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NEW MYRSINOL-RELATED POLYFUNCTIONAL PENTACYCLIC DITERPENE ESTERS FROM ROOTS OF *EUPHORBIA PROLIFERA*[1]

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ABSTRACT.—Five *Euphorbia* substances, SPr1-SPr5, were isolated from the roots of *Euphorbia prolifera*. They were found to have similar structures but were inactive in a mouse ear inflammation assay. By nmr analysis and after single-crystal X-ray crystallography the structure of SPr5 was established as a hexaester (tetraacetate-benzoate-propionate) of a hitherto unknown polyfunctional pentacyclic diterpene parent alcohol, structurally related to myrsinol. As judged from its nmr spectra, SPr4 is an analogue of SPr5, carrying an isobutyrate substituent in place of a benzoate ester functionality. SPr1-SPr3 were partially characterized by their mass spectra as esters of diterpene parent alcohols possibly related to the myrsinol structure. SPr1-SPr5 may represent one of the product lines branching off the proposed main route of biogenesis of the oligocyclic diterpenoid skin irritants and tumor promoters occurring in many, but not all, of the species in the plant families Thymelaeaceae and Euphorbiaceae.

Euphorbia prolifera Buch-Ham. (Euphorbiaceae), indigenous to Yunnan province in southwestern People's Republic of China, is used in folk medicine for the treatment of inflammation and tumors (3). Many species of the plant families Thymelaeaceae and Euphorbiaceae are known to contain skin-irritant and tumor-promoting principles of the diterpene ester type (4–6). From the latex of *E. prolifera*, in ongoing studies on irritant, tumor-promoting, and antineoplastic compounds as potential constituents of Chinese medicinal plants in the plant families mentioned above, skin-irritant tigliane type diterpene esters have been isolated (1). In this paper the isolation, characterization, and structural elucidation are reported of five new non-irritant diterpene esters [*Euphorbia* substances SPr1-SPr5; for the terminology used see Sosath *et al.* (7)] from the roots of *E. prolifera*.

RESULTS AND DISCUSSION

Euphorbia substances SPr1-SPr5 were isolated, by means of cc and subsequent tlc (Table 1). They turned out to be practically non-irritant to the mouse ear (Table 1), in contrast to the *Euphorbia* factors Pr1-Pr5 obtained from the latex of *E. prolifera* (1). SPr5 was obtained in crystalline form.

The mass spectrum of SPr5 exhibited a molecular ion at *m/z* 726 with prominent fragment peaks at *m/z* 604, 122, and 105, which were compatible with the presence of a benzoate ester substituent, whereas the peak at *m/z* 606 could be explained by sequential loss of two acetic acid moieties from the molecular ion. The ^{13}C-nmr spectrum of SPr5 revealed six carbonyl groups of the ester moieties (ca. δ 166–174) and one isolated carbonyl function (δ 204.11). From the ^{1}H-nmr spectrum, the presence was evident of four acetates (δ 2.06–1.65; 4s, each 3H) and one propionate (δ 2.33, 2H, q, J=7.5 Hz; δ 1.12, 3H, t, J=7.5 Hz) as ester substituents in the molecule of SPr5.

[1]Part 7 in the series "Oligocyclic and Macrocyclic Diterpenes in Thymelaeaceae and Euphorbiaceae Occurring and Utilized in Yunnan (Southwest China)." For part 6, see Wu *et al.* (1).

[2]Part of the Dr. rer. nat. Thesis of D.W. (1989), see Wu (2).

TABLE 1. Chemical and Biological Characterization of the *Euphorbia* Substances SPr1-SPr5 Obtained by Preparative Tlc from Fractions F1–F3 of the Separation Procedure.

Compound	Prep. Tlc		Yield		Irritant Activity IU^{24c} [μg/ear]	Analytical Tlc			Molecular Ion [*m/z*][f]
	fraction	mobile phase[a]	[mg]	[%][b]		R_f	mobile phase[a]	stain[d]	
SPr1.....	F3	E	78	0.022	>1000	0.11	C	brownish	(653)
SPr2.....	F1	A	45	0.013	>1000	0.33	D	brownish	(606)
SPr3.....	F1	A	17	0.005	>1000	0.31	D	brownish	(620)
SPr4.....	F2	A	85	0.024	>1000	0.30	C	brownish	692
SPr5.....	F2	A	87	0.024	>1000	0.10	B	brownish	726

[a]Si gel plates, A: petroleum ether-Me_2CO (7:3), B: $CHCl_3$-Me_2CO (7:3), C: cyclohexane-Me_2CO (3:1), D: cyclohexane-EtOAc (7:3), E: petroleum ether-Me_2CO (2:1).

