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THREE FURANO-DITERPENES FROM THE BARK OF *CROTON CAMPESTRIS*

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Key Word Index—*Croton campestris*; Euphorbiaceae; furano-clerodanes; diterpenes; velamone; velamolone; velamolone acetate.

Abstract—Three furano-diterpenes have been isolated from the dichloromethane extract of *Croton campestris* St Hil. roots. Their structures have been established by spectroscopic methods. The compounds were named velamone (*ent*-15,16-epoxy-2-oxo-3,13(16),14-clerodatrien), velamolone (*ent*-15,16-epoxy-20-hydroxy-2-oxo-3,13(16),14-clerodatrien) and velamolone acetate (*ent*-15,16-epoxy-20-acetoxy-2-oxo-3,13(16),14-clerodatrien).

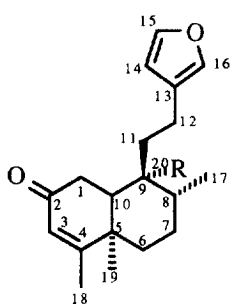
INTRODUCTION

The use of natural products in the treatment of diseases has been an important component of medical therapy for centuries [1]. Plants of the Euphorbiaceae family have been used in many countries as a folk remedy, and claimed to be effective for the treatment of a variety of illnesses, amongst which are wounds [2], cancers [3], inflammation and infection [4]. References to their uses have appeared in the literature of many countries [5]. In Brazilian folk medicine, preparations for external and internal uses containing extracts from the root cortices of *Croton campestris*, called "Velamo do campo", are used as a potent purgative, and to drain and to treat the syphilis. They have been successfully employed in the treatment of bile duct infections [6–9].

The present investigation was carried out to study the diterpenes occurring as major constituents in the dichloromethane extract of *C. campestris* roots. Previously, only two alkaloids were isolated from this species [10]. In this paper, we report on the structural elucidation of three clerodanes (**1**–**3**) on the basis of their spectral data.

RESULTS AND DISCUSSION

Repeated silica gel columns chromatography of the dichloromethane extract (bark), using a mixture of toluene-EtOAc as eluent, allowed the isolation of three new diterpenes in the form of clear yellowish resins. These diterpenes, with a furanoid clerodane skeleton were called velamone (**1**), velamolone (**2**) and velamolone acetate (**3**).



1 R = Me
2 R = CH_2OH
3 R = CH_2OAc

Velamone (**1**) on CI mass spectroscopy (reagent gas: CH_4) exhibited a base peak $[M+H]^+$ at *m/z* 301 indicative of the formula $C_{20}H_{28}O_2$ and gave rise to fragmentation ions at *m/z* 329 $[M+C_2H_5]^+$ and 341 $[M+C_3H_5]^+$. This formula was confirmed by the ^1H and ^{13}C NMR data (Table 1).

The IR spectrum of (**1**) showed a weak hydroxyl absorption band at 3423 cm^{-1}. Peaks at 2921, 1595 and 874 cm^{-1} suggested the presence of a furan ring system [11–13]. Peaks at 1671 and 1725 cm^{-1} revealed, respectively, a double bond (C = C) and a conjugated ketone (C = O).

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Table 1. ^{13}C NMR (CD_3OD, 100.62 MHz) and ^1H NMR (CD_3OD, 400.31 MHz) data for compounds 1–2*$^{\Delta}$

