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FLAVONOIDS OF ROOTS OF *Glycyrrhiza uralensis* GROWING IN SIBERIA

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The composition of phenolic components of roots and rhizomes of Glycyrrhiza uralensis *Fisch. from Siberia is studied. A total of 15 components belonging to 8 structural types including 4′-methylglucoliquiritigenin, which was previously unknown in licorices, is found. The components in samples collected from various regions of Siberia are practically identical.*

Key words: *Glycyrrhiza uralensis*, extracts, phenolic components, flavanonic glycosides.

The total flavonoids isolated by alcohol extraction of roots and rhizomes of *Glycyrrhiza glabra* L. and *G. uralensis* Fisch. are known to be the active principle of the antiulcer preparation "liquiriton" [1]. The most representative species of licorice in eastern Russia is ural licorice (*G. uralensis* Fisch.), the range of which includes Altai, western and central Siberia, and Transbaikal. Ural licorice is rather widely distributed over Mongolia and China [2]. The chemistry and biological activities of the very important components of licorice have been reviewed [3]. Flavonoids of the aerial and subterranean parts of ural licorice that was exported from China have been studied by Japanese [4-12] and Chinese researchers [13].

Licorice from Siberian populations has not been systematically studied. While studying Siberian licorice, we considered the importance of obtaining data on the qualitative and quantitative composition of the flavonoids owing to the necessity of standarizing the antiulcer and other medicinal preparations, the activity of which is due to the aforementioned flavonoids. We studied three root and rhizome samples of ural licorice collected in Buryatiya (sample 1), Khakasiya (sample 2), and Novosibirsk district (sample 3).

The plant material was extracted using the solvents listed in Table 1. The components extracted by methanol and butanol were distributed in EA—water, which separated the reserve sugars and also isolated isoprenylated and glycoside-containing compounds. Table 1 shows that the total extracted substances from various samples are very similar.

TABLE 1. Yield of Extracted Substances from *G. glabra*, %

Solvent for extraction	Sample		
	1	**2**	**3**
Hexane	0.84	0.85	0.88
Diethylether	3.30	3.32	2.86
Methyl-*t*-butylether (MTBE)	1.05	1.08	1.19
Ethylacetate (EA)	0.80	0.83	0.93
Methanol	11.54	9.02	10.02
n-Butanol	1.52	1.96	2.95
Distribution of methanol extract in EA—water, %	30/70	38/62	24/76

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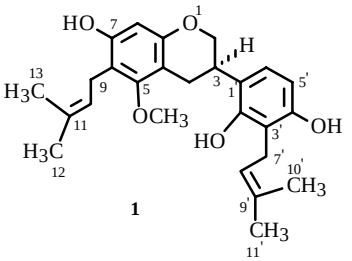

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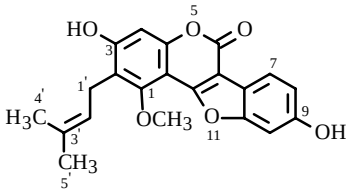

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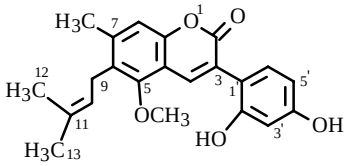

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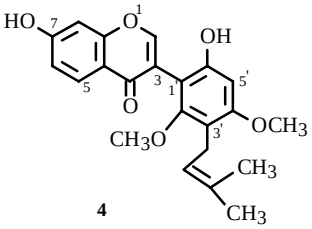

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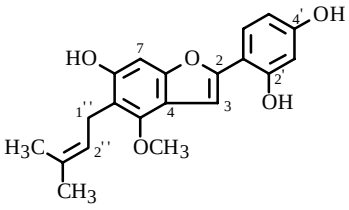

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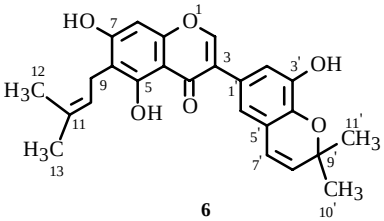

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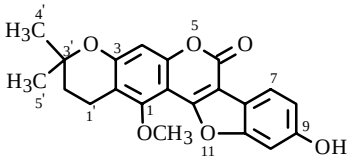

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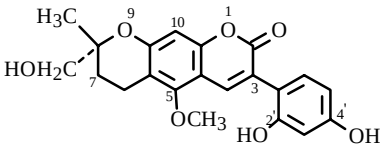

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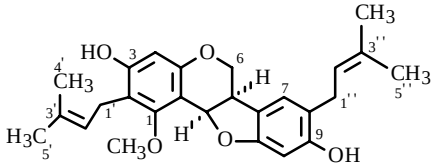

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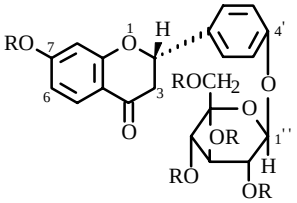
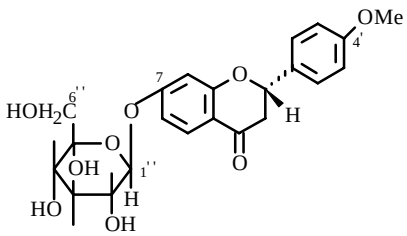


