Spectroscopic Elucidation of Glycobismines, First Naturally Occurring Binary Acridone Alkaloids containing a Carbon–Carbon Linkage

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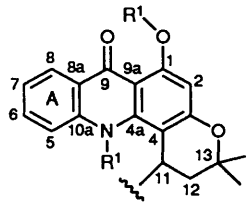
The structures of glycobismine-A **1**, -B **4** and -C **4'**, novel binary acridone alkaloids, from *Glycosmis citrifolia* (Willd.) Lindl. (Rutaceae) have been elucidated by spectroscopic studies using ^1H–^{13}C long-range COSY and/or HMBC experiments. In a preliminary communication we reported the structure of glycobismine-A **1** as the first naturally occurring binary acridone alkaloid having a carbon–carbon linkage. This paper deals with the complete and detailed spectroscopic structural elucidation of compound **1** and the structures of two additional new binary acridone alkaloids, glycobismine-B **4** and -C **4'**.

Isolation of many kinds of monomeric acridone alkaloids from plants of the genus *Glycosmis* has been reported.[2,3] In our continuing investigation of the acridone alkaloids,[2,4] we describe here the isolation and structural elucidation of three novel binary acridone alkaloids which we have named glycobismine-A **1**,[1] -B **4** and -C **4'** from *Glycosmis citrifolia* (Willd.) Lindl. (Rutaceae) collected in Taiwan.[2] Glycobismine-A **1** is the first example of naturally occurring binary acridone alkaloids containing a carbon–carbon linkage between the two halves of the molecule.[1]

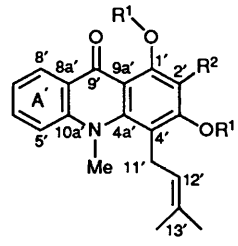
An ethanolic extract of the root and stem barks of *G. citrifolia* was fractionated by a combination of silica gel column and preparative TLC to give new binary alkaloids which we have named glycobismines, along with new and known monomeric acridones as previously reported.[2]

Structure of Glycobismine-A **1**.[1]—Glycobismine-A **1** was isolated as yellow needles, m.p. 256–258 °C, $[\alpha]_D$ ±0 in chloroform, from the dried stem bark of the plant, and showed no CD absorption in the range between 200 and 400 nm in ethanol. The molecular formula was determined as $C_{37}H_{34}N_2O_6$ by high-resolution mass spectrometry. The 1-hydroxy-9-acridone nuclei in the molecule was suggested by the UV absorptions at λ_{max} 246, 282, 336, 372 and 423 nm, IR bands at ν_{max} 3360, 1630, 1600 and 1580 cm^{-1}, and ^1H NMR signals at δ_H 16.53 and 14.12 due to two strongly hydrogen-bonded hydroxy protons, besides two deuterium-exchangeable proton signals at δ_H 9.17 and 6.01.[5] The ^1H and ^{13}C NMR spectra (Table 1) showed the presence of a prenyl group, an *N*-methyl group, and a lone aromatic proton. Treatment of glycobismine-A **1** with methyl iodide in the presence of anhydrous potassium carbonate in acetone gave the *N,O,O,O*-tetramethyl derivative **2**, which showed methyl signals at δ_H 2.80, 3.40, 3.48, 3.99 and 4.07, and at δ_C 43.18, 43.88, 56.16, 61.37 and 62.89 in the ^1H and ^{13}C NMR spectra, respectively, along with other signals analogous to those of the original alkaloid.

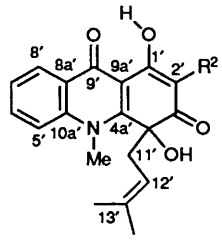
Further, structural analyses using proton decoupling experiments and ^1H–^1H correlation spectroscopy (COSY) of compound **1** revealed the presence of two pairs of four-spin proton systems due to two 1,2-disubstituted aromatic rings assigned to ring-A and -A' having no substituent in the acridone nuclei. A three-spin proton system at δ_H 4.99 (dd, *J* 8.5 and 10.8), 2.48 (dd, *J* 10.8 and 13.7) and 2.24 (dd, *J* 8.5 and 13.7) was assignable to protons due to a 4-equatorially substituted 2,2-dimethyl-3,4-dihydropyran ring fused with an acridone nucleus. An angular



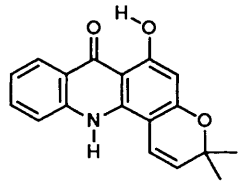
A =



1; R^1 = H, R^2 = **A**
2; R^1 = Me, R^2 = **A**
3; R^1 = R^2 = H



4; R^1 = H, R^2 = **A**
4'; R^1 = H, R^2 = **A**
(diastereoisomer of **4**)
5; R^2 = H



