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Article

Macrocarpane, a New Sesquiterpene Skeleton from the Leaves of *Porcelia macrocarpa*

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O óleo volátil e o extrato hexânico das folhas de *Porcelia macrocarpa* (Warm.) R.E. Fries, Annonaceae, foram submetidos a fracionamentos cromatográficos. Nove sesquiterpenos (*α-cubebeno*, *α-copaeno*, *germacreno*-D, *biciclogermacreno*, *γ-cadineno*, *δ-cadineno*, *espatulenol*, *globulol* e *cis-cubenol*) além de um diterpeno (fitol) foram identificados no óleo volátil, correspondendo a 83.1% (em massa) do total de constituintes. Dois sesquiterpenos (*espatulenol* e *macrocarp-11(15)-en-8-ol*), o último apresentando um novo esqueleto estrutural, um diterpeno (*fitol*), além de dois esteróides (*sitosta-5,25-dien-3β-ol* e *sitosta-5,22,25-trien-3β-ol*), foram isolados do extrato hexânico. As substâncias foram caracterizadas através de experimentos de RMN de ^{1}H e ^{13}C, incluindo análise bidimensional, além de espectrometria de massas.

The volatile oil and the hexane extract from the leaves of *Porcelia macrocarpa* (Warm.) R.E. Fries, Annonaceae, were submitted to chromatographic separations. Nine sesquiterpenes (*α-cubebene*, *α-copaene*, *germacrene*-D, *bicyclogermacrene*, *γ-cadinene*, *δ-cadinene*, *spathulenol*, *globulol* and *cis-cubenol*) and one diterpene (*phytol*) were identified in the volatile oil (83.1 % in weight). Two sesquiterpenes (*spathulenol* and *macrocarp-11(15)-en-8-ol*), the latter with a novel skeleton, one diterpene (*phytol*) and two steroids (*sistosta-5,25-dien-3β-ol* and *sitosta-5,22,25-trien-3β-ol*) were isolated from the hexane extract. These compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy, including bidimensional analysis and mass spectrometry.

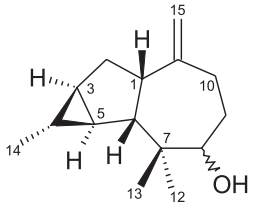
Keywords: macrocarpane, spathulenol, steroids, sesquiterpenes, *Porcelia macrocarpa*

Introduction

The chemical constitution of *Porcelia macrocarpa* was described in four previous papers. In the first one, acetogenins from seeds were reported.[1] Two other papers report the presence of amides, lignanamides and alkaloids from the branches.[2,3] The polar constituents, such as aminoacids, trimethylamonium salts and glycosilated flavonoids were also described.[4] From our previous studies with *P. macrocarpa* we describe herein the identification of nine sesquiterpenes and one diterpene in the volatile oil and two sesquiterpenes, one diterpene and two steroids in the hexane extract, both from the leaves. The ^{1}H and ^{13}C NMR data of **10**, including bidimensional analysis, showed a novel sesquiterpene skeleton, which was denominated macrocarpane.

Results and Discussion

The hexane extract of the leaves of *P. macrocarpa* was submitted to chromatographic separations to give two sesquiterpenes: *spathulenol* (**7**) and *macrocarp-11(15)-en-8-ol* (**10**), one diterpene: *phytol* (**11**) and two steroids: *sistosta-5,25-dien-3β-ol* (**12**) and *sitosta-5,22,25-trien-3β-ol* (**13**). The sesquiterpene **7**, the diterpene **11** and the steroids **12** and **13** were identified by analysis of their ^{1}H and ^{13}C NMR data and comparison with data described in the literature.[5-8]



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The EIMS spectrum of **10** showed the molecular ion peak at *m/z* 220 Da. The ^{13}C NMR spectra (BBD and DEPT 135°) displayed fifteen signals refering to three methyls, four methylenes, six methines, as well as two quaternary carbon atoms, suggesting the occurrence of an oxygenated sesquiterpene with molecular formula $C_{15}H_{24}O$. The signals at δ 146.0 (C) and δ 105.7 (CH_2) were indicative of an exocyclic methylenic group, and the single signal at the carbinolic region [δ 77.4 (CH)] confirms the presence of one hydroxyl group in the molecule of **10**.