10 (R=H), **10a** (R=Ac)



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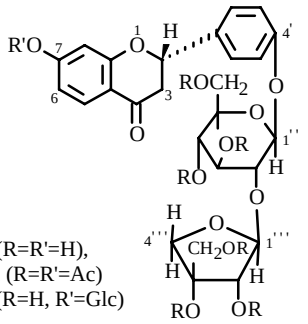
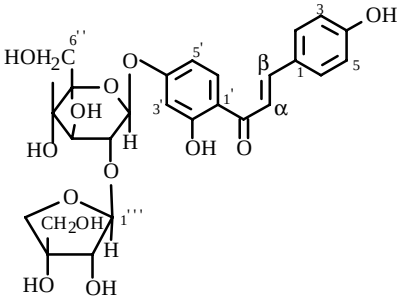


12 (R=R'=H),
12a (R=R'=Ac)
13 (R=H, R'=Glc)



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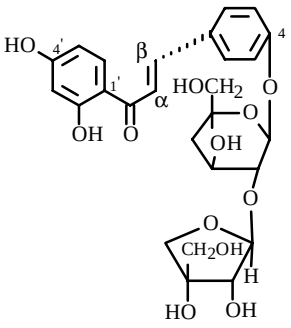

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PMR spectra were recorded for all extracts and their acetates. The positions and intensities of the signals demonstrated that the extracts obtained from the various licorice samples were practically identical. This conclusion enabled primarily sample 1 to be used in the experiments on the isolation of the pure compounds.

The hexane extract was analyzed according to the literature method [14]. Neutral (62%) and polar lipids were present. The composition of the lipid fractions is beyond the scope of the present study. However, the PMR and GC—MS data show that the neutral lipids of ural licorice contain sterols such as β-sitosterol, stigmasterol, brassicasterol, and diosgenin. The lipid

fraction contains saturated and unsaturated fatty acids (~20%) with predominance of C_{18}-compounds.

The PMR spectrum of the total flavonoids extracted by diethylether contains signals characteristic of prenyl substituents: singlets at 1.66, 1.68, 1.75, and 1.79 ppm; doublets at 3.30 and 3.33 ppm, and triplets coupled to them at 5.20 and 5.30 ppm. Column chromatography on silica gel of the ether extract of sample 1 was used to isolate the pure phenolic components: licoricidin (**1**), glycyrol (**2**), glycyrin (**3**), licoricone (**4**), and licocoumarone (**5**). It should be emphasized that the spectra of the ether extracts of samples 2 and 3 contain all signals characteristic of compounds **1**-**5**. In particular, the signals already noted for prenyl groups are accompanied by signals of methoxy groups at 3.45-4.05 ppm; signals at 2.80, 3.30, 3.98, and 4.15 ppm that are characteristic of protons on C-2, C-3, and C-4 of the isoflavan system; and signals of C-2 protons of isoflavone, C-3 of benzofuran, and C-4 of coumarin, which are located at 8.01, 7.20, and 7.96 ppm, respectively.

Further extraction of samples 1-3 by methyl-*t*-butylether (MTBE) produced a mixture of prenylated phenolic compounds. Chromatography of these isolated licoricone (**4**), gancaonin H (**6**), isoglycyrol (**7**), licopyranocoumarin (**8**), and 1-methoxyficifolinol (**9**) [5-8].

A characteristic feature of the EA extracts is the presence of glycosides [15], which can be inferred from signals in the PMR spectra corresponding to flavanonic glycosides (δ ~2.80, dd; ~3.08, dd, J_{gem} 15-17 Hz, H-3α and -β; ~5.50, dd, H-2; ~5.0, d, and 4.0-3.5, m, carbohydrate fragment). Chromatography of the EA extracts isolated **1**, **2**, **6**, **7**, and **9** in addition to the glycosides liquiritin (**10**), 4′-methylglucoliquiritigenin (**11**), and the 4′-apioside of liquiritin (**12**) [16]. The last compound was characterized as the heptaacetate **12a**. We demonstrated for the first time the presence of **11** in the licorice (5% yield of the EA extract of sample 1). Its structure was found by COSY and COLOC spectra that revealed $^{13}C—^{1}H$ and $^{1}H—^{1}H$ couplings. The location of the methyl group on the O of C-4′ was confirmed by analyzing ^{13}C NMR spectra recorded using single resonance. The spectrum taken with selective decoupling shows that the C-7 signal retains its CH coupling whereas that of C-4′ undergoes a narrowing of the multiplet owing to decoupling of the spin—spin interaction with the methyl protons.

