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A New Isoflavone and the Corresponding Isoflavanone of Licorice Root[1]

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As the constituents of Sinkiang licorice root (*Glycyrrhiza* spp., Leguminosae), two new isoflavonoid compounds, licoisoflavone B (V) and licoisoflavanone (XII), were isolated as acetates and their structures were determined by chemical and spectroscopic studies. Besides them, isoliquiritigenin, formononetin, 7,4′-dihydroxyflavone, echinatin (III) and glabrol (IV) were also isolated.

Keywords——licorice root; Sinkiang licorice; *Glycyrrhiza* spp.; licoisoflavone B; licoisoflavanone; echinatin; isoflavone; isoflavanone; PMR

In the previous paper,[3] we reported the isolation of unusual chalcones, licochalcones A (I) and B (II), from Sinkiang licorice, *Glycyrrhiza* spp. (Leguminosae). Since these chalcones have no hydroxyl at 2′-position but a methoxyl at 2-position, they were assumed to be produced biosynthetically by the reverse route of ordinary chalcones; the A ring of these chalcones is formed by the shikimic acid pathway and the B ring by the acetate-malonate pathway. Therefore, we named retrochalcones to these unusual chalcone. This hypothesis was actually proved[4] by the radioisotopic tracer experiments in the biosynthesis of echinatin (III) in the callus induced from *Glycyrrhiza echinata* L.[5]

Further investigation of Sinkiang licorice revealed the presence of isoliquiritigenin,

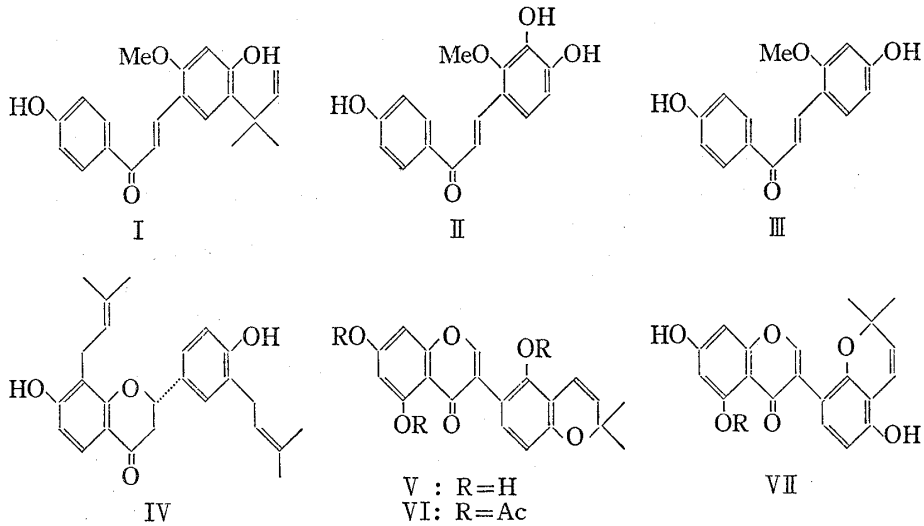

Chart 1

1) Part XLIV in the series of *Chemical Studies on the Oriental Plant Drugs*. Part XLIII: T. Kinoshita, T. Saitoh, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **26**, 141 (1978).
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3) T. Saitoh and S. Shibata, *Tetrahedron Lett.*, **1975**, 4461.
4) T. Saitoh, S. Shibata, U. Sankawa, T. Furuya, and S. Ayabe, *Tetrahedron Lett.*, **1975**, 4463.
5) T. Furuya, K. Matsumoto, and M. Hikichi, *Tetrahedron Lett.*, **1971**, 2567.

formononetin, 7,4′-dihydroxyflavone, glabrol (IV)[6] and echinatin. Although the content of echinatin in Sinkiang licorice is low, its coexistence with licochalcones A and B positively supports that all these chalcones are biosynthesized by the same route.

In addition, two new compounds have been isolated. The following section deals with their structure elucidation.

The ethyl acetate extract of the root bark was roughly separated into about 30 fractions by silica gel chromatography. One of the fractions was further purified by silica gel and Sephadex LH-20 chromatography. A fraction thus obtained showed a homogeneous spot on a thin-layer chromatogram but was revealed to be a mixture of two compounds by proton magnetic resonance (PMR) spectroscopy. After acetylation of the mixture with acetic anhydride and pyridine at room temperature, the separation of the two compounds was carried out by rapid chromatography on silica gel and finally on Sephadex LH-20. Thus, licoisoflavone B and licoisoflavanone were isolated as acetates in crystalline state.

