Large-Scale Synthesis of Methyl *cis*-9,*trans*-11-Octadecadienoate from Methyl Ricinoleate

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ABSTRACT: The conjugated linoleic acid methyl *cis*-9,*trans*-11-octadecadienoate has been prepared on a large scale from methyl ricinoleate. Methyl ricinoleate was purified from castor esters by a partition method. It was converted to the mesylate, which was reacted with a base (1,8-diazabicyclo[5.4.0]-undec-7-ene) to give a product that contained 66% of the desired ester. Two urea crystallizations produced a product containing 83% methyl *cis*-9,*trans*-11-octadecadienoate, the identity of which was confirmed by gas chromatography linked to mass spectrometry and by Fourier transform infrared spectroscopy. The remaining impurities were methyl *cis*-9,*cis*-11- and *cis*-9-,*trans*-12-octadecadienoate.

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KEY WORDS: Castor oil, conjugated linoleic acid (CLA), ricinoleic acid, synthesis.

cis-9,*trans*-11-Octadecadienoic acid (9*c*,11*t*-18:2), sometimes called conjugated linoleic acid (CLA), is the most abundant of the possible positional and geometric isomers that may exist in nature and is of interest because of its anticarcinogenic and anticholesterolemic properties (1–5). CLA is found naturally in milk, dairy products, and meat from ruminants because of its formation as an intermediate of biohydrogenation by anaerobic bacteria in the rumen (6–8). In addition, isomers of CLA are produced by free-radical-induced isomerization of linoleic acid (9) or during commercial hydrogenation of vegetable oils (10). 9*c*,11*t*-18:2 is believed to be the most biologically active CLA isomer (1,2).

Some commercial sources of CLA appear to be complex mixtures of isomers, produced by alkaline isomerization of linoleic acid. To further study the metabolic pathways and physiological effects of 9*c*,11*t*-18:2, it is desirable to obtain substantial amounts of this compound in a relatively pure form, ideally by simple methods from readily accessible materials. Here, the authors describe such a method, starting from methyl 12-hydroxyoctadec-*cis*-9-enoate (ricinoleate) of castor oil, by an approach adapted from Gunstone and Said (11).

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MATERIALS AND METHODS

Castor oil and chemical reagents were purchased from Sigma-Aldrich (Poole, Dorset, UK). Analytical thin-layer chromatography (TLC) was performed on 0.25-mm pre-coated silica gel plates that contained a fluorescent indicator (Merck, Darmstadt, Germany).

Analytical gas chromatography. Gas-chromatographic analyses were performed with a Hewlett-Packard HP 5890 Series II (Hewlett-Packard Ltd., Wokingham, United Kingdom), equipped with a splitless/split injector and a flame ionization detector. The temperature of both the injector and detector was 250°C. Hydrogen was the carrier gas. The analyses were performed with two columns (fused-silica capillary) of different polarities. The first was coated with BPX-70TM (SGE Ltd., Melbourne, Australia; 50 m × 0.33 mm i.d.), and the second with carbowax (Chrompack UK Ltd., London, 30 m × 0.25 mm i.d.). The oven temperature was programmed to increase from 60 to 170°C at 20°C·min^{-1} for the BPX column and from 170 to 220°C at 4°C·min^{-1} for the carbowax column.

Gas chromatography–Fourier transform infrared (GC–FTIR) spectroscopy. The gas-phase infrared spectra were obtained with a Bruker IFS 85 Fourier-transform infrared spectrometer, connected to a Carlo Erba (Massy, France) 5160 gas chromatograph, equipped with an on-column injector and a flame ionization detector. Both were maintained at 300°C. The BPX-70 capillary column was used. The interface consisted of a gold-coated light-pipe (20 cm × 0.8 mm i.d.) maintained at 250°C. Helium was the carrier gas. The oven temperature was programmed to increase from 60 to 250°C at 10°C·min^{-1}; it was then held isothermally to complete the analyses. The spectral resolution was fixed at 8 cm^{-1}, and 12 interferograms were collected per second.

