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Venalstonine and dioxokopsan derivatives from *Kopsia fruticosa*

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Abstract

Two new venalstonine derivatives, viz., venacarpines A and B, and one new dioxokopsan derivative, kopsorinine, in addition to the kopsifolines A–F, and 11 other known alkaloids, were isolated from a Malayan *Kopsia* species. The structures of the new alkaloids were determined using NMR and MS analysis.

Keywords: *Alstonia* species; Apocynaceae; Indole alkaloids

1. Introduction

About 17 species of the genus *Kopsia* are found in Malaysia (Markgraf, 1972; Sevenet et al., 1994), of which a considerable number have been investigated (Awang et al., 1992; Uzir et al., 1997; Kam, 1999; Kam et al., 1994, 1995, 1996a,b, 1997, 1998, 1999a,b,c, 2001). We have previously reported the presence of the kopsifolines (A–F, **1**–**6**), which are distinguished by a novel carbon skeleton, from a sample identified as *Kopsia fruticosa* (Middleton, Leiden) (Kam and Choo, 2003, 2004). We now wish to report further isolation of three new alkaloids, including new venalstonine and dioxokopsan derivatives from the same plant.

2. Results and discussion

The leaf extract provided a series of novel indoles, viz., kopsifolines A–F (**1**–**6**) (Kam and Choo, 2003, 2004) and three new indole alkaloids, venacarpine A (**7**), venacarpine B (**8**), and kopsorinine (**9**). The stem-bark extract gave in addition to (**9**), 11 other known alkaloids as detailed in Section 3.

Compound (**7**) was obtained as a colourless oil, with $[\alpha]_D$ +18° (*c* 0.43, $CHCl_3$). The UV spectrum showed absorption maxima at 219, 244, and 280 nm typical of an dihydroindole chromophore. The IR spectrum showed bands at 3359 and 1710 cm^{-1} due to *N*H and conjugated ester carbonyl functions, respectively. The ESI-MS spectrum showed a $[MH]^+$ at *m*/*z* 379, and HRFABMS of **7** gave the formula $C_{22}H_{22}N_2O_4$. The ^1H NMR spectrum of **7** (Table 1) showed the presence of two aromatic hydrogens, a *N*H, a methylenedioxy, a conjugated methyl ester function, and three olefinic hydrogens. Two of the olefinic hydrogens are readily assigned to H(14) and H(15) {δ 5.93 and 5.80, respectively} while the remaining olefinic H is due to the vinylic H(17) {δ 6.86}.These assignments are in agreement with the COSY spectrum which indicated the presence of NCH_2CH_2, $NCH_2CH{=}CH$, and CH_2CH_2 fragments, in addition to the isolated vinylic H(17). These partial structures, except for the presence of an additional unsaturation, are characteristic of an aspidofractinine compound, e.g., venalstonine (**11**). The ^{13}C NMR spectrum (Table 1) showed a total of 22 carbon signals, of which 12 are found in the low-field region. The resonance at δ 164.7 is assigned to the ester carbonyl linked to C(16), while the olefinic carbon signals at δ 133.3 and 145.7 are attributed to C(16) and C(17), respectively. These assignments are in agreement with the HMBC spectrum {three-bond correlations from H(21) and H(17) to C(19), and from H(18) to C(16)} and revealed structure **7** for venacarpine A.

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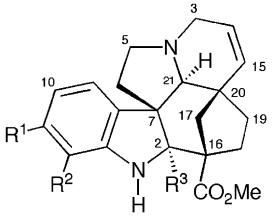
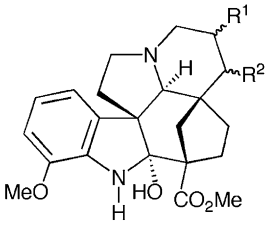
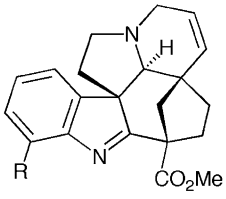
Table 1
NMR spectral data for **7–9**[a]

