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CLERODANE DITERPENES FROM *ARISTOLOCHIA* SPECIES

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Key Word Index—*Aristolochia brasiliensis*; *A. esperanzae*; Aristolochiaceae; diterpenes; clerodanes.

Abstract—The investigation of *Aristolochia brasiliensis* and *A. esperanzae* afforded 12 clerodane derivatives, including the following six novel ones: rel (5*S*, 8*R*, 9*S*, 10*R*)-2-oxo-*ent*-3-cleroden-15-oic acid, rel (5*S*, 8*R*, 9*S*, 10*R*)-2-oxo-*ent*-clerod-3,13-dien-15-oic acid methyl ester, (5*R*, 8*R*, 9*S*, 10*R*)-*ent*-3-cleroden-15-oic acid, rel (5*S*, 8*R*, 9*S*, 10*R*)-*ent*-clerod-3,13-dien-15-oic acid, (2*S*, 5*R*, 8*R*, 9*S*, 10*R*)-2-hydroperoxy-*ent*-3-cleroden-15-oic acid methyl ester and (2*S*, 5*R*, 8*R*, 9*S*, 10*R*)-2-hydroperoxy-*ent*-clerod-3,13-dien-15-oic acid methyl ester. The structures were assigned on the basis of spectral data and derivatization by chemical reactions. The occurrence of this type of diterpene has not previously been reported in Aristolochiaceae.

INTRODUCTION

Phytochemical studies on species of Aristolochiaceae have shown phenanthrene type alkaloids and arylpropanoids to be common chemical constituents of this family [1–3]. As far as we know, the registry of diterpenes is restricted to *Aristolochia elegans* Mart. and *A. triangularis* Cham. from which kaurane diterpernoids were isolated [4, 5].

In this paper we report the isolation of 12 clerodane diterpenes, six of which appear to be new derivatives, from the stems of *A. brasiliensis* Mart. et Zucc. and the roots of *A. esperanzae* Kuntz. The structural assignments based on physical and spectral data are presented and discussed.

RESULTS AND DISCUSSION

The crude petrol extract from the stems of *A. brasiliensis* afforded by preparative TLC the diterpenes, **1a**, **2a**, **3a**, **3c** and **4**. After methylation of this extract **1c** and **2b** were also isolated. The chromatographic resolution of a methylated acetone extract from the roots of *A. esperanzae* led to the isolation of **1b**, **1c**, **3b**, **3d**, **5a** and **5b**.

Analysis of ^{13}C NMR (Table 1) as well as IR, ^{1}H NMR and mass spectral data of **1a** led to its identification as 2-oxokolavenic acid [6]. On treatment with diazomethane compound **1a** afforded **1b** which was identified by spectral comparison with methyl 2-oxokolavenoate [6]. The identification was confirmed by ^{13}C NMR analysis.

The mass spectrum of **1c** recorded $[M]^+$ at *m/z* 334 ($C_{21}H_{34}O_3$) and a fragment ion at *m/z* 205. The loss of 129 amu, suggested that **1c** had a saturated side chain structurally analogous to that of **1b**. By comparison of CD curves, IR, ^{1}H NMR and mass spectral data, **1c** was identified as the methyl ester of 2-oxopopulifolic acid [7, 8].

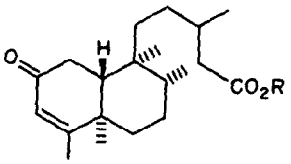
The methylation of a chromatographic fraction obtained from the crude extract of *A. brasiliensis* yielded a mixture of **1c** and **2b** in a 3:1 ratio as evidenced by ^{1}H NMR and ^{13}C NMR. From the ^{13}C NMR spectrum of this mixture, the data for **2b** were deduced after subtraction of the signals corresponding to **1c**. The ^{13}C NMR chemical shift values assigned to **2b** were compared with those reported for oxodeoxysagittariol [9] leading to the proposed C-8 and C-9 relative configuration as well as to the *cis*-stereochemistry at the junction of rings A and B. Similarly, compound **2a**, which was isolated together with relatively small amounts of **1a**, had its structure elucidated by taking **2b** as a model.

The spectral properties of **3a** and **3b** permitted their identification as kolavenic acid [9–11] and methyl kolavenoate [10–12], respectively. The ^{13}C NMR chemical shift values recorded for **3a** are not in accord with those reported in a previous study [9]. The data now presented for the carbons of the bicyclic system are compatible with those attributed to agelasine-B (**6**) [13] and the values assigned to C-11 and C-12 are in accord with those of **1a** (Table 1).

