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Two Antimicrobial Flavanones from the Leaves of *Glycyrrhiza glabra*

HIROSHI FUKUI,*,[a] KATSUMI GOTO[b] and MAMORU TABATA[a]

Faculty of Pharmaceutical Sciences, Kyoto University,[a] Yoshida, Kyoto 606, Japan and The Garden of Medicinal Plants, Kyoto Pharmaceutical University,[b] Hino, Fushimi, Kyoto 601–13, Japan

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A new prenylated flavanone named licoflavanone was isolated, together with pinocembrin, from the leaves of *Glycyrrhiza glabra* var. *typica* as an antimicrobial agent.

Keywords——*Glycyrrhiza glabra*; Leguminosae; flavonoid; flavanone; licoflavanone; pinocembrin; antimicrobial activity

Introduction

Roots of *Glycyrrhiza* species, including *G. glabra* var. *typica*, *G. glabra* var. *glandulifera* and *G. uralensis*, are not only important as crude drugs but also are used widely as a sweetener or a spice.[1] Although many triterpenoids and flavonoids have been isolated from these roots,[2] there have been few reports on chemical constituents in the aerial parts.[3] This paper describes the isolation and structure elucidation of antimicrobial substances in the leaves of *G. glabra* var. *typica*.

Results and Discussion

Air-dried leaves of *G. glabra* var. *typica* collected in Turkey were extracted with MeOH. Bioassay-directed fractionation of the MeOH extract resulted in the isolation of two antimicrobial substances; pinocembrin (**1**) and a new flavanone (**2**) named licoflavanone.

Compound **1** was identified as pinocembrin, which had previously been isolated for *G. glabra*,[3] *Hymenoclea monogyra*[4] and the family Pinaceae,[5] based on its physicochemical properties.[6]

Licoflavanone (**2**), $C_{20}H_{20}O_5$, was concluded to be a derivative of 5,7-dihydroxyflavanone from the close correspondence of its ultraviolet (UV) spectra in MeOH, methanolic base, aluminum chloride and sodium acetate with those of naringenin.[7] This was supported by the presence of two proton signals (δ 5.95, 2H, s) characteristic of C_6-H and C_8-H of 5,7-dihydroxyflavanone and by the diagnostic separation of signals due to C_2-H and C_3-2H of ring C into three double-doublets in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum.[8] In addition, the NMR spectrum indicated the presence of a 3,3-dimethylallyl group directly attached to a benzene ring. Acetylation of **2** yielded a triacetate (**3**). The mass spectrum (MS) of **2** showed fragmentation peaks arising from a retro Diels–Alder reaction[6a] at *m/z* 153 and 188, indicating the location of the dimethylallyl and the third hydroxyl groups on ring B. Their respective locations at C-3′ and C-4′ were determined from the characteristic splits of the ring B signals in the NMR spectrum, showing an ABC system similar to that of glabrol,[9] and by the quantitative conversion of **2** to cyclolicoflavanone (**4**) upon treatment

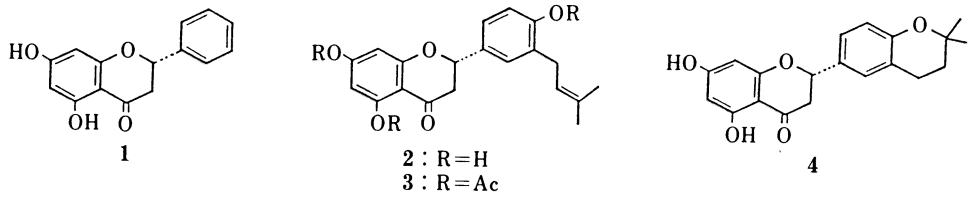

Chart 1

TABLE I. Antimicrobial Activities of Pinocembrin (**1**), Licoflavanone (**2**) and Cyclolicoflavanone (**4**)

Compound	MIC (μg/disc)		
	Bacillus subtilis	*Stapohylococcus aureus*	*Candida albicans*
Pinocembrin (**1**)	64	125	32
Licoflavanone (**2**)	32	64	250
Cycloflavanone (**4**)	125	250	250

with methanolic hydrogen chloride.[9] The S-configuration at C-2 was revealed by the negative Cotton effect at 298 nm (trough) and 273 nm (peak).[10] Therefore, the structure of licoflavanone (**2**) was proved to be (2*S*)-3′-(3,3-dimethylallyl)-4′,5,7-trihydroxyflavonone.

