Cyclopropane Fatty Acid Metabolism: Physical and Chemical Identification of Propane Ring Metabolic Products in the Adipose Tissue[1]

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Abstract

Twelve male weanling rats were distributed equally into 3 groups and placed on fat-free diets. The diets of groups 1 and 2 were supplemented with 0.54% of racemic methyl *cis*-9,10-methylene octadecanoate (CMO) and racemic methyl *trans*-9,10-methylene octadecanoate (TMO), respectively. Group 3 served as a control. Gas liquid chromatography (GLC) analyses of the adipose tissue methyl esters indicated at the level fed, that cyclopropane fatty acids do not affect normal fatty acid metabolism as has been shown for cyclopropene fatty acids. GLC analyses of groups 1 and 2 revealed the presence of a different unidentified fatty acid for each of the acids fed in addition to the CMO and TMO acids themselves. Each of the unidentified acids and the CMO and TMO acids were isolated and purified by preparative GLC. The absolute identity of the CMO and TMO acids fed and isolated from body fat was established by IR, NMR, and mass spectra. The biodegradation products of the CMO and TMO esters were shown to be *cis*- and *trans*-3,4-methylene dodecanoic acid, respectively. Unequivocal proof of structure was established through synthesis followed by comparison of IR, NMR, and mass spectra and melting points, GLC retention times, and elemental analyses with those obtained for the degradation products. Neither member of the racemic mixtures of either the *cis* or the *trans* cyclopropane acids was preferentially utilized by the rat as shown by the lack of optical activity in the degradation products and the CMO and TMO acids isolated from the body fat. The accumulations of the 3,4-methylene dodecanoic acids in the adipose tissue of the rats fed CMO and TMO cyclopropane fatty acids suggest the inability of the beta oxidation enzyme system to proceed past the cyclopropane ring in a fatty acid chain. The synthesis of *cis*- and *trans*-3-dodecenoic acids, intermediates in the synthesis of the 3,4-methylene dodecanoic acids, and the geometrical cyclopropane isomers are discussed.

Introduction

CYCLOPROPANE FATTY ACIDS occur in several microorganisms as major lipid components. Lactobacillic acid was first isolated in 1950 from *Lactobacillus arabinosus* by Hofmann et al. (1). Since then the number of bacterial species from which these fatty acids have been found now number more than a dozen (2–9). These organisms are present in large numbers in ruminants and to a smaller degree in the intestinal flora of monogastric animals. Since ruminants derive much of their nutrition from the digestion of microorganisms, the possibility exists that their meats and edible by-products could contain cyclopropane fatty acids. These fatty acids also occur in microorganisms used in the preparation of several dairy products.

Varma et al. (10), and Shenstone and Vickery (11) have shown that cyclopropene fatty acids can be easily hydrogenated. Cottonseed oil, one of the major sources of cooking and salad oils and margarines, contains 1–2% cyclopropene fatty acids (12). It is among some 3 doz species of plants whose oil contains these acids and are potential sources of cyclopropane fatty acids.

Phelps (12) has reported the observation by several workers that cyclopropene fatty acids affect normal fatty acid metabolism in the rat. More recently, Reiser and Raju (13) have demonstrated that cyclopropene fatty acids inhibit dehydrogenation of stearic to oleic acid, resulting in an increase of the former.

In a search of the literature we have been unable to find any information with regard to the fate of dietary cyclopropane fatty acids in higher animals or their effect, if any, on normal fatty acid metabolism. In view of these deficiences in our knowledge of the metabolism of dietary cyclopropane fatty acids routinely consumed by human beings, research in this area to supply such information was conducted. This report gives an account of the results obtained using two cyclopropane fatty acids.

Analytical Methods

Analytical Gas Chromatography. A Research Specialties Company Model 600 gas chromatograph equipped with an argon ionization detector was used in this study. A 6 ft x 0.25 in. U-type copper column packed with 20% diethylene glycol succinate polyester (DEGS) on 60–80 mesh Chromosorb W was used for analytical determinations. The column was operated isothermally at 180C. The detector was maintained at 225C while the injection port and outlet were operated at 250C. The flow rate of the carrier gas was 60–70 ml/min.