		Compound 1				Compound 2				
	C	$\delta(^{13}C)$	$\delta(^1H)$		HMBC°		$\delta(^{13}C)$	$\delta(^1H)$		HMBC°
1	CH_2	35.8	2.33 (14.1) 2.45 (3.5)	AB*d* (17.6)	(H-10)	1	37.7	2.47 (3.0) 2.88 (14.4)	AB*d* (17.8)	(H-3) (H-10)
2	C = O	203.0	—	—	(H-10) (H-1)	2	203.6	—	—	(H-1) (H-18)
3	CH	126.5	5.72 (1.3)	—	(H-18)	3	125.6	5.71 (1.3)	*d*	(H-18)
4	Cq	176.4	—	—	(H-18) (H-19)	4	176.3	—	—	(H-19) (H-18)
5	Cq	41.3	—	—	(H-1) (H-3) (H-6) (H-10) (H-18) (H-19)	5	41.4	—	—	(H-1) (H-3) (H-18) (H-19)
6	CH_2	36.6	1.43 (12.5) (4) 1.89 (12.5) (1.3)	*m*	(H-19)	6	37.1	1.44 (13) 1.66	*ddd*	(H-19)
7	CH_2	27.9	1.55	*m*	(H-6) (H-8) (H-17)	7	28.0	1.55	*m*	(H-17)
8	CH	37.2	1.63 (6.3)	*m*	(H-7) (H-17) (H-20)	8	37.0	1.67	*m*	(H-17) (H-20)
9	Cq	39.9	—	—	(H-10) (H-17) (H-7) (H-20) (H-11)	9	43.8	—	—	(H-11) (H-17) (H-20)
10	CH	47.1	2.01 (14.1) (3.5)	*dd*	(H-1) (H-7) (H-19) (H-20)	10	47.2	2.02 (14.3) (3)	*m*	(H-1) (H-19) (H-20)
11	CH_2	39.4	1.56 (9.4)	*m*	(H-12) (H-20)	11	33.4	1.74 (4.5) 1.92	AB*d* (12.5)	(H-12) (H-20)
12	CH_2	18.8	2.1 (9.4) 2.3 (14.0)	—	(H-11)	12	18.4	2.17 2.36 (4.5)	*m* (14)	(H-11)
13	Cq	125.7	—	—	(H-12) (H-14) (H-15) (H-16)	13	126.6	—	—	(H-12) (H-14) (H-15) (H-16)
14	CH	111.8	6.27 (1.8) (0.9)	*dd*	(H-15) (H-16) (H-12)	14	111.9	6.32 (1.6) (0.8)	*dd*	(H-12) (H-16)
15	CH	144.0	7.36 (1.7)	*t*	(H-14) (H-16)	15	144.0	7.38 (1.6)	*dd*	(H-14) (H-16)
16	CH	139.7	7.25 (1.7) (0.9)	*m*	(H-12) (H-14) (H-15)	16	139.8	7.29 (1.6) (0.9)	*dd*	(H-12) (H-14) (H-15)
17	CH_3	16.1	0.88 (6.3)	*d*	(H-8)	17	16.8	0.92(6.5)	*d*	—
18	CH_3	19.2	1.94 (1.3)	*d*	(H-3)	18	19.3	1.95 (1.3)	*d*	(H-3)
19	CH_3	18.6	1.16	*s*	(H-10)	19	18.1	1.25	*s*	
20	CH_3	18.2	0.85	*s*	(H-8) (H-11)	20 CH_2O H	65.5	3.59 (11.7) 3.90	AB	(H-11)

s = singlet *t* = triplet *d* = doublet *m* = multiplet

* = δ in ppm from TMS as int. standard

Δ = carbon-hydrogen connectivity established by HMQC methods

° = observed correlation by HMBC

The skeleton was established by the NMR spectra (Table 1). The ^{13}C *J*-modulated spectrum indicated that 20 carbons are present in the molecule as four methyls, five methylenes, six methines and five quaternary carbons. Characteristic NMR signals for a furan ring were present in the ^1H and ^{13}C NMR spectra (δ ^1H 6.27 *dd*, 7.36 *t*, 7.25 *m* and δ ^{13}C 118.8, 144.0, 139.7 and 125.7 C*q*). Considering the quaternary carbons (δ 41.3 and 39.9), the CH group (δ 47.1) and their heteronuclear correlations, we could assign these signals respectively to C-5, C-9 and C-10 of a decalin moiety. The most deshielded carbon of the spectrum (δ 203.0) correlated in the HMBC with H-1 and H-10: its chemical shift agreed with a ketone that we could locate on position 2; this fact justified that protons H-1 (δ 2.33 and 2.45, 2H, AB*d*, J_{AB} = 17.6 Hz, $J_{H_{1A}-H_{1B}}$ = 14.1 Hz and $J_{H_{1B}-H_{10}}$ = 3.5 Hz) were deshielded. The signals (3H) observed at δ_H 0.88, 1.94, 1.16 and 0.85 were assigned respectively to methyl groups 17, 18, 19 and 20, characteristic of clerodanes [14]. The protons of Me-18 were weakly coupled to H-3 ($^4J_{H_{18}-H_3}$ = 1.3 Hz) through the C-3 = C-4 double bond. The correlations observed between Me-18 and H-3 in ^1H-^1H COSY experiments (data not shown) confirmed this result. A Homonuclear NOESY experiment showed that the methyls 17, 19 and 20 were on the same side of the *trans* decalin.

The stereochemistry of velamone was established by using the CD exciton chirality method [15]. **1** showed a negative first and a positive second Cotton effect ($\Delta\varepsilon_{346}-0.71$, $\Delta\varepsilon_{243}+2.51$) that corroborated the *ent*-clerodane configuration [16–18].

