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A 3,4-*SECO*-8βH-FERNADIENOIC ACID AND OTHER CONSTITUENTS FROM *EUPHORBIA CHAMAESYCE*

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Key Word Index—*Euphorbia chamaesyce*; Euphorbiaceae; whole herb; triterpene; cycloart-23*Z*-en-3β, 25-diol; 3,4-*seco*-8βH-ferna-4(23), 9(11)-dien-3-oic acid.

Abstract—A new *seco*-triterpene-dienoic acid was isolated, together with three known triterpenes, glutinol, 3β-hydroxymultiflor-8-en-7-one and cycloart-23*Z*-en-3β, 25-diol, from the whole herb of *Euphorbia chamaesyce*, and the structure was established as 3,4-*seco*-8βH-ferna-4(23),9(11)-dien-3-oic acid on the basis of chemical and spectral evidence.

INTRODUCTION

Recently, we reported the structure of 3,4-*seco*-oleana-4(23), 18-dien-3-oic acid, isolated together with the known lupeol, butyrospermol, 11α,12α-oxidotaraxerol and 3β-hydroxy-30-nor-lupan-20-one from the methylene chloride extract of the whole herb of *Euphorbia chamaesyce* L. [1]. A further search for constituents in the extract led to the isolation of another new 3,4-*seco*-triterpene acid (**4**), in addition to three known triterpeniods (**1**–**3**). This paper deals with the characterization of the above compounds.

RESULTS AND DISCUSSION

Two of the known compounds were identified by direct comparison with authentic samples of glutinol (**1**) [2] and 3β-hydroxymultiflor-8-en-7-one (**2**) [3]. The remaining one was proved to be cycloart-23*Z*-en-3β,25-diol (**3**), as its physical and spectral data were in good agreement with those for the compound isolated from *Juncus effusus* [4] except for the ^{1}H NMR signals of the *cis*-disubstituted olefinic protons, which had been reported to be δ5.59 (2H, *m*, $W_{1/2}$ = 8 Hz) and are now shown to be δ5.60 (2H, *dd*, *J* = 3.1 and 4.1 Hz).

Compound **4** was assigned the molecular formula $C_{30}H_{48}O_2$ by its HR-EI mass spectrum. The IR spectrum showed the presence of a carboxyl group, a terminal methylene group and a trisubstituted double bond [ν_{max} cm^{-1}: 1708, 1648 and 900 (>C=CH$_2$) and 1617 and 821 (−CH=C<)], while the UV spectrum exhibited only a terminal absorption. In the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), compound **4** revealed signals due to four quaternary methyl groups, two secondary methyl groups [δ_H0.83 and 0.89 (each 3H, *d*, *J* = 6.5 Hz)], a vinylic methyl group [δ_H1.71 (3H, *s*)], a terminal methylene group [δ_H 4.78 and 4.90 (each 1H, *d*, *J* = 1.8 Hz); δ_C113.1 (*t*) and 146.0 (*s*)], a trisubstituted double bond [δ_H5.33 (1H, *ddd*, *J* = 5.8, 2.0 and 2.0 Hz); δ_C 119.3 (*d*) and 141.7 (*s*)] and a carboxyl group [δ_C180.7 (*s*)]. The DEPT spectrum indicated that the carbon skeleton of **4** was composed of seven methyls, nine methylenes, five methines, four quaternary sp^3 carbons, one sp^2 methylene, one sp^2 methine, two quaternary sp^2 carbons and one carboxyl carbon. These data suggested **4** to be an unknown tetracyclic triterpene-dienoic acid. Treatment of **4** with diazomethane afforded the corresponding methyl ester (**4a**) [ν_{max} 1742 cm^{-1}; δ_H3.65 (3H, *s*, CO$_2$Me)]. In the EI mass spectrum of **4** there were two significant fragment ion peaks due to [M–C$_3$H$_7$]$^+$ (ion *a*) and [M–CH$_2$CH$_2$CO$_2$H]$^+$ (ion *b*) at *m*/*z* 397.3095 and 367.3369, respectively, as well as those at *m*/*z* 411.3263 and 367.3369 in **4a**, suggesting **4** to have an isopropyl group and a propionic acid moiety in the molecule. Information on the structure was obtained by employing two-dimensional (2D) ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, long range ^{1}H–^{13}C COSY and EI mass spectral experiments. The 2D long range ^{1}H–^{13}C COSY data provided the carbon framework of **4**, as shown in Fig. 1. In the HR-EI mass spectrum (Scheme 1), compound **4** showed a predominant fragment ion peak at *m*/*z* 339.3047, which was assigned to ion *c* caused by the elimination of an ethylene molecule from the B-ring of ion *b*. The most characteristic feature of the fragmentation of **4** was the appearance of two predominant peaks at *m*/*z* 372.3028 (ion *d*) and 359.2945 (ion *f*, base peak) arising from a retro-Diels–Alder cleavage of the B-ring, followed by the fission of either the C-6/C-7 or C-7/C-8

