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A KETO FATTY ACID FROM *SMILAX MACROPHYLLA* SEED OIL

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Key Word Index—*Smilax macrophylla*; Liliaceae; seed oil; keto fatty acid; 9-keto-octadec-*cis*-13-enoic acid.

Abstract—A hitherto unknown keto fatty acid, 9-keto-octadec-*cis*-13-enoic acid, has been isolated from *Smilax macrophylla* seed oil in appreciable amounts (26.03%). Its identification was based on chemical and spectroscopic evidence.

INTRODUCTION

In continuation of our research work on keto fatty acids [1, 2], it was found that seed oil of *Smilax macrophylla* contains a novel keto fatty acid (26.03%) as a component of its seed fat triacylglycerols. *Smilax macrophylla* is a large climber. In some parts of India, roots of this plant are used as a substitute for sarsaparilla in the treatment of venereal diseases. The roots are also used in the treatment of dysentery and urinary complaints [3].

RESULTS AND DISCUSSION

Smilax macrophylla seed oil responded to the DNPH test [4], indicating the presence of a keto group. The IR spectrum of the corresponding methyl esters exhibited characteristic double carbonyl peaks at 1740 cm^{-1}

$$\underset{\text{(ester }-\overset{\overset{\|}{O}}{C}-\text{)}}{} \text{ and } 1705\ cm^{-1} \underset{\text{(chain }-\overset{\overset{\|}{O}}{C}-\text{)}}{}.$$

The IR spectrum also showed bands at 715 and 1620 cm^{-1} for *cis*-double bonds. However, IR and UV spectra of the oil showed no evidence of *trans*-conjugation. The ^1H NMR spectrum of the isolated methyl ester of the keto acid exhibited a multiplet at δ 5.38 (2H, –CH=CH–), a second multiplet at δ 2.25 (2H, $-CH_2-CO_2^-$) and a third multiplet at δ 2.10

$$\underset{\text{(4H, }-\overset{\overset{\|}{O}}{C}-CH_2-\text{, }-CH_2-\overset{\overset{\|}{O}}{C}-\text{)}}{},$$

apart from the usual proton signals.

The acid on reduction with Pd–C furnished 9-keto-octadecanoic acid (mp 81–82°); its methyl ester had mp 43–44°. Oxidation [5] of the unsaturated acid with $KMnO–NaIO_4$ in *tert*-butanol, gave pentanoic acid (bp 185–186°) (*p*-bromophenacyl ester, mp 75–76°). However, azelaic acid (mp 105–106°) (*p*-bromophenacyl ester, mp 130–131°) was also obtained during the degradation. There was no depression in the mixed melting point of the *p*-bromophenacyl ester prepared from authentic pentanoic acid and the ester of the pentanoic acid obtained from the degradation. Furthermore, both esters had the same R_f value. The mixed melting point of the C_5 and C_6 acid was 71–73°. The *p*-bromophenacyl esters of the C_5, C_6 and C_7 acids melting points 75°, 72° and 72°, respectively [6]. The methyl pentanoate had the same GC R_t as that of an authentic sample.

The structure of the keto acid was further supported by its mass spectrum, which gave a $[M]^+$ at *m/z* 310 (3.7%) and ions at *m/z* 153 (6%) and 185 (12%); α-cleavage fragments on either side of the keto group were observed at *m/z* 168 (6%) and 200 (12%) (McLafferty cleavage ions on both sides of the keto group). These four ions thus locate the keto group at C-9. Furthermore, an allylic cleavage at *m/z* 267 (84%) and 97 (12%) was observed. The other important ions were observed at *m/z* 154 (12%), 139 (24%), 261 (60%), 290 (42%), 92 (24%), 95 (26%), 51 (42%), 64 (30%) and 71 (base peak). All these observations showed that the original acid is 9-keto-octadec-*cis*-13-enoic acid (**1**).

