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A NEW CASBANE-TYPE DITERPENOID FROM *CROTON NEPETAEFOLIUS*[1]

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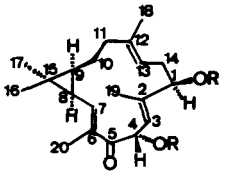
ABSTRACT.—The structure of a new macrocyclic diterpene isolated from the stem of *Croton nepetaefolius* has been determined as the casbane diterpenoid **1** by ^{1}H- and ^{13}C-nmr spectroscopy, including 2D nmr experiments (^{13}C, ^{1}H-COSY, and ^{1}H, ^{1}H-COSY). The arylpropanoid **3**, the acetophenone **4**, and sucrose have also been isolated from the same plant.

In the course of our continuing study of plants of the *Croton* genus (1,2), we reinvestigated a specimen of *Croton nepetaefolius* Baill. (Euphorbiaceae). Observations of the use of this plant by rural populations led to its first chemical investigation (3).

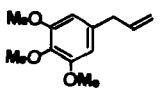
Hexane and EtOH extraction of the finely ground dried stem followed by chromatography afforded large amounts of a new macrocyclic diterpenoid **1**, as well as one arylpropanoid **3**, one acetophenone derivative **4**, and sucrose. Two other macrocyclic diterpenoids, also belonging to the casbane series, have not been fully elucidated.

The molecular formula, $C_{20}H_{30}O_3$, of **1** was determined on the basis of its molecular ion at *m/z* 318 and nmr data, mainly the noise-decoupled and DEPT ^{13}C-nmr spectra (Table 1). Formation of the diacetate **2** showed the presence of two secondary [**1** δ_H 4.11 (dd, J = 6.6, 9.0 Hz, H-1), 5.25 (d, J = 9.6 Hz, H-4), δ_C 77.62 (d, CH-1), 67.71 (d, CH-4); **2** δ_H 5.22 (dd, J = 8.7, 11.8 Hz, H-1), 6.21 (d, J = 9.3 Hz, H-4), δ_C 77.90 (d, CH-1), 70.30 (d, CH-4)] hydroxyl groups (Tables 1 and 2), whereas the remaining oxygen atom belongs to a conjugate carbonyl group (ν max 1690 cm^{-1}, δ_C 199.61).

The molecular formula requires six degrees of unsaturation. The signals at δ_C 143.76 (C-2) and 125.49 (CH-3), 134.51 (C-6) and 145.01 (CH-7), and 137.42 (C-12) and 119.51 (CH-13) in the ^{13}C-nmr spectrum (Table 1) revealed the existence of three trisubstituted double bonds. With these functionalities



1 R=H
2 R=Ac



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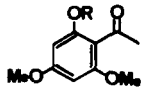


4 R=H
5 R=Ac

[(OH)$_2$(C=O) (C=CH)$_3$], the two remaining degrees of unsaturation were ascribed to two carbocyclic systems, clearly revealing **1** as a dicyclic diterpene. The quaternary carbon (C-15)

[1]Based on the MS thesis presented by V.L.A.M. to Universidade Federal do Ceará (1988); for a preliminary communication see *Cienc. Cult. (Sao Paulo)*, **40** (Suplemento), 568 (1988).

TABLE 1. Comparison of ^{13}C-nmr Spectral Data of Macrocyclic Diterpenes **1** and **2**, and **7** and **8**.[a]

Carbon	Compound				
	1	**2**	DEPT[b]	**7**	**8**[c]
C-1	77.62	77.90	CH	47.56	44.36
C-2	143.76	142.58	C	137.92	140.89
C-3	125.49	121.95	CH	128.67	124.02
C-4	67.71	70.30	CH	68.32	71.39
C-5	199.61	198.93	C	199.02	199.21
C-6	134.51	135.64	C	133.41	134.89
C-7	145.01	142.58	CH	145.06	142.73
C-8	27.98	27.76	CH	27.47	27.43
C-9	35.61	35.23	CH	34.57	34.02
C-10	25.31	25.50	CH_2	22.89	22.96
C-11	39.77	39.76	CH_2	31.70	31.78
C-12	137.43	138.54	C	137.31	140.35
C-13	119.51	118.13	CH	127.08	123.34
C-14	31.55	29.47	CH_2	70.21	72.53
C-15	27.60	26.55	C	27.31	26.70
C-16	15.99	15.85	CH_3	16.03	16.14
C-17	29.13	29.00	CH_3	28.99	29.05
C-18	15.32	15.40	CH_3	22.83	22.81
C-19	10.10	11.89	CH_3	17.83	18.58
C-20	11.97	11.88	CH_3	11.57	11.61
OC=O	—	170.54	C	—	170.22
	—	169.71	C	—	169.76
Me	—	21.20	CH_3	—	21.26
	—	20.73	CH_3	—	20.72

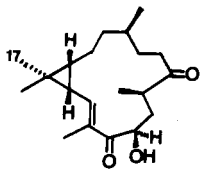
[a]Chemical shifts in δ (ppm) and TMS as internal standard.

