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SUPINENOLONES A, B AND C, FERNANE TYPE TRITERPENOIDS FROM *EUPHORBIA SUPINA*

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Key Word Index—*Euphorbia supina*; Euphorbiaceae; whole herb; triterpenoids; 3β,7α-dihydroxyfern-8-en-11-one; 3β,11β-dihydroxyfern-8-en-7-one; 3β-hydroxyfern-8-en-7,11-dione; ^{13}C NMR; HRMS.

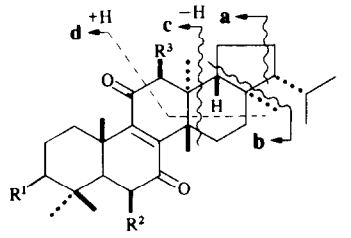
Abstract—Three new triterpenoids, named supinenolones A, B and C, have been isolated from *Euphorbia supina*. Their structures were established as 3β,7α-dihydroxyfern-8-en-11-one, 3β,11β-dihydroxyfern-8-en-7-one and 3β-hydroxyfern-8-en-7,11-dione, respectively, on the basis of chemical and spectral evidence.

INTRODUCTION

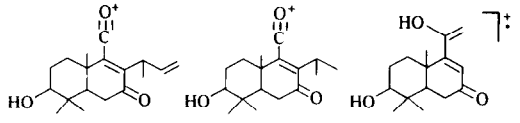
In the course of a search for the biologically active constituents of *Euphorbia supina* Rafin., a toxic annual weed which is used as a folk medicine [1, 2], we have isolated and characterized 21 biogenetically interesting triterpenoids [3–8] including spirosupinanonediol [4] and the known compound, loliolide [8]. Spirosupinanonediol has the novel skeletal system of 7(8→9) *abeo*-9*S*-D:C-*friedo*-B′:A′-neogammacerane, a migrated fernane, for which we gave the name of 'spirosupinane'. Further examination of the neutral benzene extract of the whole herb of this plant by silica gel column chromatography furnished three new triterpenoids, which we have named supinenolones A, B and C. The present paper deals with the structure elucidation of the above compounds, starting with supinenolone C because of explanatory convenience.

RESULTS AND DISCUSSION

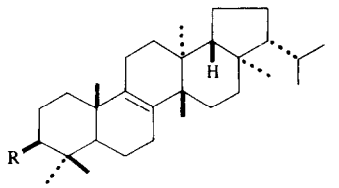
Supinenolone C (**1a**), $C_{30}H_{46}O_3$ $[M]^+$ at *m/z* 454.3445), showed an UV absorption band characteristic for a transoid-ene-dione chromophore [λ_{max} 272 nm (ε 8500)]. Its ^{1}H and ^{13}C NMR spectra established the presence of six tertiary methyl groups, one isopropyl group, a C-3α carbinolic methine group, a tetrasubstituted double bond and two keto-groups (see Tables 1 and 2). On acetylation, it gave an acetate (**1b**). Deuteration of **1b** afforded a tetradeutero-acetate (**1c**), indicative of the presence of a $-CH_2-CO-\underset{|}{C}=\underset{|}{C}-CO-CH_2-$ grouping in the molecule of **1a**. The physical and IR spectral data of **1b** were closely similar to those of 3β-acetoxyfern-8-en-7:11-dione [9] which has previously been synthesized from motiol (fern-7-en-3β-ol), a triterpene component of *Rhododendron linearifolium*. Furthermore, in the high resolution mass spectrum (HRMS) of **1a**, the presence of seven characteristic fragment peaks at *m/z* 384.2669 $[C_{25}H_{36}O_3]^+$ (ion **a**), 369.2430 $[C_{24}H_{33}O_3]^+$ (ion **b**), 301.1806 $[C_{19}H_{25}O_3]^+$ (ion **c**), 290.1867 $[C_{18}H_{26}O_3]^+$ (ion **d**), 289.1803 $[C_{18}H_{25}O_3]^+$ (ion **e**), 277.1829 $[C_{17}H_{25}O_3]^+$ (ion **f**), and 250.1575 $[C_{15}H_{22}O_3]^+$ (ion **g**) [10], besides peaks at *m/z* 411 $[M-C_3H_7]^+$ and 396 $[M-Me-C_3H_7]^+$ supported the above deduction.



1a R¹ = OH, R² = R³ = H₂
1b R¹ = OAc, R² = R³ = H₂
1c R¹ = OAc, R² = R³ = D₂
1d R¹ = OAc, R² = H₂, R³ = O



e **f** **g**



4a R = OH
4b R = OAc

The synthesis of **1b** was carried out using fern-8-en-3β-ol (**4a**), which is the most abundant triterpene constituent in this plant [6]. Acetylation of **4a** followed by chromium trioxide oxidation of the resulting acetate (**4b**) in acetic acid afforded, together with a small amount of 3β-acetoxy-fern-8-en-7,11,12-trione (**1d**), the acetoxy-dione identical in all respects with **1b**. Thus, the structure of supinenolone C was shown to be 3β-hydroxyfern-8-en-7,11-dione (**1a**).

