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Diterpenoids from *Euphorbia aleppica* Linn.

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Two new pentacyclic diterpenes, euphoreppinol **1** and 3,14,15,17-tetra-*O*-acetyl-5-*O*-lactoyleuphoreppinol-7-yl isopropenylacetate **2** have been isolated from the whole plants of *Euphorbia aleppica*. Their structures have been elucidated by spectroscopic methods.

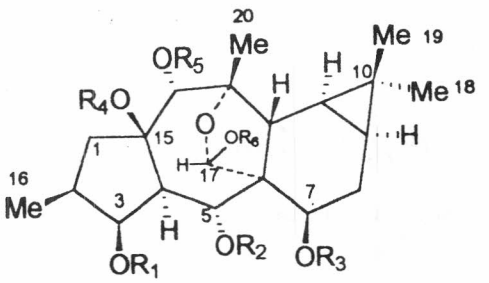
A number of bioactive diterpenoids have been isolated from the family Euphorbiaceae[1–3]. Many of them are skin irritants, such as the tetracyclic tigliane ingenane and tricyclic daphnane esters and some are tumor promoters. We have reported earlier the isolation and structural elucidation of four new diterpenoids from *E. petiolata*, indigenous to Jordan and six new diterpenes with a novel carbon skeleton from *E. aleppica*[4–6]. We have now isolated two more new diterpenes possessing the same carbon skeleton, named euphoreppinol **1** and 3,14,15,17-tetra-*O*-acetyl-5-*O*-lactoyleuphoreppinol -7-yl isopropenylacetate **2**, from the petroleum ether (60-90°C)-diethyl ether-methanol (1:1:1) extract of the whole plants of *E. aleppica*. This paper deals with the structural characterisation of these compounds. Biogenetic relationship of the terpenoid constituents has also been proposed.

Results and Discussion

Compound **1**, a colourless gum, $[\alpha]^{18}_{D}$ +2.1° (*c* 0.35, MeOH), was deduced to have the molecular formula $C_{20}H_{32}O_7$ (5 degrees of unsaturation) on the basis of FAB mass spectrum ($[M+1]^+$ at m/z 385; $[M+Li]^+$ at m/z 391; and $[M+Na]^+$ at m/z 407) together with the combination of ^{13}C NMR and DEPT spectra (20 carbons: 4×CH_3, 2×CH_2, 10×CH, 4×C). In addition to the quasimolecular ion peaks $[M+Li]^+$ and $[M+Na]^+$, the FAB MS exhibited significant fragments at m/z 349, 331, 313 and 279 (base peak) produced by the successive loss of six hydroxyl groups. The ^1H NMR spectrum of **1** showed signals for a secondary methyl group (δ 1.05) and three tertiary methyl groups (δ 0.94, 1.02, 1.44), as well as five signals for oxygen-bearing methine protons (δ 3.34, s; 4.14, t; 4.36, d; 4.40,dd; 5.53, s). The above ^1H NMR feature (also see Table I) along with the ^{13}C NMR spectral data (Table II) suggested compound **1** to be a polyhydroxy diterpene alcohol with the same pentacyclic diterpenoid skeleton as that of euphoreppine-A **4** and euphoppine-A **5**[6], and the locations of the six hydroxyl groups were also straight forward.

The relative configuration of **1** was established by comparison of similar or identical coupling constants ($J_{1,2}$=10.1 Hz, $J_{1',2}$=$J_{2,3}$=$J_{3,4}$=3.3 Hz, $J_{4,5}$=10.5 Hz, $J_{7,8}$=3.1 Hz, $J_{7,8'}$=12.4 Hz, and $J_{11,12}$=8.3 Hz) of compound **1** with those of euphoreppines A,B[5], euphoppines A-D[6] and enukolurine[7]. Thus, the structure of **1** was completely established as euphoreppinol (cf. **Figure 1**), which is regarded as the parent alcohol of the diterpene esters, the euphoreppines A, B and the euphoppines A-D.

