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BUNTANBISMINE, A BISACRIDONE ALKALOID FROM *CITRUS GRANDIS F. BUNTAN*

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Key Word Index—*Citrus grandis* f. *buntan*; Rutaceae; stem bark acridone alkaloid; bisacridone; buntanbismine.

Abstract—A C–C linked bisacridone alkaloid, buntanbismine, was isolated from the stem bark of *Citrus grandis* f. *buntan*. Its structure has been characterized by spectral analyses and chemical transformation.

INTRODUCTION

The isolation of acridone alkaloids and coumarins from the stem bark of *Citrus grandis* f. *buntan* (Chinese name, buntan) has been reported previously [1, 2]. During the separation of the lower polar fraction of the acetone extract, an unidentified compound **b** has also been obtained [1]. The present paper deals with the structural elucidation of this compound as buntanbismine (**1**) by spectral analyses and chemical transformation.

RESULTS AND DISCUSSION

Buntanbismine (**1**) was isolated as red needles (mp > 300°) in 0.00055% yield. High-resolution FAB mass spectrometry determined the molecular formula of **1** as $C_{35}H_{32}N_2O_9$. The UV spectrum showed maxima at 202, 251 (sh), 271, 289 (sh), 331 nm, typical for the 9-acridone chromophore [3–6]. The absorption bands at 3407 and 1638 cm^{-1} in the IR spectrum together with downfield signals at δ 15.10 and δ 14.21 (each 1H, disappearing with D_2O) in the ^1H NMR spectrum (Table 1) corresponded to the presence of two strongly intramolecular hydrogen-bonded hydroxyl protons. Thus, a binary 1-hydroxy-9-acridone structure was suggested for **1**. In the aromatic region of the ^1H NMR spectrum of **1**, there were two sets of mutually coupled proton systems. One at δ 6.74 (*d*, $J = 7.4$ hz), 7.04 (*t*, $J = 7.4$ Hz) and 7.61 (*d*, $J = 7.4$ Hz) was assignable to H-6, H-7 and H-8, respectively the other at δ 7.19 (*d*, $J = 9.0$ Hz) and 8.10 (*d*, $J = 9.0$ Hz) for H-7′ and H-8′, respectively. These assignments were determined through the lower-field signals of H-8 and H-8′, which were deshielded by the adjacent carbonyl group. The ^1H NMR spectrum also showed one characteristic *N*-methyl at δ 3.66, three aryl methoxyls at δ 3.06, 3.96 and 4.07, two D_2O-exchangeable acridone NH and phenolic OH at δ 9.51 and 11.06. The remaining signals occurred two oxygen-linked methyls at δ 1.52 (*s*) and 1.61 (*s*), one methylene at δ 1.78 (*m*) and 2.18 (*m*), and one benzylic proton at δ 5.01 (*dd*, $J = 7.0$, 11.0 Hz) and were deduced to be a dimethyldihydropyran ring residue. In order to determine the location of these substituents, acetylation of **1** with acetic anhydride in pyridine was undertaken and gave a monoacetylated compound (**1a**) and a diacetylated compound (**1b**) after preparative TLC.

The M_r of **1a** was 42 amu more than that of **1**, that is, a hydrogen atom was replaced by an acetyl group. Comparing the ^1H NMR spectrum of **1a** with that of **1**, the hydroxyl signal at δ 11.06 disappeared, being replaced by acetyl absorption at δ 1.94 (Table 1). In addition, the doublet signal in **1** at δ 6.74 (H-6) was shifted significantly to lower field in **1a** at δ 7.06. Hence, the hydroxyl group can be placed at C-5. In an NOE difference experiment of **1a** (Fig. 1), irradiation of the *N*-methyl (δ 3.58) caused a 1.8 and 13.5% enhancement of signals at δ 1.94 (5-OAc) and 5.0 (H-11) which revealed a dimethyldihydropyran ring angularly-fused to the upper acridone, with a singlet aromatic proton assigned to H-2. On further examination of the ^{13}C NMR spectrum of **1a**, a doublet ^{13}C signal appearing at δ 99.15 could be assigned to C-2 in this angular pyranoacridone; on the other hand, the other doublet ^{13}C signal in the same region at 87.9 should be inferred for C-4′ [7, 8]. Irradiation of the two methoxyl singlets at δ 3.08 and 4.03 resulted in 16.1 and 9.0% increase of the signals at δ 5.82 (H-4′) and 6.98 (H-7′), respectively. However, no effect was

Table 1. ^{1}H NMR spectral data of compounds **1**, **1a** and **1b** (multiplicity, *J*, Hz)