EXPERIMENTAL

General. IR: 1% CCl_4 soln. ^1H NMR: 90 MHz in $CDCl_3$ with TMS as int. standard. Chemical shifts were measured in δ downfield from TMS. MS were obtained by GC-MS at 70 eV, source temp. 150°. GC was carried

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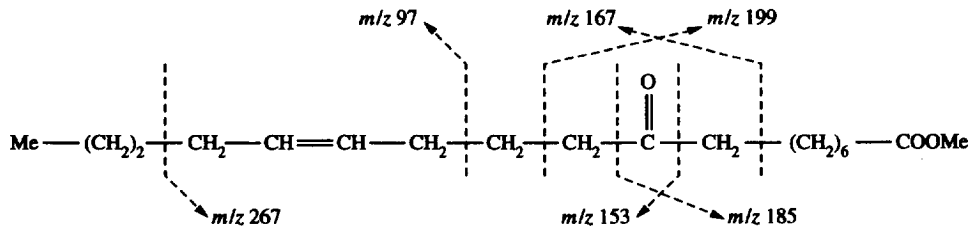

Scheme 1. Mass spectral fragmentation of the methyl ester of compound **1**.

out using a column of 15% DEGS with temp. of injector, detector and oven of 240°, 240° and 190°, respectively. N_2 flow was 30 ml min^{-1}.

Extraction and isolation. Air-dried seeds were extracted with petrol. Analyt. values were determined according to AOCS methods [7] and are listed in Table 1. The oil did not respond to the Halphen [8] and picric acid TLC [9] tests, indicating the absence of cyclopropenoid and epoxy fatty acids, respectively. However, the oil responded to the DNPH test [4], showing the presence of a keto group. Me esters were prepd by refluxing the oil in MeOH in an acidic medium. Saponification of oil was carried out by stirring overnight with 0.8 M alcoholic KOH. Non-saponifiable matter was removed by extracting with Et_2O. The mixed fatty acids were partitioned according to method of ref. [10] between petrol and 80% MeOH. A sample of pure keto acid was obtained by prep. TLC.

Identification of keto acid (**1**). Analysis, C 73.35% (required 73.52%), H 11.05% (required 11.03%), molecular formula $C_{19}H_{34}O_3$. IR: 1740 cm^{-1} ($-CO_2Me$) and 1705 cm^{-1} (chain –CO–), *cis*-double bond absorption at 715 and 1620 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.90 *s* (3H, Me), 1.28 *br s* (16H, $-CH_2-$chain), 1.95 *m* (4H, $-CH_2-C{=}C-CH_2$), 2.10 *m*

$$\begin{matrix} & O & \\ & \| & \\ (4H,\ -CH_2-&C-&CH_2), \end{matrix}$$

2.25 *m* (2H, CH_2-CO_2), 3.75 *s* (3H, OMe), 5.38 *m* (2H, –CH=CH–). MS: *m/z* 310 $[M]^+$, see Scheme 1. Hydrogenation was carried out using 10% Pd–C in EtOH (5 ml) to give 9-keto-octadecanoic acid (mp 81–82°). ^1H NMR: δ 0.90 (3H, Me). 2.10 (4H, $-CH_2-CO-CH_2-$), 2.25 (2H, CH_2-CO_2), 3.75 (3H, OMe). MS *m/z* 312 $[M]^+$, α-cleavage fragments *m/z* 153 and 185.

Table 1. Analytical data and component fatty acids of *Smilax macrophylla* seed oil

Oil content in seeds	9.3%
Unsaponifiable matter	2.36%
Saponification value	200.66
Iodine value	62.3
2,4-DNPH TLC test	+ve
Picric acid TLC test	−ve
Halphen test	−ve
Fatty acids	*Percentage composition*
Myristic	15.23
Stearic	15.79
Oleic	42.95
9-Keto-octadec-*cis*-13-enoic	26.03

Position of double bond. Oxidation of the unsatd acid was carried out in *t*-BuOH (20 ml). A soln of acid (0.25%) was treated with a soln of $NaIO_4$ (200 mg) in 20 ml H_2O and $KMnO_4$ (1 ml) in the presence of K_2CO_3 (60 mg). The mixt. was stirred at room temp. for 24 hr and the soln then decolourized with $NaHSO_3$, followed by acidification with HCl. The fatty acids were extracted with Et_2O, the solvent removed and the acids treated with 1% H_2SO_4 in MeOH (20 ml). The mixt. was refluxed for 1 hr and then extracted with Et_2O. The extracts were dried (Na_2SO_4) and the solvent removed under red. pres. GC analysis was carried out using a column of 15% DEGS with temp. at injector, detector and oven of 240°, 240° and 190°, respectively. N_2 flow rate was 30 ml min^{-1}. Me pentanoate was obtained as one of the products and had the same R_t as that of an authentic sample.

Position of keto group. Hydrogenation and oxidation of the keto acid was carried out as described in the text. GC analysis of the product showed Me azeleate to be one of the products.

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