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CHEMICAL MODIFICATION OF THE NATURAL FLAVONOID MYRICETIN

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A review is presented of our own results on the synthesis of nitro-, amino-, azo-, and bromo- derivatives of myricetin, as a result which 19 new derivatives have been obtained. The compounds synthesized are of interest as substances with potential antitumoral, antimicrobial, antifungal, antioxidant, and antitubercular activities.

Flavonoids, as the broadest group of phenolic compounds and important components of the vegetable organism, take an active part in oxidative—reductive processes, the development of immunity, and the protection of plants from unfavorable effects of ultraviolet rays and low temperatures. On the human and animal organisms the majority of them exhibit a capillary-strengthening action and lower the permeability of hematoparenchymatous barriers. This action lies at the basis of the pharmacological, prophylactic, and therapeutic effects of these compounds [1—8].

Features of the structure of flavonoid molecules are determined the variety of their hydroxylation, methylation, methoxylation, and O- and C-glycosylation of the aromatic nuclei *A* and *B*, and also acylation.

In our view, one of the natural flavonoids available in practice is myricetin (**1**). As natural sources of myricetin we have proposed the roots of the sea-lavender *Limonium salsa* (Willd.) and the oil of the buds of the balsam poplar (*Populus balsamifera*). The yield of myricetin from the sea-lavender roots amounts to 2.3%, calculated on the air-dry raw material.

In view of the possibility of using myricetin as a renewable chemical material and the features of its molecule we have subjected (**1**) to electrophilic and nucleophilic exchange reactions, condensations, and the opening of the pyrone ring. In this way we have obtained nitro-, amino-, and bromo- derivatives of myricetin and, using these as intermediates have synthesized hydrazones (Schemes 1 and 2).

We have studied the possibility of using (**1**) in the synthesis of azo compounds, the diazo components of these being sulfonamide reagents with given bacterial properties that are used in the treatment of infectious diseases (Scheme 3).

The structures of the compounds obtained have been confirmed by elementary analysis, qualitative reactions, and spectral characteristics (IR, UV, NMR).

When an alcoholic solution of myricetin was heated in ammonia, the pyrone ring opened with the formation of 3,3′,4′,5,5′,7-hexahydroxy-α-hydroxychalcone (**2**) and 1-(2,4,6-trihydroxyphenyl)-3-(3,4,5-trihydroxyphenyl)propane-1,2-dione (**3**) with a total yield of 86% (see Scheme 1).

The UV spectrum of derivative (**2**) contained the absorption maxima that are characteristic for chalcones. The IR spectra of the derivatives obtained by the opening of the pyrone ring show characteristic intense absorption bands of functional groups confirming the formation of two isomeric products present in keto-enol tautomerism. The presence of an absorption band in the 1660 cm^{-1} region is characteristic for the carbonyl group of the enol form, and one at 1720 cm^{-1} for the C=O groups of α-diketones. Broadened absorption bands at 3400 and 3200 cm^{-1} corresponded to the hydroxy groups of the enolic form (**2**).

The action of *o*-phenylendiamine on 3,3′,4′,5,5′,7-hexahydroxy-α-hydroxychalcone (**2**) formed 2-(3,4,5-trihydroxybenzyl)-3-(2,4,6-trihydroxyphenyl)quinoxaline (**4**) with a yield of 27%.

The nitration of 3,3′,4′,5,5′,7-hexahydroxyflavone with nitric acid in glacial acetic acid gave a 77% yield of 3,3′,4′,5,5′,7-hexahydroxy-2′,6,6′,8-tetranitroflavone (**5**).

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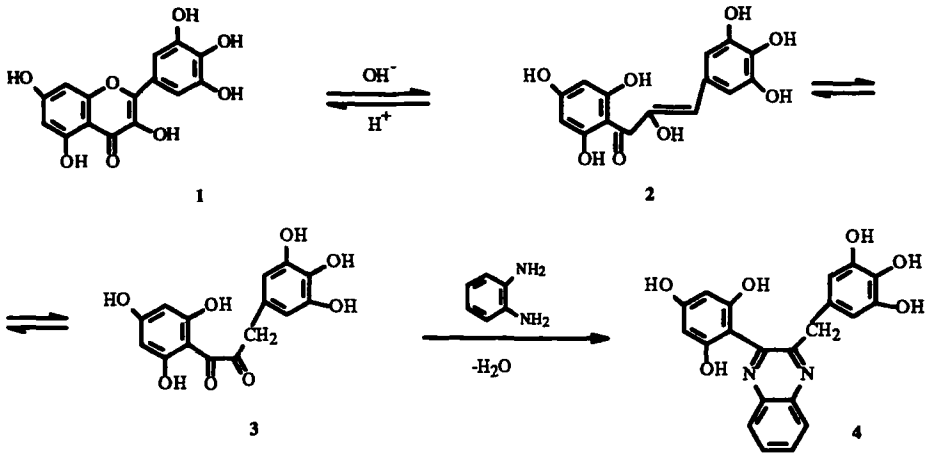


Scheme 1. Production of a chalcone and a quinoxaline from myricetin.

The reduction of 2′,6,6′,8-tetranitromyricetin (**5**) was conducted with tin in methanolic hydrochloric acid. The hydrochloride of 2′,6,6′,8-tetraamino-3,3′,4′,5,5′,7-hexahydroxyflavone (**6**) was formed with a yield of 77%.

