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NEW PRENYLATED ISOFLAVANONES FROM THE ROOTS OF *Glycyrrhiza glabra*

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Two new prenylated isoflavanones have been isolated from the roots of Glycyrrhiza glabra *L. along with the known compounds cetoleic acid, β-sitosterol, stigmasterol, lanast-5,24-dien-3β-D-glucuronopyranoside, and glucuronic acid. The structures of the prenylated isoflavanones have been established as 8-isoprenyl-7,4′-dihydroxylicoisoflavanone (glabraisoflavanone A) and 7,3′-dihydroxy-8-isoprenyl-4′-cyclogeranioloxyisoflavanone (glabraisoflavanone B) on the basis of spectral data analyses and chemical reactions.*

Key words: glabraisoflavanone A, glabraisoflavanone B, *Glycyrrhiza glabra*, prenylated isoflavanones, structure elucidation.

Glycyrrhiza glabra L. (Fabaceae), commonly known in trade as liquorice, is a perennial herb distributed in subtropical and warm temperate regions of the world [1]. The roots are reported to have anti-inflammatory, demulcent, expectorant, and tonic properties and are prescribed to treat sore throat, bronchial catarrh, cough, gastric and duodenal ulcers, dyspepsia, allergic reactions, arthritis, rheumatism, tuberculosis, and liver toxicity [1, 2]. The important phytoconstituents isolated from the roots included glycyrrhizin, liquiritic acid, glabranins A and B, glycyrrhetol, glabrolide, formononetin, liquiritin, isoliquertin, and other phenolic compounds [3–9]. This present paper describes the isolation and characterization of the known compounds cetoleic acid (**1**), *β*-sitosterol (**2**), stigmasterol (**3**), lanast-5,24-dien-3*β*-D-glucuronopyranoside (**6**), glucuronic acid (**7**) and of two new prenylated isoflavanones (**4**, **5**) from the roots of *G. glabra* of northern India.

Compound **4,** designated as glabraisoflavanone A, was obtained as a pale yellow crystalline product from chloroform eluates. Its UV λ_{max} at 211, 294 nm and the presence of the O-CH_2-CH-CO sequence in its ^{1}H and ^{13}C NMR spectra (Table 1) accounted for its isoflavanone structure. The presence of a bathochromic shift after addition of sodium acetate in the UV spectrum indicated a free hydroxyl group at C-7. It was assigned a molecular formula of $C_{25}H_{28}O_4$ (*m/z* 392.1652) from HREIMS. A set of three AMX patterns of double doublets in the ^{1}H NMR spectrum at δ 4.45 (J = 10.5, 7.5 Hz), 4.62 (J = 11.3, 5.2 Hz), and 3.91 (J = 5.2, 10.5 Hz) for the aliphatic proton signals and carbon signals at δ 70.83 (C-2), 51.12 (C-3), and 196.89 (C-4) were consistent with compound **4** being an isoflavanone derivative [10, 11]. The ^{1}H and ^{13}C NMR spectra indicated the presence of one 3-methylbut-2-enyl, one hydroxyl, and dimethyl pyranosyl substituents on the isoflavanone skeleton.

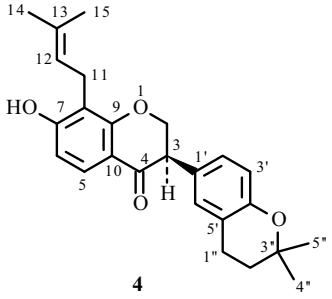

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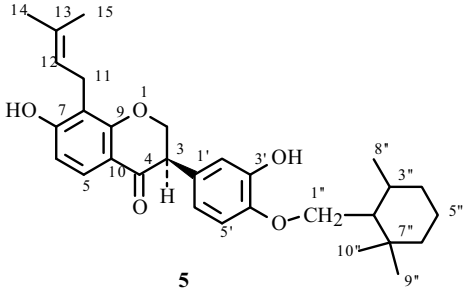
TABLE 1. ^{1}H NMR and ^{13}C NMR Values of Glabraisoflavanones A (**4**) and B (**5**)