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orientation of the dihydropyran ring with respect to the acridone nucleus was shown by the appearance of a cross-peak due to three-bond ^1H–^{13}C long-range connectivity between a typical lower field hydrogen-bonded hydroxy proton at δ_H 14.12 and a carbon (C-2) at δ_C 98.18 bearing a lone aromatic proton at δ_H 6.26 (1 H, singlet) in ^1H–^{13}C long-range COSY, together with an observation of the nuclear Overhauser effect (NOE) between signals at δ_H 9.17 (1 H, singlet) and 4.99 (1 H, double doublet) due to NH and 11-H, respectively. From these results, the structure of the upper structural unit of glycobismine-A **1** was assigned to the dihydro derivative of de-*N*-methylnor-

Table 1 ^{1}H and ^{13}C NMR spectroscopic data of glycobismines and related compounds

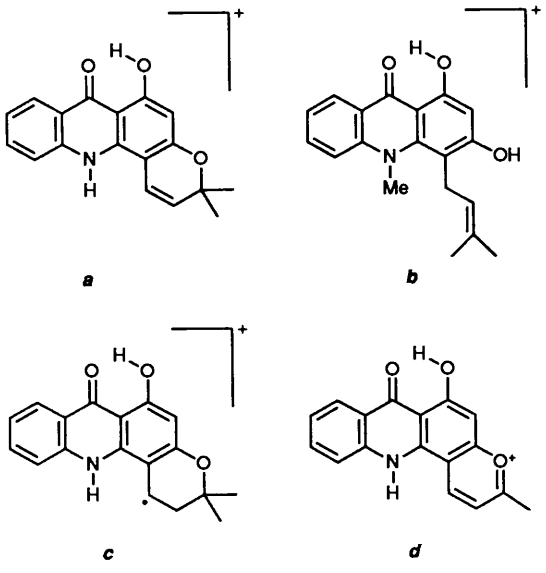
	Glycobismine-A **1**		Glycobismine-B **4**		Glycobismine-C **4'**		Glycocitrine-II **3**		**5**	
No.	δ_H	δ_C	δ_H	δ_C	δ_H	$\delta_C[(CD_3)_2SO]$	δ_H	$\delta_C[(CD_3)_2SO]$	δ_H	δ_C
1		163.29		162.27		160.85				
1-OH	14.12 (s)		13.98 (s)		14.00 (br s)					
2	6.26 (s)	98.18	6.25 (s)	98.19	6.23 (br s)	96.55				
3		160.95		161.05		160.38				
4		97.11		99.70		99.55				
4a		140.10		139.73		139.33				
5	7.07 (d, 8.3)	116.60	7.17 (d, 7.8)	116.06	7.20 (br d, 7.8)	117.03				
6	7.50 (t, 8.3)	133.59	7.49 (t, 7.8)	133.22	7.50 (br t, 7.8)	133.14				
7	7.15 (t, 8.3)	121.81	7.10 (t, 7.8)	121.24	7.12 (br t, 7.8)	121.16				
8	8.24 (d, 8.3)	125.79	8.23 (d, 7.8)	125.91	8.23 (br d, 7.8)[b]	124.52[b]				
8a		120.00		119.88		118.65				
9		181.33		181.23		180.07				
9a		105.62		105.15		139.33				
NH	9.17 (s)		9.11 (s)		9.55 (br s)					
10a		140.48		140.34		103.84				
11	4.99 (dd, 8.5, 10.8)	23.64	4.50 (dd, 8.5, 10.5)	23.48	4.45 (t, 8.8)[c]	24.93				
12	2.24 (dd, 8.5, 13.7), 2.48 (dd, 10.8, 13.7)	38.34	1.98 (dd, 8.5, 13.2), 2.37 (br t, 11.6)	36.83	1.99 (br dd, 8.8, 13.7), 2.71 (dd, 13.7, 10.8)	36.23				
13		76.88		75.70		75.61				
13-Me	1.38 (3 H, s), 1.56 (3 H, s)	23.16, 29.60	1.31 (3 H, s), 1.51 (3 H, s)	22.85, 29.77	1.30 (3 H, s), 1.54 (3 H, s)	22.42, 28.98				
1'		159.19		*a*		*a*		162.33		175.75
1'-OH	16.53 (s)		18.59 (s)		17.37 (br s)		14.59 (s)		16.34 (s)	
2'		108.83		107.97		107.54	6.30 (s)	96.57	5.52 (s)	95.52
3'		161.55		193.25		*a*		164.29		194.56
3'-OH	6.01 (s)									
4'		106.89		79.22		77.92		104.92		79.54
4'-OH			5.15 (s)		5.50 (br s)				5.25 (s)	
4a'		145.61		158.49		158.10		146.82		158.87
5'	7.42 (d, 8.8)	122.00	7.78 (d, 8.8)	116.89	7.69 (br d, 7.8)	118.04	7.41 (t, 8.3)	116.99	7.81 (d, 8.8)	116.77
6'	7.74 (t, 8.8)	134.40	7.89 (t, 8.8)	134.84	7.78 (br t)	134.46	7.71 (d, 8.3)	133.81	7.88 (t, 8.8)	134.40
7'	7.34 (t, 8.8)	116.41	7.65 (t, 8.8)	126.73	7.50 (br t, 7.8)	126.28	7.29 (t, 8.3)	121.23	7.60 (t, 8.8)	126.25
8'	8.43 (d, 8.8)	126.17	8.57 (d, 8.8)	126.32	8.33 (br d, 7.8)[b]	124.61[b]	8.36 (dd, 1.7, 8.3)	124.93	8.48 (d, 8.8)	126.31
8a'		121.14		124.48		123.47		120.46		125.01
9'		181.75		177.90		176.84		180.34		178.07
9a'		106.44		105.77		105.44		105.82		106.50
NMe	3.81 (3 H, s)	43.85	4.37 (3 H, s)	39.56	4.39 (3 H, br s)	39.70[c]	3.84 (3 H, s)	43.28	4.44 (3 H, s)	39.48
10a'		146.50		140.97		140.69		145.26		141.09
11'	3.29 (2 H, br t, 5.9)	27.29	2.71 (2 H, d, 6.8)	42.82	2.87 (2 H, br s)	*a*	3.48 (2 H, d, 6.0)	26.50	2.74 (2 H, d, 7.3)	42.66
12'	5.25 (br s)	122.32	4.97 (br s)	115.47	5.08 (br s)	115.57	5.41 (br t, 6.0)	124.74	5.01 (t, 7.3)	115.52
13'		135.38		137.21		135.29		130.62		137.85
13'-Me	1.68 (3 H, s), 1.73 (3 H, s)	18.15, 25.60	1.38 (3 H, s), 1.62 (3 H, s)	17.95, 25.98	1.48 (3 H, br s), 1.74 (3 H, br s)	16.88, 23.96	1.83 (3 H, s), 1.84 (3 H, s)	17.83, 25.39	1.40 (3 H, s), 1.65 (3 H, s)	17.62, 25.90

