Cis-Trans Isomerization of Unsaturated Fatty Acid Methyl Esters Without Double Bond Migration

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ABSTRACT

Trans isomerization of monoenoic and dienoic fatty acid methyl esters has been carried out with thiols and diphenylphosphine in the presence of azobisisobutylnitrile. The equilibrium mixture contained 75-80% *trans* double bonds and there was no migration of the double bonds.

INTRODUCTION

Unsaturated fatty acids can be isomerized from the *cis* to the *trans* configuration by various catalysts, including univalent atoms, molecules with odd electrons, free radicals and paramagnetic substances in general (1,2). Both free radical and ionic mechanisms have been suggested and the formation of addition products and transitional forms as intermediates has been postulated. These intermediates are free to rotate about the axis of the former double bond. After such rotation, dissociation from the catalyst permits reestablishment of the double bond with consequent formation of the geometrical isomer. Side reactions concurrent with the *cis-trans* isomerization include addition (1,3), polymerization (4), hydrogen transfer and shifting of the double bond (5-7). Several studies have demonstrated that reagents isomerizing unsaturated fatty acids differ greatly in their selectivity towards the formation of by-products. Recently, Gunstone and Ismail (7) used thiyl radicals produced by photolysis of diphenyl sulfide and prepared *trans* octadecenoic acids free from positional isomers. In the present paper, monoenoic and dienoic fatty acid methyl esters have been isomerized *cis-trans* with thiyl or phosphinyl radicals produced by radical initiators. The method has been shown to give efficiently and cleanly a definitive *cis-trans* equilibrium and to be totally free of double bond migration. Other advantages that may be cited are: mild reaction conditions with a high yield of product, ready applicability of the reaction to small scale preparation of labeled compounds and simplicity of procedure.

MATERIALS AND METHODS

Methyl palmitate, oleate, petroselenate, linoleate, *cis* vaccenate, elaidate and 11-*cis*, 14-*cis* eicosadienoate were purchased from the Hormel Institute, Austin, Minnesota. Gas liquid chromatography (GLC) indicated that they contained less than 1% impurities. Thin layer chromatography (TLC) on Silica Gel G impregnated with silver nitrate (8), also showed that these esters were pure. Thiophenol, diphenyl sulfide and dodecanethiol were supplied by Aldrich Chemical Co., Milwaukee, Wis., and diphenylphosphine by Orgmet, Inc., Hampstead, N.H. These reagents were fractionally distilled and a center cut used in this study. Solvents were A. R. grade and distilled before use. Azobisisobutylnitrile (AIBN) was used as received (Eastman Chemical Products, Inc. Rochester, N.Y.) 1-^{14}C-oleic and 1-^{14}C-linoleic acids (specific activity 9.0 mc/mmole and 16.1 mc/mmole, respectively) were purchased from Tracerlab, Waltham, Mass. They were purified by chromatography on acid-treated Florisil (9).

The reactions were carried out in glass vials having constricted necks for sealing. In a typical experiment (Exp. 2, Table I), 3 mg of AIBN, 0.2 ml of benzene, 110 mg (1 mmole) of thiophenol, 296 mg (1 mmole) of methyl oleate and 50 mg methyl palmitate, serving as a nonreactive internal standard, were placed in a vial. The reaction mixture was then deaerated by freezing, evacuating and thawing, sealed under vacuum, placed in a constant temperature sand bath preheated to 65 C and maintained at this temperature. Similar conditions were used for other experiments (Table I). At a given time interval, each sample was removed from the bath, cooled, an equivalent amount of silver nitrate in aqueous solution was added to precipitate the thiophenol or diphenylphosphine and the methyl esters were extracted with petroleum ether. They were passed through a silicic acid column and subjected to further analysis. In certain cases the methyl esters were purified by preparative GLC (10% Apiezon L on Celite 545) prior to their analysis.

GLC employing packed columns (15% Apiezon L in chromosorb W 100-200 mesh treated with hexamethyl disilazane at 210 C, or 10% diethylene glycol succinate on chromosorb W 60-80 mesh at 165 C) indicated depletion of catalyst and absence of side reaction products.

