Chemical Constituents of *Glycosmis citrifolia* (WILLD.) LINDL. Structures of Four New Acridones and Three New Quinolone Alkaloids

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The structures of two new dimeric acridone alkaloids, glycobismine-D (1) and -E (2), having a novel linkage as binary acridones, three monomeric acridones, glycocitrine-IV (3), -V (4), and -VI (5), and three quinolone alkaloids, glycocitlone-A (6), -B (7), and -C (8) from *Glycosmis citrifolia* (WILLD.) LINDL. (Rutaceae) have been elucidated by spectrometric studies.

Key words acridone alkaloid; quinolone alkaloid; *Glycosmis citrifolia*; glycobismine; glycocitrine; glycocitlone

We previously reported the isolation of many kinds of acridone alkaloids including three binary ones from *Glycosmis citrifolia* (WILLD.) LINDL. (Rutaceae).[1—4] In our continuing studies of the constituents of this plant,[5] we report here the isolation and structural elucidation of two additional binary acridone alkaloids, three monomeric acridones in addition to three new quinolone alkaloids called glycobismine-D (**1**) and -E (**2**), glycocitrine-IV (**3**), -V (**4**), and -VI (**5**), and glycocitlone-A (**6**), -B (**7**), and -C (**8**), respectively. Binary acridone alkaloids isolated this time are shown to have a novel linkage.

An acetone or methanol extract of root and stem bark of the plant was treated with hexane and the residue was dissolved in a mixture of dichloromethane and acetone (10 : 1). The soluble components were chromatographed on silica gel eluted by hexane–acetone followed by repeatedly preparative TLC to afford new alkaloids along with known compounds.

Structure of Glycobismines Glycobismine-D (**1**), $[\alpha]_D$ $\pm 0°$ (MeOH), was obtained as a yellow oil. The molecular formula $C_{35}H_{30}N_2O_{10}$ was established by the analysis of high resolution (HR)-FAB-MS. The dimeric 1-hydroxy-*N*-methyl-9-acridone skeleton in the molecule was suggested by observations of the following spectral data: 1) characteristic UV absorptions[6,7] (see Experimental) and IR bands at λ_{max} 3217 and 1630 cm^{-1}, 2) two lower-field ^{1}H-NMR signals at δ_H 13.97 and 14.32 due to strongly hydrogen-bonded hydroxyl protons, 3) two carbonyl carbon signals at δ_C 181.71 and 182.29 in the ^{13}C-NMR spectrum, 4) two *N*-methyl signals at δ_H 3.72 and 3.07; δ_C 46.67 and 47.59. As remaining ^{1}H-NMR signals, three 3H singlets due to two methoxyls (δ_H 3.94 and 3.79) and a quarternary methyl (δ_H 1.65) attached to an oxygenated carbon, three pairs of AB type doublets at δ_H 6.62, 6.28 (J=16.1 Hz), δ_H 7.93, 7.03 (J=8.8 Hz), and δ_H 4.45, 4.06 (J=11.0 Hz) assignable to a *trans*-oriented olefinic and an *ortho*-located aromatic, and an oxygen-linked isolated methylene protons, respectively, were observed. Further, the results of H–H correlation spectroscopy (COSY) spectrum showed the presence of three vicinal aromatic protons at δ_H 7.76 (1H, br d, J=8.1 Hz), 7.07 (1H, t, J=8.1 Hz), and 7.02 (1H, br d, J=8.1 Hz). The connectivities of these moieties were deduced by three-bond C–H long-range correlations in the ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum summarized by arrows in Fig. 1.

The upper-half structural unit of the molecule shown in Chart 1 was proposed by the following correlations. The deshielded lower-field proton signal at δ_H 7.93 (H-8) in *ortho*-coupled two-spin proton system showed three-bond correlation with a carbonyl carbon at δ_C 181.71 (C-9), an oxygenated carbon at δ_C 147.83 (C-6), and an angular carbon at δ_C 138.82 (C-10a) which was further correlated with *N*-methyl proton at δ_H 3.72. Another *ortho*-coupled proton signal at δ_H 7.03 (H-7) was correlated with another oxygenated carbon at δ_C 132.36 (C-5), which further was correlated with the isolated methylene proton at δ_H 4.45 (H-4″). This methylene carbon signal at δ_C 70.84 (C-4″) correlated with a quarternary methyl proton at δ_H 1.65 (3″-CH_3). From these results, the 2-substituted 2-methyl-1,4-dioxane ring fused at C-5 and C-6 on the upper acridone A ring was assigned. The other C–H long-range correlations between C-2 (δ_C 94.11) bearing a lone proton at δ_H 6.36 (H-2) and a hydrogen-bonded proton at δ_H 13.97, and H-2 (δ_H 6.36) and C-4 (δ_C 129.90) having three-bond correlation with a methoxy proton at δ_H 3.79 (4-OCH_3), and observation of an 18% nuclear Overhauser effect (NOE) enhancement between H-2 (δ_H 6.36) and another methoxy signal at δ_H 3.94 (3-OCH_3) showed the substitution pattern on C ring in the upper-half acridone nucleus as shown in Chart 1.