Values are in ppm (δ_H and δ_C). ^{1}H and ^{13}C NMR spectra were recorded at 270 or 400 MHz and 100 MHz, respectively, in $CDCl_3$, unless otherwise stated. Each proton signal corresponds to 1 H, unless otherwise stated. Figures in parentheses are coupling constants (J) in Hz. [a] No signal was detected for overlapping or broadening of the signal. [b] May be interchanged. Assignments of ^{13}C signals were determined by ^{1}H–^{13}C COSY, ^{1}H–^{13}C long-range COSY and/or HMBC spectrometry. [c] Overlapped signals.

acronycine (A, R^1 = H)[6] having an equatorially oriented substituent at C-11.

On the other hand, in the ^1H and ^{13}C NMR spectra of compound **1**, a signal pattern, excluding signals due to the upper half was similar to that of glycocitrine-II **3**[2b,d] isolated from the same plant, taking into account some chemical-shift differences and lack of a lone aromatic proton signal at δ_H 6.33 in the spectrum of compound **3** (Table 1). Moreover, in the lower half of compound **1**, the location of the prenyl moiety at C-4' was confirmed by NOE experiments as follows: Irradiation of the *N*-methyl signal at δ_H 3.81 produced 9 and 7% enhancements of signals at δ_H 3.29 (2 H, br t, 11'-H) and 5.25 (1 H, br s, 12'-H), respectively, along with signals at δ_H 7.42 (5'-H). The location of a hydroxy group at C-3' was deduced from the presence of a three-bond correlation between the hydroxy proton at δ_H 6.01 and C-4' at δ_C 106.89 which have a two-bond correlation with the methylene proton at δ_H 3.29 on the prenyl moiety. These spectral data led us to assign the structure of the 2-substituted glycocitrine-II **3** to the lower structural unit.

These structural features were supported by a mass spectroscopic analysis. In the electron impact mass spectrum (EI-MS) of compound **1**, principal fragment ions were shown at *m/z* 602, 309, 294, 293, 278 and 241. The molecular ion at *m/z* 602 was considered to give rise to two ions from the halves of the molecules at *m/z* 293 (***a***) and *m/z* 309 (***b***). The ion at *m/z* 294 included an ion ***c*** and/or the ion derived from ***b*** by the elimination of a methyl radical, and an ion of *m/z* 241 also from the same ion ***b*** by elimination of $\cdot CH_2CH{=}CMe_2$, followed by transfer of a hydrogen atom. A strong ion of *m/z* 278 was considered to have the structure ***d*** derived from a loss of a methyl radical from ion ***a***.



The linkage of the two acridone nuclei between C-11 and C-2' in the upper and lower halves of the molecule, respectively, was further adduced from the following results of ^1H–^{13}C long-range correlations: The methine proton (11-H) at δ_H 4.99 showed three-bond correlations with hydroxylated carbons (C-1' and C-3') at δ_C 159.19 and 161.55, respectively, and a two-bond connectivity with C-2' at δ_C 108.83.

