Phytochemistry, Vol 23, No 10, pp 2247–2249, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

AN 8-HYDROXYOCTADECA-*CIS*-11,14-DIENOIC ACID FROM *MIRABILIS JALAPA* SEED OIL

MOHAMMAD SHAMIM AHMAD, ABDUL RAUF, JAMAL MUSTAFA and SHEIKH MOHAMMAD OSMAN

Department of Chemistry, Aligarh Muslim University, Aligarh-202001, India

(*Revised received* 5 *March* 1984)

Key Word Index—*Mirabilis jalapa*, Nyctaginaceae, seed oil, 8-hydroxyoctadeca-*cis*-11,14-dienoic acid

Abstract—A fatty acid, found as a minor component in the seed oil of *Mirabilis jalapa*, is shown to be the hitherto unknown 8-hydroxyoctadeca-*cis*-11,14-dienoic acid

INTRODUCTION

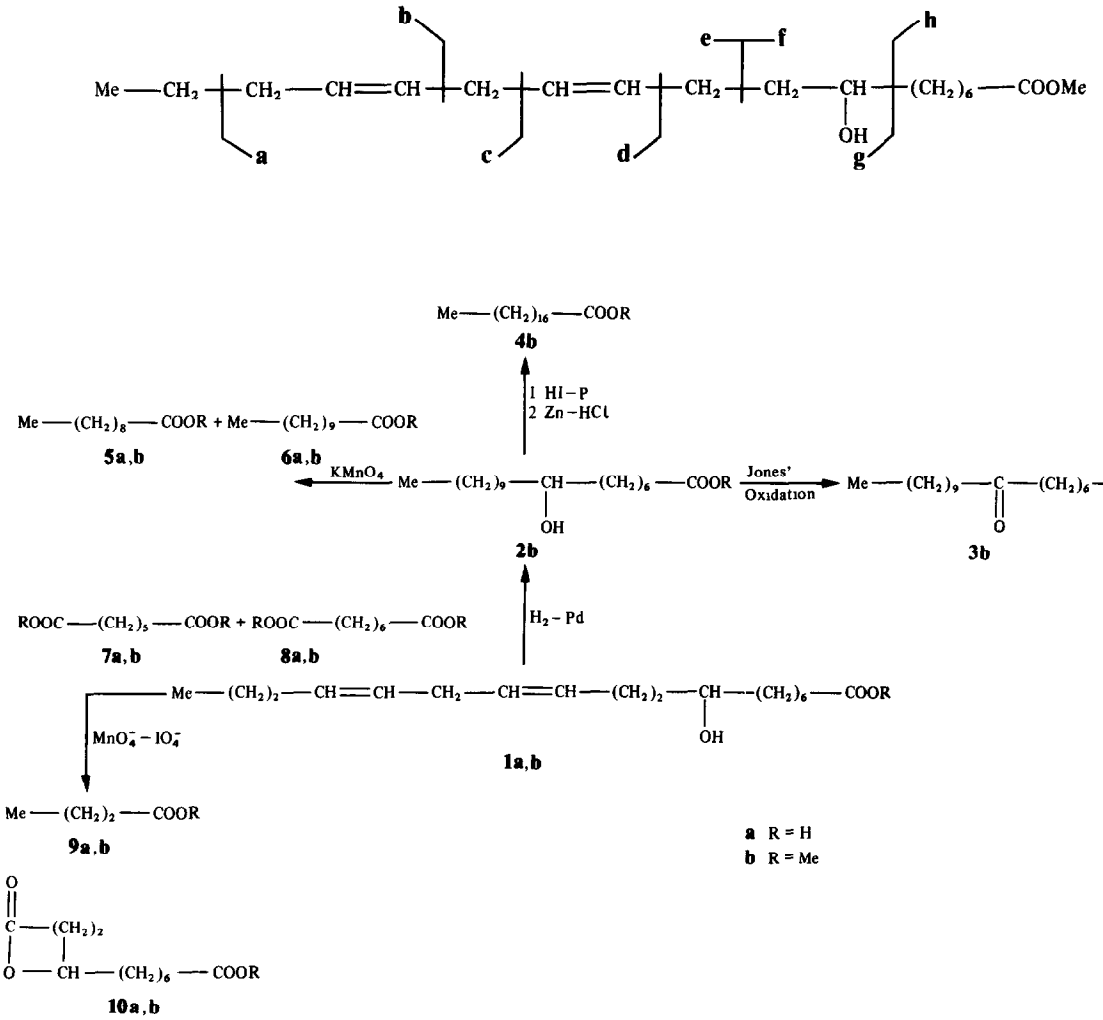
In continuation of our previous papers [1–4] on unusual hydroxy acid-containing seed oils, it was found that the oil of *Mirabilis jalapa* contains an oxygenated acid as a component of the seed fat glycerides As only some analytical constants have been reported [5] for this seed oil, the present work was undertaken The oil was found to contain a new hydroxydiolefinic acid, characterized as 8-hydroxyoctadeca-*cis*-11,14-dienoic acid by spectral and chemical methods

