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NEW HYDROXY FATTY ACID FROM SEED OIL OF *BALIOSPERMUM AXILLARE*

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Key Word Index—*Baliospermum axillare*; Euphorbiaceae; seed oil; 11,13-dihydroxytetracos-*trans*-9-enoic acid.

Abstract—A fatty acid, found as a minor component in the seed oil of *Baliospermum axillare*, is shown to be the hitherto unknown 11,13-dihydroxytetracos-*trans*-9-enoic acid (axillarenic acid).

INTRODUCTION

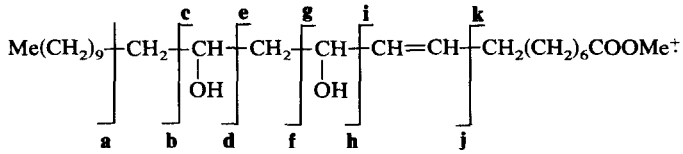
The seed oils of a relatively large number of species of Euphorbiaceae have been studied and it is already evident that unusual fatty acids occur in this family. Recent investigations have disclosed the occurrence of hydroxy acids with mono-, di- and tri-unsaturation (conjugated or non-conjugated) and without unsaturation.

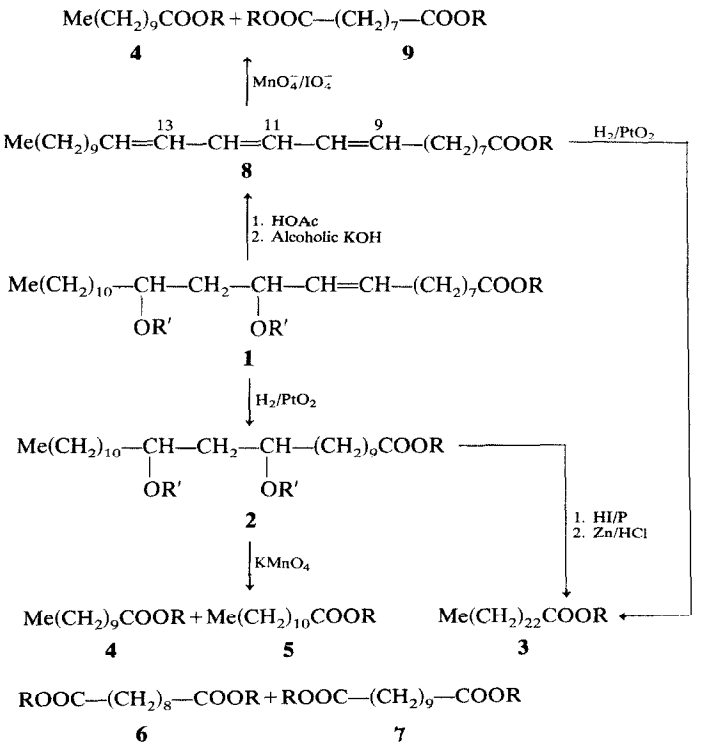
Baliospermum axillare [1] is a stout leafy undershrub, native to Dehra Dun (India) and grows in shady places along the sub-Himalyan forest tracts, where it often forms a considerable portion of the undergrowth. The seeds, which resemble those of the castor oil plant, but smaller, are used as a drastic purgative and are rich in oil. This paper reports the occurrence of a new non-vicinal dihydroxy monounsaturated acid as a component of the seed fat glycerides. This unusual acid, present to the extent of 2.8%, has been characterised as 11,13-dihydroxytetracos-*trans*-9-enoic acid by spectral and chemical methods.