Licoisoflavone B triacetate (VI), mp 181—182°, $C_{26}H_{22}O_9$ (M^+ 478) gave an infrared (IR) band at 1650 cm^{-1}. The mass spectrum showed the presence of three phenolic acetoxyls which gave three singlet signals at δ 2.14, 2.32 and 2.36 in the PMR spectrum, and the PMR also revealed the presence of a chromene ring δ 1.42 (6H, s, 2 × CH_3), 5.60 (1H, d, J=10 Hz) and 6.20 (1H, d, J=10 Hz). The isoflavone character of the compound was indicated by the ultraviolet (UV) spectrum and a characteristic PMR signal at δ 7.68. As for aromatic protons, two pairs of AB doublets were observed with the coupling constants of 8.5 Hz (*ortho*-splitting) and 2.5 Hz (*meta*-splitting). As the parent compound showed a dark colored $FeCl_3$ reaction and no PMR signal low enough to be assigned to 5-H (*peri*-position to 4-carbonyl), a hydroxyl should locate at 5-position. From these results, along with biosynthetical consideration, structure V or VII can be assigned to licoisoflavone B.

From a kind of Chinese licorice roots, licoisoflavone A (VIII) has been isolated, and treatment of VIII with methanolic hydrochloric acid yielded cyclolicoisoflavones A_1 (IX) and A_2 (XI).[1] Hydrogenation of licoisoflavone B triacetate with Pd-C in ethyl acetate afforded dihydrolicoisoflavone B triacetate identified with cyclolicoisoflavone A_1 triacetate (X). Therefore, the structure of licoisoflavone B is represented by V.

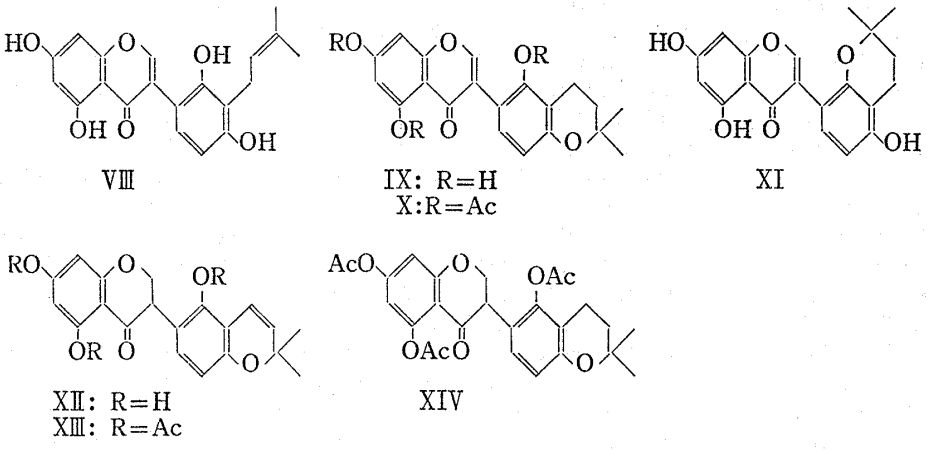

Chart 2

Licoisoflavanone triacetate (XIII), mp 172—173°, $C_{26}H_{24}O_9$ (M^+ 480). From the analysis of the PMR spectrum the substituents of this compound were found to be the same with those of licoisoflavone B triacetate (VI). The characteristic singlet for 2-H in the PMR, however, did not appear but a broad doublet (J=8 Hz) counted for two protons and a triplet (J=8 Hz) for one proton were observed. The IR band which should be noted is one at 1700 cm^{-1}.

6) T. Saitoh, T. Kinoshita, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **24**, 752 (1976).

These data suggested that the compound is a derivative of isoflavanone which is rarely occurring in nature,[7] and it would be an isoflavanone corresponding to licoisoflavone B (V).

Licoisoflavone B triacetate (VI) was hydrogenated in a mixture of acetic acid and ethyl acetate in the presence of PtO_2 to give dihydrolicoisoflavone B triacetate (X), 1650 cm^{-1}, and a tetrahydro compound, 1697 cm^{-1}, and the latter was identical with dihydrolicoisoflavanone triacetate (XIV) derived from licoisoflavanone triacetate (XIII) by hydrogenation with Pd-C. Consequently, licoisoflavanone is represented by the structure XII. Although licoisoflavanone has an asymmetric carbon, it was isolated as a racemate like other natural isoflavanones with an exception of sophorol.[8]

Experimental

Extraction and Isolation——The root bark of dried Sinkiang licorice roots imported from China was extracted with *n*-hexane, and then extracted with ethyl acetate. The AcOEt extract was subjected to rough separation with silica gel column chromatography into about 30 fractions. Each fraction was re-chromatographed on silica gel and/or Sephadex LH-20. Thus, isoliquiritigenin, formononetin, 7,4′-dihydroxyflavone, echinatin (III) and glabrol (IV) were isolated. As for two new compounds, licoisoflavone B (V) and licoisoflavanone (XII), isolation was carried out by rapid column chromatography on silica gel and on Sephadex LH-20 after acetylation because usual isolation methods were not effective. The two parent compounds give same *Rf* value on a thin-layer chromatography (TLC) plate.

