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Gas chromatographic properties of cyclic dienoic fatty acids formed in heated linseed oil

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Abstract

Cyclic dienoic fatty acids isolated from heated linseed oil were examined by capillary gas chromatography on a variety of stationary phases. Equivalent chain lengths were determined for CP-Wax 52CB, BPX70, CP-Sil 84 and OV-275 phases. Not all cyclic dienes could be resolved on any one column. Identification and quantification of all components was possible only by analyses on CP-Wax 52CB together with BPX-70 or CP-Sil 84 columns.

Keywords: Linseed oil; Heated oils; Stationary phases, GC; Fatty acids; Dienoic fatty acids, cyclic; Dienes

1. Introduction

Cyclic dienoic fatty acids are formed from linolenic acid during heating of linseed oil to temperatures of 200°C and above [1]. The mixtures have been simplified by hydrogenation and some of the skeletal structures have been determined by gas chromatography–mass spectrometry (GC–MS) [2–5]. By comparison to synthetic compounds, these structures were verified and the configurations of the rings were assigned. Studies on the native methyl esters by GC–MS and GC–Fourier Transform Infrared Spectroscopy (FTIR) allowed the degree of unsaturation and geometry of the double bonds, respectively, of many of the dienoic acids to be determined [1].

Recently the complete structures of all sixteen cyclic dienes were elucidated by GC–MS of the picolinyl ester and dimethyloxazoline (DMOX) derivatives of native, hydrogenated and deuterated fractions, isolated by silver ion HPLC of a total cyclic diene mixture [6]. Eight fatty acids contained a cyclopentenyl ring formed either between C-10 and C-14 of the original linolenic acid chain with double bonds at C-12 and C-15 or between C-11 and C-15 with double bonds at C-9 and C-12 (Table 1). The remaining eight fatty acids contained a cyclohexenyl ring formed between C-10 and C-15 with double bonds at either C-8 and C-12 or C-12 and C-16. Each of the four basic structures was represented by the four possible isomers arising from differences in configuration of the ring and/or the double bond in the straight chain. The fatty acids were quantified by analysing each silver ion HPLC fraction by GC in the presence of an internal standard.

In the present study, the GC properties of a total mixture of cyclic diene methyl esters were examined on a variety of stationary phases with a view to

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Table 1
Equivalent chain length values of cyclic dienoic fatty acid methyl esters derived from linolenic acid

Peak[a]	Ring size	Ring position[b]	Double bond positions[b]	Double bond configuration[c]	Ring configuration	ECL values[d]			
						OV-275	CP-Sil 84	BPX70	CP-Wax 52CB
a	5	11–15	9, 12	*Z*	*trans*	18.43	18.9	18.8	18.38
b	5	10–14	12, 15	*E*	*trans*	18.29	18.8	18.61	18.46
c	5	11–15	9, 12	*E*	*trans*	18.32	18.83	18.68	18.46
d	5	10–14	12, 15	*Z*	*trans*	18.47	18.95	18.83	18.49
e	5	10–14	12, 15	*E*	*cis*	18.41	18.96	18.84	18.58
f	5	11–15	9, 12	*E*	*cis*	18.46	19.03	18.95	18.58
g	5	11–15	9, 12	*Z*	*cis*	18.93	19.47	19.39	18.84
h	5	10–14	12, 15	*Z*	*cis*	18.87	19.43	19.34	18.88
i	6	10–15	8, 12	*E*	*cis*	18.91	19.6	19.55	19.01
j	6	10–15	8, 12	*Z*	*trans*	19.04	19.68	19.64	19.01
k	6	10–15	8, 12	*E*	*trans*	19.07	19.7	19.65	19.12
l	6	10–15	8, 12	*Z*	*cis*	19.34	20.01	19.94	19.26
m	6	10–15	12, 16	*E*	*trans*	19.31	19.98	19.87	19.43
n	6	10–15	12, 16	*E*	*cis*	19.38	20.1	19.98	19.52
o	6	10–15	12, 16	*Z*	*trans*	19.59	20.27	20.21	19.6
p	6	10–15	12, 16	*Z*	*cis*	19.85	20.58	20.51	19.84
18:2($n-6$)						18.77	19.18	18.98	18.58

Structural assignments from Dobson et al. Ref. [6].
[a] According to labelling of peaks on gas chromatograph of total cyclic compounds (Fig. 1).
[b] Positions numbered according to original positions in linolenic acid.
[c] Double bond in straight-chain.
[d] ECL values determined isothermally at 140, 160, 180 and 170°C for OV-275, CP-Sil 84, BPX70 and CP-Wax 52 CB columns, respectively.

identifying and quantifying the components without having to first fractionate the mixture by HPLC.

