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Note

Semi-preparative high-performance liquid chromatography and spectroscopic characterisation of eight geometric isomers of leukotriene A methyl ester

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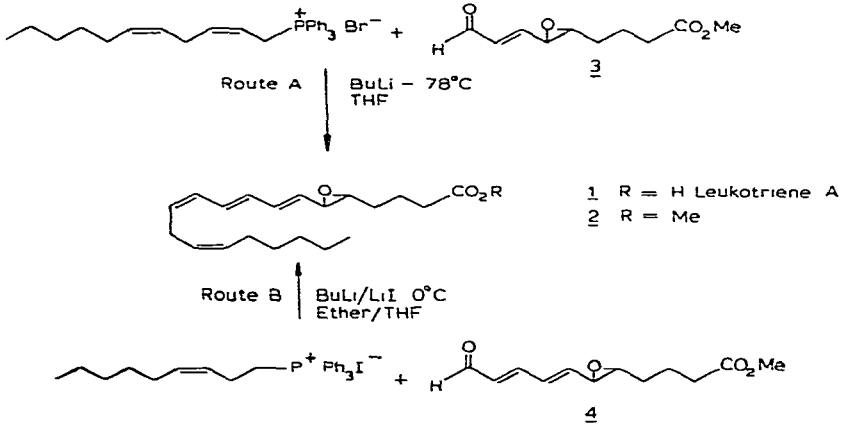
Leukotriene A (1) is now recognised as the precursor of leukotriene B, a compound with potent chemotactic activity for polymorphs, and leukotrienes C and D. The latter compounds have recently been shown[1] to be related to SRS-A which is thought to be the main mediator in human asthma. The biological potency of these compounds is resulting in intense synthetic activity in this new pathway of arachidonic acid metabolism[2,3]. The stereochemistry of these molecules must be characterised to enable satisfactory comparison of natural and synthetic compounds, as well as explore their structure–activity relationships.

The synthesis initially used to prepare the methyl ester of leukotriene A (2), using a 9 carbon epoxide (3) has been described previously[4–6] (route A). A route similar to that described by Corey[6], utilizing an 11 carbon epoxide (4), was also used (route B). Both reactions have yielded a number of isomers of 2.



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Due to the complexity of the reaction product, a high-performance liquid chromatographic (HPLC) separation and mass spectrometry (MS) and ultraviolet (UV) characterisation of the eluate peaks was required, prior to stereochemical assignments.

This paper describes the chromatographic separation and MS and UV characterisation of eight geometric isomers of 2 and an isomeric tetraene molecule (5).

EXPERIMENTAL

Reagents

The Spherisorb S5W and S5NH column packing material was supplied by Phase Separations (Queensferry, Great Britain). HPLC grade hexane was purchased from Fisons (Loughborough, Great Britain), analytical-reagent grade diethyl ether from May and Baker (Dagenham, Great Britain) and analytical-reagent triethylamine from BDH (Poole, Great Britain).

Instrumentation

Chromatography was performed with a constant-flow Milton Roy constametric IIG pump and a Cecil 212 variable wavelength UV detector set at 276 nm for analytical separations and 296 nm to reduce sensitivity for preparative work. The detector was fitted with a 10-mm path length flow-cell for analytical chromatography and a 1-mm path length flow-cell for preparative chromatography. Samples were injected using a Rheodyne variable volume valve injector fitted with a 20-μl loop for analytical separations, and a 2.0-ml loop for preparative separations.

Chromatography

All stainless-steel columns were packed in a vertically upwards mode from a methanol slurry of the packing material. Analytical chromatography was performed on 12.5 cm × 5 mm I.D. columns packed with either Spherisorb S5W or S5NH material. The isomers were eluted with hexane–diethyl ether–triethylamine (95:5:0.5) at a flow-rate of 1.0 ml/min. Preparative separations were carried out on 50 cm × 8 mm I.D. columns packed with the same materials. The identical eluting solvent was used at a flow-rate of 5.0 ml/min.

