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A New Isoflavone from Licorice Root[1]

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A new isoflavone, licoisoflavone A, was isolated from the root of *Glycyrrhiza* spp. (Leguminosae), and the structure I was formulated on the basis of chemical and spectroscopic studies.

Keywords——*Glycyrrhiza* spp.; licoisoflavone A; dehydrogenation; acid-catalysed cyclisation; licorice root; isoflavone; PMR; licoricidin

Licorice (genus: *Glycyrrhiza*; family: Leguminosae) imported to Japan from China is generally classified into Sinkiang licorice (Shinkyo Kanzo in Japanese), Sipei licorice (Seihoku Kanzo) and Tongpei licorice (Tohoku Kanzo) by the trade names. The original plant of Tohoku Kanzo has been assigned to *G. uralensis* Fisch. et Dc.

Previously we discussed a close relationship between Tohoku Kanzo and Seihoku Kanzo from chemotaxonomical point of view.[3] It was based on the co-occurrence of licoricidin (VIII), licoricone, glycyrol and 5-O-methylglycyrol in both species of licorice. Besides them, Seihoku Kanzo contains two flavonols, kumatakenin and licoflavonol, and a 3-arylcoumarin, glycyrin, as the main constituents which have not been isolated from Tohoku Kanzo.[1,3] Further investigation afforded a new isoflavone, licoisoflavone A, as a minor constitient of Seihoku Kanzo.

Licoisoflavone A (I) was obtained as pale yellow needles of mp 111—113°, whose molecular formula was assigned to $C_{20}H_{18}O_6$ by means of elementary and mass analyses. The ultraviolet (UV) absorption at 265.5 nm (log ε=4.47) and the infrared (IR) absorption at 1650 cm^{-1} (C=O) indicated an isoflavone structure.

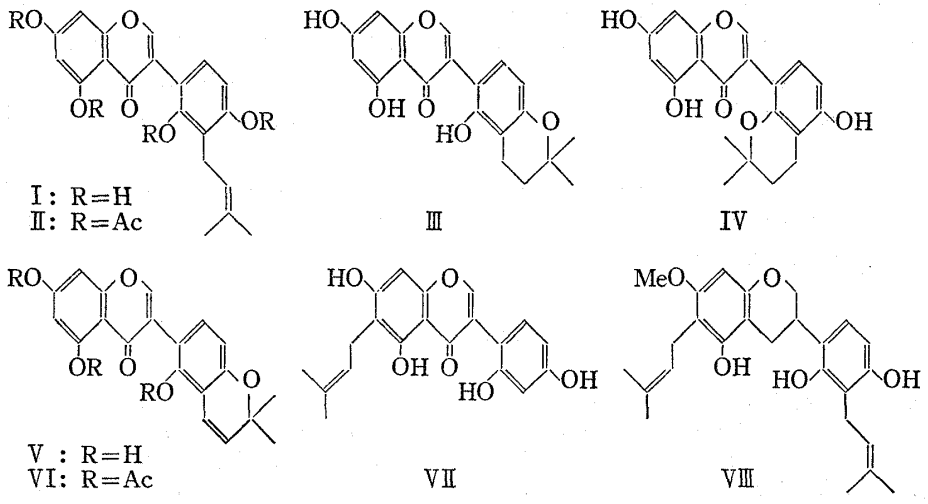

Chart 1

1) Part XLIII in the series of *Chemical Studies on the Oriental Plant Drugs*. Part XLII: T. Kinoshita, T. Saitoh, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **26**, 135 (1978).
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3) T. Saitoh, T. Kinoshita, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **24**, 1242 (1976).

The proton magnetic resonance (PMR) spectrum showed signals assignable to γ,γ-dimethylallyl at δ 1.64 (3H, s), 1.73 (3H, s), 3.26 (2H, d, 7Hz) and 5.20 (1H, t, 7 Hz). In aromatic region were observed signals of *ortho*- and/or *meta*-coupled protons at δ 6.20 (1H, d, 2 Hz), 6.36 (2H, two doublets were overlapped, 2 and 8 Hz) and 6.74 (1H, d, 8 Hz), along with a singlet at δ 8.06, characteristic of isoflavone 2-H. Moreover, the spectrum also showed signals of four hydroxyls exchangeable with D_2O at δ 8.42, 9.22, 10.76, and 12.72. The lowest-field signal was assigned to 5-OH, strongly chelated with 4-carbonyl. Acetylation with acetic anhydride and pyridine yielded a tetraacetate (II), mp 136—138°, whose PMR spectrum exhibited signals of four acetoxyls at δ 2.11, 2.28, 2.34, and 2.39. These results indicated that licoisoflavone A is a tetrahydroxyisoflavone having a γ,γ-dimethylallyl group. The UV spectrum with shift reagents proved the presence of free 5- and 7-hydroxyls since marked bathochromic shifts were observed by the addition of $AlCl_3$ and NaOAc, respectively. Therefore, *meta*-coupled protons were assigned to 6- and 8-H on A ring. The remaining hydroxyls and γ,γ-dimethylallyl group locate in B ring, along with *ortho*-coupled aromatic protons.

