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Two Flavonoids from *Clarisia racemosa*

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Clarisia racemosa Ruiz et Pavon é uma árvore da família Moraceae comumente encontrada na Região Amazônica. Seu cerne possui uma coloração amarelo-vivo que exposta à luz solar e ao ar atmosférico escurece tornando-se castanho amarelo-escuro. Dos extratos hexânico, clorofórmico e etanólico desta espécie, cromatografados em coluna de gel de sílica, foram isolados dois flavonóides (artocarpina e isoartocarpina), uma mistura de β-sitosterol, estigmasterol e campesterol. Estes esteróides tiveram sua presença confirmada nas frações mais apolares nos extratos hexânico e clorofórmico, por co-injeção em CGAR e CGAR-EM com uma mistura certificada desses três esteróides em três colunas cromatográficas de diferentes polaridades.

Two prenylflavones were isolated from *Clarisia racemosa,* an arboreal Moraceae species fairly common in the Amazonia region and found to be artocarpin and isoartocarpin. Their structures were elucidated on the basis of ^{1}H and $^{13}CNMR$, MS, UV and IR spectral data and chemical transformations. In addition, the known compounds β-sitosterol, stigmasterol and campesterol were identified. These steroids were confirmed in the more apolar fractions of the hexane and chloroform extracts through HRGC by co-injection of a standard mixture of these compounds in three chromatographic columns of different selectivities.

Keywords: Clarisia racemosa, *Moraceae, artocarpin, isoartocarpin, prenylflavones,* β*-sitosterol, stigmasterol, campesterol, HRGC*

Introduction

Clarisia racemosa Ruiz et Pavon (= *Olmedia erythroriza* Hub.) is an arboreal Moraceae species fairly common in the Amazônia region. It is known as "guariúba" in the State of Amazonas, "oiticica" in the south of Bahia and in the north of Espírito Santo, and "tatajuba amarela" in the state of Pará. In other parts of Latin America it is also known as "murere" (Bolívia), "moral", "aji" and "guariúba" (Colômbia), "pituca" and "matapolo" (Equador), and "capinuri" and "murere" (Peru). Its heartwood is bright yellow, which turns to brown when it is exposed to sunlight. It is moderately resistant to decay and is used in building and carpentry[1,2].

In bioassays, the heartwood of "guariúba" has been shown to be resistant to attack by fungi and insects. In contact with soil it exhibits medium durability[3].

Experimental Details

Mps: uncorr. CC: Merck silica gel (0.05 - 0.02 mm); TLC Merck silica gel H, G or $PF_{254+366}$; NMR 200 and 300 MHz for proton resonance and 50 and 75 MHz for carbon resonance; HRGC-MS: Hewlett Packard model 5987A, with H_2 (2 mL/min) as carrier gas in a 25 m, 0.25 μm, 0.3 mm i.d., glass capillary column (SE-54, OV-17 and OV-31-OH).

Plant Material

Clarisia racemosa was collected in January 1987 from the ZF-II Reserve near Manaus. This species was identified by Dr. Arthur Araujo Loureiro, and a reference specimen is held at the Wood Department of the INPA (Xiloteca of INPA (no. 8859)).

Extraction

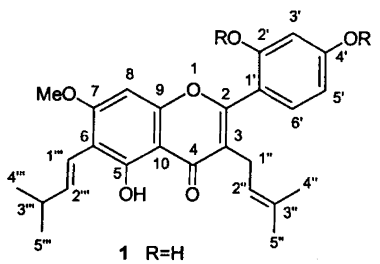
The heartwood was cut into small pieces and was ground in a hammer mill. The dust obtained was extracted successively with hexane, chloroform and ethanol. The crude extracts were evaporated under reduced pressure to afford three extracts: hexane (8.26 g; 0.25% by weight of extract), chloroform (16.20 g; 0.49%), and ethanol (405.00 g; 12.26%).

