Journal of Natural Products
Vol. 53, No. 6, pp. 1436-1440, Nov-Dec 1990

VISMIAEFOLIC ACID, A NEW TRITERPENE FROM *VOCHYSIA VISMIAEFOLIA*

FRANCISCA W.L. ARAÚJO, MIRIAN P. SOUZA,

Laboratório de Produtos Naturais, Universidade Federal do Ceará, Caixa Postal 12.200, 60.021, Fortaleza, Ceará, Brazil

and RAIMUNDO BRAZ FILHO*

Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, Caixa Postal 74.541, 23851, Seropédica, Rio de Janeiro, Brazil

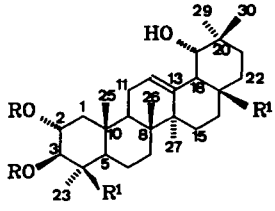
ABSTRACT.—Two dicarboxylated triterpenes were isolated from the branches of *Vochysia vismiaefolia*. The structures of these acids have been established by chemical and spectrometric methods as bartogenic acid [**1**] and the new isomer 2α,3β,19α-trihydroxyurs-12-ene-24,28-dioic acid (vismiaefolic acid) [**2**].

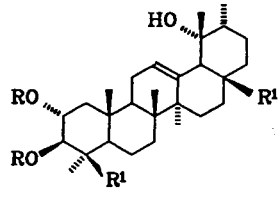
The Vochysiaceae family includes the genera *Callisthene, Erisma, Qualea, Salvertia,* and *Vochysia* of South America, with most species found in Brazil. The genus *Erismadelphus*, with only one species, is the only one not found in America, and its natural habitat is tropical Africa (1,2). The literature mentions that the juice of some *Vochysia* species is used to obtain a domestic wine (3).

Previous chemical examinations of *Vochysia* reported the occurrence of ellagic acids, physion, 2,6-dimethoxy-1,4-benzoquinone, a rutinosylellagic acid, several flavones, and their glucosides (1,4), but no record of triterpenes was found. In this paper we report the occurrence of the new triterpene vismiaefolic acid [**2**] in *Vochysia vismiaefolia* Spruce ex Warm., along with 3-*O*-β-D-glucopyranosyl-β-sitosterol and bartogenic acid [**1**] not described before in the Vochysiaceae family. Bartogenic acid [**1**] was previously reported in *Barringtonia speciosa* Forst and *Barringtonia acutangula* Gaertn. (Barringtoniaceae) (5).

RESULTS AND DISCUSSION

Compound **1** was acetylated and methylated to give, respectively, **1a** ($[M]^+$ 602) and **1b** ($[M]^+$ 546). The dimethyl ester **1b** furnished the diacetyl derivative **1c** ($[M]^+$ 630) and **1d** after reduction with $AlLiH_4$ and acetylation. The ^1H nmr (200 MHz, $CDCl_3$) of **1d** revealed the two methylenic protons of C-24 as a broad signal at δ 4.18, along with signals at δ 5.20 (td, $J = 10, 5$, H-2β) and 4.80 (d, $J = 10$, H-3α). These data are in accordance with the stereochemistry 2α,3β,24-$(OAc)_3$ shown in formula **1**. In the 2α,3β,23-$(OAc)_3$ configuration the two-proton signals appear as two doublets





1	R=H, R^1=COOH	**2**	R=H, R^1=COOH
1a	R=Ac, R^1=COOH	**2a**	R=Ac, R^1=COOH
1b	R=H, R^1=COOMe	**2b**	R=H, R^1=COOMe
1c	R=Ac, R^1=COOMe	**2c**	R=Ac, R^1=COOMe
1d	R=Ac, R^1=CH_2OAc	**2d**	R=Ac, R^1=CH_2OAc

with chemical shifts about δ 3.5 and 3.8 ($J = 12$ Hz), characterized as a clear AB system (6). Thus, ^{1}H- and ^{13}C-nmr spectra (Table 1) of these compounds provided support for the structure of triterpenoid **1** and helped to identify it as bartogenic acid (5).

The molecular formula $C_{30}H_{46}O_7$ of the new triterpene **2** was determined through the diacetate **2a** by a combination of low resolution mass spectrometry, *m/z* 602 (1%)

TABLE 1. ^{13}C-nmr Spectral Data[a] for **2a**, **2b**, **2d**, **1a**, and **1d** Compared with the Models **3** and **4**.

