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New triterpene isolated from *Eschweilera longipes* (Lecythidaceae)

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ABSTRACT

The phytochemical studies of *Eschweilera longipes* Miers (Lecythidaceae) have led to the identification of a new triterpene 3β, 24-dihydroxyfriedelane, the known 1β, 2β, 3β, 19β-tetrahydroxyurs-12-en-28-oic acid (1β-hydroxyeucaphic acid) besides the saponin sitosterol 3βO-βD-glucopyranoside. The structures were established from the IR, NMR and mass spectra data including 2D NMR experiments of natural substances and of the acetyl derivative of the new triterpene.

Key words: *Eschweilera longipes*, Lecythidaceae, triterpenoids.

INTRODUCTION

Lecythidaceae is a pantropical family (about 25 genera and 400 species) with the greatest concentration of genera in tropical South America (Brito 1986).

Species of this family have been reported as showing pharmacological activities and the chemical study of some species as *Petersianthus macrocarpus*, *Barringtonia acutangula* and *Cereya arborea*, allowed the identification of pentacyclic triterpenes, saponins, elagic acid and indolo[2,1-b]quinazolinic alkaloids (Pant and Rastogi 1979, Das and Mahato 1983, Pal et al. 1991, Massiot et al. 1992 and Bergman 1989).

Eschweilera longipes Miers is a tree that occurs in the north and north-east of Brazil and has been used in the wood industry and in construction. Only triterpenes have been found in the *Eschweilera* genera. Two previous papers report the isolation of ten known triterpenes along with sitosterol, stigmasterol, α-tocopherol and tocotrienol from *E. longipes* (Carvalho et al. 1998) and three pentacyclic triterpenoids which were isolated from the bark and leaves of *E. rabeliana* (Carvalho et al. 1995).

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MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURE

Mp's are uncorrected. NMR spectra were measured in Pyridine-d_6, $MeOD_4$ or $CDCl_3$ solutions and recorded on a Bruker (200 and 500 MHz for ^{1}H and 50.3 and 100 MHz for ^{13}C, respectively) and on a GEOL (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer using TMS as internal standard. High resolution mass spectra were obtained using a VG Auto Spec-300 spectrometer; FT-IR spectra were recorded in KBr disks on a Perkin-Elmer 1600 spectrometer. Chromatography was performed using Aldrich silica gel with 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

The wood and leaves were collected in the Amapá State. A voucher specimen (n° 00358) is deposited in the Amapaense Herbarium HAMAB of the Museu Angelo Moreira da Costa Lima-IEPA, Macapá, Amapá, Brazil.

Extraction and isolation

The dried leaves (0.7Kg) were extracted exhaustively by CH_2Cl_2 maceration at room temperature. The solvent was removed under vacuum to yield a residue (10.38g). This residue was chromatographed on silica gel column starting with CH_2Cl_2 and successive mixtures of CH_2Cl_2-EtOAc, EtOAc-MeOH and finally, MeOH as eluent to afford 70 fractions of 50 mL each. The 23-40 fractions, eluted with EtOAc-MeOH (9:1), gave a colourless solid (**1**, 70mg, mp 340°C) which is insoluble in $CDCl_3$. The derivative **1a** was prepared dissolving **1** in a mixture of pyridine and Ac_2O (1:1) and the solution was allowed to stand for 24h at room temperature. The usual work-up gave a residue which was dried under vacuum and crystallized from AcOEt to yield the diacetate (**1a**, 65mg, mp 318°C).

The dried wood (1.0 Kg) was extracted exhaustively by MeOH maceration at room temperature. The solvent was removed under vacuum to yield a residue (47.7g). This residue was dissolved in MeOH:H_2O (8:2) and extracted with dichloromethane. The fraction CH_2Cl_2 was chromatographed on silica gel column using CH_2Cl_2 and successive mixtures of CH_2Cl_2-EtOAc, EtOAc-MeOH and finally, MeOH as eluent to afford 220 fractions of 50mL each. The 9-17 fractions, eluted with CH_2Cl_2, gave a colourless solid (**2**, 40mg, mp 285°C) soluble in MeOH. The 70-82 fractions, eluted with EtOAc-MeOH (9:1), gave colourless solid (**3**, 40mg, mp 290°C). The derivative **3a** was prepared dissolving **3** in a mixture of pyridine and Ac_2O (1:1) and working up as usual.