Isolation of the constituents

Chromatography on silica gel (Merck, 0.05 - 0.02 mm) of the hexane and chloroform extracts yielded a mixture of steroids which were identified by comparing their spectral data and chromatographic properties with authentic samples. Chromatography of the chloroform extract also yielded **1**, mp 174-176 °C (10% weight of extract). The artocarpin **1** was also found in the hexane and ethanol extracts.

2',4'-Dihydroxy-7-methoxy-3-γ,γ-dimethylallyl-6-(trans-3-methylbut-1-enyl) flavone

IR ν^{Kbr}_{max} cm^{-1}: 3380, 2980, 2950, 2880, 1660, 1620, 1480, 1450, 1350, 1270, 1250, 1210, 1160, 1140, 1100, 1080, 1040, 980, 840 and 820. UV λ_{max}^{MeOH} (log ε) 316 (4.21), 279 (4.66); ($AlCl_3$) 348 (4.23), 292 (4.59); ($AlCl_3$ + HCl) 342 (4.23), 294 (4.59); (NaOMe) 356 (4.15), 276 (4.66); (NaOAc) 322 (4.23), 278 (4.71); (NaOAc + H_3BO_3) 321 (4.21), 278 (4.65). EIMS m/z (rel. int.) 436 ($M^{+\bullet}$ 52), 393 (100), 381 (30), 337 (63), 309 (12), 284 (21), 241 (18), 234 (10), 202 (16), 189 (18), 173 (17), 148 (36) and 88 (11). ^1H NMR: see Table 1. ^{13}C NMR: see Table 2.



Methylation of 1

Treatment of 1 with CH_2N_2 yielded yellow crystals of **1a** mp 150-152 °C. IR ν_{max}^{KBr} cm^{-1}: 3350, 2960, 2920, 2860, 1650, 1625, 1575, 1480, 1450, 1350, 1310, 1275, 1245, 1220, 1170, 1145, 1085, 1040, 975, 845, 820, 805 and 745. EIMS m/z (rel. int.) 464 ($M^{+\bullet}$ 45), 421 (100), 365 (25), 333 (30), 256 (10), 233 (27), 175 (17), 165 (5), 112 (5) and 79 (5). ^1H NMR: see Table 1. ^{13}C NMR: see Table 2. The same product **1a** was obtained when **1** was treated with dimethyl sulphate.

Acetylation of 1

Artocarpin **1** (40 mg) was dissolved in Ac_2O (2 mL) and a catalytic amount of DMAP was added. The mixture was

Table 1. ^1H NMR data of **1**, **1a** and **1b** in $CDCl_3$ and TMS as internal standard (chemical shifts in δ and J in Hz)

H	1	1a	1b
3'	6.57 (s)	6.55 (s)	7.15 (s)
5'	6.52 (d, J = 8.5)	6.57 (d, J = 8.3)	7.14 (d, J = 9.3)
6'	7.20 (d, J = 8.5)	7.26 (d, J = 8.3)	7.48 (d, J = 9.3)
8	6.35 (s)	6.35 (s)	6.35 (s)
1''	3.13 (d, J = 6.6)	3.03 (d, J = 6.5)	3.07 (d, J = 6.5)
2''	5.15 (t, J = 6.6)	5.10 (t, J = 6.5)	5.08 (t, J = 6.5)
4''	1.46 (br, s)	1.40 (s)	1.42 (s)
5''	1.61 (br, s)	1.61 (s)	1.62 (s)
1'''	6.54 (d, J = 16.8)	6.58 (d, J = 16.3)	6.57 (d, J = 16.4)
2'''	6.70 (dd, J = 16.8, 6.8)	6.72 (dd, J = 16.3, 6.4)	6.72 (dd, J = 16.4, 6.7)
3'''	2.48 (m)	2.55 (m)	2.49 (m)
4''', 5'''	1.11 (d, J = 6.8)	1.13 (d, J = 6.8)	1.17 (d, J = 6.7)
HO-5	13.49 (s)	13.78 (s)	13.50 (s)
HO	5.43 (s)	–	–
HO	5.84 (s)	–	–
MeO	3.88 (s)	3.88 (s)	3.88 (s)
MeO	–	3.79 (s)	–
MeO	–	3.79 (s)	–
OAc	–	–	2.12 (s)
OAc	–	–	2.33 (s)

Table 2. ^{13}C NMR data of **1, 1a** and **1b** compared with the models 4-6 in $CDCl_3$ (**1, 1a** and **1b**) and DMSO-d_6 (4,6,7) and TMS as internal standard (chemical shifts in δ)*.

