III) ions in the high spin state (S=5/2) with the rather symmetrical electron density distribution.



Fig.2. Plot of the  $\chi_M T$  product of **1** as a function of temperature. The solid line is a fit of the experimental data to the HDVV model; see the text for the fitting parameters.

The magnetic properties of compounds 1 has been measured in the range of temperatures 300–2.0 K. The substance is characterized by a gradual reduction of the  $\chi_M T$  product with the lowering of temperature (Fig. 2) thus indicating an antiferromagnetic interaction between the iron (III) ions. Lower than  $\sim 30$  K the plateau exists.

For interpretation of magnetic properties of 1 the HDVV model for Fe<sub>4</sub> was used with the following scheme of interaction:



The original least-squares fitting computer program FDHM [11, 12] with a Full Diagonalisation of Hamiltonian Matrix approach was employed to fit  $(\chi T)_{exp}$  vs. T, in order to obtain the exchange couplings constants. The program uses the spin Hamiltonian operator:

$$\mathbf{H}_{total} = \mathbf{H}_{HDVV} + \mathbf{H}_{Z} \tag{1}$$

$$\mathbf{H}_{HDVV} = -2\mathbf{J}(\mathbf{S}_{Fe1}\mathbf{S}_{Fe2} + \mathbf{S}_{Fe3}\mathbf{S}_{Fe4}) - 2\mathbf{J}'(\mathbf{S}_{Fe1}\mathbf{S}_{Fe3} + \mathbf{S}_{Fe2}\mathbf{S}_{Fe4}) - 2\mathbf{J}''(\mathbf{S}_{Fe1}\mathbf{S}_{Fe4} + \mathbf{S}_{Fe2}\mathbf{S}_{Fe3})$$
(2)

where 
$$\mathbf{H}_{Z} = g \boldsymbol{\mu}_{\mathrm{B}} \sum_{i=1}^{4} \mathbf{S}_{i}^{z} B,$$
 (3)

 $S_i = 5/2$  is the spin operator of the *i*-th ion,  $J_i$  are the exchange parameters,  $\mu_B$  is the Bohr magneton, g is the electronic g-factor for the tetranuclear compound, B is the magnetic field strength. The magnetic susceptibility data of 1 was calculated from the spin-coupled wave function by using a simplified form of Van Vleck equation. The better fitting was obtained at the following values of exchange parameters:  $J = J_{12} = J_{34} = -71.86 \text{ cm}^{-1}$ ;  $J' = J_{13} = J_{24} = +0.18 \text{ cm}^{-1}$ ;  $J'' = J_{14} = J_{23} = -2.46 \text{ cm}^{-1}$ ,  $\rho_{\text{paramagn, impur.}} = 2.5 \%$  and  $\theta_{\text{Curie Weiss}} = 2.46 \text{ K}$ , and  $F_{\text{qualit, factor}} = 1.46 \cdot 10^{-5}$ . High magnetic EPR measurements of 1 indicates the presence of S=0 ground state with low-lying S=1 excited at the advantage of S=0 ground state with low-lying S=1 excited at the second state of S=0 ground state with low-lying S=1 excited state of S=0 ground state state of S=0 ground state with low-lying S=1 excited state of S=0 ground state with low-lying S=1 excited state of S=0 ground state state state state of S=0 ground state state

state centred around  $g = 2.0054 \pm 0.0001$  (Fig.3). This result is in a good agreement with the above mentioned scheme of



Fig. 3. W – band powder EPR spectrum of 1 at 4 K.

exchange interactions leading to antiferromagnetic coupling between iron(III) ions in a high spin state: Fe1-Fe2 and Fe3-Fe4, respectively. As the individual iron(III) ions are in a high spin state (S=5/2) that has been shown by our investigations of the Mossbauer spectrum of 1, the existence of a ground state spin singlet for 1 is a simple consequence of  $\mathbf{D}_{_{5/2}} \otimes \mathbf{D}_{_{5/2}}$  direct product of the rotation group representations

$$\left(\mathbf{D}_{5/2} \otimes \mathbf{D}_{5/2} = \sum_{S=0}^{5} D_{s}\right)_{o}$$

Here  $D_0$  and  $D_1$  correspond to ground singlet spin state exited triplet spin state of dimmers Fe1-Fe2 and Fe3-Fe4 characterized by the same exchange integral ( $J = J_{12} = J_{34} = -71.86 \text{ cm}^{-1}$ ) according to magnetic susceptibility data.

#### Experimental

Synthesis. The title compound was synthesised by the reaction of  $Fe(NO_3)_3 \cdot 9H_2O$  with 2 equivalents of  $Ca(CHCl_2COO)_2 \cdot 6H_2O$  in methanol, followed by evaporation of solvent at room temperature [13]. Dissolving in tetrahydrofuran(THF) the obtained solid gives are d-orange solution which crystals of  $[Fe_4Ca_2(\mu_3-O)_2(CHCl_2COO)_{12}(THF)_6]$  are obtained from. A brown-orange crystalline material has been separated after 2 weeks, washed with heptane and air dried. The yield is high (~ 87%). Calc. (Found) for  $C_{48}H_{60}Cl_{24}Fe_4Ca_2O_{32}$ : C, 25.03(24.94); H, 2.63(2.47); Fe, 9.70(9.92); Ca, 3.48(3.37)%.

*The Mossbauer spectra* were acquired using a constant acceleration spectrometer with symmetrical waveform. <sup>57</sup>Co (1.0 MBq) source at room temperature has been used.

*Variable temperature susceptibility* studies were recorded with an Oxford Instruments Vibrating Sample Magnetometer (VSM) working between 0 and 12 T and in the 1.5–350 K temperature range.

*EPR measurements*. Polycrystalline powder EPR spectra were recorded at frequencies ranging from 90–270 GHz at the high-field electron magnetic resonance facility at the National High Magnetic Field Laboratory in Tallahassee (USA), as described elsewhere [14, 15]. The sample's temperature was varied from 300 to 4 K.

*Crystal data* for 1: (130 K)  $C_{48}H_{60}Ca_2Fe_4Cl_{24}O_{32}$ ,  $M_r = 2303.32$ , triclinic, space group P $\overline{1}$ , a = 13.475(3), b = 14.678(3), c = 24.152(5) (Å),  $\alpha = 84.99(3)$ ,  $\beta = 79.21(3)^\circ$ ,  $\gamma = 63.57(3)$  (°), V = 4202.0(15) Å<sup>3</sup>, Z = 2,  $\rho_{calcd} = 1.820$  g/cm<sup>3</sup>, MoK<sub> $\lambda$ </sub> radiation ( $\lambda = 0.71073$  Å,  $\mu = 1.639$  mm<sup>-1</sup>), R = 0.0615 ( $F^2 > 2\sigma$ ),  $R_w = 0.1518$  (for 14773 data and 994 refined parameters); (293 K)  $C_{48}H_{60}Ca_2Fe_4Cl_{24}O_{32}$ ,  $M_r = 2303.32$ , triclinic, space group P $\overline{1}$ , a = 13.611(3), b = 14.919(3), c = 12.784(3) (Å),  $\alpha = 81.28(3)$ ,  $\beta = 69.73(3)^\circ$ ,  $\gamma = 63.55(3)$  (°), V = 2180.0(8) Å<sup>3</sup>, Z = 1,  $\rho_{calcd} = 1.754$  g/cm<sup>-3</sup>, MoK<sub> $\lambda$ </sub> radiation ( $\lambda = 0.71073$  Å,  $\mu = 1.580$  mm<sup>-1</sup>), R = 0.0618 ( $F^2 > 2\sigma$ ),  $R_w = 0.1581$  (for 8532 data and 466 refined parameters).

X-ray diffraction data were collected with a Kuma KM4CCD diffractometer using graphite-monochromated Mo- $K_{\alpha}$  radiation. The crystal was placed 60 mm from the CCD detector. For both temperatures, more than hemisphere of reciprocal space was covered by combination of six sets of exposures; each set had a different  $\varphi$ -angle (0, 90, 180, 270, 45, 135) and each exposure of 30s covered 0.75° in  $\omega$ . Coverage of the unique set is 99.2% complete up to  $2\Theta = 52^{\circ}$ . The unit cell determination and data integration were carried out using the CrysAlis package of Oxford Diffraction. Intensity data were corrected for the Lorentz and polarization effects. The structure was solved by direct methods and refined with the SHELX program by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were placed at calculated positions and refined as riding atoms in the subsequent least squares model refinements. For better fitting of the electron density, the positions of some of the  $-CHCl_2$  groups and one THF ligand were refined taking into account disordered models in the combination with the available tools (PART, DFIX and SADI) in SHELXL97 [16, 17]. The atoms belonging to the minor component of the disordered fragment were refined only isotropically. CCDC 692342 (130 K), CCDC 692959 (293 K) contain the supplementary crystallographic data for this structure. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or <u>deposit@ccdc.cam.ac.uk</u>

#### Conclusions

In conclusion the technique of synthesis of new hexanuclear bis( $\mu_3$ -oxo) carboxylates containing {Fe<sub>4</sub>Ca<sub>2</sub>O<sub>2</sub>} core is developed. Mossbauer spectrum of this complex at room temperature shows one doublet characteristic for Fe(III) positions in a high spin state (S=5/2). In the range of temperature 300-20 K between iron (III) ions there is an antiferromagnetic exchange interaction in the two isosceles triangular skeletons with the following values of exchange integral (J = J<sub>12</sub> = J<sub>34</sub> = -71.86 cm<sup>-1</sup>). High magnetic EPR measurements of **1** indicates the presence of S=0 ground state with low-lying S=1 excited state centred around g = 2.0054 ±0.0001.

#### Supporting information available.

Listing crystallographic parameters in CIF format, atomic coordinates, bond distances and angles, thermal parameters and hydrogen atom positions for the title complex are available free of charge from authors.

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# $\begin{array}{c} \text{MIXED-LIGANDS } \mu_3\text{-}OXO \text{ TRINUCLEAR CARBOXYLATES} \\ [\text{Fe}_3O(\text{CH}_2\text{BrCOO})_{1.5}(\text{CH}_2\text{CICOO})_{4.5}(\text{H}_2\text{O})_3]\text{Br}_{0.75}\text{Cl}_{0.25} \text{ 5H}_2\text{O} \text{ and } [\text{Fe}_3O(\text{BrCH}_2\text{COO})_6(\text{H}_2\text{O})_3] \\ \text{NO}_3\cdot 2.63\text{H}_2\text{O} \end{array}$

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**Abstract:** Two novel  $\mu_3$ -oxo-centered carboxylate-bridged triiron complexes [Fe<sub>3</sub>O(BrCH<sub>2</sub>COO)<sub>6</sub>(H<sub>2</sub>O)<sub>3</sub>] NO<sub>3</sub>·2.63H<sub>2</sub>O (1) and [Fe<sub>3</sub>O(CH<sub>2</sub>BrCOO)<sub>1.5</sub>(CH<sub>2</sub>ClCOO)<sub>4.5</sub>(H<sub>2</sub>O)<sub>3</sub>]Br<sub>0.75</sub>Cl<sub>0.25</sub> 5H<sub>2</sub>O (2) were synthesized and their structures were characterized by X-ray crystallography. The opportunity of mixed-ligand complex formation in iron(III)-bromoacetic acid system was shown. The first co-ordination sphere of the iron atom in compound 2 includes two different carboxylate anions, CH<sub>2</sub>BrCOO<sup>-</sup> and CH<sub>2</sub>ClCOO<sup>-</sup> in the capacity of *syn-syn-* bidentate-bridged ligands, while Br and Cl<sup>-</sup> anions being in the ratio 1:1, formulate the external sphere of the complex. The IR spectra, thermic analysis and magnetic properties of complexes were studied.

Keywords: mixed-ligands complexes; Iron(III) carboxylates; crystal structure, magnetic properties

#### Introduction

Oxo-centered carboxylate–bridged trinuclear complexes of the type  $[M_3(\mu_3-O)(RCOO)_6L_3]^{n+}$  represent an important class of compounds in transition metal chemistry [1]. The study of trinuclear carboxylates of iron(III) covers a large number of publications. Extensive structural and physicochemical studies of these compounds have been crucial for increasing understanding of bonding and magnetic interactions between proximate metal centers, topics with implications ranging from metalloprotein structures [2-12] to industrial catalysis and molecular materials [13-16]. The relatively large metal-metal distances preclude the possibility of direct metal-metal bonding and the complexes are particularly interesting and are useful models for systematically studying weak metal-metal interactions in multi-nuclear metal complexes.

The oxotrinuclear complexes are known with a wide variety of metal ions, bridging carboxylate anions and monodentate terminal ligands as well as with mixed-valence [17-20] and mixed-metal combinations [21-25]. The clusters which contain various bridge carboxylate ligands in the first coordination sphere are less studied. Among these there can be mentioned compounds with composition  $[Fe_3O(RCOO)_n(B)_{6-n} L_3]^{m+}$ : where RCOOH are different aminoacids,  $B = H_2PO_3^-$ , n = 3, m = 4,  $A = NO_3^-$  [26]; RCOO= CH\_3COO<sup>-</sup>,  $B = C_2H_5COO^-$ , n = 3, m = 1 [27]. Since, the variations in the bridging carboxylates and the monodentate ligands can influence structural and electronic properties of the complexes [28-30], synthesis and study of such complexes remain attractive.

In the present paper we have studied the formation of new complexes in different conditions of iron(III)bromoacetic acid system which is only described by one structural work in literature [31]. The structure, IR spectra, thermic analysis and magnetic properties of complexes have been investigated.

#### **Results and discussion**

The reaction of bromoacetic acid with iron(III) nitrate or chloride in molar ratio 2:1, at sodium alkaline presence, results to the trinuclear compounds with following composition:  $[Fe_3O(BrCH_2COO)_6(H_2O)_3]NO_3 \cdot 2.63H_2O$  (1) and  $[Fe_3O(CH_2BrCOO)_{1.5}(CH_2CICOO)_{4.5}(H_2O)_3]Br_{0.75}Cl_{0.25} \cdot 5H_2O$  (2). Compound 1 is also formed in the case when iron(III) carbonate was used for neutralization of carboxylic acid. In the reaction containing Cl<sup>-</sup> as anion, the formation of the mixed-ligand complex is revealed. The complexes are crystalline solids with red-brown colour. The molecular and crystal structures have been established by single X-ray structural analysis for both complexes. Crystal data, data collection parameters and refinement for 1 and 2 are presented in table 1, while selected bonds lengths and angles – in table 2.
Both 1 and 2 compounds have the similar molecular structure typical for trinuclear iron(III) carboxylates containing a  $\mu_3$ -O bridge. The iron atoms are described as an almost equilateral triangle and each of them is coordinated by four O atoms of bridging carboxyl groups and the water molecule in the *trans* position to the  $\mu_3$ -bridging oxygen atom.

The crystal **1** consists of trinuclear complex cations  $[Fe_3O(CH_2BrCOO)_6(H_2O)_3]^+$ ,  $NO_3^-$  anions and solvent water molecules in 1:1:2.63 ratio. The outer sphere anions  $NO_3^-$  occupy two different systems of special positions on the two-fold axis with the occupancy factor of 0.5. Two solvent water molecules O(6w) and O(7w) are also located on the two-fold axis with the same occupancy factor. The general position of solvent water molecule O(4w) statistically alternates with the position of one of  $NO_3^-$  groups in 1:1 ratio. The structure of the complex cation  $[Fe_3O(CH_2BrCOO)_6(H_2O)_3]^+$  with the labeling scheme is depicted in figure 1. Three iron atoms define an equilateral



Figure 1. Molecular structure of the complex 1.

triangle with the  $\mu_3$ -O atom in its centre. The geometrical characteristics of the  $[Fe_3O]^{7+}$  core are well compared with those found in  $[Fe_3O(CH_2BrCOO)_{1.5}(CH_2CICOO)_{4.5}(H_2O)_3]^+$  (2) and in the other iron carboxylate complexes [18, 31, 32]. The average Fe-Fe distances are equal to 3.307(1) and 3.31(1) Å, while Fe-O(1) adopts the values 1.912(5) and 1.902(3) Å in 1 and 2, respectively. Six carboxylate groups with *syn-syn* configuration bridge the pairs of Fe(III) atoms in the cluster. The Fe-O<sub>COO</sub> distances are in the range 2.007(5)-2.039(5)Å for 1 and 1.976(5)-2.043(5)Å for 2. For both complexes each of the Fe atom exhibits (6O) slightly distorted octahedral environment with very similar geometric characteristics (table 1).

