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ALIPHATIC HYDROXYKETONES AND DIOSGENIN FROM *COSTUS SPECIOSUS* ROOTS*

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Key Word Index—*Costus speciosus*; Costaceae; roots; 24-hydroxytriacontan-26-one; 24-hydroxyhentriacontan-27-one; methyl triacontanoate; diosgenin; sitosterol.

Abstract—Two new compounds, B and C, isolated from the roots of *Costus speciosus* have been characterized as 24-hydroxyhentriacontan-27-one and 24-hydroxytriacontan-26-one by spectral data and chemical studies. Methyl triacontanoate, diosgenin and sitosterol have also been isolated and identified.

INTRODUCTION

Diosgenin is an important intermediate for the synthesis of steroidal drugs. Its occurrence in the rhizomes of *Costus speciosus* Sm. (Costaceae) was first reported by Das Gupta and Pandey [1]. It occurs both in the free and glycosidic form with varying percentages in different parts of this plant, viz. rhizome (free 0.15%, glycosidic 2.55%) [2], seeds (free 0.64%, glycosidic 2.13%) [3], stems (free 0.19%, glycosidic 0.52%), flowers (free 0.19%, glycosidic 1.04%), leaves (free 0.09%, glycosidic 0.3%) [2]. In the present study, we have found the roots of this plant to be an additional source of diosgenin (free 0.003%, glycosidic 0.32%) and a more detailed investigation was undertaken.

RESULTS AND DISCUSSION

Five crystalline compounds (A, B, C, D and E) were isolated by silica gel chromatography of the *n*-hexane extract of the roots of this plant.

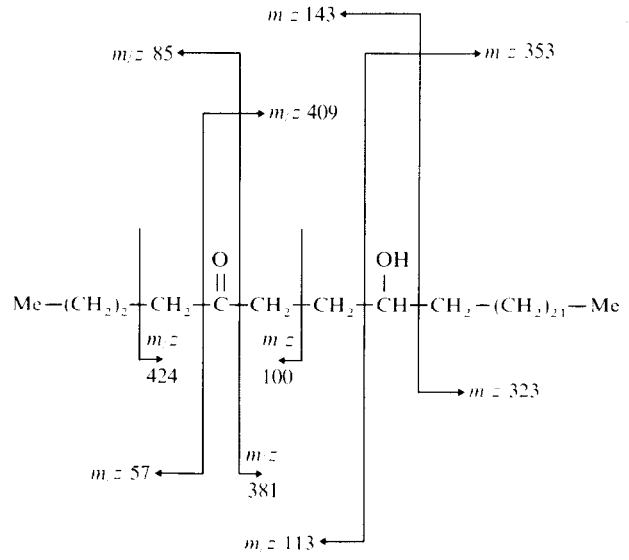
Compound B, mp 84–85°, obtained in traces, had IR absorption bands at 3440 (OH), 1715 (CO), 2920, 2860, 1468, 735 and 725 cm^{-1} (long chain) and gave a positive 2,4-dinitrophenylhydrazine test. The mass spectrum of this compound displayed M^+ at *m/z* 466 which establishes the molecular formula as most probably $C_{31}H_{62}O_2$. The location of the CO at C-27 is deduced from the presence of α-fission ions at *m/z* 57 (base peak), 409, 85, 381 and β-fission ions, involving McLafferty rearrangement, at *m/z* 424 and 100 [4]. The ion at *m/z* 58 is obtained by double rearrangement and is characteristic of a ketone having a γ-H in both alkyl fragments. The significant ions at *m/z* 113, 353, 143, 323 are due to the α-fission of the hydroxyl group, which is assigned at C-24. The straight chain nature of this ketone is further supported by the absence of an $M^+ - 15$ ion [5] and the presence of an $M^+ + 1$ peak is characteristic of its unsymmetrical nature [6, 7]. The data obtained strongly suggested the structure 24-hydroxyhentriacontan-27-one (**1**) for B.

