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Short Report

Steroids and Flavonoids of *Porcelia macrocarpa*

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Três misturas de esteróides (**1a+1b**, **2a+2b** e **3a+3b**) e dois flavonóides glicosilados (**4** e **5**) foram isolados do extrato etanólico dos galhos da *Porcelia macrocarpa* (Warm.) R. E. Fries (Annonaceae). Os esteróides Estigmasta-4,25-dien-3-ona (**2a**) e (22*E*)-Estigmasta-4,22,25-trien-3-ona (**2b**) são substâncias novas. As estruturas foram determinadas com base na análise de dados espectrais de RMN e de massas e por comparação com dados de substâncias da literatura usadas como modelos.

Three mixtures of steroids (**1a+1b**, **2a+2b** and **3a+3b**) and two flavonoid glycosides (**4** and **5**) were isolated from the ethanol extract of branches of *Porcelia macrocarpa* (Warm.) R. E. Fries (Annonaceae). The steroids Stigmasta-4,25-dien-3-one (**2a**) and (22*E*)-Stigmasta-4,22,25-trien-3-one (**2b**) are news. The structures were elucidated by NMR and MS data and comparison with literature data of model compounds.

Keywords: Porcelia macrocarpa, Annonaceae, steroids, flavonoids

Introduction

Porcelia macrocarpa is a botanical species from Annonaceae family and the only one of the genus occurring in Brazil.[1] This species has a very diversified chemical composition, revealed by isolation of acetogenins, lignanamides, hydroxycinnamoyltyramines, sesquiterpenes and alkaloids, including azapolycyclics, from different parts, previously investigated, of the same specimen.[2-6]

This paper describes the isolation and identification of three steroid mixtures and two flavonoid glycosides from the ethanol extract of the branches of *Porcelia macrocarpa*.

Results and Discussion

The ether soluble part from the ethanol extract of the branches of *Porcelia macrocarpa* was submitted to a partition between hexane and aqueous methanol. Chromatographic separation of the hexane phase afforded two steroid mixtures (**M1** and **M2)** and the hydroalcoholic phase gave, after the same procedure, one mixture of steroid glycosides (**M3**). An insoluble material precipitated in the interface between diethyl ether and water, during the partition from the ethanol extract, afforded two flavonoid glycosides **4** and **5**.

A GCMS analysis of the **M1** mixture showed two compounds **1a** and **1b** in a 5:2 relationship, where the major compound presents a molecular peak $[M]^+$ at *m/z* 412 and the minor one at m/z 410. The ^{1}H NMR spectrum (Table 1) of the mixture **M1** showed signals at δ 3.51 (m), 5.34 (m) and between δ 0.6 and 1.6 attributed to H-3, H-6 and methyl hydrogens, respectively, from a Δ^5-3β-hydroxy sterol with an allylic methyl group.[7] Signals at δ 5.23-5.17 and 4.72-4.62 suggested additional double bonds in the steroidal nucleus. The ^{1}H and ^{13}C NMR data agree with the structure of Δ^5 sterols [δ_C 71.8 (oxygenated CH-3), 140.7 (C-5) and 121.7 (CH-6)] containing additionally one (**1a**, $[M]^+$ at *m/z* 412) and two double bonds (**1b**, $[M]^+$ at *m/z* 410) located at the side chain. The side chain of both steroids sustain an ethyl group at C-24, as in sitosterol. The presence in the mixture of two methylidene groups ($C{=}CH_2$) was deduced by ^{13}C signals corresponding to sp^2 non-hydrogenated and methylene carbon atoms [δ_C 147.5 and 111.4 (**1a**); 148.6 and 109.5 (**1b**)]. Thus, the second double bond of **1a** was placed at C-25 to justify the ^{13}C signals and the allylic methyl group in the ^{1}H NMR (Table

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Table 1. ^{1}H NMR data for **1a, 1b, 2a, 2b, 3a** and **3b** (200 MHz, $CDCl_3$)

C	1a+1b	2a+2b	3a+3b[a]
3	3.51	-	3.91
4	-	5.72	-
6	5.34	-	5.33
22/23	5.17-5.23	5.19-5.23	5.28-5.31
27	4.62-4.72 (0nly **1b**)	4.65-4.70 (0nly **2b**)	4.53-4.59 (0nly **3b**)
Me-26	1.56 or 1.46	1.65 or 1.57	1.61 or 1.72
glucopyranosyl	-	-	4.26-5.06

[a] Compounds **3a + 3b** were measured in C_5D_5N.

