生 藥 学 雑 誌
Shoyakugaku Zasshi
47(3), 326~329 (1993)

Phenolic Constituents of *Glycyrrhiza* Species. 11[1)] A New Prenylated 3-Arylcoumarin, Gancaonin W, from Licorice

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(Received January 7, 1993)

A new prenylated 3-arylcoumarin, gancaonin W, was isolated from Xibei licorice (*Glycyrrhiza* species) and the structure was elucidated as 5-methoxy-3′-prenyl-7,2′,4′-trihydroxy-3-arylcoumarin (**1**).

Keywords——*Glycyrrhiza*; Leguminosae; prenylated 3-arylcoumarin; gancaonin W

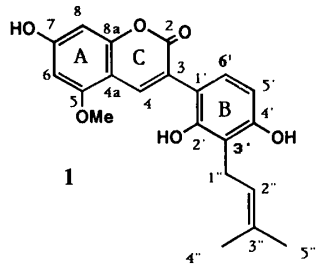
Licorice, the roots and rhizomes of various species of the genus *Glycyrrhiza* (Leguminosae), has been used as one of the most important crude drugs for a long time. Commercial Chinese licorice in Japan mainly consists of the Dongbei licorice (東北甘草, from northeastern China, *G. uralensis*), the Xibei licorice (西北甘草, from northwestern China), and Xin Jiang licorice (新疆甘草, from Xin Jiang Autonomous Region of China, mainly *G. inflata*).[2,3)] Each of these licorices can be distinguished from each other as they contain characteristic coumarin and flavonoids (the Dongbei licorice contains licoricidin and glycyrol, the Xibei licorice, licoricidin, glycyrol, and kumatakenin,[4)] and Xin Jiang licorice, licochalcone A[2)]). Accordingly, the original plants of the Dongbei licorice and Xin Jiang licorices can be easily identified by these coumarin and/or flavonoids. On the other hand, chemical identification of the original plants of the Xibei licorice is not easy as its phenolic components resembles those of Dongbei licorice. So is the identification by morphological and anatomical methods.[5)]

Demizu *et al.* reported that the high performance liquid chromatography (HPLC) profiles of the extracts of the Dongbei licorice and the Xibei licorice closely resembled each other (both contained four coumarin derivatives, glycycoumarin, glycyrin, glycyrol, and isoglycyrol)[6)] and that the original plant of the Xibei licorice might also be *G. uralensis*.[6)]

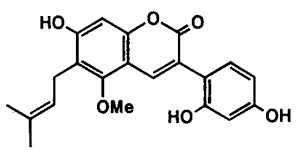
In the northwestern region of China, five *Glycyrrhiza* species, *i.e. G. uralensis*, *G. glabra*, *G. inflata*, *G. eurycarpa*, and *G. aspera*, grow.[7)] In the previous papers,[8,9)] isolations of glycycoumarin, glycyrol, isoglycyrol, licoricidin, kumatakenin, and glycyrin[10)] from *G. aspera* were reported. These compounds were also isolated from the Xibei licorice.[11-13)] This fact suggested that one of the original plants of the Xibei licorice might be *G. aspera*. For the confirmation of this possibility, we studied the minor phenolic constituents of the Xibei licorice, and isolated a new prenylated 3-arylcoumarin, gancaonin W (**1**). This paper deals with the characterization of the new prenylated 3-arylcoumarin.

Results and Discussion

Gancaonin W (**1**), yellow prisms, mp 205–211°C, $C_{21}H_{20}O_6$ was negative to methanolic ferric chloride test. Its UV spectrum resembled those of 3-arylcoumarin derivatives,[6,8,14,15)] such as glycycoumarin (**2**).[15)] The ^{1}H-NMR spectrum showed the signals of AB type aromatic protons, AXY type aromatic and olefinic protons, three protons of a methoxyl group, protons in a prenyl (γ,γ-dimethylallyl) group, and three protons of three hydroxyl groups. The signals of the ^{13}C-NMR spectrum were assigned by comparing the spectrum with those of glycycoumarin (**2**), synthetic 3-arylcoumarins (**3** and **4**), and licoisoflavone A (**6**)[16)] as shown in TABLE I. The chemical shifts of five oxygenated aromatic carbon atoms (δ 154.86–163.04) of **1** indicated that these carbon atoms are at *meta*-positions to each other.[16)] The chemical shift of the methoxyl carbon (δ 56.67) suggested that the *ortho*-position(s) of the methoxyl group is nonsubstituted carbon(s).[16)] Therefore, the methoxyl group is at C-4′ or C-5 or C-7 position. The chemical shifts of the B ring carbon signals of **1** were similar to those of the relevant carbons of lico-



1



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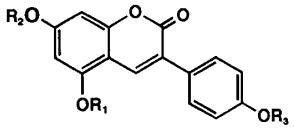

3: R_1= R_2= R_3=H
4: R_1= Me, R_2= R_3= H
5: R_1= Me, R_2= R_3= Ac

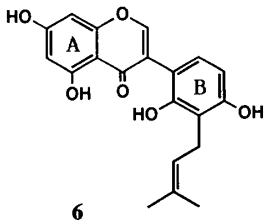

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Chart 1.