In the IR spectrum of substance (**6**) there were absorption bands in the regions of 1640 and 1600 cm^{-1} characterizing the presence of C=O and C=C groups. The absence of the absorption at 1320—1380 cm^{-1} that is characteristic for NO_2 groups witnessed the reduction of all the nitro groups in compound (**5**).

Within the project of synthesizing compounds possessing pronounced antitubercular, antimicrobial, and antiphlogistic activities, we have studied the condensation of myricetin and its derivatives with phenylhydrazine and dinitrophenylhydrazine.

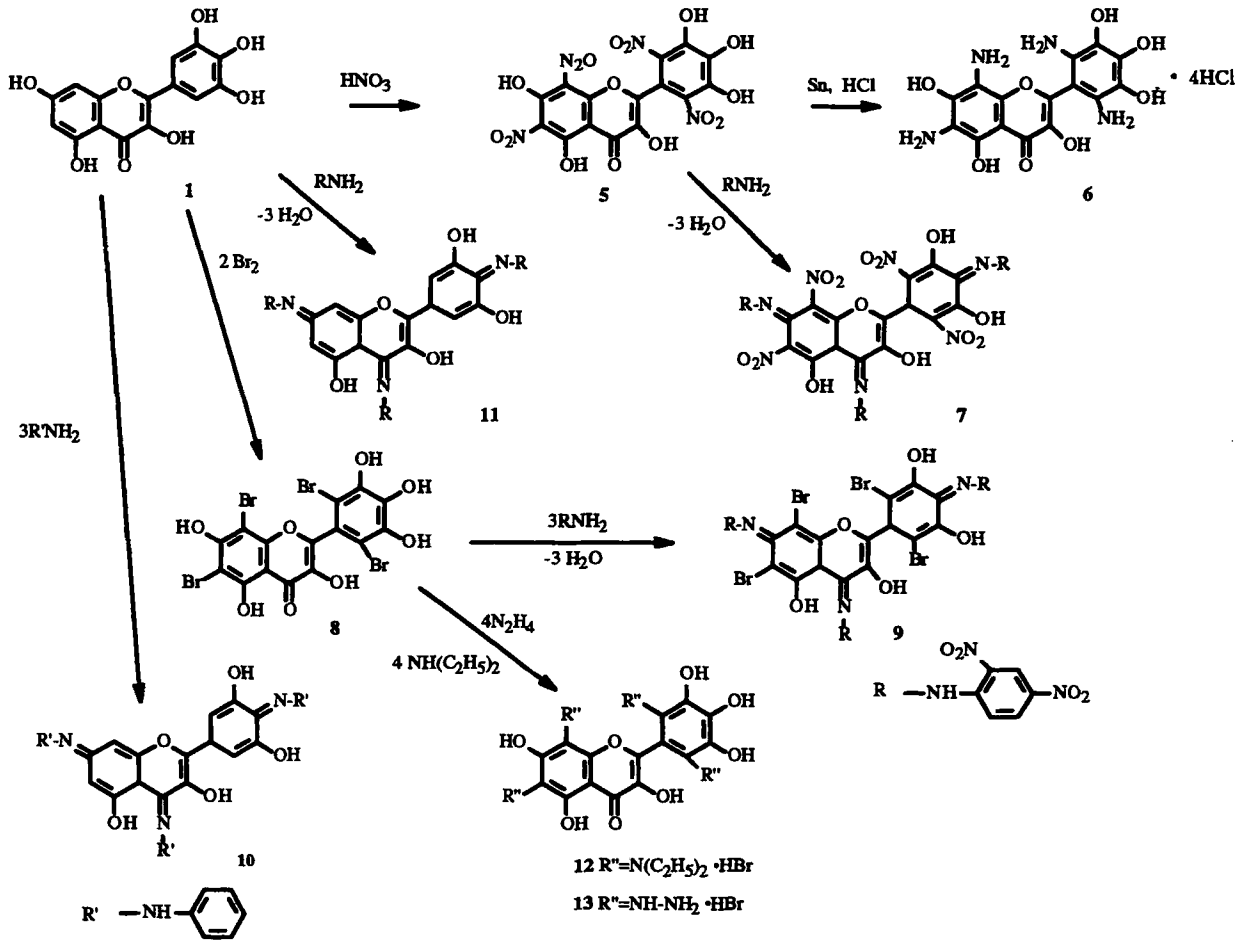
The condensation of 2′,6,6′,8-tetranitromyricetin and dinitrophenylhydrazine in glacial acetic acid led to 4,4′,7-tris(dinitrophenylhydrazono)-3,3′,5,5′-tetrahydroxy-2′,6,6′,8-tetranitroflavone (**7**) with a yield of 40%. Absorption bands were observed in the UV spectrum at 257 and 332 nm. In the IR spectrum there were absorption bands at 1680 and 1580 cm^{-1}, characterizing the presence of C=N and C=C bonds, respectively, and that of a nitro group at 1320 cm^{-1}, while there was no absorption band characteristic for a C=O group.

