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Obtusifoliol and related steroids from the whole herb of *Euphorbia chamaesyce*

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Abstract

Two new ergostane-type steroids were isolated together with two known compounds, obtusifoliol and 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trine-3β-ol, from the whole herb of *Euphorbia chamaesyce*. The structures of the new compounds were established as 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one and 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-7,11-dione on the basis of chemical and spectral evidence.

Keywords: *Euphorbia chamaesyce*; Euphorbiaceae; Steroids; 3β-Hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one; 3β-Hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-7,11-dione

1. Introduction

Previously, it was reported that *Euphorbia supina* Rafin., an annual weed native to North America and introduced into Japan, contained two unusual fernane triterpenoids named spirosupinanonediol [7(8→9)*abeo*-9*S*-D:C-*friedo*-B′:A′-neogrammaceran-8-one-3*S*,7*S*-diol (Matsunaga et al., 1984) and neospirosupinanetrione [7(8 → 9)*abeo*-9*R*-D:C-friedo-B′:A′-neogammaceran-3,7,8-trione] (Tanaka, & Matsunaga, 1991), together with 30 other triterpenoids including five oxygenated fernanes named supinenolones A (3β,7α-dihydroxyfern-8-en-11-one), B (3β,11β-dihydroxyfern-8-en-7-one), C (3β-hydroxyfern-8-en-7,11-dione), D (fern-8-en-3,7,11-trione) and E (3β-hydroxyfern-8-en-7-one) (Tanaka, & Matsunaga, 1989, 1991) and five 3,4-*seco*-adiananes named espinendiols A and B, espinenoxide, *trisnor*-isoespinenoxide and espinanoxide (Tanaka, Matsunaga, Ishida, & Shingu, 1989). Plausible biosynthetic pathways were also proposed to the above two spirosupinanes from supinenolone E via epoxidation of the Δ^8-double bond and subsequent cleavage of the epoxy ring involving transformation of the C-7/C-8 bond to the C-9 position (Tanaka, & Matsunaga, 1991). These results aroused our phytochemical and biological interest in the constituents of *Euphorbia chaemaesyce* L., native to the torrid zone of the world and now becoming naturalized in Japan. Recently, we isolated 3,4-*seco*-oleana-4(23)-18-dien-3-oic acid (Tanaka, Ida, Kita, Kamisako, & Matsunaga, 1994) and 3,4-*seco*-8β*H*-ferna-4(23),9(11)-dien-3-oic acid (Tanaka, Ida, Kita, Kamisako, & Matsunaga, 1996), as well as butyrospermol, cycloart-23*Z*-en-3β,25-diol, lupeol, glutinol, 11α,12α-oxidotaraxerol, 3β-hydroxy-30-nor-lupan-20-one and 3β-hydroxymultiflor-8-en-7-one, from the methylene chloride extract of the whole herb of *E. chamaesyce*. Further examination of this extract led to the isolation of two known and two new steroids and this paper deals with their structural elucidation.

2. Results and discussion

The known compounds were confirmed as obtusifo-

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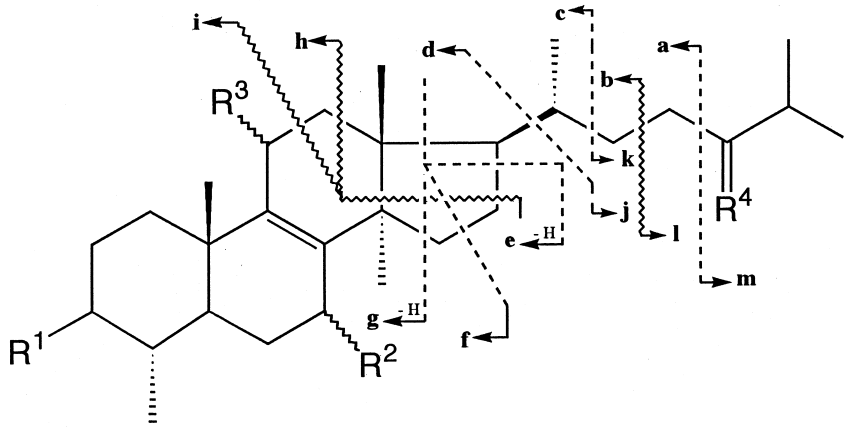
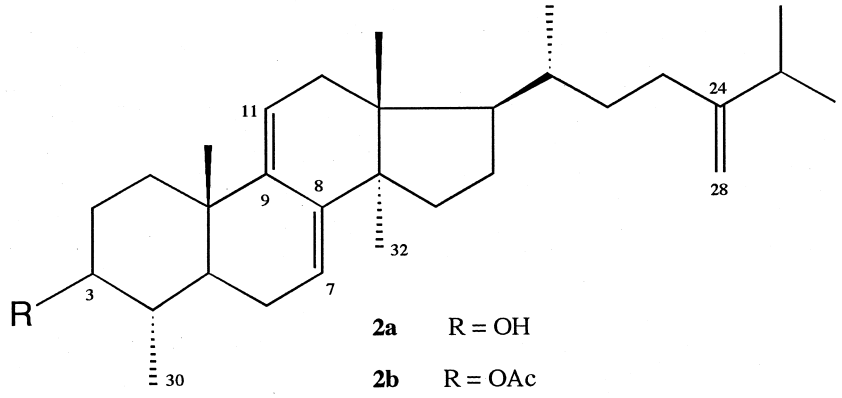
liol [4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-3β-ol] (**1a**) and 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol (**2a**), respectively, based on the physical and spectral data of the corresponding acetates, **1b** and **2b**, which were in good agreement with literature values (Gonzalez, Breton, & Garcia, 1958; Gonzalez, Breton, Dergado Martin, & Fraga, 1972; Itoh, Kikuchi, Shimizu, Tamura, & Matsumoto, 1981; Akihisa, Yokota, Takahashi, Tamura, & Matsumoto, 1989; Akihisa, Kokke, Yokota, Tamura, & Matsumoto, 1990). An assignment of the ^{13}C NMR signals of **2a** is shown in Table 2 and is based on ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY analyses.

