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9-HYDROXYDODECANOIC ACID, AN ACID FROM *BLEPHARIS SINDICA* SEED OIL

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Key Word Index—*Blepharis sindica*; Acanthaceae; seed oil; 9-hydroxydodecanoic acid.

Abstract—A hitherto unknown hydroxy acid has been isolated from *Blepharis sindica* seed oil and has been characterized as 9-hydroxydodecanoic acid by IR, NMR and mass spectral studies. The structure of this acid was further supported by its chemical transformations.

INTRODUCTION

Published information concerning hydroxy fatty acids from plants has been reviewed by Downing [1]. The occurrence of short chain hydroxy fatty acids in seed oils is a rare phenomenon. However, the only seed oil of the Proteaceae to contain a hydroxy acid (3.8% monohydroxymyristic acid) is the seed fat of *Embothrium coccinium* [2]. Other short chain hydroxy fatty acids, containing one or more hydroxyl groups bound glycosidically to sugar moieties, have been reported in the seed oils of the Convolvulaceae [3, 4].

In our search for new natural fatty acids reported [5–7] earlier from this laboratory, the seed oil of *Blepharis sindica*, not so far examined, was found to contain an oxygenated fatty acid as a minor constituent. In this paper we report the isolation and proof of the structure of a new short chain acid, 9-hydroxydodecanoic acid, present in seed oil triacylglycerols.

RESULTS AND DISCUSSION

Preliminary TLC analysis of the seed oil, as well as that of its methyl esters, indicated the presence of an oxygenated acid. This was confirmed by a strong hydroxyl absorption near 3400 cm^{-1} in the IR spectra of the oil and its esters. The R_f value observed on TLC of the oxygenated ester approximated to that expected for a monohydroxy ester. Partitioning of the total fatty acids in hexane–methanol yielded an acid, mp 46–47° (**1a**). The molecular composition ($C_{12}H_{24}O_3$) of **1a** suggested a monohydroxy compound. Compound **1a** on catalytic hydrogenation remained unchanged (mp 46–47°) indicating that it is a saturated acid. Reductive removal of the hydroxyl of **1b** by hydrogen iodide–phosphorus [8] yielded a saturated methyl ester. Hydrolysis gave an acid, mp 43–44°, characterized as dodedecanoic acid by mmp and co-TLC with authentic samples as well as by GC. These data established a normal C_{12} skeleton for the acid. The acetylated product of **1b** showed two sharp IR bands at 1230 and 1020 cm^{-1}, thus confirming the presence of a hydroxyl moiety. The ^1H NMR spectrum of **1b** showed signals at δ 0.9 (*t*, terminal Me), 1.3 (*br s*, chain CH_2), 3.66 (*s*, ester Me), 3.4 (1H, *br m*, CHOH) and 2.1 (1H, *s*, OH, D_2O exchangeable). The absence of a signal at δ 5.3 (olefinic protons) indicated **1b** to be a saturated compound.

Me —— $(CH_2)_2$ —— CH(OH) —— $(CH_2)_7$ —— CO_2R

1a R = H
1b R = Me

Me —— $(CH_2)_2$ —— C(=O) —— $(CH_2)_7$ —— CO_2Me

2

Jones oxidation of **1b** gave an oxo-derivative, **2**. The IR spectrum of **2** gave a double carbonyl peak at 1740 and 1720 cm^{-1}. Its ^1H NMR spectrum gave, besides the usual signals found in long chain esters, a complex multiplet at δ 2.4 ($-CH_2COCH_2, CH_2COOMe$).

Oxidative degradation of **1b** with potassium permanganate in acetic acid [9] followed by GC of the methylated product showed the formation of methyl butyrate and methyl 1,9-nonanedioate which supported the position of the hydroxyl group at C-9.

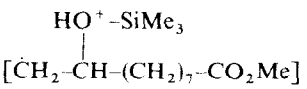
Further confirmation of structure **1b** comes from the study of the mass spectrum of its trimethylsilyl derivative [10]. The spectrum of **3** had the $[M+1]^+$ peak at *m/z* 303. This indicated that the hydroxyl group is attached at the C-9 position in a fatty acid chain of 12 carbon atoms. The α-cleavage on either side of the TMSi group gave the diagnostic mass ions at *m/z* 259 and 145 indicative of the position of the hydroxyl group. Other significant ions were *m/z* 274 $[M-28]^+$, 271 $[M-31]^+$, 260, 259 $[M-43]^+$, 215 $[M-87]^+$, 213 $[M-89]^+$, 201, 187, 155,

m/z 145 ←

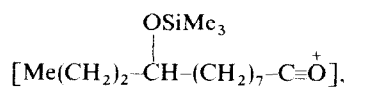
Me $(CH_2)_2$ — CH(OTMS) — $(CH_2)_7CO_2Me$ → *m/z* 259

3

145, 129, 103, 75 and 73 (base peak). The peaks at *m/z* 73 (base peak) and 75 are due to the trimethylsilyl ion $[SiMe_3]^+$ and its rearranged product, as previously reported [11], in the spectra of TMSi derivatives. The low intensity fragment ions at *m/z* 274 and 271 are attributed to



and



respectively.

