

1102. The Oxygenated Acids of Isano (Boleko) Oil.

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Onguekoa Gore seed oil contains four monohydroxy-acetylenic acids having the hydroxyl group adjacent to the unsaturated system. *cis*-9,10-Epoxystearic acid is a minor component of the oil.

ISANO or boleko oil, the seed oil of the tree *Onguekoa Gore* (family Olacaceae), contains a number of acetylenic and monohydroxy-acetylenic acids as components of its glycerides. These acids have not all been fully characterised, despite numerous investigations of the oil.^{1,2} Gunstone and Sealy³ have shown that isano oil contains five acetylenic acids, four hydroxy-acetylenic acids, and a dihydroxystearic acid, in addition to the usual range of saturated and unsaturated acids. The structures of most of these acids were unambiguously elucidated. This paper is complementary to that of Gunstone and Sealy, and confirms their conclusions. In addition, *cis*-9,10-epoxystearic acid is shown to be a constituent of isano oil.

Isolation Procedures.—The mixed esters were prepared by mild hydrolysis of the oil and reaction of the resulting acids with diazomethane. Four types of ester (see Table) were detected and identified, by comparison with standards, by thin-layer chromatography,

Composition of isano oil (% wt. as methyl esters).

Non-oxygenated acids ("normal," ca. 20%; acetylenic, ca. 40%)	60.6
<i>cis</i> -9,10-Epoxystearic acid	1.4
Unknown	0.7
8-Hydroxy-acetylenic acids * (9a,11a 13.7%; 9a,11a,13e 0.9%; 9a,11a,17e 20.2%; 9a,11a,13e 1.2%)	36.0

* These are all C₁₈-acids. The symbols indicate acetylenic bond (a) and ethylenic bond (e), and the structures are assigned on the basis of Gunstone and Sealy's work.³ The proportions are different from those for the oil examined by Gunstone and Sealy, although the general pattern is similar, but such variations in the compositions of seed oils from different sources are relatively common.

and were separated by adsorption column chromatography to give a non-oxygenated ester fraction, partially resolved into "normal" esters and acetylenic esters, an epoxy-ester fraction (E, 2.1%), and a hydroxy-acetylenic ester fraction (HA).

The Epoxy-ester Fraction.—Thin-layer chromatography on silicic acid impregnated with silver nitrate⁴ demonstrated two components of this fraction and these were separated on a column of silver nitrate-impregnated silicic acid.⁵ The first component to be eluted (E1, 1.4% of mixture) had migration characteristics identical with those of methyl *cis*-9,10-epoxystearate on thin layers of silicic acid, and, after treatment with anhydrous hydrogen bromide in ether, the product migrated like *threo*-9(or 10)-bromo-10(or 9)-hydroxystearate. The second component (E2, 0.7% of mixture) did not react with ethereal hydrogen bromide under the same conditions and is therefore unlikely to be an epoxy-compound. Its structure has not been determined.

Component E1, on hydrolysis, gave *cis*-9,10-epoxystearic acid which, on acetolysis and hydrolysis, gave *threo*-9,10-dihydroxystearic acid, whose structure was verified by periodate-permanganate oxidation,⁶ which gave only azelaic and nonanoic acids. Component E1 is, therefore, methyl *cis*-9,10-epoxystearate, which may be the natural precursor of the

¹ Riley, *J.*, 1951, 1346; Kaufmann, Baltés, and Herminghaus, *Fette u. Seifen*, 1951, **53**, 534; Black and Weedon, *J.*, 1953, 1785, and references therein; Meade, in "Progress in the Chemistry of Fats and Other Lipids," ed. Holman, Lundberg, and Malkin, Pergamon Press, London, 1957, Vol. 4, pp. 49, 50.

² Seher, *Annalen*, 1954, **589**, 222.

³ Gunstone and Sealy, preceding paper.

⁴ (a) Morris, *Chem. and Ind.*, 1962, 1238; (b) Mangold and Morris, paper communicated to Internat. Society for Fat Research Congress, London, April 1962.

⁵ de Vries, *Chem. and Ind.*, 1962, 1049.

⁶ von Rudloff, *J. Amer. Oil Chemists' Soc.*, 1956, **33**, 126.

threo-9,10-dihydroxystearic acid characterised by Gunstone and Sealy.³ *cis*-9,10-Epoxy-stearic acid occurs in *Tragopogon porrifolius* seed oil⁷ and in the oils of certain fungi⁸ but this is the first report of its occurrence in isano oil.