Compound **3a** was assigned the molecular formula $C_{30}H_{48}O_2$ (HREIMS). The UV and IR spectra showed absorption bands for a hydroxyl group, a conjugated enone (λ_{max} 253 nm) and a terminal methylene group. The ^{1}H and ^{13}C NMR and DEPT spectra (Tables 1 and 2) revealed signals of three quaternary and two secondary methyl groups, an isopropyl group (δ_H 1.025, 1.030 (each 3H, d) and 2.25 (1H, sept.)), nine methylene groups, four methine groups, three sp^3 quaternary carbons, a hydroxymethine group (δ_H 3.15 (1H, ddd); δ_C 75.3 (d)), a terminal methylene group (δ_H 4.66 (1H, d) and 4.72 (1H, s); δ_C 106.0 (t) and 156.7 (s)) and a conjugated enone including a tetrasubstituted double bond (δ_C 139.5 (s), 164.8 (s) and 198.1 (s)). Acetylation of **3a** afforded a monoacetate (**3b**), in which the carbinol methine proton signal was shifted to δ 4.39 (ddd). In the EIMS spectrum, **3a** exhibited fragment peaks due to ions **a**–**d** and **j**–**m**, along with peaks which had lost one atom of hydrogen from ions **a** and **b** and two atoms of hydrogen from ions **c**, **d** and **j**–**m**, indicating it to have the same side chain **1a** and **2a** (see Section 3). Moreover, a peak corresponding to ion **i** was observed as a predominant peak at *m/z* 247.1681 $[C_{16}H_{23}O_2]^+$, together with peaks attributable to ions **e**, **f** and **g** at *m/z* 301, 288 and 273, re-

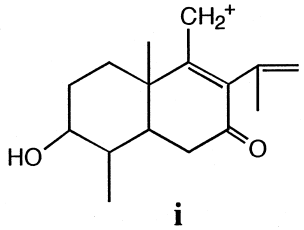


	R^1	R^2	R^3	R^4
1a	OH	H_2	H_2	CH_2
1b	OAc	H_2	H_2	CH_2
3a	OH	:O	H_2	CH_2
3b	OAc	:O	H_2	CH_2
4a	OH	:O	:O	CH_2
4b	OAc	:O	:O	CH_2
4c	OAc	:O	:O	:O

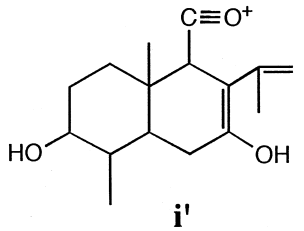


2a R = OH

2b R = OAc



i



i'

Table 1
500 MHz ^{1}H NMR spectra data of **3a**, **3b**, **4a**, **4b** and **4c**

H	3a	3b	4a	4b	4c
1α	1.41 m	1.44 m	1.16 m	1.18 m	1.18 m
1β	1.87 dt (13.1, 3.0)	1.87 dt (13.0, 3.5)	2.85 dt (13.5, 3.8)	2.86 dt (13.5, 3.8)	2.85 dt (13.7, 3.8)
2α	1.92 ddt (12.4, 4.8, 3.0)	1.97 m	1.89 m	1.93 m	1.94 m
2β	1.60 m	1.57 m	1.61 m	1.60 m	1.61 m
3α	3.15 ddd (11.3, 9.5, 4.8)	4.39 ddd (11.0, 11.0, 4.8)	3.14 ddd (11.0, 11.0, 4.8)	4.40 ddd (11.0, 11.0, 4.8)	4.39 ddd (11.0, 11.0, 4.8)
4β	1.46 m	1.68 m	1.54 m	1.75 m	1.76 m
5α	1.55 ddd (11.6, 13.6, 3.7)	1.62 m	1.45 m	1.54 ddd (15.2, 11.1, 3.1)	1.53 ddd (15.0, 11.5, 3.0)
6α	2.48 dd (16.5, 3.7)	2.48 dd (16.5, 3.7)	2.51 dd (15.2, 3.1)	2.52 dd (15.2, 3.1)	2.53 d (15.0, 3.0)
6β	2.19 dd (16.5, 13.6)	2.18 dd (16.5, 13.5)	2.28 dd (15.2, 15.2)	2.29 dd (15.2, 15.2)	2.28 dd (15.0, 15.0)
11α	2.39 ddd (21.0, 7.5, 3.0)	2.37 ddd (21.0, 6.0, 4.0)	–	–	–
11β	2.31 m	2.27 m	–	–	–
12α	1.79 m	1.79 m	2.77 dd (16.2, 1.1)	2.79 dd (15., 1.2)	2.78 d (16.0, 0.8)
12β	1.79 m	1.79 m	2.64 d (16.2)	2.65 d (15.8)	2.62 d (16.0)
15α	2.05 m	2.06 m	2.14 m	2.13 m	2.16 ddd (12.5, 10.0, 2.5)
15β	1.73 m	1.73 m	1.75 m	1.74 m	1.75 m
16α	1.98 m	1.98 m	2.01 m	2.01 m	2.02 m
16β	1.34 m	1.34 m	1.36 m	1.36 m	1.42 m
17α	1.47 m	1.45 m	1.70 m	1.70 m	1.70 m
18	0.68 s	0.68 s	0.82 s	0.82 s	0.80 s
19	1.19 s	1.20 s	1.30 s	1.32 s	1.31 s
20β	1.40 m	1.39 m	1.40 m	1.42 m	1.39 m
21	0.94 d	0.96 d (6.5)	0.93 d (6.5)	0.93 d (6.5)	0.89 d (6.4)
22	1.16 m	1.14 m	1.17 m	1.16 m	1.26 m
22	1.58 m	1.58 m	1.59 m	1.57 m	1.79 m
23	1.88 m	1.89 m	1.89 m	1.88 m	2.39 ddd (17.0, 9.5, 6.5)
23	2.10 m	2.12 ddd (15.0, 11.0, 4.0)	2.11 m	2.11 m	2.49 dd (17.0, 10.0, 5.0)
25	2.25 septet (6.9)	2.23 septet (7.0)	2.23 septet (6.9)	2.23 septet (6.9)	2.62 septet (6.9)
26	1.025 d (6.9)[a]	1.025 d (7.0)[a]	1.025 d (6.9)[a]	1.026 d (6.9)[a]	1.09 d (6.9)
27	1.030 d (6.9)[a]	1.030 d (7.0)[a]	1.030 d (6.9)[a]	1.031 d (6.9)[a]	1.09 d (6.9)
28a	4.66 d (1.4)	4.66 d (1.4)	4.66 d (1.2)	4.66 d (1.2)	–
28b	4.72 s	4.72 s	4.73 s	4.72 s	–
30	0.99 d (6.2)	0.86 d (6.2)	1.02 d (6.4)	0.88 d (6.4)	0.88 d (6.4)
32	0.93 s	0.93 s	1.19 s	1.20 s	1.19 s
OAc	–	2.06 s	–	2.07 s	2.06 s

