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OXO ACIDS AND BRANCHED FATTY ACID ESTERS FROM RHIZOMES OF *COSTUS SPECIOSUS*

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Key Word Index—*Costus speciosus*; Costaceae; rhizomes; tetradecyl 13-methylpentadecanoate; tetradecyl 11-methyltridecanoate; 14-oxotricosanoic acid; 14-oxoheptacosanoic acid; 15-oxooctacosanoic acid; 5α-stigmast-9(11)-en-3β-ol; diosgenin; sitosterol; triacontanol; triacontanoic acid.

Abtract—Five new compounds, isolated from the rhizomes of *Costus speciosus* have been characterized as tetradecyl 13-methylpentadecanoate, tetradecyl 11-methyltridecanoate, 14-oxotricosanoic acid, 14-oxoheptacosanoic acid and 15-oxo-octacosanoic acid by spectral and chemical studies. Triacontanol, 5α-stigmast-9(11)-en-3β-ol, triacontanoic acid, sitosterol and diosgenin have also been isolated and identified.

INTRODUCTION

Costus speciosus is a rich source of diosgenin which is used for the synthesis of steroidal drugs. The compound has been reported to occur in all parts of this plant in varying percentages [1–4]. During a large scale isolation of diosgenin from the rhizomes of the plant it was of interest to investigate the compounds from the hexane soluble fraction since they have not been examined.

RESULTS AND DISCUSSION

Ten compounds (**1–10**) were isolated by silica gel chromatography, followed by prep. TLC of the *n*-hexane extract of the rhizomes of *C. speciosus*.

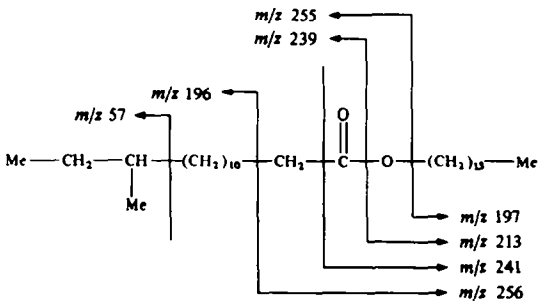
Compound **1**, mp 60–62°, had IR absorption bands at 1730 (ester CO) and 730, 720 cm^{-1} (long chain). The mass spectrum displayed an $[M]^+$ at *m/z* 452, which established the molecular formula as $C_{30}H_{60}O_2$. The ^1H NMR spectrum of **1** displayed a triplet at δ0.88 and a doublet at δ0.80 for two terminal and one branched methyl groups, respectively. A methylene group adjacent to the CO function was seen as a triplet at δ2.28. Another triplet was observed at δ4.00 for –CH–O–CO– protons. Alkaline hydrolysis of **1** afforded an alcohol, mp 41–43°, identified as tetradecanol (IR, mass spectrometry, lit. mp 39–39.5° [5]) and an acid, mp 65–66°, which had an $[M]^+$ at *m/z* 256 ($C_{16}H_{32}O_2$) and showed IR bands for acid [6] at 1700, 3300–2500 and 920 cm^{-1}. The mass spectrum of the acid had a β-fission ion at *m/z* 60 which is characteristic of a terminal carboxyl group. The presence of an $[M-15]^+$ ion at *m/z* 241 showed the branched chain nature of this acid [7]. The base peak at *m/z* 57 favoured the position of branching at C-13. In the ^1H NMR spectrum the terminal methyl group was observed as a triplet at δ0.88 and the branched methyl as a doublet at δ0.80. The methylene group adjacent to the carboxylic group was seen as a triplet at δ2.28. These data suggested the structure of the acid as 13-methylpentadecanoic acid.

On the basis of its hydrolysis products, **1** should therefore be tetradecyl 13-methylpentadecanoate. The mass spectral fragmentation was consistent with the proposed structure. The ions at *m/z* 255, 241, 239, 213, 197 and at *m/z* 256 and 196 were due to α- and β-fissions, respectively, of the ester group. The base peak at *m/z* 57 again supported branching at C-13 in the acid moiety of this ester.

