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Labdane dimers from *Xylopia aromatica*[1]

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Abstract

From the stem bark of *Xylopia aromatica* (Annonaceae), have been isolated two new labdane dimers as their methyl esters, together with the known compounds *ent*-labda-8(17),13(16),14-trien-18-oic acid, sitosterol and stigmasterol. The structures of the dimers were elucidated on the basis of detailed spectroscopic analyses. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

We have been working over the last ten years with *Xylopia aromatica* (Annonaceae) (Moraes & Roque, 1988; Vestri, Moraes, & Roque, 1992; Martins, Alvarenga, Roque, & Felício, 1995). Natural products of several classes have been isolated from this plant, including potent biologically active acetogenins (Colman-Saizarbitoria et al., 1994, 1995; Fournier et al., 1994). Adducts of kaurane and labdane diterpenes have also been isolated from other *Xylopia* species (Waterman, 1989; Vilegas, Felicio, Roque, & Gottlieb, 1991), but no labdane dimers have been detected, thus far, from this genus.

We recently reported the isolation of a sesquiterpene dimer from the leaves of this plant (Martins, Osshiro, Roque, Marks, & Gottlieb, 1998) and in the present paper we describe the isolation of two new labdane dimers (**2** and **3**), together with the diterpene *ent*-labda-8(17),13(16),14-trien-18-oic acid (**1**), sitosterol and stigmasterol from its bark.

The occurrence of a diastereomer of the dimer **2**, from the fruits of *Juniperus oxycedrus* L. (Cupressaceae) was previously reported (Teresa, Feliciano, & Corral, 1974), but this, to our knowledge, is the only report on a labdane dimer from the Annonaceae.

2. Results and discussion

From a dichloromethane extract of the stem bark of a specimen of *X. aromatica* collected in Minas Gerais, Brazil, were isolated labdane acid **1**, two labdane dimers, sitosterol and stigmasterol. During the isolation procedures, the dimers were methylated with CH_2N_2 and thus they were obtained as their methyl esters (**2**, **3**).

The ^{1}H NMR spectrum of **2** is very similar to that of **1** in the region between δ 0.60 and δ 2.40. This spectrum showed three methyl signals at δ 0.67, 0.68 and 1.11 (6H), besides the methoxyl signal at δ 3.63 (6H) (Table 1). The signals at δ 4.51 and 4.80 were assigned to an exocyclic double bond, integrated for two protons each, when compared with the broad singlet at δ 5.33, corresponding to one proton, and assigned to an endocyclic double bond. The absence of a signal at δ 6.35

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Table 1
^{1}H NMR spectral data for **1**–**4** in $CDCl_3$ (200 MHz). Assignments were confirmed by the ^{1}H–^{1}H COSY spectra

H[a]	**1**	**2**	**3**	**4**
14	6.35 dd[b]	5.33 br s	5.31 br s	2.93 br s
15_E	5.19 d[b]	–	–	–
15_Z	5.03 d[b]	–	–	–
16′a	4.96 br s	4.72 br s	–	4.70 br s
16′b	4.98 br s	4.72 br s	–	4.70 br s
17a (17′a)	4.84 br s	4.80 br s	4.76 br s	4.80 br s
17b (17′b)	4.56 br s	4.51 br s	4.41;4.46 br s	4.50 br s
19 (19′)	1.13 s	1.11 s	1.08 s	1.12 s
20	0.70 s	0.67 s	0.64 s	0.69 s
20′	–	0.68 s	0.64 s	0.69 s
OMe	–	3.63 s	3.59 s	3.64 s

[a] The signals for all compounds corresponding to other protons overlapped in the region between δ 1.1 and δ 2.4.

[b] Coupling constants (J in Hz) H-14, H-15_E (17.6); H-14, H-15_Z (10.8).