Preparative Gas Chromatography. A Wilkens Instrument Company Aerograph A-90-P gas chromatograph equipped with a thermal conductivity detector was used. On-column injection with the aid of a 6 in. needle was used when sample size exceeded 0.1 ml. A 17 ft x 0.375 in. aluminum column packed with 30% SE 30 (Analytical Engineering Laboratories, Hamden, Conn.) on 45–60 mesh firebrick was used for the isolation of methyl *cis- and trans*-9,10-methylene octadecanoates and their respective degradation products from body fat. The column temperature was manually programmed from 80–290C. A 9 ft x 0.375 in. aluminum column packed with 20% DEGS on 60–80 mesh Chromosorb W was used to isolate methyl *cis*-3-dodecenoate and methyl *cis*- and *trans*-3,4-methylene dodecanoate. The column was operated isothermally at 190C. A flow rate of approximately 200 ml/min of helium through the column was used in all

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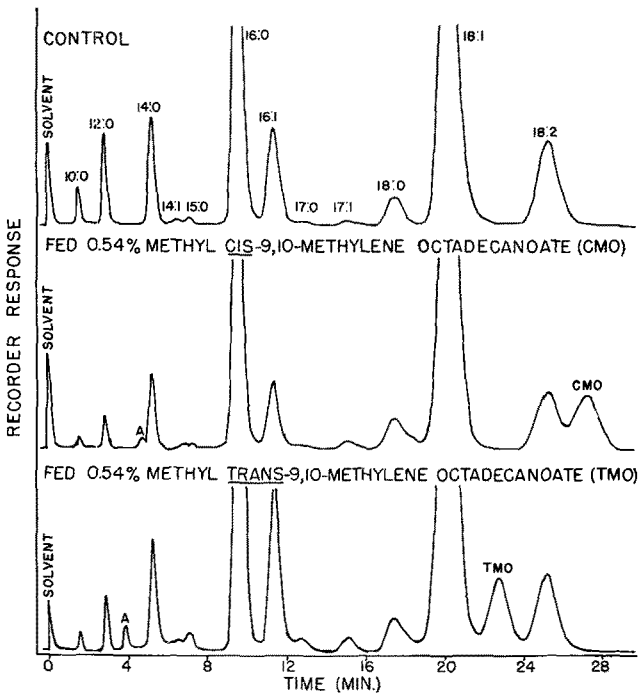

FIG. 1. A representative chromatogram of rat adipose tissue methyl esters for each of the 3 groups after 5 weeks. The small peaks labeled *A* are degradation products for each of the isomeric cyclopropane acids fed.

cases. A fraction collector designed in this laboratory (14) was used.

Infrared Spectroscopy. Infrared spectra were obtained with a Beckman Instruments IR8 infrared spectrophotometer. The solvent-free liquid esters were sandwiched between two optically-ground rock salt plates. The cell holder was then tightened until a film of the desired thickness was obtained.

Nuclear Magnetic Resonance Spectroscopy. A Varian A-60 spectrometer was used to obtain the spectra of the cyclopropane fatty esters. Unless otherwise noted, the spectra were obtained on approximately 0.4 ml of solvent-free sample, later marked with tetramethylsilane (TMS). The TMS peak was assigned the value of zero parts per million (ppm) of the total magnetic field. Those values to the right of the TMS peak (high applied field) were given negative signs, while those to the left were given positive signs.

Mass Spectroscopy. The mass spectrographic data reported were obtained on a Consolidated Electrodynamics Corp. (CEC) Model 21-103 mass spectrometer and recorded on an oscillograph or with the CEC peak digitizer. The ionizing voltage was 70 v.

Optical Rotatory Dispersion Spectroscopy. Optical rotatory dispersion data was obtained with an Instrument Engineer Company Rudolph RSP5 instrument. A 0.1% solution of the cyclopropane esters in hexane was used to obtain spectra from 230–400 μ. The cell employed had a 1 cm light path.

Elemental Analyses. Elemental analyses of the cyclopropane esters and their degradation products were run by Galbraith Laboratories, Inc., Knoxville, Tenn.

