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Short Report

A New Pentacyclic Triterpene Isolated from *Myroxylon balsamum* (syn. *Myroxylon peruiferum*)

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Um novo triterpeno pentacíclico, o 11α-metoxi-β-amirina (**1**) foi isolado de *Myroxylon balsamum* (L.) Harms (sin. *Myroxylon peruiferum* L.f.). A estrutura desta substância foi elucidada pela análise de seus dados de IV, EM, RMN ^{1}H e ^{13}C. RMN bidimensional foi também utilizada para definir a estrutura e atribuir os deslocamentos químicos dos átomos de hidrogênio e carbono do novo triterpeno.

The new pentacyclic triterpene, 11α-methoxy-β-amyrin (**1**), was isolated from *Myroxylon balsamum* (L.) Harms (syn. *Myroxylon peruiferum* L.f.). Its structure was established on the basis of IR, MS, ^{1}H NMR, ^{13}C NMR. 2D NMR experiments were also used to establish the structure and the hydrogen and carbon chemical shift assignments of the new triterpene.

Keywords: *Myroxylon balsamum (Myroxylon peruiferum)*, Fabaceae, 3β-hydroxy-11α-methoxyolean-12-ene

Introduction

Myroxylon balsamum (L.) Harms (syn. *Myroxylon peruiferum* L.f.), family Fabaceae, is a large size tree, with white flowers, winged and aromatic fruits. Its dispersion is wide, being spread from the South of Mexico until the North of Argentina. In Brazil, its distribution includes a vast range of the forest region of the country, being very common in the States of Bahia, Paraná and Mato Grosso. It is known as "cabriuna" and "cabriúna - vermelha"[1].

The red-brown wood shows fine stripes, is a little rough and has a peculiar scent due to essential oil. It is a heavy wood and is resistant to deterioration, being widely used in carpentry. The hurtled log supplies a well-known exudate known as balsam of Peru or Tolu or a red oil which is quite rich in vanilla. The oil was used formerly in popular medicine as expectorant for breathing affections and as a sedative in cases of cystitis. Now its use is limited to the perfumery industry and as sedative pills for coughs[1-2]. Chemically, the balsam is a mixture of free acids, especially benzoic and cinnamic, and benzyl benzoate. This plant also furnishes a resinous fraction containing monoterpenoids, sesquiterpenoids, alcohols and phenylpropanoids derivatives[2]. From trunk wood were isolated isoflavones, pterocarpan, coumestans, flavanone, isoflavanones and arylbenzofuran[3,4].

In this paper we report the isolation and structural determination of a new pentacyclic triterpene **1** from the leaves of a specimen of *Myroxylon balsamum* (Fabaceae) collected in Espirito Santo State, Brazil. The structure was established by spectral analysis of **1** and its acetyl derivative **1a**, mainly through ^{1}H and ^{13}C NMR spectra, including homonuclear ^{1}H-^{1}H COSY and heteronuclear ^{1}H detected (inverse method) ^{1}H-^{13}C HMQC $^1J_{CH}$ and ^{1}H-^{13}C HMBC $^nJ_{CH}$ (n=2 and 3) 2D shift-correlated spectra[5].

Results and Discussion

The pentacyclic triterpene **1** was obtained as colorless crystals whose molecular formula $C_{31}H_{52}O_2$ (6 unsaturations) was deduced by comparative analysis of ^{13}C NMR (31 singlet signals) and DEPT-^{13}C NMR data[6] (Table 1) [θ = 90º : 6 CH, including one sp^2 (δ_C 121.51, CH-12) and two sp^3 oxygenated (δ_C 78.61, CH-3, and 75.85, CH-11); θ = 135º : 9 CH_2 and 9 CH_3 including one methoxy group (δ_C 53.67, MeO-11)] and LREIMS [*m/z* 424, 100% (M-MeOH]. The DEPT results [6 CH, 9 CH_2 and 9 CH_3 = $C_{24}H_{51}$] indicated that one exchangeable hydrogen (hydroxy group) was present, in accordance with the absorption at ν_{max} 3380 cm^{-1} observed in the IR spectrum and a monoacetyl [δ_H 2.03 (*s*)] derivative (**1a**) obtained by treatment with Ac_2O in the presence of pyridine (see experimental). The

^{13}C NMR spectra of **1** revealed signals at δ_C 121.51 (CH-12) and 149.67 (C-13), indicating the presence of a double bond. Because the unsaturation number is 6, **1** must therefore be a pentacyclic triterpene.

