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A NEW DIHYDROXY ACID FROM THE OIL OF *PEGANUM HARMALA*

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Key Word Index—*Peganum harmala*; Rutaceae; seed oil; 9,14-dihydroxy octadecanoic acid.

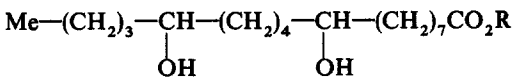
Abstract—The seed oil of *Peganum harmala* contains a previously unknown dihydroxy acid characterised as 9,14-dihydroxy octadecanoic acid. *P. harmala* is the first higher plant found to contain this non-vicinal diol acid (3.2%) in its triglycerides.

INTRODUCTION

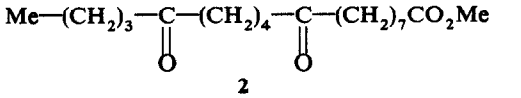
Peganum is a genus of herbaceous plants belonging to Rutaceae, a family of chemotaxonomic interest as Rutaceae plants are known to contain furanocoumarins and pentacyclic triterpenes [1]. Only vicinal dihydroxy acids have been reported [2–5] but a non-vicinal 9,14-dihydroxy octadeca-10,12-dienoic acid was reported as an artifact in *Aleurites fordii* seed oil [6]. This paper reports for the first time the occurrence of a new non-vicinal dihydroxy acid as a component of the seed fat glycerides. Structural details were elucidated through chromatographic techniques, IR, NMR, chemical degradation etc., and confirmed by MS studies.

RESULTS AND DISCUSSION

Results which were obtained in a continuing screening programme at this laboratory indicated a minor oxygenated fatty acid component in the seed oil of *Peganum harmala*. IR spectra of the oil, as well as its methyl ester, showed a hydroxyl band at 3350 cm^{-1}. TLC of the esters also revealed a component that was distinctly more polar than a monohydroxy ester standard. The R_f approximated that expected for dihydroxy esters. Partitioning of the total fatty acids in hexane–methanol yielded an acid, mp 89–90° (**1a**). Elemental analysis corresponded to the formula $C_{18}H_{36}O_4$ suggesting a dihydroxy compound. Attempted catalytic hydrogenation of **1a** resulted in the complete recovery of the starting material, indicating that **1a** is a saturated hydroxy acid. Reductive removal of the two hydroxyls in **1b** by hydrogen iodide–phosphorus resulted in a product which was identified unequivocally as methyl octadecanoate by mp, mmp, GLC and co-TLC with authentic methyl stearate. This established a normal C_{18} skeleton for **1a**. Boric acid co-TLC with standards of 9,10-dihydroxyoctadecanoic acid and 9-hydroxyoctadecanoic acid indicated that the dihydroxy acid was non-vicinal. The ester **1b** on acetylation gave a product whose IR spectrum showed two sharp bands at 1230 and 1020 cm^{-1}, thus confirming the presence of hydroxyl moiety. NMR spectrum of **1b**, mp 65–66° showed, besides the signals usually found in long chain fatty esters, a broad proton singlet at δ 2.1 (disappeared in D_2O) attributable to hydroxyl protons. The two methine protons associated with hydroxyl bearing carbons were observed at δ 3.37. The absence of a signal for olefinic protons in the region δ 5.4 indicated **1b** to be a saturated compound.



1a R = H
1b R = Me



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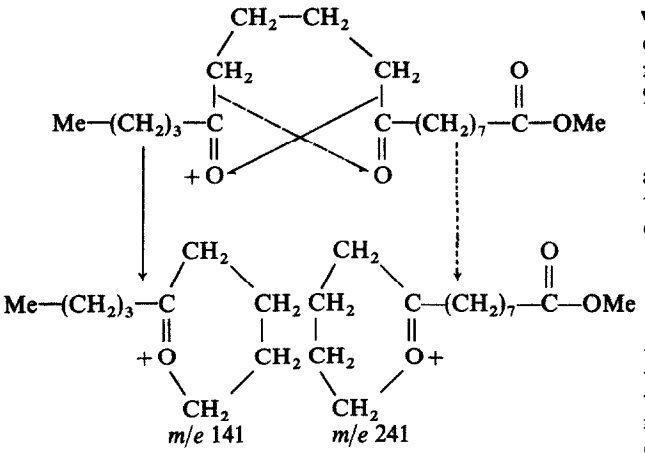
The positions of the two hydroxyls in the fatty acid chain were located by first converting **1b** to the dioxo ester **2** and then carrying out Beckmann rearrangement. The acidic fractions obtained from the hydrolysed products were isolated, methylated and identified by GLC. The cleavage products obtained, pentanoic acid and nonanedioic acid, placed the hydrolysis at C-9 and C-14 on a normal C_{18} skeleton.

