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Mechanism of Reaction of Flavone Derivatives with Hydroxyl Radical by Semiempirical Methods

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Abstract — Semiempirical quantum-chemical calculations were used to study the reactivity of the $C^2=C^3$ bond in flavones in reaction with hydroxyl radical. The preferred pathway was found to be addition of the radical by C^3. Increased reactivity in the reaction in question of the hydroxy groups on C^3 and $C^{4'}$ in polyhydroxyflavones was revealed.

The present notion of flavonoids as potent antioxidants is based on experimental data which show that these compounds are capable of binding active oxygen forms. The increased interest in this problem is primarily due to the fact that flavonoids by functioning as "traps" for free radicals regulate peroxide oxidation of membrane lipids [1].

According to data in [2], humans take up with vegetable food up to 1 g of various flavonoids daily, which creates in tissues and biological fluids considerable pharmacological concentrations of these compounds. The structural diversity and widespread natural occurrence have formed the basis for the scientifically substantiated application of flavonoids and flavonoid-containing medicinal plants for correction of more than 40 types of biochemical processes [3].

Such species as singlet oxygen, superoxide radical anion ($O_2^{\cdot-}$), hydroxyl and peroxyl radicals ($HO^{\cdot}$, $HO_2^{\cdot}$, $RO_2^{\cdot}$), as well as hydrogen peroxide (H_2O_2) take part, along with peroxide oxidation, in tissue oxygen assimilation processes, microsomal oxidation, *etc.* [4, 5]. It is proved that increased concentration of active oxygen forms hinders peroxide oxidation of membrane lipids and favors pathochemical processes leading to cell damage. It is assumed that $HO^{\cdot}$ radical can selectively concentrate near DNA, which results in hydroxylation of the latter [6].

In this connection we considered it of interest to study interaction of flavonoids of various structure with active oxygen forms.

The antiradical activity of flavonoids toward free radicals generated *in vitro* is well-documented [1, 7, 8]. Thus the photochemical reaction of flavonoids with singlet oxygen gives rise to chromone heteroring cleavage. Probably, this process is to a certain extent related to the metabolism of natural chromones [9].

In the present work we studied the reactivity of flavone derivatives toward hydroxyl radical. To this end, we used the experimental data of Husaine *et al.* [10] on the activity of flavonoids toward $HO^{\cdot}$ radical.

Table 1 lists the flavonoids studied in [10] in order of decreasing activity. Hydroxyl radicals were generated by UV irradiation of reaction mixtures in the presence of H_2O_2.

Flavones and reaction intermediates were calculated by three semiempirical methods: MNDO, AM1, and PM3 (MOPAC 6.0 package) using parametrization of the PM3 method [11, 12]. Comparison of the experimental and calculated data allowed a number of structure–reactivity relations to be revealed for this series of compounds and probable mechanisms of the reaction in question to be proposed.

There are two most probable pathways flavonoids can react with hydroxyl radical. The first suggests addition of the radical species at the C^2–C^3 bond of the pyrone fragment (Scheme 1).

In view of the fairly high electrophilicity of $HO^{\cdot}$ radical [5, 6], the probability of the addition at C^3 is much higher, which agrees well with the electron density distribution in the propenone fragment [13]. Thus, intermediate **A** formation is preferred. We suggest that intermediates **A** and **B** are formed by concurrent processes, which is consistent with the results of hydroxylation of unsaturated compounds under UV irradiation [14].

