

GAS CHROMATOGRAPHIC AND SPECTROSCOPIC ANALYSIS OF EPOXIDISED CANOLA OIL

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ABSTRACT. Peroxyformic acid prepared *in-situ* was employed for epoxidation of canola oil in the presence of toluene. Gas chromatographic analysis of the product revealed the following species: C16:0; C18:0; C18:1; C18:2; C18:3; monoepoxy C18:0; monoepoxy C18:1; monoepoxy C18:2; diepoxy C18:0; diepoxy C18:1 and triepoxy C18:0, that were well separated and easily quantified. Result also revealed that epoxidation of canola oil was almost complete after 8 h using 4.0 moles of 85% formic acid in excess hydrogen peroxide. The ¹H and ¹³C NMR of the products are discussed.

KEY WORDS: Epoxidation, Canola oil, Epoxidised canola oil, Peroxyformic acid

INTRODUCTION

Since 1861, when Berthelot *et al.* described the preparation and characterization of epoxides, epoxy compounds have continued to attract continued interests [1]. Generally epoxidation can be defined as the formation of an oxirane group by olefinic or aromatic double bonds [2]. Epoxidised vegetable oil and esters derived from them have found important applications as stabilizers and plasticizers in polyvinylchloride and also as detergent and flavour precursors [3, 4]. Biological important oxiranes include leukotriene A (LTA), the biogenetic precursors of the leukotrienes LTC, LTD and LTE which are important natural mediators of allergic asthma [5]. Arene oxides have also been employed as intermediates in the metabolism of aromatic compounds to phenols [6]. Up till date vernolic acid (cis-12,13-epoxyoleic) is still the only natural epoxy acid of potential value and it occurs in high levels in seeds oil of *Vernonia anthelmintica*, *Vernonia galamensis*, *Stokesia laevis*, *Cephalocroton cirdofanus*, *Euphobia lagascae*, *Erlangea tomentosa*, *Crepis aura* and *Crepis biennis* [7]. However to meet demand for epoxidised oil, they are produced on an industrial scale from mainly soybean and linseed oil. Some other oils that have been investigated for production of epoxidised oils include safflower, cottonseed, olive and canola [8], sunflower [9], and rubberseed oil [10]. The composition of such epoxidised oils follows from that of the starting material when it is assumed that the oils are completely epoxidised and there is no residual unsaturation. Oleic, linoleic and linolenic esters are thus converted into mono-, di- and tri-epoxy esters. However in reality, residual unsaturation remains and different epoxy products are obtained. Literature review reveals that there has been little or no attempt to analyse the products in terms of their various epoxy acids. In this work, we report the epoxidation of canola oil and analysis of the epoxy products obtained with the aim of identifying and quantifying the different epoxy acids present in the epoxidised oil.

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EXPERIMENTAL*Materials*

Brand-named canola oil was purchased locally. The oil was flushed with nitrogen and stored at -20 °C until needed. Based on the bottle label, the oil was additive free. Tetramethylammonium hydroxide (TMAH, 25 wt % in methanol), CDCl_3 , HCOOH (99.7%), H_2O_2 (30%), were obtained from Merck (Darmstadt, Germany). Toluene was purchased from Fluka (Seelze, Germany).

Epoxidation of canola oil

Epoxidations of canola oil were carried out with peroxy formic acids generated *in situ*. The reaction mixtures used in the epoxidation reactions are as shown in Table 1.

Table 1. The reaction mixtures used in the epoxidation reactions of canola oil.

Experiment	HCOOH (mol)	Toluene (mL)	H_2O_2 (mL)	Oil (g)
A	2	50	100	10
B	2.5	50	100	10
C	3.0	50	100	10
D	4.0	50	100	10
E	5.0	50	100	10
F	7.0	50	100	10
G	10.0	50	100	10

10 g of canola oil was dissolved in 50 mL of toluene and calculated volumes of formic acid added while stirring at 10-15 °C. Hydrogen peroxide (30%), which had been pre-equilibrated at the same temperature, was then added while keeping the temperature between 10-15 °C. After addition of hydrogen peroxide, the reaction was allowed to continue at room temperature overnight. Reaction mixture was then allowed to settle and aqueous layer drawn off. The organic layer was repeatedly washed with warm water until free from acid. The remaining traces of water and toluene were removed under reduced pressure in a rotary evaporator. Having determined the optimum concentration of formic acid required for efficient epoxidation of the oil, the fixed concentration was employed while varying the period of epoxidation in order to determine the best conditions for epoxidation of canola oil.