RESULTS AND DISCUSSION

As a part of our screening programme on unusual fatty acid-containing seed oils, it was found that the oil of *Baliospermum axillare*, not previously examined, contains an oxygenated acid as a minor component. The UV spectrum of the oil, as well as that of its methyl esters, showed the absence of conjugation in the component acids. The oxygenated component, isolated as its methyl ester (**1b**) (Scheme 1), had a mp of 120–122°. Its IR spectrum showed hydroxyl absorption (3450 cm^{-1}) and a *trans* double bond (965 cm^{-1}). The iodine number of **1b** indicated the presence of one double bond and microanalysis corresponded to the formula $C_{25}H_{48}O_4$, suggesting that **1b** is a dihydroxy monoenoate. The ester **1b** on acetylation gave a product which showed two sharp bands at 1230 and 1020 cm^{-1} in its IR spectrum, thus confirming the presence of the hydroxyl group. The ^1H NMR spectrum of **1b** gave signals at δ 6.2 *d* (2H, $-C\underline{H}=C\underline{H}-$, $J = 15$ Hz), 3.6 *s* (3H, $COOC\underline{H}_3$), 3.4 *brm* (2H, unresolved multiplet, $2\times C\underline{H}-OH$), 2.2 *br* (2H, $2\times CH-O\underline{H}$, disappeared on addition of D_2O), 2.1 *br* (2H, $-\underset{H}{CH}-C\underline{H}_2-\underset{OH}{CH}$), 1.3 *brs* (chain-$C\underline{H}_2$), and 0.9 *t*-like (3H, terminal-$C\underline{H}_3$). After shaking with D_2O the signal at δ 2.2 disappeared with a small change in the signal at 3.4. The J value of the vinylic protons strongly suggests the *trans* configuration of the double bond. The MS of **1b** showed a M^+ at *m/e* 412 (4.8), 55 (base peak), and associated peaks at *m/e* 413(1.6, $M^+ + 1$), 411 (1, $M^+ - 1$), 381 (0.5, $M^+ - 31$), 380 (1, $M^+ - 32$), and 353 (1, $M^+ - 59$), corresponding to the loss of a COOMe group. Other significant peaks at *m/e* 271 (1.6, $M^+ - \mathbf{a}$), 257 (1, $M^+ - \mathbf{b}$), 255 (1, $M^+ - \mathbf{k}$), 229 (1.2, $M^+ - \mathbf{i}$), 227 (1, $M^+ - \mathbf{d}$), 213 (4.8, $M^+ - \mathbf{f}$), 199 (2, $M^+ - \mathbf{g}$), 185 (2, $M^+ - \mathbf{e}$), 183 (1, $M^+ - \mathbf{h}$), 157 (2.4, $M^+ - \mathbf{j}$), 155 (1, $M^+ - \mathbf{c}$) are considered to arise from the cleavages indicated below.

Confirmation of the position of the hydroxyl groups and the chain length was obtained after hydrogenation of **1b** to **2b** which analysed for $C_{25}H_{50}O_4$ and had an IR absorption at 3450 cm^{-1}(OH); IR showed no *trans* absorption. Reductive removal of two hydroxyls in **2b** by hydrogen iodide–phosphorus furnished **3b** which was identified as methyl tetracosanoate by mp, mmp, GLC and elemental analysis. This established a normal C_{24} skeleton for **1a**. Oxidation of **2b** by permanganate in acetic acid gave a mixture of monobasic (**4a**





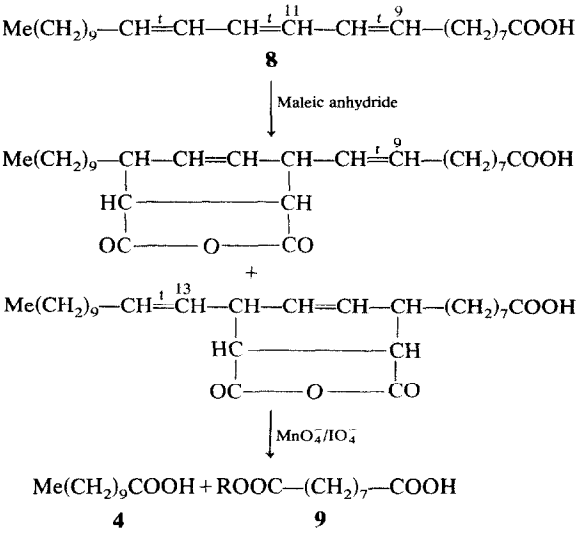
Where R = R′ = H (**1a** to **9a**)
R = CH_3, R′ = H (**1b** to **9b**)
R = CH_3; R′ = Ac (**1c**)

Scheme 1.

and **5a**) and dibasic (**6a** and **7a**) acids. The identified fragments were undecanoic, dodecanoic, decanedioic and undecanedioic esters. Hence the hydroxyl groups must have been on C-11 and C-13 in a normal C_{24} skeleton.