From these data, coupled with the results of three- and two-bond ^1H–^{13}C long-range connectivities in the COSY spectrum as shown by the arrows in Fig. 1, the structure of glycobismine-A was proposed as **1**, corresponding to a binary acridone consisting of de-*N*-methylnoracronycine **6**[6] and glycocitrine-II **3**,[2b,d] both of which co-occurred in the same plant.[2d]

Isolation of two bisacridone alkaloids having an ether linkage between the two molecular halves has been reported from the Rutaceous plants.[7] Glycobismine-A **1** is the first example of a carbon–carbon linked bisacridone alkaloid isolated from a natural source.*

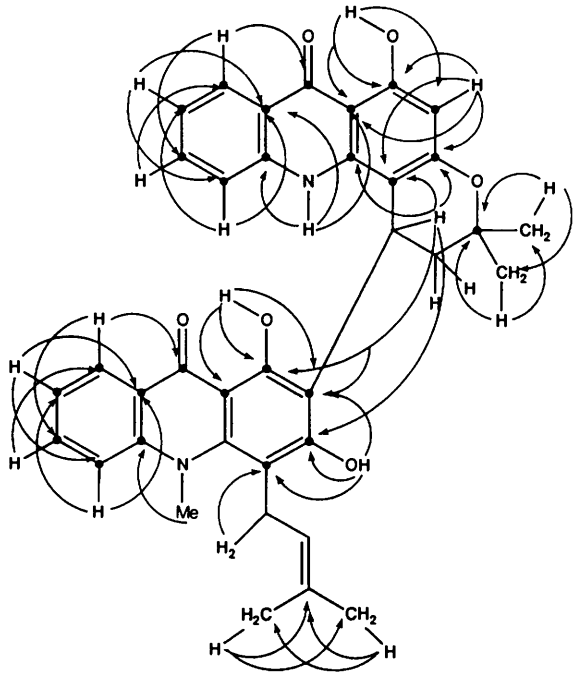


Fig. 1 ^1H–^{13}C Long-range correlations in the long-range COSY spectrum of compound **1**

In vitro anti-malarial activity of glycobismine-A **1** comparable to that of chloroquine diphosphate has been reported by one of us (H. F.).[9]

Structure of Glycobismine-B **4**.—Glycobismine-B **4** was isolated as a yellow oil, $[\alpha]_D$ ±0 in chloroform and also showed no CD absorption in the range between 200 and 400 nm in methanol. The molecular formula was established as $C_{37}H_{34}N_2O_7$ by high-resolution mass analysis of a fast-atom-bombardment mass spectrum (FAB-MS). The UV spectrum exhibited absorption bands at λ_{max} 213sh, 240, 273, 295sh, 334 and 392sh, characteristic of the 9-acridone nucleus.[5] The IR spectrum showed bands at ν_{max} 1640 and 1610 cm^{-1} which were also reminiscent of an acridone nucleus.

The ^1H and ^{13}C NMR spectra (Table 1) showed the presence of two strongly hydrogen-bonded hydroxy groups (δ_H 13.98 and 18.59), an *N*-methyl (δ_H 4.37; δ_C 39.56), a prenyl [δ_H 2.71 (2 H), 4.97, 1.38 (3 H) and 1.62 (3 H)], two tertiary methyls attached to an oxygen-linked carbon [δ_H 1.31 and 1.51 (each 3 H, s); δ_C 75.70], and a lone aromatic proton [δ_H 6.25 (s)]. The results of the analysis of the ^1H–^1H COSY spectrum together with coupling constant values of the ^1H NMR signals revealed the presence of two pairs of four-spin and one three-spin proton system due to non-substituted A and A' rings in the acridone nuclei and a $-CHCH_2-$ group in the 2,2-dimethyldihydropyran ring having an equatorially oriented substituent. Close resemblance of chemical shifts and multiplicities of proton and carbon signals due to the upper-half acridone nucleus between the NMR spectra of compounds **1** and **4** (Table 1), together with the occurrence of significant mass fragment ions at *m/z* 293, 294 and 278 ascribed to ions ***a***, ***c*** and ***d***, respectively, as the same in the EI-MS of **1** indicated that the structure of compound **4** in

* The C–C-linked oligomers of noracronycine, one of the monomeric acridones, have been reported to be prepared by treatment of noracronycine with HCl or H_2SO_4 in methanol (ref. 8).

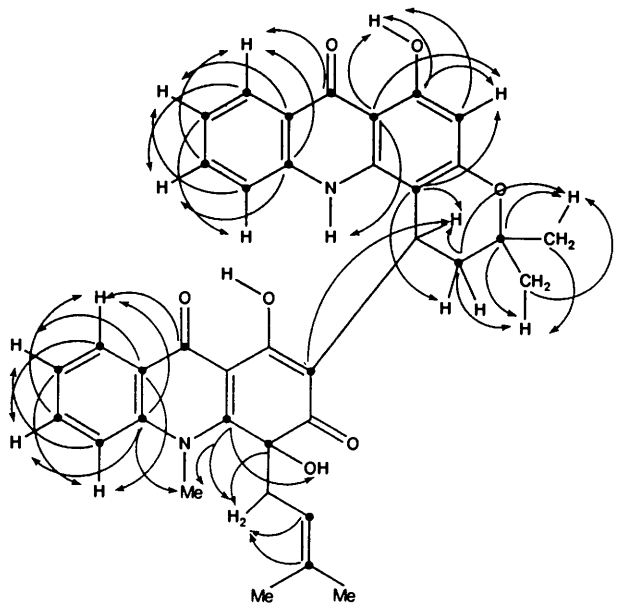


Fig. 2 ^{13}C–^{1}H Long-range correlations in the HMBC spectrum of compound **4**