The contents of the methanol extracts were distributed in EA—water (1:1 by vol.). Chromatography of the EA extract isolated **6**-**8**, **10**, and **1**. Liquiritin (**10**) crystallized spontaneously from the aqueous layer of the methanol extract of sample 1. Evaporation of the mother liquor, acetylation of the solid, and chromatography isolated liquiritin pentaacetate **10a** and liquiritin 4′-apioside peracetate **12a**.

The final extraction of the roots by butanol isolated another fraction of the total flavonoids, which were separated by the distributive extraction described above. The components of the EA extract included compounds **7** and **8**. Treatment of the water extract isolated glycosides **10** and **12** in addition to glucoliquiritin 4′-apioside **13**. The water extract of the butanol extract characteristically contains chalcone glycosides licuraside **14** and isoliquiritin apioside **15** (up to 10% of the fraction mass), the principal phenolic components of glabrous licorice [17].

We note in discussing the structure of certain components that the C-2-(*S*) configuration of liquiritin (**10**) is suggested by chiro-optical data. The circular optical dichroism (CD) spectrum has maxima for the $n \rightarrow \pi^*$ transition at $[\theta]_{338}$ $+4.2 \cdot 10^3$ and $[\theta]_{297}$ $-6.2 \cdot 10^3$ ($c = 5.1 \cdot 10^{-3}$ M, MeOH) [18]. The absolute configuration (+)-(*R*)-licoricidin (**1**) was also confirmed by CD ($c = 1.3 \cdot 10^{-3}$ M, MeOH) $[\theta]_{292}$ 0, $[\theta]_{286}$ $+1.2 \cdot 10^3$, $[\theta]_{275}$ 0, $[\theta]_{270}$ $-1.0 \cdot 10^3$, $[\theta]_{245}$ 0 [4, 19].

The structure of **9** is determined by comparing its spectral and optical characteristics with those of other pterocarpane derivatives. Thus, the absolute configuration can be established from the sign of the optical rotation angle [20]. Levorotary pterocarpanes have the configuration 6a *R*,11a *R*.

Thus, 15 flavonoids are isolated from roots of ural licorice from the Siberian population. These include the previously unobserved 4′-methylglucoliquiritigenin. A comparison of the flavonoid conpositions of the ural and glabrous licorices indicates that the former has a high content of isoflavone (licoricone, gancaonin H) and coumestane (glycyrol, isoglycyrol, methoxyficifolinol) types. Such compounds as licoricidin (**1**), glycyrol (**2**), and isoglycyrol (**7**) occur in the roots in significant quantities (isolated in yields of 0.42, 0.28, and 0.32%, respectively). The qualitative composition of the flavonoids that is practically independent of the region in which ural licorice was grown is noteworthy. The low content of chalcones is characteristic of ural licorice. Our data and that of Japanese researchers [21] suggest that the flavonoid compositions of ural licorice from Siberian and Chinese populations are similar.

The difference in the qualitative flavonoid composition of ural and glabrous licorice makes additional pharmacological studies advisable.

EXPERIMENTAL

Freshly distilled solvents and pure-grade reagents were used. Melting points were determined on a Kofler apparatus. IR spectra were recorded on a Vector 22 spectrometer in KBr pellets; UV spectra, on a Specord UV-Vis spectrophotometer in ethanol (c 10^{-4} M). A solution of KOH (0.1 N) in ethanol was used for basicification. Molecular weights and elemental compositions were determined on a high-resolution mass spectrometer (Finnigan MAT 8200). NMR spectra were obtained on Bruker AC 200 (working frequency 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C) and Bruker DRX 500 (working frequency 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C) spectrometers in $CDCl_3$, CD_3OD, $CDCl_3$—CCl_4, $(CD_3)_2SO$, or $(CD_3)_2CO$. The multiplicity of signals in the ^{13}C NMR spectra was determined using standard methods for recording spectra in the J-modulation mode (JMOD) and with off-resonance irradiation of protons. Signals in the NMR spectra were assigned using various types of H—H and C—H shift correlation spectroscopies (COSY, COLOC, CORRD, NOESY). Specific rotations ($[\alpha]_{580}$) were measured on a Polamat A polarimeter in ethanol at room temperature. Optical circular dichroism curves were recorded on a JASCO J 500A spectropolarimeter.

Phenolic components were separated using column chromatography on silica gel KSK (0-140 μm). The purity of the desired products was determined by HPLC using a Milikhrom-4 microcolumn liquid chromatograph from the Orlovskii PO "Nauchpribor". The chromatography conditions were: steel column 2×64 mm, Nucleosil C-18 sorbent (5 μm), ~20°C, elution rate 100 μl/min, UV detection at 240, 260, and 320 nm. The eluents were mixtures of MeOH (85%) and 0.05 M H_3PO_4 (15%) for analyzing **1-9** and MeOH (70%), 0.05 M H_3PO_4 (15%), and H_2O (15%) for analyzing **10-13**. Raw licorice was stored at the collection site as a working supply suitable for mass procurement. Roots of sample 1 came from Zaigraevskii region of Buryatiya in August, 1997; sample 2, from Askizskii region of Khakasiya in August, 1999; sample 3, from Karasukskii region of Novosibirsk district in September, 1998.