Gas chromatography

Fatty acid methyl esters of the ordinary oil (FAMES) and the epoxidised oil (EFAMES) were prepared by shaking 50 mg of sample dissolved in 10 mL of toluene with 0.2 mL of TMAH. 4 mL of water was added, reaction mixture allowed to settle and 1 μL of the toluene layer injected into a Hewlett Packard GC (model CP 9000) equipped with split/splitless injection port and flame ionization detector and fitted with a J&W scientific fused silica column DB5, 25 x 0.25 mm, $\mu_f = 0.25 \mu\text{m}$. The injection port and detector temperatures were 250 °C and 280 °C, respectively. The oven temperature was programmed as follows; 120 °C for 3 min, then temperature increased at 4 °C/min till 260 °C and at 260 °C for 5 min. Identification of the peaks was done on a Hewlett Packard HP 5973 Mass Spectrometer (mass selective detector) interfaced with a HP 6890 series GC (Agilent Technologies, Waldron, GmbH). The mass spectrometer parameters used were electron impact energy 70 eV, transfer line 270 °C, source temperature

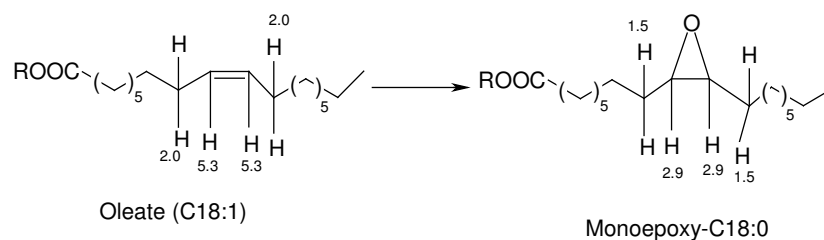
250 °C, emission current 0.5 mA, cycle time 0.6 sec/scan from 45 to 400 a.m.u, electron multiplier voltage 1.7 kV and pre-amp sensitivity 10^{-7} Amp/V.

Spectroscopic analysis

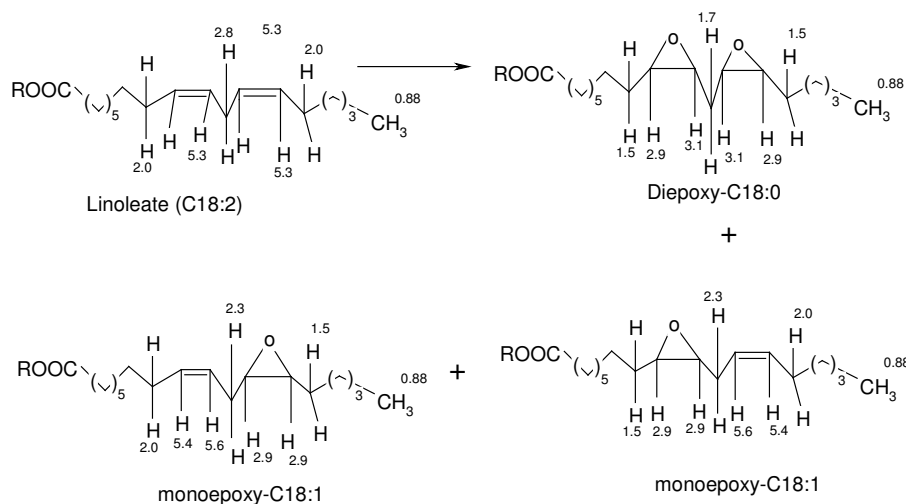
Infrared spectrum of the oil and epoxidised products (liquids) were recorded on a Fourier transform infrared spectrometer Tensor 27 FT/IR – H1026302 (Bruker optic, GmbH, Germany). The frequency and intensity of each band was obtained automatically by using the find peak command of the instrument OPUS software. ^1H NMR spectra at 400 MHz and ^{13}C NMR spectra at 100.6 MHz were obtained for the samples dissolved in deuteriated chloroform (CDCl_3) using a Bruker Avance-400 (Bruker Instrument, Inc. Karlsruhe, Germany) Fourier Transform spectrometer.