2. Experimental

2.1. Materials and reagents

Standard fatty acid methyl esters were purchased from Sigma (Poole, UK).

2.2. Isolation of cyclic dienes

The cyclic fatty acid dienes were isolated from a linseed oil heated at 275°C under nitrogen [7]. Briefly, the oil was converted into fatty acid methyl esters and the non-polar fraction was isolated by column chromatography on silicic acid. This was submitted to urea fractionation; the adducts contained the usual fatty acids, while the non-adducted fraction contained a mixture of the cyclic fatty acids (96% pure) together with some linoleate.

Fractionation of the total cyclic dienes was achieved by silver ion HPLC as previously described [6].

2.3. Derivatization procedures

Butyl esters were prepared by base-catalysed trans-esterification of the methyl esters [8]. DMOX derivatives were prepared according to Fay and Richli [9]. They were purified through a small column of Florisil using hexane–acetone (94:6, v/v) as eluant.

2.4. Analytical gas chromatography

Fatty acid methyl esters were examined on a Hewlett-Packard 5890 Series II Plus (Hewlett-Packard, Wokingham, UK) capillary gas chromatograph, fitted with split/spitless injection and connected to a HP 3365 Series II Chemstation. The injector was used in the split mode with a split ratio of 50:1 and the flow rate was 1 ml/min. Hydrogen was the carrier gas. Several capillary columns were used; DB-5 (5% phenyl 95% dimethyl polysiloxane, 25

m×0.25 mm I.D., and 0.2-μm film thickness; J and W Scientific, Folsom, CA, USA), CP-Wax 52CB (100% polyethylene glycol 20M, 25 m×0.25 mm I.D. and 0.2-μm film thickness; Chrompack, London, UK), BPX70 (70% cyanopropyl 30% dimethyl polysilphenylene-siloxane, 50 m×0.22 mm I.D. and 0.25-μm film thickness; SGE, Milton Keynes, UK), CP-Sil 84 (90% cyanopropyl 10% phenyl polysiloxane, 25 m×0.22 mm I.D. and 0.2-μm film thickness; Chrompack) and OV-275 (dicyanoallylsilicone, 25 m×22 mm I.D. and 0.2-μm film thickness; Chrompack). Equivalent chain-length (ECL) values were calculated from isothermal runs at 140, 160, 170 and 180°C for OV-275, CP-Sil 84, CP-Wax 52CB and BPX70 columns, respectively. Temperature programming was used in practical separations.

2.5. Gas chromatography–mass spectrometry

Fatty acids were subjected to GC–MS in the form of DMOX derivatives on a Hewlett-Packard Model 5890 gas chromatograph attached to a Model 5970 Mass Selective Detector. A BPX70 (50 m×0.22 mm I.D. and 0.25–μm film thickness) capillary column was used isothermally at 180°C.

3. Results

The GC peaks corresponding to the sixteen cyclic dienes have been designated a–p. Peak q was unidentified but was not a cyclic diene. On a non-polar column (DB-5) the cyclic dienes were poorly separated into only ten peaks. On a CP-Wax 52CB column, with an initial temperature of 160°C for 5 min followed by a programme at 0.5°C/min to 175°C, peaks a, k, m, n, o and p were totally resolved, the pairs b+c/d and g/h, were partially resolved and pairs b/c, e/f and i/j were completely unresolved (Fig. 1A). Peak l was partially separated from minor unidentified peaks and additionally the pair e/f was only partially resolved from linoleate. At 130°C for 5 min, followed by a programme at 0.5°C/min to 165°C, linoleate was completely resolved from this pair, but h overlapped with the unidentified peak q, whereas at 170°C isothermal they were not at all resolved. Also, at the lower temperature, peak c eluted before the overlapping peaks b and d. The ECL values of all cyclic dienes were determined at 170°C and ranged from 18.38 to 19.84 (Table 1). For components that overlapped, precise ECL values were determined by running silver ion HPLC fractions composed of a limited number (1–5) of cyclic dienes. This approach was subsequently used for determining ECL values for other columns.