1 and 2). The peak at *m/z* 84 (19%) attributed to fragment **1a**-F originated by a McLafferty rearrangement (Table 3 and Figure 1) is in accordance with this suggestion. The compound **1a** has been isolated before from the marine green alga *Codium iyengarii*.[8] The low relative intensity of the peak at *m/z* 84 (3%) observed in the mass spectrum of **1b** is justified by the presence of another peak at *m/z* 138 (43%) attributed to the fragment **1b**-D formed also by a McLafferty rearrangement involving the additional double bond localized between the carbon atoms C-22 and C-23 (Table 3 and Figure 1). This third double bond is present only in **1b,** as indicated by the ^{1}H NMR multiplet integration at δ 5.23-5.17 and by the molecular ion. The CH signals at δ 137.2 and 130.0 observed in the ^{13}C NMR spectrum (Table 2) are in accordance with the proposed structure. Compound **1b** is also described in the literature.

Table 2. ^{13}C NMR spectral data of steroids **1a, 1b, 2a, 2b, 3a** and **3b** (50.3 MHz, $CDCl_3$)

C	1a	1b	2a	2b	3a[d]	3b[d]
1	37.2	37.2	35.7	35.7	38.0	38.0
2	31.6	31.6	34.0	34.0	30.7	30.7
3	71.8	71.8	199.6	199.6	79.1	79.1
4	42.3	42.3	123.8	123.8	40.4	40.3
5	140.7	140.7	171.7	171.7	141.4	141.4
6	121.7	121.7	32.9	32.9	122.4	122.4
7	31.9	31.9	32.0	32.0	32.6[a]	32.6
8	31.9	31.9	35.6	35.5	32.5	32.5
9	50.1	50.1	53.8	53.8	50.8	50.8
10	36.5	36.5	38.6	38.6	37.4	37.4
11	21.1	21.1	21.0	21.0	21.8	21.8
12	39.7	39.6	39.6	39.5	39.8	39.8
13	42.3	42.3	42.3	42.3	43.0	42.8
14	56.7[a]	56.8[a]	56.0[b]	56.0[a]	57.3[b]	57.3[a]
15	24.3	24.3	24.2	24.2	25.0	25.0
16	28.1	28.7	28.1	28.6	28.9[c]	29.5
17	56.0[a]	55.8[a]	55.9[b]	55.8[a]	56.7[b]	56.5[a]
18	11.8	12.1[b]	12.0	11.9[b]	12.5	12.6[b]
19	19.4	19.4	17.4	17.4	19.9	19.9
20	35.5	40.2	35.6	40.6	35.7	40.9
21	18.6	20.8[c]	18.6	20.7[c]	19.4	21.5[c]
22	33.6	137.2	33.6	137.0	34.5[a]	138.1
23	29.4	130.0	29.4	130.2	30.2[c]	130.8
24	49.5	52.0	49.5	52.0	50.2	52.8
25	147.5	148.6	147.5	148.5	148.2	149.1
26	177.0	20.2[c]	17.8	20.2[c]	18.4	20.8[c]
27	111.4	109.5	111.4	109.6	112.4	110.6
28	26.5	25.7	26.7	26.7	27.2	26.5
29	12.1	12.1[b]	12.1	12.1[b]	12.8	12.9[b]

[d] Compounds **3a + 3b** were measured in C_5D_5N. Assignments with the same symbol ([a,b,c]) in the column are interchangeable. Signals for carbons of the glucosyl moiety in compounds **3a** and **3b** occurred at δ 103.0 (C-1'), 75.8 (C-2'), 78.6 (C-3'), 72.2 (C-4'), 78.9 (C-5'), 63.3 (C-6').

