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ALKALOIDS AND QUASSINOIDS OF *BRUCEA MOLLIS* VAR. *TONKINENSIS*

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Key Word Index—*Brucea mollis* var. *tonkinensis*; Simaroubaceae; roots; bruceollines C and G; canthin-6-one alkaloids; β-carboline alkaloids; quassinoids.

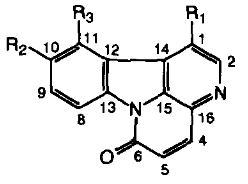
Abstract—Two new canthin-6-one alkaloids, bruceollines C and G, were isolated from the root bark and root wood of *Brucea mollis* var. *tonkinensis*, together with three known canthin-6-one alkaloids, 11-hydroxycanthin-6-one, 1-hydroxy-11-methoxycanthin-6-one and 11-hydroxy-1-methoxycanthin-6-one, and four known β-carboline alkaloids, 1-hydroxymethyl-β-carboline, 1-ethyl-β-carboline, 1-(2′-hydroxyethyl)-β-carboline and β-carboline-1-propionic acid, and six known quassinoids, brucein B, brucein D, brusatol, bruceoside B, yadanziolide A and soulameanone. Their structures were elucidated based on the spectral and chemical evidence.

INTRODUCTION

In our phytochemical investigation of the Simaroubaceae, we recently reported six new alkaloids from the root wood of *Brucea mollis* Wall. var. *tonkinensis* Lecomte [1, 2]. The present paper deals with the isolation and structural elucidation of two new canthin-6-one alkaloids, named bruceollines C (**1**) and G (**2**), and three known canthin-6-one alkaloids, four known β-carboline alkaloids and six known quassinoids, which were isolated from the root bark and root wood of this species.

RESULTS AND DISCUSSION

Bruceolline C (**1**) was obtained as a yellow amorphous powder and assigned the molecular formula $C_{16}H_{12}N_2O_4$ (HR-mass spectrum) in combination with the ^{13}C NMR spectrum (Table 1). The IR spectrum indicated the presence of a hydroxyl group (ν_{max} 3426 cm^{-1}) and the UV spectrum was similar to that of 1-hydroxy-11-methoxycanthin-6-one (**3**) [3], which suggested that **1** was an oxysubstituted canthin-6-one alkaloid. The ^{1}H NMR spectrum (Table 2) showed two methoxyl signals at δ3.90 (3H, *s*) and 4.25 (3H, *s*), an aromatic singlet signal at δ8.65 (1H, H-2), two pairs of *ortho*-coupled signals at δ6.79 (1H, *d*, *J* = 9.9 Hz, H-5), 8.05 (1H, *d*, *J* = 9.9 Hz, H-4) and at δ7.25 (1H, *d*, *J* = 8.8 Hz), 8.20 (1H, *d*, *J* = 8.8 Hz) which can be attributed to the two aromatic protons of the 8,9- or 10,11-positions. The



	R1	R2	R3
1	OMe	OH	OMe
2	H	H	O-glc
3	OH	H	OMe
4	H	H	O-glc-$\overset{6\text{-}1}{}$glc

Table 1. ^{13}C NMR (100 MHz, DMSO-d_6) spectral data for compounds **1** and **2**

Position	1	2
1	151.4	118.7
2	124.4	145.6
4	138.7	139.6
5	132.2	127.8
6	159.1	158.6
8	111.9	109.6
9	119.0	131.9
10	143.3	111.5
11	148.8	154.3
12	118.4	114.9
13	139.1	139.4
14	126.5	127.9
15	129.8	130.9
16	131.7	135.0
1′	—	100.6
2′	—	72.2
3′	—	77.0
4′	—	69.7
5′	—	76.5
6′	—	60.7
OMe-1	61.7	—
OMe-11	57.1	—

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Table 2. ^1H NMR (400 MHz, DMSO-d_6) spectral data for compounds **1** and **2**

