Investigation efficiency of Alhagi Pseudalhagi flavonoids dimerization process

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Abstract: Flora of Republic of Kazakhstan have more than 6000 species of medicinal plants, among of which the plant Alhagi Pseudalhagi are the object of fixed attention as a source of essential oil, flavonoids, steroids, vitamins, organic acids, tannin, etc., possessing by wide spectrum of biological activity. Development of methods separation from it the individual compounds, that can be used as a raw material for more efficiency new biological substances, are the most perspective in the present time. In this work are investigated processes of electrodimerization and cross oxidation for obtainment the 8 new compounds based extracted flavonoids. This article contain data of fulfillment of dimerization processes with purpose of obtainment with maximum yield the dimmers based on Oroxyline, 3-Methylquercetine, Isoramnetine and Kemipherid – flavonoids, allocated from Alhagi pseudalhagi, as well as results of investigation of physiological activity of obtained products. The most optimal medium, optimal parameters have been established for effective dimerization process conducting where maximal yield of synthesized compounds are registered. Also, medicobiological testing, implemented in the laboratory of Tumor Radiobiology into Kazakh Scientific Research Institute of Oncology and Radiology, revealed high antiradiation activity of Oroxiline-7-0-7-Glaucinide.

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1. Introduction

Recently plants flavonoids became object of scientists attention, because they are potent physiologically-active substances [1-6]. It is known, that many dimeric derivatives reveal more high physiological action, than initial compounds, particularly, binding of aromatic rings into polycyclic system are more efficient: diphenyl are more physiologically active than benzol, phenantren and anthracene – than diphenyl, many biflavonoids – than initial flavonoids [7, 8]. Flavonoids dimerization products have practical interest, because they can be easy obtained by electrochemical synthesis and they have high characteristics of physiological activity. More detailed the process of electrochemical; dimerization, it's mechanism and kinetics are described into paper [9], where the optimum condition have been determined and the recommendation for choice of process implementation have been given.

Camel's-thorn, Alhagi pseudalhagi Fabiaccae family, was used as a medicinal plant from old-time. Diversity of names: camel's-thorn, camel plant, alhagal, Yandak, Jantak, tugu-tecen etc., testifies that camel's-thorn was known and valued by many people [10].

It's medicinal properties were known, but in official medicine it is practically not used, because active principle of this plant were not revealed and it's pharmacological activity adequate tests were not carried out.

2. Material and Methods

Seed, flowers, leaf, stems, so, all herbage of Alhagi pseudalhagi, collected in phase of flowerage (May) and fruitage (September) in South-Kazakhstan area serve as the objects of research.

Collected raw material was dried in the shadow till residual moisture 8-12%, and was crushed on handmill prior to particles with size less than 1 mm.

The raw material pretreatment consisted of next stages: seed deffating by way of processing by Benzol or petroleum ether in ratio of 1:5: removal of pigments and other lyophilic substances from plant superstructure block have been implemented by Chloroform by infusion at room temperature in the course of 3-4 hours. Then raw material was dried, and was exposed to extraction by various solvents [11-13].

For research have been used a mathematical of synthesis optimization process by Rosenbork method. Synthesis was carried out at controlled anode potential with use of potentiostat, and as a reference electrode are used chlor-silver electrode. Quantity of electricity was determined by coulometer. Identification of synthesized compounds was conducted by use of IR-,

UV-, and PMR- spectroscopy. Medicobiological testing was conducted on white outbred mice.

3. Results

Purpose of given researches – to achieve maximum electrodimerization process selectivity, based on these data, to obtain dimmers on the basis of Oroxyline, 3-Methylquercetine, Isoramnetine and Kempherid – flavonoids, allocated from Alhagi pseudalhagi with maximum yield and to investigate physiological activity of obtained products. For this purpose 7 tests series on the investigation of various factors influence on the dimerization process have been implemented. Resulting factors are presented in the table 1.

Table 1. Factors influencing on the dimerization process

Factor	Factor meaning (limits)
1. Solvent nature	Ethyl spirit, nitromethan, acetonitrile
2. Electrolyte nature , M	$LiClO_4$, C_2H_5COONa, Tetrapropylammonium bromide, Hexaftuorineantimonat
3. Current density, A/см^2	0,005-0,02
4. Substrate concentration, M	$10^{-5} – 10^{-3}$
5. Anode material	Platinum, glasscarbon, lead dioxide
6. Electrolyte concentration, M	0,1-1,0
7. Quantity of missing electricity , Q	80-200% от Q_{reop}

Solvent and background electrolyte carry out very important function in electrochemical experiment [14]. Usage of nonaqueous solvents appreciably extended the variability of possible electrochemical reactions, because many compounds are oxidized (or are recovered) more difficulty than water and therefore can be modified into more broad field of potentials. To avoid experimental difficulties, the solvents with high dielectric constant have been chosen, that at test temperature are being into liquid condition, with low vapour pressure, polar and nonpolar, protonic and aprotonic. Such properties as solubility, electrochemical and chemical stagnation have been accounted at choice of electrolyte. Tests results on the study of influence of solvent and electrolyte nature are presented into table 2.

