Phytochemistry, Vol 28, No 6, pp 1699–1702, 1989
Printed in Great Britain

0031–9422/89 $3.00+0.00
Pergamon Press plc

LOLIOLIDE AND OLEAN-12-EN-3β,9α,11α-TRIOL FROM *EUPHORBIA SUPINA*

REIKO TANAKA and SHUNYO MATSUNAGA*

Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan

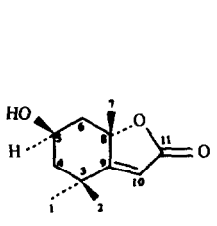
(*Received* 27 *September* 1988)

Key Word Index—*Euphorbia supina*; Euphorbiaceae; monoterpene; loliolide; triterpene; olean-12-en-3β,9α,11α-triol

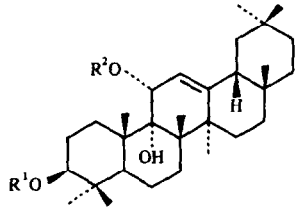
Abstract—Olean-12-en-3β,9α,11α-triol was isolated together with the known monoterpene lactone, loliolide, from the whole herb of *Euphorbia supina*.

INTRODUCTION

Euphorbia supina Rafin., an annual weed used as a folk medicine in Taiwan [1], contains various biogenetically interesting triterpenoids such as hopane [2], spirosupinane [3], dammarane [4], fernane [5, 6], friedelane, multiflorane, oleanane, simiarane, taraxastane and taraxerane [5] derivatives. Further examination of the neutral benzene extract [2] of the whole herb by silica gel column chromatography resulted in the isolation of a new triterpene triol (**2**) as a minor constituent, together with the known monoterpene lactone, loliolide (**1**) [7–19]. This paper deals with the isolation and characterization of compounds **1** and **2**.



1



2 $R^1 = R^2 = H$

2a $R^1 = R^2 = Ac$

RESULTS AND DISCUSSION

Compound **1**, $C_{11}H_{16}O_3$ (HRMS), contained a hydroxyl group and an α,β-unsaturated-γ-lactone ring (IR and UV spectra). The ^1H and ^{13}C NMR spectra indicated signals due to three tertiary methyl groups, a secondary hydroxyl group, a trisubstituted ethylene bond and a lactone carbonyl (Tables 1 and 2). Its physical and spectral data coincided with those of loliolide which has previously been isolated from a considerable number of plant species including several algae [7–19], and compound **1** was identified by direct comparison with authentic material [9]. Detailed analysis of its ^{13}C NMR spectrum employing ^1H–^1H 2D COSY, ^1H–^{13}C 2D COSY and long range ^1H–^{13}C 2D COSY experiments indicated that assignments of carbon signals for C-1, C-2, C-4, C-6 and C-7 in compound **1** already given in the literature [11, 12, 18, 19] should be corrected as presented in Table 2.

Compound **2**, $C_{30}H_{50}O_3$ (M^+ at *m/z* 458.3760) was isolated as a minor component. Its ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed signals for eight tertiary methyl groups, two secondary hydroxymethine groups, a tertiary hydroxyl group and a trisubstituted olefinic bond. Acetylation of compound **2** gave a diacetate (**2a**) in which the tertiary hydroxyl group (IR 3450 cm^{-1}; δ79.38) still remained unchanged. The ^1H NMR signal patterns of methyl resonances in compounds **2** and **2a** suggested that **2** might be an olean-12-ene derivative [20]. One of the two hydroxymethine groups in compound **2**, which showed a ^1H NMR signal at δ3.24 (*dd*), was probably in the usual C-3β position. The second one was an allylic hydroxymethine which exhibited an ^1H NMR signal at δ4.53 (*d*, $J = 2.9$ Hz) with the same coupling constants as the trisubstituted olefinic proton. In compound **2a**, the above two hydroxymethine signals were shifted to δ4.47 (*dd*) and 5.74 (*d*), respectively. The doublet nature of the allylic hydroxymethine proton signal indicated that there was only one hydrogen atom on the neighbouring carbons combined with this function. If compound **2** has a tertiary hydroxyl group at C-9 in the olean-12-ene skeleton, the second hydroxyl can reasonably be located at the less hindered 11α-position, because the signal in question shows a relatively small coupling constant. Both ^{13}C NMR and EI-mass spectra of compounds **2** and **2a** strongly supported this assumption. Signals at δ78.99 in **2** and 79.38 in **2a** were attributed to the tertiary carbinol at C-9 (Table 2). In the mass spectrum (Scheme 1), compound **2** showed characteristic fragment ion peaks arising from the cleavage of the B and C rings at *m/z* 287.2380 (ion **a**) and 273.2218 (**b**) and at *m/z* 234.1977 (**c**), 219.1722 (**d**) and 189.1625 (**e**), respectively, together with peaks at *m/z* 440 $[M-H_2O]^+$, 425 $[M-H_2O-Me]^+$, 422 $[M-2H_2O]^+$ and 407 $[M-2H_2O-Me]^+$, indicating the presence of the three hydroxyl groups at C-3, C-9 and C-

