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PENTACYCLIC DITERPENE ESTERS FROM *EUPHORBIA ALEPPICA*

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Key Word Index—*Euphorbia Aleppica*; Euphorbiaceae; diterpenoids; euphoreppines A and B.

Abstract—Two pentacyclic diterpene esters, euphoreppines A and B with a novel carbon skeleton, have been isolated from whole plants of *Euphorbia aleppica*, and their structures have been elucidated by spectroscopic methods (MS, IR, 1D and 2D NMR techniques). In addition, four known compounds have also been obtained.

INTRODUCTION

Euphorbia species have afforded many polyfunctional diterpenoids with the tetracyclic tigliane, ingenane skeletons and the tricyclic daphnane skeleton [1, 2]. Most of them are skin irritants and many are tumour promoters of skin [1, 2]. Moreover, non-irritant macrocyclic diterpenoids with lathyane and jatrophane skeletons have been found, which are considered to be biogenetic precursors of the irritants [3]. No investigation on the plant *E. aleppica* has been reported up to now.

RESULTS AND DISCUSSION

Six compounds were isolated by repeated column chromatography and preparative TLC from the petrol diethyl ether–methanol (1 : 1 : 1) extract of *E. aleppica*. Their structures were identified as sitosterol [4], daucosterol [4], α-euphol [5] and kaempferol-3-*O*-L-rhaside [6], as well as two diterpenoids, named euphoreppine A (**1**) and euphoreppine B (**2**) with a novel carbon skeleton.

Euphoreppine A (**1**), was assigned the molecular formula $C_{35}H_{48}O_{13}$ on the basis of elemental analysis and the FAB mass spectrum ($[M + 1]^+$, m/z 677). Its IR spectrum showed strong absorption for ester carbonyl (1736 cm^{-1}) and trisubstituted double bond (1651 cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra (Tables 1 and 2) indicated the presence of five acetyl groups (δ2.01, 1.99, 1.96, 1.95, 1.64, each 3H, *s*) and one tigloyl group (δ6.73 [1H, *q*]; 1.67 [6*H*, *m*]), which was supported by the fragment peaks at m/z: 617, 557, 517, 457, 397, 337, 83 (tigloyl) and 43 (acetyl) in the FAB mass spectrum. In addition to the signals of ester moieties, the ^{1}H NMR spectrum showed one secondary methyl group (δ0.68) and three tertiary methyl groups (δ1.17, 0.96, 0.91) as well as five signals of oxygen-bearing methine protons (δ6.45, *s*; 5.71, *d*; 5.52, *s*; 4.96, *t*; 4.90, *dd*). The ^{13}C NMR and DEPT spectra (Table 2) revealed that the basic carbon skeleton consisted of four methyls, two methylenes, 10 methines and four quaternary carbons. Among the 20 carbons, there was no sp^2 carbon atom. To accommodate 12 degrees of unsaturation compound **1** was proposed to be a pentacyclic diterpene ester with five acetyl groups and one tigloyl group. A quaternary carbon (δ18.9) in the ^{13}C NMR spectrum indicated the presence of a gem-dimethyl cyclopropane ring [7], which was further supported by proton signals (δ0.87 [1H, *m*]; 0.75 [1H, *m*]) in the ^{1}H NMR spectrum. Further 2D NMR experiments of ^{1}H–^{1}H cosy and ^{1}H–^{13}C cosy showed the partial structures, which were separated by quaternary carbon (Fig. 1, I–VIII). The connection of these partial structures was achieved by consideration of the ^2J and ^3J couplings: C-6/H-12; C-10/H-18, H-19, H-12; C-13/H-20, H-17; C-15/H-14, H-3; C-3/H-16; C-5/H-4, H-12; C-14/H-20, H-12; C-17/H-5 in the 2D NMR ^{1}H–^{13}C COLOC spectrum. These NMR experiments led to a pentacyclic diterpene skeleton for **1** with 5/7/6/5/3 membered rings. Therefore, comparison of the ^{1}H and ^{13}C NMR spectral data for **1** with those for myrsinol and its derivation [8] revealed that they have the same A, B, C and D rings except that a cyclopropane ring (E) of **1** instead of a double bond between C-8 and C-9 in the D ring and an isopropylene group attached at C-11 of myrsinol. Because of the presence of an additional E ring compound **1** was confirmed as a new pentacyclic diterpene ester with a novel skeleton.

The ^{1}H–^{13}C COLOC spectrum of **1** displayed cross peaks between the ester carbonyl carbons and methines bearing-oxygen (δ_c170.7/H-3; 169.6/H-14; 169.6/H-17; 169.0/H-5; 165.5/H-7). Consequently, the positions of attachment of the various esters were assigned.

