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DITERPENES FROM THE FRUITS OF *XYLOPIA AROMATICA**

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Key Word Index—*Xylopia aromatica*; Annonaceae; diterpene; atisane; labdane; trachylobane; kaurane.

Abstract—Fruits of *Xylopia aromatica* contain atisane, labdane, kaurane and trachylobane diterpenes besides three new *nor*-diterpenes characterized as degraded products. The *ent*-labdan-13,16-diol-8(17),14-dien-18-oic acid, *ent*-trachyloban-3β-ol, *ent*-atisan-16α,18-diol and *ent*-atisan-16α-ol-18-oic acid are novel natural products. The monoacetate and the methyl ester respectively of the two last compounds are known as trachylobane degradation products.

INTRODUCTION

As part of a phytochemical investigation of Brazilian *Xylopia* species (Annonaceae), we are studying *X. aromatica* Lam. (Mart.) also known as *X. grandiflora* St. Hil. This species is a tree of common occurrence in several regions of Brazil. Its fruits are used as a spice substitute for *Piper nigrum* [1]. However, there are no reports about the chemical constitution of the fruits.

RESULTS AND DISCUSSION

The triglycerides of the hexane extract of the green fruits of *Xylopia aromatica* were precipitated by methanol. The methanol solution was submitted to several chromatographic separations followed by acetylation and diazomethane methylation of some fractions which afforded the labdanes **1**–**3**; the kauranes **4** and **5**; the trachylabane **6**; the atisanes **7**–**9**; the *nor*-atisanes **11**–**13**, the sesquiterpene, spatulenol [2] and stigmasterol.

Compounds **1** and **2** showed spectral data identical with those reported in the literature [3, 4]. The ^{13}C NMR spectrum of **1** (Table 1) also agreed with the proposed structure. The new labdane **3** ($C_{21}H_{34}O_4$) showed some common spectral features with **1** and **2**. The IR spectrum showed absorption bands corresponding to a methyl ester function (1730, 1250 cm^{-1}), terminal double bonds (3080, 1645, 990, 920, 895 cm^{-1}) and to a hydroxyl group (3430 cm^{-1} large). The ^1H NMR spectrum (Table 2) characterized the same carbocyclic system as that present in **1**. It also showed an AB pattern corresponding to oxymethylenic protons and multiplets due to vinyl and vinylidene groups. The ^{13}C NMR spectrum of **3** (Table 1) revealed the presence of a tertiary hydroxyl and confirmed the presence of the other groups. Comparison of the ^{13}C NMR data of **3** with those of manool [5]established the side chain structure. The decoupled spectrum also revealed that **3** was a mixture of the C-13 epimers. The kauranes **4** and **5** (isolated as their acetates) were identified by their spectral data [6, 7].

Compound **6**, which analysed for $C_{22}H_{32}O_2$, was obtained from the an acetylated fraction. The ^1H NMR spectrum (Table 2) revealed the presence of four methyl groups, the acetyl moiety and a multiplet centred at δ 0.62 which suggested a cyclopropane ring. These data agree with the structure of an acetylated trachylobane diterpene. The position of the acetyl group was determined by the comparison of the ^{13}C NMR data (Table 1) of **6** with those of related compounds [8].

The ^{13}C NMR spectral data of **7**–**9** indicated that they were atisane diterpenes [9]. Compound **7** was previously isolated from *Helianthus annus* [10]. This compound, the acetate **8a** and the methyl ester **9a**, were obtained previously from trachylobane degradation [11]. The C-16 stereochemistry of the semisynthetic products was not established. To unequivocally determine the configuration at C-16 a ^{13}C NMR spectrum of **7** with $Eu(DPM)_3$ was determined. The results (Table 1) indicated that the hydroxy group was located on the same side of the molecule as H-9. As all three atisane compounds differ only at C-18, the structures [**8** and **9**] were established from their ^{13}C NMR data. Compound **10** was not isolated from the fruits of *X. aromatica*, but the *nor*-diterpenes **11** and **12** are, without doubt, artifacts. The ^{13}C NMR data (Table 1) were conclusive in the determination of the structures. The decomposition of C-4 kaurene aldehydes giving alcohols and hydroperoxides is well described [12]. The aldehyde **10** was obtained by the oxidation of **8** and then left to stand for three hr. Analysis by TLC confirmed the presence of **11** and **12** as decomposition