The relative amounts of *cis* and *trans* isomers were conveniently measured by capillary GLC using either 200 ft Apiezon L, or diethylene

TABLE I

Cis, Trans-Isomers Equilibria of Unsaturated Fatty Acid Methyl Esters by Thiyl and Phosphinyl Radicals

Exp.	Methyl ester[a]	Reagent[a]	Solvent[a]	Temp. C	Time hr	Isomer distribution % by GLC[b] All *trans*	*cis, trans*	All *cis*	Double bond movement[c]
1	Oleate	Thiophenol	None	65	6	79.		20.8	~0
2	Oleate	Thiophenol	Benzene	65	6	77.6		22.	~0
3	Oleate	Thiophenol	Cyclohexane	65	6	79.8		18.9	~0
4	Oleate	Thiophenol	CCl_4	65	6	81.5		19.2	~0
5	Oleate	Diphenyl-phosphine	Benzene	75	6	79.1		21.3	~0
6	Oleate	Diphenyl-phosphine	CCl_4	75	6	78.1		20.9	~0
7	Oleate	Dodecane-thiol	Benzene	65	6	50.8		49.0	~0
8	Oleate	Diphenyl sulfide	Benzene	65	6	3.6		96.2	~0
9	Petroselenate	Thiophenol	Benzene	65	6	78.5		23.2	~0
10	Vaccenate (*cis*)	Thiophenol	Benzene	65	6	77.6		21.5	~0
11	Elaidate	Thiophenol	Benzene	65	6	76.8		23.5	~0
12	Linoleate	Thiophenol	Benzene	65	8	50.8	40.2	10.5	<2%
13	Linoleate	Thiophenol	Cyclohexane	65	8	50	38.7	12.1	<2%
14	Linoleate	Diphenyl-phosphine	Benzene	75	8	49.5	39.8	11.3	<2%
15	Eicosa-9,12-dienoate	Thiophenol	Benzene	65	8	48	40	12.8	<2%

[a]The reaction mixture contained: 1mmole of reagent per 1 equivalent double bond, 0.2 ml solvent and 3 mg of AIBN.

[b]Expressed as percentage of the total area under the peaks on the chromatogram tracing.

[c]Migration by ozonolysis.

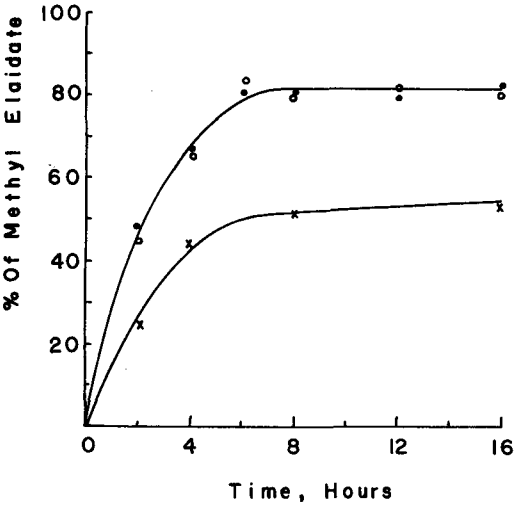

FIG. 1. *Trans* isomerization of methyl oleate with thiophenol (●--●), diphenylphosphine (o--o) and dodecanethiol (x--x). Experiments 2, 5 and 7 (Table I), respectively.

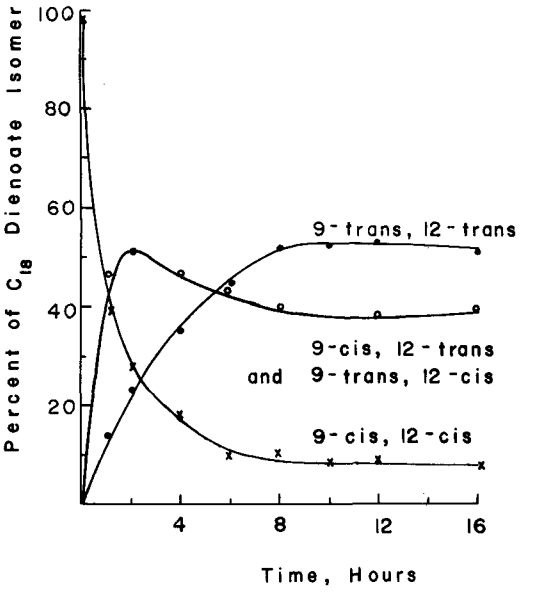

FIG. 2. Isomer composition of randomly *cis-trans* isomerized methyl linoleate using thiophenol catalyst. Experiment 12 in Table I.

glycol succinate capillary columns (10,11).

