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5α-STIGMAST-11-EN-3β-YL PALMITATE AND 24-ETHYL-5α-CHOLESTA-8(14),25-DIEN-3β-YL STEARATE, TWO STERYL ESTERS FROM *CRYPTOCORYNE SPIRALIS* RHIZOMES

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Key Word Index—*Cryptocoryne spiralis*; Araceae; rhizomes; steryl esters; 5α-stigmast-11-en-3β-yl palmitate; 24-ethyl-5α-cholesta-8(14),25-dien-3β-yl stearate.

Abstract—Two new steryl esters, 5α-stigmast-11-en-3β-yl palmitate and 24-ethyl-5α-cholesta-8(14),25-dien-3β-yl stearate have been isolated from the rhizomes of *Cryptocoryne spiralis*. Their structures have been established on the basis of chemical and spectroscopic studies.

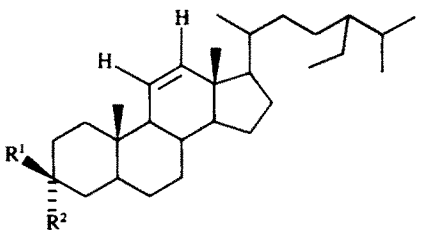
INTRODUCTION

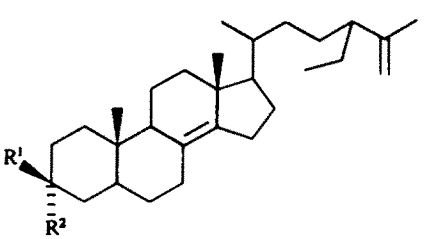
In an earlier investigation, we reported two new oxo-fatty acid esters, ethyl 14-oxotetracosanoate and 15-oxoeicosanyl 14-oxoheptadecanoate from the hexane extract of the rhizomes of *Cryptocoryne spiralis* Fisch. (Araceae) [1]. More recently, two new oxo-fatty acids, 22-oxononacosanoic acid and 26-oxohentriacontanoic acid have been characterized by us from the same source [2]. Our continued interest in this plant has led to the isolation of a palmityl ester (**1a**) of a new sterol (**1b**) and a stearyl ester (**2a**) of another new sterol (**2b**).

RESULTS AND DISCUSSION

From the hexane extract of 3.5 kg air dried rhizomes, 67 mg of **1a** and 5 mg of **2a** were obtained. Compound **1a**, mp 90–91°, $[\alpha]_D$ −22° ($CHCl_3$), showed positive Liebermann–Burchard (LB) and tetranitromethane (TNM) tests. A molecular ion peak was not observed in the mass spectrum of **1a**, instead it showed an ion at *m/z* 396 $[M - \text{palmitic acid}]^+$. It had IR bands at 1732, 1262 and 1238 cm^{-1} for an ester group. The ^1H NMR spectrum displayed two singlets at δ0.69 and 0.71 for the C-18 and C-19 angular methyl groups. A broad multiplet at δ4.50 ($W_{1/2}$ = 20 Hz) was observed for the C-3 axial methine [3, 4]. A characteristic double doublet was observed at δ5.06 (J = 2 and 6 Hz) and 5.28 (J = 3.5 and 6 Hz) for Δ^{11}-double bond [5]. The *cis* nature of this double bond was evident from the IR bands at 1660, 1410 and 695 cm^{-1} [6] and the J values for the C-11 and C-12 protons [7].

Hydrolysis of **1a** afforded palmitic acid, and a sterol (**1b**), $[M]^+$ 414, ν_{max} at 3420 and 1055 cm^{-1}, suggesting an equatorial hydroxy group [8] and 1660, 1410 and 695 cm^{-1} for a *cis*-disubstituted double bond. The ^1H NMR spectrum displayed a broad multiplet for the C-3 methine proton at δ3.48 ($W_{1/2}$ = 20 Hz). The C-18 and C-19 angular methyl groups resonated at δ0.69 and 0.70, respectively (calc. by the rules of Zürcher. C-18: 0.75; C-19: 0.775) [9]. The characteristic ions in the mass spectrum suggesting a stigmastane skeleton [10, 11] were observed at *m/z* 273 $[M - \text{side chain (sc)}]^+$, 255 $[M - sc - H_2O]^+$, 231 $[M - sc - \text{ring D}]^+$ and 213 $[231 - H_2O]^+$. The presence of ions at *m/z* 201 and 159, resulting from $[M - sc - \text{ring A}]^+$ and the loss of 54 mass units from 213,





1a $R^1 = C_{16}H_{31}O_2$, R^2 = H
1b R^1 = OH, R^2 = H
1c R^1 = OAc, R^2 = H
1d $R^1 = R^2$ = O

