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OXINDOLE ALKALOIDS FROM *ALSTONIA MACROPHYLLA*

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Key Word Index—*Alstonia macrophylla*; Apocynaceae; bark; oxindole alkaloids; N_b-demethylalstophyllal oxindole; alstonal.

Abstract—Two new oxindole alkaloids, N_b-demethylalstophyllal oxindole and alstonal, together with three known alkaloids, N_b-demethylalstophylline oxindole, alstonisine and talcarpine, have been isolated from the bark of *Alstonia macrophylla*. Structures of the alkaloids were assigned by spectral methods.

INTRODUCTION

In recent years, *Alstonia macrophylla* [1] from Sri Lanka [2–6], the Philippines [7] and Thailand [8] has received much attention. Investigations on this species from the various countries have revealed that there are diversities in the composition of the chemical constituents, macroline-related oxindole alkaloids being documented as one of those predominating. However, little previous, chemical work has been done on Malaysian *A. macrophylla*. Herein, we report the isolation and structural elucidation of two new alkaloids, N_b-demethylalstophyllal oxindole (**1**) and alstonal (**2**), together with three known alkaloids, N_b-demethylalstophylline oxindole (**3**) [6], alstonisine (**4**) [9] and talcarpine (**5**) [10]. Structural determinations were carried out with the aid of NMR and mass spectral data.

RESULTS AND DISCUSSION

A crude alkaloidal mixture was isolated from ethanolic extracts of the bark of *A. macrophylla*. Repeated chromatography of this mixture furnished alkaloids **1**–**5**. The three known compounds, **3**–**5** were readily identified by comparison of their spectral properties with literature data [6, 9, 10].

HREI mass spectrometry of N_b-demethylalstophyllal oxindole (**1**) showed a $[M]^+$ at m/z 368.1742 (calc. 368.1736) consistent with the molecular formula $C_{21}H_{24}N_2O_4$, which is identical with the molecular formula of N_b-demethylalstophylline oxindole (**3**) [6]. Furthermore, the identical fragmentation pattern (m/z 190, 179, 161, 136, 118 and 56) observed in the EI mass spectra of **1** and **3**, suggested that these two molecules are closely related in structure.

Analysis of the ^1H and ^{13}C NMR spectra of **1** revealed congruence with **3** in rings A, B, C and D; however, substantial differences in ring E were observed (Table 1). The main differences were the presence of the aldehyde function at C-20, together with the vinyl methyl (18-Me) in **1** as compared to the presence of an acyl group at C-20 and the vinyl proton (H-21) in **3**. The vinyl aldehyde group was characterized by the signal at δ189.5 (C-21) and a 1H singlet at δ9.8 (H-21), whilst the carbon resonances at δ16.7 and δ171 in **1** were attributed to C-18 and C-19, respectively. Data from the 2D-NMR of **1** further supported the proposed assignments. A HMQC experiment performed on **1** disclosed correlation of the singlet at δ9.8 (H-21) to the δ189.5 (C-21) resonance, whereas the δ16.7 (C-18) resonance corresponded to the 3H singlet at δ2.24 (18-Me). Long-range coupling (J^2) between the 18-Me resonance and the C-19 signal, in the HMBC spectrum of **1** further supported the proposed structure.

The HREI mass spectrum of **2** showed a $[M]^+$ at m/z 338.1638 (calc. 338.1630), suggesting the molecular formula $C_{20}H_{22}N_2O_3$. The ^1H and ^{13}C NMR of **2** relates well to the structure of **4** (Table 2), particularly the unsubstituted oxindole moiety, whereas the structural features in ring E of **2** are in accordance to the assignments made for ring E in structure **1**. Hence, **2** is readily deduced to be the 11-demethoxy derivative of N_b-demethylalstophyllal oxindole (**1**).

EXPERIMENTAL

Plant material. Collected during the fruiting season in October 1993 from Sabah, Malaysia. The species was identified by Leopold Madani from the Forest Research Centre, Sepilok, Sabah, and a voucher specimen (No. San. 138326) is deposited at the Forest Research Centre, Sepilok.