From presented results follows that minimal yields have been received into medium of Methyl alcohol, because therein along with basic reaction occur the secondary reactions of methoxylation and formation of dimeric methoxiderivatives. The solvent Nitromethane and acetonitrile with additive $LiClO_4$ being should be considered as more acceptable as a electrolyte. Low output in the presence of tetrapropylammonium are explaned by these fact that all halogenides are sufficiently easy oxidized into anode field and therefore it is possible the occurring of side reactions.

Results on study of yield influence from water quantity into composition of reaction medium are being in the good compliance with data of polarization research and study of physical-chemical properties of flavonoids: with increase of water content from 0 till 10% the yield of dimeric products are increased on 7-10%, achieving maximum at water content 10%, and herewith flavonoids oxidation mechanisms are not changed, electron number on each stage approximately equal 1.

Table 2. Solvent and electrolyte nature influence on dimeric products yield

Solvent	Electrolyte, 0,1 M	Current density, A/см^2	B B, %			
			Oroxyline	Kempherid	3-methylquercetin	Isoramnetin
Methyl alcohol	C_2H_5COON	0,004	22,8	24,7	30,2	21,3
		0,008	27,2	24,9	32,3	30,1
	$LiClO_4$	0,004	32,8	37,1	32,4	35,3
		0,008	41,2	43,2	40,1	43,2
Nitromethane	$LiClO_4$	0,004	40,3	40,0	41,9	40,3
		0,008	45,2	47,3	49,1	52,8
Nitromathane: water (95:5)	$LiClO_4$	0,004	47,3	42,1	45,0	42,1
		0,008	49,9	51,2	52,3	56,1
Nitromathane: water (90:10)	$LiClO_4$	0,004	49,4	47,3	49,9	50,1
		0,008	52,7	54,8	55,7	58,3
	Tetrapropyl-ammonium bromide	0,004	30,2	37,3	38,3	37,9
		0,008	28,7	27,1	29,1	28,3
Nitromethane: water (80:20)	$LiClO_4$	0,004	35,4	31,2	32,3	40,0
		0,008	18,3	19,4	24,1	36,8
Acetonitrile	$LiClO_4$	0,004	47,2	46,1	48,3	49,4
		0,008	50,4	50,9	52,1	56,1
	Hexafluorineantimonit	0,004	49,3	47,4	48,1	41,2
		0,008	52,8	51,3	53,8	52,4

At water content of 20% and above the yield of end product deeply decreases, and sharp current growth are observed on the voltampere curves, quantity of electrons increases on each stage up to 3-5, that testifies about change of reaction mechanism. Herewith the large quantity of gum-like products are formed. So for subsequent investigations the system Nitromethane:water (90 : 10) · $LiClO_4$ have been chosen.

At introduction of results of many electrochemical synthesizes into production the complex problem arise – rational selection of electrode materials, properties of which define direction, rate, economics and constructive execution of these processes. Complex, difficult-to compatible requirements to properties of anode materials are proffered: catalytical activity, stability at anode polarization and oxidizing medium, high electric conductance, good mechanical properties, and, cheap cost. To all requirements, apart from last, are satisfied anodes from platinum metals group that on catalytical, electrochemical and corrosive properties considerable suppress all. But their dearness and deficiency stipulate the necessity of more cheap materials choice, that possess by above mentioned properties. Main complexity of anode materials problem lies that at polarization the anode surface are exposed to deep change into oxygen-containing medium because of oxygen allocation: electrochemical activity and stability of anodes are being in direct dependence from nature and condition of surface oxide film. Catalytical properties of metaloxide anodes are defined by thermodynamical characteristics of oxide phase – by bond energy of oxygen on surface and into volume, by energy of defect formation, stipulated by nonstoichiometry, energy of oxygen ions motion activation into grating. Usually if bond energy of oxygen into oxide is high, the activation energy of oxidizing process is larger [15].

