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TERPENOID AND ALKALOID COMPOUNDS FROM THE SEEDS OF *MONODORA BREVIPES**

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Key Word Index—*Monodora brevipes*; Annonaceae; monoterpenes; sesquiterpenes; diterpenes; clerodane derivatives; alkaloids; proaporphines; 3-formyl indole.

Abstract—The seeds of *Monodora brevipes* have yielded five known compounds, the proaporphine alkaloids crotsparine and stepharine, 3-formylindole, the sesquiterpene (−)-α-cadinol and the monoterpene 1α,6β-dihydroxy-4α-*p*-menth-2-ene, and three novel clerodane diterpenes. The latter have been characterized on the basis of extensive spectroscopic analysis as 2-oxo-18-acetoxy-10α,17α,19α,20β-(−)-cleroda-3,13(16),14-triene, and the related acetals 2,18,19-triacetoxy-(−)-cleroda-13(16),14-diene-18,19-oxide and 2-isobutoxy-18,19-diacetoxy-(−)-cleroda-13(16),14-diene-18,19-oxide (stereochemistry not resolved).

INTRODUCTION

Monodora brevipes Benth. (Annonaceae) is a shrub or small tree found throughout the forest zone of west and central Africa [2]. The only previous phytochemical report is for a species allied to *M. brevipes* which yielded the aporphine isoboldine [3]. Other species of *Monodora* have been found to contain alkaloids, notably 1-benzyltetrahydroisoquinoline derivatives [4, 5] but also simple prenylated indoles [4, 6, 7]. In this paper we report the isolation of a range of terpenoid compounds, as well as typical alkaloids, from the seeds of *M. brevipes*.

RESULTS AND DISCUSSION

The ground seeds of *M. brevipes* were extracted with petrol, then chloroform, and finally methanol. TLC analysis showed petrol and chloroform extracts to be identical. These were collectively subjected to column chromatography over silica gel eluting with petrol containing increasing amounts of ethyl acetate. This yielded seven compounds (A–G) of which all except B (sitosterol) were further purified by circular preparative TLC. Two further compounds (H, I) were obtained from the methanol extract. Of the isolates A was characterized as (−)-α-cadinol [8], F as 1α,6β-dihydroxy-4α-*p*-menth-2-ene [9, 10], G as 3-formylindole [11] and H and I as the proaporphine alkaloids stepharine and crotsparine [12]. Identification was based on full comparison of spectral and physicochemical data with those published.

Compound D analysed by EIMS for $C_{22}H_{32}O_3$ including an acetoxy moiety (δ2.12, δ_c 170.1 *s*, 20.6 *q*). The IR spectrum showed the presence of two carbonyl bands (1740, 1660 cm^{-1}) the second of which was an α,β-unsaturated system (δ6.01 *br s* for α-H, δ_c 198.5 *s*, 125.2 *d*, 163.6 *s*) substituted at the β-position. Five further olefinic protons occurred as isolated exomethylene and styryl systems which, together with a major fragement at *m/z* 81 $[C_6H_9]^+$, suggested partial structure **1**. Other features of the ^1H NMR spectrum were (i) an ABX system centred at δ2.70, 2.60 (AB) and 1.94 (X) suggesting a –CO–CH_2–CH– system, (ii) three methyl resonances as singlets at δ1.33 and 0.87 and a doublet at 0.97 and (iii)an isolated AB quartet centred at δ4.76 indicating CH_2–O–Ac. These data suggested a clerodane (kolavane) nucleus and this was supported by a second major ion at *m/z* 263 (**2**) which would be formed together with **1** by the typical C-9/C-11 fission of this type of diterpene [13]. On this basis structure **3** was indicated but with stereochemistry at C-5, C-8, C-9 and C-10 unresolved.