RESULTS AND DISCUSSION

The chromatographic fractionation of the dichloromethane extract from the leaves of *Eschweilera longipes* afforded two triterpene, **1** and **2**, besides the saponin sitosterol 3-βO-β-D-glucopiranoside (**3**).

The IR spectrum of **1** showed absorption bands attributed to hydroxyl (ν_{OH}3450 cm^{-1}), $\nu_{C\text{-}O}$ (1100 and 1050 cm^{-1}) and very strong $\nu_{C\text{-}H}$ at 2950 and 2960 cm^{-1} suggesting a terpenoid with primary and secondary alcohol. The difficulty to dissolve it in $CDCl_3$ led to prepare the acetyl derivative treating it with pyridine and Ac_2O (1:1).

The ^1H NMR spectrum of **1a** displayed singlet signals for seven tertiary methyl groups of a pentacyclic triterpene and two signals at 1.96 (s, 3H) and 2.00 (s, 3H) of acetyl groups. The signals at 4.40 and 4.60 (d, J = 13 Hz) are typical of two methylene hydrogens. The H-3 was represented by the signal at 4.94 (br d, J = 2.4 Hz). The comparative analysis of HBBD and DEPT ^{13}C NMR spectra was used to recognize the signals corresponding to six quaternary carbons, two monoxigenated (δ_{CH}74.5 and δ_{CH2}65.1) besides signals of seven methyl, eleven methylene, four methyne groups and two acetyl groups (δ170.1, 170.0, 21.2 and 21.1). Those data allowed to propose the molecular formula $C_{30}H_{50}O_2$ $(O{=}C\text{-}CH_3)_2$ that was confirmed by HRMS with M^+ 528.41740 Da [calcd for $C_{30}H_{35}(O_2CCH_3)_2$ 528.41808]. Thus, these spectral data, the 2D experiments (^1H-^1H-COSY, ^1H-^{13}C-COSY-$^nJ_{CH}$, n=1,2,3) and comparison with ^{13}C NMR spectroscopic values described in the literature for acetyl friedelinol (Carvalho et al. 1995, Mahato and Kundu 1994 and Ahmad and Atta-ur-Rahman 1994) show the absence of signal at 15.7 (CH_3-24) in the fridelinol. This observation and the difference of the C-5 and C-6 chemical shift of **1** and those of acetyl friedelinol led to locate one acetyl group at C-24. The prominent peaks in the HRMS at *m/z* 455 (**1b**, 21,5%, M- CH_2OCOCH_3), 395 [**1c**, 8,3%, M- (CH_2OCOCH_3 + $HOCOCH_3$)], 344 (**1d**, 33,9%, $C_{25}H_{44}$), 274 (**1e**, 17,3%, $C_{20}H_{34}$) 255 (**1f**, 15%) and 205 (**1g**, 23.8%, $C_{15}H_{25}$), Figure 1, also suggested the presence of two acetyl groups in the C-3 and C-24 carbons. The NOE observed between H-24/H-25, H-24/H-23, H-24/H-1 in the NOESY spectra of **1a** (3β, 24-diacetylfriedelane) was used

