J. Braz. Chem. Soc., Vol. 11, No. 2, 187-190, 2000.
Printed in Brazil

Short Report

Further Diterpenoids Isolated from *Pterodon polygalaeflorus*

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Dos frutos de *Pterodon polygalaeflorus* (Leguminosae) foram isolados o novo diterpeno 6α-hidroxivouacapano e os conhecidos diterpenóides 6α,7β,14β,19-tetraidroxivouacapano, 6α,7β-diidroxivouacapan-17β-oato de metila e o flavonóide taxifolina. As estruturas foram determinadas com base em análise espectroscópica, inclusive RMN bidimensional, transformação química do novo diterpeno e comparação com dados da literatura.

From fruits of *Pterodon polygalaeflorus* (Leguminosae) the new diterpene 6a-hydroxyvouacapane, the known diterpenoids vouacapane-6α,7β,14β,19-tetraol and methyl 6α,7β-dihydroxyvouacapan-17β-oate and the flavonoid taxifolin were isolated. Structural determinations were accomplished by spectroscopic analysis, including two-dimensional NMR, chemical transformation of the new diterpene and comparison with literature data.

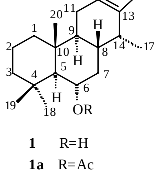
Keywords: *Pterodon polygalaeflorus*, Leguminosae, diterpenoids, flavonoid

Introduction

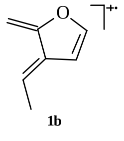
In continuation of our systematic studies of the Brazilian northeast medicinal plants, we have undertaken a study of *Pterodon polygalaeflorus* Benth. (Leguminosae), which is used in folk medicine against bronchytis, amigdalytis and as tonic. We have already reported diterpenoids[1] and the volatile constituents[2] from this plant. In continuation to this phytochemical investigation, we now report the isolation and structure determination of a new diterpene 6α-hydroxyvouacapane (**1**) and of the known compounds vouacapane-6α,7β,14β,19-tetraol (**2**)[3], methyl 6α,7β-dihydroxyvouacapan-17β-oate (**3**)[4] and the flavonoid taxifolin (**4**, 5,7,3',4'-tetrahydroxydihydroflavonol)[5] , isolated from the fruits. The structures of these compounds were deduced by spectral analysis, mainly ^{1}H and ^{13}C NMR, including 2D-NMR experiments [^{1}H-^{1}H-COSY, ^{13}Cx^{1}H-COSY-$^1J_{CH}$ (HETCOR) and ^{13}Cx^{1}H-COSY-$^nJ_{CH}$(n=2 and 3, COLOC)], chemical transformation of the new diterpene (**1**) and comparison with literature data.

Results and Discussion

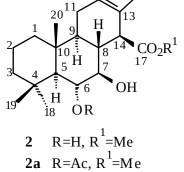
The hot hexane extract of coarsely ground fruits of *P. polygalaeflorus* was submitted to aqueous ethanol alkaline hydrolysis. After usual work-up, the insaponifiable fraction obtained was chromatographed on silica gel column to furnish a new diterpene 6α-hydroxyvouacapane (**1**), methyl 6α,7β-dihydroxyvouacapan-17β-oate (**2**), previously isolated from *Pterodon emarginatus*[4], vouacapane-6α,7β,14β,19-tetraol (**3**)**,** which was also isolated from the seeds of a specimen of *P. polygalaeflorus* collected in Minas Gerais-Brazil[6], and the flavonoid **4**, known as taxifolin (5,7,3',4', tetrahydroxydihydroflavonol)[5]. Compound **1** was not previously obtained as a natural product, although it was prepared from its acetylated derivative **1a,** which had been isolated from *Dipterix lacynifera*[7].