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Table 1. ^{1}H NMR spectral data for compounds **4**, **4a**, **5**, **6** and **7** ($CDCl_3$, TMS)*

	δ_H				
H	**4**	**4a**	**5**	**6**	**7**
H-1	1.74, 1.98		1.14, 1.89		1.80, 2.07
H-2	2.50		1.44, 1.55		2.39, 2.72
H-3	—		1.11, 1.36		—
H-4	—		—		—
H-5	2.02		1.26		1.38, 1.86
H-6	1.47, 1.97		1.72, 1.56		1.36
H-7	1.44, 1.63		1.60, 1.33		1.56, 1.66
H-8	2.03		2.06		2.10 *dd* (3, 6)
H-9	—		—		—
H-10	—		—		—
H-11	5.33 *ddd* (5.8, 2.0, 2.0)	5.32 *ddd* (5.8, 2.0, 2.0)	5.29 *ddd* (5.1, 2.4, 2.4)	5.13 *dd* (3.6, 3.6)	5.29 *br d* (6)
H-12	1.79 (α), 1.56 (β)		1.62, 1.51		1.52, 1.72
H-13	—	—	—	—	—
H-14	—	—	—	—	—
H-15	1.42 (α), 1.32 (β)		1.40, 1.32		1.27, 1.50
H-16	1.66 (α), 1.38 (β)		1.65, 1.40		1.41, 1.68
H-17	—	—	—	—	—
H-18	1.61		1.56		1.62
H-19	1.27, 1.37		1.35		1.37
H-20	1.22, 1.85		1.21, 1.83		1.22, 1.85
H-21	0.99		0.97		0.98
H-22	1.43		1.45		1.47
H-23	4.78 *d* (1.8)	4.78 *d* (1.8)	0.847	0.898	1.07
	4.90 *t* (1.8)	4.89 *t* (1.8)	—	—	—
Me-24	1.71	1.71	0.888	0.830	1.07
Me-25	1.02	1.01	1.053	1.020	1.22
Me-26	0.78	0.77	0.733	0.898	0.81
Me-27	0.85	0.84	0.822	0.830	0.80
Me-28	0.76	0.75	0.759	0.762	0.77
Me-29	0.89 *d* (6.5)	0.89 *d* (6.5)	0.894 *d* (6.4)	0.898 *d* (6.7)	0.89 *d* (6)
Me-30	0.83 *d* (6.5)	0.83 *d* (6.5)	0.830 *d* (6.4)	0.830 *d* (6.7)	0.83 *d* (6)
CO_2Me	—	3.65	—	—	—

*Measured at 400 MHz. Data for compounds **5**, **6** and **7** cited from refs [13], [14] and [15], respectively.

bond accompanying the rearrangement of a H-6 to C-11. Ion *d* provided a satellite peak at *m/z* 221.1547 (ion *e*) by the further fission of the D-ring; as well as ion *f* there were two satellite peaks at *m/z* 209.1565 (ion *g*) and 207.1385 (ion *h*). Fragment ion peaks due to the D- and E-rings were observed at *m/z* 205.1956 (ion *i*) and 191.1799 (ion *j*). Similarly, peaks corresponding to ions *c* – *j* were confirmed in the spectrum of **4a**. All these data suggested **4** to be a 3,4-*seco*-4(23),9(11)-dien-3-oic acid derived from either fern-9(11)-en-3-ol [5, 6] or arbor-9(11)-en-3-ol [7–10] via oxidative fission of the C-3/C-4 bond in the plant [11]. The ^{1}H and ^{13}C NMR data for **4** were compared with those for the known 8α*H*-fern-9(11)-ene (**5**) [12, 13], 8β*H*-fern-9(11)-ene (**6**) [14] and arborinone (**7**) [15] for the purpose of confirming the genuine skeletal system, although nothing has yet been published on the ^{13}C NMR data for **6**. It revealed that, owing to anisotropies by the propionic acid and isopropenyl groups, the ^{13}C NMR signals of C-9 and C-11 in **4** were shifted by – 10 and + 3.7 ppm, respectively, from those of **5**, as well as those of **7** by – 5.7 and + 3.7 ppm, respectively. The signals of C-5–C-8 and C-10, composed of the B-ring, and C-27 in **4** exhibited significant paramagnetic shifts in comparison with those of **5**. Except for C-10, the above carbons resonated at higher magnetic field than those of corresponding signals of **7**, suggesting **4** to be not the 3,4-*seco*-arbor-9(11)-en-3-oic acid, but the 8α*H*- or 8β*H*-isomer of 3,4- *seco*-fern-9(11)-en-3-oic acid. However, no effective information could be obtained to solve the stereochemistry by comparison of the ^{1}H NMR data for **4** with those for **5** and **6**.