Experimental

Synthesis of Long-Chain Cyclopropane Acids. Racemic methyl *cis*-9,10-methylene octadecanoate (CMO) and racemic methyl *trans*-9,10-methylene octadecanoate (TMO) were synthesized from methyl oleate and methyl elaidate, respectively, according to the procedure of Simmon and Smith (15). Yields in excess of 65% were obtained for each of the isomers. It was later learned that yields in excess of 90% could be achieved by the addition of one half the amount originally used of the zinc-copper catalyst and methylene iodide after 24 hr and continuation of the reaction for 24 additional hours at 65–70C. The *cis* unsaturated acid always gave the corresponding *cis* cyclopropane acid without contamination with the *trans* isomer. The *trans* acid also showed no contamination with the *cis* form. The synthetic mixtures were used to prepare the respective animal diets without further purification, since gas liquid chromatography (GLC) analyses indicated the corresponding unsaturated ester to be the only other material present. Preparative GLC using the 17 ft x 0.375 in. SE-30 column was used to isolate the two geometric cyclopropane isomers more than 98% pure, as determined by analytical GLC analyses, for physical and chemical analyses. The melting point of the racemic *cis* isomer was 35.6–36.5C. [Simmon and Smith (15) reported mp 36–38C, and Hofmann et al. (16) reported a mp 38.6–39.6C.] The racemic *trans* isomer gave a mp 28.4–28.9C [the melting point reported by Hofmann et al. (17) was 33.6–35.0C].

Analysis: $C_{20}H_{38}O_2$; calculated: C, 77.42; H, 12.26; O, 10.32
Found: (*cis* isomer) C, 77.21; H, 12.25; O, 10.48
Found: (*trans* isomer) C, 77.57; H, 12.49; O, 10.14

IR, NMR and mass spectra were obtained for the compounds. These analyses revealed the presence of no impurities.

Synthesis of Methyl Cis-3-dodecenoate. Three-dodecynoic acid was synthesized starting with 1-decyne, according to the procedure employed by Knight and Diamond (18) for the preparation of 3-octynoic acid. Selective hydrogenation of 3-dodecynoic acid to yield *cis*-3-dodecenoic acid was carried out according to the procedure described by Cram and Allinger (19). The methyl ester was prepared by treating the acid with diazomethane. The ester was partially purified by elution from a short silicic acid column with diethyl ether. Preparative GLC was used to isolate more than 95% pure methyl *cis*-3-dodecenoate for the synthesis of racemic methyl *cis*-3,4-methylene dodecanoate. The contaminating material was the *trans* isomer. An unsuccessful attempt was made to synthesize 3-dodecynoic acid by the addition of methyl bromacetate to sodium decynide in liquid ammonia. The matter was not pursued further.

Synthesis of Methyl Trans-3-dodecenoate. Methyl *trans*-3-dodecenoate was prepared from methyl *cis*-3-dodecenoate by catalytic isomerization. The nitrous acid procedure of Litchfield et al. (20) failed to produce any appreciable isomerization. Possibly a *cis* double bond in the 3-position is rendered practically invulnerable to attack by the nitrous acid through the formation of a semistable ring structure formed between carbon-5 and the carbonyl oxygen. Instead, selenium isomerization (21) was used. A sample of the selenium catalyzed equilibrium mixture consisting of 64% of the *trans* and 36% of the *cis* isomer as determined by GLC analysis of the *cis* and *trans* cyclopropane acids prepared from them, was oxidized by the periodate-permanganate procedure of Von Rudloff (22). GLC analysis of the oxidation products showed the mixture to contain more than 90% nonanoic acid indicating no appreciable double bond migration during isomerization.

Synthesis of Racemic Methyl Cis- and Trans-3,4-Methylene Dodecanoates. The isomerized mixture consisting of 64% methyl *trans*-3-dodecenoate and 36% methyl *cis*-3-dodecenoate was used to synthesize methyl

trans-3,4-methylene dodecanoate and methyl *cis*-3,4-methylene dodecenoate, respectively, according to the procedure of Simmon and Smith (15). Methyl *cis*-3-dodecenoate was used to synthesize racemic methyl *cis*-3,4-methylene dodecanoate by the same procedure. Preparative GLC using the 9 ft x 0.375 in. DEGS column was used to isolate the two racemic geometrical isomers more than 95% pure as determined by GLC, IR, NMR, and mass spectrographic analyses.