The relative configuration as **1** can be deduced from the results of NOE difference experiments. A convenient

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Table 1. ^{1}H NMR spectral data for compounds **1** and **2** (400 MHz, TMS, $CDCl_3$). Chemical shift δppm (coupling constants *J*, Hz)

H		1	2	H	1	2
1	β	2.25 *dd* (8.4, 15.1)	2.35 *dd* (8.0, 15.0)	17	6.45 *s*	6.58 *s*
1	α	1.60 *dd* (3.5, 15.1)	1.70 *dd* (3.5, 15.0)	18	0.96 *s*	1.04 *s*
2		1.91 *m*	1.89 *m*	19	0.91 *s*	1.01 *s*
3		4.96 *t* (3.5)	5.10 *t* (3.5)	20	1.17 *s*	1.27 *s*
4		3.0 *dd* (3.5, 10.5)	3.15 *dd* (3.5, 10.7)		Acetyl moieties	
5		5.71 *d* (10.5)	5.83 *d* (10.7)		2.01 *s*, 1.99 *s*,	2.07 *s*, 2.04 *s*
7		4.90 *dd* (3.0, 10.0)	5.01 *dd* (3.0, 10.8)		1.96 *s*, 1.95 *s*,	2.01 *s*
8	α	1.80 *ddd* (3.0, 3.0, 15.0)	1.87 *m*		1.64 *s*	1.76 *s*
8	β	1.20 *ddd* (7.5, 10.0, 15.0)	1.23 *m*		(*E*)-Methyl-2-butenoyl	
9		0.87 *m*	0.96 *m*		6.73 *q* (7.0)	6.98 *q* (6.5)
11		0.75 *m*	0.85 *m*		1.67 *m*	6.83 *q* (6.8)
12		2.43 *d* (7.5)	2.56 *d* (7.3)			1.77–1.86 *m*
14		5.52 *s*	5.67 *s*			1.77–1.86 *m*
16		0.69 *d* (7.0)	0.75 *d* (6.8)			

Table 2. ^{13}C NMR spectral data of compounds **1** and **2** (100 MHz, $CDCl_3$)

C	1	2	DEPT	C	1	2	DEPT
1	44.5	44.7	CH_2	19	15.5	15.8	CH_3
2	36.3	36.8	CH	20	24.7	25.0	CH_3
3	76.7	76.7	CH		Acetyl moieties		
4	51.1	51.4	CH		170.7, 22.3	170.6, 22.6	
5	66.7	66.9	CH		170.1, 20.7	170.1, 21.0	
6	56.0	56.3	C		169.6, 20.4	169.6, 21.3	
7	71.5	71.7	CH		169.6, 20.7		
8	24.8	25.0	CH_2		169.0, 21.0	168.9, 21.0	
9	23.2	23.5	CH		(*E*)-Methyl-2-butenoyl		
10	18.9	19.1	C		165.5 (CO)	165.7 (CO)	
11	18.5	18.5	CH		138.3 (CH)	138.2 (CH)	
12	37.7	37.6	CH		128.4 (C)	128.0 (C)	
13	87.6	88.4	C		14.0 (CH_3)	14.1 (CH_3)	
14	72.7	72.7	CH		11.6 (CH_3)	11.9 (CH_3)	
15	88.0	89.5	C			166.5 (CO)	
16	14.2	14.2	CH_3			138.9 (CH)	
17	97.5	97.6	CH			128.8 (C)	
18	27.4	27.8	CH_3			14.5 (CH_3)	
						12.2 (CH_3)	

point of reference is H-3, which is assumed to be α. Irradiation of H-3 produced a strong NOE at H-2 (15%) and H-4 (13.5%). Models indicated that such an effect is possible only with an α-configuration of H-2 and H-4. Irradiation of H-17 gave a significant NOE at H-4 (14%), indicating the tetrahydrofurane ring (C-ring) below the molecular plane and the H-17 close to H-4. The large coupling constant ($J_{4,5}$ = 10.5 Hz), along with the observation of NOEs from H-5 of both H-12 (10%) and 15-$OCOCH_3$ (3.5%) requires *trans* A and B rings and a β-configuration of H-5 [9]. Furthermore, irradiation of H-14 caused an NOE of H-12 (5%), 3H-20 (3.5%) and H-1 (3%). These results indicate H-14, 3H-20 and H-1 with β-orientation. Irradiation of H-12 gave a significant NOE at 3H-19 (15%) and H-8′ (4%), indicating the E-ring and H-8′ with β-orientation. NOE effects of H-8 (5%) and H-9 (3%) on irradiation of H-7 indicated an α-configuration for H-7 and H-8. Thus, the structure of euphoreppine A was assigned as **1**.