The complete structure was established by employing NOE difference (NOED) and NOESY experiments (Fig. 2). Selective irradiation on the Me-26 signal fur-

Table 2. ^{13}C NMR spectral data for compounds **4**, **4a**, **5** and **7** ($CDCl_3$, TMS)*

	δ_C			
C_n	**4**	**4a**	**5**	**7**
1	35.6	36.4	41.49	36.6
2	29.5	29.6	19.56	34.8
3	180.7	175.5	42.43	217.1
4	146.0	146.5	33.64	47.6
5	51.0	51.1	44.88	53.2
6	24.1	25.7	19.53	26.3
7	19.4	19.4	17.90	22.6
8	40.4	40.5	39.98	41.0
9	141.7	142.3	151.68	147.4
10	41.2	41.3	38.05	39.3
11	119.3	119.4	115.60	115.6
12	36.73	36.84	36.78	36.1
13	36.66	36.77	36.74	36.7
14	38.6	38.7	37.69	38.2
15	29.5	29.6	29.28	29.6
16	35.9	36.0	36.19	35.8
17	42.8	42.9	42.97	42.8
18	52.0	52.1	52.02	52.0
19	20.2	20.2	20.15	20.1
20	28.2	28.3	28.23	28.2
21	59.6	59.8	59.68	59.6
22	30.8	30.9	30.80	30.7
23	113.1	113.3	32.80	22.0†
24	25.6	24.2	21.68	25.5†
25	22.6	22.7	25.06	21.6
26	15.7	15.7	15.84	16.9
27	16.1	16.2	15.43	15.3
28	14.1	14.1	14.00	13.9
29	22.1	22.2	22.14	22.1‡
30	23.0	23.1	23.02	23.0‡
$CO_2\underline{Me}$	—	51.7	—	—

*Measured at 125 MHz. Data for compounds **5** and **7** cited from refs [13] and [15], respectively.

†‡Assignments in each column may be interchanged.

nished 6.3% NOE for the signal of H-8. Irradiation of the signal of Me-25 afforded 15.0, 4.0, 3.2, 2.8 and 1.8% NOE enhancements for the signals of H-11, H-2, H-23, H-8 and Me-24, respectively. Cross-correlation was also observed between signals of Me-27 and Me-28 and between those of H-6β and H-8 in the NOESY experiment. The above results indicated that the B/C-rings in **4** must have a twisted-boat/twisted-chair conformation joined with H-8β, and thus Me-25 geminal to a bulky propionic acid group at C-10 comes sterically close to H-11. Accordingly, the structure of **4** was proved as 3,4-*seco*-8β*H*-ferna-4(23), 9(11)-dien-3-oic acid [3,4-*seco*-8β*H*-D:C-friedo-B′ : A′-neogammacera-4(23), 9(11)-dien-3-oic acid], which has not been described previously in the literature.

EXPERIMENTAL

General. Mps: uncorr. Optical rotations: $CHCl_3$; UV: EtOH, IR: KBr discs; ^1H NMR (500 and 300 MHz) and ^{13}C NMR (125 and 74.5 MHz): $CDCl_3$ with TMS as int. standard; EI-MS (probe): 70 eV; CC: Kieselgel 60 and Alumina 90 (70–230 mesh, Merck); TLC: Silica gel HF_{254} and PF_{254} (Merck).

