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New 3,4-Seco *ent*-Kaurenes from *Croton caracasana* Flowers

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This paper is dedicated to Professor P. Joseph-Nathan for his 65th birthday.

Two new 3,4-seco-*ent*-kaurenes, caracasine (**1**) and caracasine acid (**2**), were isolated from non-polar extracts of the flowers of *Croton caracasana* (Euphorbiaceae), together with six known terpenes, stigmasterol (**4**), stigmastenone (**5**), 2,6-dimethylocta-3,7-diene-2, 6-diol (**6**), spathulenol (**7**), caryophyllene oxide (**8**), and aromadendrene (**9**), and the flavonoid tribuloside (**10**). The chemical structures were determined by spectroscopic means and chemical correlations. All isolated compounds are being described for the first time for this species.

Keywords: *Croton caracasana*, Euphorbiaceae, seco *ent*-kaurenes, terpenoids, flavonoids.

The *Croton* genus is the second largest in the plant family Euphorbiaceae, with more than 1200 species distributed in the tropical and neotropical regions of the world [1,2]. Many *Croton* species have shown pharmacological activities, such as anti-inflammatory [3,4], antinociceptive [5,6], anticancer [7] and, hypoglycemic [8,9]. This genus is well known chemically for the common presence of different types of diterpenes, such as clerodanes and kaurenes [10-14], flavonoids [15] and alkaloids [16-18].

C. caracasana Pittier is a shrub that grows in the northern part of Venezuela. To the best of our knowledge neither phytochemical nor pharmacological study of this plant has been reported. As part of our continuing work on *Croton* species from Venezuela [19-22], we report here the isolation and characterization of two new seco *ent*-kaurenes (**1** and **2**), in addition to six known terpenoids and one flavonoid from the apolar extracts of *C. caracasana* flowers.

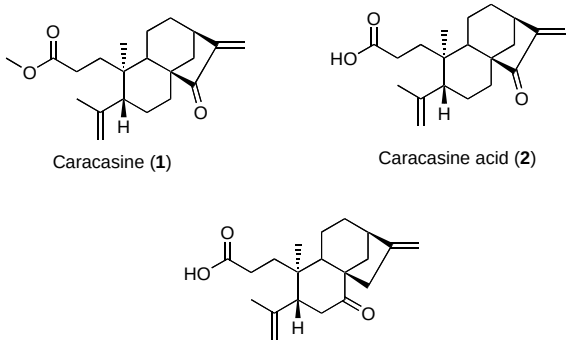

Figure 1: Structures of 3,4-seco *ent*-kaurenes: caracasine (**1**), caracasine acid (**2**), and geayine (**3**).

Caracasine (**1**) was obtained as a yellow syrup; the molecular formula was determined as $C_{21}H_{30}O_3$ from the HRCIMS (m/z 330.4651) and from ^{13}C NMR spectroscopic data, including DEPT. The FT-IR spectrum showed a strong band for the carbonyl groups, which indicated the presence of an ester and ketone groups (1735, 1707 cm^{-1}), and olefin carbons at 1642 cm^{-1} and 894 cm^{-1}.

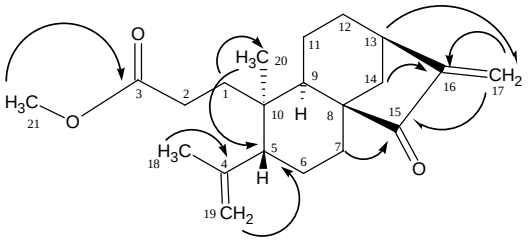

Figure 2: Key correlations in the HMBC spectrum of caracasine (**1**).

The ^{13}C NMR spectrum displayed 21 signals, which were assigned by HMQC and DEPT 135 experiments as the resonances of three methyl (including methoxy) groups, nine methylenes, including two exo-methylenes, three methines, four quaternary carbons and two carbonyls belonging to an ester group at δ 174.3 and one ketone at δ 210.5. The ^{1}H NMR spectrum of **1**, showed two methyl singlets at δ 1.05 and 1.73, and one methoxy group at δ 3.61. The presence of four singlet signals at δ 4.66, 4.87, 5.24, and 5.93 were in good agreement with the presence of two exomethylene groups in the isolated compound. An HMBC spectrum established the positions of all the functional groups in the skeleton, especially that of the ketone group, which was corroborated by the correlation between the carbon at δ 210.0 and the hydrogen signals at δ 1.26 (H-7), 2.36 (H-14), 5.24, and 5.94 (H-17), which unequivocally located this group at C-15, and the olefins on the carbons C-16 and C-4. By comparison with NMR spectroscopic data of closely related structures we could conclude that the ^{1}H and ^{13}C NMR spectrum of **1** (Table 1) were very similar to those of the known compound geayine (**3**), the first 3,4 seco-*ent*-kaurene isolated from *C. geayi* [23]. Compound **1** differed from **3** by the presence of one additional methoxy group and the large difference in the chemical shift between their respective ketone groups, suggesting a different position for this group in the new compound. With all the spectroscopic data, the structure of caracasine (**1**) was assigned as shown in Figure 1.