RESULTS AND DISCUSSION

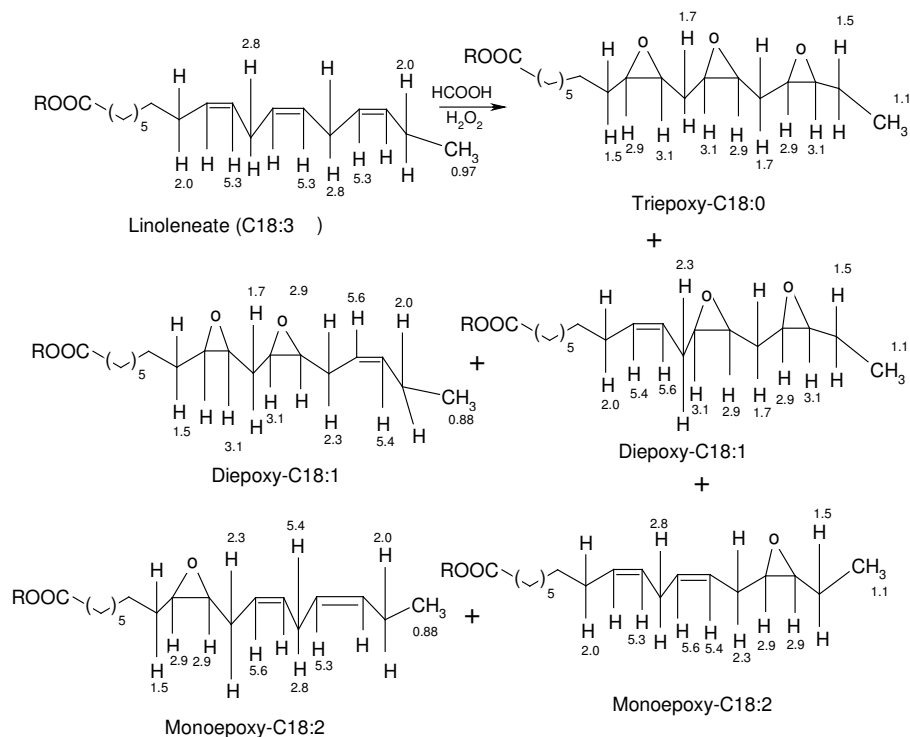
For oil like canola oil containing oleic, linoleic and linolenic esters, different products are possible on epoxidation. These products are illustrated in Schemes 1, 2 and 3 showing some possible epoxy compounds from oleate, linoleate and linolenate.



Scheme 1



Scheme 2



Scheme 3

N.B. Numbers attached to the protons indicate their region of absorption in the ^1H NMR spectra.

All epoxidation reactions were carried out in the presence of organic solvent (toluene and at room temperature. This was because degradation of the oxirane product has long been recognized as a serious problem in most epoxidation process. Findley *et al.* reported that the rate of ring opening of 9,10-epoxystearic acid was 1% per hour at 25 °C and 100% at 65-100 °C in 1-4 h and recommended that by-product formation could be minimized by operating at moderated temperature and by using a suitable solvent such as heptane or toluene [11]. Gan *et al.*, have also reported that presence of organic solvents appeared to have some stabilizing effects on oxirane formed by peroxyformic or peroxyacids epoxidation of oils [12]. Carrying out epoxidation reactions at room temperature and in presence of organic solvent therefore minimized side and bye products and ring opening reactions especially to hydroxyl compounds [9].

The GC-MS of methyl esters of epoxidised canola oil revealed the presence of the following: C16:0, C18:0, C18:1, C18:2, C18:3, monoepoxy C18:0, monoepoxy C18:1 (from C18:2), monoepoxy C18:2 (from C18:3), diepoxy C18:0, diepoxy C18:1 and triepoxy C18:0 which are well separated so that quantitation of each component is possible when needed. Table 2 presents the per cent composition of the FAME and EFAMES prepared using increasing concentration of formic acid. The results reveal that as the concentration of formic acid increased, the level of epoxidation also increased. Epoxidation was almost complete using 4.0 moles of formic acid. Increasing the concentration of formic acid further to 10.0 mF only caused a marginal increase, 0.5, 0.9 and 1.0% in the level of triepoxy, diepoxy and monoepoxy

products. The results further show that using very high concentration of formic acid did not overcome the problem of residual olefins as products like monoepoxy C18:1 from linolenic still persisted in the mixture. Based on these results, we would recommend epoxidation of canola oil with 4 moles of formic acid under the conditions described in the experimental.