Supinenolones A (**2a**) and B (**3a**) had the same molecular formulae, $C_{30}H_{48}O_3$, in the high resolution mass spectra (HRMS), and also showed similar UV absorption

Table 1. 300 M Hz ^{1}H NMR chemical shifts of compounds **1a**, **1b**, **2a**, **2b**, **3a**, **3b**, **4a** and **4b** in $CDCl_3$

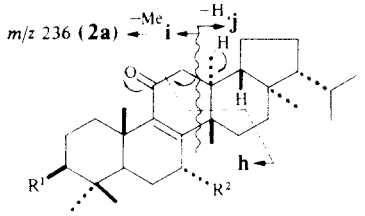
	1a	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**
23-Me	1.02	0.90	1.05	0.83	0.99	0.87	1.00	0.87
24-Me	0.90	0.98	0.85	0.88	0.92	0.98	0.78	0.88
25-Me	1.25	1.27	1.18	1.22	1.19	1.11	0.953	0.97
26-Me	1.27	1.28	1.11	1.11	1.36	1.35	0.949	0.76
27-Me	1.04	1.03	0.99	0.97	0.81	0.81	0.77	0.76
28-Me	0.76	0.77	0.79	0.77	0.74	0.73	0.76	0.75
29-Me	0.84 *d* *J* 6.5	0.84 *d* *J* 6.5	0.84 *d* *J* 6.5	0.84 *d* *J* 6.5	0.83 *d* *J* 6.5	0.82 *d* *J* 6.5	0.83 *d* *J* 6.5	0.83 *d* *J* 6.5
30-Me	0.90 *d* *J* 6.5	0.90 *d* *J* 6.5	0.90 *d* *J* 6.5	0.89 *d* *J* 6.5	0.89 *d* *J* 6.5	0.88 *d* *J* 6.5	0.89 *d* *J* 6.5	0.89 *d* *J* 6.5
H-1	1.15 *m* 2.50 *dt* *J* 12.3 3.2	1.17 *m* 2.51 *dt* *J* 12.3 3.2	1.03 *m* 2.53 *dt* *J* 12.3 3.2	1.21 *m* 2.50 *dt* *J* 12.3 3.2	1.61 *m* 2.32 *dt* *J* 12.3 3.2	1.66 2H, *m*		
H-3α	3.29 *dd* *J* 10.3 6.2	4.58 *dd* *J* 10.8 6.2	3.33 *dd* *J* 10.0 6.7	4.58 *dd* *J* 10.0 6.7	3.32 *dd* *J* 10.2 6.7	4.56 *dd* *J* 10.2 6.7	3.25 *dd* *J* 11.5 5.3	4.50 *dd* *J* 11.5 5.3
H-6α	2.47 *dd* *J* 18.5 8.0	2.48 *dd* *J* 18.5 8.0	1.71 *m*	1.66 *m*	2.44 *dd* *J* 18.5 8.0	2.45 *dd* *J* 18.5 8.0		
H-6β	2.54 *dd* *J* 18.5 11.7	2.54 *dd* *J* 18.5 11.7	1.82 *m*	1.82 *m*	2.50 *dd* *J* 18.5 11.7	2.50 *dd* *J* 18.5 11.7		
H-7β			4.41 *br s* *W*/2 8.0	5.46 *dd* *J* 3.1 1.9				
H-11α					4.72 *dd* *J* 15.2 7.2	5.73 *dd* *J* 15.2 7.2		
H-12α	2.30 *d* *J* 18.7	2.36 *d* *J* 18.7	2.20 *d* *J* 18.7	2.21 *d* *J* 18.7	2.02 *dd* *J* 18.7 9.8	2.23 *dd* *J* 18.7 9.8		
H-12β	2.19 *d* *J* 18.7	2.16 *d* *J* 18.7	2.10 *d* *J* 18.7	2.12 *d* *J* 18.7	1.48 *m*	1.42 *m*		
H-15	1.63 *m* 2.48 *dt* *J* 12.1 4.4	1.65 *m* 2.47 *dt* *J* 12.1 4.4	1.49 *m* 2.21 *dt* *J* 12.1 4.4	1.25 *m* 1.96 *dt* *J* 12.1 4.4	1.43 *m* 2.41 *dt* *J* 12.1 4.4	1.39 *m* 2.39 *dt* *J* 12.1 4.4		
OCOMe		2.07		2.06		2.05		2.05
OCOMe				2.07		2.07		

bands for an α,β-conjugated enone system (see Experimental). The ^{1}H and ^{13}C NMR spectra established the presence in both compounds of six tertiary and two secondary methyl groups, two hydroxymethine groups, a tetrasubstituted double bond and a keto-group (Tables 1 and 2). On acetylation, compounds **2a** and **3a** gave different diacetates, **2b** and **3b** respectively, whereas on chromium trioxide oxidation in pyridine both compounds gave the same trione (**2c**) with a transoid enedione chromophore [λ_{max} 272 nm (ε 8 500)]. These results, together with the absence of any ethylenic proton signals in the IR and ^{1}H NMR spectra, indicated that **2a** and **3a** must have the same carbon skeleton involving a γ-hydroxy-α,β-conjugated enone system with a tetrasubstituted double bond. The CD spectra of both compounds showed similar multiple cotton effect curves (see Experimental). Partial acetylation of **2a** gave two different monoacetates, **2d** and **2e**, together with both **1b** and **2b**, while mild oxidation with chromium trioxide in pyridine afforded the hydroxy-dione identical in all respects with

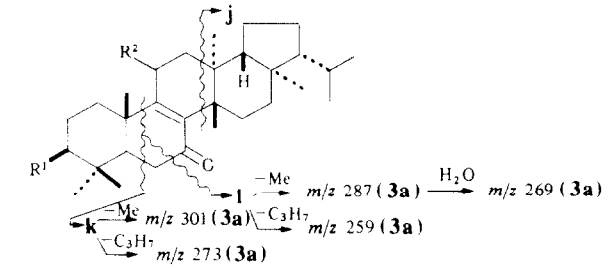
Table 2. 74.5 MHz ^{13}C NMR chemical shifts of compounds **1a**, **1b**, **2a**, **2b**, **3a**, **3b** and **4a** in $CDCl_3$