Extraction and isolation of compounds. The isolation of 3, 4-*seco*-oleana-4(23), 18-dien-3-oic acid and four known triterpenes from residues I–IV, fractionated by CC from the CH_2Cl_2 extract of the dried whole herb of cultured *E. chamesyce* L. (5.516 kg), has previously been reported [1]. A residue (4.87 g) collected from the above CC, which had eluted between residues II and III (fr. nos: 85–101, each fr. 1 l), was subjected to repeated CC on silica gel (300 g) to give gultinol (**1**), 178 mg, mp 210–212° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 62.8° (*c* 0.55) (lit. [2]: mp 210–213°, $[\alpha]_D^{23}$ 63.3°); IR ν_{max} cm^{-1}: 3490, 1643, 972, 826; ^1H NMR: δ 0.85 (Me-25), 0.95 (Me-29), 0.99 (Me-30), 1.00 (Me-27), 1.04 (Me-23), 1.09 (Me-26), 1.14 (Me-24), 1.16 (Me-28), 3.47 (1H, *t*, *J* = 3.1 Hz, H-3α), 5.63 (1H, *br d*, *J* = 5.9 Hz, H-6); EI-MS: *m/z* 426 $[M]^+$, from the frs eluted with C_6H_6–$CHCl_3$ (5:1). The above compound

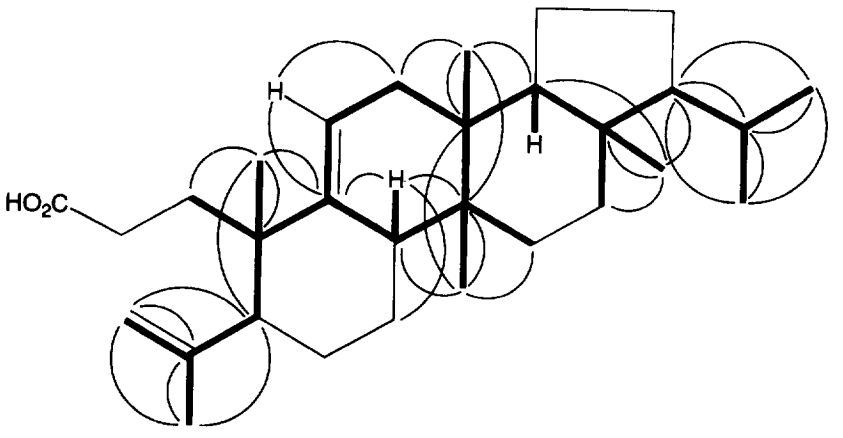
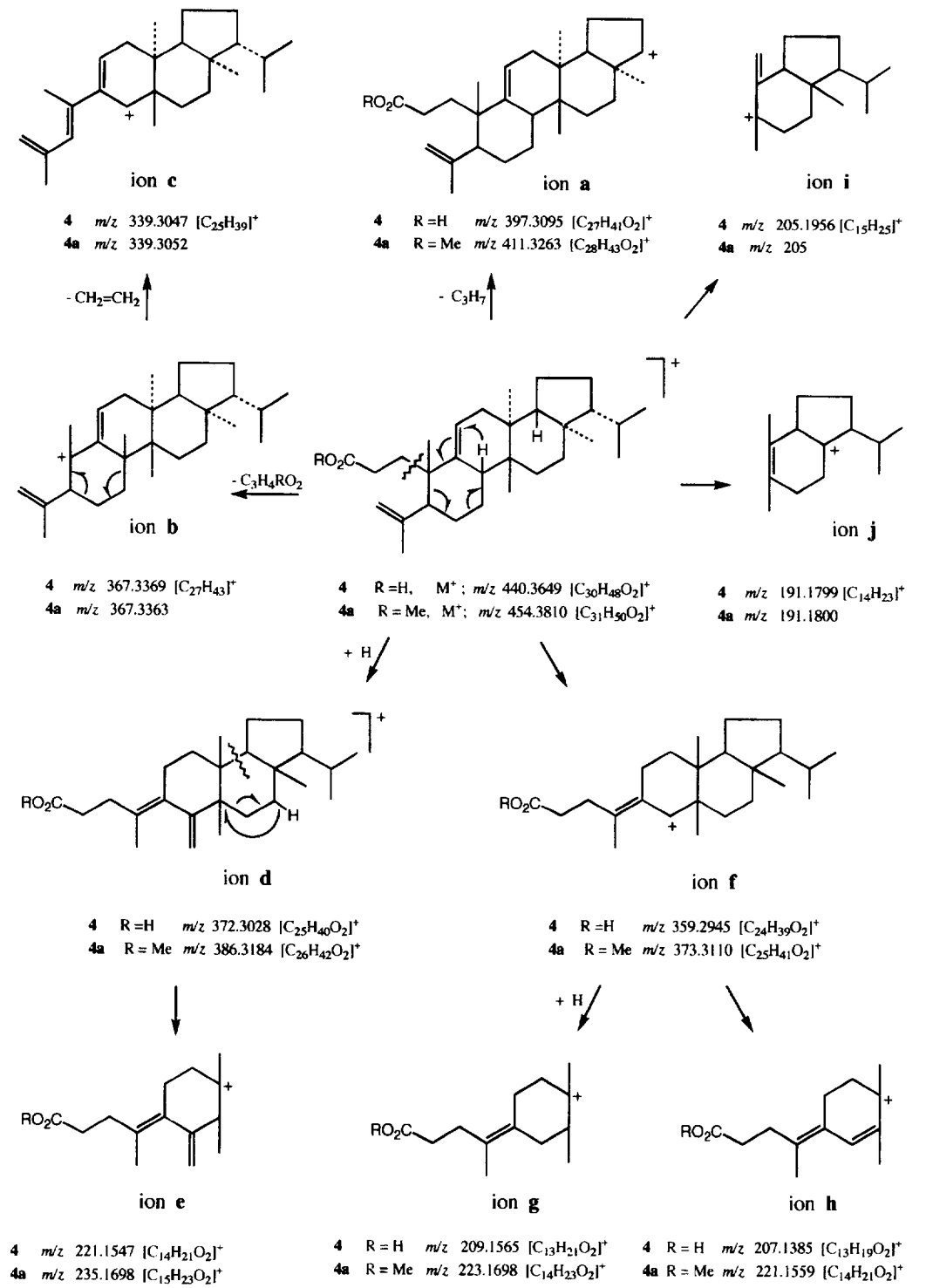
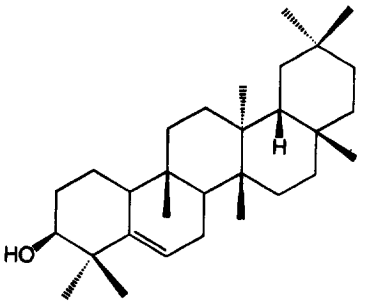