RESULTS AND DISCUSSION

The UV spectrum of the oil, as well as that of its methyl esters, showed the absence of conjugation The IR spectrum of the oil and of its methyl esters showed a hydroxyl band at 3360 cm^{-1} TLC of the ester also revealed a component which was 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 prep TLC This was further purified by column chromatography which yielded a brown viscous ester (**1b**) Since there was no absorption in the IR at 967 cm^{-1}, all double bonds must be *cis* Elemental analysis corresponded to the molecular formula $C_{19}H_{34}O_3$, suggesting a monohydroxy acid with two double bonds The ester (**1b**) on acetylation gave a product whose IR spectrum showed strong bands at 1230 and 1020 cm^{-1} The ^1H NMR spectrum gave signals at δ5 39 *m* (4H, 2 × –CH=CH–), 3 61 *s* (3H, COOMe), 3 38 *m* (2H, –CH–OH, after D_2O exchange the peak reduces to its half), 2 72 *t* (2H, =CH–C<u>H</u>$_2$–CH=, J = 7 Hz), 2 22 *m* (6H, allylic to double bond and α to ester grouping), 1 35 *br s*, (chain-C<u>H</u>$_2$–) and 0 92 *t* (3H, Me, J = 6 Hz) Its acetate derivative showed no unusual features apart from the expected but significant signal at δ2 02 *s* (3H, Ac) and 4 8 *m* (1H, C<u>H</u>-OAc) A signal at δ3 38 was not observed The appearance of a peak at δ0 92 in the NMR spectrum as a clear triplet for the terminal methyl with a J value of 6 Hz clearly demonstrated that one double bond is present at C-14 Hopkins [6] reported a similar effect in *cis*-12-hexadecenoic acid This was further supported by the observation of Frost *et al* [7] Since the two double bonds are methylene interrupted, the other double bond is therefore located at C-11 The methine proton attached to the hydroxyl group appeared at δ3 38, thereby showing that the position of the hydroxyl group is neither at C-10 nor C-2 If it was then the methine proton signal would be more downfield and it would be only a four proton signal at δ2 22 The structure of the hydroxy acid (**1a**) was further substantiated by the mass spectrum of its methyl ester (**1b**) It gave an $[M]^+$ peak at m/z 310 ($C_{19}H_{34}O_3$, M^+, 1), along with other peaks at m/z 311 $[M+1]^+$ (2), 309 $[M-1]^+$ (2), 308 $[M-2]^+$ (3), 281 [**a**] (1), 279 $[M-31]^+$ (19), 262 $[279-17]^+$ (3), 261 $[279-18]^+$ (4), 187 [**f**] (11), 185 $[187-2]^+$ (14), 167 [**g**] (55), 155 $[\mathbf{f}-32]^+$ (22), 149 $[\mathbf{g}-18]^+$ (97), 143 [**h**] (7), 123 [**e**] (12), 113 $[\mathbf{f}-74]^+$ (13), 109 [**d**] (15), 83 [**c**] (46), 69 [**b**] (49) and 55 (base peak) Some of the assignments were supported by accurate mass measurements