On CP-Sil 84 and BPX70 columns (Fig. 1B) the order of elution of many cyclic dienes was different from that on the CP-Wax 52CB (Fig. 1B). On the 25-m CP-Sil 84 column, with an initial temperature of 160°C for 3 min followed by a temperature programme at 2°C/min to 185°C, peaks f, i, n, o and p were completely resolved, a/d+e, b/c, g/h and l/m were present as partially resolved pairs and the pairs d/e and j/k overlapped completely. The unidentified peak, q, was not at all resolved from peaks g and h. Linoleate was completely resolved from all cyclic dienes. At an isothermal temperature of 150°C, peaks b and c, and l and n merged so that they were barely resolved, although the separation of g, h and q was enhanced. ECL values were calculated for the cyclic dienes run isothermally at 160°C and ranged from 18.80 to 20.58 (Table 1). Resolution, especially of peaks b and c, was improved, and q was completely separated from g and h, on a longer (50 m) column (BPX70) of similar polarity at an isothermal temperature of 160°C (Fig. 1B). An unidentified minor component appeared as a shoulder on peak n and, by comparison to the peak area on the CP-Wax 52CB column, a minor unidentified component must overlap with peak p. ECL values were calculated for cyclic dienes run isothermally at 180°C and ranged from 18.61 to 20.50 (Table 1).

On the most polar column tested (OV-275) the resolution was inferior to that of the other polar columns such that, at an isothermal temperature of 140°C, peaks b and c were poorly resolved, e and f overlapped with a and d respectively, g completely overlapped with, and h was poorly resolved from, i, and l overlapped with m and n. ECL values were determined at this temperature and ranged from 18.29 to 19.85 (Table 1).

Butyl esters were examined on CP-Sil 84 and CP-Wax 52CB columns and the pattern of elution of

