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ALIPHATIC CONSTITUENTS FROM *DUBOISIA MYOPOROIDES*

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Key Word Index—*Duboisia myoporoides*; Solanaceae; leaves and stems; 6-methyldotriacontane; hentriacontanyl tetratriacontanoate; 3-hydroxydotriacontan-28-one; 4-pentatriacontanone.

Abstract—Four new compounds, isolated from the leaves and stems of *Duboisia myoporoides* have been characterized as 6-methyldotriacontane, hentriacontanyl tetratriacontanoate, 3-hydroxydotriacontan-28-one and 4-pentatriacontanone, respectively, by spectral data and chemical studies.

INTRODUCTION

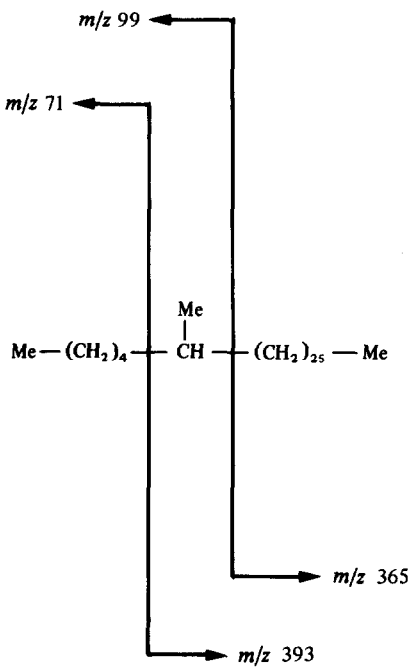
Duboisia myoporoides is indigenous to Australia from where it has been introduced to India. It has been successfully cultivated at C.I.M.A.P. Experimental Farm at Bangalore for its high tropane alkaloid contents especially hyoscine and hyoscyamine which have mydriatic, anticholinergic and antispasmodic properties. During a large scale isolation of hyoscine and hyoscyamine from this plant, it was of interest to examine the non-alkaloidal compounds since such compounds have not been reported from this species.

RESULTS AND DISCUSSION

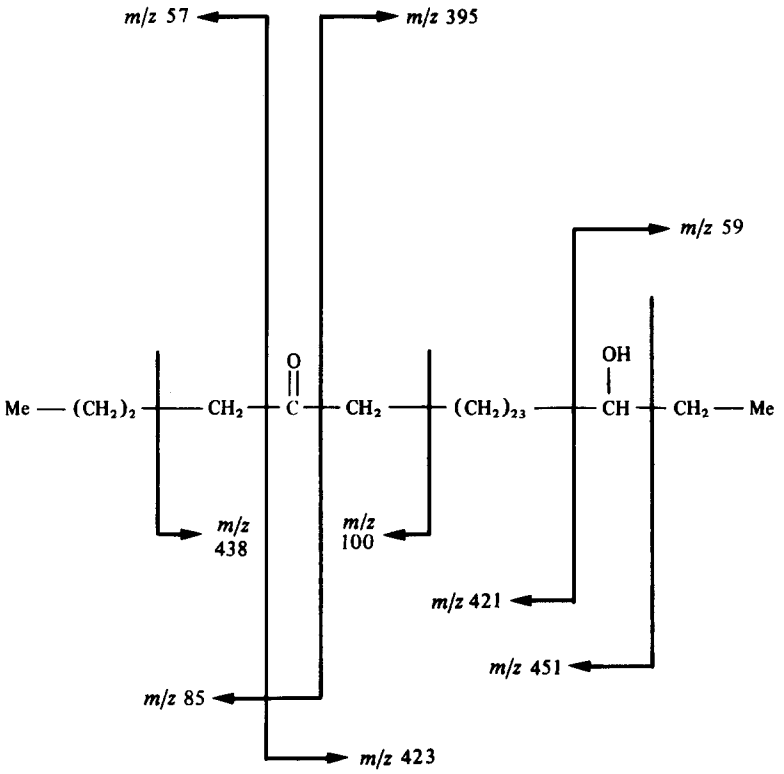
Silica gel CC of the *n*-hexane extract of the plant afforded four crystalline compounds **1**–**4**. Compound **1**, mp 64° showed IR bands at 2950, 2910, 2840, 1460, 728 (long chain), 1378 cm^{-1} (Me). The mass spectrum of the compound had a $[M]^+$ at *m/z* 464 suggesting the molecular formula $C_{33}H_{68}$. The presence of an ion at *m/z* 449 $[M-Me]^+$ and the ratio of abundances of $[M-Me]^+/[M]^+$ indicated the compound to be branched having a methyl group as substituent [1]. A thorough examination of the mass spectrum of the compound indicated that the intensity of C_nH_{2n+1} peaks, after a maximum at $n = 6$, steadily declined up to $n = 27$, followed by a strong peak at $n = 28$ (*m/z* 393). The intense peak at $n = 28$ $[M-71]^+$ suggested the attachment of the methyl group at C-6 [2]. The ^1H NMR spectrum of the compound displayed a triplet at δ 0.90 ($J = 6$ Hz) for the two terminal methyl groups and a doublet at δ 0.76 ($J = 8$ Hz) for the methyl group at C-6. These data led us to characterize this compound as 6-methyldotriacontane (**1**).

