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FRUTOIC ACID, A DIMERIC KAURANE DITERPENE FROM *XYLOPIA FRUTESCENS*

JACQUELINE A. TAKAHASHI, MARIA AMÉLIA D. BOAVENTURA, JOAQUIM DE CARVALHO BAYMA* and ALAÍDE B. OLIVEIRA†‡

Departamento de Quimica, Instituto de Ciências Exatas, UFMG, Caixa Postal 702, CEP 31270-901, Belo Horizonte, MG-Brazil; *Departamento de Química, Universidade Federal do Pará, Rua Augusto Correa s/n, CEP 6075-110. Belém, Pará-Brazil; †Faculdade de Farmácia, UFMG, Av. Olegário Maciel 2360, CEP 30180-112, Belo Horizonte, MG-Brazil

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Key Word Index—*Xylopia frutescens*; Annonaceae: kaurane diterpenes; diterpenic dimers; frutoic acid.

Abstract—Sitosterol and six diterpenes were isolated from green fruit, stem bark and leaves of *Xylopia frutescens* and characterized as *ent*-15α-acetoxy-kaur-16-en-19-oic acid (xylopic acid), *ent*-kaur-16-en-19-oic acid (kaurenoic acid), *ent*-16β-hydroxy-kaurane, *ent*-kaur-16-en-19-ol, *ent*-16β-hydroxy-kauran-19-oic acid and *ent*-16β,17-dihydroxy-kauran-19-oic acid. Acutifloric acid, a diterpenic dimer, and its 7β-hydroxy-derivative, a novel compound named frutoic acid, were also isolated.

INTRODUCTION

Xylopia frutescens Aubl. is a tree found in central and southern America, Africa and Asia [1]. In Brazil, its seeds are used in folk medicine as a bladder stimulant and against rheumatism, among other uses [2]. The genus *Xylopia* has long been known as a source of kaurane diterpenes [3, 4]. More recently, some diterpenic dimers have been isolated from *Xylopia* species [5, 6]. In this paper we report on a phytochemical study of green fruit, stem bark and leaves of *X. frutescens*. Sitosterol and six known kaurane diterpenes (**1**–**6**) were isolated. Acutifloric acid (**7**), a diterpenic dimer, and its 7β-hydroxy-derivative (**8**), a novel compound, were also isolated. To the latter we have assigned the trivial name, frutoic acid.

RESULTS AND DISCUSSION

Column chromatography of the hexane and ethanol extracts of green fruit, stem bark and leaves of *X. frutescens* over silica gel, eluting with an *n*-hexane, dichloromethane, ethyl acetate and methanol polarity gradient, afforded sitosterol and the kaurane diterpenes *ent*-15α-acetoxy-kaur-16-en-19-oic acid (xylopic acid, **1**), *ent*-kaur-16-en-19-oic acid (kaurenoic acid, **2**), *ent*-16β-hydroxy-kaurane (**3**), *ent*-kaur-16-en-19-ol (**4**), *ent*-16β-hydroxy-kauran-19-oic acid (**5**) and *ent*-16β,17-dihydroxy-kauran-19-oic acid (**5**). These compounds were identified by comparing their spectral data with those reported in the literature [7–9]. Two diterpenic dimers (**7** and **8**) were also isolated. Acutifloric acid (**7**) has already been isolated from *Xylopia acutiflora* [5]. The spectral data of our compound are in accordance with those described in the literature [5]. Frutoic acid (**8**) was isolated from the hexane extract of the stem bark after successive separations by column chromatography over silica gel. Recrystallization from ethanol afforded plates, mp 274–276°. A low-resolution mass spectrum indicated the empirical formula $C_{40}H_{60}O_4$. The IR spectrum indicated the presence of hydroxyl groups (3440 and 3180 cm^{-1}), carbonyl (1740 cm^{-1}), carboxylic acid (1700 cm^{-1}) and an exocyclic double bond (1640 and 880 cm^{-1}). The ^1H NMR spectrum ($CDCl_3$, 360 MHz) revealed five tertiary methyl resonances at δ0.70, 0.80, 0.88, 1.04 and 1.26. The presence of the exocyclic double bond was confirmed by two singlets (1H each) at δ4.68 and 4.86. A one-proton singlet at δ5.49 revealed a third olefinic proton. All these data are in accordance with those reported for acutifloric acid [5]. However, the ^1H NMR spectrum of **8** showed an additional signal at δ4.11 (*dd*, $J = 5.7$ and 9.0 Hz), indicating further hydroxylation in the molecule. This was confirmed by the presence of a signal at δ75.0 in the ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of **8** when compared with the data from acutifloric acid (**7**) [5] (Table 1). The location of the additional hydroxyl group at C-7 was established by changes in the ^{13}C NMR spectrum, as the signal associated with C-6 of the kaurane ring showed a downfield shift, whilst that assigned to the C-7 methylene had been replaced by a secondary alcohol signal at δ75.0. The multiplicity of the secondary alcohol ^1H NMR resonance and the γ-gauche effect over C-5 when compared with

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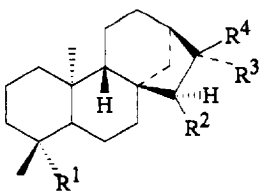
‡Author to whom correspondence should be addressed.