In addition, GLC provided a quantitative estimate of the isomerized fatty acid methyl esters. Methyl palmitate was used as an internal standard and calibration curves of methyl palmitate and oleate were constructed based on their corresponding peak areas in chromatograms obtained by GLC. From the slope of the calibration curve and the known weight of added methyl palmitate, the amount of *cis* or *trans*, or both, in any given sample could be determined in the reaction products. Alternately, the amount of isolated *trans* bonds was determined in KBr pellets from the absorbance measurements of 675 cm^{-1} (*cis*) and 962 cm^{-1} (*trans*) on an IR spectrometer using a calibration curve.

In order to determine the position of the double bond(s), methyl esters were cleaved to aldehydes and aldehydo-esters by reductive ozonolysis (12). Cleavage products were identified by TLC and quantified by temperature programmed GLC (12). When required, configurational isomers were separated by argentation chromatography either on columns (13) or plates (8).

Radioactive samples were isomerized in the same way and the reaction products analyzed by argentation chromatography (8,13). In a typical experiment conducted in a capillary tube, 1 μmole of methyl 1-^{14}C-linoleate (16.1 μC/μmole) reacted with 2 μmoles thiophenol in 10 μl benzene in the presence of a small crystal of AIBN. The tube was sealed and heated in a sand bath at 65 C for 8 hr. The sample was taken up in hexane and a small aliquot was mixed with 50 mg of methyl linoleate isomerized under similar conditions (Exp. 12, Table I) and the mixture was subjected to column argentation (13) and thin layer argentation (8) chromatography. Radioactivity was monitored in the column effluent and in the Silica Gel scrapings. Counts were made in a scintillation counter.

RESULTS AND DISCUSSION

The results of the rate studies with equivalent amounts of methyl oleate and thiophenol, dodecanethiol or diphenylphosphine are shown in Figure 1. The per cent of isolated *trans* bonds were determined from IR and capillary GLC data. Thiophenol gave a much faster reaction over dodecanethiol, probably due to the composite radical formed from addition of the thiol to the double bond (15,16) which was resonance stabilized in the case of thiophenol. By analogy diphenylphosphine gave similar rates to thiophenol. After 5 hr an oleic:elaidic acid equilibrium was obtained and the final product contained 80-82% *trans* bonds. This value closely agreed with the equilibrium ratio of elaidic:oleic when either selenium or nitrous acid was used as catalyst (17). Using the calibration curve of the internal standard, recoveries of better than 97% of monoenoic acids were obtained pointing to the absence of side reactions. Capillary GLC and double bond cleavage

