Two New Dimeric Acridone Alkaloids from *Glycosmis citrifolia*

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Two new acridone dimers, glycobismines-F (1) and -G (2), were isolated from the roots of *Glycosmis citrifolia* collected in Taiwan. The structures of the new compounds were determined based on spectral analysis.

Key words   acridone dimer; *Glycosmis citrifolia*; glycobismine-F; glycobismine-G; Rutaceae

The genus *Glycosmis* (Rutaceae-Aurantioideae) has been reported to be a rich source of various types of alkaloids.[1] *Glycosmis citrifolia* (Willd.) Lindl.[2] is used for the treatment of skin itch, scabies, and ulcers[3] as folk medicine. Previously, we reported the isolation and structure elucidation of many monomeric and dimeric acridone alkaloids and quinolone alkaloids from *G. citrifolia* collected in Taiwan.[1,4] In continuing our studies of the constituents of this plant, we report here the isolation and structure elucidation of two additional new dimeric acridone alkaloids, glycobismines-F (**1**) and -G (**2**) (Fig. 1). Glycobismine-G (**2**) has a novel ether linkage among dimeric acridones.

An acetone extract of the root was chromatographed on silica gel, followed by repeated preparative TLC to furnish new alkaloids along with known compounds. Glycobismine-F (**1**), racemate, was obtained as a yellow oil. The molecular formula $C_{38}H_{32}N_2O_9$ was determined using high-resolution (HR)-FAB-MS. The IR and UV absorption spectra (see Experimental) indicated the presence of a 1-hydroxy-9-acridone skeleton[5] in the molecule. The ^1H- and ^{13}C-NMR spectra showed two low-field signals at δ_H 14.49 and 14.35 due to hydrogen-bonded hydroxyl protons, two carbonyl carbon signals at δ_C 182.1 and 183.1, and two *N*-methyl signals at δ_H 3.79 and 3.47, δ_C 49.3 and 48.9, suggesting the presence of two 1-hydroxy-*N*-methylacridone skeletons in the molecule. In the aromatic proton region, an *ortho*-coupled AB type [δ_H 7.85, 7.05 (each 1H, d, $J=8.8$ Hz)], a continuously located ABC type [δ_H 7.74 (1H, dd, $J=7.8$, 1.0 Hz), 7.25 (1H, dd, $J=7.8$, 1.0 Hz), 7.15 (1H, t, $J=7.8$ Hz)], and two isolated [δ_H 6.09, 6.25 (each 1H, s)] proton signals were observed. Further, three 3H singlets due to quarternary methyls (δ_H 1.49, 1.46, 1.65), two pairs of doublets at δ_H 6.70, 5.66 ($J=9.8$ Hz) and δ_H 6.86, 6.53 ($J=16.1$ Hz) assignable to *cis*- and *trans*-oriented olefinic protons, respectively, and isolated methylene protons at δ_H 4.25 and 4.57 (each 1H, d, $J=11.2$ Hz) appeared. The connectivities of these moieties were deduced based on C–H long-range correlations in the heteronuclear multiple-bond connectivity (HMBC) spectrum (Fig. 2). The following C–H long-range correlations were more significant in the structure determination of the upper acridone unit: between C-2 bearing a lone H-2 and a hydrogen-bonded OH; between H-2 and C-4 which further correlated with a *cis*-olefinic H-12; and between an oxygenated C-3 (δ_C 161.8) and another *cis*-olefinic H-11 which also correlated with the quarternary C-13 having dimethyls and C-4a which correlated with the *N*-methyl proton. In addition, in the nuclear