C	1a	1b	2a	2b	3a	3b	4a
1	34.42	34.06	34.14	33.92	33.67	33.58	35.46
2	27.43	23.80	27.93	24.19	27.18	23.66	27.96
3	78.06	79.56	78.62	80.11	78.25	79.49	79.02
4	38.64	37.51	38.38	37.29	38.99	37.96	38.92
5	47.97	48.02	45.18	45.63	48.00	48.30	50.47
6	36.70	36.51	28.63	25.54	36.85	36.70	20.36
7	199.99	199.51	64.24	67.19	200.68	199.76	27.23
8	151.92	150.93	157.95	154.63	142.20	145.30	134.28
9	156.01	155.76	142.58	144.88	160.19	156.01	134.28
10	38.16	37.97	38.59	38.16	39.47	39.03	37.60
11	201.14	201.06	199.93	199.59	65.88	68.37	19.14
12	51.14	51.10	50.80	50.83	42.05	38.67	30.26
13	38.47	38.44	43.33	43.41	39.38	39.03	41.08
14	41.59	41.56	37.54	37.68	40.51	40.63	36.73
15	26.41	26.36	27.23	25.99	25.63	25.85	26.98
16	35.80	35.76	35.83	35.66	35.71	35.80	35.94
17	42.57	42.55	42.71	42.66	42.27	42.46	42.92
18	50.80	50.77	51.90	51.84	51.13	51.31	52.75
19	20.40	20.38	20.29	20.32	20.21	20.34	18.90
20	28.93	27.90	28.02	28.04	27.96	28.07	28.38
21	59.54	59.50	59.52	59.46	59.58	59.66	59.77
22	30.66	30.62	30.66	30.66	30.56	30.66	30.77
23	27.51	27.39	28.18	27.79	27.36	27.29	28.07
24	14.87	15.92	16.07	16.86	14.96	16.07	15.54
25	17.93	17.96	17.96	18.07	20.01	22.90	22.08
26	21.78	20.72	23.94	23.86	23.73	22.90	22.08
27	20.79	21.75	19.73	19.61	16.94	17.68	15.83
28	14.02	14.02	13.85	13.91	14.55	14.47	14.64
29	22.90	22.87	22.90	22.90	22.78	22.82	22.99
30	22.03	22.01	22.06	22.00	21.85	21.97	22.08
OCOMe	—	21.22	—	21.27	—	21.22	—
OCOMe	—	—	—	21.38	—	21.44	—
OCOMe	—	—	—	169.98	—	170.06	—
OCOMe	—	170.88	—	171.02	—	170.82	—

supinenolone C (**1a**), along with **2f**. These facts indicated that compounds **2a** and **3a** must be either the 7-hydroxy or the 11-hydroxy derivatives of **1a**. The HRMS data allowed us to discriminate the structural difference between **2a** and **3a**. Compound **2a** showed four predominant peaks due to the cleavage of the rings C and D at *m/z* 277.1804 $[C_{17}H_{25}O_3]^+$ (ion **h**) 251.1645 $[C_{15}H_{23}O_3]^+$ (ion **i**), 236.1805 $[\mathbf{i}-Me]^+$ and 205.1958 $[C_{15}H_{25}]^+$ (ion **j**), besides peaks at *m/z* 413.3015 $[M-C_3H_7]^+$ and 395 $[M-H_2O-C_3H_7]^+$. In contrast, compound **3a** showed eight characteristic fragment peaks due to α- and β-cleavage of the C-7 keto-group in the B ring involving rupture of the C(9)–C(10) bond at *m/z* 316.2385 $[C_{21}H_{32}O_2]^+$ (ion **k**), 302.2239 $[C_{20}H_{30}O_2]^+$ (ion **l**), 301.2157 $[\mathbf{k}-Me]^+$, 287.2020 $[\mathbf{l}-Me]^+$, 273.1908 $[\mathbf{k}-C_3H_7]^+$, 269.1862 $[\mathbf{l}-Me-H_2O]^+$, 259.1745 $[\mathbf{l}-C_3H_7]^+$, and 205.1943 $[C_{15}H_{25}]^+$ (ion **j**), together with peaks at *m/z* 413.3063 $[M-C_3H_7]^+$ and 395 $[M-H_2O-C_3H_7]^+$. The above results clearly indicated that **2a** and **3a** had the 7-hydroxy-Δ^8-en-11-one and the 11-hydroxy-Δ^8-en-7-one grouping, respectively, in the fernane skeleton. In the 90 MHz ^{1}H NMR spectra of **2a** and **3a**, the signals for one of the two hydroxymethine groups appeared at δ3.33 (*dd*, *J* = 11 and 7 Hz) and were attributed to the C-3α axial proton, which exhibited acetylation shift at δ4.63 (*dd*, *J* = 11 and 7 Hz), 4.58 (*dd*, *J* = 11 and 7 Hz) and 4.56 (*dd*, *J* = 11 and 7 Hz) in **2b**, **3b** and **2d**, respectively. The signal for the second group appeared at δ4.43 (*m*, *W*/2 = 8 Hz) in **2a** and 4.75 (*dd*, *J* = 15 and 7 Hz) in **3a** and were due to the allylic hydroxymethine protons of C-7β (eq) and C-11α (ax), respectively, on the half-chair environments of the B/C rings. These signals were shifted to δ5.53 (*m*, W/2 8 Hz, H-7β) and 5.75 (*dd*, *J* = 15 and 7 Hz, H-11α) in **2b** and **3b**, respectively. Therefore, supinenolones A and B must have the structures of 3β,7α-dihydroxyfern-8-en-11-one (**2a**) and 3β,11β-dihydroxyfern-8-en-7-one (**3a**), respectively. Conclusive evidence for the structures was obtained by the synthesis of compound **2a** from fern-8-en-3β-ol (**4a**) [7]. Chromium trioxide oxidation of **4a** in acetic acid afforded the trione identical with **2c**. Lithium aluminium hydride reduction of **2c**, followed by partial acetylation provided two monoacetates, **2d** and **2e**, together with **1b** and **2b**. Alkaline hydrolysis of the synthetic **2b**, **2d** and **2e** gave the keto-diol identical in all respects with supinenolone A (**2a**). Consequently, the structures of supinenolones A and B were established to be **2a** and **3a**. Detailed analyses of the ^{1}H and ^{13}C NMR signals for compounds **1a**, **1b**, **2a**, **2b**, **3a**, **3b** and **4a** have also been carried out employing proton decoupling, DEPT, 2D ^{1}H–^{1}H COSY, NOESY,



