Terracinolides A and B, Two Bishomoditerpene Lactones with a Novel Carbon Framework from *Euphorbia terracina*†

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A methanolic extract of *Euphorbia terracina* L. has been shown to contain two peracylated polyhydroxy terpenoid lactones with a novel C_{22} carbon framework. These metabolites, which have been named terracinolides A (**1**) and B (**2**), are based on the same parent compound, but differ in the nature of one of the acyl residues. This novel skeletal system is formally derived from the jatrophane framework by addition of a two-carbon fragment on C-17 (jatrophane numbering).

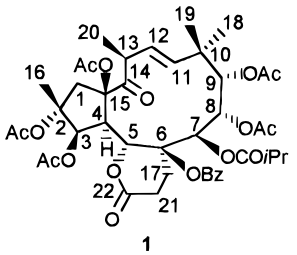
Introduction

Many species of the large spurge family (Euphorbiaceae) have long been noted for their toxicological effects on animals and humans.[2] When broken or cut, the aerial parts of some of these species, most particularly those of the genus *Euphorbia*,[3] excrete a milky fluid, which causes a number of physiological effects, including skin irritation, tumor promotion, and proinflammatory properties.[2] These biological responses have in many cases been traced back to the presence of specific types of diterpenes, most particularly phorbol derivatives.[4] Further diterpenes with remarkable biological properties are those displaying the jatrophane framework. The antitumor agent jatrophone, present in certain *Jatropha* species, is an archetypal example of this class of compounds.[5]

In the present paper, we describe the isolation and structural elucidation of two novel C_{22} terpenes (bishomoditerpenes) isolated from an extract of air-dried aerial parts of *Euphorbia terracina* L.[6]

Results and Discussion

The plant extract was partitioned into three main fractions by chromatography on reverse-phase silica gel. The middle polarity fraction was further subjected to silica gel chromatography and reverse-phase HPLC. This led to the isolation of compounds **1** (terracinolide A, 240 mg, 0.013% based on dry weight) and **2** (terracinolide B, 178 mg, 0.010% based on dry weight).



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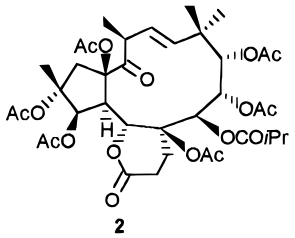

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Compound **1**, $C_{43}H_{54}O_{17}$, mp 240−242 °C, $[\alpha]_D$ +39°, displayed a broad and complex IR carbonyl absorption consisting of several partially overlapped lactone, ester, and ketone bands. No hydroxyl band was visible in the IR spectrum. ^1H and ^{13}C NMR data (see Table 1) revealed the presence of one ketone carbonyl, one *trans* disubstituted C=C bond, and eight ester-type carbonyls. Seven ester residues were easily identified by NMR as one benzoate, one isobutyrate, and five acetate groups. The eighth carbonyl signal was likely due to a lactone

Table 1. NMR Data for Terracinolide A (1)[a]

atom no.	^1H	NOE with (H no.)	^{13}C	HMBC with (H no.)
1α	3.07 d[b] (17)	13	49.4	3, 16
1β	2.56 d (17)			
2	−		86.9	1, 16
3	5.87 d[b] (3.8)	4, 16	78.5	1
4	3.92 dd (9.5, 3.8)		45.8	1, 5
5	5.91 d (9.5)	21β, Bz[c]	72.4	
6	−		80.8	17
7	6.32 s	4, 8, 11	67.6	5, 9
8	5.77 s	7, 9, 19, Bz[c]	67.7	
9	4.98 s	8, 18, 19	81.7	18, 19
10	−		40.2	9, 11, 12, 18, 19
11	6.00 d (16)	7, 13, 18	135.3	9, 13, 18, 19
12	5.48 dd (16, 10)	19, Bz[c]	130.3	13, 20
13	3.90 dq (10, 6.5)		43.1	11, 20
14	−		202.4	1, 13, 20
15	−		90.4	1, 5
16	1.55 s	1β, 3	18.3	1
17α	2.66 ddd (15, 15, 4.5)		26.9	21
17β	1.88 dd[b] (15, 6.5)	8, Bz[c]		
18	0.92 s	9, 11, 19	26.1	9, 11, 19
19	1.42 s	8, 12, Bz[c]	23.1	9, 11, 18
20	1.18 d (6.5)	1, 12, 13	20.6	13
21α	2.52 dd[b] (15, 4.5)		29.1	
21β	3.47 ddd (15, 15, 6.5)	5		
22	−		172.2	5

[a] ^1H Chemical shifts (in ppm) are followed by coupling constants (Hz) in parentheses. The stereochemical descriptors α and β have the usual meaning (hydrogen pointing downwards and upwards, respectively). ^1H signals of the acyl groups: acetates, 2.26 s, 2.04 s, 2.03 s, 1.99 s, 1.98 s; isobutyrate, 2.60 qq (7, 7), 1.25 d (7), 1.18 d (7); benzoate, 7.90 dd (8, 1.5), 7.60 tt (8, 1.5), 7.47 dt (8, 1.5). ^{13}C signals: acetates, 169.9, 169.8, 169.1, 169.0, 168.5, 22.4, 21.1, 20.9, 20.8, 20.6; isobutyrate, 174.8, 34.2, 18.6, 17.9; benzoate, 166.2, 133.6, 130.5, 129.6, 128.2. [b] Slightly broadened by a small, nonresolved coupling. [c] Ortho hydrogens of the benzoate group.

