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Pimarane Diterpenes and a Sesquiterpene from *Salzmmania nitida*

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ABSTRACT

Two new terpenoids, (+)-3-oxo-thermarol and 11-acetoxyeudesman-4α-methyl-5α-ol along with the (+)-thermarol were isolated from the aerial parts of *Salzmmania nitida*. The structures and unambiguous ^{1}H and ^{13}C chemical shift assignments were established by spectroscopic means, including homo and heteronuclear techniques.

Key words: *Salzmmania nitida*, Rubiaceae, diterpenes, eudesmane.

INTRODUCTION

The Rubiaceae family has been shown to be one of much interest in phytochemical investigation due to the presence of species produced biologically active compounds such as alkaloids, flavonoids, antraquinones, saponins and triterpenes. *Chinchona*, *Coffea*, *Psychotria and Rubia* species are genera of this family known due to biosynthesize interesting naphthoquinones and alkaloids such as quinine, caffeine, emetine (Bruneton 1995, Evans 1991). Continuing our phytochemical investigation of plants from the northeastern region of Brazil, we recently reported the first study of *Salzmmania nitida* D.C. (Rubiaceae), describing the structures of triterpenes isolated from this plant (Alves et al. 2000). This species is a monotypic plant and common in the "restinga" (a kind of sand bank covered with vegetation) of the Northeast. This work describes the isolation and structural determination of two pimarane diterpenes besides 5α-hydroxy-4α-methyl-11-O-acethyl-eudesmane obtained from *Salzmania nitida* (Rubiaceae).

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MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURE

Mp's are uncorrected. NMR spectra were recorded on Bruker AC-200 (^{1}H: 200 MHz, ^{13}C: 50,3 MHz) and AMX 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) spectrometers using approximately 10-15 mg of sample dissolved in 0,5 ml of $CDCl_3$in 5 mm NMR tubes. Residual $CHCl_3$ (δ_H 7.24) and $^{13}CDCl_3$ (δ_C 77.00) signals were used as references. Homonuclear 2D (^{1}H-^{1}H-COSY and NOESY) and heteronuclear 2D {^{1}H-^{13}C-COSY-$^{n}J_{CH}$ [n=1, HMQC (modulated with J_{CH} 130 Hz); and n=2 and 3, HMBC (modulated with J_{CH} = 9.0 Hz)]} spectra were ob-

tained with standard pulse sequences. FT-IR spectra were recorded using KBr disks or NaCl film on a Perkin-Elmer 1600 spectrometer. Mass spectra were obtained using a VG Auto Spec-300 spectrometer. Chromatography was performed using Aldrich silica gel with a suitable granulation for column and preparative TLC. The visualization of spots was done by UV (254 and 366 nm) and exposure to iodine vapor.

PLANT MATERIAL

Salzmania nitida D.C. (Rubiaceae) was collected in January 1998 in the surroundings of Santa Rita, State of Paraíba, Brazil, and identified by botanist Dr. Maria de Fátima Agra of the Universidade Federal da Paraíba. A Voucher specimen (Agra 2986) is deposited at the Herbarium Prof. Lauro Pires Xavier (JPB), Universidade Federal da Paraíba.

ISOLATION OF CONSTITUENTS

The dried and ground aerial parts of *S. nitida* (3.40 Kg) were extracted in a Soxhlet apparatus with 95% EtOH. The solvent was removed under vacuum to yield a residue (180.0 g). This residue was dissolved in MeOH-water(9:1) and subjected to partition with $CHCl_3$ and AcOEt. The residue from the AcOEt fraction (80.3 g) was submitted to a C.C. on silica gel using hexane-$CHCl_3$ (1:1) as eluent. 76 fractions of 50 mL each were obtained. Fractions 10-30 and 35-55 yielded **1** (0.10 g), and **2** (0.15 g), respectively, after recrystallization from methanol. Fractions 57-76 were submitted to preparative TLC using hexane:AcOEt (8:2) to yield the sesquiterpene **3** (0.010 g, oil).