2a $R^1 = R^2 = OH$
2b $R^1 = R^2 = OAc$
2c $R^1 = R^2 = O$
2d $R^1 = OAc$, $R^2 = OH$
2e $R^1 = OH$, $R^2 = OAc$
2f $R^1 = O$, $R^2 = OH$



3a $R^1 = R^2 = OH$
3b $R^1 = R^2 = OAc$

2D ^1H–^{13}C COSY, and 2D long range ^1H–^{13}C COSY techniques. In the ^1H–^1H COSY experiment, the C-7β proton signal in **2a** correlated with the methylene proton signals at C-6, and the C-11α proton signal in **3a** correlated with the methylene proton signals at C-12. Moreover, in the 2D long range ^1H–^{13}C COSY spectrum, the signal of the C-11 carbonyl carbon in **2a** clearly exhibited coupling to the methylene proton signals at C-12, whereas the C-7 carbonyl signal in **3a** was coupled to the methylene proton signals at C-6. All the above data supported well the structures of **2a** and **3a**. Assignments are listed in Tables 1 and 2.

To the best of our knowledge, compounds **1a**, **2a** and **3a** have not yet been described in the literature, although **1b** has been synthesized once [9]. So far 3β-hydroxylanost-8-en-7,11-dione [11] was the only naturally occuring triterpenoid having the Δ^8-en-7,11-dione chromophore in the molecule. This compound was isolated from wool wax along with 3β-hydroxylanost-8-en-7-one, 3β-hydroxylanostan-7-one and 3β-hydroxylanostan-7,11-dione [12]. Investigation of the physiological activities of compounds **1a**, **2a** and **3a** are now in progress.

EXPERIMENTAL

General. Mps: uncorr. Optical rotations: $CHCl_3$; CD: MeOH; UV: aldehyde-free EtOH; IR: KBr discs, unless otherwise noted; ^1H NMR (90 or 300 MHz) and ^{13}C NMR (74.5 MHz): $CDCl_3$ with TMS as int. standard; EIMS: double focussing mass spectrometer (accelerating voltage of 3–6.5 kV; ionizing potential 70 eV). CC: Kieselgel 60 and alumina 90 (Merck, 70–230 mesh); TLC: silica gel HF_{254} and PF_{254} (Merck).

Extraction and isolation of compounds: The extraction of the whole herb (10 kg) and the preliminary CC of the resulting neutral C_6H_6 extract (1.15 kg) has already been reported [5]. After germanicol and sitosterol had been eluted, fractionation of the column was continued with C_6H_6–$CHCl_3$ (1:1), $CHCl_3$ and $CHCl_3$–EtOAc (10:1). All the above fractions were combined (86.0 g) on the basis of the TLC analytical results and twice rechromatographed over silica gel and alumina. Elution with C_6H_6 and C_6H_6–$CHCl_3$ (10:1) yielded an yellowish brown gum (1.491 g) from the latter eluant. Successive elution of the column with C_6H_6–$CHCl_3$ (3:1–1:1) afforded two colourless crystals, **2a** (885 mg) and **3a** (521 mg), respectively. Further elution with C_6H_6–$CHCl_3$ (1:1) and $CHCl_3$ afforded fractions containing spirosupinanonediol [4], 3β-hydroxyhexanordammaran-20-one [5], 3,4-*seco*-adianane derivatives [7] and olean-12-en-3β,9α,11α-triol [8], respectively, which have already been reported. Repeated CC of the gummy material on alumina furnished **1a** (1.282 g) as a pale yellow solid from the fractions eluted with C_6H_6–$CHCl_3$ (1:1).

Supinenolone C (**1a**). Mp 209–210°, $[\alpha]_D^{23}$ −3.0° (*c* 0.63), HRMS: *m/z* 454.3446 (M^+, $C_{30}H_{46}O_3$ requires 454.3444); R_f 0.69 (C_6H_6–$CHCl_3$–EtOAc, 2:2:1); λ_{max} 272 nm (ε 8500) (–OC–C=C–CO–); ν_{max} cm^{-1} 3450 (OH), 1670 (>C=C–C=O), 1390, 1370, 1108, 1065, 1043; ^1H and ^{13}C NMR: see Tables 1 and 2; EIMS *m/z* (rel. int.): 454 $[M]^+$ (100), 439 $[M-Me]^+$ (55), 436 $[M-H_2O]^+$ (7), 426 $[M-CO]^+$ (6), 421 $[M-Me-H_2O]^+$ (6), 411 $[M-C_3H_7]^+$ (4), 396 $[M-Me-C_3H_7]^+$ (3), 393 $[M-H_2O-C_3H_7]^+$ (3), 384 [ion **a**] (13), 369 [ion **b**] (4), 301 [ion **c**] (4), 290 [ion **d**] (13), 289 [ion **e**] (11), 277 [ion **f**] (22), 250 [ion **g**] (6), 189 (4).

