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A NEW HYDROXYOLEFINIC ACID FROM *PLANTAGO MAJOR* SEED OIL

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Key Word Index *Plantago major*; Plantaginaceae; greater plantain; seed oil; 9-hydroxy-*cis*-11-octadecenoic acid.

Abstract—A new isomer of ricinoleic acid has been found as a minor constituent (1.5%) of the seed oil of *Plantago major*. This previously unknown β-hydroxyolefinic acid, 9-hydroxy-*cis*-11-octadecenoic, was characterized by IR, ^{1}H NMR and oxidative cleavage, and the structure was supported by MS.

INTRODUCTION

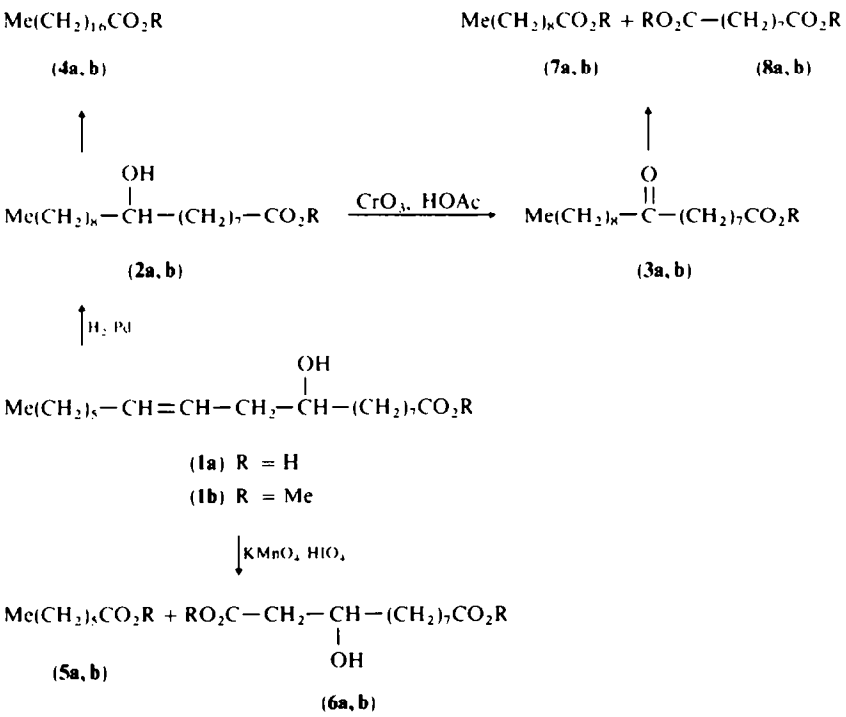
Hydroxyolefinic fatty acids have long been known to be seed oil constituents [1]. Those found have either a β-hydroxyolefinic (ricinoleic acid) or a γ-hydroxyolefinic (strophanthus acid) unsaturation. Previous work on *Plantago major* seed oil [2, 3] reported that it contained the usual fatty acids. On re-investigation of this oil, it was found to contain an unusual hydroxyolefinic acid. We now report a previously unknown hydroxyolefinic acid, 9-hydroxy-*cis*-11-octadecenoic acid, occurring as a minor component of the seed acylglycerols.

RESULTS AND DISCUSSION

P. major is a perennial herb grown in hilly areas and its seeds are used medicinally as a substitutes for isapaghul (*P. ovata*). During the course of a screening programme in this laboratory, a minor oxygenated fatty acid in the seed of *P. major* was detected. The IR spectrum of the oil, as well as that of its methyl ester, showed an OH band at 3500–3300 cm^{-1}, which is attributed to an OH group β to a double bond, due to the hydrogen bonding interaction between the OH group and the π electrons of the β-unsaturated centre [4]. TLC of the ester also revealed a component which is more polar than an ordinary non-oxygenated ester standard. The R_f approximated to that expected for an unsaturated monohydroxy ester. A concentrate of the hydroxy ester was obtained by preparative TLC. This was subsequently purified by column chromatography which yielded a brown viscous ester (**1b**). The IR spectrum showed no *trans* unsaturation. Elemental analysis corresponded to the molecular formula $C_{19}H_{36}O_3$ suggesting a monohydroxyolefinic compound. The ester (**1b**) on acetylation gave a product whose IR spectrum showed strong bands at 1235 and 1025 cm^{-1}. The ^{1}H NMR spectrum gave signals at δ 5.35 *m* (2H, $-CH=CH-$), 3.6 *s* (3H, $-COOCH_3$), 3.3 *br* (1H, $-CH-OH$), 2.75 (1H, $CH-OH$, disappeared on addition of D_2O), 2.2 (6H, $-CH_2-CH=CH-CH_2$ and $-CH_2-COOCH_3$), 1.3 *br s* (chain $-CH_2$) and 0.88 *t* (3H, $-CH_3$). Its acetate derivative showed no unusual features apart from the two expected, but significant, signals at δ 1.87 *s* (3H, $-OCOCH_3$) and 4.8 (1H, $CH-OAc$). The signals at δ 3.3 and 2.75 were not observed.