Positions	δ_H (J/Hz)		δ_C	
	4	**5**	**4**	**5**
2a	4.45 (dd, J = 10.5, 7.5)	4.61 (dd, J = 11.8, 6.3)	70.83	71.23
2b	4.62 (dd, J = 11.3, 5.2)	4.59 (dd, J = 11.8, 4.5)		
3	3.91 (dd, J = 5.2, 10.5)	2.38 (dd, J = 11.5, 4.5)	51.12	49.21
4	–	–	196.89	197.46
5	7.98 (d, J = 9.6)	7.66 (d, J = 9.42)	129.85	130.23
6	6.28 (d, J = 9.6)	6.26 (d, J = 9.42)	107.41	109.33
7	–	–	166.75	165.82
8	–	–	110.36	106.92
9	–	–	156.53	155.39
10	–	–	111.32	112.63
11	3.17 (d, J = 7.0)	3.31 (d, J = 7.1)	22.14	21.68
12	5.43 (dd, J = 7.0, 7.0)	5.52 (dd, J = 7.1, 7.1)	123.29	121.63
13	–	–	133.16	135.25
14	1.72 br.s	1.79 br.s	17.87	18.36
15	1.70 br.s	1.77 br.s	22.91	22.72
1′	–	–	123.33	122.63
2′	6.97 (dd, J = 2.82, 8.4)	6.83 (d, J = 3)	117.81	119.21
3′	6.91 (d, J = 8.4)	–	115.36	151.18
4′	–	–	157.40	156.33
5′	–	8.37 (d, J = 8.6)	136.27	120.48
6′	7.61 (d, J = 2.82)	6.81 (dd, J = 8.6, 3)	120.91	119.27
1″	2.49 m, 2.03 m	3.43 (d, J = 10.5)	35.63	63.25
		3.38 (d, J = 10.5)		
2″	1.13 m, 1.11 m	1.68 m	28.27	47.81
3″	–	1.62 m	78.38	36.43
4″	1.03 br.s	1.54 m, 1.30 m	19.36	33.18
5″	0.96 br.s	0.88 m, 0.86 m	21.45	27.79
6″	–	1.24 m, 1.30 m	–	23.83
7″	–	–	–	41.66
8″	–	1.12 (d, J = 6.9)	–	19.60
9″	–	1.21 br.s	–	21.08
10″	–	1.17 br.s	–	20.57

The presence of three *ortho*-coupled aromatic protons at δ 7.98 (J = 9.6 Hz), 6.28 (J = 9.6 Hz), and 6.91 (J = 8.4 Hz) assignable to H-5, H-6, and H-3′, one *meta*-coupled doublet at δ 7.61 (J = 2.82 Hz), and an *ortho*, *meta*-coupled double doublet at δ 6.97 (J = 2.82, 8.4 Hz) ascribable to H-6′ and H-2′, respectively, suggested location of the isoprenyl unit at C-8 and dimethyl pyranosyl moiety at C-4′, C-5′. In the mass spectrum of **4** the ion fragment at *m*/*z* 188 $[C_{13}H_{16}O]^+$ resulting from retro-Diels–Alder cleavage of ring C indicated the placement of one hydroxy and one 3-methylbut-2-enyl groups in ring A and the dimethyl pyranosyl moiety in ring B [12] with the biogenetically expected oxygenations at C-5 and C-7; the 3-methylbut-2-enyl group could either be at C-6 or C-8. In the HMBC spectrum, the signal at δ 5.43 (dd, J = 7.0, 7.0, H-12) showed a correlation with C-11 and C-8, and the signal at δ 3.17 (d, J = 7.0 Hz, H_2-11) with C-12, C-13, C-8, C-7, and C-9, supporting the location of the 3-methylbut-2-enyl group at C-8. The H-3 signal at δ 3.91 showed a correlation with C-2, C-1′, C-2′, C-3′, and C-6′, indicating the presence of the dimethyl pyranosyl group at C-4′, C-5′. The ^{13}C NMR values of **4** were compared with the related isoflavanones [13–15]. The positive Cotton effect at 322 nm due to the $n\rightarrow\pi^*$ transitions for the carbonyl group and the equatorial position of ring B was in agreement with the *trans*-diaxial relationship between H_b-2 and H-3 and accounted for the (3*R*) absolute configuration on the basis of the octant rule modified for the cyclic aryl ketones [16]. On the basis of the spectral data analysis, the structure of **4** is 8-isoprenyl-7,4′-dihydroxylicoisoflavanone.