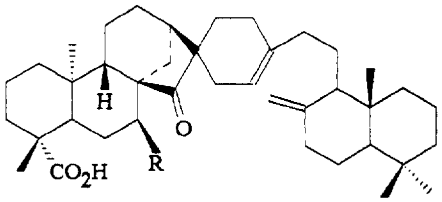
Table 1. ^{13}C NMR data for compounds 7–9 ($CDCl_3$, 125 MHz)

C	7*	8	9†
1	39.7	39.7	39.6
2	19.3	19.1	19.2
3	38.3	38.2	38.2
4	43.6	43.3	43.1
5	56.2	52.8	47.7
6	20.6	26.5	26.4
7	30.6	75.0	72.4
8	52.5	53.6	52.2
9	51.7	51.5	48.1
10	40.0	39.9	39.5
11	18.7	18.7	18.6
12	34.8	34.7	33.9
13	36.6	36.9	35.7
14	35.4	32.5	33.5
15	227.1	228.3	223.2
16	50.4	50.6	51.4
17	25.2	24.7	24.3
18	27.8	28.7	28.3
19	183.5	180.2	177.5
20	15.2	15.2	15.0
1′	39.1	39.2	39.0
2′	19.0	19.0	18.6
3′	42.3	42.2	42.2
4′	33.4	33.4	33.5
5′	55.7	55.7	55.6
6′	24.4	24.2	24.9
7′	37.6	37.7	37.6
8′	148.5	146.9	148.3
9′	55.7	55.9	55.8
10′	39.5	39.1	39.3
11′	21.4	19.2	21.5
12′	36.0	35.3	35.9
13′	138.5	138.8	137.7
14′	117.5	121.8	117.4
15′	26.7	27.6	26.8
16′	26.0	20.0	25.8
17′	106.2	106.0	106.1
18′	33.6	33.5	33.5
19′	21.6	21.4	21.3
20′	14.4	14.4	14.4
OMe	—		51.0
OCOMe	—	—	21.3
OCOMe			170.5

*Ref. [2].
†Ref. [6].



	R^1	R^2	R^3	R^4
(1)	CO_2H	OAc		CH_2
(2)	CO_2H	H		CH_2
(3)	CH_3	H	OH	CH_3
(4)	CH_2OH	H		CH_2
(5)	CO_2H	H	OH	CH_3
(6)	CO_2H	H	OH	CH_2OH



(7) R = H : Acutifloric acid [5]
(8) R = OH : Frutoic acid
(9) R = OAc [6]

7 indicated that 8 had the 7β-equatorial position. The corresponding acetate derivative of 9 has been previously isolated from *Xylopia emarginata* [6]. Its ^{13}C NMR data (Table 1) are in accordance with those reported for 8.

It has been postulated that diterpenic dimers, such as 7 and 8, probably arise from a Diels–Alder cyclization between a kaurane unit and a labdane unit [5]. Vilegas and collaborators [6] have attempted to cyclize these two units under several chemical conditions without success. This is a good indication that these dimers isolated from *Xylopia* species are not artifacts and indeed have a natural origin.

EXPERIMENTAL

General. ^{1}H NMR spectra were determined at 80 and 360 MHz. ^{13}C NMR spectra were determined at 125 MHz. IR spectra were measured as KBr discs. TLC spots were detected by spraying with 2% ceric sulphate soln in 50% H_2SO_4 and heating at 100°. Silica gel for chromatography was Merck 7734.

Plant material. Xylopia frutescens Aubl. was collected in Belém, Pará State, Brazil. A voucher specimen (BHCB 16188) has been deposited at the herbarium of the Instituto de Ciências Biológicas (UFMG), Belo Horizonte, Minas Gerais, Brazil.

Extraction and isolation. Green fruit, stem bark and leaves were dried and ground. Ground fruit (793 g) were successively extracted with hexane and EtOH in a Soxhlet apparatus. From these extracts a yellowish powder precipitated (5.5 g). Selective recrystallization from EtOAc–$CHCl_3$ (9 : 1) afforded 2.1 g of 1 and 1 g of 2. The residue of the hexane extract (40.7 g) was fractionated by silica gel CC with gradient elution starting with hexane and finishing with MeOH–EtOAc (9:1), to give **1** (1.6 g), **2** (4.5 g), **3** (800 mg) and **4** (10 mg) as crystalline compounds. The residue from the EtOH extract of the fruit afforded **6** (44 mg) and an unidentified compound.

Ground stem bark (3.25 kg) was extracted with EtOH in a Soxhlet apparatus. The EtOH extract (67.8 g) was exhaustively refluxed with hexane, giving a soluble portion which was chromatographed on silica gel using the elution gradient described above. An additional 660 mg of **1** and 40.5 mg of **2** were obtained from this CC. Compound **8** (365 mg) and sitosterol (49 mg) were also obtained as crystalline compounds after recrystallization. The residue from the hexane extraction (35 g) was chromatographed on a silica gel column affording an additional 84.2 mg of **3** and 15.6 mg of **5**.

Ground leaves were extracted with EtOH at room temp. The EtOH extract was extracted with CH_2Cl_2. The soluble portion (130.5 g) was treated with active charcoal and then chromatographed on a silica gel column, yielding **8** as a crystalline powder (105 mg).

Frutoic acid (**8**). Plates, mp 274–276° (from EtOH–$CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3850, 3440, 3180, 3160, 2900, 1740, 1700, 1640, 1420, 1150, 880. ^{1}H NMR ($CDCl_3$, 360 MHz): δ0.70 (3H, *s*, 20'-CH_3), 0.80 (3H, *s*, 19'-CH_3), 0.88 (3H, *s*, 18'-CH_3), 1.04 (3H, *s*, 20-CH_3), 1.26 (3H, *s*, 18-CH_3), 4.11 (1H, *dd*, J = 5.7 and 9.0 Hz, H-7), 4.68 (1H, *s*, 17'-H_A), 4.86 (1H, *s*, H-17'$_B$), 5.49 (1H, *s*, 14'-H). $C_{40}H_{60}O_4$ ($[M]^+$ *m/z* 604). ^{13}C NMR ($CDCl_3$, 125 MHz): Table 1.

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