MEDICINAL PLANTS

ISOLATION, CHEMICAL ANALYSIS, AND STUDY OF THE HEPATOPROTECTOR AND BILIGENIC ACTIVITY OF TOTAL FLAVONOID PREPARATIONS FROM *Thermopsis dolichocarpa* AND *Vexibia alopecuroides*

V. N. Syrov,[1] M. P. Yuldashev,[1] M. I. Mamutova,[1] Z. A. Khushbaktova,[1] and É. Kh. Batirov[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 35, No. 1, pp. 29 – 32, January, 2001.

Original article submitted April 10, 2000.

Some flavonoids were reported to produce a positive pharmacotherapeutic effect in cases of hepatobiliary pathology [1, 2]. In the course of a broad chemico-biological investigation devoted to flavonoids of the plants occurring in Central Asia, we observed a high yield of these substances from the plants *Thermopsis dolichocarpa* and *Vexibia alopecuroides*. It was decided not only to qualitatively characterize the isolated preparations, but to evaluate their efficacy as potential means of treating disorders in the functional state of liver metabolism in cases of toxic damage.

EXPERIMENTAL CHEMICAL PART

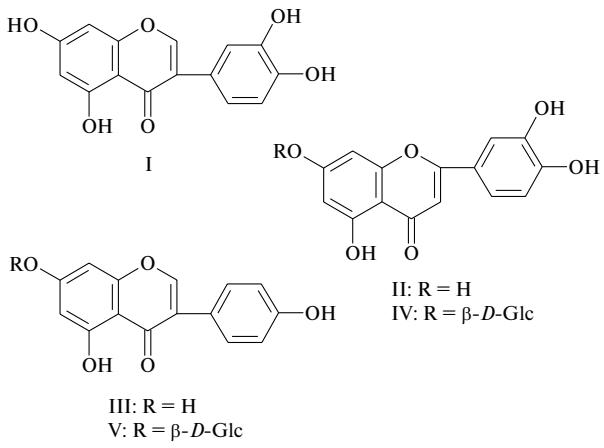
The column chromatography was effected using a 100/250 μm silica gel fraction (Chemapol, Czech Republic). TLC analyses were performed on Silufol UV-254 plates (Kavalier, Czech Republic) eluted in various chloroform – methanol solvent systems: (1) 97 : 3; (2) 19 : 1; (3) 9 : 1; (4) 17 : 3; and (5) 4 : 1.

The mass spectra were obtained using an MX-1310 instrument with an electron-impact ionization source operated at an electron beam energy of 50 eV. The IR spectra were measured on a UR-20 spectrophotometer (Carl Zeiss – Jena, Germany) using samples pelletized with KBr. The UV spectra were recorded on a Hitachi EPS-3T spectrophotometer (Japan) in ethanol solutions. The ^{1}H NMR spectra were measured on a Tesla BS-567A spectrometer (Czech Republic) operated at a working frequency of 100 MHz. The measurements were performed using Py-d_5 as the solvent; the chemical shifts (δ, ppm) were determined relative to the peaks of HMDS internal standard.

The samples of *Thermopsis dolichocarpa V. Nikitin* (aboveground parts) were collected during the fructification period in the Gissar district of Tajikistan. The roots of *Vexibia alopecuroides (L.) Yakovl*. were collected at the Kibrai village (Tashkent district, Uzbekistan) at the end of the vegetation period.

Isolation of the total flavonoid fraction from the *Thermopsis dolichocarpa* herbs. A sample (2.0 kg) of crushed air-dry aboveground material was triply extracted with water to remove carbohydrates, alkaloids, and other hydrophilic substances. Then the material was extracted eight times with an 80% aqueous ethanol solution, after which the extract volume was reduced to 3 liter by evaporation. In order to remove chlorophyll and waxy components, the ethanol – water extract was washed with chloroform (5 × 800 ml).

Flavonoids from *T. dolichocarpa* aboverground parts



The purified extract was allowed to stand for one day, after which the precipitate was separated by filtration, washed

[1] Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan.

with chloroform (0.4 liter), and dried at 95 – 100°C to obtain 52 g of the total dry flavonoid extract (2.6% of the initial air-dry material weight).

