n-Alkanes and α-Palmitin from *Paspalum scrobiculatum* Seeds[#]

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Hexane and methanol extracts of *Paspalum scrobiculatum* seeds have yielded several known fatty acids, sterols, unusual straight chain hydrocarbons which are well known for their insect pheromone activity along with the antitumour glyceride, α-palmitin.

Introduction

Paspalum is a large genus of grasses with about 14 species occurring in India of which *P. scrobiculatum* is the common weed of plains of India (Anonymous, 1966). It grows vigorously in the cultivated crops especially in the paddy fields. The plant is used as fodder and its seeds are eaten by poor folk during the scarcity of food. The seeds have been found to possess several biological activities ranging from tranquillizer (Bhide, 1962, 1963), caseinolytic, tryptic, chymotryptic inhibitions (Udupa *et al.,* 1984) to starch binder on tablets (Wahi *et al.*, 1985). The seeds have earlier been examined to yield long chain alcohols and common sterols (Sharma *et al.,* 1972) and carbohydrate constituents (Paramhans and Tharnathan, 1980). In continuation of our program of investigating medicinal and aromatic plants of India (Dixit and Misra, 1997; Misra and Ahmad, 1997; Misra *et al.,* 1997), we have examined n-hexane and methanol extracts of the seeds of *P. scrobiculatum* which have yielded the antitumour compound α-palmitin (**1**) and unusually occurring n-alkanes (**2, 3**) along with common fatty acids (linoleic, oleic, palmitoleic and palmitic) and sterols (β-sitosterol, stigmasterol and β-sitosterol glucoside). The structure of known acids and sterols were confirmed by comparison of their data with the literature while **1**, **2** and **3** was determined mainly by NMR and chemical transformation which are discussed in this paper.

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Materials and Methods

Plant material

The seeds (4 kg) of *P. scrobiculatum* were procured from the local market in May 1998 and were authenticated by the botanists of our institute.

General experimental procedures

After grinding, the seeds were successively extracted with n-hexane (4 l) and MeOH (3 l) three times by soaking overnight. After solvent removal and column chromatography on silica gel, using n-hexane and EtOAc (up to 10%), eight main fractions were obtained. Fr. 1 (n-hexane) gave **2** and **3** (1.6 g, Rf. 10.0, n- hexane) and fr. 4 (n-hexane-EtOAc, 49:1 v/v) gave palmitic acid (26 mg), fr. 6 (n-hexane- EtOAc, 9:1 v/v) gave a mixture, a part of which after further column chromatography yielded a mixture (26 mg) of oleic, linoleic and palmitoleic acid (GLC, GC-MS). After further separations fr. 8 yielded β-sitosterol (20 mg) and stigmasterol (15 mg). GLC and GC-MS of Fr. 1 (dissolved in n-hexane) was done on OV-101 (2m × 3 mm) under the following temperature conditions: 60 °C for 5 min., then 5°/min to 200 °C with injector temperature at 200 °C and detector at 220 °C. RT of **2** was 2.71 and of **3** was 3.10 (ratio **2** to **3**, 19:1). Similarly, the GLC and GC-MS conditions for fatty acid methyl esters were as follows: on HP-5 (15m × 0.5 mm) under the following temperature conditions: 150° for 2 min, then 8°/min to 270 °C.

After column chromatography on silica gel, with n-hexane- EtOAc the MeOH extract yielded 10 fractions. Fr.1 gave a mixture of linoleic and oleic

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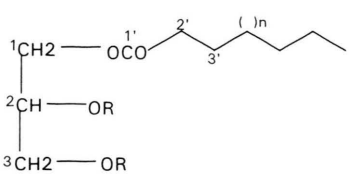
acids (100 mg); fr. 3 after TLC ($CHCl_3$) a mixture of β-sitosterol and stigmasterol (58 mg); fr. 6, n-hexane- EtOAc, 1:3 (v/v), after crystallization afforded **1** (32 mg, Rf 0.6, n-hexane-EtOAc, 3:2); fr. 10 after crystallization gave β-sitosterol glucoside (45 mg).

