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24β-METHYLCHOLESTA-5,22*E*,25-TRIEN-3β-OL AND 24α-ETHYL-5α-CHOLEST-22*E*-EN-3β-OL FROM *CLERODENDRUM FRAGRANS*

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Key Word Index—*Clerodendrum fragrans*; Verbenaceae; sterol; 24β-methylcholesta-5,22*E*,25-trien-3β-ol; 24α-ethyl-5α-cholest-22*E*-en-3β-ol.

Abstract—Two minor sterols isolated from *Clerodendrum fragrans* were identified as 24β-methylcholesta-5,22*E*,25-trien-3β-ol and 24α-ethyl-5α-cholest-22*E*-en-3β-ol of which the former has so far been detected only in a marine sponge. The other sterols identified in the plant were clerosterol, 22*E*-dehydroclerosterol and several other common sterols.

INTRODUCTION

Plants of the *Clerodendrum* species are known to contain 24β-ethylcholesta-5,25-dien-3β-ol (**1f**, clerosterol) and 22*E*-dehydroclerosterol (**1g**), 24β-ethylsterols possessing a Δ^{25}-bond, as the major sterols [1–3]. This constitutes the characteristic feature of this species since the great majority of higher plants contain 24α-alkylsterols (24*R* if a saturated or Δ^{25}-unsaturated sterol, 24*S* if the Δ^{22} derivative) which lack a Δ^{25}-bond as the major sterols represented by 24α-ethylcholest-5-en-3β-ol (24α-**1d**, sitosterol) [4]. Thus, we considered it worthwhile to undertake detailed analysis on the sterol constituents of *Clerodendrum* species. This paper describes the thorough investigation on the sterols of the leaves and stems of *C. fragrans* (Vent.) R. Br. which led to the isolation and identification of two very rare sterols, 24β-methylcholesta-5,22*E*,25-trien-3β-ol (**1c**) and 24α-ethyl-5α-cholest-22*E*-en-3β-ol (24α-**2e**), in addition to 24α-**1d**, **1f**, **1g** and several other sterols.

RESULTS AND DISCUSSION

Two sterols **1c** and **2e** were isolated as the acetyl derivatives from *C. fragrans* by virtue of the procedure described in the Experimental section. The mass spectrum of **1c**-acetate showed $[M - HOAc]^+$, the highest mass ion, at *m/z* 378 ($C_{28}H_{42}$) and other prominent fragmentation ions at *m/z* 363 $[M - HOAc - Me]^+$, 282 $[M - C_7H_{12}$ (C-20, 22 vinylic cleavage with 1H transfer) $- HOAc]^+$, 255 $[M - C_9H_{15}$ (side chain) $- HOAc]^+$, 253 (255 – 2H) and 213 $[M - C_9H_{15} - C_3H_6$ (part of ring D) $- HOAc]^+$ indicating that it was an acetate of a C_{28}-sterol with three double bonds, one of which was in the skeleton and the other two were in the C_9 side chain [5–7]. Lack of an ion corresponding to the molecular ion suggests that the skeletal double bond was located at C-5 [5], whereas the ion at *m/z* 282 indicates one of the two side chain double bonds was located at C-22 [7]. The ^{1}H NMR spectrum (Table 1) of **1c**-acetate displayed the signals at δ0.690 (3H, *s*, 18-H_3), 1.020 (3H, *s*, 19-H_3), 2.032 (3H, *s*, 3β-OAc), 1.012 (3H, *d*, 21-H_3), 4.60 (1H, *m*, 3α-H) and 5.37 (1H, *m*, 6-H), which are characteristic for Δ^5-3β-yl acetate [2, 7, 8]. Other signals observed were a multiplet around δ5.24 (2H), which can be assigned to the 22-H and 23-H olefinic protons, and two broad singlets at δ4.692 and 4.707 (each 1H), typical for a terminal methylene group [2, 7, 8]. A doublet at δ1.080, highly deshielded by its surrounding two double bonds, is due to the methyl protons at C-28. An isolated multiplet at δ2.71 (1H), shifted considerably downfield, is attributed to the proton at C-24 which is located in a bis-allylic position. Irradiation of this multiplet collapsed the dublet at δ1.080 (28-H_3) into singlet, and also simplified the multiplet at δ5.24 (22-H, 23-H). Finally, a methyl singlet at δ1.677 is ascribed to the allylic methyl protons at C-27 [2, 7, 8]. The stereochemistry at C-22 was established to be *E* (*trans*) since the 21-H_3 doublet is displayed at δ1.00–1.01 when it occurs together with a *E*-Δ^{22} double bond, whereas a *Z* (*cis*) double bond shifts it to δ0.94–0.95 [9]. From the foregoing, **1c**-acetate was regarded as 24-methylcholesta-5,22*E*,25-trien-3β-yl acetate. Upon hydrolysis, the acetate gave free sterol **1c** ($[M]^+$, *m/z* 396; $C_{28}H_{44}O$). The ^{1}H NMR (Table 1) and MS data of **1c** agreed well with those of 24β-methylcholesta-5,22*E*,25-trien-3β-ol cited in the literature [7], and hence **1c** was confirmed to be 24β-methylcholesta-5,22*E*,25-trien-3β-ol. The 24β-configuration was supported from the ^{1}H NMR comparison (Table 1) of the hydrogenated **1c**-acetate, *i.e.*, 24β-methylcholest-5-en-3β-ol (24β-**1b**, 24β-methylcholesterol) acetate ($[M - HOAc]^+$, *m/z* 382), with both of the C-24 epimers of **1b**-acetate.