the upper half was the same as that in the molecule of compound **1**, and this was supported by ^{1}H–^{13}C long-range relationships in the ^{1}H detected heteronuclear multiple bond connectivity (HMBC) spectrum summarized by arrows in Fig. 2.

On the other hand, the ^{1}H and ^{13}C NMR spectra of compound **4**, excluding signals due to the upper half, showed additional signals due to a carbonyl carbon (δ_C 193.25), a tetra-substituted carbon (δ_C 79.22) and a hydroxy group (δ_H 5.15), besides an *N*-methyl, a prenyl and a strongly hydrogen-bonded hydroxy group described previously. In the HMBC spectrum of compound **4** (Fig. 2), the appearance of ^{1}H–^{13}C three-bond correlations between a hydroxy proton at δ_H 5.15 and an angular carbon (C-4'a) at δ_C 158.49 which correlated to both the *N*-methyl (δ_H 4.37) and the methylene protons (11'-H_2, δ_H 2.71) suggested the location of the hydroxy and prenyl groups at C-4', which was also supported by the observation of a nuclear Overhauser enhancement (NOE) between 11'-H_2 and *N*-methyl protons. Moreover, the signal pattern of the ^{1}H NMR spectrum was found to be similar to that of compound **5**,[10] one of the oxidation products of glycocitrine-II **3**[2b,d] with *m*-chloroperbenzoic acid (MCPBA), taking into account some differences in chemical shifts and a lack of a singlet (δ_H 5.52) due to 2'-H (Table 1) in compound **5**, suggesting the structure of the lower half and the location of the linkage at C-2' in the molecule **4**. The linkage in the upper and lower halves at C-11 and C-2', respectively, was also indicated by an observation of ^{1}H–^{13}C two-bond correlation between C-2' and 11-H in the HMBC spectrum (Fig. 2). These spectral results, together with those of the ^{1}H–^{13}C long-range correlations shown by arrows in Fig. 2, led us to propose structure **4** for glycobismine-B, except for the relative stereochemistry between C-11 and C-4'.

Structure of Glycobismine-C **4'**.—Glycobismine-C **4'**, a yellow oil, was also obtained as a racemate; $[\alpha]_D$ ±0 in chloroform and no CD absorption in the range 200–400 nm. The molecular formula $C_{37}H_{34}N_2O_7$ was found to be the same as that of compound **4** by HR-FAB-MS. The UV and IR spectra (see Experimental section) also showed absorptions characteristic of 9-acridones.[5] The ^{1}H NMR spectrum also revealed the presence of two acridones having no substituent on the A- and A'-ring, two strongly hydrogen-bonded hydroxy groups, an *N*-methyl, and a prenyl group. The presence of an angularly oriented dimethyldihydropyran ring was suggested by ^{1}H and ^{13}C NMR signals (Table 1) and the appearance of ^{1}H–^{13}C three-bond connectivities between a typical lower field hydrogen-bonded hydroxy proton at δ_H 14.00 and C-2 (δ_C 96.55) bearing a lone aromatic proton at δ_H 6.23 (1 H, s) in the HMBC spectrum. The overall signal pattern of the ^{1}H NMR spectrum of compound **4'** was similar to that of compound **4**, except for a line broadening of signals in the spectra in $CDCl_3$, even in $(CD_3)_2SO$ solution at 70 °C, and some chemical-shift differences.

On the basis of the aforementioned results, together with good similarity of the mass fragmentation and ^{1}H–^{13}C long-range correlations in the HMBC spectrum of compound **4'** with those of its isomer **4**, glycobismine-C **4'** was considered to be a diastereoisomer of glycobismine-B **4**.*

Some significant differences in chemical shifts of the ^{1}H NMR spectrum of glycobismine-C **4'** compared with that of compound **4** were observed as follows: (a) paramagnetic shifts of an equatorial 12-H, 11'-H and NH signals by 0.34, 0.16 and 0.44 ppm, respectively, and (b) diamagnetic shifts (0.24–0.11 ppm) of proton signals on the lower acridone A'-ring (5'-, 6'-, 7'- and 8'-H). On the basis of these spectral data, attempts at assignment of the relative stereochemistries in isomers **4** and **4'** by using Dreiding models were unsuccessful.

Glycobismines **1**, **4** and **4'** can be considered to occur biogenetically by an oxidative coupling or acid-catalysed reaction between the corresponding acridone derivatives. Since these alkaloids were obtained as racemates, the possibility of a coupling reaction occurring during isolation procedures cannot be excluded. However, at present it is impossible to give a definite answer to whether these alkaloids are artefacts or true natural metabolites.

