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4′-DEHYDROXYCABENEGRIN A-I FROM ROOTS OF *HARPALYCE BRASILIANA**

GRAÇA LÚCIA DA SILVA, FRANCISCO JOSÉ DE ABREU MATOS and EDILBERTO ROCHA SILVEIRA†

Curso de Pós-Graduação em Química Orgânica. Departamento de Química Orgânica e Inorgânica, Laboratório de Produtos Naturais, Universidade Federal do Ceará, Cx. Postal 12.200, Fortaleza-CE, 60.021-970. Brazil

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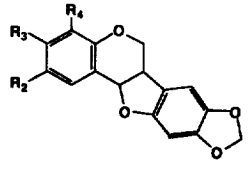
Key Word Index—*Harpalyce brasiliana*; Leguminosae; roots; 'erva-de-cobra'; prenylated pterocarpane; triterpene; 4′-dehydroxycabenegrin A-I; betulinic acid.

Abstract—An ethanol extract from roots of *Harpalyce brasiliana* yielded betulinic acid and a prenylated pterocarpane, 3-hydroxy-4-(4-isopentenyl)-8(9)-methylenedioxi-6a*R*, 11a*R*-dihydro-6H-benzofuro[3,2-c][1]benzopyran (4′-dehydroxy-cabenegrin A-I), very similar to the already known potent snake venom antidote, cabenegrin A-I. Structural determination, including the absolute stereochemistry, was accomplished by spectral analysis particularly CD and 2D NMR techniques. Chemical derivatization and comparison with literature data were also helpful for structural elucidation. © 1997 Elsevier Science Ltd

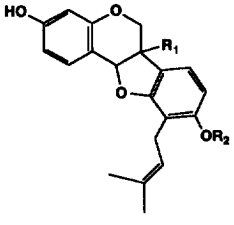
INTRODUCTION

Traditional medicine in northeast Brazil has used a tincture of some plant roots for the treatment of snake bites. Such a plant is *Bredemeyera floribunda*, which has been subject of a phytochemical analysis previously reported by our group [1], where xanthones were the major isolated compounds. In continuation of our efforts to analyse anti-snake venom-reputed plant material from Brazil, we have undertaken the analysis of an ethanol extract of the roots of *Harpalyce Brasiliana*, a shrub popularly designated as 'erva-de-cobra' (Portuguese: snake's herb).

It is worth mentioning that Nakagawa and co-workers have reported the isolation and synthesis of two prenylated pterocarpanes, designated cabenegrin A-I (**1**) and cabenegrin A-II (**2**) from a hydroalcoholic beverage called 'Especifico Pessoa', available to plantation workers in the Amazon jungle as an oral antidote against snake and spider venom [2, 3], but prepared at Pessoa's factory in Sobral, Ceará State, north east Brazil. The designation cabenegrin came after their belief that this antidote was prepared from a plant called 'cabeça-de-negro' (Portuguese: black's head) designated after a legend stating that lizards bitten when fighting with snakes run to a dark round rhizome, eat a piece of it and then come back to continue fighting. However, according to them, they have never been able to identify the plant because there are more than 10 plant species in South America popularly designated in the same way [2].



	R_2=	R_3=	R_4=
1.	H	OH	$CH_3(CH_2OH)C=CH\text{-}CH_2$-
2.	$CH_3(CH_2OH)C=CH\text{-}CH_2$-	OH	H
3.	CH_3CO_2-	OMe	H
6.	$(CH_3)_2C=CH\text{-}CH_2$-	OH	H
7.	H	OH	$(CH_3)_2C=CH\text{-}CH_2$-
8.	H	OMe	$(CH_3)_2C=CH\text{-}CH_2$-
9.	-O-$C(CH_3)_2$-CH=CH-		H
10.	H	-O-$C(CH_3)_2$-CH=CH-	



	R_1=	R_2=
4.	OH	Me
5.	H	H

* This work forms part of the MSc Thesis of Graça Lucia da Silva, presented at the Departamento de Química Orgânica, Universidade Federal do Ceará, Fortaleza, CE, Brazil, 1994.

† Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

Silica gel chromatography of the water-insoluble portion of the ethanol extract of ground dried roots of *H. brasiliana* yielded a resinous substance designated HBRE-1, homogeneous on TLC. Its IR spectrum showed a broad and intense absorption centred at 3400 cm^{-1} (ν_{O-H}), a shoulder absorption near 3000 cm^{-1} ($\nu_{=C-H}$), skeletal bands at 1600 and 1500 cm^{-1} (Φ-H), C-O stretching at 1150 ($\nu_{=C-O}$) and 1090–1050 cm^{-1} (ν_{Csp^3}-O) and also = C-H bending at 780–940 cm^{-1}.

