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A DITERPENE FROM *EUPHORBIA MADDENI*

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Key Word Index—*Euphorbia maddeni*; Euphorbiaceae; euphornin; jatrophone diterpene.

Abstract—From the anticancer-active $CHCl_3$ extract of the plant *Euphorbia maddeni*, a polyacylated diterpene of jatrophone type, euphornin, has been isolated and its structure elucidated by physico-chemical methods.

INTRODUCTION

Euphorbia maddeni Boiss. is a glabrous, annual herb distributed over the western Himalayas at a height of about 2000 m. The $CHCl_3$ extract of the defatted plant material exhibited anticancer activity in PS system (200 mg/kg, T/C 134). Consequent isolation studies on this fraction by repeated chromatography on Si gel led to the isolation of a new diterpene named euphornin. This substance is under anticancer testing and the present paper describes its structure elucidation.

RESULTS AND DISCUSSION

Euphornin (**1**) was assigned the molecular formula $C_{33}H_{44}O_9$ on the basis of elemental analysis and CI mass spectrum ($M + 1 - H_2O$, *m/z* 567). Its IR spectrum showed absorptions for benzoyl (1705, 1600, 1500, 1280 cm^{-1}) and acetyl groups (1740, 1245 cm^{-1}) which were supported by the CIMS fragments at *m/z* 525 (567 − ketene), 507 (567 − HOAc), 465 (507 − ketene), 447 (507 − HOAc), 405 (447 − ketene), 403 ($525 - C_6H_5COOH$) and by the ^1H NMR spectrum which showed signals for the protons of three acetoxymethyl groups (δ 1.85, 1.73 and 1.41) and one benzoyl group (δ 7.16 *m*, 8.29 *m*). These observations indicated that the parent substance had the molecular formula $C_{20}H_{34}O_5$ and was a polyhydroxy-diterpene. This was further confirmed by elemental analysis and the high resolution mass spectrum of deacyl euphornin (**2**), *m/z* 336.230 ($M^+ - H_2O$) which corresponded to $C_{20}H_{32}O_4$.

The ^1H NMR spectrum of euphornin showed three olefinic protons (δ 5.08, *d*; 5.87; *dd*; 6.06, *br. d*) which retained their respective chemical shift values in deacyl euphornin (5.11, *d*; 5.60, *dd*; 5.87 *br. d*) confirming the presence of two double bonds in the molecule. The ^1H NMR spectrum also contained signals due to two tertiary methyls (1.06, 0.93), two secondary methyls (1.01, *d*, $J = 6.5$ Hz; 1.07, *d*, $J = 7$ Hz) and an olefinic methyl (1.49, *d*, $J = 1.5$ Hz). Its IR spectrum exhibited absorptions for a hydroxyl group (3500, 1040 cm^{-1}), but it could not be acetylated, indicating its tertiary nature. This was confirmed by the observation of $M - H_2O$ ions in the mass spectra of the substance and its derivatives. Therefore, the molecular structure of deacyl euphornin included two rings, two double bonds and five hydroxyl groups.

On acetylation deacyl euphornin gave a tetraacetyl derivative whose IR spectrum showed strong bands at 1720 and 1225 cm^{-1} (OCOMe) and the mass spectrum exhibited fragment ions at *m/z* 480, 402, 360, 342 as a result of the loss of four acetoxyl groups as ketene or acetic acid. Its ^1H NMR spectrum was in conformity with that of euphornin except that the H-12 signal at δ 5.18, which was more downfield in euphornin (5.53), indicated the attachment of the benzoate moiety at C-12.

The ^1H NMR spectral data of the tetraacetate led to the elucidation of the carbon skeleton and the relative configurations at the asymmetric centres. Irradiation of the olefinic signal at δ 5.85 collapsed the signal at 2.51 to a doublet of quartets and the doublet at 5.08 to a singlet. Irradiation at 2.51 collapsed the doublets at 1.07 and 4.92 to singlets thus indicating the sequence **A**. Further spin decoupling starting with the olefinic signal at 5.90 led to the sequence **B**, which had to be linked with the sequence **C**, as on irradiation at 5.90 the signal at 5.18 was sharpened. The latter was coupled with signals at 2.09 and 1.85, which also coupled with the broadened doublet at 5.13. Furthermore, the fragments **D** and **E** must be present. If a normal diterpene skeleton was present, only structure **3** was in agreement with these findings.