(+)-8β, 19-dihydroxy-3-oxopimar-15β-ene, (+)-3-oxo-thermarol (**1**): M.P. 158 ^{o}C, $[\alpha]_D$ = + 22.60 ($CHCl_3$, c 0.045). ^{1}H and ^{13}C NMR data in Table I.

(+)-8β, 19-dihydroxy-pimar-15β-ene, (+)-thermarol (**2**): M.P. 148 ^{o}C, $[\alpha]_D$ = +8.28 ($CHCl_3$, c 0.052). ^{1}H and ^{13}C NMR data in Table I.

11-acetoxyeudesman-4α-methyl-5α-ol (**3**): oil; δ_H ($CDCl_3$, 500 MHz): 2.2 (H-7, m), 1.93 (H-4, H-1, m), 1.70 (H-2, 6, 9, m), 1,45 (H-2, 3, 8, m), 1.30 (H-1, 3, 9, m), 1.2 (H-6, 8, m), 1.95 (s, $\underline{H}_3CCO$), 1.42 and 1.49 (3H, s, H-12 and 13), 0.84 (3H, s, H-14), 0.77 (3H, d, 7.0 Hz, H-15); δ_C ($CDCl_3$, 125 MHz): 170.5 ($H_3C\underline{C}O$), 85.2 (C-11), 73.2 (C-5), 41.1 (C-10), 39.4 (C-7), 36.5 (C-9), 32.9 (C-4), 32.0 (C-1), 30.0 (C-6), 29.7 (C-3), 23.4 and 23.2 (C-12 and C-13), 22.9 (C-2), 22.4 (C-8), 22.1 ($H_3\underline{C}CO$), 15.2 (C-14), 15.9 (C-15).

RESULTS AND DISCUSSION

The analysis of ^{13}C NMR [HBBD and DEPT (θ: 135 and 90°)] spectra of **1** allowed to identify three methyl, nine methylene, three methyne and five quaternary carbons. The IR spectra of this compound showed bands at ν_{max} 1715 cm^{-1} ($\nu_{C=O}$), 3470 cm^{-1} (ν_{OH}) and 3080, 1630, 975 and 910 cm^{-1} characteristic of a vinyl group. These observations together with a peak at m/z = 320($M^{+\cdot}$) in the mass spectrum are in agreement with the molecular formula $C_{20}H_{32}O_3$for **1**. These data are in accordance with an oxo-pimarane diterpene. The ^{1}H NMR spectrum shows singlets at δ_H 0.92, 0.98 and 1.28 for three methyl groups, two signals at δ_H 3.45 (*d*, 11.0 Hz, 1H) and 4.05 (*d*, 11.0 Hz, 1H) for a hydroxymethylene group and three double doublets at δ_H 5.13 (9.0 Hz and 2.0 Hz, 1H), 5.19 (16.0 Hz and 2.0 Hz, 1H) and 5.98 (16.0 Hz, 9.0 Hz, 1H) of the identified vinyl group. These groups were also confirmed by cross-peaks in the 2D (^{1}H-^{13}C-COSY, $^1J_{CH}$) NMR spectrum between those signals and carbon-13 chemical shifts at 16.5, 22.3, 32.5 (CH_3), 66.0 (CH_2), 112.8 (CH_2) and 147.9 (CH) relative to connections for methyl groups, hydroxymethylene and vinyl hydrogen, respectively. The HMBC analysis showed $^{2,3}J_{CH}$ cross-peak correlation as described in Table I. The NOE observed between H_3C-20, H-1 (eq) and H_2C-19 in ^{1}H-^{1}H-NOESY experiment, along with the considerable shielding effect on C-18 (22.3) and deshielding on C-4 (51.1), were used to confirm the presence of a carbonyl at C-3 of ring A. The location of the hydroxyl group at C-8 was confirmed by the chemical shift at 72.0 ppm besides the signals of long-range

TABLE I

^{1}H and ^{13}C NMR data of (+)-3-oxo-thermarol (1) and (+)-thermarol (2) using 1D and 2D [$^{1}J_{CH}$, ^{1}H-^{13}C-COSY (HMQC) and $^{2,3}J_{CH}$, (HMBC)].