		F F F F F F F F F F F F F F F F F F F
Compound	I	II
formula	$C_{12}H_{23}Br_{6}Fe_{3}NO_{21.50}$	$C_{12}H_{28}Br_{2.21}Cl_{4.79}Fe_{3}O_{21}$
formula weight	1172.32	1022.19
crystal system, space group	Orthorhombic, C222 <sub>1</sub>	Monoclinic, P2 <sub>1</sub> /n
<i>a</i> , Å	18.778(4)	12.580(3)
b, Å	19.061(4)	14.554(3)
<i>c</i> , Å	18.393(4)	17.947(4)
α, deg	90.0	90.0
β, deg	90.0	96.13(3)
γ, deg	90.0	90.0
$V, Å^3$	6583(2)	3267.1(13)
Z, $\rho_{calcd}$ , g cm <sup>-3</sup>	8, 2.366	4, 2.018
λ, Å	0.71073	0.71073
Т, К	120	150

Crystallographic data collection and structure determinations for complexes I and II

$\mu(MoK_{\alpha}), cm^{-1}$	8.669	4.485
GOOF for $F^2$	1.067	1.042
${}^{a}R\left[I>2\sigma(I)\right]$	0.0440	0.0570
$^{b}wR$ (all data)	0.0532	0.0717
$\Delta \rho_{\rm max}$ and $\Delta \rho_{\rm min}$ , eÅ <sup>3</sup>	1.576 and -1.209	1.458 and -0.888

 ${}^{a}R = \sum ||F_{o}| - |F_{c}|| / \sum |F_{o}|. {}^{b}wR = [\sum w(|F_{o}|^{2} - |F_{c}|)^{2} / \sum w |F_{o}|^{2} |^{2}]^{1/2}$ 

# Selected bond lengths (Å) and angles (°) in structures 1 and 2.

Bond Compound 1 2 Fe1-01 1.912(5) 1.902(3)Fe1-O2 2.005(4)2.005(5)Fe1-08 2.008(5)1.980(4)Fe1-06 2.022(5) 2.037(4)Fe1-O4 2.023(5)2.023(4)Fe1-O1w 2.051(5)2.073(4)Fe2-O1 1.904(5)1.913(3) Fe2-O10 2.008(5)2.048(4)Fe2-O7 2.009(5) 2.014(4)Fe2-09 2.039(4)2.011(5) Fe2-O12 2.023(5) 2.012(4)Fe2-O2w 2.094(5) 2.030(4)Fe3-O1 1.915(5) 1.919(3) Fe3-O11 2.016(5) 2.005(4)Fe3-O3 2.018(4)2.020(5)Fe3-O13 2.028(5)1.998(4) Fe3-O3w 2.031(5)2.031(4)Fe3-O5 2.037(5)2.044(4)Angle 1 2 97.7(2) 96.96(15) O1-Fe1-O2 01-Fe1-08 95.9(2) 94.56(16) O2-Fe1-O(8 166.1(2)168.47(16) O(1-Fe1-O6 95.99(15) 92.6(2) O2-Fe1-O6 89.1(2) 84.32(17) 08-Fe1-O6 92.6(2) 93.97(17) 93.28(15) 01-Fe1-O4 91.5(2) O2-Fe1-O4 90.3(2)91.99(17) 08-Fe1-O4 87.0(2) 87.87(17) 06-Fe1-O4 175.8(2)170.38(16) O1-Fe1-O1w 178.0(2) 177.92(16) 84.40(16) O2-Fe1-O1w 83.7(2) 84.10(17) O8-Fe1-O1w 82.6(2) 06-Fe1-O1w 85.71(15) 88.7(2)O4-Fe1-O1w 87.1(2) 85.08(16) O1-Fe2-O10 98.9(2) 97.07(15) O1-Fe2-O7 94.8(2) 94.35(15) 168.48(15) O10-Fe2-O7 166.1(2)O1-Fe2-O9 95.1(2) 94.66(15) O10-Fe2-O9 86.9(2) 85.83(16) O7-Fe2-O9 94.0(2) 91.73(17) O1-Fe2-O12 95.1(2) 93.43(15) O10-Fe2-O12 89.60(16) 89.2(2) O7-Fe2-O12 91.25(17) 87.5(2)

Table 2

O9-Fe2-O12	169.5(2)	171.14(16)
O1-Fe2-O2w	179.0(2)	178.48(16)
O10-Fe2-O2w	81.3(2)	83.84(15)
O7-Fe2-O2w	85.0(2)	84.77(16)
O9-Fe2-O2w	83.9(2)	86.62(16)
O12-Fe2-O2w	85.9(2)	85.35(15)
O1-Fe3-O11	97.9(2)	96.13(15)
O1-Fe3-O3	96.6(2)	97.56(15)
O11-Fe3-O3	165.4(2)	166.27(16)
O1-Fe3-O13	92.0(2)	93.97(15)
O11-Fe3-O13	88.6(2)	88.11(17)
O3-Fe3-O13	89.3(2)	92.01(16)
O1-Fe3-O3w	179.5(2)	176.52(15)
O11-Fe3-O3w	81.6(2)	80.63(15)
O3-Fe3-O3w	83.8(2)	85.66(15)
O13-Fe3-O3w	88.0(2)	87.20(16)
O1-Fe3-O5	91.4(2)	93.05(15)
O11-Fe3-O5	89.5(2)	86.91(17)
O3-Fe3-O5	91.8(2)	91.29(16
O13-Fe3-O5	176.3(2)	171.79(16)
O3W-Fe3-O5	88.6(2)	85.55(16)

In the crystal structure of **1** all components interact via a system of O-H...O, C-H...O, O-H...Br and C-H...Br hydrogen bonds that lead to the formation of 3D supramolecular aggregate. The structural functions of two nitrate anions are different. One of them is located in the closed cages, which are formed by the packing of trinuclear cations in the crystal (figure 2a). Thus, it is connected directly or through O(4w) by hydrogen bonds with the coordinated water molecules of four next complexes. It results in the layers running perpendicular to the *c* axis in the unit cell and being additionally stabilized by the intermolecular C-H...Br contacts. The second NO<sub>3</sub><sup>-</sup> group is located in the space between these layers and consolidate bromine atoms, co-ordinated O(1w), O(2w) and solvated O(5w), O(6w), O(7w) water molecules (figure 2b). O(7w) water molecule realises its donor functions in the links with O(2w) and bromine atoms, thus provides the interaction between four  $\mu_3$ -oxo clusters in the crystal.



Figure 2. *a* and *b*. Structural function of two  $NO_3^-$  anions in crystal 1.

The final chemical composition and structure of compound **2** was only proved by X-ray analysis. The first co-ordination sphere of iron includes two different carboxylate anions,  $CH_2BrCOO^-$  and  $CH_2ClCOO^-$  in the capacity of *syn-syn-* bidentate ligands being in 1:3 ratio, while Cl<sup>-</sup> and Br anions are counter-ions.

The presence of the bromide anion as counter-ion can be only explained by the suggestion that in the reactive medium in the conditions of high concentration of chlorine ions the partial replacement of bromine atoms in bromoacetic acid molecule by chlorine takes place. Iron(III) chloride and monobromoacetic acid were used as the initial substances for the complex preparation. Thus, the final complex composition for **2** corresponds to the formula  $[Fe_3O(CH_2BrCOO)_{1.5}(CH_2CIOO)_{4.5}(H_2O)_3]Br_{0.75}CI_{0.25}$ ·5H<sub>2</sub>O.

The packing of the complexes **2** in the crystal affords the closed cavities, which are filled by the water tetramer associates,  $[H_2O]_4$  as showed in figure 3. They are bound with the nearest trinuclear complexes by the hydrogen bonds through bromine (or chlorine) atoms of acid residue, and co-ordinated water molecules. Each  $[H_2O]_4$  tetramer is built



Figure 3. Fragment of the crystal structure of the complex 2.

of O(7w) and O(8w) water molecules and their two-fold axis related counterparts, O(7w)' and O(8w)' molecules. Water tetramers are combined into the 3D network (Figure 4) by the hydrogen bonds with the participation of the solvent water molecules O(6w), O(4w) and outer sphere Br anions. The hydrogen bonds in which O(1w), O(3w), O(4w), O(6w) water molecules and the oxygen atoms of one nitrate group defined by (N(1) atom are involved facilitate the formation of infinite ribbons running along c axis in the unit cell. The ribbons are linked via the C-H...O and O-H...Br interactions, which cause the formation of two-dimensional network (layers).



Figure 4. 3D network formed by water molecules in the crystal structure of 2.

The thermic decomposition of the nitrate complex includes 5 overlapping processes. The first step is endothermic and is characterized by  $t_{initial} = 50-60$  °C;  $t_{max} = 140$  °C;  $t_{final} = 160$  °C. The mass loss corresponds to ~15 %. The obtained data show that in this range of the temperatures the complex decomposition occurs with the removal of outer sphere and inter-sphere water molecules, and the decomposition of bridge monobromoacetate ligands begins. Next three processes

are exothermic ones and correspond to the loss of mass till 69 %. The fifth, terminal process occurs at 330-420 °C and apparently corresponds to the iron(III) oxide formation (the residue is 22 - 21 %).

The thermic decomposition of the second compound differs essentially from the first one. The process mainly occurs in the range of the temperatures of 40 - 180°C with the maximum at 100 °C. Apparently, this temperature interval ensures the removal of the ligands with the formation of iron oxyhalogenides. This endothermic process corresponds to the mass loss of ~ 34 %. In the range 180 - 340 °C the further mass loss of 7 % takes place. Unexpectedly on the TGA curve the endothermic peak appears in the range 400 - 420 - 440 °C, which does not correspond to the mass change. We suggest the possibility of phase transition of the decomposition products in this temperature interval. In the range of 560 - 730 °C the further loss of the substance equal 5-6% is observed that may correspond to the removal of the definite part of iron halogenides. Even at 800 °C the stabilization of the decomposition process does not occur.

The iron(III) oxide should be considered as a final product of the thermic decomposition of compounds **1** and **2**. The first two decomposition steps for the studied iron(III) carboxylates correspond to the removal of water molecules and carboxylate ions with the formation of the product characterized by the tetrahedral environment of the central metal *via* anions ( $O^{2-}$ ), ( $Cl^{-1}$ ), ( $Br^{-1}$ ). Apparently each cluster keeps its framework {Fe<sub>3</sub>O}, where chloride and bromide anions complete the coordination number of each iron cation till four. Further increase of the temperature results in the removal of iron chloride and/or bromide with the simultaneous formation of iron(III) oxide. The additional study could help to clarify the mechanism of decomposition of these compounds.

IR spectra of the synthesized substances exhibit all the main characteristic frequencies typical for the carboxylate anions, C-Hal bonds and water. The assignment of the absorption bands was carried out according with [33]. In the range of  $3650 - 3000 \text{ cm}^{-1}$  the wide band v(OH) is characteristic for the H-bonded water molecules. On the background of this wide band two peaks of the medium intensity are exhibited at 3570 and  $3540 \text{ cm}^{-1}$ , which can be attributed to the non-coordinated water molecules. The appearance of a band at 920 cm<sup>-1</sup> is natural and corresponds to the deformation vibrations of OH moiety,  $\delta$ (HOH). Coordinated carboxyl groups exhibit the frequencies at 1600 µ 1415 cm<sup>-1</sup> for (1) and at 1608 µ 1426 cm<sup>-1</sup> for (2) which correspond to the bridge COO<sup>-</sup> moieties ( $\Delta v = 172 - 185 \text{ cm}^{-1}$ ). The single band of the medium intensity at 1220 cm<sup>-1</sup> for (1) and two splitting absorption bands at 1260-1256;  $1232-1220 \text{ cm}^{-1}$  for **2**, can be attributed to the combination of the vibrations  $\delta$ (OH) +  $\delta$ (C-O) of two these moieties, O-H and (C-O) [33].

Complex 2 is characterized by the band at 794 cm<sup>-1</sup> of the medium intensity, which is absent in the IR spectrum of 1, and it can be attributed to the stretching vibrations v(C-Cl). The stretching vibrations v(C-Br) at ~570 cm<sup>-1</sup> are characteristic for the both complexes. The very weak band at 850 cm<sup>-1</sup> in the IR spectrum of 1 corresponds to the  $v_2(NO_3^{-1})$  frequency of nitrate anion. The band at 680 ± 5cm<sup>-1</sup> is present in the spectra of both 1 and 2 and it is assigned to the bending vibrations of  $\delta(OCO)$ , while the band at 725 ± 5 cm<sup>-1</sup> is assigned to the -  $\rho$  (COO) [1, 33]. In the 960 - 850 cm<sup>-1</sup> range of the spectrum there are three bands: 950, 928 cm<sup>-1</sup> for 2 and 950 cm<sup>-1</sup> for 1 assigned to v(C-C) and  $\delta$  (O-H) at 885 ± 5 cm<sup>-1</sup>. Asymmetric vibrations of the {Fe<sub>3</sub>O} core are identified at about 600 – 605 cm<sup>-1</sup>( $v_{as}$ (Fe<sub>3</sub>O)). Thus, the positions of the absorption bands v(COO) and  $v_{as}$ (Fe<sub>3</sub>O) in the IR spectra of iron carboxylates confirm the coordination mode of the acid residues to the central metal atom in according with X-ray data.

Magnetic measurements for the complex 1 were made at the range 300 K - 2 K and the results are shown in the figure 5. The magnetic moments of complexes 1 and 2 per one iron atom were found to be 3.28 and 3.17 B.M. (RT) respectively, that is essentially lower than the pure spin value for iron(III) ( $\mu_{ef}$ = 5.92 B.M.). These observations



Figure 5. The temperature dependence of the experimental and theoretical values of  $\mu_{ef}$  for complex 1.

indicate the antiferromagnetic interaction between the paramagnetic metal ions. The temperature dependence of magnetic properties of (1) was described by Heisenberg-Dirac-van Vleck (HDVV) approximation  $\mathbf{H} = -2J \Sigma (\mathbf{S}_i \mathbf{S}_j)$  [34, 35] for complex with  $D_{3h}$  symmetry. The best fit between experimental and theoretical data have been obtained by the value of (-J) equal to ~40 cm<sup>-1</sup>, which is in good concordance with the respective parameter for other trinuclear oxobridged iron(III) carboxylates [32, 36].

#### Experimental

General: The following compounds Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O, monobromacetic acid, sodium hydroxide were obtained from commercial sources and used as received. The carbon and hydrogen content was determined by standard micromethods in the microanalytical group of the Institute of Chemistry of the Academy of Sciences of Moldova. IR spectra of polycrystalline samples were recorded (4000-400cm<sup>-1</sup>) as oil mulls on a Specord M-75 spectrophotometer. TG studies were performed on a Paulik-Paulik-Erdey derivatograph in air, with platinum crucible, Al<sub>2</sub>O<sub>3</sub> as calibration standard and at a speed of heating equal to 5 °C/min. DTG - 1/5; DTA - 1/10; TG - 100/100. T<sub>max</sub> =1000 °C. Variable temperature susceptibility was measured with an Oxford Instruments Vibrating Sample Magnetometer (VSM) working between 0 and 12 T and in the 1.5–350.0K temperature range. The diamagnetic Pascal's constants were used to correct the magnetic values [35]. To determine the value of  $\mu_{eff}$  the relation ( $\mu_{eff}$ )<sub>M</sub> =  $\sqrt{8c}_M T$  (B.M.) was used. The J parameter was calculated by least squares fitting using the experimental data fitting Program "Minsk".