Extraction and isolation. Dried ground bark of *A. macrophylla* Wall. was extracted exhaustively with 95% EtOH at room temp. The EtOH extract was concd *in vacuo* and acidified with 5% HCl. Filtration to remove

Table 1. ^{1}H (270 MHz) and ^{13}C NMR (68 MHz) spectral data for compounds **1*** and **3** in $CDCl_3$

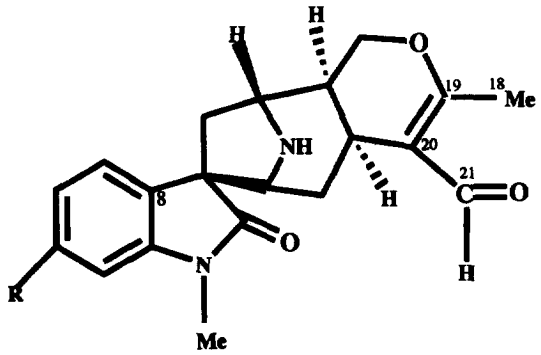
	1		**3**	
Position	δC	δH	δC	δH
2	183.1	—	183.1	—
3	63.7	3.09–3.21 *m*	63.7	3.10–3.22 *m*
5	56.3	3.66 *br d* (7)	56.3	3.66 *br d* (7)
6	42.1	2.12 *d* (14)	42.1	2.13 *d* (14)
		2.49 *dd* (14, 7)		2.50 *dd* (14, 7)
7	56.3	—	56.4	—
8	120.9	—	120.9	—
9	126.3	8.16 *d* (8)	126.2	8.17 *d* (8)
10	106.4	6.80 *dd* (8, 2)	106.3	6.80 *dd* (8, 2)
11	160.0	—	160.1	—
12	96.5	6.40 *d* (2)	96.6	6.45 *d* (2)
13	145.3	—	145.3	—
14	30.8	1.52 *ddd* (14, 12, 3.5)	31.1	1.52 *ddd* (14, 12, 3.5)
		2.20–2.34 *m*		2.17–2.30 *m*
15	24.0	3.30–3.42 *m*	24.2	3.31–3.42 *m*
16	37.0	1.90–2.07 *m*	37.0	1.90–2.07 *m*
17	68.7	4.26 *ddd* (11, 4, 1.5)	68.4	4.25 *ddd* (11, 4, 1.5)
		4.50 *t* (11)		4.45 *t* (11)
18	16.7	2.24 *s*	24.9	2.24 *s*
19	171.0	—	196.7	—
20	118.2	—	121.8	—
21	189.5	9.85 *s*	157.7	7.62 *s*
N-Me	26.2	3.16 *s*	26.2	3.16 s
O-Me	55.6	3.86 *s*	55.6	3.85 *s*

*Assignments based on COSY, HMQC and HMBC.

Table 2. ^{1}H (270 MHz) and ^{13}C NMR (68 MHz) spectral data for compounds **2*** and **4** in $CDCl_3$

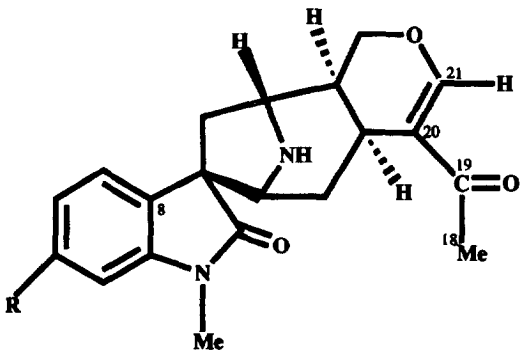
	2		**4**	
Position	δC	δH	δC	δH
2	182.5	—	182.5	—
3	63.8	3.10–3.20 *m*	63.8	3.15–3.20 *m*
5	56.4	3.66 *d* (7)	56.4	3.68 *d* (7)
6	41.9	2.17 *d* (14)	41.9	2.19 *d* (14)
		2.51 *dd* (14, 7)		2.52 *dd* (14, 7)
7	56.9	—	56.9	—
8	129.1	—	129.1	—
9	107.9	6.86 *dd* (7, 1.5)	107.9	6.86 *dd* (7, 1.5)
10	123.4	7.30 *dd* (7, 1.5)	123.4	7.30 *dd* (7, 1.5)
11	127.9	7.32 *dd* (7, 1.5)	127.9	7.32 *dd* (7, 1.5)
12	125.6	8.24 *dd* (7, 1.5)	125.6	8.25 *dd* (7, 1.5)
13	143.9	—	144.0	—
14	30.8	1.54 *ddd* (15, 11.5, 3)	31.0	1.47–1.58 m
		2.22–2.34 *m*		2.20–2.33 *m*
15	23.9	3.33–3.39 *m*	24.2	3.35–3.41 *m*
16	37.0	1.90–2.00 *m*	37.0	1.90–2.00 *m*
17	68.6	4.27 *ddd* (11, 4, 1.5)	68.4	4.26 *ddd* (11, 4, 1.5)
		4.50 *t* (11)		4.45 *t* (11)
18	16.7	2.23 *s*	24.9	2.24 *s*
19	170.9	—	196.6	—
20	118.1	—	121.8	—
21	189.5	9.85 *s*	157.6	7.62 *s*
N-Me	26.2	3.19 *s*	26.2	3.19 s

*Assignments based on COSY, HMQC and HMBC.