Experimental

M.p.s were measured on a micromelting point hot-stage apparatus (Yanagimoto). ^{1}H and ^{13}C NMR spectra were recorded on GX-270 (JEOL) and GX-400 (JEOL) spectrometers, respectively, for solutions in $CDCl_3$, unless otherwise stated. Chemical shifts are shown in δ-values with tetramethylsilane as internal reference. *J*-Values are given in Hz. ^{1}H–^{13}C long-range COSY and HMBC spectra were measured at *J* 5 and 8 Hz, respectively, on the GX-400. EI-, HR- and FAB-MS measured on an M-80 (Hitachi) or a JMS-HX-110 (JEOL) spectrometer having a direct inlet system. UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) for solutions in methanol, IR spectra on an IR-810 (JASCO) for samples in $CHCl_3$, optical rotations on a DIP-181 (JASCO) for solutions in $CHCl_3$ at 25 °C ($[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}), and CD spectra on a J-600 (JASCO) for samples in ethanol or methanol. Preparative TLC (PLC) was carried out on Kieselgel 60 F_{254} (Merck).

Isolation of Glycobismine-A **1**, *-B* **4** *and -C* **4'**.—The dried root and stem bark (7 kg) of *Glycosmis citrifolia* (Willd.) Lindl. (Rutaceae) was collected in Heng Chun Tropical Botanical Garden (Kehg-Ting Botanical Garden), Taiwan, and extracted with ethanol under reflux. The ethanolic extract was treated under the procedure previously reported.[2] The methanolic mother liquor of atalaphyllidine, one of the known monomeric acridone alkaloids, was treated with $CHCl_3$ to obtain glycobismine-A **1** as yellow prisms (150 mg). The mother liquor after

* The possibility of conformational isomers was excluded, because the temperature-dependent variability of signal resonances could not be observed in ^{1}H NMR [in $(CD_3)_2SO$] spectra measured at 27, 40, 60 and 70 °C for both alkaloids (**4** and **4'**).

filtration of crystalline **1** was subjected to silica gel PLC with a mixture of $CHCl_3$–benzene–acetone (5:4:1) as developing solvent. Glycobismine-B **4** (10 mg) and -C **4'** (4 mg) were isolated, with R_f values 0.44 and 0.42, respectively.

Glycobismine-A **1**. Yellow needles, m.p. 256–258 °C; $[\alpha]_D$ ±0 (*c* 0.80, $CHCl_3$); CD (EtOH): no absorption in range 200–400 nm; λ_{max}(MeOH)/nm (log ε) 219sh (4.64), 235sh (4.72), 246 (4.75), 282 (4.73), 300sh (4.62), 336sh (4.13), 372 (4.20) and 423 (4.02); $\nu_{max}(CHCl_3)/cm^{-1}$ 3410, 3360br, 1630, 1600, 1580 and 1545; $\delta_H(CDCl_3)$ and $\delta_C(CDCl_3)$ (see Table 1). Difference NOE: Irradiation of NH (δ_H 9.17)—4% enhancement of 1'-OH (δ_H 16.53), 12% enhancement of 5-H (δ_H 7.07) and 12% enhancement of 11-H (δ_H 4.99); irradiation of NMe (δ_H 3.81)—19, 9 and 7% enhancement of 5'-H (δ_H 7.42), 11'-H (δ_H 3.29) and 12'-H (δ_H 5.25), respectively; *m/z* (%) 602 (M^+, 12), 309 (52), 294 (57), 293 (30), 278 (100), 266 (10), 254 (14), 252 (19) and 241 (61) (Found: M^+, 602.2444. $C_{37}H_{34}N_2O_6$ requires M, 602.2415; Found: 309.1355. $C_{19}H_{19}NO_3$ requires *m/z*, 309.1363; Found: 293.1037. $C_{18}H_{15}NO_3$ requires *m/z*, 293.1050).