2a $R^1 = C_{18}H_{35}O_2$, R^2 = H
2b R^1 = OH, R^2 = H

respectively, through collapse of ring A in a retro-Diels–Alder reaction [12, 13] proved that it was not a Δ^5-sterol. Ions at *m/z* 246 and 231 (ring D fission) were in support for the absence of a double bond in ring D [14]. Thus the mass spectrum indicated the absence of a double bond in rings A, B and D and the side chain. The possibility of a Δ^6-double bond instead of a Δ^{11} was excluded since in the Δ^6-compound the C-18 methyl signal would be expected to appear in the ^{1}H NMR spectrum at its usual position of δ0.64. In **1a**, the C-18 methyl signal was slightly shifted downfield at δ0.69 because of the adjacent Δ^{11}-double bond. Calculation of the chemical shifts of the C-18 and C-19 methyls by the rules of Zürcher (see above) also favoured a Δ^{11}-double bond.

Acetylation of **1b** afforded a monoacetate (**1c**), $[M]^+$ at *m/z* 456, ν_{max} 1725 and 1245 cm^{-1}. The singlet nature of the latter 'acetate band' established the equatorial orientation of the C-3 acetoxyl group [15, 16]. Jones oxidation of **1b** afforded a ketone (**1d**). Its UV and IR spectra lacked absorption for an α,β-unsaturated ketone indicating the absence of a double bond at C-5. Unlike a $\Delta^{9(11)}$-sterol [17], **1a** did not respond to NBS oxidation.

Finally, hydrogenation of **1b** with Pd on activated charcoal yielded a dihydro compound, identified as stigmastanol by comparison with an authentic sample (mmp, IR, NMR, MS). Based on the data described above, the structure of this compound can be represented by **1a**.

Compound **2a**, mp 75–77°, obtained in trace, showed positive LB and TNM tests. It had a molecular ion at *m/z* 678 $[C_{47}H_{82}O_2]^+$ and base peak at *m/z* 394 [M – stearic acid]$^+$ which showed it to be a doubly unsaturated sterol ester. The ions at *m/z* 253 $[394 - C_{10}H_{19}(sc) - 2H]^+$ and 213 [394 – sc – 42 of ring D]$^+$ indicated the presence of a C_{10} side chain with one double bond [18]. The other double bond should be present in the sterol nucleus. An IR band at 880 cm^{-1} (terminal methylene of isopropenyl function) [19] and ^{1}H NMR signals at δ1.54*s* and δ4.67*m* and 4.71*m* supported the Δ^{25}-double bond [20] in this compound. Since no other signals corresponding to a double bond were observed in the ^{1}H NMR spectrum, the double bond in the sterol nucleus should be tetrasubstituted either at C-8 (9) or at C-8 (14). Signals for the angular methyls at δ0.83 (C-18) and 0.78 (C-19) were in good agreement with the values calculated for $\Delta^{8(14)}$-sterols (calc. by the rules of Zürcher. C-18: 0.84, C-19: 0.71). The presence of an appreciable ion at *m/z* 228 formed from the *m/z* 394 fragment by the total loss of the side chain and 27 mass units from ring D further supported the location of double bond at C-8(14) [12]. As in **1a**, α-orientation of H-3 in **2a** was supported by a broad multiplet at δ4.57 ($W_{1/2}$ = 20 Hz). Therefore, the data now available suggest the most plausible structure of this compound as **2a**.

The existence of the rare Δ^{11}-double bond in **1a** makes this compound a potentially interesting natural source for the synthesis of 11-oxo steroids of medicinal value.

EXPERIMENTAL

Mps are uncorr. The IR spectra were recorded in KBr and UV spectra in MeOH. The 300 MHz NMR spectra for compounds **1a** and **1b** and the 400 MHz spectrum for compound **2a** were obtained in $CDCl_3$ with TMS as internal standard. TLC was carried out on silica gel G and the spots were visualized by exposure to I_2 vapour or spraying with LB reagent. The homogeneity of the sterols was checked on $AgNO_3$–silica gel TLC in at least three different solvent systems.

Plant material. Plant material was purchased from the local market, and a voucher specimen has been deposited in the Botany Department of our Institute.

Extraction and isolation of compounds. Air dried and milled rhizomes (3.5 kg) were extracted with EtOH (7 × 2.5 l.). The extract was concd to 250 ml, diluted with H_2O (500 ml) and extracted with *n*-hexane (6 × 500 ml), $CHCl_3$ (5 × 500 ml) and *n*-BuOH (6 × 200 ml). The hexane extract was freed of solvent and the residue (27.85 g) was chromatographed over silica gel (1200 g, 60–120 mesh, BDH). Elution was carried out in hexane, hexane–C_6H_6 (3:1, 1:1, 1:3), C_6H_6 and C_6H_6–$CHCl_3$ (3:1). Fractions collected were 250 ml each and they were monitored by TLC.

