

MONO AND DITERPENES FROM SEEDS OF *XYLOPIA SERICEA*Jacqueline A. Takahashi[#], Henriete S. Vieira, Maria Amélia D. Boaventura

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A monoterpene, 3 β ,6 β -dihydroxy-p-menth-1-ene has been isolated from the seeds of *Xylopi* *sericea* along with four kaurane, one beyerene, one atisene and four trachylobane diterpenoids, including the trachyloban-18- and 19-methyl esters. The X-ray crystal structure of methyl *ent*-trachyloban-18-oate was determined in order to make an unambiguous distinction between the 18- and 19-esters. The ¹³C NMR data for *ent*-15 α -hydroxy-trachyloban-19-oic acid has been revised.

Keywords: monoterpene; kaurane diterpenes; X-ray crystallography.

INTRODUCTION

The genus *Xylopi* (Annonaceae) includes about 160 species and is a well-known source of kaurane and trachylobane diterpenoids¹. In our search for Brazilian plants rich in diterpenoids², which could be used in obtention of compounds with biological activity via microbial biotransformation³, we have carried out a phytochemical investigation of *Xylopi* *sericea* St. Hill. The composition of the essential oil of this plant has been analysed previously⁴. In this paper we report the isolation of ten diterpenoids*, *ent*-kaur-16-en-19-oic acid (**1**), *ent*-15 α -acetoxykaur-16-en-19-oic acid (**2**), *ent*-kauran-16 β -ol (**3**), *ent*-16 β -hydroxykauran-19-oic acid (**4**), methyl *ent*-15-atisene-19-oate (**5**) methyl *ent*-beyer-15-en-19-oate (**6**), and four diterpenes of the *ent*-trachylobane series, *ent*-15 α -hydroxytrachyloban-19-oic acid (**7**), its acetylated methyl ester (**8**), *ent*-trachyloban-19-oic acid (**9**), its methyl ester (**10**) and methyl *ent*-trachyloban-18-oate (**11**).

In order to make an unequivocal distinction between compounds **10** and **11**, the later was analysed by X-ray crystallography. A monoterpene, 3 β ,6 β -dihydroxy-p-menth-1-ene (**12**) was also isolated.

EXPERIMENTAL

General experiments methods

¹H NMR spectra were determined at 360 or 400 MHz for solutions in deuteriochloroform or deuterio-pyridin. ¹³C NMR and 2D NMR spectra were determined at 100 or 125 MHz. TMS was used as an internal standard. Mass spectra were determined at 70 eV. IR spectra were measured using KBr discs. TLC spots were detected by spraying with 2% ceric sulfate soln. in 50% sulfuric acid and heating at 100°C. Silica gel for chromatography was Merck 7734.

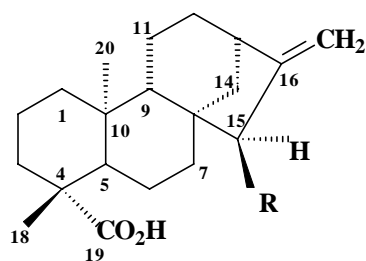
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* Substituents which are above the plane of this paper are referred to as β and those which are below this plane are referred to as α . The function of the *ent* prefix is to invert the stereochemical descriptor in the name that follows. Systematically, then, these substituents are termed *ent*- α and *ent*- β respectively⁵.

