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Diterpene adducts from branches of *Xylopia emarginata*☆

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1. Subject and source

The branches of *Xylopia emarginata* (2300 g) were collected in August 1995 in Campo Grande, Mato Grosso do Sul State, Brazil. The plant material was identified by Prof. Renato Mello Silva from the Instituto de Biociências, Universidade de São Paulo, in which herbarium (SPF) a voucher specimen (number 101499) has been deposited.

In the present work, the crude CH_2Cl_2 extract from branches of *X. emarginata* was submitted to acid/base extraction and then chromatographed over silica gel to give a mixture of two diterpene adducts which were characterized by spectrometric analysis, mainly mass spectrometry and NMR, and comparison with literature data of model compounds.

2. Previous work

X. emarginata is a large tree which grows in Brazil in Amazonas, Mato Grosso and Mato Grosso do Sul States (Pio Correa, 1984). This species has been extensively studied in which several terpenoids, flavonoids and alkaloids have been isolated from the leaves and fruits (Moreira et al., 2003; Brochini et al., 1999). Besides these compounds, diterpene Diels–Alder adducts were also detected in the stem bark (Vilegas et al., 1991).

3. Present study

Dried and powdered branches (530 g) were exhaustively extracted with CH_2Cl_2, yielding, after solvent evaporation *in vacuo*, 8.0 g of crude extract. This extract was dissolved in hexane and extracted with NaOH 10% aqueous solution

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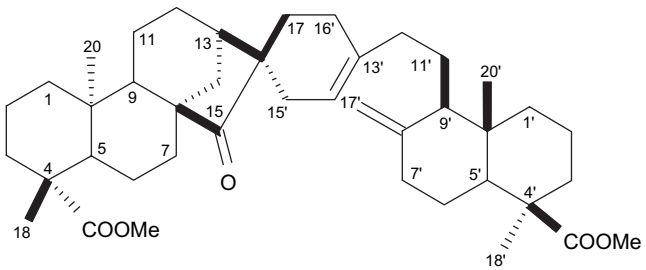
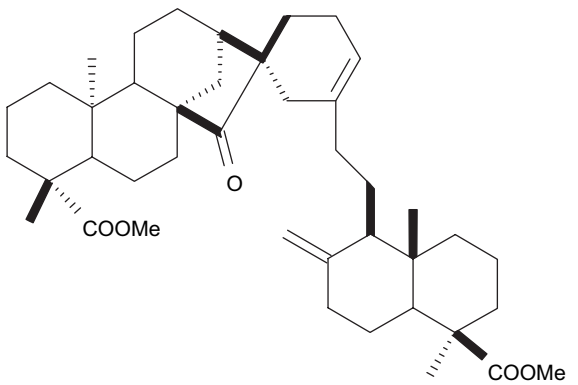
to yield hexane and NaOH/H_2O phases. The alkaline aqueous phase was acidified with HCl 10% and then partitioned between H_2O and CH_2Cl_2. The organic phase was methylated with diazomethane and chromatographed on silica gel eluted with CH_2Cl_2 to give four fractions (A–D). Fraction C (145 mg) was subjected to CC on silica gel eluted with hexane:CH_2Cl_2 1:1 to yield 25 mg of mixture composed of **1** + **2** (yield 0.31%) (Fig. 1).