Fig. 1. 2D long range ^1H–^{13}C COSY correlations of compound **4**.



Scheme 1. Mass spectrometric fragmentation of compounds **4** and **4a**.

was identified by direct comparison (mmp, co-TLC, IR, ^{1}H NMR and EI-MS) with an authentic sample [2]. Further elution of the column with C_6H_6–$CHCl_3$ (2:1) furnished an amorphous mass (0.38 g), which was re-chromatographed by CC on 10% $AgNO_3$-impregnated alumina (40 g). Elution with *n*-hexane–C_6H_6 (1:1) afforded 3β-hydroxymultiflor-8-en-7-one (**2**), 33 mg, mp 202–205° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 35° (*c* 0.26) (lit. [3]: mp 203–205°, $[\alpha]_D$ + 33.8°); UV: λ_{max} 255 nm (ε 4800), IR ν_{max} cm^{-1}: 3426, 1658, 1177, 1105; ^{1}H NMR: δ0.89



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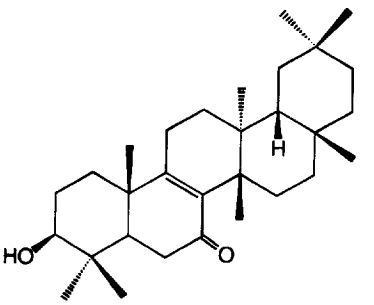

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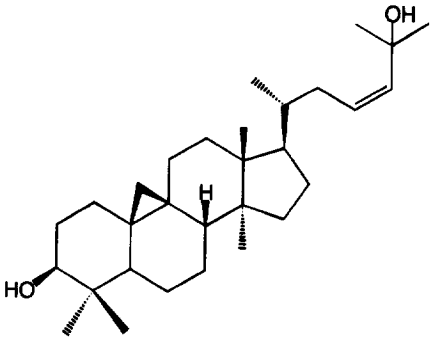

