

## BOETICOL, A NEW TETRACYCLIC TRITERPENE FROM *EUPHORBIA BOETICA*

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**ABSTRACT.**—Boeticol [**1**], a new tetracyclic triterpene, was isolated from the acetone extract of *Euphorbia boetica*. Its structure was established as 19(10→9)-*abeo*-8 $\alpha$ ,9 $\beta$ ,10 $\alpha$ -eupha-5,24-dien-3 $\beta$ -ol, based on nmr spectroscopic data obtained using a combination of one- and two-dimensional techniques. The known triterpene euphol was also isolated.

*Euphorbia boetica* Boiss. (Euphorbiaceae), an herb endemic to Europe, is commonly encountered in the southern regions of Portugal. It produces a large amount of latex which has an irritant effect on the skin and mucous membranes.

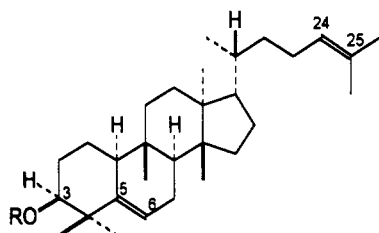
In the present study, a Me<sub>2</sub>CO extract of the air-dried powdered whole plant has been investigated. The crude extract was saponified and the non-saponifiable part was chromatographed on a Si gel column. A further fractionation yielded both a new tetracyclic triterpene, named boeticol [**1**], which was established as 19(10→9)-*abeo*-8 $\alpha$ ,9 $\beta$ ,10 $\alpha$ -eupha-5,24-dien-3 $\beta$ -ol, and the known compound euphol, which was identified by comparison of its spectroscopic data with those described in the literature (1).

The molecular formula of boeticol [**1**] (C<sub>30</sub>H<sub>50</sub>O) was deduced by mass spectrometry (molecular ion peak at *m/z* 426) and elemental analysis. Its <sup>1</sup>H-nmr spec-

trum showed the presence of five tertiary methyl groups ( $\delta$  0.80, 0.83, 0.87, 1.05, and 1.14), one secondary methyl group ( $\delta$  0.85, d, *J* = 6.3 Hz), two methyl groups attached to an sp<sup>2</sup> carbon ( $\delta$  1.60 and 1.68), two olefinic protons ( $\delta$  5.09, m, *J* = 6.5 Hz and 1.4 Hz;  $\delta$  5.63, d, *J* = 6.0 Hz), and a proton attached to C-3 as a narrow triplet ( $\delta$  3.46, *J* = 2.9 Hz) characteristic of an equatorial position with equal couplings to the H-2 protons (2,3).

The signal at  $\delta$  5.63 in the <sup>1</sup>H-nmr spectrum of **1** was ascribed to the olefinic proton at C-6. This assignment was based on the observed fragmentation pattern in the mass spectrum, with strong peaks at *m/z* 274 (63%), 259 (50%), and 134 (80%), resulting from retro-Diels-Alder cleavage in ring B, typical of triterpenoids with C-5, C-6 unsaturation (4). The <sup>13</sup>C-nmr spectrum, with signals at  $\delta$  121.86 and 142.01, confirmed the location of this double bond (5). The other olefinic proton was assigned to H-24 based on the <sup>1</sup>H- and <sup>13</sup>C-nmr spectra of **1**. The fragment at *m/z* 313 [*M* - side-chain - 2H]<sup>+</sup> confirmed the existence of a C-8 side-chain containing one double bond (6). The long-range selective INEPT, COSY, HMQC, and NOESY experiments revealed an *abeo*-euphol-type triterpene skeleton (7).

The assignment of chemical shifts of the side-chain carbons of **1** was based on literature data (8). Having assigned the



**1** R = H  
**1a** R = Ac

carbons of the side-chain, the chemical shifts of the attached protons were easily determined from the HMQC nmr spectrum (Table 1).

The methyl singlets of the skeleton of **1** at  $\delta$  1.05 and 1.14 were assigned to Me-4 $\alpha$  and Me-4 $\beta$  based on the differences observed after acetylation and by comparison with the values reported for 10 $\alpha$ -cucurbitadienol (**9**). The other three tertiary methyl groups were identified through the long-range selective INEPT, HMQC, and COSY nmr experiments performed on **1**.

