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3β-HYDROXYHEXANORDAMMARAN-20-ONE FROM *EUPHORBIA SUPINA*

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Key Word Index—*Euphorbia supina*; Euphorbiaceae; whole herb; 3β-hydroxyhexanordammaran-20-one; ^{13}C NMR.

Abstract—A new tetracyclic hexanortriterpene ketol, 3β-hydroxy-22,23,24,25,26,27-hexanordammaran-20-one, has been isolated from the whole herb of *Euphorbia supina*.

INTRODUCTION

Euphorbia supina Raf. has been documented for use as a folk medicine for treatment of gastroenteric diseases such as diarrhoea, for a styptic, and for healing suppurated swellings [1, 2]. Previously, we reported from the neutral benzene extract of the whole herb the isolation and structure elucidation of motiol, hopenol-B [3] and spirosupinanonediol [4], a triterpenoid keto-diol bearing a novel skeletal system of 7(8 → 9) *abeo*-9*S*-D:C-*friedo*-B′:A′-neogammacerane for which the name spirosupinanone has been given. We now reports the further isolation and characterization of a new natural product, 3β-hydroxy-22,23,24,25,26,27-hexanordammaran-20-one (**1**) from the same plant.

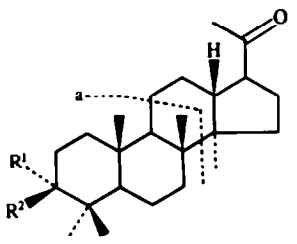
RESULTS AND DISCUSSION

The neutral benzene extract of the dried whole herb on repeated silica gel CC afforded compound **1** (0.0104% of the dried herb) from the fractions eluted with a mixture of benzene and chloroform (1:1). Subsequent elution of the above column with the same eluant yielded spirosupinanonediol [4].

Compound **1** gave molecular formula $C_{24}H_{40}O_2$ (high resolution mass spectrometry) and showed a change in colour from yellow to pink-red upon Liebermann–Burchard test. Its IR and ^1H NMR spectra exhibited the presence of five tertiary methyl groups containing a *gem* dimethyl group ($\nu_{max}cm^{-1}$: 1380, 1360; δ0.77–0.98), one methylketone attached to a methine group ($\nu_{max}cm^{-1}$: 1687 (–C(=O)–); δ2.25 (3H, *s*) and 2.58 (1H, *dt*)] and an axial hydroxymethine proton [$\nu_{max}cm^{-1}$: 3350, 1020; δ3.22 (1H, *dd*)], while no signal arising from an ethylenic linkage has been observed. These data suggested that compound **1** may be a tetracyclic triterpene ketol which lost 2-methylpentyl moiety from the usual side chain.

Compound **1**, on acetylation gave a keto-acetate (**2**), while oxidation with chromium trioxide in pyridine furnished a dione (**3**). The MS analyses of compounds **1–3** played a significant role for confirming the nature of the tetracyclic ring system (see Experimental). Compound **1** showed fragment ions due to cleavage of C-ring and the concomitant loss of one molecule of water at *m/z* 207.1738 ($C_{14}H_{23}O$ requires 207.1748; fragment *a*) and *m/z* 189.1579 ($C_{14}H_{21}$ requires 189.1642; $[a-H_2O]^+$), providing evidence for dammarane skeleton [5] and excluding euphane or lanostane ring system [6]. The loss of the methylketone by rupture of the C-20–C-22 bond gave a prominent peak at *m/z* 317.2825 ($C_{22}H_{37}O$ requires 317.2843), which on the further loss of one molecule of water furnished base peak at *m/z* 299.2691 ($C_{22}H_{35}$ requires 299.2737). Other features of the spectrum were ions at *m/z* 342.2887 ($C_{24}H_{38}O$ requires 342.2921; $[M-H_2O]^+$) and *m/z* 327.2686 ($C_{23}H_{35}O$ requires 327.2686; $[M-H_2O-Me]^+$). The ^{13}C NMR signals of compounds **1–3** were also in accord with those of the carbon skeleton of dammarane triterpenes [7–9] (see Table 1). Compound **2** was identified by direct comparison with the authentic sample of 3β-acetoxyhexanordammaran-20-one [10].

Though compound **2** (hollongdione) was isolated once from the oleoresin of *Dipterocarpus pilosus* (Dipterocarpaceae) [11] and compound **1** has been prepared by



1 R¹ = H, R² = OH
2 R¹ = H, R² = OAc
3 R¹, R² = O

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Table 1. ^{13}C NMR spectra of compounds 1–3

C	1	2	3
1	39.087	38.778	39.958
2	27.341	23.688	34.085
3	78.792	80.843	217.885
4	38.946	37.913	47.429
5	55.862	55.947	55.381
6	16.238	18.124	19.614
7	35.546	35.462	34.872
8	40.492	40.495	40.373
9	50.692	50.605	50.046
10	37.176	37.117	36.895
11	21.215	21.243	21.712
12	25.599	25.571	25.599
13	45.156	45.134	45.097
14	50.074	50.049	49.933
15	31.556	31.559	31.528
16	25.964	25.961	25.995
17	54.317	54.258	54.148
18	15.567*	15.845*	15.792*
19	15.371*	15.567*	15.280*
20	212.518	212.378	212.153
21	29.934	30.070	30.042
28	28.015	27.956	26.695
29	16.242	26.495	21.019
30	15.876*	16.298*	16.403*
OCOMe	—	21.331	—
OCOMe	—	171.018	—

*The signals may be interchanged.

chemical conversion of dammarenediol-II [12], dipterocarpol [13], hydroxyhopanone [14] and lupeol [10], this is the first isolation of **1** in nature.