CrO_3 oxidation of **1** gave acids which were methylated with CH_2N_2. GC of these methyl esters showed the presence of methyl heptacosanoate and methyl hexacosanoate, the corresponding acids of which were formed by the cleavage on both sides of the tertiary C atom at C-6.

Compound **2**, mp 80–81° had IR absorption bands at 2950, 2910, 2840, 1460, 725, 715 (long chain), 1390 (Me), 1735 cm^{-1} (ester group). The mass spectrum of this compound indicated a $[M]^+$ ion at *m/z* 942 corresponding with $C_{65}H_{130}O_2$. The ^1H NMR spectrum of the compound showed two triplets, $J = 8$ Hz at δ 3.96 and 2.20 for methylene protons of $-CH_2-O-CO-$ and $-COCH_2-$, respectively. Alkaline hydrolysis of this ester gave an acid, mp 90°, $[M]^+$ 508, $C_{34}H_{68}O_2$, identified as tetratriacontanoic acid by comparison of literature data [3] and an alcohol, mp 85°, $[M]^+$ 452, $C_{31}H_{64}O$, identified as hentriacontanol by comparison with an authentic sample. This compound, therefore, was identified as hentriacontanyl tetratriacontanoate.



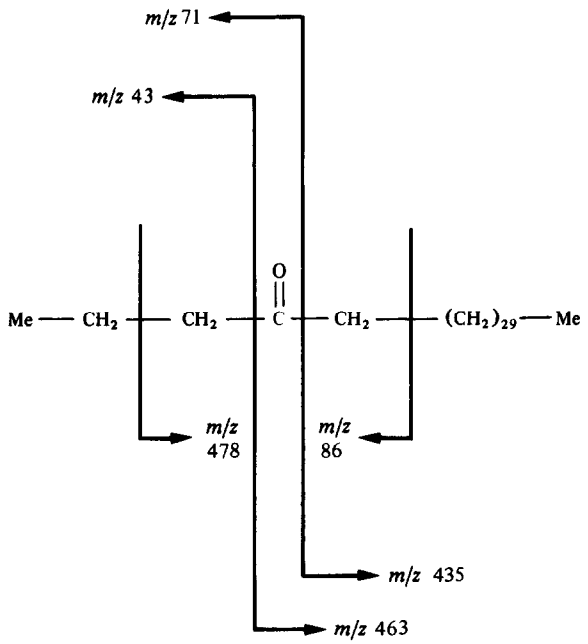
Compound **3**, mp 75–76°, exhibited IR absorption bands at 3440 (OH), 1705 (CO), 2950, 2910, 2840, 1460, 725, 715 (long chain), 1370 cm^{-1} (Me) and gave a positive 2,4-dinitrophenylhydrazine test. The mass spectrum of **3** indicated an $[M]^+$ at *m/z* 480 suggesting the molecular formula $C_{32}H_{64}O_2$. The location of the CO group at C-28 was established by the presence of prominent α-fission ions at *m/z* 57, 423, 85, 395 and β-fission ions, involving McLafferty rearrangement, at *m/z* 438 and 100 [4]. The ion at *m/z* 58 was due to the double rearrangement,



characteristic of a ketone having a γ-H in both alkyl fragments [4]. Similarly the hydroxyl group was placed at C-3 because of the significant ions at *m/z* 59, 421 and 451. ^{1}H NMR spectrum of **3** showed a triplet, $J = 6$ Hz at δ 2.34 for the four protons of the two CH_2 groups adjacent to the carbonyl function and a multiplet at δ 2.80 for the –C<u>H</u>(OH)– proton. The above data strongly suggested the structure of this compound as 3-hydroxydotriacontan-28-one (**3**). Treatment of **3** with Ac_2O–pyridine furnished a keto-acetate, mp 73°, showing IR bands at 1730, 1705 and 1250 cm^{-1}.