The ^1H NMR spectrum showed three methyl groups at δ 0.79 (s), δ 0.93 (s) and δ 0.93 (d, *J* 3.0 Hz), the latter being linked to a methinic carbon atom. This spectrum also showed a signal at δ 0.51 (m), suggesting the presence of a cyclopropane ring in the structure of **10**. The observation of a double-multiplete at δ 48.6, in the gated ^{13}C NMR spectrum with *J* 163 Hz, characteristic of three member ring,[9] confirms the cyclopropane ring in the molecule. The HMQC spectrum showed long-range correlations between carbons and hydrogens (Table 1). These results associated with those showed in ^1H-^1H COSY spectrum indicated the connectivities in the molecule, and suggested a decahydroazulene derivative with a cyclopropane ring. The mutual long range coupling between the signals at δ 1.48 (H-1) and δ 4.82 (H-15), δ 2.28 (H-10) and δ 1.29 (H-5), the latter being a W coupling, corroborates the suggested structure and indicated the β orientation to cyclopropane ring. This spectrum also showed the coupling between the hydrogens at C-9 and those at C-10 and C-8; thus, the two methyl groups should be at C-7. The presence of these groups interrupted the ^1H-^1H connectivities in the seven member ring. Therefore, the cyclopropane group could only be at the five-carbon ring. The mutual coupling observed among the signals at δ 0.51 (H-4) and δ 1.29 (H-5), and δ 1.18 (H-3) located the cyclopropane ring between C-3/C-5. This situation was corroborated by the correlations between H-3/H-2 and H-3/H-6, observed in the ^1H-^1H COSY spectrum. HMBC long range correlations confirm the proposed structure. The small coupling constants of H-1 ($W_{½}$ = 8 Hz) indicated a *cis* ring junction between the five and seven member rings. The chemical shift of methyl group at C-4 (δ 21.8) suggested the presence of this group at a position, free of δ interactions. This sesquiterpenic skeleton has not been described in the literature yet, and was denominated *macrocarpane*. Therefore, the structure of **10** was defined as *macrocarp-11(15)-en-8-ol*.

The biosynthetic pathway proposed to **10** should involve a himachalane derivative which was not detected in this study, but has been isolated from *Ferula latipinna* (Umbelliferae),[10] which could be formed by cyclization of bicyclogermacrene (Figure 1). In the volatile oil, the absence of **10** and the reduced amount of **7** (3.0%) could be explained by the predominance of germacrene-D (37.8%) and bicyclogermacrene (27.5%). In the hexane extract, these sesquiterpenes were not detected, indicating that **7** and **10** should be formed from **4**,[11] corroborating the proposed biosynthetic pathway.

The volatile oil from the leaves of *P. macrocarpa* was submitted to chromatographic separation and the fractions obtained were analysed by GC.[12] The sesquiterpenes **3**, **4**, **7**, **8** and **9** and the diterpene **11** were characterized as the

Table 1. ^1H and ^{13}C NMR data for sesquiterpene **10** (500 and 125 MHz, δ, $CDCl_3$)

	^1H-^1H coupling	$^1H^a$ (multiplicity, *J*/Hz)	$^{13}C^b$	HMBC
1	1.29, 4.82, 2.28	1.48 (m)	57.8	1.96, 4.79, 4.82
2	1.96, 1.18	1.02 (dd, 12.0; 4.0)	42.7	
	1.02, 1.18	1.96 (dd, 12.0; 7.0)		
3	0.93, 1.96, 1.29, 1.02, 0.51	1.18 (m)	24.6	
4	0.93, 1.18, 1.29	0.51 (m)	48.6	0.93
5	1.48, 1.18	1.29 (m)	24.4	
6	0.51, 0.79, 1.18	0.93 (br dd, 10.0; 4.0)	32.4	0.93
7	-	-	59.1	0.79
8	1.75, 1.43	3.51 (dd, 12.0; 5.5)	77.4	0.79
9	3.51, 2.28, 2.02, 1.75	1.43 (dq, 12.0; 5.5)	31.1	
	2.02, 1.43, 3.51, 2.28	1.75 (td, 12.0; 5.5)		
10	2.28, 1.75, 1.43, 4.79	2.02 (ddd, 14.0; 12.0; 5.5)	34.0	4.79
	2.02, 1.43, 1.75, 1.48	2.28 (br dd, 14.0; 5.5)		
11	-	-	146.0	
12	0.93	0.79 (s)	13.8	
13	-	0.93 (s)	21.9	0.93
14	0.51	0.93 (d, 3.0)	21.8	
15	1.48	4.82 (br s)	105.7	
	2.02	4.79 (br s)		

[a]…500 MHz; [b]…125 MHz.

