Alkaloids from the Stems of *Glycosmis pentaphylla*

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A new simple carbazole alkaloid, 4-(7-hydroxy-3-methoxy-6-methyl-9*H*-carbazol-4-yl)but-3-en-2-one (**1**), and two new dimeric carbazole alkaloids, bisglybomine B (**2**) and biscarbalexine A (**3**), together with seven known alkaloids, were isolated from the stems of *Glycosmis pentaphylla*. Their structures were elucidated by spectroscopic methods, especially 2D-NMR techniques.

Introduction. – *Glycosmis* (Rutaceae) is a genus of glabrous shrub, distributed in warm and temperate regions of the world including eleven species in China [1]. *Glycosmis pentaphylla* is a tree native to the south and southwest of Yunnan Province, P. R. China, which can grow up to 1.5–5 m, and it is widely used as a folk medicine in the treatment of protracted diseases, sourness, numbness, and certain other diseases [2]. Previous phytochemical investigations on this plant have resulted in the isolation of alkaloids including of the acridone [3], carbazole [4], quinolone [5], and quinazoline type [6], as well as of isoflavone diglycosides [7] and hydroquinone diglycoside acyl esters (= hydroquinone *O*-acyldiglycosides) [8]. Carbazole alkaloids represent a large family of plant constituents obtained from the genera *Murraya*, *Glycosmis*, and *Clausena* belonging to Rutaceae. Biological activities of carbazole alkaloids were reported such as cytotoxic [9], anti-HIV [10], antifungal [11], and anti-tumor-promoting activity [12]. In the course of our search for new bioactive lead compounds from Rutaceae plants [13], we investigated the chemical constituents of the stems of *G. pentaphylla*. As a result, A new simple carbazole alkaloid, 4-(7-hydroxy-3-methoxy-6-methyl-9*H*-carbazol-4-yl)but-3-en-2-one (**1**), and two new dimeric carbazole alkaloids, bisglybomine B (**2**) and biscarbalexine A (**3**), together with the seven known alkaloids **4**–**10** (*Fig.*), were isolated from the stems of *Glycosmis pentaphylla*. Their structures were elucidated by spectroscopic methods, especially 2D-NMR techniques. This paper describes the structural investigation of these natural products.

Results and Discussion. – Compound **1** was isolated as a yellow powder, and its molecular formula was determined as $C_{18}H_{17}NO_3$ by HR-EI-MS (*m*/*z* 295.1207), indicating eleven degrees of unsaturation. The ^{1}H-NMR spectrum of **1** displayed the presence of two isolated aromatic H-atoms at δ(H) 6.84 and 7.88 (*s*, 2 H), two aromatic *ortho* H-atoms at δ(H) 7.37 and 7.06 (2*d*, each $J = 8.8$ Hz), two *trans*-positioned olefinic H-atoms at δ(H) 8.56 (*d*, $J = 16.4$ Hz) and 7.30 (*d*, $J = 16.0$ Hz), one MeO, and at δ(H)