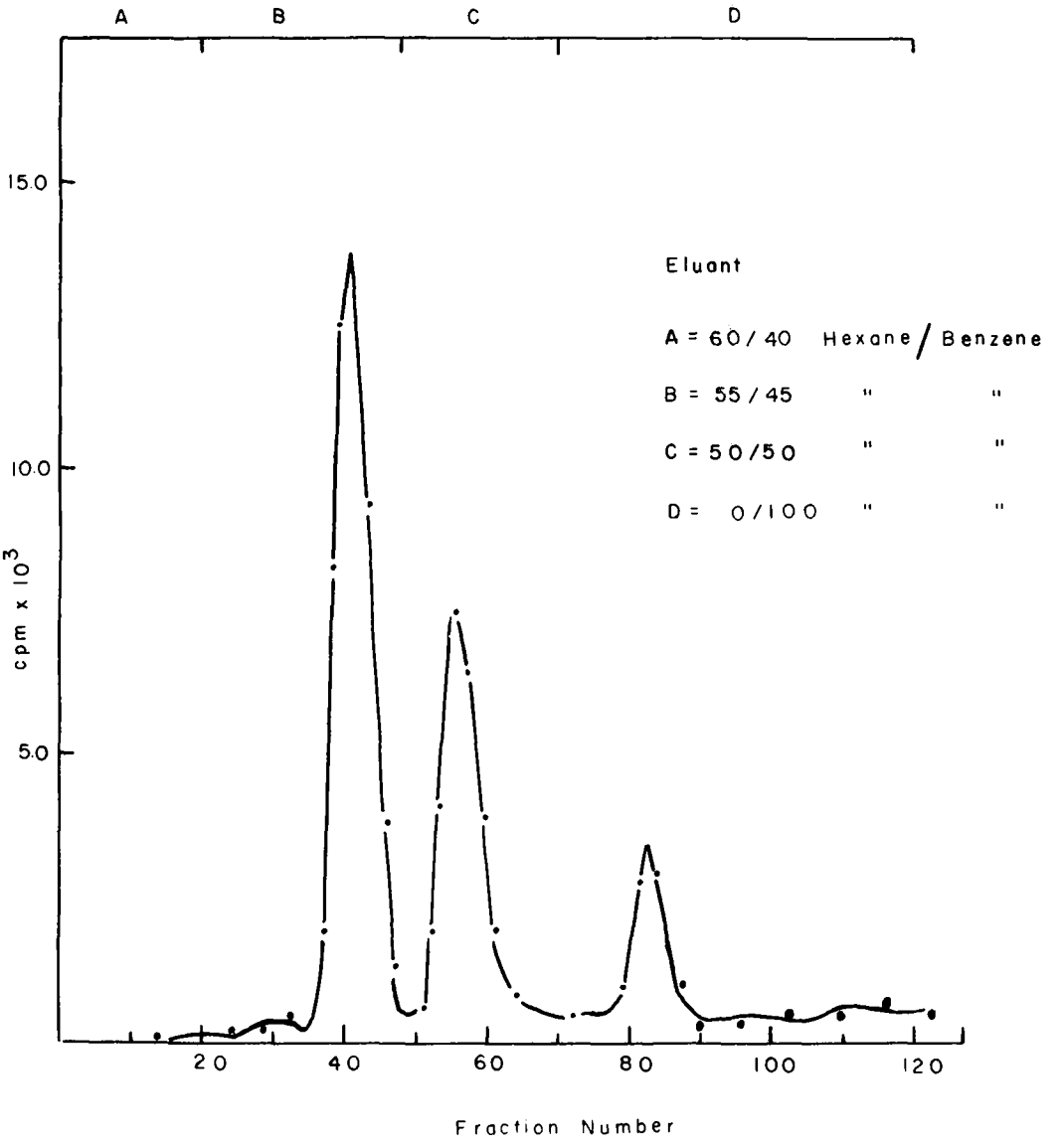

FIG. 3. Liquid-solid chromatography of isomerized methyl 1-^{14}C-linoleate. Eluted in sequence 9-*trans*, 12-*trans*; mixture of 9-*cis*, 12-*trans* and 9-*trans*, 12-*cis*; and 9-*cis*, 12-*cis* methyl octadecadienoate. Prior to chromatography, the radioactive sample was diluted with carrier methyl linoleate isomerized under similar conditions.

analyses indicated absence of detectable amounts of positional isomers.

Data on methyl linoleate isomerized with thiophenol showed (Fig. 2) that about 75% *trans* bonds were present at equilibrium. *Trans* bonds were determined from IR data after the correction suggested by Scholfield et al. (18) was applied. Capillary GLC indicated that the actual isomer content found at equilibrium was approximately 10% 9-*cis*, 12-*cis*; 17% 9-*trans*, 12-*cis*; 18% 9-*cis*, 12-*trans* and 53% 9-*trans*, 12-*trans* methyl octadecadienoate. Ultraviolet, GLC and double bond cleavage analyses indicated absence of conjugated as well as positional isomers. Yields based on the internal standard approximated 94% to 95% for the recovered stereoisomers. However, on longer reaction times or in excess of catalyst, or both, the overall yield decreased and the formation of by-products was noticed (Sgoutas, manuscript in preparation).