C	1	1a	1b	4[8]	5[11]	6[10]	7[9]
2	158.7	158.9	157.9	159.0	158.2	158.6	158.9
3	121.6	121.5	121.6	119.4	121.1	120.2	119.4
4	182.3	182.4	182.9	181.7	182.3	181.8	181.8
5	159.8	158.9	158.0	161.1	157.3	151.9	155.0
6	110.1	109.8	109.8	105.5	104.8	98.0	97.9
7	163.0	162.9	163.0	161.6	162.6	162.0	161.7
8	89.6	89.8	89.5	98.0	94.7	104.4	105.5
9	155.3	156.4	156.1	155.0	156.5	160.7	160.3
10	108.4	105.2	105.1	103.6	104.9	100.5	103.4
1'	112.6	114.8	120.8	111.3	114.8	110.9	111.3
2'	160.2	160.4	152.5	160.3	160.5	156.7	156.5
3'	103.9	98.6	116.3	102.8	98.7	103.0	109.7
4'	156.3	158.3	148.9	156.5	159.1	161.0	161.2
5'	105.1	104.6	119.8	106.7	104.6	107.0	106.7
6'	131.6	131.3	132.6	130.9	131.3	131.3	131.2
1''	24.3	24.0	23.9	23.9	24.1	–	23.5
2''	121.0	121.4	123.1	122.2	122.6	–	121.7
3''	133.0	131.8	130.9	130.9	131.8	–	131.2
4''	25.2	25.6	25.6	25.6	25.6	–	25.4
5''	17.6	17.5	17.6	17.5	17.5	–	17.3
1'''	115.7	115.8	115.9	–	–	–	21.1
2'''	147.7	148.1	147.6	–	–	–	122.1
4'''	32.9	33.1	33.1	–	–	–	130.7
5'''	22.6	22.7	22.7	–	–	–	25.4
6'''	22.6	22.7	22.7	–	–	–	17.3
OMe	55.9	55.8	55.9	–	–	–	–
OMe	–	55.7	–	–	–	–	–
OMe	–	55.6	–	–	–	–	–
OAc	–	–	168.5	–	–	–	–
OAc	–	–	168.3	–	–	–	–
OAc	–	–	21.1	–	–	–	–

*The multiplicity of carbon signals were deduced by the comparative analysis of ^{13}C NMR-PND and ^{13}C NMR-DEPT.

left for 4 h at room temperature and the usual work-up yielded **1b** as yellow crystals (42 mg; 95%), mp 136-138 °C. IR ν_{max}^{KBr} cm^{-1}: 3350, 2960, 2860, 1765, 1635, 1620, 1585, 1470, 1445, 1350, 1300, 1255, 1240, 1200,1165, 1150, 1120, 1040, 1020, 990, 975, 915, 855, 830, 805, 795, 775, 740, 725 and 700. EIMS m/z (rel. int.): 520 ($M^{+\bullet}$ 37), 477 (100), 465 (20), 405 (22), 379 (47), 337 (57), 295 (7), 233 (5), 179 (12), 147 (7), 105 (5) and 91 (5). ^1H NMR: see Table 1. ^{13}C NMR: see Table 2.