By irradiating H-6 ( $\delta$  5.63), enhancements were observed at a quaternary carbon ( $\delta$  40.97), two methines ( $\delta$  49.47 and 44.57), and a methylene ( $\delta$  25.25). The quaternary and the methylenic carbons were assigned as C-4 and C-7, respectively. The lower field methine is correlated with a proton resonance at  $\delta$  2.05 and the other methine with a proton peak at  $\delta$  1.64. It was determined that the downfield methine was H-10, close to the  $\Delta^5$  double bond, while the methine at  $\delta$  1.64 was H-8. Irradiation of the methyl singlet at  $\delta$  0.83 gave responses at both methines, C-8 and C-10, at a quaternary carbon ( $\delta$  35.15), and at a methylenic carbon ( $\delta$  35.76). Hence, this methyl must be Me-9 while the quaternary and the methylenic carbons are C-9 and C-11, respectively. In the same way, excitation of the methyl singlet at  $\delta$  0.80 transferred polarization to the quaternary carbons at  $\delta$  46.12 and 47.43, the methine at  $\delta$  49.91, and the methylene at  $\delta$  30.34.

When the methyl singlet at  $\delta$  0.87 was irradiated, a signal which overlaps the Me-21 doublet at  $\delta$  0.85, responses were observed at the quaternary carbons at  $\delta$  46.12 and 47.43, at the methine C-8 ( $\delta$  44.57), at the methylene  $\delta$  34.20, at the side-chain carbons C-22 ( $\delta$  35.43) and C-20 ( $\delta$  35.22), and at the methine resonating at  $\delta$  49.91. The methine carbon at  $\delta$  49.91 is undoubtedly C-17 and was correlated by HMQC with H-17 at  $\delta$  1.53. Following the proton couplings in the D ring through the COSY spectrum, the methine proton H-17 was shown to be correlated with a methylenic proton H-16 at  $\delta$  1.31, which was further correlated with its geminal proton at  $\delta$  1.86 and with the pair of protons at C-15 ( $\delta$  1.11 and 1.20). This made the assignments of C-16, C-15, and consequently of Me-13 ( $\delta$  0.80), Me-14 ( $\delta$  0.87), and C-12, evident. The quaternary carbons C-13 ( $\delta$  46.12) and C-14 ( $\delta$  47.43) were attributed by comparison with the assignments reported for euphol (**8**) (Table 2). The complete assignment of the  $^1\text{H}$ -nmr spectrum of **1** was accomplished by means of the HMQC and COSY nmr experiments.

Boeticol [**1**] yielded a monoacetate [**1a**], which gave essentially the same  $^1\text{H}$ -nmr spectrum except for the signal due to H-3 ( $\delta$  4.69, t,  $J=2.9$  Hz) which was shifted to lower field. The  $^{13}\text{C}$ -nmr spectrum of this derivative was also very similar to that of **1** with small differences in the chemical shifts of ring A.

Examination of the NOESY spec-

TABLE 1.  $^1\text{H}$ -Nmr Chemical Shifts of Boeticol [**1**].

CH <sub>3</sub>	$\delta$ (ppm), J (Hz)	CH <sub>2</sub>	$\delta$ (ppm)	CH <sub>2</sub>	$\delta$ (ppm)	CH	$\delta$ (ppm), J (Hz)
4 $\alpha$ .....	1.05 s	1 $\alpha$ ....	1.46	12 $\beta$ ...	1.76	3 $\alpha$ ....	3.46, t, $J=2.9$
4 $\beta$ .....	1.14 s	1 $\beta$ ....	1.57	15 $\alpha$ ...	1.11	6 .....	5.63, d, $J=6.0$
9 $\beta$ .....	0.83 s	2 $\alpha$ ....	1.87	15 $\beta$ ...	1.20	8 $\alpha$ ....	1.64
13 $\alpha$ .....	0.80 s	2 $\beta$ ....	1.69	16 $\alpha$ ...	1.31	10 $\alpha$ ...	2.05
14 $\beta$ .....	0.87 s	7 $\alpha$ ....	1.66	16 $\beta$ ...	1.86	17 $\beta$ ...	1.53
21 .....	0.85, d, $J=6.3$	7 $\beta$ ....	2.07	22 $\alpha$ ...	1.12	20 .....	1.52
26 .....	1.68 br s	11 $\alpha$ ...	1.54	22 $\beta$ ...	1.57		
		11 $\beta$ ...	1.54	23 $\alpha$ ...	1.88		
27 .....	1.60 br s	12 $\alpha$ ...	1.54	23 $\beta$ ...	2.02	24 ....	5.09, m, $J=6.5, 1.4$

