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ALKALOIDS FROM *TABERNAEMONTANA DIVARICATA*

TOH-SEOK KAM* and S. ANURADHA

Department of Chemistry, University of Malaya, 59100 Kuala Lumpur, Malaysia

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Key Word Index—*Tabernaemontana divaricata*; Apocynaceae; leaves; indole alkaloids.

Abstract—An ethanolic extract of the leaves of *Tabernaemontana divaricata* (double flower variety) furnished, in addition to voaphylline, N_1-methylvoaphylline, voaharine, pachysiphine, apparicine and conophylline, two new alkaloids, (−)-mehranine and the dimeric indole conofoline. The structures of the new alkaloids were elucidated by spectral methods.

INTRODUCTION

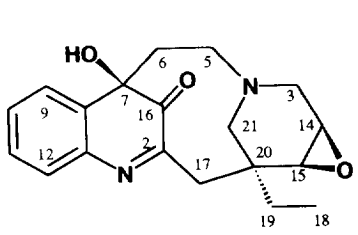
Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult. is widely cultivated as a garden plant in south-east Asia and other tropical countries. There are two distinct varieties, the single-flower and double-flower variety [1–4] and both occur widely in Malaysia. In an earlier communication [5], we reported the presence of a novel 3-quinolone alkaloid, voaharine, as well as a novel dimeric indole, conophylline, from leaf extracts of the double-flower variety. We also reported the alkaloidal composition of the leaf extract of the single-flower variety, including the presence of novel dimeric indoles [6]. We now report the full alkaloidal composition of the leaves of the double-flower variety, including the further isolation of new alkaloids.

RESULTS AND DISCUSSION

The ethanol extract of the leaves furnished a number of known alkaloids, including voaphylline, N_1-methyl-voaphylline, voaharine, pachysiphine (tabersonine-β-epoxide), apparicine, and the dimeric indole, conophylline. Voaharine (**1**) $[\alpha]_D$ − 500° ($CHCl_3$, c = 0.091) is exceptional in being in all probability a tryptamine and *seco*loganine-derived alkaloid but possessing a 3-quinolone instead of an indole chromophore [5]. The structure of the dimeric indole conophylline (**2**) was first established based on spectral data [5] and then confirmed by X-ray analysis [6]. We have now found several new minor alkaloids from a larger scale extraction of the leaves: (−)-mehranine (**3**) and a dimeric indole, conofoline (**4**). (+)-Mehranine $[\alpha]_D$ + 36° ($CHCl_3$) was reported previously from *E. coronaria* grown in West Pakistan but the stereochemistry of the chiral centres were not defined [7]. As our compound is similar to (+)-mehranine (mass spectrum and ^{1}H NMR; ^{13}C NMR not reported), except for the sign of the specific rotation ($[\alpha]_D$ − 49° ($CHCl_3$, c = 0.83)), we conclude that ours is probably the enantiomer and tentatively assign the stereochemistry as shown in **3**, assuming that it has a common biogenetic origin with the other alkaloids found in this plant. The structural assignment is further augmented by ^{13}C NMR spectral data, as well as 2-D H–H and H–C COSY experiments, which confirm the assignment of **3** as N_1-methyl-aspidospermidine-epoxide. The stereochemistry of the 14,15-epoxide function is deduced to be β in common with those of the other alkaloids occurring in the plant, as well as by the excellent agreement of the 3, 14, 15, 19, 20, and 21 carbon resonances when compared with those of tabersonine-β-epoxide [8], as well as those of several other dimeric alkaloids incorporating aspidosperma-type moieties, such as voafoline [9], ervafoline [10] and hazuntiphyllidine [11].

The second new alkaloid, conofoline (**4**) $[\alpha]_D$ − 98° ($CHCl_3$, c = 0.725), is a dimeric alkaloid as shown by its mass spectrum (FABMS $[MH]^+$ m/z 737, $C_{43}H_{52}N_4O_7$ + H). The UV spectrum indicates the presence of β-anilinoacrylate chromophores and is similar to that of conophylline [5, 6]. The ^{1}H and ^{13}C NMR spectra confirm the dimeric nature of the alkaloid, the ^{13}C NMR spectrum accounting for all 43 carbons. Examination of the ^{13}C NMR spectrum reveals that one unit of the dimer is the same 10-hydroxy-11,12-dimethoxy-tabersonine-β-epoxide unit that occurs in conophylline [5, 6]. The other unit of the dimer was readily deduced to be a 10-alkylmehranine moiety from the excellent agreement of the non-aromatic ^{13}C NMR resonances with those of (−)-mehranine discussed earlier. The attachment of the dimer at C-10 of the mehranine unit is confirmed by both the ^{1}H and ^{13}C NMR spectral fea-

*Author to whom correspondence should be addressed.



