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5α-STIGMAST-9(11)-EN-3β-OL, A STEROL FROM *COSTUS SPECIOSUS* ROOTS*

MADAN M. GUPTA, RAM N. LAL and YOGENDRA N. SHUKLA

Central Institute of Medicinal and Aromatic Plants, Lucknow 226010, India

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Key Word Index—*Costus speciosus*; Costaceae; roots; sterol; 5α-stigmast-9(11)-en-3β-ol.

Abstract—A new sterol isolated from *Costus speciosus* roots has been characterized as 5α-stigmast-9(11)-en-3β-ol by spectroscopic data and chemical studies.

INTRODUCTION

Diosgenin is an important raw material for the synthesis of steroidal drugs. It has been reported in varying amounts from different parts of *Costus speciosus*. Recently we reported the isolation and characterization of two new aliphatic hydroxyketones, and diosgenin from the roots of this plant [1]. Our continued interest in the constituents of the roots has led to the isolation of a new sterol to which we have assigned the structure, 5α-stigmast-9(11)-en-3β-ol (**1a**).

RESULTS AND DISCUSSION

The isolate, mp 132–133°, $[\alpha]_D$ +50° (positive LB and TNM tests) possessed a molecular formula $C_{29}H_{50}O$ (MS) and ν_{max} 3430 and 1050 cm^{-1} for an equatorial OH group [2], 1640 and 840 cm^{-1} for a trisubstituted double bond and 2940, 2870, 1465, 1380 cm^{-1} for CH_2 and Me functions. The ^1H NMR spectrum displayed two singlets at δ 0.66 and 1.00 indicative of C-18 and C-19 angular methyl groups, respectively, typical of a $\Delta^{9(11)}$-sterol [3–5] (calc. by the rules of Zürcher C-18 Me: δ 0.60, C-19 Me: δ 0.950) [6]. It also showed signals corresponding to C-21, C-26, C-27 and C-29 methyl groups and an olefinic proton along with a broad singlet at δ 1.42 (exchanged with D_2O) for an OH group and a broad multiplet at δ 3.12 ($W_{\frac{1}{2}}$ = 25 Hz), shifted to δ 4.44 in acetate assignable to C-3 axial methine [4, 7]. The characteristic ions in the mass spectrum suggesting a stigmastane skeleton [8, 9] were observed at *m/z* 273 $[M - \text{side chain (sc)}]^+$, 231 $[M - sc - \text{ring D}]^+$ and 213 $[231 - H_2O]^+$. The presence of ions at *m/z* 201, 342 and 159, resulting from $[M - sc - \text{ring A}]^+$ and the loss of 54 mass units from the ions *m/z* 396 $[M - H_2O]^+$ and 213, respectively, through collapse of ring A in a retro-Diels–Alder reaction [10, 11] proved that it was not a Δ^5-sterol. The mass spectrum indicated the absence of a double bond in rings A, B, D and the side-chain.

Acetylation afforded a monoacetate, ν_{max} 1725 and 1245 cm^{-1} and the singlet nature of the latter 'acetate band' established the equatorial orientation of the C-3 hydroxyl group [12–14]. The C-3 methine signal was shifted downfield by about δ 1.3. Biogenetically, one hydroxyl group could be placed at C-3 and from the $W_{\frac{1}{2}}$ (25 Hz) of the C-3 proton, the OH group had a β-configuration. The protons of the C-18 and C-19 methyl groups appeared as singlets at δ 0.64 and 1.00, respectively (calc. C-18 Me: δ 0.600, C-19 Me: δ 0.967).

Jones oxidation of **1a** furnished a ketone in which the C-18 and C-19 methyl groups appeared as singlets at δ 0.64 and 1.18, respectively (calc. C-18 Me: δ 0.634, C-19 Me: δ 1.159). Its IR and UV spectra lacked absorptions for an α,β-unsaturated ketone indicating the absence of a double bond at C-5.

Hydrogenation of **1a** with PtO_2–HOAc yielded a dihydro compound, identified as stigmastanol (mp, $[\alpha]_D$, IR, NMR, MS) by comparison of literature data [5, 15]. This indicated the stigmastanol nucleus in **1a** while the position of the double bond remained to be fixed. The possibility of Δ^7-sterol was eliminated by total and rapid hydrogenation [16, 17] and the fact that the chemical shift of the C-18 methyl group is lower in Δ^7-sterols [18].

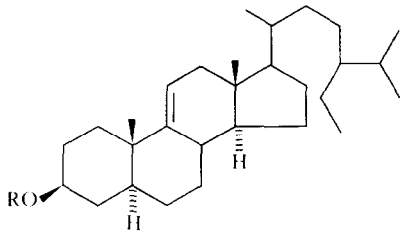
Finally the location of the double bond at the $\Delta^{9(11)}$ position was achieved by comparison of observed and calculated chemical shifts for the C-18 and C-19 methyl groups (described above) and the allylic oxidation of **1b**. The major product (**2**) isolated had ν_{max} 1705 cm^{-1} and λ_{max} 241 nm indicating an α,β-unsaturated ketone system. The minor products of this reaction could not be identified due to low yield. The location of the double bond at C-5 in **1a** was not favoured as NBS oxidation of sitosterol has been reported to give a mixture of bromohydrins and epoxides rather than the desired 7-oxo derivative [19]. The ion at *m/z* 231 [20] in **1a** and the absence of any cyclopentenone system in **2** revealed the absence of double bond in ring D.