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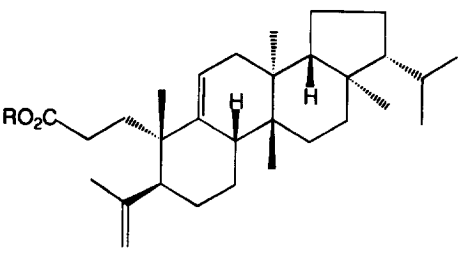
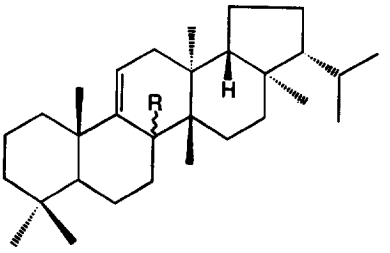
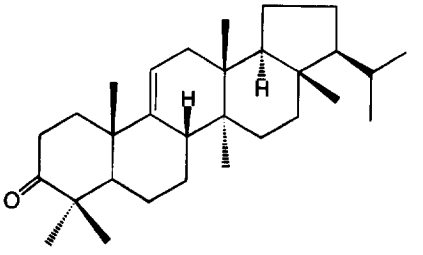


4 R = H
4a R = Me



5 R = α-H
6 R = β-H



7

(Me-24), 0.95 (Me-27), 0.96 (Me-29), 0.98 (Me-30), 0.99 (Me-23), 1.01 (Me-25), 1.15 (Me-28), 1.34 (Me-26), 3.30 (1H, *dd*, $J = 11.4$, 5.2 Hz, H-3α); EI-MS *m/z* 440 $[M]^+$, which was identified by direct comparison (mmp, co-TLC, ^{1}H NMR and EI-MS) with an authentic sample [3]. On the other hand, repeated silica gel CC of residue III was continued with $CHCl_3$ to give compound **4**, 925 mg, from the frs after 3,4-*seco*-oleana-4(23),18-dien-3-oic acid was entirely eluted [2]. Repeated alumina CC of residue IV was continued with C_6H_6–$CHCl_3$ (10: 1) to yield the known cycloart-23 *Z*-en-3β,25-diol (**3**), 129 mg, mp 199.5–202° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 43.6° (*c* 0.73) (lit. [4]: $[\alpha]_D$ + 45°), IR ν_{max} cm^{-1}: 3655 and 3324 (OH), 3038 (cyclopropane), 2970, 2931, 2866, 1467, 1377, 1358, 1224, 1163, 1104, 1051, 971, 890 (>C=CH_2); ^{1}H NMR: δ0.33, 0.55 (each 1H, *d*, $J = 4.1$ Hz, H-19), 0.81 (Me-29), 0.86 (*d*, $J = 6.3$ Hz, Me-21), 0.89 (Me-30), 0.97 (Me-18, Me-28), 1.32 (Me-26, Me-27), 3.29 (1H, *dd*, $J = 10.2$, 4.9 Hz, H-3α), 5.60 (2H, *dd*, $J = 4.1$, 3.1 Hz, H-23, H-24); ^{13}C NMR: δ14.0 (*q*, C-28), 18.1 (*q*, C-18), 18.3 (*q*, C-21), 19.3 (*q*, C-30), 20.0 (*s*, C-9), 21.1 (*t*, C-6), 25.4 (*q*, C-29), 26.0 (*s*, C-10), 26.1 (*t*, C-11), 26.4 (*t*, C-16), 28.1 (*t*, C-7), 29.9 (*q*,