Velamolone (**2**) on CI mass spectroscopy (reagent gas: CH_4) exhibited a base peak $[M+H]^+$ at *m/z* 317 indicative of a formula $C_{20}H_{28}O_3$.

The ^1H and ^{13}C NMR data of velamolone were almost identical with those of velamone (Table 1). The ^{13}C NMR *J*-modulated spectrum of compound **2** confirmed the presence of 20 carbons in the molecule but only three methyls. In the HMBC spectrum, the protons and carbons of the methylene groups (δ_C 65.5, δ 3.59 and 3.90, 2H, AB, $J = 11.7$ Hz) showed correlation with C-11 and H-11. The structure of compound **2** followed that of the compound **1** by substitution of a methyl group by an hydroxymethyl located on C-9, which is deshielded.

Velamolone acetate (**3**) gave rise to NMR data which were very close to those of velamone (Table 2), except for the deshielded C-9 ($\Delta_\delta+2.8$ ppm) and for the CH_2 on position 11, shielded on ^{13}C ($\Delta_\delta-5.5$ ppm) and deshielded on ^1H ($\Delta_\delta+0.3$ ppm) NMR. These differences came from the substitution of Me-20 in compound **1** by CH_2-O-Ac in compound **3**. The C-H correlations observed on HMBC confirmed this result. Protons H-20 correlated with C-8, C-10, C-11 and C-21 (all ^3J) and C-9 (^2J); then we noted the correlations C-20-H-11 (^3J) and C-21-H-22 (^2J).

The chemical shift assignments agreed quite closely with the reported values for diterpenes with a furanoid clerodane skeleton [19–21] and with the calculated ones [Chemintosh 3.4.4. Softshell international Ltd].

EXPERIMENTAL

General

IR: EtOH KBr; MS: NERMAG R-10-10 by DCI with CH_4; UV: EtOH; CD: soln; NMR: Bruker ARX400 spectrometer (400.13 MHz: ^1H and 100.62 MHz: ^{13}C) in FT mode. Phase sensitive proton detected one bond ($J = 156$ Hz) ^1H-^{13}C shift correlated (HMQC) spectrum: for the acquisition, 56 scans were made for each of 512 transients. Proton detected multiple bond ($J = 8$ Hz) ^1H-^{13}C shift correlated (HMBC) spectrum: for the acquisition, 48 scans were made for each of 512 transients. In every case, processing was done with cosine square filters in both dimensions and the final matrix had dimensions 1K × 1K.

Plant material

The root barks of *Croton campestris* were collected in São Paulo (Brazil) in March 1995. Voucher specimens of roots were identified by Professor Fourasté Isabelle and deposited at the herbarium of the Pharmacognosy Laboratory, Faculty of Pharmacy (Toulouse) with the number 95/024.

Extraction, fractionation and isolation

The air-dried powdered barks (1 kg) of *C. campestris* were extracted with CH_2Cl_2 (15 l) at room temp. for 2 days. Evpn of the solvents in vacuum gave 50 g of syrupy residue. The CH_2Cl_2-soluble fraction was hydrodistilled in a Clevenger-type apparatus [22] for 4 hr when a colourless oil with a warm and sweet aroma was obtained in a yield of 0,12%.

The aq. mixture with the $CHCl_2$ extract gave an emulsion which was again extracted with CH_2Cl_2 and evapd under a low pres. to dryness (49.5g of syrupy residue). A 2.5 g portion of this extract was dissolved in 6 ml of toluene and loaded on the top of a medium pres. (10 bars) silica gel column [Silica 60 Å, CC chromagel SDS, 6-35 μm; (230 × 26 mm); Büchi apparatus, pump ref. 988].

Elution begun first with toluene and continued with toluene-EtOAc-MeOH mixts (19:1:0 to 40:9:1). 40 fractions of 30 ml each were collected. Fractions were visualized on a silica Merck F_{254} TLC developed with toluene-EtOAc-MeOH (40:9:1), under UV 254 nm and by spraying 1% of 4-dimethylaminobenzaldehyde in EtOH soln ($R_f = 0.8$ (**1**) 0.6 (**2**) and 0.35 (**3**)) and combined into pools according to their similar TLC patterns; we obtained three parts [1 = 2 to 5; 2 = 13 to 18; 3 = 34 to 37]. Each portion enriched with furano diterpenes (Ehrlich positive test) was subjected to an other medium pressure (15 bars) silica column (230 × 15 mm) eluting for each fraction with mixture of toluene-EtOAc: 97:3 (F_{2-5}), 95:5 (F_{13-18}) and 85:15 (F_{34-37}). Final purification allowed isolation of compounds **1**, **2** and **3** and was performed using prep. TLC developed with hexane-EtOAc (9:1; 7:3; 1:1 respectively).