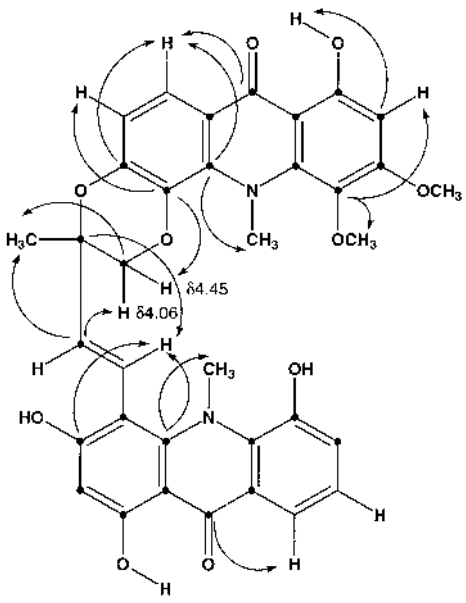

Fig. 1. C–H Long-Range Correlations in the HMBC Spectrum of **1** in $CDCl_3$

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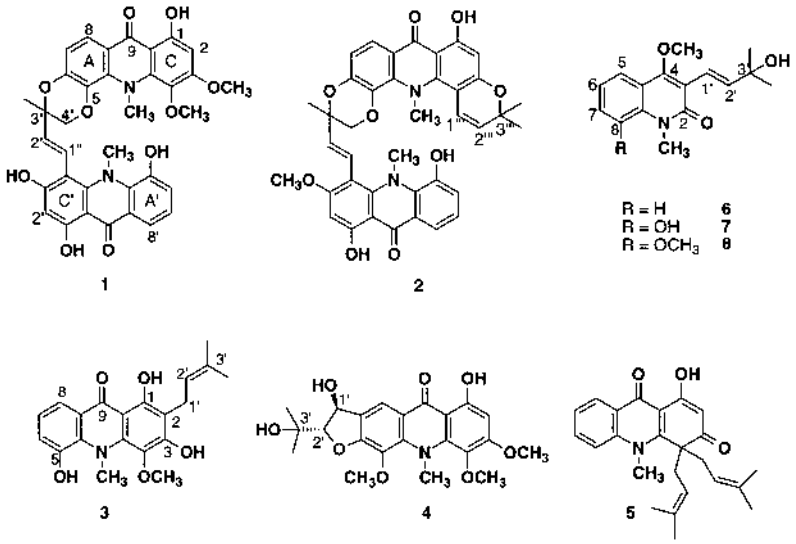

Chart 1

The structure of the lower-half unit of the molecule was also assigned by three-bond C–H long-range correlations in the HMBC spectrum. A correlation between a lower field proton in the three-spin proton system at δ_H 7.76 (H-8′) and a carbonyl carbon at δ_C 182.29 (C-9′) indicated the location of a substituent at C-5′ in the C′-ring. Further, correlation between an angular carbon at δ_C 150.29 (C-4′a) having a correlation with an *N*-methyl proton (δ_H 3.07) and an olefinic proton at δ_H 6.62 (H-1″) which further correlated with a quarternary carbon at δ_C 160.65 (C-3′), together with an observation of NOE enhancements between the *N*-methyl and olefinic protons (see Experimental), suggested the location of a disubstituted (*E*)-olefinic group at C-4′. The linkage of two acridone nuclei between C-3″ and C-2″ in the molecule was proposed by C–H long-range correlations between the olefinic carbon at δ_C 131.46 (C-2″) and 3″-methyl proton at δ_H 1.65 and the isolated methylene proton at δ_H 4.06 (H-4″), and between the quarternary carbon at δ_C 75.29 (C-3″) and the olefinic proton at δ_H 6.62 (H-1″). Observation of NOE enhancement of one of two olefinic proton signals on irradiation of *N*-methyl proton at δ_H 3.07 also showed this linkage of two acridone nuclei. Based on these results, the double-bridge linkage of the two acridone nuclei was concluded to be formally between 5,6-dihydroxy group and the end (C-4″/C-3″) of the prenyl side-chain in the upper and lower acridones of the molecule, respectively, and the structure of glycobismine-D was proposed as **1**.

Glycobismine-E (**2**), $[\alpha]_D$ ±0° (MeOH), $C_{39}H_{34}N_2O_9$, a yellow oil, was isolated as a minor base. The ^{1}H-NMR spectrum of this alkaloid was found to be similar to that of **1**, except for additional signals due to a dimethyl pyran ring [δ_H 1.58 (6H, s), 5.53 (1H, d, J=9.9 Hz), 6.64 (1H, d, J=9.9 Hz)] instead of two 3H singlets due to methoxy groups (see Table 1) on the upper-half structural unit. There was an additional 3H singlet due to a methoxy group [δ_H 3.91 (3H, s)] instead of a 1H broad signal due to a hydroxyl group (see Table 1) on the lower-half structural unit. These data suggested that the substitution pattern on each acridone nucleus and the linkage between upper and lower acridones were the same as those of **1**. In NOE experiments, irradiations of *N*-methyl signals at δ_H 3.68 and 2.98 caused 3 and 7% enhancements of the doublets at δ_H 6.64 (J=9.9 Hz, H-1‴) on the pyran ring and δ_H 6.57 (J=16.1 Hz, H-1″) on the (*E*)-double bond, respectively. Further, 22% enhancement of a lone aromatic proton singlet at δ_H 6.34 (H-2′) was observed on irradiation of a methoxy signal at δ_H 3.91 (3′-OCH$_3$). From these results, the structure of glycobismine-E was proposed as **2**.