Compound **5**, named glabraisoflavanone B, was obtained as a pale yellow amorphous powder from chloroform–methanol (49:1) eluants. Its UV λ_{max} absorption at 208 and 293 nm and ^{1}H and ^{13}C NMR spectral data indicated that **5** was also an isoflavanone derivative. It showed a molecular ion peak at *m*/*z* 478.1243, consistent with the molecular formula

$C_{30}H_{38}O_5$ having two hydroxyl groups, one 3-methylbut-2-enyl, and cyclogeranioloxy substituents. The mass spectrum exhibited ion fragments at *m*/*z* 274 and 204 resulting from a retro-Diels–Alder fragmentation [12], indicating the presence of one hydroxy and one 3-methylbut-2-enyl substituents in ring A and one each of hydroxyl and cyclogeranioloxy substituents in ring B. The three sets of double doublets of the AMX pattern at δ 4.61 (J = 11.8, 6.3 Hz), 4.59 (J = 11.8, 4.5 Hz), and 2.38 (J = 11.5, 4.5 Hz) in the ^{1}H NMR spectrum and the carbon signals at δ 71.23 (C-2), 49.21 (C-3), and 197.46 (C-4) in the ^{13}C NMR spectrum (Table 1) were assigned to the aliphatic positions of the isoflavanone. The presence of three *ortho*-coupled doublets at δ 7.66 (J = 9.42 Hz), 6.26 (J = 9.42 Hz), and 8.37 (J = 8.6 Hz), assigned to H-5, H-6, and H-5′, respectively, one *meta*-coupled doublet at δ 6.83 (J = 3.0 Hz) ascribed to H-2′, and one *ortho*, *meta*-coupled double doublet at δ 6.81 (J = 8.6, 3.0 Hz) assigned to H-6′, indicated the location of the hydroxyl group at C-7, 3-methylbut-2-enyl at C-8, and oxygenation at C-3′ and C-4′. A one-proton doublet at δ 3.31 (J = 7.1 Hz, H_2-11), a one-proton double doublet at δ 5.52 (J = 7.1, 7.1 Hz), and two three-proton broad signals at δ 1.79 (Me-14) and 1.77 (Me-15) were assigned to isoprenyl protons. Two one-proton doublets at δ 3.43 (J = 10.5 Hz) and 3.38 (J = 10.5 Hz) were attributed to oxygenated methylene protons H_2-1″. A three-proton doublet at δ 1.12 (J = 6.9 Hz) and two three-proton broad signals at δ 1.21 and 1.17 were ascribed correspondingly to secondary C-8″ and tertiary C-9″ and C-10″ methyl protons. In HMBC the H_2-11 methylene ^{1}H NMR signal at δ 3.31 correlated with the ^{13}C carbon signal C-8, C-7, and C-9; H-5′ with C-6′, C-4′, and C-1″; and H-3 with C-2, C-4, and C-1′. The positive Cotton effect at 327 nm due to the $n\rightarrow\pi^*$ transitions for the carbonyl group and the equatorial position of ring B was in agreement with the *trans*-diaxial relationship between H_b-2 and H-3 and accounted for the (3*R*) absolute configuration on the basis of the octant rule modified for the cyclic aryl ketones [16]. On the basis of the foregoing account the structure of **5** is 7,3′-dihydroxy-8-isoprenyl-4′-cyclogeranioloxy isoflavanone.