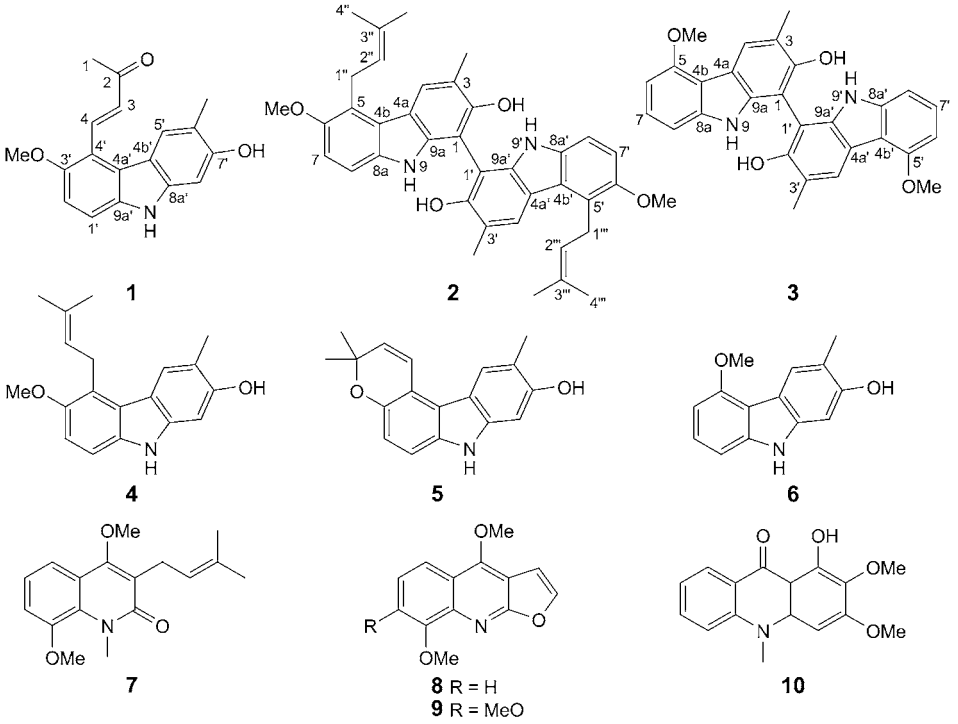

Figure. *Compounds* **1**–**10**, *isolated from* Glycosmis pentaphylla

3.96 (*s*), and two Me groups at δ(H) 2.34 and 2.50 (2*s*). Its ^{1}H- and ^{13}C-NMR spectra (*Table 1*), in conjunction with the HSQC spectrum, revealed the presence of nine quaternary C-atoms (including one C=O group at δ(C) 202.5) and six CH, one MeO, and two Me groups. A comparison of the 1D- and 2D-NMR data with those of glybomine B (**4**) [12], isolated from *Glycosmis arborea*, revealed similar C-atom signals, except for the absence of signals for the prenyl moiety at C(4′) and the presence of signals for a butenone moiety (δ(H) 8.56 (*d*, J = 16.4 Hz,1 H), 7.30 (*d*, J = 16.0 Hz, 1 H), and 2.50 (*s*, 3 H); δ(C) 140.7 (*d*), 131.0 (*d*), 202.5 (*s*), and 27.8 (*q*)). The position of the butenone moiety was deduced by a HMBC experiment. The HMBCs H–C(4) (δ(H) 8.56/C(4′) (δ(C) 124.7) and C(3′) (δ(C) 154.8) indicated that the butenone moiety was located at C(4′). Based on the above results, the structure of compound **1** was established as 4-(7-hydroxy-3-methoxy-6-methyl-9*H*-carbazol-4-yl)but-3-en-2-one.

Compound **2** was obtained as a yellow powder. The molecular formula $C_{38}H_{40}N_2O_4$ was deduced from the HR-EI-MS which showed a molecular ion at *m*/*z* 588.2993. The fragment ion $[M - C_{19}H_{20}NO_2]^+$ at *m*/*z* 294 which represented half of the molecule in the EI-MS as well as ^{1}H- and ^{13}C-NMR data (*Table 2*) suggested that the structure of **2** was a highly symmetrical carbazole-alkaloid dimer. The ^{1}H-NMR signals displayed the presence of one isolated aromatic proton at δ(H) 8.02 (*s*), a pair of aromatic *ortho* H-atoms at δ(H) 7.04 and 6.98 (2*d*, each J = 8.4 Hz), one prenyl group at δ(H) 5.34–5.41

Table 1. *^{1}H- and ^{13}C-NMR, and HMBC Data* (CD_3OD) *of Compound* **1**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC (H → C)
H–C(1′)	7.06 (*d*, *J* = 8.8)	109.7 (*d*)	C(4′a), C(3′), C(9′a)
H–C(2′)	7.37 (*d*, *J* = 8.8)	113.7 (*d*)	C(4′), C(3′)
C(3′)		154.8 (*s*)	
C(4′)		124.7 (*s*)	
C(4′a)		117.4 (*s*)	
C(4′b)		116.7 (*s*)	
H–C(5′)	7.88 (*s*)	125.2 (*d*)	C(7′), C(8′a), *Me*–C(6′), C(4′b)
C(6′)		118.6 (*s*)	
C(7′)		156.4 (*s*)	
H–C(8′)	6.84 (*s*)	96.9 (*d*)	C(7′), C(6′), C(8′a), C(4′b)
C(8′a)		143.4 (*s*)	
C(9′a)		136.6 (*s*)	
Me(1)	2.50 (*s*)	27.8 (*q*)	C(3), C(2)
C(2)		202.5 (*s*)	
H–C(3)	7.30 (*d*, *J* = 16.0)	131.0 (*d*)	C(2)
H–C(4)	8.56 (*d*, *J* = 16.4)	140.7 (*d*)	C(4′), C(3′), C(3), C(2)
Me–C(6′)	2.34 (*s*)	17.1 (*q*)	C(7′), C(6′), C(5′)
*Me*O–C(3′)	3.96 (*s*)	56.9 (*q*)	C(3′)