The Hydroxy-ester Fraction.—Thin-layer chromatography on silicic acid impregnated with silver nitrate resolved the monohydroxy-ester fraction (HA) into two components,^{4b} now shown to differ in that the more strongly adsorbed one contains a vinyl group.³ These were separated by column chromatography on silver nitrate-silicic acid, to give a non-vinyl component (HA1, 14.6%) and a vinyl component (HA2, 21.4% of total esters).

The mixed fraction (HA) had λ_{max} at 215, 227.5, 240, 253.5, 268, and 284 $m\mu$ ($E_{1\text{cm}}^{1\%}$ 47 at 268 $m\mu$). By comparison with the value of 796 at this wavelength quoted by Crombie and Williams⁹ for a C_{18} -enediynoic acid, this indicates that some 6% of this fraction contains the enediyne chromophore. The separated hydroxy-fractions (HA1 and HA2) had similar ultraviolet curves with $E_{1\text{cm}}^{1\%}$ values at 268 $m\mu$ of 57 and 44, respectively, indicating that the non-vinyl fraction contains 6.4% and the vinyl fraction 5.5% of enediyne component (*i.e.*, 0.9% and 1.2%, respectively, of total esters).

The near-infrared spectrum of the HA fractions showed a sharp symmetrical band at 3611 cm^{-1} (OH stretching), with no trace of a band near 3580 cm^{-1} . Compounds with unsaturated centres $\beta\gamma$ to a hydroxyl group show a free hydroxyl band near 3625 cm^{-1} and an associated hydroxyl band near 3580 cm^{-1} , attributed to intramolecular hydrogen bonding of the hydroxyl group to the π -electron system of the unsaturated bond, whereas vicinally unsaturated hydroxy-compounds show only a single hydroxyl band.¹⁰ Methyl 12-hydroxyoctadec-9-ynoate, for example, has two bands,^{10a} *viz.*, free hydroxyl absorption at 3623 cm^{-1} (absorptivity, 0.058) and a much stronger associated hydroxyl absorption at 3580 cm^{-1} (absorptivity, 0.101). This is strong evidence for the vicinally unsaturated hydroxyl structure in isano hydroxy-acids favoured by Gunstone and Sealy³ rather than one in which these groups are separated by a methylene group as suggested by Seher.² The high intensity of this hydroxyl absorption (absorptivity, 0.202) and its position at 3611 cm^{-1} also support this assignment.^{10a}

In a study of the stability of hydroxy-esters during gas-liquid chromatography,¹¹ it was found that the isano hydroxy-esters were dehydrated under the conditions used, as were other hydroxy-esters having $\alpha\beta$ -unsaturation but not those with $\beta\gamma$ or other unsaturation. Although some of the structural assignments made in interpreting the results of that study require re-evaluation in the light of more recent work, the isano hydroxy-esters were undoubtedly dehydrated as stated, providing further evidence for the vicinally unsaturated hydroxyl grouping.

EXPERIMENTAL

Light petroleum was the fraction of b. p. 40—60°, redistilled. Ultraviolet spectra were measured on a Unicam S.P. 500 spectrophotometer for 0.03% and 0.003% solutions in methanol. Near-infrared spectra were obtained with 0.3% solutions in carbon tetrachloride on a Beckman DK 2 recording spectrophotometer.

Silica gel G (Merck) was applied as a thin layer to glass plates (20 × 20 cm.) with the Desaga equipment. Silver nitrate-impregnated plates were prepared by mixing Silica gel G (23.75 g.) with silver nitrate (1.25 g.) in water (50 ml.), the resulting suspension being spread, as usual, over five plates to give layers with 5% (w/w) impregnation. Development was carried out with ether-light petroleum (usually 1 : 4 or 2 : 3, v/v). Separated components were observed either by spraying with a 0.2% solution of 2',7'-dichlorofluorescein in ethanol and

⁷ Chisholm and Hopkins, *Chem. and Ind.*, 1959, 1154.

⁸ Tulloch, Craig, and Ledingham, *Canad. J. Microbiol.*, 1959, 5, 485; Tulloch and Ledingham, *ibid.*, 1960, 6, 425; Tulloch, *Canad. J. Chem.*, 1960, 38, 204.

⁹ Crombie and Williams, *J.*, 1962, 2449.

¹⁰ (a) Morris, paper communicated to Internat. Society for Fat Research Congress, London, April 1962; Morris and Grundy, unpublished results; (b) Schleyer, Trifan, and Bacskai, *J. Amer. Chem. Soc.*, 1958, 80, 6691; (c) Eddy, Showell, and Zell, *J. Amer. Oil Chemists' Soc.*, 1963, 40, 92.

