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OXO FATTY ACID ESTERS FROM *CRYPTOCORYNE SPIRALIS* RHIZOMES

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Key Word Index—*Cryptocoryne spiralis*; Araceae; rhizomes; ethyl 14-oxotetracosanoate; 15-oxoeicosanyl 14-oxoheptadecanoate.

Abstract—Two new compounds, isolated from the rhizomes of *Cryptocoryne spiralis*, have been characterized as ethyl 14-oxotetracosanoate and 15-oxoeicosanyl 14-oxoheptadecanoate by spectral data and chemical studies. Hentriacontane and sitosterol have also been isolated and identified.

INTRODUCTION

In combination with other drugs, the rhizomes of *Cryptocoryne spiralis* Fisch. (Araceae) are used in the treatment of infantile vomiting, coughing, fever and abdominal complaints in adults [1]. In the past an unidentified amorphous basic substance was reported from this plant [2]. Since no systematic work has been done on this plant, a detailed investigation was undertaken.

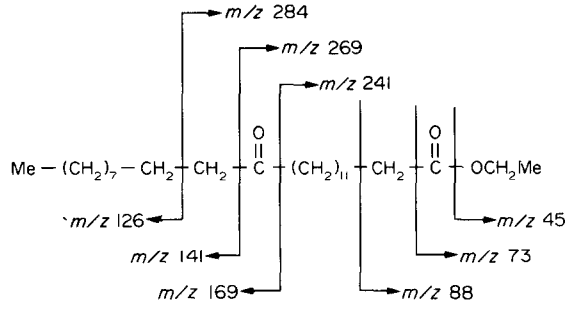
RESULTS AND DISCUSSION

Four compounds (A–D) were isolated by Si gel chromatography of the *n*-hexane extract of the rhizomes of *C. spiralis*.

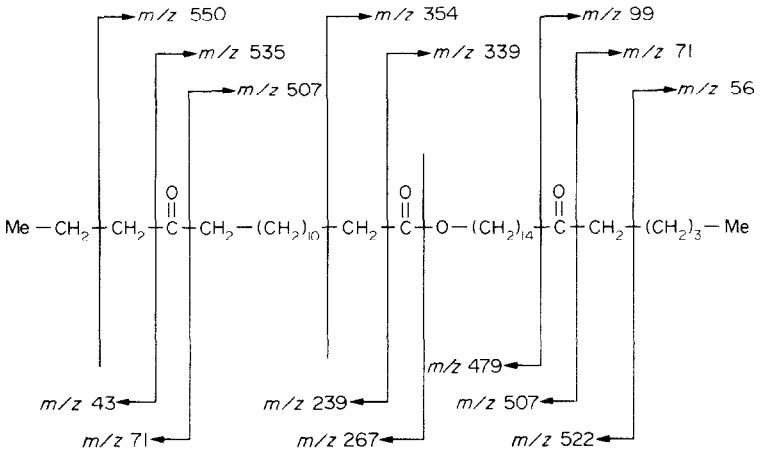
Compound B, had IR absorption bands at 1732 (ester CO), 1715 (CO) and 720 cm^{-1} (long chain), and gave a positive 2,4-dinitrophenylhydrazine test suggesting that it was a long chain saturated keto ester. The mass spectrum gave an M^+ at *m/z* 410 which, together with elemental analysis, established the molecular formula as $C_{26}H_{50}O_3$. The location of the carbonyl group was deduced to be at C-14 from the prominent α-fission ions at *m/z* 269, 241, 169, 141 and β-fission ions, involving McLafferty rearrangement, at *m/z* 284 and 126 [3]. α- and β-fission fragments characteristic of an ethyl ester moiety were observed at *m/z* 73, 45 and 88, respectively. The ion at *m/z* 58 generated by double rearrangement, is characteristic of a ketone having a γ-hydrogen in both alkyl fragments. The straight chain nature of the compound was supported by the absence of an $[M-15]^+$ ion [4] and the presence of an $[M+1]^+$ peak was characteristic of its unsymmetrical nature [5, 6]. The ^1H NMR spectrum of the compound displayed a triplet ($J = 6$ Hz) at δ1.28 for the methyl of an OCH_2Me group and a quartet at δ4.05 for CH_2 protons of an OCH_2Me group. These data, along with a strong peak at *m/z* 88, fully supported the ethyl ester structure of B [7]. The four protons of the CH_2 groups adjacent to the free carbonyl function appeared as a triplet ($J = 6$ Hz) at δ2.22.