Data from various unsaturated methyl esters

isomerized in the presence of thiophenol, diphenylphosphine and dodecanethiol are presented in Table I. The reaction was stopped at time intervals when equilibrium was attained as indicated by the rate studies (Fig. 1 and 2). For the methyl esters tested, the *cis:trans* ratio varied little with the position of the double bond and there was no pronounced solvent effect. Diphenyl sulfide was inert.

Walling and Helmreich (15) and Pellon (19) studied in detail the radical additions of thiols and phosphines to olefins and they have proposed a multistep chain mechanism. Conceivably the same scheme can describe the addition of thiyl and phosphinyl radicals to the double bond of unsaturated fatty acids. It is assumed that an intermediate radical can regenerate either the *trans-* or *cis*-unsaturated fatty acid depending upon its conformation at the time of the thiyl elimination. For methyl 1-^{14}C-linoleate, isomerized as described, the scheme of separation of the isomeric species is given in Figure 3. The radioactivity distribution was approximately 52.0% in the 9-*trans*, 12-*trans*; 41.5% in the 9-*cis*, 12-*trans* and 9-*trans*, 12-*cis*; and 6.5% in the 9-*cis*, 12-*cis* methyl octadecadienoate. The radioactivity distribution practically matched the mass distribution (Exp. 12, Table I). Data from TLC radioassay substantiated the above findings. However, the radiochemical yield was only 88% at specific activities of 16.1 μc/μmole of methyl 1-^{14}C-linoleate. When methyl 1-^{14}C-linoleate of lower specific activity (16.1 μc/mmole) was isomerized in the same way the radiochemical yield was 92.5%; practically the same as with the chemical yield. The reason for this was not investigated.

Theoretically the method should be applicable to fatty acids with any type or combination of types of polyunsaturation, whether isolated, methylene interrupted or conjugated. The only factor that would make its unqualified use unwise is the possibility that the pol unsaturated fatty acids are susceptible to rac cal polymerization.

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REFERENCES

1. Griffiths, H. N., and T. P. Hilditch, J. Chem. So *1932*, 2315.
2. Fitzpatrick, J. D., and M. Orchin, J. Am. Che Soc. *79*, 4765 (1957).
3. McCutchon, M. A., R. T. O'Connor, E. F. DuPr L. A. Goldblatt and W. G. Bickford, JAOCS *3* 115 (1959).
4. Subrahmanyam, V. V. R., and F. \ Quackenbush, Ibid. *41*, 275 (1964).
5. MacGee, J., F. H. Mattson and L. N. Bec "Essential Fatty Acids," Edited by H. M. Sinclai New York, 1958, p. 21-29.
6. Mehta, T. N., C. V. N. Rao and K. S. Rao, Indi Soap J. *21*, 37 (1955).
7. Gunstone, F. D., and I. A. Ismail, Chem. Phy Lipids *1*, 264 (1967).
8. Barrett, C. B., M. S. J. Dallas and F. B. Padle JAOCS, *40*, 580 (1963).
9. Carroll, K. K., J. Lipid Res. *3*, 388 (1962).
10. Litchfield, C., A. F. Isbell and R. Reiser, JAOC *39*, 330 (1962).
11. Litchfield, C., R. Reiser and A. F. Isbell, Ibid. *4* 302 (1963).
12. Ramachandran, S., P. V. Rao and D. G. Cornwel J. Lipid Res. *9*, 137 (1968).
13. Sgoutas, D. S., and F. A. Kummerow, Chromatog. *16*, 448 (1964).
14. Snyder, F., and N. Stephen, Anal. Biochem. 128 (1962)
15. Walling, C., and W. Helmreich, J. Am. Chem. So *81*, 1144 (1959).
16. Pallen, R. H., and C. Sivertz, Can. J. Chem. *3* 723 (1957).
17. Litchfield, C. J. E. Lord, A. F. Isbell and I Reiser, JAOCS *40*, 553 (1963).
18. Scholfield, C. R., R. O. Butterfield and H. Dutton, Anal. Chem. *38*, 1694 (1966).
19. Pellon, J., J. Am. Chem. Soc. *83*, 1915 (1961).

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