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INTERRELATIONSHIPS OF THE STRUCTURES AND ANTIOXIDANT ACTIVITIES OF SOME FLAVONOIDS FROM THE PLANTS OF CENTRAL ASIA

Z. A. Khushbaktova,[a] S. M. Yusupova,[a] M. V. Zamaraeva,[b] É. T. Tadzhibaeva,[b] V. N. Syrov,[a] É. Kh. Batirov,[a] and M. P. Yuldashev[a]

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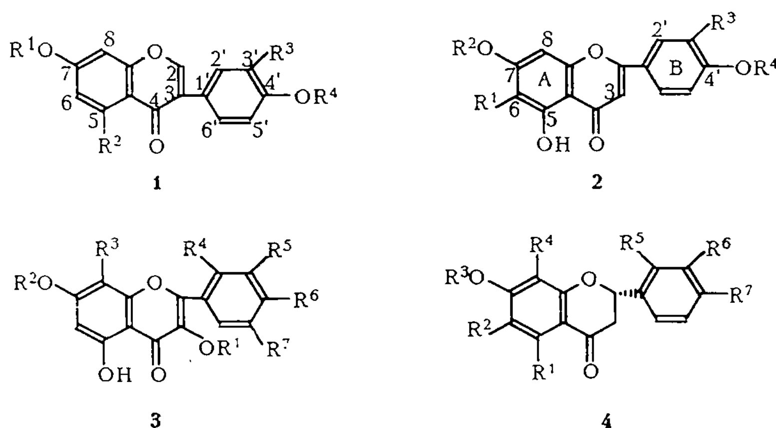
A study has been made of the influence 27 flavonoids on the processes involved in the peroxide oxidation of lipids. The interrelationship between the chemical structures of isoflavones, flavones, flavanones, and flavonols and their antioxidant activities is discussed.

Flavonoids are the most numerous class of natural phenolic compounds. The structural diversity of this class has permitted the creation from them of a number of highly effective and relatively nontoxic medicinal preparations exhibiting capillary-strengthening [1, 2], antiinflammatory [3, 4], antiallergic [5-7], hepatoprotector [8, 9], antiatherosclerotic [10], and other types of action. Since it is known that a common link in the development of many pathologies is the activation of the peroxide oxidation of lipids [11], some workers explain the broad spectrum of the biological action of phenolic compounds by the assumption that their activity is based on an antioxidant effect.

Acting as free-radical traps, flavonoids are capable of inhibiting the peroxide oxidation of lipids (POL) induced by various factors [12-14], thanks to which they are potential antioxidants. The search among flavonoids and products created from them of highly effective POL inhibitors of low toxicity with a broad application spectrum is therefore of undoubted interest.



Of the 27 flavonoids that we have studied, 5 were isoflavones, 5 were flavones, 10 were flavonols, and 7 were flavanones (scheme and Table 1). The results of the investigations showed that the majority of them powerfully inhibit the processes of iron-induced ascorbate-dependent POL. The antioxidant activities (AOAs) of these flavonoids were largely determined by their chemical structures (Fig. 1). Thus, in the isoflavone series, only orobol in a concentration of $2.5 \cdot 10^{-5}$ M caused a 67% inhibition of the formation of a product of POL — malondialdehyde (MDA) — while the other flavonoids studied exhibited a prooxidant effect under similar conditions. It is not excluded that the presence of four hydroxy groups (two

a) Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. b) Tashkent State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 350-356, May-June, 1996. Original article submitted November 21, 1994.

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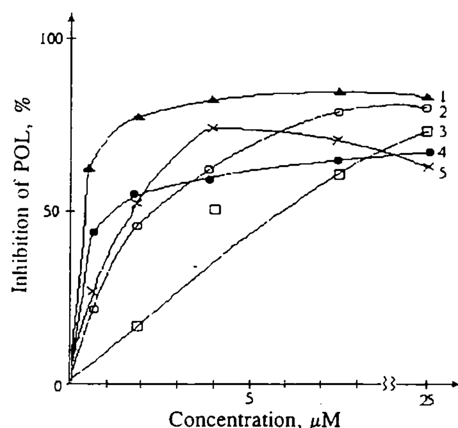


Fig. 1. Antioxidant activities of flavonoids as functions of concentration: *1*) ionol; *2*) glabrol; *3*) lehmannin; *4*) orobol; *5*) luteolin.

in the *ortho* position in ring *B*) and the absence of methoxy groups and of carbohydrate components were responsible for its manifestation of an antioxidant effect not exhibited by the other isoflavones studied.