Mass spectrometry

The required HPLC eluate was reduced in volume before transference to a sample holder tube and the sample blown to dryness. All spectra were recorded by an LKB 9000S mass spectrometer. Samples were analysed by direct insertion probe at 50°C using an ion accelerating voltage of 3.5 kV, an electron voltage of 20 eV and a source temperature of 270°C.

UV spectrometry

The UV absorption spectra were recorded in cyclohexane on a Pye Unicam SP8-100 spectrophotometer calibrated at 279.4 nm with a Holmium filter.

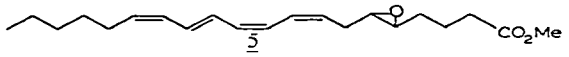
RESULTS AND DISCUSSION

Previous workers[4–6] have reported the presence of geometric isomers of the methyl ester of leukotriene A (2) associated with the stereochemistry of the 9,10- and 11,12-double bonds. As these molecules had similar R_F values in a number of thin-layer chromatography solvent systems[4], or were not considered chromatographically separable[6], no attempt was made to separate the molecules using preparative chromatographic techniques. We initially observed the presence of only three of the four potential geometric isomers[7] associated with the 9,10- and 11,12-double bonds in these leukotrienes. However, more recent reaction mixtures obtained under modified experimental conditions have shown, in addition to the presence of the fourth geometric isomer of 2, a further four minor isomers.

Chromatography

An optimum HPLC separation was obtained using a Spherisorb S5W column and an ether–hexane eluent. Triethylamine was incorporated in the solvent system as leukotriene A methyl ester is unstable under acidic conditions. Although the crude reaction mixtures (route A) were initially purified on a gravity-feed silica column, they still contained over ten components. Four main components isolated by HPLC were shown by MS and UV analysis to be isomers of leukotriene A methyl ester.

As the isomers were only just resolved under analytical conditions, a high-performance semi-preparative column was required to separate the required amounts. The use of a semi-preparative S5W column, which had an efficiency of over 50,000 theoretical plates for a *m*-nitroaniline standard, allowed the separation of up to 3 mg of crude material per injection. Isomers 2a, 2b and 2d appeared chromatographically pure on re-analysis. Isomer 2c showed a 7% impurity, identified as the tetraene (5). As these latter two compounds are chromatographically well resolved, it was assumed that 5 was formed as a rearrangement[8] product from 2c.



5

In samples obtained from route B, a minor peak eluted before isomer 2a on the semi-preparative column. This component was isolated and appeared by MS and UV analysis to be a fifth isomer (2e) of leukotriene A methyl ester.

In certain samples the chromatographic peak assigned as isomer 2a (route A and route B) was seen to have a slight shoulder on the tail of the peak. Further analytical HPLC on an S5NH column of selected semi-preparative fractions of 2a showed the presence of a further component. Semi-preparative HPLC of these frac-

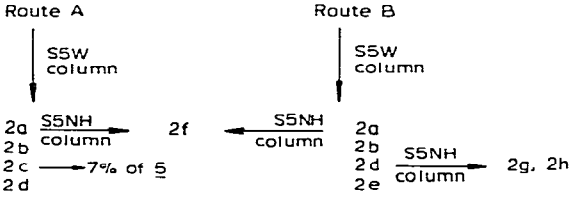


Fig. 1. Purification route used for isomers 2a–2h.

tions on an S5NH column (40,000 theoretical plates for a *m*-nitroaniline standard) and subsequent MS and UV analysis showed the presence of a sixth isomer (2f). Isomers 2b–2e were also examined on a Spherisorb S5NH column. No further impurities were observed in isomers 2b, 2c and 2e although isomer 2d was found to contain two impurities. Isomer 2d was therefore further purified on the semi-preparative S5NH column. The two impurities were isolated and were shown by MS and UV analysis to be further isomers (2g and 2h) of leukotriene A methyl ester.