Treatment of Plant Material. Air-dried and ground (2-3 mm) licorice roots (100 g) were successively extracted with hexane, diethylether, ethylacetate, methanol, and butanol using three-fold extraction (3×150 ml) in each instance with refluxing on a water bath for 3 h. The yields of total extracted substances are listed in Table 1. The hexane extract was separated and analyzed by the literature method [14]. PMR and GC—MS data were used to identify free sterols and their esters [β-sitosterol (M^+ 414), stigmasterol (M^+ 412), diosgenin (M^+ 414), brassicasterol (M^+ 398), total content ~15%]. Fatty acids (~20%, 60:40 ratio of saturated to unsaturated) are represented mainly by linoleic and oleinic (1:1) and palmitic and stearic (9:1), respectively.

Isolation of Flavonoids. Separation of Ether Extract. Column chromatography of the ether extract (2.8 g) on silica gel (70 g) (benzene—ethylacetate eluent 10:0 → 10:2) produced three fractions: 1 (benzene eluent), 0.1 g (lipid part); 2 (benzene:ethylacetate eluent, 10:0.1 → 10:0.5), 1.0 g; 3 (benzene:ethylacetate eluent, 10:0.5 → 10:2), 1.6 g. Fractions 2 and 3 were rechromatographed on silica gel ($CHCl_3$:ethanol eluent, 10:0.1 → 10:0.5) and produced six fractions each, crystallization of which from the respective solvent isolated **1** (0.20 g), **2** (0.16 g), **5** (0.06 g), **4** (0.08 g), and **3** (0.03 g).

Separation of MTBE Extract. Column chromatography on silica gel (1.05 g substance, 30 g SiO_2, $CHCl_3$:ethanol eluent, 10:0 → 10:1) successively eluted eight fractions, from which crystallization from the respective solvent isolated **7** (0.08 g), **9** (0.028 g), **8** (0.025 g), **4** (0.02 g), and **6** (0.03 g).

Separation of EA Extract. Chromatography of the EA extract (0.6 g) on silica gel (25 g) with elution by $CHCl_3$:CH_3OH (10:0.1 → 10:2) isolated **1** (0.05 g), **2** (0.03 g), **7** (0.04 g), **6** (0.03 g), **9** (0.018 g), **11** (0.03 g), and **10** (0.06 g). The extract (0.2 g) was acetylated as usual to produce a mixture of acetates (0.26 g), chromatography of which on silica gel ($CHCl_3$:ethanol eluent, 10:0 → 10:2) and subsequent crystallization of the fractions isolated licoricidin triacetate (0.022 g), glycyrol diacetate (0.018 g), compound **10a** (0.06 g), and substance **12a** (0.05 g).

Separation of Methanol Extract. Chromatography on silica gel (0.9 g) of the EA extract of the methanol extract of sample 1 ($CHCl_3$:methanol eluent, 10:0.2 → 10:2) produced successively three fractions. Fraction 1 (0.2 g) yielded **6** (0.01 g) and **1** (0.01 g); fraction 2 (0.2 g), **7** (0.03 g), **10** (0.05 g), and **8** (0.01 g). Rechromatography of fraction 3 (0.45 g) on silica gel with addition of diethylether to the solid after removal of solvent isolated **8** (0.01 g), **7** (0.02 g), and **10** (0.12 g).

The aqueous extract of the methanol extract of sample 1 (2.1 g) was filtered to remove a precipitate (0.32 g) of pure **10**. The solid after evaporation of the mother liquor under vacuum (1.72 g) was acetylated to give 1.82 g of total acetates. Chromatography on silica gel ($CHCl_3$—CH_3OH eluent, 10:1 → 10:2) isolated compound **10a** (0.25 g) and substance **12a** (0.05 g).

Separation of Butanol Extract. Distributive extraction of sample 3 in EA—water and subsequent evaporation of the EA extract under vacuum produced a fraction of phenolic compounds (0.07 g, 2.4% of the fraction mass and 0.07% of the

extract mass). According to HPLC and PMR spectra [recorded after decolorization by boiling with activated carbon (0.2 g) in ethanol (50 ml)], the fraction contains a mixture of compounds **7** and **8**. The aqueous extract of the butanol extract of sample 3 (1.62 g) was evaporated under vacuum and dissolved in CH_3OH to yield **10** (0.42 g). The contents of the mother liquor (1.2 g) were chromatographed on silica gel ($CHCl_3$—CH_3OH eluent, 10:1 → 10:5) to give a fraction (0.15 g) enriched in **12** and **13** (0.09 g), which was further purified by recrystallization from acetonitrile and ethanol.