The molecular formula of **2** was assigned as $C_{36}H_{50}O_{14}$ (12 degrees of unsaturation) by EI mass spectrum (the molecular ion peak $[M]^+$ at m/z 706), and by the combination of ^{13}C NMR and DEPT spectra (Table II). The ^1H NMR and ^{13}C NMR spectra (**Tables I** and **II**) indicated the presence of four acetoxyl groups (δ 2.10, 2.09, 2.05, 1.89, each 3H, s), a lactoyl group (δ 1.45, d, 3H; 4.84, q, 1H), and an isopropenylacetoxyl group (δ 1.26, s, 3H; 1.35, s, 2H; 5.92, 6.52 each 1H, br s), which was supported by the fragment peaks at m/z 647, 633, 617, 517, 457, 414, 354 in the EI mass spectrum. Apart from the signals of ester moieties,



1 $R_1=R_2=R_3=R_4=R_5=R_6=$ H

2 $R_1=R_4=R_5=R_6=$ -Ac, $R_2=$ - COCH(OH)-CH_3, $R_3=$ -$COCH_2$-$C(CH_3)=CH_2$

4 $R_1=R_2=R_4=R_5=R_6=$ -Ac, $R_3=$ -Tig

5 $R_1=R_2=R_4=$ -Ac, $R_5=R_6=$ -H, $R_3=$ -Tig

Figure 1—Structures of compounds **1-5**.

Table I—400 MHz ^{1}H NMR data of compounds **1** and **2**[a]

Atom No.	1[b]	2[c]
1	2.94 dd (10.1, 14.1)	2.45 dd (8.4, 15.1)
1′	—	1.69 dd (3.5, 15.1)
2	1.82 m	1.95 m
3	4.14 t (3.3)	5.17 t (3.5)
4	2.06 dd (3.3, 10.5)	3.26 dd (3.5, 10.7)
5	4.36 d (10.5)	5.94 d (10.7)
7	4.40 dd (3.1, 12.4)	5.01 dd (4.2, 8.0)
8	1.16 m	1.80 m
8′		1.61 m
9	0.74 m	0.89 m
11	0.80 dd (8.3, 9.7)	0.97 m
12	3.03 d (8.3)	2.62 d (5.5)
14	3.34 s	5.65 s
16	1.05 d (6.7)	0.82 d (6.8)
17	5.53 s	6.46 s
18	1.02 s	1.06 s
19	0.94 s	1.07 s
20	1.44 s	1.25 s
Acetoxyl		2.10 s; 2.09 s; 2.05 s; 1.89 s
Lactoyl		1.45 d (6.8)
		4.84 q (6.8)
Isopropenylacetoxyl		1.35 s
		1.26 s
		5.92 brs
		6.52 brs

[a] Chemical shifts (in δ, ppm), multiplicity, and coupling constants (Hz in parentheses).
[b] CD_3OD as solvent.
[c] $CDCl_3$ as solvent.

the ^{1}H NMR spectrum of **2** showed one secondary methyl group (δ 0.82) and three tertiary methyl groups (δ 1.25, 1.07, 1.06) as well as five signals for oxygen-bearing methine protons (δ 6.46, s; 5.65, s; 5.17, t; 5.94, d; 5.01, dd). The ^{13}C NMR and DEPT spectra revealed that the basic carbon skeleton consisted of four methyls, two methylenes, ten methines and four quaternary carbons. These NMR data suggested that compound **2** had the same pentacyclic diterpene skeleton as compounds **1, 4** and **5**. Furthermore, comparison of the ^{1}H NMR spectral data of **2** with those of **1**, and the down-field shift of the signals of oxygen-bearing methines of **2** (δ 6.46, 5.65, 5.17, 5.94, 5.01) revealed that compound **2** was a pentacyclic diterpene ester of **1** with four acetoxyl, one lactoyl and an isopro-

Table II—100 MHz ^{13}C NMR data of compounds **1** and **2**

Carbon	1[a]	2[b]	DEPT
1	48.20	44.73	CH_2
2	39.03	36.67	CH
3	79.90	77.32	CH
4	35.12	50.84	CH
5	69.83	69.93	CH
6	59.27	56.02	C
7	79.10	79.86	CH
8	25.49	24.86	CH_2
9	21.85	22.91	CH
10	20.96	18.66	C
11	21.43	18.07	CH
12	39.45	36.95	CH
13	85.87	88.88	C
14	74.83	72.87	CH
15	88.03	89.20	C
16	16.15	14.08	CH_3
17	99.52	98.00	CH
18	29.21	28.41	CH_3
19	16.53	15.70	CH_3
20	27.06	25.20	CH_3
Acetoxyl		170.32, 21.59	CO, CH_3
		169.60, 20.84	CO, CH_3
		169.60, 21.19	CO, CH_3
		168.92, 21.35	CO, CH_3
Lactoyl		172.39	CO
		69.88	CH
		17.00	CH_3
Isopropenyl-acetoxyl		163.32	CO
		139.40	C
		129.98	CH_2
		29.70	CH_2
		19.10	CH_3