Compound **2**, mp 58–61°, possessed IR bands at 1725 (ester CO), 725 and 720 cm^{-1} (long chain). The $[M]^+$ at *m/z* 424 led to the molecular formula of $C_{28}H_{56}O_2$. The ^1H NMR spectrum of **2** was similar to that of **1**. Alkaline hydrolysis of **2** afforded tetradecanol and an acid, mp 63–65°, $[M]^+$ *m/z* 228 ($C_{14}H_{28}O_2$), ν_{max} 1700, 3300–2500 and 920 cm^{-1}. Similar to the acid obtained from **1**, it had an $[M-15]^+$ fragment indicating a methyl group as a substituent. The base peak at *m/z* 57 supported the position of branching at C-11. The ^1H NMR spectrum of this acid was similar to that of the acid from **1** and it was identified as 11-methyltridecanoic acid.

Therefore, on the basis of its hydrolysis products, **2** was identified as tetradecyl 11-methyltridecanoate. The mass spectral fragmentation of **2** was consistent with the proposed structure. Thus, α-fission ions were observed at *m/z* 241, 227, 213, 211, 197 and 183 while the ions at *m/z* 256 and 168 were due to β-fission. The base peak at *m/z* 57 indicated the presence of a methyl group at C-11 of the acid moiety.

Compound **6**, mp 74–77°, gave a positive 2,4-dinitrophenylhydrazine test (DNP) and had IR absorption bands



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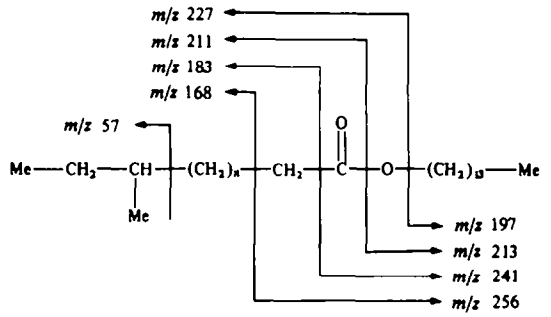

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for a COOH group at 3400–2500 (br), 1700, 1270 and 920 cm^{-1} and for a carbonyl function at 1720 cm^{-1}. The bands at 725 and 715 cm^{-1} suggested its long chain nature. The $[M]^+$ at *m/z* 368 suggested the molecular formula $C_{23}H_{44}O_3$. The ion at *m/z* 60 indicated the presence of a terminal COOH group in the compound. The position of the carbonyl group at C-14 was obtained from the prominent α- and β-fission ions (involving McLafferty rearrangement [8]) at *m/z* 241, 213, 155 and 127 and at *m/z* 256, 198 and 112, respectively. A double rearrangement ion at *m/z* 58 indicated the presence of *v* H atoms in both the alkyl fragments. The presence of an $[M+1]^+$ ion showed the unsymmetrical nature of the ketone [9, 10] whereas the straight chain nature was supported by the absence of an $[M-15]^+$ ion. The ^1H NMR spectrum of **6** showed a triplet for a terminal methyl group at δ0.88 ($J = 6$ Hz) and another triplet, integrated for six protons was at δ2.25 ($J = 6$ Hz) for three methylene groups adjacent to carbonyl and carboxylic acid functions.

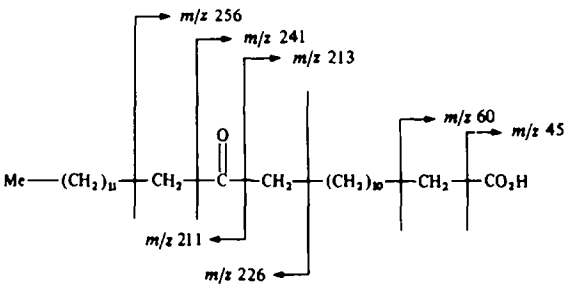
Reduction of **6** with sodium borohydride yielded a hydroxy acid, mp 80°, which lacked CO absorption but showed a band for hydroxyl at 3430 cm^{-1}. The $[M]^+$ ion was not observed, the molecule losing water to give an ion at *m/z* 352. The α-fission ions corresponding to the C-14 hydroxyl group were observed at *m/z* 243, 213, 157 and 127. The above data led us to characterize **6** as 14-oxotricosanoic acid.