The second pathway (Scheme 2) suggests involvement of substrate hydroxyls in reaction with the

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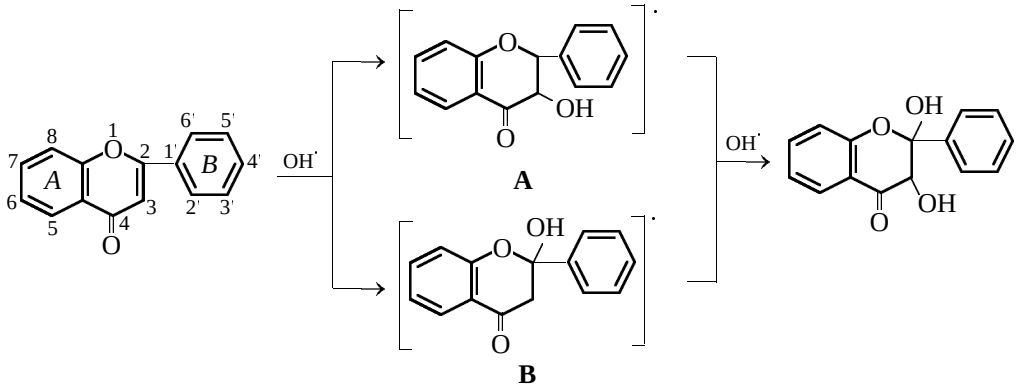
Scheme 1.



A

B

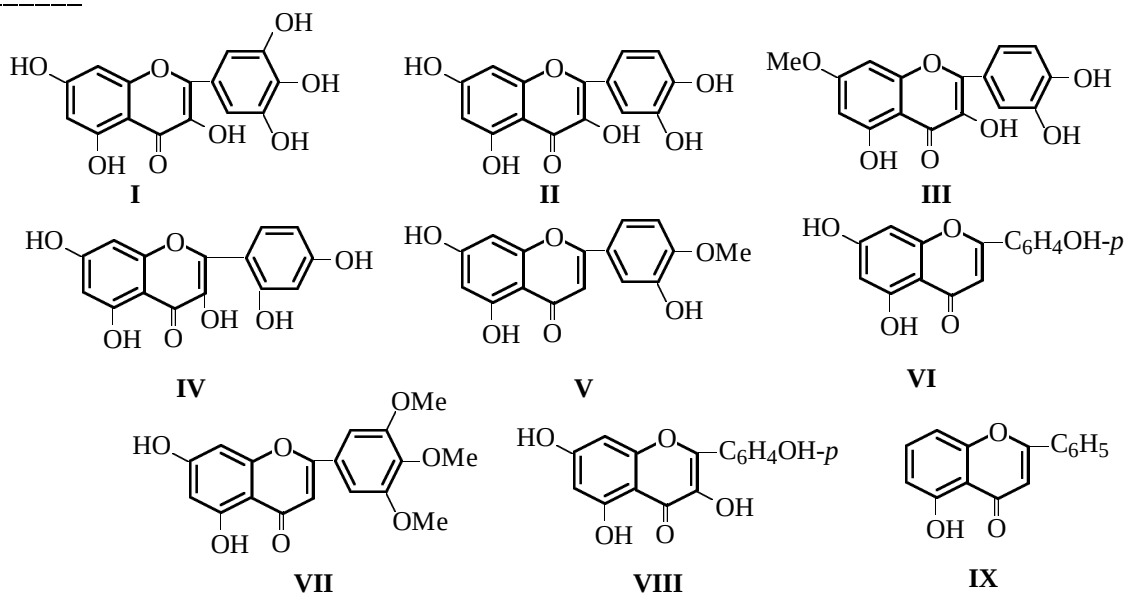
Table 1. Experimental activities (*A*) of flavones **I**–**IX** toward hydroxyl radical

Comp. no.	Compound	*A*, % [10]
I	Myricetin	50
II	Quercetin	48
III	Rhamnetin	46
IV	Morin	40
V	Diosmetin	39
VI	Apigenin	34
VII	5,7-Dihydroxy-3',4',5'-trimethoxyflavone	28
VIII	Kaempferol	20
IX	Flavone	4

radical. A number of authors left room for this mechanism in discussing the antioxidant activity of phenols and flavones [15, 16].