Analysis: $C_{14}H_{26}O_2$; calculated: C, 74.77; H, 11.50; O, 14.16
Found: (*cis* isomer) C, 74.87; H, 11.27; O, 13.96
Found: (*trans* isomer) C, 74.53; H, 11.21; O, 14.26

The uncorrected melting point for the *cis* and *trans* isomers were 9.8 to 11.4C and −9.5 to −7.8C, respectively.

Experimental Animals. Twelve male weanling albino rats of the Holtzman strain were distributed into 3 groups and placed on a fat-free diet fortified with 1% corn oil to supply essential fatty acids. The diets of groups 1 and 2 were supplemented with 0.54% CMO and TMO, respectively. Group 3 served as a control. At the end of 5 weeks the rats were sacrificed and the carcasses were stored at −20C when necessary until the lipids could be extracted and analyzed.

Extraction of Fat from the Tissue. Lipids were extracted from portions of adipose tissue of the epididymal, perirenal, and subcutaneous regions and from the liver with a 2:1 (v/v) solution of chloroform:methanol. The fat was taken to dryness in vacuo on a steam bath. The lipids were then saponified, acidified, extracted with hexane, dried over anhydrous sodium sulfate, and methylated. The resulting esters were quantitatively analyzed by GLC.

Isolation of Cyclopropane Acids Fed and Their Degradation Products. The whole carcasses were ground and the lipids extracted by refluxing with 2:1 (v/v) chloroform:methanol solution for 4 hr. Methyl esters were then prepared by refluxing with an approximately 100-fold excess of a 2% solution of sulfuric acid in methanol. When necessary, the esters were stored at −20C until needed. The CMO and TMO esters metabolic degradation products were isolated more than 97% pure by preparative GLC. On-column injection and temp programming allowed the separation according to carbon number of 0.5 to 0.75 ml of rat fat methyl ester within 25 min. The uncorrected melting points obtained for the degradation products of the *cis* and *trans* cyclopropane acids were 9.8 to 10.6C and −10.2 to −8.5C, respectively.

Analysis: $C_{14}H_{26}O_2$; calculated: C, 74.77; H, 11.50; O, 14.16
Found: (*cis* degradation product) C, 74.85; H, 11.37; O, 14.29
Found: (*trans* degradation product) C, 74.18; H, 11.27; O, 14.25

The uncorrected melting points for the CMO and TMO acids isolated from the rat fat were 34.4–35.3C and 27.9–28.4C, respectively.

Analysis: $C_{20}H_{38}O_2$; calculated: C, 77.42; H, 12.26; O, 10.32
Found: (*cis* isomer): C, 77.55; H, 12.40; O, 10.13
Found: (*trans* isomer): C, 77.48; H, 12.43; O, 10.51

GLC retention times and optical rotatory dispersion curves were obtained in addition to the IR, NMR, and mass spectra for these cyclopropane esters and their degradation products.

Materials. Methyl oleate was obtained from the Hormel Institute, Austin, Minn., and the methyl elaidate was prepared by low temp crystallization of selenium isomerized oleic acid (22). Both were more than 99% pure as determined by GLC analyses. The 1-decyne was purchased from Columbia Organic Chemical Co., Columbia, S.C. All other solvents and reagents used were reagent grade and used without further purification.

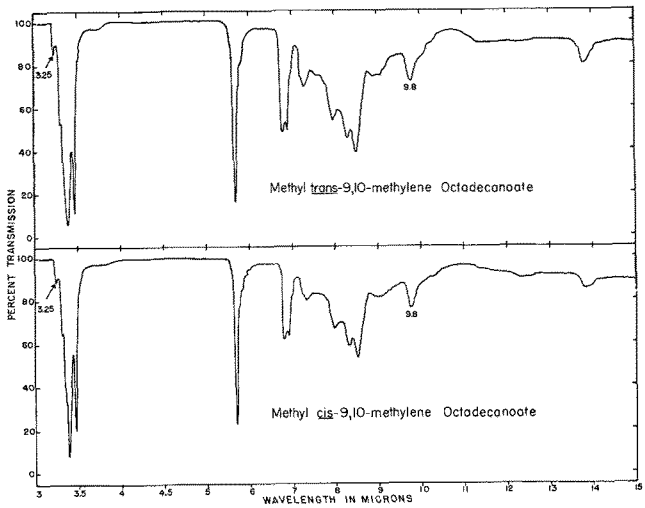

FIG. 2. IR spectra of the methyl ester of two isomeric cyclopropane fatty acids.