Compound **7**, mp 80–82°, showed a positive 2,4-DNP test and its IR and ^1H NMR spectra were similar to those of **6**. An $[M]^+$ ion at *m/z* 424 suggested the molecular formula $C_{27}H_{52}O_3$. The CO group was located at C-14 since the α-fission ions were seen at *m/z* 241, 213, 211 and the β-fission ions at *m/z* 226 and 256. Reduction of **7** with sodium borohydride yielded a hydroxy acid, mp 84–85°, *m/z* 408 $[M-H_2O]^+$. The α-fission ions at *m/z* 243, 213 and 183 were in accordance with the hydroxyl group at C-14. The data described above were fully consistent with the structure of **7** as 14-oxoheptacosanoic acid.

Compound **8**, mp 83–85°, also gave a positive 2,4-DNP test and its ^1H NMR and IR spectra were similar to those of **6** and **7**. An $[M]^+$ ion at *m/z* 438 in its mass spectrum established the molecular formula as $C_{28}H_{54}O_3$. The position of the CO group was established at C-14 since prominent α-fission ions were observed at *m/z* 241, 225, 213 and 197, and β-fission ions at *m/z* 256, 240, 198 and 182. The presence of an $[M+1]^+$ ion and the absence of one at $[M-15]^+$ suggested its unsymmetrical and unbranched nature. Reduction of **8** with sodium borohydride yielded a hydroxy acid, mp 86°, which showed an $[M-H_2O]^+$ ion at *m/z* 422. The α-fission ions at *m/z* 243, 227, 213 and 197 deduced the position of the hydroxyl group at C-14. Thus, **8** was characterized as 14-oxo-octacosanoic acid.

Compound **3**, mp 84–86°, was characterized as triacontanol by IR, NMR, mass spectrometry and comparison with an authentic sample. Compound **4**, mp 132°, was identified as 5 α-stigmast-9(11)-en-3β-ol by direct comparison with authentic material, isolated by us previously from the roots of this plant [11]. Compound **5**, mp 92–93°, was identified as triacontanoic acid (IR, NMR, mass spectrum) by comparison with an authentic sample. Compounds **9** and **10**, mps 135–136° and 200–202°, were identified as sitosterol and diosgenin, respectively, by direct comparison with authentic samples.

Oxo acids occur in milk, fat, lipid, oil, epicuticular wax, latex and rhizomes. Most natural straight chain acids,



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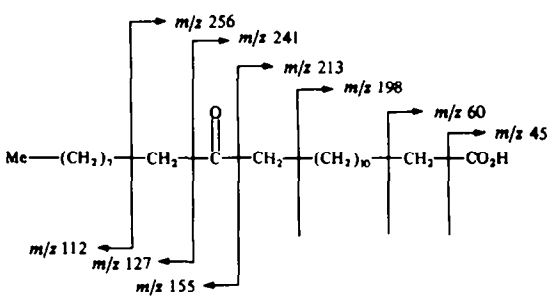

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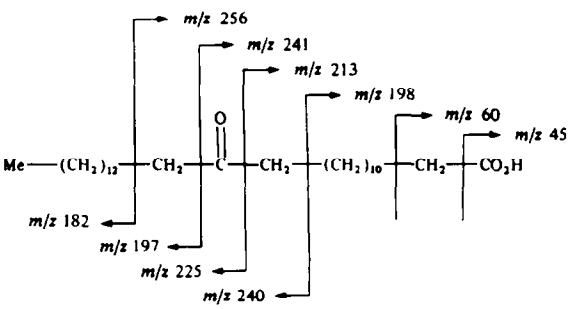

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whether saturated or unsaturated, have an even number of carbon atoms in the molecule [12]. In the even acids oxo groups are rare but are more likely to be situated on an odd carbon atom. Both these observations can be explained in terms of biosynthesis. Compounds **6–8** isolated in the present investigation do not fit in with these generalizations. However, some unusual oxo acids are reported in the literature [12–16]. Fatty acid esters serve as an essential source of energy for plant growth. The aliphatic alcohols are reported to play important roles in the metabolic pathways of a number of organisms since they can generate fatty acids [17].