The results of researches of anode materials influence on dimeric products yield at electric oxidizing of flavonoids are presented in the table 3.

Table 3. Anode materials influence on dimeric products yield at flavonoids electrooxidizing into

Material of anode	Current density, $A/см^2$	Substance yield, %			
		Oroxyline	Kempherid	3-methylquercetine	Isoramnetine
Platinum	0,004	49,4	47,3	49,9	50,1
	0,008	52,7	54,8	55,7	58,3
Lead dioxide	0,004	21,8	24,1	20,8	25,4
	0,008	17,3	15,2	16,3	15,2
Glasscarbon СУ30	0,004	50,3	48,4	52,5	50,0
	0,008	54,8	56,9	55,9	58,8

Nitromethane:water medium (90:10) + 0,1 M $LiClO_4$

From resultings it is shown that on the lead dioxide the dimeric products yields are most low. For explanation of this fact the composition of side products of flavonoids electrooxidation have been investigated: hydrooxidation reaction occur with high efficiency on lead oxide. This is explaned by presence of active particles OH_{ads} on PbO_2 surface, that easy interact to reaction with flavonoids radicals with formation of hydroxyderivatives, if current density is high, so they are easy formed.

After study of all factors influence and mathematical process optimization by Rozenbork method the dimmers yield made up: 8-8-bioroxyline-67,4%, 8-8-bikempherid-69,5%, 8-8-bi-3 methylquercetine-67,8%, 8-8-biisoramnetine-75,4%.

As result of dimerization process mathematical optimization and experimental testing of resultings the dimeric products yield reach to 70%. With purpose of yield increase, as well as for data obtainment for reaction scheme drawing up the series of synthesizes at controlled anode potential have been implemented. Resultings are presented in the table 4.

It is shown, that maximal yields, closed to qualitative, are obtained at first wave potential field, where the reaction of radicals dimerization, formed at flavonoids molecules discharge, are most probable. Hydroxyderivatives are not formed in this conditions, because anode potential are unsufficiently for this reaction. At increase of anode potential up to 1,2 –1,3 B, that meet to second wave, along with dimerization reaction begin implement the radicals hydroxylation reaction, that to led to end products (dimmers) yields decrease, and increase the yield of side products (hydroxyderivatives).

Thereby, dimerization process implementation at controlled potential allow to obtain the dimeric products with yield about 100%.

As result of flavonoids electrodimerization have been obtained the buff powders as a basic products after recrystallization from acetone. Identification have been implemented by IR, UV, and PMR – spectroscopy methods, because similar compounds have not been described in literature.

Into IR-spectrs of synthesized products the absorption edge are kept, corresponding to functional group of initial flavonoids.

UV-spectrums testify about preservation of flavonoid structure of compounds.

Into PMR-spectrums of investigated compounds are absent the signals of protons at C_8 atom (in the field of 6,45м.д.), that testify about participation of this atom into ontraflavonoids bonds (spectral characteristics are presented in the table 5).