*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR (300 M Hz) chemical shifts of compounds **1**, **2** and **2a** in $CDCl_3$

H	1	2	2a
Me-1	1 47	- -	- -
Me-2	1.27	—	—
Me-7	1 79	—	—
Me-23	- -	1.03	0 95
Me-24	—	0 80	0 86
Me-25	—	1.23	1 23
Me-26	—	1 14	1 18
Me-27	-	1 38	1 41
Me-28	—	0 85	0.83
Me-29	—	0 888	0 89
Me-30	- -	0 875	0 86
H-3	—	3 24 *dd*	4 47 *dd*
		J 11 5, 5.7	*J* 11.5, 5 7
H-4	1 53 *dd*	—	
	J 14 0, 3 5		
	1 99 *dt*		
	J 14 0, 2 5		
H-5	4 33 *dd*	—	—
	J 7 5, 3 5		
H-6	1 78 *dd*	—	- -
	J 13 5, 3 9		
	2 47 *dt*	—	—
	J 13.5, 2 5		
H-10	5 69 *s*	—	—
H-11	—	4 53 *d*, *J*, 2 9	5 74 *d*, *J* 2.9
H-12	-	5 11 *d*, *J* 2 9	5.00 *d*, *J* 2 9
OCOMe	—	—	2.04
	—	—	2 06

Table 2 ^{13}C NMR (74 5 M Hz) chemical shifts of compounds **1**, **2** and **2a** in $CDCl_3$

C	1	2	2a	3*
1	30 69	32.99	32 29	38 73
2	26.50	27 43	23 74	27.29
3	35.97	78.20	80 08	78.98
4	47 32	38 97	37 80	38.84
5	66 77	48.28	48 30	55 29
6	45 67	18.46	18 24	18.45
7	27.00	30 47	30.50	32 80
8	86.96	46 87	47.29	38.78
9	172 05	78.99	79 38	47 74
10	112 88	44.82	44.88	37 60
11	182 68	67 24	71 01	23 57
12		123 21	119 03	121.8
13		149 04	150 14	145.10
14		43.33	43 78	41.80
15		27 67	27.62	26 22
16		26 81	26 89	27 02
17		32 85	33 28	32 47
18		46.82	46 78	47 36
19		45 27	44 97	46 93
20		31.02	30 99	31.07
21		34.77	34 73	34.79
22		36 86	36 82	37 22
23		28 15	28 10	28 21
24		15 46	16.48	15 48
25		20 20	20 25	15 59
26		19.62	19 87	16 88
27		27 55	27.11	26.00
28		28 72	28 73	28 43
29		33 30	32.90	33 34
30		23 58	23.58	23 73
OCOMe			21 30	
OCOMe			21 92	
OCOMe			169.97	
OCOMe			170 99	

*β-Amyrin Data were quoted from ref. [21]

11 positions on the olean-12-ene skeleton in **2**. Although compound **2a** did not give a parent ion peak, it showed the same ion peaks, **a**–**e** (see Experimental) to **2**, together with peaks at *m/z* 482.3753 $[M-HOAc]^+$, 467 $[M-HOAc-Me]^+$, 440 $[M-HOAc-CH_2CO]^+$, 422.3395 $[M-2HOAc]^+$ and 407 $[M-2HOAc-Me]^+$. All the above results suggested the structure of compound **2** to be olean-12-en-3β,9α,11α-triol.