The GC-MS of **M2** showed two compounds, in the same proportion of **M1**, with molecular peaks at *m/z* 410 and 408 (Table 3). The ^{1}H NMR spectrum (Table 1) was very similar with that of **M1**, but presented a signal at δ 5.72 instead of the signals at δ 3.51 and 5.34, suggesting a Δ^4 conjugated double bond as in sitostenone.[9] The ^{13}C NMR spectra (Table 2) showed signals that confirmed this suggestion, when compared with literature data of model compounds.[10] The MS fragmentation followed the same pattern as **M1** (Figure1 and Table 3). Hydrogenation of **M2** mixture, at room pressure, gave the steroid sitostenone, as proved by GCMS and ^{1}H NMR spectra.[9] **M2** is then composed by a mixture of **2a** and **2b** which are described here for the first time.

The ^{1}H NMR spectrum (Table 1) of **M3** differs from that of **M1** only in the carbinolic hydrogen region. It showed signals between, δ 4.05 and 5.07, from a sugar unit. The H-3 multiplet is deshielded by 0.4 ppm suggesting that the mixture is composed by, glycopyranosyl steroids, with β configuration on the anomeric carbon, evidenced by the doublet at δ 5.02 (7.6 Hz).[11] The ^{13}C NMR (Table 2) data suggested that the

Table 3. Mass spectrometric fragments of the side-chain of steroids **1a,.1b, 2a** and **2b** *m/z* (rel. int.)

Fragments	1a	1b	2a	2b
$[M]^+$	412 (20)	410 (5)	410 (18)	408 (8)
A	273 (4)	273 (9)	271 (10)	271 (36)
B	383 (1)	381 (5)	381 (1)	379 (14)
C	272 (9)	272 (23)	270 (15)	270 (36)
D	138 (5)	138 (43)	138 (5)	138 (25)
E	271 (21)	271 (52)	269 (30)	269 (100)
F	84 (19)	84 (3)	84 (52)	84 (6)

