MONO AND DITERPENES FROM SEEDS OF *XYLOPIA SERICEA*

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A monoterpene, 3β,6β-dihydroxy-p-menth-1-ene has been isolated from the seeds of *Xylopia 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 ^{13}C NMR data for *ent*-15α-hydroxy-trachyloban-19-oic acid has been revised.

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

INTRODUCTION

The genus *Xylopia* (Annonaceae) includes about 160 species and is a well-known source of kaurane and trachylobane diterpenoids[1]. In our search for Brazilian plants rich in diterpenoids[2], which could be used in obtention of compounds with biological activity via microbial biotransformation[3], we have carried out a phytochemical investigation of *Xylopia sericea* St. Hill. The composition of the essential oil of this plant has been analysed previously[4]. In this paper we report the isolation of ten diterpenoids*, *ent*-kaur-16-en-19-oic acid (**1**), *ent*-15α-acetoxykaur-16-en-19-oic acid (**2**), *ent*-kauran-16β-ol (**3**), *ent*-16β-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*-15α-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, 3β,6β-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 deutero-pyridin. ^{13}C 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.

Extraction and isolation

Seeds (3 kg) of *Xylopia 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*-15α-acetoxy-kaur-16-en-19-oic acid (**2**, 0.42 g), *ent*-kauran-16β-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-b*eyer-15-en-19-oate (**6**, 0.021 g) and methyl ent-15α-acetoxytrachyloban-19-oate (**8**, 0.023 g). In another fraction it was detected, by NMR, together with **9**, another compound that showed the same Rf 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 $AgNO_3$), 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.

3β,6β-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-15α-hydroxytrachyloban-19-oic acid (**7**, 0.32g), the last as a powder, m.p. 265-267 °C (from EtOH), IR ν_{max}/cm^{-1}: 3450, 3200, 2900, 1700, 1500, 1300, 1150, 700.

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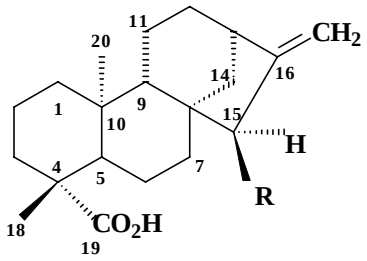
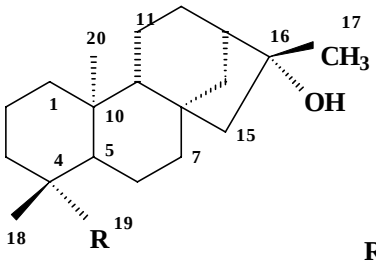
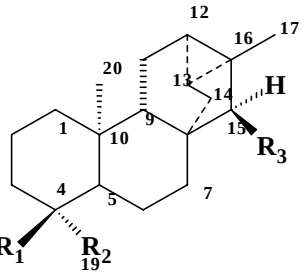
* Substituents which are above the plane of this paper are referred to as β and those which are below this plane are referred to as α. The function of the ent prefix is to invert the stereochemical descriptor in the name that follows. Systematically, then, these substituents are termed ent-α and ent-β respectively[5].



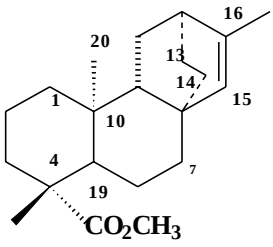
1 R = H
2 R = OAc



3 R = CH_3
4 R = CO_2H



	R_1	R_2	R_3
7	CH_3	CO_2H	OH
8	CH_3	CO_2CH_3	OAc
9	CH_3	CO_2H	H
10	CH_3	CO_2CH_3	H
11	CO_2CH_3	CH_3	H



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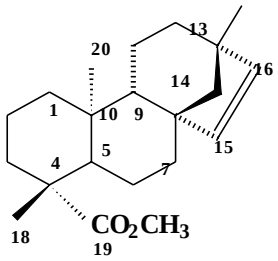

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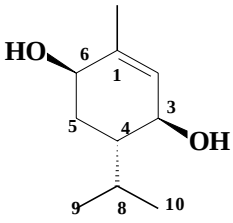

12

^{1}H NMR (C_5D_5N, 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). ^{13}C NMR (C_5D_5N, 125MHz): see Table 1. EIMS: *m/z*: 318 (M^+, $C_{20}H_{30}O_3$), 300, 285, 261, 151, 123, 105, 91, 81, 41.

Table 1. ^{13}C NMR data for compounds **7**, **10** and **11**. (50 MHz, $CDCl_3$)

compound	**7**[12]	**7**	**10**	**11**
carbon n°				
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** $C_{21}H_{32}O_2$ M_r 316.47, orthorhombic, space group $P2_12_12_1$ (No. 19), a=6.236 (2), b=11.658 (3), c=25.042 (8) A, $\alpha = \beta = \gamma = 90^o$, v=1820.5 (9) A^3, Z=4, $D_{calc.}$=1.16 g.cm^3, F(000) 696, monochromated Mo-Kα radiation λ=0.71073 A, 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 $2 < \theta < 25^o$ and $0 < h < 7$, $0 < k < 13$, $0 < l < 29$. 1170 reflections with $I > 2\sigma$ (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 [$I > 2\sigma$ (i)] were R_1 = 0.058 and wR_2 = 0.120 and R indices (all data) were R_1 = 0.109, wR_2 = 0.146. The maximum shift/esd was 0.006. The tables of crystalographic 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 ^{13}C NMR data obtained for compound **7**, when compared with the work of Harrigan and collaborators[12]. The trachylobane 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