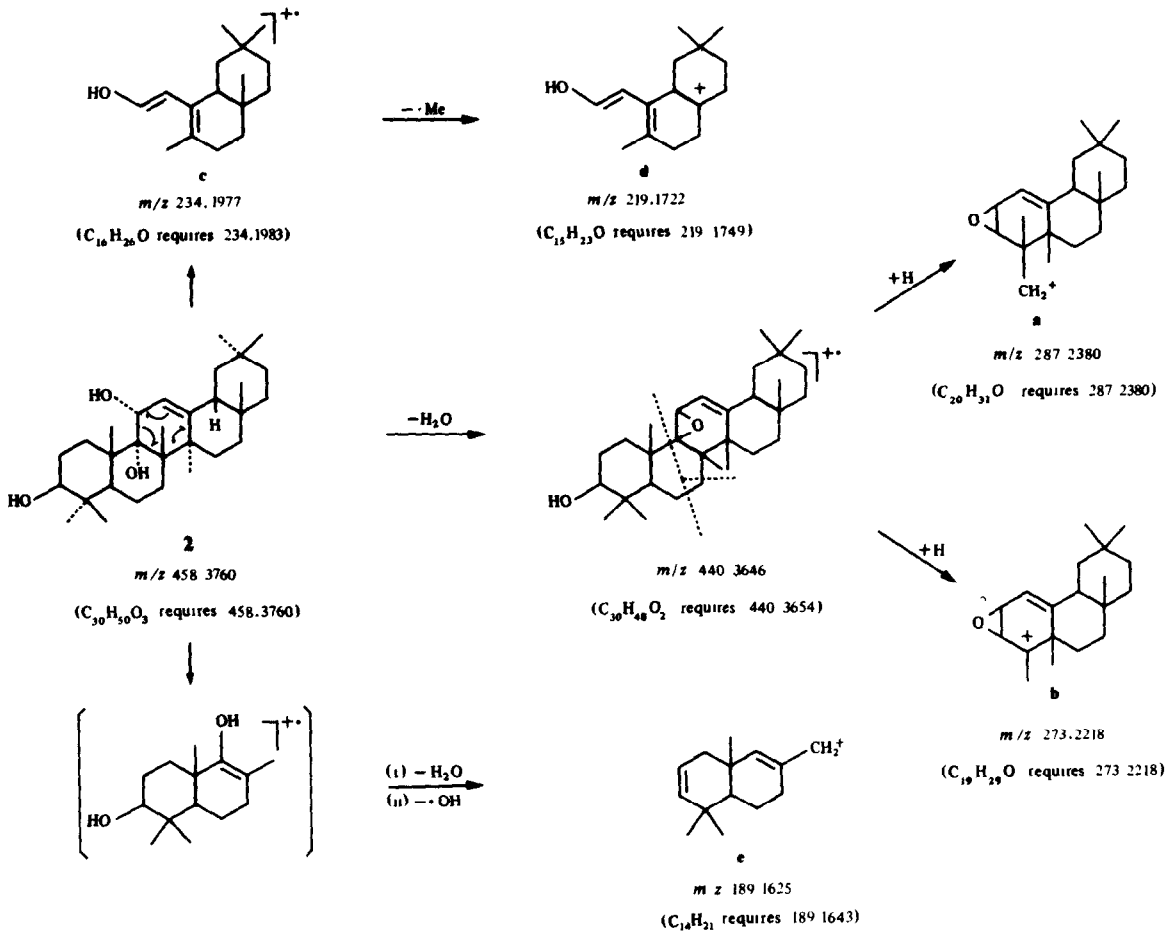
In order to confirm this structure, we attempted to apply ^{1}H–^{1}H 2D COSY, NOESY, ^{1}H–^{13}C 2D COSY and long range ^{1}H–^{13}C 2D COSY experiments to compounds **2** and **2a**. The presence of the tertiary hydroxyl group at C-9 was proved by linking of the carbon signal at position C-9 to proton signals of methyl groups at C-25 and C-26 in the 2D long range ^{1}H–^{13}C COSY spectrum of compound **2**. The configurations of both 11α-hydroxyl group and the D/E ring juncture of compound **2** were determined by the NOE experiments. Upon selective irradiation to the signal of δ1 23 (25-Me), NOEs were observed for signals of 11β-H (14.5%), 24-Me (11.3%) and 26-Me (13.4%). Irradiation of the signal at δ1.14 (26-Me) gave 14.2% NOE for the signal of 11β-H. On the other hand, a 5.1% NOE has also been observed between signals of the 27-methyl group and the 19α-proton (Fig. 1), indicating the rings D/E to be *cis* fused [22]. Unambiguous assignments of the ^{1}H and ^{13}C NMR signals for compounds **2** and **2a** are given in Tables 1 and 2. Consequently, the structure of compound **2** proved to be olean-12-en-3β,9α,11α-triol, for which this appears to be the first report