# Synthesis. [Fe<sub>3</sub>O(CH<sub>2</sub>BrCOO)<sub>6</sub>(H<sub>2</sub>O)<sub>3</sub>]NO<sub>3</sub>·2.63H<sub>2</sub>O (1)

(a) A solution of BrCH<sub>2</sub>COOH (1.39 g, 10 mmol) in distilled water (10 cm<sup>3</sup>) was neutralized with solid NaOH (0.4 g, 10 mmol). To this solution was added (dropwise) Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O (2.02 g, 5 mmol) in distilled water (20 cm<sup>3</sup>) while stirring, forming a deep red solution. The resulting solution was heated up on a water bath at temperature 50°C for 30 minutes and than cooled to room temperature. After two days the crystalline dark red precipitate was collected by filtration, washed with ethanol, and dried in air at room temperature. Yield: 1.5 g (77%). Found, %: C 12,03, H 1.90, N 1.14, Fe 14.00. Calc. for  $C_{12}H_{23}Br_6Fe_3NO_{21.50}$  (1172.32), %: C 12.28, H 1.98, N 1.19, Fe 14.29.

(b) The complex 1 was also obtained using iron carbonate by the following method. To the warm solution of BrCH<sub>2</sub>COOH (1.39 g, 10 mmol) in distilled water (20 cm<sup>3</sup>) the corresponding amount of iron carbonate was added while stirring. The equivalence point was determined proceed from the effervescence of CO<sub>2</sub>. Step-by-step the color was changing to deep red. After the termination of the CO<sub>2</sub> effervescence the heating on the water bath was continued for 15-20 minutes. The resulting solution was filtrated off and the aqueous solution of Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O (0.67 g, 1.6 mmol) was added. After two days the crystalline dark red precipitate was collected by filtration, washed with ethanol, and air dried at room temperature. Yield: 1.4 g (72%). Found, %: C 12.03, H 2.1, N 1.17, Fe 14.05. Calc. for  $C_{12}H_{23}Br_6Fe_3NO_{21.50}$  (1172.32), %: C 12.28, H 1.98, N 1.19, Fe 14.29.

 $[Fe_3O(CH_2BrCOO)_{1.5}(CH_2CICOO)_{4.5}(H_2O)_3]Br_{0.75}Cl_{0.25}$  5H<sub>2</sub>O (2). A solution of BrCH<sub>2</sub>COOH (1.39 g, 10 mmol) in distilled water (10 cm<sup>3</sup>) was neutralized with solid NaOH (0.4 g, 10 mmol). To this solution was added (dropwise) FeCl<sub>3</sub>·6H<sub>2</sub>O (1.35 g, 5 mmol) in distilled water (20 cm<sup>3</sup>) while stirring, forming a deep red solution. The resulting solution was heated up on a water bath at temperature 50°C for 30 minutes and than cooled to room temperature. After several days the microcrystalline dark red precipitate was collected by filtration, washed with ethanol, and airdried at room temperature. Yield: 1.1 g (67%). Found, %: C 13.89, H 2.70, Fe 16.07. Calc. for  $C_{12}H_{28}Br_{2.21}Cl_{4.79}Fe_3O_{21}$  (1022.19), %: C 14.08, H 2.73, Fe 16.38.

*X-ray crystallography*: All crystallographic measurements were carried out with a KM4CCD diffractometer equipped with a graphite monochromated Mo-K<sub>a</sub> radiation source. For both **1** and **2** crystals 532 frames were measured in four series, rotated by  $\varphi$ =0.75° from each other and a detector-to-crystal distance has been equal to 60 mm. All data processing was performed with the use of the Kuma Diffraction program package (Wroclaw, Poland). Intensity data were corrected for the Lorentz and polarization effects. The structure was solved by direct methods using SHELXS-86 [37] and refined by full-matrix least-squares on  $F^2$  using SHELXL-93 [38]. All measured reflections were included in the refinement process. The non-H atoms were refined with anisotropic displacement parameters. The positions of H-atoms bonded to C atoms were fixed in the idealized positions and allowed to ride. Positional parameters of the water H-atoms were obtained from the difference Fourier syntheses and verified by the geometric parameters of hydrogen bond and are summarized in Table1, while bond lengths and angles - in Table 2.

Crystallographic data for the structures in this paper have been deposited at the Cambridge Crystallographic Data Centre as supplimentary publication. CCDC reference numbers 701028 and 701029. Copies of data can be obtained on application by <deposit@ccdc.cam.ac.uk>. Main crystallographic parameters and structure refinement details are summarized in table 1, while selected bond lengths and angles in table 2.

## Conclusions

Two novel  $\mu_3$ -oxo-centered carboxylate-bridged triiron complexes [Fe<sub>3</sub>O(BrCH<sub>2</sub>COO)<sub>6</sub>(H<sub>2</sub>O)<sub>3</sub>]NO<sub>3</sub>·2.63H<sub>2</sub>O (1) and [Fe<sub>3</sub>O(CH<sub>2</sub>BrCOO)<sub>1.5</sub>(CH<sub>2</sub>ClCOO)<sub>4.5</sub>(H<sub>2</sub>O)<sub>3</sub>]Br<sub>0.75</sub>Cl<sub>0.25</sub> 5H<sub>2</sub>O (2) have been synthesized and their structures were refined by single X-ray diffraction. The opportunity of mixed-ligand complex formation in iron(III)-bromoacetic acid system was shown. In the reaction medium containing iron(III) chloride as initial, the partial replacement of bromine atoms in bromoacetic acid molecule by chlorine takes place. The first co-ordination sphere of the iron atom in compound 2 includes two different carboxylate anions, CH<sub>2</sub>BrCOO<sup>-</sup> and CH<sub>2</sub>ClCOO<sup>-</sup> in the capacity of *syn-syn*-bidentate-bridged ligands, while Br and Cl<sup>-</sup> anions being in the ratio 1:1, formulate the external sphere of the complex. The magnetic moments of the complexes 1, 2 per one iron atom founded 3.28 and 3.17 B.M.(RT) respectively, indicate the antiferromagnetic interaction between the paramagnetic metal ions with the value of -J = 40.2 cm<sup>-1</sup> calculated for the complex 1.

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# SPECIFIC "IONIC LIQUIDS" AS NEW ORGANOCATALYSTS OF BIGINELLI REACTION

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Abstract: New ionic liquids bearing an imidazolium core with a carboxy group have been prepared in an attempt to design new organocatalysts of Biginelli reaction. Trends in the properties of these compounds are discussed. The synthetized compounds represented a novel class of molted salts and may be considered as new organocatalysts for chemical reaction.

Keywords: ionic liquids, organocatalyst, Biginelli reaction.

#### Introduction

The knowledge about the three-component condensation reaction between an aldehyde, a urea or thiourea, and an easily enolizable carbonyl compound, is quite extensive, and has been described by the Italian chemist Pietro Biginelli in 1893. This reaction offers a straightforward approach to 3,4-dihydropyrimidin-2-(1*H*)-ones(thiones) and others bioactive heterocyclic compounds [1,2]. The discussed reaction has catalyzed mineral, organic or Lewis acids and microwave irradiation as well [1-4].

Herein, we describe the specific synthesis of the carboxy group containing imidazolium ionic liquids with hydrogensulphate as well as bromide as anions and their catalytic properties.

#### **Results and discussion**

A short time ago we reported about the synthesis of the mixture **2a,3a** or **2b,3b** from monochloroacetic acid and N-substituted imidazoles **1a,b**, and their application as catalysts of Biginelli reaction [5].



Scheme 1

In continuation of this approach, we are looking for ionic liquids composed of imidazolium cations with "free" carboxyl group. *Wang Z. et al* [6] proposed synthesis of compound 2a via alkylation of 1-methylimidazole 1a with chloroacetic acid under basic condition. We repeated the published procedure, but obtained the mixture of products.

We therefore decided to develop the new route to such compounds. The most logical protocol for the synthesis comprises the use the protected carboxyl group  $\alpha$ -monohalogenated acetic acid followed by removal of protecting group.



Scheme 2

In this respect, imidazolium bromide **4** has been prepared by reaction of the appropriate imidazole **1a** with *tert*-butyl bromoacetate, which was subsequently treated with catalytic amount of water solution of HBr to afford the

3-carboxymethyl-1-methyl-1*H*-imidazolium bromide **5a** according to Scheme 2. Oily salt **5b** was obtained by addition of one equivalent of 98%  $H_2SO_4$  to product **5a**.

We examined the Biginelli reaction of ethyl acetoacetate with benzaldehyde and urea (or thiourea) in the presence of 5a and 5b (0.6 mol%) at elevated temperatures (scheme 3).



Both reactions gave rise to the corresponding 3,4-dihydropyrimidin-2-(1*H*)-ones(thiones) **6a,b** which were formed in variable yield. The yield, as well as the time of the reaction, was significantly improved by the nature of the catalyst (see Table 1).

Table 1

	• •				
№ Compound	Mol. Formula	M.p. <sup>0</sup> C	Catalyst	Time (min)	Yield (%)
		Registered /		Registered /	Registered/
		Ref.		Ref.	Ref.
6a	$C_{14}H_{16}N_{2}O_{3}$	213-214/	2a,b	90 [5]	56 [5]
		233_236 [4]	3a,b	60 [5]	61 [5]
		233-230 [4]	5a	30	72
		213-214 [5]	5b	10	67
		235-236 [7]			
6b	C <sub>14</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub> S	212-213/	2a,b	90 [5]	55 [5]
	14 10 2 2	220-224 [4]	3a,b	80 [5]	67 [5]
		220-224 [4]	<b>5</b> a	60	65
		212-213 [5]	5b	10	65

Physical and analytical data of synthesized compounds

It is worth noting, that our synthesized "ionic liquids" are more effective catalysts in comparison with other early published ones [4-6].

It is reasonable to speculate that the reaction mechanism involves the condensation of urea with the aldehyde at high temperature to yield the corresponding iminium intermediate, which is then trapped by an aldol-type reaction with the enol derived from the ketoester. Such an effect may also be of significance in the action of "free" carboxy group of acetic acid and the enhancement of selectivity in the presence of imidazolium, as well as hydrogensulphate or bromine ions.

### Conclusions

In conclusion, we have synthetized two new ionic liquids bearing an imidazolium core with a carboxy group, which can be easily used as organocatalysts of Biginelly reaction. These results open new possibilities in the construction of a new type of specific "catalysts" and could be used in the future for the production of pharmaceutically interesting products without any transition metal contamination. The studies of the enantiospecific variant of the Biginelli reaction are now being investigated in our laboratory.

#### Experimental

All the used solvents were of reagent quality, and all commercial reagents were used without additional purification. Removal of all solvents was carried out under reduced pressure. Analytical TLC plates were Silufol<sup>®</sup> UV-254 (Silpearl on aluminium foil, Czecho-Slovakia). IR spectra were recorded on a Specord 75 IR instrument. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded for d<sub>6</sub>-DMSO 2-3% solution on a Bruker AC-80 (80 and 20 MHz).

N-methylimidazole 3 and tert-butyl 2-bromoacetate - reagents from Aldrich Chemical Company.

**Preparation of 3**-(*tert*-butyloxycarbonylmethyl)-1-methyl-1*H*-imidazolium bromide 4. Bromide 4 was prepared by mixing 0.82 g (0.001 mol) of N-methylimidazole 1a and 1.94 g (0.001 mol) *tert*-butyl 2-bromoacetate in 5 ml of dry MeCN at room temperature for 24 hours. The solvent was removed and obtained 2.59 g of 4. The yield was 94%. IR (v/cm<sup>-1</sup>): 1248 ((CH<sub>3</sub>)<sub>3</sub>C), 2854 (CH<sub>3</sub>) 3153, 1577 (CH=C), 1743 (C=O), 623 (Br). <sup>1</sup>H NMR ( $\delta$ , ppm): 1.37 s (9H, tert-butyl), 3.92 s (3H, Me-N), 5.29 s (2H, CH<sub>2</sub>), 7.86 s, 7.90 s, 9.39 s (3H, imidazole). Mol. For. C<sub>10</sub>H<sub>17</sub>BrN<sub>2</sub>O<sub>2</sub> Cal. C 43.34; H 6.18; N 10.11. Find C 43.24; H 6.00; N 9.87.

**Preparation of 3-carboxymethyl-1-methyl-1H-imidazolium bromide 5a.** Several drops of 45% solution of HBr in water were added to a solution of 2.77 g (0.001 mol) of **4** in MeCN (5 ml) at room temperature. The residue was stirred at room temperature for 3 hours, followed by distilling of the solvent and drying over  $P_2O_5$  and we obtained 1.79 g of **5a**. The yield was 82%. M.p. 184-185°C from ethanol. IR (v/cm<sup>-1</sup>): 3450, 3091, 2906 (OH<sup>-</sup>), 2906 (CH<sub>3</sub>), 2570, 2479 (N<sup>+</sup>), 3114, 1463, 1570 (CH=C), 1732 (C=O), 619 (Br<sup>-</sup>), <sup>1</sup>H NMR ( $\delta$ , ppm): 3.88 s (3H, Me-N), 4.97 s (2H, CH<sub>2</sub>), 4.97 s (1H, CO<sub>2</sub>H), 7.68 s, 7.70 s, 9.10 s (3H, imidazole). Mol. For. C<sub>6</sub>H<sub>9</sub>BrN<sub>2</sub>O<sub>2</sub> Cal. C 32.60; H 4.10; N 12.67. Find C 32.54; H 4.00; N 12.88.

**Preparation of 3-carboxymethyl-1-methyl-1***H***-imidazolium hydrogensulphate 5b**. Mixrure of 5a (1.97 g, 0.089 mol) and  $H_2SO_4$  (0.87 g, 0.089 mol) in 5 ml MeCN was stirred for 30 min. Distilling of the solvent followed drying over  $P_2O_5$  to give 2.34 g of yellow oil 5b. The yield was 98%. IR (v/cm<sup>-1</sup>): 3417, 3158 (OH<sup>-</sup>), 2965, 2888 (CH<sub>3</sub>), 2552 (N<sup>+</sup>), 3120, 1419 (CH=C), 1088 (HSO<sub>4</sub><sup>--</sup>), 1740 (C=O). <sup>1</sup>H NMR (δ, ppm): 3.84 (C, 3H, N-CH<sub>3</sub>), 5.09 (C, 2H, CH<sub>2</sub>-CO), 7.65 s 7.67 s 9.04 s (3H, imidazol), <sup>13</sup>C NMR (δ, ppm): 36.21 (CH<sub>3</sub>), 59.84 (CH<sub>2</sub>), 123.54, 124.04, 135.96 (CH imidazole), 168.45 (C). Mol. For.  $C_6H_{10}N_2O_6S$ . Cal. C 30.25; H 4.23; N 11.76. Find C 30.29; H 4.11; N 11.56.

General procedure of preparation of ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5carboxylate 6a and ethyl 6-methyl-2-thioxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate 6b. To a mixture of 1.06 g (0.01 mol) of benzaldehyde, 1.3 g (0.01 mol) of ethyl acetoacetate and 0.01 mol of thiourea or urea were added 0.6 mol % of appropriate catalyst. The mixture was heated at 105-120°C. On completion of reaction, the mixture was dispersed in EtOH, the residue was filtered off, washed with  $H_2O$  and dried over  $P_2O_5$ . A sample was recrystallized for analysis from EtOH. Physical, analytical and catalytically data of synthetized compounds are presented in Table.

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# **GLYCOSIDES FROM LINARIA VULGARIS MILL**

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**Abstract:** A new flavonol glycoside, 5,4'-dimethylkaempferol  $3-O-\beta-D-(6''-\alpha-L-rhamnopyranosyl)-glucopyranoside, together with three known compounds were isolated from the n-butanolic soluble fraction of underground and aerial parts of$ *Linaria vulgaris Mill*, collected on the territory of Moldova. The characterisation of these compounds was achieved by various chromatographic and spectroscopic methods (IR, UV, <sup>13</sup>C-NMR, <sup>1</sup>H-NMR and MS).

Keywords: Linaria vulgaris Mill; flavonol glycoside; linaroside V; NMR analysis.