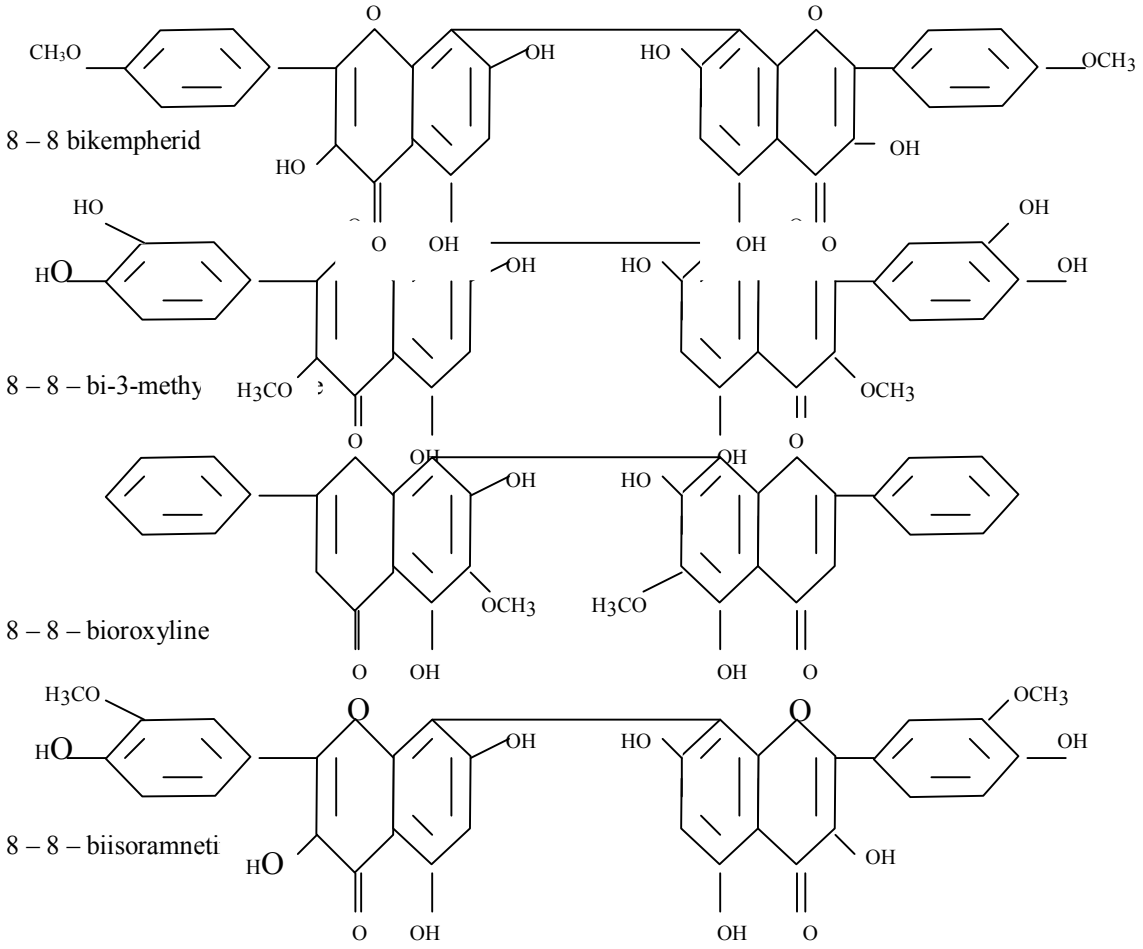
On the basis of physical-chemical characteristics, as well as spectral data, allocated products are identified by next method:

Table 4. Results of synthesis on the flavonoids basis, implemented at controlled anode potential

Flavonoid	Anode potential, B											
	0,8		0,9		1,0		1,1		1,2		1,3	
	Products yields, % *											
	Д	Г	Д	Г	Д	Г	Д	Г	Д	Г	Д	Г
Oroxyline	97,0	-	99,0	-	98,7	-	73,4	12,1	12,4	36,5	10,2	52,3
Kempherid	-	-	98,7	-	98,9	-	96,2	-	29,5	33,4	21,4	54,8
3-methylquercetine	-	-	99,1	-	99,0	-	96,0	-	32,5	38,2	24,3	49,3
Isoramnetine	-	-	45,2	-	98,5	-	97,4	-	41,4	35,2	28,9	47,1

D – dimeric product. H – hydroxyderivatives.

* BB are given in calculation for used raw material.



In Kazakh Research Institute of Oncology and Radiology (Almaty) the medicinal-biological tests of synthesized compounds have been implemented on the white mice, 8-8-bi-3-methylquercetin in dose of 320 mg/kg give high indexes of radiotread action: survivability percentage made up 81,3 at average

lifetime of 17,7 days, FID-2,3, dose 80 mg/kg stipulated 50% of survivability at average lifetime of 13,1 days, FID-1,4.

4. Discussions

From results of research can be do following conclusion:

- The maximal yield in the dimerization process will be obtained into solvent medium of Nitromethne and Acetonitrile with additive $LiClO_4$ as an electrolyte.

- With increase of water content from 0 to 10% the yield of dimeric products are increased on 7-10%, achieving maximum at water content 10%, and mechanism of flavonoids oxidation don't changed, the number of electrons on each stage approximately are equal 1. But at water content 20% the mechanisms of reaction are changed, the yield of products are sharply decreased. Therefore for futher researches have been chosen system Nitromethane:Water (90:10) · $LiClO_4$.

- The yield of dimeric products on glasscarbon in 2-4% above than on Pt. Futher synthesis with purpose of optimization have been carried out on glasscarbon.

- Conduct of dimerization process at controlled potential allow to obtain dimeric products with yield about 100%.

Thereby, after study influence of all factors and mathematical process optimization by Rosenbrock method the yield of dimmers made up: 8-8-bioroxyline-67,4%, 8-8-bikempheride-69,5%, 8-8-bi-3-methylkvercetine-67,8%, 8-8-biisoramnetine-75,4%.

Medicibiological testing shown that obtained dimmers reveal high antiradiation activity.

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