Carbon	Compound						
	2a	**2b**	**2d**	**1a**[b]	**1d**	**3**[c]	**4**[d]
C-1	45.0	46.6	44.3	43.9 (38.4)	44.7	46.1	38.5
C-2	70.4	68.2	68.2	69.6 (69.8)	69.2	67.7	26.9
C-3	79.6	83.6	79.8	78.4 (80.9)	79.8	84.2	73.3
C-4	50.2	49.3	42.7	50.2 (58.5)	42.7	42.5	52.2
C-5	56.2	56.6	55.3	55.9 (56.3)	55.9	53.3	49.6
C-6	20.6	20.3	19.4	19.2 (18.4)	19.5	18.0	19.5
C-7	33.5	33.0	32.9	32.6 (32.6)	32.4	32.0	34.5
C-8	40.2	39.9	40.4	39.7 (39.3)	40.0	40.8	39.8
C-9	47.3	48.0	47.2	45.1 (47.7)	47.7	46.8	45.9
C-10	38.9	38.3	36.7	38.7 (37.5)	35.5	39.2	26.8
C-11	24.1	24.2	23.9	23.7 (23.8)	24.1	24.9	24.1
C-12	127.6	128.8	128.6	125.2 (124.6)	125.2	127.2	125.6
C-13	139.9	138.2	138.2	142.3 (143.7)	142.5	138.2	137.6
C-14	42.2	41.3	42.7	40.8 (42.0)	40.1	41.1	40.0
C-15	29.2	28.3	24.8	28.0 (27.7)	28.4	28.6	31.1
C-16	26.3	25.6	25.6	24.0 (23.5)	25.7	25.4	25.4
C-17	48.2	48.0	41.1	47.5 (46.7)	46.6	47.7	45.7
C-18	54.5	53.4	54.7	43.4 (41.5)	44.9	55.3	52.3
C-19	72.6	73.1	73.6	80.9 (81.9)	82.5	71.9	70.4
C-20	42.3	41.4	41.6	27.7 (28.2)	27.9	41.3	39.8
C-21	26.9	27.4	26.1	34.8 (34.0)	31.9	27.7	24.8
C-22	38.3	37.4	35.7	32.0 (32.7)	31.1	37.2	36.1
C-23	26.9	26.1	27.6	26.3 (28.3)	27.8	22.9	178.2
C-24	176.3	178.3	65.5	186.0 (185.2)	65.5	64.2	9.8
C-25	14.6	14.5	16.1	14.3 (14.2)	14.2	21.8	13.8
C-26	16.9	16.5	16.3	16.0 (16.7)	17.2	14.6	14.4
C-27	24.1	23.9	23.1	25.1 (24.8)	24.9	22.3	24.6
C-28	180.5	178.2	71.2	180.7 (178.5)	70.3	180.7	178.3
C-29	24.6	24.2	24.2	26.3 (32.9)	27.8	23.0	26.9
C-30	16.7	16.0	16.2	23.6 (51.3)	23.6	15.7	14.8
OMe	—	51.4	—	— (51.7)	—	—	—
	—	51.3	—	— (51.7)	—	—	
OAc	170.3	—	170.3	170.2 —	170.3	—	—
	171.1	—	170.5	170.9 —	170.3	—	—
	20.9	—	170.7	20.1 —	170.9	—	—
	21.1	—	171.3	21.1 —	170.9	—	—
	—	—	21.0	— —	21.0	—	—
	—	—	21.0	— —	21.0	—	—
	—	—	20.9	— —	20.8	—	—
	—	—	20.8	— —	20.8	—	—

[a]Spectra were run in $CDCl_3$ (**1a**, **1d**, **2b**, **2d**, **3**) and C_5D_5N (**2a** and **4**) at 25.2 (**1a**, **2b**), 75 (**2a**), 50.3 MHz (**1d** and **2d**). Chemical shifts are given downfield from TMS. Assignments were made with the aid of the DEPT and/or 2D-shift-correlated (^{1}H ^{13}C-HETCOSY) spectra of **1a** and **1c**.

[b]Values given in brackets are the reported chemical shifts of the dimethyl ester of bartogenic acid [**1b**] as in Rao *et al.* (5).