Overhauser effect (NOE) experiment, the appearance of 12% enhancement between an *N*-methyl signal at δ_H 3.79 and H-11 suggested the presence of a [6,5-*c*] fused 2,2-dimethylpyran ring in the upper acridone unit, as shown in the structure of **1**. Further, the deshielded lower-field proton H-8 at δ_H 7.85 in the *ortho*-coupled AB-type system showed
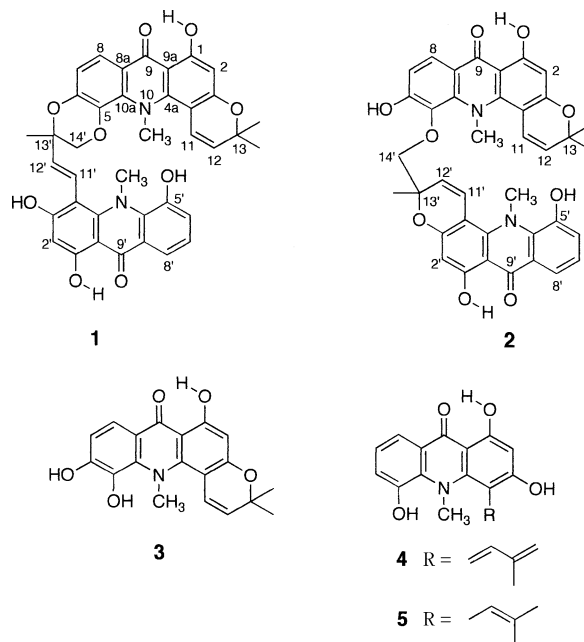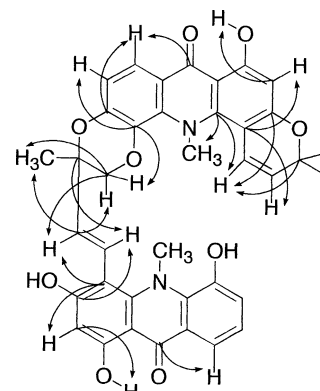

Fig. 1

Fig. 2.   Significant C–H Long-Range Correlations of **1** in the HMBC Spectrum

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Table 1. ^{1}H-NMR Data on Glycobismine-F (**1**) and Glycobismine-G (**2**)

	1	2
1-OH	14.49 (s)	14.49 (s)
H-2	6.09 (s)	6.03 (s)
6-OH		10.76 (s)
H-7	7.05 (d, J=8.8 Hz)	7.00 (d, J=8.3 Hz)
H-8	7.85 (d, J=8.8 Hz)	7.82 (d, J=8.3 Hz)
10-Me	3.79 (3H, s)	3.61 (3H, s)
H-11	6.70 (d, J=9.8 Hz)	6.23 (d, J=9.8 Hz)
H-12	5.66 (d, J=9.8 Hz)	5.26 (d, J=9.8 Hz)
13-Me	1.46 (3H, s)	1.27 (3H, s)
	1.49 (3H, s)	1.29 (3H, s)
1′-OH	14.35 (s)	14.49 (s)
H-2′	6.25 (s)	6.05 (s)
3′-OH	6.65 (s)	
5′-OH		10.46 (s)
H-6′	7.25 (dd, J=7.8, 1.0 Hz)	7.27 (dd, J=7.8, 1.5 Hz)
H-7′	7.15 (t, J=7.8 Hz)	7.23 (t, J=7.8 Hz)
H-8′	7.74 (dd, J=7.8, 1.0 Hz)	7.69 (dd, J=7.8, 1.5 Hz)
10′-Me	3.47 (3H, s)	3.73 (3H, s)
H-11′	6.86 (d, J=16.1 Hz)	6.85 (d, J=10.3 Hz)
H-12′	6.53 (d, J=16.1 Hz)	5.75 (d, J=10.3 Hz)
H-14′	4.25 (d, J=11.2 Hz)	4.04 (d, J=10.8 Hz)
	4.57 (d, J=11.2 Hz)	4.39 (d, J=10.8 Hz)
13′-Me	1.65 (3H, s)	1.61 (3H, s)

All signals correspond to 1H, unless otherwise stated.

Table 2. ^{13}C-NMR and C–H Long-range Correlations (J^3) in HMBC Spectra of Glycobismine-F (**1**) and Glycobismine-G (**2**)