In examining possible reaction centers of formation of intermediates **A** and **B** we used known methods of reactivity assessment. Comparison of bond orders with adjacent atoms ($R_{\mu\nu}$) and bond indices (N_μ) of carbon atoms as parameters relating to the reactivity of the latter toward HO$^\cdot$ radical was performed. It is known that radicals prefer to add to atoms having the lowest N_μ [17, 18]. The bond indices obtained with the PM3 parametrization (Table 2) for flavone (**IX**) allow us to determine possible reaction centers in the molecule.

As follows from Table 2, radical attack may be directed on C^3, which is supported, first, by the fact that such processes have low activation energies [19] and, second, by the high "unsaturation" of bonds of this atom with the three adjacent atoms. Analysis of the bond indices (Table 2) shows that the free-radical substitution may involve, along with C^2 and C^3,



I **II** **III**

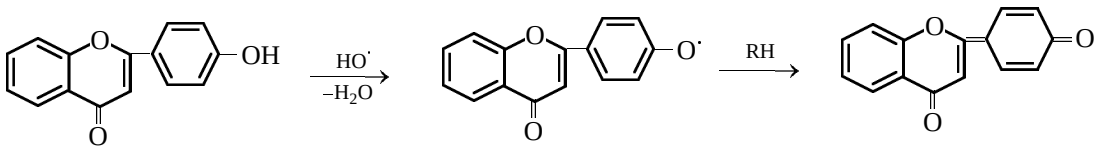
IV **V** **VI**

VII **VIII** **IX**

Table 2. Bond indices N_μ for flavones **I, V**–**VII**, and **IX**

I		**V**		**VI**		**VII**		**IX**	
position of C_ν	N_μ	position of C_ν	N_μ	position of C_ν	N_μ	position of C_ν	N_μ	position of C_ν	N_μ
6	3.647	6	3.678	6	3.679	6	3.679	3	3.706
4'	3.657	3	3.687	8	3.694	4'	3.679	8	3.770
8	3.664	8	3.693	3	3.708	8	3.694	1'	3.777
3	3.680	4'	3.720	1'	3.759	3	3.715	5	3.782
2	3.681	2'	3.722	3'	3.760	6'	3.720	6'	3.784
2'	3.711	3'	3.768	5'	3.767	2'	3.725	2'	3.786
6'	3.713	5	3.770	5	3.770	3'	3.748	4	3.801
3'	3.715	1'	3.774	4	3.776	5'	3.751	4'	3.806
5'	3.718	6'	3.774	4'	3.784	5	3.770	2	3.808
5	3.728	5'	3.775	7	3.788	4	3.777	6	3.809
7	3.744	4	3.776	2'	3.790	1'	3.786	7	3.810
1'	3.748	7	3.788	6'	3.790	7	3.788	6'	3.817
4	3.755	2	3.799	2	3.800	2	3.801	3'	3.817

Scheme 2.



the C^6 and C^8 positions of aromatic ring *A*, as well as unsubstituted positions of ring *B*. Myricetin (**I**), as the most active compound in the series in study, is characterized by a high "unsaturation" of bonds of the C^3 and C^2 atoms of the carbon skeleton.

It is known that hydroxyl radicals generated *in vitro*, for example, by the Fenton reaction, add to the benzene ring to give hydroxycyclohexadienyl radicals [5, 20]. By analogy, for flavones we can propose Scheme 3 [on an example of apigenin (**VI**)].

Adducts **C** and **D** are intermediate species. They are formed, as a rule, in the rate-limiting stage of radical aromatic substitution [20].

The calculated enthalpies of formation of radical adducts over all unsubstituted positions of the flavone nucleus in flavones and flavonols (Table 3) show that the most stable adducts are formed by the radical hydroxylation by C^3. The stabilities of radical adducts were estimated by the AM1 method, since this method proved very suitable for radical reactions (in particular, for calculation of the enthalpies of homolytic bond cleavage) [21–23].