Table 2
^{13}C NMR spectral data for **1**–**4** and COSY ^{1}H–^{13}C of **2** in $CDCl_3$. Multiplicities were established from the DEPT pulse sequence

C	**1**	**2**	**2** (^{1}H)*	**3**	**4**
1; 1′	$37.8^{\dagger}$	$37.8^{\ddagger}$	1.1	$37.7^{\dagger}$	$37.9^{\ddagger}$
2; 2′	18.4	18.4	1.5	18.3	18.5
3; 3′	37.1	37.0	1.5; 1.7	36.9	37.0
4; 4′	47.5	47.7	–	47.6	47.8
5; 5′	49.5	49.9	1.9	49.7	49.9
6; 6′	26.8	26.8	1.2; 1.4	26.7	26.9
7; 7′	$37.8^{\dagger}$	$38.0^{\ddagger}$	1.9; 2.3	$37.7^{\dagger}$	$38.1^{\ddagger}$
8; 8′	147.8	147.9	–	147.6; 147.7	147.5; 148.0
9; 9′	56.4	56.4	1.7	56.0; 56.1	56.4; 56.6
10; 10′	38.8	38.9	–	38.8; 38.9	39.1
11	22.1	$21.6^{\S}$	$1.3^{\ddagger}$	21.4	18.2
11′	–	$22.1^{\S}$	$1.6^{\ddagger}$	17.0	22.0
12	30.1	38.0	1.7; 2.3	37.9	36.0
12′	–	33.5	1.8; 2.2	39.4	33.6
13	146.9	137.9	–	137.8	60.5; 61.8
13′	–	154.9	–	214.1	154.1
14	139.0	120.0	5.3	118.8	58.2; 58.8
14′	–	40.4	2.1	46.8	36.6; 40.5
15	113.2	34.7	1.8	$27.9^{\S}$	34.1; 34.4
15′	–	27.9	1.7	$27.0^{\S}$	23.8; 24.6
16	115.6	25.7	2.0	24.9	26.3; 27.2
16′	–	$106.7^{\dagger}$	4.7	–	107.2
17; 17′	107.0	$106.7^{\dagger}$	4.5; 4.8	106.8	106.8
18; 18′	185.3	179.1	–	179.1	179.3
19; 19′	16.3	16.5	1.1	16.4	16.6
20; 20′	14.7	14.6	0.7	14.4; 14.6	14.8
OMe	–	51.6	3.6	51.7	51.9

* Approximate values obtained by HETCOR ^{1}H–^{13}C and the connectivities were confirmed by detailed analysis of HMBC spectrum.

† Overlapping signals.

‡ Interchangeable signals.

§ Interchangeable signals.

(dd) and the appearance of a broad singlet at δ 4.72 (2H) indicated a modification of the side-chain of the labdane **1**. The low resolution EIMS (30 eV) gave a molecular ion at m/z 632, twice the molecular weight of the methyl ester of **1**. These data suggested, therefore, that **2** was a dimer of the methyl ester of **1**, with the union of the monomers occurring in the side-chain. The ^{13}C NMR spectrum showed 27 signals (Table 2). 15 of them could be assigned to the carbons of partial structure **A** (Fig. 1). The high intensity of these signals was in agreement with a dimer, in which the cyclic skeleton of both monomer units was intact. The observation of a base peak at m/z 235 in the EIMS, that corresponds to the structure **A**, and of peaks due to other allylic fragmentations (Fig. 2) is in agreement with the proposal. The DEPT 135 and 90 spectra established the multiplicity of the carbon signals and a ^{13}C–^{1}H COSY established the one bond correlations between carbons and hydrogens. The elemental analysis associated with the low resolution EIMS established the molecular formula as $C_{42}H_{64}O_4$, which is in agreement with the presence of five rings and four double bounds, besides two carboxyl groups in the molecule. Thus, the union of the labdane monomers should have occurred by a Diels-Alder reaction (Fig. 1). The HMBC spectrum confirms this suggestion and defines the major constitutional isomer, formed by the observation of the correlations between H-14 and C-14′, C-12, C-15 and C-16, as well as between H-16′ and C-14′ and C-12′. The relative configuration at C-14′ was not determined.