TABLE 2.  $^{13}\text{C}$ -Nmr Data for Compounds **1** and **1a**.

Carbon	Compound	
	<b>1</b>	<b>1a</b>
1	18.78	19.46
2	27.88	25.53
3	76.41	78.63
4	40.97	39.23
5	142.01	142.29
6	121.86	119.79
7	25.25	25.04
8	44.57	44.48
9	35.15	35.08
10	49.47	49.60
11	35.76	35.74
12	30.34	30.37
13	46.12	46.07
14	47.43	47.43
15	34.20	34.14
16	28.20	28.21
17	49.91	49.92
18	15.43	15.41
19	16.51	16.35
20	35.22	35.23
21	18.94	18.84
22	35.43	35.41
23	24.69	24.67
24	125.23	125.20
25	130.91	130.92
26	25.74	25.75
27	17.69	17.69
28	19.06	19.06
29	28.97	29.13
30	25.55	25.14
CH <sub>3</sub> CO-		21.24
CH <sub>3</sub> CO-		170.86

trum of **1** showed the necessary enhancements to probe the configuration at the various asymmetric centers of the molecule. The nOe enhancement of Me-4 $\beta$  (but not Me-4 $\alpha$ ) at H-6 ( $\delta$  5.63) indicated its equatorial orientation. In the same way, the correlation of H-3 ( $\delta$  3.46) with both methyls, Me-4 $\beta$  and Me-4 $\alpha$ , confirmed its equatorial position on the  $\alpha$ -face of the compound. Furthermore, the nOe enhancement of axial Me-4 $\alpha$  ( $\delta$  1.05) at H-10 ( $\delta$  2.05), indicated that this compound exhibits an  $\alpha$  configuration at C-10 characteristic of euferyl (**7**) and of its isomer, 10 $\alpha$ -cucurbitadienol (**9**).

The stereochemistry at the B,C and C,D ring junctions may be inferred from nOe enhancements observed from Me-9 and Me-14 to H-7 $\beta$  ( $\delta$  2.07) and from Me-13 to H-8 ( $\delta$  1.64). Thus, it was concluded that Me-9, Me-14, and H-7 $\beta$ , and Me-13 and H-8, respectively, have 1,3-diaxial relationships. This in turn, confirmed that H-8 and H-10 have an  $\alpha$ -configuration, while Me-14 is  $\beta$ . The equatorial orientation of H-7 $\alpha$  was confirmed by the nOe correlation between H-6 ( $\delta$  5.63) and this proton. The  $\alpha$  configuration of the side-chain at C-17 in euferyl is also retained in boeticol [**1**] as deduced from the nOe correlation observed between Me-14 $\beta$  and H-17.

The 20*R* stereochemistry (H-20 $\beta$ ) was deduced from the chemical shift of the doublet ( $\delta$  0.85) observed in the  $^1\text{H}$ -nmr spectrum of **1** for the Me-21 protons (10,11) which are, respectively, at 0.07 and 0.05 ppm higher field than the signal presented in the spectra of 10 $\alpha$ -cucurbitadienol and euferyl where the configuration at C-20 is inverted (H-20 $\alpha$ ). Figure 1 shows the relevant nOe correlations for **1**.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mp's were obtained on a Kofler apparatus. Ir spectra were determined on a Perkin-Elmer 1310 instrument.  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra were recorded on a Varian Unity-300 nmr spectrometer with TMS as internal standard and  $\text{CDCl}_3$  as solvent. The one-bond proton-carbon HMQC nmr spectrum was acquired with carbon decoupling, a delay in the BIRD pulse optimized for an average 135 Hz coupling and as 256 $\times$ 2 K complex points. The long-range delays in the selective INEPT nmr spectrum were optimized for 4 and 8 Hz and the soft pulses were generated by a DANTE sequence. The conventional COSY and the phase-sensitive NOESY experiments were run on a Varian Unity 500 nmr spectrometer. The NOESY spectrum was acquired with a mixing time of 1 sec. Both spectra were collected as 256 $\times$ 1 K complex points. Temperature was controlled at 26 $^\circ$  for all 2D-nmr experiments. Eims were taken at 70 eV on a Kratos MS25RF mass spectrometer.