#### Introduction

*Linaria vulgaris Mill* is widely spread on the territory of Europe as well as in the Republic of Moldova. The plant is used in traditional, folk medicine and in homeopathy due to its contents of biologically active substances. In medicine, the liquid extract of *L*. vulgaris *Mill* is used as purgative [1], diuretic, stimulator of the gall-bladder secretion [2, 3]. The ointment made from it is useful in the case of skin disease [1], while the tinctures in dentistry [4]. This plant has antibacterial and fungicidal properties and it is used for treating liver and kidney diseases, tonsillitis, asthma, dermatomes, etc [5].

The previous phytochemical investigation of L. vulgaris *Mill* has been revealed the presence of alkaloids, flavonoids, triterpenoids, steroids and iridoid glycosides [6-13].

A new compound IV and three known compounds I-III have been isolated from the butanol watery extract of the plants of *L*. vulgaris *Mill*. By comparison of physical and spectroscopic properties (m.p., IR, UV, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR spectra), the known compounds were identified as antyrrinoside (I) [9], benzyl alcohol O- $\beta$ -D-glycopyranoside (II) [14] and benzyl alcohol  $\beta$ -D-(2'-O- $\beta$ -xylopyranosiyl)-glycopyranoside (III) [15, 16]. In this article, we present the isolation and structural determination of the new compound, called linaroside V, and give its <sup>13</sup>C- and <sup>1</sup>H-NMR data, which have not been reported previously.

# **Results and Discussion**

The n-butanol soluble fractions of *L. vulgaris* were fractionated on  $SiO_2$  columns. Further separation and purification was achieved by combining chromatographic methods (silica columns, Sephadex LH-20, HPLC) to yield compounds **I-IV** in a pure form.

Compound IV, named linaroside V, was obtained as yellow amorphous powder (m.p. 188-193 °C). The ESMS spectroscopy of IV resulted in the quasimolecular ion  $[M+H]^+$  at m/z 623, indicating the molecular formula of  $C_{29}H_{34}O_{15}$ . The IR spectrum showed strong absorption bands at 3420 (-OH group), 2965 (C-H bonds), 1650 (C=C aromatic ring), and 1620 cm<sup>-1</sup> (C=O), confirming the flavonoid nature of IV. The structure of linaroside V was elucidated by <sup>13</sup>C-NMR and <sup>1</sup>H-NMR spectroscopy (see Table 1). The <sup>13</sup>C- and <sup>1</sup>H-NMR spectra showed signals for aromatic ring at  $\delta$  C 122.5-128.4 ppm and  $\delta$  H 6.92-7.15 ppm, while the signals at  $\delta$  C 60.0; 55.3 ppm and  $\delta$  H 3.76; 3.85 ppm indicated two methoxy substitutions in the aglycone. The <sup>1</sup>H-NMR spectrum suggested that IV is a disaccharide on the basis of two signals in the sugar region at  $\delta$  5.12 (d, J = 7.1 Hz) and 4.56 ppm (s), corresponding to the anomeric proton of  $\beta$ -glucose and to the anomeric proton of the  $\alpha$ -rhamnose, respectively (rhamnose methyl group generates bonds at  $\delta$  17.7 in <sup>13</sup>C-NMR and 1.05 ppm in <sup>1</sup>H-NMR). The  $\alpha$ -rhamnose was concluded to be attached to C-6 of  $\beta$ -glucose moiety, which was confirmed by its <sup>13</sup>C-NMR spectrum and <sup>1</sup>H–<sup>1</sup>H COSY experiment. The HMBC spectra showed a correlation between the H-1<sup>177</sup>-rhamnosyl proton ( $\delta$  4.56) and C-6<sup>77</sup> glucose unit (66.8 ppm), H-1<sup>77</sup> glucosyl proton at  $\delta$  5.12 and aglycone C-3 ( $\delta$  142.4 ppm). The findings defined the disaccharide as a 3-O-rutinoside [17].

The <sup>13</sup>C- and <sup>1</sup>H-NMR spectral dates of **IV** have been compared with those previously described in the literature for nicotiflorin (kaempferol 3-O- $\beta$ -D-(6"- $\alpha$ -L-rhamnopyranosyl)-glucopyranoside) [17, 18]. The coincidence of signals of the sugar moieties has been revealed, except for the signals corresponding to the flavonol aglycone, due to the presence of two methyl groups in the spectra of **IV** (Table 1). Different NMR experiments indicated C-5 and C-4' methoxy substitutions in the aglycone. In HMBC experiment, the protons signals at  $\delta$  3.76 (3H, OCH<sub>3</sub>) and 3.85 (3H, OCH<sub>3</sub>) were correlated, with C-5 at 132.2 and C- 4' at 162.5 ppm, respectively. Thus, the aglycone of **IV** is 5, 4'-dimethylkaempferol. Considering all the data, the structure of the new flavonol glycoside - linaroside V is determined to be the 5, 4'-dimethylkaempferol 3-O- $\beta$ -D-(6"- $\alpha$ -L-rhamnopyranosyl)-glucopyranoside (Fig.1).

The spectral data of compounds **I-III**, including UV, <sup>13</sup>C- and <sup>1</sup>H-NMR, were verified by comparison with those previously described in the literature [9, 14, 15, 16].



Fig. 1. Structure of linaroside V

Table 1

# <sup>13</sup>C- and <sup>1</sup>H- NMR spectral data for compound IV (175 MHz, 300 MHz, CD<sub>3</sub>OD, DMSO-d<sub>6</sub>)

Position	δ C, ppm	δ H, ppm, J (Hz)
Aglycon		
2	161.8	
3	142.4	
4	182.7	
5	132.2	
6	102.1	6.92 d (2.0)
7	152.2	
8	94.0	6.93 d (2.5)
9	156.7	
10	105.9	
1'	122.5	
2'	128.4	8.03 d (8.8)
3'	114.5	7.15 d (8.2)
4'	162.5	
5'	114.5	7.15 d (8.4)
6'	128.4	8.03 d (8.6)
Glc		
1″	100.1	5.12 d (7.1)
2"	73.1	3.33 m
3″	76.3	3.32 m
4″	69.5	3.18 t (8.6)
5″	75.5	3.62 m
6″	66.8	3.47 d (10.8)
		3.89 dd (6; 9)
Rha		
1‴	100.3	4.56 s
2"'	70.3	3.66 m
3‴′	70.7	3.46 m
4"'	71.9	3.14 m
5"'	68.5	3.41 m
6"'	17.7	1,05 d (6.5)
MeO-C <sub>5</sub>	60	3.76 s
MeO-C <sub>4'</sub>	55.3	3.85 s

# Conclusion

The new flavonol glycoside called linaroside V has been isolated from *Linaria vulgaris Mill* and its chemical structure has been established by various spectroscopic methods.

# Experimental

Plant material

The plants of *Linaria vulgaris Mill* were collected in the Rebublic of Moldova in august-september 2006 and were identified by Professor Vasilii Florea (Laboratory of Medicinal Plants, Academy of Sciences of Moldova).

# General Experimental Procedures

Spectra were recorded using the following instruments: IR – on Specord 71-IR spectrophotometer, KBr; UV – on Specord UV-VIS spectrophotometer (MeOH, c=1). <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded on a Bruker DRX-spectrometer (300 MHz, 175 MHz); solvents CD<sub>3</sub>OD, DMSO-d<sub>6</sub>; TMS as internal standard. The mass-spectra were obtained on ESMS in the positive ion mode instrument. The chromatography was performed on silica gel (60-100  $\mu$ m Merck); GPC: Sephadex LH-20 (Pharmacia). Preparative HPLC: Varian ProStar 210, Varian 350 refractive index detector, Luna C-18 Phenomenex column 250 cm x 10 mm i.d. x 10  $\mu$ m, 50% MeOH, 2.0 ml min<sup>-1</sup>.

## Extraction and Isolation

The air-dried plants (2 kg) were extracted with 70% ethanol under reflux (4 l x 3) for 5 h each time. The total extract was concentrated and extracted with  $CHCl_3$  and n-BuOH. The n-BuOH soluble fraction was dried in vacuum (to afford 50 g), purified by crystallization and fractionated by combining chromatographic methods: columns (60 mm x 30 mm) on silica gel (40 x 100 µm, Merk) with a solvent system of  $CHCl_3$ -MeOH-H<sub>2</sub>O (95:5:0  $\rightarrow$  300:120:30 v/v/v), and Sephadex LH-20 using MeOH. The fractions (5 ml) were collected, characterized by TLC on Silufol. The fractions with similar R<sub>r</sub> values were recombined and further purified by reversed-phase HPLC. Four compounds (I-IV) were obtained: I -50 mg; II - 25 mg; III - 27 mg; IV - 120 mg.

Linaroside (**IV**) – yellow amorphous powder, m.p. 188-193°C,  $R_f = 0.54$  (CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O; 76:14:3); IR,  $\nu_{max}$  KBr cm<sup>-1</sup>: 3420 (oh); 2695 (C-H); 1650 (C=C); 1620(C=O). ESMS, m/z 623 (calcd for  $C_{29}H_{34}O_{15}$  [M+H]<sup>+</sup>); 476 [M+H-Rha]<sup>+</sup>; 314 [M+H-Rha-Glc]<sup>+</sup>. <sup>13</sup>C- and <sup>1</sup>H-NMR see in the Table 1.

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# PHENYLETHANOID AND IRIDOID GLYCOSIDES FROM VERONICA CHAMAEDRYS L.

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**Abstract.** Three phenylethanoid glycosides (1, 2, 3) and one iridoid glycoside (4) were isolated from aerial parts of *Veronica chamaedrys* L. (Scrophulariaceae) for the first time. On the basis of spectral analysis, the structures of these compounds were determined to be acteoside (3,4-dihydroxy- $\beta$ -phenylethoxy-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl- $\beta$ -D-glucopyranoside) (1), ehrenoside (3,4-dihydroxy- $\beta$ -phenylethoxy-O- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl- $\beta$ -D-glucopyranoside) (2), chamaedroside (3,4-dihydroxy- $\beta$ -phenylethoxy-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl- $\beta$ -D-glucopyranoside) (3) and aucuboside (4).

Keywords: Veronica chamaedrys L., Scrophulariaceae, phenylethanoid glycoside, iridoid glycoside.

#### Introduction

The genus Veronica (Scrophulariaceae), which is widely distributed in Europe and Asia, especially in the Mediterranean area, is represented by 32 species in Republic of Moldova [1, 2]. Iridoid glycosides, phenylethanoid and flavonoid glycosides have been mainly reported from different Veronica species [3 - 9]. Several Veronica species are used for the treatment of cancer, influenza, hemoptysis, laryngopharyngitis, hernia, and against cough, respiratory diseases plus as an expectorant and antiscorbutic in different countries [10 - 12].

This fact and our interest in secondary metabolites of Scrophulariaceae family prompted us to perform the phytochemical investigation of the aerial parts of *Veronica chamaedrys L*. Our investigation yielded three phenylethanoid glycosides (1, 2, 3) and one iridoid glycoside (4) (fig. 1).

#### **Results and Discussion**

Compound 1 was isolated as an amorphous powder with negative optical rotation ( $[\alpha]_{D}^{23}-81.9^{\circ}$ , c=1.67, MeOH), whose UV spectra showed  $\lambda_{max}$  at 342, 321 and 304 nm indicating its polyphenolic nature. The molecular formula  $C_{29}H_{36}O_{15}$  was determined by the [M]<sup>+</sup> ion peak at m/z 624 in the negative high resolution (HR) – FAB – MS.

Its structure was determined by corresponding shifts of <sup>1</sup>H and <sup>13</sup>C NMR spectral data.

The <sup>13</sup>C-NMR spectrum of **1** showed eight carbon signals of aglycone moiety, six of which were assignable to the aromatic system – benzenic ring with two hydroxy groups attached at C3 ( $\delta$  146.16 ppm) and C4 ( $\delta$  144.78 ppm). The <sup>13</sup>C-NMR spectrum of **1** showed the presence of residue of (E)-caffeic acid at  $\delta$  114.98 ppm ( $\alpha$ '),  $\delta$  147.56 ppm ( $\beta$ ) and carbonyl group  $\delta$  168.56 ppm. Furthermore, the caffeoyl group was positioned at C4' of the glucose on the basis of strong deshielding on the H4' proton of the inner glucose unit  $\delta$  4.92 ppm (J=9.3 Hz).

Additionally, two anomeric carbons were observed ( $\delta$  C1' 103.28 and  $\delta$  C1'' 103.26) indicating its biglycosidic structure containing D-glucopyranose and L-rhamnopyranose as sugar units. This biglycosidic structure was also confirmed by the <sup>1</sup>H-NMR spectrum of **1** where two signals of anomeric protons were observed at  $\delta$  4.48 (d, J=7.6 Hz) indicating  $\beta$ -linked D-glucopyranose and  $\delta$  5.12 (J=1.6 Hz) as  $\alpha$ -D-rhamnopyranose.

The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of **1** observed signal of methyl group at  $\delta$  1.08 ppm and  $\delta$  18.44 ppm, respectively, belonging to ramnopyranose. Ramnopyranose residue was attached to C3' of the inner glucose on the basis of the downfield shielding of 5 ppm ( $\delta$  86.71) comparing with shift of terminal glucose. Also acyl moiety was attached to inner glucose and showed eight carbon signals, six of which revealed the presence of para-disubstituted aromatic ring, similar to that of aglycone moiety (Table 1).

From the above results, compound **1** was determined as 3,4-dihydroxy- $\beta$ -phenylethoxy-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl- $\beta$ -D-glucopyranoside. The structure of compound **1** was also confirmed by comparison of the data published for phenylethanoides, previously isolated from genus *Veronica L*., and correspond to acteoside [13,14].

Compound **2** was obtained as an amorphous powder with negative optical rotation ( $[\alpha]_{D}^{23}-58^{\circ}$ , MeOH) and assigned the molecular formula  $C_{34}H_{44}O_{19}$  by (HR) – FAB – MS (from ion peak [M]<sup>+</sup> m/z 756). The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of **2** resembled those of **1** (Table 1). The <sup>13</sup>C-NMR spectrum of **2**, however, showed additionally five signals of a characteristic terminal arabinose moiety. In the <sup>1</sup>H-NMR spectrum, the coupling constant of the anomeric proton of the arabinose moiety was J=6.7 Hz ( $\delta$  4.54, d) showing the  $\alpha$ -configuration of this sugar. These data suggested that compound

**2** had three sugar units: one  $\beta$ -D-glucopyranose, one  $\alpha$ -L-rhamnopyranose and one  $\alpha$ -L-arabinopyranose. The complete assignment of all proton and carbon resonances was based on the results of 1H-1H shift correlation spectroscopy (1H-1H COSY), 1H-13C-heteronuclear multiple quantum coherence (1H-13C-HMQC) and HMBC experiments.

From the above data, the structure of **2** was determined to be 3,4-dihydroxy- $\beta$ -phenylethoxy-O- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl- $\beta$ -D-glucopyranoside, a known compound identical to ehrenoside isolated previously from the genus *Veronica L*. [15].

Compound **3** was isolated as an amorphous powder with negative optical rotation  $([\alpha]^{22}_{D} - 19.6^{\circ}, MeOH)$ . The molecular formula of compound **3** was determined as  $C_{28}H_{34}O_{14}$  by negative high-resolution (HR) – FAB – MS. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra (Table 1) revealed the presence of aromatic system confirmed by the AB-type aromatic protons (3,4-dihydroxyphenylethyl alchohol  $\delta$  6.48, 6.63, 6.64) and two methylenes which were coupled with each other ( $\alpha$ :  $\delta_{H}$  3.69, 4.05,  $\delta_{C}$  72.19;  $\beta$ :  $\delta_{H}$  2.79,  $\delta_{C}$  36.77). The signals assigned to the sugar moiety indicated the presence of an arabinose moiety. In the <sup>13</sup>C- and <sup>1</sup>H-NMR spectra, the anomeric signal was observed at  $\delta_{C}$  104.15 and  $\delta_{H}$  4.52 d (J=6.7 Hz), as an  $\alpha$ -linked L-arabinose attached to the aglycone moiety and in C4 the residue of (E)-caffeic acid. All protons in the arabinose unit were assigned unambiguously from the correlated all proton resonances with those of the corresponding carbons in the sugar unit. The downfield signals at  $\delta_{H}$  4.01, belonging to H4 of arabinose suggest that the acyl moiety was attached to C4 of arabinose. The signal at  $\delta_{C}$  74.35, arising from C4 of the arabinose moiety (downfield shift +5ppm – glycoside effect) showed that the acyl moiety was indeed attached to C4.