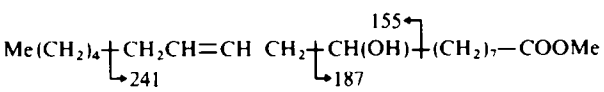
A few reactions were carried out (Scheme 1) to determine the structures of the acid. Catalytic hydrogenation of **1b** gave a solid compound, mp 53–54°, which showed no depression on a mmp determination with an authentic sample of methyl 9-hydroxyoctadecanoate (**2b**) obtained by the hydrogenation of isoricinoleic acid isolated from *Wrightia tinctoria* seed oil [5]. The hydrogenated acid (**2a**) had mp and mmp 81.5°. On chromic acid oxidation the saturated hydroxy acid (**2a**) yielded 9-ketostearic acid (**3a**), mp 80–81°, the IR spectrum of which showed a carbonyl band at 1715 cm^{-1}. Reductive removal of the OH group from the saturated ester (**2b**) by hydrogen iodide–phosphorus followed by zinc and HCl resulted in the formation of a product which was identified unequivocally as Me octadecanoate (**4b**) by mp, mmp and GC with an authentic sample. This indicated a normal C_{18} skeleton for **1a**. The position of the double bond in **1a** was established by permanganate–periodate cleavage [6]. The acidic fragments after methylation were examined by GC, which showed the presence of Me heptanoate (**5b**) by comparison of R_t with that of an authentic sample. This identification showed that the double bond was in the C-11 position, and that the OH group was present between the double bond and the carboxyl group. Further evidence for the position of the OH in the fatty acid chain was established by carrying out a Beckmann rearrangement of the oxime of 9-ketostearic acid (**3a**). The acidic fractions obtained from the hydrolysed products were isolated, methylated and identified by GC as Me-decanoate (**7b**) and diMe-nonanedioate (**8b**), placing the OH group at the C-9 position on a *n*-C_{18} skeleton. All these data were consistent with the presence of the double bond at C-11 and the OH group at C-9 in the *P. major* fatty acid.

GC analysis of the TMSi Me esters on silicone and polyester columns showed the fatty acid composition to be: 10:0 (3.5%); 12:0 (5.0%); 14:0 (5.1%); 16:0 (40.0%); 16:1 (3.1%); 18:0 (10.8%); 18:1 (14.1%); 18:2 (10.7%); 18:3 (6.1%) and 9-hydroxy-*cis*-11-octadecenoic acid

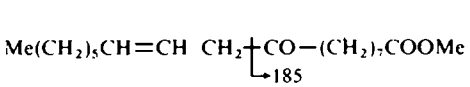


Scheme 1.

(1.5%). The structure of the hydroxy acid (**1a**) was further confirmed by the MS of its Me ester (**1b**). The MS was found to be useful in confirming the proposed structure. Significants peaks were observed at *m/e* 312 ($C_{19}H_{36}O_3$, M^+), 311, 310, 294 ($M^+ - 18$), 281 ($M^+ - 31$), 241, 240, 201, 199, 187, 185, 155 (base peak, $C_9H_{15}O_2$), 138, 127, 115 and other low mass peaks. Some of the assignments are supported by accurate mass measurements. The peak at *m/e* 241, although not very intense, is significant as it results from allylic cleavage and suggests a Δ^{11}-double bond. The ion *m/e* 187 is the second most intense peak and



helps to locate the OH at C-9 and the double bond at Δ^{11}. The alternative cleavage α to CHOH would produce a fragment ($C_{10}H_{19}O$) of *m/e* 155 but the significant 155 fragment is $C_9H_{15}O_2$ and results from the 187 fragment by loss of 32 amu units (CH_3OH). The exclusive C-9 to C-10 cleavage is due to the Δ^{11}-double bond. The M^+ possibly loses one (*m/e* 311) and then a second hydrogen atom (*m/e* 310) to furnish a keto ester. This undergoes cleavage that is both α to the keto function and allylic to produce the fragment *m/e* 185. There is no significant 153 peak resulting from the alternative α-cleavage again showing the strong influence of the double bond in promoting allylic cleavage.



EXPERIMENTAL

Preliminary analysis. Seed oil was obtained from ground seeds of *P. major* by Soxhlet extraction with petrol (40–60°); the yield was 14%. Mild saponification of the oil was accomplished by allowing a mixture of the oil with M KOH in EtOH to stand at room temp. for 24 hr. The soln was diluted with H_2O, and the unsaponifiables were extracted with Et_2O. The soap soln was cooled to 0°, acidified with ice-cold 3N HCl, and the liberated organic acids extracted with Et_2O. After the usual work-up, the mixed fatty acids were subjected to methylation (acid-catalysed esterification with MeOH). TLC of the oil, as well as that of the esters, on Si gel G plates with petrol–Et_2O (7:3) gave two spots. The oil and seed characteristics were: IV = 55.2; SV = 257.9; protein content (N × 6.25) = 26.2%; moisture content = 3.8%; RI = 1.4765 n_D^{30}. Argentation TLC was effected on Si gel G impregnated with 20% $AgNO_3$. The system petrol–Et_2O (23:2) was used for development of the esters. Spots of saturates, monoene, diene and triene and a slow moving spot similar to methyl isoricinoleate were visualized by spraying with 2′,7′-dichlorofluorecein and viewing under UV.