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EXPERIMENTAL

Plant Material. The roots of *G. glabra* were collected from the local market of Khari Baoli, New Delhi and identified by Dr. M. P. Sharma, taxonomist, Department of Botany, Faculty of Science, Jamia Hamdard (Hamdard University). A voucher specimen No. KB/ND/PRL/GG/10 was deposited in the Herbarium of the Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction and Isolation The air-dried roots (2 kg) of *G. glabra* were coarsely powdered and extracted exhaustively in a Soxhlet apparatus with methanol for 72 h. The methanolic extract was concentrated under reduced pressure in a Buchi rotavapor to obtain a dark green viscous mass. A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous dark green mass was adsorbed on a silica gel (60–120 mesh) column after being dissolved in a small quantity of methanol for preparation of slurry. The slurry (200 g) was air-dried and chromatographed over a silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, a mixture of petroleum ether and chloroform (9:1, 3:1, 1:1, and 1:3), pure chloroform, and finally a mixture of chloroform and methanol (99:1, 98:2, 96:4, 95:5, 97:3, 9:1). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions (having the same R_f values) were combined and crystallized. The isolated compounds were recrystallized to get the pure compounds.

The structures of cetoleic acid (**1**), β-sitosterol (**2**), stigmasterol (**3**), lanast-5,24-dien-3β-D-glucuronopyranoside (**6**), and glucuronic acid (**7**) were identified by analyses of their spectral data and comparison of physical data with authentic samples. The physicochemical and spectral data of the isolated compounds are reported below.

Cetoleic Acid (1). Elution of the column with petroleum ether–chloroform (3:1) furnished a colorless amorphous powder of **1**, recrystallized from chloroform–methanol (1:1), 145 mg (0.009% yield), R_f 0.21 (petroleum ether–chloroform 7:3), mp 94–98°C; UV λ_{max} (methanol): 214 nm; IR ν_{max} (KBr): 3450, 2945, 2855, 1690, 1645, 1455, 1310, 1205, 1110, 1020, 725 cm^{-1}; ^1H NMR ($CDCl_3$, δ, J/Hz): 5.57 (2H, m, H-11), 5.07 (1H, m, H-12), 2.98 (1H, d, J = 10.98, H_2-2a), 2.95 (1H, d, J = 10.98, H_2-2b), 2.35 (1H, m, H_2-10a), 2.29 (1H, m, H_2-10b), 2.03 (2H, m, H_2-13), 1.63 (2H, m, CH_2) 1.41 (2H, m, CH_2), 1.30 (4H, br.s, 2 × CH_2), 1.25 (22H, br.s, 11 × CH_2), 0.85 (3H, t, J = 6.6, Me-22). MS *m*/*z* (rel. int.): 338 $[M]^+$ ($C_{22}H_{42}O_2$) (65.3), 279 (21.61), 191 (19.5).

***β*-Sitosterol (2).** Elution of the column with chloroform gave colorless crystals of **2**, recrystallized from methanol, 420 mg (0.014% yield), R_f 0.43 (petroleum ether–chloroform–methanol 1: 4: 1), mp 137–138°C; EIMS *m*/*z:* 414 $[M^+]$ ($C_{29}H_{50}O$) (10.5).

Stigmasterol (3). Compound **3** was obtained as colorless crystals from the chloroform eluates and recrystallized from methanol, 820 mg (0.041% yield), R_f 0. 31 (chloroform–methanol 9:1), mp 168–169°C; UV λ_{max} (methanol): 226, 211 nm; IR ν_{max} (KBr): 3675, 3650, 2923, 2390, 1635, 1470, 1365, 1260, 1120 cm^{-1}; EIMS *m*/*z*: 412 $[M^+]$ ($C_{29}H_{48}O$) (6.3).