The mass spectrum of **2e**-acetate showed $[M]^+$ at *m/z* 456, corresponding to $C_{31}H_{52}O_2$, accompanied with fragmentation ions at *m/z* 441 $[M - Me]^+$, 396 $[M - HOAc]^+$ and 315 $[M - C_{10}H_{19}$ (side chain) $- 2H]^+$ indicating that it was an acetate of a C_{29}-sterol with one double bond in the C_{10} side chain [5, 6]. Other ions at *m/z* 413 $[M - C_3H_7$ (allylic cleavage of the terminal isopropyl group)$]^+$, 353 (413 – HOAc) and 344 $[M - C_8H_{16}$ (C-20, 22 vinylic cleavage with 1H transfer)$]^+$ suggest that the side chain double bond was located at C-22 [5, 10]. The ^{1}H NMR spectrum (Table 1) of **2e**-acetate showed signals

Table 1. ^{1}H NMR data of some sterols isolated from *Clerodendrum fragrans* (400 MHz, $CDCl_3$, TMS as int. standard)

Acetate	18-H_3 (*s*)	19-H_3 (*s*)	21-H_3 (*d*)	26-H_3 (*d*)	27-H_3 (*d*)	28-H_3 (*d*)	29-H_3 (*t*)	3β-OAc (*s*)	3α-H (*m*)	6-H (*m*)	22-H (*dd*)	23-H (*dd*)	24-H (*m*)
24α			0.911(6.6)†	0.851 (7.2)	0.803 (6.6)	0.774 (6.6)							
1b*	0.679	1.018					—	2.030	4.60	5.37	—	—	—
24β			0.919 (6.5)	0.855 (6.6)	0.785 (7.7)	0.775 (7.7)							
24β-**1b**‡	0.675	1.018	0.920 (6.6)	0.855 (6.6)	0.784 (6.6)	0.777 (6.6)	—	2.031	4.60	5.37	—	—	—
1c (24β)	0.690	1.020	1.012 (6.6)	1.677 (*s*)	4.692 (1H, *s*) 4.707 (1H, *s*)	1.080 (6.6)	—	2.032	4.60	5.37	5.24 (2H, *m*) 5.24 (2H *m*)		2.71
1c (24β) (3β-OH)	0.693	1.010	1.012 (6.6)	1.675 (*s*)	4.690 (1H, *s*) 4.705 (1H, *s*)	1.080 (7.1)	—	—	3.52	5.35	5.24 (2H, *m*)		2.71
1c (24β)§ (3β-OH)	0.692	1.010	1.012 (6.4)	1.676 (*s*)	4.692 (1H, *s*) 4.705 (1H, 1.3)	1.080 (6.9)	—	—	3.52	5.35	5.25 (2H, *m*)		2.71
24α-**1d**	0.679	1.020	0.922 (6.6)	0.837 (7.7)	0.815 (6.6)	—	0.847 (7.7)	2.032	4.60	5.37	—	—	—
24α-**1e**	0.696	1.020	1.021 (6.4)	0.846 (6.4)	0.796 (7.1)	—	0.804 (7.2)	2.035	4.60	5.37	5.013 (8.8, 15.1)	5.154 (8.8, 15.1)	—
1f (24β)	0.669	1.016	0.904 (6.6)	1.565 (*s*)	4.640 (1H, 2.8) 4.726 (1H, *s*)	—	0.801 (7.4)	2.031	4.60	5.37	—	—	—
1g (24β)	0.690	1.019	1.008 (5.5)	1.646 (*s*)	4.695 (2H, 0.9)	—	0.831 (7.3)	2.023	4.60	5.37	5.18 (2H, *m*)		—
24α-**2d**‖	0.647	0.818	0.905 .6.6)	0.832 (7.1)	0.811 (6.0)	—	0.843 (7.3)	2.017	4.68	—	—	—	—
24α-**2e**	0.665	0.819	1.005 (6.6)	0.844 (6.0)	0.792 (7.1)	—	0.801 (7.1)	2.019	4.68	—	5.005 (8.8, 15.4)	5.145 (8.2, 14.8)	—