The relative stereochemistry was examined by means of NOE's involving irradiation of the three methyl resonances. Irradiation of Me-19 caused significant enhancement of H-10 (5%), H-1 (δ2.70, 5.5%) and one H-18 proton (3%) so indicating a *cis* A/B ring junction. Irradiation of Me-17 again caused enhancement of H-10 (4%) so requiring placement of Me-17 on the same face as Me-19 and H-10. Finally, irradiation of Me-20 showed an appreciable enhancement only for H-3 (3%) placing it on the opposite face to Me-17 and Me-19 and requiring relative stereochemistry depicted in **4**. This is supported by the coupling constants between H-10 and H-1 protons (J = 6.5, 2.0 Hz) which are typical of a *cis* ring junction where H-10 is equatorial and Me-19 axial [14]. As it appears to be a general rule [15] that 5α-methyl-*cis*-clerodane derivatives are laevorotatory while the 5β-methyl-*cis*-clerodanes are dextrorotatory, assignment of absolute stereochemistry as in **3** appears probable. However, the occurrence of the unusual *trans* relationship between Me-17 and Me-20 introduces an element of uncertainty into this rule and the 5α-methyl configuration

*Part 28 in the series 'Chemistry of the Annonaceae'. For Part 27 see ref. [1].

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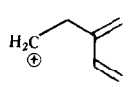

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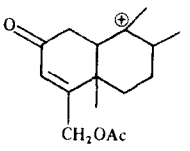

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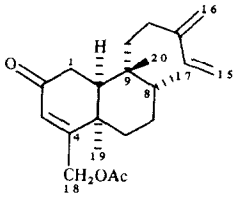

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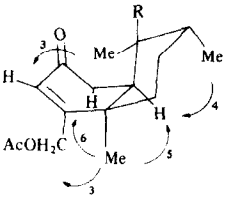

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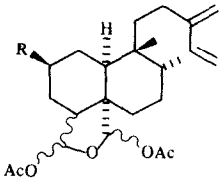

5 R = OAc
6 R = $OCCH(Me)_2$
‖
O

adopted on the basis of the OR of −33° should be regarded as tentative. ^1H and ^{13}C NMR spectra of **3** are given in Tables 1 and 2, respectively.

The EIMS of compound E showed a highest ion for $C_{22}H_{32}O_4$ but NMR studies (Tables 1 and 2) revealed three acetate substituents and the ^{13}C spectrum required 26 × C and suggested $C_{26}H_{38}O_7$. Analysis of EIMS and NMR spectra indicated the diterpenoid nature of E and a side-chain identical to **3**. Only two methyl resonances were present, as a singlet at δ0.93 and a doublet at δ0.85. These could be assigned to Me-20 and Me-17 of the clerodane system. A major feature of the ^1H NMR spectrum was three oxymethine protons at δ5.24 (*m*), 6.14 (*s*) and 6.46 (*d*). A series of decoupling experiments commencing from the δ5.24 resonance suggested the sequence $-CH_2-CH(OR)-CH_2-CH-CH(OR)$. In the ^{13}C NMR spectrum the oxymethine carbons occurred at δ70.4, 98.2 and 100.0, the highly deshielded nature of the latter two indicative of carbons substituted with two oxygen atoms. Given these requirements E was formulated as **5**, in which an acetal has been formed between the 18- and 19-Me substituents. Similar acetals have recently been reported from *Gutierrezia texana* (Asteraceae) [16] and from *Caesaria* species (Flacourtiaceae) [17, 18]. The relatively broad resonance (*ca* 25 Hz) for H-2 indicates this proton is axial. Assuming a common source for **3** and **5** suggests that the partial stereochemistry for **5** will be as shown but this is not firmly established.

Compound C differed from **5** only in the loss of one acetoxy group and its replacement by resonances attributable to an isobutoxy moiety. In comparison with **5** both the ^{13}C NMR spectrum showed small but significant changes at C-2 but not at C-18 or C-19 so allowing assignment of structure **6**.

Previous studies on *Monodora*, usually on the bark, have generally yielded only alkaloids. By contrast the most interesting of the compounds found in this investigation are the clerodanes, a type of diterpene relatively rarely encountered in the Annonaceae. The capability of *M. brevipes* to produce unusual terpenoids as well as alkaloids again illustrates the remarkable range of compounds that the Annonaceae is capable of synthesizing.