Heating of **1b** with acetic acid gave a solid acid **8a**, shown by spectral analysis to have a conjugated triene structure and no hydroxyl group. The acid **8a** analysed for $C_{24}H_{42}O_2$ and had an IR absorption at 990 cm^{-1} characteristic of the all-*trans* form. It consumed 3 mol of hydrogen to give **3a**. Oxidative cleavage of **8a** gave undecanoic (**4a**) and azelaic (**9a**) acids. This result confirms the position of the double bond at C-9 and the hydroxyls at C-11 and C-13. Because of the ease with which dehydration takes place, it is almost certain that one hydroxyl group is at C-11, where it is activated by the adjacent double bond at C-9; the second hydroxyl group is at C-13 which undergoes dehydration with the same readiness by the generated double bond at C-11. The all-*trans* structure was further confirmed by preparing its maleic anhydride adduct (Scheme 2). Two different adducts were formed in the reaction of **8a** with maleic anhydride. The IR spectrum of the adduct showed a strong band at 963 cm^{-1}, indicative of a *trans*-exocyclic double bond. Oxidative degradation of the adduct gave undecanoic (**4a**) and azelaic (**9a**) acids in substantial amounts, showing that addition of maleic anhydride had occurred at the 9,12 and 11,14 positions, forming two separate adducts.

On the basis of this physical and chemical evidence, the hydroxy acid (**1a**) was characterised as 11,13-dihydroxytetracos-*trans*-9-enoic acid for which the name axillarenic acid is proposed. GLC analysis of the silyated methyl esters on silicone and polyester columns showed the fatty acid composition to be 12:0 (4.8%); 14:0 (6.9%); 16:0 (45.4%); 16:1 (7.6%); 18:0 (4.2%); 18:1 (22.8%); 18:2 (5.5%) and 11,13-dihydroxytetracos-*trans*-9-enoic (2.8%).



Scheme 2.

EXPERIMENTAL

General methods. All mps are uncorr. IR spectra were run in CS_2 unless stated otherwise. UV measurements were made in cyclohexane sol. ^1H NMR was determined at 60 MHz in $CDCl_3$ soln containing 1% TMS. Chemical shifts are expressed in ppm (δ). MS were measured at 70 eV. GLC of Me esters was carried out as described in ref. [2].

Preliminary analysis of oil. Oil of *B. axillare* obtained by overnight extraction of the ground seeds with petrol (bp 40–60°) in a Soxhlet apparatus amounted to 33.4% (dry wt). The oil and seed characteristics were: IV (Wij's) = 47.8; SV = 166.4; $n_D^{40} = 1.5251$; protein (N × 6.25) = 38.5%; moisture content = 3.7%. Spectral characteristics of oil included a maximum at 3450 cm^{-1} in the IR, indicative of OH. UV showed no absorption. Mixed Me 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. TLC of the oil, as well as the esters, using Si gel G and development in petrol–Et_2O–HOAc (7:3:1) revealed distinct spots for oxygenated and non-oxygenated esters. Separation of the component esters on Si gel G impregnated with 12% $AgNO_3$ revealed distinct spots for saturated, monoenoic, dienoic esters, and a slow moving spot attributable to hydroxy ester. Spots were made visible by spraying with an aq. soln of perchloric acid (20%) and heating in an oven at 110° for 15 min. GLC of the Me esters was done after reaction with HMDS and TMCS [3].

