PERGAMON

Phytochemistry 55 (2000) 711–713

PHYTOCHEMISTRY

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A limonoid from *Trichilia estipulata*

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Received 20 January 1999; received in revised form 20 October 1999

Abstract

The limonoid 21,24,25,26,27-pentanor-15,22-oxo-7α,23-dihydroxy-apotirucalla(eupha)-1-en-3-one was isolated from the dichloromethane extract of the stem bark of *Trichilia estipulata*. Its structure was established by spectroscopic methods (UV, EIMS, ^{1}H and ^{13}C NMR, HMQC and HMBC). © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Trichilia estipulata; Meliaceae; Limonoid

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1. Introduction

Recently we reported the isolation of γ-hydroxybutenolide limonoids, triterpenoids, steroids, coumarins, and lignan glycosides from *Trichilia estipulata* (Cortez et al., 1997a,b). The butenolides are known to occur in families of the order Rutales (Meliaceae, Rutaceae and Cneoraceae) (Connolly, 1983). Continuing our investigation of this plant, we report the isolation of a C-25 limonoid bearing a carbon skeleton new to this class of compounds.

2. Results and discussion

Column chromatography of the dichloromethane extract of *T. estipulata* afforded four previously reported γ-hydroxybutenolide limonoids, 24-β-7-oxositosterol, 24-α-7-oxositosterol, scopoletin and isofraxidin (Cortez et al., 1998b). Continuing with the purification of the remaining fraction it was possible to isolate the limonoid **1**, which was obtained in a mixture with scopoletin. This mixture was first analyzed for the absence of natural acetate and then acetylated. The corresponding acetyl derivative was purified by semi-prep. HPLC to yield the diacetate **1a**.

The IR spectrum of compound **1a** showed absorptions at 1667 cm^{-1}, attributed to a carbonyl group of a cyclohexenone system, and at 1736 cm^{-1} (strong and broad band) due to other carbonyl groups. The EIMS spectrum displayed a molecular ion at m/z 500 in accordance with the molecular formula $C_{29}H_{40}O_7$. The ^{1}H NMR spectrum (Table 1) showed signals at δ 6.50 (1H, *brd*, $J = 10.2$ Hz, H-1), 5.88 (1H, *d*, $J = 10.2$ Hz, H-2), 5.07 (1H, *dd*, $J = 3.5$, 2.3 Hz, H-7) and 2.33 (1H, *dd*, $J = 1.4$, 0.6 Hz, H-14) (Table 1), indicating that rings A and D of **1a** were identical with those of **2** (7-acetylneotrichilenone) (Kraus et al., 1981). Also, the ^{1}H NMR spectrum of **1a** displayed five signals for methyl groups at δ 0.50 (H-18), 0.60 (H-19), 0.68 (H-30), 0.94 (H-29) and 1.11 (H-28), which were confirmed through HMQC and HMBC experiments. The difference between the NMR spectra of compound **1a** and **2** (Kraus et al., 1981), is the absence of the furan ring signals and the lack of one carbon in the side chain (C-21) of **1a**. The ^{13}C NMR spectrum for **1a** showed signals at δ 37.61 (C-20), 201.59 (C-22) and 66.63 (C-23) (Table 2) suggesting a side chain containing three carbons. A more detailed analysis of the ^{1}H NMR spectrum of **1a** permitted assignment of a double quartet at δ 2.67 ($J = 10.6$, 3.1 Hz) to proton H-17, coupled with two adjacent methylene resonances at δ 2.48 (*ddd*, $J = 19.5$, 10.6, 0.6 Hz), 1.56 (*ddd*, $J = 19.5$, 10.6, 1.4 Hz), 1.98 (*dd*, $J = 16.8$, 3.1 Hz)

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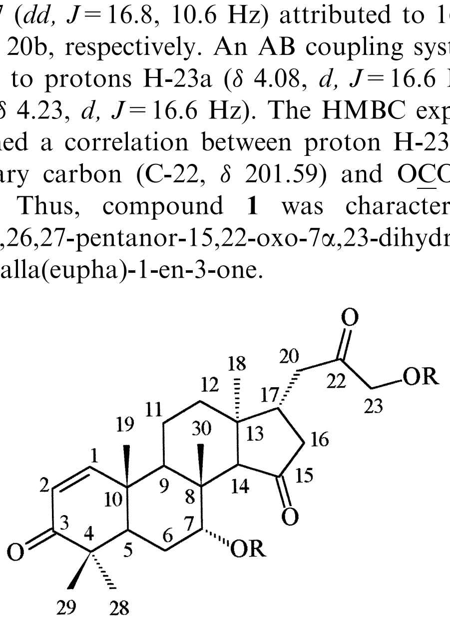
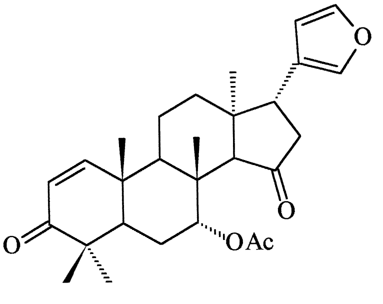
PII: S0031-9422(00)00298-3

and 1.57 (*dd, J*=16.8, 10.6 Hz) attributed to 16α, 16β, 20a and 20b, respectively. An AB coupling system was assigned to protons H-23a (*δ* 4.08, *d, J*=16.6 Hz) and H-23b (*δ* 4.23, *d, J*=16.6 Hz). The HMBC experiment established a correlation between proton H-23a and a quaternary carbon (C-22, *δ* 201.59) and $O\underline{C}OCH_3$ (*δ* 167.07). Thus, compound **1** was characterized as 21,24,25,26,27-pentanor-15,22-oxo-7α,23-dihydroxy-apotirucalla(eupha)-1-en-3-one.