Compound **2** was isolated as a pink solid, mp 118-122^{o}C, with a molecular composition of $C_{20}H_{28}O_3$, as inferred from HRCIMS (m/z 316.3561). Its IR spectrum also showed absorption bands for carbonyl and a double bond. The ^{1}H NMR spectrum included two pairs of singlet signals at δ 4.66, 4.87, 5.23 and 5.94 corresponding to two exomethylene groups. Comparison of the ^{1}H NMR and ^{13}C spectral data of **2** with those of **1** indicated that both compounds had the same basic skeleton. The twenty carbons in **2** were characterized by ^{13}C and DEPT spectral analysis, which revealed that the only difference from caracasine (**1**) was the lack of the methoxyl group and the difference in the chemical shift of the carbonyl from caracasine (**1**) at δ 174.3 to 179.5, revealing that **2** was the carboxylic acid of **1**, caracasine acid (**2**).

The known compounds stigmasterol (**4**) (33.0 mg) [24], stigmastenone (**5**) (9.6 mg) [25], 2,6-dimethylocta-3,7-diene-2,6-diol (**6**) (42.5 mg) [26], spathulenol (**7**) (15.2 mg) [27], caryophyllene oxide (**8**) (7.0 mg) [28], aromadendrene (**9**) (13.6 mg) [29], and the flavonoid tribuloside (**10**) (44.1 mg)[30] were identified by comparison of their spectroscopic data with those reported in the literature.

Experimental

General procedures: Melting points were determined on a Fisher-Jones melting point apparatus and are uncorrected. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a Bruker NMR spectrometer DRX-500. Chemical shifts are given in δ (ppm) with the solvent signals being used as reference. MS data were determined on a Varian Saturn (GC-MS) instrument at 70 eV. IR spectra were obtained on a FT-IR Thermo Nicolet Nexo 470 model. Column chromatography was carried out on silica 60 (Merck) and TLC on precoated silica gel G_{254} (Merck) plates; spots were visualized by UV (254 nm) irradiation and reaction with *p*-anisaldehyde/H_2SO_4/HOAc reagent.

Plant material: *Croton caracasana* was collected from Ocumare de la Costa, Aragua, Venezuela in June 2006 and identified by Dr Stephen Tillett. A voucher specimen with the number MYF-26701 is deposited at the Herbarium Victor Manuel Ovalles of the Pharmacy Faculty, Universidad Central de Venezuela.

Extraction and isolation: The flowers of *C. caracasana* (118.0 g) were extracted with *n*-hexane, CH_2Cl_2, EtOAc and MeOH to yield 3.20, 0.98, 0.24, and 3.4 g of residues, respectively. The *n*-hexane extract was chromatographed on CC over silica gel, using a gradient of $CHCl_3$ and increasing amounts of MeOH (up to 10%). The composition of the obtained fractions was monitored by TLC, and the chromatographically identical fractions were combined to give ten fractions (H1-H10). H5 (127.0 mg) was fractionated by silica gel column

chromatography using a gradient from CH_2Cl_2-*n*-hexane (1:1) to CH_2Cl_2-methanol (98:2) to give the compounds **1**, **4**, and **5**.

Fraction H3 (130.0 mg), which showed one major component, was purified by passage through a silica gel column, eluting with a gradient of *n*-hexane:EtOAc from 99:1 to 70:30, to provide pure compound **6**.

From H4 (166.5 mg), chromatographed on silica gel by eluting with *n*-hexane-EtOAc (9:1) to EtOAc:MeOH (9:1), the sesquiterpene spathulenol (**7**) was obtained.