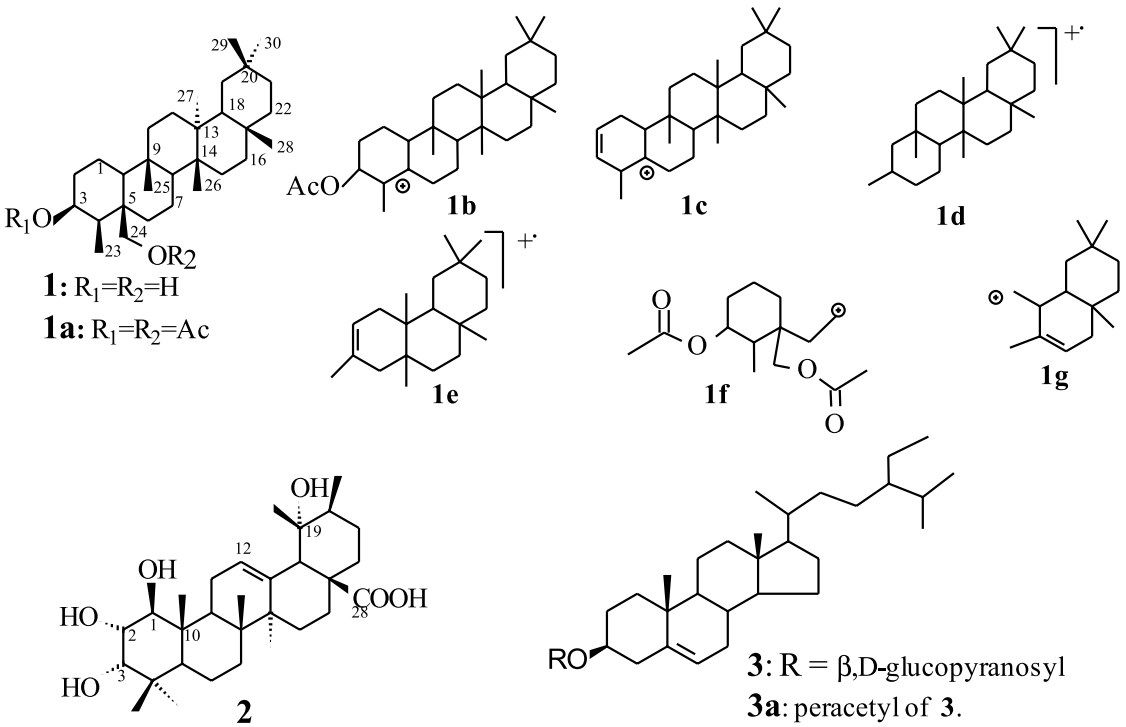

Fig. 1 – Structures for compounds isolated from *E. longipes*, acethyl derivatives and for prominent peaks in the HRMS.

to confirm the structure of the new triterpene (**1**) as 3β, 24-dihydroxyfriedelane, Figure 1. The complete ^{1}H and ^{13}C NMR (1D and 2D) assignments of **1** and **1a** are described in Table I.

Compound **2** was characterized as 1β-hydroxyeucaphic acid by analysis of IR, NMR ^{1}H and ^{13}C (HBBD and DEPT) and 2D experiments (^{1}H-^{1}H-COSY, ^{1}H-^{13}C-COSY, $^{n}J_{CH}$, n=1,2,3) and EI-MS spectra including comparison of the δ_H chemical shifts in pyridine registered in the literature (Guang et al. 1989). The NOE signal between H-1/H-5, H-1/H-9, H-2/H-24, H-2/H-25, H-3/H-24, H-3/H-23, H-11/H-25, H-18/H-29, H-18/H-12 and H-12/H-29 observed in the NOESY spectrum were used to confirm the structure of **2** as 1β, 2β, 3β, 19β-tetrahydroxyurs-12-en-28-oic acid. The better resolution of the ABC system (H-2, H-1 and H-3) and the absence of ^{13}C NMR data of **2** in the literature led us to make the complete assignment of δ_H and δ_C in methanol. The EIMS spectra data were used to confirm the structure. δ_H ($MeOD_4$, 200 MHz): 3.39 (d, 8.4Hz, H-1), 3.63 (dd, 8.4 and 3.2 Hz, H-2), 3.45 (d, 3.2 Hz, H-3), 1.3 (m, H-5), 2.1 (m, H-9), 5.20 (brs, H-12), 2.47 (s, H-18), 0.96 (s, H-23), 0.87 (s, H-24), 1.00 (s, H-25), 0.78 (s, H-26), 1.34 (s, H-27), 1.18 (s, H-29), 0.92 (d, 6.6Hz, H-30); δ_C ($MeOD_4$, 50.3 MHz): 79.9 (C-1), 70.4 (C-2), 79.3 (C-3), 39.1 (C-4), 48.2 (C-5), 18.0 (C-6), 32.8 (C-7), 41.2 (C-8), 48.0 (C-9), 37.4 (C-10), 25.2 (C-11), 129.3 (C-12), 137.3 (C-13), 43.2 (C-14), 29.4 (C-15), 28.3 (C-16), 48.2 (C-17), 53.3 (C-18), 72.2 (C-19), 41.2 (C-20), 26.9 (C-21), 37.6 (C-22), 27.7 (C-23), 21.0 (C-24), 11.6 (C-25), 16.4 (C-26), 23.5 (C-27), 180.0 (COOH), 25.3 (C-29), 15.2 (C-30); EIMS, m/z (%): 504(10%), 264(35%), 246(15%), 201(45%), 173(20%) and 146(100%).