The structure of **3c** was proposed by comparison of its spectral data with those of **2a** and **3a**. The *O*-methyl derivative **3d** was identified as populifolic acid methyl ester [7, 12]. The treatment of **3d** with *m*-chloroperbenzoic acid resulted in the oxidation of C-2; the reaction product was identified as **1c**. The formation of this carbonyl compound was explained by an acid promoted 1,2-nucleophilic rearrangement of the epoxide initially formed in the reaction [14].

The diterpene **4** was not isolated in pure form being always accompanied by a small amount of **3a**. The IR, ^{1}H NMR and mass spectra obtained for the mixture, suggested for **4** a dehydro-**3a** constitution. The A/B *cis*-fused structure was proposed on the basis of the low field ^{13}C NMR signal of a bridge head methyl carbon (δ33.2) [15].

The substance **5a** was highly unstable and it was rapidly converted to **1c**. ^{1}H NMR spectral data showed **5a** to be closely related to **3d**. The main differences were the signals in the ^{1}H NMR spectrum of **5a**, at δ 4.28 (1H, *m*) and 9.10 (1H, *br s*), the latter disappearing upon addition of D_2O. The ^{13}C NMR spectrum showed, besides the signals assigned to **1c**, the following signals at δ18.2, 22.6, 40.9, 78.4 and 116.8, suggesting that **5a** had a peroxide function linked to C-2. This assumption was confirmed by dehydration (acetic anhydride–pyridine) of **5a** to **1c** [16]. For

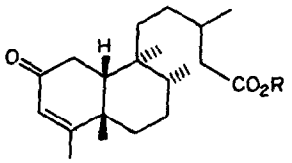


R

1a H , $\Delta^{13,14}$

1b Me , $\Delta^{13,14}$

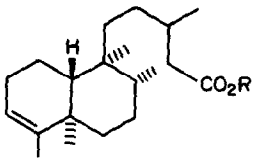
1c Me



R

2a H

2b Me , $\Delta^{13,14}$



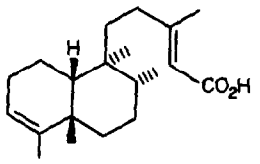
R

3a H , $\Delta^{13,14}$

3b Me , $\Delta^{13,14}$

3c H

3d Me



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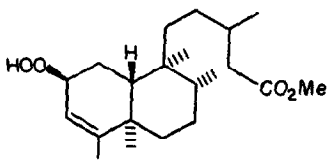
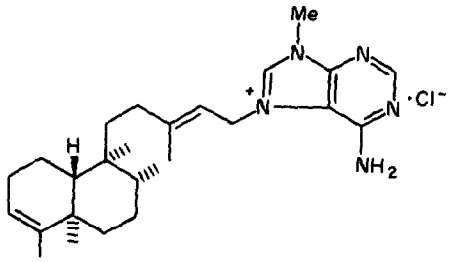


5a

5b $\Delta^{13,14}$



6

compound **5b** only the ^{1}H NMR spectrum could be recorded since **5b** also undergoes rapid decomposition. The compounds probably differ only by the saturation (**5a**) or unsaturation (**5b**) at C-13. On the basis of the rapid conversion of the peroxy derivatives **5a** and **5b** to their corresponding carbonyl derivatives **1c** and **1b**, it is suggested that the 2-oxo-3-cleroden diterpenoids could be artifacts.

EXPERIMENTAL

Plant material. A. brasiliensis was collected and identified by Dr J. E. de Paula (UnB, Brasília) in Pernambuco, Brasil, and *A. esperanzae* was collected around Araraquara, São Paulo, Brasil, by Dr G. L. Pozetti (UNESP, Araraquara) and identified by Dr C. Aranha (IAC, Campinas, São Paulo, Brasil).

Extraction and isolation of constituents. The dried and powdered stems of *A. brasiliensis* were extracted with petrol and the crude extract obtained (3.0 g) was washed with MeOH. The MeOH soluble fraction (1.4 g) was resolved by prep. TLC (silica gel, 1% $AgNO_3$, C_6H_6–EtOAc, 3:1) into **1a** (70 mg), **3a** (10 mg), **3c** (150 mg), **4** (60 mg, including a small amount. of **3a**) and a mixture of **1a** and **2a** (65 mg). The residue from the MeOH washing was treated with CH_2N_2 under standard conditions and submitted to prep. TLC (silica gel, C_6H_6–EtOAc, 9:1) yielding a terpenic fraction from which, after prep. TLC (silica gel, 1% $AgNO_3$, C_6H_6–EtOAc, 4:1), a mixture of **1c** and **2b** (163 mg) was separated. The crude petrol extract of *A. brasiliensis* also afforded lignans as described earlier [17].