C	1			2		
	δ_C	δ_H (mult., J Hz)	$^{2,3}J_{CH}$	δ_C	δ_H	$^{2,3}J_{CH}$
1	38.0	1.95(ddd, 4.5; 8.5; 14.0; H-α); 1.50(m, H-β)	C-2;5;10;20	39.7	0.85(m, Hβ); 1.70(m, Hβ)	C-2;3;10
2	34.5	2.45(dt, 8.5;14.0; Hα) 2.57(ddd, 4.5;8.5;14.0, Hβ)	C-3;10	18.3	1.45-1.55(m, 2H)	C-3;10
3	220.9	–	–	35.8	0.92(Hα p;1.80(Hβ)	C-2;4;5
4	51.1	–	–	38.9	–	
5	56.8	1.25	C-4;6;18,19	57.4	1.02(m)	C-4;7
6	18.3	1.40-1.65(2H-6)	C-7;8	18.3	1.78(Hα);1.22(Hβ)	C-5;7;8;10
7	41.5	1.78(Hα); 1.22(Hβ)	C-6;9	41.6	1.45-1.5(2H)	C-9;8
8	72.0	–	–	72.7	–	
9	55.1	0.95	C-10;12	56.6	0.88 (dd, 9.0, 3.5)	C-10;12
10	36.6	–	–	37.4	-	
11	18.6	1.46-1.60(2H-11)	C-9;12;13	18.7	1.50-1.70(2H)	C-9;13
12	36.3	2.03(Hβ); 1.25(Hα)	C-11, 13, 14;15	36.2	1.21(Hα);2.00(ddd, 10.7, 6.0, 2.9, Hβ)	C-13;15
13	36.8	–	–	36.6	–	–
14	53.0	1.26(Hα); 1.68 (dd, 2.0; 14.0, Hβ)	C-8;12;13;15	53.6	1.24(Hα) 1.65(dd, Hβ)	C-13
15	147.4	5.98(dd, 9.0; 16.0)	C-12;16	147.4	5.97(dd, 17.0,11.0)	C-12;16
16	112.8	5.13(dd, 2.0; 9.0); 5.19(dd, 2.0; 16.0)	C-13;15	112.2	5.08(dd, 11.0, 1.2) 5.13(dd, 17.0, 1.2)	C-13;15
17	32.5	0.92(s)	C-12;13;14;15	32.5	0.91(s)	C-13;14
18	22.3	1.28(s)	C-4;5;19	27.2	0.91(s)	C-3;4;5;19
19	66.0	3.45(d, 11.0); 4.05(d, 11.0)	C-3;4;5	66.5	3.48(d, 11.0) 3.08(d, 11.0)	C-3;4
20	16.5	0.98(s)	C-1;5;9;10	16.3	0.97(s)	C-1;5;9;10

coupling with H-6, H-9 and 2H-14 in the HMBC experiment. Additional signals showing correlation between 3H-20 and C-1, C-5 and C-9, 3H-17 with C-12, C-14 and C-15 together with comparison of (-)-thermarol ^{13}C NMR chemical shifts (Matsuo et al. 1976, Ramos et al. 1984) allowed to assign hydrogen and carbon chemical shifts as shown in Table I. The peaks in the mass spectrum of **1** have the same m/z values described in the literature for a thermarol derivative (Takaishi et al. 1997). The difference between the C-17 chemical shift in **1** (δ_{CH3} 32.5) and that described in the literature (δ_{CH3} 24.3) for 8β,19-dihydroxy-3-oxopimar-15α-ene (Takaishi et al. 1997) along with the NOE cross peak between H-17 and H-14α in the ^{1}H-^{1}H-NOESY spectrum led us to established an equatorial position for the C-17 methyl group, such as in the representation for (-)-thermarol (Matsuo et al. 1976). Finally, the $[\alpha]_D$ = + 22.60 ($CHCl_3$, c 0.045) allowed to define the structure of **1** as (+)-8β,19-dihydroxy-3-oxopimar-15β-ene represented for the new diterpene named (+)-3-oxo-thermarol (Fig. 1).