(*m*, 1 H), 3.95 – 4.01 (*m*, 2 H), 1.98 (*s*, 3 H), and 1.75 (*s*, 3 H), one MeO group at δ(H) 3.87 (*s*), one aromatic Me group at δ(H) 2.50 (*s*), and one NH group at δ (H) 7.49 (*s*). The ^{13}C-NMR and DEPT spectra displayed 19 C-atom signals, including those of ten quaternary C-atoms and four CH, one MeO, one CH_2, as well as three Me groups. A comparison of the NMR data of **2** with those of glybomine B (**4**) [12] isolated from *Glycosmis arborea*, revealed the presence of an additional aromatic quaternary C-atom at δ(C) 98.7 and the absence of the aromatic H-atom at δ(H) 6.77 (*s*) in **2**. This suggested that compound **2** is a symmetrical dimeric carbazole with two glybomine B units. These findings, along with the presence of a low-field signal assigned to H–C(4,4′) at δ(H) 8.02 (*s*), and the absence of a C(1,1′) signal in the ^{1}H-NMR spectrum data revealed the C(1)–C(1′) linkage between the two carbazole moieties [14]. Consequently, the structure of compound **2** was deduced as bisglybomine B.

Compound **3** was obtained as a white powder, and its molecular formula was deduced as $C_{28}H_{24}N_2O_4$ by HR-EI-MS (*m*/*z* 452.1730). The ^{1}H-NMR data was similar to those of carbalexine A (**6**) [11], except for the lack of the signal of H–C (1)). The EI-MS showed a molecular ion peak at *m*/*z* 452, and a peak at *m*/*z* 226, which suggested a symmetrical dimeric carbazole with two carbalexine A units. The linkage between the two carbazole units through C(1)–C(1′) was suggested by the lack of a signal for H–C(1,1′). Based on these results, the structure of **3** was established as biscarbalexine A.

The bis-carbazole alkaloids **2** and **3** contained previously by known monomeric carbazoles as structure subunits. All bis-carbazole alkaloids were isolated only from plants of the genus *Murraya* until 1996 [15]. *Glycosmis* species are medicinal plants which are a rich source of carbazole alkaloids; however, they rarely produce dimeric carbazoles. Less than four dimeric carbazole alkaloids have been isolated from this

Table 2. *^{1}H- and ^{13}C-NMR, and HMBC Data of Compounds* **2** *and* **3**. δ in ppm, J in Hz.