Results and Discussion

Metabolism

Experimental Animals. Low levels of the cyclopropane fatty acids were fed since they are normally consumed in small amounts. The rate of wt gain of the animals fed the cycloproane esters was not significantly different from that of the control animals in either case. The fatty acid composition was not affected noticeably apart from the deposition of the particular cyclopropane acid fed and its degradation product. In a complementary experiment, animals were sacrificed after 12, 24, and 36 days on the respective diets. A continued increase in the amount of the cyclopropane acid fed in the adipose tissue was observed. A typical chromatogram of the adipose tissue methyl esters for each of the 3 groups is shown in Figure 1. An equivalent chain length (ECL) of 18.9 and 20.1 was obtained for the TMO and CMO esters, respectively. The CMO and TMO acids represented 7–8% of the adipose tissue. Unidentified peaks (labeled *A* in Fig. 1) with an ECL of 13.05 and 13.75 were present in the adipose tissue and body fat of the rats on the TMO and CMO diets, respectively. These unidentified acids represented 0.3 to 0.6% of the adipose tissue fatty acids. It should be noted that no increase in branched or odd chain acids, which could have resulted from the cleavage of the cyclopropane ring, was observed.

GLC analyses of the fatty acids extracted from an aliquot of a 25-day composite sample of feces from each group revealed small peaks with retention times identical to those obtained for the metabolic products for each of the isomeric cyclopropane acids. However, it is not known whether these were CMO and TMO metabolic products or other fatty acids from the complexed fecal mixture with similar retention times. Each of the cyclopropane acids fed constituted 35–40% of the total fecal fatty acids.

Identification of the CMO and TMO Acids Fed and Isolated

Infrared Analyses. IR spectra of the methyl ester of the TMO and CMO acids are shown in Figure 2. Both isomers gave practically the same spectrum. Absorption bands in the region of 3.25 and 9.8 μ are characteristic of the cyclopropane ring structure. The IR spectra for the TMO and CMO esters isolated from the rat adipose tissue were identical with the respective synthetic acids.

Nuclear Magnetic Resonance Spectra. Proton reso-

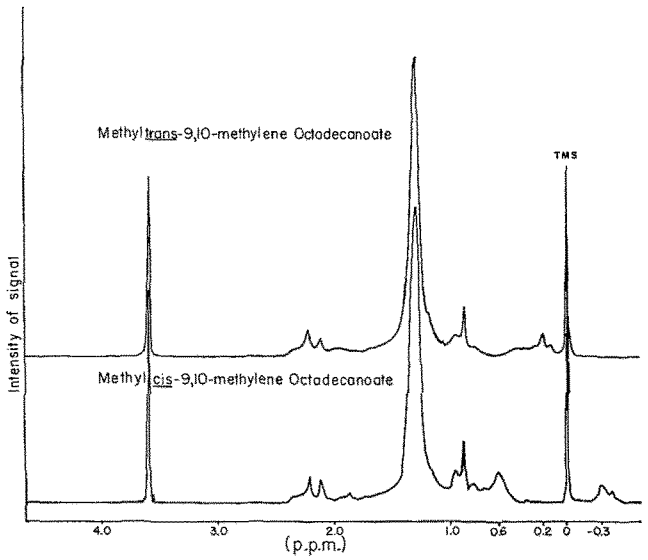

FIG. 3. Proton resonance spectra of the methyl esters of two isomeric cyclopropane fatty acids. Spectra were determined neat at 60 megacycles/sec (mc).