EXPERIMENTAL

Mps are uncorr. IR spectra were recorded in KBr and the 80 MHz NMR spectra in $CDCl_3$ with TMS as int. standard. TLC was performed on silica gel G (BDH) and the spots were visualized by exposure to I_2 vapour or by spraying with 2,4-DNPH. Sterols were visualized by spraying with Liebermann–Burchard reagent. Homogeneity of compounds was checked on TLC in at least three different solvent systems. Plant material was obtained from the experimental farm of this Institute and a voucher specimen has been deposited in the Botany Department.

Extraction and isolation of compounds. Dried and powdered rhizomes (850 g) of *C. speciosus* Sm. were extracted with EtOH (5 × 2.5 l.) at room temp. The EtOH extract was concd to 250 ml, diluted with H_2O (500 ml) and extracted with *n*-hexane (5 × 500 ml, 6.1 g). The hexane extract was chromatographed over silica gel (180 g, 60–120 mesh, BDH) eluting with hexane, hexane–C_6H_6 (9:1, 4:1, 7:3, 3:2, 1:1), C_6H_6 and C_6H_6–$CHCl_3$ (17:3). Fractions collected (50 ml) were monitored by TLC.

Tetradecyl 13-methylpentadecanoate (**1**). Earlier fractions (26–38) of the hexane–C_6H_6 (9:1) eluate furnished a solid which was purified by prep. TLC (hexane–C_6H_6, 3:1) to give a residue, 28 mg, mp 60–62° (Me_2CO–MeOH), R_f 0.45 (hexane–C_6H_6, 3:1). IR ν_{max} cm^{-1}: 2910, 2850, 1730, 1460, 1410, 1375, 1180, 730 and 720. ^{1}H NMR: δ0.88 (6H, *t*, J = 6 Hz, 2 × Me), 0.80 (3H, *d*, J = 6 Hz, –CHMe–), 1.20 [$(CH_2)_n$, *br s*], 2.28 (2H, *t*, J = 6 Hz, $-CH_2CO-$), 4.00 (2H, *t*, J = 6 Hz, $-OCOCH_2-$). MS *m/z* (rel. int.): 452 [M]$^+$ ($C_{30}H_{60}O_2$) (0.2), 437 (2), 256 (24), 255 (6), 241 (1), 239 (2), 213 (1), 211 (1), 197 (1), 196 (1), 57 (100), 43 (75).

Hydrolysis of **1**. Compound **1** (20 mg) was refluxed with 5% KOH in EtOH (20 ml) for 8 hr. The vol. was then reduced and the reaction mixture diluted with H_2O (25 ml), extracted with Et_2O (4 × 25 ml), washed with H_2O (2 × 25 ml), and dried (Na_2SO_4). Removal of solvent gave tetradecanol, 5 mg, mp 41–43° (IR, MS). The mother liquor from the above extraction was acidified with dil HCl and then extracted with Et_2O (3 × 25 ml), washed with H_2O (2 × 25 ml) and dried (Na_2SO_4). Removal of solvent furnished an acid, 10 mg, mp 65–66° (Me_2CO). IR ν_{max} cm^{-1}: 2910, 2850, 3300–2500, 1700, 1460, 1375, 1260, 920 and 720. ^{1}H NMR: δ0.88 (3H, *t*, J = 6 Hz, terminal Me), 0.80 (3H, *d*, J = 6 Hz, –CHMe–). 1.20 [$(CH_2)_n$, *br s*], 2.28 (2H, *t*, J = 6 Hz, $-CH_2CO-$). MS *m/z* (rel. int.): 256 [M]$^+$ ($C_{16}H_{32}O_2$) (1), 241 (3), 60 (60), 57 (100), 45 (15) and 43 (55).

