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ERGOSTA-5,24(24′)-DIENE-3β, 4β, 20*S*-TRIOL, AN ERGOSTANE STEROID FROM *DYSOXYLUM MALABARICUM*

T. R. GOVINDACHARI,* G. N. KRISHNA KUMARI and G. SURESH

Centre for Agrochemical Research, SPIC Science Foundation, 10 Mount Road, Madras 600 032, India

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Key Word Index—*Dysoxylum malabaricum*; Meliaceae; ergostane steroid, cycloartane triterpenoid; ergosta-5,24(24′)-diene-3β, 4β, 20*S*-triol.

Abstract—A new ergostane derivative was isolated from the leaves of *Dysoxylum malabaricum* and identified as ergosta-5,24(24′)-diene-3β,4β, 20*S*-triol in addition (24*R*)-cycloartane-3β, 24,25-triol and ergosta-5,24(24′)diene-3β, 7α-diol, were also identified. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

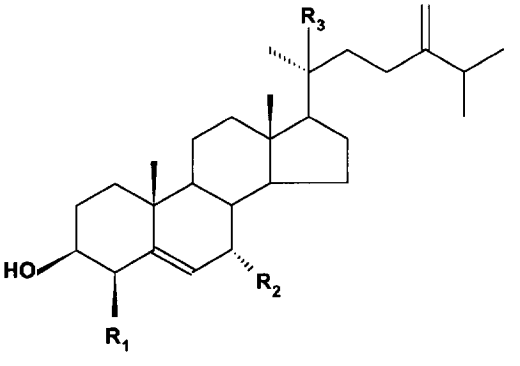
In our earlier paper [1] we reported the isolation of three dammaranes, i.e. dymalol, shoreic acid and ocotillone, from the leaves of *Dysoxylum malabaricum* Bedd. Further work has afforded a new ergostane derivative, whose structure was established as ergosta-5,24(24′)-diene-3β, 4β, 20*S*-triol (**3**) by spectral methods along with a cycloartane derivative, (24*R*)-cycloartane-3β-24,25-triol (**1**) and an ergostane steroid, ergosta-5,24(24′) diene-3β, 7α-diol (**2**), whose identities were established by comparison of their physical and spectral data with published data.

RESULTS AND DISCUSSION

The hexane extract of the leaves of *D. malabaricum* was fractionated between *n*-hexane and 95% methanol and the methanol wash on column chromatography yielded three compounds **1, 2** and **3**. Compound **1** was identified as (24*R*)-cycloartane-3β-24,25-triol which has been earlier isolated from *Mangifera indica* (Anacardiaceae) [2] and *Juncus effusus* (Juncaceae) [3]. Compound **2** was identified as ergosta-5,24(24′) diene-3β, 7α-diol earlier isolated [4] from the marine sponge *Stelodoryx chlorophylla* Levi (Myxillidae). Identities were established by a comparison of the physical data with those reported previously for these compounds and by the ^{1}H NMR data.

The structure of compound **3** was established as ergosta-5,24(24′)diene-3β 4β, 20β-triol, which is a new steroid. Compound **3** had the molecular formula $C_{28}H_{46}O_3$. Its IR spectrum had bands at 3344 (hydroxyl) and 1645 cm^{-1} (double bond). The ^{1}H NMR spectrum (Table 1) showed two secondary methyls (δ 1.03, 6H *d*, J = 6.8 Hz), and three tertiary methyls (singlets) at δ 0.87, 1.18 and 1.3. Two singlets at δ 4.67 and 4.73 indicated the presence of a >C=CH$_2$. Two signals at δ 3.5 (1 H, *td* J = 3.5, 11.2 Hz) and δ 4.12 (*d*, J = 3.5 Hz) were assigned to protons on hydroxyl-bearing carbon atoms. The ^{13}C NMR spectrum (Table 2) of compound **3** showed the presence of two methine carbons bearing oxygen at δ 72.8 and 77.6 and one quaternary carbon atom bearing oxygen at δ 75.4 (*s*), thus confirming the presence of two secondary hydroxyls and one tertiary hydroxyl. Signals at δ 22.3 (*q*) for two methyl groups and at δ 106.6, 156.2 (>C = CH$_2$) were indicative of a 24(24′)-methylene side chain sterol [5].

As in other phytosterols one of the hydroxyls was assigned to position C-3. The HOMOCOSY spectrum showed that both the hydroxyls were on adjacent carbons. So it should be a 2,3- or 3,4-dihydroxylated compound. The ^{1}H NMR signal at δ 4.12 (1H, *d*, J = 3.5 Hz) showed correlation to the other CHOH at



2 R_1 = H; R_2 = OH; R_3 = H

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*Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data of compounds **2** and **3** (300 MHz, δ values in $CDCl_3$)

H	2	3
3	3.51 *m*	3.5t *d* (*J* = 3.5, 11.2 Hz)
4	—	4.12 *d* (*J* = 3.5 Hz)
6	5.58 *d* (*J* = 5.2 Hz)	5.67 *d* (*J* = 3Hz)
7	3.79 *m*	—
18	0.76 *s*	0.87 *s*
19	1.04 *s*	1.18 *s*
21	1.01 *d* (*J* = 7 Hz)	1.3 *s*
24′	4.68 *br s*, 4.74 *br s*	4.67 *br s*, 4.73 *br s*
25	2.21 *septet* (*J* = 7 Hz)	2.23 *septet* (*J* = 6.8 Hz)
26	1.04 *d* (*J* = 7 Hz)	1.03 *d* (*J* = 6.8 Hz)
27	1.06 *d* (*J* = 7 Hz)	1.03 *d* (*J* = 6.8 Hz)