GC of the silylated methyl esters from *Blepharis* oil both on silicone and polyester columns showed the fatty acid composition to be: 10:0 (1.3%), 12:0 (0.9%), 14:0 (1.6%), 16:0 (15.6%), 18:0 (1.7%), 18:1 (65.7%), 18:2 (5.9%) and hydroxy acid (7.3%).

EXPERIMENTAL

General. All mps are uncorr. ^{1}H NMR spectra were measured in $CDCl_3$ at 60 MHz with TMS as int. standard; chemical shifts are expressed in δ-values. MS were measured using the direct insertion probe at source temp. 140° and ionization energy 75 eV. GC of the Me 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. Separations were carried out isothermally at 200° with a H_2 flow of 70 ml/min.

Preliminary analysis of oil. Oil was extracted from ground seeds with petrol (bp 40–60°); the yield was 8.2%. Mixed Me esters were prepared by NaOMe transesterification [12]. The IR spectrum of the oil as well as that of the Me esters exhibited a OH band at 3400 cm^{-1}. Both the oil and its Me esters, on analytical TLC using Si gel G developed in petrol–Et_2O (7:3), revealed two spots. The oil and seed characteristics were IV (Wijs) = 69.4; SV = 162.3; protein content (N × 6.25) = 8.17%; moisture content = 7.2%; n_D^{30} = 1.4790. Argentation TLC was carried on Si gel G containing 10% $AgNO_3$; solvent system Et_2O–petrol (2:23). This revealed distinct spots for satd monoenoic and dienoic esters, and a slow moving spot for the hydroxy ester.

Isolation of the hydroxy acid **1a**. The acid was separated by the procedure of ref. [13]. The crude acid on crystallization from petrol–Me_2CO (1:1) yielded a TLC homogeneous product, **1a**, mp 46–47°. (Found: C, 66.82; H, 11.25; $C_{12}H_{24}O_3$ requires: C, 66.65; H, 11.18%.) Treatment of **1a** with CH_2N_2 furnished the Me ester, **1b**. The volatile mono-TMSi ether derivative was prepared by treating **1b** with hexamethyldisilazane and trimethyl chlorosilane [10]. MS (75 eV), *m/z* (rel. int.): 303 $[M+1]^+$ (2.9), 274 $[M-28]^+$ (3.6), 271 $[M-31]^+$ (5.0), 260 (7.0), 259 $[M-43]^+$ (31.9), 215 $[M-87]^+$ (21.7), 213 $[M-89]^+$ (32.8), 201 (11.0), 187 (6.5), 155 (20.5), 145 (28.2), 129 (16.4), 103 (20.7), 75 (25.0) and 73 (100.0).

Characterization of **1b**. Acetylation of **1b** (45 mg) was carried out with Ac_2O–pyridine. A 50 mg portion of **1b** was hydrogenated using 10% Pd–C in HOAc. Work-up yielded **1b** unchanged.

Reduction of **1b** [7]. Monohydroxy ester **1b** (85 mg) was refluxed for 17 hr with red P (35 mg) and HCl (3 ml). Et_2O extraction of the dilute mixture, followed by washing with 5% sodium metabisulphite gave an oily product (100 mg). This was reduced by refluxing for 4 hr with granular Zn (200 mg), MeOH (5 ml) and HCl(1 ml). Usual work-up of the mixture afforded 75 mg Me dodecanoate which on saponification gave dodecanoic acid (mp 43–44°, lit. 43.5°). GC and co-TLC also indicated this acid to be dodecanoic acid.

Position of the OH group in **1a** [9]. Oxidative degradation of **1a** (0.1 g) with $KMnO_4$ in HOAc gave a mixture of monobasic and dibasic acids. After methylation with CH_2N_2, these were examined by GC and shown to be Me butyrate and Me 1,9-nonanedioate which conclusively indicated the position of the OH group at C-9.

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