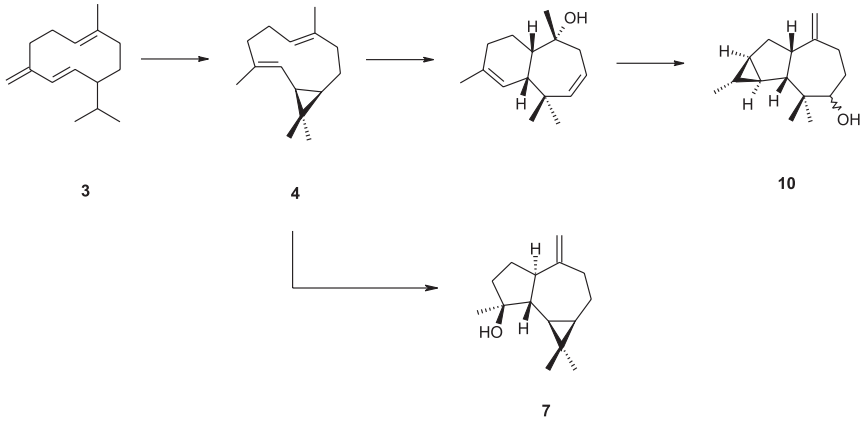

Figure 1. Hypothetic biosynthetic pathway of sesquiterpenes **7** and **10** from **3** and **4**.

main compounds in mixtures by analysis of their ^{1}H and ^{13}C NMR spectra. The comparison with the literature values confirmed the proposed structures[5,13-15]. Additionally, the crude volatile oil was submitted to analysis by GC/MS. The sesquiterpenes **1**, **2**, **5** and **6** were identified. This analysis confirms the identification of the sesquiterpenes **3**, **4** and, **7**, and the diterpene **11**, which were characterized by NMR. Using this methodology, which is a combination of three spectrometric techniques (NMR, GC, GC/MS), nine sesquiterpenes, and one diterpene (83.1 % in weight) were identified in the volatile oil (Table 2).

Table 2. Chemical constituition of the crude volatile oil from leaves of *P. macrocarpa*

component	RR_t	RI	percentage	identification	
				GC/MS	NMR
α-cubebene (**1**)	562	1351	3.1	X	
α-copaene (**2**)	581	1376	1.8	X	
germacrene-D (**3**)	712	1480	37.8	X	X
bicyclogermacrene (**4**)	734	1496	27.5	X	X
γ-cadinene (**5**)	745	1513	1.4	X	
δ-cadinene (**6**)	769	1524	2.0	X	
spathulenol (**7**)	849	1576	3.0	X	X
globulol (**8**)	857	1592	1.6		X
cis-cubenol (**9**)	954	1668	1.9		X
phytol (**11**)	1518	2114	3.0	X	X

Experimental

Plant material

All the leaves of *P. macrocarpa* (Warm.) R.E. Fries were collected from the same specimen, in March 1992 (hexane extract), and in December 2000 (volatile oil) at the Jardim Botânico of São Paulo State. A voucher specimen has been deposited in the herbarium of the Instituto Botânico, São Paulo, Brazil under reference SP76791.