The spectrometric analysis of IR, ^{1}H and ^{13}C (PND and DEPT) NMR of **3** including comparison with literature data (Chaurasia and Wichtl 1987) were used to identify the saponin **3** as sitosterol

TABLE I

^{1}H and ^{13}C NMR data of the new triterpene (1, Pyridine-D_6) and its derivative (1a, $CDCl_3$) using 1D and 2D ($^{1}J_{CH}$, ^{1}H -^{13}C-COSY and $^{2,3}J_{CH}$, COLOC).

	1a			1	
C	δc	δ_H^a ($^{1}J_{CH}$)	$^{2,3}J_{CH}$	δc	δ_H^b
1	16.3	1.45	H-3	17.1	–
2	32.1	1.90, 1.55	–	40.3	–
3	74.2	4.94(d, 2.4Hz)	H-23	74.7	5.25(brs)
4	48.5	1.5(md)	H-24, H-23	49.1	–
5	40.6	–	–	41.4	–
6	35.8	2.3(brd), 1.5(m)	H-24	36.0	2.3(d, 14 Hz)
7	17.6	1.40	–	19.3	–
8	53.1	1.3(brd)	H-27, H-6 , H-7	53.8	–
9	36.9	–	–	36.8	–
10	61.1	1.1(m)	H-25	61.6	–
11	35.7	1.2-1.5(m)	H-25	36.5	–
12	30.6	–	–	31.4	–
13	38.3	–	–	37.6	–
14	39.5	–	H-26	39.9	–
15	32.0	–	–	32.5	–
16	35.9	1.4(m), 1.0(m)	–	35.6	–
17	29.9	–	–	30.7	–
18	42.7	1.6(dd)	H-28 e H-27	43.6	–
19	35.2	1.3(m), 2.3 (13Hz)	–	35.6	2.0(dd,14.1, 2.4 Hz)
20	28.1	–	–	28.8	–
21	32.7	–	–	32.9	–
22	39.2	0.9(d), 1.4(m)	H-28	39.0	–
23	13.7	0.92(d, 7 Hz)	H-4	14.8	1.09(d, 7 Hz)
24	65.1	4.40, 4.6(d,13Hz)	–	65.8	4.61, 4.90 (d, 14 Hz)
25	18.3	0.85(s)	–	18.4	0.88(s)
26	18.6	0.96	–	18.9	1.04(s)
27	20.1	0.96	H-8	20.8	1.07(s)
28	32.0	1.13(s)	–	33.6	1.18(s)
29	35.0	0.91(s)	–	35.6	0.90(s)
30	31.7	0.96(s)	–	32.7	1.0(s)
$H_3\underline{C}$-CO	21.2	1.96, 2.0	–	–	–
H_3C-$\underline{C}O$	170.1170.0		$\underline{H}_3C$-CO	–	–

[a]Other signals were not defined. [b]Multiple signal between 1.7-1.2.

3βO-βD-glucopyranoside, Figure 1.

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RESUMO

O estudo fitoquímico de *Eschweilera longipes* Miers (Lecythidaceae) conduziu a identificação de um novo triterpeno 3β, 24-diidroxifriedelano, do ácido 1β, 2β, 3β, 19β-tetraidroxiursa-12-en-28-óico conhecido como ácido 1β-hidroxieucáfico além da saponina 3βO-βD-glucopiranosilsitosterol. As estruturas foram estabelecidas com análise de dados espectrais de IV, massas e RMN incluindo experimentos 2D das substâncias naturais e do derivado acetilado do triterpeno novo.

Palavras-chave: *Eschweilera longipes*, Lecythidaceae, triterpenoides.

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