Alkaline hydrolysis of B afforded a keto acid, mp 49–50° having IR bands at 3300–2500 (broad), 1700 and 920 cm^{-1} for a carboxyl group [8] and 1715 cm^{-1} for a carbonyl group. The mass spectrum of the acid had an M^+ at *m/z* 382 ($C_{24}H_{46}O_3$) and a significant ion at *m/z* 60 due to the β-fission of the carboxyl group. The position of the carbonyl group in compound B was again confirmed as C-14 since this acid had α- and β-fission ions at *m/z* 241, 213, 169, 141, and 256 and 126, respectively. These data suggested the structure of the acid as 14-oxotetracosanoic acid.

Sodium borohydride reduction of B afforded a hydroxy ester, mp 40–41° with IR bands at 3340 cm^{-1} for a hydroxyl group. On the basis of the above data, compound B was characterized as ethyl 14-oxotetracosanoate (**1**).



Compound C, mp 46–48°, gave a positive 2,4-dinitrophenylhydrazine test and showed IR bands at 1740 (ester CO), 1720 (CO) and 715 cm^{-1} (long chain). The mass spectrum displayed an M^+ at *m/z* 578, which established the molecular formula as most probably $C_{37}H_{70}O_4$. Alkaline hydrolysis of C afforded a keto acid and a keto alcohol. The keto acid, mp 40°, displayed an M^+ at *m/z* 284 ($C_{17}H_{32}O_3$) and had IR bands at 1705 (CO), 1720, 3400–2500 and 925 cm^{-1} (COOH). The mass spectrum of the acid exhibited a β-fission ion at *m/z* 60 which is characteristic of a terminal carboxyl group. The position of the carbonyl group was located at C-14 from the significant α- (*m/z* 241, 213, 71 and 43) and β-fission (*m/z* 256 and 86) ions. This acid, therefore, was characterized as 14-oxoheptadecanoic acid.



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The keto alcohol could not be crystallized and was purified by prep. TLC. It had an IR absorption band at 3400 cm^{-1} (OH) and the M^+ in its mass spectrum at *m/z* 312 suggested the molecular formula as $C_{20}H_{40}O_2$. The ions at *m/z* 294 and 279 could be attributed to the loss of water and water plus a methyl, respectively from the M^+ ion which is usually observed in the spectra of primary alcohols [9]. The position of the carbonyl group at C-15 was deduced from the α-fission ions at *m/z* 241, 213, 99, 71 and β-fission ions at *m/z* 256, 114, 56. These data indicated the structure of the keto alcohol as 15-oxoeicosanol.

On the basis of its hydrolysis products, C should be 15-oxoeicosanyl 14-oxoheptadecanoate (**2**). The mass spectral fragmentation was consistent with the proposed structure. The characteristic β-fission ion at *m/z* 550 and the α-fission ions at *m/z* 535, 507, 71 and 43 were due to the C-14 carbonyl group of the acid moiety. Similarly, the second carbonyl group was at C-15 in the alcohol part of the ester because of the presence of prominent α-fission ions at *m/z* 507, 479, 99, 71 and β-fission ions at *m/z* 522 and 56. The other ions at *m/z* 339, 267, 239 (α-fission) and 354 (β-fission) were due to the ester carbonyl group.

Compound A, mp 65°, obtained in traces was identified as hentriacontane (mass and IR spectra) and comparison of lit. mp [10].

Compound D, mp 137–138°, was identified as sitosterol, by direct comparison with an authentic sample.