[b]The chemical shifts of the quaternary carbon atoms of diterpenes **1** and **2** were deduced by comparative analysis of the noise-decoupled ^{13}C-nmr and DEPT spectra. Heteronuclear ^{13}C,^{1}H-COSY 2D nmr spectrum was also used for these assignments.

[c]Choi *et al.* (5) register different assignments for C-16 (δ 29.95), C-17 (δ 18.58), and C-19 (δ 16.14).

with δ_C 27.60 and the evidence of *gem*-dimethyl functionality [δ_H 1.01 (s), 1.16 (s); δ_C 15.99 (q), 29.13 (q)] suggested the presence of a *cis*-substituted cyclopropyl ring (4,5). Consequently, the other ring was deduced as a macrocyclic system with fourteen carbon atoms (C_{14}). In addition, the ^{13}C-nmr spectrum showed signals for three more methyl [δ_C 15.32 (q), 11.97 (q), and 10.10 (q)], three methylene [δ_C 39.77 (t), 31.55 (t), and 25.31 (t)], and two methine groups [δ_C 35.61 (d) and 27.98 (d)]. The ^{1}H-nmr spectrum allowed us to characterize these three methyl groups as attached to sp^2 carbons (δ_H 1.93, 1.70, and 1.59). Thus, the molecular formula $C_{20}H_{30}O_3$ was expanded to C (C=O) $(CH)_2$ $(CHOH)_2$ $(C=CH)_3$ $(CH_2)_3$ $(CH_3)_5$.

The location of the three double bonds was deduced from analysis of the high resolution ^{1}H- (300 MHz) and ^{13}C- (75 MHz) nmr spectra, through chemical shifts and multiplicity of signals, comparison with models (e.g., compound **7**) (4,5), and application of the 2D nmr techniques such as ^{1}H,^{1}H-COSY and ^{13}C,^{1}H-COSY (Table 2). The 2D nmr technique ^{1}H, ^{1}H-COSY clearly showed the respective coupling of H-1 [δ_H 4.11 (dd, J = 6.6, 9.0 Hz)] and H-13 [δ_H 4.68 (dd, J = 6.0, 3.0 Hz)] to the same methylene protons of CH_2-14. This result was used to confirm the presence of one double bond between the carbon atoms C-12 and C-13 and of one hydroxyl group at C-1, which shifted downfield by 1.11 ppm in the diacetate **2** (δ_H 4.11↦5.22 ppm). One olefinic



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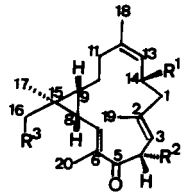
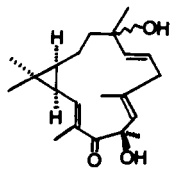


7 $R^1=R^2=OH$, $R^3=H$
8 $R^1=R^2=OAc$, $R^3=H$
9 $R^1=O$, $R^2=OH$, $R^3=H$
10 $R^1=R^2=R^3=OH$



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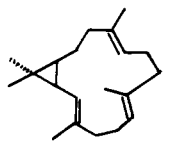

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proton appeared as a doublet at δ 6.28 ($J=9.3$ Hz) in the ^{1}H-nmr spectrum of **1** and was assigned as a proton β to a carbonyl group, in agreement with the ^{13}C-nmr spectrum [δ_C 199.61 (C=O), 134.51 (C-6), and 145.01 (CH-7)]. The two remaining downfield signals in the ^{1}H-nmr spectrum of **1** (Table 2) were attributed to H-3 [δ_H 5.17 (d, $J=9.6$ Hz)] and H-4 [δ_H 5.25 (d, $J=9.6$ Hz)] adjacent to hydroxyl and carbonyl groups, which move downfield by 0.87 ppm in the diacetate **2** (δ_H 5.25→6.12 ppm). This environment and the accurate assignment of the chemical shift of C-3 (δ_C 125.49) were confirmed by the change of the latter value to higher field in the diacetate derivative **2** (δ_C 121.95). The singlets at δ_C 143.76, 137.43, and 119.51, revealed by an

