ON THE STRUCTURE OF LICORICIDIN

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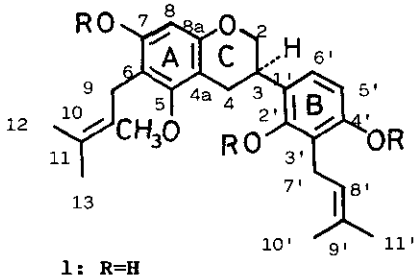
Abstract ——— A prenylated isoflavan isolated from Sipei licorice (Glycyrrhiza sp., Leguminosae, Seihoku Kanzo in Japanese) was proved to be identical with already known compound licoricidin (2). From the spectral data, the structure (2) of licoricidin should be revised to the structure (1).

Licorice, the root of various species of Glycyrrhiza (Leguminosae), has been used for a long time as a very important crude drug. The constituents of the crude drug have been studied by many investigators, and a series of prenylated flavonoids have been isolated.[1] In the course of our studies on the prenylated phenols from the crude drugs,[2] the studies were carried out on the phenolic compounds of Sipei licorice (Glycyrrhiza sp., Leguminosae, Seihoku Kanzo in Japanese).[1] From the benzene extract of the crude drug (4.8 Kg), a prenylated isoflavan (1, 500 mg) was isolated by using column chromatography (silica gel, n-hexane-benzene as eluent) and by preparative tlc (silica gel, $CHCl_3$:acetone = 10:1, developing solvent), successively.

Compound (1) was obtained as colorless needles, mp 161 °C, $[\alpha]_D^{20}$ +22.8° (c=0.412, MeOH), negative to ferric chloride test. The compound (1) showed the following spectra; ir ν_{max}^{KBr} cm^{-1}: 3520, 3395(br), 2940(br), 1616, 1606(sh), 1495; uv λ_{max}^{MeOH} nm (log ε): 210 (4.86), 230 (sh 4.48), 276 (sh 3.67), 283 (3.73); EI-Ms m/z: 424 (M^+), 221, 204 and 165. The ^1H nmr spectrum of 1 (400 MHz, acetone-d_6) showed the signals of the following protons: 1) protons in two γ,γ-dimethylallyl moieties, δ 1.65 (3H, br s), 1.66 (3H, d, J=0.9 Hz), 1.75, 1.78 (each 3H, br s), 3.24 (1H, br dd, J = 6.6 and 14), 3.30 (1H, br dd, J = 7 and 14), 3.45 (2H, br d, J = 7), 5.26 (2H, m), 2) five aliphatic protons, δ 2.73 (1H, dd, J = 11 and 16, C-4-H), 2.90 (1H, ddd, J = 2, 5 and 16, C-4-H), 3.39 (1H, m, C-3-H), 3.94 (1H, t, J = 10,



1: R=H

1a: R=Ac

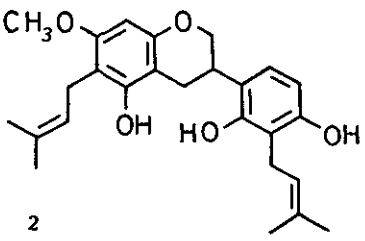


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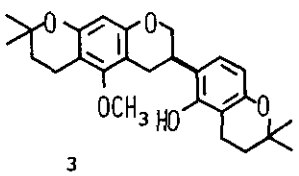
Fig. 1



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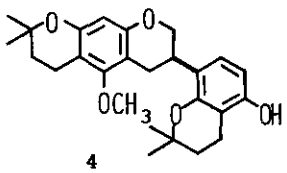

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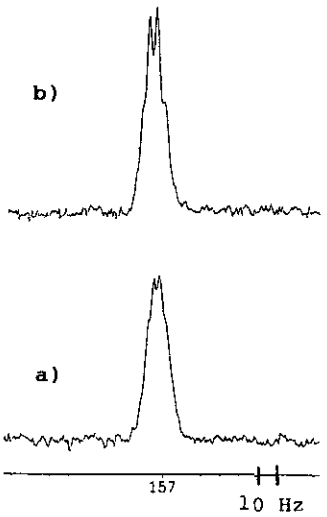

Fig. 2 LSPD spectra of **1**

a) Reference spectrum, irradiation at δ 12.17 and 12.73 ppm (triple irradiation).

b) Irradiation at δ 2.63 and 2.81 ppm (H_2-4), (triple irradiation).