We observed an analogous relationship in the flavone series. Luteolin, likewise containing four OH groups, and these in the same positions, exhibited an AOA almost equal to that of orobol (64%). This shows that the structure of the basic nucleus (isoflavone or flavone) exerts no substantial influence on the AOA. The glycosylation of the 7-OH group of luteolin, just like the methylation of the phenolic hydroxy group at C-3′ — with the formation of cynaroside and chrysoeriol, respectively — led to decreases in their AOAs. In the case of chrysoeriol — glycosylation of the 7-OH group with the formation of thermopsoside even led to the appearance of a prooxidant effect. The low activity of hispidulin is also probably due to the presence of a methoxy group.

The most pronounced AOAs of the flavonoids studied were shown by the flavonols. In this series of compounds, each containing from three to six free phenolic hydroxy groups, the percentage inhibition of the POL by individual flavonoids amounted to ~80%. Thus, the AOAs of quercetin (78.1%) and morin (80.6%), having in ring *B* two OH groups in the 3′,4′ (*ortho*) and 2′,4′ (*meta*) positions, respectively, and that of myricetin (83.4%), differing from the former by the presence of an additional OH group in position 5′, are comparable. However, in spite of the absence of hydroxy groups from its ring *B*, galangin (3,5,7-trihydroxyflavone) showed the highest AOA (86.3%). Moreover, methylation of the 3′-hydroxy group of quercetin with the formation of isorhamnetin was not reflected on its AOA (80.9%).

It is evident that the mutual positions of the OH groups in ring *B* exert no substantial influence on the manifestation of AOA by the compounds investigated.

The influence of a hydroxy group at C-3 (ring *C*) on the AOAs of the flavonoids was traced by comparing the activities of luteolin and quercetin. As can be seen from its structural formula, the latter differs from luteolin only by the presence of an additional 3-OH group, and this led to a considerable increase in activity over that of quercetin.

On the whole, the glycosylation of flavonols, just like that of flavones, diminished their antioxidant action, which can be well seen by taking as an example the actions of myrcetin 3-glucoside and of rutin. Myrcetin 3-glucoside, containing one sugar residue, exhibited an antioxidant effect 43% smaller than that of its aglycon, but the presence of two sugar residues in the rutin molecule led to a more than threefold fall in its AOA as compared with quercetin. A similar relationship was observed in the case of limocitrin, exhibiting a considerable inhibiting effect on POL processes (69.5%). The glycosylation of its 7-OH group (ring *A*) by an acetylated biose with the formation of haploside C even led to the appearance of a prooxidant effect. Haplogenin 7-O-glucoside, the molecule of which contains a sugar residue together with four OH groups, also exhibited a low activity (26.7%). The inclusion of a methoxy group in position 8 of isorhamnetin with the formation of limocitrin led to a fall in activity (see Table 1). These experimental facts are in harmony with those obtained previously, which showed that the polymethoxylation and glycosylation of flavonoids leads to a fall in their biological activity [11, 14].

It is obvious that the manifestation of a high AOA in this series of flavonoids requires the presence of free OH groups in positions 3, 5, and 7 (ring *A*), while OH groups in ring *B* have practically no effect on activity.

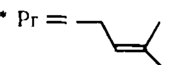
TABLE 1. Influence of the Flavonoids Investigated ($2.5 \cdot 10^{-5}$ M) on the Accumulation of MDA in a Rat Liver Homogenate and on the Hemolysis of Blood Erythrocytes

