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ACRIDONE ALKALOIDS FROM *BOSISTOA TRANSVERSA*

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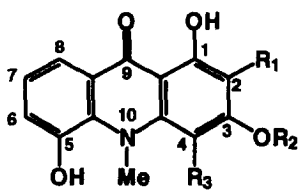
Key Word Index—*Bosistoa transversa*; Rutaceae; 2- and 4-prenylated 9-(10)-acridone alkaloids; bosistidine; bosistine; 4-(2ξ-hydroxy-3-methylbut-3-enyl)yukocritrine.

Abstract—Eight acridone alkaloids were isolated from *Bosistoa transversa*. Three of them are novel and have been identified as 1,3,5-trihydroxy-2-(2ξ-hydroxy-3-methylbut-3-enyl)-10-methylacridan-9-one (trivial name, bosistidine), 1,3,5-trihydroxy-2-(2ξ-hydroxy-3-methylbut-3-enyl)-4-(3-methylbut-2-enyl)-10-methylacridan-9-one (bosistine) and 1,3,5-trihydroxy-4-(2ξ-hydroxy-3-methylbut-3-enyl)-yukocritine. The structures were elucidated on the basis of NMR spectral data, notably NOESY and HMBC experiments.

INTRODUCTION

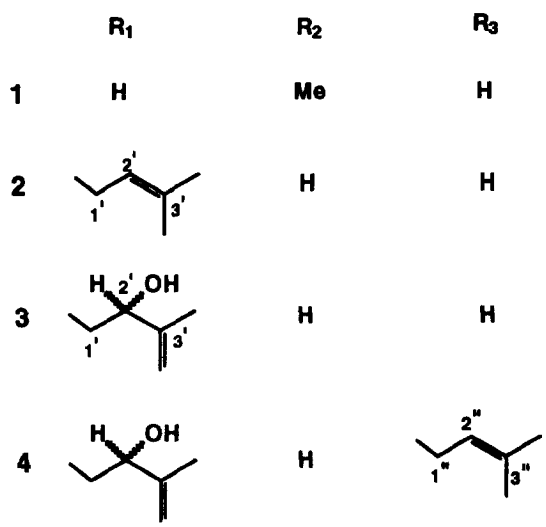
In continuation of our investigation into the chemistry of the genus *Bosistoa* [1, 2] we have undertaken a study of the aerial parts of *B. transversa* J. F. Bailey and C. T. White, a small- to medium-sized tree found in the rain forests of Queensland and northeast New South Wales [3]. In the present paper we report the isolation and identification of eight acridone alkaloids, of which three appear to be novel.

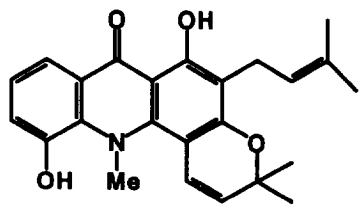


RESULTS AND DISCUSSION

By a combination of vacuum liquid chromatography (VLC), gel filtration and preparative TLC procedures, the hexane and ethyl acetate extracts of the leaf material afforded six acridones (**1**, **2** and **4**–**7**). Similar treatment of the stem bark again gave these alkaloids, plus the two additional acridones, **3** and **8**. The alkaloids were all yellow in colour and gave UV, IR and NMR spectra typical of the 1,3,5-oxygenated-*N*-methylacridan-9-one nucleus [4–6].

Five were known compounds which were identified, by direct comparison of their spectral data with that published, as citrusamine (**1**) [7], junosine (**2**) [8], *N*-methylataphylline (**5**) [9], yukocitrine (**7**) [10] and *N*-methylataphyllinine (**6**) [11]. The remaining compounds (**3**, **4** and **8**) were novel acridones, which had several NMR spectral features in common. There were strongly H-bonded hydroxyls at *ca* δ 15, three adjacent





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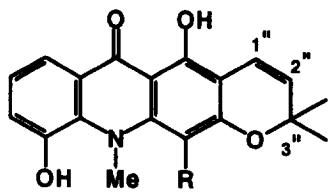

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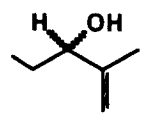
8 R = 

aromatic protons, one of which was deshielded and therefore placed *peri* to the carbonyl at H-8, an *N*-methyl group, and signals for a 2-hydroxy-3-methylbut-3-enyl moiety (Table 1).