The existence of the rare $\Delta^{9(11)}$-double bond in **1a** makes this compound a potentially interesting natural source for the synthesis of corticosteroids of medicinal value.

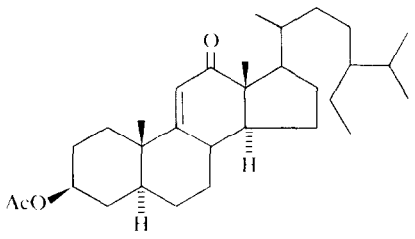
EXPERIMENTAL

Mps are uncorr. The IR spectra were recorded in KBr and UV spectra in MeOH. The 90 MHz NMR spectra were taken in $CDCl_3$ with TMS as int. standard. TLC was carried out on Si gel G and the spots were visualized by exposure to I_2 vapour or spraying with LB reagent. The homogeneity of the sterol was checked on

*Part II in the series "Studies on *Costus speciosus* Roots". For Part I see ref. [1].



1a R = H
1b R = Ac



2

$AgNO_3$–Si gel TLC in at least 4 different solvent systems. A 200 W tungsten lamp was used for irradiation.

Plant material. Plant material was cultivated at the Experimental Station, Kukrail of this Institute and a voucher specimen has been deposited in the Botany Department.

Extraction and isolation of **1a**. Dried and powdered roots (2 kg) of *C. speciosus* were extracted in the cold with MeOH (5 × 6 l.). The MeOH extract was concd to 250 ml and diluted with H_2O (250 ml). This aq. methanolic extract was extracted with *n*-hexane (5 × 250 ml). The hexane extract was freed of the solvent and the residue (12.8 g) was chromatographed over Si gel (800 g, 60–120 mesh, B.D.H.). Elution was carried out in hexane, hexane–C_6H_6 (3:1), hexane–C_6H_6 (1:1), hexane–C_6H_6 (1:3), C_6H_6 and C_6H_6–$CHCl_3$ (3:1). Fractions collected were 250 ml each and monitored by TLC.

5α-Stigmast-9(11)-en-3β-ol (**1a**). Removal of solvent from hexane–C_6H_6 (1:1) fractions afforded a residue, mp 132–133° (Me_2CO), 0.3043 g, $[\alpha]_D^{24}$ +50° ($CHCl_3$), R_f 0.73 (C_6H_6–Me_2CO, 95:5). IR ν_{max}^{KBr} cm^{-1}: 3430, 2940, 2870, 1640, 1465, 1380, 1110, 1050, 1010 and 840. ^1H NMR: δ 0.66 (3 H, *s*, 18-H_3), 1.00 (3 H, *s*, 19-H_3), 0.81 (3 H, *d*, J = 6 Hz, 21 – H_3), 0.84 (3 H, *t*, J = 6 Hz, 29 – H_3), 0.87 (6 H, *d*, J = 6 Hz, 26 – H_3, 27 – H_3), 5.05 (1 H, *m*, 11 – H), 3.12 (1 H, *m*, $W_{\frac{1}{2}}$ = 25 Hz, 3 – H), 1.42 (1 H, *bs*, OH). MS *m/z* (rel. int.): 414 $[M]^+$ ($C_{29}H_{50}O$, 16), 399 $[M - Me]^+$ (27), 396 $[M - H_2O]^+$ (39), 381 $[M - Me - H_2O]^+$ (34), 342 $[M - C_4H_8O$ of ring A$]^+$ (3), 301 $[M - C_8H_{17}]^+$ (15), 288 [9, 10 and 6, 7 bonds fission] (24), 283 $[M - C_8H_{17} - H_2O]^+$ (14), 273 $[M - C_{10}H_{21}]^+$ (9), 231 $[M - sc - ring\ D]^+$ (5), 213 $[M - sc - ring\ D - H_2O]^+$ (4), 201 $[M - sc - ring\ A]^+$ (7), 159 $[M - sc - rings\ A\ and\ D]^+$ (14), 95 $[M - H_2O - ring\ B]^+$ (100), 57 (26), 55 (41).