† Dedicated to the memory of the late Professor Félix Serratosa, deceased January, 1995.

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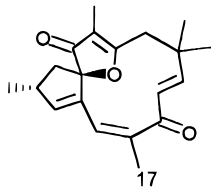
ring. The aforementioned groups accounted for all seventeen oxygen atoms of the molecule. In view of the molecular formula, the presence of three rings, including the lactone, was proposed. After discounting the contribution of the side chains, the presence of 22 skeletal carbon atoms was deduced.

To ascertain the different carbon and hydrogen connectivities, a series of spin decoupling and heteronuclear 2D-COSY experiments (HMQC/HMBC, see Table 1) was performed. This enabled us to establish the presence of a polyhydroxylated jatrophane-type carbon framework[7] bearing an additional two-carbon segment bound to C-17 (see jatrophane numbering). One of these two carbon



Jatrophone

atoms is oxidized at the carboxylic acid level and cyclized with a proximate hydroxyl group to give a δ-lactone ring. Some of the acyl residues were unambiguously located through the long-range 2D correlations (HMBC) of the corresponding carbonyl with the hydrogen atom geminal to the ester group (O═C—O—C—H).[8] Other acyl moieties (*e.g.*, the benzoyl group), however, could not be located in this way due to the absence of suitable correlations. The relative stereochemistry was deduced from the results of NOE difference experiments.

In order to assign the precise location of each ester group, a single-crystal X-ray diffraction analysis of **1** was performed.[10] This confirmed all our preliminary conclusions and gave us not only the complete structure, including relative configuration, but also insight into the three-dimensional geometry of the molecule. Interestingly, all observed NOEs fit nicely with this geometry, which would indicate that the conformation in solution does not substantially differ from that in the solid state. The solid state conformation also agreed with the geometry of the lowest energy conformer found by means of molecular mechanics calculations.[9] The fact that some acyl groups are bound to tertiary alcohol oxygens explains the missing HMBC correlations.

Considering its spectral features, the structure of compound **2**, $C_{38}H_{52}O_{17}$, mp 264−266 °C, $[\alpha]_D$ −4.3°, is closely related to that of **1**. A careful comparison of the NMR data of compound **2** (see Experimental Section) with those of **1** (Table 1) led us to conclude that both compounds were based on the same parent system and differred only in the nature of one of the seven ester moieties, in this case one isobutyrate and six acetate groups. In fact, the difference in the molecular formulas of **1** and **2** was easily accounted for by replacing the benzoyl group of the former with an acetyl residue. All NOEs and HMBC correlations were otherwise essentially identical with those observed in **1**. The structure of **2** was definitively confirmed, as in the previous case, by a single-crystal X-ray diffraction analysis.[10] The molecular conformation turns out to be almost identical with that observed in **1**.

Lactones **1** and **2** display the novel 17-ethyljatrophane carbon framework, for which we have not found any literature precedent. The absolute configuration of the compounds has not yet been determined, but we have assumed it to be the same as in other structurally close, naturally occurring jatrophane derivatives.[7]

Experimental Section

General Methods. NMR spectra were measured at 25 °C in a Varian Unity spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The signals of the deuterated solvent ($CDCl_3$) were taken as the reference (the singlet at 7.25 for ^{1}H NMR and the triplet centered at 77.00 ppm for ^{13}C NMR data). Two-dimensional experiments were performed with standard Varian software. Mass spectra were run in the electron impact mode (70 eV). Samples for IR spectral measurements were prepared as KBr pellets. Melting points are not corrected. Optical rotations were determined at 24 °C. Column chromatography was done on silica gel Merck (60−200 μm). MPLC separations were done on silica gel Macherey-Nagel (25−40 μm). Silica gel RP-2 was from Merck. Preparative HPLC was performed in the reverse-phase mode with a μBondapak C-18 column, using MeOH−water or acetonitrile−water mixtures.

Isolation of 1 and 2. The plant material was air-dried, ground, and extracted at room temperature with methanol−water 9:1. After adding silica gel RP-2 to the solution (3 g of silica gel/1 g of extract), the solvent was totally eliminated *in vacuo*. The greenish, powdery material was then placed on the top of a glass chromatographic column filled with silica gel RP-2 and eluted first with water and then with methanol−water 65:35 and finally with methanol. Only the middle fraction was investigated further, as the other two were shown to contain only sugars and highly polar compounds (water fraction) and waxes, sterols, and similar compounds of low polarity (methanol fraction).