The mixture was analyzed by LRESIMS which showed a molecular ion peak at *m/z* 647 [M + H]. These data, associated to TLC chromatographic profile, which showed one spot in several elution systems, and GC/LREIMS, indicated the presence of two compounds with similar fragmentation patterns, suggesting the occurrence of isomeric derivatives. The ^{13}C spectra (BBD and DEPT 135°) of the mixture showed 46 signals corresponding to the carbons of compounds **1** and **2**, and were analyzed following the methodology described for the identification of triterpenes in mixtures (Galegos-Olea and Roque, 1990). These spectra showed the presence of carbonyl group at δ 227.4 and carboxyl groups at δ 177.8 and 177.7, which were confirmed by analysis of IR spectrum of mixture ($\nu_{C=O}$ at 1637 and 1712 cm^{-1}). Besides these signals, sp^2 carbons were observed at δ 148.0 (C), 137.9 (C), 135.4 (C), 120.2 (CH), 117.7 (CH) and 106.4 (CH_2). These data in association with the LRESIMS and GC/LREIMS suggested a mixture of diterpene adducts similar to those previously described in the stem bark of *Xylopia amazonica* (Vilegas et al., 1991) (**M-1** and **M-2**) (Fig. 2 and Table 1). A comparison between the ^{13}C NMR chemical shifts of these reported compounds and of **1** and **2** showed high similarity, except for the signals assigned to the carbons C-3′, C-4′, C-5′, C-7′, C-11′, C-12′, C-16′, C-18′, C-19′ and C-20′. Furthermore, the signal attributed to C-20′ was observed at δ 12.6 showing a shielding of 2.1 ppm when compared with those of **M-1** and **M-2**, which could be due to diamagnetic effect caused when the carboxyl group was located at the same side of C-20′ (β-position). The comparison of the ^{13}C NMR data of **1** and **2** with those reported to the methyl 15-aldo-labd-8(17)-en-19-oate (Zdero et al., 1991) (**M-3**) which presented the carboxyl group at axial position (C-19), confirmed the proposed structure. This was also confirmed by ^1H NMR analysis whose spectrum showed a shielded signal at δ 0.50 assigned to H-20′. Therefore, the only difference between **1**, which was named emarginatine A, and **M-1**, consisted in the axial positioning of the carboxyl group in the labdane moiety. Using this comparison procedure, all the carbon atoms of compound **1** should be assigned as showed in Table 1.

As the ^{13}C NMR spectra also showed signals at δ 135.4 (C) and 120.2 (CH), these could be attributed to an isomeric derivative of diterpene adduct **1**, as observed to compounds **M-1** and **M-2**. As proposed previously (Vilegas et al., 1991),



1



2

Fig. 1. Diels–Alder adducts **1** and **2** isolated from the branches of *Xylopia emarginata*.