Supinenolone A (**2a**). Mp 309–310°, $[\alpha]_D^{23}$ −4.5° (*c* 0.81), R_f 0.45 (C_6H_6–$CHCl_3$–EtOAc, 1:1:1); HRMS: *m/z* 456.3596 (M^+, $C_{30}H_{48}O_3$ requires 456.3603); λ_{max} 255.5 nm (ε 7500) (γ-hydroxy-α,β-conjugated enone); ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1660 (sh) and 1640 (>C=C–C=O), 1385, 1370, 1365, 1120, 1078, 1020; $\nu_{max}^{CHCl_3}$ ($CHCl_3$) cm^{-1}: 3440, 1720 (sh), 1658, 1375, 1357, 1340, 1015; CD: $[\theta]_{408}$ 0°, $[\theta]_{358}$ +1730° (peak), $[\theta]_{323}$ 0°, $[\theta]_{307}$ −406° (trough), $[\theta]_{295}$ −135° (peak), $[\theta]_{264}$ −2164° (trough), $[\theta]_{254}$ −1204° (peak), $[\theta]_{220}$ −10280°; ^1H and ^{13}C NMR: see Tables 1 and 2; EIMS *m/z* (rel. int.): 456 $[M]^+$ (65), 438 $[M-H_2O]^+$ (100), 423 $[M-H_2O-Me]^+$ (23), 420 $[M-2H_2O]^+$ (30), 413 $[M-Me-C_3H_7]^+$ (8), 405 $[M-Me-2H_2O]^+$ (31), 395 $[M-H_2O-C_3H_7]^+$ (11), 317 (12), 277 [ion **h**] (39), 251 [ion **i**] (58), 236 [**i**−Me]$^+$ (22), 205 [ion **j**] (20).

Supinenolone B (**3a**). Mp 284–287°, $[\alpha]_D^{23}$ +4.6° (*c* 0.69); R_f 0.27 (C_6H_6–$CHCl_3$–EtOAc, 1:1:1); HRMS: *m/z* 456.3614 (M^+, $C_{30}H_{48}O_3$ requires 456.3603); λ_{max} 254.5 nm (ε 7900) (γ-hydroxy-α,β-conjugated enone); ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1655 (sh) and 1642 (>C=C–C=O), 1380, 1375, 1360, 1060, 1038, 1010; ν_{max} ($CHCl_3$) cm^{-1}: 3440, 1710 (sh), 1664, 1377, 1360, 1348, 1036, 1010; CD: $[\theta]_{390}$ 0°, $[\theta]_{345}$ +900° (peak), $[\theta]_{310}$ 0°, $[\theta]_{300}$ −120° (trough), $[\theta]_{283}$ −144° (peak), $[\theta]_{261}$ −2316° (trough), $[\theta]_{240}$ +1464° (peak), $[\theta]_{210}$ −8340°; ^1H and ^{13}C NMR: see Tables 1 and 2; EIMS *m/z* (rel. int.): 456 $[M]^+$ (100), 441 $[M-Me]^+$ (52), 438 $[M-H_2O]^+$ (31), 423 $[M-Me-H_2O]^+$ (90), 413 $[M-C_3H_7]^+$ (15), 405 $[M-Me-2H_2O]^+$ (21), 395 $[M-H_2O-C_3H_7]^+$ (30), 316 [ion **k**] (35), 302 [ion **l**] (47), 301 [**k**−Me]$^+$ (27), 287 [**l**−Me]$^+$ (37), 273 [**k**−C_3H_7]$^+$ (38), 269 [**l**−Me−H_2O]$^+$ (30), 259 [**l**−C_3H_7]$^+$, 205 [ion **j**] (41).

Acetylation of **1a**. Compound **1a** (40 mg) was acetylated (Ac_2O–pyridine, 1:1, 2 ml) at room temp. overnight. Work-up as usual furnished the corresponding acetate (**1b**), mp 231–234° (MeOH–$CHCl_3$) (38 mg), $[\alpha]_D^{23}$ −18.6° (*c* 0.42) (lit, [9] mp 227–228°); λ_{max} 275 nm (ε 7.500); ν_{max} cm^{-1}: 1737 (OAc), 1668 (>C=C–CO–), 1383, 1375, 1364, 1248, 1190, 1160, 1028, 1010; ^1H and ^{13}C NMR: see Tables 1 and 2; EIMS *m/z* (rel. int.): 496 $[M]^+$ (100), 481 $[M-Me]^+$

(59), 468 $[M-CO]^+$ (6), 453 $[M-C_3H_7]^+$ (11), 436 $[M-HOAc]^+$ (20), 426 [ion **a**] (23), 421 $[M-Me-HOAc]^+$ (17), 411 [ion **b**] (11), 408 $[M-HOAc-CO]^+$ (8), 369 $[\mathbf{b}-CH_2CO]^+$ (14), 343 [ion **c**] (6), 332 [ion **d**] (32), 331 [ion **e**] (21), 319 [ion **f**] (44), 301 $[\text{ion } \mathbf{c}-CH_2CO]^+$ (6), 292 [ion **g**] (18), 257 (14), 255 (11), 189 (14).

Tetradeutero-supinenolone C (**1c**). A soln of Na (25 mg) in MeOD (1.5 ml) containing D_2O (0.5 ml) was added slowly to a boiling soln of compound **1b** (4.7 mg) in MeOD (6.5 ml) under a stream of N_2 gas and the mixture was refluxed for 10 min. Evapn of the solvent *in vacuo* gave a residue, which was dissolved in dry Et_2O. Removal of the solvent afforded a solid. After twice repeating the above treatment on the residual solid, the final product was purified by prep. TLC ($CHCl_3$) to give **1c**, (4 mg) as pale yellow needles, mp 211–213° (MeOD); ν_{max} cm^{-1}: 3550 (OH), 1670 (>C=C–CO–); ^1H NMR (300 MHz): δ0.76 (3H, *s*, Me-28), 0.83 (3H, *d*, J=6.5 Hz, Me-29), 0.89 (3H, *d*, J=6.5 Hz, Me-30), 0.90 (3H, *s*, Me-24), 1.02 (3H, *s*, 23-Me), 1.256 (3H, *s*, Me-25), 1.266 (3H, *s*, Me-26), 3.30 (1H, *dd*, J 10.8 and 6.2, H-3α); EIMS *m/z* (rel. int.): 458 $[M]^+$ ($C_{30}H_{42}D_4O_3$, 100), 443 $[M-Me]^+$ (33), 440 $[M-H_2O]^+$ (5), 430 $[M-CO]^+$ (5), 425 $[M-Me]^+$ (3), 415 $[M-C_3H_7]^+$ (3), 388 [ion **a**] (10), 373 [ion **b**] (3), 303 [ion **c**] (3), 304 (3), 292 [ion **d**] (14), 291 [ion **e**] (8), 279 [ion **f**] (18), 252 [ion **g**] (8), 205 [ion **j**] (4).