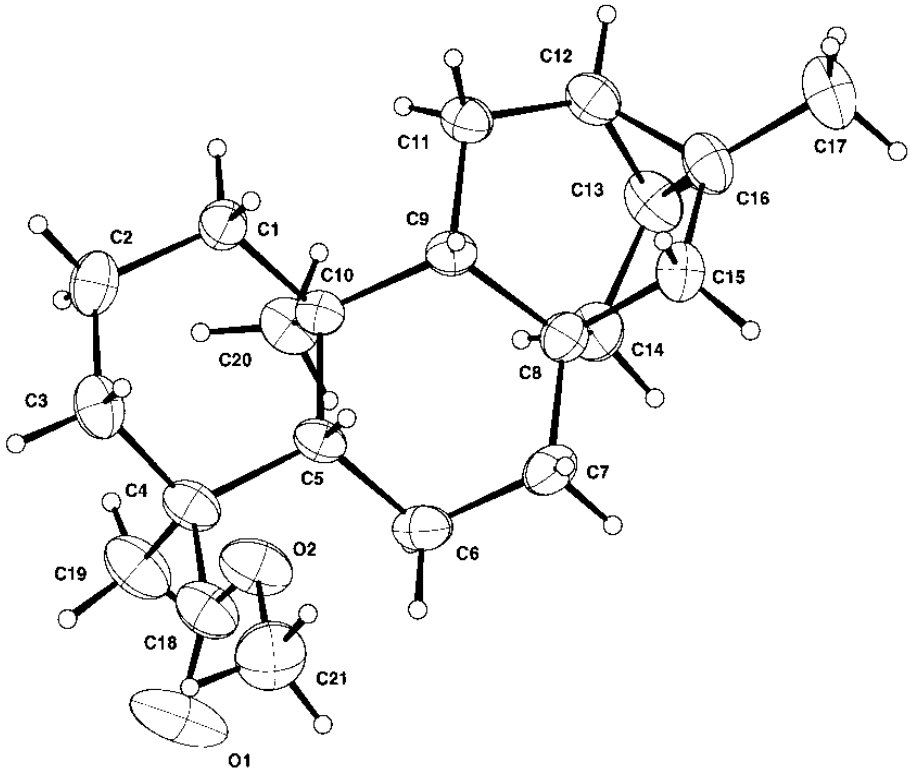

Figure 1. *X-ray crystal structure of compound* ***11***

a secondary alcohol was revealed by signals at δ_H 3.39 and δ_C 83.7 in the ^{1}H 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, 16β-hydroxy-kaur-16-en-19-oic acid (**4**).

A fraction containing compound **9**, together with a further compound with the same Rf value, was also obtained. Attempts at their separation by several chromatographic systems, including silica gel/$AgNO_3$ column chromatography, were not successful. Purification was only achieved after esterification followed by recrystalization 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 ^{1}H 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 4β-epimer of **10**[13]. 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 3β,6β-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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REFERENCES

1. Faulkner, D. F.; Lebby, V.; Waterman, P. G.; *Planta Medica* **1985**, *4*, 354.
2. Takahashi, J. A.; Boaventura, M. A. D.; Bayma, J. C.; Oliveira, A. B.; *Phytochemistry* **1995**, *40*, 607.
3. Boaventura, M. A. D.; Oliveira, A. B.; Hanson, J. R.; Hitchcock, P. B.; Takahashi, J. A .; *Phytochemistry* **1995**, *40*, 1667.
4. Craveiro, A. A.; Alencar, J. W.; Vostrowsky, O.; *J.Nat.Prod.* **1986**, *49* , 1146.
5. Croft, K. G. D.;Ghisalberti, E. L; Jefferies, P. R.; Knox, J. R.; Mahoney, T. J.; Sheppard, P. N.; *Tetrahedron* **1974**, *30*, 3663.
6. Sheldrick, G. M.; *SHELXS-86 Program for the Solution of Crystal Structures* 1985, University of Gottingen, Germany.
7. Sheldrick, G. M.; *SHELXS-93 Program for Crystal Structure Refinment* 1993, University of Gottingen, Germany.
8. Dictionary of Terpenoids, ed. J.D.Connoly and R.A.Hill, Chapman and Hall, London, 1993.
9. Fraga, B. M.; *Phytochem. Anal.* **1994**, *5*, 49.
10. Coates, R. M.; Bertram, E. F.; *J. Org. Chem.* **1971**, *36*, 2625.
11. Grande, M.; Mancheño, B.; Sanchez, M. J.; *Phytochemistry* **1991**, *30*, 1977.
12. Harrigan, G. G.; Bolzani, V. S.; Gunatilaka, A. A. L.; Kingston, D. G. I.; *Phytochemistry* **1994**, *36*, 109.
13. Hugel, G.; Lods, L.; Mellor, J. M.; Theobald, D. W.; Ourisson, G.; *Bull. Soc. Chim. France* **1965**, *10*, 2882.
14. Talapatra, S. K.; Pal, M. K.; Mallik, A. K.; Talapatra, B.; *J. Indian Chem. Soc.* **1985**, *42*, 999.