excitation beam (313 nm) was passed through a polarizing prism (Carl Zeiss) and a film polarizer was oriented parallel to the emission polarizer. The intensity of emitted fluorescence was measured at 420 nm. The slit width for both excitation and emission beams was 10 nm. The thermo-regulated cuvette was stirred and the temp monitored with a copper-constantan thermocouple. Measurements were made in both heating and cooling modes but the temp of the completion or onset of the transition did not differ by more than one degree in any sample.

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A NOVEL OXO FATTY ACID IN *PLANTAGO OVATA* SEED OIL

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Key Word Index—*Plantago ovata*; Plantaginaceae; seed oil; 9-oxooctadec-*cis*-12-enoic acid.

Abstract—*Plantago ovata* seed oil contains two oxygenated fatty acids one of which is the known 9-hydroxyoctadec-*cis*-12-enoic acid. The other is 9-oxoactadec-*cis*-12-enoic acid a new acid. The structural elucidation of this novel compound is described.

INTRODUCTION

The occurrence of keto fatty acids in natural seed oils is a rarity [1], although naturally occurring long chain hydroxy acids are widely distributed in plants [2] and microorganism [3]. *Plantago major* [4], a member of Plantaginaceae contains an unusual hydroxy fatty acid, 9-hydroxy-*cis*-11-octadecenoic, an isomer of ricinoleic acid. This prompted us to analyse another *Plantago* species, *P. ovata*, to examine the presence of any unusual fatty acid in its oil. An earlier report [5] on this seed oil indicated that it contained only the usual fatty acids. However, we identified isoricinoleic acid (**2a**) and its corresponding keto derivative (**1a**) having the same positions of double bond and oxygenated group. Identification of these two acids is described herein and is based upon spectral and chemical evidences.

RESULTS AND DISCUSSION

P. ovata oil responded to the DNP test [6], indicating the presence of an oxo function. The IR and UV spectra of the oil exhibited no absorption bands for the presence of

trans unsaturation and conjugation, respectively, in the component acids of the triacylglycerols. Direct TLC of the oil and its derived esters showed three distinct spots, two being of lower R_f value and the third moving to the solvent front. The higher iodine value of the oil than its theoretical value is consistent with the literature [7].

Crystallization of the mixed fatty acids and liberation of the solid and liquid acids from their mixed fatty acid lead salts was carried out by usual porcedures which led to the separation of saturated and unsaturated acids. Column chromatographic separation of the liquid portion furnished two oxygenated fractions (one separated with 7% ether, the other with 12% ether). The TLC pure component revealed the presence of a keto acid from the DNP test. Each oxygenated fraction was esterified with acidic methanol for chemical and spectral studies.

The IR spectrum of **1b** (7.4%) exhibited double carbonyl peaks, 1738 cm^{-1} (ester C=O) and 1717 cm^{-1} (chain C=0) and 1620 and 715 cm^{-1} (*cis* double bond) in the fatty ester. The ^1H NMR spectrum indicated an unsaturated keto ester (a multiplet at δ1.99 for $-CH_2-\underset{\underset{O}{\|}}{C}-CH_2-$ and CH_2CO_2- protons, another complex multiplet at δ2.11 for $-CH_2-C=C-CH_2-$ protons and a third multiplet at δ5.23 for $-CH=CH-$ protons) besides the usual fatty ester signals. It was not possible to measure the coupling constant of these olefinic protons, as is cited in the literature [8] for most isolated double bonds. However, mass spectrometry permitted structural elucidation sharing *m/z* 310 ($[M]^+$, 5.7%), 185 (32.1%) and 153 (14.3%) (α-cleavage ions on either side of the oxo function), 200 (8.9%) and 168 (9.9%) (McLafferty cleavage ions on both sides of the oxo function). These four ions locate the oxo group at C-9. The peak at *m/z* 110 (17.8%) is also structurally significant arising from allylic cleavage and places the double bond at C-12. Other important ions were observed at *m/z* 157 (3.5%), 154 (16.4%), 153 (13.9%), 129 (7.1%), 128 (32.1%), 125 (24.3%), 111 (16.0%), 97 (17.5%), 83 (42.8%), 69 (60.7%), and 55 (base peak). These collective spectral data thus suggested that the new oxo acid is mono unsaturated.