[a] Assignments may be interchangeble vertically.

spectively. These data suggested **3a** to be an analogue of obtusifoliol attached to a keto group not at C-11 but at C-7. This assumption was supported by the 2D ^{1}H–^{1}H COSY, HMQC, HMBC and *J*-resolved spectra. The HMBC analysis (Fig. 1) indicated **3a** to have the gross structure of 7-oxo-obtusifoliol. The NOESY data (Fig. 1) exhibited cross correlations for H-3α (with H-1α and H-5α), Me-30 (with H-3α, H-5α and H-6α), Me-19 (with H-1β, H-4β, H-6β, H-11β and Me-18), Me-18 (with H-12β, H-15β, H-20 and Me-21), Me-21 (with H-17α, H-23a and H-23b) and Me-32 (with H-15α and H-17α), indicating **3a** to have a 3β-hydroxyl group and the same 20*R*-orientation of the side chain moiety as obtusifoliol (**1a**) (Akihisa et al., 1990; Nes et al., 1998). Hence, **3a** was 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one.

Compound **4a**, obtained as pale yellow needles, was assigned the molecular formula $C_{30}H_{46}O_3$ (HREIMS). The UV spectrum showed absorption bands for a transoid ene-dione chromophore (λ_{max} 205 and 270 nm). The IR, ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) revealed the presence of three quaternary and two secondary methyl groups, an isopropyl group (δ_H 1.025 and 1.030 (each 3H, d), 2.23 (1H, sept.)), eight methylene groups, four methine groups, a hydroxymethine group (δ_H 3.14 (1H, ddd); δ_C 75.4 (d)), a terminal methylene group (ν_{max} 888 cm^{-1}; δ_H 4.66 (1H, d) and 4.73 (1H, s); δ_C 106.2 (t) and 156.4 (s)), a tetrasubstituted double bond (δ_C 151.2 and 151.5 (each s)) and two carbonyl carbons (ν_{max} 1673 cm^{-1}; δ_C 201.5 and 202.6 (each s)). Acetylation of **4a** yielded an acetate (**4b**), in which the carbinol methine proton signal was shifted to δ 4.40 (1H, ddd). The EIMS spectrum of **4a** also exhibited fragment peaks due to ions **a**–**d** and **k**–**m**, along with peaks which had eliminated one atom of hydrogen from ions **a** and **b** and two atoms of hydrogen from ions **c**, **d** and **j**–**m**, indicating it to have the same side chain as **3a** (see Section 3). A peak attributable to [ion **j** (side chain part)–CH_4]$^+$ was observed as a base peak at *m*/*z* 109.1026

Table 2
125 MHz ^{13}C NMR spectral data of compounds **2a**, **3a**, **3b**, **4a**, **4b** and **4c**