Position	1		2	
	δ_C	HMBC	δ_C	HMBC
1	165.5		163.6	
2	98.4	1-OH	97.1	1-OH
3	161.8	H-11	160.1	H-11
4	103.3	H-2, H-12	101.9	H-2, H-12
4a	148.6	H-11, 10-Me	146.9	H-11, 10-Me
5	133.9	H-7, H-14′	135.9	H-7, 6-OH, H-14′
6	148.9	H-8	155.7	H-8
7	114.9		113.8	
8	119.7		121.5	
8a	118.9	H-7	116.6	H-7
9	182.1	H-8	180.5	H-8
9a	107.3	H-2	105.6	1-OH
10-Me	49.3		47.2	
10a	139.6	10-Me, H-8	142.7	10-Me, H-8
11	121.6		119.9	
12	125.0		124.1	
13	77.3	H-11	76.2	H-11
13-Me	27.0		26.5	
	27.5		26.6	
1′	164.6		164.0	
2′	97.9	1′-OH	96.8	1′-OH
3′	164.0	H-11′	160.1	H-11′
4′	105.1	H-2′, H-12′	101.5	H-12′
4′a	151.5	H-11′, 10′-Me	147.3	H-11′, 10′-Me
5′	149.4	H-7′	148.5	H-7′
6′	120.6	H-8′	130.2	H-8′
7′	124.1		123.8	
8′	117.1	H-6′	115.3	H-6′
8′a	125.9	H-7′	123.7	H-7′
9′	183.1	H-8′	181.3	H-8′
9′a	107.8	1′-OH, H-2′	106.4	1′-OH, H-2′
10′-Me	48.9		48.6	
10′a	138.5	10′-Me, H-6′, H-8′	136.5	10′-Me, 5′-OH, H-8′
11′	125.6		122.5	
12′	131.3	H-14′, 13′-Me	126.3	H-14′, 13′-Me
13′	77.2	H-11′	79.0	H-11′
14′	71.6	H-12′, 13′-Me	75.0	13′-Me
13′-Me	23.3		22.2	

three-bond correlations with a carbonyl carbon C-9 and an oxygenated C-6 (δ_C 148.9). Another *ortho*-coupled H-7 was also correlated with oxygenated C-5 (δ_C 133.9), suggesting the presence of oxygenated substituents at C-5 and C-6 on the upper acridone nucleus. On the other hand, the structure and location of the substituents on the lower acridone unit were also determined based on C–H long-range correlations in the HMBC spectrum as follows: observation of three-bond correlations between methylene H-14′ and sp^2 C-12′, which further correlated with the 13′-CH$_3$ proton; between olefinic H-11′ and the quarternary C-13′; and between H-12′ and C-14′. Together with consideration of the chemical shift values of C-14′ and H-14′ (δ_C 71.6; δ_H 4.25, 4.57) and C-13′ (δ_C 77.2) and J-values (16.1 Hz) of H-11′ and H-12′, we proposed the structure of the (*E*)-3,4-oxygenated 3-methyl-1-butenyl side chain. The location of this side chain at C-4′ was shown by the presence of three-bond correlations between H-11′ and an oxygenated C-3′ (δ_C 164.0), between H-12′ and C-4′ which further correlated with a lone H-2′ on the carbon correlated with a hydrogen-bonded OH, and between C-5 and methylene H-14′, indicating the presence of a [5,6-*h*] oriented 2-methyl-2-substituted 1,4-dioxane ring in the molecule. In addition, an observation of 9% enhancement of olefinic H-11′ at δ_H 6.86 on irradiation of the *N*-methyl signal at δ_H 3.47 in the NOE experiment also supported the location of the side chain at C-4′. Further, correlations between a lower signal at δ_H 7.74 (H-8′) in the continuously located three proton system and a carbonyl C-9′ indicated a remaining hydroxyl group at C-5′. From these results, the structure of glycobismine-F was suggested to be **1**. Biogenetically, glycobismine-F (**1**) is considered to be formed by oxidative coupling of citracridone-III (**3**)[6] and a side chain analogue (**4**) of *O*-demethylglycocitrine-I (**5**).[7]

Glycobismine-G (**2**), a yellow oil, was isolated as a racemate. The molecular formula $C_{38}H_{32}N_2O_9$, the same as that of **1**, was determined with HR-FAB-MS. The ^{1}H- and ^{13}C-

NMR spectra (Tables 1, 2) were shown to have close similarities with those of **1**, except for differences in the J-values of olefinic proton signals (J=10.3 Hz in **2** and 16.1 Hz in **1**) in their ^{1}H-NMR spectra, indicating a similar arrangement of substituents on two acridone nuclei in **2** as that in **1**, and further the presence of an additional *cis*-oriented olefinic-H in **2**, instead of *trans*-oriented ones in **1**. In the HMBC spectrum, C–H long-range correlations (C-4′/a lone H-2′ and H-12′, C-12′/14′-methylene-H and 13′-CH$_3$, and H-11′/oxygenated C-3′ and a quarternary C-13′ revealed the presence of a [6,5-*c*] oriented pyran ring with methyl and methylene moieties at C-2. The linkage of the two acridone nuclei between C-5 in the upper and C-14′ in the lower halves was also determined by the observation of the C–H long-range correlation between the 14′-methylene proton and oxygenated C-5 (δ_C 135.9) having a correlation with H-7. These results, together with the HMBC spectral data shown in Fig. 3, established the structure **2** for glycobismine-G. Biogenetically, glycobismine-G (**2**) is also considered to be derived from the same precursors **3**[6] and **5**[7] as those of **1**.
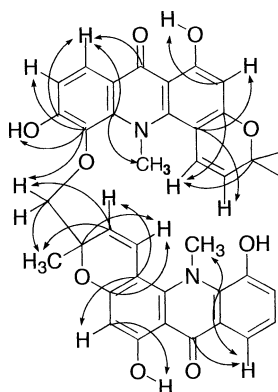
Fig. 3. Significant C–H Long-Range Correlations of **2** in the HMBC Spectrum