[c]Values are from Houghton and Lian (8).

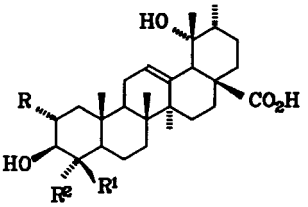
[d]Values are from Nakatani *et al.* (9).

$[M]^+$, and ^1H- and ^{13}C-nmr (broadband decoupled and DEPT) counts of ^1H and ^{13}C numbers, including 2D heteronuclear correlation ($^1H^{13}C$-HETCOSY) (7). Spectral comparison of derivatives **2a**, **2b**, and **2d** with **1a** allowed us to classify this natural product **2** as a pentacyclic triterpene of the urs-12-ene [**2a**: δ C 127.6 (C-12), 139.9 (C-13); δ H 2.99 (br s, H-18), 1.10 (d, J = 6.5 Hz, Me-30)] group (Tables 1 and 2). The peak at *m/z* 264 (44%) in the eims of **2a**, corresponding to fragment **5** (retro-Diels-Alder reaction), reinforced this deduction. The major distinction between the two natural products **1** and **2** (Tables 1 and 2) was the methyl substitution at C-19 and C-20. The chemical shifts and the observed multiplicity in the ^1H- and ^{13}C-nmr spectra (Tables 1 and 2) were consistent with the structure of the new triterpene as **2**. The assignments were based on the application of the usual shift parameters, observed multiplicities of signals (DEPT), 2D shift-correlated $^1H^{13}C$-HETCOSY, and comparison with literature data for models **3** (8) and **4** (9). The stereochemistries at C-2, C-3, and

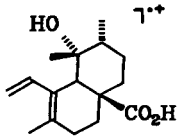
TABLE 2. $^1H^{13}C$ 2D-shift-correlated ($^1H^{13}C$-HETCOSY) Nmr Data[a] of Compound **2a** [C_5D_5N, 300 MHz (^1H) and 75.4 (^{13}C)], Along with ^1H-nmr Spectral Data of **2c** (60 MHz, $CDCl_3$) and **2d** (200 MHz, $CDCl_3$)].

Position	Compound			
	2a		**2d**	**2c**
	δc	δH	δH	δH
1	45.0	2.30 (m) 1.20 (m)		
2	70.4	6.45 (td, J = 10.3, 4.9 Hz)	5.14 (td, J = 10.5, 5)	5.50–5.80 (m)
3	79.6	5.23 (d, J = 10.3)	4.82 (d, J = 10.5)	4.72 (d, J = 10)
5	56.2	1.30 (m)		
6	20.6	2.10 (m)		
7	33.5			
9	47.3	1.80 (m)		
11	24.1	2.00 (m) 1.50 (m)		
12	127.5	5.52 (br t)	5.22 (m)	5.25 (m)
15	29.2	1.30 (m)		
16	26.3	3.08 (td, J = 12.8, 4.3) 1.90 (m)		
18	54.5	2.99 (s)		2.50 (s)
20	42.3	1.40 (m)		
21	26.9	2.00 (m) 1.40 (m)		
22	38.3	2.05 (m)		
23	16.9	1.02 (s)	0.95 (s)	1.18 (s)
24	—	— —	4.19 (s) —	
25	14.6	1.24 (s)	1.02 (s)	0.65 (s)
26	26.9	1.42 (s)	1.07 (s)	1.25 (s)
27	24.1	1.52 (s)	1.12 (s)	1.25 (s)
28		—	3.99 (d, J = 10.9) 3.64 (d, J = 10.9)	
29	24.6	1.72 (s)	1.27 (s)	1.25 (s)
30	16.7	1.10 (d, J = 6.5)	0.92 (d, J = 6.2)	0.86 (d, J = 6)
OMe	—	—	—	3.50 (s), 3.60 (s)
OAc	—	—	2.04 (s), 2.03 (s) 2.02 (s), 1.95 (s)	2.02 (s), 1.90 (s)

[a]Chemical shifts in δ, and coupling constants (J) in Hz; TMS as internal standard.