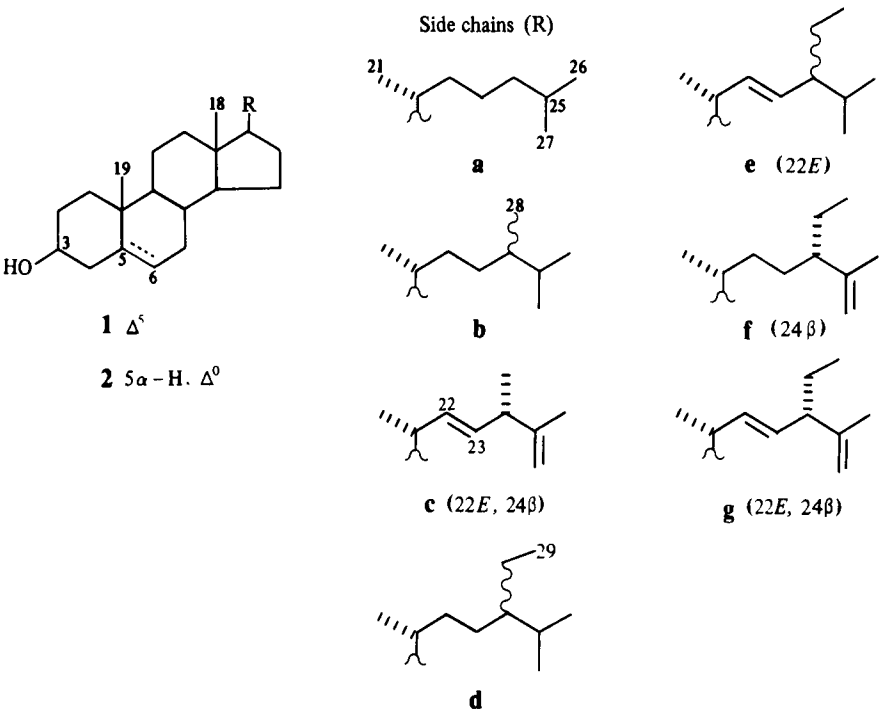
* Mixture of 24α- and 24β-epimers.

† Figures in parentheses denote *J* values (Hz) for doublet and triplet signals.

‡ Prepared from 24β-**1c**-acetate by hydrogenation.

§ Isolated from *Pseudoaxinella lunacharta* [7] (360 MHz).

‖ Prepared from 24α-**2e**-acetate by hydrogenation.



arising from 3β-acetoxy-5α-skeleton [δ0.665 (3H, *s*, 18-H_3), 0.819 (3H, *s*, 19-H_3), 2.019 (3H, *s*, 3β-OAc) and 4.68 (1H, *m*, 3α-H)] [11] and those from 24-ethyl-Δ^{22} side chain [δ1.005 (3H, *d*, 21-H_3), 0.844 (3H, *d*, 26-H_3), 0.792 (3H, *d*, 27-H_3), 0.801 (3H, *t*, 29-H_3), 5.005 (1H, *dd*, 22-H) and 5.145 (1H, *dd*, 23-H)] [8, 12]. The side chain proton signals were closely correlated with those of 24α-ethylcholesta-5,22*E*-dien-3β-ol(24α-**1e**, stigmasterol) acetate (Table 1), but differed from those of its 24β-epimer (24β-**1e**-acetate), especially in the chemical shift of 29-H_3 signal [8], which made it possible to determine the configuration at C-24 of **2e**-acetate to be α. Thus, **2e** was 24α-ethyl-5α-cholest-22*E*-en-3β-ol (24α-**2e**). The 24α stereochemistry was supported from the following evidence. Hydrogenation of **2e**-acetate afforded 24α-ethyl-5α-cholestan-3β-ol (24α-**2d**) acetate ($[M]^+$, *m/z* 458). The ^{1}H NMR data of the side chain proton signals of 24α-**2d**-acetate were closely correlated with those of 24α-**1d**-acetate (Table 1) but differed enough for differentiation from those of its 24β-epimer [8].