C	2a	3a	3b	4a	4b	4c
1	34.7 t	34.2 t	33.9 t	33.3 t	33.0 t	33.0 t
2	31.3 t	30.7 t	28.7 t	30.9 t	26.9 t	26.9 t
3	76.0 d	75.3 d	76.7 d	74.5 d	77.3 d	77.3 d
4	39.7 d	39.1 d	36.4 d	38.2 d	35.2 d	35.1 d
5	45.5 d	46.7 d	46.7 d	47.2 d	47.2 d	47.2 d
6	26.5 t	39.1 t	39.1 t	38.2 t	38.2 t	38.1 t
7	119.3 d	198.1 s	197.6 s	201.5 s	201.1 s	201.0 s
8	143.0 s	139.5 s	139.7 s	151.2 s	151.3 s	151.3 s
9	143.3 s	164.8 s	164.4 s	151.5 s	151.3 s	151.3 s
10	36.5 s	38.8 s	38.7 s	38.6 s	38.4 s	38.4 s
11	117.0 d	24.5 t	24.5 t	202.6 s	202.5 s	202.4 s
12	37.9 t	30.1 t	30.1 t	51.5 t	51.5 t	51.5 t
13	43.8 s	44.9 s	44.9 s	49.0 s	49.0 s	49.0 s
14	50.4 s	47.8 s	47.8 s	47.5 s	47.6 s	47.5 s
15	31.5 t	31.9 t	32.0 t	32.0 t	32.1 t	32.0 t
16	27.9 t	28.7 t	26.9 t	27.3 t	27.3 t	27.2 t
17	50.9 d	48.9 d	48.9 d	49.0 d	49.0 d	49.1 d
18	15.7 q	15.7 q	15.8 q	16.8 q	16.8 q	16.8 q
19	20.7 q	17.7 q	17.7 q	16.4 q	16.3 q	16.3 q
20	36.3 d	36.4 d	36.1 d	36.1 d	36.2 d	35.8 d
21	18.5 q	18.8 q	18.8 q	18.6 q	18.6 q	18.3 q
22	39.4 t	34.9 t	34.9 t	34.7 t	34.7 t	29.7 t
23	31.3 t	31.2 t	31.6 t	31.1 t	31.1 t	29.7 t
24	156.8 s	156.7 s	156.7 s	156.4 w	156.4 s	215.0 s
25	33.8 d	33.7 d	33.7 d	33.8 d	33.8 d	40.9 d
26	21.9[a] q	21.8[a] q	21.8[a] q	21.8[a] q	21.8[a] q	18.4 q
27	22.0[a] q	22.0[a] q	22.0[a] q	22.0[a] q	22.0[a] q	18.4 q
28	106.0 t	106.0 t	106.0 t	106.2 t	106.2 t	–
30	15.1 q	14.4 q	14.5 q	14.8 q	14.8 q	14.8 q
32	25.6 q	25.1 q	25.1 q	26.0 q	26.0 q	26.0 q
OCO*Me*	–	–	21.2 q	–	21.2 q	21.2 q
O*C*OMe	–	–	170.7 s	–	170.7 s	170.7 s

[a] Assignments in some column are interchangeable.

$[C_8H_{13}]^+$, although it appeared as an inconspicuous peak in **3a**. Furthermore, two characteristic peaks tentatively assigned to ions **h** and **i′** were observed at *m/z* 275 $[C_{17}H_{23}O_3]^+$ and 263.1655 $[C_{16}H_{23}O_3]^+$, respectively, as well as peaks due to ions **e**, **f** and **g**. Together with these results, the HMBC data (Fig. 2) indicated that **4a** must be 7,11-dioxo-obtusifoliol. The complete structure was obtained by the following experiments. The NOESY spectrum provided cross correlations (Fig. 2) for Me-30 (with H-3α, H-5α and H-6α), Me-19 (with H-1β, H-2β, H-4β and Me-18), Me-18 (with H-12β, H-20 and Me-21), Me-21 (with H-17α, H-23a and H-23b) and Me-32 (with H-15α and H-17α), indicating **4a** to have a 3β-hydroxyl group and the same 20*R*-orientation of side chain as obtusifoliol (**1a**) (Akihisa et al., 1990; Nes et al., 1998). Oxidation of **1a** with chromium trioxide in acetic acid furnished 3β-acetoxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-7,11-dione, together with almost the same amount of 3β-acetoxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11,24-trione (**4c**). The former product was identical in all respects with **4b** prepared from **4a**. Hence, **4a** was proved to be 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-7,11-dione.

This is the first report for the isolation of **3a** and **4a** in the literature, although the 24(28)-dihydro-derivatives of **3b** and **4b** had previously been prepared from **1a** (Barrera, Breton, Dergado Martin, & Gonzalez, 1967).

3. Experimental

3.1. General

M.p.'s: uncorr.; optical rotations: $CHCl_3$, unless otherwise noted. UV: EtOH; IR: KBr discs; ^1H (500 MHz) and ^{13}C NMR (125 MHz): $CDCl_3$ with TMS as int. standard; EIMS (probe): 70 eV; CC: Kieselgel 60 (70–230 mesh, Merck) and alumina 90 (70–230 mesh, Merck). Reversed phase MPLC: Cosmosil-40 ODS C_{18}-PREP (Nacalai Tesque Co.); equipped with Yamazen SS 50-1296 pump; TLC and prep. TLC: silica gel HF_{254} and PF_{254} (Merck).