Bromination of myricetin was carried out with bromine in glacial acetic acid at room temperature. The tetrabromo derivative (**8**) was obtained with a yield of 75%. The IR spectrum showed an absorption band at 1620 cm^{-1}, corresponding to a C=O bond, and one at 662 cm^{-1}, showing the presence of a bromine atom. The PMR spectrum lacked signals of aromatic protons in the 5—9 ppm region, but broadened signals of hydroxy groups were observed at 9.3, 10.9, and 12.7 ppm.

The condensation of 2′,6,6′,8-tetrabromo-3,3′,4′,5,5′,7-hexahydroxyflavone (**8**) with 2,4-dinitrophenylhydrazone led to 3,3′,5,5′-tetrabromo-4,4′,7-tris(dinitrophenylhydrazono)-2′,6,6′,8-tetranitroflavone (**9**) with a yield of 80%. In the UV spectrum of (**9**) there were absorption bands at 258 and 334 nm. In the IR spectrum there were absorption bands at 1580, 1530, and 1330 cm^{-1}, characterizing the presence of C=C bonds and nitro groups, the band of a bromine atom at 590 cm^{-1}, and also that of C=N bonds at 1670 cm^{-1}. The PMR spectrum showed signals of aromatic protons in the 8—9 ppm region and broadened signals of the protons of hydroxy groups at 12.8 and 9.3 ppm.

The condensation of myricetin with phenylhydrazine led to the formation of 4,4′,7-tri(phenylhydrazono)-3,3′,5,5′-tetrahydroxyflavone (**10**) with a yield of 93% (see Scheme 2). In the IR spectrum of the derivative obtained there were absorption bands at 3150 and 1650 cm^{-1}, characterizing the presence of OH groups and of C=N bonds, respectively. 4,4′,7-Tris(dinitrophenylhydrazono)-3,3′,5,5′-tetrahydroxyflavone (**11**) was obtained under analogous conditions with a yield of 40%.

The introduction of amino groups into the structure imparts to the compound new properties, the main one of which is solubility in water. In view of this we performed the aminoethylation of tetrabromomyricetin, as a result of which compound (**12**) was obtained with a yield of 60%. In the UV spectrum of the hydrobromide of 2′,6,6′,8-tetrakis(diethylamino)-3,3′,4′,5,5′,7-hexahydroxyflavone (**12**) there were absorption bands at 272 and 365 nm. The IR spectrum showed absorption bands at 3000, 1650, and 1605 cm^{-1}, characterizing the presence of OH, C=O, and C=C bonds, respectively. The hydrobromide of 2′,6,6′,8-tetrahydrazino-3,3′,4′,5,5′,7-hexahydroxyflavone (**13**) was obtained with a yield of 72% by the interaction of tetrabromomyricetin and hydrazine hydrate. In the UV spectrum of (**13**) two absorption bands were observed at 258 and 358 nm. The IR spectrum contained characteristic absorption bands at 3200 cm^{-1} (OH) and 1640 cm^{-1} (C=O).



Scheme 2. Chemical transformation of myricetin.

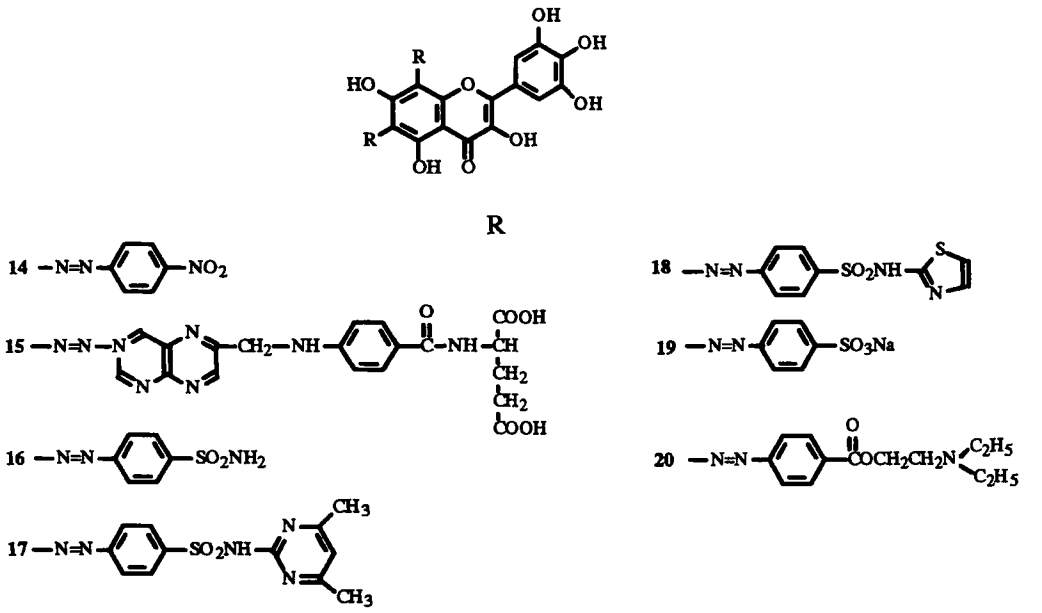
The presence of free hydroxy groups in the myricetin molecule enabled various nitrogen-containing derivatives to be obtained with retention of the system of double bonds and all the hydroxyls. By means of the azo coupling reaction we obtained a series of nitrogen derivatives of myricetin possessing enhanced bactericidal activity. As reagents for obtaining nitrogen derivatives of myricetin we used a number of sulfonamide compounds — streptocide (sulfanilamide), norsulfazole (sulfathiazole), and sulfadimezin (sulfamethazine) — and also novocaine (procaine hydrochloride), folic acid, and *p*-nitroaniline and sulfanilic acid, which possess bactericidal properties and are used in the treatment of infectious diseases. These substances were converted into diazonium salts and coupled with myricetin (see Scheme 3).