Structure of Glycocitrines Glycocitrine-IV (**3**) and -V (**4**) were obtained as yellow oils from the acetone extract. The typical UV absorptions of these alkaloids (see Experimental), an IR band at 1631 cm^{-1}, and hydrogen-bonded hydroxyl proton signals at δ_H 14.18 and 14.05 in the ^{1}H-NMR spectrum suggested the presence of an 1-hydroxy-9-acridone nucleus as a common structural unit in each molecule.

The molecular formula $C_{20}H_{21}NO_5$ of **3** was determined by HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectra showed the presence of a methoxy (δ_H 3.18, δ_C 60.07), an *N*-methyl (δ_H 3.80, δ_C 44.96), and a prenyl moiety [δ_H 3.43 (2H, d, J=7.3Hz), 5.33 (1H, m), 1.83, 1.71 (each 3H, s)] in the molecule. In the aromatic proton region of ^{1}H-NMR spectrum in acetone-d_6, observation only of clearly separated three-spin proton signals [δ_H 7.80 (1H, d, J=8.1 Hz), 7.27 (1H, d, J=8.1 Hz), and 7.14 (1H, t, J=8.1 Hz)] including a lower field signal of H-8 due to *peri*-located proton of the 9-carbonyl group suggested the structure of 2,3,4,5-tetrasubstitited 1-hydroxy-*N*-methylacridone for this alkaloid. Locations of each substituent were deduced by C–H long-range correlations in the HMBC spectrum. Correlations between the methylene proton at δ_H 3.43 (H-1′) of the prenyl moiety and C-1 (δ_C 157.82) bearing a hydrogen-bonded hydroxyl group (δ_H 14.18) and C-3 (δ_C 154.90) having a hydroxyl group (δ_H 6.67) showed the position of the prenyl group at C-2 and one of the hydroxyl groups at C-3. The proton of the hydroxyl group at δ_H 6.67 (3-OH) showed correlations both with C-4 (δ_C 127.66) having a three-bond correlation with a methoxy proton at δ_H 3.81, and C-2 (δ_C 108.91) having a two-bond correlation with the methylene proton (δ_H 3.43, H-1′) of the prenyl group, respectively, suggesting the location of a methoxyl group at C-4. Lack of the observation of NOE on irradiation of *N*-methyl protons also revealed the presence of

Table 1. ^{1}H- and ^{13}C-NMR Data for the New Dimeric Acridone Alkaloids

	1 in acetone-d_6			**2**
1-OH	13.97 (s)	14.21 (s)		14.15 (s)
1			160.19	
2	6.36 (s)	6.37 (s)	94.11	6.25 (s)
3			159.68	
3-OCH_3	3.94 (3H, s)	3.95 (3H, s)	56.21	
4			129.90	
4-OCH_3	3.79 (3H, s)	3.75 (3H, s)	60.63	
4a			141.92	
5			132.36	
6			147.83	
7	7.03 (d, 8.8)	6.99 (d, 8.9)	113.64	7.11 (d, 8.8)
8	7.93 (d, 8.8)	7.82 (d, 8.9)	119.82	7.98 (d, 8.8)
8a			117.79	
9			181.71	
9a			106.02	
N-CH_3	3.72 (3H, s)	3.74 (3H, s)	46.67	3.68 (3H, s)
10a			138.82	
1′-OH	14.32 (s)	14.36 (s)		14.34 (s)
1′			163.68	
2′	6.24 (s)	6.26 (s)	97.33	6.34 (s)
3′			160.65	
3′-OCH_3				3.91 (3H, s)
4′			103.58	
4′a			150.29	
5′			147.30	
6′	7.02 (br d, 8.1)	7.25 (br d, 7.9)	119.86	7.02 (br d, 7.7)
7′	7.07 (t, 8.1)	7.15 (t, 7.9)	123.30	7.11 (t, 7.7)
8′	7.76 (br d, 8.1)	7.73 (br d, 7.9)	117.05	7.80 (br d, 7.7)
8′a			125.10	
9′			182.29	
9a			107.44	
N′-CH_3	3.07 (s)	3.48 (s)	47.59	2.98 (3H, s)
10′a			137.17	
1″	6.62 (d, 16.1)	6.86 (d, 16.5)	125.96	6.57 (d, 16.1)
2″	6.28 (d, 16.1)	6.54 (d, 16.5)	131.46	6.36 (d, 16.1)
3″			75.29	
3″-CH_3	1.65 (3H, s)	1.65 (3H, s)	23.66	1.47 (3H, s)
4″	4.45 (d, 11.0)	4.54 (d, 11.3)	70.84	4.42 (d, 11.0)
	4.06 (d, 11.0)	4.23 (d, 11.3)		4.07 (d, 11.0)
Other	7.43 (br, OH)			6.94 (br, 5-OH)
	6.81 (br, OH)			6.64 (d, 9.9, H-1‴)
				5.53 (d, 9.9, H-2‴)
				1.58 (6H, s, 3″-CH_3)*

Values are in ppm. Figures in parentheses are coupling constants (J) in Hz. All signals correspond to 1H in the ^{1}H-NMR spectrum unless otherwise stated. NMR spectra were recorded in $CDCl_3$. * Overlapped with H_2O.

substituents at both *peri*-positions of the *N*-methyl group. From these results, we proposed the structure **3** for glycocitrine-IV.