δ 3.5 but not to any other protons, indicating that it was adjacent to a fully substituted carbon. The hydroxyls should therefore be at C-3 and C-4. The ^{1}H NMR signal at δ 3.5 was coupled also to protons in the methylene region at δ 1.95 and 1.75 in addition to the proton at δ 4.12. So the signal at δ 3.5 was assigned to the C-3 CHOH and the *J* values (3.5, 11.2 Hz) indicated that the C-3 H was axial and hence the OH was equatorial and β. The other signal at δ 4.12 was assigned to the C-4-CHOH and the *J* value (3.5 Hz) indicated an equatorial proton at C-4 and so the hydroxyl at C-4 was axial and β. The presence of an internal double bond was indicated by a signal at δ 5.67 (*d*, 1H, *J* = 3 Hz). This was further supported by the

Table 2. ^{13}C NMR spectral data for compounds **2** and **3** (75 MHz, δ values in $CDCl_3$)

C	2	3
1	37.3 *t*	37.3 *t*
2	31.6 *t*	25.6 *t*
3	71.5 *d*	72.8 *d*
4	42. 4 *t*	77.6 *d*
5	146.5 *s*	143.0 *s*
6	124.1 *d*	128.5 *d*
7	65.6 *d*	32.2 *t*
8	37.8 *d*	31.6 *d*
9	42.5 *d*	50.5 *d*
10	37.7 *s*	36.2 *s*
11	21.0 *t*	20.6 *t*
12	39.4 *t*	40.2 *t*
13	42.3 *s*	42.9 *s*
14	49.7 *d*	57.0 *d*
15	24.6 *t*	24.0 *t*
16	28.5 *t*	22.6 *t*
17	55.9 *d*	58.1 *d*
18	11.9 *q*	13.8 *q*
19	19.0 *q*	21.2 *q*
20	36.0 *d*	75.4 *s*
21	18.5 *q*	26.4 *q*
22	34.9 *t*	42.5 *t*
23	31.1 *t*	29.1 *t*
24	157.1 *s*	156.2 *s*
24′	106.2 *t*	106.6 *t*
25	34.0 *d*	34.4 *d*
26	22.3 *q*	22.3 *q*
27	22.1 *q*	22.3 *q*

Table 3. HMBC connectivities of compound **3** (75 MHz, δ values in $CDCl_3$)

C	δ (ppm)	Connected protons
3	72.8	H-4
4	77.6	H-6, H-3
5	143	19-CH_3, H-4, H-6
6	128.5	H-4
20	75.4	21-CH_3
24	156.2	H-24, H-24, H-25
24′	106.6	H-25

^{13}C NMR signals at δ 143 (*s*) and δ 128.5 (*d*). The double bond was placed at the 5,6-position on the basis of HMBC data (Table 3). The quaternary carbon at δ 143 showed long range connectivity to the 19-methyl (δ 1.18), to H-4 at δ 4.12 and the olefinic proton at δ 5.67. The carbon at δ 128.5 showed long range connectivity to H-4 at δ 4.12.

The tertiary hydroxyl was assigned to position C-20 since the 21-methyl carbon showed a downfield shift to δ 26.4 in the ^{13}C NMR spectrum. The γ-effect of this hydroxylation could be seen in the upfield shift of C-16 and C-23 signals compared to compound **2** [6]. The signal at δ 1.3 assigned to the C-21 methyl group indicated the *S*-configuration at C-20 [7]. Thus compound **3** was identified as ergosta-5,24(24′)-diene-3β, 4β, 20*S*-triol.

EXPERIMENTAL

Leaves of *D. malabaricum* Bedd were extracted as reported earlier [1] and 94 g of the material from the MeOH wash of the hexane extract was chromatographed over a silica gel column using hexane and hexane–EtOAc mixtures as eluents. Frs 20–50 contained the three dammaranes reported earlier [1]. Frs (25 ml each) 51–71 (16 g) were chromatographed over silica gel using $CHCl_3$, and $CHCl_3$ with 1% MeOH as eluents.

Isolation of compound **1**. Frs 27–28 on further purification and crystallization from EtOAc gave compound **1** (200 mg) mp 198–200°; $[\alpha]_D$ = +42.8° ($CHCl_3$ *C* = 0.07) lit. +43° [2]; $[M+1]^+$ 461.

Isolation of compound **2**. Frs 35–40 on further purification by silica gel chromatography and recrystallization from EtOAc gave compound **2** (300 mg) mp 216°; $[\alpha]_D$ − 90° ($CHCl_3$ *C* = 0.2) ^{1}H and ^{13}C NMR values are comparable with the lit. data [4]. $[M]^+$ 414.

Isolation of compound **3**. Frs 29–34 on further chromatography gave **3**, recrystallized from EtOAc (150 mg); mp 198–200° $[\alpha]_D$ − 82° ($CHCl_3$ C = 1); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3344, 1645; ^{1}H NMR and ^{13}C NMR: See Tables 1 and 2; Mass: FAB-ms, $[(M-H_2O)+1]$ requires 413.341956, obs. 413.342393.

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