GC–mass spectrometry (GC–MS). A Hewlett-Packard 5890 gas chromatograph, coupled to an HP model 5989 MS engine, was used for the GC–MS analyses. The latter was used in the electron impact mode at 70 eV with a source temperature of 250°C. The GC separation was performed on a BPX-70 capillary column as described previously, and helium was used as carrier gas. The oven temperature was programmed to increase from 60 to 190°C at 20°C·min^{-1}. Split-

less injection was used, with the injection port maintained at 250°C.

Transesterification of castor oil. The oil (100 g) was dissolved in dichloromethane (100 mL), and methanol (200 mL) containing fresh sodium (1 g) was added. The solution was refluxed for 10 min, then poured into water (500 mL) that contained 12N hydrochloric acid (16 mL). The aqueous layer was extracted with hexane (3 × 400 mL), and the hexane layer was washed with water (200 mL) that contained potassium bicarbonate (2%) and then dried over anhydrous sodium sulfate.

Isolation of methyl ricinoleate by countercurrent distribution (CCD). Hexane and 90% aq. methanol were shaken together to obtain equilibrated layers. The upper layer (3 × 600 mL) and lower layer (12 × 300 mL) were used in three separatory funnels for the separation of 100 g fatty acid methyl esters (FAME).

FAME (100 g) was shaken with 600 mL hexane and 300 mL 90% aq. methanol in the first separating funnel. The layers were separated, and the methanolic phase was removed. The hexane phase was washed with fresh 90% aq. methanol. The methanol (mobile) phases were sequentially passed through two more separating funnels, each containing 600 mL hexane (stationary phase). On the basis of a TLC examination, 12 of these methanolic phases were collected. The first 10 were combined to give fraction 1, and the eleventh and twelfth to give fraction 2; these were concentrated under reduced pressure on a rotary film evaporator (50°C). A turbid oil was formed in each case. Oil from fraction 1 was extracted with hexane (3 × 500 mL), water (850 mL) and saturated brine (250 mL). The combined organic layers were dried and concentrated under vacuum with a rotary evaporator (40°C) to yield 86.3 g esters, containing 98.5% methyl ricinoleate. The oil from fraction 2 was similarly extracted to give 2.1 g esters, containing 92% methyl ricinoleate and 8% other esters (palmitate, stearate, oleate, linoleate, and linolenate). GC analysis of the three combined hexane phases showed 2.4% methyl ricinoleate and 97% normal esters (palmitate, stearate, oleate, linoleate, and linolenate).

Preparation of 12-mesyloxyoleate. Methyl ricinoleate (80 g, 256 mmol) was stirred with methanesulfonyl chloride (65 mL, 734 mmol) and pyridine (400 mL) for 2 h at 0°C and then for 2 h at 5–10°C. Ice-cold hydrochloric acid (3 M, 1.2 L) was then added slowly with further cooling. The resulting solution was extracted by diethyl ether (3 × 700 mL) to yield methyl 12-mesyloxyoleate (89.5 g, 229 mmol, 89.4% yield) as a straw-colored oil.

Preparation of methyl octadeca-9,11-dienoates. A mixture of methyl 12-mesyloxyoleate (89 g, 228 mmol), toluene (350 mL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (145 mL) was refluxed for 4 h. The resulting solution was cooled, neutralized with acetic acid, diluted with water (600 mL), and extracted with diethyl ether (3 × 500 mL) to yield a mixture (57.5 g, 195 mmol, 85.1% yield).