Inspection of models showed that the couplings observed were in good agreement with the stereochemistry at C-1 through C-12, while that at C-14 could not be assigned with certainty. However, the absolute configuration could not be determined. The ^1H NMR spectral data of **1** and **2** (Table 1) were also in agreement with the structures proposed. Therefore, euphornin (**1**) most probably was a bicyclic jatraphone derivative.

Attempts were made to prepare other derivatives of euphornin. The catalytic hydrogenation of euphornin for 70 hr led to a crystalline hydro derivative as a result of saturation of the disubstituted double bond and hydrogenolysis of the allylic benzoate. Similar hydrogenation of deacyl euphornin for 2.5 hr showed the formation of the dihydro product. Acetonide formation from deacyl euphornin resulted in a complex mixture of products from which a crystalline derivative was obtained in about 10 % yield but this was not investigated further.

Euphorbia species are rich in diterpenes of the tigliane, ingenane and daphnane groups, which are also related to

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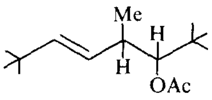
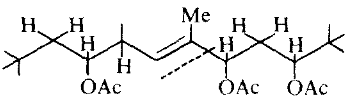
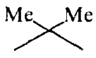


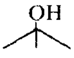
A



B **C**



D



E

the rare jatrophone group, since all these diterpenoids are biogenetically derived via casbene-type compounds [1]. Until now only three substances having the jatrophone skeleton have been isolated, namely jatrophone from *Jatropha gossypiifolia* [2] and kansuanines A and B from *Euphorbia kansui* [3,4], and since both these plants also belong to the Euphorbiaceae, the present findings may be of taxonomic significance.

Table 1. The 270 MHz ^{1}H NMR spectral data of euphornin (**1**) and its derivatives

	1 (C_6D_6)80°	**2** (acetone-d_6)	**3** (C_6D_6)80°	
Proton	Chemical shift	Chemical shift	Chemical shift	Δ*
1-H	5.08 *d*	5.11 *d*	5.08 *d*	1.04
2-H	5.87 *dd*	5.60 *dd*	5.85 *dd*	1.12
3-H	2.52 *ddq*	—	2.51 *ddq*	0.97
4-H	4.98 *d*	—	4.92 *d*	1.51
6-H	1.85 *m*	1.75 *dd*	1.70 *dd*	1.28
6′-H	2.25 *dd*	—	2.18 *dd*	1.10
7-H	1.85 *m*	—	1.85 *m*	0.55
8-H	5.12 *dd*	3.77 *br. s*	5.21 *dd*	2.28
9-H	2.69 *dd*	2.70 *dd*	2.63 *dd*	1.73
10-H	6.06 *br.d*	5.87 *br.d*	5.90 *br.d*	3.78
12-H	5.53 *dd*	3.47 *br. s*	5.18 *dd*	3.22
13-H	2.06 *ddd*	1.80 *ddd*	2.09 *ddd*	1.43
13′-H	1.85 *m*	—	1.85 *m*	1.84
14-H	5.06 *br. d*	4.27 *br. d*	5.13 *br. d*	3.07
16-H_3	1.06 *s*	1.04 *s*	1.08 *s*	0.82
17-H_3	0.93 *s*	0.86 *s*	0.95 *s*	0.56
18-H_3	1.49 *d*	1.75 *d*	1.50 *d*	0.50
19-H_3	1.01 *d*	1.02 *d*	1.00 *d*	0.19
20-H	1.07 *d*	1.05 *d*	1.07 *d*	0.60
OAc	1.85 *s*	—	1.93 *s*	2.07
	1.73 *s*	—	1.89 *s*	1.41
	1.41 *s*	—	1.82 *s*	0.79
	—	—	1.80 *s*	0.65
OCOPh	7.16 *m*	—	—	—
	8.29 *m*	—	—	—

* Chemical shift difference after addition of Eu $(fod)_3$.