Half of the product mixture (26 g) was chromatographed on a silica gel column eluted in system 2 to isolate 3.59 g (14.8% of the initial weight) of orobol (I) and 6.83 g (26.3%) of luteolin (II). Then the column was eluted in system 3 to obtain 2.91 g (11.2%) of genistein (III). Finally, the column was eluted in system 5 to isolate 2.44 g (9.4%) of cynaroside (IV) and 1.97 g (7.6%) of genistin (V).

Orobol (I). Composition, $C_{15}H_{10}O_6$; m.p., 272 – 273°C; UV spectrum (λ_{max}, nm): 264, 293 (ethanol); 269, 320 (+CH_3COONa); 270, 364 (+$AlCl_3$); 272, 370 (+$AlCl_3$ + HCl); IR spectrum (ν_{max}, cm^{-1}): 3420 – 3270 (OH), 1660 (C=O γ-pyrone), 1622, 1576 (C=C arom.); mass spectrum, *m/z* (I_{rel}, %): [M^+] 286 (100), 268 (2), 153 (30), 134 (27), 124 (17) [3 – 5].

Luteolin (II). Composition, $C_{15}H_{10}O_6$; m.p., 328 – 331°C; UV spectrum (λ_{max}, nm): 260, 274, 356 (ethanol); IR spectrum (ν_{max}, cm^{-1}): 3450 – 3300 (OH), 1658 (C=O γ-pyrone), 1612, 1584 (C=C arom.); ^{1}H NMR spectrum in Py-d_5 (δ, ppm): 6.58 (d, 1H, J 2.0 Hz, H-6), 6.67 (d, 1H, J 2.0 Hz, H-8), 6.78 (s, 1H, H-3), 7.07 (d, 1H, J 8.0 Hz, H-5′), 7.50 (bs, 1H, H-2′), 7.54 (dd, 1H, J 2.0 and 8.0 Hz, H-6′). The acetylation of compound II with acetic anhydride in the presence of pyridine leads to the formation of tetraacetate with m.p., 225 – 226°C; [M+], 454 [3, 4, 6].

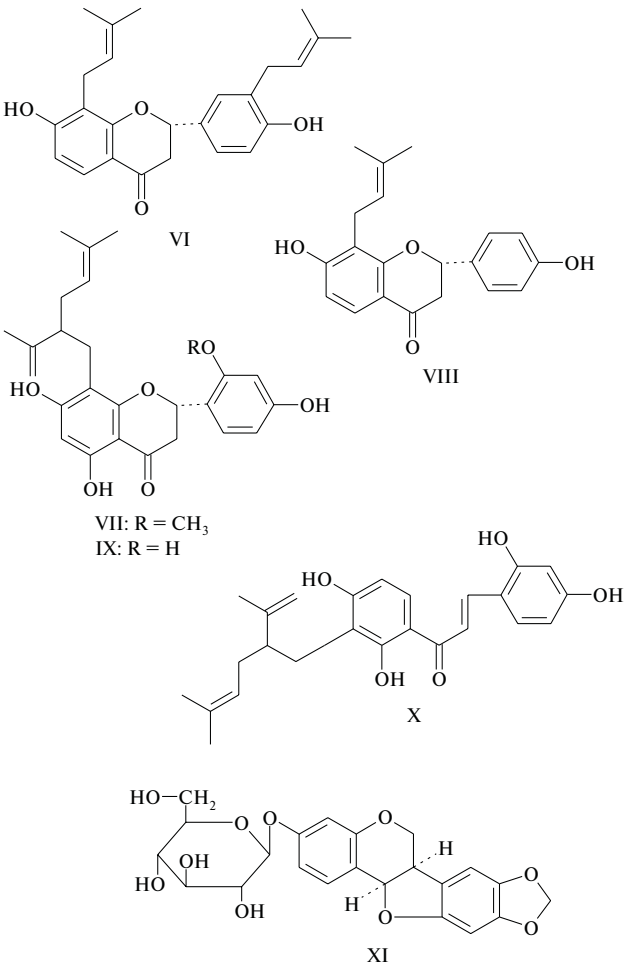
Genistein (III). Composition, $C_{15}H_{10}O_5$; m.p., 299 – 302°C; UV spectrum (λ_{max}, nm): 263, 329 (ethanol); 272, 323 (+CH_3COONa); 271, 369 (+$AlCl_3$); 272, 370 (+$AlCl_3$/HCl); 275, 328 (+CH_3ONa); mass spectrum, *m/z* (I_{rel}, %): [M+] 270 (100), 153 (69), 152 (36), 124 (11), 118 (20); ^{1}H NMR spectrum in Py-d_5 (δ, ppm): 6.46 (d, 1H, J 2.0 Hz, H-6), 6.55 (d, 1H, J 2.0 Hz, H-8), 7.06 (d, 2H, J 8.5 Hz, H-3′, H-5′), 7.54 (d, 2H, J 8.5 Hz, H-2′, H-6′), 7.92 (s, 1H, H-2) [3, 4, 7].