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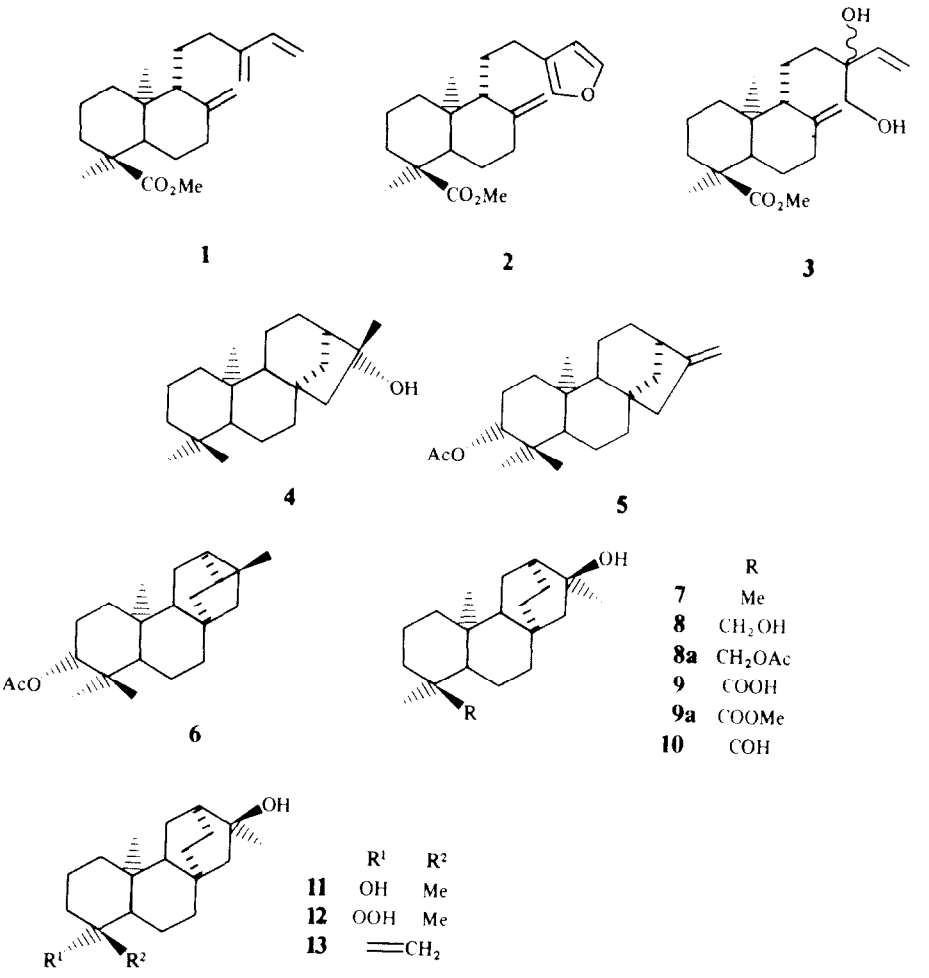

products of **10**. The *nor*-atisenol **13** was isolated from the same column fractions as **11** and **12**. This fact suggested that **13** is also an artifact, probably obtained by dehydration of **11** or **12**. The ^{1}H NMR spectrum of **13** showed absorptions due to a vinylidene group (δ 4.62 and 4.40) and in the IR spectrum the hydroxyl band at 3410 cm^{-1} was still present. The structure of **13** was finally established by its ^{13}C NMR spectrum (Table 1). The *ent*-stereochemistry of the diterpenes of *Xylopia aromatica* was deduced by the observation of the specific rotation of some products.