Licoisoflavone B Triacetate (VI)——mp 181—182° (recrystallised from EtOH). λ_{max}^{EtOH} nm (log ε): 228 (4.55), 240.5 (4.55, inf.), 245 (4.57, sh.), 249.5 (4.59), 254.5 (4.54), 260.5 (4.37, inf.), 305 (3.87). $\nu_{max}^{CHCl_3}$ cm^{-1}: 2975, 1775, 1650, 1625, 1575, 1490, 1435, 1185, 1120. *m/e*: 478 (M^+, 16.5%), 463 (M−15, 5.8%), 436 (M−42, 17.2%), 394 (M−42×2, 6.9%), 379 (M−42×2—15, 100%), 352 (M−42×3, 11.2%). *Anal.* Calcd. for $C_{26}H_{22}O_9$: C, 65.27; H, 4.64. Found: C, 65.32; H, 4.69.

Dihydrolicoisoflavone B Triacetate (X)——Licoisoflavone B triacetate (24 mg) was hydrogenated in the presence of prereduced 10% Pd–C (40 mg) in AcOEt. Removal of the solvent and recrystallisation of the residue from MeOH gave dihydroisoflavone B triacetate (X) (19.9 mg). mp 208—210°. $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1775, 1650, 1624, 1575, 1495, 1435, 1375, 1190, 1120. *Anal.* Calcd. for $C_{26}H_{24}O_9$: C, 64.99; H, 5.04. Found: C, 64.84; H, 5.06.

Acetylation of Cyclolicoisoflavone A_1 (IX)——Cyclolicoisoflavone A_1 was acetylated with acetic anhydride and pyridine in the usual manner. The product was recrystallised from MeOH, mp 209—212°, and was identified with dihydrolicoisoflavone B triacetate (X) by means of mixed mp, TLC, IR and PMR spectra.

Licoisoflavanone Triacetate (XIII)——mp 172—173° (recrystallised from EtOH), $[\alpha]_D$ 0°. λ_{max}^{EtOH} nm (log ε): 224 (4.71), 244 (4.11, sh.), 250 (4.20, sh.), 255.5 (4.28), 261 (4.27), 313 (3.82). $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000—2900, 1775, 1700, 1620, 1575, 1480, 1440, 1375, 1200. PMR (δ, $CDCl_3$, 100 MHz): 1.44 (6H, s, CH_3 of dimethylchromene), 2.30 (9H, s, 3×OAc), 3.93 (1H, t, 9 Hz, 3-H), 4.49 (2H, d, 9 Hz, 2-H), 5.59 (1H, d, 10 Hz, 3-H of dimethylchromene), 6.15 (1H, d, 10 Hz, 4-H of dimethylchromene), 6.47 (1H, d, 2.5 Hz, 8-H), 6.64 (1H, d, 8.5 Hz, 5′-H), 6.70 (1H, d, 2.5 Hz, 6-H), 6.85 (1H, d, 8.5 Hz, 6′-H). *m/e*: 480 (M^+, 14.0%), 465 (M−15, 8.9%), 438 (M−42, 20.6%), 423 (M−42−15, 35.6%), 396 (M−42×2, 10.6%), 381 (M−42×2-15, 100%), 354 (M−42×3, 8.9%). *Anal.* Calcd. for $C_{26}H_{24}O_9$: C, 64.99; H, 5.04. Found: C, 64.84; H, 4.98.

Dihydrolicoisoflavanone Triacetate (XIV)——Licoisoflavanone triacetate was hydrogenated in the presence of prereduced 10% Pd–C in EtOH. The product was recrystallised from EtOH, mp 168°. $\nu_{max}^{CHCl_3}$ cm^{-1}: 2975, 1775, 1697, 1620, 1580, 1490, 1475, 1440, 1375, 1195, 1135. *m/e*: 482 (M^+). *Anal.* Calcd. for $C_{26}H_{26}O_9$: C, 64.72; H, 5.43. Found: C, 64.58; H, 5.50.