Proton NMR analysis (Table 1) revealed two vinyl methyls as singlets at δ 1.71 and 1.78 (H-4′ and H-5′), a vinyl methylene doublet at δ 3.39 (2H, J = 6.8 Hz, H-1′), a methyne at δ 3.45 (1H, *ddd*, J = 5.0, 6.7 and 11.0 Hz, H-6a), a triplet-like absorption at δ 3.60 (1H, J = 11.0, H-6$_\beta$), a doublet of doublets centred at δ 4.20 (1H, J = 5.0 and 11.0 Hz, H-6$_\alpha$), an olefinic hydrogen triplet at δ 5.20 (1H, J = 6.8 Hz, H-2′), a benzylcarbinolic methyne doublet at δ 5.48 (1H, J = 6.7 Hz, H-11a), a broad singlet, exchangeable with D_2O, at δ 5.65 (O-H), a methylenedioxide as two narrow doublets centred at δ 5.89 (O-CH_2-O), an aromatic hydrogen, probably *ortho* to two oxygens, at δ 6.42 (1H, *s*, H-10) followed by a doublet at δ 6.51 (1H, J = 8.4 Hz, H-2), another singlet at δ 6.70 (1H, H-7) and, finally, another doublet at δ 7.22 (1H, J = 8.4 Hz, H-1).

^{13}C NMR (BB) analysis (Table 1) showed 21 carbons, whose hydrogenation pattern was determined by comparison of the BB spectrum with the DEPT 135 spectrum. Two methyls at δ 17.8 (C-5′) and 25.7 (C-4′), a non-oxygenated methylene at δ 22.3 (C-1′) and an oxygenated one at δ 66.6 (C-6), a methylenedioxide at δ 101.0, two saturated methynes, one of which was oxygenated, at δ 40.0 (C-6a) and 79.1 (C-11a), respectively, five monohydrogenated sp^2 carbons at δ 93.7 (C-10), 104.7 (C-7), 109.7 (C-2), 121.7 (C-2′) and 129.9 (C-1), and thus seven non-hydrogenated sp^2 carbons (Table 1).

The HR-mass spectrum of HBRE-1 showed a m/z 352.1304 for the $[M]^+$, compatible with the formula $C_{21}H_{20}O_5$ (calcd. for $C_{21}H_{20}O_5$, 352.1310), in complete agreement with the NMR analysis just described. Facile methylation with diazomethane confirmed its monophenolic character, suggested by IR and ^1H NMR.

The 2D ^1H,^1H-COSY spectrum showed strong correlation for the aromatic doublets (H-1 and H-2), the olefinic hydrogen and the vinyl methylene (H-1′ and H-2′), the carbinolic methyne (δ 5.48) with the non-oxygenated methyne (δ 3.45) (H-11a and H-6a), the geminal coupling between H-6a (δ 4.20) and 6b (δ 3.60), and also weaker correlation for the allylic coupling of the methyls with the olefinic hydrogen, as well for the homoallylic coupling for the methyls with the vinyl methylene. The 2D carbon-channel detected heteronuclear NMR experiment (HETCOR) gave the one-bond carbon-hydrogen correlations suggested in Table 1.

At this point, all data fitted nicely into a monophenol pterocarpane structure possessing a prenyl and methylenedioxide moieties as substituents. A literature survey on pterocarpane NMR data revealed a series of prenylated pterocarpanes with these

Table 1. ^1H and ^{13}C NMR data of compound 7.

C#	δ_C	DEPT	HETCOR	COSY*
1	104.7	CH	7.22 (*d*, J = 8.4 Hz)	
1a	112.4	C	—	
2	109.7	CH	6.51 (*d*, J = 8.4 Hz)	
3	155.5	C	—	
4	115.5	C	—	
4a	154.0	C	—	
6	66.6	CH_2	4.20 (*dd*, J = 5.0 & 11.0 Hz)-Hα	
			3.60 (*dd* = *t*, J = 11.0 Hz)-Bβ	
6a	40.0	CH	3.45 (*ddd*, J = 5.0, 6.7 & 11.0 Hz)	
7	104.7	CH	6.70 (*s*)	
7a	118.0	C	—	
8	141.5	C	—	
9	147.9	C	—	
10	93.7	CH	6.42 (*s*)	
10a	153.9	C	—	
11a	79.1	CH	5.48 (*d*, J = 6.7 Hz)	
1′	22.3	CH_2	3.39 (*d*, J = 6.8 Hz)	
2′	121.7	CH	5.20 (*t*, J = 6.8 Hz)	
3′	134.7	C	—	
4′	25.7	CH_3	1.78 (*s*)	
5′	17.8	CH_3	1.71 (*s*)	
O-CH_2-O	101.0	CH_2	5.89 (two *d*, J = 1.3 Hz)	