Synthesis of **1b**, **1d** *and* **1a**. Acetylation of fern-8-en-3β-ol (40 mg) with Ac_2O and pyridine (1:1, 2 ml) at room temp. gave the acetate **4b** (36 mg), mp 229–231° (lit. [6], mp 229–231°, which was dissolved in HOAc (90 ml) at 80° and oxidized with a soln of CrO_3 (13 mg) in HOAc (3 ml) containing two drops of H_2O with stirring at 70–80° for 7 hr. After cooling, a few drops of 5% $NaHSO_3$ soln was added to the mixture to destroy excess CrO_3. Evapn of the HOAc *in vacuo* gave a residual solid, which was dissolved in Et_2O, washed with 5% $NaHCO_3$ and H_2O, and the Et_2O layer dried over Na_2SO_4. Removal of the solvent gave a yellow residue (38 mg). Separation by prep. TLC afforded two products, 3β-acetoxy-fern-8-en-7,11-dione, mp 230–232° (MeOH–$CHCl_3$) (29 mg), $[M]^+$ at *m/z* 496, identical in all respects (mmp, co-TLC, IR, ^1H NMR, EIMS) with supinenolone C acetate (**1b**) from the non-polar zone, and 3β-acetoxyfern-8-en-7,11,12-trione (**1d**), mp 297–300° (MeOH–$CHCl_3$) (2 mg), ν_{max} cm^{-1}: 1740 (OAc), 1710 (C=O), 1678 (>C=C–C=O), 1390, 1367, 1248, 1197, 1017; EIMS *m/z* (rel. int.): 510 $[M]^+$ (100), 495 $[M-Me]^+$ (10), 482 $[M-CO]^+$ (22), 477 $[M-Me-H_2O]^+$ (15), 450 $[M-HOAc]^+$ (10), 441 $[M-CO-C_3H_5]^+$ (65), 439 $[M-CO-C_3H_7]^+$ (64), 425 $[M-CH_2CO-C_3H_7]^+$ (64), 218 (64), 151 (97), from the polar zone. Treatment of the synthetic **1b** (20 mg) in boiling KOH–EtOH (0.05 M, 30 ml) afforded the corresponding dionol, mp 209–211°, which was identified by direct comparison (mmp, co-TLC, IR, ^1H NMR, EIMS) with supinenolone C (**1a**).

Acetylation of **2a**. Compound **2a** (40 mg) was acetylated as usual (Ac_2O–pyridine, 1:1, 3 ml) at room temp., overnight, to give the diacetate **2b** (40 mg) as colourless needles, mp 206–209°, ν_{max} cm^{-1}: 1742, 1240 (OAc), 1664 (>C=C–CO–); ^1H and ^{13}C NMR: see Tables 1 and 2; EIMS *m/z* (rel. int.): 540 $[M]^+$ (13), 498 $[M-CH_2CO]^+$ (31), 480 $[M-HOAc]^+$ (76), 465 $[M-Me-HOAc]^+$ (9), 455 $[M-CH_2CO-C_3H_7]^+$ (36), 438 $[M-HOAc-CH_2CO]^+$ (16), 420 $[M-2HOAc]^+$ (100), 405 $[M-2HOAc-Me]^+$ (66), 389 (13), 377 (12), 351 (27), 335 [ion **i**] (31), 326 (23), 311 (28), 306 (23), 205 [ion **j**] (25).

Acetylation of **3a**. Compound **3a** (30 mg) was acetylated as usual (Ac_2O–pyridine 1:1, 2 ml) to give the diacetate **3b** (40 mg) as colourless needles, mp 274–276°, ν_{max} cm^{-1}: 1742, 1258 (OAc), 1675 (>C=C–CO–); ^1H and ^{13}C NMR (300 MHz): see Tables 1 and 2; EIMS *m/z* (rel. int.): 540 $[M]^+$ (12), 525 (24), 498 (32), 480 $[M-HOAc]^+$ (57), 465 (100), 438 (20), 420 $[M-2HOAc]^+$ (12), 405 (20), 395 (15), 358 [ion **K**] (8), 353 (21), 344 [ion **l**] (10), 343 $[\mathbf{k}-Me]^+$ (14), 329 $[\mathbf{l}-Me]^+$ (35), 315 $[\mathbf{k}-C_3H_7]^+$ (24), 302 $[\mathbf{l}-CH_2CO]^+$ (18), 301 $[\mathbf{l}-C_3H_7]^+$ (13), 269 $[\mathbf{l}-Me-HOAc]^+$ (70), 255 (17), 241 (23), 205 [ion **j**] (11).