The ^{1}H NMR spectrum of **3** (Table 1) shows the same spectral features as that obtained from **2**, except for the absence of the signal at δ 4.72, which was assigned to the vinyl protons at C-16′. The signals corresponding to H-17 and H-17′, that gave two broad singlets for **2**, were observed as three broad singlets in **3**, suggesting a structural modification in the side-chain of one of the monomers. The IR spectrum of **3** presented a shoulder at 1700 cm^{-1}, indicating the presence of a carbonyl group besides the carboxymethyl groups. The ^{13}C NMR spectrum of **3** (Table 2) confirmed these changes by the appearance of a signal at δ 214.1 and the absence of the signals at δ 154.9 and 106.7. Moreover, the chemical shifts of the carbons for structure **A** are all present in the spectrum of **3**; the signal of C-11′ was shielded, while those of C-14′ and C-12′ were deshielded in relation to those of **2**. All these data located the carbonyl group at C-13′. The EIMS is in agreement with the structure by the observations of $[M]^+$ at m/z 634, the base peak at m/z 235 and a peak at m/z 291 that corresponds to a α-carbonyl fragmentation between C-13′ and C-14′. Elemental analysis together with the low resolution EIMS indicated the molecular formula as $C_{41}H_{62}O_5$, required for structure **3**.

After purification, the dimer **2** undergoes decomposition, in a few weeks, affording a mixture of more polar products. Compound **3** was not detected among these products. Thus, the dimer **3** should be natural. An epoxide mixture **4** was obtained after chromatographic separation of the degraded products of **2**.

The ^1H and ^{13}C NMR spectra of mixture **4** (Tables 1 and 2) suggested the presence of an epoxide in place of the endocyclic double bond of **2**, by the absence of the signals at δ 5.33 (H-14), 137.9 (C-13) and 120.0 (C-14). The presence of signals at δ 2.93 in the ^1H NMR spectrum and at δ 58.2 to 61.8 in the ^{13}C NMR spectrum of **4**, corresponding to quaternary and methyne carbons, indicated the presence of an epoxy ring. The shielding of C-11 and C-15′, in relation to **2**, observed in the ^{13}C NMR spectrum, confirmed the position of the epoxide at C-13. The observation that in the ^{13}C NMR spectrum of mixture **4** there still persists a signal at δ 40.5 (C-14′), along with the shielded C-14′ signal (36.6), indicated that epoxidation occurred on both sides of the cyclohexene. The IR spectrum of **4** showed a band at 914 cm^{-1} assigned to the epoxy ring.

The absolute configurations of the dimers was assigned as *ent* by observation of the $[\alpha]_D^{22}$ −20 ($CHCl_3$, *c* 0.28) for the methyl ester of **1** (Khoo, Oehlschlager, & Ourisson, 1973). Since the dimers originated from **1**, their configuration should be the same. The $[\alpha]_D^{22}$ −44 ($CHCl_3$, *c* 3.73) observed for dimer **2** is in agreement with that configuration.

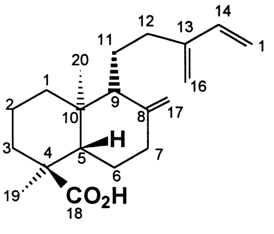
3. Experimental

3.1. General

^1H NMR (200 MHz), ^{13}C NMR (50 MHz), HOMOCOSY ^1H–^1H and HETCOR ^1H–^{13}C were performed in $CDCl_3$ on a Bruker AC 200 FT-NMR and HMBC on a Bruker DPX 300 FT-NMR. EIMS: HP 5988 A, direct inlet. IR spectra (film) were measured on a Perkin-Elmer FT-IR 1750. Silica gel 60 (Merck) was used for chromatography: 70–230 mesh for CC and 230–400 mesh for flash CC. Merck Nanograde solvents were used for extraction, partition and chromatography.