EXPERIMENTAL

Plant material. A sample of the seeds of *Monodora brevipes* was collected in the Korup National Park, Cameroun, in 1984. A

Table 1. ^1H NMR data for compounds **3**, **5** and **6** (*J* values in parentheses)

H	3	5	6
1α	2.70 *dd* (18.4, 6.5)	—	—
1β	2.60 *dd* (18.4, 2.0)	—	—
2	—	5.24 *m*	5.17 *m*
3	6.01 *br s*	2.07–2.20 *m*	2.07–2.20 *m*
10	1.94 *dd* (6.5, 2.0)	—	—
14	6.34 *dd* (17.1, 10.8)	6.41 *dd* (17.7, 10.9)	6.37 *dd* (17.7, 10.9)
15 *cis*	5.02 *d* (10.8)	5.06 *d* (10.9)	5.01 *d* (10.9)
15 *trans*	5.21 *d* (17.2)	5.24 *d* (17.7)	5.18 *d* (17.7)
16	4.95 *br s*	4.91 *s*	4.86 *s*
	4.96 *br s*	5.03 *s*	4.97 *s*
17	0.97 *d* (7.1)	0.85 *d* (8.4)	0.85 *d* (8.4)
18	4.80/4.72 AB*q* (16.8)	6.46 *d* (6.9)	6.45 *d* (6.9)
19	1.33 *s*	6.14 *br s*	6.12 *br s*
20	0.87 *s*	0.93 *s*	0.92 *s*
MeCO	2.12 *s*	2.04 *s*	1.96 *s*
		2.01 *s*	1.85 *s*
		1.91 *s*	
Me_2CHCO			2.45 *qq* (6.9)
			1.10 *d* (6.9)
			1.11 *d* (6.9)

Table 2. ^{13}C NMR data for compounds **3**, **5** and **6** (multiplicities not repeated if identical with preceding column)

C	3	5	6
1	37.1 *t*	26.9[a]	26.8[a]
2	198.5 *s*	70.4 *d*	69.9
3	125.2 *d*	29.6 *t*	26.7[a]
4	163.6 *s*	46.8 *d*	46.7
5	38.9 *s*	49.9	49.8
6	24.3 *t*	23.9	23.8
7	26.6 *t*	27.2[a]	27.2[a]
8	35.0 *d*	37.8[b]	37.7[b]
9	38.4 *s*	37.2	37.1
10	47.3 *d*	35.3[b]	35.3[b]
11	35.1 *t*	33.3	33.3
12	29.2 *t*	28.1	28.0
13	146.9 *s*	145.4	145.3
14	138.9 *d*	140.1	140.1
15	113.0 *t*	112.3	112.3
16	115.2 *t*	115.1	115.1
17	14.3 *q*	15.5	15.4
18	62.2 *t*	100.0 *d*	100.1
19	31.7 *q*	98.2 *d*	98.4
20	22.6 *q*	26.5	26.5
MeCO	170.1 *s*	169.6	169.6
		169.6	169.6
		167.8	
	20.6 *q*	21.2	21.3
		21.2	20.8
		20.9	
Me_2CHCO			18.9 *q*
			18.8 *q*
			34.0 *d*
			176.7 *s*

[a,b]Assignments in the same column interchangeable.

voucher specimen (D. W. Thomas 4814) has been deposited at the Herbarium of the Missouri Botanic Gardens, St. Louis.

Extraction and isolation of compounds. The powdered seeds (600 g) were extracted separately and successively with petrol (bp 60–80°), $CHCl_3$ and MeOH. Each extract was concd and examined by TLC as a result of which petrol and $CHCl_3$ extracts were bulked and subjected to CC over silica gel eluting with petrol and then petrol containing increasing amounts of EtOAc. Elution with 6% EtOAc gave A followed by B (sitosterol, 300 mg). Compound A was purified by circular PTLC on silica gel (toluene–EtOAc–HOAc, 96:4:1) to give α-cadinol (80 mg), Found: $[M]^+$ 222.1957; $C_{15}H_{26}O$ requires 222.1894; $[\alpha]_D$ −34° ($CHCl_3$; *c* 0.03) (ref. [8], −37°). 10% EtOAc gave C which was further purified by PTLC (solvent as before, 95:5:1) to give **6** (20 mg). 12% EtOAc gave D followed by E (both impure) which on PTLC (solvent as before, 95:5:1) gave **3** (30 mg) and **5** (10 mg). 15% EtOAc gave F which was purified by PTLC (solvent as before, 95:5:1) to give 1α,6β-dihydroxy-4α-*p*-menth-2-ene (90 mg), mp 53–55° (ref. [9], 54°), $[\alpha]_D$ −58° ($CHCl_3$; *c* 0.40); (ref. [10], −53°). Finally elution with 20% EtOAc gave G (3-formylindole, 15 mg) mp 196–198° (ref. [11], 198–199°), Found: M^+ 145.0497; C_9H_7NO requires 145.0528.