Position	**1**	**2**
1	—	8.32 *d* (4.9)
2	8.65 *s*	8.80 *d* (4.9)
4	8.05 *d* (9.9)	8.10 *d* (9.8)
5	6.79 *d* (9.9)	6.97 *d* (9.8)
8	8.20 *d* (8.8)	8.19 *d* (8.0)
9	7.25 *d* (8.8)	7.70 *t* (8.0)
10	—	7.42 *d* (8.0)
1′	—	5.20 *d* (7.7)
OMe-1	4.25 *s*	—
OMe-11	3.90 *s*	—

above data suggest that **1** has one hydroxyl and two methoxyl groups; their locations were deduced by difference NOE experiments (Fig. 1). Irradiation of the methoxyl protons at δ4.25 induced 24% NOE at the singlet proton at δ8.65 (H-2) and 4% NOE at the methoxyl protons at δ3.90. Irradiation of the other methoxyl protons, at δ3.90, induced no NOE. Acetylation of **1** with acetic anhydride in pyridine gave a monoacetate (**1a**) with one new acetyl-methyl resonance at δ2.38 and a proton at C-9 resonating downfield ($\Delta\delta = 0.3$) with respect to that of **1**. Therefore, these data indicate that the hydroxyl group was located at C-10 and the two methoxyl groups at C-1 and C-11. Thus, bruceolline C (**1**) was concluded to be 10-hydroxyl-1, 11-dimethoxycanthin-6-one.

Bruceolline G (**2**) was obtained as a pale yellow amorphous powder and assigned the molecular formula $C_{20}H_{18}N_2O_7$ by the positive FAB-mass spectral (*m/z* 460 $[M + K + Na]^+$) in combination with the ^{13}C NMR spectrum (Table 1). The IR and UV spectra were similar to those of bruceolline A (**4**) [1]. The ^1H NMR spectrum (Table 2) of **2** showed two pairs of doublets ascribed to *ortho*-coupled signals at δ8.32 (1H, *d*, $J = 4.9$ Hz, H-1), 8.80 (1H, *d*, $J = 4.9$ Hz, H-2) and at δ6.97 (1H, *d*, $J = 9.8$ Hz, H-5), 8.10 (1H, *d*, $J = 9.8$ Hz, H-4) and ABX-type signals at δ7.42 (1H, *d*, $J = 8.0$ Hz), 7.70 (1H, *t*, $J = 8.0$ Hz) and 8.19 (1H, *d*, $J = 8.0$ Hz) attributed to the three aromatic protons of an 8- or 11-substituted indolic part. The ^1H and ^{13}C NMR spectra of **2** showed an anomeric proton (δ5.20, *d*, $J = 7.7$ Hz) and carbon (δ100.6). Acid hydrolysis of **2** gave 11-hydroxycanthin-6-one (**5**) [4] and D-glucose. The latter was identified by GC as the TMSi derivative. Moreover, the EI-mass spectrum of **2** showed an abundant fragment ion at *m/z* 236 $[M - 162]^+$ due to loss of one glucose unit, which supported the hydrolysis result. A comparative study of the ^1H and ^{13}C NMR spectra of **2** with those of **4**, suggested that **2** was 11-*O*-glucopyranosylcanthin-6-one. The configuration of the anomeric position of the glucose moiety was assigned to be β from the coupling constant of the anomeric proton at 7.7 Hz. Thus, bruceolline G (**2**) was concluded to be 11-*O*-β-D-glucopyranosylcanthin-6-one.

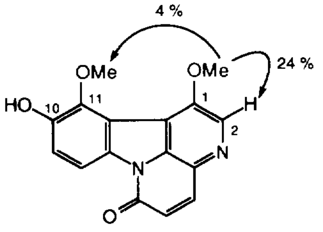

Fig. 1. NOEs of bruceolline C (**1**).

Compounds **5**, **7**, **8** and **10** were identified as 11-hydroxycanthin-6-one (**5**) [4], 1-hydroxymethyl-β-carboline (**7**) [5], 1-ethyl-β-carboline (**8**) [6] and β-carboline-1-propionic acid (**10**) [7] by direct comparison (mixed mp, IR, ^1H NMR, mass spectrometry and co-TLC) with authentic samples, respectively. Compounds **3**, **6**, **9** and **11**–**16** were identified as 1-hydroxy-11-methoxycanthin-6-one (**3**) [3], 11-hydroxy-1-methoxycanthin-6-one (**6**) [3], 1-(2′-hydroxyethyl)-β-carboline (**9**) [8], brucein B (**11**) [9], brucein D (**12**) [10], brusatol (**13**) [11], bruceoside B (**14**) [12], yadanziolide A (**15**) [13] and soulameanone (**16**) [14] by comparison with literature data (mp, IR, mass spectrometry and ^1H NMR), respectively.