Cynaroside (IV). Composition, $C_{21}H_{20}O_{11}$; m.p., 240 – 242°C; UV spectrum (λ_{max}, nm): 256, 268, 350 (ethanol); IR spectrum (ν_{max}, cm^{-1}): 3480 – 3300 (OH), 1665 (C=O γ-pyrone), 1560, 1510 (C=C arom.), 1095, 1030 (C–O bonds in glycosides); ^{1}H NMR spectrum in Py-d_5 (δ, ppm): 3.90 – 4.05 (glucose protons), 5.66 (d, 1H, J 7.0 Hz, H-1′), 6.67 (d, 1H, J 2.5 Hz, H-6), 6.77 (s, 1H, H-3), 6.84 (d, 1H, J 2.5 Hz, H-8), 7.13 (d, 1H, J 8.0 Hz, H-5′), 7.38 (dd, 1H, J 2.5 and 8.0 Hz, H-6′), 7.74 (d, 1H, J 2.5 Hz, H-2′). The acid hydrolysis of glycoside IV leads to the formation of luteolin II and *D*-glucose. The acetylation of compound IV with acetic anhydride in the presence of pyridine leads to the formation of a heptaacetyl derivative with the composition $C_{35}H_{34}O_{18}$ ([M+], 742); m.p., 121 – 123°C [3, 4, 8].

Genistin (V). Composition, $C_{21}H_{20}O_{10}$; m.p., 253 – 255°C; UV spectrum (λ_{max}, nm): 263, 329 (ethanol); 262, 330 (+CH_3COONa); 273, 373 (+$AlCl_3$); 272, 355 (+CH_3ONa); ^{1}H NMR spectrum in Py-d_5 (δ, ppm): 3.90 – 4.60 (glucose protons), 5.64 (d, 1H, J 6.5 Hz, H-1″), 6.70 (d, 1H, J 2.5 Hz, H-6), 6.90 (d, 1H, J 2.5 Hz, H-8), 7.15 (d, 2H, J 8.0 Hz, H-3′, H-5′), 7.55 (d, 2H, J 8.0 Hz, H-2′, H-6′), 8.02 (s, 1H, H-2). The acid hydrolysis of glycoside V leads to the formation of genistein III and D-glucose. The acetylation of compound V with acetic anhydride in the presence of pyridine leads to the formation of a hexaacetyl derivative with m.p., 189 – 191°C; [M^+], 684 [3, 4, 9].

Isolation of the total flavonoid fraction from the *Vexibia alopecuroides* roots. A sample (3.0 kg) of crushed air-dry roots was exhaustively (eight times) extracted with 95% aqueous ethanol, after which the extract volume was reduced to 1.5 liter by evaporation. Then the ethanol extract was diluted with water (1 : 1) and successively treated by shaking with petroleum ether, chloroform, and ethyl acetate. The chloroform tincture yields (upon solvent removal) 100 g of the total dry flavonoid extract. A half of this amount (50.0 g) was chromatographed on a silica gel column eluted in system 1 to isolate 0.75 g (1.5% of the initial weight) of glabrol (VI), 0.95 g (1.9%) of vexibidin (VII), 0.13 g (0.26%) of isobavachin (VIII), and 2.45 g (4.9%) of vexibinol (IX).