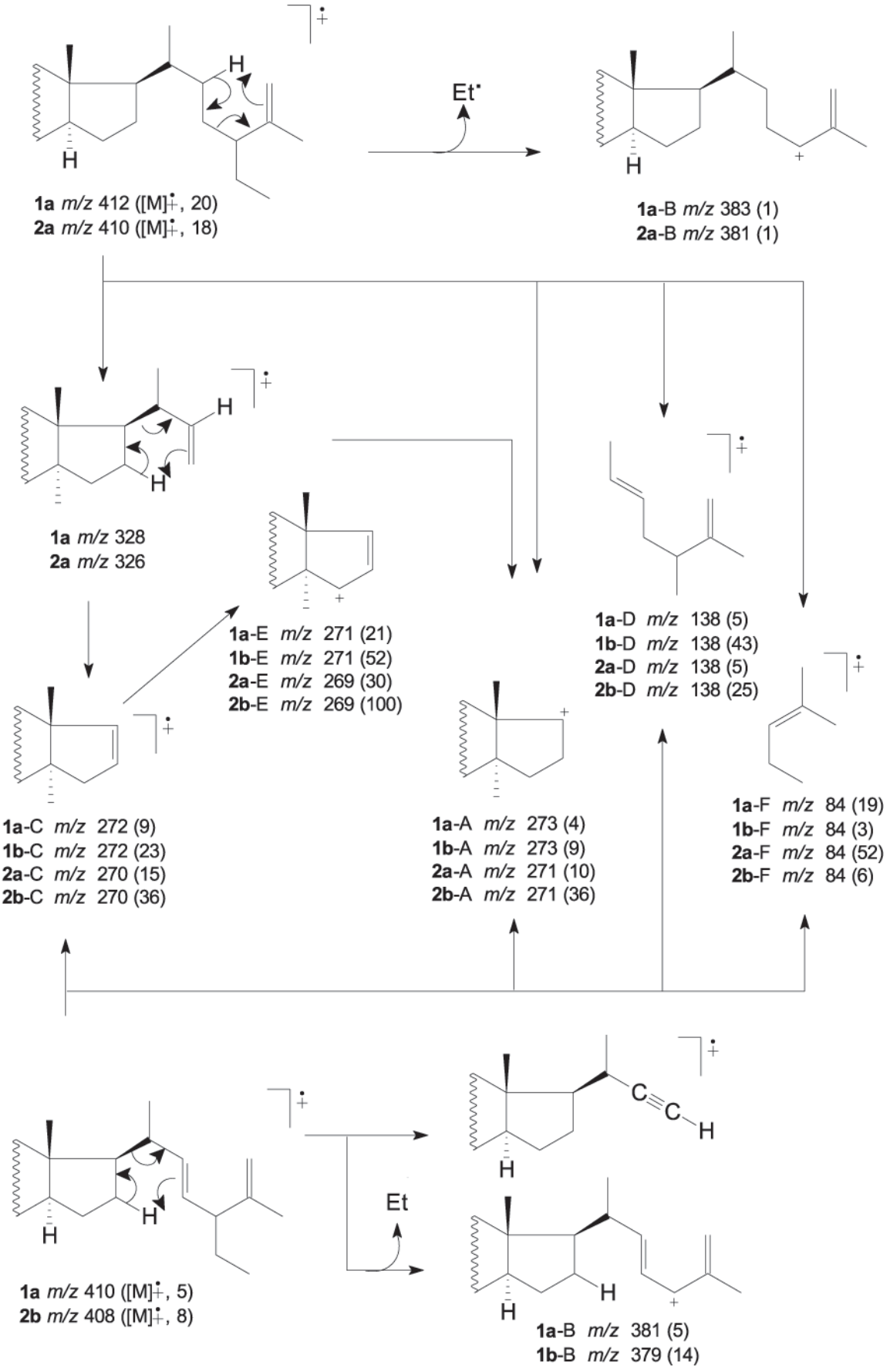


Figure 1. Proposed fragmentation patterns for side chain of steroids **1a, 1b,2a** and **2b.**

compounds are steroid glucosides.[11,12] All the other signals are compatible with the structure of **3a** and **3b**. The steroid **3a** has been isolated from *Clerodendron colebrookianum* (Verbenaceae).[11]

The ^{1}H NMR spectrum of **4** (Table 4) showed five signals in the aromatic region with the pattern of the flavonoid quercetin.[13] Besides of those a singlet at δ 3.84 (6H) and signals between δ 3.05 and δ 5.35 were assigned to methoxyl groups and sugar hydrogens respectively. A doublet at δ 0.95 suggested that rhamnose should be present in the molecule and the singlet at δ 12.5 indicated the presence of one hydroxyl at C-5 of the aglycone. The ^{13}C NMR spectrum (Table 5) showed 29 signals indicating that two sugar units and two methoxyl groups are present in the flavonoid. The chemical shifts of the sugar moiety are consistent with those of rutinosyl (rhamnopyranosyl-(1→6) glucopyranosyl). Comparison between the ^{13}C NMR data of **4** with those of 7,4'-dimethoxyquercetin (**6**)[13,14] showed that C-3 is shielded and C-2, C-4 and C-10 are deshielded in agreement with the glycosylation at C-3 of **4**.[14-16]

The ^{1}H NMR spectrum of **5** (Table 4) had the same pattern as that of **4** but the signal of C-5 OH was absent. The ^{13}C NMR spectrum showed 33 signals indicating the presence of 3 sugar units in the dimethoxyflavonoid, one of them should be linked to C-5. Signals of rutinosyl and glucosyl groups were present. Hydrolysis of **5** led to the isolation of 7,4'-dimethoxyquercetin (**6**).[14] To determine which group, glicosyl or rutinosyl is bounded to C-5, a COLOC ^{1}H-^{13}C was obtained and a long range correlation between δ 4.84, assigned to the anomeric hydrogen of glucose and δ 158.6, assigned to C-5 of the flavonoid was observed. To confirm the structure of **5** as quercetin-5-O-glucoside-3-O-rutinoside-7,4'-dimethyl ether a comparison between the ^{13}C NMR data of **4** and **5** and those of 5-glucopyranosyl luteolin and luteolin[17] was made. The difference found in the chemical shifts of rings A and C of those flavonoids confirmed the proposed structure, as showed in Table 6.