nance spectra for the methyl esters of CMO and TMO are shown in Figure 3. The absence of the corresponding unsaturated acids used in the CMO and TMO synthesis is supported by the absence of peaks in the region of 5.35 and 2.0 ppm assigned to the vinyl and allyl hydrogens, respectively (23). A doublet roughly equivalent to one hydrogen appeared in the region of −0.3 ppm for the *cis* isomer. This peak has previously been assigned to the two methylene hydrogens of the cyclopropane ring by Hopkins and Bernstein (24,25). They also suggested that the peak appearing in the region of 0.6 ppm is due to the hydrogens on carbon 9 and 10 of the cyclopropane ring. A peak at 0.6 ppm roughly equivalent to two hydrogens was obtained, in addition to what appears to be a doublet similar to that at −0.3, at 0.8 ppm. Since the two methylene hydrogens of the cyclopropane ring are more equivalent than the hydrogens on carbon 9 and 10, it would seem likely that the peak at 0.6 ppm is due to the methylene hydrogens of the ring, while the doublets at −0.3 ppm and possibly at 0.8 ppm are due

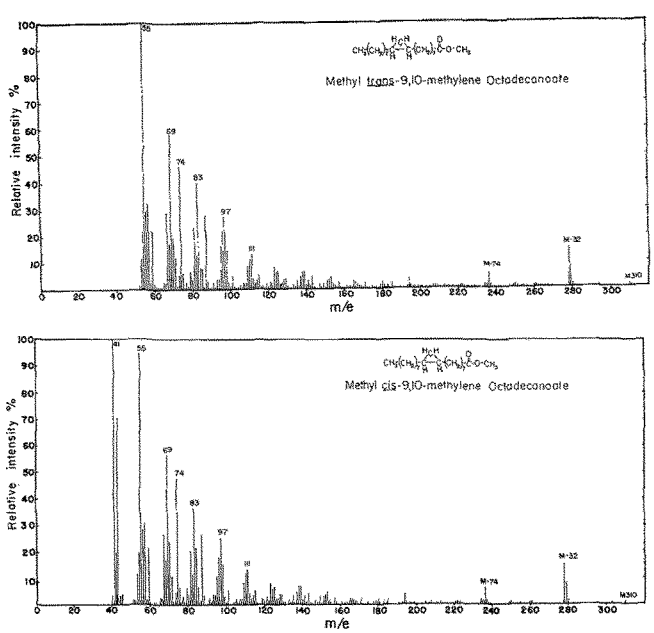

FIG. 4. Mass spectra of two isomeric cyclopropane fatty acids.

to the hydrogens on carbon 9 and 10. The NMR spectrum of the *trans* isomer, previously unreported, is easily distinguished from the *cis* isomer by a doublet equivalent to one hydrogen in the region 0.2 ppm. The peaks at −0.3, 0.6, and 0.8 ppm for the *cis* isomer are not present in the spectrum of the *trans* isomer. The NMR spectra for the methyl esters of the CMO and TMO acids isolated from the rat body fat were identical with the corresponding synthetic cyclopropane fatty esters.

Mass Spectra. Mass spectra for the previously unreported CMO and TMO methyl esters are shown in Figure 4. Identical spectra, as expected, were obtained for the two isomeric cyclopropane fatty esters. A small molecule-ion peak at m/e 310, the assumed molecular weight, was obtained for both isomers. It is noted that the acylinum ion m/e = M-32 and the ion m/e = M-116, usually obtained for monounsaturated esters other than α-β unsaturated, are present for the cyclopropane esters. The expected characteristic ion peak m/e 74, described by Ryhage and Stenhagen (26) is present. The usual hydrocarbon series of peaks corresponding to the empirical formulae $[C_nH_{2n+1}]^+$ and $[C_nH_{2n}]^+$ and $[C_nH_{2n-1}]^+$ are present. The unexpected intensity of the $[C_nH_{2n-2}]^+$ and $[C_nH_{2n-3}]^+$ ions might be due to the $[C_nH_{2n}]^+$ and $[C_nH_{2n-1}]^+$ ions containing a cyclopropane ring. The partial similarity of the cyclopropane ester spectra with that obtained for a monounsaturated ester, such as methyl oleate, might be explained in part by some degree of cleavage between carbon 9 and 10 giving rise to an unsaturated ester one carbon longer. The 2 ion peaks m/e 153 and 157 represent a simple cleavage between carbon No. 8 and 9. The ion peaks m/e 139 and 171 probably are due to a double cleavage of the propane ring, first between carbon No. 9 and the methylene carbon of the ring followed by a cleavage between carbon No. 9 and 10, accompanied by a hydrogen migration to the oxygen containing fragment. Ion peaks m/e 125 and 185, having equal intensity to ion peaks 139 and 171, are probably due to a similar type cleavage but first cleaving between carbon No. 10 and the methylene carbon of the ring.