[b]MeOH extract of the roots: 100% dry wt.

[c]IU^{24}: irritant unit, read 24 h after administration (8).

[d]Vanillin/sulphuric acid vizualization (8).

[e]Suspected molecular ions in parentheses.

For detailed structural elucidation, SPr5 was subjected to single-crystal X-ray analysis. Figure 1 presents a perspective view of the carbon skeleton of the SPr5 molecule. Its structure comprises a cyclohexanone partial structure in good accordance with the bond lengths (data not given) O-2–C-7, C-6 through C-10, and C-10–C-12–C-6.

The six ester residues detected in SPr5 represent the 5,11,14,15-tetraacetate-8-benzoate-3-propionate of a polyfunctional parent alcohol. From the usual display of the chemical structure (Figure 2, top) it is apparent that the parent alcohol of SPr5 (i.e., **1**) is closely related structurally to myrsinol (**2**; Figure 2, bottom), one of the polyfunctional diterpene parent alcohols of several 3,5,7-triesters occurring in *Euphorbia myrsinites* (9,10).

With the structure of SPr5 at hand, it was possible to establish assignments of its nmr signals, with further support from a ^1H-^1H COSY nmr experiment (data not shown). For confirmation of spatial relations, a detailed analysis of possible nOes was carried out, the results of which are compiled in Table 2.

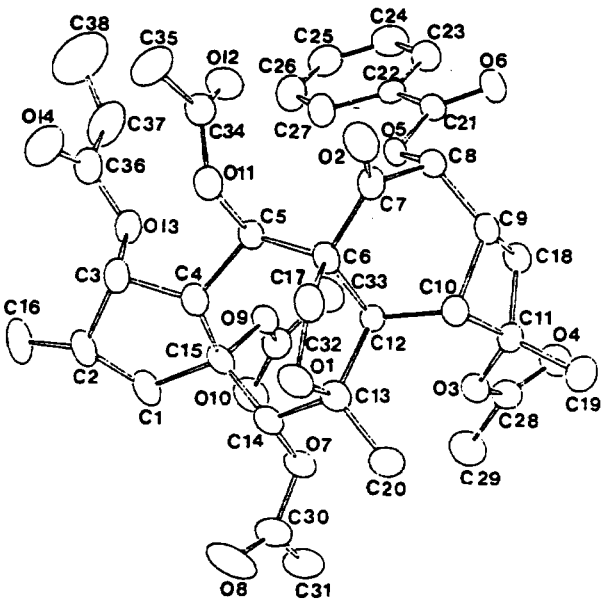


FIGURE 1. Perspective view of the structure of *Euphorbia* substance SPr5 established by single crystal X-ray analysis.

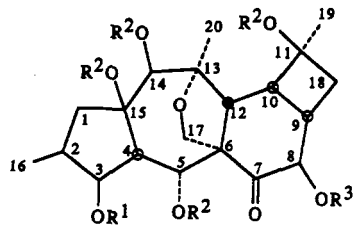
TABLE 2. Nuclear Overhauser Enhancements in the ^{1}H-Nmr Spectrum (500 MHz, $CDCl_3$) of *Euphorbia* Substance SPr5.

Proton	δ_H	Proton	δ_H	% nOe
H-5	6.09	H-12	4.06	7.8
H-3	5.46	H-4	3.03	6.1
		H-2	2.19	5.1
H-8	5.18	H-9	2.91	9.3
H-14	5.04	H-4	3.03	6.5
		H-1b	2.49	6.5
		H_3-20	1.21	2.3
H-17a	4.31	H-17b	3.56	27.6
		H-4	3.03	8.3
H-12	4.06	H-5	6.09	7.8
H-17b	3.65	H-17a	4.31	27.1
H-4	3.03	H-3	5.46	8.6
		H-14	5.04	6.8
		H-17a	4.31	5.3
		H-2	2.19	3.8
H-9	2.91	H-8	5.18	15.5
		H-18a	2.66	3.2
		H-10	2.44	2.7
H-1a	2.72	H-1b	2.49	15.5
		H_3-16	0.86	3.4
H-18a	2.66	H-9	2.91	7.9
		H-18b	2.42	19.8
		H_3-19	1.56	1.7
H_3-20	1.21	H-14	5.04	9.2
		H-10	2.44	14.6

The mass spectrum of the resinous *Euphorbia* substance SPr4 suggested a molecular ion of *m/z* 692 (Table 1). The fragment peak at *m/z* 604 ($M^+ - 88$) indicated the presence of a butyrate substituent in the compound. The ^{13}C- and ^{1}H-nmr spectra clearly demonstrated the substance to be an analogue of SPr5, with SPr4 being the 5,11,14,15-tetraacetate-8-isobutyrate-3-propionate of the same parent alcohol [**1**] as SPr5 (Figure 2, top). Thus, these two esters differ solely in their 8-acyl group.