In summary, a total of eight geometric isomers of leukotriene A methyl ester were isolated using a combination of the semi-preparative Spherisorb S5W and S5NH columns. The purification route used for each isomer is shown in Fig. 1. A separation of isomers 2a-2e and 5 on the S5W and S5NH columns is shown in Fig. 2a and 2b, respectively. The minor isomers 2g and 2h are not observed under either of these conditions as they coelute with the major isomers. To obtain isomers 2f–2h it was necessary to isolate the fractions corresponding to 2a and 2d on the S5W column and reprocess these fractions on the S5NH column. A comparison of the separations of the major isomers 2a and 2d and minor isomers 2f, 2g and 2h on a semi-preparative S5W and S5NH column is shown in Fig. 2c and 2d, respectively.

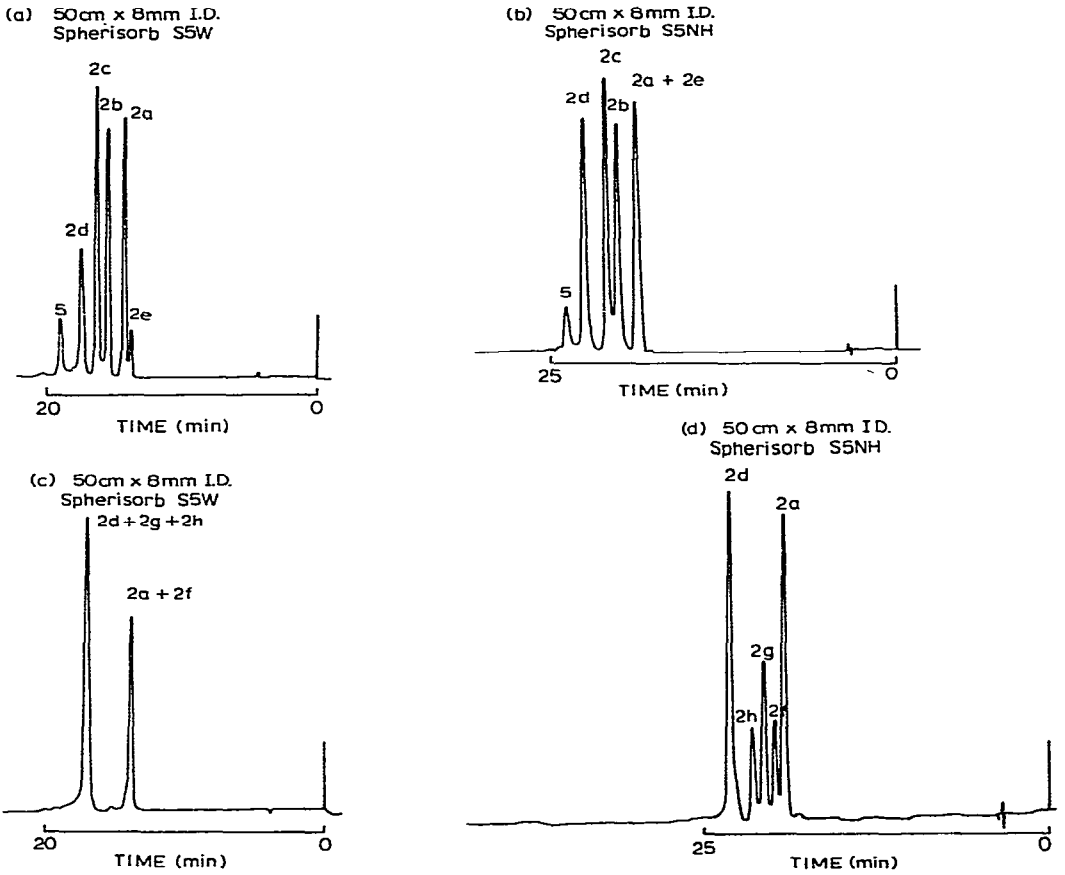


Fig. 2. Separation of isomers of leukotriene A methyl ester (2) and tetraene (5) on Spherisorb S5W and S5NH semi-preparative columns using hexane–diethyl ether–triethylamine (95:5:0.5) as eluent.