Urea adduct formation. FAME (110 g) was dissolved in a hot solution of urea in methanol (110 g urea/550 mL methanol). After cooling under nitrogen with occasional swirling, the flask was left overnight at 4°C. The urea adduct and nonadduct fractions were then separated by filtration through a Buchner funnel. The crystals were washed twice with portions of methanol saturated with urea. The mother liquor was poured into a 1% HCl solution (600 mL) and extracted with hexane (3 × 500 mL). The combined organic layers were washed twice with water (50 mL) and dried over anhydrous sodium sulfate before the solvent was removed under reduced pressure. The adduct esters were recovered by breaking up the complex with a warm 1% HCl solution (300 mL); this was cooled before being extracted twice with hexane (250 mL), dried, and concentrated under reduced pressure. FAME from each fraction was dissolved in hexane for GC analysis.

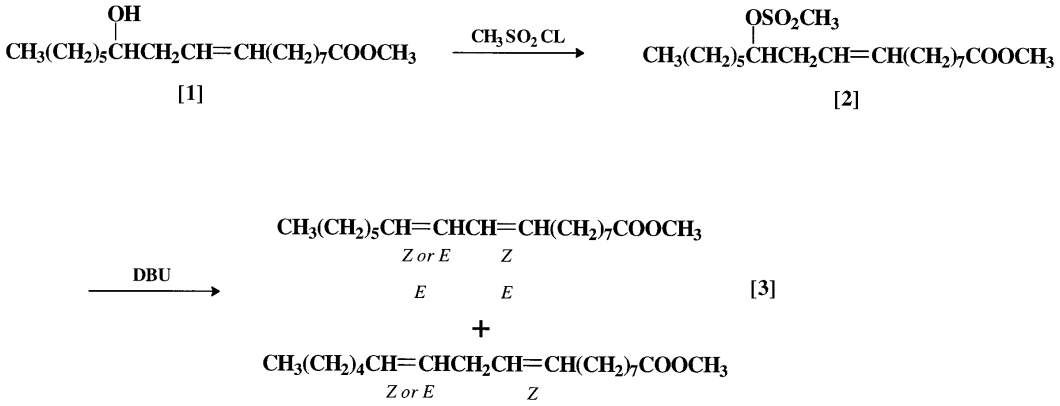
Preparation of 4,4-dimethyloxazoline (DMOX) derivatives. FAME (500 mg) was converted to the DMOX derivatives by treatment with 2-amino-2-methylpropanol (0.25 mL) in a sealed ampoule at 170°C for 8 h (12–14). The reaction mixture was cooled, dissolved in 3 mL of dichloromethane, and washed twice with 1 mL water. After drying the organic phase, the solvent was removed under a stream of N_2, and the sample was dissolved in hexane. The sample was applied to a short column of Florisil that was subsequently washed with hexane prior to elution of the DMOX derivatives with a mixture of hexane–acetone (96:4, vol/vol) (15).

Reaction of 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) with conjugated double bonds. The reaction of MTAD with a conjugated diene was carried out by simply dissolving both in dichloromethane and mixing them at 0°C. The reaction was nearly instantaneous and was stopped immediately with a fivefold excess of 1,3-hexadiene (Dobson, unpublished). The solvent was removed in a stream of nitrogen, and the sample was dissolved in hexane for GC-MS analysis.

RESULTS AND DISCUSSION

Purification of methyl ricinoleate from castor oil. GC analysis of the transesterified castor oil revealed a mixture of 85.3% methyl ricinoleate, 1.4% 16:0, 1.0% 18:0, 3.4% 18:1, 5.7% 18:2, and 0.6% 18:3. Although pure methyl ricinoleate **[1]** could be isolated from transesterified castor oil on a silica chromatographic column by using a mixture of isohexane–diethyl ether (75:25) as the developing solvent, or by preparative HPLC (11,16), these methods are only suitable on the milligram to gram scale. Recently, Tassignon *et al.* (17) have used an efficient CCD method for large-scale isolation of dimorphecolic acid methyl ester [*S*(+)9-hydroxy-10*t*,12*t*-octadecadienoate] with a 90% aq. methanol–hexane solvent system. The authors applied this method to purify methyl ricinoleate from castor esters quickly on a large scale (100 g). Only 12 methanolic phases passed sequentially through three separatory funnels with hexane; these yielded 86 g methyl ricinoleate with a purity of 98.5%.