ent-15,16-*epoxy*-2-*oxo*-3,13(16),14-*clerodatrien* (**1**)

$C_{20}H_{28}O_2$. Clear yellowish resin (30 mg). UV λmax EtOH nm (ε): 325 (42), 240 (7869), CD (EtOH) $\Delta_{\varepsilon 316}-0.71$, $\Delta_{\varepsilon 243}+2.51$; IR, ^1H and ^{13}C NMR: see text; CI-MS (reagent gas: CH_4) *m/z* (rel. int.): $[M+H]^+$ 301 (100), 109 (3), 123 (2), 149 (4), 205 (6), 257 (3), 285 (4), 302 (24), 329 (20), 341 (4).

ent-15,16-*epoxy*-20-*hydroxy*-2-*oxo*-3,13(16),14-*clerodatrien* (**2**)

$C_{20}H_{28}O_3$. Clear yellowish resin (25 mg). UV λmax EtOH nm (ε): 298 (146), 240 (16666); CD (EtOH) $\Delta_{\varepsilon 299}-0.48$, $\Delta_{\varepsilon 244}+2.72$; IR, ^1H and ^{13}C NMR: see text; CI-MS (reagent gas: CH_4) *m/z* (rel. int.): $[M+H]^+$ 317 (100), 109 (13), 203 (6), 235 (2), 349 (3), 429 (1).

Table 2. ^{13}C NMR ($CDCl_3$, 100.62 MHz) and ^{1}H NMR ($CDCl_3$, 400.13 MHz) data for compounds 1–3*[Δ]

	Compound 1				Compound 3				
	C	δ (^{13}C)	δ (^{1}H)		C	δ (^{13}C)	δ (^{1}H)		HMBC[o]
1	CH_2	35.0	2.36 (9.0)	*d*	1	36.6	2.52 (3.5) (17.6) 2.6 (14)	AB*d*	—
2	C = O	200.3	—	—	2	200.1	—	—	(H-1)
3	CH	125.5	5.69 (1.3)	*d*	3	125.7	5.71	—	(H-18)
4	Cq	172.6	—	—	4	171.9	—	—	(H-19) (H-18)
5	Cq	39.9	—	—	5	40.0	—	—	(H-1) (H-3) (H-6) (H-18) (H-19)
6	CH_2	35.6	1.34 (3.0) (12.0) 1.80 (3.0)		6	36.0	1.36 (13) 1.70.(12) (5)	*m* *td*	(H-19)
7	CH_2	26.9	1.5	*m*	7	26.9	1.56 1.68	*m* *m*	(H-17)
8	CH	36.0	1.56	*m*	8	36.2	1.56 (6.7)	*m*	(H-17) (H-20)
9	Cq	38.8	—	—	9	41.6	—	—	(H-17) (H-20)
10	CH	45.7	1.92 (9.0)	*dd*	10	45.9	2.02 (14) (3.5)	*m*	(H-1) (H-19) (H-20)
11	CH_2	38.0	1.5	*m*	11	32.5	1.70 1.86	*m*	(H-12) (H-17) (H-20)
12	CH_2	17.9	2.13 (6.5) (14.0) 2.26 (6.0)	AB*dd*	12	17.7	2.18 (14) (5) 2.30 (4.5)	AB*d*	—
13	Cq	125.0	—	—	13	124.8	—	—	(H-12) (H-15) (H-16)
14	CH	110.9	6.18 (0.8) (1.7)	*dd*	14	111.0	6.22 (09) (1.7)	*dd*	(H-12) (H-16)
15	CH	142.8	7.28 (1.6)	*t*	15	143.0	7.31 (1.7)	*dd*	(H-14) (H-16)
16	CH	138.5	7.14 (0.8) (1.6)	*m*	16	138.8	7.18 (0.9) (1.7)	*dd*	(H-12) (H-14) (H-15)
17	CH_3	15.8	0.82 (6.0)	*d*	17	16.5	0.90 (6.7)	*d*	(H-8)
18	CH_3	19.3	1.85 (1.3)	*d*	18	19.2	1.87 (1.2)	*d*	(H-3)
19	CH_3	18.4	1.08	*s*	19	17.6	1.12	*s*	(H-6)
20	CH_3	17.8	0.78	*s*	20	67.5	4.08 4.25 (20)	AB	(H-11)
					21 O-C = O	171.2	—	—	(H-20) (H-22)
					22 CH_3	21.3	2.02	*s*	—

s = singlet *t* = triplet *d* = doublet *m* = multiplet
* = δ in ppm from TMS as int. standard
Δ = carbon-hydrogen connectivity established by HMQC methods
° = observed correlation by HMBC methods

ent-15,16-*epoxy*-20-*acetoxy*-2-*oxo*-3,13(16),14-*clerodatrien* (**3**)