EXPERIMENTAL

Mps: uncorr. Optical rotations were measured in $CHCl_3$ and IR spectra in KBr discs. ^1H NMR spectra at 300 MHz were recorded in $CDCl_3$ soln using TMS as int. standard and ^{13}C NMR spectra at 75.4 MHz in $CDCl_3$ soln with TMS as int. standard. EIMS were obtained at 70 eV using direct probe insertion at 180–210°. CC was on Merck silica gel 60 (0.05–0.20 mm), TLC on Merck silica gel HF_{254} or PF_{254}.

Extraction and isolation of compound **1**. The air-dried whole herb of *E. supina* (10 kg), collected in August 1984, in the field of Minamikawachi district, Nara Pref., Japan, was cut in pieces and extracted with C_6H_6 (5 × 36 l). The C_6H_6 soln was coned to *ca* 10 l, filtered and then washed with 5% NaOH soln to remove the acidic components. After evacuation of the solvent, the resulting neutral extract (1.15 kg) was chromatographed over silica gel (12 kg) using *n*-hexane, *n*-hexane–C_6H_6, C_6H_6 and C_6H_6–$CHCl_3$ in different proportions as eluants. Fractions eluted with C_6H_6–$CHCl_3$ (1:1) were combined and the resulting amorphous solid (42.8 g) was rechromatographed twice on silica gel (800 and 100 g) to give **1** (1.251 g) from the fractions eluted with C_6H_6–$CHCl_3$ (1:1). Recrystallization of compound **1** from MeOH–$CHCl_3$ furnished colourless needles, mp 195–197°, $[\alpha]_D^{22}$ + 54.1° (c 1.33) (lit. [12] mp 194–195°, $[\alpha]_D$ + 55° (c 1.02)), R_f 0.29 (C_6H_6–$CHCl_3$–EtOAc 2:2:1). UV: end absorption, IR ν_{max} cm^{-1}: 3350, 1020 (OH), 1700 (–C=O), 1380, 1360 (*gem* dimethyl). ^1H NMR δ0.773 (3H, *s*) 0.848 (3H, *s*), 0.871 (3H, *s*), 0.977 (3H, *s*), 0.952 (3H, *s*), 2.254 (3H, *s*, H-21), 2.582 (1H, *dt*, *J* = 11, 6 Hz, H-17), 3.219 (1H, *dd*, *J* = 11.5, 5.3 Hz, H-3α). For ^{13}C NMR, see Table 1. EIMS *m/z* (rel. int.): 360 $[M]^+$ (53), 342 $[M-H_2O]^+$ (19), 327 $[M-H_2O-Me]^+$ (11), 317 $[M-MeCO]^+$ (95), 299 $[M-MeCO-H_2O]$ (91), 207 [fragment *a*] (100), 191 (67), 189 $[a-H_2O]^+$ (55), 95 (96).

Acetylation of compound **1**. Compound **1** (35 mg) was acetylated with Ac_2O–pyridine (1:1, 2 ml) as usual to give **2** (35 mg) as colourless needles (MeOH–$CHCl_3$), mp 206–209°, $[\alpha]_D^{22}$ + 61.1° (*c* 0.53) (int. [13] mp 204–205°, $[\alpha]_D$ + 67° (*c* 1.12)). IR ν_{max} cm^{-1}: 1720, 1240 (OAc), 1700 (–C=O). ^1H NMR δ0.850 (6H, *s*), 0.869 (6H, *s*), 0.982 (3H, *s*), 2.045 (3H, *s*, OAc), 2.121 (3H, *s* H-21), 2.598 (1H, *dt*, *J* = 11, 6 Hz, H-17), 4.477 (1H, *dd*, *J* = 11.5, 5.5 Hz, H-3α). EIMS *m/z* (rel. int.): 402 $[M]^+$ (23), 359 $[M-MeCO]^+$ (28), 342 $(M-HOAc]^+$ (45), 327 $[M-HOAc-Me]^+$ (13), 299 $[M-MeCO-HOAc]^+$ (100), 249 [fragment *a*] (5), 191 (75), 189 $[a-HOAc]^+$ (77), 95 (80). Compound **2** was identified by direct comparison with the authentic sample of 3β-acetoxy-22,23,24,25,26,27-hexanordammaran-20-one [11] (mmp, TLC, IR, MS and ^1H NMR).

Oxidation of compound **1** *with chromium trioxide*. Compound **1** (22 mg) in pyridine (2 ml) was oxidized with CrO_3–pyridine (22 mg, in 3 ml) at 20° for 5 hr. After work up a compound was obtained (21 mg), which was purified by prep. TLC (C_6H_6–EtOAc 5:1) to give **3** as colourless needles (MeOH–$CHCl_3$), mp 183–185°, $[\alpha]_D^{22}$ + 87.6° (*c* 0.36) (lit. [11] mp 173–175°, $[\alpha]_D$ + 98.9°). IR ν_{max} 1700 (–C=O). ^1H NMR δ0.886, 0.946, 1.026, 1.042, 1.084 (each 3H, *s*), 2.141 (3H, *s*, H-21), 2.416 (1H, *ddd*, *J* = 15.6, 7.9, 4.7 Hz, H-2_{eq}), 2.511 (1H, *ddd*, *J* = 15.6, 9.6, 7.6 Hz, H-2_{ax}), 2.601 (1H, *dt*, *J* = 11, 6 Hz, H-17). EIMS *m/z* (rel. int.): 358 $[M]^+$ (68), 315 $[M-MeCO]^+$ (100), 205 [fragment *a*] (39).

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