The CH_2Cl_2 (0.98 g) extract showed four compounds with close Rf values. However, column chromatographic separation using silica gel and eluting with 100% EtOAc to EtOAc-MeOH (8:2), gave 6 fractions. The major fractions were constituted of insoluble waxes. One of the last fractions was further purified by column chromatography, eluting with *n*-hexane-EtOAc (8:2) to EtOAc-MeOH (9:1), to obtain compounds **2** and **6**.

The EtOAc extract, showing only two major spots on TLC, was submitted to column chromatography using a mixture of EtOAc-MeOH (99:1) to (90:10) to provide tribuloside (**10**) as a pure compound.

Caracasine (**1**)

Yellow oil (13.7 mg).
$[\alpha]_D$: -3.47° (*c* 0.1, $CHCl_3$).
Rf: 0.38 (CH_2Cl_2-*n*-hexane, 9:1).
IR (KBr) γ_{max}: 2926, 2865, 1735, 1642 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$): δ 1.05 (3H, s, H-20), 1.26 (2H, m, H-7), 1.35 (1H, d, *J* = 8.35 Hz, H-9), 1.49 (2H, m, H-6), 1.62 (2H, dd, *J* = 6.75 Hz, H-1), 1.64 (2H, m, H-11), 1.73 (3H, s, H-18), 1.86 (2H, m, H-12), 2.04 (1H, dd, *J* = 9.4, 3.5 Hz, H-5), 2.10 (2H, m, H-2), 2.36 (2H, d, *J* = 12.3 Hz H-14), 3.04 (1H, brs, H-13), 3.61 (3H, s, H-21), 4.66 (1H, brs, H-19a), 4.87 (1H, brs, H-19b), 5.24 (1H, s, H-17a), 5.93 (1H, s, H-17b).
^{13}C NMR (125 MHz, $CDCl_3$): δ 18.3 (t, C-11), 21.6 (q, C-20), 23.7 (q, C-18), 24.5 (t, C-6), 28.3 (t, C-2), 32.0 (t, C-7), 32.1 (t, C-12), 33.6 (t, C-1), 36.3 (t, C-14), 38.0 (d, C-13), 41.4 (s, C-10), 43.3 (d, C-9), 49.7 (d, C-5), 51.6 (q, C-21), 51.9 (s, C-8), 113.6 (t, C-19), 114.7 (t, C-17), 146.8 (s, C-4), 149.2 (s, C16), 174.3 (C-3), 210.5 (C-15).
EI-MS m/z (rel. Int.): 330 M^+ (100), 287 (52), 243 (75), 215 (85).

Caracasine acid (**2**)

Pink solid (60.2 mg).
MP: 118-122°C.
Rf: 0.4 (*n*-hexane:EtOAc, 7:3).
$[\alpha]_D$: -1.36° (*c* 0.1, $CHCl_3$)
IR (KBr) γ_{max}: 3500, 2930, 2870, 1740, 1642 cm^{-1}.
^1H NMR (500 MHz, $CDCl_3$): δ 1.08 (3H, s, H-20), 1.26 (2H, m, H-7), 1.35 (1H, m H-9), 1.49 (2H, m, H-6), 1.62 (2H, dd, *J* = 6.7, 3.10 Hz, H-1), 1.64 (2H, m, H-11), 1.73 (3H, s, H-18), 1.86 (2H, m, H-12), 2.04 (1H, dd *J* = 9.4, 3.5, Hz, H-5), 2.10 (2H, m, H-2), 2.36 (2H, d, *J* = 12.0 Hz H-14), 3.04 (1H, brs, H-13), 3.61 (3H, s, H-21), 4.66 (1H, brs, H-19a), 4.87 (1H, brs, H-19b), 5.24 (1H, s, H-17a), 5.93 (1H, s, H-17b).
^{13}C NMR (125 MHz, $CDCl_3$): δ 18.2 (t, C-11), 21.6 (q, C-20), 23.4 (q, C-18), 24.6 (t, C-6), 28.2 (t, C-2), 31.9 (t, C-7), 32.1 (t, C-12), 33.4 (t, C-1), 36.3 (t, C-14), 37.9 (d, C-13), 41.4 (s, C-10), 43.3 (d, C-9), 49.7 (d, C-5), 51.9 (s, C-8), 114.0 (t, C-19), 114.8 (t, C-17), 146.8 (s, C-4), 149.2 (s, C-16), 179.5 (C-3), 210.5 (C-15).
EI-MS m/z (rel. Int.): 297 $[M-H_2O]^+$ (100), 287 (52), 243 (75), 215 (85).

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