Compound C, mp 81° gave a positive 2,4-dinitrophenylhydrazine test and showed IR bands at 3425 (broad, OH), 1695 (chelated CO), 2900, 2850, 1460, 735 and 725 cm^{-1}. The mass spectrum of C possesses an M^+ ion at *m/z* 452, suggesting the molecular formula $C_{30}H_{60}O_2$. The location of the CO at C-26 is obtained from the α-fission ions at *m/z* 57 (base peak), 395, 85, 367 and the β-fission ions, involving McLafferty rearrangement, at *m/z* 410 and 100 [4]. The ion at *m/z* 58 is again due to double rearrangement. The hydroxyl group at C-24 is supported by the significant α-fission ions at *m/z* 99, 353, 129. As in **1**, the absence of a $M^+ - 15$ ion further established the straight chain nature of the compound and the presence of $M^+ + 1$ ion suggested an unsymmetrical nature. Following the above observations, compound C has been characterised as 24-hydroxytriacontan-26-one (**2**).

Treatment of **2** with Ac_2O–C_5H_5N afforded a ketoacetate, mp 80°, which showed IR bands at 1730, 1700 and 1250 cm^{-1}. Jones oxidation of **2** provided a β-diketone (**3**), mp 84–85° with IR bands at 1710 (sh) 1700 (CO) and 1640 cm^{-1} (weak, CO of enol). The mass spectrum of **3** had an M^+ at *m/z* 450 ($C_{30}H_{58}O_2$). The loss of H_2O from the enol form gave an ion at *m/z* 432 ($M^+ - 18$). The location of the two CO groups at C-24 and C-26 is supported by α-fission ions at *m/z* 351, 127 and at 57, 393, 85 respectively. The corresponding β-fission ions are observed at *m/z* 408, 142, and 100. These fragmentations are shown on the structure overleaf. The above data and results are in full agreement with the assigned structure **2** for C. However, an anomaly was noted in the relative order of elution of **1** and **2**. **2** should have eluted before **1** as it is less polar and involved in intramolecular hydrogen bonding.

Compound A, mp 75°, obtained in traces, had IR absorption bands at 1730, 1370 (CO_2Me), 2910, 2850, 1460, 725 and 715 cm^{-1} (long chain). The mass spectrum of this compound is in conformity with the general fragmentation pattern for long chain methyl esters [8]. The M^+ ion at *m/z* 466 accounts for the molecular formula $C_{31}H_{62}O_2$. A significant ion at *m/z* 59 is assigned to the α-cleavage of the ester carbonyl. The base peak at *m/z* 74 is obtained by the β-cleavage with transfer of a γ-hydrogen atom (McLafferty rearrangement) resulting in the formation of an ion $CH_2{=}C(^+OH)OMe$. Alkaline hydrolysis of this ester afforded an acid, mp 92°, identified

*Part I in the series "Studies on *Costus speciosus* Roots".



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as triacontanoic acid (lit. mp 94° [9]). Compound A, therefore, is identified as methyl triacontanoate isolated previously from *Prunus persica* [10], by comparison of literature data.

Compounds D and E, mp 136° and 201°, were identified as sitosterol and diosgenin respectively, by direct comparison with authentic samples. The characterization of four additional compounds from the hexane extract of the roots is currently in progress.