Antimicrobial potencies of **1**, **2** and **4** were estimated by the paper disk method. As shown in Table I, these compounds were not so active against *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*, as we expected, and showed no activity against *Escherichia coli*. These results are similar to those reported for other flavonoids isolated from the roots of *Glycyrrhiza* species.[2a,11]

Experimental

Plant Material——*Glycyrrhiza glabra* var. *typica* was collected near Muş, Turkey in August, 1986. A specimen has been deposited in the Faculty of Pharmaceutical Sciences, Kyoto University, and in The Garden of Medicinal Plants, Kyoto Pharmaceutical University.

Isolation of Antimicrobial Substances——The air-dried leaves (200 g) of *G. glabra* var. *typica* were extracted twice with MeOH (2 l) for 3 d at room temperature. The MeOH extracts were combined and concentrated under reduced pressure to give a dark green syrup (*ca.* 16 g). The syrup was chromatographed on silica gel (500 g, Wako gel C-100, Wako Pure Chemicals, Kyoto) with a mixture of benzene–EtOAc–MeOH. The isolation was guided by bioassay of antimicrobial activities against *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*. The benzene–EtOAc (1 : 1) eluate (9.6 g) showed activity against these microorganisms. Most of the residue (9 g) was rechromatographed on silica gel (450 g, Wako gel C-300) with ethyl ether–benzene (1 : 4). Each eluate (10 g) collected was analyzed by thin layer chromatography (TLC) (silica gel, ether–benzene, 1 : 4); spots on the plate were visualized under UV light and also by spraying vanillin–H_2SO_4 followed by heating at 120 °C for 10 min. Fractions containing the same spots were combined. Two fractions (No. 23—31 and 77—110) showing antimicrobial activity gave pinocembrin (**1**, 1.9 g) and licoflavanone (**2**, 0.98 g), respectively.

Pinocembrin (**1**): Pale yellow needles, mp 198—199 °C, $[\alpha]_D^{32}$ −52° (*c*=0.188, MeOH). UV λ_{max}^{MeOH} nm (log ε); 289 (4.24), 324 (3.73); +NaOMe: 324; +$AlCl_3$: 312, 374; +$AlCl_3$+HCl: 309, 373; +NaOAc; 324; +NaOAc+H_3BO_3: 290, 329 sh. IR ν_{max}^{Nujol} cm^{-1}: 1625, 1600, 1580, 1480, 1460, 1360, 1300. MS *m/z* (rel. int.): 256.07440 (M^+, $C_{15}H_{12}O_4$, 100), 238 (8), 179 (78), 153 (17), 152 (65), 124 (28), 104 (13), 103 (12), 77 (11), 69 (14). ^{1}H-NMR (acetone-d_6, 200 MHz) δ: 2.81 (1H, dd, *J*=17.1, 3.2 Hz, H-3), 3.16 (1H, dd, *J*=17.1, 12.7 Hz, H-3), 5.56 (1H, dd, *J*=3.2, 12.7 Hz, H-2), 5.98 (1H, d, *J*=2.2 Hz, H-6 or 8), 6.01 (1H, d, *J*=2.2 Hz, H-6 or 8), 7.38—7.60 (broad signals, H of ring B).