The increased stability of the radical adducts by C^3 is probably explained by stabilizing effect of aryl ring *C* on the radical center on C^2, arising in the course of hydroxylation. Thus, not excluding the possibility of formation of adducts **C** and **D**, we can conclude, based on the data in Table 3, that C^3 is the preferred position for hydroxyl radical addition.

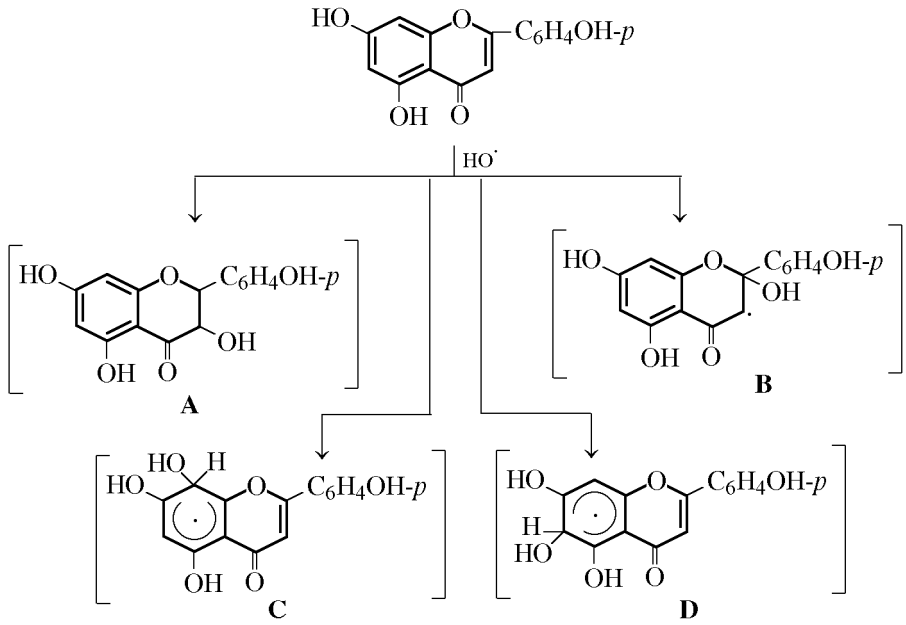
As seen from Scheme 1, the main conjugation chain in flavones includes the propenone fragment $C^2{=}C^3{-}C^4{=}O$ of the pyrone heteroring and the aryl residue (ring *B*) attached to C^2. The relation of the structure of main conjugation chain to specific types of activity has been demonstrated in a number of works on structure–biological activity relations in the flavone series [24–26].

A large body of data on the metabolism of flavones *in vivo* [27, 28] and on their chemical reactivity attests that flavones transform into the corresponding final products occurs via clevage of the $O^1{-}C^2$ and $C^3{-}C^4$ bonds in flavonols and the $O^1{-}C^2$ and $C^4{-}C^5$ bonds in flavones (Scheme 4).

Table 3. Stability of possible adducts of flavones **I**–**IX** with hydroxyl radical

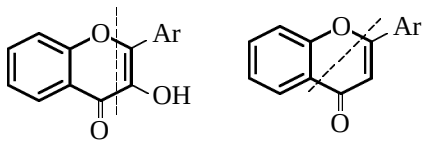
Comp. no.	Enthalpy of formation, kcal/mol				
	chromone ring *A*				ring *B*
	C^2	C^3	C^6	C^8	
I	–306.2	–309.4	–299.3	–297.8	–299.6 ($C^{2'}$)
II	–262.3	–265.2	–254.7	–253.0	–253.0 ($C^{5'}$), –256.3 ($C^{2'}$), –251.8 ($C^{6'}$)
III	–256.1	–259.1	–245.5	–244.9	–246.8 ($C^{5'}$), –250.1 ($C^{2'}$), –245.6 ($C^{6'}$)
IV	–260.2	–264.0	–247.5	–252.5	–252.4 ($C^{3'}$), –250.2 ($C^{6'}$), –254.3 ($C^{5'}$)
V	–202.1	–211.1	–206.1	–204.0	–169.9 ($C^{5'}$), –198.6 ($C^{6'}$), –173.2 ($C^{2'}$)
VI	–166.9	–172.6	–163.4	–166.6	–166.3 ($C^{2'}$), –168.0 ($C^{3'}$), –161.6 ($C^{6'}$)
VII	–230.2	–236.2	–227.0	–230.0	–231.2 ($C^{2'}$)
VIII	–215.7	–220.6	–210.4	–209.1	–211.0 ($C^{3'}$), –208.3 ($C^{2'}$), –205.6 ($C^{6'}$)
IX	–31.2	–36.8	–26.3	–28.8	–34.1 ($C^{4'}$)