Therefore, the structure of **3** was identified as 3,4-dihydroxy- $\beta$ -phenylethoxy-O-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)]-(4-O-caffeoyl)- $\alpha$ -L-arabinopyranoside, for which we proposed the name chamaedroside. Data about this compound are absent in literature.

On the basis of spectral data and phytochemical constants, compound 4 was isolated and its structure was identified as aucuboside by the comparison of its spectral data with those reported in the literature [16].



Fig. 1. Structures of isolated compounds

	C-	1	1ata (300 MII 2	их, съ <sub>з</sub> ов, ррш, а і	an 112) of gry	(tosiues (1-5)
Δσl	C	Ч	C 2	-	, C	н
1 1	131 71	11	131.92	11	131.87	11
2	117 46	6.73 d(2.1)	117 53	6 72 d (2 1)	117 48	6 64 d (2 0)
3	146 16	0.75 <b>u</b> (2.1)	146 14	0.72 4 (2.1)	146.18	0.01 a (2.0)
4	144.78		144.80		144.78	
5	116.37	6.67 d (7.8)	116.34	6.68 d (7.8)	116.38	6.63 d (8.0)
6	121.51	6.58 dd (8.0, 8.2)	121.53	6.59dd(7.9.2.1)	121.55	6.48 dd (2.8)
α	72.24	3.72 m	72.17	3.61 m	72.19	3.69 m
		4.07 m		4.05 m		4.05 m
β	36.71	2.79 t (7.2)	36.75	2.80 t (7.1)	36.77	2.79 m
Glc						
1'	103.28	4.48 d (7.6)	103.06	4.45 d (7.6)		
2'	73.86	3.18 t (8.9)	82.72	3.64 t (8.6)		
3'	86.71	4.11 t (9.1)	81.42	3.99 t (9.2)		
4'	70.84	4.92 t (9.3)	70.93	4.95 t (9.2)		
5'	75.79	3.56 m	75.94	3.54 t (9.3)		
6'	62.42	3.54 dd (12.0, 5.4)	62.51	3.56dd(12.1,6.0)		
		3.65 dd (12.1, 2.2)		3.61dd(12.1,2.0)		
Rha						
1"	103.26	5.12 (1.6)	103.25	5.20 (1.5)	103.26	5.12 (1.6)
2"	71.91	3.98 dd (3.1, 1.8)	72.16	4.01dd (3.3, 1.8)	71.91	3.98dd(3.1,1.8)
3"	72.05	3.53 m	72.06	3.56 m	72.05	3.53 m
4"	73.82	3.38 t (9.1)	73.86	3.31 t (9.5)	73.82	3.38 t (9.1)
5%	/0.66	3.52-3.54	/0.6/	3.53-3.56	70.66	$3.52 - 3.54^{a}$
6″	18.44	1.08 d (6.4)	18.52	1.11 d (6.1)	18.44	1.08 d (6.4)
Ara			104.12	4544(7)	104.15	4.52 1 (6.7)
1			104.12	4.54  d (6.7)	104.15	4.52 d (6.7)
2 2'''			75.05	3.01 l (9.4) $3.52 - 2.56^*$	70.68	3.38 dd 3.06 c
5 1'''			74.55 60.56	3.33-3.30 3.75 c	79.00	3.90 S
4 5""			66.86	3.738 3.744(12.5)	74.55 66 56	4.01  u 3 86dd(12 4 3 3)
5			00.80	3.24 d (12.5) 3.84 dd (12.5.2.9)	00.50	3.53dd(12.4, 3.3)
Acvl				5.0444(12.5,2.5)		5.5544(12.4,1.5)
1""	127.68		127 70		127 71	
2""	115.26	7.08 d (1.8)	115.26	7.07 d (2.1)	115.26	7.08 d (1.8)
3""	146.92	,,	146.88	,	146.92	(110)
4""	149.74		149.84		149.74	
5''''	116.65	6.78 d (8.2)	116.85	6.78 d (8.2)	116.65	6.78 d (8.2)
6""	123.16	6.98 dd (8.2, 1.6)	123.24	6.96 dd (8.2, 2.1)	123.16	6.98dd(8.2,1.6)
α'	114.98	6.31 d (15.6)	114.84	6.27 d (15.9)	114.98	6.31 d (15.6)
β'	147.56	7.56 d (16.1)	148.01	7.59 d (15.9)	147.56	7.56 d (16.1)
Ċ=O	168.56		168.60	· /	168.58	· · · ·

# <sup>13</sup>C- and <sup>1</sup>H- NMR spectral data (300 MHz , CD<sub>3</sub>OD, ppm, J in Hz) of glycosides (1-3)

\*Signal patterns are unclear due to overlapping

# Experimental

General experimental procedures

Melting points were measured on a Boetius table, and a specific rotation on a polarimeter on the firm Zeiss. UV spectra were recorded on UV HP 8472-A spectrometer (MeOH). NMR experiments were performed on a Bruker DRX-300 spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) at 300 K dissolving all the samples in CD<sub>3</sub>OD (Carlo Erba, 99.8 %). The standard pulse sequence and phase cycling were used for DQF-COSY, HSQC and HMBC spectra. The NMR data were processed using UXNMR software.

ESI-MS in the positive ion mode was performed using a Finnigan LCQ Deca ion trap instrument from Thermo Finnigan (San Jose, CA) equipped with Xcalibur software.

HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep  $MSC_{18}$  column (300 x 7.8 mm i.d.) and a Rheodyne injector.

Column chromatography was performed over Silica gel (0.1-0.06 mm, Merck). TLC was performed on silica gel plates (Merck precoated silica gel 60  $F_{254}$ ). Solvent systems: (1) chloroform/methanol (4:1), (2) chloroform/methanol/ water (65:35:3). All solvents for chromatographic separation were of analytical grade from Carlo Erba (Rodano, Italy). HPLC grade water (18 m $\Omega$ ) was prepared using a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA).

#### Plant Material

The aerial parts of *Veronica chamaedrys L*. have been collected in the scientific research field of Institute of Genetics and Physiology of Plants, Academy of Sciences of Moldova in May 2006 year. The voucher specimen has been deposited by Doctor in Biology Florea V.N. in Laboratory of Natural Bioregulators under the direction of Doctor in Chemistry Kintea P. K.

#### Extraction and separation

Dry powdered aerial parts (600 g) were extracted three times in boiled n-buthanol saturated with water. The received n-butanol-water extract was evaporated and then purified with chloroform. After purification it was crystallised in acetone. The residue was dried in vacuum at 40°C and summary extract of glycosides has been obtained as yellow powder in 3.7 % yield. 3g of extract have been chromatographied on silica gel column (30-500mm, 60-100µm, Merck). The column was eluted with system chloroform-methanol-water (8:2:0→20:10:1) and 4ml fractions were collected. Fractions showing identical characteristics [TLC, silica gel, chloroform-methanol (4:1)] were combined. Two subfractions, (A) and (B) were obtained, which were further separated on a  $C_{18}$  column (7,8x300mm, LiChroprep RP18, 25-40µm, XTerra Waters) using a H<sub>2</sub>O/MeOH (60-80% MeOH) isocratic. Four single compounds were isolated.

Acteoside (1): Amorphous powder,  $[\alpha]_{D}^{23}$  =81.9° (c=1.67, MeOH). HRMS *m/z* 624.452 [calcd for C<sub>29</sub>H<sub>36</sub>O<sub>15</sub> (M)<sup>+</sup>]; 488.26 [M-136]<sup>+</sup>; 324.03 [M-136-164]<sup>+</sup>; 179.06 [M-136-164-145]<sup>+</sup>. <sup>1</sup>H NMR and <sup>13</sup>C NMR see Table 1.

Ehrenoside (2): Amorphous powder,  $[\alpha]_{D}^{23}-58^{\circ}$  (MeOH). HRMS, *m/z* 756.443 [calcd for  $C_{34}H_{44}O_{19}$  (M)<sup>+</sup>]; 620.34 [M-136]<sup>+</sup>; 456.9 [M-136-164]. <sup>1</sup>H NMR and <sup>13</sup>C NMR see Table 1.

Chamaedroside (3): Amorphous powder,  $[\alpha]_{D}^{22}-19.6^{\circ}$  (MeOH). HRMS, *m/z* 595.29 [calcd for C<sub>28</sub>H<sub>34</sub>O<sub>14</sub> (M)<sup>+</sup>]; 462.25 [M-132]<sup>+</sup>; 316.15 [M-132-146]; 156.29 [M-132-146-160]<sup>+</sup>. <sup>1</sup>H NMR and <sup>13</sup>C NMR see Table 1.

#### Conclusion

Four compounds (1), (2), (3) and (4) have been isolated from aerial parts of *Veronica chamaedrys L*. for the first time. The structures of (1), (2) and (3) were elucidated as acteoside (3,4-dihydroxy- $\beta$ -phenylethoxy-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl- $\beta$ -D-glucopyranoside), ehrenoside (3,4-dihydroxy- $\beta$ -phenylethoxy-O- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl- $\beta$ -D-glucopyranoside), chamaedroside (3,4-dihydroxy- $\beta$ -phenylethoxy-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl- $\beta$ -D-glucopyranoside), chamaedroside (3,4-dihydroxy- $\beta$ -phenylethoxy-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-4-O-caffeoyl- $\alpha$ -L-arabinopyranoside) and (4) aucuboside, respectively by means of physicochemical methods.

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# ISOLATION OF *ENT*-KAUR-16-EN-19-OIC AND *ENT*-TRACHILOBAN-19-OIC ACIDS FROM THE SUNFLOWER *HELIANTHUS ANNUUS L*. DRY WASTE

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Abstract. A relatively simple method for isolation of the mixture of *ent*-kaur-16-en-19-oic (1) and *ent*-trachiloban-19-oic (2) acids from dry waste of sunflower processing has been elaborated, and it has been shown that the waste can serve as an accessible source of *ent*-kauranic and *ent*-trachilobanic diterpenoids.

Keywords: ent-kaur-16-en-19-oic acid, ent-trachiloban-19-oic acid, diterpene, isolation.

## Introduction

Many representatives of tetracyclic ent-kauranic and pentacyclic *ent*-trachilobanic diterpenoids display a diverse biological activity [1, 2]. *ent*-Kauranic compounds are widely spread in plants, being biogenetical precursors of gibberillin plants growth hormones gibberillin [3]. Pentacyclic *ent*-trachylobanic compounds occur in natural sources more seldom. However, they are also of interest since they can serve as starting compounds for biomimetic synthesis of a number of tetracyclic diterpenoids [4]. Investigations of biologically active principls of medicinal plants, especially those used in non-traditional Chinese medicine, have shown that a large spectrum of biological activities, including the anti-microbial, anti-inflammatory, the cardio-vascular, diuretic, cytotoxic and ant-AIDS ones are conditioned by the presence in these plants of *ent*-kauranic and *ent*-trachilobanic diterpenoids [5].

# **Results and discussion**

Kauranic and trachylobanic diterpenoids have a common biogenetic precursor [6]. However, according to available data, there are only two cases of common presence of kauranic and trachilobanic derivatives: in *Trahylobium verrucosum* [7] and *Helianthus annuus L*. (sunflower) [8]. *ent*-Kaur-16-en-19-oic (1) and *ent*-trachiloban-19-oic (2) acids have been isolated from sunflower inflorescence [8].

For finding accessible sources of *ent*-kauranic diterpenoids we carried out a literature search, its result being given in Table 1. It includes the name of plants that contain *ent*-kaurans, the kind of flora (wild, cultivated), the total content of *ent*-kaurans and the corresponding reference. From Table 1 data, one can see that the highest content of *ent*-kaurans in dry plants is to be found in sunflower. In the total extracts obtained from various parts of sunflower, ent-kauran6-en-19-oic acid (1) is prevalent. In the Republic of Moldova, sunflower is one of the plants that is cultivated on industrial scale, thus making it attractive as accessible source of *ent*-kaur-16-en-19-oic acid (1).

Table 1

The main sources of <i>ent</i> -kauran unerpendius.							
Plant	Natural habitat	Form habitat	Ent-kauran content (% from dry mass)	References			
Euphorbia Wangii	China	Wild flora	0,003	[9]			
Annona glabra	China, Taiwan	Wild flora	0,016	[10]			
Annona squamosa	China, Taiwan	Wild flora	0,034	[11]			
Aristolochia pubescens	Brazil	Wild flora	0,035	[12]			
Annona senegalensis	Nigeria	Cultivated flora	0,074	[13]			
Oyedaea verbesinoides	Central America	Wild flora	0,104	[14]			
Paraschistochila pinnatifolia	New Zealand	Wild flora	0,120	[15]			
Helianthus annuus	Worldwide	Cultivated flora	0,650	[8]			

The main sources of ent-kauran diterpenoids.



One of the most widely spread representatives of *ent*-kauranic diterpenoids is the *ent*-kaur-16-en-19-oic acid (1). First of all, it is of interest as a compound with a wide spectrum of biological activity. It displays activity against microbus *Bacillus sublitis*, [16, 17], *Staphylococcus aureus*, [17-19], *Micobacterium smegmatis* [17, 18], *Saccaromices cerevisiae* [17], *E. coli* and *Candida albicans* [19], as well as against very simple flagellates *Triponosoma cruzi* [20], which generate the Chagas disease. *ent*-Kaur-16-en-19-oic acid (1) also displays antifeedant activity against granaries vermin *Trilobium confusum Duv.*, *Tragoderma granarium Ev.* and *Sitophilus granarius L.* [21]. Acid (1) has been tested on cytotoxicity [13, 22]. It displays a poor activity against leukemia, but a considerably selective activity against breast cancer cells [22], moderate inhibitory properties *in vitro* against lungs tumours, central nervous system's tumours, skin and vertebrae cancer [23].

This paper contains data concerning the elaboration of a convenient method for isolation of *ent*-kaur-16-en-19-oic (1) and *ent*-trachiloban-19-oice (2) acids from dry waste of sunflower processing. The sunflower has proved to be a rich source of ent-kauranic compounds (Table 1).

Extraction of the vegetal raw material has been done by diethyl ether in a Soxhlet extractor. The solvent has been chosen by us with consideration of the fact that *ent*-kauranic and *ent*-trachilobanic diterpenoids are labile compounds that can be subjected to rearrangement of the carbon skeleton [24] during extraction at high temperature under the influence of accompanying substances of acidic character. The obtained extract has been treated with potassium hydroxide solutions of various concentrations. As a result of such treatment of the extract, the neutral compounds have been removed (control by TLC). According to experimental data, the best results for extraction of the acidic part have been realized using the 5% solution of KOH. Utilization of more concentrated alkali solutions (e.g. 10%) does not lead to the increase of output of the acidic part. When 3% and 5% of KOH solutions have been used, the obtained results practically do not differ. Utilization of sodium hydroxide solutions instead of potassium hydroxide has led to worse results. In this case, longer time has been necessary for separation of phases and, besides, some amount of neutral compounds got into the acid fraction.

The acidic part (1 g) has been subjected to separation on silica gel, in result of which the mixture of *ent*-kaur-16-en-19-oic (1) and *ent*-trachiloban-19-oic (2) acids has been obtained, their overall yield being 35%. Due to similar chromatographic properties, the mixture of acids 1 and 2 could not be resolved by flash chromatography on silica gel. The mixture of acids 1 and 2 has been separated by chromatography on columns with silica gel, impregnated with silver nitrate [25]. The ratio of *ent*-kaur-16-en-19-oic (1) and *ent*-trachiloban-19-oic (2) acids in the extract was  $\sim$ 7:2. Acids 1 and 2 have been identified by comparing their spectral and physico-chemical data and the data of their methyl esters (3) and (4), obtained by their methylation with diazomethane, with those available in the literature.