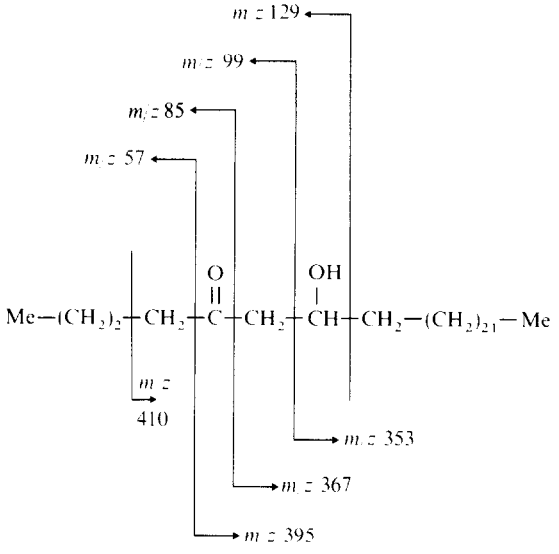
24-Hydroxyhentriacontan-27-one (**1**) and 24-hydroxytriacontan-26-one (**2**) have not been previously found in nature. However, 10-hydroxyhentriacontan-16-one [11], 13-hydroxyhentriacontan-16-one [12], 16-hydroxytriacontan-15-one [13] and 3-hydroxytriacontan-11-one [7] have been reported earlier.

EXPERIMENTAL

Mps are uncorr. IR spectra were recorded in KBr and 60 MHz NMR spectra in $CDCl_3$ with TMS as int. ref. TLC was carried out on Si gel G and the spots were visualized by exposure to I_2 vapour or spraying with 2,4-dinitrophenylhydrazine.

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 compounds. Dried and powdered roots (2 kg) of *C. speciosus* were extracted in the cold with MeOH



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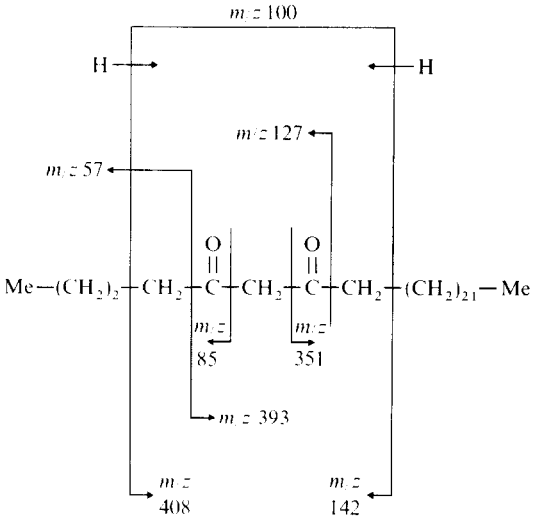

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(5 × 6 l.). The MeOH extract was concd to 250 ml, diluted with H_2O (250 ml) and extracted with *n*-hexane (5 × 250 ml) and *n*-butanol (5 × 250 ml) respectively. The hexane extract was freed of solvent, and the residue (12.8 g) was chromatographed over Si gel (800 g, 60–120 mesh, BDH). 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 (250 ml) were collected and monitored by TLC. The homogeneity of the compounds was checked on TLC in at least four different solvent systems.

Compound A (*methyl triacontanoate*). Removal of solvent from the hexane fractions afforded a residue, 10 mg, mp 75° (MeOH) (lit. [10] mp 77°) R_f 0.83 (hexane–EtOAc, 9:1). IR ν_{max} cm^{-1}: 2910, 2850, 1730, 1460, 1370, 1165, 725 and 715. MS *m/z* (rel. int.): 466 (M^+, $C_{31}H_{62}O_2$, 30), 423 (4), 395 (5), 339 (3), 255 (3), 227 (4), 199 (5), 185 (5), 143 (23), 129 (9), 115 (3), 101 (6), 99 (3), 87 (72), 85 (12), 74 (100), 71 (21), 59 (5), 57 (38), 55 (26).

Hydrolysis of methyl triacontanoate. Compound A (5 mg) was refluxed with 5% alcoholic KOH (2 ml) for 4 hr. At the end of reaction it was diluted with H_2O (20 ml), acidified with dil. HCl and then extracted with Et_2O (4 × 25 ml). The extract was washed with H_2O (2 × 50 ml) and dried (Na_2SO_4). Removal of solvent gave a residue, mp 92° (Me_2CO). IR ν_{max} cm^{-1}: 2900, 2850, 3400–2500, 1715, 1450, 1365, 1250, 925, 722 and 718, identified as triacontanoic acid, lit. mp 94° [9].