Euphoreppine B (**2**), $C_{38}H_{52}O_{13}$, a colourless gum, showed a mass spectrum, IR absorption and NMR (1D and 2D) signals nearly superimposable on those reported above for **1** except an acetyl group for **1** was replaced by a tiglate for **2**. The positions of attachment of ester groups were determined by the ^{1}H–^{13}C COLOC spectrum of **2**, and the ^{1}H and ^{13}C NMR spectral chemical shifts of **2** were assigned by 2D NMR techniques (^{1}H–^{1}H cosy, ^{1}H–^{13}C cosy and COLOC). Thus, the structure of **2** was also confimed.

EXPERIMENTAL

Mps: Uncorr.; IR film, 5DX-FTIR; ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz), Bruker AM 400

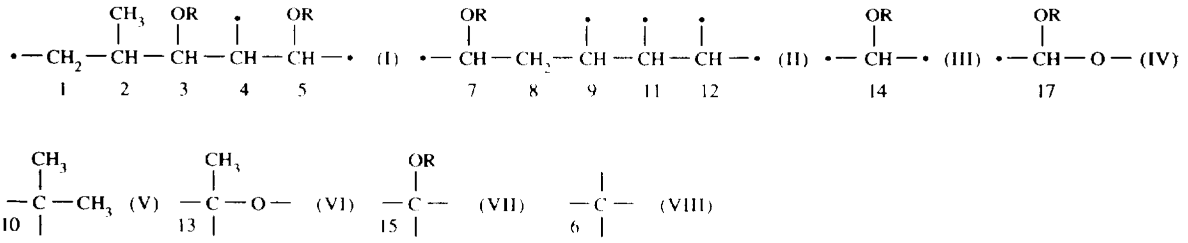

Fig. 1. Partial structures of the euphoreppine A. ●. These are quaternary carbon atoms.

FT-NMR, TMS as int. standard; MS: VG ZAB-HS, direct inlet, 70 eV.

Plant material. *Euphorbia aleppica* was collected around Irbid in Jordan (in July 2, 1993) and was identified by Dr J. Lahham; a specimen is deposited in the Herbarium of the Biology Department of Yarmouk University, Irbid, Jordan.

Extraction and isolation. The dried plants (850 g) were powdered and extracted (× 3) with petrol–Et_2O–MeOH (1 : 1 : 1) at room temp. The extract was concd (65 g) and then sepd by CC with a gradient of petrol–Me_2CO (50 : 1–1 : 5) on silica gel (400 g). Three frs were collected, and from the second fr. (petrol–Me_2CO, 5 : 1), **1** (60 mg), **2** (50 mg), sitosterol (100 mg) and α-euphol (110 mg) were obtained by repeated CC on silica gel with first CH_2Cl_2–Et_2O (15 : 1) and then petrol–Et_2OAc (2 : 1). Finally, from the third fr. (petrol–Me_2SO, 1 : 1–1 : 5), two frs were collected by CC with $CHCl_3$–MeOH (5 : 1) on silica gel. Daucosterol (20 mg) was obtained by the purification of the first fr. with $CHCl_3$–MeOH (10 : 1) and from the second fr., kaempferol-3-*O*-L-rhaside (25 mg) was obtained by prep. TLC (Silica gel GF_{254}) with $CHCl_3$–MeOH (4 : 1).

Euphoreppine A (**1**). Gum $[\alpha]_D^{24}$ −46.24° ($CHCl_3$, *c* 0.86); found: C 62.05, H 7.02; requires: C 62.13; H 7.10%; IR ν_{max}^{Film} cm^{-1}: 2935, 2874, 1736, 1651, 1457, 1371, 1226, 1121, 1073, 1010, 755, 606; FABMS *m/z*: 677 [MH^+], 617 (M-HOAc), 557 (M-2 × HOAc) 517 (617-tiglic acid), 457 (517-HOAc), 397 (457-HOAc), 337 (397-HOAc), 309, 295, 267, 249, 223, 175, 83, 43; ^1H and ^{13}C NMR: see Tables 1 and 2.

Euphoreppine B (**2**). Gum $[\alpha]_D^{24}$ −47.61 ($CHCl_3$, *c* 0.73); IR ν_{max}^{Film} cm^{-1}: 2961, 2875, 1740, 1651, 1457, 1372, 1230, 1124, 1071, 1009, 754, 607; FABMS *m/z*: 717 [MH^+], 658, 598 (658-HOAc), 558 (658-tiglic acid), 515 (558-Ac), 455 (515-HOAc), 415 (515-tiglic acid), 355 (415-HOAc), 295 (355-HOAc), 267, 223, 175, 136, 83, 43; EIMS *m/z*: 716 [M^+] 658, 598, 557, 515, 498, 455, 437, 415, 366, 223, 191, 167, 83, 43; ^1H and ^{13}C NMR: see Tables 1 and 2.

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