In bosistidine (**3**), the only additional signal in the ^{1}H NMR spectrum was for a single A-ring proton, which must be placed at either C-2 or C-4. As this proton exhibited a strong NOE interaction with the *N*-methyl, it must be assigned to C-4 and bosistidine must be **3**.

Table 1. ^{1}H NMR (400 MHz, acetone-d_6) spectral data for compounds **3** and **8**

H	3	8
1-OH	15.26 *s*	14.70 *s*
4	6.45 *s*	—
5-OH	9.40 *s*	9.36 *s*
6	7.27 *dd* (7.8 1.4)	7.28 *dd* (7.9, 1.5)
7	7.12 *t* (7.8)	7.18 *t* (7.9)
8	7.90 *dd* (7.8, 1.6)	7.90 *dd* (7.9, 1.5)
N-Me	4.05 *s*	3.78 *s*
1′	3.15 *dd* (14.4, 8.0)	3.22 *dd* (14.4, 8.0)
	2.85 *dd* (14.4, 8.0)	3.18 *dd* (14.4, 8.0)
2′	4.40 *m*	4.64 *m*
2′-OH	5.35 *s*	5.35 *s*
H_2-4′	4.96 *s*	4.66 *s*
	4.76 *s*	4.54 *s*
3′-Me	1.85 *s*	1.76 *s*
1″	—	6.72 *d* (10.0)
2″		

The ^{1}H NMR spectrum of bosistine (**4**), showed in place of an A-ring proton, an additional 3-methyl-2-enyl group substituent. The problem of placing the two different prenyl substituents at C-2 and C-4 was resolved in a number of ways. (a) In the ^{13}C NMR spectrum (Table 2), the methylene of the 3-methylbut-2-enyl side-chain appeared at δ 27.3, which is characteristic of a C-4 substituent of this type [6]. (b) A ^{1}H–^{1}H NOESY experiment revealed a strong interaction between the *N*-methyl and the olefinic proton of the 3-methylbut-2-enyl side-chain at δ 5.40, again indicating that this side-chain is at C-4. (c) In an HMBC experiment (Table 2) long-range coupling (^3J) was observed between the methylene protons H-1′a and H-1′b of the 2-hydroxy-3-methylbut-3-enyl side-chain and the carbon nuclei resonating at δ 161.2 (C-1) and 164.5 (C-3), with a ^2J-coupling with C-2 (δ 108.3).

The third new alkaloid gave a ^{1}H NMR spectrum that showed signals for a 2,2-dimethyl chromene system and a 2-hydroxy-3-methylbut-3-enyl which must be located on ring A. The orientation of the pyran ring with respect to the acridone nucleus was suggested as linear by the UV spectrum [6, 10]. This required placement of the 2-hydroxy-3-methylbut-3-enyl side-chain at C-4; this was further confirmed by the observation of NOESY cross-peaks between the *N*-methyl and the exomethylene protons of the side-chain. The chemical shifts of the chromene system protons were very similar to those of yukocitrine (**7**) [10]. On the basis of these data, **8** has been assigned.

The three novel compounds all possessed the 2-hydroxy-3-methylbut-3-enyl side-chain, which has a chiral centre. Unfortunately, insufficient quantities were isolated to permit establishment of any absolute configurations.

Our study has revealed that *B. transversa* is a major source of 1,3,5-oxygenated-2,4-prenylated acridones. Many of the compounds isolated have previously been reported from two other rutaceous genera, *Atalantia* [9, 11] and *Citrus* [7, 8, 10]. Both of these genera are members of the sub-family Aurantioideae, while *Bosistoa* is part of the tribe Zanthoxyleae (sub-family Rutoideae). The co-occurrence of this unusual type of acridone in these taxonomically distance taxa of the Rutaceae is rather surprising. *Bosistoa transversa* appears to be chemically distinct from the previously studied *Bosistoa* species, *B. brassii* [2] and *B. floydii* [1], where the major metabolites are flavonoids. No flavonoids were detected in our study.