Compound B (*24-hydroxyhentriacontan-27-one* (**1**)). Removal of solvent from the hexane–C_6H_6 (3:1) eluate afforded a residue, 5 mg, mp 84–85° (MeOH). IR ν_{max} cm^{-1}: 3440, 2920, 2860, 1715, 1468, 1180, 735 and 725. MS *m/z* (rel. int.): 466 (M^+, $C_{31}H_{62}O_2$, 6), 424 (3), 409 (1), 381 (1), 367 (1), 353 (1), 323 (2), 227 (2), 213 (2), 199 (4), 185 (4), 171 (3), 157 (3), 143 (13), 113 (12), 100 (2), 99 (10), 85 (36), 71 (62), 58 (8), 57 (100), 43 (93).

Compound C (*24-hydroxytriacontan-26-one* (**2**)). The fractions from hexane–C_6H_6 (1:3) gave a residue, 200 mg, mp 81° (Me_2CO–MeOH), R_f 0.25 (C_6H_6–Me_2CO, 17:3). IR ν_{max} cm^{-1}: 3425, 2900, 2850, 1695, 1460, 735 and 725. MS *m/z* (rel. int.): 452 (M^+, $C_{30}H_{60}O_2$, 3), 410 (1), 395 (1), 367 (2), 353 (3), 339 (3), 311 (3), 157 (4), 143 (6), 129 (38), 100 (2), 99 (10), 85 (33), 71 (50), 58 (5), 57 (100), 43 (90).

Acetylation of **2**. To **2** (50 mg) was added C_5H_5N (1 ml) and Ac_2O (1 ml) and the mixture left overnight at room temp. It was then diluted with H_2O (25 ml) and extracted with Et_2O (4 × 25 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 (Na_2SO_4). Removal of solvent gave a residue, mp 80° (Me_2CO–MeOH). IR ν_{max} cm^{-1}: 2900, 2850, 1730, 1700, 1430, 1250, 730 and 720.

Jones oxidation of **2**. **2** (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 a yellow colour in the supernatant liquid for at least 10 min. The solvent was concd to 50 ml *in vacuo*, the concentrate diluted with H_2O (100 ml) and 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 furnished a residue, mp 84–85° (Me_2CO–MeOH). IR ν_{max} cm^{-1}: 2900, 2830, 1710, 1700, 1640, 1455, 725 and 720. MS *m/z* (rel. int.): 450 (2), 432 (3), 408 (4), 393 (10), 351 (11), 142 (6), 127 (19), 100 (15), 85 (25), 57 (100).

Compound D (*sitosterol*) was eluted in C_6H_6, 235 mg, mp 136° (MeOH). Its identity was established by comparison with an authentic sample (mp, mmp, IR, NMR, MS, co-TLC).

Compound E (*diosgenin*). Fractions obtained in C_6H_6–$CHCl_3$ (3:1) afforded a residue, 60 mg, mp 201° (MeOH), identified as diosgenin by comparison with an authentic sample (mp, mmp, IR, NMR, MS, co-TLC).

Isolation of glycoside diosgenin. The *n*-BuOH extract was freed of the solvent to provide a residue (23.2176 g). Part of this (1 g) was hydrolysed with methanolic H_2SO_4 (7%, 100 ml, 4 hr). The reaction mixture was concd to 25 ml, diluted with H_2O (100 ml) and extracted with $CHCl_3$ (4 × 100 ml). This extract was washed with H_2O (2 × 100 ml), dried (Na_2SO_4) and the solvent removed to obtain crude genins (0.57 g). 100 mg of this material was subjected to prep. TLC (C_6H_6–Me_2CO, 17:3) which afforded pure diosgenin (48.2 mg).

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