The peak at m/z 281 (**a**), although very weak, is significant as it results from allylic cleavage and suggests a Δ^{14}-double bond The ion at m/z 167 (**g**) is the third most intense peak and helps to locate the hydroxyl group at C-8 due to cleavage between C-7 and C-8 Cleavage between C-8 and C-9 should give a peak at m/z 173 but it is not present in the mass spectrum Instead a peak at m/z 187 (**f**) is present indicating cleavage between C-9 and C-10 and the presence of a Δ^{11}-double bond It is also pertinent to mention here that the absence of peak at m/z 157 with a peak at m/z 143 clearly demonstrated that the hydroxyl group is present at C-8 but not at C-9

A few reactions were carried out (Scheme 1) to establish the structure of the acid Catalytic hydrogenation of **1b** gave a solid compound methyl 8-hydroxystearate (**2b**, mp 55 4°, lit [8] 55 5°), which analysed for $C_{19}H_{38}O_3$ and had IR absorption at 3440 cm^{-1} (OH) Jones' oxidation of **2b** furnished methyl 8-ketostearate (**3b**), mp 44 8° (lit [9] mp 44 5–45 1°) Reductive removal of the hydroxyl group in **2b** by hydrogen iodide–phosphorus furnished **4b**, which was identified as methyl octadecanoate by GC This confirmed a normal C_{18} chain length for **1a** Oxidation of **2b** with permanganate in acetic acid gave a mixture of monobasic (**5a** and **6a**) and dibasic (**7a** and **8a**) acids The identified fragments were decanoic, undecanoic, heptanedioic and octanedioic esters Therefore, the hydroxyl group is present on C-8 in a normal C_{18} skeleton Oxidation of **1a** with permanganate–periodate [10] and methylation of the resulting products gave material with a strong IR absorption at 1775 cm^{-1} characteristic of a γ-lactone GC analysis showed only a



Scheme 1 Reaction scheme for identification of the hydroxydienoic acid and its methyl ester

monobasic compound, methyl butyrate (**9b**). The formation of the γ-lactone itself indicates that the double bond and the hydroxyl group are separated by two methylene groups The formation of **9b** confirms that a double bond is present between C-14 and C-15

GC analysis of the TMSi methyl esters on silicone and polyester columns showed the fatty acid composition to be 14 0 (0 7%), 16 0 (22 1%), 18 0 (0 3%), 18 1 (54 3%), 18 2 (11 1%), 18 3 (7 2%) and 8-hydroxyoctadeca-*cis*-11,14-dienoic acid (4 3%)

EXPERIMENTAL

General methods All mps are uncorr IR spectra were measured as liquid films or as 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 made using a stainless steel column (2 m × 3 mm) packed with 15% DEGS on chromosorb W or one 0 6 m × 4 mm packed with 2% SE30 Separations were carried out at 200° using an FID instrument

Preliminary analysis Petrol (40–60°) extracted seed oil (yield 9 8%) of *M jalapa* seeds, was subjected mild saponification with ethanolic KOH at room temp for 22 hr After usual work-up, the mixed fatty acids were subjected to methylation (acid-catalysed esterification with MeOH) TLC of the esters on silica gel G with petrol–Et_2O (7 3) gave two spots The oil and seed characteristics were Iodine value = 98 2, Saponification value = 187 6, Protein content (N × 6.25) = 36 8%, Moisture content = 3 2% and RI = 1 4679 n_D^{30} Argentation TLC was effected on silica gel G impregnated with 20% $AgNO_3$ Petrol–Et_2O (23 2) was used for development of the esters Spots of saturates, monoene, diene and triene and a slow moving spot with an R_f value similar to Me isoricinoleate were visualized by spraying with 2′,7′-dichlorofluorescein and viewing under UV light

Isolation of hydroxy ester (**1b**) The ester was concd by prep TLC (petrol–Et_2O, 4 1) and the concentrate, further purified by CC using petrol–Et_2O (9 1), yielded a TLC homogeneous product **1b**, as an oil (Found C, 73 48, H, 10 98 Calc for $C_{19}H_{34}O_3$ C, 73 50; H, 11 04%) IR $\nu_{max}^{CCl_4}$ cm^{-1} 3360 (OH) and 1738 (COOMe) **1b** (40 mg) when heated under reflux with 0 8 N KOH in EtOH (*ca* 1 hr), followed by usual work-up, afforded the

hydroxy acid (**1a**) also an oil (32 mg) IR ν_{max} cm^{-1} 3420–3200 (OH and COOH), 1710 (COOH)