Catalytic reduction of 1 + 3

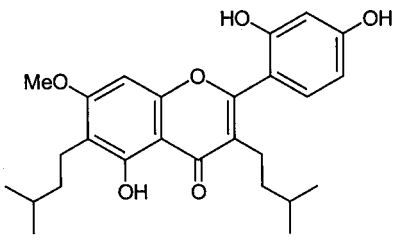
A solution of a mixture of **1** + **3** (50 mg) in methanol was hydrogenated over 10% Pd-C (100 mg) at 50 psi. After 3 h, the catalyst was filtered off and washed with EtOAc (45 mg; 90%) resulting in a brownish powder. IR ν_{max}^{KBr} cm^{-1}: 3380, 2980, 2950, 2880, 1740, 1600, 1460, 1370, 1280, 1210, 1150, 1130, 1075, 820 and 740. EIMS m/z (rel. int.): 440 ($M^{+\bullet}$ 33), 397 (20), 383 (100), 327 (55), 311 (12), 257 (2), 237 (8), 193 (6), 179 (58), 137 (8), 108 (4) and 65 (4). ^1H NMR ($CDCl_3$): δ 0.74 (6H, d, J = 6.5 Hz), 0.93 (6H, d, J = 6.5 Hz), 1.25 - 1.38 (6H, m), 2.56 (4H, m), 3.83 (3H, s), 6.33 (1H, s), 6.39 (1H, d, J = 8.5 Hz), 6.53 (1H, s) and 7.15 (1H, d, J = 8.5 Hz).

Results and Discussion

The dry ground heartwood was extracted successively with hexane, chloroform and ethanol. A mixture of steroids (β-sitosterol, stigmasterol and campesterol) was isolated from the hexane and chloroform extracts, and the constituents were identified by HRGC-MS and by co-injection with a standard mixture of these steroids in three chromatographic columns of different selectivities. The flavones were isolated from the chloroform extract. TLC and color tests with ferric chloride (green) and magnesium-concentrated HCl (orange) suggested the presence of flavonoids. The major flavonoid from the chloroform extract (≅ 10% by weight of extract) was artocarpin **(1).** The molecular formula $C_{26}H_{28}O_6$ for **1** was deduced from the HRMS, which revealed a molecular peak at 436.1865 for a calculated value of 436.1864. The presence of a hydroxyl group at C-5 was shown by a singlet signal at δ 13.49 in the ^1HNMR spectrum and by a bathochromic shift of band I (Δλ 36 nm) in the UV spectrum after the addition of $AlCl_3$. The addition of NaOAc did not affect band II, revealing the absence of a free 7-OH, and NaOMe shifted band I (Δλ 40 nm) without an increase in its intensity, indicating the presence of a free 4'-OH group. Thus the UV spectrum established that the compound is a flavone with hydroxyls at positions 5-OH and 4'-OH[4].

The dimethyl ether **(1a)** exhibited $M^{+\bullet}$ 464 and the diacetate **(1b)** $M^{+\bullet}$ 520, and both gave a green color when mixed with alcoholic $FeCl_3$, confirming the presence of a chelated hydroxyl group. The presence of two ethylenic bonds was confirmed by the formation upon hydrogenation of a single tetrahydro derivative **(2)** $M^{+\bullet}$ 440.

The ^1H NMR spectrum of **1** (Table 1) showed the presence of two groups in the molecule: γ,γ-dimethylallyl [e.g. 1: δ 3.13 (d, J = 6.6 Hz, 2H-1''), 5.15 (t, J = 6.6 Hz, H-2''), 1.46 (br,s) and 1.61 (br,s), 3H-4'' and 3H-5''], and



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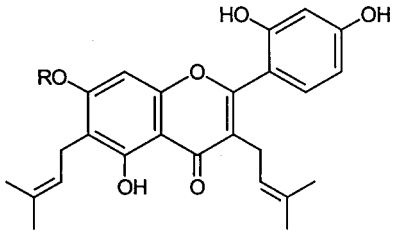
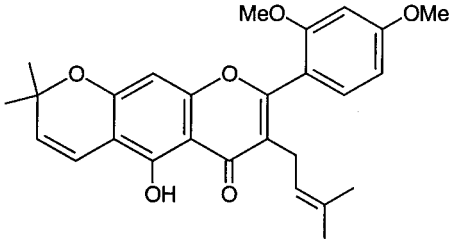