Scheme 3.



Boulton *et al.* [29] suggested that metabolic transformations are induced by oxidative processes involving active oxygen forms [29]. In this context we considered it worthwhile to examine the bond orders in

Scheme 4.



the main conjugation chain including the propenone fragment $C^2{=}C^3{-}C^4{=}O$ and aromatic ring *B*.

For four of the flavones in study there is a statistically significant linear activity–bond order coorelation (A = 7854.9$P_{O^1-C^2}$ – 8232.6, R 0.97; A = 1425.6$P_{C^3-C^4}$ – 1402.2, R 0.99). Probably, the radical addition is facilitated by increased electron density in the O^1–C^2 and C^3–C^4 bonds, i.e. by stronger electron delocalization over the main conjugation chain.

In terms of structure–reactivity relations, it seems purposeful to focus on theunsubstituted flavone, since its reaction with HO· radical involves exclusively the C=C double bond of the propenone fragment. For firm location of the reaction center (C^2 or C^3) we

Table 4. Charges on carbon atoms in flavones, au

V		**VI**		**VII**		**IX**	
position of atom	charge	position of atom	charge	position of atom	charge	position of atom	charge
3	–0.310	6	–0.307	6	–0.307	3	–0.301
6	–0.307	3	–0.305	3	–0.291	8	–0.147
8	–0.249	8	–0.248	8	–0.248	6	–0.144
5'	–0.149	3'	–0.203	6'	–0.185	5'	–0.107
2'	–0.102	5'	–0.151	2'	–0.147	3'	–0.105
1'	–0.073	1'	–0.125	4'	–0.035	4'	–0.082
6	–0.064	2'	–0.024	1'	–0.014	1'	–0.074
4'	0.029	6'	–0.020	CH_3 at $C^{4'}$	0.04	2'	–0.072
CH_3 at $C^{4'}$	0.061	4'	0.123	CH_3 at $C^{3'}$	0.042	6'	–0.063
3'	0.073	2	0.172	CH_3 at $C^{5'}$	0.047	7	–0.045
2	0.170	7	0.203	5'	0.107	5	–0.008
7	0.203	5	0.267	3'	0.113	2	0.150
5	0.268	4	0.413	2	0.154	4	0.381
4	0.416			7	0.203		
				5	0.268		
				4	0.412		

compared the contributions of the p_z orbitals of the carbon atoms into the highest occupied molecular orbital (HOMO). Analysis of the reactivity from this point of view, too, is dictated by the fairly high electrophilicity of hydroxyl radical, responsible for some specific reactions of the latter [5, 6]. The p_z-AO of C^3 contributes more into HOMO than those of the other carbon atoms and twice as much as the p_z-AO of C^2. However, using the frontier electron density as a single reactivity index may lead to a mistake because of the neglect of the total electron density orientation [20].

The distribution of Mulliken atomic charges for flavones **V**–**VII** and **IX** is given in Table 4. As seen, the preferred position for electrophilic addition in the unsubstituted flavone (**IX**) is C^3.