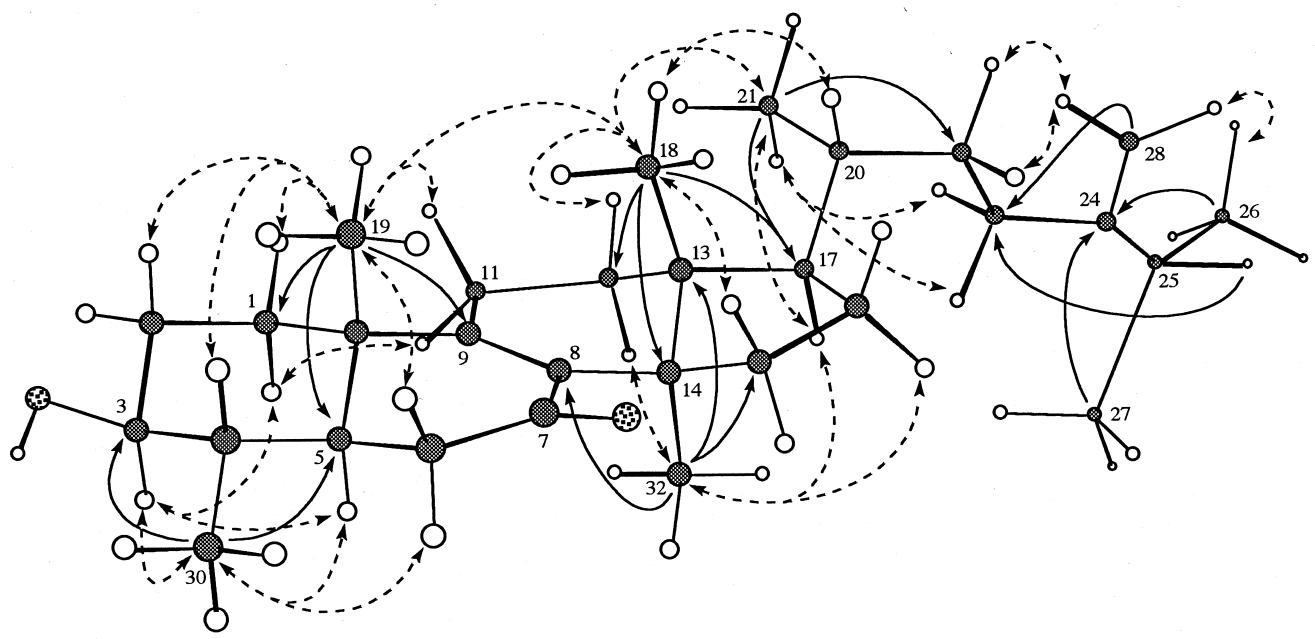


Fig. 1. HMBC (plain arrow) and selective NOESY (dashed arrow) correlations of **3a**.

3.2. Isolation of compounds

Details on isolation of 3,4-*seco*-oleana-4(23),18-dien-3-oic acid, 3,4-*seco*-8β*H*-ferna-4(23),9(11)-dien-3-oic acid and eight known constituents separated from the CH_2Cl_2 extract of the whole herb of *Euphorbia chamaesyce* L. (5.56 kg) by preliminary silica gel CC, were described previously (Tanaka et al., 1994, 1996). Continuous CC of residue **I** (32.29 g) yielded an amorphous gummy product (1.54 g) from frs eluted with *n*-hexane–C_6H_6 (2:1–1:1). Rechromatography of the product over silica gel (150 g) CC yielded an amorphous gum (141 mg) from the fr. eluted with *n*-hexane–EtoAc (7:1), which was subjected to reversed phase MPLC using Cosmosil-40 C_{18} (ODS) column. Elution with MeOH afforded obtusifoliol (**1a**), 72 mg, m.p. 142–144°C (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +73° (*c* 0.34) (lit. (Gonzalez et al., 1972) m.p. 145–146°, $[\alpha]_D$ +74°);

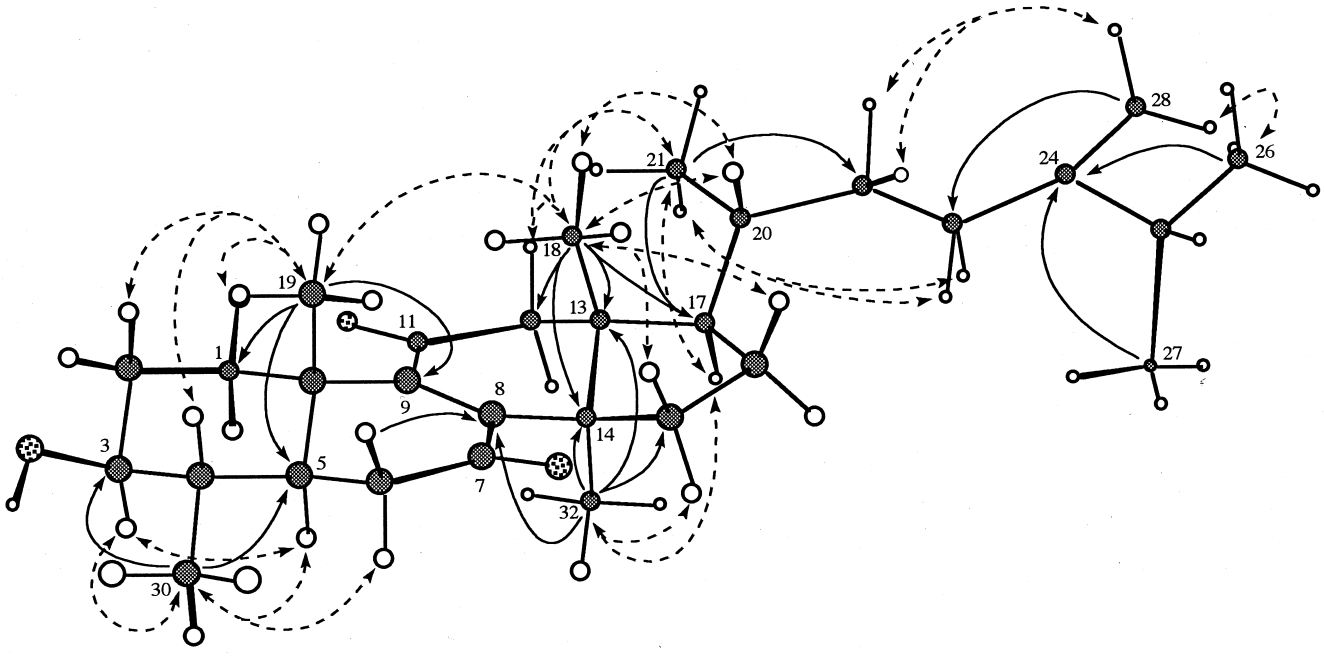


Fig. 2. HMBC (plain arrow) and selective NOESY (dashed arrow) correlations of **4a**.