3.2. Plant material

The stem bark of *Xylopia aromatica* (Lamarck) Martius was collected near Coqueiral, Minas Gerais, Brazil in February 1992. A voucher specimen has been deposited in the herbarium of the Instituto de Botânica da Secretaria do Meio Ambiente do Estado de São Paulo (SP 142.360).



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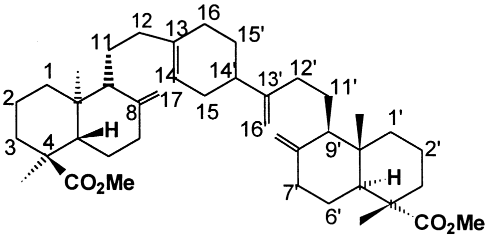

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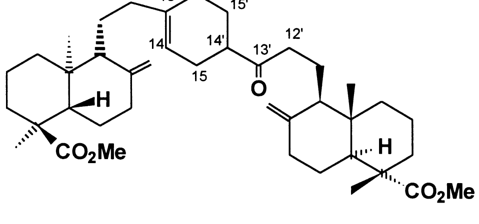

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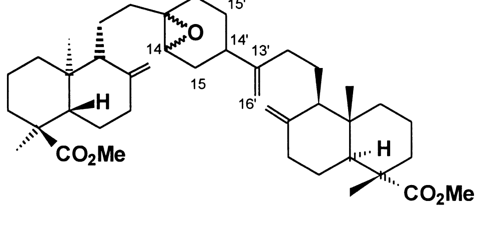

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3.3. Extraction and isolation

1.5 kg of dried, powdered stem bark were extracted with CH_2Cl_2 (3 × 3 l) at room temp. The extract was concentrated (42 g) and then partitioned between hexane and $MeOH/H_2O$ (9:1). The hexane phase, after evaporation of the solvent, was subjected to CC on silica gel with a gradient of hexane/EtOAc/MeOH to give 25 frs. A 613 mg portion of frs 3–8 (total wt 5.6 g) was subjected to chromatography (silica flash CC) using hexane/EtOAc/MeOH/HOAc (60:20:2:0.5) as eluant, to give **1** (189 mg). After methylation with CH_2N_2 frs 9–10 (total wt 1.9 g) were submitted to CC on silica eluted with increasing amounts of EtOAc in hexane to give a mixture (210 mg) containing sitosterol and stigmasterol and a fraction (358 mg) which was chromatographed on silica flash CC with hexane/EtOAc/MeOH (85:15:1) to give **2** (114 mg). From frs 11–15 (combined wt 4.8 g), **2** (654 mg) and **3** (105 mg) were obtained after methylation with CH_2N_2 and repeated CC on silica gel with first hexane/EtOAc/MeOH (gradient) and then hexane/EtOAc/MeOH (90:10:1, flash CC). After 4 weeks of storage, dimer **2** partially decomposed to give a mixture of more polar compounds. 416 mg of this mixture was chromatographed on silica flash CC with hexane/EtOAc/MeOH (90:10:1) to give **2** (109 mg) and **4** (23 mg). For the observation of the $[\alpha]_D^{22}$ −20 ($CHCl_3$, *c* 0.28) to the methyl ester of **1**, the labdane acid was methylated with CH_2N_2.