TABLE I. ^{13}C-NMR Data of 3-Arylcoumarins (**1–4**) and Licoisoflavone A (**6**) (100 MHz, in Acetone-d_6)

C	1	2[a]	3[a]		4[a]	6[b]
2	163. 61	162. 12	161. 34	(Sd, J=10 Hz)[c]	161. 13	
3	121. 93	122. 15	122. 04	(St, J=3 Hz)	122. 59	
4	138. 58	138. 15	134. 52	(D, J=162 Hz)	134. 06	
4a	104. 77	108. 15	104. 02	(St, J=8 Hz)	104. 49	
5	158. 58	156. 91	156. 59	(St, J=3 Hz)	158. 45*	
6	96. 37	115. 65	99. 28	(Dd, J=164 and 4 Hz)	95. 98	
7	163. 04	160. 34	162. 27	(St, J=3 Hz)	162. 53	
8	95. 54	99. 07	95. 08	(br Dd, J=164 and 4 Hz)	95. 56	
8a	156. 75	154. 45	156. 97	(Sdd, J=4 and 6 Hz)	156. 80	
1′	117. 51	119. 96	128. 04	(Sm)	128. 39	109. 8
2′	154. 86	157. 13	130. 47	(Dd, J=160 and 7 Hz)	130. 22	154. 2
3′	116. 53	104. 52	115. 87	(Dd, J=160 and 5 Hz)	115. 90	115. 7
4′	157. 53	159. 78	158. 30	(Stt, J=2 and 9 Hz)	158. 42*	156. 6
5′	108. 60	108. 19	115. 87		115. 90	107. 0
6′	129. 15	132. 77	130. 47		130. 22	128. 8
1″	23. 43	23. 37				
2″	124. 28	123. 61				
3″	130. 88	131. 88				
4″	17. 95	17. 97				
5″	25. 92	25. 82				
OMe	56. 67	63. 52				

[a] Signals were assigned according to the coupling patterns and coupling constants (ref. 19).
[b] Data from K.R. Markham *et al.* (in DMSO-d_6 at 30°C, ref. 16).
[c] S and D refer to singlet and doublet patterns resulting from directly bonded protons, and d, dd, t, tt, or m refer to doublet, double-doublet, triplet, triple-triplet or multiplet caused by long-range ^{13}C-^{1}H coupling.
* Assignments may be interchanged.

isoflavone A (**6**) except that of C1′. The chemical shifts of the A ring carbon signals were similar to those of the corresponding carbons of **4**. These spectral data suggest that the structure of gancaonin W is formula **1**. The position of the methoxyl group was further confirmed by the nuclear Overhauser effect (NOE) measurement of **1**: when the methoxyl protons (δ 3.97) were irradiated, an enhancement of the C-6-H signal by 16% was observed, but not of the C-8-H signal.

Thus, the structure of gancaonin W is determined as formula **1**.

The compound (**1**) was also isolated from *G. aspera*, but has not been isolated from *G. uralensis*.

Experimental

General procedures——Melting points were measured on a Yazawa micromelting point apparatus (hot-stage type) and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were measured with Hitachi R-900 (90 MHz, CW mode), JEOL JNM-GX-400, and JEOL JNM-EX-400 spectrometers with tetramethylsilane (TMS) as internal reference. Mass spectra (MS) were obtained on JEOL JMS-D-300 and JEOL JMS-DX-303 mass spectrometers. UV spectra were recorded on a Shimadzu UV-265 spectrophotometer.

Plant material and extraction——The Xibei licorice was purchased from Matuura Yakugyo Co., Nagoya (Lot. No. 714112). The botanical source of the licorice could not be identified, because this licorice was a mixture of roots and rhizomes. The method of extraction used was the same as that reported in the preceding paper.[12]

Isolation of gancaonin W (1)——The acetone extract (150 g)[12] was chromatographed on silica gel (600 g) by using successively C_6H_6 (Fr. 1–59) and C_6H_6-Et_2O=99:1 (Fr. 60–110) as eluent, each fraction (eluent volume 500 ml) being monitored by TLC. The fractions 70–88 were evaporated to give 4.6 g of residue, from which licoricone (145 mg) was obtained by recrystallization from C_6H_6-MeOH. The mother liquor (4.4 g) was rechromatographed on silica gel (100 g) successively using C_6H_6 (Fr. 1–12), C_6H_6-MeOH=199:1 (Fr. 13–16), C_6H_6-MeOH=99:1 (Fr. 17–26) as the eluent (each eluent volume 500 ml). The fractions 17–20 were evaporated to give 0.76 g of residue, which was further subjected to preparative TLC (silica gel, $CHCl_3$-acetone=4:1, C_6H_6-Et_2O=2:3, *n*-hexane-acetone=1:1) to give gancaonin W (**1**, 2 mg) as yellow prisms.

