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Note

Direct high-performance liquid chromatography separation of diastereoisomeric methyl 9,10,12-trihydroxystearates and its application to the stereochemical study of hydroperoxy cyclic peroxides

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The four diastereoisomers of methyl 9,10,12-trihydroxystearate have been previously characterised and their configurations determined[1]. Gas chromatography of trifluoroacetate (TFA) derivatives has been used to separate *threo*-10,12 and *erythro*-10,12 pairs but mixtures of all four isomeric TFA derivatives could not be separated[2]. A separation of all four isomers was obtained by thin-layer chromatography (TLC) on sodium arsenite impregnated silica gel[2].

This communication reports a high-performance liquid chromatography (HPLC) method which separates the four isomers directly as methyl esters using methyl ricinoleate as an internal standard. An autoxidation product of methyl linolenate which had been previously shown to be methyl 9-hydroperoxy-10,12-epidioxy-13-*trans*-15-*cis*-octadecadienoate and erroneously assigned a *threo*-9,10 configuration[3] was shown to have an *erythro*-9,10-*cis*-10,12-configuration after conversion to the corresponding 9,10,12-trihydroxystearate which was identical to authentic methyl (9*S*,10*R*,12*R*)-trihydroxystearate derived from methyl ricinoleate.

MATERIALS AND METHODS

HPLC separations were carried out on a Zorbax Sil 25 × 0.46 cm I.D. column (DuPont Instruments, Hitchin, U. K.) at 20°C and 1 ml/min flow rate with a Waters Assoc. Model 6000A pumping system. The column eluent was monitored at 208 nm (ester function) with a variable wavelength detector (Cecil Instruments, Cambridge, U.K.). Elution was carried out isocratically with 7% ethanol in 50% water saturated hexane as solvent.

The four diastereoisomers of 9,10,12-trihydroxystearic acid obtained by hydroxylation of ricinoleic acid[1] were donated by Professor F. G. Gunstone (University of St. Andrews, U.K.). The corresponding methyl esters were prepared with diazomethane. Methyl ricinoleate was obtained from Sigma (London, U.K.). The hydroperoxy cyclic peroxide derivative of methyl linolenate[3] was catalytically reduced to methyl 9,10,12-trihydroxystearate with Adams catalyst in ethanol for 15 min at room temperature. Hexane, HPLC grade, was supplied by Flourochem (Glossop, U.K.).

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RESULTS AND DISCUSSION

The four diastereoisomers designated α, β, γ and δ are listed in Table I together with their configurations and retention data. Under the optimum conditions which were established for their separation methyl ricinoleate had a retention time of 20 min and served as a convenient internal standard for measurement of relative retention times. A typical chromatogram of the four isomers (Fig. 1) illustrates that the γ-, α- and β-isomers are completely resolved but the δ- and β-isomers are just sufficiently resolved for identification.

TABLE I

HPLC RETENTION DATA FOR DIASTEREOISOMERIC METHYL 9,10,12-TRIHYDROXYSTEARATES

For conditions see text.

*Isomer**	*Configuration*	*Retention time (min)*	*Relative retention***
α	9*S*,10*R*,12*R* (*erythro*-9,10-*erythro*-10,12-)	14.2	0.71
β	9*R*,10*S*,12*R* (*erythro*-9,10-*threo*-10,12-)	15.2	0.76
γ	9*R*,10*R*,12*R* (*threo*-9,10-*erythro*-10,12-)	13.6	0.68
δ	9*S*,10*S*,12*R* (*threo*-9,10-*threo*-10,12-)	14.8	0.74

* Designation as originally used by Kass and Radlove[4].

** Measured relative to methyl ricinoleate (retention time 20.0 min).

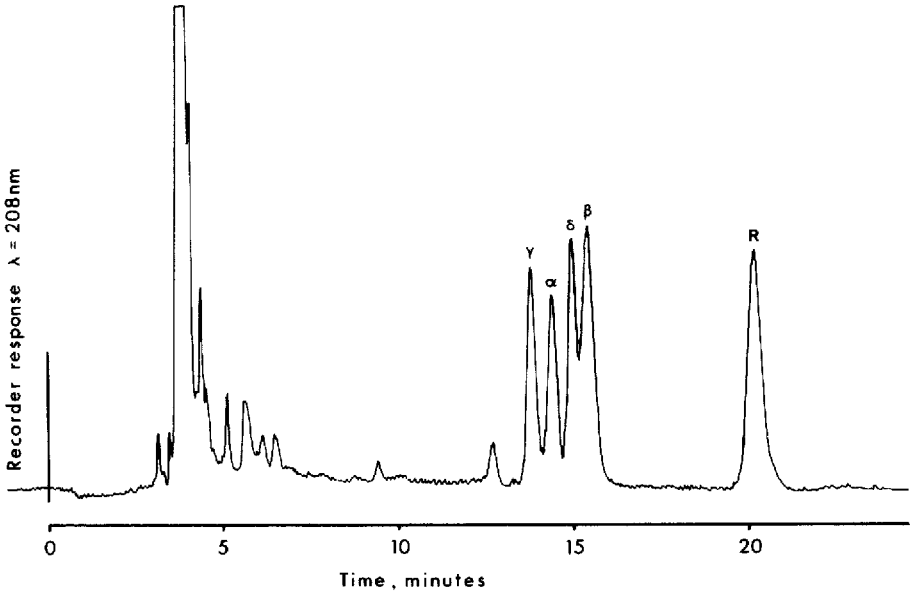
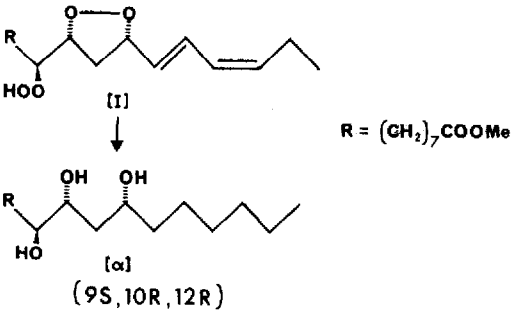


Fig. 1. HPLC separation of trihydroxystearate isomers α, β, γ and δ. R is internal standard methyl ricinoleate.

Having established the chromatographic procedure for the identification of diastereoisomeric trihydroxystearates it was used for characterisation of a specific isomer obtained by catalytic reduction of a hydroperoxy cyclic peroxide (I) derived from methyl linolenate. The stereochemistry of the hydroperoxy cyclic peroxide was



in question. Two isomeric methyl 9-hydroperoxy-10,12-epidioxy-13-*trans*-15-*cis*-octadecadienoates are obtained as products of methyl linolenate autoxidation. The major isomer (I) which is less polar on TLC is characterised by higher field shifts in the NMR spectrum for both the hydroperoxide proton (δ 9.56) and the corresponding methine proton (δ 4.21). The two isomers are obviously epimeric at C-9 and hence may be designated as *erythro*-9,10- and *threo*-9,10-isomers. We previously assigned the *threo*-9,10-structure to the major less polar isomer (I) based on arguments related to hydrogen bonding. Conversion of this compound (I) to the corresponding trihydroxystearate which was then shown by HPLC analysis to be solely the α-isomer enabled us to reassign the stereochemistry of I as *erythro*-9,10-*cis*-10,12 which is consistent with the findings of Mihelich for a similar compound derived from methyl linoleate[5]. All of the *threo* and *erythro* assignments in our previous paper[3] should therefore be reversed.

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