The methanol−water fraction was separated into three main fractions by chromatography on silica gel with hexane−Et_2O 1:1, Et_2O, and Et_2O−MeOH 6:1. The Et_2O fraction was subjected to silica gel chromatography with hexane−Et_2O mixtures of increasing polarity. The intermediate fractions were further purified using MPLC or HPLC. This allowed the isolation of **1** (240 mg) and **2** (178 mg).

Terracinolide A (1): white needles, mp 240−242 °C (from hexane−EtOAc); α_D +39 (*c* 6.35, $CHCl_3$); IR (KBr) 3067, 3052, 2982, 2878, 1763, 1748, 1744, 1732, 1462, 1373, 1319, 1219, 1084, 1038, 956, 737, 702 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* (% relative intensity) $[M]^+$ 842 (8), $[M - CO]^+$ 814 (38), $[M - CO - ketene]^+$ 772 (11), $[M - CO - ketene - HOAc]^+$ 712 (50), 652 (11), 530 (4), 442 (7), 400 (9), 382 (15), 340 (19), 322 (19), $[PhCO]^+$ 105 (100), $[C_4H_9]^+$ 71 (24); HREIMS obsd 842.3360, calcd for $C_{43}H_{54}O_{17}$ 842.3361.

Terracinolide B (2): white needles, mp 264−266 °C (from hexane−EtOAc); α_D −4.3 (*c* 2.8, $CHCl_3$); IR (KBr) 2979, 2944, 2874, 1767, 1755, 1744, 1732, 1450, 1366, 1230, 1049, 945, 737 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 (s, 1H, H-7), 5.92 (d, 1H, J = 16 Hz, H-11), 5.80 (d, 1H, J = 3.5 Hz, H-3), 5.57 (s, 1H, H-8), 5.52 (d, 1H, J = 8.5 Hz, H-5), 5.45 (dd, 1H, J = 16, 10 Hz, H-12), 4.85 (s, 1H, H-9), 3.92 (dq, 1H, J = 10, 6.5 Hz, H-13), 3.80 (dd, 1H, J = 8.5, 3.5 Hz, H-4), 3.25 (ddd, 1H, J = 15, 15, 6 Hz, H-21β), 3.08 (d, 1H, J = 17 Hz, H-1α), 2.62 (d, 1H, J = 17 Hz, H-1β), 2.53 (qq, 1H, J = 7, 7 Hz, isobutyrate

(7) See, for example: (a) Shizuri, Y.; Kosemura, S.; Ohtsuka, J.; Terada, Y.; Yamamura, S.; Ohba, S.; Ito, M.; Saito, Y. *Tetrahedron Lett.* **1984**, 1155−1158. (b) Manners, G. D.; Wong, R. Y. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2075−2081. (c) Manners, G. D.; Davis, D. G. *Phytochemistry* **1987**, *26*, 727−730. (d) Yamamura, S.; Shizuri, Y.; Kosemura, S.; Ohtsuka, J.; Tayama, T.; Ohba, S.; Ito, M.; Saito, Y.; Terada, Y. *Phytochemistry* **1989**, *28*, 3421−3436.

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(9) Performed with Macromodel, V3.5X version, coupled with Monte Carlo multiconformer search: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(10) The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

CH), 2.45 (br dd, 1H, J = 15, 4.5 Hz, H-21α), 2.40 (ddd, 1H, J = 15, 15, 4.5 Hz, H-17α), 2.26, 2.11, 2.07, 2.00, 1.99, 1.95 (s, 3H ea, acetate methyl groups), 1.85 (br m, 1H, H-17β), 1.56 (s, 3H, H-16), 1.30 (d, 3H, J = 6.5 Hz, H-20), 1.23 (s, 3H, H-19), 1.20 (d, 3H, J = 7 Hz, isobutyrate Me), 1.18 (d, 3H, J = 7 Hz, isobutyrate Me), 0.89 (s, 3H, H-18); ^{13}C NMR (100 MHz, $CDCl_3$) (consecutively from C-1 to C-22) δ 49.3, 86.9, 78.3, 45.5, 71.8, 80.2, 66.5, 67.1, 81.6, 39.9, 134.7, 130.6, 43.2, 204.1, 90.5, 18.3, 25.5, 26.2, 22.7, 20.5, 28.7, 172.1, (six acetates) 170.0, 169.9, 169.7, 169.1, 169.0, 168.5, 22.7, 22.3, 21.2, 20.9, 20.6 (× 2), (isobutyrate) 174.6, 34.1, 18.7, 17.9; EIMS (70 eV) m/z (% relative intensity) $[M]^+$ 780 (5), $[M - CO]^+$ 752 (23), $[M - CO - \text{ketene}]^+$ 710 (8), $[M - CO - \text{ketene} - HOAc]^+$ 650 (74), 590 (13), 530 (7), 442 (9), 400 (13), 382 (17), 340 (21), 322 (19), $[C_4H_9]^+$ 71 (100); HREIMS obsd 780.3210, calcd for $C_{38}H_{52}O_{17}$ 780.3204.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of **1** and **2**; ORTEP plots for terracinolides A and B (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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