Table 1. ^{13}C nmr data of licoricidin (**1**)

C	(ppm)	
2	69.46	
3	30.83	
4	26.24	
4a	113.23	
5	157.02	
6	106.93	
7	154.46	
8	98.87	
8a	153.02	**59.91**
1'	120.00	**(OCH_3)**
2'	152.97	
3'	116.23	
4'	154.46	
5'	107.44	
6'	123.86	
9, 7'	22.35,	22.54
10, 8'	123.69,	124.37
11, 9'	129.10,	129.52
12, 10'	17.57,	17.70
13, 11'	25.36,	25.40

in dmso-$\underline{d}_6$ at 60 °C.

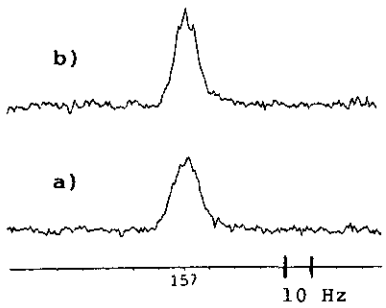

Fig. 3 LSPD spectrum of **1**

a) Gated decoupling with NOE (reference).

b) Irradiation at δ 3.62 ppm (OCH_3), (gated decoupling with NOE).

C-2-H), 4.17 (1H, ddd, J =2, 3.5 and 10, C-2-H), 3) three aromatic protons, δ 6.17 (1H, s, C-8-H), 6.45 (1H, d, J = 8, C-5'-H), 6.83 (1H, d, J = 8, C-6'-H), 4) protons in a methoxyl group, δ 3.68 (3H, s) and 5) protons in hydroxyl groups, δ 7.21, 8.17, 8.18 (each 1H, s). Work up of **1** with acetic anhydride in pyridine gave the triacetate (**1a**), mp 136-137.5°C. The compound (**1a**) showed the following spectra: EI-Ms m/z: 550 (M^+); ^1H nmr (400 MHz, in $CDCl_3$), δ 1.68 (6H, br s), 1.72, 1.74 (each 3H, br s), 2.27, 2.29, 2.31 (each 3H, s, OAc), 2.75 (1H, br dd, J = ca. 13 and 14, C-4-H), 3.08 (1H, ddd, J = ca. 2, 4 and 14, C-4-H), 3.13 (1H, m,C-3-H), 3.18, 3.20 (each 2H, br dd, J = 7 and 14), 3.70 (3H, s, OCH_3), 3.92 (1H, br t, J = ca. 10, C-2-H), 4.25 (1H, ddd, J = 2, 3 and 10, C-2-H), 5.01, 5.09 (each 1H, tm, J = < 1 and 7), 6.41 (1H, s, C-8-H), 7.00 (1H, d, J = 8.5, C-5'-H), 7.08 (1H, d, J = 8.5, C-6'-H). The above physical and spectral data of **1** and **1a** are in good agreement with those of licoricidin (**2**)[3] and its triacetate (**2a**)[3], respectively. The compound (**1**) was proved to be identical with authentic licoricidin (**2**)[3] by mixed melting point experiment and comparison of ^1H nmr spectra. The structure of licoricidin was reinvestigated as presented below.

The isoflavan skeleton for licoricidin was reconfirmed by the following long-range selective ^1H decoupling (LSPD) technique. When the C-3 proton at δ 3.34 (m, dmso-d_6) was weakly irradiated, the signal of the C-6' (δ 123.86 ppm, Table 1) changed (dd, J = 5 and 155 ⟶ d, J = 155). The assignment of the signal at δ 123.86 ppm was confirmed by the selective decoupling technique as follows. When the C-6' proton at δ 6.72 ppm (J = 8.5, dmso-d_6) was irradiated, the carbon signal at δ 123.86 ppm changed to broad singlet. The 3-(γ,γ-dimethylallyl)-2,4-dihydroxyphenyl partial structure for the B-ring was reconfirmed by the below-mentioned results. Treatment of **1** with concentrated hydrochloric acid in methanol solution gave the compounds (**3** and **4**) having two 2,2-dimethylchroman rings in each structure.[3] The compound (**3**), amorphous powder, exhibited a positive Gibbs test and showed the following spectra: EI-Ms m/z: 424 (M^+); ^1H nmr (400 MHz, acetone-d_6), δ 1.28 (3H, s), 1.29 (9H, s), 1.75, 1.82, 2.68, 2.73 (each 2H, t, J = 7), 2.76 (1H, dd, J = 11 and 16, C-4-H), 2.93 (1H, ddd, J = 2, 5 and 16, C-4-H), 3.43 (1H, m, C-3-H), 3.72 (3H, s, OCH_3), 3.96 (1H, t, J = 10, C-2-H), 4.19 (1H, ddd, J = 2, 3.5 and 10, C-2-H), 6.00 (1H, s, C-8-H), 6.32 (1H, d, J = 8.5, C-5'-H), 6.90 (1H, d, J = 8.5, C-6'-H). The compound (**4**), amorphous powder, exhibited a negative Gibbs test and showed the following spectra: EI-Ms m/z: 424 (M^+); ^1H nmr (400 MHz, acetone-d_6), δ 1.28, 1.29, 1.326, 1.331 (each 3H, s),