1-Hexadecanoyl propan-2,3-diol (**1**). M. P. 70 °C, $[\alpha]_D^{27}$ −4.85° ($CHCl_3$; 0.35), **IR** ν_{max}^{CHCl3} cm^{-1}: 3500–3300 (OH), 2931, 1734 (CO), 1456, 1394, 1188, 1084; **MS** *m/z* (rel. int.): 330 $[M]^+$ (3), 312 $[M\text{-}H_2O]^+$ (35), 239 $[C_{16}H_{31}O]^+$ (45), 135 (30), 98 (55), 69 (55), 55 (75), 43 (100); ^{1}HNMR, δ, $CDCl_3$: 0.87, t (*J* = 6.0 Hz), H-16′, 1.25, sbr, H-4′ to H-15′, 1.62, t (*J* = 6.5Hz), H-3′, 2.34, t (*J* = 6.5 Hz), H-2′, 4.16, dd (*J* = 6.0, 10.0 Hz), H-1, 3.92, p (*J* = 5.5 Hz), H-2, 3.62, dd (*J* = 6.0, 10.0 Hz), H-3; ^{13}C NMR, δ, $CDCl_3$: 14.46 (C-16′), 23.05 (C-15′), 32.29 (C-14′), 29.51, 29.62, 29.72, 29.83, 29.92, 29.98, 30.05 (C-4′ to C-13′), 25.29 (C-3′), 34.54 (C-2′), 174.73 (C-1′), 65.52 (C-1), 70.67 (C-2), 63.77 (C-3). ^{1}H NMR of **1a** , δ, $CDCl_3$: 0.88, t, (*J* = 6.5 Hz), H-16′, 1.25, sbr, H-4′ to H-15′, 1.61, t (*J* = 6.5 Hz), H-3′, 2.32, t (*J* = 6.5 Hz), H-2′, 4.31, dd (*J* = 6.0, 10.0 Hz), H-1, 5.20, p (*J* = 5.5 Hz), H-2, 4.15, dd (*J* = 6.0, 10.0 Hz), H-3.

Nonadecane (**2**) and *tetracosane* (**3**). Viscous mass, **IR** ν_{max}^{CHCl3} cm^{-1}: 2900, 1380, 1185; **MS of 2** *m/z* (rel. int.) : 268 $[M]^+$ (4.0), 239 $[M\text{-}C_2H_5]^+$ (4.0), 225 $[239\text{-}CH_2]^+$ (4.0), 211 $[225\text{-}CH_2]^+$, (4.5), 197 $[211\text{-}CH_2]^+$ (5.0), 183 (5.5), 169 (6.0), 155 (8), 141 (9), 127 (11), 113 (13), 99 (20), 97 (53), 85 (55), 71 (73), 57 (100), 43 (54), **MS of 3** *m/z* (rel. int.) : 338 $[M]^+$ (4), 309 $[M\text{-}C_2H_5]^+$ (3), 295 $[309\text{-}CH_2]^+$ (3), 281 $[295\text{-}CH_2]^+$ (4), 267 (4), 253 (4.5), 239 (4.5), 225 (4.5), 211 (5.0), 197 (5.0), 183 (5.5), 169 (6.0), 155 (8), 141 (9), 127 (11), 113 (13), 99 (18), 97 (45), 85 (55), 71 (73), 57 (100), 43 (54); ^{1}H NMR of 2 and 3, δ, $CDCl_3$: 0.88 and 0.89, t each (*J* = 6.5 Hz), terminal Me, 1.26, sbr, n CH_2. ^{13}C NMR of 2 and 3, δ, $CDCl_3$: 14.15 (2 Me), 22.72 (2 CH_2), 32.0 (2 CH_2), 30.0, 29.9, 29.8 (n CH_2).