The above data are in full agreement with the structures assigned to B and C. These compounds have not previously been found to occur naturally. However, a few oxo acids are known to occur [11]. The characterization of three additional compounds from the hexane extract of the rhizomes of this plant is currently in progress.

EXPERIMENTAL

Mps are uncorr. IR spectra were recorded in KBr pellets unless otherwise stated. The 60 MHz NMR spectra were measured in $CDCl_3$ with TMS as int. standard. TLC was carried out on Si gel G and spots were visualized by exposure to I_2 vapour or spraying with 2,4-dinitrophenylhydrazine reagent. The homogeneity of the compounds was checked by TLC using at least three different solvent systems.

Plant material. C. spiralis Fisch. was purchased from the local market and a voucher specimen has been deposited in the Botany Department of this institute.

Extraction and isolation of compounds. Dried and powdered rhizomes (2.5 kg) were extracted with EtOH (7 × 2.5 l). The EtOH 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), respectively. The hexane extract was evaporated and the residue (27.85 g) chromatographed over Si gel (1200 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.

Compound A (hentriacontane). Removal of solvent from hexane fractions (1–4) afforded a solid, 5 mg, mp 65° (Me_2CO–MeOH). IR ν_{max} cm^{-1}: 2915, 2840, 1460, 1370, 725 and 715.

Compound B (ethyl 14-oxotetracosanoate). Removal of solvent from the hexane–C_6H_6 (3:1) and hexane–C_6H_6 (1:1) fractions furnished a viscous mass which was purified by prep. TLC, 385 mg, R_f 0.77 (C_6H_6). (Found: C, 76.45; H, 11.90%. $C_{26}H_{50}O_3$ requires: C, 76.10; H, 12.20%.) IR ν_{max}^{neat} cm^{-1}: 2920, 2850, 1732, 1715, 1455, 1380, 1250, 1165, 1110, 1030 and 720. ^1H NMR: δ0.82 (3H, *t*, *J* = 6 Hz, Me), 1.28 (3H, *t*, *J* = 6 Hz, $-\underset{\underset{O}{\|}}{C}-O-CH_2-Me$), 1.20 [36H, $(CH_2)_{18}$, *br s*], 2.22 (6H, *t*, *J* = 6 Hz, $-CH_2-\underset{\underset{O}{\|}}{C}-CH_2-$, $-CH_2-\underset{\underset{O}{\|}}{C}-O-$), 4.05 (2H, *q*, *J* = 8 Hz, $-\underset{\underset{O}{\|}}{C}-O-CH_2-Me$). MS *m/z* (rel. int.): 410 $[M]^+$ ($C_{26}H_{50}O_3$) (0.25), 396 (0.89), 382 (0.24), 368 (0.45), 353 (0.21), 340 (0.24), 326 (0.16), 325 (0.19), 312 (4), 284 (20), 269 (4), 241 (10), 239 (11), 227 (2), 213 (5), 199 (6), 185 (4), 171 (4), 169 (2), 157 (22), 143 (11), 141 (2), 129 (6), 126 (2), 115 (12), 113 (5), 102 (9), 101 (100), 99 (5), 88 (99), 85 (12), 73 (35), 71 (27), 58 (5), 57 (56) and 45 (13).

Hydrolysis of **1**. Compound **1** (80 mg) was refluxed with 5% alcoholic KOH (30 ml) for 4 hr. The vol. was then reduced by half and the reaction mixture diluted with H_2O (50 ml) and acidified with dilute HCl. It was extracted with Et_2O (4 × 50 ml), washed with H_2O (2 × 50 ml) and dried (Na_2SO_4). Removal of solvent gave an acid, 25 mg, mp 49–50° (MeOH). IR ν_{max} cm^{-1}: 2910, 2840, 3300–2500, 1715, 1700, 1460, 1410, 1380, 1275, 920 and 715. ^1H NMR: δ0.82 (3H, *t*, *J* = 6 Hz, Me), 1.20 [36H, $(-CH_2)_{18}$, *br s*], 2.22 (6H, *t*, *J* = 6 Hz, $C\underline{H}_2-\underset{\underset{O}{\|}}{C}-C\underline{H}_2$, $C\underline{H}_2-CO_2H$). MS *m/z*

(rel. int.): 382 $[M]^+$ (5), 256 (5), 241 (11), 213 (6), 169 (10), 141 (16), 126 (10), 85 (100), 60 (11).