When licoisoflavone A was refluxed with a mixture of MeOH and conc. HCl, a mixture of two chroman isomers, cyclolicoisoflavones A_1 (III) and A_2 (IV), was obtained. Their formation occurred by acid-catalysed cyclisation of a γ,γ-dimethylallyl group with two alternative hydroxyls located at the *ortho*-positions. Dehydrogenation of cyclolicoisoflavone A_1 with DDQ yielded a chromene, dehydrocyclolicoisoflavone A_1 (V), whose PMR spectrum revealed the presence of Hα and Hβ protons of chromene at δ 6.68 and 5.57, respectively. On acetylation, Hα proton was shifted higher by 0.48 ppm (δ 6.20) and Hβ proton slightly lower by 0.03 ppm (δ 5.60), suggesting the presence of a *peri*-hydroxyl.[4] The UV spectra of cyclolicoisoflavones A_1 and A_2 exhibited bathochromic shifts by the addition of NaOAc and $AlCl_3$, respectively, indicating that 7- and 5-hydroxyls are still free. These results also confirmed that γ,γ-dimethylallyl group is attached to B ring, not to A ring. As cyclolicoisoflavones A_1 and A_2 gave positive (blue) and negative results towards Gibbs test, respectively, the structures III and IV have been assigned to A_1 and A_2, respectively. These spectroscopic and chemical properties are consistent with the structure I for licoisoflavone A.

Koshimizu *et al.* have isolated luteone from *Lupinus luteus* (Leguminosae) and determined. its structure to be VII.[5] It possesses the same isoflavone skeleton (2′,4′,5,7-tetrahydroxyisoflavone) with a γ,γ-dimethylallyl group as licoisoflavone A, namely both compounds are structural isomers. Therefore, their IR and UV spectra are very similar each other, but PMR spectra are entirely different in the aromatic region; licoisoflavone A exhibited a pair of *meta*-coupled and a pair of *ortho*-coupled signals while luteone showed a singlet and ABC-type signals.

In the previous paper,[3] the isolation of a compound tentatively named C-14 was described. It was identical with a known compound, licoricidin (VIII), isolated from Tohoku Kanzo (the root of *Glycyrrhiza uralensis*).[6]

Experimental

Isolation and Purification——Dried and ground licorece roots (8 kg) were percolated with *n*-hexane, and then extracted with $CHCl_3$ at room temperature. Removal of the solvent gave a brown extract (250 g). The extract was chromatographed on silica gel, eluted with C_6H_6 containing increasing amounts of $(CH_3)_2CO$ (19: 1 to 4: 1). Crude fractions A, B, C, D, E and F were obtained. Further chromatography of each crude fraction on polyamide with MeOH gave pure crystals, licoricidin (VII) from A, kumatakenin from B, glycyrol from D, licoricone from E and glycyrin from F. Elution of C with MeOH on Sephadex LH-20 yielded licoflavonol, and licoisoflavone A in a pure state. The presence of 5-O-methylglycyrol was confirmed on TLC of fraction A though it has not been isolated.

4) A. Arnone, G. Gardillo, L. Merlini, and R. Mondelli, *Tetrahedron Lett.*, **1967**, 4201.
5) H. Fukui, H. Egawa, K. Koshimizu, and T. Mitsui, *Agr. Biol. Chem.*, **37**, 417 (1973).
6) S. Shibata and T. Saitoh, *Chem. Pharm. Bull.* (Tokyo), **16**, 1932 (1968).