¹¹ Morris, Holman, and Fontell, *J. Lipid Res.*, 1960, 1, 412.

viewing under ultraviolet light, or by spraying with 50% sulphuric acid and heating at 200° to char organic materials.

Fractionation of Isano Methyl Esters.—Isano oil (10 g.) was set aside overnight at room temperature, and under nitrogen, with 2*N*-alcoholic potassium hydroxide (50 ml.). After addition of water (150 ml.) the solution was cooled to 0° and carefully acidified to pH 5 with 2*N*-hydrochloric acid; the mixed fatty acids were immediately extracted with ether, washed with water, recovered, and esterified with an excess of diazomethane in ether solution.

A portion of the esters (7.00 g.) was fractionated on a column of activated silica gel (Davison, 60–200 mesh; 75 g.). Elution was with light petroleum–ether, 20 ml. fractions being collected, monitored by thin-layer chromatography, like fractions being combined. Non-oxygenated esters (4.24 g.) were eluted with 3% ether–light petroleum, the epoxy-ester fraction (E, 0.15 g.) with 5% ether–light petroleum, and the monohydroxy-esters (HA, 2.52 g.) with 10% ether–light petroleum. No further fractions were collected after all the monohydroxy-esters had been eluted, and the dihydroxystearate described by Gunstone and Sealy³ may have been present, but remained undetected on the column.

Further Fractionation of Hydroxy-esters.—Silicic acid (Mallinckrodt, 100 mesh; 20 g.) was impregnated with silver nitrate by thoroughly mixing with a solution of silver nitrate (20 g.), in water (40 ml.), filtered, and dried at 110° overnight. A column (14 × 1.5 cm.) was prepared with a slurry of this adsorbent in light petroleum. Isano hydroxy-esters (90 mg.) were applied in 5% ether–light petroleum and eluted with increasing proportions of ether in light petroleum. The non-vinyl hydroxy-ester fraction (HA1, 36 mg.) was eluted with 10%, and the vinyl ester fraction (HA2, 53 mg.) with 20% ether–light petroleum. Fractions were monitored by thin-layer chromatography on silicic acid and on silicic acid impregnated with silver nitrate. Separation was quantitative.

The Epoxy-ester Fraction.—Fraction E (100 mg.) was fractionated on an identical column to give two components; E1 (74 mg.) was eluted with 5% and E2 (35 mg.) with 10% ether–light petroleum.

A portion of each of these was treated with an anhydrous ethereal solution of hydrogen bromide (*ca.* 5%) for 30 min. and, after the excess of hydrogen bromide had been removed by washing with water, the products were compared with the original untreated samples and with reference compounds by thin-layer chromatography on silicic acid.

A portion (10 mg.) of E1 was hydrolysed at room temperature with 2*N*-ethanolic potassium hydroxide (2.0 ml.) and carefully acidified to pH 5; the acid was recovered, and after one crystallisation from a small volume of acetone, had *m. p.*, and mixed *m. p.* with *cis*-9,10-epoxystearic acid, 57.5–58°.

A further portion (20 mg.) of E1 was refluxed with glacial acetic acid (2 ml.) for 2 hr. and the product was recovered and hydrolysed. The resultant dihydroxy-acid, crystallised twice from small volumes of ethyl acetate, had *m. p.*, and mixed *m. p.* with *threo*-9,10-dihydroxystearic acid, 93.5–94.5°.

*von Rudloff Oxidation of the Dihydroxy-acid.*⁶—The dihydroxy-acid (*ca.* 10 mg.) was dissolved in a solution of potassium carbonate (50 mg.) in water (6 ml.), and a solution of potassium permanganate (3 mg.) and potassium periodate (200 mg.) in water (10 ml.) was added. The mixture was shaken at room temperature overnight, decolourised with sulphur dioxide gas, and the acidified solution thoroughly extracted with ether. The ether solution was concentrated and the oxidation products esterified with ethereal diazomethane. The esters were examined by gas–liquid chromatography on a Pye Argon chromatograph with a 4 ft. column of ethylene glycol–adipate polyester stationary phase (10%) in Celite. Dibasic esters were studied at 170° and monobasic esters at 75° and the components of the oxidation mixture were compared with standard reference mixtures of monobasic and dibasic acid esters. Only methyl nonanoate and dimethyl azelate were present in the products of the oxidation.

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