EIMS: m/z 426 $[M]^+$, from fr. No.'s: 9–12 (each fr.: 30 ml). Acetylation of **1a** (30 mg) with Ac_2O/C_5H_5N (1:1, 3 ml) furnished the corresponding acetate (**1b**), 30 mg, m.p. 106–107°C (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +73° (lit. (Gonzalez et al., 1972) m.p. 107–108°C; $[\alpha]_D$ +79°; lit. (Itoh et al., 1981) m.p. 111–113°C), EIMS: m/z 468 $[M]^+$. Further elution of the column with the same solvent afforded 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol (**2a**), 55 mg, m.p. 152.5–154°C (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +46° (c 0.13); ^{1}H NMR: δ 0.58 (3H, s, Me-18), 0.90 (3H, s, Me-32), 0.92 (3H, d, J=6.4Hz, Me-21), 0.94 (3H, s, Me-19), 1.00 (3H, d, J=6.4Hz, Me-30), 1.028 (3H, d, J=6.9 Hz, Me-26), 1.033 (3H, d, J=6.9Hz, Me-27), 3.12 (1H, ddd, J=11.0, 10.9, 4.8 Hz, H-3α), 4.67 (1H, d, J=1.2 Hz, H-28a), 4.72 (1H, s, H-28b), 5.39 (1H, d, J=6.2 Hz, H-11), 5.42 (1H, d, J=6.6 Hz, H-7); ^{13}C NMR: see Table 2; EIMS: m/z 424 $[M]^+$, from fr. 6. Acetylation of **2a** (6 mg) with Ac_2O/C_5H_5N (1:1, 1 ml) afforded the corresponding acetate (**2b**), 6 mg, m.p. 123–124.5°C (MeOH–$CHCl_3$), (lit. (Gonzalez et al., 1958) m.p. 123–124°C), EIMS; m/z 466 $[M]^+$. All the above physical and spectral data for **1a** (Gonzalez et al., 1972), **1b** (Gonzalez et al., 1972; Itoh et al., 1981; Akihisa et al., 1989; Akihisa et al., 1990) and **2b** (Gonzalez et al., 1958) were in good agreement with those already reported in the literature, respectively. A residue (18.92 g) eluting between residues **III** and **IV** on the preliminary silica gel CC of the CH_2Cl_2 extract (fr. No.'s: 106–116, each fr.: 1 l), was fractionated by silica gel (800 g) CC to yield a gummy product (280 mg) from the fr. No.'s 55–82 eluted with $CHCl_3$ (each fr.: 200 ml) (Tanaka et al., 1994). Further CC of the gum with 10% $AgNO_3$ impregnated silica gel (20 g) afforded a crystalline solid (159 mg) from the frs eluted with C_6H_6–$CHCl_3$ (1:1), which was purified by prep. TLC (plate: 20×20 cm; solvent: $CHCl_3$–MeOH, 50:1) to furnish compound **3a** (103 mg). We also reported that alumina CC of residue **IV** (41.02 g) afforded three known triterpenes including cycloart-23Z-ene-3β,25-diol (Tanaka et al., 1996). Continuous fractionation of this column afforded compound **4a**, 31 mg, from the frs eluted with C_6H_6–$CHCl_3$ (5:1).

3.3. 3β-Hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one (*3a*)

Needles, m.p. 149–150°C (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +33° (c 0.48); HREIMS: m/z 440.3655 ($C_{30}H_{48}O_2$ requires 440.3652); UV λ_{max} (nm): 253 (ε 5000) (conj. enone); IR ν_{max} cm^{-1}: 3436 (OH), 3077 (=C–H), 2965, 2932, 1662 (>C=C–C=O), 1640 and 886 (>C=CH_2), 1456, 1418 (–CH_2CO), 1374, 1335, 1269, 1243, 1193 and 1024; ^{1}H and ^{13}C NMR: see Tables 1 and 2; EIMS m/z (rel.int.): 440 (51) $[M]^+$, 425.3423 (100, calc. for $C_{29}H_{45}O_2$: 425.3417) $[M–Me]^+$, 422 (2) $[M–H_2O]^+$, 371 (1, ion **a**) $[M–C_5H_9]^+$, 370 (2, ion **a**–H), 357 (2, ion **b**) $[M–C_6H_{11}]^+$, 356.2707 (13, ion **b**–H, calc. for $C_{24}H_{36}O_2$: 356.2713), 343 (16, ion **c**) $[M–C_7H_{13}]^+$, 341.2472 (29, ion **c**–2H, calc. for $C_{23}H_{33}O_2$: 341.2479), 315 (24, ion **d**) $[M–C_9H_{17}]^+$, 313.2179 (30, ion **d**–2H, calc. for $C_{21}H_{29}O_2$: 313.2166), 301 (30, ion **e**) $[C_{20}H_{29}O_2]^+$, 288 (20, ion **f**) $[C_{19}H_{28}O_2]^+$, 273.1832 (18, ion **g**, calc. for $C_{18}H_{25}O_2$: 273.1853), 247.1681 (83, ion **i**, calc. for $C_{16}H_{23}O_2$: 247.1697), 222.1615 (14) $[C_{14}H_{22}O_2]^+$, 215 (19), 187 (11), 175 (19), 173 (18), 135 (18), 125 (5, ion **j**, side chain) $[C_9H_{17}]^+$, 123 (11, ion **j**–2H), 121 (27), 97 (8, ion **k**) $[C_7H_{13}]^+$, 95 (16, ion **k**–2H), 83 (13, ion **l**) $[C_6H_{11}]^+$, 81 (14, ion **l**–2H), 69 (30, ion **m**) $[C_5H_9]^+$ and 67 (10, ion **m**–2H).

