Revised Structures of Glycyrol and Isoglycyrol, Constituents of the Root of *Glycyrrhiza uralensis*

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The structures of glycyrol (1) and isoglycyrol (4), which were isolated from the root of *Glycyrrhiza uralensis*, were revised on the basis of a series of irradiation experiments.

Keywords revised structure; glycyrol; isoglycyrol; *Glycyrrhiza uralensis*; Leguminosae; coumestan

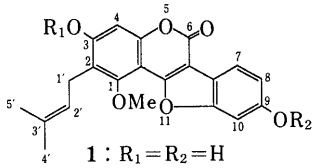
Licorice, the root of some species of *Glycyrrhiza* (Leguminosae), is one of the main ingredients in the prescriptions of traditional Chinese medicine (Kampo medicine), consisting of various types of herbal drugs. Thus, it has long been the subject of extensive chemical investigations, and a number of flavonoids, many of which contain the isoprenyl unit, have been isolated.[1-5] In a recent report dealing with bioactive principles of licorice,[5] one of the authors (T.K.) reported the isolation of glycycoumarin, a potent antimicrobial 3-arylcoumarin derivative, and licocoumarone, a 2-arylbenzofuran derivative with strong antioxidant activity, from xibei licorice (西北甘草; seihoku kanzo in Japanese). Glycyrol and its analogues, coumestan derivatives, were first isolated from licorice of Chinese origin, known in commerce as dongbei licorice (東北甘草; tohoku kanzo in Japanese), and were later shown to occur in xibei licorice.[1] Glycyrol has a similar functional group disposition to that of glycycoumarin and licocoumarone with the exception of the location of a methoxyl group. Recently, the structure of licoricidin, which is also one of the main phenolic constituents in xibei and dongbei licorices, was revised, and the methoxyl group was relocated from the 7-position to the 5-position.[6] These recent findings prompted us to reinvestigate the structures of glycyrol and its analogues, constituents of these licorices, in which a methoxyl group was assigned at the 3-position (7-position according to the previous numbering).

Results and Discussion

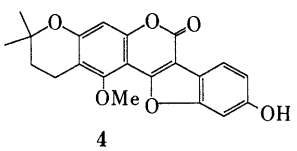
Glycyrol and its analogues were obtained from the repeated chromatographic separation of the phenolic fraction of the licorice extract over silica gel. Glycyrol (**1**) was obtained as colorless needles, mp 244 °C, 3-*O*-methylglycyrol (**3**) as colorless needles, mp 260 °C (dec.) and isoglycyrol (**4**) as colorless needles, mp 295 °C (dec.).

The previously assigned structure of glycyrol had an *ortho*-methoxyl substituent adjacent to an isolated aromatic proton. According to the empirical methoxy-proton shift rule,[7] the proton nuclear magnetic resonance (^{1}H-NMR) signal due to a methoxyl group with an *ortho*-hydrogen substituent moves upfield by 0.30 ppm or more on change of the solvent from chloroform-*d* to benzene-d_6. In the case of glycyrol dibenzyl ether (**2**), a methoxyl signal appeared at δ 3.99 in chloroform-*d*, and shifted to δ 3.77 in benzene-d_6. This finding disfavors the *ortho*-disposition of hydrogen to the methoxyl group as in the previously assigned structure of glycyrol. It also agreed with the experimental finding that irradiation of the methoxyl group at δ 3.77 induced no nuclear Overhauser effect (NOE) on a singlet proton signal at δ 6.55, while there were significant NOEs between the methoxy and 1′-H_2, 2′-H and 4′-Me (2%, 3% and 4% based on the NOE difference spectrum, respectively). The location of the methoxyl group was finally confirmed on the basis of a series of irradiation experiments, the results of which are shown in Fig. 1. In the ^{1}H-NMR spectrum of glycyrol dibenzyl ether (**2**), signals due to both benzylic methylenes overlapped in chloroform-*d*, but were resolved (δ 4.57 and 4.66) in benzene-d_6. Irradiation of the benzylic methylene at δ 4.57 resulted in a 22% NOE on a singlet proton (4-H) at δ 6.55, while 7% and 14% NOEs were observed between a double doublet (8-H) at δ 6.95 and a doublet (10-H) at δ 7.05, respectively, on irradiation of another benzylic methylene at δ 4.66. Irradiation of a singlet at δ 6.55 induced an 11% increase in the intensity of benzylic methylene signal at δ 4.57. The above results ruled out the possibility of the location of a methoxyl group at the 3-position, and thus the structure of glycyrol was revised to **1**.