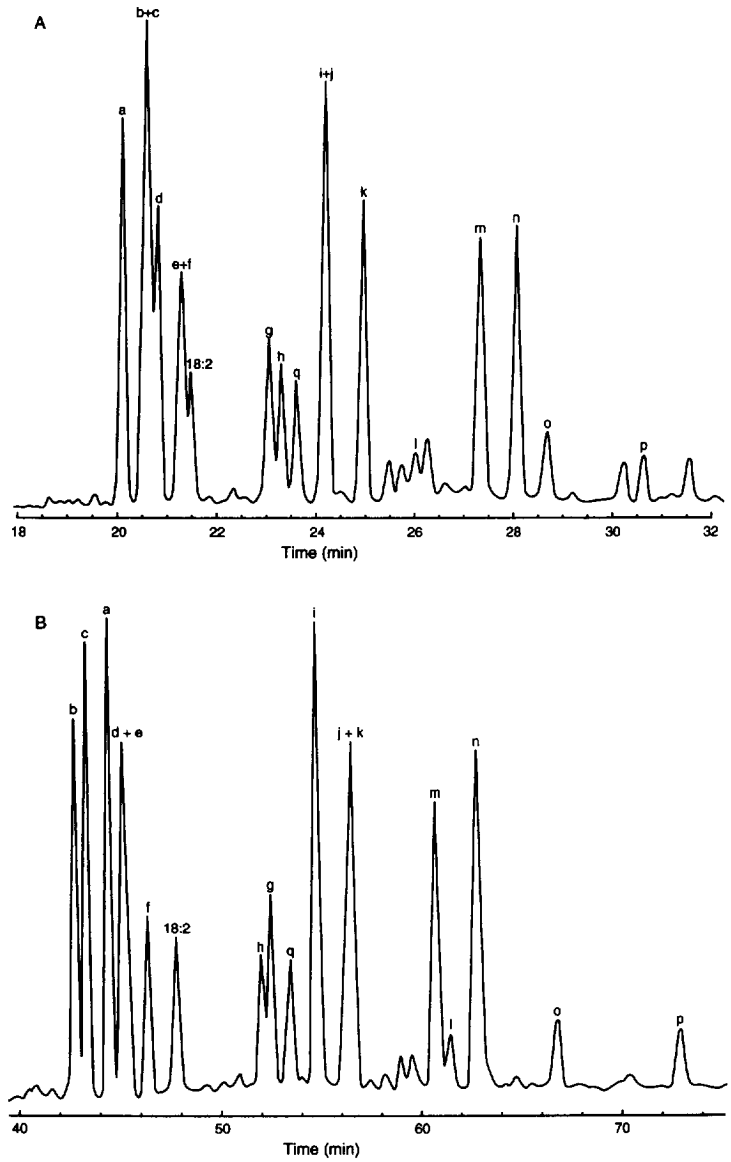

Fig. 1. Partial gas chromatographic traces of total cyclic fatty acid methyl esters derived from linolenic acid. Peaks are identified in Table 1. (A) CP-Wax 52CB (25 m) column. Initial temperature of 160°C for 5 min followed by a programme at 0.5°C/min to 75°C. (B) BPX70 (50 m) column. Isothermal temperature of 160°C.

cyclic dienes was essentially similar to those of methyl esters. On the CP-Sil 84 column, however, peaks g and h, and l and m were not resolved, under conditions (160°C for 3 min followed by a temperature programme at 2°C/min to 200°C) in which these pairs were partially resolved as methyl esters. Resolution of other peaks was similar to that for methyl esters. On the CP-Wax 52CB column (170°C for 3 min followed by a temperature programme at 2°C/min to 220°C), the separation of most peaks was similar to that for methyl esters except that e, f and linoleate were beginning to resolve although q was unresolved from i/j.

The basic structure of the majority of cyclic dienes

(ie. size and position of ring and positions of double bonds, but not configuration) was confirmed by GC–MS of the total cyclic dienes as DMOX derivatives on a BPX70 column. The mass spectra have been published previously [6]. The separations were similar in many respects to those for methyl esters on a similar column (Fig. 1B). Similar to methyl esters, peaks j and k overlapped and an unidentified component was unresolved from p. Also, peak f and linoleate, and g and h, which were at least partially resolved as methyl esters, did not separate as DMOX derivatives. However, whereas peaks d and e completely overlapped as methyl esters they were partially resolved as DMOX derivatives.

The percentage composition of the cyclic dienes was determined from analyses under two different conditions; on the BPX70 column isothermally at 160°C and on the CP-Wax 52CB with an initial temperature of 160°C for 5 min followed by a programme at 0.5°C/min to 175°C (Fig. 1). The areas of peaks a, b, c, d+e, f, g, h, i, j+k and l were measured on the former column and those of d, k, m, n, o and p were measured on the latter column. The areas from the two columns were correlated by using peak *a* as an internal standard. The areas of e and j were determined by the difference between d+e and d, and j+k and k, respectively. In Table 2, the results are compared to those previously determined by GC of silver ion HPLC fractions of the same cyclic diene sample in the presence of an internal standard [6].

Table 2
A comparison of cyclic dienoic acid compositions determined by GC and HPLC–GC

Peak	GC[a] (Mol%)	HPLC–GC[b] (Mol%)
a	10.6	10
b	8.2	7.2
c	9.8	9.6
d	7.4	7.1
e	3.5	3.7
f	4.1	4.4
g	5.0	5.3
h	3.5	3.4
i	12.5	12.5
j	1.8	2.2
k	9.4	10
l	1.5	1.6
m	9.1	9
n	9.3	9.8
o	2.6	2.4
p	1.7	1.8

[a] Calculated from GC of total cyclic dienes on CP-Wax 52CB and BPX70 columns. For GC conditions see Experimental.
[b] Data derived from Dobson et al. Ref. [6].