3 R=Me
4 R=H



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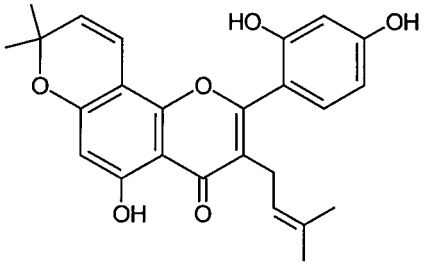

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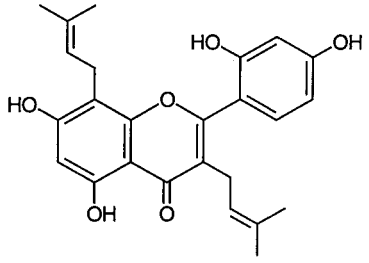

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trans-3-methylbut-1-enyl [e.g. 1: δ 6.54 (d, J = 16.8 Hz, H-1''') 6.77 (dd, J = 16.8 and J = 6.8 Hz, H-2'''), 2.48 (m, H-3'''), 1.11 (d, J = 6.8 Hz, 3H-4''' and 3H-5''')]. The presence of these groups was confirmed by the appearance of two doublets at δ 0.74 (d, J = 6.5 Hz, 6H) and 0.93 (d, J = 6.5 Hz, 6H) in the ^{1}H NMR spectrum of tetrahydro derivative **2**. In addition, the ^{1}H NMR spectrum established the substitution patterns of **1, 1a** and **1b,** showing that only B-ring proton signals are coupled (Table 1). The pattern 2', 4'-OR [R = H (1), R = Me (1a) R = Ac (1b)] of the B-ring was defined by chemical shifts of the carbons C-1' to C-6' [e.g. 1: δ 112.6 (s, C-1'), 160.2 (s, C-2'), 103.9 (d, C-3'), 156.3 (s, C-4'), 105.1 (d, C-5') and 131.6 (d, C-6')] when compared with values described for model flavones[7-11] containing the same B-ring system (Table 2). The presence of the B-ring 2',4'-dihydroxy in the natural flavone was confirmed by both the upfield shifts, compared with those of the methyne carbons C-3' and C-5' of **1** and **1a** [C-3': δ 103.9 (1) → 98.6 (1a), Δδ = 5.3 ppm; C-5': δ 105.1 (1) → 104.6 (1a), Δδ = 0.5 ppm], and the downfield shifts of these carbons after conversion of the 2',4'-diacetoxy system [C-3': δ 103.9 (1) → 116.3 (1b), Δδ = 12.4 ppm; C-5': δ 105.1 (1) → 119.8 (1b), Δδ = 14.7 ppm]. The chemical shift at δ ≅ 24 (t, CH_2-1'') is in good agreement with those previously reported in the literature for 3-γ,γ-dimethylallyl flavonoids (Table 2). The absence of an additional signal between δ 102.0 - 117.7, corresponding to CH-3 of the flavone in the ^{13}C NMR spectra of **1, 1a** and **1b,** led us to locate the γ,γ-dimethylallyl group at C-3. Finally, the upfield chemical shifts of the methyne carbon, appearing at about δ 89.5 in **1** and in the derivatives **1a** and **1b,** were attributed to a lack of substitution at C-8, and consequently to the presence of a methoxyl group at C-7 (Table 2).

In the EI-mass spectrum, two significant fragments were observed at m/z 235 $[A_1 + H]^+$ and m/z 202 $[B_1]^{\bullet+}$, which originated from the A and B rings by RDA fragmentation, establishing the substituents of the A and B rings. The localization of the prenyl group at C-6 was confirmed by intense fragments at m/z 393 (100) and 381 (30) originating in the loss of neutral fragments C_3H_7 and C_4H_7 from the $M^{+\bullet}$ at m/z 436, respectively. Furthermore, it was not observed the fragment at m/z $M^{+\bullet}$-15 typical of 8-prenylated flavone[6].