Preparation and purification of methyl octadeca-9c,11t-dienoate **[3]**. The reaction scheme adopted for the synthesis



SCHEME 1

of 9*c*,11*t*-18:2 is shown in Scheme 1. As a first step, the hydroxy group was modified to become a better leaving group, and methyl ricinoleate was transformed to the mesylate **[2]** with a good yield (80%) (11,16). Methyl 12-mesyloxyoleate can undergo competitive elimination or substitution, depending on the experimental conditions. Gunstone and Said (11) showed that elimination was the dominant reaction, and conjugated and nonconjugated methyl octadecadienoates were the major reaction products with an appropriate base. In particular, heating for 12 h with a polycyclic base 1,8-diazabicyclo[5.4.0]undec-5-ene (DBU) or 1.5-diazabicyclo[4.3.0]-undec-non-5-ene (DBN) gave 100% elimination and mainly the 9*c*,11*t*-octadecadienoate isomer, though a reaction time of 4 h may be sufficient (16). In this work, after 4 h at 110°C in toluene, a mixture containing mainly 9*c*,11*t*-18:2 (**A**, 66%), accompanied by 9*c*,11*c*- (**B**, 20.7%), 9*t*,11*t*- (**C**, 0.6%), 9*c*,12*t*- (**D**, 6.6%), and 9*c*,12*c*- (**E**, 1.5%) 18:2 isomers was obtained (Table 1). Increasing the reaction time above 4 h did not modify the ratio between the different isomers, and only a slight increase in the amount of 9*t*,11*t*-18:2 was noticeable. However, 4 h were necessary to obtain complete reaction of the 12-mesyloxoleate. The ratio of formation of 11*t*, relative to the 11*c* double bond, was close to that expected from simple thermodynamic considerations.

Structures of the different compounds were confirmed by GC–MS and GC–FTIR spectroscopy. An aliquot of the mixture was converted to DMOX derivatives, and another aliquot was used for the Diels-Alder reaction with MTAD. GC–MS analyses of the DMOX derivatives of the conjugated isomers **A**, **B**, and **C** gave the same spectra with an intense molecular ion (*m/z* 333) and the characteristic fragments shown in Figure 1. For example, a mass interval of 12 units (instead of 14) occurred between *m/z* 196 (C8) and 208 (C9) and between 222 (C10) and 234 (C11), indicating the presence of conjugated double bonds in the 9 and 11 positions. Intense ions at *m/z* 182 (C7), 262 (C13), and 276 (C14) are also characteristic (18). DMOX derivatives from compounds **D** and **E** gave spectra resembling those of 9,12-18:2 (14). To confirm the positions of the conjugated double bonds for compounds **A**, **B**, and **C**, the authors used 4-methyl-1,2,4-triazoline-3,5-dione (MTAD), which reacts selectively with conjugated systems to form a Diels-Alder adduct. Mass spectra of the adducts gave abundant fragment ions (Fig. 2), that result from the loss of an alkyl side-chain, adjacent to the ring structure; the fragments are diagnostic for the position of the conjugated diene system in the hydrocarbon chain. The latter gave rise to ions, $[M - R]^+$ and/or $[M - R']^+$, depending on the nature of the alkyl substituents (19). Thus, GC–MS spectra from the MTAD derivatives of **A**, **B**, and **C** gave the molecular ion (M+) at *m/z* 407, as expected for the MTAD adduct of methyl octadecadienoate, and abundant fragments at *m/z* 322 ($[M - 85]^+$) and 250 ($[M - 157]^+$), for fragmentation alpha to the ring structure, showing that the original conjugated double bonds were in positions 9 and 11 of the hydrocarbon chain (Dobson, unpublished). The large peak at *m/z* 290 represents loss of methanol from the ion at *m/z* = 322.