Glycocitrine-V (**4**), $[\alpha]_D$ ±0° (MeOH), $C_{22}H_{25}NO_8$, was isolated as a yellow oil. The ^{1}H- and ^{13}C-NMR spectra (Table 2) showed the presence of three methoxyls, an *N*-methyl, and two lone aromatic protons, one of which was assignable to H-8 as a consequence of its lower field chemical shift (δ_H 8.11). The presence of a 2-(2-hydroxyisopropyl)-3-hydroxydihydrofuran ring fused to the acridone nucleus was indicated by observations of two 3H singlets assignable to two methyls (δ_H 1.40 and 1.36) attached to an oxygen-linked quarternary carbon (δ_C 71.35, C-3′), and a pair of doublets [δ_H 5.48 (H-1′, J=4.4 Hz) and 4.52 (H-2′, J=4.4 Hz)] due to *trans*-oriented vicinal protons attached to oxygenated tertiary carbons (δ_C 72.42 and 98.74, respectively) in both ^{1}H- and ^{13}C-NMR spectra, together with C–H long-range correlations between C-2′ having a typical ^{13}C chemical shift (δ_C 98.74) in the 5-membered ring system (2-(1-hydroxy-1-methylethyl)-3-hydroxydihydrofuran ring) and the protons of two methyls (δ_H 1.36, H-4′, 1.40, H-5′) in the HMBC spectrum. Linear orientation of the dihydrofuran ring as shown in formula **4** was revealed by an NOE increment between a deshielded singlet (δ_H 8.11, H-8) and a doublet (δ_H 5.48, H-1′), and by three-bond C–H long-range correlation between C-1′ (δ_C 72.42) and H-8 (δ_H 8.11) having further correlations with a carbonyl C-9 (δ_C 181.48) and an oxygen-linked C-6 (δ_C 156.84). Furthermore, observation of C–H long-range correlations between C-2 (δ_C 94.12) bearing a lone H-2 (δ_H 6.37) and a hydrogen-bonded 1-hydroxy proton (δ_H 14.05), and 17% NOE between H-2 (δ_H 6.37) and a methoxyl signal (δ_H 3.95, 3-OCH_3) indicated the location of two methoxyls at C-3 and C-4 in the acridone nucleus. Based on these findings, the structure of glycocitrine-V was concluded to be **4**.

Glycocitrine-VI (**5**) was obtained as a yellow oil from the methanol extract. The molecular formula was established as $C_{24}H_{27}NO_3$ by HR-EI-MS. The UV spectrum exhibited absorption bands at λ_{max} 203, 229, 286, 296, and 340, similar to those of the 9-acridone nucleus. The IR and ^{13}C-NMR spectra showed the presence of two carbonyl groups in the molecule (ν_{max} 1655 and 1618; δ_C 195.59 and 178.32). In the ^{1}H-NMR spectrum, signals due to a strongly hydrogen-bonded hydroxyl proton at δ_H 16.07, a lone aromatic proton at δ_H 5.67 (1H, s), and two prenyl groups at δ_H 3.29 (2H, dd, J=15.1, 6.4 Hz), 2.94 (2H, dd, J=15.1, 7.3 Hz), 4.68 (2H, m), 1.50 (6H, s), and 1.47 (6H, s) were observed. Appearance of a pair of four-spin proton signals [at δ_H 8.54 (1H, dd, J=1.5, 7.8 Hz), 7.86 (1H, br t, J=7.8 Hz), 7.75 (1H, br d, J=7.8 Hz), and 7.60 (1H, br t, J=7.8 Hz)] including a lower field proton signal assignable to H-8 which was deshielded by 9-carbonyl group and correlated with the 9-carbonyl carbon by three-bond in the HMBC spectrum, indicated the presence of non-substituted A-ring in the molecule. Further analysis of the HMBC spectrum revealed three-bond correlations of a hydrogen-bonded proton signal at δ_H 16.07 with C-2 (δ_C 101.20) attached to a singlet proton at δ_H 5.67 (H-2), and two methylene proton signals (δ_H 3.29, H-1′) in prenyl moieties both with the other carbonyl carbon at δ_C 195.59 (C-3) and with δ_C 162.60 (C-4a) which was correlated with *N*-methyl proton signal at δ_H 4.22. From these data, the structure of glycocitrine-VI was proposed as **5**.

Structure of Glycocitlone-A (6), -B (7), and -C (8) These alkaloids were obtained as yellow oils or amorphous powder having molecular formula $C_{16}H_{19}NO_3$, $C_{16}H_{19}NO_4$, and $C_{17}H_{21}NO_4$, respectively. These differences in molecular formula were assumed due to an additional substituent of a hydroxyl or a methoxy group compared with **6**, respectively. Common structural unit, 3-[(*E*)-3-hydroxy-3-methyl-1-butenyl]-4-methoxy-*N*-methyl-2-quinolone, of these alkaloids was shown by the following spectral data: (a) a strong UV band at λ_{max} 296—307 nm accompanied by some minor bands, (b) an IR band at λ_{max} 1620—1633 cm^{-1} and a ^{13}C-NMR signal at δ_C 163.05—164.45 due to an amide carbonyl group, (c) an *N*-methyl signal at δ_H 3.74—4.00 and δ_C 29.65—35.53, and an *O*-methyl signal at δ_H 3.85—3.92 and δ_C 60.75—61.13 accompanied by a lower field aromatic proton signal at δ_H 7.44—7.90 (H-5) , and (d) NOE enhancement of this deshielded proton signal (H-5) on irradiation of the methoxy signal δ_H 3.85—3.92, suggesting a 4-methoxy-*N*-methyl-2-quinolone skeleton, (e) a pair of doublets at δ_H 6.83—6.89 and 7.14—7.26 (J=16.1 Hz) assignable to *trans*-oriented olefinic proton, an overlapped dimethyl singlet at δ_H