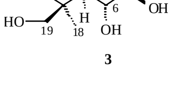
1 R=H
1a R=Ac



1b



2 R=H, R^1=Me
2a R=Ac, R^1=Me
2b R=R^1=H



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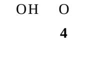

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The identification of the known compounds **2-4** involved the analysis of ^{1}H NMR (1D and 2D ^{1}H-^{1}H-COSY) and ^{13}C NMR (HBBD and DEPT) spectral data (Table 1) and comparison with literature values[1,5,6]. The comparative analysis of the chemical shifts of the hydrogen and carbon atoms of **2** and methyl 6α-acetoxy-7β-hydroxyvouacapan-17β-oate (**2**a) and 6α,7β-dihydroxyvouacapan-17β-oic acid (**2**b), which were also isolated from *Pterodon polygalaeflorus*[1], revealed, as anticipated, the modifications in the chemical shifts of the hydrogen H-6 [**2**: δ_H 4.79 (*dd*, *J*=10.7, 8.9 Hz); **2**a: δ_H 5.25 (*dd*, *J*=11.5, 9.2 Hz)] and carbons C-5 [$\Delta\delta_C$ = 55.8 (**2**) - 54.9 (**2**a) = 0.9 ppm], C-6 [$\Delta\delta_C$ = 74.3 (**2**) - 75.5 (**2**a) = - 1.2 ppm] and C-7 [$\Delta\delta_C$ = 82.8 (**2**) - 81.2 (**2**a) = 1.6 ppm] as a consequence of the absence of the γ-effect (C-5 and C-7) and β-effect (C-6) of the carbonyl carbon of the 6a-acetoxy group in **2** (Table 1).

Compound **1** was obtained as colorless needles after crystallization from methanol. The HREIMS spectrum showed the molecular ion at *m/z* 302.2126 corresponding to the molecular formula $C_{20}H_{30}O_2$ (*m/z* 302.2238, Δ*m/z* = 0.0112 daltons). This data together with the presence of signals for three quaternary [δ_H 0.89 (*s*,3H-20), 1.07(*s*, 3H-19) and 1.17 (*s*, 3H-18)] and one tertiary [δ_H 0.99 (*d*, *J*=6.9 Hz, 3H-17)] methyl groups, one carbinolic hydrogen at δ_H 3.94 (*td*, *J*=7.1, 3.6 Hz, H-6) and two hydrogens at δ_H 6.17 (*d*, *J*=1.6 Hz, H-15) and 7.20 (*d*, *J*=1.6 Hz, H-16) of a 2,3-disubstituted furan observed in the ^{1}H NMR spectrum allowed us to classify this diterpene as a vouacapanoid, the same class of diterpenoids which was reported previously[1]. This deduction was confirmed by comparative analysis of the HBBD- and DEPT-^{13}C NMR, which revealed the presence of signals corresponding to four quaternary (δ_C 149.48, 122.14, 38.58 and 33.39), seven methine (δ_C 140.42, 109.42, 70.02, 60.02, 44.46, 35.30 and 30.97), five methylene (δ_C 43.69, 42.15, 39.81, 22.42 and 18.42) and four methyl (δ_C 37.01, 22.57, 17.36 and 15.41) carbon atoms. The presence of one secondary hydroxyl group was indicated by ^{1}H NMR of the monoacetate derivative (**1a**), which revealed downfield shift for carbinolic hydrogen H-6 [$\Delta\delta_H$ 5.12 (**1**a) - 3.93 (**1**) = 1.19 ppm].

The localization of the hydroxyl group at C-6 was deduced by the spin-spin interaction of hydrogen H-6 [δ_H 3.94 (*td*, *J*=7.1, 3.6 Hz)] with H-5 [δ_H 0.91 (*d*, *J*=7.1 Hz)] and 2H-7 (δ_H 1.90 and 1.40) revealed by homonuclear 2D ^{1}H-^{1}H-COSY spectrum and the chemical shift of C-5 (δ_C 60.02) that was assigned on the basis of heteronuclear 2D direct CH [^{13}Cx^{1}H-COSY$^1J_{CH}$: δ_C 60.02 (C-5) correlated with H-5 (δ_H 0.91)] and via long range coupling CH [^{13}Cx^{1}H-COSY-$^nJ_{CH}$ (n = 2 and 3) COLOC]: δ_C 60.02 (C-5) correlated with 3H-18 (δ_H 1.17)] spectra. Additional confirmation of the presence of a hydroxyl group at C-6 was obtained from the ^{13}C NMR spectrum of the acetyl derivative of **1**a: i) the