EXPERIMENTAL

Mps uncorr. UV: MeOH. IR: $CHCl_3$. EIMS: probe (90–130°) at 70 eV. FABMS: nitrobenzyl alcohol matrix. NMR: run in $CDCl_3$ or Me_2CO-d_6. NOESY and HMBC [12] expts were obtained on a Bruker AMX-400 instrument using standard Bruker microprograms.

Table 2. ^{1}H and ^{13}C NMR chemical shift data (400 MHz, acetone-d_6) and selected ^{2}J and ^{3}J H–C couplings for compound **4**

Position	δ_H	δ_C	^{2}J	^{3}J
1	13.39 *s*	161.2		
2	—	108.4		
3	10.29 *s*	164.5		
4	—	110.1		
4a	—	150.5		
5a	—	139.1		
5	9.25 *s*	148.4		
6	7.27 *d* (8.0)	120.5	123.7, 148.4	117.3, 139.1
7	7.15 *t* (8.0)	123.7	117.3, 120.5	125.9, 148.4
8	7.77 *dd* (8.0, 1.2)	117.3	123.7, 125.9	120.5, 139.1, 183.5
8a	—	125.9		
9	—	183.5		
9a	—	105.0		
N-Me	3.69 *s*	48.5		139.1, 150.5
1'a	2.85 *dd* (14.4, 8.0)	30.0	77.5, 108.4	161.2, 164.5
1'b	3.19 *dd* (14.4, 8.0)	—		
2'	4.39 *dd* (8.0, 2.1)	77.5		
2'-OH	6.08 *br s*	—		
3'	—	148.2		
4'	4.82 *s*	110.5		
	5.04 *s*	—		
3'-Me	1.86 *br s*	18.7	148.2	77.5, 110.5
1''	3.56 *m*	27.3	110.0, 125.4	164.5
2''	5.40 *m*	125.4		
3''	—	131.5		
3''-Me	1.78 *s*	18.2	131.5	25.9, 125.4
3''-Me	1.69 *s*	25.9	131.5	18.2, 125.4

deposited at the Australian National Herbarium, Canberra.

Extraction and isolation of alkaloids. Ground leaves (400 g) and stem bark (80 g) were separately extracted (Soxhlet) with hexane, then EtOAc and finally MeOH. The hexane extract of the leaves (4 g) was fractionated by VLC over silica gel, eluting with petrol (bp 60–80°) containing increasing amounts of EtOAc. The 10–20% EtOAc eluate was passed through a column of Sephadex LH-20, eluting with $CHCl_3$, followed by prep. TLC (silica gel, $CHCl_3$–EtOAc, 9:1) to give **5** (10 mg) and **6** (5 mg). The EtOAc extract was passed through a column of Sephadex LH-20 followed by prep. TLC (silica gel, $CHCl_3$–EtOAc, 7:3) to give **1** (6 mg), **2** (4 mg), **4** (5 mg) and **7** (4 mg).

The hexane extract of the stem bark, when treated in an identical manner, gave two further alkaloids **3** (5 mg) and **8** (3 mg), together with **5** (20 mg) and **6** (7 mg). The EtOAc extract gave **1** (12 mg), **2** (12 mg), **4** (10 mg) and **7** (8 mg) on separation following the same procedures as used for the leaf extract.

Citrusamine (**1**). Yellow, amorphous. Found: $[M]^+$ 271.0841; $C_{15}H_{13}NO_4$ requires 271.0845. The spectral data (UV, IR, NMR, MS) were in agreement with that published [7].

Junosine (**2**). Orange needles from $CHCl_3$–MeOH, mp 220° (lit. [8] 210–213°). Found: $[M]^+$ 325.1325; 3 - *enyl*) - 10 - *methylacridan* - 9 - *one* (*bosistidine*) (**3**). Yellow, amorphous. $[\alpha]_D$ −15.1 (*c* 0.0018, $CHCl_3$). Found: $[M]^+$ 341.1263; $C_{19}H_{19}NO_5$ requires 341.1263. UV λ_{max} nm (log ε): 235 (4.20), 260 (4.27), 280 (4.35), 335 (3.90), 410 (3.50). IR γ_{max} cm^{-1}: 3400, 1630, 1560. ^{1}H NMR: Table 1. HREIMS: *m/z* (rel. int.): 341 (44), 323 (15), 308 (31), 270 (100), 236 (12).