Tetradecyl 11-methyltridecanoate (**2**). Removal of solvent from later fractions (45–88) of the hexane–C_6H_6 (9:1) eluate, furnished a solid which after purification by prep. TLC gave a residue, 23 mg, mp 58–61° (Me_2CO–MeOH), R_f 0.42 (hexane–C_6H_6, 3:1). IR ν_{max} cm^{-1}: 2910, 2840, 1725, 1460, 1410, 1380, 1180, 725 and 720. ^{1}H NMR: δ0.88 (6H, *t*, J = 6 Hz, 2 × Me), 0.80 (3H, *d*, J = 6 Hz, –CHMe–), 1.20 [$(CH_2)_n$, *br s*], 2.28 (3H, *t*, J = 6 Hz, $-COCH_2-$), 4.00 (2H, *t*, J = 6 Hz, $-CH_2-O-CO$). MS *m/z* (rel. int.): 424 [M]$^+$ ($C_{28}H_{56}O_2$) (0.5), 409 (3), 256 (40), 241 (1), 227 (1), 213 (1), 211 (2), 197 (2), 183 (5), 168 (5), 57 (100), 43 (70).

Hydrolysis of **2**. Compound **2** (15 mg) was refluxed with 5% KOH in EtOH (20 ml) for 8 hr. After usual work up 4 mg tetradecanol were obtained, mp 41–43° together with 7 mg of an acid, mp 63–65° (Me_2CO). IR ν_{max} cm^{-1}: 2910, 2850, 3300–2500, 1700, 1460, 1380, 1250, 920, 725 and 720. ^{1}H NMR: δ0.88 (3H, *t*, J = 6 Hz, terminal Me), 0.80 (2H, *d*, J = 6 Hz, –CHMe–), 1.20 [$(CH_2)_n$, *br s*], 2.28 (2H, *t*, J = 6 Hz, $-CH_2COOH$). MS *m/z* (rel. int.): 228 [M]$^+$ ($C_{14}H_{28}O_2$) (0.5), 213 (4), 60 (50), 57 (100), 45 (20), 43 (45).

Triacontanol (**3**). Eluted in the hexane–C_6H_6 (4:1) fractions 101–109, 15 mg, mp 84–86°. Identified by comparison with authentic material (mmp, IR, NMR, MS).

5α-Stigmast-9(11)-en-3β-ol (**4**). Fractions 160–190 of the hexane–C_6H_6 (7:3) eluate afforded a residue, 15 mg, mp 132°, identified by comparison with an authentic sample of the sterol (mmp, IR, NMR, MS, co-TLC).

Triacontanoic acid (**5**). Obtained in fractions 191–205 of the hexane–C_6H_6 (3:2) eluate, 250 mg, mp 92–93°. Identified by comparison with an authentic sample (mmp, IR, NMR, MS).

14-Oxotricosanoic acid (**6**). Fractions (220–235) of the hexane–C_6H_6 (1:1) eluate furnished a residue which was purified by prep. TLC (C_6H_6–Me_2CO, 19:1), 14 mg, mp 74–77° ($CHCl_3$–MeOH), R_f 0.60 (C_6H_6–Me_2CO, 19:1). IR ν_{max} cm^{-1}: 2910, 2840, 3400–2500, 1720, 1700, 1455, 1410, 1380, 1270, 1120, 1070, 920, 725, 715. ^{1}H NMR: δ0.88 (3H, *t*, J = 6 Hz, terminal Me), 1.22 [$(CH_2)_n$, *br s*], 2.25 (6H, *t*, J = 6 Hz, $-C\underline{H}_2COC\underline{H}_2-$, $-C\underline{H}_2COOH$). MS *m/z* (rel. int.): 368 [M]$^+$ ($C_{23}H_{44}O_3$) (1) 256 (8), 241 (4), 213 (5), 198 (5), 155 (5), 127 (5), 112 (10), 60 (30), 58 (4), 57 (95), 45 (8), 43 (100).

Reduction of **6**. Compound **6** (8 mg) was dissolved in MeOH (5 ml) and $NaBH_4$ (5 mg) added gradually. The mixture was then stirred at room temp. for 4 hr. It was then diluted with H_2O (25 ml), extracted with Et_2O (4 × 25 ml), washed with H_2O (2 × 25 ml) and dried (Na_2SO_4). Removal of solvent gave a residue, 5 mg, mp 80° (Me_2CO). IR ν_{max} cm^{-1}: 3430, 2910, 2840, 3400–2500, 1700, 1450, 1380, 1260, 1120, 1070, 930 and 715. MS *m/z* (rel. int.): 352 [M − H_2O]$^+$ (5), 243 (8), 213 (7), 157 (5), 127 (6), 60 (40), 57 (95), 45 (2), 43 (100).