Table 1. ^{1}H (200 MHz) and ^{13}C (50.3 MHz) NMR spectral data for diterpenes **2**, **2a** and **2b** in $CDCl_3$ and TMS as internal standard, including $^1J_{CH}$ (fully coupled) of **2** obtained by gated experiment.*

	2		2a		2b
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C
C					
4	33.4 (*s*)	-	33.1	-	32.9
10	38.4 (*s*)	-	38.7	-	37.1
12	150.7 (*s*)	-	150.5	-	150.1
13	113.3 (*s*)	-	113.4	-	113.2
17	175.9 (*s*)	-	175.5	-	
CH					
5	55.8 (*d*, *J*=121.5)		54.9	1.25 (*d*, *J*=11.5)	55.2
6	74.3 (*d*, *J*=141.4	4.79 (*dd*, *J*=10.7,8.9)	75.5	5.25 (*dd*, *J*=11.5, 9.2)	73.4
7	82.8 (*d*, *J*=139.1)		81.2	3.37 (*dd*, *J*=9.2, 14.3	82.2
8	41.1 (*d*, *J*=129.2)		42.5	2.35	40.4
9	48.1 (*d*, *J*=130.6)		47.9	1.48	47.9
14	46.7 (*d*, *J*=132.9)		46.4		45.8
15	108.4 (*dd*, *J*=173.7,13.3)	6.11 (*d*, *J*=1.9)	108.1	6.16 (*d*, *J*=2.0)	108.2
16	141.3 (*dd*, *J*=202.6,10.2)	7.20 (*d*, *J*=1.9)	141.4	6.23 (*d*, *J*=2.0)	140.40
CH_2					
1	39.4 (*t*, *J*=125.9)		39.2	1.7-1.0	38.9
2	18.4 (*t*, *J*=124.0)		18.3	1.5-1.0	17.9
3	43.5 (*t*, *J*=125.5)		43.4	1.2-0.9	43.0
11	21.9 (*t*, *J*=129.0)	2.63 (*dd*, *J*=16.3,5.7) 2.50-2.30	22.0	2.67 (*dd*, *J*=16.5, 5.1) 2.5-2.3	21.3
CH_3					
18	36.5 (*q*, *J*=124.7)	1.04 (*s*)	36.2	1.02 (*s*)	35.8
19	22.4 (*q*, *J*=125.7)	0.98 (*s*)	22.5	0.94 (*s*)	21.7
20	15.6 (*q*, *J*=124.9)	1.14 (*s*)	15.4	1.07 (*s*)	14.9
MeO-17	52.3 (*q*, *J*=146.8)	3.71 (*s*)	52.3	3.72 (*s*)	-

*Multiplicity of signals of carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR. Chemical shifts and coupling constants of hydrogen atoms obtained from ^{1}H NMR.

signals of the carbon atoms C-5 (δ_C 57.35) and C-7 (δ_C 37.34) revealed, as anticipated, the γ-effects of the carbonyl carbon of the acetoxyl group [C-5: $\Delta\delta_C$ = 57.35 (**1**a) - 60,02 (**1**) = - 2.67 ppm; C-7: $\Delta\delta_C$ = 37.34 (**1**a) - 42.15 (**1**) = - 4.81 ppm]; ii). The C-6 signal showed the downfield shift = $\Delta\delta_C$ = 72.62 (**1**a) - 70,02 (**1**) = 2.6 ppm as a consequence of the β-effect of the carbonyl carbon atom (Table 2). The presence of a tertiary methyl group at C-14 [δ_H 0.99 (*d*, *J*=6.9 Hz) and δ_C 17.37] was confirmed by 2D carbon-hydrogen correlation ($^3J_{CH}$) of the 3H-17 (δ_H 0.99) with the carbon atom C-13 (δ_C 122.14) observed in the ^{13}Cx^{1}H-COSY-$^nJ_{CH}$ (n = 2 and 3) spectrum.