In addition to the above, six sterols were isolated as the acetyl derivatives from *C. fragrans* which were identified as the acetates of cholesterol (**1a**), a mixture of 24α- and 24β-**1b** (24α:24β = 7:3), **1d**, **1e**, **1f** and **1g**. Identification of these sterols was performed based on the GC, ^{1}H NMR [8] and MS data with the exception of **1a**-acetate which was identified by GC and MS. Composition of *C. fragrans* sterols was determined on the basis of argentation TLC, GC and ^{1}H NMR data as the acetyl derivatives as follows: **1a** (0.7%), 24α-**1b** (1.1%), 24β-**1b** (0.4%), **1c** (24β) (2.3%), 24α-**1d** (1.8%), 24α-**1e** (6.4%), **1f** (24β) (17.3%), **1g** (24β) (67.6%) and 24α-**2e** (2.4%).

This study has, thus, demonstrated the occurrence of two very rare sterols, **1c** and 24α-**2e**, in addition to several other sterols in *C. fragrans*. Sterol **1c** has heretofore been reported to occur only in a marine sponge, *Pseudoaxinella lunachatra* [7], and this study seems to be the first instance for its detection in a plant. Although sterol **2e** (24ξ) has previously been detected in three plants: *Bupleurum falcatum* [13], *Dictyostelium discoideum* [14] and *Lycopersicon esculentum* [15], this study is considered to be the first case of its unambiguous characterization including the determination of the stereochemistry at C-24.

EXPERIMENTAL

Mp: uncorr. Argentation TLC: silica gel–$AgNO_3$ (4:1) developed × 3 with CCl_4–CH_2Cl_2 (5:1); HPLC: I: Partisil 5 ODS-2 column (Whatman; 25 cm × 10 mm i.d.) or II: Altex Ultrasphere ODS column (Beckman; 5 μm; 25 cm × 10 mm i.d), MeOH as mobile phase (flow rate, 4 ml/min) in both systems; GC: OV-17 SCOT glass capillary column (30 m × 0.3 mm i.d.), column temp. 260°. RR_t on HPLC and GC expressed relative to cholesterol (**1a**) acetate. EIMS (70 eV): probe; ^{1}H NMR: 400 MHz, $CDCl_3$, TMS as int. standard; Acetylation: Ac_2O–pyridine at room temp. overnight; Hydrolysis: 5% KOH in EtOH at room temp. overnight; Hydrogenation: EtOH over pre-reduced PtO_2 at atoms. pressure and temp. overnight. The acetates of following sterols: **1a**, a mixture of 24α- and 24β-**1b**, 24α- and 24β-**1d**, 24α- and 24β-**1e**, **1f**, **1g** and **2d**, were used as the reference specimens [8, 12]. Leaves and stems of *C. fragrans* were collected locally in India.

Isolation of sterols. Air-dried and powdered leaves and stems (950 g) of *C. fragrans* were extracted with MeOH in a Soxhlet extractor for 48 hr. After removal of the solvent, extracted lipid (70 g) was treated with cold Me_2CO. The Me_2CO soluble part (37 g) was refluxed with 5% KOH in EtOH for 2 hr and then extracted with Et_2O which gave unsaponifiable lipid (11.0 g). CC of the unsaponifiable lipid on silica gel (200 g) [hexane (1.3 l), hexane–Et_2O (9:1, 1 l), hexane–Et_2O (6:1, 1 l), hexane–EtOAc (4:1, 1 l) and then MeOH (1.5 l) as eluants] gave the sterol mixture (810 mg) [eluted with hexane–EtOAc (6:1)]. (The elution was monitored by TLC on precoated silica gel.) This was

acetylated, and the acetate mixture (843 mg) was subjected to argentation TLC to give four bands (referred to as bands 1–4 in the order of polarity, beginning with the least polar). The least polar fraction (12.5 mg) from band 1 (R_f 0.70–0.78) was a mixture which on further argentation TLC afforded two fractions: fraction 1A (2.5 mg) from the less polar band and fraction 1B (4.3 mg) from the more polar band. Fraction 1A was 24α-**2e**-acetate. Fraction 1B was a mixture of three components from which was isolated the acetates of **1a**, **1b** (C-24 epimeric mixture) and 24α-**1d** by HPLC. The fractions from band 2 (R_f 0.63–0.70) and 3 (R_f 0.41–0.5), on further argentation TLC, gave 24α-**1e**-acetate (30.1 mg) and **1f**-acetate (89.8 mg), respectively. A fraction (215.4 mg) from the most polar band 4 (R_f 0.14–0.41) was a mixture of two steryl acetates, and a portion (90 mg) of this fraction was subjected to HPLC giving **1c**-acetate (1.0 mg) and **1g**-acetate (48.8 mg).

