Botocudorol, a New Bisabolene Type *nor*-Sesquiterpenoid from *Sparattanthelium botocudorum*

Ronaldo N. de Almeida, José M. Barbosa-Filho

Laboratório de Tecnologia Farmacêutica-UFPb
58059 João Pessoa, Paraíba, Brasil

Victor M. Rumjanek and Raimundo Braz-Filho*

Departamento de Química ICE - UFRRJ
Caixa Postal 74541, 23851 Seropédica, RJ, Brasil

Received: April 30, 1991; September 9, 1991

Do extrato etanólico-acquoso (95%) das folhas de *Sparattanthelium botocudorum* foram isolados e identificados 5-hidroxi-3,4',7-trimetoxiflavona(1) e 4α,10α-dihidroxiguaja-6-eno (2) e um novo *nor*-sesquiterpeno, que foi denominado botocudorol (3). Dados espectrais foram usados nas determinações estruturais. As atribuições dos deslocamentos químicos dos átomos de ^{1}H e ^{13}C apoiaram-se na análise dos resultados fornecidos por experiências APT, DEPT, ^{1}H x ^{1}H-HOMOCOSY e 1Hx ^{13}C-HETECOSY($^{1}J_{CH}$).

From the 95% ethanolic extract of the leaves of *Sparattanthelium botocudorum* were isolated and identified 5-hidroxy-3,4',7-trimethoxyflavone(1), 4α,10α-dihydroxyguaja-6-ene (2) and a new *nor*-sesquiterpene, botocudorol (3). Spectral data were used in the structure determination. The ^{1}H and ^{13}C NMR chemical shifts have been assigned on the basis of APT, DEPT, ^{1}H x ^{1}H-HOMOCOSY and ^{1}H x ^{13}C-HETECOSY experiments.

Key words: Sparattanthelium botocudorum: *Hernandiaceae; Flavonol; sesquiterpenes.*

Introduction

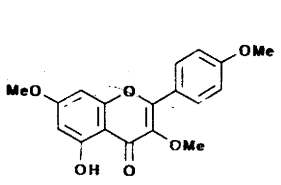
Sparattanthelium botocudorum Mart, family Hernandiaceae, is a shrub widely distributed in the coastal region of Paraíba State and is commonly known as "canela-brava". A survey of the literature showed that alkaloids have been reported from *Sparattanthelium uncigerum*, the only species of the genus studied so far[1]. In this paper we wish to describe the isolation and characterization of two known compounds, a flavonol (1) and a sesquiterpene (2), along with a new bisabolene type *nor*-sesquiterpenoid, botocudorol (3) from *Sparattanthelium botocudorum*.

Experimental

General experimental procedures. Melting points were measured using a Kofler block and are uncorrected. ^{1}H (80 and 200 MHz) and ^{13}C (20 and 50.3 MHz) NMR spectra were obtained in $CDCl_3$ solution, on either a Varian FT-80A or a Bruker AC-200 equipment. TMS was used as an internal standard and the chemical shifts are given in δ values (ppm). EIMS were determined with a GC/MS system and relative intensities of peaks are reported with reference to the most intense peak higher than *m/z* 100. IR spectra were recorded on a Perkin-Elmer 467 spectrometer. For TLC, Kieselgel 60 F_{254} was used. Commercial Merck Si gel (60-230 mesh ASTM) was used for CC.

Plant material. The leaves of *Sparattanthelium botocudorum* were collected in the proximity of the city of João Pessoa by Maria de Fátima Agra, Universidade Federal da Paraíba, João Pessoa. A voucher specimen (n° 5799) is deposited at the Herbarium of the Universidade Federal da Paraíba, João Pessoa, PB, Brazil.

Extraction and isolation of constituents. The pulverized and air-dried plant material (11 kg) was percolated with 95% EtOH at room temperature, and the EtOH extract was concentrated *in vacuo* to furnish a residue (800 g). This residue was suspended in 10% AcOH and extracted with $CHCl_3$. The $CHCl_3$ solution was concentrated to give the $CHCl_3$-soluble fraction (48 g) which was chromatographed on Si gel (700 g) column to give the combined fractions 15/16, 46/62 and 78/85, eluted with C_6H_6-$CHCl_3$ (8:2); C_6H_6-$CHCl_3$ (1:1) and C_6H_6-$CHCl_3$ (1:2), respectively. These three fractions were further purified by Si gel TLC to furnish 1 (22 mg), 2 (62 mg) and 3 (115 mg), respectively.