The 2D carbon-hydrogen correlation experiments modulated with $^1J_{CH}$ established the hydrogen chemical shift correlations with those of the carbon atoms and also $^2J_{CH}$ and $^3J_{CH}$ couplings to detect hydrogen resonance correlated with not directly bound carbon signals, along with the application of the usual shift parameters and multiplicity of signals, were used to complete the assignment of the ^{1}H and ^{13}C NMR spectra of **1** (Table 2).

The location of the hydroxyl group at chiral carbon C-6 in the equatorial position was deduced on the basis of the *J*-value (7.1 Hz) observed for the signal of the H-6 [δ_H 3.94 (*dd*, *J*=7.1, 3.6 Hz)], downfield shift [$\Delta\delta_H$ = 5.12 (**1a**) - 3.94 (**1**) = 1.18 ppm] observed in the ^{1}H NMR spectrum of the monoacetate **1a** (Table 2). The monoacetate **1a**, obtained by acetylation of **1** with Ac_2O/Py, was identified by a comparative study of the ^{1}H and ^{13}C NMR spectra with the natural product isolated from *Dipteryx lacunifera*[7].

Confirmation of **1** as a natural product (and not as a hydrolysis product) was obtained by preparation of a new hexane extract from fruits (1.5 kg) collected from the same specimen. The residue (16 g) was submitted to column chromatography on silica gel eluted with hexane, $CHCl_3$ and EtOAc. The residue (5.3 g) from the $CHCl_3$ elution was submitted to a preparative TLC affording **1** (12.7 mg).

The relative configuration of **1** was also deduced on the basis of the $[\alpha]_D$= + 26.6 ± 0.05 (*c* 1.0, $CHCl_3$) compared with value described in the literature for this compound obtained of the acetyl derivative **1a** by treatment with $LiAlH_4$[7].

Table 2. ^{1}H (200 MHz) and ^{13}C (50.3 MHz) NMR data for diterpene **1** and acetyl derivative **1a**, in $CDCl_3$ and TMS as internal standard. The chemical shifts are in δ (ppm) and coupling constants (*J*) in Hz are described in parentheses.*

	1					
	^{13}C x^{1}H-COSY -$^1J_{CH}$		^{13}C x^{1}H-COSY -$^nJ_{CH}$		**1a**	
	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C	δ_H
C						
4	33.39	-	3H-18		33.03	-
10	38.58	-	3H-20		38.82	-
12	149.48	-	H-11		149.30	-
13	122.14	-		3H-17	122.15	-
AcO-6		-			170.41	-
CH						
5	60.02	0.91 (*d*, *J*=7.1)		3H-18	57.35	
6	70.02	3.94 (*td*, *J*=7.1, 3.6)	2H-7		72.62	5.12 (*td*, *J*=4.5,12.0)
8	35.30	1.90		H-6	34.88	
9	44.46	1.40		3H-20	44.51	
14	30.97	2.70-2.55 (*m*)			30.82	
15	109.42	6.17 (*d*, *J*=1.6)			109.48	
16	140.42	7.20 (*d*, *J*=1.6)			140.52	
CH_2						
1	39.81	1.60 1.07			39.61	
2	18.42	1.40			18.36	
3	43.69	1.40 1.10			43.45	
7	42.15	1.90 1.40			37.34	
11	22.42	2.54 (*dd*, *J*=6.8, 16.7) 2.27 (*dd*, *J*=10.3, 16.7)			22.42	
CH_3						
17	17.37	0.99 (*d*, *J*=6.9)			17.36	0.96 (*d*, *J*=7.3)
18	37.01	1.17 (*s*)			37.34	1.14 (*s*)
19	22.57	1.07 (*s*)			22.63	1.04 (*s*)
20	15.41	0.89 (*s*)			15.35	0.98 (*s*)
AcO-6	-	-			22.00	2.04 (*s*)

*Multiplicity of signals of carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR. Chemical shifts and coupling constants of hydrogen atoms obtained from ^{1}H NMR.