The interaction of myricetin with freshly prepared diazonium salts of sulfonamide derivatives was carried out in ethanol at 25°C. The following azo coupling products were obtained: 3,3′,4′,5,5′,7-hexahydroxy-6,8-bis(*p*-nitrobenzeneazo)flavone (**14**), the product of the coupling of myricetin with folic acid (**15**), 6,8-bis(*p*-sulfamoylbenzeneazo)-3,3′,4′,5,5′,7-hexahydroxyflavone (**16**), 6,8-bis[*p*-(4,6-dimethylpyrimidinylsulfamoyl)benzeneazo]-3,3′,4′,5,5′,7-hexahydroxyflavone (**17**), 6,8-bis[*p*-(1,3-thiazol-2-ylsulfamoyl)benzeneazo]-3,3′,4′,5,5′,7-hexahydroxyflavone (**18**), the sodium salt of 6,8-bis(*p*-sulfobenzeneazo)-3,3′,4′,5,5′,7-hexahydroxyflavone (**19**), and the diethylaminoethyl ester of 6,8-bis(*p*-carboxybenzeneazo)-3,3′,4′,5,5′,7-hexahydroxyflavone (**20**).

In the PMR spectra of compounds (**14—20**) there were signals characteristic for aromatic protons in the 5—9 ppm region and for the protons of hydroxy groups in the 9—13 ppm region. Thus, for the sodium salt of 6,8-bis(*p*-sulfobenzeneazo)-3,3′,4′,5,5′,7-hexahydroxyflavone (**19**) we observed signals in the regions of 7.3 ppm (2H, s, H-2′ and H-6′) and 9.3 and 10.9 ppm (OH). In the PMR spectrum of 6,8-bis(*p*-nitrobenzeneazo)-3,3′,4′,5,5′,7-hexahydroxyflavone (**14**) we observed signals at 7.3 ppm (H-2′ and H-6′) and 7.7-8 ppm (aromatic protons) and broadened signals at 9.3, 10.9 and 12.7 ppm, corresponding to the protons of hydroxy groups.

TABLE 1. Characteristics of the UV Spectra of the Azo Derivatives of Myricetin (14) and (16)

Solutions and additives	Bands	Myricetin (1)		6,8-Bis(*p*-nitrobenzeneazo) myricetin (14)		6,8-bis(*p*-sulfamoylbenzeneazo) myricetin (16)	
		λ_{max}	$\Delta\lambda$	λ	$\Delta\lambda$	λ	$\Delta\lambda$
$2\cdot10^{-5}$ M solution in	I	380		395		365	
abs. methanol	II	255		290		253	
Sodium acetate	I	408	28	405	10		
	II	260	5	293	3		
Sodium ethanolate	I	310	-70	355	-40	338	-27
	II	255	0	290	0	244	9
Sodium acetate +	I	405	25	415	20	380	15
boric acid	II	257	2	292	2	256	3
zirconyl oxychloride	I	475	95	485	90		
	II	269	5	297	7		



Scheme 3. Products of the azo coupling of myricetin with diazonium salts of some amines.

The results obtained on recording UV spectra with ionizing and complex-forming additives showed the presence of a flavonoid structure and of free hydroxy groups. Myricetin and its derivatives gave a stable coloration with zirconium oxychloride that did not disappear on the addition of citric acid, which showed the presence of an OH group in the C-3 position (Table 1). On the addition of sodium acetate a shift by 10—15 nm in the short-wave direction was observed, which showed the presence of an OH group at C-7.

In addition, the 3′,4′,5′-trihydroxy groups in ring *B* formed a complex with boric acid which on ionization with sodium acetate showed a bathochromic shift by 22—25 nm of the maximum of band II, while the observation of two maxima in the UV spectrum showed the presence of an *ortho*-dihydroxy grouping in ring *B*.

Thus, as a result of the chemical modification of myricetin, 19 of its derivatives having various heteroatoms — nitrogen, bromine, and sulfur — in their compositions have been synthesized. The substances obtained are of interest as compounds with high biological activities — antioxidant, antitumoral, antimicrobial, antitubercular, and antifungal.

EXPERIMENTAL

Melting points were determined on a Kofler stage, IR spectra were taken in KBr on a UR-20 instrument, and UV spectra on a Specord UV-vis. NMR spectra were recorded on Varian 300 (300 MHz) and Tesla BS 567 A (60 MHz) instruments with the solvent DMSO-d_6, 0 — TMS, δ-scale. The chromatographic purification of the compounds obtained was performed on KSK silica gel. The course of the reactions and the purity of the substances obtained were monitored by thin-layer chromatography on Silufol UV-254 plates. The plates were developed with ferric ammonium alum or were viewed in UV light.

