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ISOPRENYLATED FLAVONOIDS FROM HAIRY ROOT CULTURES OF *GLYCYRRHIZA GLABRA*

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Key Word Index—*Glycyrrhiza glabra*; Leguminosae; licorice; hairy root; flavonoids; prenylated flavonoids; licoagrochalcone A; licoagrocarpin.

Abstract—Two new prenylated flavonoids, licoagrochalcone A and licoagrocarpin, were isolated from the hairy root cultures of *Glycyrrhiza glabra* along with eight known flavonoids. On the basis of spectroscopic evidence, the structures of the new compounds were elucidated as 3-prenyl-2′,4,4′-trihydroxychalcone and (6a*R*, 11a*R*)-4-prenyl-3-hydroxy-9-methoxypterocarpan, respectively.

INTRODUCTION

The roots of *Glycyrrhiza* species containing glycyrrhizin as the main sweetening principle have long been used as one of the most important crude drugs in China and Europe. In recent years, the antimicrobial [1–3] and antioxidant [4] activities of flavonoids isolated from *G. glabra* were reported. A study on establishment of the hairy root culture system of *G. glabra* has been reported by Leena *et al.* [5]. However, there are no work on the secondary metabolites of *G. glabra* hairy root cultures. In our studies on hairy root cultures, we also established several clones of *G. glabra* hairy root induced by *Agrobacterium rhizogenes* pRi 15834; pBI 121 (GUS). In this paper, we report the isolation of two new flavonoids, named licoagrochalcone A and licoagrocarpin, together with eight known flavonoids from *G. glabra* hairy root.

RESULTS AND DISCUSSION

The hairy root cultures of *G. glabra* were induced from axenic young plants by direct infection with *A. rhizogenes* pRI 15834; pBi 121 (GUS) according to the previous papers [6]. The opines, agropine and mannopine, were detected from the hairy roots by paper electrophoresis. Mass culture was carried out on woody plant medium. After 4 weeks culture, the hairy roots were harvested. The dried roots were extracted with methanol and then partitioned with ethyl acetate and water. The ethyl acetate extract was chromatographed on a silica gel column and further purified by ordinary-phase HPLC to give two new flavonoids, licoagrochalcone A (**4**) and licoagropin (**10**), and eight known flavonoids, isobavachalcone (**1**) [7], 4-hydroxylonchocarpin (**2**) [8], kanzonol B (**3**) [9], abssinone II (**5**) [10], glabrol (**6**) [11], euchrenone a5 (**7**) [12], xambioona (**8**) [13, 14], 3-hydroxyglabrol (**9**) [15].

Licoagrochalcone A (**4**) was obtained as a yellow powder. Its molecular formula, $C_{20}H_{20}O_4$, was established by the HR-FAB mass spectrum. The ^{1}H NMR spectrum of **4** showed two one-proton doublets at δ 7.73 and 7.83 (J = 15.5 Hz) characteristic of trans-olefinic protons of chalcone. The spectrum also exhibited the presence of a prenyl group at δ 1.74, 1.76 (each 3H, *d*, J = 0.5 Hz), 3.38 (2H, *d*, J = 7.0 Hz), 5.38 (1H, *m*) and chelated OH group at δ 13.66. Furthermore, two ABX-type aromatic proton signals appeared at δ 6.94 (*d*, J = 8.5 Hz), 7.58 (*dd*, J = 8.5, 2.5 Hz), 7.63 (*d*, J = 2.5 Hz) due to A ring protons and δ 6.37 (*d*, J = 2.5 Hz), 6.47 (*dd*, J = 8.5, 2.5 Hz), 8.10 (*d*, J = 8.5 Hz) due to B ring protons. The ^{13}C NMR spectrum indicated the presence of 20 carbon signals and a carbonyl group at δ 192.8. These data suggested that **4** is a prenylated chalcone derivative. The structure of **4** was presumed to be 3-prenyl 2′,4,4′-trihydroxychalcone by a comparison with the ^{1}H and ^{13}C NMR data of kanzonol B (**3**). In order to confirm this substitution pattern and also for more accurate assignment of ^{13}C NMR data, the HMBC spectrum was measured to give long-range correlation as shown in Fig. 1. These data supported the structure of **4**.