#### Conclusions

Thus, we have elaborated a simple method for isolating the mixture of *ent*-kaur-16-en-19-oic (1) and *ent*-trachiloban-19-oic (2) acids from dry waste of sunflower processing and their chromatographic separation. It has been shown that this waster can serve as an accessible source of *ent*-kaurenic and *ent*-trachilobanic diterpenoids. The novelty of this method consists in the direct isolation of the above mentioned diterpenic acids in their individual forms without having to methylate the acid mixture and chromatographic separation of their methyl esters as it has been done earlier [7,8].

# Experimental

IR spectra were recorded on a Bio-Rad FTS 7 spectrophotometer.<sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> on Bruker WM 300 (300 MHz) and Bruker AC 80 (80 MHz) spectrometers; chemical shifts are given in ppm and are referenced to chloroform (CHCl<sub>3</sub>) as internal standard ( $\delta = 7.26$  ppm for proton and  $\delta = 77.0$  ppm for carbon). Optical rotations were measured in chloroform on a Jasco P 2000 polarimeter, using a 10 cm cell. Commercial Merck Si gel 60 (70–230 mesh ASTM) was used for flash chromatography, and Merck precoated SiO<sub>2</sub> gel plates were used for TLC. The chromatograms were sprayed with 0.1% solution of cerium(iv) sulfate in 2N sulfuric acid, and heated at 80 °C

for 5 min to detect the spots. Treatment of reaction mixtures in organic solvents included the extraction by diethyl ether, washing of the extract with water up to neutral reaction, drying over anhydrous  $Na_2SO_4$ , filtering, and solvent removal in vacuum.

**Obtaining of the extract from dry wastes of sunflower.** Dry wastes of sunflower (800 g) have been preliminarily crumbled up and extracted in the soxlet by diethyl ether during 2 hours. After evaporation of the solvent, the 85 g of extract was obtained in the form of a hard brown mass which, when heated at ~50°C, melts. The obtained extract dissolves readily in diethyl ether, benzene, chloroform, but badly in petrol ether. A part of the extract (9.3 g) has been dissolved in diethyl ether and treated with aqueous solution of alkali KOH (5% solution, 40 mL). The aqueous phase has been separated, acidified with 10% solution of  $H_2SO_4$  (20 mL), extracted with ether, washed with brine up to the neutral reaction and concentrated in the vacuum, giving 7.5 g of yellow oil.

A portion of the extract (6.8 g) has been separated on column with silica gel (140 g) by gradient elution with the mixture of petrol ether and ethylacetate, affording the mixture of *ent*-kaur-16-en-19-oic (1) and *ent*-trachiloban-19-oic (2) acids (2.37 g, 35% yield).

Chromatographic separation of the mixture of ent-kaur-16-en-19-oic (1) and ent-trachiloban-19-oic (2) acids. The mixture of acids 1 and 2 (860 mg) has been separated on the column with  $SiO_2/AgNO_3$  (38 g). Elution with 2% solution of ethylacetate in petrol ether resulted in obtaining of 192 mg of ent-trachiloban-19-oic acid (2) (22% yield) and 640 mg of ent-kaur-16-en-19-oic acid (1) (74% yield).

ent-*Kaur-16-en-19-oic acid (1)*, colourless crystals, m.p. 177-179°C (from hexane),  $[\alpha]_D$  -105° (c 2.3; CHCl<sub>3</sub>). IR liquid film, (v, cm<sup>-1</sup>): 2937, 1686, 1258, 874, 794, 635, 530. <sup>1</sup>H NMR (300 MHz,  $\delta_H$ ): 0.95 (3H, s, H<sub>3</sub>-20), 1.00-1.16 (4H, m, 2CH<sub>2</sub>), 1.24 (3H, s, H<sub>3</sub>-19), 1.46-2.18 (10H, m), 2.64 (1H, bd. s, H-13), 4.74 (1H, s, H<sub>A</sub>-17), 4.80 (1H, s, H<sub>B</sub>-17). <sup>13</sup>C NMR (75 MHz,  $\delta_C$ ): 15.6 (q, C-20), 18.5 (t, C-11), 19.1 (t, C-2), 21.9 (t, C-6), 29.0 (q, C-18), 33.2 (t, C-14), 37.8 (t, C-3), 39.7 (s, C-10), 39.7 (t, C-12), 40.7 (t, C-1), 41.3 (s, C-4), 41.3 (t, C-7), 43.8 (d, C-13), 44.3 (s, C-8), 49.0 (t, C-15), 55.1 (d, C-9), 57.1 (d, C-5), 103.4 (t, C-17), 155.9 (s, C-16), 184.8 (s, C-19). Literature data [26]: , m.p. 179-181°C,  $[\alpha]_D$  -110° (c 3.0; CHCl<sub>3</sub>).

**ent-***Trachiloban-19-oic acid (2)*, colourless crystals, m.p. 124-126°C (from hexane),  $[\alpha]_D$  -43° (c 1.5; CHCl<sub>3</sub>). IR liquid film, (v, cm<sup>-1</sup>): 2846, 1691, 1443, 1261, 1178, 1022, 798, 630, 535. <sup>1</sup>H NMR (300 MHz,  $\delta_H$ ): 0.56 - 0.58 (2H, m, cyclopropane protons), 0.81-0.83 (2H, m), 0.87 (3H, s, 20-H3), 0.97-1.02 (3H, m), 1.13 (3H, s, 17-H<sub>3</sub>), 1.21 (3H, s, 18-H<sub>3</sub>), 1.23-2.14 (14H, m). <sup>13</sup>C NMR (75 MHz,  $\delta_C$ ): 12.5 (q, C-20), 18.7 (t, C-2), 19.8 (t, C-11), 20.6 (t, C-12), 20.7 (q, C-17), 21.8 (t, C-6), 22.4 (s, C-16) 24.3 (d, C-13), 28.9 (q, C-18), 33.2 (t, C-14), 37.9 (t, C-3), 38.9 (s, C-10), 39.3 (t, C-7), 39.4 (t, C-1), 40.8 (s, C-8), 43.7 (s, C-4), 50.4 (t, C-15), 52.8 (d, C-9), 57.0 (d, C-5), 184.5 (s, C-19). Literature data [27]: m.p. 125-127°C,  $[\alpha]_D$  -52° (c 0.8, CHCl<sub>3</sub>).

*Methyl ester of ent-kaur-16-en-19-oic acid (3). ent*-Kaur-16-en-19-oic acid (1) (70 mg) has been methylated by an excess of diethyl ether solution of diazomethane. The residue (71.6 mg), obtained after distillation of the solvent, was chromatographied on a column with silica gel (1.2 g). By the mixture of petrol ether and etylacetate (97:3), 68.2 mg (93% yield) of methyl ester of *ent*-kaur-16-en-19-oic acid (3) have been eluted, colourless crystals, m.p. 76-77.5°C (from CH<sub>3</sub>OH),  $[\alpha]_D$  -101° (c 1.8; CHCl<sub>3</sub>). IR liquid film, (v, cm<sup>-1</sup>): 1720, 1670, 1240, 1225, 1190, 1145. <sup>1</sup>H NMR (80 MHz,  $\delta_H$ ): 0.84 (3H, s, H<sub>3</sub>-20), 1.15 (3H, s, H<sub>3</sub>-18), 2.62 (1H, bd.s, H-13), 3.62 (3H, s, CO<sub>2</sub>Me), 4.72 (1H, s, H<sub>A</sub>-17), 4.78 (1H, s, H<sub>B</sub>-17). Literature data [8]: m.p. 73.5-74.5°C,  $[\alpha]_D$  -104° (CHCl<sub>3</sub>).

*Methyl ester of ent-trachiloban-19-oic acid (4). ent-Trachiloban-19-oic acid (2)* (40 mg) has been methylated by an excess of diethyl ether solution of diazomethane. After the solvent evaporation, the residue (39.8 mg), was chromatographied on the column with silica gel (0.7 g). By the mixture of petrol ether and etylacetate (97:3), (37.2 mg, 89% yield) of methyl ester of *ent-* trachiloban-19-oic acid (4) have been eluted, colourless crystals, m.p. 107-109°C (from CH<sub>3</sub>OH),  $[\alpha]_{\rm D}$  -65.4° (c 1.2, CHCl<sub>3</sub>). IR liquid film, (v, cm<sup>-1</sup>): 1720, 1261, 1230, 1200, 1162, 1150. <sup>1</sup>H NMR (80 MHz,  $\delta_{\rm H}$ ): 0.55 - 0.60 (2H, m, cyclopropane protons), 0.85 (3H, s, 20-H<sub>3</sub>), 1.08 (3H, s, 17-H<sub>3</sub>), 1.13 (3H, s, 18-H<sub>3</sub>), 3.60 (3H, s, CO<sub>2</sub>Me). Literature data [7]: m.p. 110-112°C,  $[\alpha]_{\rm D}$  -41° (CHCl<sub>3</sub>), [8]: m.p. 98-100°C,  $[\alpha]_{\rm D}$  -70.5° (CHCl<sub>3</sub>).

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# THE THEORETICAL STUDY OF SOME REACTIONS WITH THE PARTICIPATION OF $\dot{O}H$ AND $H\dot{O}_2$ RADICALS

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**Abstract.** The geometry of the  $H_2O_2$ ,  $\dot{O}H$ ,  $H\dot{O}_2$ ,  $O_2$  particles and the energy profiles of the initial reactions between them, that are supposed to take place during the process of decomposition of hydrogen peroxide, were studied by *ab initio* calculations. It was shown that the differences between the energies of reactants and the products of all analysed reaction are positive values and correlate with the experimental data.

Keywords: the radicals reactions, decomposition of hydrogen peroxide, the thermodynamic stability

#### Introduction

The study of the elementary reactions that take place in the process of the  $H_2O_2$  decomposition is of a great importance both from the theoretical point of view (in order to understand the mechanisms of the numerous processes in the chemical and biological systems, in the processes of photolysis, redox transformations of different organic substances, etc.) and from the practical point of view – the use of such compounds in many processes of oxidation. The knowledge of the mechanism of these reactions also presents interest for creating new catalytic systems for the oxidation of the organic substances.

In researches [1-3] it is supposed, that both at the direct decomposition of the hydrogen peroxide and at its decomposition in the presence of the complexes of transitional metals, at certain intermediate stages the following reactions take place:

$$H_2O_2 + H_2O_2 \to H_2O + H_2O + O_2$$
 (I)

$$H_2O_2 + OH \to H_2O + HO_2 \tag{II}$$

$$H_2O_2 + HO_2 \to H_2O + O_2 + OH \tag{III}$$

$$\dot{HO_2} + H\dot{O_2} \rightarrow H_2O_2 + O_2$$
 (IV)

$$H\dot{O}_2 + \dot{O}H \rightarrow H_2O + O_2$$
 (V)

$$\dot{O}H + \dot{O}H \rightarrow H_2O_2$$
 (VI)

In this paper it was proposed to study if the reactions I-VI really take place and if they are profitable from the thermodynamic point of view. For the realization of this goal the quantum-chemical calculations of the possible

geometries of the  $H_2O_2$ ,  $HO_2$ , OH,  $H_2O$  and  $O_2$  particles, the ways of interactions between them and the total energies of all systems were carried out.

#### Method of calculation

All calculations were performed with the help of the GAMESS programme [4], using MO LCAO Hartree-Fock-Roothaan SCF method in the restricted-open Hartree-Fock (ROHF) approximation with the STO-6G basis set for atomic functions.

### **Results and discussions**

#### Calculation of the geometry of the investigated particles.

For every particle in reactions I-VI,  $(H_2O_2, HO_2, OH, H_2O)$  and  $O_2$  the most propitious geometry was found and the total energies were calculated. In all cases it was considered that the spatial nuclear configuration of the investigated molecules corresponds to  $C_1$  symmetry point group.

The values of equilibrium geometrical parameters and the total energies of the studied particles, obtained as a result of the geometry optimization, are presented in Table 1.

	R(O-H), (Å)	< <i>(HOO)</i>	R(O-O), (Å)	Е, (а.е.и.)
$H_2O_2$	1.00 (0.94 [6])	100.67 (99.5 [6])	1.40 (1.44[6])	-150.8595
HÔ <sub>2</sub>	1.00 (0.99 [5])	103.89 (104.96 [5])	1.35 (1.33 [5])	-149.6171
$H_2O$	0.99 (0.96 [5])	100 (104.45 [5])	-	-75.6812
ĊН	1.01 (0.97 [5])	-	-	-75.0775
<i>O</i> <sub>2</sub>	-	-	1.22 (1.20 [5])	-149.0504

The values of geometrical parameters and the total energies.

Note that calculated values of the bond lengths (R) and the valence angles of investigated particles are in a rather good agreement with those obtained by other authors (Table 1).

# Interaction of the studied particles.

Further, the optimization of the geometrical configuration of the pairs of molecules that take part in the **I-VI** reactions and were at the beginning in the immediate vicinity was done. In all the investigated reactions, the atoms of oxygen, at the beginning of optimization, are in a plane, a tendency that is kept to all along, this not being true for the atoms of hydrogen. A general analysis of these reactions, the way of approaching particles to each other, and the evolution of reactions are described and analysed below.

**Reaction**  $H_2O_2 + H_2O_2$ . Approaching these two molecules to each other and optimizing them together, one can see (Fig. 1) that at the beginning of reaction two hydrogen bonds appear between them (O1-H3 and O2-H4). Then there takes place the transfer of the (H3) and (H4) atoms towards the (O1) and (O2) atoms that provokes the breakage of the (O1-O2) bond ending up with the formation of two molecules of water and one molecule of oxygen. The dotted lines in Fig.1 show the hydrogen bonds and the weaker bonds between atoms of oxygen that are formed as a result of the interaction and are broken as a result of the reaction.



Fig. 1. The reaction of a pair of the hydrogen peroxide molecules with the formation of two molecules of water and a molecule of oxygen.

According to the quantum-chemical data (Table 2), the conclusion can be draw that the investigated reaction takes place as it is supposed in the literature [7]. So, as a result of this process the reaction takes place the following way:

$$\boldsymbol{H}_2\boldsymbol{O}_2 + \boldsymbol{H}_2\boldsymbol{O}_2 \rightarrow \boldsymbol{H}_2\boldsymbol{O} + \boldsymbol{H}_2\boldsymbol{O} + \boldsymbol{O}_2$$

**Reaction**  $H_2O_2 + \dot{O}H$ . Further, the interaction of the hydrogen peroxide with the  $\dot{O}H$  radical was analysed. At the beginning, the formation of the (O3-H1) hydrogen bond between the radical and the molecule of peroxide occurs that causes the transfer of the atom of hydrogen (H1) towards the radical of hydroxyl. During the reaction, the formation of a molecule of water and of a hydroperoxyl radical takes place (Fig. 2), the latter participates further in reaction III.



Fig. 2. The way of obtaining of the  $HO_2$  radical due to the interaction of the hydrogen peroxide with the OH radical.

Comparing the obtained results (Table 2) with the experimental ones [8-9], the conclusion was drawn that they are in a good concordance. The final reaction is:

$$H_2O_2 + \dot{O}H \rightarrow H_2O + H\dot{O}_2$$

**Reaction**  $H_2O_2 + HO_2$ . Another reaction that has been investigated from the thermo-dynamic point of view

is the interaction of the hydrogen peroxide with the hydroperoxyl radical  $H\dot{O}_2$  [10]. When these two particles approach with each other the formation of the (O2-H3) hydrogen bond takes place followed by the transfer of the (H3) atom

from the hydroperoxyl radical to the hydrogen peroxide that causes the (O1-O2) bond breaking, resulting in a new OH radical and two stable molecules: oxygen and water. Fig. 3 presents a general look of the development of the reaction and the obtained products:



Fig. 3. The best way of approaching of the  $H\dot{O}_2$  radical to the hydrogen peroxide to obtain the  $\dot{O}H$  radical.

$$H_2O_2 + HO_2 \rightarrow H_2O + O_2 + OH$$

Both the obtained results (Table 2) and those from the literature confirm that this transformation takes place with the energy gain.