Table 2. ^{1}H- and ^{13}C-NMR Data for the New Acridone and Quinolone Alkaloids

	3 in acetone-d_6)			**4**		**5**	
1-OH	14.18 (s)		14.39 (s)	14.05 (s)		16.07 (s)	
1		157.82			160.31		173.72
1-NMe							
2		108.91		6.37 (s)	94.12	5.67 (s)	101.20
3	6.67 (s, OH)	154.90			159.59		195.59
3-OMe				3.95 (3H, s)	56.20		
4		127.66			130.04		58.63
4-OMe	3.81 (3H, s)	60.07	3.82 (3H, s)*	3.77 (3H, s)	60.59		
4a		138.98			141.66		162.60
5	6.21 (br, OH)	146.26			133.06	7.75 (br d, 7.8)	116.34
5-OMe				3.99 (3H, s)	60.33		
6	7.10 (br)	119.84	7.27 (d, 8.1)		156.84	7.86 (br t, 7.8)	134.24
7	7.10 (br)	122.54	7.14 (t, 8.1)		126.84	7.60(br t, 7.8)	125.86
8	7.92 (br)	118.19	7.80 (d, 8.1)	8.11 (s)	117.94	8.54 (dd, 1.5, 7.8)	126.57
8-OMe							
8a		125.00			119.32		124.83
9		182.07			181.48		178.32
9a		106.16			105.72		110.00
9-NMe	3.80 (3H, s)	44.96	3.74(3H, s)*	3.77 (3H, s)	46.96	4.22 (3H, s)	38.93
10a		136.60			143.58		141.43
1′	3.43 (d, 7.3)	21.77	3.38 (d, 7.4)	5.48 (d, 4.4)	72.42	3.29 (2H, dd, 15.1, 6.4) 2.94 (2H, dd, 15.1, 7.3)	38.61
2′	5.33 (m)	121.94	5.31 (m)	4.52 (d, 4 .4)	98.74	4.68 (2H, m)	117.07
3′		132.27			71.35		135.54
3′-OH							
4′	1.71 (3H, s)	25.80	1.64 (3H, s)	1.36 (3H, s)	24.99	1.50 (6H, s)	17.93
5′	1.83 (3H, s)	17.83	1.79 (3H, s)	1.40 (3H, s)	25.62	1.47 (6H, s)	25.70

	6		**7**		**8**	
1-OH						
1						
1-NMe	3.74 (3H, s)	29.65	4.00 (3H, s)	35.53	3.94 (3H, s)	35.52
2		163.05		164.45		164.09
3		117.21*		120.59		117.17
3-OMe						
4		160.65		160.97		160.44
4-OMe	3.92 (3H, s)	61.13	3.85 (3H, s)	60.75	3.87 (3H, s)	60.94
4a		117.82*		116.67		120.16
5	7.90 (dd, 1.5, 8.1)	123.78	7.44 (br d, 8.1)	115.16	7.51 (dd, 1.5, 8.1)	116.15
5-OMe						
6	7.27 (br t, 8.1)	122.13	7.05 (t, 8.1)	123.06	7.18 (t, 8.1)	122.60
7	7.57 (br t, 8.1)	130.69	6.96 (br d, 8.1)	118.20	7.06 (dd, 1.5, 8.1)	113.81
8	7.37 (br d, 8.1)	114.09		145.56		148.51
8-OMe					3.90 (8-OMe)	56.59
8a		138.83		128.62		130.30
9						
9a						
9-NMe						
10a						
1′	6.85 (d, 16.1)	116.54	6.89 (d, 16.1)	116.86	6.83 (d, 16.1)	116.53
2′	7.26 (d, 16.1)	144.20	7.14 (d, 16.1)	144.10	7.24 (d, 16.1)	144.36
3′		71.58		71.63		71.50
3′-OH	1.71 (s)				1.99 (s)	
4′	1.46 (3H, s)	29.93	1.44 (3H, s)	29.60	1.45 (3H, s)	29.86
5′	1.46 (3H, s)	29.93	1.44 (3H, s)	29.60	1.45 (3H, s)	29.86

Values are in ppm. Figures in parentheses are coupling constants (J) in Hz. All signals correspond to 1H in the ^{1}H-NMR spectrum unless otherwise stated. NMR spectra were recorded in $CDCl_3$. * Assignments may be reversed.

1.44—1.46 due to geminal dimethyls attached to an oxygenated carbon (δ_C 71.50—71.63) coupled with the appearance of mass fragment ion of [M^+-59] produced by loss of [–$C(CH_3)_2OH$] from molecular ion, suggesting the presence of (*E*)-3-hydroxy-3-methyl-1-butenyl moiety in the molecule, (f) NOE enhancements of signals of an olefinic proton at δ_H 6.83—6.89 (H-1′) on irradiation of a methoxy signal at δ_H 3.85—3.92 (4-OCH_3), and (g) C–H long-range correlations between a carbonyl C-2 (δ_C 163.05—164.45) with *N*-methyl proton (δ_H 3.74—4.00) and an olefinic proton (δ_H 6.83—6.89, H-1′), indicating the location of the side chain at C-3.