The location of the methoxy group at CH-11 (δ_C 75.85 and δ_H 3.84) and not at CH-3 (δ_C 78.61 and δ_H 3.23) was defined by heteronuclear long-range couplings between CH-11 (δ_C 75.85) and the methoxyl hydrogens (δ_H 3.23, $^3J_{CH}$), CH-12 (δ_C 121.51) and H-11 (δ_H 3.84, $^2J_{CH}$) and C-13 (δ_C 149.67) and H-11 (δ_H 3.84, $^3J_{CH}$) observed in the HMBC spectrum (Table 1). Other heteronuclear long-range couplings are summarized in Table 1.

The ^1H NMR spectrum of **1** (Table 1) showed nine singlet signals corresponding to one methoxy [δ_H 3.23 (MeO-11)] and eight tertiary methyl groups [δ_H 1.21 (3H-27), 1.04 (3H-25), 1.00 (3H-23), 0.99 (3H-26), 0.91 (3H-29), 0.89 (3H-30), 0.83 (3H-28) and 0.80 (3H-24)].

The position of H-11 in **1** [δ_H 3.84 (*dd*, *J* = 3.5 and 8.9 Hz)] was defined as axial (H-11β) on the basis of its coupling constant with H-9 (δ_H 1.69, *d*, *J* = 8.9 Hz). The equatorial orientation of the hydroxyl group at CH-3 (H-3 axial) was deduced by comparative analysis of the chemical shifts corresponding to CH_2-1 (δ_C 39.26), CH_2-2 (δ_C 27.30), CH-3 (δ_C 78.61), CH-5 (δ_C 55.02), CH_3-24 (δ_C 15.44) of **1** and the model triterpenoids **2** and **3** supporting a HO-3β (H-3α) and HO-3α (H-3β) [**2** (HO-3β)/**3** (HO-3α): δ_C 39.5/33.5 (CH_2-1), 27.4/25.4 (CH_2-2), 78.7/76.0 (CH-3), 39.0/37.0 (C-4), 55.1/48.8 (CH-5) and 15.5/22.4 (CH_3-24)][7], since superimposition of the signals was observed for H-3 (δ_H 3.23) and the methoxyl group (δ_H 3.23) in the ^1H NMR. The shielding revealed by the chemical shifts corresponding to carbon signals of CH_2-1, CH-5 and CH_3-24 of **3** can be justified by γ-effects attributed to the hydroxyl group at an axial position (H-3β equatorial). This deduction was confirmed by ^1H NMR and ^1H-^1H COSY spectra (200 MHz) of the monoacetyl derivative **1a**, which revealed the signal corresponding to H-3 as a double doublet [*J* = 8.2 (axial-axial interaction) and 5.0 Hz] at δ_H 4.49 [$\Delta\delta_H$ = 4.49 (**1a**) – 3.23 (**1**) = 1.26 ppm].

^1H and ^{13}C NMR assignments (Table 1) of the pentacyclic triterpene **1** were determined on the basis of HMQC

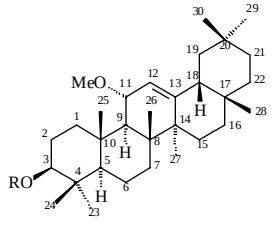
Table 1. ^1H and ^{13}C NMR spectral data for **1** ($CDCl_3$).*

	HMQC		HMBC	
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
4	39.00	-	3H-23; 3H-24	H-2
8	43.05	-	H-9; 3H-26	3H-27
10	38.12	-	H-1a; H-9; 3H-25	H-11
13	149.67	-		H-11; 3H-27
14	41.70	-	3H-27	H-12; 3H-26
17	32.30	-	3H-28	H-16
20	31.07	-	H-19; 3H-29; 3H-30	
CH				
3	78.61	3.23	H-2	2H-1; 3H-23; 3H-24
5	55.02	0.78		H-1; 3H-23; 3H-24; 3H-25
9	51.56	1.69 (*d*, *J*=8.9)	H-11	H-12; 3H-25; 3H-26
11	75.85	3.84 (*dd*, *J*=3.5, 8.9)	H-9	MeO-11
12	121.51	5.34 (*d*, *J*=3.5)	H-11	H-18
18	46.76	1.99		H-12; 3H-28
CH_2				
1	39.26	1.91 (*td*, *J*=13.9, 3.3); 1.23		3H-25
2	27.30	1.63		
6	18.24	1.48; 0.96		
7	33.08	1.49; 1.31		3H-26
15	26.13	1.25; 1.00		3H-27
16	26.60	2.01; 1.69		3H-28
19	46.36	1.65; 1.08		3H-29; 3H-30
21	34.51	1.15; 1.00		3H-29; 3H-30
22	36.84	1.46; 1.23		3H-28
CH_3				
23	28.03	1.00 (*s*)		3H-24
24	15.44	0.80 (*s*)		3H-23; H-5
25	18.04	1.04 (*s*)		H-9
26	16.76	0.99 (*s*)		
27	25.07	1.21 (*s*)		H-18
28	28.35	0.83 (*s*)		
29	33.11	0.91 (*s*)		
30	23.52	0.89 (*s*)		H-19; 3H-29
MeO-11	53.67	3.23 (*s*)		H-11