* Observed correlations connected by arrows.

features. However, the methylenedioxide substituent is, for all surveyed cases, in the benzofuran portion; we found an example of a non-prenylated pterocarpane, the 2-acetoxypterocarpin, **3** [4], with both positions C-2 and C-3 oxygenated, that could accommodate a methylenedioxide moiety. On the other hand, the isoprenyl moiety appears commonly in either the benzofuran (as in crystacarpin (**4**) and phaseollidin (**5**) [5]) or the benzopyran portion of the pterocarpane system (as in cabenegrin I-A (**1**) [2] and edunol (**6**) [4, 6]). Thus, two structures, which would accommodate the observed NMR data. A long-range inverse-detected (hydrogen-channel detection) heteronuclear 2D NMR experiment (HMBC) allowed the observation of the correlations shown by the arrows in Fig. 1. For the final structure and unambiguous complete assignment of the NMR data for HBRE-1, the most important correlations were those for H-6 (δ 4.20), H-1′ (δ 3.39) and H-1 (δ 7.22), all with the same carbon 4a (δ 154.0). Indeed, the ^{13}C NMR data of cabenegrin A-I are very similar to those from HBRE-1, suggesting the same substitution pattern. From our data one can maybe suggest that the correlations not made by Nakagawa [2] for carbons 4 and 1a, in cabenegrin A-I, are δ 112.6 and 115.0, respectively.

Circular dichroism analysis of HBRE-1 yielded a spectrum with the same profile observed for cabenegrin A-I (**1**) [2] and edunol (**6**) [6], indicating the same absolute stereochemistry for all three substances. Thus, HBRE-1 is the 3-hydroxy-4-(4-isopentenyl)-8(9)-methylenedioxi-6a*R*, 11a*R*-dihydro-6H-benzofuro[3,2-c][1]benzopyran or 4′-dehydroxycabenegrin A-I (**7**).

Recently, Machado and co-workers [7], have reported the isolation of the three pterocarpanes, **6**, **9** and **10**, from *H. brasiliana* from the same region. Thus, because of the vicinity of the plant collection site and the 'Especifico Pessoa' preparation factory, we strongly believe that 'erva-de-cobra' and not 'cabeça-de-negro' is one of the major materials used for the 'Especifico Pessoa' preparation analysed by Nakagawa and co-workers [2].

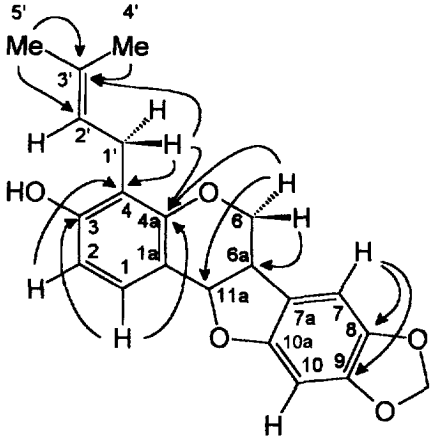


Fig. 1. Long-range ^{1}H, ^{13}C NMR correlations observed by HMBC for compound 7.

EXPERIMENTAL

General. IR: KBr (solids), NaCl discs (oil films). ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR: Bruker ACE-200; ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR: Varian Unity 400. $CDCl_3$. δ 7.24 for ^{1}H and δ 77.0 for ^{13}C as int. references.

Plant material. Harpalyce brasiliana Benth roots were collected in Chapada da Ibiapaba, CE, Brazil, during the flowering stage. A voucher specimen (# 14.841) representing the collection was identified by Dr Afrânio G. Fernandes (Botanist, Departamento de Biologia, UFCE) and has been stored at Herbarium Prisco Bezerra of the Departamento de Biologia, Universidade Federal do Ceará, Brazil.