5α-Stigmast-11-en-3β-yl palmitate (**1a**). Removal of solvent from hexane–C_6H_6 (3:1) early fractions afforded a residue, purified by preparative TLC, mp 90–91° (Me_2CO), 0.067 g, $[\alpha]_D$ –22° ($CHCl_3$), R_f 0.64 (hexane–C_6H_6, 3:1). IR ν_{max} cm^{-1}: 2900, 2840, 1732, 1660, 1460, 1410, 1360, 1262, 1238, 720 and 695. ^{1}H NMR: δ0.69 (3H, *s*, 18-H$_3$), 0.71 (3H, *s*, 19-H$_3$), 0.98 (3H, *d*, *J* = 6 Hz, 21-H$_3$), 0.84 (3H, *t*, *J* = 6 Hz, 29-H$_3$), 0.88 (6H, *d*, *J* = 6 Hz, 26-H$_3$, 27-H$_3$), 4.50 (1H, *m*, $W_{1/2}$ = 20 Hz, H-3), 5.06 (1H, *dd*, *J* = 2 and 6 Hz, H-12), 5.28 (1H, *dd*, *J* = 3.5 and 6 Hz, H-11), 2.20 (2H, *t*, *J* = 8 Hz, –CH_2CO_2–), 1.20 (*br s*, $(CH_2)_n$). MS *m/z* (rel. int.): 396 $[M - C_{16}H_{32}O_2]^+$ (93), 381 [396 – Me]$^+$ (10), 288 [M – 9, 10 and 6, 7 bond fission]$^+$ (8), 256 $[C_{16}H_{32}O_2]^+$ (13), 255 $[396 - C_{10}H_{21}]^+$ (45), 239 [Me–$(CH_2)_{14}$–C≡O]$^+$. (5), 228 [255–C_2H_3 of ring D]$^+$ (9), 213 [255 – ring D]$^+$ (16), 201 [255 – ring A]$^+$ (5), 159 [213 – ring A]$^+$ (24), 95 [396 – ring B fission]$^+$ (41), 85 (24), 57 (86), 43 (100).

Hydrolysis of compound **1a**. Compound **1a** (48 mg) was refluxed with 5% alcoholic KOH (20 ml) for 4 hr. The solvent was reduced to half and it was then diluted with H_2O (50 ml), extracted with Et_2O (4 × 50 ml), washed with H_2O (2 × 50 ml) and dried (Na_2SO_4). Removal of solvent furnished an alcohol (**1b**), 21 mg, mp 122–123° (Me_2CO), $[\alpha]_D$ –26° ($CHCl_3$). IR ν_{max} cm^{-1}: 3420, 2920, 2850, 1660, 1460, 1410, 1380, 1055, 695. ^{1}H NMR: δ0.69 (3H, *s*, 18-H$_3$), 0.70 (3H, *s*, 19-H$_3$), 0.98 (3H, *d*, *J* = 6 Hz, 21-H$_3$), 0.84 (3H, *t*, *J* = 6 Hz, 29-H$_3$), 0.88 (6H, *d*, *J* = 6 Hz, 26-H$_3$, 27-H$_3$), 3.48 (1H, *m*, $W_{1/2}$ = 20 Hz, H-3), 5.06 (1H, *dd*, *J* = 2 and 6 Hz, H-12), 5.28 (1H, *dd*, *J* = 3.5 and 6 Hz, H-11). MS *m/z* (rel. int.): 414 $[M]^+$ ($C_{29}H_{50}O$) (16), 399 [M – Me]$^+$ (2), 381 [M – Me – H_2O]$^+$ (9), 329 [M – C_6H_{13} of sc]$^+$ (8), 315 [M – C_7H_{15} of sc]$^+$ (5), 301 [M – C_8H_{17} of sc]$^+$ (3), 288 [M – B ring fission]$^+$ (3), 283 [301 – H_2O]$^+$ (4), 273 [M – sc]$^+$ (12), 255 [273 – H_2O]$^+$ (30), 246 [273 – C_2H_3 of ring D]$^+$ (3), 231 [273 – ring D]$^+$ (11), 228 [246 – H_2O]$^+$ (7), 213 [231 – H_2O]$^+$ (26), 201 [273 – ring A]$^+$ (7), 159 [231 – ring A]$^+$ (30), 105 [288 – ring D – sc]$^+$ (34), 99 $[C_7H_{15}]^+$ (18), 95 [M – H_2O – ring B fission]$^+$ (45), 57 (100), 43 (88). The mother liquor from the above extraction was acidified with dil. HCl and then extracted with Et_2O (4 × 50 ml), washed with H_2O (2 × 50 ml) and dried (Na_2SO_4). Removal of solvent furnished palmitic acid, $C_{16}H_{32}O_2$, $[M]^+$ at *m/z* 256, 10 mg, mp 65° (mmp, MS).