$C_{22}H_{30}O_4$. Clear yellowish resin (20 mg). UV λmax EtOH nm (ε): 320 (1127), 225 (28571); CD (EtOH) $\Delta_{\varepsilon\ 329}-1.38$, $\Delta_{\varepsilon\ 243}+4.10$: 309; IR, ^{1}H and ^{13}C NMR: see text; CI-MS (reagent gas: CH_4) *m/z* (rel. int.): $[M+H]^+$ 359 (85), 301 (39), 317 (24), 331 (12), 349 (16), 375 (44), 391 (100), 409 (32), 419 (15), 431 (5).

REFERENCES

1. Hartewell, J. L., *Lloydia*, 1969, **32**, 156.
2. Zheng-Pin, C., Cai, Y. and Phillipson, J. D. *Planta Medica*, 1994, **60**, 541.
3. Rizk, A. F. M. *Botanical Journal of Linnean Society*, 1987, **94**, 293.
4. Bettolo, R. M. and Scarpati, M. L. *Phytochemistry*, 1979, **18**, 520.
5. Kupchan, S. M., Vohida, I., Branfman, A. R., Dailey, R. G. J. R. and Fei, B. Y. *Science*, 1976, **191**, 571.
6. Pio Correa, M. Diccionário das plantas úteis do Brasil e das exóticas cultivadas, Vol. VI. Rio de Janeiro, Impressa Nacional, 1926, p. 399.
7. Penna, M. Diccionário brasileiro das plantas

medicinais, Plantas brasilieras, 2ᵉ édisão, 1930, 120.
8. Balbach, A. Flora nacional na medicina, Plantas medicinais, 8ᵉ edisão, 1934, 843.
9. Grange, Père A. Plantes médicinales de la flore amazonienne, Poconéol nº1, 6, 21, 25, 101, 1983.
10. Ribeiro Prata, E. M., Paulo, M. Q. and Souza Parito, A. R. M., *Review Bras. Farm.*, 1993, **74** (2), 3641.
11. Hideji, I., Ichiara, Y., Kogima, H., Watamabe, K. and Takeya, Y. *Phytochemistry*, 1989, **28**, 1140.
12. Mc Chesney, J. P. and Clark, A. M. *Journal of Natural Products*, 1991. **54**(6), 1625.
13. Krebs, H. and Ramiarantsoa, H, *Phytochemistry*, 1995, **41**(2), 561.
14. Moulis, C, Déterminations structurales de terpénoïdes de *Croton levatii* Guill.-Thèse Nº 1876-Toulouse, France, 1994.
15. Harada, N. and Nakanishi, K., Circurlar Dichroic Spectroscopy Exciton Coupling in Organic Stereochemistry. University Sciences Books, Mill Valley, CA, 1983.
16. Hussein, A. A., C. De La Torre, M., Jimeno, M.-L., Rodriguez, B., Bruno, M., Piozzi, F. and Servettaz, O. *Phytochemistry*, 1996, **43**(4), 835.
17. C. De La Torre, M., Rodriguez, B., Bruno, M., Piozzi, F., Savona, G., Vassallo, N. and Servettaz, O., *Phytochemistry*, 1994, **38**(1), 181.
18. Rogers, D., Unal, G. G., Wiliams, D. J., Ley, S. V., Sim, G. A., Joshi, B. S. and Ravindranath, K. R., *Journal of the Chemical Society, Chemical Communication*, 1979, 97.
19. Mc Chesney, J. D. and Silveira, E. R. *Phytochemistry*, 1989, **28**(12), 3411.
20. Monti, H., Tiliacos, N. and Faure, R. *Phytochemistry*, 1995, **42**(6), 1653.
21. Cai, Y., Chen, P. N. and Phillipson, J. D., *Phytochemistry*, 1992, **34**(1), 265.
22. Pharmacopée Européenne, 3ᵉ édition, Edition du Conseil de l'Europe-Jouve Paris (2.8.12) 121. 1997.