[a] CD_3OD as solvent.
[b] $CDCl_3$ as solvent.

penylacetoxyl groups. The positions of attachment of the various esters were assigned by comparing the ^{1}H NMR and ^{13}C NMR spectral data of **2** with those of euphoreppines-A **4**, B[5] and euphoppines A **5**-D[6], together with the cross peaks (C-172.39/H-5 and C-163.32/H-7) in the HMBC experiment. The nearly identical NMR data and coupling pattern ($J_{1,2}$=8.4 Hz, $J_{1',2}$=$J_{2,3}$=$J_{3,4}$=3.5 Hz, $J_{4,5}$=10.7 Hz) of **2** and the known diterpenes compounds **4** and **5** implied that **2** had the same stereochemistry as euphoreppine-A **4** and euphoppine-A **5**. Consequently, compound **2** was identified as 3,14,15,17-tetra-*O*-acetyl-5-*O*-lactoyleuphoreppin-7-yl isopropenylacetate.

The polyfunctional parent alcohol **1** and its hexa-esters **2** and **3** are structurally comparable with myrsinol **6** and its derivative **7**[8–11]. The 13,17-ether bridge in the compounds **1-7** may be biogenetically derived from the 6,17-epoxylathyrol **8**[12], which may be considered an oxygenated product of a macrocyclic precursor (lathyrane type diterpenes) of the tigliane, ingenane and daphnane diterpenes[11,13]. Therefore, components **1-3**, myrsinol **6** and sprol **7** may represent one of the product lines branching off the main route of the biosynthesis of the skin irritant and tumor promoting diterpenes occurring in many species of the plant family *Euphorbiaceae*[13] (cf. **Figure 2**).

Experimental Section

General. All optical rotations were ditermined on a JASCO-20C automatic recording spectropolarimeter . Mass spectra were recorded on a VG ZAB-HS mass spectrometer at 70 eV electron im-