14-Oxoheptacosanoic acid (**7**). The acid was obtained by purification of fractions 245–255 of the hexane–C_6H_6 (1:1) eluate by prep. TLC (C_6H_6–Me_2CO, 19:1), 12 mg, mp 80–82° ($CHCl_3$–MeOH), R_f 0.55 (C_6H_6–Me_2CO, 19:1). IR ν_{max} cm^{-1}: 2910, 2840, 3400–2500, 1725, 1705, 1455, 1410, 1380, 1260, 1120, 1060, 920, 725 and 715. ^{1}H NMR: δ0.88 (3H, *t*, J = 6 Hz, terminal Me), 1.22 [$(CH_2)_n$, *br s*], 2.25 (6H, *t*, J = 6 Hz, $-C\underline{H}_2COC\underline{H}_2-$, $-C\underline{H}_2COOH$). MS *m/z* (rel. int.): 424 [M]$^+$ ($C_{27}H_{52}O_3$) (1), 256 (10), 241 (3), 226 (2), 213 (5), 211 (1), 60 (45), 58 (6), 57 (80), 45 (5), 43 (100).

Reduction of **7**. Compound **7** (8 mg) was dissolved in MeOH (5 ml) and $NaBH_4$ (5 mg) added gradually. The reaction mixture was then stirred at room temp. for 4 hr. After usual work up a hydroxy acid was obtained, 5 mg, mp 84–85° (Me_2CO). IR ν_{max} cm^{-1}: 3440, 2910, 2840, 3400–2500, 1705, 1455, 1380, 1260, 1125, 1070, 925, 715. MS *m/z* (rel. int.): 408 [M − H_2O]$^+$ (4), 243 (10), 213 (6), 183 (10), 60 (50), 57 (90), 45 (4), 43 (100).

14-Oxo-octacosanoic acid (**8**). Fractions 260–273 from the hexane–C_6H_6 (1:1) eluate when purified by prep. TLC (C_6H_6–Me_2CO, 19:1), afforded a residue, 15 mg, mp 83–85° ($CHCl_3$–MeOH), R_f 0.40 (C_6H_6–Me_2CO, 19:1). IR ν_{max} cm^{-1}: 2910, 2840, 3400–2500, 1715, 1700, 1450, 1415, 1380, 1290, 1125, 1075, 940, 730 and 720. ^{1}H NMR: δ0.88 (3H, *t*, J = 6 Hz, terminal Me), 1.22 [$(CH_2)_n$, *br s*], 2.25 (6H, *t*, J = 6 Hz, $-C\underline{H}_2COC\underline{H}_2-$, $-C\underline{H}_2COOH$). MS *m/z* (rel. int.) : 438 [M]$^+$ ($C_{28}H_{54}O_3$) (0.5), 256 (6), 241 (2), 240 (3), 225 (2), 213 (6), 198 (1), 197 (6), 182 (4), 60 (15), 58 (55), 57 (55), 45 (5), 43 (100).

Reduction of **8**. Compound **8** (9 mg) was dissolved in MeOH (5 ml) and $NaBH_4$ (5 mg) added gradually and stirred for 4 hr. After usual work up 5 mg of a hydroxy acid were isolated, mp 85–86° (Me_2CO). IR ν_{max} cm^{-1}: 3450, 2910, 2840, 3400–2500, 1700, 1450, 1380, 1280, 1120, 1070, 940, 730, 715. MS *m/z* (rel. int.): 422 $[M-H_2O]^+$ (5), 243 (8), 227 (10), 213 (6), 197 (12), 60 (60), 57 (85), 45 (2), 43 (100).

Sitosterol (**9**). Eluted in the C_6H_6 fractions 320–350, 40 mg, mp 135–136° (MeOH). Identified by IR, MS, mmp, co-TLC.

Diosgenin (**10**). Obtained from the C_6H_6–$CHCl_3$ (17:3) fractions, 90 mg, mp 200–202° (MeOH). Identified by mmp, co-TLC, MS, IR.

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