3,3′,4′,5,5′,7-Hexahydroxy-α-hydroxychalcone (2). With stirring, 15 ml of ammonia was added dropwise to a solution of 2 g of myricetin (1) in 30 ml of ethyl alcohol. Then the reaction mixture was heated at 60°C for 1.5 h, cooled to room temperature, and evaporated under vacuum. The residue was treated with ether (2 × 25 ml). The crystals that had deposited were washed with acetone and dried in the air. This gave 1.72 g of a crystalline substance with mp 138—139°C. Yield 86%.

Elementary analysis. Found, %: C 56.9; H 3.57; $C_{15}H_{12}O_8$. Calculated, %: C 56.26; H 3.75.

Paper chromatography: R_f 0.43 (BAW, 4:1:5), 0.08 (15% AcOH).

Thin-layer chromatography on Silufol: R_f 0.7 (ethyl acetate—petroleum ether, 3:1).

UV spectrum (EtOH, λ, nm): 253, 374.

IR spectrum (KBr, ν, cm^{-1}): 850, 1570, and 1610 (C=C); 1660 (C=O); 3200, 3400 (OH).

PMR spectrum (DMSO-d_6, δ, ppm, 300 MHz): 6.1 s (H-5), 6.25 s (H-3′), 6.83 br.s (2H, H-2 and H-6).

2-(3,4,5-Trihydroxybenzyl)-3-(2,4,6-trihydroxyphenyl)quinoxaline (4). Dropwise, 0.5 g of *o*-phenylenediamine in 15 ml of alcohol was added to 1 g of 3,3′,4′,5,5′,7-hexahydroxy-α-hydroxychalcone (2) in 30 ml of alcohol, and the mixture was heated at 50°C with stirring for 2 h. After cooling, the precipitate that had deposited was filtered off and recrystallized from alcohol. This gave 400 mg of substance (4) with mp 210—211°C. Yield 26.7%.

Elementary analysis. Found, %: C 65.32; H 4.08; N 3.83; $C_{21}H_{16}N_2O_6$. Calculated, %: C 64.28; H 4.54; N 3.57.

3,3′,4′,5,5′,7-Hexahydroxy-2′,6,6′,8-tetranitroflavone (5). Dropwise, 2.5 ml (0.54 mole) of nitric acid (d = 1.37) in 10 ml of glacial acetic acid was added to a solution of 5 g (0.015 mole) of myricetin in 20 ml of glacial acetic acid. The reaction mixture was stirred at room temperature for 3 h and was then evaporated under vacuum and the residue was extracted with ether. The ethereal solution was evaporated, and the residue was dissolved in ethyl acetate and precipitated with petroleum ether. The precipitate was filtered off and dried. This gave 6.3 g of a yellow crystalline substance with mp 150—151°C. Yield of (5) 77%.

Elementary analysis. Found, %: C 35.67; H 1.21; N 10.87; $C_{15}H_6N_4O_{16}$. Calculated, %: C 36.14; H 1.24; N 11.24.

Paper chromatography: R_f 0.71 (15% CH_3COOH; 0.51 (BAW, 1:4:5).

UV spectrum (EtOH, λ, nm): 270, 382.

IR spectrum (KBr, ν, cm^{-1}): 1580, 1640 (C=C); 1710 (C=O); 1320, 1530 (NO_2); 820, 3250, 3430 (OH).

PMR spectrum. No signals of aromatic protons in the 5—9 ppm region.

Hydrochloride of 2′,6,6′,8-Tetramino-3,3′,4′,5,5′,7-hexahydroxyflavone (6). A solution of 0.1 g (0.0003 mole) of tetranitromyricetin in 3 ml of methanol was treated with 0.44 g of tin, 2.7 ml of hydrochloric acid (d = 1.17) was added dropwise, and the mixture was stirred at 50—60°C for 5 h. After the end of the reaction, the solution was filtered from the unchanged tin, the mother solution was evaporated to dryness, and the residue was extracted with ether to eliminate the starting material and products of its partial reduction.

A light yellow crystalline substance with mp 196—198°C was obtained. Yield 77%.

Its qualitative composition was investigated by the chromatographic method on paper and on Silufol plates in various solvent systems: butanol—acetic acid—water (4:1:5) — R_f 0.60, acetic acid 15% — R_f 0.58, revealed in the form of a dark yellow spot. A Beilstein test was positive.

Elementary analysis. Found, %: C 33.12; H 2.87; N 11.02; Cl 25.98; $C_{15}H_{18}N_4Cl_4O_8$. Calculated, %: C 24.45; H 3.14; N 10.68; Cl 26.72.

IR spectrum (KBr, ν, cm^{-1}): 1030—1230, 1450, 1640 (C=O); 1600 (C=C); 2400—2800.

4,4′,7-Tris(dinitrophenylhydrazono)-3,3′,5,5′-tetrahydroxy-2′,6,6′,8-tetranitroflavone (7). A solution of 0.5 g (0.771 mole) of the nitro derivative of myricetin in 2 ml of ethanol was treated with 0.6 g (0.003 mole) of dinitrophenylhydrazine dissolved in 120 ml of glacial acetic acid. After being stirred at 100°C for 5 h, the reaction mixture was evaporated under vacuum, and the precipitate that deposited was recrystallized from ethyl alcohol. This gave the

crystalline substance (7), with decomp. p. 181—183°C. Yield 40%, Paper chromatography: R_f 0.86 (BAW, 4:1:5), 0.77 (15% AcOH).