CrO_3 *oxidation of* **2a** *and* **3a**. (i) A soln of CrO_3 (50 mg) in pyridine (1.5 ml) containing one drop of H_2O was gradually added to a soln of compound **2a** (23 mg) in pyridine (5 ml) with stirring at 5°. The mixture was then kept at room temp. for 17 hr. Work-up as usual furnished a yellow solid (30 mg), which was purified by prep. TLC (C_6H_6–$CHCl_3$–EtOAc, 2:2:1) to give the trione **2c** (19 mg), as pale yellow needles, mp 209–212° (MeOH–$CHCl_3$), *m/z* 452.3289 ($C_{30}H_{44}O_3$ requires 452.3290); λ_{max} 271 nm (ε 8600); ν_{max} cm^{-1}: 1770, 1660 (>C=C–C=O); ^1H NMR (90 MHz); δ0.74 (3H, *s*), 0.82 (3H, *d*, J=6.5 Hz), 0.85 (3H, *s*), 0.89 (3H, *d*, J=6.5 Hz), 0.99 (3H, *s*), 1.09 (3H, *s*), 1.27 (3H, *s*), 1.46 (3H, *s*), 2.24 (2H, *m*), 2.44–2.83 (4H, *m*); EIMS *m/z* (rel. int.): 452 $[M]^+$ (100), 437 $[M-Me]^+$ (68), 424 $[M-CO]^+$ (9), 409.2754 $[M-C_3H_7]^+$ (11), 382.2500 [ion **a**] (28), 367 [ion **b**] (21), 299 [ion **c**] (5), 288.1732 [ion **d**] (30), 287 [ion **e**] (12), 275.1635 [ion **f**] (47), 248.1395 [ion **g**] (15), 205 [ion **j**] (11), 201 (15). (ii) Compound **3a** (20 mg) in C_5H_5N (5 ml) was oxidized with a soln of CrO_3 (45 mg) in pyridine (1.5 ml) containing one drop of H_2O at 5° for 17 hr and the mixture was treated as described above to give pale yellow needles, mp 208–211° (MeOH–$CHCl_3$) (16 mg). It was identified by direct comparison (mmp, co-TLC, UV, IR, ^1H NMR, EIMS) with the sample of **2c** derived from **1a**.

Partial acetylation of **2a**. Compound **2a** (20 mg) was acetylated with Ac_2O–pyridine (1:1, 4 ml) at 0° for 1 hr. After work-up as usual, the resulting crude product (21 mg) was separated by prep. TLC (C_6H_6–$CHCl_3$– EtOAc, 2:2:1) to give two monoacetates, **2d** (6 mg) and **2e** (5 mg), together with **2b** (7mg), mp 207–209°. (i) Compound **2d**: mp 315–316° (MeOH–$CHCl_3$), ν_{max} cm^{-1}: 1745 and 1242 (OAc), 1640 (>C=C–C=O), 1030, 1012 (C–O); ^1H NMR (90 MHz): δ0.80 (3H, *s*), 0.84 (3H, *d*, J=6.5 Hz), 0.89 (3H, *d*, J=6.5 Hz), 0.93 (6H, *s*), 0.98 (3H, *s*), 1.12 (3H, *s*), 1.21 (3H, *s*), 2.06 (3H, *s*, OAc), 2.43–2.63 (2H, *m*, H-12), 4.42 (1H, *m*, $W/2$ 8 Hz, H-7β), 4.58 (1H, *dd*, J=11, 6 Hz, H-3α); EIMS *m/z* (rel. int.): 498 $[M]^+$ (91), 480 (80), 465 (9), 456 (5), 455 (12), 438 (42), 420 (99), 405 (78), 377 (23), 333 (30), 319 [ion **h**] (73), 293 [ion **i**] (100), 278 $[\mathbf{i}-Me]^+$ (35), 215 $[\mathbf{i}-HOAc-H_2O]^+$ (39), 205 [ion **j**] (42). (ii) Compound **2e**: amorphous solid, ν_{max} cm^{-1}: 1738 and 1232 (OAc), 1689 (>C=C–CO), 1018 (C–O); ^1H NMR (90 MHz): δ0.77 (3H, *s*), 0.81 (3H, *s*), 0.83 (3H, *d*, J=6.5 Hz), 0.89 (3H, *d*, J=6.5 Hz), 0.95 (3H, *s*), 0.98 (3H, *s*), 1.12 (3H, *s*), 1.20 (3H, *s*), 2.08 (3H, *s*, OAc), 2.37–2.62 (2H, *m*, H-12), 3.27 (1H, *dd*, J=11, 6 Hz, H-3α), 5.53 (1H, *m*, $W/2$ 8 Hz, H-7β); EIMS *m/z* (rel. int.): 498 $[M]^+$ (8), 456 $[M-CH_2CO]^+$ (36), 438 $[M-HOAc]^+$ (100), 423 $[M-HOAc-Me]^+$ (23), 420 $[M-HOAc-H_2O]^+$ (26), 413 $[M-C_3H_7-CH_2CO]^+$ (28), 405 $[420-Me]^+$ (35), 377 $[420-C_3H_7]^+$ (9), 351 (14), 339 (33), 311 (16), 293 [ion **i**] (21), 264 (23), 215 $[\mathbf{i}-HOAc-H_2O]^+$ (23), 205 [ion **j**] (28).

Partial oxidation of **2a** *with* CrO_3. Compound **2a** (23 mg) in pyridine (5 ml) was oxidized with a soln of CrO_3 (25 mg) in pyridine (1 ml) containing one drop of H_2O at 0–10° for 1.5 hr. Then, several drops of 5% $NaHSO_3$ soln was added quickly to the mixture with stirring. Removal of the C_5H_5N *in vacuo* below 40° afforded a residue, which was dissolved with Et_2O and neutralized. Evapn of the solvent and successive separation of the resulting residue (22 mg) on prep. TLC (C_6H_6–$CHCl_3$–EtOAc 2:2:1) gave zones 1–4 in the order of their polarity. Individual elution of zones 1 and 2 with $CHCl_3$ furnished the

trione, mp 209–211° ($MeOH–CHCl_3$) (2 mg), and the hydroxy-dione, mp 199–201° ($MeOH–CHCl_3$) (9 mg), which were identified by direct comparison (mmp, co-TLC, IR, ^1H NMR, EIMS) with **2c** and **1a**, respectively. Elution of zone 3 with $CHCl_3$ gave **2f**, mp 314–315° ($MeOH–CHCl_3$) (3 mg), λ_{max} 255 nm (ε 7500), ν_{max} cm^{-1}: 3365 (OH), 1715 (>C=O), 1640 (>C=C–C=O); EIMS *m/z* (rel. int.): 454 $[M]^+$ (60), 436 $[M-H_2O]^+$ (54), 421 $[M-H_2O-Me]^+$ (31), 393 $[M-H_2O-C_3H_7]^+$ (16), 357 (14), 351 (18), 340 (15), 326 (11), 301 (11), 289 (20), 275 [ion **h**] (41), 249 [ion **i**] (100), 234 $[i-Me]^+$ (22), 205 [ion **j**] (23), 191 (20), 189 (19). Elution of zone 4 with $CHCl_3$ gave **2a**, mp 309–310° (6 mg).