3.4. Acetylation of *3a*

A soln of compound **3a** (21.5 mg) in Ac_2O/C_5H_5N (1:1, 2 ml) was kept at room temp. overnight. The reaction mixture was poured into ice water and the resulting precipitate was extracted with Et_2O. Evapn of the neutral Et_2O layer yielded a solid, which was purified by prep. TLC (plate: 20 × 20 cm; solvent: $CHCl_3$–MeOH, 100:1) to afford the corresponding acetate (**3b**), 18.5 mg, m.p. 148.5–150°C (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +28° (c 0.18); HREIMS: m/z 482.3743 ($C_{32}H_{50}O_3$ requires 482.3757); UV λ_{max} (nm): 253 (ε 9600); IR ν_{max} cm^{-1}: 3077 (=C–H), 2963, 2876, 1729 and 1246 (OAc), 1649 (>C=C–C=O), 1641 and 886 (>C=CH_2), 1468, 1421 (–CH_2CO), 1374, 1335, 1194 and 1027; ^{1}H and ^{13}C NMR: see Tables 1 and 2; EIMS m/z (rel. int.): 482 (41) $[M]^+$, 467.3531 (100) $[M–Me]^+$, 422.3522 (4) $[M–HOAc]^+$, 413 (3, ion **a**–H), 399 (8, ion **b**), 398.2809 (13, ion **b**–H, calc. for $C_{26}H_{38}O_3$: 398.2819), 385 (19, ion **c**), 383.2605 (28, ion **c**-2H, calc. for $C_{25}H_{35}O_3$, 383.2584), 357 (26, ion **d**), 355 (30, ion **d**–2H), 343 (30, ion **e**), 330 (19, ion **f**), 315.1931 (18, ion **g**, calc. for $C_{20}H_{27}O_3$, 315.1959), 289.1782 (55, ion **i**, calc. for $C_{18}H_{25}O_3$, 289.1802), 264.1713 (10) $[C_{16}H_{24}O_3]^+$, 215 (23), 187 (10), 175 (16), 173 (16), 135 (18), 123 (16, ion **j**–2H) [side chain, 2H]$^+$, 121 (25), 97 (8, ion **k**), 95 (18, ion **k**–2H), 83 (12, ion **l**), 81 (16, ion **l**–2H), 69 (31, ion **m**) and 67 (12, ion **m**–2H).

3.5. 3β-Hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-7,11-dione (*4a*)

Pale yellow needles, m.p. 118–120°C (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +102° (c 0.92); HREIMS: m/z 454.3449 ($C_{30}H_{46}O_3$ requires 454.3445); UV λ_{max} (nm): 205 and 270 (ε 4500 and 6200) (transoid ene-dione); IR ν_{max} cm^{-1}: 3392 (OH), 3033 (=C–H), 2965, 2931, 2871, 1673 (O=C–C=C–C=O), 1643 and 888 (>C=CH_2), 1459, 1430 and 1421 (–CH_2CO), 1377, 1345, 1232, 1207, 1017 and 947; ^{1}H and ^{13}C NMR: see Tables 1

and 2; EIMS m/z (rel. int.): 454 (55) $[M]^+$, 436.3342 (7, calc. for $C_{30}H_{44}O_2$: 436.3339) $[M–H_2O]^+$, 385 (2, ion **a**), 384 (2, ion **a**–H), 371 (12, ion **b**), 370.2525 (46, ion **b**–H, calc. for $C_{24}H_{34}O_2$: 370.2507), 357.2430 (8, ion **c**, calc. for $C_{23}H_{33}O_3$: 357.2428), 355 (2, ion **c**-2H), 329 (11, ion **d**), 327 (4, ion **d**–2H), 315 (8, ion **e**), 302.1883 (35, ion **f**, calc. for $C_{19}H_{26}O_3$: 302.1880), 289 (4), 287 (4, ion **g**) $[C_{18}H_{23}O_3]^+$, 275 (13, ion **h**) $[C_{17}H_{23}O_3]^+$, 263.1655 (22, ion **i′**, calc. for $C_{16}H_{23}O_3$: 263.1646), 241 (11), 236 (10), 187.0767 (19) $[C_{12}H_{11}O_2]^+$, 123 (11, ion **j**–2H), 121 (10), 109.1026 (100, ion **j**–CH_4, calc. for C_8H_{13}: 109.1017), 97 (8, ion **k**), 95 (13, ion **k**–2H), 83 (17, ion **l**), 81 (15, ion **l**–2H), 69 (43, ion **m**) and 67 (12, ion **m**–2H).

3.6. Acetylation of *4a*

A soln of compound **4a** (13 mg) in Ac_2O/C_5H_5N (1:1, 2 ml) was kept at room temp. overnight. Work up as described above yielded a crystalline mass, which was purified by prep. TLC (plate: 20 × 20 cm; solvent: $CHCl_3$–MeOH, 100:1) to furnish the corresponding acetate (**4a**), 12 mg, m.p. 137.5–139°C (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +89° (*c* 0.33); IR ν_{max} cm^{-1}: 3034, 1641 and 888 (>C=CH_2), 2963, 2893, 1736 and 1243 (OAc), 1677 (O=C–C=C–C=O), 1457, 1430 (–CH_2CO), 1376, 1363, 1205 and 1029; ^1H and ^{13}C NMR: see Tables 1 and 2; EIMS (rel. int.): m/z 496 (33) $[M]^+$, 481 (2) $[M–Me]^+$, 436 (4) $[M–HOAc]^+$, 426 (2, ion **a**–H), 413 (14, ion **b**), 412 (23, ion **b**–H), 399 (4, ion **c**), 397 (3, ion **c**–2H), 371 (9, ion **d**), 369 (3, ion **d**–2H), 352 (6) [412-HOAc], 344 (33, ion **f**), 331 (5, ion **g**), 317 (13, ion **h**) $[C_{19}H_{25}O_4]^+$, 305 (20, ion **i′**), 289 (9), 278 (10), 256 (10), 241 (14), 187 (25), 123 (18, ion **j**–2H), 121 (17), 109 (100, ion **j**, CH_4), 97 (14, ion **k**), 95 (28, ion **k**–2H), 83 (33, ion **l**), 81 (42, ion **l**–2H), 69 (75, ion **m**) and 67 (20, ion **m**–2H).