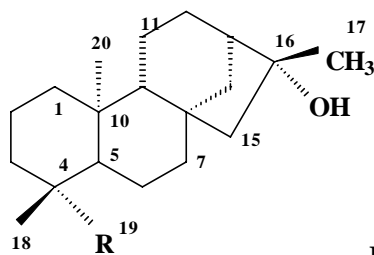
Extraction and isolation

Seeds (3 kg) of *Xylopi* *sericea* were collected in Caratinga, Minas Gerais State, Brazil. After dried, they were powdered and repeatedly extracted with hexane in a Soxhlet apparatus. The hexane extract (105 g) was subjected to column chromatography on silica gel in hexane by use of an increasing gradient of ethyl acetate and methanol. The less polar fractions containing crystalline material were further purified by column chromatography on silica using the same solvent system to give *ent*-kaur-16-en-19-oic acid (**1**, 1.11 g), *ent*-15 α -acetoxykaur-16-en-19-oic acid (**2**, 0.42 g), *ent*-kauran-16 β -ol (**3**, 0.015 g), and the major constituent *ent*-trachyloban-19-oic acid (**9**, 11.4 g). These compounds were identified by their m.p., IR and NMR data. One fraction containing a mixture of compounds was esterified with ethereal diazomethane and further submitted to column chromatography on silica (petrol/ethyl acetate 9:1) to give compounds methyl *ent*-15-atisene-19-oate (**5**, 0.045 g), methyl *ent*-beyer-15-en-19-oate (**6**, 0.021 g) and methyl *ent*-15 α -acetoxytrachyloban-19-oate (**8**, 0.023 g). In another fraction it was detected, by NMR, together with **9**, another compound that showed the same R_f value, both cocrystallized, the former being the major component of the mixture (c. 95%). After several unsuccessful attempts of purification by column chromatography (Si gel and Si gel impregnated with AgNO₃), this fraction was treated with ethereal diazomethane and the esters mixture was recrystallized with ethanol/chloroform (9:1). Two kind of crystals were detected and they were carefully separated by hand in accordance with their different morphology and successively recrystallized. Using this procedure, 0.82 g of methyl *ent*-trachyloban-19-oate (**10**) (plates) were obtained as well as 0.07 g of methyl *ent*-trachyloban-18-oate (**11**) (thin needles). The methyl ester **11**, m.p. 107-109°C (lit.¹⁰ 112°C), was analysed by X-ray crystallography.

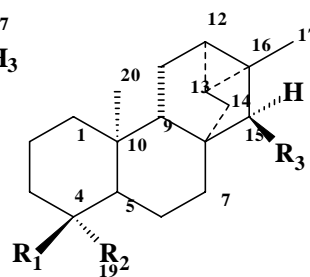
3 β ,6 β -dihydroxy-p-menth-1-ene (**12**, 0.11 g) was isolated from the fractions eluted with hexane/ethyl acetate (1:1) and identified by its NMR spectra. The same fractions gave *ent*-16 β -hydroxykauran-19-oic acid (**4**, 0.11g) and *ent*-15 α -hydroxytrachyloban-19-oic acid (**7**, 0.32g), the last as a powder, m.p. 265-267 °C (from EtOH), IR $\nu_{\max}/\text{cm}^{-1}$: 3450, 3200, 2900, 1700, 1500, 1300, 1150, 700.



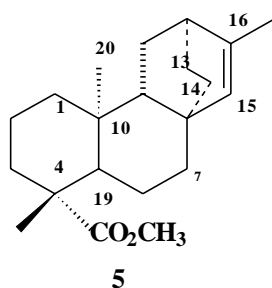
1 R = H
2 R = OAc



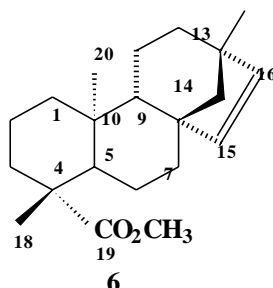
3 R = CH₃
4 R = CO₂H



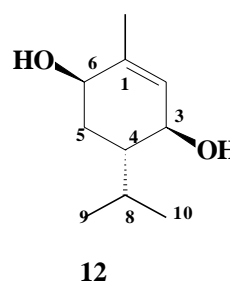
	R ₁	R ₂	R ₃
7	CH ₃	CO ₂ H	OH
8	CH ₃	CO ₂ CH ₃	OAc
9	CH ₃	CO ₂ H	H
10	CH ₃	CO ₂ CH ₃	H
11	CO ₂ CH ₃	CH ₃	H



5



6



12

¹H NMR (C₅D₅N, 360MHz): δ_H 0.62 (1H, m, H-12), 0.76 (1H, dd, *J* = 3.0 and 7.5 Hz, H-13), 0.97 (3H, s, H-20), 1.16 (H, s, H-17), 1.18 (3H, s, H-18), 3.39 (1H, s, H-15). ¹³C NMR (C₅D₅N, 125MHz): see Table 1. EIMS: *m/z*: 318 (M⁺, C₂₀H₃₀O₃), 300, 285, 261, 151, 123, 105, 91, 81, 41.