Licoagrocarpin (**10**) a white powder, was analysed for $C_{21}H_{22}O_4$ from its HR-FAB mass spectrum. The

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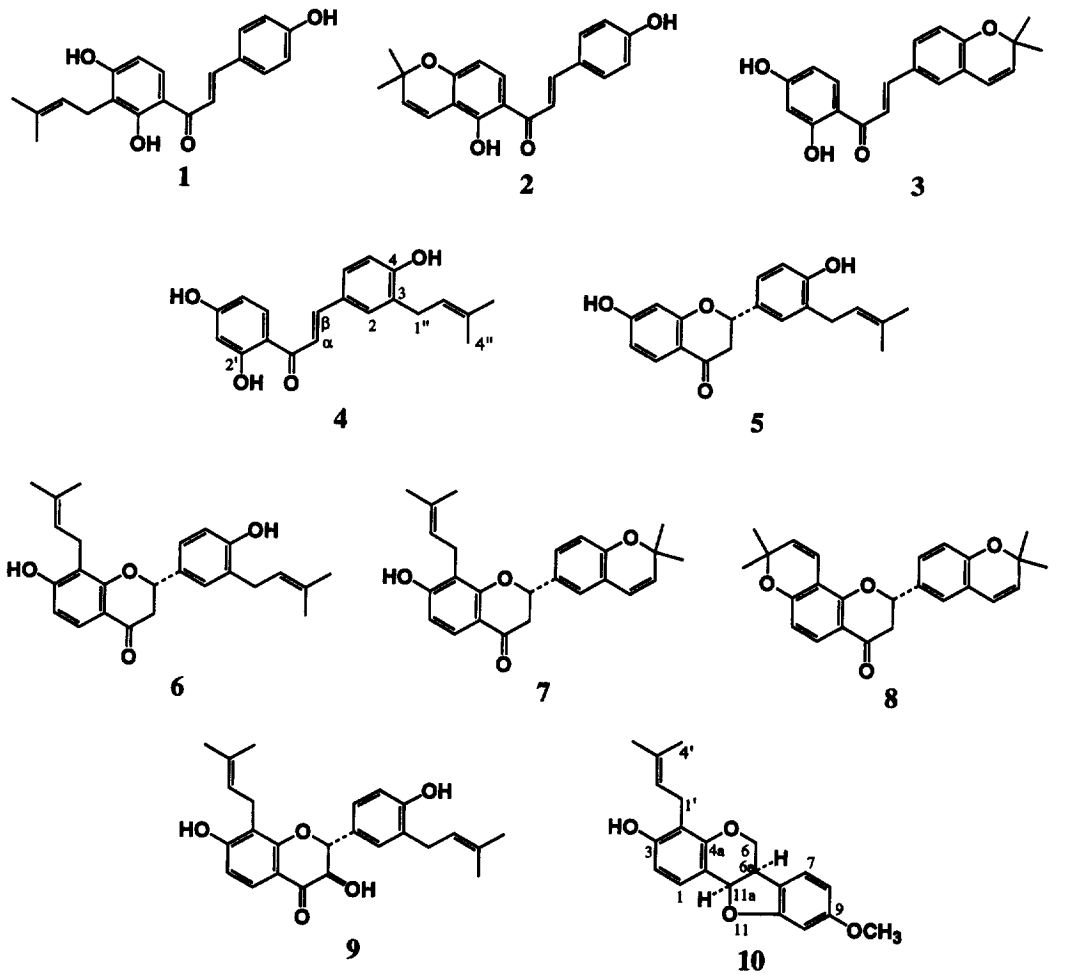

^{1}H NMR spectrum of **10** showed a set of four proton signals at δ 3.60 (1H, *m*, H_β-6), 3.60 (1H, *m*, H-6a), 4.34 (1H, *m*, H_α-6) and 5.53 (1H, *ddd*, J = 6.0, 2.0, 0.5 Hz, 11a-H), indicating that **10** is a pterocarpan. In addition, the ^{1}H NMR spectrum exhibited the presence of a prenyl group at δ 1.63 (3H, *d*, J = 0.5 Hz), 1.75 (3H, *d*, J = 0.5 Hz), 3.33 (2H, *d*, J = 7.0 Hz), 5.54 (1H, *m*) and a methoxyl group at δ 3.75. The ^{1}H NMR spectrum further showed the *ortho*-coupled aromatic protons at δ 6.61 (*d*, J = 8.5 Hz), 7.17 (*dd*, J = 8.5, 0.5 Hz) and the protons in an ABX spin system at δ 6.38 (*d*, J = 2.5 Hz), 6.46 (*dd*, J = 8.5, 2.5 Hz), 7.24 (*br d*, J = 8.5 Hz). These aromatic protons were assigned by the decoupling experiment, suggesting that the proton at C-11a is coupled with *ortho*-coupled H-1 (δ 7.17) and the proton at C-6a with H-7 (δ 7.24) through ^4J, respectively. The position of the methoxyl group was assigned to be located at C-9 by NOE measurement: the enhancement of the 8-H [δ 6.46 (1H, *dd*, J = 8.5, 2.5 Hz)] was observed by 5%