$NaBH_4$ *reduction of* **1**. Compound **1** (50 mg) was dissolved in MeOH (0.5 ml) and $NaBH_4$ (10 mg) was gradually added. The reaction mixture was stirred for 3 hr at room temp. At the end of reaction it was 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 gave a solid, 20 mg, mp 40–41° (Me_2CO). IR ν_{max} cm^{-1}: 3340, 2920, 2850, 1732, 1460, 1375, 1265, 1050 and 720.

Compound C [*15-oxoeicosanyl 14-oxoheptadecanoate* (**2**)]. Removal of solvent from fractions (151–180) of hexane–C_6H_6 (1:3) furnished a solid, 70 mg, mp 46–48° (Me_2CO–MeOH). R_f 0.45 (C_6H_6). IR ν_{max} cm^{-1}: 2920, 2840, 1740, 1720, 1450, 1380, 1260, 1150, and 715. MS *m/z* (rel. int.): 578 $[M]^+$ ($C_{37}H_{70}O_4$) (6), 550 (13), 535 (1), 522 (1), 507 (1), 492 (1), 479 (1), 354 (4), 339 (2), 267 (6), 239 (18), 149 (100), 99 (6), 71 (60), 58 (6), 57 (90), 56 (16), 43 (63).

Hydrolysis of **2**. Compound **2** (40 mg) was refluxed with 5% alcohol KOH (20 ml) for 4 hr. After partial evaporation the reaction mixture was 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 gave a keto alcohol (purified by prep. TLC), 8 mg. IR ν_{max}^{neat} cm^{-1}: 3400, 2915, 2840, 1720, 1460, 1375, 1270, 1115, 1070, 735 and 718. MS *m/z* (rel. int.): 312 $[M]^+$ ($C_{20}H_{40}O_2$) (0.52), 294 $[M-H_2O]^+$ (0.52), 281 (1), 279 $[M-H_2O+Me]^+$ (6), 269 (1), 267 (2), 256 (7), 253 (2), 241 (1), 239 (2), 225 (2), 213 (5), 211 (3), 197 (3), 185 (5), 183 (3), 171 (4), 169 (4), 157 (4), 155 (5), 143 (5), 141 (5), 129 (12), 127 (6), 115 (9), 114 (2), 113 (18), 101 (5), 99 (14), 73 (30), 71 (61), 58 (6), 57 (100), 56 (24), 45 (8) and 43 (84). The mother liquor from the above extraction was acidified with dilute 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 a keto acid, 10 mg, mp 40° (Me_2CO). IR ν_{max} cm^{-1}: 2915, 2840, 3400–2500, 1720, 1705, 1460, 1410, 1375, 1275, 925, 735 and 720. MS *m/z* (rel. int.): 284 $[M]^+$ ($C_{17}H_{32}O_3$) (3), 270 (3), 256 (16), 242 (1), 241 (2), 239 (2), 214 (2), 213 (9), 211 (1), 200 (2), 199 (3), 186 (1), 185 (9), 183 (2), 172 (2), 171 (8), 169 (3), 158 (1), 157 (8), 155 (5), 144 (1). 143 (8), 141 (5), 130 (3), 129 (26), 127 (6), 116 (4), 115 (10), 113 (9), 102 (3), 101 (9), 99 (11), 88 (3), 87 (22), 86 (3), 74 (31), 73 (64), 71 (53), 60 (55), 58 (8), 57 (100), 45 (10) and 43 (95).

Compound D (*sitosterol*). Eluted in hexane–C_6H_6 (1:3) fractions (184–210), 50 mg, mp 137–138° (MeOH). Its identity was established by comparison with an authentic sample (mp, mmp, IR, NMR, MS, co-TLC).

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