Further chemical evidence was obtained to support the identity of the new acid (**1a**). TLC pure, 9-hydroxyctadec-*cis*-12-enoic acid (isoricinoleic), isolated from *Wrightia tinctoria* seed oil, was oxidised using Jones reagent [9] to give 9-oxooctadec-*cis*-12-enoic acid. Its TLC mobility, IR and NMR spectra were identical to those of **1b**. The position of the double bond in **1a** was established by permanganate-periodate cleavage [10]. The acidic fragments after methylation were examined by GC, which showed the presence of methyl hexanoate by comparison of R_t with that of an authentic sample. This observation confirmed the position of the double bond at C-12. Thus, the structure of this new oxo acid is 9-oxooctadec-*cis*-12-enoic acid.

$$Me(CH_2)_4CH=CH(CH_2)_2-\underset{\underset{O}{\|}}{C}(CH_2)_7CO_2R$$

(1)

1a; R = H whereas 1b; R = Me

$$Me(CH_2)_4CH=CH(CH_2)_2\underset{\underset{OH}{|}}{CH}(CH_2)_7CO_2R$$

(2)

2a; R = H whereas 2b; R = Me

$$Me(CH_2)_4CH=CH(CH_2)_2\underset{\underset{OTMSi}{|}}{CH}(CH_2)_7CO_2Me$$

(3)

The IR and ^1H NMR spectra of **2b** (15.5%) indicated that the second oxygenated fraction is an unsaturated hydroxy compound. Further confirmation of its structure was obtained by converting it to its TMSi derivative (**3**). The mass spectram of **3** gave a peak at *m/z* 384 ($[M]^+$). Characteristic ions at *m/z* 259 (21.4%) and 227 (27.5%) appeared from the cleavage between C-9 and C-10 and C-8 and C-9. Moreover, the latter ion *m/z* 227 was also obtained from that at *m/z* 259 due to loss of methanol. The formation of these ions of *ca* equal intensity are characteristic when the TMSi group and the double bond are separated by two methylene groups [11]. Other important peaks were found at *m/z* 368 ($[M-15]^+$, 1.1%), 368 ($[M-CH_4]^+$, 6.1%), 353 ($[M-31]^+$, 0.7%), 294 ($[M-90]^+$, 39.9%), 273 ($[M-C_8H_{15}]^+$, 1.4%), 230 (TMSi transfer to C-9, 6.8%) and 73 ($[M-311]^+$, base). Thus, collective spectral and chemical evidences confirmed the structue of **2b** as methyl 9-hydroxyoctadec-*cis*-12-enoate.

EXPERIMENTAL

General. All mps are uncorr. NMR were run in $CDCl_3$ at 60 MHz with TMS as int. st. MS were measured at 70 eV. GC of silylated Me esters was carried out on a stainless steel column (2 m × 3 mm) packed with 15% DEGS or a 60 cm × 4 mm column of 2% SE 30 at 200°.

Preliminary analysis. Seed oil of *P. ovata* was obtained after 8 hr. Soxhlet extn of ground seeds with petrol (bp 40–60°), yield 8%. Me esters were prepared by refluxing the oil under N_2 with acidic MeOH and GC analysis as TMSi esters 16:0, 18.8%; 18:0, 4.0%; 18:1, 46.1%; 18:2, 8.1%; isoricinoleic, 15.5% and oxo acid, 7.4%. Determination of I_2 value = 82; sap. value = 196 and RI n_D^{30} 1.4815 were based on AOCS procedures [12]. Oxo esters were identified by running duplicate TLC samples in hexane–Et_2O (9:1) and spraying one half with 2,4-dinitrophenyl hydrazine, which gives yellow hydrazone spots without heating. Subsequent fuming with ammonia gave more easily detectable red brown keto spots and also revealed a hydroxy compound by the fluorescein technique.

The total fatty acids (5 g) dissolved in 25 ml EtOH were mixed with a boiling soln of $Pb(OAc)_2$ (3.5 g in 25 ml EtOH and 0.1 ml HOAc. Crystallization of the lead salt at 15° and liberation of solid acids (saturated acids) (~ 20%) and liquid acids (unsaturated acid) (~ 80%) was performed by usual methods.