**Reaction**  $H\dot{O}_2 + H\dot{O}_2$ . The interaction between two  $H\dot{O}_2$  molecules can be considered as a process of

interruption of the  $H\dot{O}_2 + H\dot{O}_2$  radical reactions. When combining two  $H\dot{O}_2$  radicals, first of all the (O3-H2) hydrogen bond is formed, followed by the transfer of the (H2) atom. So, the formation of the molecules of the hydrogen peroxide and the oxygen (Fig. 4) takes place.



Fig. 4. The reaction of two  $H\dot{O}_2$  radicals resulting in the formation of the molecules of the oxygen and peroxide.

This reaction takes place as it is described in the literature [11], the final reaction being:

$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2$$

**Reaction**  $H\dot{O}_2 + \dot{O}H$ . Another studied reaction is the interaction between  $\dot{O}H$  hydroxyl radical and  $H\dot{O}_2$  radical [12]. If we make a general analysis of this reaction, it will be noticed that, first, there occurs the formation of the (O2-H1) hydrogen bond, and then the transfer of the (H1) hydrogen atom with the formation of a molecule of water and oxygen. (Fig. 5)



Fig. 5. The interaction of the  $\dot{O}H$  and  $H\dot{O}_2$  radicals

The interaction of these radicals can also be considered as a stage of interruption of the studied reactions followed by the obtaining of the molecules of water and oxygen that are stable.

$$HO_2 + OH \rightarrow H_2O + O_2$$

**Reaction**  $\dot{O}H + \dot{O}H$ . From the experimental data [3] it is known that  $\dot{O}H$  radical is more suitable to be combined with another  $\dot{O}H$  radical, followed by the formation of a molecule of the hydrogen peroxide. The theoretical analysis of this reaction demonstrates that the bond of two OH radicals can occurs. The scheme of the development of this reaction is presented in Fig. 6. . H1



Fig. 6. The recombination of the  $\dot{O}H$  radicals to the hydrogen peroxide

Analyzing the processes I-VI we can conclude that the reactants and the products of reactions are separated by rather significant energetical barrier  $\Delta E_{1}$ , followed by an energetical gain  $\Delta E_{2}$  (Table 2). Using the calculated values of the total energy for the investigated systems and calculating the energy of the reaction according to the formula:

$$\Delta E_2 = \sum_i E_i^{(prod.)} - \sum_j E_j^{(react.)}$$

it is possible to draw the energetical profile of the reactions (Fig. 7). According to the scheme (Fig. 7) the products of the reactions are energetically more profitable than the initial substances. The values of the total energy gains are presented in Table 2.



Energetical barrier  $\Delta E_{\cdot}$  and energetical gain  $\Delta E_{\cdot}$ 

Table 2

Reaction	$\Delta E_1$ (kcal/mol)	$\Delta E_2$ (kcal/mol)					
Ι	493.95	32.28					
II	77.71	20.29 (32.2 [7])					
III	144.82	2.08 (30 [13])					
IV	240.75	2.99 (41 [7])					
V	115.07	23.29 (70 [7])					
VI	149.66	20.98 (51 [7])					

Fig. 7. Schematic drawing of the energetical profile of considered reactions

From the Table 2 it is seen that the obtained values of the barriers are too large. The reason is that the energies of transition states were calculated without re-optimization of their geometry. So these values can be considered only as semi-quantitative estimations.

Proceeding from the obtained data for the energies of the reactions  $\Delta E_2$  one can note a rather good correlation between the theoretical and experimental results for reactions II, V and VI (Table 2).

These processes were also studied from the point of view of the charge transfer between the studied particles during the reactions. The calculated charges of the reaction products in all the cases are equal to zero. This agrees with the experimental data which stands that only neutral particles are forming.

# Conclusions

- 1. It was proved that the differences between the energies of the reactants and the products of all analysed reactions are positive values that correlates with the experimental data.
- 2. Obtained theoretical results show that the products of all reactions I-VI have total charge equal to zero.

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# *AB INITIO* STUDY OF THE POSSIBLE SINGLE-CENTER UNITS FOR BINUCLEAR IRON COMPLEX [Fe,(bpym)<sub>3</sub>Cl<sub>4</sub>]

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**Abstract:** In present work we study two possible single-center units for binuclear iron complex  $Fe_2(bpym)_3Cl_4 - [Fe(bpym)_3]^{2+}$  and  $Fe(bpym)_2Cl_2$ . The obtained ground states for both studied systems are singlet states. In the case of  $Fe(bpym)_2Cl_2$  the lowest excited states were calculated to be  $240 \text{cm}^{-1}$  (triplet) and  $660 \text{cm}^{-1}$  (quintet) above the ground state and so are placed according to Lande rule. These states could be populated at room temperatures. For  $[Fe(bpym)_3]^{2+}$  first excited state was found to be about  $6000 \text{cm}^{-1}$  above the ground state and so cannot be populated at normal temperatures.

Keywords: iron complexes, bipyrimidine complexes, magnetic properties, ab initio study.

## Introduction

The binuclear and polynuclear transition metal complexes are widely used as models of natural enzymatic systems as well as models for molecular magnets. These complexes could be paramagnetic or diamagnetic in dependence of the bridging ligand(s) and ligand environment, manifesting the ferromagnetic or antiferromagnetic interactions between localized metal spins, respectively.

In order to explain magnetic properties of such complexes the Heisenberg-Dirac-Van Vleck model is widely used (see, for instance [1, 2]). This model takes into account so-called localized spin values. These are nothing else than values supposed according to the electronic configurations of the metal centers and ligand field. These assumptions make sense when they give good fit to experimental data and there are no possibilities to measure experimentally or calculate theoretically the exact spin values on centers. At the same time these presumptions can give only very general understanding of the way interactions take place by. The exact on-center spin values (measured or calculated) can give a much better comprehension about mechanism of these processes.

Considering the binuclear system mentioned above ( $[Fe_2(bpym)_3Cl_4]$ , see Fig. 1) we can choose one center and nearest ligands to form a single-center monomeric unit (marked with circle in Fig.1)



Fig. 1.  $\text{Fe}_2(\text{bpym})_3\text{Cl}_4$  binuclear complex. Monomeric unit  $\text{Fe}(\text{bpym})_2\text{Cl}_2$  is marked with a circle around, dotted line shows the C<sub>2</sub> axis.

This unit seems to be symmetrical. The highest symmetry group possible in this case is  $C_2$ . The main symmetry axis is also shown in Fig.1.

The monomeric unit shown before was selected as first possible monomer. The second monomer considered is  $[Fe(bpym)_3]^{2+}$  complex. It is well-known compound with similar ligand environment (only two chlorine atoms are substituted by bipyrimidine molecule). The magnetic properties of this monomer are described in experimental works [3, 4]. Both monomeric units are shown on **Fig.2**. Because of the similarity of ligand environment the metal atoms in binuclear compound most likely have the same electronic configuration (and magnetic characteristics) as in these single-center molecules.



Fig. 2. Fe(bpym)<sub>2</sub>Cl<sub>2</sub> (1) and [Fe(bpym)<sub>3</sub>]<sup>2+</sup> (2) systems with atom numbering.

# **Methods of calculations**

Geometry configurations of the systems under consideration were optimized using *ab initio* (ROHF) gradient methods realized in PC GAMESS [5] quantum chemistry package. Geometry optimizations were performed for states with total spin value (S) equal to 0 and 1.

Electronic structure calculations were performed using ROHF method with and without taking in consideration configuration interactions (CI). CI basis is discussed below.

In all calculations STO-6G (Slater-Type Orbital approximated by 6 Gauss-type orbitals) orbital basis set was used.

### **Results and discussion**

The first studied compound is  $Fe(bpym)_2Cl_2$  neutral complex with  $C_2$  symmetry as it was mentioned before. The second compound is the  $[Fe(bpym)_3]^{2+}$  complex. The highest possible point group for this case is  $D_3$  (see Fig.2). Three axes  $C_2$  can be selected to go through each one of three bipyrimidine molecules centers and iron atom and  $C_3$  axis could be selected to go through iron atom and to be perpendicular to the plane in which three  $C_2$  axes lay (in Fig.2  $C_3$  axis is perpendicular to the image plane).

The geometry data on these complexes are given in Table.1. and Table.2. There are presented calculated geometry data for both complexes and experimental data for  $[Fe(bpym)_3]^{2+}$  reported by de Munno et al in [3] and van Albada G.A. et al in [4].

Bond distances and angles in Fe(bpym) <sub>2</sub> Cl <sub>2</sub> (1)										
	R	(Fe – A),	Å	$\angle$ (N1 – Fe – A), deg			$\angle$ (N1 – Fe – A), deg $\angle$ (Cl1 – Fe – A), deg			g
А	N1	N2	Cl1	N2	N1'	N2'	Cl1'	N1	N2	N2'
Calculated	1.892	1.876	2.196	83.72	174,96	92.64	93.61	89.96	89.21	176.04

Table 2

Table 1

Bond distances and angles in [Fe(bpym) <sub>3</sub> ] <sup>2+</sup> (2)						
	R (Fe-N1), Å	$\angle$ (N1–Fe–N2), deg	$\angle$ (N1–Fe–N1'), deg	$\angle$ (N1–Fe–N2'), deg		
Exp [3], [4]	1.970, 1.968-1.975	81.0, 81.04-81.66	88.31-95.76	173.90-175.41		
Calculated	2.043	80.16	91.05	172.60		

From tables data it is seen that calculated geometry (at least for complex 2) is in good accordance with experimental data.

Calculated one-electron states which were used for CI calculations are presented on Fig.2. Their analytical forms are given in Table 3 and Table 4. It is seen that in both cases many active orbitals include large contributions from iron d-orbitals.



Fig.3. One-electron states of monomers  $Fe(bpym)_2Cl_2$  (1) and  $Fe(bpym)_3^{2+}$  (2). Electrons are placed according to the ground states obtained.

Table 3

MO LCAO for complex 1					
Orbital No, Sym	, Sym Composition				
118 a	$0.13d_{x^2-y^2}^{Fe} + 0.23d_{z^2}^{Fe} + 0.24d_{xy}^{Fe} + 0.17p^{p_{1}p_{ym}}$				
117 b	0.18p <sup>bipym</sup>				
116 b	0.18p <sup>blpym</sup>				
115 a	0.17 <sup>p<sup>blpym</sup>p<sup>blpym</sup></sup>				
114 b	$0.29d_{x^2-y^2}^{Fe} + 0.5d_{z^2}^{Fe} + 0.52d_{xy}^{Fe} + 0.1p^{bipym}$				
113 a	0.23p <sup>bipym</sup> (LUMO)				
112 a	0. $17d_{x^2-y^2}^{Fe} + 0.29d_{z^2}^{Fe} + 0.31d_{xy}^{Fe} + 0.18p^{pupym}$ (HOMO*)				
111 b	0. 5 <i>p<sup>Ul</sup></i>				
110 b	0.45 <i>p<sup>Cl</sup></i>				
109 a	0. 68 <i>p<sup>Cl</sup></i>				

Table	4
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	MO LCAO for complex 2		
Orbital No.,	Composition		
Sym			
138 e	$-0.20d_{\chi^2-y^2}^{Fe} - 0.14d_{yz}^{Fe} + 0.15p^{iig}$		
137 e	$0.19d_{xy}^{Fe} + 0.14d_{xz}^{Fe} + 0.15p^{iig}$		
136 <sub>a</sub> 2	0.15p <sup>itg</sup> 0.15p <sup>itg</sup> (LUMO)		
135 e	$0.78d_{x^2-y^2}^{Fe} + 0.54d_{yz}^{Fe} 0.78d_{x^2-y^2}^{Fe} + 0.54d_{yz}^{Fe} (\text{HOMO}^*)$		
134 e	$0.79d_{xy}^{Fe} + 0.54d_{xz}^{Fe}$		
<b>133</b> <sup>a</sup> l	$0.98d_{z^2}^{Fe}$		
* <b>HOMO</b> levels are shown according to ROHF calculations results.			

The main criteria for choosing the MOs for CI basis were their composition (main contributing AOs) and the energy gap between them and HOMO (LUMO) level. The size of the CI basis was also chosen so that the possible number of configurations to be big enough.

For complex 1 it is seen that HOMO levels are two degenerated states with main contribution of  $d^{Fe}$  orbitals as well as HOMO-1 level. LUMO is composed from p-orbitals of the three bipyrimidine ligands. HOMO and LUMO levels are very well separated ( $\Delta E \approx 6.8 \text{eV}$  (0,25a.u.))

In the case of the complex 2 the main contributors to the most active levels are d-orbitals of the iron atom, p-orbitals of the chlorine atoms and p-orbitals of the nitrogen atoms of the bipyrimidine ligand.

The CI calculations for  $Fe(bpym)_2Cl_2$  were performed using 108 core orbitals and 10 active with 8 electrons which give 22100 determinants with  $S_z=0$ . In case of the  $Fe(bpym)_3^{2+}$  we have used 132 core orbitals and 6 active with 6 electrons which give 400 determinants with  $S_z=0$  (175 with S=0; 189 with S=1; 35 with S=2 and 1 with S=3).

The active orbitals in both cases were selected to include highest occupied levels that contain contributions from metal d-orbitals and so these orbitals can be considered "magnetic orbitals" (see [1]).



Fig.4. Lowest multielectronic states of the (1) Fe(bpym)<sub>2</sub>Cl<sub>2</sub> and (2) [Fe(bpym)<sub>3</sub>]<sup>2+</sup>. Only the most contributing electronic configurations are presented with corresponding coefficients. Orbitals populations are shown by arrows.

The multielectronic states of the complexes 1 and 2 are presented on Fig.4. In case of the complex 1 we have very interesting picture. The levels are placed according to Lande rule. In this case the ground state is singlet. The first excited state which is triplet-one is only 240 cm<sup>-1</sup> above the ground state and the second excited state is a quintet at 660cm<sup>-1</sup> above the ground state. In this case the lowest excited states could be populated at normal temperatures.

In case of complex 2 ground state is singlet too. This result is in accordance with experimental data given both in [3] and [4]. The gap between the ground and the first excited state is about 6100cm<sup>-1</sup> and so the excited states are not populated at normal temperatures.

## Conclusions

The results obtained show that values of the localized spins on centers in  $Fe_2(bpym)_3Cl_4$  complex cannot be presupposed to be equal to 2. The both studied single-center units were shown to have singlet ground states in accordance with experimental data and cannot be used in Heisenberg model. This means, that the only way to successfully develop any suitable model that will describe not only the behavior of the complete system, but the internal interactions that take place there, is to proceed with the magnetic properties of polynuclear complexes quantitatively by both the experimental or theoretical *ab initio* mean.

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# SYNTHESIS, CHARACTERIZATION AND CRYSTAL STRUCTURE OF BIS-(2-HYDROXYBENZALDEHYDE)DIAMINOGUANIZONE

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**Abstract.** The new ligand, bis(2-hydroxybenzaldehyde)diaminoguanizone (1) has been synthesized and characterized by elemental analysis, IR and <sup>1</sup>H NMR spectroscopies. The crystal structure of the compound was determined by X-ray diffraction. The ligand  $C_{15}H_{15}N_5O_2\cdot C_2H_5OH$  crystallizes in the monoclinic space group  $P2_1/c$  with unit cell parameters a = 8.9102(3), b = 10.0357(3), c = 19.7618(6) Å,  $\beta = 98.385(2)^\circ$ , Z = 4, V = 1748.21(9) Å<sup>3</sup>, R1 = 0.040. The amino form of the ligand adopts a planar conformation stabilized by two intramolecular hydrogen bonds of the type O–H···N, in which the H atoms of the central amino group are directed to the lone-pair regions of the azomethine nitrogen atoms.

#### Keywords: Schiff base, guanidine, X-ray diffraction

#### Introduction

Carbohydrazones and thiocarbohydrazones are the next homologues of semicarbazones and thiosemicarbazones, which are well-known for their biological activity. Owing to the presence of different kinds of potential donor sites they are interesting ligand species capable of generating mononuclear, dinuclear and even tetranuclear complexes [1-4]. X-ray diffraction structures of these compounds revealed the presence of different conformers (*cis*, *cis* or *cis*, *trans*) differing in position of hydrazine group with respect to C=X (X = S, O).