Characterization of **1b** Acetylation of **1b** (63 mg) with Ac_2O–pyridine gave a product which showed strong IR bands at 1735 (COOMe and OCOMe), 1230 and 1025 cm^{-1} (acetate) A portion of **1b** (180 mg) was hydrogenated, using 10% Pd–C in EtOAc (2 ml), to give Me 8-hydroxystearate (**2b**), as a white solid (141 mg) mp 55 4° (lit [8] 55 5°) (Found C, 72 53, H, 12 12 Calc for $C_{19}H_{38}O_3$ C, 72 56, H, 12 18%) IR ν_{max} cm^{-1} 3440 (OH), 1735 (COOMe) Jones' oxidation of **2b** (20 mg) furnished Me 8-ketostearate (**3b**), mp 44 8° (lit [9] 44 5–45 1°)

Reductive removal of OH *group* (**2b**) [11] Me 8-hydroxystearate (**2b**, 40 mg) was refluxed for 17 hr with red P (18 mg) and HI (1 2 ml) Et_2O extraction of the dil mixture followed by washing with 5% $NaHSO_3$ gave an oily product (37 mg) This was reduced by refluxing for 4 hr with granular Zn (95 mg), MeOH (2 4 ml) and HCl (0 46 ml) Usual work-up of the mixture afforded 14 mg of semi-solid ester (**4b**) GC analysis with authentic samples showed it to be Me stearate Oxidative degradation [12] of **2b** (100 mg) with $KMnO_4$ in HOAc gave a mixture of monobasic (**5a** and **6a**) and dibasic (**7a** and **8a**) acids After methylation with CH_2N_2 these were examined by GC and shown to be Me decanoate, Me undecanoate, Me heptanedioate and Me octanedioate

Position of double bond in **1a** [10] Compound **1a** (130 mg), K_2CO_3 (150 mg) and *t*-BuOH (40 ml) were treated with a soln of $NaIO_4$ (450 mg) in 40 ml H_2O and $KMnO_4$ (1 3 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 butyrate (**9b**) The IR of the mixture showed a strong band at 1775 cm^{-1} which confirms the presence of a γ-lactone (**10b**)

Acknowledgements—We are extremely grateful to Professor W Rahman, for providing necessary facilities and to CSIR (Government of India) for financial assistance This research was financed in part by a grant from USDA under PL-480

REFERENCES

1 Ahmad, I, Ahmad, F and Osman, S M (1977) *Phytochemistry* **16**, 1761
2 Husain, S, Ahmad, M U and Osman, S M (1980) *Phytochemistry* **19**, 75
3 Ahmad, M S, Ahmad, M U and Osman, S M (1980) *Phytochemistry* **19**, 2137
4 Ahmad, I, Sherwani, M R K, Hasan, S Q, Ahmad, M S, and Osman, S M (1983) *Phytochemistry* **22**, 493
5 Barclay, A S and Earle, F R (1974) *Econ Botany* **28**, 195
6 Hopkins, C Y (1965) *Progress in the Chemistry of Fats and other Lipids* (Holman, R T, ed) Vol III, p 213 Pergamon Press, Oxford
7 Frost, D J and Gunstone, F D (1975) *Chem Phys Lipids* **15**, 53
8 Markley, K S (ed) (1960) *Fatty Acids Their Chemistry, Properties Production and Use*, Part 1, p 72 Interscience, New York
9 Markley, K S (ed) (1967) *Fatty Acids Their Chemistry, Properties Production and Use*, Part 4, p 2549 John Wiley, New York
10 Christie, W W (1973) *Lipid Analysis*, p 137 Pergmon Press, New York
11 Christie, W W, Gunstone, F D and Prentice, H G (1963) *J Chem Soc* 5768
12 Bharucha, K E and Gunstone, F D (1957) *J Chem Soc* 613