PLANT MATERIAL.—The plant material was collected in July 1992 at Coruche, Portugal, and identified by Dr. Teresa Vasconcelos. A voucher

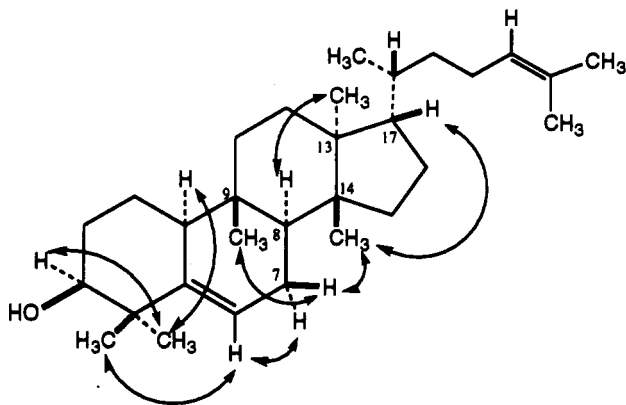


FIGURE 1. Relevant nOe correlations observed for **1**.

specimen has been deposited at the Herbarium of the Instituto Superior de Agronomia, University of Lisbon.

**EXTRACTION AND ISOLATION.**—The air-dried whole plant (2.3 kg) was extracted with  $\text{Me}_2\text{CO}$  ( $4 \times 12$  liters) at room temperature for 3 days. Each extract was filtered on a Buchner funnel and evaporated under reduced pressure at low temperature ( $40^\circ$ ). The combined extracts gave a residue of 164 g.

**Saponification.**—A 10% KOH solution in MeOH (1 liter) was added to the total extract. The mixture was left at room temperature for 36 h. After concentration of the MeOH at reduced pressure, the residue was suspended in 1 liter of  $\text{H}_2\text{O}$  and extracted 4 times with 0.5 liters of hexane followed by extraction 3 times with 0.5 liter of ether. The combined extracts (hexane and ether) containing the non-saponifiable part were dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated, yielding a residue of 40 g. The non-saponifiable part was then dissolved in 750 ml of hot  $\text{Me}_2\text{CO}$  and cooled. The precipitate was filtered off (10 g of a  $\text{Me}_2\text{CO}$ -insoluble part). The filtrate was evaporated giving 30 g of the  $\text{Me}_2\text{CO}$ -soluble part. Separation of the latter extract (30 g) was performed by cc on Si gel (1.3 kg; Merck No. 9385) with hexane/EtOAc mixtures of increasing polarity. The fraction containing **1** (2.5 g; hexane and hexane/EtOAc, 9:1) was chromatographed twice on Si gel eluted with hexane/EtOAc mixtures. After prep. tlc (hexane/EtOAc, 8.5:1.5), 14 mg of boeticol [**1**] were obtained.

Euphol was obtained from a fraction (1.1 g) eluted with hexane-EtOAc (9:1). Cc of this fraction yielded 320 mg of euphol.