3.4. Ent-methylisoozate dimer (2)

Gum; $[\alpha]_D^{22}$ −44 ($CHCl_3$, *c* 3.73); EIMS 30 eV, *m/z* (rel. int.): 632 $[M]^+$ (4), 617 $[M–CH_3]^+$ (5), 573 $[M–CO_2Me]^+$ (9), 572 $[M–HCO_2Me]^+$ (10), 557 [572-

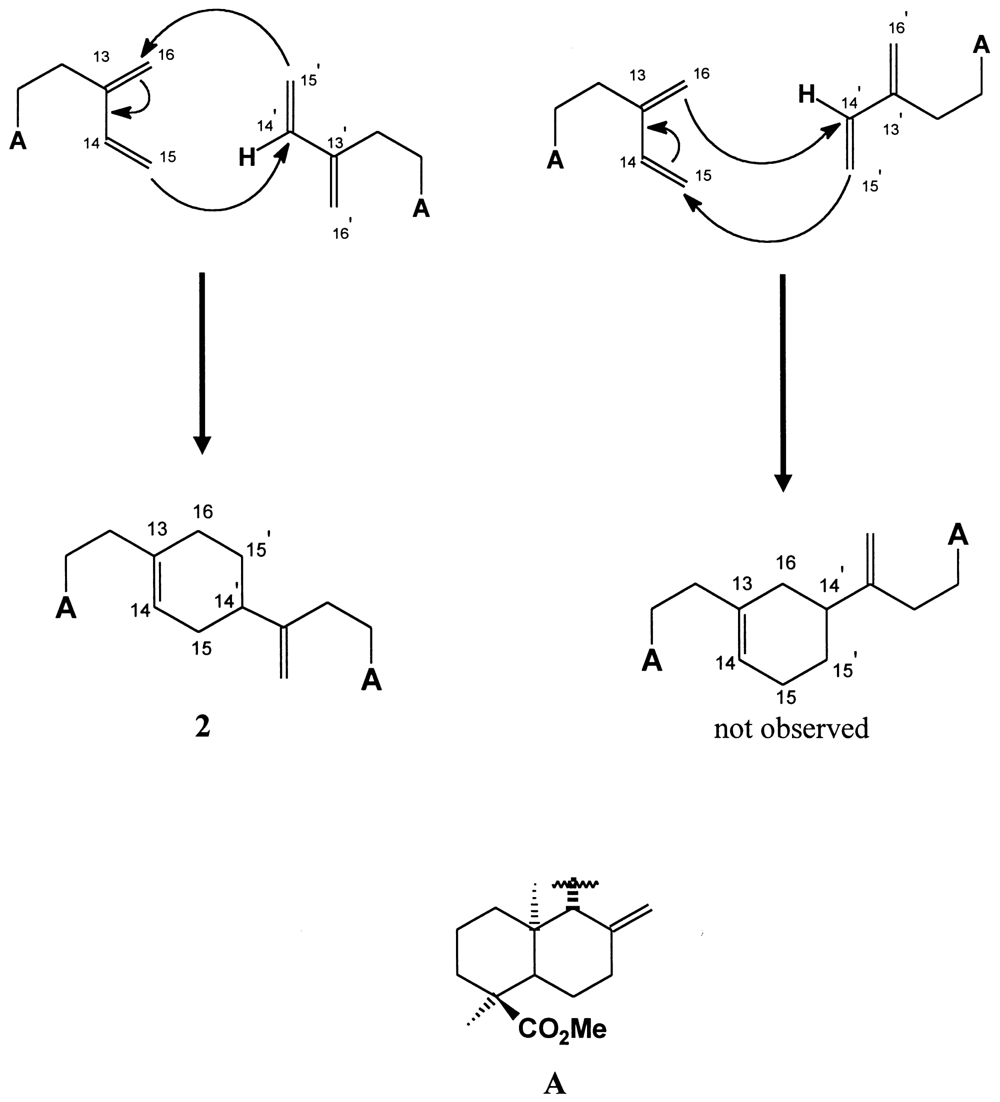
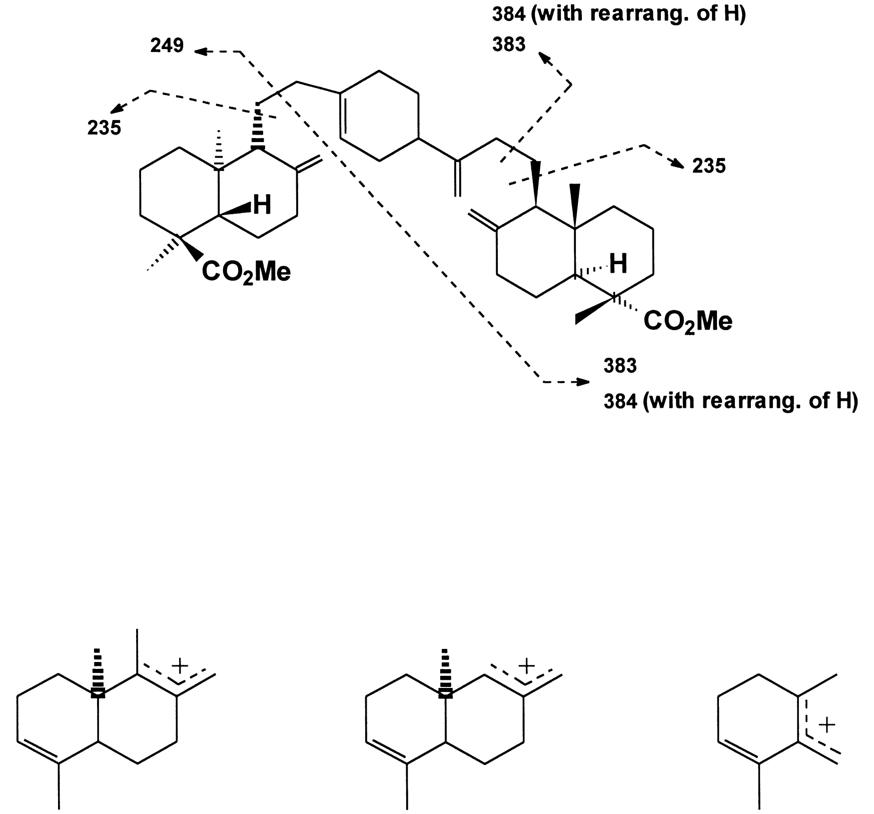