Grabraisoflavanone A (4). Further elution of the column with chloroform afforded pale yellow crystals of **4**, which were recrystallized from methanol, 990 mg (0.0495% yield), R_f 0.17 (chloroform–methanol 49:1), mp 107–109°C; CD (MeOH; C 2.2×10^{-2}): $[Q]_{322}$ = + 425; UV λ_{max}: 211, 294 nm (log ε 5.2, 5.8); λ_{max} (AcONa): 210, 321 nm; IR ν_{max} (KBr): 3490, 2928, 2888, 1700, 1614, 1557, 1507, 1397, 1277, 1231, 1126, 1005, 937 cm^{-1}; ^1H NMR: (Table 1); ^{13}C NMR: (Table 1); +ve FAB MS *m*/*z* (rel. int): 392 $[M]^+$ ($C_{25}H_{28}O_4$) (10.5), 323 (18.6), 260 (47.3), 204 (11.3), 188 (35.7), 162 (100), 161 (13.2). HREIMS *m*/*z*: 392.1652 $[M]^+$.

Glabraisoflavanone B (5). Elution of the column with chloroform–methanol (49:1) furnished a pale yellow amorphous powder of **5**, which was recrystallized from methanol, R_f 0.17 ($CHCl_3$–MeOH 8.9:1.1), mp 160–162°C; UV λ_{max} (MeOH): 208, 293 nm (log ε 4.1, 5.3); λ_{max} (AcONa): 209, 325 nm; IR ν_{max} (KBr): 3447, 2930, 2850, 1700, 1614, 1507, 1458, 1397, 1231, 1126, 1000, 835 cm^{-1}; ^1H NMR: (Table 1); ^{13}C NMR: (Table 1); +ve ion FAB MS *m*/*z*: 478 $[M]^+$ ($C_{30}H_{38}O_5$) (15.8), 409 (48.6), 339 (12.1), 274 (27.9), 247 (21.3), 231 (16.2), 204 (43.5) 162 (100), 139 (18.5), 155 (32.9). HREIMS *m*/*z*: 478.1243 $[M]^+$.

Lanast-5,24-dien-3*β*-D-glucuronopyranoside (6). Elution of the column with chloroform–methanol (19:1) furnished a colorless amorphous powder of **6**, which was recrystallized from methanol, 540 mg (0.027% yield), R_f 0.28 ($CHCl_3$–MeOH 9:1), mp 134–136°C; CD (MeOH: C 1.5×10^{-2}): $[Q]_{322}$ = + 563; UV λ_{max} (methanol): 208, 324 nm; IR ν_{max} (KBr): 3400, 2927, 2855, 1700, 1614, 1457, 1370, 1225, 1225, 1120, 1037 cm^{-1}; ^1H NMR ($CDCl_3$, δ, J/Hz): 5.36 (2H, br.s, H-5, H-24), 5.09 (1H, br.s, H-1′), 4.68 (1H, br.s, H-5′), 4.59 (1H, m, H-3′), 4.28 (1H, m, H-4′), 3.66 (1H, m, H-2′), 2.31 (2H, m, H_2-7), 2.05 (2H, m, H_2-23), 1.68 (3H, br.s, Me-26), 1.60 (3H, br.s, Me-27), 1.25 (3H, br.s, Me-19), 1.02 (3H, br.s, Me-29), 0.96 (3H, d, J = 6.84, Me-21), 0.82 (3H, br.s, Me-28), 0.78 (3H, br.s, Me-30), 0.76 (3H, br.s, Me-18); +ve ion FAB MS *m*/*z*: 602 $[M]^+$ ($C_{36}H_{58}O_7$).

Glucuronic Acid (7). Elution of the column with the chloroform–methanol (9:1) mixture furnished colorless crystals of **7**, which were recrystallized from methanol, 980 mg (0.044% yield), R_f 0.19 ($CHCl_3$–MeOH 9.5:0.5), mp 163–165°C; IR ν_{max} (KBr): 3510, 3460, 3320, 2920, 2950, 1460, 1310, 1205 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 5.17 (1H, br.s, H-1), 4.56 (1H, m, H-5), 3.87 (1H, m, H-3), 3.41 (1H, br.s, H-4), 3.17 (1H, br.s, H-2).

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