Elementary analysis. Found, %: C 44.21; H 2.18; N 19.21; $C_{33}H_{20}N_{12}O_{20}$. Calculated, %: C 43.80; H 2.21; N 18.58.

UV spectrum (EtOH, λ,nm): 330, 260.

NMR spectrum (DMSO-d_6, δ, ppm, 300 MHz): broad signals at (ppm) 9.3 and 13 (OH) and 7.3 (H-2′ and H-6′).

2′,6,6′,8-Tetrabromo-3,3′,4′,5,5′,7-hexahydroxyflavone (8). A solution of 2 g (0.006 mole) of myricetin in 30 ml of glacial acetic acid was treated dropwise with 1.6 ml (0.03 mole) of bromine in 30 ml of glacial acetic acid. The reaction mixture was stirred at room temperature for 3 h and was then evaporated under vacuum. The residue was washed with distilled water to eliminate traces of acid and bromine. It was then filtered off and recrystallized from 20% alcohol. This gave 3 g of yellow acicular crystals with mp 225—226°C. The yield of 2′,6,6′,8-tetrabromo-3,3′,4′,5,5′,7-hexahydroxyflavone (8) was 75%.

Elementary analysis. Found, %: C 28.93; H 0.85; Br 48.31; $C_{15}H_6O_8Br_4$. Calculated, %: C 28.34; H 0.95; Br 50.43.

Paper chromatography: R_f 0.41 (15% AcOH, Silufol), 0.72 (methanol—AcOH—H_2O, 90:5:5), 0.82 (ethyl acetate—THF—H_2O, 1:5:5), 0.68 (benzene—ethyl acetate—formic acid—water, 9:21:6:5). On all the chromatograms the substance appeared in the form of a single spot.

UV spectrum (EtOH, λ, nm): 220, 273.

IR spectrum (KBr, ν, cm^{-1}): 850, 1580 (C=C); 1620 (C=O); 662 (Br); 3580, 3420 (OH).

PMR spectrum (DMSO-d_6, δ, TMS, 60 MHz): no signals of aromatic protons in the 5—9 ppm region; 9.3, 10.9 and 12.7 ppm, br. signals (OH).

3,3′,5,5′-Tetrabromo-4,4′,7-tris(dinitrophenylhydrazono)-2′,6,6′,8-tetrahydroxyflavone (9). With stirring, a solution of 4.7 g (0.024 mole) of 2,4-dinitrophenylhydrazine in 200 ml of glacial acetic acid was added over 30 min to a solution of 5 g (0.008 mole) of 2′,6,6′,8-tetrabromo-3,3′,4′,5,5′,7-hexahydroxyflavone in 50 ml of ethanol. The reaction mixture was heated at 100°C for 5 h and was then evaporated under vacuum. The crystals that deposited on cooling were filtered off, washed with a small amount of water, and recrystallized from ethanol. This gave 7.4 g of a yellow crystalline substance with mp 190—192°C. The yield of 3,3′,5,5′-tetrabromo-4,4′,7-tris(dinitrophenylhydrazono)-2′,6,6′,8-tetrahydroxyflavone was 80.1%.

Elementary analysis. Found, %: C 34.21; H 1.07; N 14.1; Br 26.53; $C_{33}H_{16}O_{17}N_{12}Br_4$. Calculated, %: C 33.92; H 1.15; N 14.18; Br 26.55.

UV spectrum (EtOH, λ,nm): 258, 334.

IR spectrum (KBr, ν, cm^{-1}): 860, 1580 (C=C); 1330, 1530 (NO_2); 590 (C-Br); 1670 (C=N); 1440, 3360 (N—H); 3200 (OH).

NMR spectrum (DMSO-d_6, δ, ppm, 60 MHz); 8—9 (arom. H); 12.8, 9.3 br. s (OH).

4,4′,7-Tri(phenylhydrazono)-3,3′,5,5′-tetrahydroxyflavone (10). To a solution of 1 g (0.0031 mole) of myricetin in 80 ml of glacial acetic acid was added 3 g (0.0186 mole) of phenylhydrazine acetate in ethyl alcohol. The reaction was conducted at 95—90°C for 5 h. After cooling, the precipitate that had deposited was recrystallized from alcohol, giving compound (10) with mp 110—112°C. Yield 93%.

Elementary analysis. Found, %: C 68.33; H 4.36; N 13.97; $C_{33}H_{26}N_6O_5$. Calculated, %: C 67.58; H 4.44; N 14.33.

IR spectrum (KBr, ν, cm^{-1}): 3150 (OH); 690, 1540 (C=C); 1650(C=N); 1440.

Hydrobromide of 2′,6,6′,8-tetrakis(diethylamino)-3,3′,4′,5,5′,7-hexahydroxyflavone (12). At 25°C, with stirring, a solution of 0.475 g (0.0065 mole) of $HN(C_2H_5)_2$ in 10 ml of alcohol was added dropwise over 20 min to a solution of 1 g (0.0015 mole) of tetrabromomyricetin in 10 ml of alcohol. The mixture was evaporated under vacuum and the product obtained after recrystallization from alcohol was washed with acetone and chloroform to eliminate the hydrobromide and resinification products. Crystals were obtained with mp 145—146°C (R_f 0.68 in the acetone—water, 8:2, system).