Substance	Source	Accumulation of MDA, nmole/mg of protein	Inhibition of POL, %	P	Hemolysis, %
1	2	3	4	5	6
Control	–	20.19	–		
Isoflavones 1					
Orobol $R^1=R^4=H$, $R^2=R^3=OH$	Thermopsis dolichocarpa V.Nikitin [16]	6.64±0.285	67.11	<0.001	4.94
Formononetin $R^1=R^2=R^3=H$, $R^4=CH_3$	Cicer mogoltavicum A.Korov [17]	25.0±0.386	+23.82	<0.01	
Genistin $R^1=\beta$-*D*-Glc, $R^2=OH$, $R^3=R^4=H$	Thermopsis alterniflora Rgl. et Schmalh [18]	23.27±0.194	+15.26	<0.02	
Ononin $R^1=\beta$-*D*-Glc, $R^2=R^3=H$, $R^4=CH_3$	Cicer mogoltavicum A.Korov [17]	24.80±0.188	+22.85	<.0.02	
Biochanin A $R^1=R^3=H$, $R^2=OH$, $R^4=CH_3$	Cicer mogoltavicum A.Korov [17]	22.18±1.734	+9.85	<0.5	
Flavones 2					
Luteolin $R^1=R^2=R^4=H$, $R^3=OH$	Ferula varia (Shrenk) Trautv. [19]	7.32±1.155	63.74	<0.001	2.63
Chrysoeriol $R^1=R^2=R^4=H$, $R^3=OCH_3$	Thermopsis alterniflora Rgl. et Schmalh [18]	18.07±0.375	10.50	<0.1	
Cynaroside $R^1=R^4=H$, $R^2=\beta$-*D*-Glc, $R^3=OH$	Ferula varia (Shrenk) Trautv. [19]	12.43±1.450	38.44	<0.02	
Thermopsoside $R^1=R^4=H$, $R^2=\beta$-*D*-Glc, $R^3=OCH_3$	Thermopsis alterniflora Rgl. et Schmalh [18]	21.66±0.940	+7.28	>0.5	
Hispidulin $R^1=OCH_3$, $R^2=R^3=R^4=H$	Saussurea elegans Ledeb. [20]	21.03±1.720	4.16	>0.5	
Flavonols 3					
Galangin $R^1=R^2=R^3=R^4=R^5=R^6=R^7=H$	Glycyrrhiza glabra L. [24]	2.76±1.01	86.33	<0.001	4.25
Quercetin $R^1=R^2=R^3=R^4=R^7=H$, $R^5=R^6=OH$	Obtained by hydrolysis from rutin [21]	4.42±0.130	78.11	<0.001	18.45
Rutin $R^1=\beta$-*D*-Glc-α-*L*-Rha, $R^2=R^3=R^4=R^7=H$, $R^5=R^6=OH$	Lagonychium farctum (Banks et Soland) Bobr [21]	15.32±0.315	24.15	<0.01	
Morin $R^1=R^2=R^3=R^5=R^7=H$, $R^4=R^6=OH$	Synthetic compound [22]	3.91±0.950	80.63	<0.001	8.24
Myricetin $R^1=R^2=R^3=R^4=H$, $R^5=R^6=R^7=OH$	Lagonychium farctum (Banks et Soland) Bobr. [21]	3.28±1.050	83.46	<0.001	
Myricetin 3-glucoside $R^1=\beta$-*D*-Glc, $R^2=R^3=R^4=H$, $R^5=R^6=R^7=OH$	Lagonychium farctum (Banks et Soland) Bobr.[21]	12.05±1.575	40.30	<0.001	12.36
Isorhamnetin $R^1=R^2=R^3=R^4=R^7=H$, $R^5=OCH_3$, $R^6=OH$	Obtained by the hydrolysis of isorhamnetin 3-O-glucoside [24]	3.85±1.150	80.93	<0.001	2.47
Limocitrin $R^1=R^2=R^4=R^7=H$, $R^3=R^5=OCH_3$, $R^6=OH$	Haplophyllum perforatum (M.B.) Kar. et Kir. [25]	6.15±0.380	69.54	<0.001	2.97
Haplogenin 7-glucoside $R^1=R^4=R^7=H$, $R^2=\beta$-*D*-Glc, $R^3=R^6=OH$, $R^5=OCH_3$	Haplophyllum perforatum (M.B.) Kar. et Kir. [25]	14.79±2.290	26.75	<01	

TABLE 1 (continued)

Substance	Source	Accumulation of MDA, nmole/mg of protein	Inhibition of POL, %	P	Hemolysis, %
1	2	3	4	5	6
Haploside C $R^1=R^4=R^7=H$, $R^2=\beta$-*D*-(6-OAc)-Glc-α-*L*-Rha, $R^3=R^5=OCH_3$, $R^6=OH$	Haplophyllum perforatum (M.B.) Kar. et Kir. [26]	21.15+0.760	+4.75	>0.5	
Flavanones 4					
Pinocembrin $R^1=OH$, $R^2=R^3=R^4=R^5=R^6=R^7=H$	Glycyrrhiza glabra L. [24]	19.91+0.370	1.39	>0.5	
Glabranin $R^1=OH$, $R^2=R^3=R^5=R^6=R^7=H$, $R^4=Pr^*$	Glycyrrhiza glabra L. [24]	20.38+0.52	0.94	<0.1	
Isoglabranin $R^1=OH$, $R^3=R^4=R^5=R^6=R^7=H$, $R^2=Pr^*$	Glycyrrhiza glabra L. [24]	17.88+0.57	11.44	<0.1	
Isobavachin $R^1=R^2=R^3=R^5=R^6=H$, $R^4=Pr^*$, $R^7=OH$	Vexibia alopecuroides (L.) Yakovl. [27]	19.62+0.390	2.82	>0.5	
Glabrol $R^1=R^2=R^3=R^5=H$, $R^4=R^6=Pr^*$, $R^7=OH$	Vexibia alopecuroides (L.) Yakovl. [27]	3.87+1.760	80.83	<0.001	7.743
Vexibinol $R_1=R_5=R_7=OH$, $R_2=R_3=R_6=H$, $R_4=Lav^{**}$	Vexibia alopecuroides (L.) Yakovl. [27]	13.37+0.50	33.80	<0.001	
Lehmannin $R_1=R_2=R_3=R_6=H$, $R_4=Lav^{**}$, $R_5=R_7=OH$	Ammothamnus lehmannii Bunge [28]	5.35+1.590	73.49	<0.001	14.83