Properties of Pure Compounds. (+)-(R)-Licoricidin (2′4′7-Trihydroxy-5-methoxy-3′,6-diisopentenyl-isoflavan) (1). Mp 158-162°C (ether), $[\alpha]_{580}$ +20° (*c* 1.0, MeOH). UV spectrum [λ_{max}, nm (log ε)]: 212 (4.45), 276 (3.58), 283 (3.64). ^1H and ^{13}C NMR data are analogous to those reported in the literature [4]. Triacetate of (+)-(R)-licoricidin, mp 130-132°C (ethanol), $[\alpha]_{580}$ +38° (*c* 0.5, $CHCl_3$). PMR spectrum ($CDCl_3$, δ, ppm, J, Hz): 1.65, 1.66, 1.70, 1.72 (all s, 12H, 4×CH_3, H-10′, 11′, 12, 13), 2.23, 2.27, 2.28 (all s, 9H, 3×C$\underline{H}_3$CO), 2.75 (1H, dd, J_{gem} = 14.0, $J_{3,4}$ = 13.2, H-4), 3.04 (1H, dd, J = 2.0, 4.2 and 14.0, H-4), 3.17 (1H, m, H-3), 3.19 (2H, dm, J_{gem} = 14.0, $J_{7',8'}$ = 7.0, H-7′), 3.20 (2H, dm, J_{gem} = 14.0, $J_{9,10}$ = 7.0, H-9), 3.67 (3H, s, OC$\underline{H}_3$), 3.90 (1H, t, J = 10.1, H-2), 4.21 (1H, ddd, J = 2.0, 3.2, 10.1, H-2), 5.01 (1H, tm, $J_{10,9}$ = 7.0 and 0.8, H-10), 5.09 (1H, tm, $J_{8',7'}$ = 7.0 and 0.9, H-8′), 6.39 (1H, s, H-8), 6.98 (1H, d, $J_{5',6'}$ = 8.5, H-5′), 7.08 (1H, d, $J_{6',5'}$ = 8.5, H-6′).

Glycyrol (3,9-Dihydroxy-1-methoxy-2-isopentenylcoumestane) (2). Mp 244-248°C (acetone). IR spectrum (ν, cm^{-1}): 1580, 1620, 1640, 1715, 3350, 3460. UV spectrum [λ_{max}, nm (log ε)]: 227 (4.36), 244 (4.12), 255 (4.27), 348 (4.16), 360 (4.10). ^1H and ^{13}C NMR data are analogous with those reported in the literature [5]. Glycyrol diacetate, mp 195-196°C (ethanol). PMR spectrum ($CDCl_3$, δ, ppm, J, Hz): 1.68, 1.79 (both s, 6H, 2×C$\underline{H}_3$, H-4′, 5′), 2.32, 2.35 (both s, 2×C$\underline{H}_3$CO), 3.36 (2H, dm, J_{gem} = 14.0, $J_{1',2'}$ = 6.2, H-1′), 3.99 (3H, s, OC$\underline{H}_3$), 5.09 (1H, t, $J_{1',2'}$ = 6.2, H-2′), 7.08 (1H, s, H-4), 7.20 (1H, dd, $J_{8,7}$ = 8.6, $J_{8,10}$ = 2.0, H-8), 7.53 (1H, d, $J_{10,8}$ = 2.0, H-10), 7.72 (1H, d, $J_{7,8}$ = 8.6, H-7).

Glycyrin [3-(2′,4′-Dihydroxyphenyl)-5,7-dimethoxy-6-isopentenylcoumarin] (3) [9]. Mp 208-211°C (acetone). UV spectrum [λ_{max}, nm (log ε)]: 250 (4.22), 259 (4.25), 352 (4.28). Glycyrin diacetate, mp 140-142°C (ethanol). ^1H and ^{13}C NMR data are analogous to those reported in the literature [9].

Licoricone [7,6′Dihydroxy-2′,4′-dimethoxy-3′-(isopentenyl)-isoflavone] (4). Mp 254-257°C (acetone) [7]. IR spectrum (ν, cm^{-1}): 1505, 1580, 1610, 1625, 3150, 3500. UV spectrum [λ_{max}, nm (log ε)]: 238 (4.31), 244 (4.24), 284 (4.03), 304 (3.87), 248 (4.38), 254 (4.31), 305 (4.16), 330 (3.83) (ethanol + KOH). Licoricone diacetate, mp 141-143.5°C (acetone). ^1H and ^{13}C NMR data are analogous to those reported in the literature [7].

Licocoumarone [2-(2′,4′-Dihydroxyphenyl)-6-hydroxy-5-isopentenyl-4-methoxybenzofuran] (5). Mp 182-185°C (acetone) [10]. ^1H and ^{13}C NMR data are analogous to those reported in the literature [10].

Gancaonin H [7-Hydroxy-6-isopentenyl-3-(3′-hydroxy-9′-dimethyl-7′,8′-dehydrochromen-1′-yl)-isoflavone] (6). Mp 204-207°C (acetone). UV spectrum [λ_{max}, nm (log ε)]: 268 (4.15), 332 (3.94). ^1H and ^{13}C NMR data are analogous to those reported in the literature [5].