Elementary analysis. Found, %: C 28.56; H 3.75; N 6.05; Br 35.02; $C_{23}H_{30}N_4Br_4O_8$. Calculated, %: C 29.83; H 3.24; N 7.02; Br 34.57.

UV spectrum (EtOH, λ, nm): 272 and 365.

IR spectrum (KBr, ν, cm^{-1}): 3000, 3150, 3430 (OH); 1650 (C=O); 1605 (C=C); 1305, 2850, 1370, 2970, 2400, 2800.

Hydrobromide of 2′,6,6′,8-Tetrahydrazino-3,3′,4′,5,5′,7-hexahydroxyflavone (13). At 3—5°C, with stirring, a solution of 0.033 g (0.0065 mole) of hydrazine hydrate in 10 ml of alcohol was added dropwise over 20 min to a solution of 1 g (0.0015 mole) of tetrabromomyricetin in 10 ml of ethyl alcohol. The precipitate that deposited was filtered off. After

recrystallization from alcohol, crystals with mp 109—110°C were obtained. Yield 72.5%

Elementary analysis. Found, %: C 27.89; H 3.01; N 14.73; Br 42.32; $C_{15}H_{22}N_8Br_4O_8$. Calculated, %: C 27.56; H 2.89; N 14.69; Br 41.99.

UV spectrum (EtOH, λ, nm): 258 and 358.

IR spectrum (KBr, ν, cm^{-1}): 3200 (OH); 1640 (C=O); 950 (C=C); 1300, 2930, 2400—2800 (H—Br).

Preparation of 6,8-Bis(*p*-nitrobenzeneazo)-3,3′,4′,5,5′,7-hexahydroxyflavone (14). With stirring, a solution of 2.5 g of sodium nitrite in 200 ml of cold water was added dropwise to a mixture of 7.0 g of *p*-nitroaniline, 24 ml of 30% hydrochloric acid, and 120 g of ice. During diazotization the temperature was kept at from 0 to 5°C. After the addition of the whole of the sodium nitrite solution, stirring was continued for another 0.5 h.

The coupling of myricetin with the diazonium salt was carried out at ratios of 1:4.5 and 1:2.5. A solution of 1 g of myricetin in 20 ml of alcohol was added with stirring to a freshly prepared solution of the diazo compound. The brown precipitate that deposited was filtered off and treated with benzene. Paper chromatography showed the presence of a flavonoid structure in the precipitate. The mother solution and the benzene extract mainly contained the starting materials. After the benzene treatment, the precipitate was carefully washed with water and then with butanol and ether and was recrystallized from 20% ethanol. A paper chromatogram of the substance in the BAW (4:1:5) system gave a single spot with R_f 0.41; on Silufol in the acetone—benzene (1:3) system it had R_f 0.11, mp 220—222°C. Yield 70%

Elementary analysis. Found, %: C 51.56; H 4.78; N 18.48; $C_{27}H_{16}N_6O_{12}$. Calculated, %: C 51.26; H 4.48; N 17.98.

UV spectrum (EtOH, λ, nm): 290 and 395.

The PMR spectrum lacked singlets of protons in the 6.1 ppm (H-6) and 6.3 ppm (H-8) regions, while protons (H-2′ and H-6′) were recorded in the 7.7—8 ppm region and benzene ring protons in the 7.7—8 ppm region. Protons of hydroxy groups were shown at 9.3, 10.9, and 12.7 ppm.

6,8-Bis(*p*-sulfamoylbenzeneazo)-3,3′,4′,5,5′,7-hexahydroxyflavone (16). The diazotization of streptocide (sulfanilamide) was carried out under condition analogous to those of the preceding experiment. The resulting diazonium salt was coupled with myricetin in alcoholic solution in a ratio of 2.5:1 at 23—25°C. The substance was freed from streptocide with ether, and on rechromatography it appeared in the form of a single spot with R_f 0.33. After drying, 0.812 g of a crystalline substance with mp 290—292°C was obtained. Yield 73%.

UV spectrum (EtOH, λ, nm): 253 and 365.

Elementary analysis. Found, %: C 36.39; H 8.19; N 17.16; $C_{27}H_{20}N_6S_2O_{10}$. Calculated, %: C 36.01; H 8.02; N 16.81.

Molecular mass: found (Rast) 688 c.u.; calculated 684 c.u.

6,8-Bis[*p*-(4,6-dimethylpyrimidinylsulfamoyl)benzeneazo]-3,3′,4′,5,5′,7-hexahydroxyflavone (17). The diazotization of sulfadimezin (sulfamethazine) and coupling with myricetin were carried out under conditions analogous to those of the preceding experiments. Starting materials: 3.5 g of sulfadimezin, 12 ml of 30% HCl, 15 ml of H_2O, 60 g of ice, 1.75 g of sodium nitrite, 1.3 g of sodium acetate, and 1 g of myricetin. Coupling was conducted at a ratio of diazonium salt to myricetin of 2.5:1. The reaction mixture was left overnight. A precipitate deposited, and this was purified first with water and then with butanol. Thin-layer chromatography on Silufol in acetone: R_f 0.51; in acetone—water (1:4): R_f 0.46. The substance was soluble in acetone and DMSO, and sparingly soluble in benzene, alcohols, and hot water, decomp. p. 210—213°C. Yield 81% (0.79 g).