The dried and powdered roots (143.0 g) of *A. esperanzae* were successively extracted with *n*-hexane, Me_2CO and EtOH. The crude Me_2CO extract, dissolved in aq. MeOH, was partitioned into fractions I, II and III as described in a previous paper [17]. Fraction I (5.5 g) was submitted to CC (silica gel, *n*-hexane) yielding a terpenic sample (1.5 g) which was methylated (CH_2N_2, standard conditions) and resolved by prep. TLC (silica gel, *n*-

Table 1. ^{13}C NMR spectral data (δ) of clerodane derivatives (20 MHz, $CDCl_3$)

C	1a	1b	1c	2a	2b	3a	3b	3c	3d	4
1	35.4*	35.6*	35.6*	35.1*	35.4	17.3	17.5	17.4	17.5	18.6
2	200.0	200.2	200.0	199.1	200.3	27.5	27.1	27.6	27.1	24.1
3	125.3	125.4	125.5	128.5	128.5	120.5	120.0	120.6	120.0	123.3
4	172.3	172.7	172.6	168.6	167.5	144.5	143.7	144.4	143.7	139.8
5	39.8**	39.9**	39.9**	38.6***	38.6**	38.3**	38.0*	38.3*	38.0*	37.9
6	34.8*	34.9*	34.8*	36.8**	36.7*	36.4*	36.4	36.5	36.4	37.6*
7	26.7	26.9	26.9	29.0	28.9	26.9	26.4	27.0	26.4	28.9
8	36.1	36.1	36.1	37.3	36.6	36.4*	36.0	36.3	36.0	37.5
9	38.7**	38.7**	38.8**	39.3***	39.3**	38.9**	38.3*	38.7*	38.3*	38.9
10	45.6	45.8	45.6	45.7	45.7	46.6	46.0	46.6	46.1	44.9
11	34.2*	34.0*	34.9*	35.4*	34.0	35.0	34.2	35.1**	35.0**	35.0
12	35.9*	35.9*	35.9*	36.2**	36.8*	36.9*	37.7	35.6**	35.8**	37.0*
13	162.6	160.3	30.9	30.7	160.3	164.4	160.8	31.0	30.6	164.5
14	115.1	115.3	41.3	41.4	115.2	114.9	114.5	41.7	41.0	115.1
15	171.2	167.1	173.4	178.7	167.0	172.0	166.5	179.8	172.8	172.4
16	19.3	19.1	19.8	19.9	19.1	19.5	18.5	19.9	19.5	19.6
17	15.5	15.7	15.7	16.0	15.9	15.9	15.5	16.1	15.5	16.1
18	18.2	18.4	18.4	20.5	20.5	18.3	18.0	18.4	18.0	20.0
19	18.7	18.9	18.9	32.1	32.1	20.0	19.5	20.0	19.5	33.2
20	17.6	17.9	18.0	18.0	17.8	17.9	17.8	18.1	17.8	17.9
OMe	—	50.8	51.4	—	50.7	—	50.1	—	50.6	—

*,**,***May be interchanged.

hexane–EtOAc, 9:1) into **1b** (471 mg), **1c** (187 mg), **3b** (150 mg), **3d** (360 mg), **5a** (37 mg) and **5b** (134 mg).

Rel (5S, 8R, 9S, 10R)-2-*oxo*-ent-3-*cleroden*-15-*oic acid* (**2a**). Colourless oil, MS *m/z* (rel. int.): 320 $[M]^+$ (< 1) 205 (12), 189 (7), 137 (13), 136 (11), 135 (24), 124 (21), 123 (31), 122 (18), 121 (41), 109 (46), 107 (29), 95 (100); IR ν_{max}^{film} cm^{-1}: 3600–3100, 1705, 1680, 1640, 1370, 1250; ^{1}H NMR (60 MHz, CCl_4): δ7.65 (1H, *br s*, COOH), 5.70 (*m*, H-3, H-14), 2.17 (*s*, H-16), 1.90 (*br s*, H-18), 1.10–0.80 (*m*, H-16, H-17, H-19).