The MeOH extract was concd and shaken with 1 M HCl. The aq. phase was made alkaline (pH 9, NH_4OH) and partitioned into $CHCl_3$. The $CHCl_3$-soluble material was then subjected to CC over silica gel, eluting with $CHCl_3$ containing increasing amounts of MeOH. Elution with 5% MeOH gave a gum (H) which on circular PTLC (silica gel, solvent, $CHCl_3$–MeOH, 23:2) gave stepharine (25 mg), Found: $[M]^+$ 297.1362; $C_{18}H_{19}NO_3$ requires 297.1365, $[\alpha]_D$ +142° ($CHCl_3$; *c* 0.002) (ref. [12], +143°). Identical treatment of the eluate obtained with 5% MeOH yielded I (crotsparine, 50 mg), Found: M^+ 283.1194; $C_{17}H_{17}NO_3$ requires 283.1208; $[\alpha]_D$ +178° (MeOH; *c* 0.03) (ref. [12], +180°).

2-Oxo-18-acetoxy-10α,17α,19α,20β-(−)-cleroda-3,13(16),14-triene (**3**). Oil, $[\alpha]_D$ −33° ($CHCl_3$; *c* 0.02). Found: $[M]^+$ 344.2369; $C_{22}H_{32}O_3$ requires 344.2351. UV λ_{max} nm: 225. IR ν_{max} cm^{-1}: 3080, 1740, 1660, 1200. ^1H NMR (360 MHz, $CDCl_3$), see Table 1. ^{13}C NMR (90.56 MHz, $CDCl_3$), see Table 2. EIMS *m/z* (rel. int.): 344 $[M]^+$ (15), 263 (11), 221 (18), 203 (19), 120 (30), 107 (22), 105 (18), 95 (22), 93 (21), 81 (30), 67 (69), 43 (100).

2β,18ξ,19ξ-Triacetoxy-4ξ,17α,19α,20β-(−)-cleroda-13(16),14-diene-18,19-oxide (**5**). Oil, $[\alpha]_D$ −67° ($CHCl_3$; *c* 0.01). UV λ_{max} nm: 225. IR ν_{max} cm^{-1}: 3080, 1770, 1740, 1640, 1230, 1140. ^1H NMR (250 MHz, $CDCl_3$) see Table 1). ^{13}C NMR (90.56 MHz, $CDCl_3$), see Table 2. EIMS *m/z* (rel. int.): 360 (3), 356 (3), 263 (7), 203 (8), 191 (12), 189 (12), 160 (27), 145 (15), 135 (16), 133 (18), 131 (11), 123 (20), 121 (25), 119 (27), 109 (23), 107 (26), 105 (26), 93 (26), 81 (42), 67 (23), 42 (100).

2β-Isobutoxy-18ξ,19ξ-diacetoxy-4ξ,17α,19α,20β-(−)-cleroda-13(16),14-diene-18,19-oxide (**6**). Oil, $[\alpha]_D$ −12° ($CHCl_3$; *c* 0.04). UV λ_{max} nm: 225. IR ν_{max} cm^{-1}: 3080, 1760, 1740, 1660, 1240, 1140. ^1H NMR (360 MHz, $CDCl_3$), see Table 1. ^{13}C NMR (90.56 MHz, $CDCl_3$), see Table 2. EIMS *m/z* (rel. int.): 389 (2), 342 (6), 300 (13), 291 (14), 283 (18), 203 (29), 191 (22), 180 (22), 173 (22), 161 (14), 159 (31), 145 (15), 133 (10), 131 (12), 121 (10), 119 (21), 107 (23), 105 (28), 93 (24), 81 (32), 67 (20), 57 (22), 43 (100).

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