TABLE 2. Comparison of ^{1}H-nmr Spectral Data of Macrocyclic Diterpenes **1** and **2**, (300 MHz) and **7** and **8** (360 MHz).[a]

Proton	1[b]	2[b]	7	8
H-1	4.11 (dd, $J=9$, 6.6)	5.22 (dd, $J=11.8$, 8.7)	2.44 (dd, $J=12$, 2.5)	2.46 (dd, $J=12$, 3)
			2.01 (m)	2.15 (m)
H-3	5.17 (d, $J=9.6$)	5.38 (dq, $J=9.3$, 1.8)	5.11 (br d)	5.40 (d, $J=10$)
H-4	5.25 (d, $J=9.6$)	6.12 (d, $J=9.3$)	5.22 (d, $J=10$)	6.24 (br d)
H-7	6.28 (d, $J=9.3$)	6.26 (d, $J=9.3$)	6.25 (d, $J=10$)	6.24 (br d)
H-8	1.53 (dd, $J=8.1$, 9.3)	1.52 (dd, $J=8.4$, 9.3)	1.51 (dd, $J=10$, 8)	1.54 (dd, $J=10$, 8)
H-9	1.25–1.16 (m)	1.26–1.10 (m)	1.11 (m)	1.13 (m)
H-10	1.25–1.16 (m)	1.25–1.00 (m)		2.10 (m)
	0.85–0.70 (m)		0.83 (m)	0.91 (m)
H-11	2.44–2.03 (m)	2.24–2.00 (m)	2.25 (m)	2.44 (m)
	1.80–1.60 (m)	1.75–1.60 (m)	1.87 (m)	1.83 (m)
H-13	4.68 (dd, $J=6.3$)	4.48 (m)	5.11 (br d)	5.08 (d, $J=10$)
H-14	2.29 (m)	2.31 (m)	4.18 (m)	5.29 (m)
H-16	1.01 (s)	1.07 (s)	0.96 (s)	1.04 (s)
H-17	1.16 (s)	1.14 (s)	1.14 (s)	1.18 (s)
H-18	1.59 (br s)	1.54 (br s)	1.70 (br s)	1.76 (br s)
H-19	1.70 (br s)	1.65 (br s)	1.76 (d, $J=1.5$)	1.78 (d, $J=1.3$)
H-20	1.90 (s)	1.88 (s)	1.87 (d, $J=0.7$)	1.90 (s)
OAc	—	2.08 (s)	—	1.99 (s)
	—	2.09 (s)	—	2.14 (s)

[a]Chemical shifts in δ (ppm), J in Hz, and TMS as internal standard.

[b]Homonuclear ^{1}H, ^{1}H-COSY 2D-nmr and heteronuclear ^{13}C, ^{1}H-COSY 2D-nmr spectra were also used for these assignments.

APT experiment, were assigned to C-2, C-12, and C-13, respectively (Table 1).

Confirmatory evidence for the other protonated carbons of the fourteen-membered ring was obtained from the heteronuclear ^{13}C, ^{1}H (^{1}J) two-dimensional chemical shifts (^{13}C, ^{1}H-COSY 2D nmr). The ^{1}H-nmr spectra assignments of **1** and **2**, including the spin-spin interactions, were deduced by homonuclear ^{1}H,^{1}H-COSY 2D nmr (Table 2). These techniques allowed an unambiguous assignment of the ^{1}H- and ^{13}C-nmr spectra of **2** (Tables 1 and 2). Comparative analysis of the ^{13}C-nmr spectra of **1** and **2** revealed the change of the signal C-14 (δ_C 31.55 and 29.47, respectively) in agreement with the expectation. Additional analysis of the ^{13}C-nmr shifts of the methyl carbons C-20 (δ_C 11.97), C-19 (δ_C 10.10), C-18 (δ_C 15.32), of methylene carbon C-11 (δ_C 39.77), and of methine carbon C-4 (δ_C 67.7) allowed us to postulate the stereochemistry of the three double bonds at the 2, 6 and 12 positions as all *E* (4,5).