Table 4. ^{1}H NMR data of flavonoids **4**, **5** and **6** (200 MHz, δ, multiplicity, *J*/Hz DMSO-d_6)

H	**4**	**5**	**6**
6	6.35 (s)	6.83 (d, 2.2)	6.33 (d, 1.6)
8	6.67 (s)	6.91 (d, 2.2)	6.70 (d, 1.4)
2'	7.52 (d, 1.9)	7.53 (d, 2.0)	7.70 (s)
5'	7.02 (d, 8.7)	7.01 (d 8.8)	7,07 (d, 8.3)
6'	7.70 (d, 8.7)	7.73 (d, 8.8; 2.0)	7.68 (dd, 8.4)
1^G	5.30 (d, 7.0)	5.24 (d, 7.1)	-
1^R	4.40 (sl)	4.39 (sl)	-
6^R	0.95 (d, 6.0)	0.96 (d, 5.7)	-
$1^{G'}$	-	4.84 (d, 7.2)	-
OMe-4'	3.84 (s)	3.85 (s)	3.84 (s)
OMe-7	3.84 (s)	3.87 (s)	3.85 (s)
5-OH	12.5 (s)	-	12.4 (s)

G =Glucosyl; R =Rhamnopyranosyl

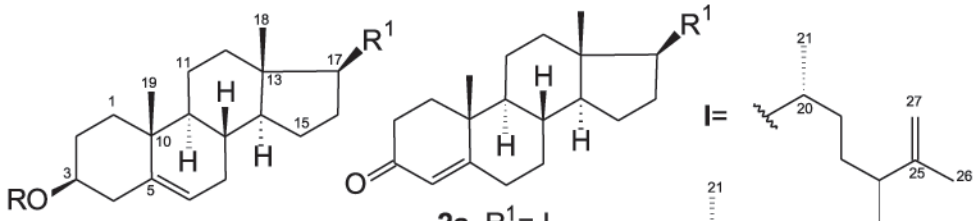
Table 5. ^{13}C NMR data of flavonoids **4**, **5** and **6** (200 and 50.3 MHz, δ, DMSO-d_6)

C	**4**	**5**	^{1}H x ^{13}C - COLOC (**5**)	**6**
2	157.1[a]	155.0	7.53 (H-2'), 7.73 (H-6')	147.0
3	134.1	136.2	-	136.7
4	177.8	173.5	-	176.3
5	161.2	158.6	6.83 (H-6), 4.84 ($H\text{-}1^{G'}$)	160.6
6	98.3	102.7	6.91 (H-8)	97.8
7	165.5	164.1	6.83 (H-6), 6.91 (H-8), 3.87 (OMe-7)	165.2
8	92.6	96.0	6.83 (H-6)	92.2
9	156.7[a]	158.0	6.91 (H-8)	156.4
10	105.3	109.2	6.83 (H-6), 6.91 (H-8)	104.3
1'	121.9	121.8	7.53 (H-2')	123.6
2'	116.1	116.1	7.73 (H-6')	115.0
3'	146.1	146.1	7.53 (H-2'), 7.01 (H-5')	146.4
4'	150.5	150.3	7.53 (H-2'), 7.73 (H-6'), 3.85 (OMe-4')	149.7
5'	111.6	111.4	-	112.0
6'	122.7	122.7	7.01 (H-5')	120.1
1^G	101.5	101.6	-	-
2^G	74.3	74.4	-	-
3^G	76.7	76.0	-	-
4^G	70.2	70.1	-	-
5^G	76.1	76.0	-	-
6^G	67.2	67.1	-	-
1^R	101.1	101.1	-	
2^R	70.7	70.7	4.39 ($H\text{-}1^R$)	-
3^R	70.9	70.9	-	-
4^R	72.1	72.2	-	-
5^R	68.6	68.6	4.39 ($H\text{-}1^R$)	-
6^R	18.0	18.1	-	-
$1^{G'}$	-	103.9	-	-
$2^{G'}$	-	73.9	-	-
$3^{G'}$	-	76.8	-	-
$4^{G'}$	-	70.1	-	-
$5^{G'}$	-	77.9	-	-
$6^{G'}$	-	61.2	-	-
OMe-4'	55.9	56.0	-	55.9[b]
OMe-7	56.4	56.5	-	56.3[b]

Assignments with the same symbol in the column are interchangeable G=glucosyl; R=Rhamnopyranosyl.