The isolation of *nor*-diterpenes from *Xylopia aromatica* poses the question about the origin of some products isolated from plants. As far as we know, only very few C-4 alcohols and no hydroperoxide have been reported as natural products. But this fact does not exclude the possibility that other nor-diterpenes could have been formed by an aldehyde degradation.

Diterpenes were reported previously from African species of *Xylopia* and alkaloids have been found only in American and Asiatic species. These observations led Waterman [13] to suggest a taxonomic difference among these species.

Another interesting features of the chemical study of *Xylopia aromatica* is the presence of atisanes. These diterpenes have not been previously isolated from the genus *Xylopia* nor from other Annonaceae species. More over, atisanes were not found in the Magnoliiflorae superorder where the Annonaceae family is classified. They occur mainly in the more evolved family Asteraceae and to a less extent in the families Lamiaceae, Apiaceae, Polypodiaceae, Euphorbiaceae, Erythroxylaceae and Ranunculaceae. In this last family the alkaloidal atisin diterpenes were isolated from the genus *Aconitum*. Atisenes with no oxidation function are also found in Liliaceae (Monocotyledone) and Araucariaceae (Gymnosperma). Except for the Asteraceae family, the occurrence of atisane is limited to a genus or even to only a species in each family.

EXPERIMENTAL

Plant material. The green fruits of *Xylopia aromatica* were collected near Mogi-Guaçu, São Paulo State, Brazil. A voucher specimen has been deposited in the herbarium of Instituto de Botânica (SP 142.360).

Extraction and isolation. Dried fruits (260 g) were extracted with hexane at room temp. The extract (60 g) was dissolved in hot MeOH, left overnight at 4° and filtered. The solvent was evapd from the MeOH soln and the residue (50 g) was filtered on

Table 1. ^{13}C NMR spectra data for the diterpenes (20 MHz, $CDCl_3$, δ)

C	1	3	6	7	Δ	8	9	11	12*	13
1	37.8	37.9	37.7	39.7†	−0.1	38.7†	38.5†	39.0†	39.8†	39.2†
2	18.3	18.5	23.3	18.2	−0.2	17.2	17.3	17.3	19.0	22.7
3	36.9	37.1	81.1	42.2	−0.2	35.3	36.9	40.9	35.6	36.0
4	47.6	47.8	38.0	33.1	−0.2	37.3	47.4	71.8	82.5	105.1
5	49.7	50.0	55.3	56.5	−0.1	49.9	50.7	54.6	65.4	52.1
6	26.7	26.9	19.9	18.7	0.0	18.3	21.5	17.7	17.9	21.0
7	37.8	38.1	38.9	39.4†	−0.1	39.1†	39.0†	38.8†	40.3†	39.4†
8	147.7	148.0‡	40.8	33.9	+0.8	33.0	34.0	33.6	34.0	33.6
9	56.4	57.4	53.1	51.3	+1.1	51.1	51.7	50.3	50.8	48.6
10	38.8	39.3	37.7	37.7	+0.2	37.3	37.1	37.3	37.9	39.2
11	22.0	16.8‡	19.8	23.3	+1.3	23.1	23.0	22.9†	24.0†	23.9†
12	30.0	37.9‡	21.3	38.0	+1.0	37.7	38.0	37.8	38.6	37.9
13	146.8	76.2‡	24.3	24.1	+0.6	23.9	24.0	23.9†	24.4†	24.0†
14	138.9	140.9‡	33.5	27.3	+0.6	27.1	27.0	27.4	28.1	27.1
15	112.9	115.2	50.4	57.8	+0.6	57.5	57.5	57.7	58.6	57.7
16	115.3	69.0	22.6	72.1	+7.0	72.0	72.0	72.0	70.8	72.1
17	106.7	107.0‡	20.6	30.5	+2.7	30.3	30.5	30.3	31.1	30.4
18	174.0	179.4	28.0	33.4	+0.1	72.0	179.4	30.6	25.4	151.1
19	16.4	16.6	16.6	21.7	−0.2	18.3	16.5	—	—	—
20	14.6	14.7	14.0	13.9	+0.1	14.0	14.3	13.2	14.5	12.3
OMe	51.6	51.9	—	—	—	—	51.3	—	—	—
OCOMe			170.9					—	—	—
OCOMe	—	—	21.1	—	—	—	—	—	—	—