Licoisoflavone A (I)——Licoisoflavone A was recrystallised from aqueous MeOH to form pale yellow prisms, mp 111—113°. It gave a brown color with $FeCl_3$ and positive Gibbs test (blue). λ_{max}^{EtOH} nm (log ε): 265.5 (4.47). $\lambda_{max}^{EtOH+NaOAc}$ nm: 277, 332. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 272, 305 (inf.). ν_{max}^{KBr} cm^{-1}: 3340, 2900, 1650, 1605, 1501, 1447. PMR (δ, d_6-DMSO, 100 MHz): 1.64 (3H, s, CH_3 of γ,γ-dimethylallyl), 1.73 (3H, s, CH_3 of γ,γ-dimethylallyl), 3.26 (2H, d, 7 Hz, $-CH_2$ of γ,γ-dimethylallyl), 5.20 (1H, t, 7 Hz, –CH= of γ,γ-dimethylallyl), 6.20 (1H, d, 2 Hz, 6-H), 6.36 (2H, two doublets were overlapped, 8 and 2 Hz, 5′- and 8-H). 6.74 (1H, d, 8 Hz, 6′-H), 8.06 (1H, s, 2-H), 8.24, 9.22, 10.76 and 12.72 (1H each, s or broad s, 2′-, 4′-, 7- and 5-OH, exchangeable with D_2O). *m/e*: 354 (M^+). *Anal.* Calcd. for $C_{20}H_{18}O_6 \cdot CH_3OH$: C, 65.27; H, 5.74. Found: C, 65.07; H, 5.52.

Licoisoflavone A Tetraacetate (II)——Licoisoflavone A (50 mg) was acetylated in the usual manner with acetic anhydride (1.5 ml) and pyridine (1.5 ml). The reaction mixture was poured into ice-water and the pecipitates were collected and recrystallised from EtOH to give a tetraacetate as colorless needles (54 mg) mp 136—138°. λ_{max}^{EtOH} nm (log ε): 242 (4.43), 300 (3.84). $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920, 1768, 1653, 1625, 1485, 1432. PMR (δ, $CDCl_3$, 100 MHz): 1.67 (3H, s, CH_3 of γ,γ-dimethylallyl), 1.71 (3H, s, CH_3 of γ,γ-dimethylallyl), 2.11, 2.28, 2.34 and 2.39 (3H each, s, OAc), 3.20 (2H, d, 7 Hz, $-CH_2$ of γ,γ-dimethylallyl), 4.98 (1H, t, 7 Hz, –CH= of γ,γ-dimethylallyl), 6.81 (1H, d, 2 Hz, 6-H), 6.97 (1H, d, 8 Hz, 5′-H), 7.11 (1H, d, 2 Hz, 8-H), 7.73 (1H, s, 2-H). *m/e*: 522 (M^+). *Anal.* Calcd. for $C_{28}H_{26}O_{10}$: C, 64.38; H, 4.99. Found: C, 64.51; H, 5.02.

Cyclolicoisoflavones A_1 (III) and A_2 (IV)——A mixture of licoisoflavone A (41 mg), MeOH (8 ml) and conc. HCl (2 ml) was refluxed for 4.5 hr. The reaction mixture was then diluted with H_2O (50 ml) and extracted with ether. The ether solution on evaporation furnished a crystalline material, which was chromatographed on silica gel to give cyclolicoisoflavones A_1 and A_2.

Cyclolicoisoflavone A_1 was recrystallised from aqueous MeOH to form colorless plates (18 mg), mp 125—126°. λ_{max}^{EtOH} nm: 265. $\lambda_{max}^{EtOH+NaOAc}$ nm: 277.5, 332. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 270, 309 (inf.). PMR (δ, CD_3OD, 100 MHz): 1.32 (6H, s, CH_3 of dimethylchroman), 1.81 (2H, t, 7 Hz, 3-H of dimethylchroman), 2.72 (2H, t, 7 Hz, 4-H of dimethylchroman), 6.21 (1H, d, 2 Hz, 6-H), 6.30 (1H, d, 2 Hz, 8-H), 6.32 (1H, d, 8 Hz, 5′-H), 6.86 (1H, d, 8 Hz, 6′-H), 7.95 (1H, s, 2-H). *Anal.* Calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.54; H, 5.18. Gibbs test: blue.