The mixture was also submitted to GC–FTIR spectroscopy. Three isomeric methyl octadeca-9,11-dienoates were readily distinguished by differing adsorption in the region 900–1000 cm^{-1}, that is, **A** = *cis*, *trans*, 985 and 950 cm^{-1}, **B** = *cis*, *cis*, negligible, and **C** = *trans*, *trans*, 988 cm^{-1} (20). Compound **D** was linoleic acid, and compound **E** was a geometrical isomer, 9*c*,12*t*-18:2.

Two successive urea crystallizations were employed to enrich 9*c*,11*t*-18:2 from its geometric and positional isomers. In particular, the technique allowed for the elimination of 35% of 9*t*,11*t*-18:2 and 79% of 9*c*,11*c*-18:2; molecular models confirmed that these were more linear than 9*c*,11*t*-18:2. Hence, when a ratio of urea/FAME equal to 1:1 (w/w) was used, 9*t*,11*t*-18:2 and 9*c*,12*c*-18:2 formed crystalline adducts, while 9*c*,11*t*-18:2 primarily remained in the mother liquor.

TABLE 1
Fatty Acid Composition (wt% of the total) of the Reaction Product and of the Materials Purified by Urea Adduction

	9*c*,11*t*	9*c*,11*c*	9*t*,11*t*	9*c*,12*t*	9*c*,12*c*
Reaction product	66.0	20.7	0.6	6.6	1.5
First urea adduct	75.0	20.7	0.4	6.9	1.7
Second urea adduct	83.0	4.4	0.4	6.7	2.4

FIG. 1. Electron impact mass spectrum of 4,4-dimethyloxazoline (DMOX) derivative of 9*c*,11*t*-18:2 (**A**).