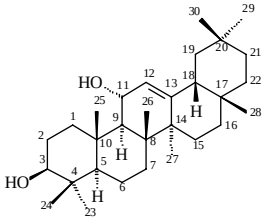
*Chemical shifts (δ_H and δ_C) and ^1H coupling constants (*J* in Hz, in parenthesis) obtained from the one dimensional ^1H and ^{13}C NMR spectra. Multiplicity of signals of carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Homonuclear ^1H-^1H COSY and heteronuclear ^1H-^{13}C HMQC $^1J_{CH}$ and ^1H-^{13}C HMBC $^nJ_{CH}$ (n=2 and 3) 2D NMR spectra were also used for these assignments.

and HMBC data and comparison to the known triterpene **2** [11α-hydroxy-β-amyrin, isolated from callus tissues of *Stauntonia hexaphylla* (Lardizabalaceae)][8]. The ^{13}C NMR spectra of **1** (Table 1), the 11-*O*-methyl ether of **2**, showed signals that matched closely with those of the triterpene **2** (recorded in $CDCl_3$)[8], revealing significant differences only in the chemical shifts of the methine CH-11 [δ_C 75.85 (**1**) and 81.70 (**2**)] and the quaternary C-13 [δ_C 149.67 (**1**) and 153.20 (**2**)] carbon atoms. These different chemical shifts may be justified by the presence of a hydrogen bond involving the hydroxy group at CH-11 of **2** and the solvent pyridine-d_5, and not in $CDCl_3$ as reported in the literature[8]. The ^{13}C and ^1H chemical shifts of the methine CH-11 [δ_C 76.6 and δ_H 3.87 (*dd*, *J* = 3.5 and 8.6 Hz)] of camaldulensis acid (3β,30-dihydroxy-11α-methoxyurs-12-en-28-oic acid, **4**, recorded in CD_3OD), isolated from leaves of *Eucalyptus camaldulensis* var. *obtusa* (Myrtaceae)[9], and **3** (δ_C 68.4)[7] were also used in this analysis.

Thus, the structure of the new triterpenoid isolated from *Myroxylon balsamum* was established as 3β-hydroxy-11α-methoxyolean-12-ene (**1**). The prominent peaks at *m/z* 255 (60 %) and 271 (16 %) observed in the mass spectrum, which could result from cleavage involving the rings B and D, respectively, in accordance with the formation of a diene after MeOH elimination[10], were also used in this structural elucidation. To the best of our knowledge, this triterpene **1** is hitherto unreported.



1 R=H
1a R=Ac



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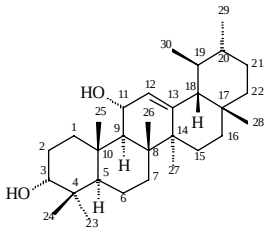

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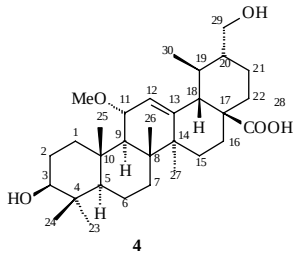

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Other 11-hydroxy or 11-methoxy pentacyclic triterpenes described in the literature are 28-oic acids: 2α,3β,23-trihydroxy-11α-methoxyurs-12-en-28-oic acid [isolated from resin of *Shorea robusta* (Dipterocarpaceae)][11], 11α-hydroxy-betulinic acid [isolated from leaves of *Licania pyrifolia* (Crysobalanaceae)][12] and 11α-hydroxytormentic acid [2α,3β,11α,19α-tetrahydroxyurs-12-en-28-oic acid, isolated from aerial parts of *Rosa laevigata* (Rosaceae)][13].