In the ^{1}H-NMR spectrum of **6**, observations of a four-spin proton system at δ_H 7.90 (1H, dd, J=1.5 and 8.1 Hz), 7.27 (1H, br t, J=8.1Hz), 7.57 (1H, br t, J=8.1 Hz), and 7.37 (1H, br d, J=8.1 Hz) assignable to H-5, -6, -7, and H-8, respectively, and a 15% NOE enhancement of a doublet at δ_H 7.37 (H-8) on irradiation of the *N*-methyl signal at δ_H 3.74 suggested the structure of glycocitlone-A to be **6**.

The molecular formula $C_{16}H_{19}NO_4$ of **7** showed the presence of an additional hydroxyl group in the molecule as compared with that of **6**. Appearance of a three-spin proton system at δ_H 7.44 (1H, br d, J=8.1 Hz), 7.05 (1H, t, J=8.1 Hz), and 6.96 (1H, br d, J=8.1 Hz), instead of a four-spin system in the spectrum of **6**, coupled with an observation of NOE between 4-methoxy group (δ_H 4.00) and a lower field aromatic proton signal at δ_H 7.44 (H-5) as mentioned above showed the location of hydroxyl group at C-8. From these results, the structure of glycocitlone-B was assigned as **7**.

The molecular formula $C_{17}H_{21}NO_4$ and the striking resemblance of ^{1}H-NMR spectral feature of **8** to that of **7**, except for an additional methoxy signal at δ_H 3.90 (8-OCH_3), which was irradiated to cause a 12% NOE enhancement at the doublet (δ_H 7.06, H-7), revealed the structure **8** as the *O*-methylated analogue of **7**.

Known alkaloids described below were also isolated and characterised by analyses of their spectral data. Acridone alkaloids: glycobismine-A,[4] glycocitrine-II,[1,2] *des*-*N*-methylnoracronycine,[8] glycocitrine-III,[9] noracronycine,[8] glycofolinine,[10] *des*-*N*-methylacronycine.[8] Quinolone alkaloids: glycosolone,[11] glycophylone.[12] Furoquinoline alkaloids: 4,8-dimethoxyfuro[2,3-*b*]quinoline,[13] skimmianine,[14] iso-γ-fagarine.[15]

Experimental

^{1}H- and ^{13}C-NMR, H–H COSY, NOE, and HMBC (J=8 Hz) spectra were recorded on an A-400 or A-600 (JEOL) spectrometer in $CDCl_3$, unless otherwise stated. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. MS were taken by M-80 (Hitachi), HX-110 (JEOL), or JMS-700 (JEOL) spectrometer having direct inlet system. UV spectra were recorded on a V-550 UV/VIS spectrophotometer (JASCO) in MeOH, IR spectra on a FT/IR-230 (JASCO) in $CHCl_3$, and optical rotations on a DIP-370 (JASCO) in $CHCl_3$ at 25 °C, and CD spectra on a J-600 (JASCO) in MeOH. Preparative TLC was done on Kieselgel 60 F_{254} (Merck).

Plant Material Root and stem bark of *Glycosmis citrifolia* (Willd.) Lindl. (Rutaceae) were collected at Pen-Lin, Tainan Hsien, Taiwan in March, 1997. The herbarium specimen has been deposited in the Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, R. O. C.

Extraction and Isolation Dried root and stem bark of the plant (3.51 kg) was extracted with acetone at room and refluxed temperature, and then MeOH refluxed condition. The acetone extract under reflux was treated with a mixture of CH_2Cl_2 and acetone (10 : 1), and soluble components were chromatographed over silica gel successively eluted with an appropriate mixture of hexane, acetone, CH_2Cl_2, and then MeOH. The CH_2Cl_2–acetone (4 : 1—3 : 2) eluates were submitted to silica gel preparative TLC with appropriate combinations of hexane, EtOAc, benzene, acetone, iso-Pr_2O, $CHCl_3$, CH_2Cl_2, and MeOH as developing solvents repeatedly to obtain new compounds, glycobismine-D (**1**) (3.2 mg), glycobismine-E (**2**) (1.0 mg), glycocitrine-IV (**3**) (8.5 mg), glycocitrine-V (**4**) (4.4 mg), glycocitlone-A (**6**) (4.1 mg), glycocitlone-B (**7**) (37.4 mg), and glycocitlone-C (**8**) (20.1 mg) along with known compounds, *des*-*N*-methylnoracronycine (16.8 mg), glycocitrine-II (78.6 mg), glycocitrine-III (0.5 mg), glycosolone (4.0 mg), noracronycine (1.3 mg), glycophylone (3.1 mg), skimmianine (5.7 mg), glycofolinine (3.3 mg), 4,8-dimethoxyfuro[2,3-*b*]quinoline (16.3 mg), glycobismine-A (2.4 mg), *des*-*N*-methylacronycine (129.8 mg), and iso-γ-fagarine (36.7 mg). Glycocitrine-VI (**5**) (3.0 mg) was obtained from the MeOH refluxed extract by treatments analogous to those mentioned above.