EXPERIMENTAL

General. Mps: uncorr. NMR spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C, using TMS as int. standard.

Plant material. Root bark (2.5 kg) and root wood (8.5 kg) of *B. mollis* Wall var. *tonkinensis* Leconte was collected in Haicao, Hainan, China, in December 1991 and identified by Mr Gi Ouyang (Hainan People's Hospital) and Mr Fu Ze-Hai (Pharmaceutical Society of Hainan). A voucher specimen has been deposited in the Department of Pharmacognosy, School of Pharmaceutical Sciences, Toho University, Japan.

Extraction and isolation. Dried root bark (2.5 kg) was extracted with CH_2Cl_2 (35 l) and MeOH (33 l) under reflux conditions. The CH_2Cl_2 and MeOH extracts were concd under red. pres. to give residues of 34 and 81 g, respectively. The CH_2Cl_2 extract (34 g) was chromatographed on silica gel (350 g) and eluted with a step-wise increase in MeOH content in CH_2Cl_2 (5, 10, 20, 50 and 100%). The alkaloid frs obtained were repeatedly chromatographed by prep. MPLC (silica gel) with $CHCl_3$–MeOH (100:1 and 50:1). Further purification by silica gel (NH-type) CC eluted with 5 and 10% MeOH gave a new alkaloid named bruceolline C (**1**; 2 mg) and five known alkaloids **3** (42 mg), **5** (43 mg), **6** (35 mg), **8** (3 mg) and **9** (23 mg). The MeOH extract (81 g) was chromatographed on Diaion HP-20 (1 kg) and eluted with a step-wise increase in MeOH content in H_2O (10, 20, 50, 80 and 100%). The frs (28–37) obtained by elution with H_2O–MeOH (9:1) afforded a mixt. of quassinoids (3 g), which was repeatedly chromatographed on silica gel to give three known quassinoids, **12** (109 mg), **13** (170 mg) and **15** (89 mg).

Dried root wood (8.5 kg) was extracted with hexane (80 l), CH_2Cl_2 (80 l) and MeOH (80 l). The hexane, CH_2Cl_2 and MeOH extracts were concd under red. pres. to give residues of 20, 21 and 208 g, respectively. The MeOH extract (208 g) was chromatographed on Diaion HP-20 (1.5 kg) and eluted with a step-wise increase in MeOH content in H_2O (10, 20, 50, 80 and 100%). Frs 1 (11.8 g) and 2 (20.5 g) were obtained by elution with MeOH and fr. 1 was repeatedly chromatographed on silica gel and eluted with a step-wise increase in MeOH content in $CHCl_3$ (1, 2, 5, 10, 20, 50 and 100%) to afford frs 3 (1.2 g), 4 (1.1 g) and 5 (2.4 g). Fr. 3 was chromatographed on ODS and eluted with H_2O–MeOH (4:1, 3:1, 2:1 and 1:1) to give compounds **7** (3 mg), **11** (5 mg) and **16** (38 mg). Fr. 4 was subjected to prep. MPLC (silica gel) with $CHCl_3$–MeOH (15:1, 10:1 and 5:1) to give compounds **8** (3 mg), **9** (10 mg) and **14** (18 mg). Fr. 5 was chromatographed on silica gel and eluted with a step-wise increase in MeOH content in $CHCl_3$ (1, 2, 5, 10, 20, 50 and 100%). The fr. eluted with 10% MeOH gave a new alkaloid named bruceolline G (**2**; 3 mg) and compound **10** (4 mg). Fr. 2 was repeatedly chromatographed on silica gel and eluted with a step-wise increase in MeOH content in $CHCl_3$ (1, 2, 5, 10, 20, 50 and 100%); the fr. eluted with 2% MeOH gave compound **13** (176 mg).