Acetylation of **1a**. Compound **1a** (0.1364 g) was dissolved in pyridine (2 ml) and Ac_2O (2 ml) was added. It was left overnight at room temp. and then diluted with cold H_2O (50 ml) and extracted with Et_2O (4 × 50 ml). The Et_2O extract was washed successively with dil. HCl (2 × 50 ml), H_2O (2 × 50 ml), $NaHCO_3$ soln (2 × 50 ml) and H_2O (2 × 50 ml) and dried over Na_2SO_4. Removal of solvent gave a residue, mp 99–101° (Me_2CO), 0.1182 g, $[\alpha]_D^{24}$ +66° ($CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2900, 2850, 1725, 1450, 1365, 1245, 1025. ^1H NMR: δ 0.64 (3 H, *s*, 18 – H_3), 1.00 (3 H, *s*, 19 – H_3), 0.81 (3 H, *d*, J = 6 Hz, 21 – H_3), 0.84 (3 H, *t*, J = 6 Hz, 29 – H_3), 0.87 (6 H, *d*, J = 6 Hz, 26 – H_3, 27 – H_3), 1.98 (3 H, *s*, OCOMe), 5.05 (1 H, *m*, 11 – H), 4.44 (1 H, *m*, $W_{\frac{1}{2}}$ = 25 Hz, 3 – H). MS *m/z* (rel. int.): 456 $[M]^+$ ($C_{31}H_{52}O_2$, 4), 441 $[M - Me]^+$ (3), 396 $[M - AcOH]^+$ (28), 381 $[M - Me - AcOH]^+$ (33), 343 $[M - C_8H_{17}]^+$ (4), 342 [M – ring A fission]$^+$ (5), 288 [ring B fission] (8), 283 $[M - C_8H_{17} - AcOH]^+$ (20), 273 $[M - C_{10}H_{21} - ring\ D]^+$ (5), 213 $[M - sc - ring\ D - AcOH]^+$ (6), 201 $[M - sc - ring\ A]^+$ (9), 159 $[M - sc - rings\ A, D]^+$ (21), 95 $[M - AcOH - ring\ B]^+$ (72), 81 (60), 60 (5), 43 (C_3H_7, 100), 42 (12).

Jones oxidation of **1a**. Compound **1a** (50 mg) was dissolved in Me_2CO (250 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 50 ml *in vacuo* and the concentrate diluted with H_2O (100 ml), extracted with Et_2O (4 × 50 ml). The Et_2O extract was washed with H_2O (2 × 50 ml) and dried (Na_2SO_4). Removal of solvent provided a residue, mp 79–82° (Me_2CO–MeOH), 37 mg. IR ν_{max}^{KBr} cm^{-1}: 2980, 2940, 2870, 1718, 1470, 1380, 820. ^1H NMR: δ 0.64 (3 H, *s*, 18 – H_3), 1.18 (3 H, *s*, 19 – H_3), 0.81 (3 H, *d*, J = 6 Hz, 21 – H_3), 0.84 (3 H, *t*, J = 6 Hz, 29 – H_3), 0.91 (6 H, *d*, J = 6 Hz, 26 – H_3, 27 – H_3), 5.05 (1 H, *m*, 11 – H), 2.32 (4 H, *m*, 2 – H_2, 4 – H_2). MS *m/z* (rel. int.): 412 $[M]^+$ ($C_{29}H_{48}O$, 6), 397 $[M - Me]^+$ (2), 299 $[M - C_8H_{17}]^+$ (7), 141 $[C_{10}H_{21}]^+$ (7), 113 $[C_8H_{17}]^+$ (12), 109 $[M - ring\ B]^+$ (6), 99 $[C_7H_{15}]^+$ (16), 85 $[C_6H_{13}]^+$ (44), 57 (100), 43 $[C_3H_7]^+$ (72).

Hydrogenation of **1a**. Compound **1a** (50 mg) in AcOH (100 ml) was hydrogenated with PtO_2 (25 mg) for 4 hr. The catalyst was filtered off and the removal of solvent under red. pres. provided a residue, mp 134–136° (Me_2CO), 27 mg, $[\alpha]_D$ +30° ($CHCl_3$), identified as stigmastanol.

Allylic oxidation of **1b**. Compound **1b** (0.051 g) was dissolved in dioxan (20 ml) and *N*-bromosuccinimide (0.055 g) was added. This mixture was stirred at room temp. for 1 hr under irradiation. The reaction mixture was concd to 10 ml under red. pres. and H_2O (50 ml) added. It was then extracted with Et_2O (3 × 75 ml) and washed with H_2O (2 × 50 ml). The Et_2O extract was dried (Na_2SO_4) and the solvent removed. The residue (0.0278 g) obtained showed 6 spots on TLC (C_6H_6) and was sepd by prep. TLC. The major product (0.005 g) resisted crystallization. IR ν_{max}^{KBr} cm^{-1}: 2900, 2850, 1725, 1705, 1450, 1360, 1240 and 865. UV λ_{max} 241 nm. MS *m/z* (rel. int.): 470 $[M]^+$ ($C_{31}H_{50}O_3$, absent), 442 $[M - CO]^+$ (12), 382 $[442 - AcOH]^+$ (13), 343 $[442 - C_7H_{15}]^+$ (3), 301 $[442 - sc]^+$ (3), 274 $[442 - sc - C_2H_3$ of ring D$]^+$ (4), 259 $[442 - sc - ring\ D]^+$ (4), 187 $[442 - sc - ring\ A]^+$ (7), 149 $[M - sc - rings\ A, B]^+$ (100), 145 $[442 - sc - rings\ A, D]^+$ (12), 95 $[M - AcOH - ring\ B]$ (47).

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