**Boeticol [**1**].**—Obtained as an oil:  $[\alpha]_D^{20} + 30^\circ$  ( $c=0.070$ ,  $\text{CHCl}_3$ ); ir  $\nu$  max (film) 3450, 2925, 2860, 1460, 1380, 1090, 970, 825  $\text{cm}^{-1}$ ;  $^1\text{H}$ -nmr data, see Table 1;  $^{13}\text{C}$ -nmr data, see Table 2; eims

$m/z$  426  $[\text{M}]^+$  (7), 411  $[\text{M}-\text{CH}_3]^+$  (5), 408  $[\text{M}-\text{H}_2\text{O}]^+$  (4), 393  $[\text{M}-\text{CH}_3-\text{H}_2\text{O}]^+$  (5), 313  $[\text{M}-\text{C}_8\text{H}_{15}-2\text{H}]^+$  (16), 295  $[\text{M}-\text{C}_8\text{H}_{15}-2\text{H}-\text{H}_2\text{O}]^+$  (6), 274 (63), 259 (50), 255 (4), 231 (9), 205 (16), 189 (14), 187 (11), 173 (17), 163 (40), 161 (29), 152 (22), 150 (25), 134 (80), 123 (58), 121 (53), 107 (49), 95 (60), 81 (51), 69 (100); *anal.*, found C 84.29%, H 11.88%; calcd for  $\text{C}_{30}\text{H}_{50}\text{O}$ , C 84.44%, H 11.81%.

**Boeticol acetate [**1a**].**—Boeticol [**1**] (7 mg) was acetylated with  $\text{Ac}_2\text{O}$ -pyridine (1:1) at room temperature overnight. The usual workup gave pure **1a**: ir  $\nu$  max (film) 2925, 2850, 1735, 1460, 1380, 1245, 825  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr ( $\text{CDCl}_3$ )  $\delta$  0.80 (3H, s,  $\text{CH}_3$ -13 $\alpha$ ), 0.83 (3H, s,  $\text{CH}_3$ -9 $\beta$ ), 0.86 (3H, d,  $J=6.3$  Hz,  $\text{CH}_3$ -21), 0.88 (3H, s,  $\text{CH}_3$ -14 $\beta$ ), 1.04 (3H, s,  $\text{CH}_3$ -4 $\alpha$ ), 1.07 (3H, s,  $\text{CH}_3$ -4 $\beta$ ), 1.60 (3H, br s,  $\text{CH}_3$ -26), 1.68 (3H, br s,  $\text{CH}_3$ -27), 2.01 (3H, s, OAc-3 $\beta$ ), 4.69 (1H, t,  $J=2.9$  Hz, H-3 $\alpha$ ), 5.09 (1H, m,  $J=6.5$  and 1.4 Hz, H-24), 5.57 (1H, d,  $J=6.0$  Hz, H-6).  $^{13}\text{C}$ -nmr data, see Table 2; eims  $m/z$  468  $[\text{M}]^+$  (2), 453  $[\text{M}-\text{CH}_3]^+$  (1), 408  $[\text{M}-\text{CH}_3\text{COOH}]^+$  (9), 393  $[\text{M}-\text{CH}_3-\text{CH}_3\text{COOH}]^+$  (6), 355  $[\text{M}-\text{C}_8\text{H}_{15}-2\text{H}]^+$  (14), 295  $[\text{M}-\text{C}_8\text{H}_{15}-2\text{H}-\text{CH}_3\text{COOH}]^+$  (10), 274 (37), 259 (43), 255 (4), 231 (5), 205 (9), 189 (15), 173 (25), 163 (34), 161 (29), 150 (20), 134 (58), 123 (59), 121 (51), 107 (42), 95 (52), 81 (44), 69 (100).

**Euphol.**—White crystals: mp  $114\text{--}115^\circ$  ( $\text{Me}_2\text{CO}/\text{MeOH}$ );  $[\alpha]_D^{20} + 31^\circ$  ( $c=0.130$ ,  $\text{CHCl}_3$ ); ir  $\nu$  max (KBr) 3450, 2925, 2860, 1460, 1380, 1090, 970, 825  $\text{cm}^{-1}$ ;  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr and ms data as described in the literature (1).

#### ACKNOWLEDGMENTS

We are grateful to Junta Nacional de Investigação Científica for financial support (Project No. PBIC/C/CEN/1086/92). We also thank Dr. Teresa Vasconcelos of the Instituto Superior de Agronomia and Dr. Ana Margarida Madureira,

respectively, for the identification and collection of the plant. We are also grateful to Professor Carlos Geraldes for access to 500 MHz nmr facilities.

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Received 13 July 1994