1,3,5 - *Trihydroxy* - 2 - (2ξ - *hydroxy* - 3 - *methylbut* - 3 - *enyl*) - 4 - (3 - *methylbut* - 2 - *enyl*) - 10 - *methylacridan* - 9 - *one* (*bosistine*) (**4**). Yellow, amorphous. $[\alpha]_D$ −4.1 (*c* = 0.0013, $CHCl_3$). Found: $[M]^+$ 409.1879; $C_{24}H_{27}NO_5$ requires 409.1879. UV λ_{max} nm (log ε): 222 (4.16), 267 (4.36), 350 (4.15), 410 (3.55). IR γ_{max} cm^{-1}: 3580, 1710, 1625, 1573, 1455, 1285, 1040. ^{1}H and ^{13}C NMR: Table 2. HREIMS: *m/z* (rel. int.): 409 (12), 338 (85), 295 (5), 282 (100).

N-*Methylataphylline* (**5**). Orange needles from petrol–EtOAc, mp 187–190° (lit. [9] 190–193°). Found: $[M]^+$ 393.1926; $C_{24}H_{27}NO_4$ requires 393.1940. Spectral data (UV, IR, NMR, MS) were in agreement with that published [9].

N-*Methylataphyllinine* (**6**). Orange needles from petrol–EtOAc, mp 195° (lit. [11] 195–196°). Found: $[M]^+$ 391.1178; $C_{24}H_{25}NO_4$ requires 391.1793. Spectral data (UV, IR, NMR, MS) were in agreement with that published [11].

Yukocitrine (**7**). Yellow oil. Found: $[M]^+$ 323.1155;

Yellow oil. $[\alpha]_D$ +16.4 (*c* 0.0005, $CHCl_3$). Found: FABMS $[M+1]^+$ 408 = $C_{24}H_{25}NO_5$. UV λ_{max} nm (log ε): 225 (4.15), 266 (4.30), 300 (4.65), 305 (34.15), 335 (3.55), 405 (3.55). IR γ_{max} cm^{-1}: 3320, 3010, 1650, 1608, 1555. ^1H NMR: Table 1. FABMS: *m/z* (rel. int.): 408 $[M+1]^+$, 336 (100), 165 (21).

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REFERENCES

1. Auzi, A. A., Gray, A. I., Hartley, T. G., Waigh, R. D. and Waterman, P. G. (1994) *Phytochemistry* **36**, 535.
2. Parsons, I. C., Gray, A. I. and Waterman, P. G. (1992) *J. Nat. Prod.* **56**, 46.
3. Hartley, T. G. (1977) *J. Arnold Arbor.* **58**, 426.
4. Groger, D. (1988) *Pharmazie* **43**, 815.
5. Diment, J. A., Ritchie, E. and Taylor, W. C. (1967) *Aust. J. Chem.* **20**, 1723.
6. Furukawa, H., Yogo, M. and Wu, T. S. (1983) *Chem. Pharm. Bull. (Tokyo)* **31**, 3084.
7. Ju-ichi, M., Inoue, M., Ito, C. M., Furukawa, H. and Kajiura, I. (1987) *Heterocycles* **26**, 1873.
8. Ju-ichi, M., Inoue, M., Ito, C. M. and Aoki, K. (1986) *Heterocycles* **24**, 1595.
9. Govindachari, T. R. and Viswanathan, N. (1970) *Tetrahedron* **26**, 2905.
10. Takemura, Y., Uchida, H., Ju-ichi, M., Ito, C., Nakagawa, K., Ono, T. and Furukawa, H. (1992) *Heterocycles* **34**, 2123.
11. Shah, J. S. and Sabata, B. K. (1982) *Indian J. Chem.* **21**, 16.
12. Bax, A. and Summers, M. F. (1986) *J. Am. Chem. Soc.* **108**, 2093.