Elementary analysis. Found, %: C 51.79; 51.8; H 3.08; 3.07; N 14.86; $C_{39}H_{32}N_{10}S_2O_{12}$. Calculated, %: C 52.29; H 5.57; N 15.63.

Molecular mass (Rast): found 883, 886 c.u.

On the basis of spectral characteristics, elementary analysis, chromatography, and qualitative reactions, the substance was identified as 6,8-bis[*p*-(4,6-dimethylpyrimidinylsulfamoyl)benzeneazo]-3,3′,4′,5,5′,7-hexahydroxyflavone.

6,8-Bis[*p*-(1,3-thiazol-2-ylsulfamoyl)benzeneazo]-3,3′,4′,5,5′,7-hexahydroxyflavone (18). Diazotization and coupling of the diazonium salt of norsulfazole (sulfathiazole) with myricetin were carried out under conditions analogous to those of the preceding experiments. Starting materials: 3.5 g of norsulfazole, 12 ml of 30% HCl, 60 g of ice, 145 ml of water, 1.75 g of sodium nitrite, 1.3 g of sodium acetate and 1 g of myricetin.

The purification and isolation of the desired product were carried out in the following way. After the elimination of the unchanged diazonium salt of norsulfazole, the mother solution was extracted with butanol. The butanol extract was evaporated under vacuum and the residue was recrystallized from alcohol. A brown crystalline substance with mp 178—180°C was obtained. Yield 82% (2.01 g).

Elementary analysis. Found, %: C 47.03; H 2.87; N 12.97; $C_{33}H_{22}N_8S_4O_{12}$. Calculated, %: C 46.58; H 2.59; N 13.18.

Sodium Salt of 6,8-Bis(*p*-sulfobenzeneazo)-3,3′,4′,5,5′,7-hexahydroxyflavone (19).

Diazotization of Sulfanilic Acid. With cooling (0°C) and continuous stirring, a solution of 1 g (0.13 mole) of sodium nitrite in 12 ml of water was added in portions to a solution of 2.5 g (0.01 mole) of sulfanilic acid in 15 ml of 2 N sodium hydroxide solution until the appearance of nitrite ion was shown by starch-iodide paper, and the reaction mixture was left for 1 h, timed from the moment of verifying the presence of nitrite ion. Then it was poured into 20 ml of 2 N hydrochloric acid.

Azo Coupling of Myricetin with the Diazonium Salt of Sulfanilic Acid. With continuous stirring, a solution of 1 g (0.003 mole) of myricetin in 30 ml of ethyl alcohol was added in portions to the solution of the diazonium salt of sulfanilic acid. After being stirred for 1 h and treated with 15 g of sodium chloride, the reaction mixture was left at 0°C for 3 h. The light brown precipitate that deposited was filtered off, washed with a small amount ice water, and recrystallized from aqueous acetone, followed by chromatography on a column of alumina (height of the absorption layer 2 m). The eluate was evaporated and the residue was crystallized from aqueous ethanol. This gave 1.58 g of a crystalline substance with mp 350—351°C. The yield of the sodium salt of 6,8-bis(*p*-sulfobenzeneazo)-3,3′,4′,5,5′,7-hexahydroxyflavone was 95%.

Elementary analysis. Found, %: C 48.03; H 2.51; N 5.24; $C_{21}H_{13}O_{11}SNa$. Calculated, %: C 48.09; H 2.48; N 5.32.

UV spectrum (EtOH, λ, nm): 269.

IR spectrum (KBr, ν, cm^{-1}): 850, 1600 (C=C); 1640 (C=O); 3450 (OH); 1240 (C—N); 1440, 2060 (N=N); 1180 (S=O).

NMR spectrum (DMSO-d_6, δ, ppm, TMS, 60 MHz): 6—8 (arom. protons), 7.3 s (2H), 9.3, 12.9 and 10.9 (OH).

6,8-Bis{*p*-[2-(diethylamino)ethoxycarbonyl]benzeneazo)}-3,3′,4′,5,5′,7-hexahydroxyflavone (20). The conditions for obtaining the diazonium salt of novocaine (procaine hydrochloride) and coupling it with myricetin were the same as in the preceding experiments. Starting materials: 3.5 g of novocaine, 12 ml of 30% HCl, 60 g of ice, 145 ml of water, 1.75 g of sodium nitrite, and 1.3 g of sodium acetate. The ratio of the diazonium salt of novocaine to myricetin was 2.5:1.

The diazonium salt that had not reacted was eliminated, the mother solution was evaporated under vacuum and, after recrystallization, a substance with R_f 0.16 in BAW (4:1:5) was obtained.

Elementary analysis. Found, %: C 61.05; H 5.56; N 11.22; $C_{41}H_{44}N_6O_{12}$. Calculated, %: C 60.59; H 5.42; N 10.34.

Unlike myricetin, the azo derivatives fluoresced brown in UV light (myricetin fluoresces yellow), which shows substitution at C-8. A positive reaction with a 1% solution of $FeCl_3$ showed the presence of an OH group in the C-5 position. A positive reaction with FAA characterizes the vicinal position of the hydroxyls of ring *B*.

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