Table 2. Effect of concentration of formic acid on the per cent composition of epoxidation products (EFAME) of canola oil.

C [*]	P	L	Ln	O	E	S	M _L	M _{Ln}	M	D _L	D _{Ln}	T	TE	Others
0 mF	5.1	19.6	9.3	59	4.0	1.7								
2.0 mF	5.1	0.9	-	4.0	-	1.7	5.9	5.5	56.1	12.9	3.0	0.7	84.1	4.3
2.5mF	5.1	0.7	-	2.5	-	1.7	4.9	4.6	58.0	13.6	3.5	0.9	85.5	4.5
3.0 mF	5.1	-	-	0.5	-	1.7	2.7	2.6	60.0	14.9	4.6	1.5	86.3	6.4
4.0 mF	5.1	-	-	-	-	1.7	1.9	1.8	62.0	16.6	5.5	2.1	89.9	3.3
5.0 mF	5.1	-	-	-	-	1.7	1.0	1.0	62.0	17.3	5.6	2.4	89.3	3.9
7.0 mF	5.1	-	-	-	-	1.7	0.8	0.8	63.0	17.3	5.6	2.4	89.9	3.3
10.0 mF	5.1	-	-	-	-	1.7	0.7	0.6	63.0	17.5	5.9	2.6	90.3	2.9

P, palmitic; L, linoleic; Ln, linolenic; O, oleic; E, elaidic; S, stearic; M_L, monoepoxy C18:1; M_{Ln}, monoepoxy C18:2; M, monoepoxy C18:0; D_L, diepoxy C18:0; D_{Ln}, diepoxy C18:1; T, triepoxy C:18:0; C^{*}, concentration (M) of HCOOH; TE, total epoxy products. All values are in percentage.

Using 4 moles of formic acid, we investigated the effect of time on the epoxidation of canola oil. The per cent composition of the FAME and EFAMES are presented in Table 3. The results show that it would require 30 min, 2 h and 4 h to produce the first traces of monoepoxy, diepoxy and triepoxy products in canola oil. The results further show no substantial difference in product composition for epoxidations carried out for 8 and 24 h.

Table 3. Effect of time on the per cent composition of epoxidation products (EFAME) of canola oil.

ET (h)	P	L	Ln	O	E	S	M _L	M _{Ln}	M	D _L	D _{Ln}	T	TE	Others
0	5.1	19.6	9.3	59	4	1.7	-	-	-	-	-	-	-	1.3
0.5	5.1	17.6	8.3	52.8	3.6	1.7	1.1	0.7	3.1	-	-	-	3.1	6.0
1	5.1	14.9	6.6	45.9	3.1	1.7	3.2	2.0	9.7	-	-	-	9.7	7.8
2	5.1	8.0	2.9	28.7	2.2	1.7	7.9	7.0	31.8	1.5	0.7	-	34	2.5
3	5.1	2.2	-	10.1	-	1.7	7.6	7.0	51.7	9.7	1.2	-	62.6	3.7
4	5.1	0.6	-	5.1	-	1.7	4.3	4.2	58.2	11.7	2.4	0.9	81.7	5.8
5	5.1	-	-	2.6	-	1.7	2.3	2.3	60.3	13.2	3.5	1.1	82.7	7.9
6	5.1	-	-	-	-	1.7	2.1	2.1	60.9	14.8	4.5	1.7	86.1	7.1
8	5.1	-	-	-	-	1.7	2.0	1.9	62	16.2	5.1	2.0	89.2	4.0
24	5.1	-	-	-	-	1.7	1.9	1.8	62	16.6	5.5	2.1	89.9	3.3

P, palmitic; L, linoleic; Ln, linolenic; O, oleic; E, elaidic; S, stearic; M_L, monoepoxy C18:1; M_{Ln}, monoepoxy C18:2; M, monoepoxy C18:0; D_L, diepoxy C18:0; D_{Ln}, diepoxy C18:1; T, triepoxy C18:0; TE, total epoxy products; ET, time (h) of epoxidation. All values are in percentage.