Chemical shifts in ppm *J*(Hz): 1,2 = 15; 2,3 = 8; 3,4 = 3.5; 3,20 = 7; 6,6′ = 12; 6,7 = 12; 6′,7 = 7; 7,8 = 4.5; 8,9 = 5; 9,10 = 10; 10,18 = 1.5; 12,13 = 3.5; 13,13′ = 16; 13,14 = 6.5; 13′,14 = < 1. All signals have been assigned by systematic spin decoupling experiments.

EXPERIMENTAL

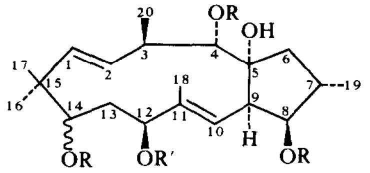
Mps are uncorr. The plant material from Kullu, Himachal Pradesh, India, was collected and identified by Dr. K. K. Singh of C.D.R.I. and a voucher specimen (No. 5708) has been preserved in the Institute's herbarium. The dry whole plant (3.5 kg) was exhaustively extracted with C_6H_{14}, followed by $CHCl_3$. The $CHCl_3$ extract (52 g) was chromatographed on Si gel in C_6H_6 containing increasing amounts of MeOH. The residue from the C_6H_6–MeOH (9:1) eluate was rechromatographed on Si gel when the C_6H_{14}–Et_2O (3:2) eluate showed the presence of a single spot on TLC, R_f 0.35 (C_6H_{14}–Et_2O, 1:1) which was concd and crystallized from Et_2O to yield euphornin, 0.25 g.

Euphornin. Colourless needles, mp 180°, $[\alpha]_D$ −25° (*c*, 1.3 MeOH); λ_{max}^{MeOH} nm: 228 (log ε 4.16). ν_{max}^{KBr} cm^{-1}: 3500 (OH), 2980, 2880, 1740, 1705 (—OCOR), 1600, 1500 (aromatic), 1370, 1280, 1245, 1138, 1040, 965, 720. CIMS (*i*-butane) *m/z* (rel. int.): 567 $[M + 1 - H_2O]^+$ (6.15), 525 $[567 - \text{ketene}]^+$ (50.41), 507 $[567 - HOAc]^+$ (78.75), 465 $[507 - \text{ketene}]^+$ (100), 447 $[507 - HOAc]^+$ 36.15, 405 $[447 - \text{ketene}]^+$ (23.1), 403 $[525 - C_6H_5COOH]^+$ (15.7), 387 (7.32), 343 (71.6), 325 (31.0), 265 (15.3). (Found: C, 67.54; H, 7.23. $C_{33}H_{44}O_9$ requires: C, 67.81; H, 7.54 %).

Deacyl euphornin. Euphornin (100 mg) was refluxed in 5 % methanolic KOH soln for 1.5 hr. The reaction mixture was diluted with H_2O and extracted with Et_2O to give a residue (50 mg) which crystallized from MeOH–Et_2O as colourless needles, mp 186°. $[\alpha]_D$ −67.3° (*c*, 1.2 MeOH). ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2900, 2825, 1438, 1055, 983, 920, 760. MS *m/z* (rel. int.): 336.230 $[M^+ - H_2O]^+$ (3), ($C_{20}H_{32}O_4$), 318 $[336 - H_2O]^+$ (9), 300 $[318 - H_2O]^+$ (4), 222.126 $[C_{13}H_{18}O_3]^+$ (33), 96.094 $[C_7H_{12}]^+$ (100). (Found: C, 68.69; H, 9.61. $C_{20}H_{34}O_5$ requires C, 68.19; H, 9.66 %). The aq. phase was acidified, extracted with Et_2O and the solvent layer was evapd to give a residue (18 mg) which crystallized from hot H_2O, mp 120° and was identified as benzoic acid.