Characterization of oxo component (**1b**). Analysis (Found: C, 73.00; H, 11.10; $C_{19}H_{34}O_3$ requires: C, 73.52; H, 11.03%); IR (nujol): 1738 (COOMe), 1717 ($\underset{\underset{O}{\|}}{C}$), 1660 and 715 cm^{-1} (*cis*, >C=C<); NMR, δ0.89 *dt* (3H, terminal Me), 1.29 *br s* (16 H, chain–CH_2–), 1.99 *m* (6 H, $-CH_2-CO-CH_2-$ and $-CH_2-CO_2$) 2.11 *m* (4 H, $-CH_2-CH=CH\ CH_2-$), 3.6 *s* (3H, $-CO_2Me$), 5.23 *m* (2 H, –CH=CH–). MS: *m/z* 310.

Characterization of hydroxy component (**2b**). Analysis (Found: C, 73.02; H, 11.4; $C_{19}H_{36}O_3$ requires: C, 73.07; H, 11.5%); IR (neat): 3600 (OH); 720 (*cis* >C=C<) cm^{-1}; NMR: δ0.88 *dt* (3H, terminal Me); 1.29 *br s* (20 H, chain CH_2–), 2.14 *m* (6 H, $-CH_2-CH=CH-CH_2-$ and $-CH_2-CO-$), 3.45 *m* (1H, –CHOH); 5.28 *m* (2H, –CH=CH–) and 6.05 *br s* (1H –CHOH–); MS (as TMSi derivative): *m/z* 384.

Hydrogenation. **2b** (200 mg) was hydrogenated using 10% Pd–C in EtOAc (2 ml) to give Me 9-hydroxystearate as a white solid (176 mg), mp, mmp, 52–53°. (Found: C, 72.45, H, 12.00,

$C_{19}H_{38}O_3$ requires: C, 72.61, H, 12.10%). IR (CCl_4): 3448 (OH), 1735 (COOMe) cm^{-1}. The corresponding acid had mp and mmp 81.5°.

Removal of the OH group [13] in Me 9-hydroxystearate (40 mg) was done as described earlier [14], which afforded 13 mg of a solid ester, mp 35°. GLC analysis and co-TLC indicated the material to be Me stearate.

Position of double bond in **1a** [10]. **1a** (50 mg), K_2CO_3 (63 mg) and *t*-BuOH (20 ml) were treated with a soln of $NaIO_3$ (200 mg) in 20 ml H_2O and $KMnO_4$ (0.6 ml of 0.057 M soln). The mixt. was stirred at room temp for 24 hr, reduced with $NaHSO_3$, acidified with HCl and extd 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. It showed one component, Me hexanoate and the other could be the γ-keto diester. The identity of the former was based on comparison of the R_t with that of an authentic sample.

Jones oxidation of isoricinoleic acid. **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 oxo ester (68 mg) recovered. This olefinic oxo ester was subjected to co-TLC, IR and NMR analysis with **1b**.

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VOLATILE MONO- AND SESQUITERPENEOIDS FROM *KLEINIA PENDULA*

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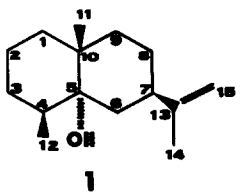
Key Word Index—*Kleinia pendula*; Compositae; essential oil; sesquiterpene; 4αH-eudesm-5α-ol.

Abstract—From the steam volatile oil of the aerial parts of *Kleinia pendula*, in addition to the known mono- and sesquiterpenoids, a biologically active sesquiterpene alcohol, 4αH-eudesm-5α-ol, was isolated. Its structure was determined by spectral analysis including 2D-NMR techniques.

INTRODUCTION

Several species of *Kleinia*, a genus generally found in tropical and sub-tropical regions, have been shown to be rich sources of oxygenated sesqui- and tri-terpenoids [1–4]. No chemical studies have been so far published on *Kleinia pendula* (Forsk) DC, a species widely distributed on the southern part of Somalia where it is known as Hadoli. The finely chopped aerial parts of the plant are



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