Flavonoids from *V. alopecuroides* roots



Then the column was eluted in system 3 to obtain 0.11 g (0.22%) of ammothamnidin (X). Finally, the column was eluted in system 4 to isolate 0.97 g (1.94%) of trifolirhizin (XI).

Glabrol (VI). Composition, $C_{25}H_{25}O_4$; m.p., 136 – 137°C; UV spectrum (λ_{max}, nm): 288, 312 (ethanol); 256, 284, 323, 330 (+CH_3COONa); 294, 329, 334 (+CH_3ONa); 287, 313 (+$AlCl_3$); IR spectrum (ν_{max}, cm^{-1}): 3390 (OH), 1662 (C=O γ-pyrone), 1602, 1588, 1516 (C=C); mass spectrum, *m/z* (I_{rel}, %): [M^+] 392 (93), 377 (5), 349 (70), 337 (18), 205 (36), 204 (30), 189 (16), 189 (20), 149 (100), 133 (40). The acetylation of compound VI with acetic anhydride in the presence of pyridine leads to the formation of a diacetyl derivative with m.p., 106 – 107°C [10, 11].

Vexibidin (VII). Composition, $C_{26}H_{30}O_6$; m.p., 156 – 157°C; UV spectrum (λ_{max}, nm): 293, 340 (ethanol); 294, 341 (+CH_3COONa); 244, 332 (+CH_3ONa); 311, 376 (+$AlCl_3$); mass spectrum, *m/z* (I_{rel}, %): [M^+] 438 (8), 315 (76), 285 (2.9), 219 (18), 203 (2), 191 (9), 177 (8), 166 (14), 165 (100), 151 (22), 150 (10), 137 (9A), 135 (6), 123 (14). The acetylation of compound VII with acetic anhydride in the presence of pyridine leads to the formation of a triacetyl derivative with m.p., 77 – 78°C. The methylation of compound VII with diazomethane yields a dimethyl ester of compound VII; mass spectrum, *m/z* (I_{rel}, %): [M^+] 466 [3, 12].

Isobavachin (VIII). Composition, $C_{20}H_{20}O_4$; m.p., 203 – 204°C; UV spectrum (λ_{max}, nm): 288, 312 (ethanol); 289, 338 (+CH_3COONa); 290, 349 (+CH_3ONa); 290, 311 (+$AlCl_3$); IR spectrum (ν_{max}, cm^{-1}): 3280 (OH), 1645 (C=O), 1585, 1520 (C=C); ^{1}H NMR spectrum in Py-d_5 (δ, ppm): 1.59 (bs, =C–CH_3), 1.72 (bs, =C–CH_3), 2.80 – 3.05 (m, H-3), 3.54 (d, J 7.0 Hz, Ar–$\underline{CH}_2$–), 5.16 – 5.68 (m, H-2, =$\underline{CH}$–CH_2–), 6.81 (d, J 8.6 Hz, H-6), 7.10 (d, J 9.0 Hz, H-3′, H-5′), 7.43 (d, J 9.0 Hz, H-2′, H-6′), 7.89 (d, J 8.6 Hz, H-5) [10, 13].

Vexibinol (IX). Composition, $C_{25}H_{28}O_6$; m.p., 173 – 175°C; UV spectrum (λ_{max}, nm): 292, 339 (ethanol); mass spectrum, *m/z* (I_{rel}, %): [M^+] 424 (14), 409 (3), 408 (3), 407 (5), 406 (18), 391 (7), 389 (3), 363 (7), 338 (3.9), 337 (10), 302 (17), 301 (75), 284 (23), 283 (100), 219 (13), 166 (5), 165 (37), 139 (5), 137 (4), 136 (6), 124 (6.8), 123 (9), 109 (13), 107 (6). The acetylation of compound VII with acetic anhydride in the presence of pyridine leads to the formation of a tetraacetyl derivative with m.p., 70 – 72°C. The methylation of compound IX with an ether solution of diazomethane yields a trimethyl ester of compound IX, which is identical to the aforementioned dimethyl ester of vexibidin [12].