TABLE I

k' VALUES OF LEUKOTRIENE A METHYL ESTER ISOMERS

Isomer	*k' values*	
	Spherisorb S5W column	*Spherisorb S5NH column*
2a	3.0	4.3
2b	3.3	4.7
2c	3.5	4.9
2d	3.8	5.3
2e	2.8	4.3
2f	3.0	4.6
2g	3.8	4.7
2h	3.8	4.9
5	4.3	5.9

The k' values of 2a–2h and 5 on the S5W and S5NH columns are shown in Table I. Over 10 mg of isomers 2a–2c, 5 mg of isomer 2d and approximately 20 μg of the minor isomers 2e–2h were isolated. The purity of the isomers isolated was $>99\%$ on re-analysis, with the exception of 2c which contained the tetraene (5). All eight isomers were characterised by MS and UV although only the major isomers were analysed by nuclear magnetic resonance (NMR).

Mass spectrometry

Isomers 2a–2h show very similar electron impact mass spectra (Fig. 3 for 2b) with the molecular ion having a significant relative abundance. Although the mass

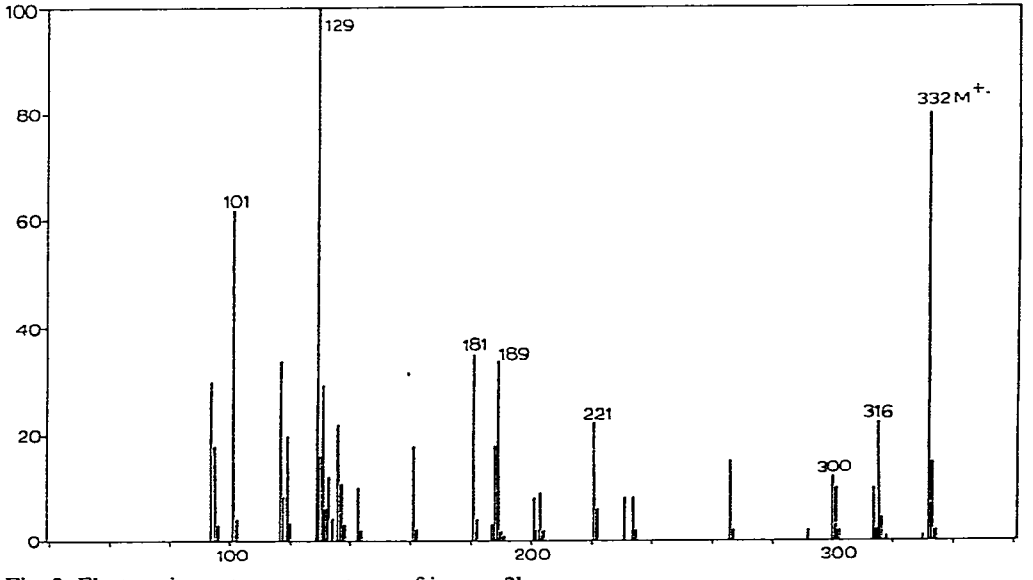
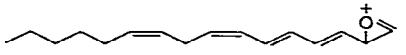
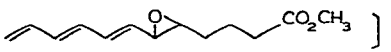
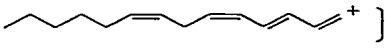
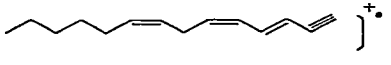
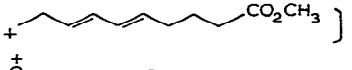
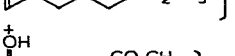
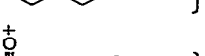
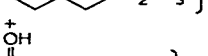
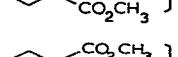
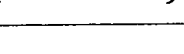


Fig. 3. Electron impact mass spectrum of isomer 2b.