Compound **4**, mp 74–75°, had IR absorption bands at 2910, 2840, 1460, 725, 715 (long chain), 1710 (CO), 1390 cm^{-1} (Me) and gave a positive DNPH test. Its molecular formula, $C_{35}H_{70}O$, was suggested by the observation of the $[M]^+$ at *m/z* 506. α-Fission ions at *m/z* 43, 463, 71, 435 and β-fission fragments, involving McLafferty rearrangement, at *m/z* 86 and 478 [4] led to the establishment of the position of the CO group at C-4. As in **3**, the ion at *m/z* 58 was due to double rearrangement. Absence of an $[M - 15]^+$ ion indicated the straight chain nature of this ketone. ^{1}H NMR spectrum of this compound displayed a triplet, $J = 6$ Hz at δ 2.30 for the two CH_2 groups adjacent to the CO function. The data obtained above suggested the structure of this compound as 4-pentatriacontanone (**4**).

The structure of compounds **1**–**4** as 6-methyldotriacontane, hentriacontanyl tetratriacontanoate, 3-hydroxydotriacontan-28-one and 4-pentatriacontanone, respectively, are in full agreement with data now available. These compounds have not previously been found in nature. The characterization of five additional compounds from the hexane extract is currently in progress.



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 silica gel G and the spots were visualized by exposure to I_2 vapour or DNP-spray. Plant material was cultivated at the Experimental Farm, Bangalore of this Institute and a voucher

specimen has been deposited in the Botany department.

Extraction and isolation of compounds. Dried and powdered leaves and stems (3 kg) of *D. myoporoides* R. Br. were extracted with *n*-hexane (5 × 7.5 l). The hexane extract was freed of solvent and the residue (40 g) was chromatographed over silica gel (1.2 kg, 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, C_6H_6–$CHCl_3$ (3:1), C_6H_6–$CHCl_3$ (1:1), C_6H_6–$CHCl_3$ (1:3), $CHCl_3$ and $CHCl_3$–MeOH (19:1). Fractions (250 ml) were collected and monitored by TLC.

Compound **1** (*6-methyldotriacontane*). Removal of solvent from hexane-fractions (3–9) afforded a residue, 2.5 g, mp 64° (Me_2CO). IR ν_{max} cm^{-1}: 2950, 2910, 2840, 1460, 1378, 728 and 718. ^1H NMR: δ 0.90 (6H, *t*, *J* = 6 Hz, H_3-1, H_3-32), 0.76 (3H, *d*, *J* = 8 Hz, H_3-6), 1.45 (1H, *m*, H-6), 1.20 (58H, *s*, $(CH_2)_{29}$). MS *m/z* (rel. int.): 464 $[M]^+$ ($C_{33}H_{68}$, 0.92), 449 (2.50), 436 (3), 421 (3), 408 (2), 393 (6), 379 (2), 365 (2), 351 (2), 337 (2), 323 (3), 309 (3), 295 (3), 281 (3), 267 (4), 253 (4), 239 (4), 225 (5), 211 (5), 197 (6), 183 (7), 169 (8), 155 (9), 141 (11), 127 (14), 113 (18), 99 (23), 85 (55), 71 (72), 57 (100), 55 (24), 43 (62).

CrO_3 *oxidation of* **1**. To **1** (500 mg) in HOAc (10 ml) was added CrO_3 (200 mg) and the mixture heated at 80–90° for 4 hr. Unreacted material floating at the surface was taken out with spatula. HOAc was removed under vacuum and the residue diluted with H_2O (100 ml) and extracted with $CHCl_3$ (4 × 100 ml). The $CHCl_3$ extract was washed with H_2O (2 × 50 ml) and dried (Na_2SO_4). Removal of solvent gave a residue, 300 mg, mp 75–85° ($CHCl_3$–MeOH). IR ν_{max} cm^{-1}: 3600–3200, 2910, 2840, 1705, 1460, 1380, 1255, 728, 718. Acids (250 mg) were methylated with CH_2N_2–Et_2O overnight at room temp. The solvent was removed to provide a residue, mp 60–64° (MeOH). IR ν_{max} cm^{-1}: 2940, 2910, 2840, 1725, 1460, 1375, 1210, 715.