The infrared spectra of ordinary and epoxidised canola oil show the characteristic C-H stretching between 2800-3000 cm⁻¹. In the epoxidised oil the highest frequency of absorption of this group attributed to the vinyl C-H is absent. This indicates the total absence of vinyl groups in the epoxidised oil. Common to both oils are the carbonyl absorption at 1744 cm⁻¹, the characteristic triglyceride ester triplet at 1237, 1160 and 1097 cm⁻¹ and the sharp absorption at 724 cm⁻¹ assigned to C-H bending of saturated carbon chain more than seven carbon atoms long. The epoxidised oil in addition to the above shows a distinct doublet absorption at 823 cm⁻¹

characteristic of the C-O-C oxirane stretch usually observed in the naturally epoxidised vegetable oils.

The ^1H and ^{13}C NMR of ordinary canola oil and canola oil epoxidised using these optimal conditions (4.0 moles formic acid, 8 h, room temperature, and excess hydrogen peroxide) present various peaks. The peaks assignments of resonances obtained in the ^1H NMR of ordinary and epoxidised canola oil are presented in Table 4. Canola oil presents two sets of peaks from 4.0-4.4 ppm produced by the four methylene protons on the glycerol centre while the peak at 5.25 ppm is produced by the β -methine proton of glycerol. The peaks at 5.3 – 5.5 ppm are produced by olefinic protons. The peaks in the region 2.2-2.3 ppm indicate the presence of methylene groups next to carboxyl group. Peaks in the region 2.7-2.8 ppm correspond to methylene groups surrounded by double bonded carbon atoms. The huge peak at 1.2-1.4 ppm is due to methylene groups surrounded by single bonded atoms. Presence of linolenic acid is indicated by the peak at 0.97 ppm corresponding to the terminal homoallylic methyl groups of n-3 polyunsaturated fatty acids (PUFA). The chemical shift is slightly more downfield than other terminal methyl signals at 0.88 ppm which appears in saturated and non n-3 PUFA. ^1H NMR of epoxidised canola oil presents some new peaks in the region 2.9 – 3.2 ppm which indicate the presence epoxy group protons. The peak at 1.5 ppm corresponds to the presence of methylene group next to epoxy band while the peak at 1.7 ppm corresponds to methylene group sandwiched between two epoxy groups. The peaks at 1.0-1.1 ppm correspond to the terminal methyl of triepoxystearate. ^{13}C NMR spectrum of canola oil presents peaks at 172 -173 ppm (C-1); 125-133 ppm (C=C), 62 ppm (C-3 of esterified glycerol); 68 ppm (C-2 of esterified glycerol); 14 – 31 ppm (various CH_2) and ^{13}C NMR spectrum of epoxidised canola oil shows all these and in addition presents new peaks between 56-58 ppm ascribable to epoxy ring carbons.

Table 4. Peaks assignments of resonances obtained in the ^1H NMR of ordinary and epoxidised canola oil.

Absorption region (ppm)	Assignment
2.3 – 2.4	- CH_2COO^-
1.6	- $\text{CH}_2\text{-C-COO}^-$
1.2 – 1.4	- $\text{CH}_2\text{-C}$
1.95 – 2.1	- $\text{CH}_2\text{C} =$
2.7 – 2.8	= $\text{CCH}_2\text{C} =$
5.3 – 5.5	= CH
0.88	Terminal methyl
0.97	Terminal methyl homoallylic in linolenic fatty acid
4.0 – 4.4	Glycerol methylenes
5.25	Glycerol methine
2.9 – 3.2	Epoxy protons
1.5	CH_2 - epoxy
1.7	Epoxy -CH_2 - epoxy
1.0 – 1.1	Terminal methyl of triepoxystearate

CONCLUSION

An almost complete epoxidation of canola oil was effected by treating the oil with 4.0 moles of 85% formic acid in excess hydrogen peroxide for 8 h. The epoxidised oil did not suffer any oxirane ring cleavage to the hydroxylated oil by the method employed. Gas chromatographic analysis of the epoxidised oil revealed the following specific species in the oil; C16:0; C18:0; C18:1; C18:2; diepoxy C18:0; diepoxy C18.1; and triepoxy C18:0.

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