Position	**2** ($CDCl_3$)			Position	**3** ((D_6)acetone)		
	δ(H)	δ(C)	HMBC		δ(H)	δ(C)	HMBC
C(1,1′)		98.7 (*s*)		C(1,1′)		102.1 (*s*)	
C(2,2′)		151.3 (*s*)		C(2,2′)		152.2 (*s*)	
C(3,3′)		117.3 (*s*)		C(3,3′)		117.5 (*s*)	
H–C(4,4′)	8.02 (*s*)	125.8 (*d*)	C(2,2′), C(9a,9a′), C(4a,4a′), *Me*–C(3,3′)	H–C(4,4′)	8.04 (*s*)	122.4 (*d*)	C(2,2′), C(9a,9a′), C(4b,4b′), *Me*–C(3,3′)
C(4a,4a′)		117.4 (*s*)		C(4a,4a′)		116.4 (*s*)	
C(4b,4b′)		123.1 (*s*)		C(4b,4b′)		113.6 (*s*)	
C(5,5′)		124.5 (*s*)		C(5,5′)		156.0 (*s*)	
C(6,6′)		150.9 (*s*)		H–C(6,6′)	6.70 (*d*, $J = 8.0$)	100.5 (*d*)	C(8,8′), C(5,5′), C(4b,4b′)
H–C(7,7′)	7.04 (*d*, $J = 8.4$)	111.3 (*d*)	C(6,6′), C(5,5′), C(8a,8a′)	H–C(7,7′)	7.17 (*t*, $J = 8.0$)	125.6 (*d*)	C(8,8′), C(5,5′), C(8a,8a′)
H–C(8,8′)	6.98 (*d*, $J = 8.4$)	108.2 (*d*)	C(4b,4b′), C(8a,8a′), C(6,6′)	H–C(8,8′)	6.92 (*d*, $J = 8.0$)	104.9 (*d*)	C(6,6′), C(4b,4b′)
C(8a,8a′)		134.9 (*s*)		C(8a,8a′)		142.0 (*s*)	
H–N(9,9′)	7.49 (*s*)		C(4b,4b′), C(4a,4a′), C(8a,8a′), C(9a,9a′)	H–N(9,9′)	9.62 (*s*)		
C(9a,9a′)		139.0 (*s*)		C(9a,9a′)		138.9 (*s*)	
CH_2(1″,1‴)	3.95–4.01 (*m*)	25.8 (*q*)	C(5,5′), C(3″,3‴), C(6,6′)	O*H*–C(2,2′)	7.29 (*s*)		
H–C(2″,2‴)	5.34–5.41 (*m*)	122.4 (*d*)	C(1″,1‴), C(4″,4‴)	*Me*–C(3,3′)	2.46 (*s*)	17.4 (*q*)	C(3,3′), C(4,4′), C(2,2′)
C(3″,3‴)		132.5 (*s*)		*Me*O–C(5,5′)	4.11 (*s*)	55.7 (*q*)	C(5,5′)
Me(4″,4‴)	1.98 (*s*)	18.3 (*q*)	*Me*–C(3″,3‴), C(2″,2‴), C(3″,3‴)				
Me–C(3″,3‴)	1.75 (*s*)	25.7 (*q*)	C(2″,2‴), C(3″,3‴), C(4″,4‴)				
OH–C(2,2′)	5.26 (*s*)		C(1,1′), C(2,2′), C(3,3′)				
Me–C(3,3′)	2.50 (*s*)	16.8 (*q*)	C(3,3′), C(4,4′), C(2,2′)				
*Me*O–C(6,6′)	3.87 (*s*)	57.9 (*q*)	C(6,6′)				

genus. Bisisomahanine isolated from *G. stenocarpa* represented the first dimeric prenylated pyranocarbazole alkaloid with a 1,1′-linkage [16]. In the present study, compounds **2** and **3** which present a 1,1′-linkage between two carbazole moieties, were isolated from *G. pentaphylla* for the first time. In addition, glybomine B (**4**) [12], glycoborinine (**5**) [17], carbalexine A (**6**) [11], 4,8-dimethoxy-1-methyl-3-(3-methylbut-2-en-1-yl)quinolin-2(1*H*)-one (**7**) [17], 4,8-dimethoxyfuro[2,3-*b*]quinoline (**8**) [12], skimmianine (**9**) [17], and arborinine (**10**) [12] were identified by comparison of their ^{1}H- and ^{13}C-NMR data with those reported in the literature.

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Experimental Part

General. TLC: Precoated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Co., Ltd.*, P. R. China). Column Chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Co*., *Ltd.*, P. R. China) and C_{18} reversed-phase silica gel (SiO_2; *YMC Co., Ltd.*, Japan). HPLC: *Ultimate-3000* HPLC system (*Dionex Co.*, *California*, USA), *Ultimate-3000* pump, *Ultimat-3000* variable-wavelength detector; column *Waters 5C_{18}-MS-II* (10 × 250 mm); t_R in min. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *DRX-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS and HR-EI-MS: *Finnigan-MAT-95* mass spectrometer (70 eV); in *m*/*z* (rel. %).

Plant Material. The stems of *G. pentaphylla* were collected from Xishuangbanna Prefecture, Yunnan Province, P. R. China. The plant material was identified by Mrs. *Ying-Hong Zhao*, Xishuangbanna Prefecture National Medicine Research Institute.