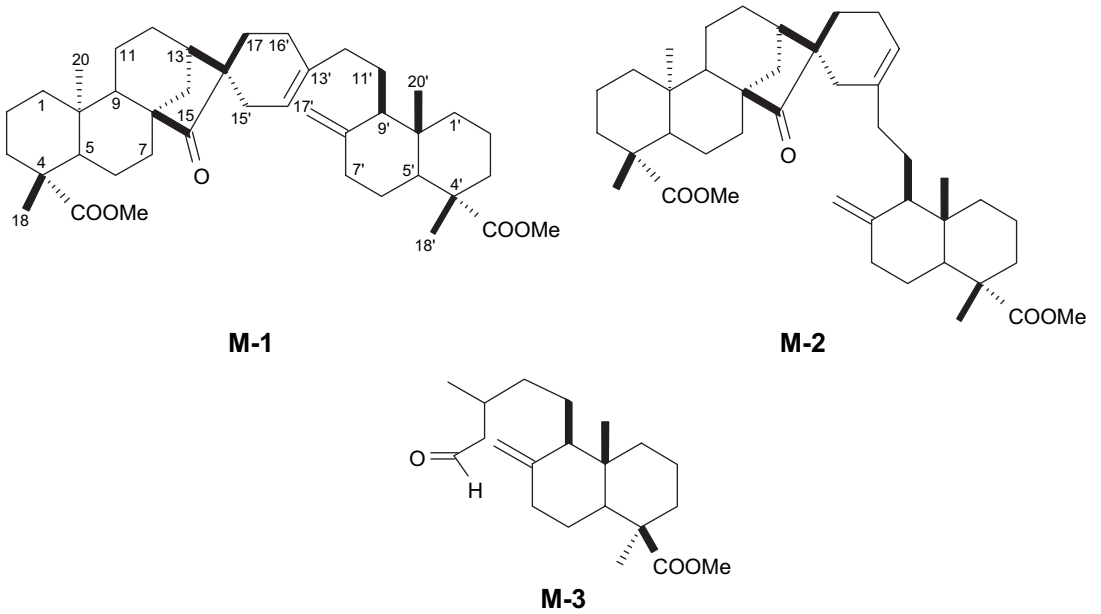


Fig. 2. Model compounds **M-1**, **M-2** and **M-3** used in the structure elucidation of compounds **1** and **2**.

in the case of **1** the precursors were labda-8(17),13,15-trien-19-oic acid and 15-oxo-kaur-16-en-19-oic acid, this last one isolated from leaves of *X. emarginata* (Moreira et al., 2003), which could be conducive to the formation of two major Diels–Alder diterpene adducts (Vilegas et al., 1991). Therefore, the comparison of spectral data to model derivatives and those reported here to compound **2**, confirmed its structure to be the same as that of **M-2** but containing the carboxyl group at axial position (C-19′), as observed to **1**. This proposal could be confirmed by analysis of LRESIMS and LREIMS after chromatographic separation of **1** and **2** due to similar fragmentation pattern observed for both compounds. Thus, **2** was named emarginatine B. All these data are in full accordance with those published for other labdane–kaurane derivatives (Vilegas et al., 1991; Hasan et al., 1985).

*3.1. Mixture of emarginatine A (**1**) and emarginatine B (**2**)*

White amorphous solid. LRESIMS (positive mode): *m/z* 647 [M + H]. IR ν_{max} (KBr) cm^{-1} (mixture): 2971, 1712, 1637, 1438, 1024, 981.

*3.2. Emarginatine A (**1**)*

LREIMS 70 eV, *m/z* (rel. int.): 330 [Retro-Diels–Alder (RDA)] (3), 316 [RDA] (3), 301 (7), 300 (4), 299 (5), 286 (4), 285 (5), 274 (23), 273 (12), 271 (7), 270 (4), 257 (7), 256 (4), 255 (8), 235 (20), 241 (7), 227 (6), 215 (27), 213 (13), 199 (13), 189 (22), 175 (21), 173 (20), 149 (29), 121 (100), 109 (58), 107 (69), 81 (65), 79 (51); ^{1}H NMR (300 MHz, $CDCl_3$): 0.50 (s, H-20′), 0.89 (s, H-20), 1.19 (s, H-18′), 1.23 (s, H-18), 3.61 (s, OMe), 3.65 (s, OMe), 4.51 (s, H-17′), 4.82 (s, H-17′), 5.25 (br s, H-14′); ^{13}C NMR (75 MHz, $CDCl_3$) (see Table 1).

*3.3. Emarginatine B (**2**)*

LREIMS 70 eV, *m/z* (rel. int.): 330 [RDA] (4), 316 [RDA] (3), 301 (9), 300 (5), 299 (7), 286 (6), 285 (5), 274 (29), 273 (14), 271 (4), 270 (6), 257 (9), 256 (4), 255 (9), 241 (6), 235 (23), 227 (8), 215 (31), 213 (16), 199 (12), 189 (26), 175 (22), 173 (21), 149 (28), 121 (100), 109 (61), 107 (72), 81 (64), 79 (53); ^{1}H NMR (300 MHz, $CDCl_3$): 0.50 (s, H-20′), 0.89 (s, H-20), 1.19 (s, H-18′), 1.23 (s, H-18), 3.61 (s, OMe), 3.67 (s, OMe), 4.47 (s, H-17′), 4.82 (s, H-17′), 5.40 (br s, H-14′); ^{13}C NMR (75 MHz, $CDCl_3$) (see Table 1).