3 R=OH, R^1=Me, R^2=CH_2OH
4 R=H, R^1=COOH, R^2=Me



5
m/z 264 (44%)

C-4 were also confirmed by chemical shifts and coupling constants [δ H 5.14 (td, J = 10.5 and 5 Hz, H-2), 4.82 (d, J = 10.5 Hz, H-3), 4.19 (br s $AcOCH_2$-24)] revealed by ^{1}H nmr of **2d**. The doublet signal of axial H-3 in the epimer $AcOCH_2$-23 appears at about δ 5.00 (6). These data were used to establish the axial position for the COOH group at C-4. The assignment of an axial (**1**–**3**) or equatorial position (**4**) for COOR or CH_2OR at C-4 can also be deduced by chemical shifts of C-3 and C-5 in the ^{13}C-nmr spectra, which show the expected shielding γ effect of the oxygen atoms when one of these groups is equatorially oriented (Table 1). From the comparison of the ^{13}C-nmr spectra of C-11 to C-22, C-29, and C-30 of **2a**, **2b**, and **2d** with those of models **3** (8) and **4** (9), the configurations at C-19 and C-20 were deduced (Table 1). Thus, the new triterpene isolated from *V. vismiaefolia* was characterized as 2α,3β,19α-trihydroxyurs-12-ene-24,28-dioic acid [**2**].

Finally, the previous assignment of chemical shifts for C-1, C-4, C-29, and C-30 of the dimethyl ester of bartogenic acid (5) must be reexamined and those of C-5 and C-18 of **3** (8) interchanged (Table 1). These necessary modifications emerged after comparative analysis of ^{13}C-nmr spectra of **2a** [assignments based on DEPT and ^{1}H^{13}C-HETCOSY (Table 2)], **2b**, **2d**, and the models **3** (8) and **4** (9). As observed in Table 1, the chemical shift of C-1 in these triterpenes with patterns 2α,3β-dihydroxy-24-oic and their 2α,3β-di-*O*-acetyl-24-oic derivative appeared at about δ 46 and δ 44, respectively, a situation in which the position of absorption of C-4 is about δ 50. In the presence of the OH group at C-19α, the chemical shift of the 29-methyl group cannot be at δ 32.9 because it is shielded by the γ effect of the OH group (Table 1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Ir spectra were measued on a Perkin-Elmer 1320 spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ or pyridine-d_5, using TMS as internal standard, employing Varian EM-360 (60 MHz), Varian VXR-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz), and Bruker AC-200 (^{1}H, 200 MHz; ^{13}C, 50.3 MHz) spectrometers. Low resolution mass spectra were obtained on a Finnigan-3200 instrument operating at 70 eV.

PLANT MATERIAL.—Branches of *V. vismiaefolia* were collected in Manaus, Brazil, and a voucher specimen was deposited in the herbarium of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil.

EXTRACTION AND ISOLATION.—The pulverized, air-dried plant material (3 kg) was percolated with EtOH (8 liters) at room temperature, and the EtOH extract was concentrated in vacuo to afford a reddish-brown solid (88 g). This material was submitted to coarse chromatography over Si gel (500 g), using C_6H_{14}, $CHCl_3$, Me_2CO, and MeOH, successively. The fraction eluted with Me_2CO (25 g) was chromatographed on a Si gel column and eluted with $CHCl_3$ gradually enriched with Me_2CO and afterwards with MeOH. The fractions eluted with $CHCl_3$/Me_2CO (10 to 15%) were mainly composed of compound **1**, and those following contained essentially **2**, which was rechromatographed in a similar manner as described above and recrystallized from MeOH to give **2** (400 mg) purified. The fractions eluted with MeOH furnished 3-*O*-β-D-glucopyranosyl-β-sitosterol (280 mg) and an additional amount of **2** (130 mg). The 3-*O*-β-D-glucopyranosyl-β-sitosterol was identified by direct comparison with an authentic sample.

2α,3β,19α-TRIHYDROXYOLEAN-12-ENE-24,28-DIOIC ACID (BARTOGENIC ACID) [**1**].—Mp >300°; spectral data identical to those previously published (5); ^{13}C nmr see Table 1. The dimethyl ester **1b**, mp 273–278°, obtained by methylation with CH_2N_2, and the dimethyl ester diacetate **1c**, mp 194–197°, prepared by reaction of **1b** with Ac_2O/pyridine as usual, were used for confirmation (5).