Extraction and Isolation. The air-dried stems of *G. pentaphylla* (24.5 kg) were powered and extracted three times with EtOH at r.t., and the EtOH extract (586 g) was suspended in 90% H_2O/MeOH and then successively extracted with petroleum ether, AcOEt, and BuOH. The AcOEt extract (91 g) was subjected to CC (SiO_2, cyclohexane/AcOEt 9:1, 8:2, 7:3, 1:1, 3:7, and 0:1): *Frs. 1–10*. *Fr. 7* (7.0 g) was subjected to CC (SiO_2, cyclohexane/$CHCl_3$ 7:3 → 0:1 and $CHCl_3$/acetone 95:5, 9:1, 8:2, and 0:1): *Frs. 7.1–7.8*. *Fr. 7.6* (1 g) was purified by CC (*ODS,* H_2O/MeOH 8:2 → 1:9): *Frs. 7.6.1–7.6.8*. *Fr. 7.6.6* (32 mg) was further purified by prep. HPLC (MeOH/H_2O 63:37, 3 ml/min): **1** (6 mg; t_R 16.4) Compounds **9** (46.2 mg) and **10** (4 mg) were crystallized from *Fr. 7.5* and *Fr. 7.4*, resp. *Fr. 7.1* (319.3 mg) was purified by CC (SiO_2, cyclohexane/$CHCl_3$ 50:1, 20:1, and 10:1 → 0:1) and then further purified by CC (SiO_2, $CHCl_3$/acetone 98:2): **8** (26 mg). *Fr. 6* (5.1 g) was subjected to CC (*MIC*, H_2O/MeOH 8:2 → 3:7): *Frs. 6.1–6.4*. *Fr. 6.2* (800 mg) was purified by CC (*ODS*, H_2O/MeOH 7:3 → 0:1): *Frs. 6.2.1–6.2.5*. *Fr. 6.2.4* (86.8 mg) was purified by CC (SiO_2, $CHCl_3$/acetone 9:1) and further purified by prep. HPLC (MeOH/H_2O 83:17 (0–10 min) and 94:6 (10–20 min) 3 ml/min): **4** (19.1 mg; t_R 9.6) and **2** (2.1 mg; t_R 18.0) *Fr. 6.2.3* (45.7 mg) was purified by CC (SiO_2, $CHCl_3$/acetone 9:1) and further purified by prep. HPLC (MeOH/H_2O 69:31 (0–12 min) and 8:2 (12–22 min), 3 ml/min): **3** (3.3 mg; t_R 11.5) and **6** (5.2 mg; t_R 21.7). *Fr. 6.2.5* (131.4 mg) was subjected to CC (SiO_2, $CHCl_3$/acetone 9:1) and then further purified by prep. HPLC (MeOH/H_2O 7:3, 2.5 ml/min): **5** (36.5 mg; t_R 25.3). *Fr. 3* (2.3 g) was subjected to CC (SiO_2, cyclohexane/acetone 95:5 → 3:7): *Frs. 3.1–3.8*. *Fr. 3.4* (700.3 mg) was purified by CC (*ODS*, H_2O/MeOH 1:1 → 0:1): **7** (20 mg).

*4-(7-Hydroxy-3-methoxy-6-methyl-9*H*-carbazol-4-yl)but-3-en-2-one* (**1**): Yellow powder. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-EI-MS: 295.1207 ($C_{18}H_{17}NO_3^+$; calc. 295.1208).

Bisglybomine B (*=6,6′-Dimethoxy-3,3′-dimethyl-5,5′-bis(3-methylbut-2-en-1-yl)[1,1′-bi-9*H*-carbazole]2,2′-diol*; **2**): Yellow powder. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 588 (100), 294 (23), 278 (9), 236 (10), 55 (9). HR-EI-MS: 588.2993 ($C_{38}H_{40}N_2O_4^+$; calc. 588.2988).

*Biscarbalexine A (=5,5′-Dimethoxy-3,3′-dimethyl[1,1′-bi-9*H*-carbazole]-2,2′-diol*; **3**): White powder. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 452 (100), 437 (22), 226 (77), 183 (22). HR-EI-MS: 452.1730 ($C_{28}H_{24}N_2O_4$; calc. 452.1736).

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