Ammothamnidin (X). Composition, $C_{25}H_{28}O_5$; m.p., 112 – 114°C; UV spectrum (λ_{max}, nm): 231, 261, 321, 390 (ethanol); 398 (+CH_3COONa); 395 (+CH_3COONa/H_3BO_3); 439 (+CH_3ONa); 392 (+$AlCl_3$); IR spectrum (ν_{max}, cm^{-1}): 3430 – 3145 (OH), 1628 (C=O), 1615, 1557, 1522 (C=C); ^{1}H NMR spectrum in Py-d_5 (δ, ppm): 1.43, 1.47, 1.78 (3s, 3CH_3–C=), 2.27 (m, H-3″), 3.08 (m, H-1″, H-2″), 4.62, 4.72 (2bs, 2H-9″), 5.10 (m, H-4″), 6.35 – 6.70 (m, H-3, H-5, H-5′), 7.62 (d, 8.0 Hz, H-6), 7.87 (d, J 9.0 Hz, H-6′), 8.02 (d, J 16 Hz, H-α), 8.70 (d, J 16 Hz, H-β). The acetylation of compound X with acetic anhydride in the presence of pyridine leads to the formation of a tetraacetyl derivative with the composition $C_{33}H_{36}O_9$ [14].

Trifolirhizin (XI). Composition, $C_{22}H_{24}O_{10}$; m.p., 140 – 142°C; UV spectrum (λ_{max}, nm): 279, 286, 312 (ethanol); IR spectrum (ν_{max}, cm^{-1}): 3370 – 3220 (OH), 1625, 1580 (C=C arom.), 1076, 1045, 1020 (C–O), 936 (OCH_2O); ^{1}H NMR spectrum in Py-d_5 (δ, ppm): 3.06 – 3.85 (m, H-6), 3.86 – 4.53 (sugar residue protons, H-6a), 5.44 (d, J 7.0 Hz, H-1′), 5.55 (d, 1H, J 6.0 Hz, H-11a), 5.85 (bs, OCH_2O), 6.58 (s, H-10), 6.78 (s, H-7), 7.06 (m, H-2, H-4), 7.42 (d, J 9.0 Hz, H-1). The acetylation of compound XI with acetic anhydride in the presence of pyridine leads to the formation of a tetraacetyl derivative with the composition $C_{30}H_{30}O_{14}$ ([M^+], 614). The acid hydrolysis of compound XI leads to the formation of inermin and *D*-glucose.

EXPERIMENTAL BIOLOGICAL PART

The experiments were performed on white mongrel male rats weighing 150 – 180 g (6 – 8 animals in each group). The total flavonoid extract from *T. dolichocarpa* and *V. alopecuroides* (preparations I and II, respectively) were introduced to the test animals in a preliminarily selected single effective daily dose (50 mg/kg, p.o.) over a period of five days. The model toxic liver damage was induced by paracetamol in a dose of 2.5 g/kg introduced in the form of a 25% aqueous suspension during the first two days via a special intragastric tube [16]. After termination of the treatment with flavonoid preparations I and II, the animals were decapitated under light ether narcosis. The efficacy of the treatment was evaluated by its effect upon the activity of alanine (AlAT) and aspartate (AsAT) aminotransferases [17] and alkaline phosphatase (AlkP) in the blood serum [18] and by the content of malonic dialdehyde (MDA) [19] and glycogen [20] in liver homogenates.

For evaluating the biligenic activity of preparations I and II, a part of the animals from each group (a total of not less than six) were narcotized with barbamyl (100 mg/kg, i.p.) and bile samples were taken every hour over a 4-h period of time using a catheter inserted into the common bile duct. The samples were analyzed for the concentration of bile acids [21], cholesterol [22], and bilirubin [23], after which their total amounts were determined. In addition, the test animals in all experimental series were characterized by the cholate – cholesterol coefficient. The experimental data were statistically processed in terms of the Student *t*-criterion.