Position	**7**		**8**		**9**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	–	71.0	–	70.3	–	69.2
3	3.26 ddd (19, 3, 2) 3.73 dt (19, 2)	47.3	3.29 ddd (19, 3, 2) 3.77 m	47.2	3.78 ddd (19, 3, 2) 4.55 dt (19, 2)	41.8
5	2.46 td (9, 7) 2.99 td (9, 1)	53.1	2.48 td (9, 7) 3.00 ddd (9, 8, 1)	53.1	– –	167.4
6	1.63 dt (13, 9) 2.13 ddd (13, 7, 1)	37.8	1.65 ddd (13, 9, 8) 2.19 ddd (13, 7, 1)	37.1	2.96 t (1) –	62.1
7	–	61.5	–	62.3	–	56.1
8	–	132.1	–	134.4	–	130.6
9	6.71 d (8)	115.1	6.90 br d (8)	115.1	7.19 dd (8, 1)	122.6
10	6.32 d (8)	100.0	6.78 t (8)	120.3	6.83 td (8, 1)	120.1
11	–	147.7	6.69 dd (8, 1)	109.4	7.13 td (8, 1)	128.8
12	–	129.1	–	145.9	6.72 br d (8)	111.7
13	–	132.9	–	136.7	–	150.7
14	5.93 dt (10, 2)	131.9	5.94 dt (10, 2)	129.1	5.68 m	124.3
15	5.80 ddd (10, 3, 2)	126.7	5.80 ddd (10, 3, 2)	126.5	5.68 m	131.9
16	–	133.3	–	138.8	2.88 dt (11, 1)	53.6
17	6.86 s –	145.7	6.84 s –	145.5	1.36 ddd (16, 3, 1) 2.04 ddd (16, 11, 2)	33.4
18	1.29 m 1.93 m	28.4	1.25 td (12, 2) 1.91 m	28.1	1.89 m 1.89 m	23.4
19	1.29 m 1.51 m	32.1	1.32 td (12, 5) 1.50 m	32.2	1.57 m 1.77 m	30.6
20	–	37.8	–	37.5	–	34.1
21	3.29 br s	68.2	3.36 br s	67.9	3.91 d (2)	63.2
22	–	–	–	–	–	204.7
CO_2Me	3.79 s	51.6	3.80 s	51.6	–	–
CO_2Me	–	164.7	–	164.7	–	–
OCH_2O	5.86 d (1) 5.92 d (1)	100.6	–	–	–	–
12-OMe	–	–	3.84 s	55.2	–	–
NH	4.41 br s	–	4.41 br s	–	–	–

[a] Assignments based on COSY, HMQC and HMBC.



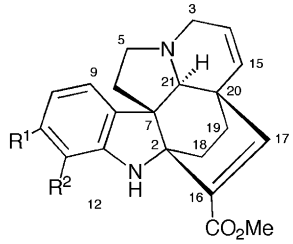


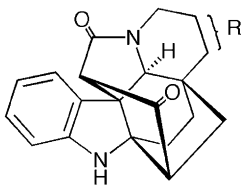


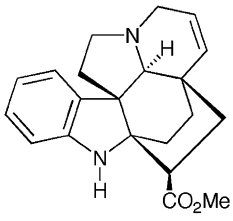
1 R^1 = H, R^2 = OMe, R^3 = OH
6 R^1 = OMe, R^2 = H, R^3 = H

2 R^1 = H, R^2 = αOH
3 R^1 = βOH, R^2 = αOH

4 R = H
5 R = OMe







7 R^1, R^2, = OCH_2O
8 R^1 = H, R^2 = OMe

9 R = $\Delta^{14,15}$
10 R = Nil

11

Venacarpine B (**8**) was obtained as a colourless oil, with $[\alpha]_D$ +12° (*c* 0.20, $CHCl_3$). The UV and IR spectra are similar to that of **7**, indicating an aspidofractinine compound with similar functionalities. The EI mass spectrum showed a molecular ion at *m/z* 364 which analyzed for $C_{22}H_{24}N_2O_3$. The ^1H NMR spectrum of **8** (Table 1) is generally similar to that of **7**, except for changes caused by the replacement of the 11,12-methylenedioxy substituent by a 12-methoxy group. These are also reflected in the ^{13}C NMR spectrum. The position of aromatic substitution was determined by the ^1H NMR coupling pattern, the ^{13}C NMR carbon shifts, and NOE {NOEs between H(9)/H(21), 12-OMe/H(11), *N*H}.

Kopsorinine (**9**) was obtained as a light yellowish oil, with $[\alpha]_D$ +14° (*c* 0.35, $CHCl_3$). The UV spectrum (220, 245, 280 nm) was characteristic of a dihydroindole while the IR spectrum showed bands due to *N*H (3358 cm^{-1}) and various carbonyl functions (1720, 1710 cm^{-1}). The latter are assigned to lactam and ketone carbonyls from the corresponding carbon shifts observed at δ 167.4 and 204.7, respectively. The COSY spectrum showed the following fragments, $NCH_2CH{=}CH$, CH_2CH_2, $CHCH_2$, reminiscent of an aspidofractinine ring system. The absence of the usual C(5)–C(6) fragment, coupled with the presence of a ketone carbonyl in place of a methyl ester group, and an isolated methine α to a carbonyl function {δ 2.96, H(6)}, suggested a kopsine derivative. The lactam carbonyl is placed at position 5, which is also in accord with the HMBC data {three-bond correlations from H(3) and H(21) to C(5)}, as well as from its effect on the C(3) hydrogens which have been shifted downfield. Kopsorinine (**9**) is therefore the 14,15-dehydro derivative of the known 5,22-dioxokopsan (**10**) (Achenbach and Biemann, 1965; Kam et al., 1999a,b,c).