Compared to studies on symmetrical Schiff bases of thiocarbohydrazones and carbohydrazones with salicyladehyde [5, 6], no data concerning the crystal structure of the title compound has been reported so far, although the synthesis of this Schiff base has been well documented [7]. Several metal chelates of bis(salicylidene) thiocarbohydrazone and carbohydrazone have been studied [8-9]. Complexation behavior of the nitrogen analogue **1** toward metal ions would be an interesting object of study. The present report can also be considered as an extension of investigations on salicylaldehyde aminoguanizone and its metal complexes reported elsewhere [10, 11]. In order to study the complexation behavior of 1,5-(2-hydroxybenzaldehyde)diaminoguanizone with first and second row transition metal ions, we synthesized the ligand **1** and determined its crystal structure.

# Materials and physical measurements

2-Hydroxybenzaldehyde (Aldrich) and diaminoguanidine hydrochloride (Aldrich) were used as received. Elemental analyses were carried out using Vario EL III CHNOS Elemental Analyzer. Infrared spectra were recorded on a Perkin-Elmer FTIR spectrophotometer. NMR spectra were performed on a Bruker 80 spectrometer in DMSO- $d_6$  solution using TMS as internal reference.

#### X-ray crystallography

The data collection was done on a Bruker X8 APEX II CCD diffractometer using graphite monochromated  $MoK_{\alpha}$  radiation ( $\lambda$ =0.71073 Å) at 100(2) K. The data were processed using SAINT software [12]. The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were placed at calculated positions and refined as riding atoms in the subsequent least-squares model refinements.

# **Results and Discussion**

Selected bond lengths (Å) and angles (deg) are listed in Table 1. As shown in Figure 1, the molecule 1 adopts a planar conformation which is stabilized by two intramolecular hydrogen bonds O1–H···N1 [O1–H 0.891. H···N1 1.873, O1···N1 2.665 Å,  $\angle$ O1–H···N1 147.09°] and O2–H···N5 [O2–H 0.912. H···N5 1.808, O2···N5 2.646 Å,  $\angle$ O2–H···N5 151.75°], as also reported for sulfur and oxygen analogs [8, 9] The H atoms of the central amino group (the hydrogens attached to N3) are directed to the lone-pair regions of the azomethine nitrogen atoms N1 and N5. Similar situation was found in 1,3-diaminoguanidinium cation [13], where the corresponding H atoms of the central amino group were found to be directed to the lone-pairs of the terminal aminogroups. The N4–C8 bond of 1.3179(19) Å is shorter than N2–C8 bond of 1.3591(19) Å as a result of proton migration from N4 to the N3 atom. Recent theoretical studies on electron

delocalization in diaminoguanidine [14] showed larger stability of the amino form over the other possible tautomeric forms. The X-ray data were deposited at the Cambridge Crystallographic Data Center No. CCDC 702169.



Fig. 1 The molecular structure of 1·EtOH. Dashed lines denote intramolecular hydrogen bonds.

Selected bond lengths (Å) and bond angles (deg)								
N1-C7	1.282(2)	C8-N4	1.318(2)	N3-C8-N4	126.9(1)	C8-N2-N1	116.1(1)	
N1-N2	1.367(2)	N4-N5	1.389(2)	N4-C8-N2	114.5(1)	C8-N4-N5	113.4(1)	
N2-C8	1.359(2)	N5-C9	1.288(2)	N3-C8-N2	118.7(1)			
C8-N3	1.333(2)							

## Experimental

2-Hydroxybenzaldehyde (1.21 g, 10 mmol) in EtOH (20 ml) was added to diaminoguanidine monohydrochloride (0.62 g, 5 mmol) and Na<sub>2</sub>CO<sub>3</sub> (0.26 g, 5 mmol) in H<sub>2</sub>O (5 ml). The solution obtained was refluxed for 1 h. The yellow solid precipitated on cooling the solution was filtered off, washed with EtOH, dietyl ether and dried in air. X-ray diffraction quality single crystals of bis(2-hydroxybenzaldehyde)diaminoguanizone were grown in ethanol. Yield: 1.01 g, 70%; m. p. 205°C. Anal. Calcd for  $C_{15}H_{15}N_5O_2$  (%): C, 60.60; H, 5.09; N, 23.56. Found: C, 60.08; H, 5.25; N, 23.44. IR (cm<sup>-1</sup>): 3467 (NH), 1622 (C=N), 1262 (C–N). <sup>1</sup>H NMR ( $\delta$ : ppm): 8.29 (s, –CH=), 6.73-7.57 (m, 8H, aromatic ring protons).

Table 2

Table 1

Crystal Data and Details of Data Collection for 1.

Empirical formula	C <sub>17</sub> H <sub>21</sub> N <sub>5</sub> O <sub>2</sub>
fw	343.39
space group	monoclinic
space group	$P2_1/c$
a, Å	8.9102(3)
<i>b,</i> Å	10.0357(3)
<i>c</i> , Å	19.7618(6)
$\beta$ , deg	98.385(2)
<i>V</i> , Å <sup>3</sup>	1748.21(9)
Ζ	4
$\rho_{\rm calcd}  {\rm g} \cdot {\rm cm}^{-3}$	1.305
crystal size, mm <sup>3</sup>	$0.35 \times 0.25 \times 0.22$
T, K	100(2)
$R1^a$	0.0402
$wR2^b$	0.1001
$\mathrm{GOF}^{c}$	0.976

<sup>*a*</sup>  $R1 = \sum ||Fo| - |Fc|| / \sum |Fo|$ , <sup>*b*</sup>  $wR2 = \{\sum [w (Fo^2 - Fc^2)^2] / \sum [w (Fo^2)^2] \}^{1/2}$ . <sup>*c*</sup> GOF =  $\{\sum [w (Fo^2 - Fc^2)^2] / (n-p) \}^{1/2}$ , where *n* is the number of reflections and *p* is the total number of parameters refined.

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# SYNTHESIS OF PINONIC ACID NEW DERIVATIVES

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**Abstract:** The method for synthetising new derivatives of the pinonic acid using, at the key step, the reaction of condensation with epichlorohydrin, has been elaborated. It has been shown that, in the presence of catalytic amount of tetrabutylammonium chloride, the reaction is directed towards the formation of etherchlorohydrin, which, later on, is easily transformed into epoxides as well as chloromethyldiketones with good overall yield.

Keywords: pinonic acid, chlorohydrin, epoxide.

# Introduction

Chlorohydrins, epoxides as well as 2-chloromethylketones are widely used in synthetising various, practically important substances. One of the perspective directions towards obtaining such type compounds in an optically active form may involve the use of pinonic acid 2 [1] as chiral matrix.

## **Results and discussions**

To realize this approach, we have investigated the possibility of regioselective transformation of acid 2 using epichlorohydrin 3.

Heating of 2 in 3 leads to an unidentified complex mixture of products. The probable reason of this phenomenon is the proceeding of the reaction of oxirane cycle disclosure according to both Sn2 and Sn1-mechanisms.

It has been established that the addition of catalytic quantities of tetrabutylammonium chloride directs the reaction towards the formation of chlorohydrins **4** according to the scheme.



In IR-spectrum of mixture 4 can observe characteristic bands of carbonyl group of methylketone and ester groups at 1715 and 1770 cm<sup>-1</sup>, respectively. The spectrum is added by signals at 770 cm<sup>-1</sup>-(C-Cl) and 3600 cm<sup>-1</sup> (OH). A distinctive feature of NMR spectra of diastereomers 4 is the doubling of all peaks due to the fact that the initial epichlorohydrin 3 represented a mixture of stereo isomers.
Alcohols 4 are oxidized by Jones's reagent into ketone 5. Its structure is confirmed by the availability of maximum of two carbonyl and carboxylic ester groups (1715, 1735 cm<sup>-1</sup>) in the IR-spectrum, as well as of characteristic signals of protons of four methylene groups, of one methylketone and two methyls of gem-dimethyl groups in the <sup>1</sup>H NMR-spectrum.

In comparison with carbon spectrum of initial 4, the signal of the carbon atom of the formed carbonyl group and the absence of the carbon atom signal at hydroxyl group in the area of 69.37 ppm, is observed in the <sup>13</sup>C NMR-spectrum of product 5 in the weak field area (195.65 ppm).

Boiling of chlorohydrin 4 in KOH ethanol solution leads, with 68% yield, to oxide 6 whose structure results from spectral data. Present in its IR-spectrum are the frequencies characteristic for keto-, carboxylic ester and oxirane groups, while in the <sup>1</sup>H NMR-spectrum available are the three protons signals of gem-dimethyl and acetyl groups. Besides, available in the spectrum are multiplet signals of the protons of oxirane fragment connected with carboxyl group.

### Conclusions

Thus, it has been shown that utilization of tetrabutylammonium chloride allows performing the chemo- and regioselective functionalization of pinonic acid 2 up to chlorohydrins, later converted into 2-chlorodiketone and epoxides. The utilizing the synthesized compounds for preparing the practically important optically active substances are now under investigation.

#### Experimental

All the used solvents were of reagent quality, and all commercial reagents have been used without additional purification. Removal of all solvents has been carried out under reduced pressure. Analytical TLC plates were Silufol® UV-254 (Silpearl on aluminium foil, Czecho-Slovakia). Melting points has been determined on the heating table "Boetius" and not corrected. IR spectra have been recorded on a Specord 75 IR instrument. <sup>1</sup>H and <sup>13</sup>C NMR spectra have been recorded for CDCl<sub>3</sub> 2-3% solution on a Bruker AC-80 (80 and 20 MHz).

(-)- $\alpha$ -Pinene 1– reagent from Fluka Chemical Company.  $n_{\rm D}^{20}$  1.466,  $[\alpha]_{\rm D}^{20}$ -58.48° (c 0.046, CHCl<sub>3</sub>). Acid 2 has been synthesized from 1 by method [1]. M.p. 64-67°C from hexane.  $[\alpha]_{\rm D}^{20}$ -74.03° (c 0.0199, CHCl<sub>3</sub>).

## Preparation of 3-chloro-2-hydroxypropyl-2-[(1S,3R)-3-acetyl-2,2-dimethylcyclobutyl]acetate 4.

5 g (27 mmol) of acid 2 and 0.1 g of tetrabutylammonium chloride in 5 ml of epichlorohydrin 3 has been kept during 1 hour at 120°C. Then, the excess of epichlorohydrin has been distilled off in vacuum. The residue has been dissolved in 100 ml of ether, washed with saturated solution NaCl (3x20 ml), dried over Na<sub>2</sub>SO<sub>4</sub>, distilled off the ether and obtained 5.9 g yellowish oil-like product 7:3 mixture of diastereoisomers 4. The yield was 79%. IR ( $\nu/cm^{-1}$ ): 770 (C-Cl), 1375, 1380 CMe<sub>2</sub>), 1715 (COCH<sub>2</sub>), 1740 (COOCH<sub>2</sub>), 3600 (OH). <sup>1</sup>H NMR of the major isomer ( $\delta$ , ppm, J/Hz): 0.79, 1.21 s,s(6H, CMe,), 1.93 s(3H, COCH,), 1.70-2.89 m (6H, 7CH,, 4CH,, 5CH, 1CH), 3.02-3.12 m (1H, OH), 3.44-4.25 m (5H, COOCH<sub>2</sub>, C<u>H</u>-OH, CH<sub>2</sub>-Cl). <sup>13</sup>C NMR of the major isomer (δ, ppm): 207.62 (<sup>2</sup>C), 172.65 (<sup>3</sup>C), 69.37 (C-OH), 65.02 (COOCH<sub>2</sub>), 54.04 (1C), 45.66 (C-Cl), 43.14 (°C), 37.82 (°C), 34.76 (<sup>4</sup>C), 30.06 (<sup>10</sup>C), 22.91 (<sup>8</sup>C, <sup>9</sup>C), 17.22 (<sup>7</sup>C). Found, %: C 56.34, H 7.58. C<sub>13</sub>H<sub>21</sub>ClO<sub>4</sub>. Calculated, %: C 56.42, H 7.65.

## Preparation of (-)-3-chloro-2-oxopropyl-2-[(1S,3R)-3-acetyl-2,2-dimethylcyclobutyl]acetate 5.

Jones's reagent prepared from 7 g CrO<sub>2</sub>, 6 ml H<sub>2</sub>SO<sub>4</sub> and 10 ml H<sub>2</sub>O has been added to solution of 2.8 g (10 mmol) of 4 in 15 ml acetone at by drops stirring, and has been stirred for 3 hours. Then, 30 ml of *i*-propyl alcohol has been added and left overnight. The following day, the mixture has been extracted by means of diethyl ether (5x50 ml), the extract has been washed with NaNCO, solution (2x30ml) and with saturated NaCl solution (2x30 ml), dried over  $Na_2SO_4$ , distilled off the ether and have obtained 2.01 g yellow oil-like product 5. The yield was 73%.  $[\alpha]_D^{20}$ -14.09° (c 0.1, CHCl<sub>2</sub>). IR (ν/cm<sup>-1</sup>): 760 (C-Cl), 1380, 1385 CMe<sub>2</sub>), 1715 (C=O), 1735 (COOCH<sub>2</sub>). <sup>1</sup>H NMR (δ, ppm, J/Hz): 1.16, 1.24 s,s (6H, CMe<sub>2</sub>), 2.05 s (3H, COCH<sub>3</sub>), 1.33-2.90 m (6H, <sup>7</sup>CH<sub>2</sub>, <sup>4</sup>CH<sub>2</sub>, <sup>5</sup>CH, <sup>1</sup>CH), 4.18 m (2H, CH<sub>2</sub>Cl), 4.89 m (2H, COOCH<sub>2</sub>). <sup>13</sup>C NMR (δ, ppm): 208.33 (<sup>2</sup>C), 195.65 (O=<u>C</u>-CH<sub>2</sub>), 172.87 (<sup>3</sup>C), 64.24 (COO<u>C</u>H<sub>2</sub>), 53.87 (<sup>1</sup>C), 46.01 (C-Cl), 43.14 (°C), 37.64 (°C), 34.33 (°C), 29.99 (1°C), 22.83 (°C, °C), 17.11 (°C). Found, %: C 56.80, H 7.02. C<sub>12</sub>H<sub>10</sub>ClO<sub>4</sub>. Calculated, %: C 56.83, H 6.97.

# Preparation of 2-oxiranylmethyl-2-[(1\$,3R)-3-acetyl-2,2-dimethylcyclobutyl]acetate 6.

Solution of 1.35 g (4.9 mmol) of 5 and 0.41 g (7.3 mmol) of KOH in 20 ml of EtOH have been boiled under refluxing during 5 hours. Then, the ethanol has been distilled off, the residue has been diluted with 20 ml of water and extracted with CHCl<sub>2</sub> (5x10 ml). The extract has been washed with 10 ml of saturated NaCl solution, dried over Na<sub>2</sub>SO<sub>2</sub>, chloroform has been distilled off and 0.8 g of yellow oil-like 7:3 mixture of diastereoisomers 6 have been obtained. The yield was 68%. IR (v/cm<sup>-1</sup>): 1380, 1385 (CMe<sub>2</sub>), 1710 (COCH<sub>2</sub>), 1735 (COOCH<sub>2</sub>), 3040 (epoxi). <sup>1</sup>H NMR of major isomer (δ, ppm, J/Hz): 1.05, 1.14 s,s (6H, CMe<sub>2</sub>), 1.90 s (3H, COCH<sub>2</sub>), 1.79-2.74 m (8H, <sup>7</sup>CH<sub>2</sub>, <sup>4</sup>CH<sub>2</sub>, <sup>5</sup>CH, <sup>1</sup>CH, CH, epoxi), 3.35-3.44 m (1H, CH epoxi), 3.83-4.10 m (2H, CO<sub>2</sub>CH<sub>2</sub>). Found, %: C 64.83, H 8.42. C<sub>13</sub>H<sub>20</sub>O<sub>4</sub>. Calculated, %: С 64.98, Н 8.39.

#### References

[1]. Gudima, A. Research in chemistry: achievements and prospects. Chişinău, 2003, V.II, p. 103-105.