Isolation of hydroxy ester (**1b**). The ester was concd by prep. TLC (petrol–Et_2O; 4:1) and the concentrate, further purified by column chromatography using petrol–Et_2O (19:1), yielded a TLC homogeneous product **1b**. 0.05 g of **1b** when heated under reflux with 0.8N KOH in EtOH (1 hr), followed by usual work-up, afforded the hydroxy acid (**1a**) as an oil (0.047 g). (Found: C, 72.38; H, 11.31. Calc. for $C_{18}H_{34}O_3$: C, 72.48; H, 11.41%). IR (CCl_4) cm^{-1}. 3450–3200 (OH and COOH), 1710 (COOH).

Characterization of **1b**. Ester 1b had IR maxima at 3500–3300 cm^{-1} (OH). Acetylation of **1b** (60 mg) with Ac_2O–pyridine gave a product which showed strong bands at 1730 (COOMe and O—CO—Me), 1235 and 1025 cm^{-1} (acetate). A portion (200 mg) of **1b** was hydrogenated, using 10%

Pd-C in EtoAc (2 ml), to give Me 9-hydroxystearate (**2b**), as a white solid (176 mg), mp, mmp 52–53°. (Found: C, 72.50; H, 11.97. Calc. for $C_{19}H_{38}O_3$: C, 72.61; H, 12.10%). IR (CCl_4) cm^{-1}: 3448 (OH) 1735 (COOMe). Its acid (**2a**) had mp and mmp 81.5°.

Reductive removal of OH *group* (**2b**) [7]. Me 9-hydroxystearate (**2b**) (40 mg) was refluxed 17 hr with red P (18 mg) and HI (1.2 ml). Et_2O extraction of the diluted mixture followed by washing with 5% $NaHSO_3$ gave an oily product (42 mg). This was reduced by refluxing for 4 hr with granular Zn (100 mg), MeOH (2.3 ml) and HCl (0.45 ml). Usual work-up of the mixture afforded 13 mg of a solid ester (**4b**), mp 35°. GC analysis and co-TLC indicated this material to be Me octadecanoate (**4b**).

Position of double bond in **1a** [6]. **1a** (100 mg), K_2CO_3 (125 mg) and *t*-BuOH (40 ml) were treated with a soln of $NaIO_3$ (400 mg) in 40 ml H_2O and $KMnO_4$ (1.2 ml of 0.057 M soln). The mixture was stirred at room temp. for 24 hr, reduced with $NaHSO_3$, acidified with HCl and extracted with Et_2O. The Et_2O soln after usual work up gave a semi-solid which was treated with CH_2N_2–Et_2O soln and then subjected to GC. GC analysis showed one component to be Me heptanoate (**5b**) and the other could be the β-hydroxy diester (**6b**). The identity of **5b** was confirmed by comparison of the R_f value with that of an authentic sample of Me heptanoate.

Position of the OH *group in* **2b** [4]. **2b** (75 mg) was dissolved in HOAc (1.5 ml) and oxidized at room temp. with CrO_3 (75 mg). After 1 hr, H_2O (15 ml) was added, excess oxidant destroyed by SO_2 and the keto ester (**3b**) (68 mg) was recovered, mp and mmp 80–81°. IR (CCl_4) cm^{-1}: 1715 (CO). **3b** (50 mg) was refluxed for 2 hr with hydroxylamine HCl (90 mg) and fused NaOAc (85 mg) in 80% EtOH (2.5 ml). Usual work up afforded the oximes (50 mg).

Beckmann rearrangement of oximes. The oxime (45 mg) was heated to 100° with H_2SO_4 (0.2 ml) for 1 hr. 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 the dibasic acid subsequently with Et_2O. After methylation with CH_2N_2, these were examined by GC with appropriate standards and shown to be Me decanoate (**7b**) and diMe nonanedioate (**8b**).

General methods. All mps are uncorr. IR spectra were measured as liquid films or 1% solns in CCl_4. ^1H NMR spectra were run in $CDCl_3$ at 60 MHz with TMS as int. standard; chemical shifts are expressed in ppm (δ). MS were measured using the direct-insertion probe at a source temp. of 140° and an ionization energy of 75 eV. GC of Me esters were obtained using a stainless steel packed column (2 m × 3 mm) coated with DEGS (15% on Chromosorb W) or a 60 cm × 4 mm column of SE 30 (2%). The separations were carried out isothermally at 200° using FID.

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