Isoglycyrol [9-Hydroxy-1-methoxy-(2,2′-dimethylchroman)-2,3:2′,3′-coumestane] (7). Mp 302-304°C (acetone). IR spectrum (ν, cm^{-1}): 1590, 1618, 1635, 1715, 3380, 3450. UV spectrum [λ_{max}, nm (log ε)]: 227 (4.35), 244 (4.31), 255 (4.12), 347 (4.30), 360 (4.30). ^1H and ^{13}C NMR data are analogous to those reported in the literature [5].

Licopyranocoumarin {7,8-Dihydro-3-(2′,4′-dihydroxyphenyl)-8-hydroxymethyl-5-methoxy-8-methyl-2H,6H-benzo[1,2-b:5,4-b′]-dipyran-2-one} (8). Mp 135-137°C (ethanol), $[\alpha]_{580}$ +10° (*c* 0.7, acetone) [8]. IR spectrum (ν, cm^{-1}): 1570, 1615, 1695. UV spectrum [λ_{max}, nm (log ε)]: 211 (4.29), 260 (4.16), 353 (4.22). ^1H NMR data are analogous to those reported in the literature [8]. ^{13}C NMR spectrum (δ_C, ppm): 17.62 (t, C-6), 21.99 (q, CH_3), 27.79 (t, C-7), 62.63 (q, OCH_3), 68.51 (t, C$\mathbf{H}_2$–OH), 79.21 (s, C-8), 100.84 (d, C-10), 104.54 (d, C-3′), 108.81 (d, C-5′), 109.21 (s, C-5a), 113.51, 115.80 (both s, C-1′, 3), 122.98 (s, C-4a), 132.98 (d, C-6′), 138.83 (d, C-4), 154.92, 156.65, 157.87, 159.39, 159.92 (all s, C-2′, 4′, 5, 9a, 10a), 162.23 (s, C-2). Licopyranocoumarin triacetate, mp 85-88°C (ether). ^1H NMR data are analogous to those reported in the literature [10].

1-Methoxyficifolinol (3,9-Dihydroxy-1-methoxy-2,8-diisopentenyl-11b,6a-dihydrocoumestane) (9). Mp 127-130°C (ethanol), $[\alpha]_{580}$ -134° (*c* 1.5, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1580, 1625, 1640, 3360, 3450. UV spectrum [λ_{max}, nm (log ε)]: 235 (4.37), 290 (3.97). ^1H and ^{13}C NMR data are analogous to those reported in the literature [6, 7].

Liquiritin [4′-O-(β-D-Glucopyranosyl)-7-hydroxyflavan-4-one] (10). Mp 210-213°C (ethanol), $[\alpha]_{580}$ -54.7° (*c* 0.5, CH_3OH) [22]. IR spectrum (ν, cm^{-1}): 1580, 1620, 1640, 3300. UV spectrum [λ_{max}, nm (log ε)]: 215 (4.22), 217 (4.19), 230 (4.12), 277 (3.89), 312 (3.66), 215 (4.20), 259 (3.69), 278 (3.20), 338 (4.01), 401 (2.86) (ethanol + KOH). ^1H and ^{13}C NMR data are analogous to those reported in the literature [22]. Liquiritin acetate (**10a**), mp 195-196°C (ethanol), $[\alpha]_{580}$ +38° (*c* 0.5, $CHCl_3$). PMR spectrum ($CDCl_3$, δ, ppm, J, Hz): 2.02, 2.03, 2.05, 2.07, 2.29 (all s, 15H, 3H×5, C$\underline{H}_3$–Ac), 2.82 (1H, dd,

J_{gem} = 17.0, $J_{3\beta,2}$ = 3.2, H-3β), 3.08 (1H, dd, J_{gem} = 17.0, $J_{3\alpha,2}$ = 12.8, H-3α), 3.95 (1H, m, H-5″), 4.20 (1H, dd, J_{gem} = 12.0, $J_{6'',4''}$ = 2.2, H-6″), 4.38 (1H, dd, J_{gem} = 12.0, $J_{6'',5''}$ = 5.1, H-6″), 5.20 (4H, m, H-1″, 2″, 3″, 4″), 5.50 (1H, dd, $J_{2,3\alpha}$ = 12.8, $J_{2,3\beta}$ = 3.2, H-2), 6.83 (1H, d, $J_{8,6}$ = 2.1, H-8), 6.75 (1H, dd, $J_{6,5}$ = 8.6, $J_{6,8}$ = 2.1, H-6), 7.07 (2H, d, J_{vic} = 8.6, H-3′, 5′), 7.44 (2H, d, J_{vic} = 8.6, H-2′, 6′), 7.91 (1H, d, $J_{5,6}$ = 8.6, H-5). ^{13}C NMR spectrum (δ_C, ppm): 20.27 (s, $\underline{C}H_3$×4), 20.79 (s, CH_3), 44.07 (t, C-3), 61.61 (t, C-6″), 66.20 (d, C-4″), 70.97 (d, C-2″), 72.10, 72.42 (both d, C-3″, 5″), 79.44 (d, C-2), 98.67 (d, C-1″), 110.99 (d, C-8), 115.56 (d, C-6), 118.72 (s, C-4a), 117.02 (d, C-3′, 5′), 127.68 (d, C-2′, 6′), 128.07 (d, C-5), 133.45 (s, C-1′), 157.06 (s, C-4′), 162.22 (s, C-8a), 156.60 (s, C-7), 166.41, 169.07, 169.23, 169.80, 170.17 (all s, C–Ac), 190.28 (s, C-4).