4. Discussion

Sixteen cyclic dienes, in a purified total cyclic diene fraction, from a heated linseed oil have recently been fully characterised [6]. In an examination of the GC properties of the methyl esters, in the present study, separation of all components was not achieved under any one set of conditions. Poor separations were attained on the non-polar (DB5) column and the very polar (OV-275) column. Different components were resolved on each of two polar columns, CP-Wax 52CB and BPX70 (or CP-Sil 84), but analyses on both columns could be used to quantify all cyclic dienes. The percent composition agreed well with that previously determined by GC, in the presence of an internal standard, of silver ion HPLC fractions of the cyclic dienes [6] (Table 2). Indeed, GC of the total cyclic dienes is a more rapid method and requires less sample than that involving prior HPLC fractionation.

For most of the cyclic dienes, separation was superior as their methyl esters compared to butyl esters, although better resolution of certain straight-chain positional isomers has been achieved with the latter derivatives [10].

Several structural features, namely the size, position and configuration of the ring, and position and configuration of the double bond in the chain, may influence the retention characteristics of the cyclic dienes. On both CP-Wax 52CB and BPX70 columns, cyclopentenyl fatty acids were retained less than cyclohexenyl fatty acids. Within the cyclopentenyl acids, all of those with a *trans* ring had shorter retention times than those with a *cis* ring whereas for cyclohexenyl fatty acids the major factor influencing retention was the position of the double bond in the chain. In this case, fatty acids with a double bond in position 8 were retained less than those with a double bond at position 16; an exception was the reversal of peaks l and m on the BPX70 column. Indeed for straight-chain octadecenoic acids, ECL

values increase as the double bond shifts from C-7 towards C-16 [11]. For the cyclopentenyl fatty acids, the 15-isomer usually eluted before the corresponding 9-isomer (a and d were the exception) on the BPX70 column, but there was no pattern on the CP-Wax 52CB column. It is perhaps suprising that the 9-isomer did not elute ahead of the 15-isomer. However a difference in double bond position was always accompanied by a difference in ring position which may also affect retention time.

For fatty acids differing only in the configuration of the double bond in the chain, in agreement with the behaviour of configurational isomers of straight chain fatty acids on polar columns [12], the *E* isomer eluted before the *Z* isomer in most cases; the difference in ECL between the members of a pair was surprisingly large. The exceptions were the pairs a/c and j/k where the orders were reversed on the CP-Wax 52CB; also j/k did not resolve on the BPX70 column. For fatty acids which differed only in the ring configuration, the *trans* isomer eluted before the *cis* isomer not only for the cyclopentenyl acids, as mentioned above, but also for most cyclohexenyl acids; i/k were the exception.

It is now intended to apply the GC method to metabolic studies in which small amounts of total cyclic dienes will be extracted and purified from tissue samples.

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References

[1] J.L. Sebedio and A. Grandgirard, Prog. Lipid Res., 28 (1989) 303.
[2] W.R. Michael, Lipids, 1 (1967) 359.
[3] W.R. Michael, Lipids, 1 (1967) 365.
[4] B. Potteau, P. Dubois and J. Rigaud, Ann. Technol. Agric., 27 (1978) 655.
[5] A. Zeman and H. Scharmann, Fette Seifen Anstrichm., 75 (1973) 32.
[6] G. Dobson, W.W. Christie, E.Y. Brechany, J.L. Sebedio and J.L. Le Quere, Chem. Phys. Lipids, 75 (1995) 171.
[7] J.L. Sebedio, J. Prevost and A. Grandgirard, J. Am. Oil Chem. Soc. 64 (1987) 1026.
[8] W.W. Christie, Gas Chromatography and Lipids, Oily Press, Ayr, 1989, Ch. 4, p. 70.
[9] L. Fay and U. Richli, J. Chromatogr. 541 (1991) 89.
[10] W.W. Christie, Lipid Technol., 7 (1995) 64.
[11] W.W. Christie, J. Chromatogr. 447 (1988) 305.
[12] A. Strocchi, Riv. Ital. Sostanze Grasse, 63 (1986) 99.