These data established that compound **1** is the 2',4'-dihydroxy-7-methoxy-3-γ,γ-dimethylallyl-6-(*trans*-3-methylbut-1-enyl) flavone (1), known as artocarpin, previously isolated from *Artocarpus heterophyllus*[7].

The other flavone **(3)** was isolated together with artocarpin **1**. The only significant difference observed between the two compounds is the replacement of the *trans*-3-methyl-but-1-enyl moiety in **1** by a γ,γ-dimethylallyl at C-6 in **3**. The chemical shift at δ 21.4, due to a methylene group of the prenyl moiety at C-6 in **3,** is identical to those described for flavonoids with the same substitution pattern in the A-ring (Table 2). This chemical shift (CH_2-1'': δ 21.4) was also used to confirm the location of the γ,γ-dimethylallyl at C-3 (and *trans*-3-methylbut-1-enyl at C-6)

of **1,** since the carbon signal of a methylene group in the γ,γ-dimethylallyl group absorbs at δ 23.5-24.3 when localized at C-3 (Table 2: **1-7**) and at δ 21.4 at C-6 *ortho*-dioxygenated **(3),** revealing, as anticipated, major γ-effects promoted by the oxygen of the carbonyl function and aromatic carbon atom C-1'. Catalytic reduction of the mixture (10 Pd-C, 50 psi, ethyl acetate) afforded a single compound, the tetrahydro derivative **2.**

In spite of exhaustive attempts, it was not possible to isolate **3** as a pure compound, either by preparative TLC or by column chromatography, as minor amounts of **1** always remained.

In an earlier work on a specimen of this same species collected in the Linhares reserve, Rio Doce, Espírito Santo, Gottlieb et al described the isolation of the β-sitosterol and 3,5-dihydroxy-4-methoxystilbene[5]. In contrast to these results, our studies did not show any evidence for the presence of this stilbene.

The structure reported initially as isoartocarpin in Devon & Scott Handbook was renamed as cicloartocarpin[12]. Therefore, we gave the name isoartocarpin to the flavonoid with structure **3,** due to the similarity to artocarpin.

Acknowledgments

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References

1. A.A. Loureiro, F.M. Silva and J.C. Alencar, *Essências Madeireiras da Amazônia* (CNPq/INPA, 1979), vol.1.
2. *New Marketable Species in South America: Guariuba.* Technical note drafted by the Centre Technique Forestier Tropical (CTFT) in collaboration with INPA (Brazil).
3. M.F.C. Cardias and M.A. Jesus, Série Técnica n° 13, CPPF/INPA (1985).
4. T.J. Mabry, K.R. Markham and M.B. Thomas, *The systematic identification of flavonoids* (Springer-Verlag, Berlin, 1970).
5. O.R. Gottlieb, R.A. Lima, P.H. Mendes and M.T. Magalhães, *Phytochemistry* **14,** 1674 (1975).
6. M. Takayama, T. Fukai, Y. Harro and T. Nomura, *Heterocycles* **33,** 405 (1992).
7. K. Venkataraman, *Phytochemistry* **11,** 1571 (1972).
8. E. Wenkert and H.E. Gottlieb, *Phytochemistry* **16,** 1811 (1977).
9. T. Nomura, T. Fukai and M. Katayanagi. *Chem. Pharm. Bull.* **26,** 1453 (1978).
10. T. Nomura, T. Fukai, S. Yamada and M. Katayanagi, *Chem. Pharm. Bull.* **26,** 1394 (1978).
11. I. Messana, F. Ferrari and M.C.M. Araújo, *Planta Médica* **53,** 541 (1987).
12. P.M. Nair, A.V. Rama Rao and K. Venkataraman, *Tetrahedron Letters* **2,** 125 (1964).