TABLE 1. Effect of the Total Flavonoid Extracts from *Thermopsis dolichocarpa* (Preparation I) and *Vexibia alopecuroides* (Preparation II) on Some Parameters of Liver Metabolism and Functioning in Rats with Paracetamol Hepatitis Model

Characteristic	Intact rats	Hepatitis (paracetamol)	Paracetamol + preparation I	Paracetamol + preparation II
AlAT***	0.94 ± 0.08	2.84 ± 0.46*	1.16 ± 0.12**	0.98 ± 0.10**
AsAT***	1.34 ± 0.18	2.06 ± 0.22*	1.38 ± 0.06**	1.32 ± 0.06**
AlkP, U/liter	216.4 ± 24.8	308.6 ± 16.2*	222.4 ± 12.4**	214.6 ± 10.8**
MDA, mmole/(mg protein)	0.496 ± 0.018	0.854 ± 0.046*	0.532 ± 0.020**	0.512 ± 0.032**
Glycogen, mg%	2088 ± 96	834 ± 56*	1706 ± 64*,**	1806 ± 94**
Bile secretion rate, mg/(min 100 g)				
1st hour	5.12 ± 0.14	3.64 ± 0.18*	5.16 ± 0.20**	5.50 ± 0.38**
2nd hour	4.44 ± 0.12	3.50 ± 0.16*	5.80 ± 0.24*,**	5.10 ± 0.20*,**
3rd hour	4.38 ± 0.10	3.30 ± 0.14*	4.50 ± 0.18**	4.80 ± 0.24**
4th hour	4.20 ± 0.10	3.18 ± 0.16*	4.30 ± 0.16**	4.76 ± 0.14*,**
Total bile, mg/(100 g 4 h)	1088 ± 84	817.2 ± 28*	1182 ± 88**	1209 ± 96**
Bile acids, mg%	876.4 ± 40.4	582.8 ± 32.6*	882.4 ± 42.6**	998.6 ± 54.2**
Cholesterol, mg%	18.8 ± 2.2	15.4 ± 1.4	18.6 ± 2.6	20.4 ± 2.8
Bilirubin, mg%	22.2 ± 3.0	17.8 ± 1.4	26.8 ± 2.4**	28.3 ± 3.0**
Total bile acids, mg/(100 g 4 h)	9.535 ± 0.650	4.763 ± 0.362*	10.429 ± 0.690**	12.073 ± 0.720*,**
Total cholesterol, mg/(100 g 4 h)	0.204 ± 0.012	0.125 ± 0.006*	0.219 ± 0.014**	0.246 ± 0.018**
Total bilirubin, mg/(100 g 4 h)	0.242 ± 0.018	0.145 ± 0.010*	0.317 ± 0.026*,**	0.342 ± 0.028*,**
Cholate – cholesterol coefficient	46.6 ± 4.2	35.5 ± 2.2*	47.4 ± 4.8**	48.9 ± 5.2**

* $p < 0.05$ relative to intact control
** $p < 0.05$ relative to untreated control
*** Aminotransferase activity in mmole PVA per ml serum for 1 h incubation.

RESULTS AND DISCUSSION

As seen from the data summarized in Table 1, the introduction of paracetamol for two days in a dose of 2.5 g/kg markedly increases the activity of serum enzymes (AlAT, AsAT, AlkP), which is accompanied by a significant increase of the MDA content and a decrease in the glycogen level in liver homogenates. The rats treated with paracetamol also exhibited a decrease in the rate of bile production, in the amount of total bile secreted over a 4-h period of time, and in the concentration of bile acids. Serious disorders were developed in the cholesterol excretion and bilirubin metabolism, which is clearly revealed by the results of calculation of the total amounts of cholesterol and bilirubin excreted during the experiment. Similar data describing a pronounced hepatotoxic effect of paracetamol in large doses were reported in [16, 24].