The remaining resinous *Euphorbia* substances SPr1-SPr3 were characterized by mass spectrometry only. From fragmentation patterns they might tentatively be considered pentaesters (acetates and butyrates, in the case of SPr1, also a nicotinate) of diterpene parent alcohols structurally related to myrsinol [**2**]. The overall structural relationship of the polyfunctional parent alcohol **1** of SPr4 and SPr5 with myrsinol [**2**] is evident (see Figure 2). The 13,17-ether bridge may relate both **1** and **2** to 6,17-epoxylathyrol (**3**, Figure 2, bottom) occurring in the seed oil of *Euphorbia lathyris*. Compound **3** may be considered an oxygenated product of a macrocyclic precursor of the hypothetical oligocyclic diterpenes tigliane, ingenane, and daphnane, as shown previously for **2** (9,11). It is of considerable plant physiological and biogenetic interest that in the latex of *E. prolifera*, mainly tetracyclic tigliane-type (1) diterpene esters occur, whereas in the roots of the same plant, pentacyclic myrsinol-type diterpene esters predominate. They may represent one of the product lines branching off the main route of biosynthesis of oligocyclic diterpenes which, after proper functionalization, show up as the parent alcohols of the skin irritants and tumor promoters occurring in many species of the plant families Thymelaeaceae and Euphorbiaceae (6).

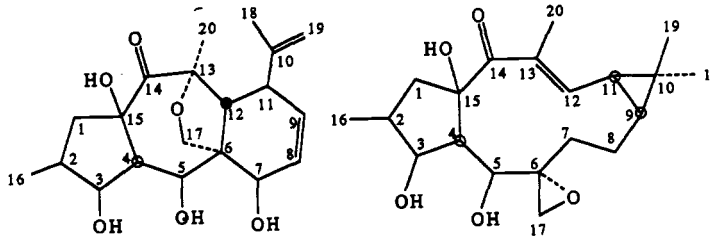
In previous investigations, it was noted that the profiles of diterpenoid compounds present in different plant parts of species in the Euphorbiaceae may differ considerably.



Euphorbia substance SPr5: R^1=propionyl, R^2=acetyl, R^3=benzoyl

Euphorbia substance SPr4: R^1=propionyl, R^2=acetyl, R^3=isobutyryl

1 $R^1=R^2=R^3=H$ (parent alcohol)



2 **3**

FIGURE 2. Top: Chemical structures of *Euphorbia* substances SPr5, SPr4 and of their parent alcohol **1**. Bottom: Chemical structures of myrsinol [**2**] and 6,17-epoxylathyrol [**3**]. The numbering of C-atoms in both **2** and **3** was taken from (10).

Hence, it would be of great interest to pinpoint, by plant physiology techniques, the site(s) of biosynthesis of skin-irritant and non-irritant diterpene esters. Some investigators assume this to take place in lacticifers although they do not occur in all genera of Euphorbiaceae (12), and not at all in the genera of Thymelaeaceae, which produce bioactive diterpene esters. More information about the site of biosynthesis and transportation of bioactive diterpene esters would be especially important because different parts of Euphorbiaceae species are utilized in the human environment and their use must be considered an inherent risk of promoting activity (depending on their content) by skin-irritant diterpene esters (6).

In forthcoming investigations of the new *Euphorbia* substances SPr1-SPr5, their interaction with protein kinases C, and, stimulated by local folk medicinal utilization (13), their therapeutic validity will be studied.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The chromatographic and spectroscopic methods used were essentially as described in a previous paper (14). The 500 MHz ^{1}H- and the 125.5 MHz ^{13}C-nmr spectra were measured in $CDCl_3$ on a Bruker AM-500 spectrometer. Chemical shifts refer to TMS (δ=0.00) as internal standard.

PLANT MATERIAL.—The sample of *E. prolifera* was identified by Prof. Wu Zheng Yi, Director, Kunming Institute of Botany, Academia Sinica, Kunming, Yunnan, People's Republic of China, where a voucher specimen is deposited. Roots (collected in December 1987 in Yunnan) were air-dried, yielding 6 kg of starting material for the isolation procedure.