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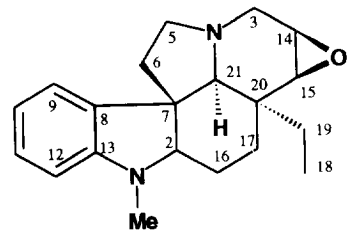

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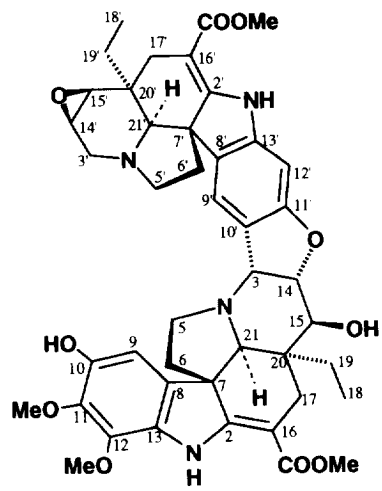

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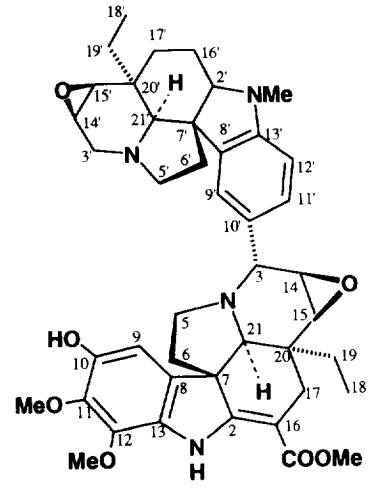

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Table 1. ^{1}H NMR data for compounds **2–4***

H	2	4	H	3	4
2	—	—	2′	3.34 *d* (4)	3.47 *d* (4)
3	4.81, *d* (8)	4.52 *s*	3′	2.40 *d* (13)	2.3–2.5 *m*
				3.54 *d* (13)	3.59 *d* (13)
5	2.7–3.1 *m*	2.5–2.6 *m*	5′	2.1–2.3 *m*	2.2–2.3 *m*
	2.7–3.1 *m*	2.8-3.0 *m*		3.1–3.3 *m*	3.1–3.3 *m*
6	1.68, *dd* (11, 4)	1.5–1.6 *m*	6′	1.6–1.8 *m*	1.6–1.8 *m*
	1.9–2.2 *m*	1.9–2.1 *m*		2.1–2.3 *m*	2.3–2.5 *m*
9	5.55, *s*	5.67 *s*	9′	7.01 *d* (7)	7.00 *s*
10	—	—	10′	6.63 *t* (7)	—
11	—	—	11′	7.07 *t* (7)	7.04 *d* (8)
12	—	—	12′	6.38 *d* (7)	6.42 *d* (8)
14	3.25, *d* (4)†	3.42 *d* (4)	14′	3.33 *d* (4)	3.34 *d* (4)
15	3.05, *d* (4)†	3.25 *d* (4)	15′	2.95 *d* (4)	2.94 *d* (4)
16	—	—	16′	1.2–1.3 *m*	1.3–1.5 *m*
	—	—		1.6–1.8 *m*	1.7–1.9 *m*
17	2.40, *d* (16)	2.57 *d* (15)	17′	1.4–1.5 *m*	1.5–1.6 *m*
	2.74, *d* (16)	2.73 *d* (15)		1.6–1.8 *m*	1.7–1.9 *m*
18	0.71, *t* (7)	0.74 *t* (7)	18′	0.81 *t* (7)	0.78 *t* (7)
19	0.7–0.9 *m*	0.9–1.0 *m*	19′	1.2–1.3 *m*	1.2–1.4 *m*
	1.1–1.3 *m*	0.9–1.0 *m*		1.2–1.3 *m*	1.2–1.4 *m*
21	2.60 *s*	2.75 *s*	21′	2.25 *s*	2.32 *s*
OMe	3.77 *s*	3.78 *s*	—	—	—
10–OH	5.19 *s*	5.24 *s*	—	—	—
11–OMe	3.82 *s*	3.84 *s*	—	—	—
12–OMe	3.86 *s*	3.87 *s*	—	—	—
NH	8.79 *s*	8.75 *s*	N′-Me	2.74 *s*	2.81 *s*

*$CDCl_3$, 270 MHz.