Table 1. ¹³C NMR data for compounds **7**, **10** and **11**. (50 MHz, CDCl₃)

compound	7 ¹²	7	10	11
carbon n ^o				
1	39.7	39.3	39.5	38.4
2	18.3	18.2	18.7	17.3
3	38.4	38.3	38.1	37.7
4	43.5	43.6	43.7	47.4
5	56.6	56.2	57.0	51.7
6	19.2	18.7	21.8	22.4
7	36.7	36.4	39.2	38.3
8	46.0	40.9	40.7	40.8
9	42.3	42.2	52.7	53.2
10	41.2	37.9	38.6	36.8
11	22.0	21.3	19.7	19.5
12	20.0	19.8	20.5	20.5
13	20.9	21.2	24.2	24.2
14	30.8	30.3	33.1	33.4
15	82.7	83.7	50.3	50.3
16	25.3	25.0	22.4	22.9
17	18.5	17.9	20.5	20.5
18	28.9	28.6	28.7	179.4
19	179.8	177.9	178.1	16.5
20	13.0	12.6	12.3	14.9
21			51.1	50.5

Crystallographic data and structure determination for compound **11** C₂₁H₃₂O₂ M_r 316.47, orthorhombic, space group P2₁2₁2₁ (No. 19), a=6.236 (2), b=11.658 (3), c=25.042 (8) Å, α = β = γ = 90°, v=1820.5 (9) Å³, Z=4, D_{calc}=1.16 g.cm⁻³, F(000) 696, monochromated Mo-Kα radiation λ=0.71073 Å, μ = 0.07 mm⁻¹. Data were collected using a crystal of size 0.40 x 0.20 x 0.15 mm on an Enraf-Nonius CAD4 diffractometer. A total of 1872 reflections were collected for 2 < θ < 25° and 0 < h < 7, 0 < k < 13, 0 < l < 29. 1170 reflections with I > 2σ (I) were used in the refinement. There was no crystal decay and no absorption correction was applied. The structure was solved by direct methods using SHELXS-86 and SHELX-93^{6,7}. The non-hydrogen atoms were refined anisotropically by full matrix least squares. Hydrogen atoms were included in riding mode with U_{iso} = 1.2U_{eq}(C) or 1.5U_{eq}(C) for methyl groups. The final R indices [I > 2σ (i)] were R₁ = 0.058 and wR₂ = 0.120 and R indices (all data) were R₁ = 0.109, wR₂ = 0.146. The maximum shift/esd was 0.006. The tables of crystallographic data have been deposited with the Cambridge Crystallographic Data Centre.

RESULTS AND DISCUSSION

The hexane extract of the seeds of *X. sericea* was subjected to column chromatography on silica gel eluting with a gradient of solvents of increasing polarity from n-hexane and ethyl acetate to methanol. Further purification by silica column chromatography of the crude fractions led to the isolation of compounds **1-4**, **7** and **9** (major constituent). One impure fraction was esterified with ethereal diazomethane to afford, after chromatography on silica gel column, compounds **5**, **6** and **8**. The spectroscopic data of these compounds are in accordance with the literature^{8,9,10,11}, but there were found some differences in the ¹³C NMR data obtained for compound **7**, when compared with the work of Harrigan and collaborators¹². The tricyclic skeleton of compound **7** was confirmed by signals at δ_H 0.62 and 0.76 (H-12 and H-13, respectively)⁹. The presence of