Rel (5S, 8R, 9S, 10R)-2-*oxo*-ent-clerod-3,13-*dien*-15-*oic acid methyl ester* (**2b**). Colourless oil, MS *m/z* (rel. int.): 334 $[M]^+$ $C_{21}H_{34}O_3$, (4), 333 (3), 206 (23), 205 (62), 203 (16), 189 (25), 163 (36), 161 (37), 135 (20), 123 (20), 121 (20), 119 (21), 95 (21), 82 (100); IR ν_{max}^{film} cm^{-1}: 1720, 1640, 1220, 1140; ^{1}H NMR (60 MHz, $CDCl_3$), (**1c** + **2b**): δ5.65 (*br s*, H-3), 2.09 (*br s*, H-16), 1.80 (*br s*, H-18), 1.06 (*br s*, H-19), 0.96 (*d*, *J* = 6.0 Hz, H-16), 0.80 – 0.50 (*m*, H-17, H-20).

(5R, 8R, 9S, 10R)-ent-3-*cleroden*-15-*oic acid* (**3c**). Colourless oil, MS *m/z* (rel. int.): 306 $[M]^+$ (15), 291 (10), 263 (39), 205 (12), 192 (57), 191 (100), 190 (82), 189 (92), 163 (57), 120 (45), 109 (67); IR ν_{max}^{film} cm^{-1}: 3600–3000, 1705, 1380, 1250; ^{1}H NMR (60 MHz, CCl_4): δ9.35 (1H, *br s*, COOH), 5.10 (1H, *br s*, H-3), 1.60 (3H, *br s*, H-18), 1.00 (3H, *s*, H-19), 0.97 (3H, *d*, *J* = 6.5 Hz, H-16), 0.83 (3H, *d*, *J* = 6.0 Hz, H-17), 0.78 (3H, *s*, H-20).

Rel (5S, 8R, 9S, 10R)-ent-*clerod*-3,13-*dien*-15-*oic acid* (**4**). Colourless oil (contaminated with **3a**), MS *m/z* (rel. int.): 304 $[M]^+$ (1), 191 (27), 189 (10), 136 (20), 123 (33), 122 (21), 121 (35), 109 (32), 107 (54), 95 (100); IR ν_{max}^{film} cm^{-1}; 3600–3000, 1700, 1640, 1440, 1250; ^{1}H NMR (60 MHz, CCl_4): δ9.40 (1H, *br s*, COOH), 5.68 (1H, *br s*, H-14), 5.21 (1H, *m*, H-3), 2.17 (3H, *br s*, H-16), 1.60 (3H, *br s*, H-18), 1.00 (3H, *s*, H-19), 0.83 (3H, *d*, *J* = 6.0 Hz, H-16), 0.78 (3H, *s*, H-20).

(2S, 5R, 8R, 9S, 10R)-2-*hydroperoxy*-ent-3-*cleroden*-15-*oic acid methyl ester* (**5a**). Colourless gum, IR ν_{max}^{film} cm^{-1}: 3420, 1730, 1650, 1440, 1220, 1170; ^{1}H NMR (60 MHz, CCl_4): δ9.10 (1H, *br s*, OH), 5.10 (1H, *m*, H-3), 4.28 (1H, *m*, H-2), 3.68 (3H, *s*, OMe), 1.60 (3H, *br s*, H-18), 0.97 (3H, *d*, *J* = 6.0 Hz, H-16), 0.95 (3H, *s*, H-19), 0.80 (3H, *d*, *J* = 6.0 Hz, H-17), 0.77 (3H, *s*, H-20).

(2S, 5R, 8R, 9S, 10R)-2-*hydroperoxy*-ent-*clerod*-3,13-*dien*-15-*oic acid methyl ester* (**5b**). Gum, ^{1}H NMR (60 MHz, CCl_4): δ5.60 (2H, *br s*, H-14, OH) 5.10 (1H, *m*, H-3), 4.25 (1H, *m*, H-2), 3.60 (3H, *s*, OMe), 2.13 (3H, *br s*, H-16), 1.60 (3H, *br s*, H-18), 0.93 (3H, *s*, H-19), 0.85 (3H, *d*, *J* = 6.0 Hz, H-17), 0.73 (3H, *s*, H-20).

Compounds **1a**, **1b**, **1c**, **3a**, **3b** and **3d** were identified through a comparison of their spectral data with those reported in the literature [6–12]. The hitherto unreported ^{13}C NMR data of compounds **1b**, **1c**, **3b** and **3d** are listed in Table 1.

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