Similar electron density distribution is also characteristic of diosmetin (**V**), whereas in apigenin (**VI**) and 5,7-dihydroxy-3',4',5'-trimethoxyflavone (**VII**) more electron density is localized on C^6. However, as mentioned above, the substitution by C^6 is less probable than by C^3 in view of the activation energies of the two reactions [5, 19].

Comparing the results of quantum-chemical calculations with the reactivities toward $HO^{\cdot}$ radical one can state that the most significant contribution in the reactivity is from the main conjugation chain. This conclusion is consistent with the conclusion of Timergazin and Khursan [21] that the propenone fragment is the main pharmacophoric group.

Table 5. Entalpies of formation of radicals like C–$O^{\cdot}$ (AM1), kcal/mol

Comp. no.	Position of hydroxy group						
	3	5	7	2'	3'	4'	5'
I	19.1	33.0	30.3		20.7	19.1	24.0
II	19.4	32.7	30.0		20.7	18.8	
III	19.4	32.8			24.0	18.6	
IV	20.4	33.8	31.1	23.9		24.2	
VIII	20.4	34.3	31.1			23.7	
V		34.7	30.6		25.7		
VI		35.2	31.1			26.0	
VII		34.7	30.6				

The involvement of hydroxy groups in binding of $HO^{\cdot}$ radical and active oxygen forms at all should also be taken into account, since there is experimental evidence for the antiradical and antioxidant activity of polyphenols [15, 25].

The reactivity of phenolic hydroxy groups was estimated by analysis of the entalpies of radical formation by hydrogen abstraction from various hydroxy groups of flavonoids (Table 5).

As seen from Table 5, the least energy consuming are the homolytic cleavages of the hydroxy groups on C^3 and $C^{4'}$. This finding suggests that the reaction by these positions is energetically most favored.

We also examined activity–OH bond order relations for the series of compounds in study. The activity of the compounds was found to increase with decreasing P_{OH} of the hydroxy groups on C^3 and $C^{4'}$, which implies serviceability of this reactivity index in analysis of radical cleavage of phenolic OH bond.

Since $HO^{\cdot}$ radicals were generated under UV irradiation [10], and the wavelength chosen for photolysis (λ 254 nm) corresponds to the short-wave absorption maximim of flavonoids, we consider it reasonable to include in reactivity analysis triplet excited states [30].