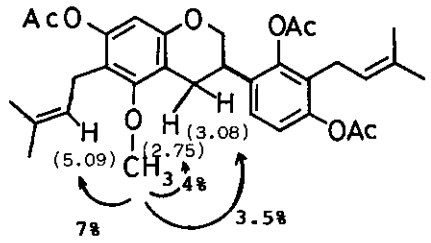

Fig. 4 NOE values for **1a**

1.75, 1.81, 2.68, 2.69 (each 2H, t, J = 7), 2.77 (1H, dd, J = 11 and 16, C-4-H), 2.89 (1H, ddd, J = 2, 5 and 16, C-4-H), 3.35 (1H, m, C-3-H), 3.72 (3H, s, OCH_3), 3.93 (1H, t, J = 10, C-2-H), 4.17 (1H, ddd, J = 2, 3.5 and 10, C-2-H), 6.00 (1H, s, C-8-H), 6.38 (1H, d, J = 8, C-5'-H), 6.84 (1H, d, J = 8, C-6'-H).

The location of the methoxyl group at C-5 position of **1** was confirmed from the three kinds of experimental results: 1) Dhami and Stothers reported that the ^{13}C nmr signal of the di-orthosubstituted methoxyl carbon nucleus appears at δ ca. 60 ppm, while that of the mono-orthosubstituted methoxyl carbon nucleus appears at δ ca. 55 ppm.[4] In the ^{13}C nmr spectrum of **1** (Table 1), the signal of the methoxyl carbon atom appeared at δ 59.91 ppm indicating that the methoxyl group of **1** seems to be di-orthosubstituted. 2) The LSPD experiment was carred out where the signal at δ 157.02 ppm (br s, C-5, Table 1) changed (Fig. 2) when the C-4 protons at δ 2.63 and 2.81 ppm (dmso-d_6) were weakly irradiated. When the methoxyl protons at δ 3.62 (dmso-d_6) was irradiated, the signal at δ 157.02 ppm changed (Fig. 3). 3) The nuclear Overhauser effect (NOE) measurement of **1a** was carried out and NOE was observed at the C-4 (δ 2.75 and 3.08) and olefinic (δ 5.09) protons (Fig. 4) when the methoxyl proton signal (δ 3.70) was irradiated.

The absolute configuration of **1** was confirmed as (R)-configuration at the C-3 position by the CD spectrum (c=5.9x10^{-5} mol/l, MeOH) as follows:[5] $[\theta]_{292}$ 0, $[\theta]_{286}$ +1.35x10^6, $[\theta]_{275}$ 0, $[\theta]_{270}$ -6.78x10^5, $[\theta]_{245}$ 0. All these results indicate that the structure (**1**) for licoricidin was confirmed and the structure (**2**) should be revised to **1**.[6,7]

ACKNOWLEDGMENT

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REFERENCES AND FOOTNOTES

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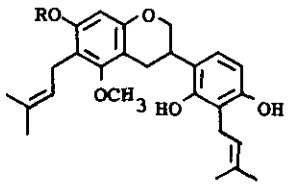
5. a): K. Kurosawa, W. David Ollis, B.T. Redman, I.O. Sutherland, H.M. Alves, and O. R. Gottlieb, Phytochemistry, 1978, **17**, 1423, b): F.R. van Heerden, E.V. Brandt, and D.G. Roux, J. Chem. Soc., Perkin I, 1978, 137.

6. One of our coworkers (M.T.) reported this result in his graduation thesis (School of Science, Toho University, 1988, February, 23rd).

7. After completion of our work, we found the abstract papers of 56th Annual Meeting of the Chemical Society of Japan, reported by Prof. S. Yamamura and his coworkers, Keio University (Y. Shizuri, K. Uchida, and S. Yamamura, Abstract papers, p. 1154, April, 1988). In the abstract paper, Yamamura's group reported two kinds of isoflavans, named licorisoflavans A (**5**) and B (**6**).



5: R=CH_3
6: R=H

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