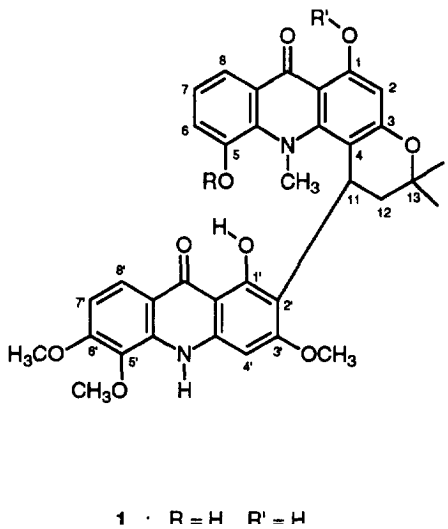
	1*	**1a**†	**1b**†
1-OH or OAc	14.21 (*s*)	13.93 (*s*)	2.49 (*s*)
H-2	6.53 (*s*)	6.33 (*s*)	6.52 (*s*)
N-Me	3.66 (*s*)	3.58 (*s*)	3.53 (*s*)
5-OH or OAc	11.06 (*s*)	1.94 (*s*)	1.92 (*s*)
H-6	6.74 (*d*, 7.6)	7.06 (*dd*, 7.8 and 1.2)	6.99 (*dd*, 7.8 and 2.1)
H-7	7.04 (*t*, 7.6)	7.14 (*t*, 7.8)	7.08 (*t*, 7.8)
H-8	7.61 (*d*, 7.6)	8.15 (*dd*, 7.8 and 1.2)	8.08 (*dd*, 7.8 and 2.1)
H-11	5.01 (*dd*, 11.0 and 7.0)	5.00 (*dd*, 12.0 and 7.0)	5.02 (*dd*, 11.0 and 6.6)
H-12	1.78 (*m*)	1.74 (*dd*, 13.0 and 12.0)	1.75 (*m*)
	2.18 (*m*)	2.20 (*dd*, 13.0 and 7.0)	2.24 (*m*)
13-Me	1.52 (*s*)	1.48 (*s*)	1.49 (*s*)
	1.61 (*s*)	1.56 (*s*)	1.56 (*s*)
1′-OH	15.10 (*s*)	14.81 (*s*)	14.80 (*br s*)
3′-OMe	3.06 (*s*)	3.08 (*s*)	3.06 (*s*)
H-4′	6.16 (*s*)	5.82 (*s*)	5.82 (*s*)
NH	9.51 (*s*)	8.45 (*s*)	8.41 (*s*)
5′-OMe	3.96 (*s*)	4.01 (*s*)	4.01 (*s*)
6′-OMe	4.07 (*s*)	4.03 (*s*)	4.03 (*s*)
H-7′	7.19 (*d*, 9.0)	6.98 (*d*, 9.3)	6.93 (*d*, 9.0)
H-8′	8.10 (*d*, 9.0)	8.11 (*d*, 9.2)	8.12 (*d*, 9.0)

*DMSO-d_6.
†$CDCl_3$.

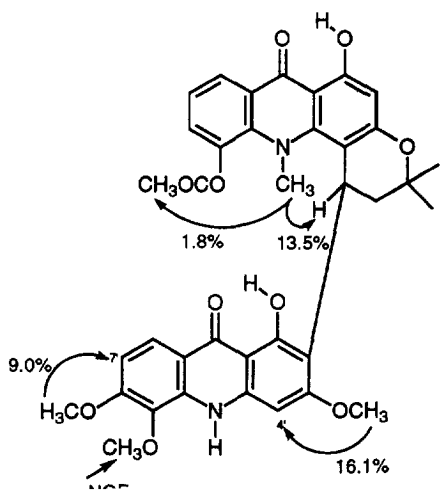
half of **1a**. The EI-mass spectrum further confirmed the structure of **1a**. A base peak at *m/z* 301 was responsible for the lower half acridone plus a hydrogen atom, as well as a fragment ion peak at *m/z* 366 for the upper half acridone. Based on the above analyses of **1** and **1a**, the linkage of two acridone units was concluded to be between C-11 and C-2′ in the upper and lower halves of this molecule and the structure of buntanbismine was established as **1**.

A notable spectral feature of buntanbismine (**1**) was an abnormal upfield shift of the 3′-methoxyl signal (δ 3.06) which was probably due to the effect of the anisotropic ring current of the upper half acridone in the three-dimensional conformation. The preferred conformation was suggested that two non-parallel acridones caused the 3′-methoxyl group in the lower half acridone located above the upper half acridone.

Compound **1b** exhibited another acetyl group at δ 2.49 and a downfield shift of H-2 (δ 6.33 in **1a**, 6.52 in **1b**) in the ^{1}H NMR spectrum (Table 1) together with



1 : R = H, R' = H



a relatively intense fragment peak at *m/z* 301 in the mass spectrum. The diacetate of **1** obviously involved substitution of C-1 and C-5 of the upper acridone.