Isolation of oxygenated ester **1b**. The mixed Me esters were fractionated by Si gel column chromatography. Elution with petrol–Et_2O (19:1) (15 ml fractions collected) gave the non-oxygenated esters and subsequent elution with petrol–Et_2O (17:3) gave the oxygenated ester. TLC-monitored fractions were combined and crystallized from petrol–Me_2CO (1:1) at 0° to yield pure hydroxy ester, **1b**, mp 120–122°. I_2 number determination indicated the presence of one double bond and the ester gave the following analysis: C, 72.69; H, 11.70; calc. for $C_{25}H_{48}O_4$: C, 72.76; H, 11.73%. Ester **1b** had IR maxima 3450 (OH), 1740 (COOMe), 1190, 1170, 1110, 1090 (C–O), and 965 cm^{-1} (*trans* olefin). Acetylation of **1b** with Ac_2O–C_5H_5N gave a product, **1c**, which showed IR absorption bands at 1230 and 1020 cm^{-1} (acetate).

Characterisation of **1b**. Ester **1b** (0.3 g) dissolved in EtOH (25 ml) was hydrogenated at 1 atm for 0.5 hr with platinum oxide catalyst. Filtration and evapn of the solvent gave the dihydro ester **2b** (0.285 g), mp 128–129°. (Found: C, 72.30; H, 11.98. Calc. for $C_{25}H_{50}O_4$: C, 72.41; H, 12.15%). **2b** (0.17 g) was refluxed for 17 hr with red P (0.07 g) and HI (6 ml) [4]. The mixture was diluted with H_2O and extracted with Et_2O. Combined Et_2O extracts were washed with 5% $NaHSO_3$ and dried (Na_2SO_4). The resulting product, obtained on evapn of the solvent, was reduced by refluxing for 4 hr with Zn (0.4 g), MeOH (10 ml) and HCl (2 ml). The usual work-up of the mixture afforded a solid ester **3b** (0.152 g), mp and mmp 59–60°. GLC analysis indicated this compound to be Me tetracosanoate. (Found: C, 78.34; H, 13.12. Calc for $C_{25}H_{50}O_2$: C, 78.47; H, 13.17%. Oxidative degradation of **2b** (0.1 g) with $KMnO_4$ in HOAc [5] gave a mixture of monobasic (**4a** and **5a**) and dibasic (**6a** and **7a**) acids. After methylation with CH_2N_2, these were examined by GLC and shown to be Me undecanoate, Me dodecanoate, Me decanedioate and Me undecanedioate.

Dehydration of **1b** *to* **8a**. Ester **1b** (1 g) was refluxed with HOAc (12 ml) for 4 hr; the solvent was removed under red. pres. and the residue was heated with N alcoholic KOH (20 ml) for 1 hr. The crude acid, on successive crystallisations from Me_2CO and EtOAc, afforded glistening pellets **8a** (0.92 g), mp 66–67°. (Found: C, 79.38; H, 11.47. Calc. for $C_{24}H_{42}O_2$: C, 79.50; H, 11.68%). It showed UV absorption maxima at 260, 270, 280 nm (all-*trans* conjugated triene). Ester **8b** had an IR maxima at 990 cm^{-1}, characteristic of the all-*trans* form. There was no OH absorption.

Characterisation of **8a**. Triene acid **8a** (0.1 g) on hydrogenation consumed 3 mol of H_2. Usual work-up afforded **3a** (0.086 g), mp and mmp 83–84.5°. (Found: C, 78.06; H, 13.11. Calc. for $C_{24}H_{48}O_2$: C, 78.19; H, 13.13%). **8a** (0.2 g) was added to maleic anhydride (0.2 g) in C_6H_6 (20 ml) and refluxed (under N_2) for 5 hr. Evapn the solvent gave the maleic adduct, which after several crystallizations from petrol–Et_2O (4:1) melted at 93–94°. (Found: C, 72.87; H, 9.51. Calc. for $C_{28}H_{44}O_5$: C, 73.0; H, 9.63%). The IR spectrum showed a small but distinct band at 963 cm^{-1} (*trans*-exocyclic double bond). The adduct was oxidized by $KMnO_4$–periodate [6] and the fragments were identified by GLC after methylation with CH_2N_2. Identified fragments were undecanoic (**4a**) and azelaic (**9a**) acids. There was some evidence of other compounds at longer R_t, possibly epoxide fragments. Oxidation of a portion of **8a** also gave **4a** and **9a** in *ca* equal amounts, together with small amounts of unidentified impurities.

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