†δ Values are for tabersonine-β-epoxide (pachysiphine).

tures of the aromatic portion of the mehranine unit, which show that the aromatic moiety is monosubstituted at C-10. The dimer is deduced to be connected from C-3 of the oxygenated tabersonine-β-epoxide moiety from examination of the ^{1}H and ^{13}C NMR spectral data (single H on C-3) and is further confirmed by HMBC experiments (long-range ^3J H-C correlation from H-3 to C-9'). With the present availability of HMBC data for conofoline, our earlier assignments for the quaternary aromatic carbon resonances of the oxygenated tabersonine-β-epoxide unit in conophylline [5, 6], which were based on comparison with pandicine [12], now require revision (Table 2). The stereochemistry at C-3 of the tabersonine-epoxide unit is readily ascertained from the NMR signal attributed to H-3 (δ4.52), which is a singlet requiring the H-3/H-14 dihedral angle to be *ca* 90°, an arrangement possible only if H-3 is β. This conclusion finds additional support from the similarity of the C-3 shift (δ58.3) to those in the dimers, conophylline (δ59.5) and criophylline (δ58.1) [13], which also have C-3α substitution. Furthermore, this stereochemistry is also the sterically less hindered arrangement compared with the case where H-3 is α. Based on the above reasoning, the structure of conofoline is as shown in **4**. Conofoline might also have been isolated independently from another *Tabernaemontana* species by another group at Reims, France (M. Zeches, personal communication). We have succeeded in growing a single crystal and hope to further confirm the structure by X-ray analysis in the near future.

Table 2. ^{13}C NMR data for compounds **2–4***

C	2	4	C	3	4
2	164.3	165.3	2′	73.3	73.5
3	59.5	58.3	3′	53.1	52.9
5	46.0	47.7	5′	53.7	53.7
6	41.8	42.4	6′	41.1	41.1
7	54.4	54.4	7′	51.3	51.4
8	133.5	133.7	8′	136.7	136.7
9	103.9	103.9	9′	121.3	123.0
10	143.5	143.7	10′	117.1	122.1
11	138.7	138.6	11′	127.6	128.2
12	136.8	136.7	12′	106.5	106.0
13	128.8	128.4	13′	150.1	149.9
14	52.1†	54.5	14′	53.0	52.9
15	56.3†	56.6	15′	57.3	57.3
16	90.7	90.7	16′	19.9	19.9
17	22.2	23.5	17′	23.4	23.8
18	7.4	7.5	18′	7.5	7.4
19	26.4	26.8	19′	27.7	28.1
20	37.1†	36.8	20′	34.6	34.7
21	65.3	61.7	21′	67.7	67.4
CO_2Me	168.8	168.8	NMe	31.5	31.6
CO_2Me	51.1	50.9	—	—	—
11-OMe	61.0	60.8	—	—	—
12-OMe	60.5	60.6	—	—	—

*$CDCl_3$, 67.8 MHz, assignments based on HETCOR and HMBC.

†δ Values are for tabersonine-β-epoxide (pachysiphine).

EXPERIMENTAL

Plant material. Plant material was collected from Petaling Jaya, Malaysia, and voucher specimens are deposited at the Herbarium, Department of Botany, University of Malaya and the Herbarium, Forest Research Institute of Malaysia.

Extraction and isolation. Extraction of plant material was carried out in the usual manner [6, 14]. Alkaloids were isolated by CC, prep. TLC and centrifugal TLC on silica gel. Solvent systems used were $CHCl_3$ with increasing proportions of MeOH, Et_2O with increasing proportions of EtOAc and hexane with increasing proportions of EtOAc. The yields (g kg^{-1}) of the alkaloids isolated were as follows: voaphylline (0.058), N_1-methylvoaphylline (0.148), voaharine (**1**) (0.009), pachysiphine (0.003), apparicine (0.042) conophylline (**2**) (0.032), (−)-mehranine (**3**) (0.014) and conofoline (**4**) (0.045).

Conophylline (**2**). $[\alpha]_D$ −143° ($CHCl_3$, *c* 0.091).

(−)-*Mehranine* (**3**). $[\alpha]_D$ −49° ($CHCl_3$, *c* 0.831). UV, λ_{max}^{EtOH} nm (log ε): 308 (2.97), 258 (3.45), 214 (3.53). EIMS (probe) 70 eV, *m/z* (rel. int.): 310 $[M]^+$ (100), 281 (10), 199 (29), 170 (14), 166 (23), 158 (52), 155 (8), 144 (58), 138 (30), 123 (11) and 108 (21). ^{1}H and ^{13}C NMR: see Tables 1 and 2.

Conofoline (**4**). Mp 196–198°. $[\alpha]_D$ −97° ($CHCl_3$, *c* 0.725). UV λ_{max}^{EtOH} nm (log ε): 205 (3.16), 230 (3.68), 313 (3.76), 339 (3.84). FABMS (glycerol), *m/z*: found 737.33 $[MH]^+$, calc. for $C_{43}H_{52}N_4O_7$ + H 737.91 ^{1}H and ^{13}C NMR: see Tables 1 and 2.

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