Fig. 1. Proposed formation of dimer **2** by Diels-Alder cyclization.



m/z 189 [249-HCO_2Me]$^+$ *m/z* 175 [235-HCO_2Me]$^+$ *m/z* 121

Fig. 2. Diagnostic EIMS fragment ions (*m*/*z*) of dimer **2**.

CH_3]$^+$ (12), 384 (14), 383 (9), 323 (11), 249 (18), 235 (100), 189 (30), 175 (38), 121 (52); IR ν_{max}^{film} cm^{-1}: 3079, 1729, 1643, 1244, 891; ^1H and ^{13}C NMR (Tables 1 and 2). Found: C, 79.03; H, 9.74. $C_{42}H_{64}O_4$ requires: C, 79.74; H, 10.12%.

3.5. *Ent-13′-nor-13′-oxomethylisoozate dimer (**3**)*

Gum; EIMS 30 eV, *m*/*z* (rel. int.): 634 [M]$^+$ (2), 399 (12), 371 (5), 291 (16), 274 (27), 273 (15), 249 (31), 248 (72), 235 (100), 231 (19), 189 (24), 175 (25), 149 (12), 121 (48); IR ν_{max}^{film} cm^{-1}: 3070, 1720, 1700, 1630, 1240; ^1H and ^{13}C NMR (Tables 1 and 2). Found: C, 76.97; H, 9.76. $C_{41}H_{62}O_5$ requires: C, 77.60; H, 9.80%.

3.6. *Ent-13-epoximethylisoozate dimer (**4**)*

Gum; IR ν_{max}^{film} cm^{-1}: 3080, 1728, 1643, 1245, 914, 891; ^1H and ^{13}C NMR (Tables 1 and 2).

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