ISOLATION AND EXTRACTION.—Air-dried roots (6 kg) from *E. prolifera* were chopped and extracted with

MeOH. After filtration the residue was reextracted twice by the same procedure. All filtrates were combined and subjected to rotary evaporation, yielding 351 g (100%) of a dry residue. Water was added and the resulting suspension was extracted three times with $CHCl_3$. After drying (Na_2SO_4) the combined $CHCl_3$ phases and removal (rotary evaporation) of the volatile constituents, 64.5 g (18.4%) of an oily material remained. This was partitioned in petroleum ether-MeOH-H_2O (1.5/1/0.05 liters of each), employing a separatory funnel. The upper phase was discarded; the lower phase was washed four times each with 1 liter of fresh pre-saturated upper phase. Finally, the MeOH/H_2O layer was subjected to rotary evaporation leaving 28 g (8%) of an oily residue. This was chromatographed on a Si gel column using petroleum ether-Me_2CO (19:1). Fractions showing R_f values (Si gel) ranging from ca. 0.05 (in petroleum ether-Me_2CO, 3:2) to 0.4 (in petroleum ether-Me_2CO, 7:3) were pooled. After evaporation of the volatile constituents, 16.5 g (4.7%) of a dry residue was obtained. This material was further separated on a Si gel column (1.5 kg) starting with petroleum ether-Me_2CO (9:1) as eluent. After passage of 2 liters, the composition of the mobile phase was changed to 4:1. Finally, guided by tlc, three fractions (F1–F3) were generated: F1 [2.1 g (0.6%) of a resin, range of R_f values: 0.3–0.4 in petroleum ether-Me_2CO, 7:3], F2 [2.8 g (0.8%) of a resin, range of R_f values: 0.2–0.3, same solvent system as used for F1] and F3 [2.5 g (0.7%) of a resin, range of R_f values: 0.05–0.2 in petroleum ether-Me_2CO, 3:2]. Each of the fractions F1-F3 was further separated by tlc, affording five tlc homogeneous *Euphorbia* substances, SPr1-SPr5, of which SPr5 and SPr4 represented the major components (Table 1). SPr1-SPr4 were obtained as resins, whereas SPr5 could be crystallized.

Euphorbia *substance SPr5*.—Colorless prisms (petroleum ether/Me_2CO), mp 217–220°; uv (MeOH) λ max (log ε) 229 (4.03), 273 (2.94), 281 (2.82), 310 (1.95) nm; ir (KBr) ν max 1740, 1605, 1585, 1370, 1270, 1260, 720 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 8.23 (2H, dd, J_1=1.5 Hz, J_2=7.5 Hz, H-2′, H-6′), 7.60 (1H, tt, J_1=1.5 Hz, J_2=7.5 Hz, H-4′), 7.43 (2H, tt, J_1=J_2=7.5 Hz, H-3′, H-5′), 6.09 (1H, dd, J_1=1.5 Hz, J_2=11 Hz, H-5), 5.46 (1H, dd, J_1=J_2=4 Hz, H-3), 5.18 (1H, d, J=7.5 Hz, H-8), 5.04 (1H, s, H-14), 4.31 (1H, d, J=10 Hz, H-17a), 4.06 (1H, d, J=12.5 Hz, H-12), 3.65 (1H, dd, J_1=1.5 Hz, J_2=10 Hz, H-17b), 3.03 (1H, dd, J_1=4 Hz, J_2=11 Hz, H-4), 2.91 (1H, dddd, J_1=7.5 Hz, J_2=J_3=9 Hz, J_4=10 Hz, H-9), 2.72 (1H, dd, J_1=10.5 Hz, J_2=16 Hz, H-1a), 2.66 (1H, ddd, J_1=3.5 Hz, J_2=9 Hz, J_3=13 Hz, H-18a), 2.49 (1H, dd, J_1=9 Hz, J_2=16 Hz, H-1b), 2.44 (1H, ddd, J_1=3.5 Hz, J_2=9 Hz, J_3=12.5 Hz, H-10), 2.42 (1H, dd, J_1=10.5 Hz, J_2=13 Hz, H-18b), 2.33 (2H, q, J=7.5 Hz, H_2-2″), 2.19 (1H, m, H-2), 2.06, 2.00, 1.96, 1.65 (3H each, s, 4×CH_3CO), 1.56 (3H, s, H_3-19), 1.21 (3H, s, H_3-20), 1.12 (3H, t, J=7.5 Hz, H_3-3″), 0.86 (3H, d, J=7.5 Hz, H_3-16); ^{13}C nmr ($CDCl_3$, 125.5 MHz) δ 204.11 (s, C-7), 173.60, 170.56, 169.79, 168.93, 168.43, 166.20 (each s, 6 ester CO), 133.30 (d, C-4′), 130.16 (d, C-2′, C-6′), 130.02 (s, C-1′), 128.27 (d, C-3′, C-5′), 90.03 (s, C-15), 89.56 (s, C-13), 81.64 (d, C-14), 73.84 (d, C-8), 77.00 (s, C-11), 77.00 (d, C-3), 68.36 (d, C-5), 67.35 (t, C-17), 62.09 (s, C-6), 51.05 (d, C-4), 43.15 (t, C-1), 42.05 (d, C-10), 41.04 (d, C-12), 36.72 (t, C-18), 36.15 (d, C-2), 30.57 (d, C-9), 27.46 (t, C-2″), 24.04, 23.04, 22.23, 21.64, 21.25, 20.79 (each q, 6×CH_3), 13.94 (q, C-16), 9.04 (q, C-3″); eims (70 eV, 210°) *m/z* $[M]^+$ 726, 667, 606, 604, 546, 533, 502, 490, 446, 442, 368, 325, 309, 297, 207, 191, 181, 149, 122, 105, 77.