Since chemical correlation between glycyrol and isoglycyrol has already been discussed,[1a] the structure of isoglycyrol was also revised to **4**. As for the structure of "5-*O*-methylglycyrol" (**3**) (1-*O*-methylglycyrol based on the current numbering of coumestans), it remains unchanged but should be renamed 3-*O*-methylglycyrol.



1: $R_1=R_2=H$
2: $R_1=R_2=CH_2Ph$
3: $R_1=Me$, $R_2=H$



4

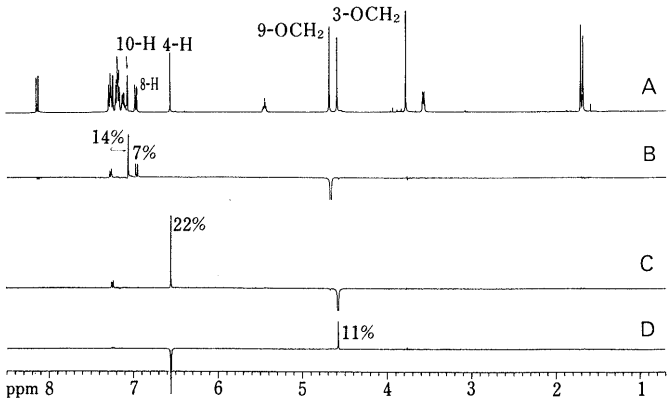


Fig. 1

Experimental

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra with JEOL FX-90A (^{1}H, 90 MHz; ^{13}C, 22.5 MHz) and JEOL JMN GSX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometers with tetramethylsilane (TMS) as an internal standard; mass spectra (MS) with a JEOL JMS-D300 mass spectrometer; infrared (IR) spectra with a JASCO A-302 spectrometer; ultraviolet (UV) spectra with a Shimadzu UV-240 spectrophotometer.

Extraction and Isolation The commercially available dongbei locorice (tohoku kanzo; root of *Glycyrrhiza uralensis*) was extracted, and the chromatographic separation of the extract proceeded as described in the literature[1] to give glycyrol (**1**), 3-*O*-methylglycyrol (**3**) and isoglycyrol (**4**). These were identified by comparison with authentic samples.

Glycyrol (1) Colorless needles from EtOH, mp 244 °C. UV λ_{max}^{MeOH} nm: 226, 244, 253 sh, 346, 355 sh. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1715 (C=O), 1610, 1595, 1365, 1300, 1100. ^{1}H-NMR (90 MHz, DMSO-d_6) δ: 1.65 (3H, s, 5′-Me), 1.77 (3H, s, 4′-Me), 3.33 (2H, d, 1′-H_2, overlapped with H_2O), 3.90 (3H, s, OMe), 5.20 (1H, br t, J=5.4 Hz, 2′-H), 6.78 (1H, s, 4-H), 6.95 (1H, dd, J=2, 8.4 Hz, 8-H), 7.17 (1H, d, J=2 Hz, 10-H), 7.72 (1H, d, J=8.4 Hz, 7-H). ^{13}C-NMR (22.5 MHz, DMSO-d_6) δ: 17.7 (5′-C), 22.0 (1′-C), 25.4 (4′-C), 62.3 (OMe), 98.5 (4-C), 99.2 (10-C), 99.7 (1a-C), 102.2 (6a-C), 114.0 (8-C), 114.3 (7a-C), 119.7 (2-C), 120.4 (7-C), 122.4 (2′-C), 130.8 (3′-C), 152.9 (1-C), 153.8 (4a-C), 156.1, 156.9, 157.4, 158.1, 159.4. EI-MS m/z: 366 (M^+).