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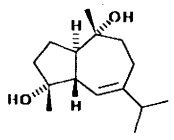
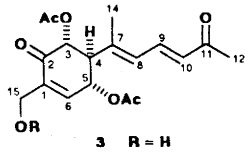
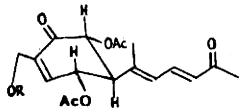


2 5β-H

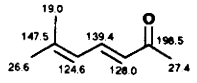


3 R = H

3a R = Ac



3b



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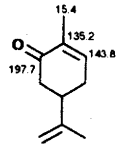

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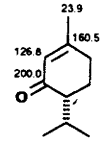

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5-Hydroxy-3,4',7-trimethoxyflavone (**1**). MP 143-145°C (benzene) (lit[2], mp 142-144°C). Spectral data (UV, IR ^{1}H and ^{13}C NMR and EIMS) are in agreement with the reported values.[2,3]

4α-10α-Dihydroxy-5β-H-guaja-6-ene (**2**). Viscous oil. IR, ^{1}H NMR and EIMS data are consistent with those recorded in the literature;[4] ^{13}C NMR (50.3 MHz, $CDCl_3$. TMS) δ149.6 (C-7), 121.3 (C-6), 80.2 (C-4), 75.3 (C-10), 50.6 (C-5), 50.3 (C-1), 42.7 (C-3), 40.4 (C-9), 37.3 (C-11), 25.1 (C-8), 22.5 (C-15), 21.5 (C-2), 21.4 (C-14), 21.3 (C-12), 21.2 (C-13).

Botocudorol (**3**). Viscous oil; υ(KBr,max) cm^{-1}: 3400, 1735, 1725, 1670, 1645, 1610, 1570, 1360, 1220, 1023; λ(MeOH,max, ε)nm: 227 (14.000), 287 (21.700); ^{1}H and ^{13}C NMR: see Table 1; EIMS *m/z* (rel.int.) 350 (M^{+}, absent), 332(1), 290(2), 272(1), 230(17), 215(5), 212(4), 202(8), 201(6), 187(22), 174(6), 159(12), 109(98), 43(100).

Acetate of **3** (**3a**). Compound **3** (10 mg) was dissolved in pyridine (1 ml) and Ac_2O (2 ml) was added, and the solution was allowed to stand for 24 h at room temperature. The usual workup afforded **3a** (7 mg), viscous oil; ^{1}H and ^{13}C NMR: see Table 1.

Results and discussion

The known compounds (1 and 2) were identified by comparison of spectral properties with literature data, as well as by ^{13}C NMR spectra (see experimental). 5-Hydroxy-3,4',7-trimethoxyflavone (**1**) has been found in *Cheilanthes longissima*[2], *Cheilanthes farinosa*[5], *Aframamum giganteum*[6], *Sideritis dasygnaphala*[7], *Sideritis bolleana*[7], *Aesculus turbinata*[8], *Ostrya virginiana*[9] and *Betula nigra*[9]. 4α-10α-Dihydroxy-5β-H-guaja-6-ene was also isolated earlier from *Silphium pefoliatum*[4] and *Silphium terebinthinaceum*[4].

The molecular formula $C_{18}H_{22}O_7$ for the *nor*-sesquiterpenoid botocudorol (**3**) was determined by a combination of low resolutions MS, and ^{1}H and ^{13}C NMR (broadband decoupled, APT and DEPT), including 2D homonuclear (^{1}H x ^{1}H-HOMOCOSY) and heteronuclear (^{1}H x ^{13}C-

Table 1. *^{1}H (200 MHz) and ^{13}C (50.3 MHz) NMR data of natural product* **3** *and its acetate derivative* **3a**, *run in $CDCl_3$ solution and TMS as internal standard.*[a]

C	δC	δH	δC	δH
1	137.8	-	137.7	-
2	192.1	-	192.1	-
3	72.5	5.46 (*d*, 13.1)	72.5	5.59 (*d*, 13.2)
4	56.7	3.15(*dd*,10.1,13.1)	56.7	3.17 (*dd*, 10.8, 13.2)
5	68.1	5.88 (*d*, 10.1)	68.1	5.88 (*d*, 10.8)
6	142.2	6.77 (*br* s)	142.9	6.75(*br s*)
7	142.6	-	142.8	-
8	129.4	6.07 (*d*, 11.3)	129.3	6.09 (*d*, 11.0)
9	137.7	7.34 (*dd*, 15.3, 11.3)	137.6	7.34 (*dd*, 15.3, 11.0)
10	131.0	6.13 (*d*, 15.3)	130.1	6.15 (*d*, 15.3)
11	198.8	-	198.8	-
12	27.6	2.28 (*s*)	27.5	2.29 (*s*)
13	-	-	-	-
14	13.4	1.87 (*br s*)	13.4	1.89 (*br s*)
15	59.4	4.30(*br s*)	59.3	4.78 (*br s*)
OH	-	-		-
OAc	169.9 20.5	2.10 (*s*)	169.9 20.5	2.12 (*s*)
OAc	169.8 20.4	2.02(*s*)	169.8 20.3	2.05 (*s*)
OAc	- -	-	169.8 20.3	2.10 (*s*)

[a] Chemical shifts are given downfield from TMS. Values of coupling constants (J) in Hz are reported in brackets. Assignments were made with the aid of the APT, DEPT and 2D-shift-correlated homonuclear (^{1}H x ^{1}H-HOMOCOSY) and heteronuclear [^{1}H x ^{13}C-HETECOSY, optimized for one bond (^{1}JCH)].