Table 6. Differences found in the carbon chemical shifts of rings A and C between **5** and **4** and those between 5-glucopyranosyl luteolin and luteolin

C	$\Delta\delta$ 5-glycopyranosyl luteolin-luteolin	$\Delta\delta$ **5-4**
2	-2.8	-2.1
3	+2.2	+2.1
4	-6.0	-4.3
5	-2.7	-2.6
6	+4.4	+4.4
7	-3.0	-1.4
8	+3.2	+3.4
9	+1.4	+1.3
10	+4.3	+3.9

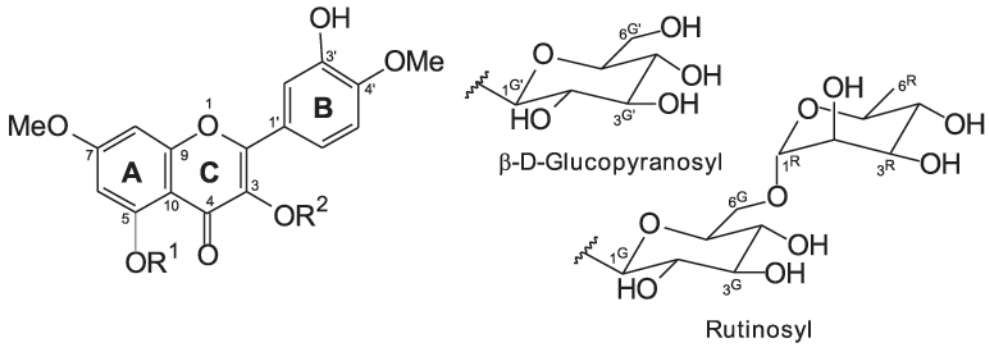


2a R^1= I
2b R^1= II

I=

II=

1a R = H; R^1= I
1b R = H; R^1= II
3a R = β-D-Glucopyranosyl; R^1= I
3b R = β-D-Glucopyranosyl; R^1= II



β-D-Glucopyranosyl

Rutinosyl

4 R^1= H; R^2= Rutinosyl
5 R^1= β-D-Glucopyranosyl; R^2= Rutinosyl
6 R^1= R^2= H

Experimental

General procedure

The low resolution mass spectra were obtained in a INCOS 50 Finnigan-Mat instrument operating at 70 eV coupled to a GLC 3400 Varian, capillary column (DB-5, 30 m x 0.25 mm), det. 280°. Temperature programming from 120 °C to 180 °C, at 10°/min, then 40°/min to 300 °C. The ^{1}H and ^{13}C NMR spectra, using $CDCl_3$, DMSO-d_6 as solvent and TMS as internal reference, were run in a Bruker AC 200. For the column separations Merck silica gel, 63-200 μm was used.

Plant material

The branches of *P. macrocarpa* (Warm.) R.E. Fries were collected at Instituto of Botânica de São Paulo in June, 1991. A voucher specimen is deposited in the herbarium of the Instituto de Botânica, São Paulo, Brazil under reference SP76791.

Extraction and isolation of the compounds

Dried and powdered branches (800 g) of *P. macrocarpa* were extracted with EtOH. The EtOH extract, after concentration in vacuum, was partitioned between Et_2O/H_2O (1:2) giving a water-soluble fraction (31 g), an ether soluble (20 g) and an insoluble interface (2.0 g). The ether soluble part was then partitioned between MeOH-H_2O (9:1) and hexane.

The hexane phase (10 g) was chromatographed on silica gel column eluted with hexane with increasing amounts of EtOAc. Two fractions were eluted with hexane-EtOAc (9:1). These fractions were, separately, submitted to further purification on silica gel column, eluted with hexane-EtOAc (95:5). The less polar fraction afforded a mixture of steroids (8 mg, **2a+2b**) and the more polar fraction afforded another mixture of steroids (64 mg, **1a+1b**).