24β-*Methylcholesta*-5,22E,25-*trien*-3β-*ol* (**1c**) *acetate* Mp 149.1–151.2°; RR_t = 1.20 (GC), 0.71 (HPLC-I); MS: *m/z* 378.3241 ($[M-HOAc]^+$, $C_{28}H_{42}$, rel. int. 100%, requires 378.3284), 363.3034 ($C_{27}H_{39}$, 7%), 282.2371 ($C_{21}H_{30}$, 7%), 255.2124 ($C_{19}H_{27}$, 26%), 253.1941 ($C_{19}H_{25}$, 13%), 213.1656 ($C_{16}H_{21}$, 7%).

24β-*Methylcholesta*-5,22E,25-*trien*-3β-*ol* (**1c**). Mp 133.0–134.0°; MS: *m/z* 396.3401 ($[M]^+$, $C_{28}H_{44}O$, rel. int. 20%, requires 396.3390), 381.3168 ($C_{27}H_{41}O$, 3%), 378.3266 ($C_{28}H_{42}$, 5%), 363.3056 ($C_{27}H_{39}$, 4%), 326.2664 ($C_{23}H_{34}O$, 3%), 314.2574 ($C_{22}H_{34}O$, 5%), 309.2598 ($C_{23}H_{33}$, 6%), 300.2431 ($C_{21}H_{32}O$, 30%), 285.2327 ($C_{20}H_{29}O$, 12%), 283.2407 ($C_{21}H_{31}$, 9%), 271.2059 ($C_{19}H_{27}O$, 76%), 255.2091 ($C_{19}H_{27}$, 48%), 253.1950 ($C_{19}H_{25}$, 8%), 241.1988 ($C_{18}H_{25}$, 4%), 239.1910 ($C_{18}H_{23}$, 5%), 229.1998 ($C_{17}H_{25}$, 6%), 215.1791 ($C_{16}H_{23}$, 13%), 213.1659 ($C_{16}H_{21}$, 18%), 81.0701 (C_6H_9, 100%).

24α-*Ethyl*-5α-*cholest*-22E-*en*-3β-*ol* (24α-**2e**) *acetate*. Mp 139.5–142.0°; RR_t = 1.44 (GC); MS *m/z* 456.3948 ($[M]^+$, $C_{31}H_{52}O_2$, rel. int. 42%, requires 456.3965), 441.3742 ($C_{30}H_{49}O_2$, 4%), 413.3386 ($C_{28}H_{45}O_2$, 4%), 396.3766 ($C_{29}H_{48}$, 21%), 381.3586 ($C_{28}H_{45}$, 3%), 358.2824 ($C_{24}H_{38}O_2$, 7%), 353.3209 ($C_{26}H_{41}$, 31%), 344.2743 ($C_{23}H_{36}O_2$, 32%), 329.2449 ($C_{22}H_{33}O_2$, 8%), 316.2414 ($C_{21}H_{32}O_2$, 27%), 315.2289 ($C_{21}H_{31}O_2$, 37%), 302.2288 ($C_{20}H_{30}O_2$, 10%), 257.2264 ($C_{19}H_{29}$, 58%), 255.2086 ($C_{19}H_{27}$, 18%), 241.1950 ($C_{18}H_{25}$, 7%), 229.1991 ($C_{17}H_{25}$, 10%), 215.1797 ($C_{16}H_{23}$, 16%), 43.0540 (C_3H_7, 100%).

Physical properties of the other C. fragrans *sterols*. Acetyl derivatives. **1a**: Mp 117.0–119.0°, RR_t = 1.00 (GC), 1.00 (HPLC-I and -II); mixture of 24α- and 24β-**1b**: mp 139.1–140.2°; RR_t = 1.31 (GC), 1.14 (HPLC-II); 24β-**1b** (prepared from 24β-**1c**-acetate by hydrogenation): mp 144.5–148.0°; RR_t = 1.31 (GC); 24α-**1d**: mp 122.4–124.0°; RR_t = 1.63 (GC), 1.26 (HPLC-II); 24α-**1e**: mp 144.0–146.0°; RR_t = 1.43 (GC), 1.06 (HPLC-II); **1f** (24β): mp 127.4–128.5°; RR_t = 1.64 (GC), 0.86 (HPLC-I); **1g** (24β): mp 151.2–153.0°; RR_t = 1.49 (GC), 0.80 (HPLC-I); 24α-**2d** (prepared from 24α-**2e**-acetate by hydrogenation): mp 131.0–132.0°; RR_t = 1.66 (GC). For the ^{1}H NMR data of the *C. fragrans* sterols, see Table 1.

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