Bruceolline C (10-*hydroxy*-1,11-*dimethoxycanthin*-6-*one*) (**1**). Amorphous yellow powder. IR ν_{max}^{KBr} cm^{-1}: 3426, 1661, 1622, 1587, 1523, 1413, 1343, 1278, 1217, 1131, 1074. UV λ_{max}^{MeOH} nm (log ε): 276 sh (3.37), 286 (3.45), 350 (3.35), 370 (3.35). UV $\lambda_{max}^{MeOH+HCl}$ nm (log ε): 276 sh (3.37), 286 (3.45), 350 (3.35), 370 (3.35). UV $\lambda_{max}^{MeOH+NaOH}$ nm (log ε): 275 sh (3.21), 287 (3.32), 310 (3.28), 340 sh (3.32), 350 (3.35). HRMS *m/z*: 296.0794 $[M]^+$ (calc. for $C_{16}H_{12}N_2O_4$: 296.0794). EIMS *m/z* (rel. int.): 296 $[M]^+$ (26), 281 (13), 256 (5), 223 (8), 205 (9), 185 (8), 167 (12), 157 (6), 149 (100). ^1H and ^{13}C NMR: see Tables 1 and 2.

Acetylation of **1**. Bruceolline C (**1**, 1 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) at room temp. for 2 hr. After MeOH (10 ml) was added, the reaction mixt. was evapd *in vacuo* to give the monoacetate (**1a**, 1 mg). Amorphous yellow powder. IR ν_{max}^{KBr} cm^{-1}: 1754, 1678, 1623, 1590, 1506, 1434, 1347, 1272, 1249, 1222, 1124, 1033, 1002. EIMS *m/z* (rel. int.): 338 $[M]^+$ (15), 310 (5), 296 (100), 281 (32), 253 (5), 235 (10), 195 (4). ^1H NMR (DMSO-d_6): δ2.38 (3H, *s*, 10-OAc), 3.91 (3H, *s*, 11-OMe), 4.26 (3H, *s*, 1-OMe), 6.84 (1H, *d*, $J = 9.9$ Hz, H-5), 7.54 (1H, *d*, $J = 8.8$ Hz, H-9), 8.11 (1H, *d*, $J = 9.9$ Hz, H-4), 8.69 (1H, *s*, H-2), 8.40 (1H, *d*, $J = 8.8$ Hz, H-8).

Bruceolline G (11-O-β-D-*glucopyranosylcanthin*-6-*one*) (**2**). Amorphous pale yellow powder. IR ν_{max}^{KBr} cm^{-1}: 3422, 1655, 1637, 1560, 1543, 1498, 1441, 1271, 1073. UV λ_{max}^{MeOH} nm (log ε): 230 sh (3.88), 242 sh (3.86), 250 sh (3.86), 268 sh (3.46), 315 (3.60), 352 sh (3.55), 369 (3.70), 398 (3.62). UV $\lambda_{max}^{MeOH+HCl}$ nm (log ε): 230 sh (3.89), 242sh (3.86), 250sh (3.84), 268sh (3.49), 315 (3.62), 352 sh (3.55), 369 (3.72), 398 (3.64). UV $\lambda_{max}^{MeOH+NaOH}$ nm (log ε): 230 sh, (3.90), 242 sh (3.86), 250 sh (3.84), 268 sh (3.46), 315 (3.63), 352 sh (3.55), 369 (3.72), 398 (3.63). Positive FAB-MS *m/z*: 460 $[M + K + Na]^+$, 237 $[M - glc + 2H]^+$, 236 $[M - glc + H]^+$. EIMS *m/z* (rel. int.): 237 $[M - glc + 2H]^+$ (22), 236 (100), 208 (39), 179 (12), 153 (6). ^1H and ^{13}C NMR: see Tables 1 and 2.

Acid hydrolysis of **2**. Bruceolline G (**2**, 10 mg) was heated in MeOH with 1N HCl (2 ml) for 6 hr. After removal of MeOH, the ppt. was filtered and washed with H_2O to give the aglycone (11-hydroxycanthin-6-one). The filtrate was extracted with EtOAc (2 ml × 3), then neutralized with ion-exchange resin (Amberlite MB-3) and evapd under red. pres. The residue was treated with *N*-trimethylsilylimidazole at 90° for 1 hr and H_2O added to the reaction mixt. to decompose excess reagent; the reaction product was extracted with hexane (1 ml × 3). The hexane soln was analysed by GC, whereby the TMSi derivative was identified as D-glucose. GC was carried out using a stainless steel column (3 mm × 1 m) packed with 2% SE-30 on Chromosorb-W (60–80 mesh) with N_2 carrier gas at a flow rate of 30 ml min^{-1}. The aglycone was identified by direct comparison (mmp, IR, ^1H NMR, MS and co-TLC) with authentic 11-hydroxycanthin-6-one (**5**).

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