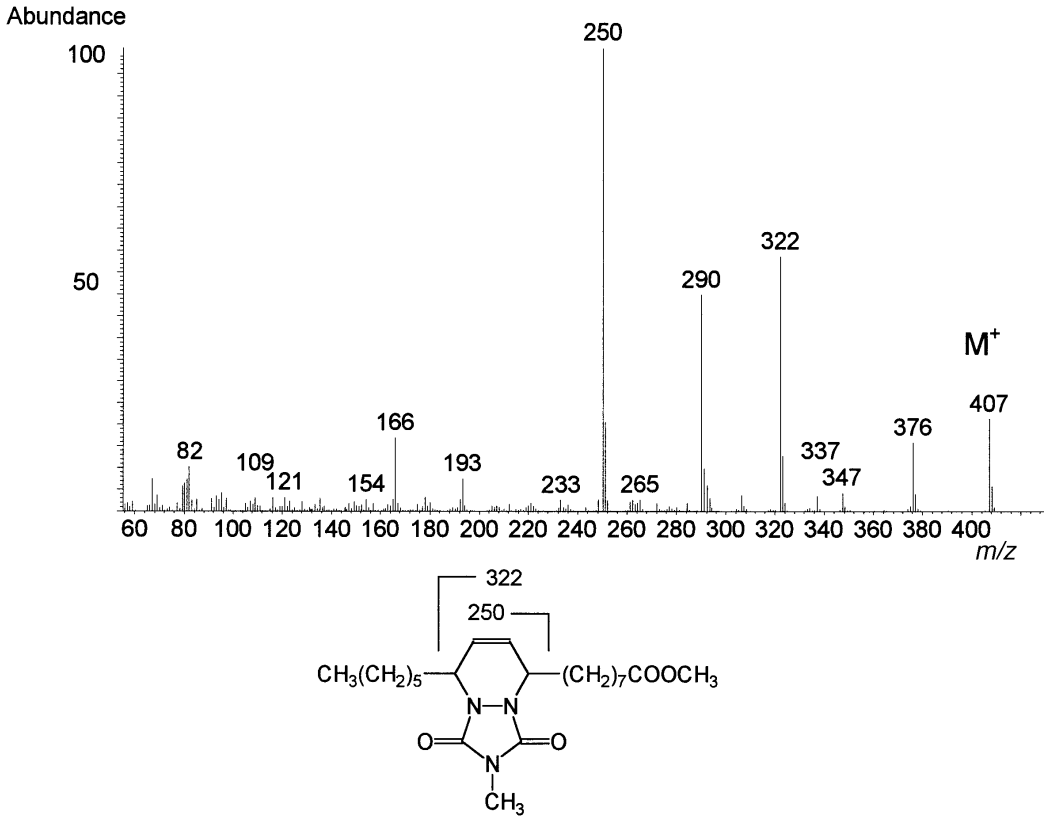


FIG. 2. Mass spectrum of MTAD derivative of 9*c*,11*t*-18:2 (**A**).

Thus, urea complexation with 110 g of FAME and 110 g of urea gave 87 g of a nonadduct fraction and 22 g of an adduct fraction. GC analysis of the nonadduct fraction showed an increase in the amount of 9*c*,11*t*-18:2 to 75%, with other components as listed in Table 1, while the adduct fraction contained only 38.4% 9*c*,11*t*-18:2. A second urea complexation with 87 g of FAME and 87 g of urea gave 55 g of a nonadduct fraction and 28 g of adduct fraction. GC analysis of the nonadduct fraction showed that 9*c*,11*t*-18:2 comprised 83.0% of the total (compared to 59% in the adduct).

In conclusion, the authors have developed a simple and rapid method to prepare 9*c*,11*t*-18:2 from methyl ricinoleate on a sufficient scale for nutritional experiments. The distribution of the 9*c*,11*t* isomers in this fraction (9*c*,11*t*-18:2, 83%; 9*c*,11*c*-18:2, 4.4%; and 9*t*,11*t*, 0.4%) is similar to what is usually found in dairy products (21). This fraction will be of great interest to test the biological activity of such a mixture, considering that previous studies were effected on fractions that did not reflect the isomeric composition found in dairy products.