Experimental

Optical rotations were measured on a JASCO DIP-360 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on an A-400 or A-600 (JEOL) spectrometer. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. HMBC spectra were measured at $J=5$ and 8 Hz on the JEOL A-600 spectrometer. EI- and HR-MS were recorded with a JMS-HX 110 spectrometer. UV spectra were recorded on a Shimadzu UV 160A spectrometer in EtOH, and IR spectra were recorded on a Shimadzu IR-450 spectrometer in CHCl$_3$. For column chromatography, Wakogel 60 was used. Preparative TLC was done on Kiesel gel 60 F$_{254}$ (Merck).

Isolation The roots (2.5 kg) of *G. citrifolia* (WILLD.) LINDL. collected at Pen-Lin, Tainan Hsien, Taiwan, were extracted with acetone for 30 h under reflux (8 l×3) and evaporated under reduced pressure. The acetone extract (114 g) was subjected to silica gel column (7.5×30 cm) chromatography and eluted with toluene, CH$_2$Cl$_2$, CH$_2$Cl$_2$–acetone (9 : 1), CH$_2$Cl$_2$–acetone (8 : 2), and acetone. The CH$_2$Cl$_2$–acetone (9 : 1) eluate (28.5 g) was separated by silica gel column chromatography, centrifugal chromatography, and finally repeated PTLC [solvent; isopropyl ether, acetone–CHCl$_3$ (1 : 9), acetone–benzene (3 : 7), AcOEt–*n*-hexane (1 : 1)] to give glycobismine-F (**1**) (16.5 mg) and -G (**2**) (4.5 mg).

Glycobismine-F (**1**): Yellow oil, $[\alpha]_D \pm 0°$ ($c=0.174$, CHCl$_3$). IR (CHCl$_3$) cm^{-1}: 3300, 1630, 1608, 1572. UV λ_{max} (EtOH) nm: 270, 282 (sh), 316 (sh), 340 (sh), 406. HR-FAB-MS m/z: 661.2137 [M+H]$^+$ (Calcd for C$_{38}$H$_{33}$N$_2$O$_9$: 661.2186). ^{1}H- and ^{13}C-NMR (acetone-d_6): Tables 1 and 2. NOE: irradiation at δ_H 3.79 (10-CH$_3$); 12% enhancement at δ_H 6.70 (H-11); irradiation at δ_H 3.47 (10′-CH$_3$); 9% enhancement at δ_H 6.86 (H-11′).

Glycobismine-G (**2**): Yellow oil, C$_{38}$H$_{32}$N$_2$O$_9$, $[\alpha]_D \pm 0°$ ($c=0.13$, CHCl$_3$). IR (CHCl$_3$) cm^{-1}: 3260 (br), 1631, 1591, 1571. UV λ_{max} (MeOH) nm: 270, 284 (sh), 296 (sh), 344 (sh), 406. HR-FAB-MS: m/z: 661.2209 [M+H]$^+$ (Calcd for C$_{38}$H$_{33}$N$_2$O$_9$: 661.2186). FAB-MS m/z: 661 [M+H]$^+$, 613, 580, 551, 460, 412, 379. EI-MS m/z: 446, 339, 323, 308, 293, 257. ^{1}H- and ^{13}C-NMR (DMSO-d_6): Tables 1 and 2. NOE: irradiation at δ_H 3.73 (10′-CH$_3$) and 9% enhancement at δ_H 6.85 (H-11′); irradiation at δ_H 3.61 (10-CH$_3$) and 9% enhancement at δ_H 6.23 (H-11).

References and Notes

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