3. Experimental

3.1. General

Optical rotations were determined on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a Perkin–Elmer 1600 Series FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-LA 400 spectrometer at 400 and 100 MHz respectively. ESI-MS were obtained on a Perkin–Elmer API 100 instrument. EIMS, HREIMS, and HRFABMS were obtained courtesy of Professor G. Pattenden, School of Chemistry, University of Nottingham, UK.

3.2. Plant material

Plant material was collected in Johor, Malaysia (November, 1996) and was identified by Dr. D. Middleton, Rijksherbarium, University of Leiden, Leiden, The Netherlands. Herbarium voucher specimens (K 651) are deposited at the Herbarium, University of Malaya, Kuala Lumpur, Malaysia and at Leiden.

3.3. Extraction and isolation

Extraction of the ground material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid as has been described in detail elsewhere (Kam and Tan, 1990). The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH (12 fractions), followed by rechromatography of appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal TLC were: Et_2O/hexane (1:1), Et_2O (NH_3-saturated), 2% MeOH/Et_2O, 3% MeOH/Et_2O (NH_3-saturated), MeOH/$CHCl_3$ (2%, and 2%, NH_3-saturated), $CHCl_3$ (NH_3-saturated), $CHCl_3$/hexane (3:1, NH_3-saturated), and EtOAc (NH_3-saturated). The yields (g kg^{-1}) of the alkaloids isolated from the leaves are as follows: **1** (0.367), **2** (0.011), **3** (0.0014), **4** (0.0016) **5** (0.0025), **6** (0.001), **7** (0.016), **8** (0.003), **9** (0.001), and 15α-hydroxykopsinine (0.015) (Langlois et al., 1979; Zeches et al., 1984). The yields of the alkaloids isolated from the stem-bark are as follows: **9** (0.021), 15α-hydroxykopsinine (0.035), kopsinine (0.131) (Goh et al., 1989), 16-*epi*kopsinine (0.003), venalstonine (0.023) (Ahond et al., 1974), kopsanone (0.002) (Zheng et al., 1989), Ψ-akuammigine (0.03) (Henry, 1932; Joule and Smith, 1962), 16-*epi*deacetylakuammiline (0.021) (Kan et al., 1993), lonicerine (0.112) (Naranjo et al., 1970), pleiocarpamine (0.002) (Akinyole and Court, 1980), 16-hydroxymethylpleiocarpamine (0.009) (Kan et al., 1993), and picramicine (0.001) (Hinshaw et al., 1971).

Venacarpine A (**7**), colourless oil, $[\alpha]_D$ +18° ($CHCl_3$, *c* 0.43). UV (EtOH) λ_{max} (log ε) 219 (4.46), 244 (4.00), 280 (3.53) nm; IR (dry film) υ_{max} 3359, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; Selected HMBC H(9), H(18), H(5), NH/C(7); OCH_2O/C(11), C(12). ESI-MS *m/z* 379 $[MH]^+$; HRFABMS *m/z* 379.1666 (calc. for $C_{22}H_{22}N_2O_4$ + H, 379.1658).

Venacarpine B (**8**), colourless oil, $[\alpha]_D$ +12° ($CHCl_3$, *c* 0.20). UV (EtOH) λ_{max} (log ε) 216 (4.27), 243 (3.93), 284 (3.50) nm; IR (dry film) υ_{max} 3399, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 364 $[M]^+$ (84), 336 (100), 281 (67), 249 (13), 199 (11); HREIMS *m/z* 364.1786 (calc. for $C_{22}H_{24}N_2O_3$, 364.1787).

Kopsorinine (**9**), light yellowish oil, $[\alpha]_D$ +14° ($CHCl_3$, *c* 0.35). UV (EtOH) λ_{max} (log ε) 220 (4.49), 245 (3.95), 280 (3.39) nm; IR (dry film) υ_{max} 3358, 1720, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS *m/z* 319 $[MH]^+$; HRFABMS *m/z* 319.1445 (calc. for $C_{20}H_{18}N_2O_2$ + H, 319.1447).

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