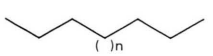
Results and Discussion

α-Palmitin (glycerol-1-hexadecanoate, **1**) has been a compound of synthetic interest (Baer and Fischer, 1945; Quinn *et al.*, 1967; Schlenk, 1965) to study the synthesis and properties of triglycerides. Later on **1** was isolated from fungi (Kato *et al.*, 1969) showing antitumour activity. To the best of our knowledge, this is the first report of the occurrence of α-palmitin from plant sources. We have recorded the spectral data of **1** and its derivative **1a** to substantiate its structure. In its ^{1}H NMR spectrum there were, a triplet (*J* = 6.0 Hz) at δ 0.87, a broad singlet at δ 1.25 followed by two triplets (*J* = 6.0 Hz) at δ 1.62 and 2.34 indicating that **1** contains a straight chain of an aliphatic acid. It also showed three down field signals, a 2H double doublet at δ 3.62 (*J* = 6.0, 10.0 Hz), a 1H pentet at δ 3.92 (*J* = 5.0 Hz) and another 2H double doublet at δ 4.16 (*J* = 6.0, 10.0 Hz). The irradiation of the pentet at δ 3.92 allowed the collapse of both the double doublets into the respective doublets. These data clearly established that **1** is a 1,2,3-trioxygenated compound having an alkoxy side chain attached to one of these oxygenated carbons. Its ^{13}C NMR also showed two triplets at δ 63.77 and 65.52 and a doublet at δ 70.67. The characteristic signals for an alkoxy chain were also present (see Materials and Methods), which substantiated that **1** is an alkoxy triol. The acetylation of **1** gave **1a** having two singlets at δ 2.07 and 2.08 for two acetates. The doublet at δ 3.62 shifted to 4.15 and pentet at δ 3.92 shifted to 5.20. Whereas the doublet at δ 4.16 did not show remarkable shifting. The signals for alkoxy chain were also nearly same as in case of **1**. The mass spectrum of **1** showed a weak fragment of $[M]^+$ at *m/z* 330 followed by the fragment at *m/z* 312 for $[M\text{-}H_2O]^+$. These data clearly supported that **1** is 1-hexadecanoyl-propane-2,3-diol.



1, R = H, n = 11

1a, R = OAc, n = 11



2, n = 13
3, n = 18

The ^1H NMR of alkanes (**2**, **3**) showed the overlapping triplets at δ 0.88 for terminal methyls and a broad singlet at δ 1.26 for CH_2. The presence of no other signal than above in the spectrum clearly indicated it to be an alkane. The ^{13}C NMR also showed typical signals for a straight chain alkane at δ 14.15 (q), 22.72 (t), 32.0 (t) and overlapping triplets at δ 30.0 to 29.8. The GLC (Experimental) analysis confirmed that it was a mixture of two compounds (19:1). GC-MS of these alkanes clearly established that it was mixture of nonadecane (**2**) and tetracosane (**3**). The MS of **2** and **3** showed $[M]^+$ at *m/z* 338 for $C_{24}H_{50}$ and at *m/z* 268 for $C_{19}H_{40}$ with the clear pattern of fragments for n-alkanes.

Some of n-alkanes are the valuable components of important essential oils to enhancing tremendously the odour of the oil when present in low concentration. Nonadecane (**2**) and tetracosane (**3**) have also been reported to be present in the high priced essential oils like rose, orange, apple etc. On the other hand, **3** has also been found to be active as copulation releaser pheromone in female whitemarked tussock moth (*Orgyia leucostigma*). Similarly **2** is also a sex pheromone of the female fall cankerworm, *Alsophila pometaria*, male southern stinkbug, *Nezara viridula* and female tigermoth, *Holomelina lamae* (Anonymous, 1994). Therefore, a pheromone activity testing of these hydrocarbons from the seeds of *P. scrobiculatum* should be carried out with other common insects to make them commercially useful in insect control by biological means.

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