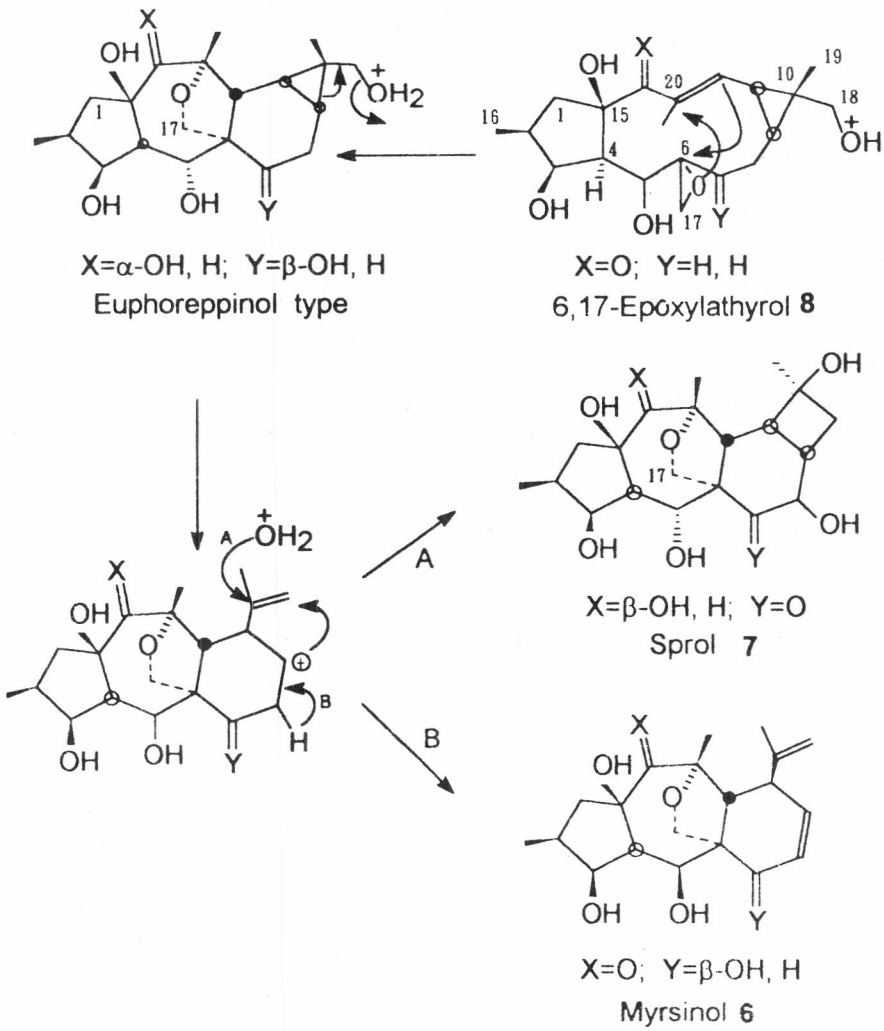


Figure 2—Proposed biogenesis of myrsinol and related compounds.

pact ionization. IR spectra were run on a Nicolet 170 SX FT-IR instrument. ^{1}H NMR (400 MHz), ^{13}CNMR (100.16 MHz) and HMBC spectra were measured on a Bruker AM 400 FT-NMR spectrometer with TMS as internal standard.

The whole parts of *E. aleppica* L (Euphorbiaceae) were collected near Irbid, along the Irbid-Amman Highway in Jordan (in February, 1993) and were identified by Dr Jamil Lahham. A voucher specimen (No. 5601) is deposited in the Herbarium of the Biology Department of Yarmouk University, Irbid, Jordan.

Extraction and isolation of diterpenoids. The air-dried whole plants (850g) of *Euphorbia aleppica* were pulverized and extracted with petroleum ether (60-90°) -Et_2O-MeOH (1:1:1) three times at room temperature (each process lasting 4 days). After evaporation *in vacuo*, the crude extract (65g) was chromatographed over a silica gel column (400 g, 200-300 mesh) and eluted with a gradient of petroleum ether-acetone (from 50:1 to 1:5). According to differences in composition indicated by TLC, three fractions were collected. From the second fraction (8 g) eluted with petroleum ether-acetone (5:1-1:1), a mixture including **2** was obtained by repeated CC over silica gel eluting first with CH_2Cl_2-Et_2O (15:1) and then with petroleum ether-Et_2OAc (1:2). The mixture were further separated by HPLC (eluent: *n*-hexane-acetone, 3:1) to obtain pure compound **2** (9 mg). Finally, from the third fraction (petroleum ether-acetone, 1:1-1:5), **1** (5.6 mg) was obtained by repeated CC over silica gel using $CHCl_3$-MeOH (10:1) as eluent.

Euphoreppinol 1: A colourless gum, $[\alpha]^{18}_D$+2.1° (*c* 0.35, MeOH); FABMS: m/z 407 $[M+Na]^+$, 391 $[M+Li]^+$, 385 $[M+H]^+$, 349, 331, 313, 279, 245, 227, 215, 205, 107, 91, 77; ^{1}H NMR (CD_3OD) data (Table I); ^{13}C NMR (CD_3OD) data (Table II).

3, 14,15,17-Tetra-*O*-acetyl-5-*O*-lactoyleuphorep-pin-7-yl isopropenylacetate 2: A colorless gum, $[\alpha]^{18}_D$ −35.6° (*c* 0.52, $CHCl_3$); IR ($CHCl_3$): 3460 (OH), 2921, 2851, 1742 (COO), 1496, 1459, 1371, 1228, 1114, 1097, 1009, 756 cm^{-1}; EIMS (%): m/z 706 (0.2%, M^+), 647 (0.2), 633 (0.5), 617 (0.5), 517 (18), 457 (10), 414 (15), 354 (24), 343 (30), 308 (42), 266 (73), 223 (90), 191 (100), 174 (83), 133 (50), 97 (55), 83 (35), 73 (45), 43 (100); ^{1}H NMR ($CDCl_3$) data (Table I); ^{13}C NMR ($CDCl_3$) data (Table II).

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