It was established that the flavonoid preparations I and II decrease or eliminate the paracetamol-induced hepatitis, which is manifested by normalization of the enzymatic activity in the blood serum, the mechanisms of lipid peroxidation and glycogen synthesis in the liver, and the bile secretion processes (see Table 1). These results indicate that the total flavonoid extracts from *Thermopsis dolichocarpa* and *Vexibia alopecuroides* possess pronounced hepatoprotector properties.

REFERENCES

1. V. A. Baraboi, *Biological Action of Plant Phenolic Compounds* [in Russian], Naukova Dumka, Kiev (1984).
2. A. D. Gordienko, *Farmatsiya*, No. 3, 75 – 78 (1993).
3. T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer-Verlag, Berlin (1970).
4. L. K. Klyshev, V. A. Bandyukova, and L. S. Alyukina, *Plant Flavonoids* [in Russian], Nauka, Alma-Ata (1978).
5. M. P. Yuldashev, E. Kh. Batirov, and V. M. Malikov, *Khim. Prir. Soedin.*, No. 4, 547 – 548 (1990).
6. S. V. Teslov and L. S. Larina, *Khim. Prir. Soedin.*, No. 5, 666 – 667 (1973).
7. E. Kh. Batirov, F. Kiyamitdinova, and V. M. Malikov, *Khim. Prir. Soedin.*, No. 1, 111 – 112 (1986).
8. Sh. V. Abdullaev, A. Sattikulov, E. Kh. Batirov, et al., *Khim. Prir. Soedin.*, No. 1, 104 (1983).
9. M. P. Yuldashev, E. Kh. Batirov, A. D. Vdovin, et al., *Khim. Prir. Soedin.*, No. 3, 352 – 359 (1989).
10. S. S. Yusupova, E. Kh. Batirov, Sh. V. Abdullaev, and V. M. Malikov, *Khim. Prir. Soedin.*, No. 2, 250 – 251 (1984).
11. T. Saitoh, T. Kinoshita, and Sh. Shibata, *Chem. Pharm. Bull.*, **24**(4), 752 – 755 (1976).
12. E. Kh. Batirov, S. S. Yusupova, Sh. V. Abdullaev, et al., *Khim. Prir. Soedin.*, No. 1, 35 – 41 (1985).
13. V. K. Bhalla, U. R. Nayak, and S. Dev, *Tetrahedron Lett.*, No. 20, 2401 – 2406 (1968).

14. É. Kh. Batirov, S. S. Yusupova, A. Sattikulov, et al., *Khim. Prir. Soedin.*, No. 4, 516 – 524 (1987).
15. S. S. Yusupova, E. Kh. Batirov, F. Kiyamitdinova, and V. M. Malikov, *Khim. Prir. Soedin.*, No. 5, 639 – 640 (1986).
16. N. P. Skakun and V. V. Shman'ko, *Farmakol. Toksikol.*, **47**(4), 105 – 108 (1984).
17. S. Reitman and S. Frankel, *Am. J. Clin. Pathol.*, **28**, 56 – 63 (1957).
18. O. A. Bessey, O. H. Lowry, and M. Brock, *J. Biol. Chem.*, **164**(1), 321 – 329 (1946).
19. V. N. Orekhovich (ed.), *Modern Methods in Biochemistry* [in Russian], Meditsina, Moscow (1977), pp. 66 – 68.
20. S. Lo, J. C. Russell, and A. W. Taylor, *J. Appl. Physiol.*, **28**(2), 234 – 236 (1970).
21. Ya. I. Karbach, *Biokhimiya*, **26**(2), 305 – 309 (1961).
22. S. M. Drogovoz, *Vopr. Med. Khim.*, **20**(4), 397 – 400 (1074).
23. N. P. Skakun, *Probl. Endokrinol.*, **2**(6), 75 – 80 (1956).
24. M. F. Dixon, J. Nimmo, and L. F. Prescott, *J. Pathol.*, **103**(4), 225 – 229 (1971).