Licoflavanone (**2**): Pale yellow needles, mp 134—135 °C. $[\alpha]_D^{32}$ −13° (*c*=0.25, MeOH). ORD (*c*=0.004) $[\phi]^{25}$ (nm): 0 (340), −14900 (298) (trough), 0 (288), +14000 (273), +8500 (250) (last wave length measured). UV λ_{max}^{MeOH} nm (log ε): 287 (4.13), 323 sh (3.57); +NaOAc: 323; +$AlCl_3$: 312, 374; +$AlCl_3$+HCl: 309, 373; +NaOAc: 324;

+NaOAc+H_3BO_3: 290, 329 sh. IR ν_{max}^{Nujol} cm^{-1}: 3540, 1620, 1590, 1490, 1450, 1330, 1260. MS *m/z* (rel. int.): 340.13080 (M^+, $C_{20}H_{20}O_5$, 60), 188 (18), 170 (16), 175 (100), 153 (49), 133 (29). ^{1}H-NMR (acetone-d_6, 200 MHz) δ: 1.75 (6H, s, C=C(CH_3)$_2$), 2.72 (1H, dd, J=3.0, 17.0 Hz, H-3), 3.18 (1H, dd, J=12.9, 17.0 Hz, H-3), 3.35 (2H, d, J=7.3 Hz, C$\underline{H}_2$CH=C), 5.36 (1H, t, J=7.3 Hz, -$CH_2C\underline{H}$=C), 5.42 (1H, dd, J=3.0, 12.9 Hz, H-2), 5.95 (2H, s, H-6, 8), 6.89 (1H, d, J=8.3 Hz, H-3′), 7.21 (1H, dd, J=8.3, 2.0 Hz, H-1′), 7.28 (1H, d, J=2.0 Hz, H-6′).

Acetate of 2 (3)——Acetylation of **2** (30 mg) with Ac_2O and pyridine at room temperature and purification by preparative TLC (silica gel, ether : benzene = 1 : 9) afforded **3** (25 mg) as an amorphous solid. UV λ_{max}^{MeOH} nm (log ε): 258 (4.10), 313 (3.66). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1680, 1610, 1420, 1360, 1230. MS *m/z* (rel. int.): 466 (14), 423 (51), 381 (73), 339 (40), 188 (25), 175 (100), 153 (60). ^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 1.70, 1.76 (each 3H, br s, =C(CH_3)$_2$), 2.30, 2.32, 2.38 (each 3H, s, OAc × 3), 2.72 (1H, dd, J=2.9, 17.0 Hz, H-3), 3.03 (1H, dd, J=13.5, 17.0 Hz, H-3), 3.26 (2H, d, J=7.0 Hz, -C$\underline{H}_2$CH=C), 5.22 (1H, br t, J=7.0 Hz, -$CH_2C\underline{H}$=C), 5.46 (1H, dd, J=2.9, 13.5 Hz, H-2), 7.08 (1H, br d, J=9.0 Hz, H-3′), 7.30 (2H, br signal, H-2′, 6′).

Cyclolicoflavanone (4)——A mixture of **2** (30 mg), MeOH (6 ml) and concentrated HCl (2 ml) was refluxed for 5 h. The reaction mixture was diluted with H_2O (40 ml) and extracted with ether. The ether extract was subjected to preparative TLC (silica gel, ether : benzene = 1 : 4, *Rf* = 0.65—0.70) and **4** was crystallized from aqueous MeOH as platelets (28 mg). mp 107 – 108 °C. UV λ_{max}^{MeOH} nm (log ε): 287 (4.17), 323 sh (3.76). IR ν cm^{-1}: 3470, 3360, 1630, 1610, 1500. MS *m/z* (rel. int.): 340 (M^+, 36), 188 (17), 175 (100), 153 (18), 133 (23). ^{1}H-NMR (acetone-d_6, 200 MHz) δ: 1.32 (6H, s, CH_3 of chroman), 1.83 (2H, t, J=6.8 Hz, CH_2 of chroman), 2.70 (1H, dd, J=2.9, 17.2 Hz, H-3), 2.81 (2H, t, J=6.8 Hz, benzylic CH_2 of chroman), 3.18 (1H, dd, J=12.9, 17.2 Hz, H-3), 5.41 (1H, dd, J=2.9, 12.9 Hz, H-2), 5.96 (2H, s, H-6, 8), 6.75 (1H, br d, J=9.0 Hz, H-3′), 7.20—7.30 (2H, br signal, H-2′, 6′).

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