Glycobismine-D (**1**): A yellow oil. $[\alpha]_D$ ±0° (c=0.031, MeOH). CD (MeOH, 200—400 nm): No absorption. UV λ_{max} (log ε) nm: 202 (4.61), 225 (4.39), 265 (4.73), 294 (sh) (4.46), 335 (4.25), 405 (3.82). IR ν_{max} cm^{-1}: 3217 (br), 1630. EI-MS *m/z* (%): 312 (50), 297 (32), 269 (64), 257 (100), 244 (12). FAB-MS *m/z*: 639 $(M+H)^+$. NOE (acetone-d_6): Irradiation at δ_H 3.94 (3-OMe)→18% enhancement at δ_H 6.37 (H-2); irradiation at δ_H 1.65 (3″-Me)→3% enhancements at δ_H 4.23 (H-4″), δ_H 6.54 (H-2″), and δ_H 6.86 (H-1″); irradiation at δ_H 3.48 (N-Me)→2% enhancement at δ_H 6.86 (H-1″); irradiation at δ_H 3.75 (4-OMe)→no enhancement at any protons; irradiation at δ_H 3.74 (N-Me)→no enhancement at any protons. HR-FAB-MS *m/z* 639.1981 $(M+H)^+$ (Calcd for $C_{35}H_{30}N_2O_{10}$+H: 639.1979).

Glycobismine-E (**2**): A yellow oil. $[\alpha]_D$ ±0° (c=0.031, MeOH). UV λ_{max} nm: 202, 211, 269, 292 (sh), 317, 340, 407. IR ν_{max} cm^{-1}: 3359 (br), 1624. EI-MS *m/z* (%): 312 (52), 297 (32), 281 (31), 269 (62), 257 (97), 244 (100), 236 (31), 229 (20). FAB-MS *m/z*: 675 $(M+H)^+$. NOE: Irradiation at δ_H 3.68 (N-Me)→3% enhancement at δ_H 6.64 (H-1‴); irradiation at δ_H 3.91 (3′-OMe)→22% enhancement at δ_H 6.34 (H-2′); irradiation at δ_H 2.98 (N′-Me)→7% enhancement at δ_H 6.57 (H-1″). HR-FAB-MS *m/z* 675.2349 $(M+H)^+$ (Calcd for $C_{39}H_{34}N_2O_9$+H: 675.2342).

Glycocitrine-IV (**3**): A yellow oil. UV λ_{max} (log ε) nm: 202 (4.44), 227 (sh) (4.21), 270 (4.50), 287 (sh) (4.36), 315 (4.09), 336 (sh) (4.00), 412 (3.63). IR ν_{max} cm^{-1}: 3496, 3282 (br), 1631, 1604. EI-MS *m/z* (%): 355 (M^+, 84), 340 (54), 312 (13), 300 (30), 284 (100), 271 (18), 256 (37), 242 (**9**). NOE: Irradiation at δ_H 3.81 (N-Me)→no enhancement at any protons. HR-MS *m/z* 355.1395 (Calcd for $C_{20}H_{21}NO_5$: 355.1419). HMBC C–H correlations: C-1→1-OH, H-1′; C-2→1-OH, H-1′, 3-OH; C-3→H-1′, 3-OH; C-4→3-OH, 4-OCH_3; C-4a→N-CH_3; C-5→H-7; C-6→H-8; C-8a→H-7; C-9a→1-OH; C-10a→N-CH_3, H-6; C-2′→H-1′, H-4′, H-5′; C-3′→H-1′, H-4′, H-5′; C-4′→H-2′, H-5′; C-5′→H-2′, H-4′.

Glycocitrine-V (**4**): A yellow oil. $[\alpha]_D$ ±0° (c=0.043). CD (MeOH, 200—400 nm): No absorption. UV λ_{max} (log ε) nm: 202 (4.22), 226 (sh) (4.00), 263 (4.27), 274 (4.28), 334 (3.84), 399 (3.40). IR ν_{max} cm^{-1}: 3589, 3396 (br), 1631. EI-MS *m/z* (%): 431 (M^+, 43), 416 (100), 340 (16). NOE: Irradiation at δ_H 3.95 (3-OMe)→17% enhancement at δ_H 6.37 (H-2); irradiation at δ_H 3.77 (N-Me)→3% enhancement at δ_H 3.99 (5-OMe); irradiation at δ_H 3.99 (5-OMe)→4% enhancement at δ_H 3.77 (N-Me); irradiation at δ_H 8.11 (H-8)→2% enhancement at δ_H 5.48 (H-1′); irradiation at δ_H 5.48 (H-1′)→2% enhancement at δ_H 8.11 (H-8). HR-MS *m/z* 431.1579 (Calcd for $C_{20}H_{25}NO_8$: 431.1579). HMBC C–H correlations: C-1→1-OH, H-2; C-2→1-OH; C-3→3-OCH_3; C-4→H-2, 4-OCH_3; C-4a→N-CH_3; C-5→5-OCH_3; C-6→H-8; C-9→H-8; C-9a→1-OH, H-2; C-10a→N-CH_3, H-8; C-1′→H-8; C-2′→H-4′, H-5′; C-3′→H-4′, H-5′; C-4′→H-5′; C-5′→H-4′.