Deacyl euphornin tetraacetate. A soln of deacyl euphornin (10 mg) in dry $CHCl_3$, Ac_2O (0.1 ml) and *p*-dimethylaminopyridine (3 mg) was allowed to stand for 3 days. The reaction product was purified on a Si gel column to give a colourless, amorphous powder (10 mg), ν_{max}^{KBr} cm^{-1}: 3460 (OH), 3000, 2920, 1720, (–OCOMe), 1430, 1360, 1225, 1005, 742, 705. MS *m/z* (rel. int.); 480 $[M - \text{ketene}]^+$ (1), 462 $[480 - H_2O]^+$ (1), 402 $[462 - HOAc]^+$ (6), 360 $[402 - \text{ketene}]^+$ (10), 342 $[402 - HOAc]^+$ (100). CIMS (*i*-butane) *m/z* (rel. int.): 505 $[M + 1 - H_2O]^+$ (1), 463 $[M + 1 - HOAc]^+$, 445 $[505 - HOAc]^+$, 403 $[463 - HOAc]^+$ (52), 343 $[403 - HOAc]^+$ (100), 283 $[343 - HOAc]^+$ (91).

Hydrogenation of euphornin. Euphornin (30 mg) in alcoholic soln and PtO_2 (35 mg) were shaken in a H_2 atmosphere. After 3 hr, TLC of the reaction mixture showed that the original substance was absent but two spots had appeared and further



1 R = Ac; R′ = COPh
2 R = R′ = H
3 R = R′ = Ac

continuation of the reaction showed a gradual disappearance of the lower spot. After 70 hr when the reaction mixture contained only a single upper spot, it was worked up and afforded a colourless, amorphous powder, (15 mg). ^{1}H NMR(C_6D_6): δ 0.85, 0.92 (3 H each, *s*, 2 × Me), 0.95, 0.98 (3 H each, *d*, *J* = 6 Hz, 2 × CH—Me), 1.47 (3 H, *d*, *J* = 1.5 Hz, C=C–Me), 1.58 and 1.73 (*m*), 1.81, 1.86, 1.96 (3 H each, *s*, OCOMe), 2.04, 2.13 (*m*), 2.35 (*dd*, *J* = 12, 8 Hz), 2.50 (1 H, *dd*, *J* = 11, 4 Hz, 9-H), 2.63 (*m*), 4.87 (1 H, *d*, *J* = 2 Hz, 4-H), 5.2 (*d*), 5.28 (*t*), 5.7 (*d*, *J* = 6.5 Hz) 6.23 (1 H, *br. d*, *J* = 11 Hz, 10-H), MS *m/z* (rel. int.): 472 (2.13), 422 (1.9), 412 (1.48), 404 (3.26), 362 (15.7), 344 (100),. 302 (7.72), 284 (8.7).

Hydrogenation of deacyl euphornin. Deacyl euphornin (7 mg) and PtO_2 (5 mg) in EtOH were shaken in a H_2 atmosphere for 2.5 hr. The reaction product (6 mg) crystallized from MeOH–C_6H_6 as colourless needles of the dihydro derivative, mp 90°. MS *m/z* (rel. int.): 338 $[M^+ - H_2O]^+$ (3.8), 320 $[338 - H_2O]^+$ (23), 302 $[320 - H_2O]^+$ (3.4), 249 (3.9), 235 (7.2), 233 (5.1), 207 (4), 193 (12), 191 (8.3), 183 (3.8), 177 (3.4), 175 (4.5), 167 (13), 163 (11.7), 157 (16.6), 155 (15.2), 149 (30.4), 139 (24.6), 137 (30.1), 135 (24.1), 127 (20.8), 122 (59.6), 111 (12.1), 109 (36.1), 107 (18.4), 95 (100), 93 (61.1), 69 (35.9), 55 (38.6). The hydrogenation of deacyl euphornin tetraacetate in alcoholic soln with PtO_2 gave a dihydrotetraacetate. MS *m/z* (rel. int.): 464 $[M - H_2O - \text{ketene}]^+$ (1), 404 $[464 - \text{HOAc}]^+$ (4), 344 $[404 - \text{HOAc}]^+$ (100), 284 $[344 - \text{HOAc}]^+$ (6).

Attempted acetonide formation of deacyl euphornin. Deacyl euphornin (35 mg) in Me_2CO (10 ml) and *p*-TSA (35 mg) were stirred for 5 hr. The reaction mixture was neutralized and extracted with Et_2O. The residue from the solvent layer showed six spots on TLC and was chromatographed on Si gel in $CHCl_3$–MeOH (95:5). The major component was obtained as colourless needles from C_6H_6–Et_2O, 3 mg, mp 105°.

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