The relative configuration of the chiral carbons of **1** was assigned on the basis of comparison of ^{1}H- and ^{13}C-nmr spectral data for **1** and **2** with data for model compounds **7** and **8** (5). The relative configuration of these models was established by analysis of the X-ray crystallographic parameters of **7** (5). Thus, the chemical shift and coupling constant data of H-3, H-4, H-7, H-8, and H-9 of **1** vs. **7** and **2** vs. **8** are closely similar (Table 2). These spectral data are consistent with similar stereochemistries of the corresponding chiral carbons (C-4, C-8, and C-9) and double bonds (Δ^2-*E* and Δ^6-*E*) in these compounds. The comparison of the chemical shifts of C-4 to C-9 and C-15 to C-17 to those of model compounds helped with this deduction (Table 1). Inspection of Dreiding models indicates that these carbons in **1** and **7** may adopt conformations with approximately identical dihedral angles, independent of the *cis*-substituted cyclopropyl ring located at β (compound **1**) or α (compound **7**) orientations. The chemical shifts of C-10 (δ_C 25.31) and C-11 (δ_C 39.77) of **1**, each shifted upfield by 2.42 and 8.07 ppm (to δ_C 22.89 and 31.70, respectively) in **7** (5), suggested that the stereochemistry of the cyclopropane moiety of **1** has an antipodal relationship to that of **7**, due to the δ effect of the methyl group at C-15 and the quaternary C-15 carbons on the signals for C-10 and C-11, respectively. The difference observed in the chemical shifts of C-10 between **1** and **7** ($\Delta\delta = 8.07$ ppm) cannot be justified only by *E* (compound **1**) and *Z* (compound **7**) isomerism of the double bond at C-12. Additional significant differences between the ^{1}H- and ^{13}C-nmr spectra of these compounds were observed in the ^{1}H and ^{13}C signals of the remaining carbons C-3 to C-10, C-18, and C-19 (Tables 1 and 2), as anticipated by the stereochemistry of the double bond at C-12 (*E* for **1** and *Z* for **7**) and the localization of the hydroxyl group (1-OH for **1** and 14-OH for **7**). The analogous allylic position and the chemical shifts of the carbinolic protons H-1 (**1** δ_H 4.11) and H-14 (**7** δ_H 4.18) were used to determine the stereochemistry of the hydroxyl group at C-1 as β (compound **1**).

These data led to the deduction of structure **1** (relative configuration) for the new diterpene isolated from *C. nepetaefolius*. Confirmation of structure **1** was obtained by an X-ray structure determination of the acetate **2**.[2]

Casbane-type diterpenes have only been found in four species belonging to three genera of the family Euphorbiaceae, namely, *Ricinus communis, Agrostistachys hookeri* (4,5), *Croton nitens* (6)

[2]Personal communication from Dr. Arthur G. Schultz (Department of Chemistry, Rensselaer Polytechnic Institute Troy, New York 12180-3590, USA). Details of the X-ray structural elucidation may be obtained directly from Dr. Schultz.

and *C. nepetaefolius* (present work). The diterpenes **7**, **9**, and **10**, with Δ^{12}-*Z*-stereochemistry and 4,14-dioxygenated, and compound **11**, with Δ^{13}-*E*-stereochemistry and 4,12-dioxygenated, were isolated from *A. hookeri* (4,5). In the other species these compounds have Δ^{12}-*E*-stereochemistry (compounds **1** and **12**) and are 1,4-dioxygenated (compounds **1** and **6**). These structural differences are easily recognized by analysis of ^{1}H- and ^{13}C-nmr spectral data (Table 1). The biogenetic significance of these compounds has been commented on previously (4,5).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot plate and are uncorrected. The ir spectra were recorded as KBr discs on a Perkin-Elmer 720 model spectrometer. ^{1}H nmr and ^{13}C nmr were determined in $CDCl_3$ on Varian EM 360 (^{1}H 60 MHz) or XL-100 (^{1}H 100 MHz, ^{13}C 25.2 MHz) or VXR-300 (^{1}H 300 MHz, ^{13}C 75 MHz) spectrometers; TMS was used as an internal standard; chemical shifts are reported in δ ppm units and coupling constants (J) in Hz. Mass spectra were obtained with a Micro Mass 12 or Hewlett Packard 55954 spectrometer, operating at 70 eV. Si gel was used for tlc plates (0.2 mm thick) and cc.