Δ = δ [7 + Eu (DPM)$_3$] − δ 7.
*C_5D_5N as solvent.
†Signals interchangeable.
‡Double signals.

silica gel (50 g) with hexane, CH_2Cl_2 and EtOAc. The hexane fraction (22 g) was submitted to CC (silica gel, 450 g) and eluted with hexane–EtOAc in mixtures of increasing polarity. The eluates (125 ml) were combined on the basis of TLC analysis and the combined fractions purified by repeated CC, prep. TLC and argentation TLC on silica gel. Some fractions before purification were methylated with CH_2N_2 or acetylated with Ac_2O–pyridine. This procedure yielded in order of increasing polarity: **1** (500 mg), **2** (10 mg), **7** (1.8 g), spatulenol (20 mg), **6** (20 mg), **5** (35 mg), **9** (100 mg), **3** (30 mg) and **8** (90 mg).

The CH_2Cl_2 fraction (13 g) submitted to the same procedure gave: **1** (60 mg), **2** (10 mg), **7** (200 mg), **4** (30 mg), **5** (30 mg), **6** (15 mg), stigmasterol (200 mg), **13** (20 mg), **12** (60 mg), **9** (280 mg), **11** (100 mg) and **8** (180 mg).

Characterisation of compounds. The molecular formulae were deduced by low resolution MS jointly with hydrogen and carbon counts by NMR spectra.

ent-*Lab*-8(17), 14-*dien*-13,16-*diol*-18-*oic acid methyl ester* (**3**). Colourless oil. IR ν_{max}^{film} cm^{-1}: 3430, 3080, 2930, 2860, 1730, 1645, 1450, 1250, 990, 920, 895, MS *m/z* (rel. int.) 332 [M]$^+$ (2), 314 (3),

Table 2. ^1H NMR spectral data for the diterpenes (60 MHz, $CDCl_3$, δ, TMS)

H	3*	6	8	9a	11	12	13*
3	—	4.40 *dd*	—	—	—	—	—
13	—	0.62 *m*	—	—	—	—	—
14	5.00–4.60 *m*	—	—	—	—	—	—
15	5.75–4.60 *m*	—	—	—	—	—	—
16	3.38 *br s*	—	—	—	—	—	—
17	4.86 *br s* 4.52 *br s*	0.75 *s*	1.28 *s*	1.28 *s*	1.26 *s*	1.26 *s*	1.22 *s*
18	—	1.10 *s*	3.42 *d* 3.10 *d*	—	1.18 *s*	1.26 *s*	4.62 *br s* 4.40 *br s*
19	1.07 *s*	0.75 *s*	0.78 *s*	1.15 *s*	—	—	—
20	0.68 *s*	0.93 *s*	0.98 *s*	0.97 *s*	1.10 *s*	1.08 *s*	0.72 *s*
OMe	3.67 *s*	—	—	3.62 *s*	—	—	—
OH	2.89 *br s*	—	1.98 *br s*	—	—	—	—
OAc	—	1.94 *s*	—	—	—	—	—

*CCl_4 as solvent.