Compound **2** (*hentriacontanyl tetratriacontanoate*). Removal of solvent from hexane–C_6H_6 (3:1) eluates (25–60) furnished a residue, 270 mg, mp 80–81° (Me_2CO). IR ν_{max} cm^{-1}: 2950, 2910, 2840, 1735, 1460, 1390, 725, 715. ^1H NMR: δ 0.82 (6H, *t*, *J* = 6 Hz, terminal Me), 1.20 (120H, *s*, $(CH_2)_{60}$), 3.96 (2H, *t*, *J* = 8 Hz, $-CH_2-O-CO$), 2.20 (2H, *t*, *J* = 8 Hz, $-OC-CH_2-$). MS *m/z* (rel. int.): 942 $[M]^+$ ($C_{65}H_{130}O_2$, 1), 635 (1), 621 (1), 593 (1), 509 (1), 495 (1), 481 (1), 467 (1), 453 (2), 439 (1), 434 (1), 425 (3), 411 (1), 406 (2), 397 (5), 383 (1), 378 (1), 369 (7), 355 (1), 350 (1), 341 (2), 327 (1), 322 (1), 313 (10), 113 (10), 99 (12), 85 (37), 71 (60), 60 (10), 57 (100), 44 (5), 43 (85). The compound (100 mg) was refluxed with 5% alcoholic KOH (50 ml) for 5 hr. At the end of the reaction the mixture was dil with H_2O (100 ml) and extracted with Et_2O (4 × 50 ml). The extract was washed with H_2O (2 × 50 ml) and dried (Na_2SO_4). Removal of solvent gave a residue, mp 85° ($CHCl_3$–Me_2CO) identified as hentriacontanol (mmp, IR, MS, Co-TLC). MS *m/z* (rel. int.): 452 $[M]^+$ ($C_{31}H_{64}O$, 0.1), 434 $[M-H_2O]^+$ (5), 406 $[M-46]^+$ (6), 378 (5), 350 (5), 322 (7), 141 (8), 127 (9), 113 (10), 99 (15), 85 (35), 71 (55), 57 (100), 55 (75), 43 (80). The aq. layer left after the above extraction was acidified with dil. HCl and extracted with Et_2O (4 × 50 ml). The extract was washed with H_2O (2 × 50 ml) and dried (Na_2SO_4). Removal of solvent provided a residue, mp 90° ($CHCl_3$–MeOH), identified as tetratriacontanoic acid. IR ν_{max} cm^{-1}: 3500–3000, 2910, 2850, 1705, 1460, 1375, 1260, 728, 720. MS *m/z* (rel. int.): 508 $[M]^+$ ($C_{34}H_{68}O_2$, 2), 448 $[M-60]^+$ (2), 60 (55).

Compound **3** (*3-hydroxydotriacontan-28-one*). Removal of solvent from hexane–C_6H_6 (3:1 and 1:1) fractions (73–120) provided a residue, 45 mg, mp 75–76° (Me_2CO). IR ν_{max} cm^{-1}: 3440, 2950, 2910, 2840, 1705, 1460, 1370, 725, 715. ^1H NMR: δ 0.86 (6H, *t*, *J* = 6 Hz, H_3-1, H_3-32), 1.20 (48H, *s*, $(CH_2)_{24}$), 2.80 (1H, *m*, H-3), 2.0 (4H, *d*, *J* = 6 Hz, H_2-2, H_2-4), 2.34 (4H, *t*, *J* = 6 Hz, H_2-27, H_2-29). MS *m/z* (rel. int.): 480 $[M]^+$ ($C_{32}H_{64}O_2$, 0.5), 451 (2), 438 (2), 423 (3), 421 (2), 395 (3), 183 (1), 169 (1), 155 (1), 141 (1), 127 (5), 113 (4), 100 (4), 99 (5), 85 (28), 71 (53), 59 (100), 58 (60), 57 (40), 43 (65). Compound **3** (20 mg) was added to pyridine (1 ml), Ac_2O (1 ml) and the mixture left overnight at room temp. When worked up it gave a residue, mp 73° (Me_2CO). IR ν_{max} cm^{-1}: 2910, 2840, 1730, 1705, 1460, 1455, 1370, 1250, 720, 710.

Compound **4** (*4-pentatriacontanone*). Hexane–C_6H_6 (1:1 and 1:3) fractions (121–156) when freed of the solvent gave a residue, 19 mg, mp 74° (Me_2CO). IR ν_{max} cm^{-1}: 2910, 2840, 1710, 1460, 1390, 725, 715. ^1H NMR: δ 0.82 (6H, *t*, *J* = 6 Hz, H_3-1, H_3-35), 1.20 (60H, *s* $(CH_2)_{30}$), 2.30 (4H, *t*, *J* = 6 Hz, H_2-3, H_2-5). MS *m/z* (rel. int.): 506 $[M]^+$ ($C_{35}H_{70}O$, 0.4), 478 (3), 463 (2), 435 (3), 155 (2), 141 (2), 127 (5), 113 (5), 99 (7), 86 (3), 85 (35), 71 (73), 58 (40), 57 (100), 43 (93).

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