R

1 OMe
2 H



R

3 OMe
4 H

non-alkaloidal material, followed by basification with 37% NH_4OH to pH 10, released the alkaloids which were taken up in $CHCl_3$ to give a total crude alkaloid yield of 0.0083 g kg^{-1} bark. The $CHCl_3$ extract (crude alkaloid mixt.) was then fractionated by CC. Elution with $CHCl_3$–MeOH (99:1) afforded mixts of alkaloids **1**–**5**. Further fractionation performed on a Chromatotron with hexane–Et_2O–MeOH (7:2:1) and fractional recrystallization resulted in the isolation of **1**–**5**.

N_b-*Demethylalstophyllal oxindole* (**1**). Needles (petrol–Me_2CO), 58 mg, mp 190–191°. $[\alpha]_D$ + 174° ($CHCl_3$; *c* 0.063). HREIMS, *m/z* 368.1742 (calc. 368.1736 for $C_{21}H_{24}N_2O_4$). EIMS (probe) 70 eV, *m/z* (rel. int.): 368 [M^+] (100), 190 (27), 179 (39), 161 (14), 136 (12), 118 (8), 56 (8).

Alstonal (**2**). Needles (petrol–Me_2CO), 48.5 mg, mp 198–199°. $[\alpha]_D$ + 167° ($CHCl_3$; *c* 0.062). HREIMS *m/z* 338.1638 (calc. 338.1630 for $C_{20}H_{22}N_2O_3$). EIMS (probe) 70 eV, *m/z* (rel. int.): 338 [M^+] (100), 179 (36), 160 (26), 136 (12), 118 (6).

N_b-*Demethylalstophylline oxindole* (**3**). Prisms (petrol–Me_2CO), 60 mg, mp 205–206°. $[\alpha]_D$ + 126° ($CHCl_3$; *c* 0.068). HREIMS *m/z* 368.1745 (calc. 368.1736 for $C_{21}H_{24}N_2O_4$). EIMS (probe) 70 eV, *m/z* (rel. int.): 368 [M^+] (100), 190 (32), 179 (38), 161 (11), 136 (13), 118 (8), 56 (19).

Alstonisine (**4**). $[\alpha]_D$ + 90° ($CHCl_3$; *c* 0.022). HREIMS *m/z* 338.1625 (calc. 338.1630 for $C_{20}H_{22}N_2O_3$). EIMS (probe) 70 eV, *m/z* (rel. int.): 338 [M^+] (100), 179 (49), 160 (42), 136 (14), 118 (11).

Talcarpine (**5**). Prisms (petrol–Me_2CO) 52 mg, mp 185–186°. $[\alpha]_D$ − 49° ($CHCl_3$; *c* 0.077). HREIMS *m/z* 338.1994 (calc. 338.1993 for $C_{21}H_{26}N_2O_2$). EIMS (probe) 70 eV, *m/z* (rel. int.) 338 [M^+] (86), 310 (52), 225 (28), 197 (100), 181 (22), 170 (30). ^1H NMR (270 MHz, $CDCl_3$) δ: 9.94 (1H, *d*, *J* = 3 Hz, H-21), 7.48 (1H, *br d*, *J* = 7 Hz, H-9), 7.28 (1H, *br d*, *J* = 7 Hz, H-12), 7.19 (1H, *br t*, *J* = 7 Hz, H-11), 7.09 (1H, *br t*, *J* = 7 Hz, H-10), 4.13 (1H, *t*, *J* = 11 Hz, H-17), 3.92–4.05 (2H, *m*, H-3, H-9), 3.89 (1H, *dd*, *J* = 11, 5 Hz, H-17), 3.62 (3H, *s*, *N*-1Me), 3.24 (1H, *dd*, *J* = 16 Hz, 7 Hz, H-6), 2.89 (1H, *d*, *J* = 7 Hz, H-5), 2.41–2.55 (1H, *m*, H-14), 2.32 (3H, *s*, *N*-4 Me), 2.15–2.24 (1H, *m*, H-15), 2.00–2.09 (1H, *m*, H-16), 1.78 (1H, *br s*, H-20), 1.40–1.48 (1H, *m*, H-14), 1.30 (1H, *d*, *J* = 6.8 Hz, H-18). ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ: 204.7 (C-21), 136.9 (C-13), 132.6 (C-2), 126.3 (C-8), 120.9 (C-11), 118.9 (C-10), 118.1 (C-9), 108.7 (C-12), 106.6 (C-7), 69.4 (C-19), 68.8 (C-17), 54.5 (C-20), 54.4 (C-5), 53.5 (C-3), 41.7 (*N*-4 Me), 39.4 (C-16), 30.0 (C-14), 28.9 (*N*-1Me), 27.0 (C-15), 22.4 (C-6), 19.2 (C-18).

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