EXPERIMENTAL

General. Mps: uncorr optical rotations: $CHCl_3$. UV: EtOH. IR: KBr discs. ^{1}H NMR (300 M Hz) and ^{13}C NMR (74.5 M Hz): $CDCl_3$ with TMS as int. standard. EIMS (probe). 70 eV. CC: silica gel 60 (70–230 mesh, Merck) and alumina 90 (70–230 mesh, Merck). TLC. silica gel HF_{254} and PF_{254} (Merck).

Extraction and isolation of compounds. The preliminary CC of the neutral C_6H_6 extract (1.15 kg) of the dried whole herb of *E supina* (10 kg) has already been reported [2, 4] After 3β-hydroxyhexanordammaran-20-one [4] has been eluted, the column was further washed with $CHCl_3$ and $CHCl_3$–EtOAc (10·1) to give an amorphous solid (43 2 g), which was dissolved in a mixture of C_6H_6–$CHCl_3$ (1 1) and the soln was subjected twice to CC on silica gel (1 8 and 0 8 kg) Elution of the column with a mixture of C_6H_6–$CHCl_3$ (1·1) furnished loliolide (**1**) (314 mg). Subsequent CC afforded compound **2** (12 mg) from the fractions eluted with $CHCl_3$.

Loliolide (**1**) Mp 148.5–149° (*n*-hexane–$CHCl_3$). $[\alpha]_D^{23}$ −87° ($CHCl_3$, *c* 0.66) {lit. [19] mp 149 5°, $[\alpha]_D^{20}$ −93.2° (MeOH, *c* 2 0)} HRMS M^+ at *m/z* 196 1102 ($C_{11}H_{16}O_3$ requires 196.1099), UV λ_{max}^{EtOH} nm 217 (ε11 000); IR ν_{max}^{KBr} cm^{-1}· 3435, 2965, 2940, 2915, 2870, 1740, 1720, 1620, 1163, 1095, 1020, 958, 865,



Scheme 1.

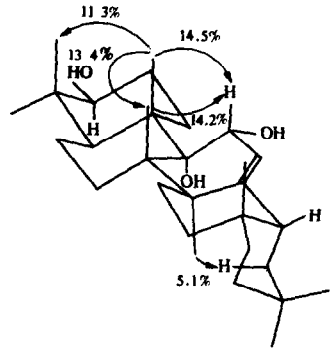


Fig. 1. NOE difference spectrum of **2**.

EIMS *m/z* (rel. int.): 196 $[M]^+$ (40), 181 (10), 178 (78), 163 (27), 153 (21), 140 (54). The above data were in good agreement with those already reported for loliolide [7–19], and compound **1** was identified by direct comparison (mmp, co-TLC, IR, ^{1}H NMR, ^{13}C NMR and EIMS) with authentic material [9].