Synthesis of **2c** *from fern-8-en-3β-ol* (**4a**). A soln of CrO_3 (30 mg) in HOAc (6 ml) containing 3 drops of H_2O was gradually added to a soln of **4a** (25 mg) in HOAc (20 ml) at 80° with stirring. The reaction was allowed to proceed for 15 hr at 70–80°. Evapn of the HOAc *in vacuo* yielded a residue, which was extracted with Et_2O and neutralized. Removal of the solvent afforded a yellow solid (26 mg). Purification with prep. TLC furnished yellow needles (20 mg), mp 208–211°, identical in all respects (mmp, co-TLC, IR, ^1H NMR, EIMS) with **2c**.

Synthesis of **2a**. A soln of the synthetic **2c** (40 mg), which was prepared from **4a**, in dry Et_2O (20 ml) was gradually added into a soln of $LiAlH_4$ (40 mg) in dry Et_2O (30 ml) with stirring. The mixture was then kept at room temp. After standing for 24 hr, a few drop of H_2O and 5% H_2SO_4 were carefully added to the reaction mixture to destroy excess $LiAlH_4$ and the resulting Et_2O layer was neutralized and dried. Removal of the solvent afforded a mixture of keto-alcohols (40 mg). Due to the poor separation of the mixture of TLC, it was subjected to mild acetylation (Ac_2O–pyridine, 1:1, 10 ml) at 0° for 1 hr. Work-up as usual gave an amorphous residue (41 mg) exhibiting four main spots on TLC. Separation of the residue by prep. TLC ($C_6H_6–CHCl_3$–EtOAc, 2:2:1) provided four acetates, **1b** (6 mg), mp 231–234° {ν_{max} cm^{-1}: 1737, 1668, 1255; ^1H NMR: δ4.63 (1H, *dd*, *J* = 10, 7 Hz, H-3α); EIMS *m/z* 496 $[M]^+$ (100)}, **2b** (13 mg), mp 206–209° {ν_{max} cm^{-1}: 1742, 1664, 1240; ^1H NMR (90 MHz): δ2.06 and 2.07 (each 3H, *s*, 2 × OAc), 4.63 (1H, *dd*, *J* = 10, 7 Hz, H-3α), 5.53 (1H, *m*, W/2 8 Hz, H-7β); EIMS: *m/z* 540 $[M]^+$ (12)}, **2d** (11 mg), mp. 314–315° {ν_{max} cm^{-1}: 3365, 1715, 1640, 1242; ^1H NMR (90 MHz): δ4.41 (1H, *m*, *W*/2 8 Hz, H-7β), 4.58 (1H, *dd*, *J* = 10, 7 Hz, H-3α); EIMS: *m/z* 498 $[M]^+$ (92)}, and **2e** (9 mg) {amorphous solid, ν_{max} cm^{-1}: 3450, 1732, 1660, 1240; ^1H NMR (90 MHz): δ3.27 (1H, *dd*, *J* = 11, 7 Hz, H-3α), 5.53 (1H, *m*, W/2 8 Hz, H-7β); EIMS: *m/z* 498 $[M]^+$ (9)}, in the order of their polarity. All the above compounds were identified by direct comparison (mmp, co-TLC, IR, ^1H NMR, EIMS) with **1b**, **2b**, **2d** and **2e** derived from natural **1a** and **2a**, respectively. Separate hydrolysis of **2a** (10 mg), **2d** (9 mg), and **2e** (8 mg) in boiling KOH–EtOH (0.05 M, 20 ml) for 1 hr followed by work-up as usual furnished the same keto-diol identical in all respects (mmp, co-TLC, IR, EIMS) with authentic supinenolone A (**2a**).

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REFERENCES

1. Nanba, T. and Mikage, M. (1983) *Poisonous Plants*, p. 74. Hoikusha, Tokyo.
2. Okuda, T. (1986) *Encyclopedia of Natural Medicine*, p. 312. Hirokawa, Tokyo.
3. Matsunaga, S. and Morita, R. (1983) *Phytochemistry* **22**, 605.
4. Matsunaga, S., Morita, R., Ishida, T., Inoue, M., Shigi, M. and Miyamae, A. (1984) *J. Chem. Soc., Chem. Commun.* 1128.
5. Tanaka (nee Morita), R., Matsuda, M. and Matsunaga, S. (1987) *Phytochemistry* **26**, 3365.
6. Tanaka, R. and Matsunaga, S. (1988) *Phytochemistry* **27**, 3579.
7. Tanaka, R., Matsunaga, S., Ishida, T. and Shingu, T. (1989) *Tetrahedron Letters* **30**, 1661.
8. Tanaka, R. and Matsunaga, S. (1989) *Phytochemistry* **28**, 1699.
9. Yamada, T., Nakamura, S., Goto, T. and Hirata, Y. (1965) *Nippon Kagaku Kaishi* **86**, 1310.
10. Talapatra, S. K., Sengupta, S. and Tarapatra, B. (1968) *Tetrahedron Letters* 5963.
11. Milburn, A. H., Turner, E. V. and Woodford, F. P. (1956) *J. Chem. Soc.* 1740.
12. Brieskorn, C. H. and Dertinger, G. (1968) *Tetrahedron Letters* 6237.