Hydrogenation of Licoisoflavone B Triacetate (VI)——Licoisoflavone B triacetate (40 mg) was hydrogenated in the mixture of AcOEt–AcOH (2: 1) in the presence of prereduced PtO_2 (20 mg). Removal of the catalyst and the solvent afforded licoisoflavanone triacetate and a small amount of dihydrolicoisoflavone B triacetate (X). The former compound was identified with the compound XIV obtained from natural source by mixed mp, TLC, IR and PMR spectra.

Isoliquiritigenin——mp 193.5—195°. ν_{max}^{KBr} cm^{-1}: 3380, 1626, 1607, 1585, 1543, 1510, 1370, 1343, 1320, 1287. PMR (δ, d_6-Me_2CO, 100 MHz): 6.33 (1H, d, 2.5 Hz, 3′-H), 6.42 (1H, d.d, 8 and 2.5 Hz, 5′-H), 6.87 (2H, d, 8 Hz, 3- and 5-H), 7.66 (1H, d, 16 Hz, Hα), 7.68 (2H, d, 8 Hz, 2- and 6-H), 7.82 (1H, d, 16 Hz, Hβ), 8.04 (1H, d, 8 Hz, 6′-H), 9.28 (2H, broad singlet, 4- and 4′-OH, exchangeable with D_2O), 13.50 (1H, s, 2′-OH, exchangeable with D_2O). It was identical with an authentic sample in all aspects.

7) E. Wong, The Isoflavonoids in "The Flavonoids," edited by J.B. Harborne, T.J. Mabry, and H. Mabry. Chapman and Hall, London, 1975.

8) H. Suginome, *J. Org. Chem.*, **24**, 1655 (1959).

Formononetin——mp 257—258.5° (recrystallised from MeOH). Monoacetate: mp 172° (recrystallised from MeOH). PMR (δ, $CDCl_3$, 100 MHz): 2.35 (3H, s, OAc), 3.83 (3H, s, OMe), 6.98 (2H, d, 8.5 Hz, 3′- and 5′-H), 7.15 (1H, d.d, 8.5 and 2.5 Hz, 6-H), 7.48 (1H, d, 2.5 Hz, 8-H), 7.49 (2H, d, 8.5 Hz, 2′- and 6′-H), 7.96 (1H, s, 2-H), 8.30 (1H, d, 8.5 Hz, 5-H). It was identified with authentic formononetin and its acetate.

7,4′-Dihydroxyflavone——mp 300°, λ_{max}^{EtOH} nm: 225 (inf.), 257.5, 268, 310, 322, 327 (sh.). *m/e*: 254 (M^+). Diacetate: mp 192—193°, PMR (δ, $CDCl_3$, 100 MHz): 2.35 (3H, s, OAc), 2.37 (3H, s, OAc), 6.76 (1H, s, 3-H), 7.14 (1H, d.d, 8.5 and 2.5 Hz, 6-H), 7.24 (2H, d, 8.5 Hz, 3′- and 5′-H), 7.38 (1H, d, 2.5 Hz, 8-H), 7.89 (2H, d, 8.5 Hz, 2′- and 6′-H), 8.20 (1H, d, 8.5 Hz, 5-H).

Echinatin (III)——mp 217—219°, PMR (δ, d_4-MeOH, 100 MHz): 3.85 (3H, s, OMe), 6.39 (1H, d.d, 8.5 and 2.5 Hz, 5-H), 6.42 (1H, d, 2.5 Hz, 3-H), 6.83 (2H, d, 8.5 Hz, 3′- and 5′-H), 7.50 (1H, d, 16 Hz, Hα), 7.52 (1H, d, 8.5 Hz, 6-H), 7.89 (2H, d, 8.5 Hz, 2′- and 6′-H), 7.95 (1H, d, 16 Hz, Hβ). It was identical with a synthetic echinatin.

Synthesis of Echinatin (III)——*p*-Hydroxyacetophenone (1 mmol: 136 mg) and 4-hydroxy-2-methoxybenzaldehyde (1 mmol: 152 mg) prepared from resorcinol monomethyl ether by the Gatterman reaction were dissolved in 50% KOH (3 ml), and the mixture was kept at room temperature for 7 days. Acidification with conc. HCl under cooling gave solid which was collected by filtration and was crystallised from dil. MeOH, 195 mg (yield: 72%), mp 218—219.5°.

Glabrol (IV)——mp 90° (recrystallised from dil. MeOH). It was identical with an authentic sample.

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