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REFERENCES

1. Ha, Y.S., J. Storkson, and M.W. Pariza, Inhibition of Benzo(a)pyrene-Induced Mouse Forestomach Neoplasia by Conjugated Dienoic Derivatives of Linoleic Acid, *Cancer Res. 54*:1097–1101 (1990).
2. Ip, C., S.F. Chin, J.A. Scimeca, and M.W. Pariza, Mammary Cancer Prevention by Conjugated Dienoic Derivatives of Linoleic Acid, *Ibid. 51*:6118–6124 (1991).
3. Ip, C., S.F. Chin, J.A. Scimeca, and H.J. Thompson, Conjugated Linoleic Acid. A Powerful Anticarcinogen from Animal Fat Sources, *Cancer Suppl. 74*:1050–1054 (1994).
4. Nicolosi, R.J., K.V. Courtemanche, L. Laitinien, J.A. Scimeca, and P.J. Huth, Effect of Feeding Diets Enriched in Conjugated Linoleic Acid on Lipoproteins and Aortic Atherogenesis in Hamsters, *Circulation 88*:Suppl. 2458 (1993).
5. Lee, K.N., D. Kritchevsky, and M.W. Pariza, Conjugated Linoleic Acid and Atherosclerosis in Rabbits, *Atherosclerosis 108*:19–25 (1994).
6. Kepler, C.R., and S.B. Tove, Biohydrogenation of Unsaturated Fatty Acids, *J. Biol. Chem. 242*:5606–5692 (1967).
7. Hughes, P.E., W.J. Hunter, and S.B. Tove, Biohydrogenation of Unsaturated Fatty Acids: Purification and Properties of *cis*-9, *trans*-11 Octadecadienoate Reductase, *Ibid. 257*:3643–3649 (1982).
8. Chin, S.F, J.M. Storkson, W. Liu, K.J. Albright, and M.W. Pariza, Conjugated Linoleic Acid (9,11- and 10,12-Octadecadienoic Acid) Is Produced in Conventional But Not Germ-Free Rats Fed Linoleic Acid, *J. Nutr. 124*:694–701 (1994).
9. Cawood, P., D.G. Wickens, S.A. Iversen, J.M. Braganza, and T.C. Dormandy, The Nature of Diene Conjugation in Human Serum, Bile and Duodenal Juice, *FEBS Lett. 59*:280–283 (1983).
10. Banni, S., B.W. Day, R.W. Evans, F. Corongiu, and B. Lombardi, Liquid Chromatographic-Mass Spectrometric Analysis of Conjugated Diene Fatty Acids in a Partially Hydrogenated Fat, *J. Am. Oil Chem. Soc. 71*:1321–1325 (1994).
11. Gunstone, F.D., and A.I. Said, Methyl 12-Mesyloxyoleate as a Source of Cyclopropane Esters and of Conjugated Octadecadienoates, *Chem. Phys. Lipids 7*:121–134 (1971).
12. Fay, L., and U. Richli, Localisation of Double Bond in Polyunsaturated Fatty Acids by Gas Chromatography-Mass Spectrometry After 4,4-Dimethyloxzoline Derivatisation, *J. Chromatogr. 541*:89–98 (1991).
13. Luthria, D.L., and H. Sprecher, 2-Alkenyl-4,4-Dimethyloxazolines Derivatives for the Structural Elucidation of Isomeric Unsaturated Fatty Acids, *Lipids 28*:561–564 (1993).
14. Zhang, J.Y., Q.T. Yu, B.N. Liu, and Z.H. Huang, Chemical Modification in Mass Spectrometry IV. 2-Alkenyl-4,4-Dimethyloxalines as Derivatives for the Double Bond Location of Long-Chain Olefinic Acids, *Biomed. Mass. Spectrom. 15*:33–44 (1988).
15. Dobson, G., and W.W. Christie, Structural Analysis of Fatty Acids by Mass Spectrometry of Picolinyl Esters and Dimethyloxazoline Derivatives, *Trends Anal. Chem. 15*:130–137 (1996).
16. Bascetta, E., F.D. Gunstone, and C.M. Scrimgeour, Synthesis, Characterisation, and Transformation of a Lipid Cyclic Peroxide, *J. Chem. Soc. Perkin Trans. I*:2199–2205 (1984).
17. Tassignon, P., P. de Waard, T. de Rijk, H. Tournois, D. de Wit, and L. de Buyck, An Efficient Countercurrent Distribution Method for the Large-Scale Isolation of Dimorphecolic Acid Methyl Ester, *Chem. Phys. Lipids 71*:187–196 (1994).
18. Spitzer, V., F. Marx, and K. Pfeilsticker, Electron Impact Mass Spectra of Some Conjugated Diene and Triene C_{18} Fatty Acids, *J. Am. Oil Chem. Soc. 71*:873–876 (1994).
19. Young, D.C., and P. Vouros, Gas Chromatography–Mass Spectrometry of Conjugated Dienes by Derivatization with 4-Methyl-1,2,4-Triazoline-3,5-Dione. *J. Chromatogr. 522*: 295–302 (1990).
20. Hopkins, C.Y., Fatty Acids with Conjugated Unsaturation, *Topics in Lipid Chemistry,* edited by F.D. Gunstone, Elek Books, London, 1972, Vol. 3, pp. 37–85.
21. Ha, Y.S., N.K. Grimm, and M.W. Pariza, Newly Recognized Anticarcinogenic Fatty Acids: Identification and Quantification in Natural and Processed Cheese, *J. Agric. Food Chem. 37*: 75–81 (1989).

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