Extraction and isolation. Ground, sun-dried, roots (5 kg) yielded a dark brown viscous EtOH extract (337 g) after solvent evapn. An aliquot (115 g) was suspended in H_2O and the insoluble material (65 g) after filtration under vacuum was mixed with 100 g of silica gel and coarsely eluted with hexane–$CHCl_3$ (1:1), $CHCl_3$, ETOAc, MeOH and, finally, EtOH. The less polar frs eluted with hexane–$CHCl_3$ (1:1) and $CHCl_3$ showed a similar TLC behaviour, and were mixed (18.5 g) and submitted to successive CC on silica gel to yield 35 mg of pure HBRE-1 (**7**). $[\alpha]_D$ −95.3° (*c* 3.45 $CHCl_3$). IR ν_{max} cm^{-1}: 3400 (*br*), 3050–2860, 1600, 1500, 1480, 1450, 1380, 1350, 1300, 1160, 1100, 1080, 1060, 1050, 950, 900, 795, 780. EI-MS *m/z* (rel. int.): 352 $[M]^+$ (100), 351 (12, $[M-H]^+$), 335 (5, $[M-OH]^+$), 296 (87, $[M-CH_2=CMe_2]^+$), 175 (18), 162 (40), 147 (25), 135 (30), 115 (25), 86 (39), 84 (65). ^{1}H and ^{13}C NMR: Table 1.

Methyl derivative (**8**). Compound **7** (10 mg) was dissolved in Et_2O (5 ml) and treated with excess CH_2N_2–Et_2O to yield **8** (8 mg), as a colourless solid, mp 104–107°. IR ν_{max} cm^{-1}: no ν_{O-H} 2850–3050, 1610, 1500, 1480, 1150, 1050, 1000. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.29 (1H, *d*, *J* = 8.0 Hz, H-1), 6.69 (1H, *s*, H-7), 6.64 (1H, *d*, *J* = 8.0 Hz, H-2), 6.40 (1H, *s*, H-10), 5.88 (2H, *d*, O-CH_2-O), 5.48 (1H, *d*, *J* = 7.0 Hz, H-11a), 5.13 (1H, *t*, *J* = 6.7 Hz, H-2′), 4.23 (1H, *dd*, *J* = 5.0 and 11.0 Hz, H-6α), 3.81 (3H, *s*, OMe), 3.58 (1H, *t*, *J* = 11.0, H-6β), 3.48 (1H, *ddd*, *J* = 5.0, 7.0 and 11.0 Hz, H-6a), 3.31 (2H, *d*, *J* = 6.7, H-1′). ^{13}C NMR (50 MHz, $CDCl_3$): Table 2.

Betulinic acid. Rechromatography over silica gel of the more polar frs and recrystallization from MeOH, yielded 800 mg of a colourless solid, mp 274–277°, whose spectral data were similar to betulinic acid (mp lit. [8]). The acetyl derivative of betulinic acid obtained after treatment with Ac_2O–pyridine, mp 268–272° (lit. [8]) was also used to confirm its identity.

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Table 2. ^{13}C NMR data comparison between compound **7**, its methyl derivative **8** and model compounds from the literature, **1** [2], **4** [4], **5** [5] and **6** [4]

	7	8	1	4	6	5
C#			[2]	[4]	[4]	[5]
1	129.9	129.1	129.15	132.4	130.8	132.4
1a	112.4	112.3	112.63*	110.4	110.7	112.6
2	109.7	109.8	109.60	113.2	121.6	110.0
3	155.5	158.0	155.08	158.8	155.9	158.5
4	115.5	114.9	115.01*	103.8	102.4	—
4a	154.0	154.5	154.20	157.3	153.9	157.4
6	66.6	66.7	66.72	69.8	65.6	66.6
6a	40.0	40.1	40.16	77.3	39.6	40.1
7	104.7	104.7	104.73	120.8	104.9	121.6
7a	118.0	118.0	118.04	120.0	118.2	118.7
8	141.5	142.0	141.67	104.5	140.8	108.9
9	147.9	147.9	148.06	160.2	147.2	156.5
10	93.7	93.7	93.77	114.3	92.9	110.9
10a	153.9	154.0	154.20	155.9	153.6	155.5
11a	79.1	79.2	79.10	84.6	78.0	78.2
1′	22.3	22.3	21.88	22.7	22.7	23.1
2′	121.7	121.6	123.49	122.3	122.8	122.3
3′	134.7	131.5	136.07	131.5	130.8	134.4
4′	25.7	25.8	68.72	25.6	25.2	25.6
5′	17.8	17.8	13.74	17.7	17.3	17.9
$O\underline{C}H_2O$	101.0	101.2	101.22	—	100.8	—
$\underline{C}H_3$-O	—	55.8	—	56.2	—	—

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