N,O,O,O-*Tetramethylglycobismine-A* **2**. Compound **1** (5 mg) was dissolved in acetone (5 cm^3) and the solution was refluxed with methyl iodide (2 cm^3) for 7 h in the presence of anhydrous K_2CO_3 (30 mg). The solution was filtered and the filtrate was concentrated. The residue was subjected to PLC to give compound **2** (5 mg) as a pale yellow oil, λ_{max}(MeOH)/nm 210, 272, 303sh, 325sh and 394; $\nu_{max}(CHCl_3)/cm^{-1}$ 1620, 1595 and 1560; δ_H(400 MHz; $CDCl_3$) 8.44 (1 H, d, *J* 6.8), 8.16 (1 H, d, *J* 6.9), 7.67 (1 H, t, *J* 6.9), 7.27 (2 H, m), 7.13 (1 H, t, *J* 6.9), 6.99 (1 H, t, *J* 6.9), 6.48 (1 H, d, *J* 6.8), 6.38 (1 H, s), 5.16 (1 H, dd, *J* 7.8 and 10.8), 4.52 (1 H, br s), 4.07 (3 H, s), 3.99 (3 H, s), 3.48 (3 H, s), 3.40 (3 H, s), 3.08 (2 H, m), 2.80 (3 H, s), 2.17 (1 H, m), 1.88 (1 H, d, *J* 10.8), 1.60 (3 H, s), 1.54 (3 H, s), 1.47 (3 H, s) and 1.44 (3 H, s); δ_C(25 MHz; $CDCl_3$) 177.91, 177.38, 164.10, 160.24, 160.06, 157.90, 150.29, 148.54, 145.73, 145.20, 133.15, 132.27, 131.92, 127.01, 126.54, 125.66, 124.96, 124.03, 122.15, 121.63, 121.28, 117.77, 116.48, 116.19, 115.60, 112.33, 106.48, 95.89, 75.23, 62.89, 61.37, 56.16, 43.88, 43.18, 41.42, 29.84, 29.72, 28.61, 25.33, 22.93 and 17.84; *m/z* (%) 658 (M^+, 73), 643 (100), 338 (22), 337 (10), 320 (53), 308 (47), 307 (17), 306 (51), 292 (58), 278 (26) and 262 (24).

Glycobismine-B **4**. A yellow oil; $[\alpha]_D$ ±0 (*c* 0.20, $CHCl_3$); CD (MeOH): no absorption in range 200–400 nm; λ_{max}(MeOH)/nm 213sh, 240, 273, 295sh, 334 and 392sh; $\nu_{max}(CHCl_3)/cm^{-1}$ 3360, 1640, 1610 and 1590; $\delta_H(CDCl_3)$ and $\delta_C(CDCl_3)$ (see Table 1); δ_H[400 MHz; $(CD_3)_2SO$; 70 °C] 13.96 (1 H, s, 1-OH), 6.03 (1 H, s, 2-H), 8.13 (1 H, overlapped d, *J* 7.8, 5-H), 7.22 (1 H, t, *J* 7.8, 6-H), 7.20 (1 H, t, *J* 7.8, 7-H), 8.45 (1 H, d, *J* 7.8, 8-H), 9.56 (1 H, br s, NH), 4.53 (1 H, dd, *J* 8.8 and 10.8, 11-H), 2.35 (1 H, t, *J* 12.7, 12-H), 1.98 (1 H, dd, *J* 8.8 and 12.7, 12-H), 1.46 (3 H, s, 13-Me), 1.28 (3 H, s, 13 or 13'-Me); 18.20 (1 H, br s, 1'-OH), 6.24 (1 H, br s, 4'-OH), 7.25 (1 H, d, *J* 8.8, 5'-H), 8.00 (1 H, t, *J* 8.8, 6'-H), 7.63 (1 H, t, *J* 8.8, 7'-H), 8.13 (1 H, overlapped d, *J* 8.8, 8'-H), 4.46 (3 H, s, NMe), 2.83 (2 H, m, 11'-H), 4.77 (1 H, br s, 12'-H), 1.34 (3 H, s, 13'-Me) and 1.28 (3 H, s, 13' or 13-Me). Each ^1H NMR spectrum taken at 27, 40 and 60 °C was the same as that measured at 70 °C. Difference NOE: Irradiation of NMe (δ_H 4.37)—15 and 5% enhancement of 5'-H (δ_H 7.78) and 11'-H (δ_H 2.71), respectively; irradiation of NH (δ_H 9.11)—13 and 16% enhancement of 5-H (δ_H 7.17) and 11-H (δ_H 4.50), respectively; *m/z* (FAB) 619 (M^+ + H) (Found: 619.2461. $C_{37}H_{35}N_2O_7$ requires *m/z*, 619.2444); *m/z* (EI, %) 600 ($M^+ - H_2O$, 11), 586 (27), 571 (10), 323 (17), 307 (29), 294 (53), 293 (83), 278 (100), 257 (40), 242 (37), 228 (26), 227 (46) and 214 (32) (Found: 600.2289. $C_{37}H_{32}N_2O_6$ requires *m/z*, 600.2259; Found: 323.1182. $C_{19}H_{17}NO_4$ requires *m/z*, 323.1157; Found: 294.1133. $C_{18}H_{16}NO_3$ requires *m/z*, 294.1129).