Glycyrol Dibenzyl Ether (2) Glycyrol dibenzyl ether was prepared by heating a mixture of glycyrol (**1**) (36 mg), benzyl bromide (70 mg) and K_2CO_3 (138 mg) in dry acetone (10 ml) under reflux for 4 h. The reaction mixture was worked up in the usual manner, and 35 mg of the dibenzyl ether was obtained. Colorless needles from benzene–MeOH, mp 157—158 °C. IR ν_{max}^{KBr} cm^{-1}: 1735 (C=O), 1615, 1590, 1110. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.70 (3H, s, 5′-Me), 1.72 (3H, s, 4′-Me), 3.50 (2H, d, J=7.3 Hz, 1′-H_2), 3.99 (3H, s, OMe), 5.17 (4H, s, OCH_2), 5.20 (1H, t, J=7.3 Hz, 2′-H), 6.86 (1H, s, 4-H), 7.14 (1H, dd, J=2.2, 8.4 Hz, 8-H), 7.27 (1H, d, J=2.2 Hz, 10-H), 7.3—7.4 (10H, m, Ph-H), 7.98 (1H, d, J=8.4 Hz, 7-H). ^{1}H-NMR (400 MHz, benzene-d_6) δ: 1.67 (3H, s, 5′-Me), 1.69 (3H, s, 4′-Me), 3.55 (2H, d, J=6.7 Hz, 1′-H_2), 3.77 (3H, s, OMe), 4.57 (2H, s, 3-OCH_2), 4.66 (2H, s, 9-OCH_2), 5.44 (1H, t, J=6.7 Hz, 2′-H), 6.55 (1H, s, 4-H), 6.95 (1H, dd, J=2.1, 8.5 Hz, 8-H), 7.05 (1H, d, J=2.1 Hz, 10-H), 7.1—7.3 (10H, m, Ph-H), 8.14 (1H, d, J=8.4 Hz, 7-H). ^{13}C-NMR (100 MHz, benzene-d_6) δ: 18.2 (5′-C), 23.3 (1′-C), 26.1 (4′-C), 62.7 (OMe), 70.7 (3-O-CH_2), 70.8 (9-O-CH_2), 97.8 (4-C), 98.1 (10-C), 102.3 (1a-C), 104.7 (6a-C), 114.6 (8-C), 117.6 (7a-C), 121.7 (2-C), 122.1 (7-C), 123.6 (2′-C), 127.9—129.0 (Ph-C), 131.9 (3′-C), 136.8, 136.9, 154.5 (1-C), 154.6 (4a-C), 157.0, 158.0, 158.8, 158.9, 160.3. EI-MS m/z: 546 (M^+).

3-*O*-Methylglycyrol (3) Colorless needles from benzene–acetone, mp 260 °C (dec.). UV λ_{max}^{MeOH} nm: 229, 246, 344, 355 sh. IR ν_{max}^{KBr} cm^{-1}: 3250 (OH), 1700 (C=O), 1620, 1110. ^{1}H-NMR (90 MHz, DMSO-d_6) δ: 1.65 (3H, s, 5′-Me), 1.76 (3H, s, 4′-Me), 3.3 (2H, br d, J=7 Hz, 2′-H_2), 3.90 (3H, s, OMe), 5.14 (1H, br t, J=7 Hz, 3′-H), 6.96 (1H, dd, J=2.0, 8.4 Hz, 8-H), 6.98 (1H, s, 4-H), 7.17 (1H, d, J=2.0 Hz, 10-H), 7.71 (1H, d, J=8.4 Hz, 7-H). ^{13}C-NMR (22.5 MHz, DMSO-d_6) δ: 17.9 (5′-C), 22.2 (1′-C), 25.6 (4′-C), 56.6 (OMe), 62.7 (OMe), 96.5 (4-C), 98.7 (10-C), 100.9 (1a-C), 103.0 (6a-C), 114.3 (7a-C), 120.9 (2- and 7-C), 122.2 (2′-C), 131.8 (3′-C), 153.2, 153.4, 156.4, 157.2, 157.8, 158.0, 160.8. EI-MS m/z: 366 (M^+).

Isoglycyrol (4) Colorless needles from EtOH, mp 295 °C (dec.). UV λ_{max}^{MeOH} nm: 223 sh, 246, 254 sh, 347, 356 sh. IR ν_{max}^{KBr} cm^{-1}: 3250 (OH), 1700 (C=O), 1625, 1355, 1080, 840. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.34 (6H, s, 2 × Me), 1.83 (2H, t, J=6.7 Hz, CH_2), 2.82 (2H, t, J=6.7 Hz, CH_2), 3.96 (3H, s, OMe), 6.71 (1H, s, 4-H), 6.96 (1H, dd, J=2.1, 8.3 Hz, 8-H), 7.17 (1H, d, J=2.1 Hz, 10-H), 7.71 (1H, d, J=8.3 Hz, 7-H). ^{13}C-NMR (22.5 MHz, DMSO-d_6) δ: 16.2 (CH_2), 26.4 (2 × Me), 31.0 (CH_2), 61.4 (OMe), 76.0 (–C–O), 98.5 (4-C), 100.3 (1a-C), 100.6 (10-C), 102.8 (6a-C), 113.0 (8-C), 114.0 (7a-C), 114.2 (2-C), 120.5 (7-C), 152.7, 153.5, 156.2, 157.0, 157.3, 157.7. EI-MS m/z: 366 (M^+).

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