Experimental

General experimental procedures

Mps: uncorr. Optical rotation was obtained on a Perkin-Elmer 341 digital polarimeter; NMR: ^{1}H(200 MHz) and ^{13}C (50.3 MHz) in $CDCl_3$ (**1** and **2**), $CDCl_3$ + acetone-d_6 (**3** and **4**) and TMS as int. standard; EIMS: 70 eV.; CC: silica gel (Merck 0.05-0.20 mm); TLC: silica gel H or G (Merck) and spots were visualized by exposure to iodine vapour.

Plant material

Pterodon polygalaeflorus Benth. was collected in May 1993 in Joaquim Pires, Piauí State, Brazil. A voucher specimen (Nº 8808) representing the collection was identified by Dr Afranio G. Fernandes (Botanist, Departamento de Biologia, UFC) and has been deposited at Herbario Prisco Bezerra of the Departamento de Biologia, Universidade Federal do Ceará, Brazil.

Extraction and isolation of the constituents

The powdered fruits (5 kg) were extracted with hexane in a Sohxlet apparatus to yield 500 g of a yellowish oil that was designated EH. The yellowish oil EH (100 g) was submitted to alkaline hydrolysis according to usual procedure, KOH-H_2O-EtOH (1:1) under reflux for 30 min. Column chromatography of the ppt (60 g) obtained after addition H_2O yielded 4 fractions designated EHH (8.6 g) eluted with hexane, EHC (17.6 g) eluted with $CHCl_3$, EHAE (21.3 g) eluted with EtOAc and EHM (10.0 g) eluted with methanol. Fraction EHAE (21.3 g) was chromatographed on a silica gel column: the fraction eluted with $CHCl_3$ yielded 6α-hydroxyvouacapane **1** (380 mg) after recrystallization from MeOH**;** the fraction eluted with $CHCl_3$-EtOAc (9:1) furnished methyl 6α,7β-dihydroxyvouacapan-17β-oate (**2**, 150 mg) after recrystallization from MeOH; the fraction eluted with $CHCl_3$-EtOAc (2:8) afforded vouacapane-6α,7β,14β,19-tetraol (**3**, 80 mg) after recrystallization from MeOH; and the fraction eluted with EtOAc yielded taxifolin (**4,** 5,7,3',4'-tetrahydroxydihydroflavonol, 100 mg) after recrystallization from MeOH.

*6α-Hidroxyvouacapane (**1**)*

M. p. 147.6-148.3 °C, needles from MeOH. $[\alpha]_D = +26.6 \pm 0.05$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$) and ^{3}C NMR (50 MHz, $CDCl_3$): Table 2. HREIMS *m/z* (rel. int.): 302.2126 ($C_{20}H_{30}O_2$, *m/z* 302.2246, $[M]^{\cdot+}$, 30); 284.2024 ($C_{20}H_{28}O$, *m/z* 284.2140, $[M - H_2O]^{\cdot+}$, 89); 108.0550 (C_7H_8O, *m/z* 108.0575, **1b**, 100).

Acetylation of **1**

6α-Acetoxyvouacapane (**1a**) was obtained by treatment of **1** (105 mg) with Ac_2O (5 mL) and pyridine (1.5 mL). The usual work-up followed by CC on silica gel [hexane:$CHCl_3$ 1:1 as eluent] of the crude product furnished **1a** (100 mg) as an oil fr. homogeneous by TLC. ^{1}H NMR (200 MHz, $CDCl_3$) and ^{13}C NMR (50 MHz, $CDCl_3$): Table 2. HREIMS *m/z* (rel. int.): 344.2794 ($C_{22}H_{32}O_3$, *m/z* 344.2396, $[M]^{\cdot+}$, 31); 316.2465 ($C_{21}H_{32}O_2$, *m/z* 316.2404, $[M\text{-}CO]^{\cdot+}$, 10.5), 285.2315 ($C_{20}H_{29}O$, *m/z* 285.2220, [M-AcO·], 20), (C_7H_8O, *m/z*. 108.0575, **1b**, 47.5).

Acknowledgments

The authors are grateful to Brazilian agencies FINEP, CAPES, CNPq and FUNCAP for financial support and to Prof. Afrânio Gomes Fernandes (Universidade Federal do Ceará) for collection and identification of the plant material.

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Received: February 22, 1999