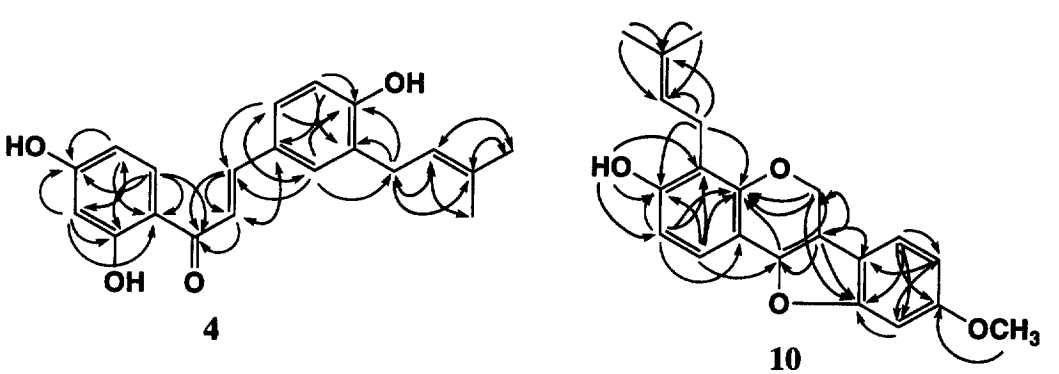

Fig. 1. ^{1}H-^{13}C Long-range correlation by HMBC of flavonoids **4** and **10**.

and the 10-H [δ 6.38 (1H, *d*, $J = 2.5$ Hz)] by 8% when the methoxyl protons were irradiated. The planar structure of **10** was further confirmed by the analysis of the HMBC spectrum (Fig. 1). Two chiral centres at C-6a and C-11a in **10** were determined to be 6a-*R*, 11a-*R* configurations on the basis of a comparison of $[\alpha]_D$ and CD spectrum [16]. Thus, licoagrocarpin (**10**) is (6a*R*, 11a*R*)-4-prenyl-3-hydroxy-9-methoxypterocarpan.

In conclusion, we have isolated two new prenylated flavonoids and eight known flavonoids from the hairy root of *G. glabra*. The known flavonoids, glabrol (**6**), euchrenone a5 (**7**) [17], xambioona (**8**) and 3-hydroxyglabrol (**9**), were previously obtained from the root of *G. glabra*.

EXPERIMENTAL

General. IR: KBr. The OR were measured with a JASCOP DIP-370 digital polarimeter in a 0.5 dm length cell, while the CD spectra were recorded on a JASCO J-720 spectropolarimeter. For HPLC, Waters model 510 HPLC system was used. CC was carried out using Wako-gel C-200. TLC was conducted on Kieselgel $60F_{254}$ plates (Merck).

Mass culture of G. glabra hairy root. The methods of induction of hairy root culture of *G. glabra* were performed according to the previous paper [6]. The opines of hairy roots were detected by paper electrophoresis. Mass culture was carried out on woody plant medium containing 2% sucrose at 25° in the dark, at 50 rpm on a rotary shaker, using 500 ml Erlenmeyer flask containing 200 ml medium.

Extraction and separation procedures of flavonoids. After 4 weeks culture, the hairy roots were harvested. The freeze-dried hairy roots (658.75 g) were extracted with MeOH, × 5. Evapn of the solvent under red. pres. from the combined extract gave the MeOH extract (198.31 g). The extract was then partitioned between EtOAc and H_2O. Removal of the solvent from the EtOAc phase under red. pres. below 40° yielded the EtOAc extract (50.66 g). The EtOAc extract was subjected to silica gel chromatography (Wako gel C-200, *ca* 1500 g) and eluted with *n*-hexane–Me_2CO (3:2) to give seven frs. Further purification of frs 2 and 3 was achieved by repeated HPLC to afford isobavachalcone (**1**, 39.6 mg), 4-hydroxylonchocarpin (**2**, 18.2 mg), kanzonol B (**3**, 15.7 mg), licoagrochalcone A (**4**, 4.0 mg), abssinone II (**5**, 46.1 mg), glabrol (**6**, 647.3 mg), euchrenone a5 (**7**, 62.9 mg), xambioona (**8**, 5.2 mg), 3-hydroxyglabrol (**9**, 6.7 mg), and licoagrocarpin (**10**, 5.2 mg).