2α,3β,19α-TRIHYDROXYURS-12-ENE-24,28-DIOIC ACID (VISMIAEFOLIC ACID) [**2**].—Mp >300°.

2α,3β-DI-*O*-ACETYL-19α-HYDROXYURS-12-ENE-24,28-DIOIC ACID [**2a**].—Compound **2** (250 mg) was dissolved in pyridine (4 ml) and Ac_2O (4 ml), and the solution was allowed to stand for 24 h at room temperature. The usual workup produced diacetate **2a** (180 mg) crystallized from $CHCl_3/Me_2CO$, mp 271–276°; ir ν max (KBr) 3530, 2980, 2940, 1740, 1700, 1270 cm^{-1}; ^{13}C nmr see Table 1; ^1H nmr see Table 2; eims (%) $[M]^+$ 602 (1), 279 (12), 264 (44), 246 (55), 219 (40), 201 (100), 133 (57).

DIMETHYL 2α,3β,19α-TRIHYDROXYURS-12-ENE-24,28-DIOATE [**2b**].—A sample of **2** (230 mg) was methylated with CH_2N_2 in Et_2O to give **2b** (190 mg): mp 265–270°; ^{13}C nmr see Table 1.

DIMETHYL 2α,3β-DI-*O*-ACETYL-19α-HYDROXYURS-12-ENE-24,28-DIOATE [**2c**].—The diacetate **2a** (250 mg) was methylated with CH_2N_2 in Et_2O to give **2c** (200 mg), mp 194–197°, after crystallization from $CHCl_3/Me_2CO$: ^1H nmr see Table 2.

2α,3β,24,28-TETRA-*O*-ACETYLURS-12-EN-19α-OL [**2d**].—The sample of **2b** (100 mg) in dry Et_2O was treated with $LiAlH_4$ (500 mg in dry Et_2O), and the mixture was stirred for 2 h at room temperature and allowed to stand for 24 h. The excess $LiAlH_4$ was destroyed by addition of EtOAc and HCl until acidified (10). The precipitate collected by filtration was washed with MeOH, and the filtrates were evaporated. The residue (80 mg) was acetylated and purified by chromatography over a Si gel column to give **2d** (45 mg): ^{13}C nmr see Table 1; ^1H nmr see Table 2.

ACKNOWLEDGMENTS

This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP), and Coordenação de Aperfeiçoamento de Pessoal do Ensino Superior (CAPES). The authors thank Dr. Charles D. Hufford, Department of Pharmacognosy, University of Mississippi, for the nmr (^1H, 300 MHz; ^{13}C, 75 MHz), and Dr. José Guilherme Soares Maia, Departamento de Química, Universidade Federal do Pará, Belém, Brazil, for obtaining the crude EtOH extract.

LITERATURE CITED

1. D.B. Corrêa, E. Birchal, J.E.V. Aguilar, and O.R. Gottlieb, *Phytochemistry*, **14**, 1138 (1975).
2. A.B. Joly, "BOTÂNICA-Introdução a taxonomia vegetal," Editora Nacional, São Paulo, Brazil, 1977, p. 423.
3. G.M. Barroso, "Sistemática de Angiospermas do Brasil," UFV Imprensa Universitária, Viçosa, Brazil, 1984, Vol. 2, p. 343.
4. J.L.C. Lopes, J.N.C. Lopes, and H.F. Leitão Filho, *Phytochemistry*, **18**, 362 (1979).
5. G.S.R.S. Rao, S. Prasanna, V.P.S. Kumar, and B. Yadagiri, *Indian J. Chem.*, **25B**, 113 (1986).
6. H. Kojima and H. Ogura, *Phytochemistry*, **28**, 1703 (1989).
7. J.K.M. Sanders and B.K. Hunter, "Modern NMR Spectroscopy-A Guide for Chemists," Oxford University Press, Oxford, 1988, pp. 100–108, 238–241, 253–256.
8. P.J. Houghton and L.M. Lian, *Phytochemistry*, **25**, 1939 (1986).
9. M. Nakatani, Y. Miyazaki, T. Iwashita, H. Naoki, and T. Hase, *Phytochemistry*, **28**, 1479 (1989).
10. B. Singh and R.P. Rastogi, *Phytochemistry*, **7**, 1385 (1968).

Received 13 February 1990