Cyclolicoisoflavone A_2 was recrystallised from aqueous MeOH to form colorless prisms (16 mg), mp 260—261°. λ_{max}^{EtOH} nm: 260, 291 (sh.). $\lambda_{max}^{EtOH+NaOAc}$ nm: 264, 268 (inf.), 325. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 263, 268 (inf.). PMR (δ, CD_3OD, 100 MHz): 1.26 (6H, s, CH_3 of dimethylchroman), 1.78 (2H, t, 6 Hz, 3-H of dimethylchroman), 2.69 (2H, t, 6 Hz, 4-H of dimethylchroman), 6.17 (1H, d, 2 Hz, 6-H), 6.29 (1H, d, 2 Hz, 8-H), 6.34 (1H, d, 8 Hz, 5′-H), 6.86 (1H, d, 8 Hz, 6′-H), 7.79 (1H, s, 2-H). *Anal.* Calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.72; H, 5.19. Gibbs test: negative.

Dehydrocyclolicoisoflavone A_1 (V)——Cyclolicoisoflavone A_1 (20 mg) and DDQ (2,3-dichloro-5,6-dicyanobenzoquinone-1,4) (13 mg) were heated under reflux in C_6H_6 (15 ml) for 2 hr. After cooling the reaction mixture was filtered and the filtrate was evaporated. The residue was recrystallised from aqueous MeOH to give dehydrocyclolicoisoflavone A_1 (13 mg) as needles, mp 245—248° (dec.). PMR (δ, CD_3OD, 100 MHz): 1.39 (6H, s, CH_3 of dimethylchromene), 5.57 (1H, d, 10 Hz, 3-H (Hβ) of dimethylchromene), 6.18 (1H, d, 2 Hz, 6-H), 6.32 (2H, two doublets were overlapped, 8 and 2 Hz, 5′- and 8-H), 6.68 (1H, d, 10 Hz, 4-H (Hα) of dimethylchromene), 6.88 (1H, d, 8 Hz, 6′-H), 7.98 (1H, s, 2-H).

Dehydrocyclolicoisoflavone A_1 Triacetate (VI)——Dehydrocyclolicoisoflavone A_1 was acetylated with acetic anhydride and pyridine in the usual manner. Colorless needles (from aqueous EtOH), mp 186—188°. λ_{max}^{EtOH} nm (log ε): 228 (4.55), 240 (4.55, inf.), 244 (4.57, sh.), 249.5 (4.59), 255 (4.53), 261 (4.36, inf.), 305 (3.87). $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 1775, 1650, 1625, 1575, 1470, 1435, 1180, 1110. PMR (δ, $CDCl_3$, 100 MHz): 1.40 (6H, s, CH_3 of dimethylchromene), 2.14, 2.32 and 2.36 (3H each, OAc), 5.60 (1H, d, 1 Hz, 3-H (Hβ) of dimethylchromene), 6.20 (1H, d, 10 Hz, 4-H (Hα) of dimethylchromene), 6.66 (1H, d, 8 Hz, 5′-H), 6.78 (1H, d, 2 Hz, 6-H), 6.93 (1H, d, 8 Hz, 6′-H), 7.16 (1H, d, 2 Hz, 8-H), 7.68 (1H, s, 2-H). *m/e*: 478 (M^+). *Anal.* Calcd. for $C_{26}H_{22}O_9$: C, 65.32; H, 4.69. Found: C, 65.27; H, 4.64.

Licoricidin (VIII)——Licoricidin was recrystallised from $CHCl_3$–ether to form colorless needles, mp 154—156°. $[\alpha]_D^{22.5} = +20°$ ($c = 1.0$, MeOH). λ_{max}^{EtOH} nm: 281 (sh.), 284. ν_{max}^{KBr} cm^{-1}: 3100, 2900, 1625, 1608, 1581, 1505. PMR (δ, CD_3OD, 100 MHz): 1.66 and 1.76 (6H each, CH_3 of γ,γ-dimethylallyl), 2.8 (2H, m, 4-H_2), 3.2—3.4 (5H, $-CH_2$ of γ,γ-dimethylallyl and 3-H), 3.67 (3H, s, 7-OMe), 3.88 (1H, t, 10 Hz, 2-Hax), 4.19 (1H, d.d, 10 and 3.5 Hz, 2-Heq), 5.21 (2H, t, 7 Hz, –CH= of γ,γ-dimethylallyl), 6.10 (1H, s, 8-H), 6.36 (1H, d, 8 Hz, 5′-H), 6.77 (1H, d, 8 Hz, 6′-H). *m/e*: 424 (M^+).

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