EXPERIMENTAL

General. Mps, uncorr. UV: MeOH. IR: KBr. ^{1}H and ^{13}C NMR: $CDCl_3$ with TMS as int. ref. except where noted. MS: direct inlet.

Plant material, extraction and isolation. The isolation procedure was as described in refs [1, 2]. The unidentified compound **b** from the Me_2CO extract of the stem bark of *C. grandis* f. *buntan* (2 kg) was determined as **1** (10 mg).

Buntanbismine (**1**). Red needles ($CHCl_3$), mp > 300°. FAB-HRMS: calcd for $C_{35}H_{33}N_2O_9$, *m/z* 625.2186 $[M+H]^+$, found 625.2172. UV λ_{max} nm (log ε): 202 (4.01), 251 (3.85, sh), 271 (4.10), 289 (2.78, sh), 331 (3.29). IR υ_{max} cm^{-1}: 3407, 1638, 1607, 1567. FABMS *m/z* (ref. int.): 625 ($[M+H]^+$, 80), 609 (7), 324 (100), 323 (12), 314 (17), 308 (7), 302 (17), 301 (5). *Acetylation of buntanbismine* (**1**). Buntanbismine (**1**) (10 mg) was dissolved in a soln containing Ac_2O (2 ml) and pyridine (2 ml) and then refluxed for 1 hr. After cooling, the resulting mixt. was extracted with Et_2O and washed with 5% HCl, 5% $NaHCO_3$ and satd. NaCl, successively. The Et_2O soln was dried ($MgSO_4$) and concd *in vacuo*. The crude product was purified by prep. TLC to give two compounds, **1a** (6 mg) and **1b** (4 mg).

5-*Acetylbuntanbismine* (**1a**). Yellow needles ($CHCl_3$), mp > 250°. EI-HRMS: calcd for $C_{37}H_{34}N_2O_{10}$, *m/z* 666.2215 $[M]^+$, found 666.2213. UV λ_{max} nm: 219, 229, 255 (sh), 272, 333, 392. IR υ_{max} cm^{-1}: 3369, 1764, 1637, 1585, 1559. EIMS *m/z* (rel. int.): 666 ($[M]^+$, 17), 651 (39), 366 (16), 365 (19), 323 (22), 308 (61), 301 (100), 293 (28), 286 (24). ^{13}C NMR: δ 21.1 (*q*), 22.5 (*q*), 28.1 (*d*), 29.5 (*q*), 39.5 (*t*), 46.5 (*q*), 55.4 (*q*), 56.3 (*q*), 61.1 (*q*), 76.0 (*s*), 87.9 (*d*), 99.2 (*d*), 103.9 (*s*), 104.8 (*s*), 107.3 (*s*), 107.7 (*s*), 110.3 (*s*), 114.9 (*s*), 121.8 (*d*), 122.0 (*d*), 123.3 (*d*), 125.1 (*s*), 128.1 (*d*), 133.8 (*s*), 135.0 (*s*), 139.8 (*s*), 140.0 (*s*), 141.0 (*s*), 150.4 (*s*), 154.7 (*s*), 160.5 (*s*), 161.9 (*s*), 162.9 (*s*), 164.0 (*s*), 168.9 (*s*), 181.0 (*s*), 181.2 (*s*).

1,5-*Diacetylbuntanbismine* (**1b**). Yellow syrup. UV λ_{max} nm: 215, 263 (sh), 327, 387. IR υ_{max} cm^{-1}: 3291, 1769, 1696, 1606, 1561. EIMS *m/z* (rel. int.): 708 ($[M]^+$, 59), 666 (100), 651 (64), 365 (18), 350 (23), 323 (40), 314 (38), 301 (61). ^{13}C NMR: δ 21.2 (*q*), 21.4 (*q*), 22.5 (*q*), 28.6 (*d*), 29.5 (*q*), 39.5 (*t*), 46.1 (*q*), 55.5 (*q*), 56.3 (*q*), 61.1 (*q*), 75.6 (*s*), 87.8 (*d*), 104.1 (*d*), 107.7 (*d*), 108.8 (*d*), 109.9 (*s*), 112.0 (*s*), 112.2 (*s*), 115.0 (*s*), 121.8 (*d*), 122.1 (*d*), 127.5 (*d*), 127.5 (*s*), 133.9 (*s*), 135.1 (*s*), 139.4 (*s*), 139.9 (*s*), 141.1 (*s*), 149.5 (*s*), 151.8 (*s*), 154.8 (*s*), 159.9 (*s*), 160.6 (*s*), 164.1 (*s*), 169.1 (*s*), 170.2 (*s*), 177.0 (*s*), 181.0 (*s*).

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