Glycobismine-C **4'**. A yellow oil; $[\alpha]_D$ ±0 (*c* 0.10, $CHCl_3$); CD(MeOH): no absorption in range 200–400 nm; λ_{max}(MeOH)/nm 213sh, 243, 272, 295sh, 332 and 392sh; $\nu_{max}(CHCl_3)/cm^{-1}$ 3310, 1645, 1615 and 1590; $\delta_H(CDCl_3)$ and δ_C[$(CD_3)_2SO$] (see Table 1); δ_H[400 MHz; $(CD_3)_2SO$; 70 °C] 13.98 (1 H, s, 1-OH), 6.01 (1 H, s, 2-H), 7.43 (1 H, d, *J* 7.6, 5-H), 7.62 (1 H, t, *J* 7.6, 6-H), 7.20 (1 H, t, *J* 7.6, 7-H), 8.11 (1 H, overlapped d, *J* 7.6, 8-H), 9.71 (1 H, s, NH), 4.45 (1 H, overlapped, 11-H), 2.01 (1 H, dd, *J* 8.8 and 13.7, 12-H), 2.52 (1 H, overlapped, 12-H), 1.26 (3 H, s, 13-Me), 1.46 (3 H, s, 13-Me); 17.26 (1 H, s, 1'-OH), 6.32 (1 H, br s, 4'-OH), 8.11 (1 H, overlapped d, *J* 7.6, 5'-H), 7.97 (1 H, t, *J* 7.6, 6'-H), 7.66 (1 H, t, *J* 7.6, 7'-H), 8.32 (1 H, d, *J* 7.6, 8'-H), 4.47 (3 H, s, NMe), 2.81 (2 H, br, 11'-H), 4.72 (1 H, br, 12'-H), 1.24 (3 H, s, 13'-Me) and 1.41 (3 H, s, 13'-Me). Each ^1H NMR spectrum taken at 27, 40 and 60 °C was the same as that measured at 70 °C. Difference NOE: Irradiation of NMe (δ_H 4.39)—13, 3 and 5% enhancement of 5'-H (δ_H 7.69), 4'-OH (δ_H 5.50) and 11'-H (δ_H 2.87), respectively; irradiation of NH (δ_H 9.55)—14 and 18% enhancement of 5-H (δ_H 7.20) and 11-H (δ_H 4.45); *m/z* (FAB) 619 (M^+ + H) (Found: 619.2450. $C_{37}H_{35}N_2O_7$ requires *m/z*, 619.2444); *m/z* (EI, %) 600 ($M^+ - H_2O$, 12), 586 (31), 571 (11), 543 (9), 323 (13), 307 (32), 294 (63), 293 (90), 278 (100), 257 (57), 242 (55), 228 (32), 227 (32) and 214 (45) (Found: 600.2287. $C_{37}H_{32}N_2O_6$ requires *m/z*, 600.2259; Found: 323.1202. $C_{19}H_{17}NO_4$ requires *m/z*, 323.1157; Found: 294.1102. $C_{18}H_{16}NO_3$ requires *m/z*, 294.1129).

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References

1 Preliminary communication, H. Furukawa, T.-S. Wu, C.-S. Kuoh, T. Sato, Y. Nagai and K. Kagei, *Chem. Pharm. Bull.*, 1984, **32**, 1647.
2 T.-S. Wu and H. Furukawa, (*a*) *Heterocycles*, 1982, **19**, 825; (*b*) p. 1047; (*c*) p. 1227; (*d*) T.-S. Wu, H. Furukawa, C.-S. Kuoh and K.-S. Hsu, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1681.
3 T. R. Govindachari, B. R. Pai and P. S. Subramaniam, *Tetrahedron*, 1966, **22**, 3245; K. Rastogi, K. S. Randhir and S. P. Popli, *Phytochemistry*, 1980, **19**, 945; I. H. Bowen, K. P. W. C. Perera and J. R. Lewis, *Phytochemistry*, 1980, **19**, 1566; V. Kumar, J. Reisch and A. Wickramasinghe, *Aust. J. Chem.*, 1989, **42**, 1375.
4 H. Furukawa, C. Ito, T. Mizuno, M. Ju-ichi, M. Inoue, I. Kajiura and M. Omura, *J. Chem. Soc., Perkin Trans. 1*, 1990, 1593 and references cited therein; C. Ito, S. Tanahashi, Y. Tani, M. Ju-ichi, M. Omura and H. Furukawa, *Chem. Pharm. Bull.*, 1990, **38**, 2586; M. Ju-ichi, Y. Takemura, C. Ito and H. Furukawa, *Heterocycles*, 1991, **32**, 1781.
5 J. Reisch, K. Szendri, E. Minker and I. Novak, *Pharmazie*, 1972, **27**, 208; R. D. Brown and F. N. Lahey, *Aust. J. Sci. Res., Ser. A*, 1950, **3** 593.
6 M. T. Fauvel, J. Gleye, C. Moulis and I. Fouraste, *Planta Med. Phytother.*, 1978, **12**, 207.
7 A. W. Fraser and J. R. Lewis, *J. Chem. Soc., Chem. Commun.*, 1973, 615.
8 S. Funayama and G. A. Cordell, *J. Nat. Prod.*, 1986, **49**, 210 and references cited therein.
9 H. Fujioka, Y. Nishiyama, H. Furukawa and N. Kumada, *Antimicrobial Agents Chemother.*, 1989, **33**, 6.
10 C. Ito, T. Ono, K. Hatano and H. Furukawa, *Chem. Pharm. Bull.*, 1993, **41**, in the press.

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