4′-Methylglucoliquiritigenin [7-O-(β-D-Glucopyranosyl)-4′-methoxyflavan-4-one] (11). Mp 173-175°C (ethanol), $[\alpha]_{580}$ -42.5° (*c* 0.8, CH_3OH). IR spectrum (ν, cm^{-1}): 837, 1014, 1074, 1512, 1608, 1664, 3386. UV spectrum [λ_{max}, nm (log ε)]: 215 (4.24), 230 (3.74), 276 (3.39), 314 (3.42), 335 (2.76), 218 (4.16), 229 (4.03), 278 (3.88), 314 (3.87), 350 (3.07) (ethanol + KOH). PMR spectrum [$(CD_3)_2CO$, δ, ppm, J, Hz]: 2.76 (1H, dd, J_{gem} = 16.8, $J_{3\beta,2}$ = 2.5, H-3β), 3.05 (1H, dd, J_{gem} = 16.8, $J_{3\alpha,2}$ = 12.5, H-3α), 3.50 (4H, m, H-2″, 5″), 3.66 (3H, s, OC$\underline{H}_3$), 3.72 (1H, dd, J $_{gem}$= 12.0, J $_{6'',5''}$= 5.0, H-6″), 3.92 (1H, dd, J_{gem} = 12.0, $J_{6'',5}$ = 2.0, H-6″), 4.96 (1H, d, $J_{1'',2''}$ = 7.2, H-1″), 5.47 (1H, dd, $J_{2,3\alpha}$ = 12.5, $J_{2,3\beta}$ = 2.5, H-2), 6.39 (1H, d, $J_{8,6}$ = 2.0, H-8), 6.53 (1H, dd, $J_{6,5}$ = 8.5, $J_{6,8}$ = 2.0, H-6), 7.16 (2H, d, J_{vic} = 8.6, H-3′, 5′), 7.46 (2H, d, J_{vic} = 8.6, H-2′, 6′), 7.75 (1H, d, $J_{5,6}$ = 8.6, H-5). ^{13}C NMR spectrum (δ_C, ppm): 44.93 (t, C-3), 56.25 (q, OCH_3), 62.51 (t, C-6″), 71.37 (d, C-4″), 74.90 (d, C-2″), 77.97, 78.15 (both d, C-3″, 5″), 80.68 (d, C-2), 102.20 (d, C-1″), 103.86 (d, C-8), 111.83 (d, C-6), 115.02 (s, C-4a), 117.83 (d, C-3′, 5′), 128.78 (d, C-2′, 6′), 129.86 (d, C-5), 134.44 (s, C-1′), 159.20 (s, C-4′), 165.38 (s, C-8a), 166.78 (s, C-7), 193.24 (s, C-4). Found, %: C 61.4, H 5.7. $C_{22}H_{24}O_9$. Calc., %: C 61.1, H 5.6.

Liquiritin 4′-β-D-apiofuranoside heptaacetate {4′-O-[β-D-(2′″,3′″,4′″-Tri-O-acetyl)-apiofuranosyl-(1′″→2″)-β-D-(3″,4″,6″-tri-O-acetyl)-glucopyranosyl]-7-acetoxyflavan-4-one} (12a). Mp 145-148°C (ethanol), $[\alpha]_{580}$ +48° (*c* 0.5, ethanol). UV spectrum [λ_{max}, nm (log ε)]: 215 (4.03), 230 (3.90), 278 (3.25), 312 (3.85), 255, 277, 310, 340 (ethanol + KOH). PMR spectrum ($CDCl_3$, δ, ppm, J, Hz): 1.96, 1.97, 1.99, 2.01, 2.03, 2.05, 2.07, 2.13 (all s, 7×CH_3), 2.78 (1H, dd, J_{gem} = 17.0, $J_{3\beta,2}$ = 2.4, H-3β), 2.99 (1H, dd, J_{gem} = 17.0, $J_{3\alpha,2}$ = 12.8, H-3α), 3.80 (4H, m, H-2″, 5″), 3.70 (1H, dd, J_{gem} = 12.0, $J_{6'',5''}$ = 5.2, H-6″), 3.93 (1H, dd, J_{gem} = 12.0, $J_{6'',4''}$ = 2.1, H-6″), 4.06 (1H, m, H-2′″), 4.19 (1H, dd, J_{gem} = 9.5, $J_{\beta4''',2'''}$ = 2.0, H-β4′″), 4.25 (1H, dd, J_{gem} = 9.5, $J_{\alpha4''',2'''}$ = 1.7, H-α4′″), 4.96 (1H, d, $J_{1'',2''}$ = 7.2, H-1″), 5.02 (1H, d, J_{gem} = 10.2, H-5′″), 5.22 (1H, d, J_{gem} = 10.2, H-5′″), 5.38 (1H, br. s, $W_{1/2}$ = 4.4, H-1′″), 5.46 (1H, dd, $J_{2,3\alpha}$ = 12.8, $J_{2,3\beta}$ = 3.2, H-2), 6.42 (1H, d, $J_{8,6}$ = 2.1, H-8), 6.54 (1H, dd, $J_{6,5}$ = 8.8, $J_{6,8}$ = 2.1, H-6), 7.15 (2H, d, J_{vic} = 8.6, H-3′, 5′), 7.43 (2H, d, J_{vic} = 8.6, H-2′, 6′), 7.79 (1H, d, $J_{5,6}$ = 8.8, H-5). ^{13}C NMR spectrum (δ_C, ppm): 20.43, 20.45, 20.47, 20.53, 20.54 (all s, 7×CH_3), 44.04 (t, C-3), 61.93 (t, C-6″), 63.06 (t, C-5′″), 71.86 (d, C-4″), 72.86, 73.90 (both d, C-2″, 4′″), 76.37, 76.43, 76.80 (all d, C-3″, 5″, 2′″), 79.21, 79.80 (both d, C-2, 3′″), 99.37 (d, C-1″), 103.29 (d, C-8), 110.62, 108.79 (both d, C-1′″, 6), 115.23 (s, C-4a), 117.12 (d, C-3′, 5′), 127.60 (d, C-2′, 6′), 129.25 (d, C-5), 133.51 (s, C-1′), 156.81 (s, C-4′), 163.14 (s, C-8a), 163.36 (s, C-7), 169.09, 169.55, 169.96 (all s, 7×C=O), 190.47 (s, C-4).