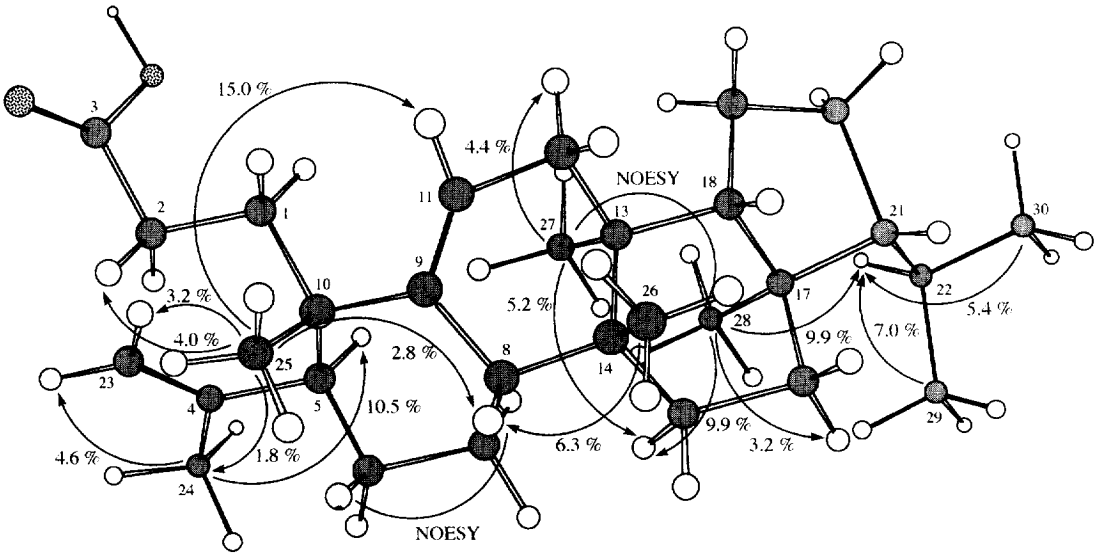


Fig. 2. NOED and NOESY correlations of compound **4**.

C-26 and 27), 30.0 (*t*, C-19), 30.4 (*t*, C-2), 32.0 (*t*, C-1), 32.8 (*t*, C-15), 35.6 (*t*, C-12), 36.4 (*d*, C-20), 39.0 (*t*, C-22), 40.5 (*s*, C-4), 45.3 (*s*, C-13), 47.1 (*d*, C-5), 48.0 (*d*, C-8), 48.8 (*s*, C-14), 52.0 (*d*, C-17), 70.8 (*s*, C-25), 78.8 (*d*, C-3), 125.6 (*d*, C-24), 139.4 (*d*, C-23), EI-MS: *m/z* 442 $[M]^+$, exhibiting physical and spectral data almost identical to those already published [4].

Compound **4**. Needles, mp 227–229° (sublim.) (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ − 52° (*c* 0.93); HR-EI-MS: *m/z* 440.3649 $[M]^+$ ($C_{30}H_{48}O_2$ requires 440.3651); IR ν_{max} cm^{-1}: 3418, 2929, 2888, 1708 (COOH), 1648 (>C=CH_2), 1617 (>C=CH−), 1471, 1452, 1381, 1365 (gem. dimethyl), 1297, 1226, 900 (>C=CH_2) and 821 (>C=CH−); ^1H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m/z* (rel. int.): 440 (64) $[M]^+$, 425 (10) [M − Me]$^+$, 397 (6) [ion *a*], 372 (4) [ion *d*], 367 (86) [ion *b*], 359 (100) [ion *f*], 339.3047 (75) [ion *c*], 229 (8), 221 (19) [ion *e*], 209 (20) [ion *g*], 207 (29) [ion *h*], 205 (15) [ion *i*], 191 (10) [ion *j*].

Methyl ester of **4**. A soln of CH_2N_2 in Et_2O (0.7%, 20 ml) was added to a soln of compound **4** (20 mg) in Et_2O (5 ml) and the mixt. was kept at room temp. for 1 hr. Removal of the solvent *in vacuo* yielded a residue, which was purified by prep. TLC (plate: 0.5 mm thick; solvent: $CHCl_3$) to give the corresponding methyl ester (**4a**), mp 102.5–104.5° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ − 48° (*c* 0.38), 21 mg, as needles; HR-EI-MS: *m/z* 454.3810 $[M]^+$ ($C_{31}H_{50}O_2$ requires 454.3808); IR ν_{max} cm^{-1}: 2953, 2839, 1742 (CO_2Me), 1647 (>C=CH_2), 1617(>C=CH−), 1452, 1436, 1381, 1375, 1288, 1277, 1171, 1017, 897 (>C=CH_2) and 821(>C=CH−); ^1H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m/z* (rel. int.): 454 (46) $[M]^+$, 439 (10) [M − Me]$^+$, 423 (5), 411 (6) [ion *a*], 386 (2) [ion *d*], 373 (23) [ion *f*], 367 (100) [ion *b*], 339 (24) [ion *c*], 235 (12) [ion *e*], 223 (11) [ion *g*], 221 (26) [ion *h*], 205 (17) [ion *i*], 191 (13) [ion *j*].

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