Gancaonin W (1)——Mp 205–211°C (from C_6H_6-acetone), UV λ_{max} (MeOH) nm: 210 (log ε 4.70), 260 (4.12), 358 (4.16), (MeOH+NaOAc): 260 (4.11), 275 (sh 4.06), 365 (4.11). EI-MS (probe) 70 eV, *m/z* (rel. int.): 369 $[M+1]^+$ (28%), 368 $[M]^+$ (100), 351 (11), 325 (29), 313 (97), 312 (88), 284 (36). HR-MS, *m/z* 368.1261 $[M]^+$, ($C_{21}H_{20}O_6$ requires: 368.1260). ^{1}H-NMR (400 MHz, acetone-d_6) δ 1.64, 1.77 (each 3H, br s, Me-3″), 3.43 (2H, br d, J=7 Hz, H_2-1″), 3.97 (3H, s, OMe), 5.32 (1H, br t, J=7 Hz, H-2″), 6.46 (1H, dd, J=0.6 and 2 Hz, H-8), 6.48 (1H, d, J=2 Hz, H-6), 6.52 (1H, d, J=8 Hz, H-5′), 6.97 (1H, d, J=8 Hz, H-6′), 7.95 (2H, br s, OH×2), 8.00 (1H, d, J=0.6 Hz, H-4), and 8.39 (1H, br s, OH).

Synthesis of 3——5,7,4′-Trihydroxy-3-arylcoumarin (**3**) was synthesized by the procedure described.[17]

Synthesis of 4——A mixture of 2,4-dihydroxy-6-methoxy-benzaldehyde[18] (20 mg, 0.12 mmol), 4-hydroxyphenylacetic acid (40 mg, 0.26 mmol), and dry NaOAc (1 g) was refluxed in acetic anhydride (2 ml) for 2 h. The reaction mixture was poured into ice water, and then extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and evaporated. The residue was crystallized from MeOH to give 7,4′-diacetoxy-5-methoxy-3-arylcoumarin (**5**) as colorless prisms (26 mg, 46%). A mixture of **5** (20 mg), 28% ammonia water (1 ml) and dioxane (2 ml) was left at room temperature for 15 h. The reaction mixture was diluted with H_2O (50 ml) and neutralized with CH_3-COOH, then extracted with EtOAc. The EtOAc layer was washed with H_2O and evaporated. The residue was purified by preparative TLC (silica gel, $CHCl_3$-EtOAc=5:3) to give 7,4′-dihydroxy-5-methoxy-3-arylcoumarin (**4**) as yellow prisms (10 mg, 65%).

7,4′-Diacetoxy-5-methoxy-3-arylcoumarin (5)——Mp 202–210°C (MeOH). EI-MS; *m/z* 369 $[M+1]^+$ (3%), 368 $[M]^+$ (13), 326 (24), 284 (100), 256 (11), 241 (13). HR-MS, *m/z* 368.0862 $[M]^+$ ($C_{20}H_{16}O_7$ requires: 368.0896). ^{1}H-NMR (90 MHz, $CDCl_3$); δ 2.31 (6H, s, OAc×2), 3.29 (3H, s, OMe), 6.50 (1H, d, J=2 Hz, H-6), 6.72 (1H, br d, J=2 Hz, H-8), 7.14 (2H, d, J=8.5 Hz, H-3′ and H-5′), 7.33 (2H, d, J=8.5 Hz, H-2′ and H-6′), 8.11 (1H, br s, H-4).

7,4′-Dihydroxy-5-methoxy-3-arylcoumarin (4)——Mp 324–326°C (acetone); EI-MS, *m/z* 285 $[M+1]^+$ (19%), 284 $[M]^+$ (100), 256 (21), 241 (46), 213 (8). HR-MS, *m/z* 248.0707 $[M]^+$ ($C_{16}H_{12}O_5$ requires: 284.0685). ^{1}H-NMR (400 MHz, acetone-d_6); δ 3.96 (3H, s, OMe), 6.39 (3H, dd, J=0.6, 2 Hz, H-8), 6.44 (1H, d, J=2 Hz, H-6), 6.90 (2H, d, J=9 Hz, H-3′ and H-5′), 7.60 (2H, d, J=9 Hz, H-2′ and H-6′), 8.01 (1H, d, J=0.6 Hz, H-4), 8.59 (1H, br s, OH), 9.50 (1H, br s, OH).

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