TABLE II

RELATIVE ABUNDANCES OF SIGNIFICANT IONS IN THE MASS SPECTRA OF 2a–2h AND 5

Ion m/e value	*Ion assignment for isomers 2a–2h*	*Isomers*								
		2a	*2b*	*2c*	*2d*	*2e*	*2f*	*2g*	*2h*	*5*
332	$M^{+\cdot}$	80	80	86	100	83	81	86	42	100
316	$[M\text{-}O]^+$	3	22	32	4	3	3	3	2	1
314	$[M\text{-}H_2O]^{+\cdot}$	6	10	2	8	23	1	2	1	1
301	$[M\text{-}OCH_3]^+$	8	10	7	15	8	6	8	3	4
300	$[M\text{-}HOCH_3]^{+\cdot}$	7	12	6	5	8	3	3	4	1
231		6	8	8	5	3	3	5	1	11
221		33	22	14	21	3	10	9	1	3
189		37	34	30	20	9	20	12	3	36
188		25	18	20	8	9	12	5	2	10
181		44	35	22	33	3	16	10	1	1
143		13	10	26	2	5	8	5	11	70
131		30	29	44	10	12	24	10	4	56
129		100	100	100	95	100	100	100	100	41
117		35	34	48	8	11	35	11	4	78
101		60	62	63	50	47	79	47	24	17

spectra can be seen to be characteristic of the molecules concerned, they cannot be used to distinguish between individual isomers. Table II indicates that all molecules show a common elimination of [O], $[H_2O]$, $[OCH_3]$ and $[CH_3OH]$ from the molecular ion. The base ion (*m/e* 129) probably has an acylium ion structure, whereas the *m/e* 131 ion arises from a transannular cleavage[9] with hydrogen transfer. α-Cleavage[10] leads to the formation of intense and diagnostic ions of *m/e* 101 and 231 or *m/e* 143 and 189.

The mass spectrum of the conjugated tetraene 5 differs from those of the other eight isomers in that the *m/e* 131 ion is more favourably formed than the *m/e* 129 ion. α-Cleavage of the 5–6 carbon bond occurs in preference to that of the 4–5 carbon bond, as shown by the increased relative abundance of the *m/e* 143 ion over the *m/e* 101 ion.

UV spectroscopy

The UV absorption maxima in cyclohexane of the eight isomers 2a–2h and 5

TABLE III

UV ABSORPTION MAXIMA OF LEUKOTRIENE A METHYL ESTER ISOMERS

Isomer	$\lambda_{max.}$ *(nm)*			
2a	268.0(sh)	277.5	287.0(sh)	
2b	271.0(sh)	280.5	292.5	
2c	271.5(sh)	280.0	291.5	
2d	267.0	277.5	289.5	
2e	269.0	277.5	288.5	
2f	270.5	281.5	293	
2g	271.0	281.0	292.5	
2h	270.0	281.5	293.5	
5	281.0(sh)	292.0	305.0	320

are listed in Table III. Isomers 2a–2h exhibited a characteristic triene chromophore and 5 was characteristic of a tetraene. The similarity of certain isomers suggests that they are isomeric about the C-14 double bond although it is difficult to draw definite conclusions.

TABLE IV

STEREOCHEMISTRY FOR ISOMERS 2a–2d

Isomer	*Epoxide*	*Double bonds*			
	5,6	*7–8*	*9–10*	*11–12*	*14–15*
2a	*trans*	*trans*	*cis*	*trans*	*cis*
2b	*trans*	*trans*	*trans*	*cis*	*cis*
2c	*trans*	*trans*	*cis*	*cis*	*cis*
2d	*trans*	*trans*	*trans*	*trans*	*cis*

Stereochemistry

After initial MS and UV characterisation of 2a–2d larger samples were collected for NMR analysis. The detailed ^{1}H NMR spectra assignment for 2a–2c determined at 270 MHz have been reported previously[7]. The isomer 2d was later shown by 360 MHz ^{1}H NMR to be the 7,9,11-*trans*-14-*cis*-isomer. The full double bond stereochemistry for 2a–2d is shown in Table IV.

It is envisaged that the use of semi-preparative HPLC will have wide applications in the purification of further members of the leukotriene family. Further transformations of compounds 2a–2d to isomers of leukotrienes B, C, D and E and measurement of their biological activities will be the subject of future communications.

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