Table 1
^{13}C NMR chemical shifts of compounds **1** and **2** and model derivatives **M-1**, **M-2** and **M-3** (75 MHz, δ, $CDCl_3$)

Position	1	2	M-1	M-2	M-3
1	39.7	39.7	39.7	39.7	–
2	19.1	19.1	19.1	19.1	–
3	37.9	37.9	37.9	37.9	–
4	43.7	43.7	43.7	43.7	–
5	56.0	56.0	56.0	55.9	–
6	26.7	26.7	26.7	26.7	–
7	32.6	32.6	32.6	32.6	–
8	53.5	53.5	53.5	53.5	–
9	51.1	51.1	50.0	50.0	–
10	39.9	39.9	39.8	39.8	–
11	18.8	18.8	18.8	18.8	–
12	35.0	35.0	34.9	34.9	–
13	36.5	36.5	35.4	35.4	–
14	34.9	34.9	34.9	34.9	–
15	227.4	227.4	227.4	227.4	–
16	51.7	50.4	51.7	50.4	–
17	22.9	25.2	22.9	25.1	–
18	28.7	28.7	28.6	28.6	–
19	177.8	177.8	179.3	179.3	–
20	15.1	15.1	15.1	15.1	–
1′	38.2	38.2	38.1	38.1	38.1
2′	19.9	19.9	18.5	18.5	19.9
3′	39.2	39.2	36.8	37.1	39.1
4′	44.3	44.3	47.7	47.7	44.2
5′	56.4	56.4	50.0	50.0	56.3
6′	26.3	26.3	26.7	26.7	26.1
7′	36.2	36.2	37.8	37.8	36.0
8′	148.0	148.0	147.8	147.8	148.1
9′	56.3	56.3	56.3	55.9	56.2
10′	40.2	40.2	39.8	39.8	40.3
11′	20.3	20.3	21.1	21.1	21.1
12′	38.8	38.8	36.7	36.0	38.7
13′	135.4	137.9	135.2	137.9	28.8
14′	120.2	117.7	120.2	117.4	50.7
15′	26.7	26.7	26.8	26.8	203.0
16′	20.3	20.3	25.0	26.0	20.1
17′	106.4	106.4	106.9	106.9	106.2
18′	28.8	28.8	179.3	179.3	28.7
19′	177.7	177.7	16.3	16.5	177.7
20′	12.6	12.6	14.7	14.7	12.5
OMe	51.7/51.2	51.7/51.2	51.7	51.7	51.1

4. Chemotaxonomic significance

The occurrence of diterpene dimers in *Xylopia* species has previously been reported. Acutifloric acid, the first adduct originating from Diels–Alder reaction between labdane and kaurane derivatives, was isolated from *Xylopia acutiflora* (Zdero et al., 1991). Four other adducts were isolated from *X. amazonica* and *X. emarginata* (Vilegas et al., 1991) in which the authors also isolated the precursor diterpenes 15-oxo-kaur-16-en-19-oic and labda-8(17),13,15-trien-18-oic acids. In order to be sure that adducts were natural and not artifacts, these compounds were submitted to a Diels–Alder reaction, under several conditions. However, no adducts were formed indicating that the isolated compounds were natural products. Although the occurrence of kaurane–labdane derivatives had not been reported in others *Xylopia* species after the works of Hasan et al. (1985) and Vilegas et al. (1991), other types of adducts have also been described. From *Xylopia frutenscens* was isolated from the frutoic acid, which was formed from two kaurane derivatives (Takahashi et al., 1995). Similarly, from *Xylopia aromatica*, two labdane dimers have been described (Martins et al., 1999). In the present work, we describe the occurrence of two other labdane–kaurane

derivatives from *X. emarginata*, in which the dimers were formed by a Diels–Alder reaction between 15-oxo-kaur-16-en-19-oic and labda-8(17),13,15-trien-19-oic. Therefore, since the occurrence of diterpene adducts has been restricted to *Xylopia* genus in Annonaceae, this profile suggests that these compounds should be considered a chemotaxonomic marker in this genus.

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