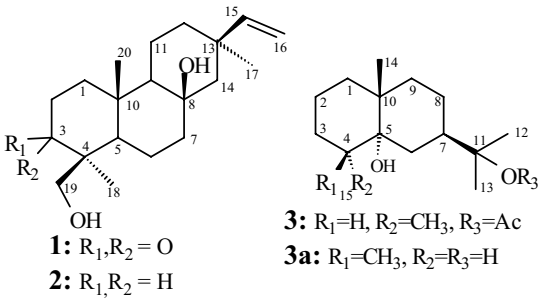

Fig. 1 – Compounds isolated from *Salzmmania nitida.*

Spectral analysis for **2** led us to identify 20 carbon signals including three methyl, ten methylene, three methyne and four quaternary carbons including two oxigenated carbons (H_2C-O and C-O). The molecular formula of **2**, $C_{20}H_{34}O_2$, was compatible with $M^{+\cdot}$ 306 observed in the mass spectrum. The ^{13}C NMR spectra data besides the peaks observed in the mass spectrum of **2** were identical to those of (-)-thermarol (Matsuo et al. 1976, Ramos et al. 1984). The cross peaks of NOE between H-9/H-12, H-1/H-20, H-12/H-9, H-17, H-14/H-17, H-19(δ_H 3.48)/H-1,H-20 and H-17/H-14α, H-15 observed in the NOESY spectra along with information from homonuclear ^{1}Hx^{1}H-COSY were used to confirm the structure for **2**. Furthermore, this analysis allowed us to make the first detailed assignment of all hydrogen chemical shifts of thermarol, Table I. The optical rotation of **2**, $[\alpha]_D$ = +8.28 ($CHCl_3$, c 0.052), has the same sign of (+)-thermarol prepared by reduction of 8β-Hydroxypimar-15-en-19-oic acid, isolated from *Taxodium mucronatum* (Ramos et al. 1984) (Fig. 1). So, the optical rotation permitted to identify **2** as being the natural enantiomer compound (+)-thermarol.

The acetyl derivative of eudesmanediol **3** was identified by IR and NMR spectral analysis besides comparison with data for 5,11-dihydroxy-eudesmane and 11-Acetoxyeudesman-4-ol isolated from *Cryptomeria japonica* (Su et al. 1995) and from *Ursinia* species (Jakupovic et al. 1992). The HMQC and HMBC experiments were useful to assign the carbon and hydrogen chemical shift of **3** (see experimental). The analysis of carbon-13 chemical shifts confirmed the location of the tertiary hydroxyl and the acetyl groups in the carbon with chemical shifts at δ73.5 (C-5) and 85,2 (C-11), respectively, together with comparison with values for **3a** described in literature (Su et al. 1995). The shielding γ-effect of an axial HO-5 on C-15 (δ_{CH3} 15.9, $\Delta\delta$ = 6.6 ppm) and on C-7 (δ_{CH} 39.4, $\Delta\delta$ = 5.8 ppm) besides the expected deshielding effect on C-10 (δ_C 41.1, $\Delta\delta$ = 3.5 ppm) were used to justify the α-orientation for HO-5 and H_3C-15. The chemical shift of C-11 (δ_C 85.2) and of 12/13 methyl groups (δ_{CH3} 23.4/23.2) are in agreement with an acetoxy group ($\delta_{C=O}$ 170.5 and δ_{CH3} 22.1) at C-11. On the basis of these data, the new sesquiterpene (**3**) was identified as 11-acetoxyeudesman-4α-methyl-5α-ol (Fig. 1).

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RESUMO

O estudo fitoquímico de *Salzmmania nitida* D.C. (Rubiaceae) conduziu ao isolamento e identificação de dois novos terpenoides, (+)-3-oxo-thermarol e 11-acetoxi-4alfa-metil-5alfa-eudesmanol além do (+)-termarol. As estruturas foram estabelecidas com base na análise de espectros de IV, Massas e RMN de ^{1}H e ^{13}C (1D e 2D).

Palavras-chave: *Salzmmania nitida*, Rubiaceae, diterpenos, eudesmano.

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