Olean-12-en-3β,9α,11α-triol (**2**). Mp 242–244° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ −11° (*c* 0.33, $CHCl_3$); R_f 0.31 (MeOH–$CHCl_3$, 20:1); IR ν_{max}^{KBr} cm^{-1}: 3630–3100 (OH), 2920, 2860, 1640, 1465, 1450, 1380, 1360, 1040, 1020, 852; EIMS *m/z* (rel. int.): 458 $[M]^+$ (2), 440 (38), 425 (7), 422 (12), 407 (3), 287 (ion **a**, 14), 273 (ion **b**, 18), 271 (16), 234 (ion **c**, 18), 219 (ion **d**, 12), 189 (ion **e**, 13), 135 (100)

9α-Hydroxy-olean-12-en-3β,11α-yl diacetate (**3**): Compound **2** (6 mg) was acetylated (Ac_2O–pyridine, 1:1, 2 ml) at room temp. overnight. Working-up as usual afforded a residue, which was recrystallized from MeOH to give a diacetate (**2a**), mp 115–117°, $[\alpha]_D^{23}$–42° ($CHCl_3$, *c* 0.40); IR ν_{max}^{KBr} cm^{-1}: 1727, 1640, 1386, 1360, 1240, 1045, 1010, 872; EIMS: *m/z* (rel. int.): 482 (100), 467 (2), 440 (2), 422 (16), 407 (1), 287.2343 (ion **a**, 6), 273 2232 (ion **b**, 18), 234.1975 (ion **c**, 12), 219 1753 (ion **d**, 10), 189.1649 (ion **e**, 43), 135 (81)

Acknowledgements—The authors are grateful to Dr T. Wada (Shionogi Pharmaceutical Industry Co. Ltd) for a gift of the authentic sample of loliolide. Thanks are also due to Miss M. Nabae of this University for measurements of NMR spectra.

REFERENCES

1. Nanba, T. and Mikage, M. (1983) *Poisonous Plants*, p. 74. Hoikushya, Tokyo.
2. Matsunaga, S. and Morita, R. (1983) *Phytochemistry* **22**, 605.
3 Matsunaga, S., Morita, R, Ishida, T, Inoue, M., Shigi, M. and Miyamae, A. (1984) *J. Chem. Soc, Chem. Commun.* 1128.

4. Tanaka (née Morita), R., Matsuda, M. and Matsunaga, S. (1987) *Phytochemistry* **26**, 3365
5. Tanaka, R and Matsunaga, S (1988) *Phytochemistry* **27**, 3579
6. Tanaka, R., Matsunaga, S. and Ishida, T (1988) *J Chem Soc., Chem. Commun* (in press)
7. Suchy, M., Herout, V (1961) *Collect Czech Chem Commun.* **26**, 690
8. Hodges, R. and Porte, A. L (1964) *Tetrahedron* **20**, 1463
9. Wada, T. (1965) *Chem. Pharm. Bull* **13**, 43.
10. Goshal, S., Singh, A K and Chaudhuri, R (1976) *J. Pharm. Sci.* **65**, 1549
11. Toth, G., Haznagy, A. and Bula, E. (1976) *Pharmazie* **31**, 51
12. Behr, D., Wahlberg, I, Nishishida, T and Enzela, C. R (1979) *Acta Chem Scand.* **B33**, 701
13 Pettit, G R., Helard, C L., Ode, R H, Brown, P, Gust, D. J and Michel, C (1980) *J Nat. Prod.* **43**, 752
14 Rao, Ch. B and Pullaiah, K. Ch. (1982) *Indian J Chem Sect B* **21**, 605.
15 Klok, J., Baas, M, Cox, H. C, de Leeuw, J W. and Schenck, P A (1984) *Tetrahedron Letters* **25**, 5577
16. Kuniyoshi, M (1985) *Bot. Marina* **28**, 501
17 Okunade, A L. and Wiemer, D F (1985) *J Nat Prod* **48**, 472.
18 Valdes III, L J. (1986) *J Nat Prod* **49**, 171
19 Willuhn, G and Westhaus, R G (1987) *Planta Med.* **53**, 304
20 Tanaka, R and Matsunaga, S (1988) *Phytochemistry* **27**, 2273.
21. Knight, S. A. (1974) *Org. Magn. Reson.* **6**, 603
22. Begly, M. J., Crombie, W. M L. and Whiting, D A. (1986) *J Chem Soc Perkin I* 1905