REFERENCES

1. Pincemail, J., Deby, C., and Lion, Y., *Proc. 7th Hung. Bioflavonoid Symp.*, Budapest, 1986, pp. 423–436.
2. Kuhau, J., *World Rev. Nutr. Diet.*, 1976, vol. 24, pp. 117–191.
3. Macander, P.J., in *Plant Flavonoids in Biology and Medicine*, New York: Liss, 1986, pp. 489–792.
4. Golikov, S.N., Sanotskii, I.V., and Tiunov, L.A., *Obshchie mekhanizmy toksicheskogo deistviya* (General Mechanisms of Toxic Effect), Leningrad: Meditsina, 1986, pp. 157–160.
5. Nonhebel, D., Tedder, J., and Walton, J., *Radicals*, Cambridge: Cambridge Univ. Press, 1979. Translated under the title *Radikaly*, Moscow: Mir, 1982, pp. 125, 180, 233–239.
6. Pryor, W.A., *Free Rad. Biol. Med.*, 1988, vol. 4, no. 4, pp. 219–223.
7. Das Mukul and Ray Prasanta, K., *Biochem. Int.*, 1988, vol. 17, no. 2, pp. 203–209.
8. Luk'yanchuk, V.D., Savchenkova, L.V., and Semenova, I.A., *Eksp. Klin. Farmakol.*, 1997, no. 1, pp. 62–64.
9. *Comprehensive Organic Chemistry*, Barton, D.H. and Ollis, W.D., Eds., Oxford: Pergamon, 1979, vol. 4. Translated under the title *Obshchaya organicheskaya khimiya*, Moscow: Khimiya, 1985, vol. 9.
10. Husaine, S.R., Cillard, J., and Cillard, P., *Phytochemistry*, 1987, vol. 26, no. 9, pp. 2489–2491.
11. Dewar, M.J.S., Zoebisch, E.G., and Stewart, J.J.P., *J. Am. Chem. Soc.*, 1985, vol. 107, no. 12, pp. 3902–3909.
12. Stewart, J.J.P., *J. Comput. Chem.*, 1989, vol. 10, pp. 221–223.
13. Oganesyan, E.T., Sarkisov, L.S., Ivchenko, A.V., Prokopov, A.A., and Pogrebnyak, A.V., *Khim.-Farm. Zh.*, 1996, no. 2, pp. 33–35.
14. *Uspekhi organicheskoi khimii* (Advances in Organic Chemistry), Moscow: Inostrannaya Literatura, 1963, vol. 1, pp. 144–147.
15. Slobodan, V.J., Steen, S., Yukihiko, H., and Michael, G.S., *J. Chem. Soc., Perkin Trans. 2*, 1996, no. 40, pp. 2497–2504.
16. Abramova, Zh.I. and Oksengendler, G.I., *Chelovek i protivookislitel'nye veshchestva* (Humans and Antioxidants), Leningrad: Nauka, 1985, pp. 38–39.
17. Minkin, V.I., Simkin, B.Ya., and Minyaev, R.M., *Teoriya stroeniya molekul* (Theory of Molecular Structure), Rostov-on-Don: Feniks, 1997, pp. 305, 327.
18. Pullman, B. and Pullman, A., *Quantum Biochemistry*, New York: Interscience, 1963.
19. *Biokhimicheskaya farmakologiya* (Biochemical Pharmacology), Sergeev, P.V., Ed., Moscow: Vysshaya Shkola, 1982.
20. Gorelik, M.V. and Efros, L.S., *Osnovy khimii i tekhnologii aromaticheskikh soedinenii* (Fondations of Chemistry and Engineering of Organic Compounds), Moscow: Khimiya, 1992, pp. 120, 123, 131.
21. Timergazin, K.K. and Khursan, S.L., *Izv. Ross. Akad. Nauk, Ser. Khim.*, 1996, no. 9, pp. 2190–2193.
22. Timergazin, K.K. and Khursan, S.L., *Izv. Ross. Akad. Nauk, Ser. Khim.*, 1996, no. 12, pp. 2858–2861.
23. Yamamura, T., Suzuki, K., Yamagychi, T., and Nishiyama, T., *Bull. Chem. Soc. Jpn.*, 1997, vol. 70, pp. 413–419.
24. Oganesyan, E.T., Yakovenko, V.I., Khachatryan, M.M., Pershkov, S.R., and Cherevatyi, V.S., *Khim.-Farm. Zh.*, 1986, vol. 20, no. 6, pp. 696–702.
25. *Rastitel'nye lekarstvennye sredstva* (Plant Medicinals), Maksyutina, N.P., Ed., Kiev: Zdorov'ya, 1985, pp. 99–101.
26. Tsukerman, S.V., Surov, Yu.N., and Lavrushin, V.F., *Zh. Obshch. Khim.*, 1968, vol. 38, no. 38, pp. 524–529.
27. Harborne, J.B. and Marby, T.J., *The Flavonoids: Advances in Research*, New York: Chapman and Hall, 1982, pp. 681–711.
28. Griffiths, L.A. and Smith, G.E., *J. Biochem.*, 1972, vol. 130, no. 1, pp. 141–151.
29. Boulton, D.W., Walle, U.K., and Walle, T.J., *Pharm. Pharmacol.*, 1999, vol. 51, no. 3, p. 353.
30. Jurd, L., *The Chemistry of Flavonoid Compounds*, Gessman, T.A.L., Ed., New York: Pergamon, 1962, p. 103.