* Pr =



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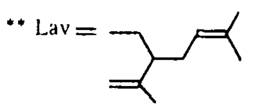

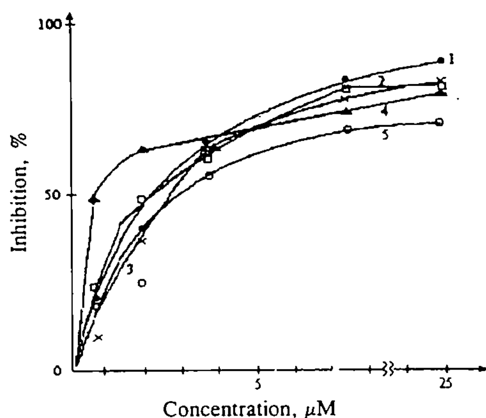

Fig 2. Concentration dependence of the antioxidant activity of flavonols: *1*) galangin; *2*) isorhamnetin; *3*) morin; *4*) quercetin; *5*) limocitrin.

Investigation of the compounds of the flavanone group showed that individual representatives of this series possess high AOAs. Thus, lehmannin — a 5-deoxyflavanone — inhibited POL by 73.49%, while vexibinol, containing an additional 5-OH group, showed a decrease (33.3%) in AOA. Isobavachin, which is also a 5-deoxyflavavone and contains a prenyl group in ring *A*, exhibited a low activity. However, the introduction of a second prenyl group into its molecule (ring *B*) with the formation of glabrol led to a fivefold increase in activity. No analogous relationship was observed in the 5,7-dihydroxyflavanone series: compounds with prenyl groups (glabranin, isoglabranin, isobavachin) showed a low activity. Apparently, in the manifestation of AOA by 5-deoxyflavanones, which, as is known, are less stable compounds than 5-hydroxyflavanones [15], a definite role is played by the number and positions of prenyl groups.

The membranotropic activities and the concentration dependence of the antioxidant effects of the ten flavonoids exhibiting the most pronounced inhibiting action on POL were studied.

The results of the investigation of membranotropic activities, performed on erythrocytes in *in vitro* experiments, showed that in the concentrations exhibiting the highest AOAs they caused no appreciable hemolytic effect (see Table 1), which indirectly characterizes them as nontoxic.

The concentration of these compounds affected their AOAs only in the range of 0.5-7.5 μM (Figs. 1 and 2). A further increase in concentration did not lead to an enhancement of the inhibition of POL, which reached values of not more than 80%, depending on the compound. The absence of 100% inhibition of the POL process with an increase in the concentration of the compounds is apparently a result of both the antioxidant and the prooxidant action of the flavonoids, since phenolic antioxidants not only disrupt the the kinetic chains of free-radical oxidation but also initiate the oxidation of the substrate [14].

Analysis of the results obtained showed a definite dependence of the AOAs of the compounds on their content of carbohydrate components and of methoxy and prenyl groups and on the presence, number, and positions of OH groups. However no clear dependence of the AOA on membership of a particular group of flavonoids was detected, this apparently being connected with the formation of reactive phenoxyl radicals [14], which makes its revelation difficult.

EXPERIMENTAL

The flavonoid compounds that we studied were isolated by column chromatography or by acid hydrolysis from the plants given in Table 1, which grow in the territory of Central Asia. We have described the sources of these substances and the methods of isolation in detail previously [16-28].

Antioxidant and hemolytic activities were determined under *in vitro* conditions on a liver homogenate and on the blood of random-bred white rats.

The influence of the flavonoids investigated on the accumulation of MDA was evaluated under the conditions of iron-induced ascorbate-dependent POL in a rat liver homogenate. Peroxide oxidation of the lipids was induced with 10 μM $FeSO_4$

in the presence of 200 μM ascorbate in a medium containing 145 mM KCl, 25 mM Tris-HCl, pH 7.4. The accumulation of POL products was determined by the color reaction with thiobarbituric acid [29]. The hemolytic activities of the most active flavonoids were determined on rat blood erythrocytes (2% suspension). The optical densities of the supernatants (absorption maximum of hemoglobin) obtained after the centrifugation of the erythrocytes that had been incubated with the compounds concerned at 37°C for 30 min were determined on a spectrophotometer at 540 nm, and then the percentage hemolysis of the erythrocytes was calculated.

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