Liquiritin 4′-β-D-apiofuranoside-7-β-D-glucopyranoside {4′-O-[β-D-Apiofuranosyl-(1′″→2′″)-β-D-glucopyranosyl]-7-O-(β-D-glucopyranosyl)-flavan-4-one} (13). Mp 155-158°C (ethanol), $[\alpha]_{580}$ -48° (*c* 0.5, CH_3OH) [11]. IR spectrum (ν, cm^{-1}): 1070, 1250, 1450, 1520, 1660, 3300. UV spectrum [λ_{max}, nm (log ε)]: 217 (4.10), 230 (3.86), 277 (3.66), 315 (3.39), 218 (4.12), 277 (4.20), 310 (3.45) (ethanol + KOH). PMR spectrum [$(CD_3)_2CO$, δ, ppm, J, Hz]: 2.77 (1H, dd, J_{gem} = 16.5, $J_{3\beta,2}$ = 2.8, H-3β), 3.08 (1H, dd, J_{gem} = 16.5, $J_{3\alpha,2}$ = 12.8, H-3α), 3.20-3.53 (8H, m, H-2″- 5″ and 2′″- 5′″), 3.64 (1H, d, J_{gem} = 9.5, H-β4′″), 3.68 (1H, br. s, $W_{1/2}$ = 5.4, H-2′″), 3.73 (2H, m, H-6″, 6′″), 3.83 (2H, m, H-6″, 6′″), 3.93 (1H, d, J_{gem} = 9.5, H-α4′″), 4.95 (1H, d, $J_{1'',2''}$ = 7.2, H-1″), 4.98 (1H, d, $J_{1''',2'''}$ = 7.0, H-1′″), 5.36 (1H, br. s, $W_{1/2}$ = 5.4, H-1′″), 5.50 (1H, dd, $J_{2,3\alpha}$ = 12.8, $J_{2,3\beta}$ = 2.8, H-2), 6.40 (1H, d, $J_{8,6}$ = 2.1, H-8), 6.54 (1H, dd, $J_{6,5}$ = 8.8, $J_{6,8}$ = 2.1, H-6), 7.17 (2H, d, J_{vic} = 8.5, H-3′, 5′), 7.46 (2H, d, J_{vic} = 8.5, H-2′, 6′), 7.75 (1H, d, $J_{5,6}$ = 8.8, H-5). ^{13}C NMR spectrum (δ_C, ppm): 44.92 (t, C-3), 60.77 (t, C-6′″), 62.51 (t, C-5′″), 71.36, 72.01, 72.55 (all d, C-4″, 2″, 4′″), 73.43, 73.73, 74.28, 74.88, 76.51, 76.68, 76.84 (all d, C-3′″, 2″″, 6″, 4″″, 5″, 3″, 2′″), 77.96, 78.14, 80.66 (all d, C-3″″, 5′″, 2), 99.14 (d, C-1′″), 102.18 (d, C-1″), 103.85 (d, C-8), 110.20 (d, C-1″″), 111.83 (d, C-6), 115.03 (s, C-4a), 117.83 (d, C-3′, 5′), 128.76 (d, C-2′, 6′), 129.86 (d, C-5), 134.42 (s, C-1′), 159.19 (s, C-4′), 165.37, 163.36 (both s, C-7, 8a), 193.23 (s, C-4).

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