301 (4), 273 (6), 247 (11), 241 (9), 191 (25), 189 (13), 151 (25), 149 (15), 121 (100). ^{13}C and ^{1}H NMR see Tables 1 and 2.

ent-*Trachyloban-3β-acethoxy* (**6**). Colourless solid, IR ν_{max}^{KBr} cm^{-1}: 2960, 2860, 1725, 1450, 1375, 1250, 1080. MS *m/z* (rel. int.): 330 $[M]^+$ (34), 315 (10), 288 (10), 274 (48), 270 (30), 255 (62), 227 (21), 199 (38), 105 (99), 45 (100). ^{13}C and ^{1}H NMR see Tables 1 and 2.

ent-*Atisan*-16α,18-*diol* (**8**). Colourless crystals, mp 160–162° (hexane). IR ν_{max}^{KBr} cm^{-1}: 3320, 2940, 2860, 1450, 1370, 1040, 1020. MS *m/z* (rel. int.): 306 $[M]^+$ (0), 288 (5), 274 (4), 257 (64), 201 (8), 187 (36), 175 (100). ^{13}C and ^{1}H NMR see Tables 1 and 2. $[\alpha]_{578}^{25°} = -45°$, $[\alpha]_{546}^{25°} = -54°$ ($CHCl_3$, *c* 0.02 g/ml).

ent-*Atisan*-16α-*ol*-18 *oic acid* (**9**). Colourless crystals, mp 205–207° (hexane). ^{1}H NMR (60 MHz, CCl_4): δ 1.00 (*s*, 3H), 1.19 (*s*, 3H), 1.28 (*s*, 3H), 4.35 (OH). MS *m/z* (rel. int.): 320 $[M]^+$ (0), 302 (14), 287 (18), 274 (3), 259 (13), 216 (9), 201 (11), 187 (22), 105 (34), 79 (38), 43 (100).

ent-*Atisan*-16α-ol-18-*oic methyl ester* (**9a**). Colourless crystals mp 140–142° (hexane). IR ν_{max}^{KBr} cm^{-1}: 3450, 2920, 2840, 1725, 1450, 1390. ^{1}H and ^{13}C NMR see Tables 1 and 2. $[\alpha]_{578}^{25°} = -24°$, $[\alpha]_{546}^{25°} = -36°$ ($CHCl_3$, *c* 0.010 g/ml).

ent-18-nor-*Atisan*-4β,16α-*diol* (**11**). Colourless crystals mp 130–132° (hexane). IR ν_{max}^{KBr} cm^{-1}: 3390, 2920, 1440, 1380, 1360, 1110, 900. MS *m/z* (rel. int.): 292 $[M]^+$ (0), 277 (12), 224 (29), 259 (57), 246 (9), 241 (46), 231 (30), 213 (15), 201 (15), 189 (100), 161 (47). ^{1}H and ^{13}C NMR see Tables 1 and 2 $[\alpha]_{578}^{25°} = -35°$, $[\alpha]_{546}^{25°} = -44°$, ($CHCl_3$, *c* 0.020 g/ml).

ent-18-nor-*Atisan*-4β-*hydroperoxide*-16α-*ol* (**12**). Colourless crystals mp 150–152° (CH_2Cl_2). IR ν_{max}^{KBr} cm^{-1}: 3390, 3150, 2930, 1440, 1360, 1180, 1140, 900. MS *m/z* (rel. int.): 308 $[M]^+$ (10), 273 (15), 257 (100), 255 (4), 241 (3), 229 (7), 215 (10), 187 (47), 175 (61), 159 (32), 147 (37), 145 (34).

ent-19-nor-*Atisan*-4(18)-*en*-16α-*ol* (**13**). Colourless, oil IR ν_{max}^{film} cm^{-1}: 3400, 3080, 2920, 1640, 1440, 1370, 1130, 1110, 900, 890. ^{1}H and ^{13}C NMR see Tables 1 and 2.

Oxidation of compound **8**. A soln of CrO_3 (100 g) in Me CO_2H (3 ml) was added to **8** (40 mg). The mixture was left at room temp for 12 hr and then H_2O was added. Work-up in the usual way gave 30 mg of **10** (IR ν_{max}^{film} cm^{-1}: 3400, 2920, 2860, 1720, 1450, 1390). Compound **10** decompose very rapidly.

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