PLANT MATERIAL.—A specimen of *C. nepetaefolius* was collected in Cocalzinho-Viçosa-Ceará, Brazil, and identified by Afrânio Fernandes (Departamento de Biologia, Universidade Federal do Ceará, Fortaleza). A voucher specimen (number 3163) of the plant is kept at the Herbarium of the Department of Biology of the University.

EXTRACTION AND ISOLATION.—Dried and pulverized material of the stem (2.6 kg) of *C. nepetaefolius* was extracted successively with hexane and EtOH at room temperature. Removal of solvents in vacuo yielded 45.4 g (yellow oil) and 65.4 g, respectively. A portion (30.4 g) of the hexane fraction was chromatographed on a Si gel (600 g) column, and four fractions were collected: A (1.1 g), B (16.1 g), C (11.0 g), and D (2.1 g), eluted with hexane, $CHCl_3$, EtOAc, and MeOH, respectively. Fraction B (0.3 g) was rechromatographed on preparative tlc on Si gel and yielded **3** (0.05 g) and **4** (0.08 g). Fraction C (9.0 g) was further chromatographed on a Si gel column. The $CHCl_3$/Me_2CO (50%) eluate (3.34 g) on preparative tlc separation afforded **1** (0.155 g). The residue of the EtOH extract (65.4 g) was mixed with sand washed with H_2O and extracted with Et_2O. After removal of solvent, the residue (39.6 g) was chromatographed over a column of Si gel. Fractions 2–8 and 14–19, eluted with hexane/$CHCl_3$ (10%) and hexane/$CHCl_3$ (50%), were purified by preparative tlc on Si gel (60 HF) to give two diterpenes that were designated Ct-7V (0.07 g) and Ct-6V (0.063 g), respectively; fractions 20–27, eluted with $CHCl_3$/EtOAc (20%) were rechromatographed over a column of Si gel and eluted with $CHCl_3$/EtOAc (50%) to afford **1** (1.436 g); the other fractions (28–55) were acetylated with Ac_2O and pyridine in the usual manner and chromatographed over a column of Si gel. The two fractions eluted with hexane/$CHCl_3$ (50%) and $CHCl_3$ furnished Ct-8Ac (0.19 g) and sucrose octaacetate (0.145 g), respectively. The work of structure elucidation of Ct-6V, Ct-7V, and Ct-8VAc is proceeding.

CASBENE DITERPENOID **1**.—Oil; ir ν max (neat) 3400, 1690, 1610, 1020 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 318 (3%), 300 (10%), 282 (3%), 218 (4%), 150 (25%), 136 (54%), 123 (56%), 121 (61%), 109 (100%), 107 (85%), 95 (76%).

ACETYLATION OF **1**.—Compound **1** (0.473 g) was treated overnight with Ac_2O (8 ml) and pyridine (4 ml) at room temperature, and the reaction mixture was worked up as usual to give the diacetate **2**: colorless derivative after crystallization from hexane/MeOH; mp 149–151°; ir ν max (KBr) 1730, 1650, 1620, 1230, 1020 cm^{-1}; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 402 (3%), 342 (29%), 282 (55%), 218 (18%), 165 (22%), 150 (72%), 138 (24%), 136 (58%), 135 (66%), 121 (72%), 109 (65%), 107 (100%).

IDENTIFICATION OF ELEMICIN [**3**].—Oil; ir, ^{1}H-nmr, and eims data are in agreement with literature values (7).

IDENTIFICATION OF 2-HYDROXY-4,6-DIMETHOXYACETOPHENONE [**4**].—Mp, ir, and ^{1}H-nmr data are in agreement with literature values (3).

2-*O*-ACETYL-4,6-DIMETHOXYACETOPHENONE [**5**].—Compound **4** (0.07 g) was treated overnight with Ac_2O (1 ml) and pyridine (1 ml) at room temperature, and the reaction mixture was worked up as usual to furnish the monoacetate **5**: ^{1}H nmr ($CDCl_3$) δ 6.32 (1H, d, J = 2.0, H-3), 6.20 (1H, d, J = 2.0, H-5), 3.78 (3H, s, OMe), 3.74 (3H, s, OMe), 2.45 (3H, s, Ac), 2.21 (3H, s, Ac); eims (rel. int.) *m/z* $[M]^+$ 238 (8%), 196 (18%), 181 (100%), 151 (3%).

SUCROSE OCTAACETATE.—Oil; spectral data, including ^{13}C nmr, are in agreement with literature values (8).

ACKNOWLEDGMENTS

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