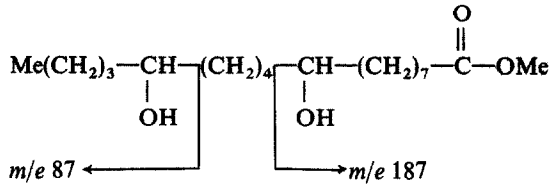
Confirmation of structure **1a**, based on the results of Beckmann rearrangement, was obtained by NMR and MS studies of **1b** and its two derivatives, the dioxo ester (**2**) and the di-TMS ether.

Jones oxidation of **1b** gave a dioxo derivative **2**. IR spectrum of **1b** gave a double carbonyl peak at 1740 and 1720 cm^{-1}. Its NMR spectrum gave the usual signals at δ 0.9 (*t*, terminal Me), 1.3 (*brs*, chain methylene) and 3.61 (*s*, ester methyl). More informative was a complex multiplet observed at δ 2.4 which integrated for ten protons. As keto groups are known to influence α-methylene protons which produce a signal similar to those protons adjacent to a carboxyl moiety, this signal was therefore attributed to eight protons α to the two oxo groups and two protons adjacent to an ester carbonyl. The MS of **1b** showed the characteristic ions expected for the 9,14-dihydroxy structure. Significant peaks were found at *m/e* 330 (M^+), 312, 281, 253, 187, 173, 155, 109, 87, 74 and 43 (base peak). The prominent peaks at *m/e* 187 and 87 support the 9,14-dihydroxy structure as these fragments would arise by the α-cleavage at the sites



Scheme 1.

of hydroxyl functions as shown below:



The MS of **2** had the molecular ion peak at *m/e* 326 and other ions at *m/e* 185 $[\overset{+}{O}{\equiv}C{-}(CH_2)_7CO_2CH_3]$, *m/e* 169 $[Me{-}(CH_2)_3{-}\underset{\underset{O}{\|}}{C}{-}(CH_2)_4{-}C{\equiv}\overset{+}{O}]$ and *m/e* 85 $[Me(CH_2)_3{-}C{\equiv}\overset{+}{O}]$ attributed to the α-cleavages at the C-9 and C-14 oxo groups. The fragment ions at *m/e* 241 and 141 of low intensity were also observed. The geneses these ions may be explained as shown in Scheme 1.

The ions at *m/e* 241, 185, 141 and 85 clearly established the positions of the oxo groups at C-9 and C-14. The other significant ions at *m/e* 242, 200 and 184, 100 (of low intensity) may be attributed to an additional mode of fragmentation occurring in keto esters [7] where these ions could arise from McLafferty rearrangement of γ-hydrogens on either side of carbonyl functions in the chain. Thus the MS of **2** is in full accord with the structure assigned to **1a** on chemical grounds.

Additional evidence to confirm the structure **1b** was also obtained by the MS study of its di-TMS ether derivative. Diagnostic ions were observed at *m/e* 459 [M—Me] and 443 [M—MeO]. An abundant ion at *m/e* 259 $[Me{-}\underset{\underset{+OTMS}{\|}}{C}{-}(CH_2)_7{-}CH{=}O]$ and a less prominent ion at *m/e* 159 $[Me(CH_2)_3{-}\underset{\underset{+OTMS}{\|}}{CH}]$ unequivocally established the positions of two hydroxyls at C-9 and C-14 of the fatty acid chain. The peaks corresponding to the trimethylsilyl ion, *m/e* 73, base peak $[Si^{+}Me_3]$ and the rearranged ion, *m/e* 75 $[HO^{+}{=}SiMe_2]$ were also found as previously reported [8] in the spectra of TMS derivatives. Another abundant peak was found at *m/e* 147 $[Me_3SiO^{+}{=}SiMe_2]$ which is known [7] to result from an intramolecular TMS rearrangement process that occurs when two TMS groups are at remote points in a long chain compound. The appearance of this TMS rearrangement ion was found to be in accordance with the structure 9,14-dihydroxyoctadecanoic acid (**1a**) assigned to the new non-vicinal diol acid.

GLC analysis of the silylated methyl esters on silicone and polyester columns showed the fatty acid composition to be 16:0 (10.1%); 18:0 (2.2%); 18:1 (27.4%); 18:2 (55.7%); 18:3 (1.3%) and 9,14-dihydroxystearic (3.2%).