HETECOSY) correlations[10]. In addition to the presence of two acetoxyl groups in the ^1H [δ 2.10 (s, 3H) and 2.02 (s, 3H) and ^{13}C [δ 169.9 (s, COO), 169.8 (s, COO), 20.5 (q, CH_3) and 20.4 (q, CH_3)] NMR spectra, the remaining signals could be assigned to $(C=O)_2$, $(C=CH)_2$, (CH=CH), (CH_2OR), $(CH\text{-}OR)_2$, (CH) and $(CH_3)_2$.

The IR spectrum of compound **3** exhibited absorption bands at 3400 (OH), 1735 ($OCOCH_3$), 1725 ($OCOCH_3$), 1670 (α, β-unsaturated ketone), 1645 (α, β, γ, δ-unsaturated ketone), 1610 (C=C) and 1570 (C=C). The ^1H NMR spectrum revealed signals at δ 6.13 (d J=15,34 Hz, H-10)m 7,34 (dd, J=15.3 and J=11.3 Hz, H-9), 6.07 (d, J=11.3 Hz, H-8), 1.87 (br s, CH_3-7) and 2.28 (s, CH_3-11) which are characteristic of the 7-methyl-7,9-dien-11-one system [λ(MeOH,max)287 (ε 21.700)nm; calculated value: 215 + 30 + (2 x 18)= 281 nm]. The coupling constant (J=15.3 Hz) corresponding to the spin-spin interaction of H-9 with H-10 indicated a *trans* relationship between these two protons. The presence of this α, β, γ, δ-unsaturated methyl ketone system was confirmed by selective double resonance and ^1H x ^1H-HOMOCOSY experiments and by ^{13}C NMR spectra (broadband decoupled, APT and DEPT) which showed signals at δ 198.8 (s, C-11), 142.6 (s, C-7), 137.7 (d, CH-9), 131.0 (d,CH-8), 129.4 (d, CH-10), 27.6 (q, CH3-10 and 13.4 (q, CH3-7). The assignments were made with the aid of the 2D-shift-correlated heteronuclear(^1H x ^{13}C-HETECOSY) and comparison with model compounds, 6-methylhepta-3,5-dien-2-one(**4**)[11], **5** and **6**[12]. The α,β-unsaturated tetrasubstituted ciclohexenone ring [λ(MeOH,max)227, (14.000)nm] was diagnosed by the chemical shifts and multiplicities of signals of the ^1H[δ 5.46 (d, J=13.1 Hz, H-3, 3.15 (dd, J=10.1 and 13.1 Hz, H-4), 5.88 (d, J=10.1 Hz, H-5) and 6.77 (br s, H-6)] and 13C [d 137.8 (s, C-1), 192.1 (s, C-2), 72.5 (d, CH-3), 56.7 (d, CH-4), 68.1 (d, CH-5) and 142.2 (d, CH-6)] NMR. The existence of a primary allylic alcohol was recognized by a broad singlet at δ 4.30 which resulted in a 0.48 ppm paramagnetic shift upon acetylation(**3a**). The multiplicities of the signals corresponding to methine sp^3 carbon atoms and the coupling constant values were used for the assignment of an axial position for H-3 (J=13.1 Hz), H-4 (J=10.1 and J=13.1 Hz) and H-5 (J=10.1 Hz). Consequently, the two acetoxy groups and the methyl-7,9-dien-10-one system are placed at equatorial positions (**3**). These assignments were also supported by NMR techniques as described above (Table 1). Finally, the *E*-configuration for the double bond between C-7 and C-8 was indicated by upfield chemical shifts of C-14 (d 13.4) and C-9 (δ 137.7), showing an expected γ-effect. The considerable upfield shift of C-14 (13.4 ppm) in comparison with that observed for the analogous methyl group of the model compound **4** (19.0 ppm) is consistent with an additional effect of C-3 and C-5. This deduction was confirmed by NOE idifference spectra[10] of **3a** performed with irradiation on methyl signal at δ 1.87 (br s, CH3-7) which revealed H-3 (δ 5.49), H-5 (δ 5.88) and H-9 (δ 7.34) signal enhancements of 3.7, 3.5 and 3.4 %, respectively. The abscence of an NOE at the H-4 during this experience shows that the C-7 is in a *trans* relationship with this proton. Thus the complete assignment of the relative configuration as shown in **3b** was deduced for the new *nor*-sesquiterpenoid isolated from *Sparattanthelium botocudorum*. This *rel* - 3α, 5α,-diacetoxy-15-hydroxy-13-*nor*-4α-H-bisabola-6, 7*E*, 9*E*-trien-2, 11-di-one was named botocudorol.

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