Experimental

General Experiments Procedures

EIMS were recorded on a VG Platform II mass spectrometer. ^1H (400 MHz), ^{13}C (100 MHz), ^1H-^{13}C HMQC $^1J_{CH}$ and ^1H-^{13}C HMBC $^nJ_{CH}$ (n=2 and 3 spectra were recorded using on a Bruker ARX-400 spectrometer, in $CDCl_3$ as solvent; residual $CHCl_3$ (δ_H 7.24) and the central peak of the triplet of $CDCl_3$ (δ_C 77.00) were used as internal references. The multiplicities of the carbon signals were deduced by comparative analysis of the HBBD- and DEPT-^{13}CNMR spectra. Heteronuclear ^1H and ^{13}C connectivities were deduced by ^1H-^{13}C HMQC $^1J_{CH}$ [spin-spin coupling of carbon and hydrogen via one bond ($^1J_{CH}$ 145.0 Hz)] and ^1H-^{13}C HMBC $^nJ_{CH}$ [n=2 and 3, spin-spin interaction of carbon and hydrogen via two ($^2J_{CH}$) and three ($^3J_{CH}$) bonds, optimized for $^nJ_{CH}$ of 9 Hz]. IR spectra with KBr plates were obtained on a FTIR Perkin-Elmer 1600/1605 spectrometer.

Plant material

The leaves of *Myroxylon balsamum* were collected at Reserva Florestal de Linhares, Companhia Vale do Rio Doce (CVRD), Espirito Santo State, Brazil, during September 1996. A voucher specimen has been deposited in the CVRD Herbarium (voucher no CVRD-483).

Extraction and Isolation

The air dried and powdered leaves (328.0 g) of *Myroxylon balsamum* were successively extracted with hexane, EtOAc and MeOH at room temperature and the solvents removed under vacuum to yeld 36.1g (rich in waxes and carotenoids), 17.4 g and 5.25 g of residues, respectively. The residue (17.4) obtained from the EtOAc solution was chromatographed on a of silica gel column eluting with hexane/CH_2Cl_2 mixtures of increasing polarity; a total of 45 fractions (*ca.* 100 mL each one) were collected and combined of the basis of TLC comparison. The fractions 18-22, eluted with hexane-CH_2Cl_2 (1:1), furnished **1** (92.0 mg) after recristalization from $CHCl_3$.

3β-Hydroxy-11α-methoxyolean-12-ene (**1**)

Colorless crystals, mp 172-173° C, $[\alpha]_D$ + 12.4° (*c* 0.73, CH_2Cl_2); IR (KBr) υ_{max} 3380, 1461,1384, 1044, 915, 731 cm^{-1}; EIMS *m/z* (rel. int.) 456 ($M^{+\cdot}$ abs.), 425 (25, M-MeO·), 424

(100, M-MeOH), 409 (51, M–MeOH-Me·), 271 (16), 255 (60), 253 (6); ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR: Table 1.

3β-Acetoxy-11α-methoxyolean-12-ene
*(**1a**, acetyl derivative of* **1**)

A sample of **1** (18.0 mg) was treated with Ac_2O (2.0 mL) and dry pyridine (1.0 mL) at room temperature overnight. After the usual work-up, the crude product was chromatographed on a silica gel column eluting with $CHCl_3$ to furnish the acetyl derivative **1a** (16.0 mg) as colorless crystals, mp 149-151° C, $[\alpha]_D$ + 5.7° (c 0.965, CH_2Cl_2); EIMS (rel. int.) 498 ($M^{+\cdot}$ abs.), 466 (100, M-MeOH), 451 (8, M – MeOH - Me·), 391 (14, M - MeOH - Me· - AcOH), 255 (42), 253 (10); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 5.30 (*br s*, H-12), 4.49 (*dd*, *J* = 8.2 and 5.0 Hz), 3.88 (*dd*, *J* = 8.8 and 3.6 Hz), 3.18 (*s*, MeO-11), 2.03 (*s*, AcO-3), 1.23 (*s*, 3H-23), 1.18 (*s*, 3H-14), 1.04 (*s*, 3H-25), 0.98 (*s*, 3H-26), 0.86 (*s*, 3H-27, 3H-28, 3H-29), 0.81 (*s*, 3H-30).

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