EXPERIMENTAL

Preliminary analysis of peganum oil. Seed oil of *P. harmala* was obtained by an 18 hr Soxhlet extraction of the ground seeds with petrol (bp 40–60°); the yield was 15.6%. Mixed methyl esters were prepared by refluxing the oil (under N_2) with 1% H_2SO_4 in MeOH for 3 hr and were recovered by the usual Et_2O extraction. Both the oil and the esters exhibited hydroxyl band in the IR at 3350 cm^{-1}. TLC of the oil as well as the esters using Si gel G and developed in petrol–Et_2O (7:3) revealed two spots. The oil and seed characteristics were: IV = 130.0; SV = 187; Protein content (N × 6.25) = 12%, Moisture content = 6.9%. RI = 1.4850 n_D^{31}. Argentation TLC was effected on Si gel G impregnated with 12% $AgNO_3$; solvent system Et_2O–petrol (2:23) used for development. Three distinct spots for monoene, diene and triene and a slow moving spot, were visualized by spraying with 2′,7′-dichlorofluorescein and viewing under UV light.

Isolation of hydroxy acid **1a**. The hydroxy acid fraction was concd in 80% aq. MeOH by a three-funnel-six-withdrawal distribution against hexane as stationary solvent. This concentrate on crystallization in petrol–Me_2CO (1:1) yielded a TLC homogeneous product, **1a**, mp 89–90°. Anal, found: C, 69.37; H, 11.48, requires: C, 68.85; H, 11.4. Treatment of **1a** with CH_2N_2 furnished the ester, **1b**, mp 65–66°. The volatile di-TMS ether (**4**) derivative was prepared by treating **1b** with hexamethyldisilazane and trimethyl chlorosilane [9].

Characterization of compound **1b**. Ester **1b** had IR maxima at 3350 cm^{-1} (OH). Acetylation of **1b** with Ac_2O–C_5H_5N gave a product which showed two absorption bands at 1230 and 1020 cm^{-1}. A 50 mg portion of **1b** was hydrogenated using 10%, Pd-C in glacial acetic acid. Work up yielded **1b** unchanged.

Boric acid TLC of compound **1a**. Co-TLC analysis of **1a** with authentic 9-hydroxy and 9,10-dihydroxy stearic acids was done on Si gel G impregnated with boric acid [2]. On developing the plate in hexane–Et_2O [7:3] and subsequent charring with CrO_3–H_2SO_4, three distinct spots of R_f 0.08, 0.28 and 0.44 were observed. Acid **1a** migrated in between the two hydroxy acids.

Reductive removal of hydroxyl groups of **1b** [10]. Dihydroxy ester **1b** (85 mg) was refluxed 17 hr with red phosphorus (35 mg) and hydroiodic acid (3 ml). Et_2O extraction of the diluted mixture followed by washing with 5% Na metabisulphite gave an oily product (100 mg). This was reduced by heating at reflux 4 hr with granular Zn (200 mg), MeOH (5 ml) and HCl (1 ml). The usual work up of the mixture afforded a 75 mg of solid ester, mp 37.5°. GLC analysis and co-TLC indicated this material to be methyl stearate.

Positions of the hydroxyl groups in **1a** [11]. **1b** (50 mg) was dissolved in HOAc (1 ml) and oxidized at room temp. by CrO_3 (50 mg). After 1 hr H_2O (10 ml) was added, the excess of oxidant was destroyed by SO_2 and dioxo ester (**2**) (45 mg) was recovered. This was refluxed for 2 hr with hydroxylamine hydrochloride (85 mg) and fused Na acetate (80 mg) in 80% EtOH (2 ml). Usual work up afforded the oximes (45 mg).

Beckmann rearrangement. Oximes (45 mg) were heated to 100° with H_2SO_4 (0.2 ml) for 1 hr to effect Beckmann rearrangement. After cooling, H_2O (1 ml) was added, and the mixture boiled to hydrolyse the amides. The resulting monobasic acid was extracted with petrol and dibasic acid subsequently with Et_2O. After methylation with CH_2N_2, these were examined by GLC with appropriate standards and shown to be methyl valerate and methyl azelate.

General methods. All mp's are uncorr. NMR spectra were run in $CDCl_3$ at 60 MHz with TMS as internal standard, chemical shifts are expressed in ppm (δ). MS were measured using the direct-insertion probe at source temp. 140° and ionization energy 75 eV. GLC of the methyl esters were obtained using a stainless steel packed column (2 m × 3 mm) coated with 15% DEGS or a 60 cm × 4 mm column of 2% SE 30. The separations were carried out isothermally at 200°, chart speed 0.76 m/hr with a hydrogen flow of 70 ml/min.

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