1 **R=H**

1a **R=Ac**



2

3. Experimental

3.1. General experimental procedures

IR (Film, BOMEM-FT-IR, Michaelson-102 spectrophotometer); UV (Perkin–Elmer, 550 SE model spectrophotometer); MS (low resolution) on a HP-2576 instrument (70 eV). All NMR experiments were recorded using a BRUKER DRX400 model spectrometer, with TMS as internal standard in a degassed sample in $CDCl_3$. For HMBC, 9 Hz coupling constant was used for long-range correlation. Semi-prep. HPLC was carried out on a Waters/Millipore model 510 equipped with a UV detector (254 nm). Purification was on silica hypersil (5 µm, 250×7 mm) at a flow rate of 2 ml min^{-1}.

3.2. Plant material

The plant was collected in Cachoeiro do Itapemirim, Espirito Santo state, Brazil. Identification of the plant was done by Professor Dr. José Rubens Pirani, and a voucher specimen (No. 2500 Pirani) is deposited in the Herbarium of São Paulo University, Biosciences Institute.

3.3. Extraction and isolation

The extraction procedure was described previously (Cortez et al., 1998). The dichloromethane extract (8 g) was subjected to repeated chromatography over silica gel 60 (63–230 µm, Merck), eluted with mixtures of solvents of increasing polarity: CH_2Cl_2, CH_2Cl_2–EtOAc, EtOAc, and MeOH. Fraction F-1 (54 mg) was acetylated with Ac_2O in pyridine and rechromatographed using silica gel CC with CH_2Cl_2–EtOAc–MeOH (50:5:3) as eluent. Further purification by semi-prep. HPLC with CH_2Cl_2 yielded **1a** (1.5 mg).

3.4. 21,24,25,26,27-Pentanor-15,22-oxo-7α,23-diacetate-apotirucalla(eupha)-1-en-3-one (1a)

Viscous oil, $[\alpha]_D^{25}$ (c 0.015, $CHCl_3$)=−37°; IR (film, ν_{max}) 2938, 1736, 1667, 1400, 1239 and 1034 cm^{-1}. UV ($CHCl_3$) λ_{max} nm (ε): 243 (1833); ^1H NMR spectral data: see Table 1; ^{13}C NMR spectral data: see Table 2;

Table 1
^1H NMR spectral data of **1a** ($CDCl_3$, 400 MHz, δ)[a]

H	1[a]	HMBC
1	*brd* 6.50 (10.2)	
2	*d* 5.88 (10.2)	
5	*dd* 2.05 (13.3, 2.4)	C-10
6α	*ddd* 1.87 (14.8, 3.5, 2.4)	
6β	*ddd* 1.39 (14.8, 13.3, 2.3)	
7	*dd* 5.07 (3.5, 2.3)	
9	*ddd* 1.08 (6.2, 2.2, 0.5)	
11α	*m* 0.85–0.94	
11β	*m* 0.85–0.94	
12α	*dtb* 1.49 (16.7, 2.5)	
12β	*m* 1.15–1.28	
14	*dd* 2.33 (1.4, 0.6)	
16α	*ddd* 1.56 (19.5, 10.6, 1.4)	
16β	*ddd* 2.48 (19.5, 10.6, 0.6)	
17	*dq* 2.67 (10.6, 3.1)	
18	*s* 0.50	C-13, C-12,
19	*s* 0.60	C-1, C-5
20a	*dd* 2.00 (16.8, 3.1)	
20b	*dd* 1.57 (16.8, 10.6)	
23a	*d* 4.08 (16.6)	C-22
23b	*d* 4.23 (16.6)	C-22, $O\underline{C}OCH_3$
28	*s* 1.11	C-4, C-3
29	*s* 0.94	C-4, C-3, C-28
30	*s* 0.68	C-9, C-14, C-13
−OAc (7)	*s* 1.75	C-6
−OAc (23)	*s* 1.70	

[a] Correlations were obtained from analysis of ^1H–^1H COSY and ^1H–^1H COSY long-range spectra.

Table 2
^{13}C NMR spectral data of **1a** ($CDCl_3$, 50 MHz, δ), and **2** (7-acetylneotrichilenone) ($CDCl_3$, 22.63 MHz, δ)[a]

C	**1a**	**2**
1	155.62	157.83
2	124.70	125.98
3	200.58	204.41
4	42.70	44.11
5	44.07	45.15
6	20.95	22.26
7	72.06	73.36
8	39.55	41.08
9	45.95	47.10
10	39.40	39.59
11	16.23	17.68
12	32.79	34.45
13	37.99	42.16
14	60.17	61.33
15	215.47	218.61
16	42.33	43.23
17	34.24	38.00
18	25.72	27.89
19	17.71	19.31
20	37.61	122.89
21		140.28
22	201.59	110.83
23	66.63	143.01
28	26.22	27.37
29	19.83	21.13
30	16.50	18.20
$-\mathrm{O\underline{C}OCH_3}$	167.07	169.30
$-\mathrm{OCO\underline{CH_3}}(7)$	18.55	21.13
$\mathrm{OCO\underline{CH_3}}(23)$	19.55	

[a] Chemical shifts were obtained from $^{13}C\{^1H\}$ DEPT135 and HMQC experiments.

EIMS m/z (rel. int.): 500 $[M]^+$ (13), 458 (16), 457 (29), 440 (59), 441 (17), 427 (12), 425 (29), 385 (5), 383 (11), 341 (17), 262 (21), 261 (100); Found: C, 69.3, H, 8.1; $C_{29}H_{40}O_7$ requires: C, 69.6, H, 8.0.

Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Financiadora de Estudos e Projetos (FINEP). One of us (D.A.G.C.) acknowledges Fundação Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for the award of a scholarship.

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