Extraction and isolation of the compounds

Dried powdered leaves (750 g) of *P. macrocarpa* were extracted with hexane three times at room temperature. The concentrated hexane extract was partitioned between MeOH/H_2O (9:1, v/v) and hexane. The hexanic part (10 g) was submitted to precipitation with MeOH from which were obtained the waxy material (9 g), and the MeOH soluble material (1 g). The MeOH/H_2O phase (6 g) and the MeOH soluble material (1 g) were pooled together, chromatographed on silica gel column and eluted with increasing amounts of EtOAc in hexane, from which were obtained four groups (I-IV), after TLC on silica gel analyses. The group I (351 mg) was submitted to flash chromatography on silica gel and eluted with hexane-EtOAc (6:4, v/v) yielding **7** (77 mg). Group II (720 mg) was methylated with diazomethane, submitted to flash chromatography on silica gel and eluted with hexane-EtOAc (97:3, v/v) giving two mixtures A (359 mg), and B (30 mg). The ^{1}H NMR spectrum of A showed the occurrence of fatty acid esters. Fraction B was applied to a Sephadex LH-20 column and eluted with hexane-CH_2Cl_2 (1:4, v/v) to yield **11** (10 mg). Group III (483 mg) was submitted to flash chromatography on silica gel and eluted with hexane-[CH_2Cl_2-MeOH (98:2, v/v)] (6:4, v/v) to give two mixtures C (30 mg) and D (200 mg). Mixture C was comprised of two steroids (**12** and **13**). Mixture D was applied to a Sephadex LH-20 column and eluted with hexane-CH_2Cl_2 (1:4, v/v) to give a mixture of one sesquiterpene and steroidal material. The purification by prep. TLC on silica gel with 15% of $AgNO_3$ [hexane-EtOAc (6:4, v/v)] yielded **10** (15 mg).

The fresh plant material (2250 g) was hydrodistilled

using a Clevenger type apparatus to give the crude volatile oil (735 mg; yield 0.03%). Part of this material (500 mg) was submitted to flash chromatography on silica gel and eluted with CH_2Cl_2 and CH_2Cl_2-MeOH (95:5 and 9:1) affording mixtures containing **1** + **3** + **4** (fr 4, 137 mg), **3** + **4** (fr 6, 25 mg), **11** (fr 34, 16 mg), **7** (fr 38, 12 mg), **8** + **9** (fr 39, 52 mg). The remaining part of the crude volatile oil was subjected to analysis by GC/MS to identify the sesquiterpenes **1**, **2**, **5** and **6**.

General

Silica gel 60 (Merck) was used for chromatography: 63-200 μm for CC, 40-63 μm for flash chromatography, PF_{254} (5-45 μm) for preparative TLC. IR spectra were obtained as KBr pellets in a Perkin-Elmer Infrared Spectrometer model 1750. Sephadex LH-20 (Sigma) was used for molecular exclusion chromatography. NMR spectra were recorded at 300 and 500 MHz for ^{1}H and 75 and 125 MHz for ^{13}C on Brüker DPX-300 and DRX-500 spectrometers using $CDCl_3$ as solvent and internal standard. The GC analysis was performed in a Hewlett-Packard 5890 series II (using helium as carrier gas) equipped with a FID detector and a capillary column HP-5, crosslinked 5% phenyl in 95% methyl silicone (30 m x 0.32 mm, film thickness 0.25 μm) with a automatic injector (HP 7673) and an electronic integrator (HP 3396A). The temperature programming was from isothermal 100 °C for 2 min, 100°-240 °C at 5°C.min^{-1}, then isothermal at 240 °C for 5 min. The (FID) injector and detector temperatures were 180 °C and 260 °C, respectively. The GC/MS analyses were carried out in an EIMS 70 eV Hewlett-Packard HP-5973 coupled with a Hewlett-Packard HP-6890 with a DB-50 column (30 m x 0.25 mm, film thickness 0.25 μm) using the same temperature programming conditions describe above. The identification of the compounds was done by comparing retention indices (determined relatively to the retention times of a series of n-alkanes) and mass spectra to those of authentic samples.

*Macrocarp-11(15)-en-8-ol (**10**)*: colourless oil. IR (KBr) ν_{max} cm^{-1}: 3402 (OH), 3108 (cyclopropane ring), 2945 (C-H), 1637 (>C=CH$_2$), 919, 888. EIMS (70 eV) m/z (rel. int.) 220 $[M]^+$(58), 202 $[M\text{-}H_2O]^+$(7), 187 $[C_{14}H_{19}]^+$(17), 177 $[C_{12}H_{17}O]^+$(22), 159 $[C_{12}H_{15}]^+$(100), 145(24), 135(16), 133(27), 131(32), 121(16), 119(32), 117(30), 109(28), 107(23), 105(48), 95(14), 93(44), 91(55), 81(23), 79(32), 77(31), 69(13), 67(32), 65(14), 57(12), 55(36), 53(22). ^{1}H and ^{13}C NMR: see table 1.

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