3.7. Synthesis of *4b* and *4c* from *1b*

A soln of CrO_3 (33.3 mg) in HOAc (5 ml) containing 3 drops of H_2O was gradually added into a soln of obtusifoliol acetate (**1b**) (30 mg) in HOAc (15 ml) under stirring at 80°C and then the reaction was continued for 4 h. After cooling, a few drops of 5% $NaHSO_3$ were added into the mixture to destroy excess CrO_3. Evapn of HOAc in vacuo gave a residue, which was dissolved in $CHCl_3$ (20 ml) and the organic layer was washed with sat. $NaHCO_3$ and H_2O and dried (Na_2SO_4). Removal of the solvent in vacuo yielded a yellow residue (31 mg) showing two spots on a TLC plate. Separation of the residue by prep. TLC (plate: 20 × 20 cm, 0.5 mm; solvent: $CHCl_3$–MeOH, 100:1), furnished 3β-acetoxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-7,11-dione (**4b**), as pale yellow needles, m.p. 138–139°C (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +89° (*c* 0.30), 12 mg, EIMS: m/z 496 $[M]^+$, TLC: R_f 0.56 (plate: 0.25 mm; solvent: $CHCl_3$–MeOH 100:1) and 3β-acetoxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11,24-trione (**4c**), 11 mg, as an amorphous pale yellow powder, $[\alpha]_D^{23}$ +100° (*c* 0.16); TLC: R_f 0.34 (plate: 0.25 mm; solvent: $CHCl_3$–MeOH: 100:1), UV λ_{max} (nm): 219 and 268 (ε 5300 and 5500); IR ν_{max} cm^{-1}: 2963, 2876, 1733 and 1246 (OAc), 1712 (C=O), 1673 (O=C–C=C–C=O), 1467, 1429 and 1420 (CH_2CO), 1377, 1100 and 1030; ^1H and ^{13}C NMR: see Tables 1 and 2; EIMS m/z (rel. int.): 498 (44) $[M]^+$, 438 (11) $[M–HOAc]^+$, 413 (7, ion **b**), 399 (7, ion **c**), 371 (6, ion **d**), 370 (10, ion **d**–H), 357 (5, ion **e**), 355 (6, ion **e**–2H), 344 (7, ion **f**), 331 (6), 329 (6, ion **g**), 317 (20 ion **h**), 305 (10, ion **i′**), 304 (13), 289 (11), 241 (13), 127 (20, ion **j**) $[C_8H_{15}O]^+$, 85 (12, ion **l**), 83 (18, ion **l**–2H) and 71 (100, ion **m**). The former product was identified by direct comparison (m.p., co-TLC, $[\alpha]_D$, UV, IR, ^1H and ^{13}C NMR and EIMS) with **4b** derived from compound **4a**.

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References

Akihisa, T., Kokke, W. C. M. C., Yokota, T., Tamura, T., & Matsumoto, T. (1990). *Phytochemistry*, *29*, 1647.

Akihisa, T., Yokota, T., Takahashi, N., Tamura, T., & Matsumoto, T. (1989). *Phytochemistry*, *28*, 1219.

Barrera, J. B., Breton, J. L., Dergado Martin Jr, & Gonzalez, A. G. (1967). *Annales de Fisica y Quimica*, *63B*, 191.

Gonzalez, A. G., Breton, J. L., & Garcia, P. A. (1958). *Annales de Fisica y Quimica*, *54B*, 93.

Gonzalez, A. G., Breton, J. L., Dergado Martin, J., & Fraga, B. M. (1972). *Annales de Quimica*, *68*, 203.

Itoh, T., Kikuchi, Y., Shimizu, N., Tamura, T., & Matsumoto, T. (1981). *Phytochemistry*, *20*, 1929.

Matsunaga, S., Morita, R., Ishida, T., Inoue, M., Shigi, M., & Miyamae, A. (1984). *Journal of the Chemical Society, Chemical Communications*, *1984*, 1128.

Nes, W. D., Koike, K., Jia, Z., Sakamoto, Y., Satu, T., Nikaido, T., & Griffin, J. F. (1998). *Journal of American Chemical Society*, *120*, 5870.

Tanaka, R., & Matsunaga, S. (1989). *Phytochemistry*, *28*, 3149.

Tanaka, R., & Matsunaga, S. (1991). *Phytochemistry*, *30*, 293.

Tanaka, R., Ida, T., Kita, S., Kamisako, W., & Matsunaga, S. (1994). *Phytochemistry*, *36*, 129.

Tanaka, R., Ida, T., Kita, S., Kamisako, W., & Matsunaga, S. (1996). *Phytochemistry*, *41*, 1163.

Tanaka, R., Matsunaga, S., Ishida, T., & Shingu, T. (1989). *Tetrahedron Letters*, *30*, 1661.