Euphorbia *substance SPr4*.—Ir (KBr) ν max 1745, 1370, 1257, 1230, 1170, 1143, 1088, 1023, 973, 930 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 5.88 (1H, dd, J_1=1.5 Hz, J_2=11 Hz, H-5), 5.46 (1H, dd, J_1=J_2=4 Hz, H-3), 5.27 (1H, d, J=7.5 Hz, H-8), 5.03 (1H, s, H-14), 4.23 (1H, d, J=10 Hz, H-17a), 4.07 (1H, d, J=12.5 Hz, H-12), 3.60 (1H, dd, J_1=1.5 Hz, J_2=10 Hz, H-17b), 2.94 (1H, dd, J_1=4 Hz, J_2=11 Hz, H-4), 2.84 (1H, dd, J_1=10.5 Hz, J_2=16 Hz, H-1a), 2.77 (2H, q, J=7 Hz, H_2-2″), 2.75 (1H, m, H-18a), 2.75 (1H, m, H-9), 2.50 (1H, m, H-1b), 2.40 (1H, dd, J_1=10.5 Hz, J_2=13 Hz, H-18b), 2.25 (1H, m, H-10), 2.23 (1H, m, H-2′), 2.22 (1H, m, H-2), 2.19, 2.10, 2.10, 1.91 (3H each, s, 4×CH_3CO), 1.63 (3H, s, H_3-19), 1.29 (1H, d, J=7 Hz, H-3′), 1.24 (1H, d, J=7 Hz, H-4′), 1.19 (3H, s, H_3-20), 1.09 (3H, t, J=7 Hz, H_3-3″), 0.86 (3H, d, J=7.5 Hz, H_3-16); eims (70 eV, 210°) *m/z* $[M]^+$ 692, 633, 604, 573, 547, 499, 357, 308, 297, 235, 205, 191, 181, 149.

Euphorbia *substances SPr1-SPr3*.—*Euphorbia* substance SPr1: eims (70 eV, 200°) *m/z* 653, 593, 533, 470, 399, 336, 276. *Euphorbia* substance SPr2: eims (70 eV, 200°) *m/z* 606, 546, 505, 357, 297, 233, 232. *Euphorbia* substance SPr3: eims (70 eV, 220°) *m/z* 620, 560, 519, 500, 472, 459, 412, 399, 371, 311, 293, 232.

X-RAY CRYSTAL STRUCTURE ANALYSIS OF *EUPHORBIA* SUBSTANCE SPR5[1].—For X-ray crystal structure analysis, a radiation wavelength (Mo-K_α) of 0.7101 Å was used. Scan mode: ω/2θ, scan range: 1–25°, collected number of reflections: 3759, observed number of reflections with $I \geq 3.0\ \sigma_I$: 2040, $R = \Sigma|F_o|-|F_c|/$

[1]Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

$\Sigma|F_o|$:0.049. The independent reflections were measured on an Enraf-Nonius CAD 4. Computations were carried out on a PDP-II computer using the SDP program.

Figure 1 shows a single molecule of SPr5. The space group was identified as $P2_12_12_1$, with a=9.195 (1) Å, b=12.402 (2) Å, c=32.915 (3) Å; $\alpha=\beta=\gamma=90°$; D_c=1.251 gcm^{-3}; V=3753 $Å^3$; μ=0.897 cm^{-1}.

BIOLOGICAL ASSAYS.—For irritant activity on the mouse ear, the irritant unit (IU^{24}) was read 24 h after administration (8). Materials exhibiting an IU^{24} >1000 μg/ear are considered inactive.

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