Glycocitrine-VI (**5**): A yellow oil. UV λ_{max} nm: 203, 229, 286, 296, 340. IR ν_{max} cm^{-1}: 3394, 3020, 1655, 1618. EI-MS *m/z* (%): 377 (M^+, 59), 334 (8), 308 (100), 294 (20), 292 (15), 280 (18), 266 (40), 252 (18), 240 (40). NOE: Irradiation at δ_H 4.22 (N-Me)→21% enhancement both at δ_H 7.75 (H-5) and 3.29 (H-1′). HR-MS *m/z* 377.1993 (Calcd for $C_{24}H_{27}NO_3$: 377.1991). HMBC C–H correlations: C-1→1-OH; C-2→1-OH; C-3→H-1′; C-4→H-2, H-1′; C-4a→H-1′, N-CH_3; C-5→H-7; C-6→H-8; C-7→H-5; C-8→H-6; C-8a→H-5, H-7; C-9→H-8; C-9a→1-OH, H-2; C-10a→H-6, N-CH_3; C-2′→H-1′, H-4′, H-5′; C-3′→H-1′, H-4′, H-5′; C-4′→H-2′, H-5′; C-5′→H-2′, H-4′.

Glycocitlone-A (**6**): A yellow oil. UV λ_{max} (log ε) nm: 230 (4.46), 286 (sh) (3.93), 296 (4.02), 309 (4.00), 331 (3.85), 343 (3.90), 358 (3.73). IR ν_{max} cm^{-1}: 3421 (br), 1622. EI-MS *m/z* (%): 273 (M^+, 25), 255 (40), 240 (37), 230 (38), 223 (63), 214 (100), 199 (63). NOE: Irradiation at δ_H 3.74 (N-Me)→15% enhancement at δ_H 7.37 (H-8); Irradiation at δ_H 3.92 (4-OMe)→5 and 4% enhancements at δ_H 7.90 (H-5) and 6.85 (H-1′), respectively. HR-MS *m/z* 273.1340 (Calcd for $C_{16}H_{19}NO_3$: 273.1365). HMBC C–H correlations: C-2→N-CH_3, H-1′; C-3→H-2′; C-4→H-5, 4-OCH_3, H-1′; C-4a→H-6, H-8; C-5→H-7; C-6→H-8; C-7→H-5; C-8a→H-5, H-7, N-CH_3; C-2′→H-4′, H-5′; C-3′→H-1′, H-2′, H-4′, H-5′; C-4′→H-5′; C-5′→H-4′.

Glycocitlone-B (**7**): A yellow amorphous powder. UV λ_{max} (log ε) nm: 202 (4.41), 225 (4.25), 267 (4.21), 296 (4.23), 307 (4.25), 410 (3.30). IR ν_{max} cm^{-1}: 3286 (br), 1633. EI-MS *m/z* (%): 289 (M^+, 3), 272 (5), 246 (5), 230 (100), 215 (16), 186 (4). NOE: Irradiation at δ_H 3.85 (4-OMe)→3, 2, and 2% enhancements at δ_H 7.44 (H-5), 6.89 (H-1′), and 7.14 (H-2′), respectively; irradiation at δ_H 4.00 (NMe)→no enhancement at any protons. HR-MS *m/z* 289.1307 (Calcd for $C_{16}H_{19}NO_4$: 289.1314). HMBC C–H correlations: C-2→N-CH_3, H-1′; C-3→H-2′; C-4→H-5, 4-OCH_3, H-1′; C-4a→H-6; C-7→H-5; C-8→H-6; C-8a→H-5, H-7, N-CH_3; C-1′→H-2′; C-2′→H-4′, H-5′; C-3′→H-1′, H-2′, H-4′, H-5′; C-4′→H-2′, H-5′; C-5′→H-2′, H-4′.

Glycocitlone-C (**8**): A yellow oil. UV λ_{max} (log ε) nm: 230 (4.10), 258

(4.05), 307 (3.88), 321 (sh) (3.81), 350 (3.39). IR ν_{max} cm^{-1}: 3421 (br), 1620. EI-MS *m/z* (%): 303 (M^+, 19), 285 (29), 270 (20), 253 (54), 244 (100), 229 (66), 214 (42). NOE: Irradiation at δ_H 3.87 (4-OMe)→6, 4, and 2% enhancements at δ_H 7.51 (H-5), 6.83 (H-1′), and 7.24 (H-2′), respectively; irradiation at δ_H 3.94 (N-Me)→no enhancement at any protons; irradiation at δ_H 3.90 (8-OMe)→12% enhancement at δ_H 7.06 (H-7). HR-MS *m/z* 303.1468 (Calcd for $C_{17}H_{21}NO_4$: 303.1471). HMBC C–H correlations: C-2→N-CH_3, H-1′; C-3→H-2′; C-4→H-5, 4-OCH_3, H-1′; C-4a→H-6; C-5→H-7; C-7→H-5; C-8→ H-6, 8-OCH_3; C-8a→H-5, H-7, N-CH_3; C-2′→H-1′, H-4′, H-5′; C-3′→H-1′, H-2′, H-4′, H-5′; C-4′→H-5′; C-5′→H-4′.

Acknowledgements This work was partly supported by Grants-in-Aid for a Scientific Research (No. 09672173, H.F.) and the High-Tech Research Center Project from the Japan Society for the Promotion of Science and the Ministry of Education, Science, Sports and Culture of Japan, respectively.

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