Licoagrochalcone A (**4**). Yellow powder. UV λ_{max}^{MeOH} nm (log ε): 298sh (3.84), 382 (4.34). IR ν_{max}^{KBr} cm^{-1}: 3430, 1630, 1560, 1500. EI MS *m/z* (%): 324 ($[M]^+$, 73), 323(31), 188(32), 175(100), 137(87), 133(51). HR-EI MS: Calcd for $C_{20}H_{20}O_4$ $[M]^+$, 324.1362; Found: 324.1366. ^{1}H NMR (400 MHz, acetone-d_6): δ 1.74 (3H, *d*, $J = 1.0$ Hz, H_3-5″), 1.76 (3H, *d*, $J = 0.5$ Hz, H_3-4″), 3.38 (2H, *d*, $J = 7.0$ Hz, H-1″), 5.38 (1H, *m*, H-2″), 6.37 (1H, *d*, $J = 2.5$ Hz, H-3′), 6.47 (1H, *dd*, $J = 8.5$, 2.5 Hz, H-5′), 6.94 (1H, *d*, $J = 8.5$ Hz, H-5), 7.58 (1H, *dd*, $J = 8.5$, 2.5 Hz, H-6), 7.63 (1H, *d*, $J = 2.5$ Hz, H-2), 7.73 (1H, *d*, $J = 15.5$ Hz, H-α), 7.83 (1H, *d*, $J = 15.5$ Hz, H-β), 8.10 (1H, *d*, $J = 8.5$ Hz, H-6′), 13.66 (1H, *s*, 2′-OH). ^{13}C NMR (100 MHz, acetone-d_6): δ 17.9 (C-4″), 25.9 (C-5″), 29.1 (C-1″), 103.8 (C-3′), 108.6 (C-5′), 114.5 (C-1′), 116.3 (C-5), 118.0 (C-α). 123.3 (C-2″), 127.6 (C-1), 129.2 (C-6), 129.7 (C-3), 131.8 (C-2), 132.8 (C-3″), 133.2 (C-6′), 145.6 (C-β), 158.7 (C-4), 165.5 (C-4′), 167.6 (C-2′), 192.8 (C=O).

Licoagrocarpin (**10**). Powder. $[\alpha]_D^{24}$ −78.6° (MeOH; *c* 0.51). CD (MeOH, *c* 0.96×10^{-4}, 24°): $\Delta\varepsilon_{305}$ 0, $\Delta\varepsilon_{287}$ +8.18 (pos. max.), $\Delta\varepsilon_{257}$ 0, $\Delta\varepsilon_{237}$ −18.21 (neg. max.), $\Delta\varepsilon_{225}$ 0. UV λ_{max}^{MeOH} nm (log ε): 284 (3.87), 290sh (3.71). IR ν_{max}^{KBr} cm^{-1}: 3420, 1620, 1600. EI MS *m/z* (%): 338 ($[M]^+$, 100), 282(92), 148(28). HR-EI MS: Calcd for $C_{21}H_{22}O_4$ ($[M]^+$), 338.1518; Found: 338.1507. ^{1}H-NMR (400 MHz, acetone-d_6): δ 1.63 (3H, *d*, $J = 0.5$ Hz, H_3-5′), 1.75 (3H, *d*, $J = 0.5$ Hz, H_3-4′), 3.33 (2H, *d*, $J = 7.0$ Hz, H-1′), 3.60 (1H, *m*, H_β-6), 3.60 (1H, *m*, H-6a), 3.75 (3H, *s*, OCH_3), 4.34 (1H, *m*, H_α-6), 5.53 (1H, *ddd*, $J = 6.0$, 2.0, 0.5 Hz, H-11a), 5.54 (1H, *m*, H-2′), 6.38 (1H, *d*, $J = 2.5$ Hz, H-10), 6.46 (1H, *dd*, $J = 8.5$, 2.5 Hz, H-8), 6.61 (1H, *d*, $J = 8.5$ Hz, H-2), 7.17 (1H, *dd*, $J = 8.5$, 0.5 Hz, H-1), 7.24 (1H, *br d*, $J = 8.5$ Hz, H-7). ^{13}C NMR (100 MHz, acetone-d_6): δ 17.8 (C-4′), 22.9 (C-1′), 25.8 (C-5′), 40.4 (C-6a), 55.7 (OCH_3), 67.5 (C-6), 80.1 (C-11a), 97.1 (C-10), 106.8 (C-8), 109.9 (C-2), 113.1 (C-11b), 116.7 (C-4), 120.6 (C-6b), 123.9 (C-2′), 125.8 (C-7), 129.7 (C-1), 131.0 (C-3′), 155.4 (C-4a), 156.8 (C-3), 161.8 (C-10a), 162.1 (C-9).

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