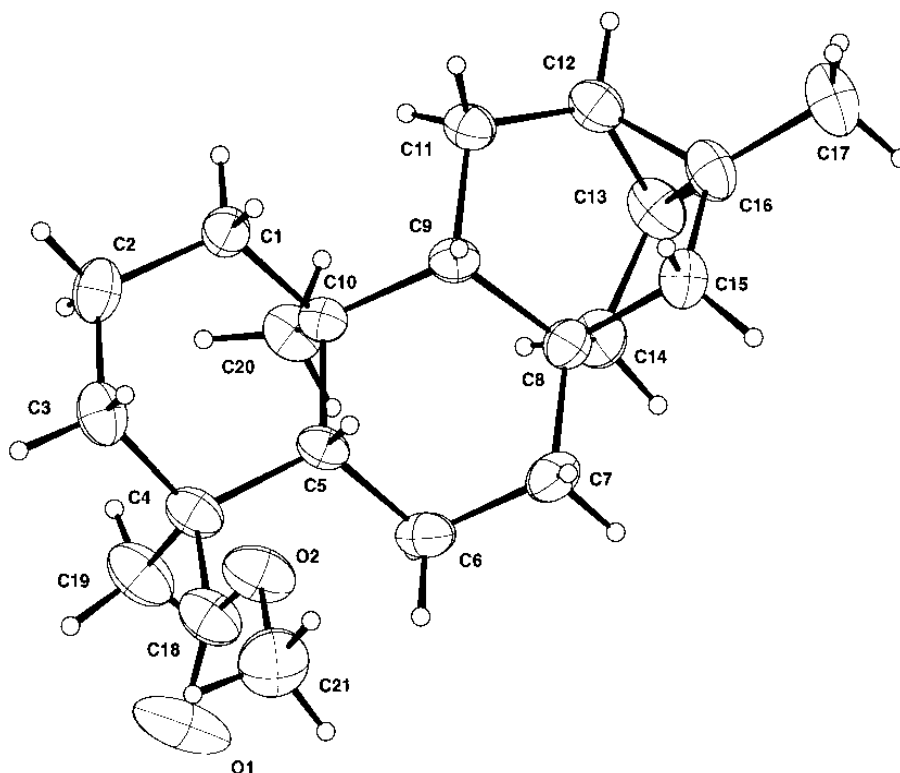


Figure 1. X-ray crystal structure of compound 11

a secondary alcohol was revealed by signals at δ_{H} 3.39 and δ_{C} 83.7 in the ^1H and ^{13}C NMR spectra. The location of the hydroxyl group at C-15 was confirmed by the observation of an n.O.e. enhancement of H-15 (4.9 %) on irradiation of the H-17 methyl group resonance (δ_{H} 1.16). This also gave an n.O.e. enhancement to the cyclopropane signals (H-12 and H-13). The stereochemistry of this hydroxyl group was confirmed by the γ -gauche shift for C-9. However, the position of the signal assigned to C-8 (reported δ_{C} 46.0,¹² found 40.9) was different (see Table 1). Careful inspection of the ^{13}C NMR spectrum for compound 7 revealed four quaternary non-oxygenated carbon signals at δ_{C} 25.0, 37.9 (co-incident with a methylene signal), 40.9 and 43.6. These were assigned to carbons 16, 10, 8 and 4, respectively by comparison with the carbon assignments for *ent*-trachyloban-19-oic acid (9). We believe that the co-incident signal at δ_{C} 37.9 may not have been noticed previously as the earlier assignment was carried out on a mixture of two diterpenes. In fact the data reported for C-8 of compound 6 might fit better for C-8 of the other component of the mixture, 16 β -hydroxy-kaur-16-en-19-oic acid (4).

A fraction containing compound 9, together with a further compound with the same R_f value, was also obtained. Attempts at their separation by several chromatographic systems, including silica gel/AgNO₃ column chromatography, were not successful. Purification was only achieved after esterification followed by recrystallization from ethanol/chloroform (9:1) and careful manual separation of the two types of crystals present in this sample. This procedure was repeated several times to afford 820 mg of compound 10 (plates) together with another substance (compound 11), which crystallized as thin needles. The ^1H NMR spectrum of compound 11 contained three methyl signals at δ_{H} 0.94, 1.11 and 1.12. The presence of an ester group was revealed by a signal at δ_{C} 179.4 in the ^{13}C NMR spectrum. A downfield shift for the signal assigned to C-4 led us to consider that the compound was the 4 β -epimer of 10¹³. The configuration at C-4 was confirmed by the X-ray crystal structure of 11 (see Figure 1). A comparison of the ^{13}C NMR data for compounds 10 and 11 is given in Table 1.

A monoterpene, identified as 3 β ,6 β -dihydroxy-p-menth-1-ene (12) was isolated from the fractions eluted with hexane:ethyl

acetate (1:1). Compound 12 was isolated previously from *Eupatorium erythropappum*¹⁴. The isolation of this monoterpene from *Xylopia* species has not been described previously.

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