Acetylation of compound **1b**. Compound **1b** (5 mg) was treated with pyridine–Ac_2O (1 ml each) and left overnight at room temp. The mixture was then diluted with H_2O (20 ml) and extracted with Et_2O (5 × 20 ml). The Et_2O extract was washed successively with dil. HCl (2 × 25 ml), H_2O (2 × 25 ml), $NaHCO_3$ soln (2 × 25 ml) and H_2O (2 × 25 ml) and dried (Na_2SO_4). Removal of solvent yielded a residue (**1c**), mp 128–130° (Me_2CO), 3 mg; IR ν_{max} cm^{-1}: 2910, 2840, 1725, 1660, 1460, 1410, 1370, 1275, 1260, 1235, 695. MS *m/z*: 456 $[M]^+$ ($C_{31}H_{52}O_2$).

Jones oxidation of compound **1b**. Compound **1b** (10 mg) was dissolved in Me_2CO (50 ml) and 8 N chromic acid added

dropwise with constant shaking. Completion of the reaction was indicated by the persistence of the yellow colour in the supernatant liquid even after 10 min. The Me_2CO was concd to 10 ml *in vacuo* and the concentrate diluted with H_2O (50 ml) and extracted with Et_2O (4 × 25 ml). The Et_2O extract was washed with H_2O (2 × 25 ml) and dried (Na_2SO_4). Removal of solvent provided a residue which resisted crystallization, 4 mg; IR ν_{max} cm^{-1}: 2920, 2840, 1725, 1460, 1375. MS *m/z*: 412 $[M]^+$ ($C_{29}H_{48}O$).

N-*Bromosuccinimide oxidation of* **1a**. Compound **1a** (10 mg) was dissolved in dioxan (5 ml) and NBS (5 mg) was added. This mixture was stirred at room temp. for 1 hr under irradiation. After usual work up it afforded unreacted **1a**.

Hydrogenation of compound **1b**. Compound **1b** (5 mg) in EtOH (10 ml) was stirred with 5% Pd–C (5 mg) under H_2 at room temp. and atm. pres. After the completion of the reaction the catalyst was filtered off and the removal of solvent under red. pres. provided a residue, 4 mg, mp 134–136° (Me_2CO), identified as stigmastanol.

24-*Ethyl*-5α-*cholesta*-8(14),25-*dien*-3β-*yl stearate* (**2a**). Removal of solvent and repeated preparative TLC of the latter fractions from hexane–C_6H_6 (3:1) afforded a residue, mp 75–77° (Me_2CO), 5 mg, R_f 0.60 (hexane–C_6H_6, 3:1). IR ν_{max} cm^{-1}: 2900, 2840, 1728, 1630, 1455, 1410, 1370, 1260, 880 and 710. ^1H NMR: δ0.83 (3H, *s*, 18-H_3), 0.78 (3H, *s*, 19-H_3), 0.95 (3H, *d*, *J* = 6 Hz, 21-H_3), 0.88 (3H, *t*, *J* = 6 Hz, 29-H_3), 1.54 (3H, *s*, 26-H_3), 4.67 (1H, *m*, H-27a), 4.71 (1H, *m*, H-27b), 4.57 (1H, *m*, $W_{1/2}$ = 20 Hz, H-3), 2.30 (2H, *t*, *J* = 8 Hz, $-CH_2CO_2-$), 1.25 (*br s*, $(CH_2)_n$). MS *m/z* (rel. int.): 678 $[M]^+$ ($C_{47}H_{82}O_2$) (8), 663 $[M-Me]^+$ (6), 394 $[M-C_{18}H_{36}O_2]^+$ (100), 379 $[394-Me]^+$ (30), 353 $[394-41$ of sc$]^+$ (10), 310 $[394-(C_6H_{11}+H)]^+$ (2), 296 $[394-(C_7H_{13}+H)]^+$ (30), 286 [9,10- and 6,7-bond fission]$^+$ (17), 284 $[C_{18}H_{36}O_2]^+$ (5), 283 $[394-C_8H_{15}]^+$ (8), 255 $[394-C_{10}H_{19}]^+$ (30), 253 $[394-C_{10}H_{19}-2H]^+$ (10), 267 $[Me-(CH_2)_{16}-C{\equiv}O]^+$. (5), 228 $[255-C_2H_3$ of ring D$]^+$ (10), 213 $[255-$ring D$]^+$ (15), 201 $[255-$ring A$]^+$ (20), 159 [213 −ring A]$^+$ (24), 95 [M − stearic acid − B ring fission]$^+$ (70), 69 $[C_5H_9]^+$ (99), 57 (93).

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