The MeOH-H_2O layer (10 g) was chromatographed on silica gel column eluted with $CHCl_3$ with increasing amounts of MeOH. A mixture of steroid glycosydes (27 mg, **3a+3b**) precipitated from the $CHCl_3$-MeOH (9:1) eluate. The insoluble interface (2.0 g) was chroma-

tographed on silica gel column eluted with $CHCl_3$ and increasing amounts of MeOH. The fractions $CHCl_3$-MeOH (7:3) and (1:1) treated with MeOH, gave insoluble materials which were, respectively, the flavonoid glicosydes **4** (12 mg) and **5** (168 mg).

Hydrogenation of **M1** (**2a+2b**). The mixture of **2a+2b** (3 mg) dissolved in $CHCl_3$ was maintained under hydrogen atmosphere, for one hour, using Pd/C as catalyst to give, after filtration and evaporation of the solvent, 2 mg of sitostenone.

Stigmast-4-en-3-ona (sitostenone)

EIMS *m/z* 412 $[M]^+$(15), 370 (10), 327(5), 289(15), 229(50), 124(100). ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 5.75 (s, 1H), 2.35-2.45 (m, 2H), 1.15 (s, 3H), 0.93 (d, 3H), 0.83-0.88 (2d,6H), 0.73(s, 3H).

Acid hydrolysis

Flavonoid **5** (50 mg) were hidrolyzed with 20 mL of 2 mol L^{-1} HCl at 80 °C for 1 h. The precipitate was filtrated, washed with water and after drying afforded 19 mg of **6**.

*(3β)-Stigmasta-4,22,25-trien--3ol (**1b**)*

^{1}H NMR: See Table 1; ^{13}C NMR: See Table 2; EIMS *m/z* 410 $[M]^+$(5), 381 (5), 300 (17), 273 (9), 272 (23), 271 (52). 255 (27), 213 (18), 187 (12), 185 (15), 173 (18), 171 (13), 163 (19), 161 (23), 159 (41), 147 (34), 145 (48), 143 (21), 138 (43), 137 (42), 133 (47), 131 (29), 129 (17), 123 (16), 121 (32), 191 (38), 109 (95), 105 (58), 95 (100), 84 (3), 79 (65), 69 (31), 67 (64), 57(21), 55(87).

*Stigmasta-4,25-dien-3-ona (**2a**)*

IR (KBr) ν_{max} /cm^{-1}: 1677; ^{1}H NMR: See Table 1; ^{13}C NMR: See Table 2; *Stigmasta-4,25-dien-3-ona.* 410 (18), 381 (1), 327 (37), 312 (8), 297 (20), 281 (20), 271 (10), 270 (15), 269 (30), 245 (19), 229 (14), 207 (54), 149 (17), 148 (12) 147 (26), 138 (5), 135 (18), 134 (11), 133 (25), 124 (26), 123 (20), 121 (21), 119 (20), 109 (22), 107 (25), 105 (26), 95 (32), 93 (27), 91 (32), 84 (52), 83 (20), 81 (33), 79 (32), 69 (40), 67 (32), 55 (100).

*(22E)-Stigmasta-4,22,25-trien-3-ona (**2b**)*

IR (KBr) ν_{max}/cm^{-1}: 1677; ^{1}H NMR: See Table 1; ^{13}C NMR: See Table 2; EIMS *m/z* 408 $[M]^+$(8), 379 (14), 299 (28), 298 (26), 283 (17), 281 (24), 271 (36), 270 (36), 269 (100), 253 (27), 229 (15), 207 (54), 177 (19), 175 (21), 161 (25), 159 (19), 149 (32), 147 (44), 145 (25), 138 (25), 137 (37), 135 (29), 133 (24), 131 (22), 124 (22), 123 (26), 121 (36), 119 (29), 110 (24), 109 (86), 107 (44), 105 (43), 95 (86), 93 (63), 91 (63), 84 (6), 81 (92), 79 (66), 77 (35), 69 (32), 67 (68), 55 (99)53 (23).

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