Melting Point. The melting points of the synthetic CMO were in close agreement with that reported independently by two other workers (15,16). However, the melting point obtained for the TMO was 4–5C lower than previously reported (17). This difference in melting points could have been due to lack of absolute purity. The melting points of the TMO and CMO acids isolated from the rat adipose tissue were approximately 1C lower than the corresponding racemic synthetic acids.

Elemental Analyses. Data obtained from the elemental analyses of each of the isomeric cyclopropane methyl esters were in close agreement with the calculated values.

Optical Rotatory Dispersion Analyses. One of the members of some racemic mixtures is sometimes preferentially utilized by living organisms. However, it is not known whether this is the case with racemic mixtures of long-chain fatty acids. Since racemic cyclopropane esters were fed, either preferential absorption or utilization would have rendered the cyclopropane acids isolated from the rat body fat optically active. Optical rotatory dispersion curves of each of the cyclopropane esters indicated no optical activity at the concentration used. This suggests that each of the optical isomers of a racemic cyclopropane fatty

acid mixture can be metabolized equally well by the rat.

Metabolic Products of the CMO and TMO Fatty Acids Fed

Two different unknown fatty acids were observed in the adipose tissue of the rats fed the diets containing CMO and TMO fatty acids. Following a series of chemical and physical analysis on the relatively pure metabolic products, they appeared to be *cis*- and *trans*-3,4-methylene dodecanotic acids resulting from beta oxidation of the CMO and TMO fatty acids fed. However, due to the lack of sufficient reference literature of this nature, the exact structure could not be unequivocally proven. This difficulty was overcome through the synthesis and purification of methyl *cis*- and *trans*-3,4-methylene dodecanoates for comparative purposes. *Infrared Analysis.* A comparison of the IR spectra of the synthetic methyl *cis*- and *trans*-3,4-methylene dodecanoates with those of the degradation products of the respective CMO and TMO fatty acids fed, is shown in Figures 5 and 6. The spectrum of each of the synthetic cyclopropane esters is practically identical with its corresponding metabolic product. Absorption bands at 3.25 and 9.8μ, characteristic of the cyclopropane ring, are present in each of the spectra. There are some basic differences between the spectra of the *cis* isomer (Fig. 5) and the *trans* isomer (Fig. 6) which were not observed between the geometrical isomers of methyl 9,10-methylene octadecanoate in Figure 2. The absorption band in the region of 7.25μ of the *cis* isomer is greatly reduced in intensity or shadowed by overtones of the adjacent regions of the *trans* isomer. The absorption bands in the region of 7.63μ and 8.4μ are reduced in intensity in the case of the *trans* isomer. Each of the IR spectra of the methyl *cis*- and *trans*-3, 4-methylene dodecanoates is distinguished from the indistinguishable spectra of the CMO and TMO esters by the absorption band in the region of 7.63μ. The former is further distinguished by the lack of the intensity of absorption in the region of 7.35μ, present in the latter. The absorption at 8.35 for the latter has apparently shifted and now appears at 8.4μ (and much reduced intensity in the case of the *cis* isomer) in the former.

Nuclear Magnetic Resonance Spectra. Nuclear magnetic resonance spectra of the two synthetic cyclopropane esters and the degradation products isolated from the rat body fat are shown in Figure 7. The spectra for the metabolic degradation products were obtained with a 10% solution in carbon tetrachloride. The spectra for the synthetic cyclopropane esters were obtained neat. The spectra of the methyl *cis*- and *trans*-3,4-methylene dodecanoates are indentical with the CMO and TMO metabolic degradation products. The *cis* isomer is easilly distinguished from the *trans* isomer by a doublet at -0.2 ppm. They are further distinguished by a doublet at 0.35 in the latter, not present in the former. A quadruplet appeared in the region of 2.25 ppm for the *trans* isomer while the expected α-hydrogen doublet was obtained for the *cis* isomer. The quadruplet is apparently due to spin coupling of the α-hydrogens with the hydrogen on carbon number 4 associated with the cyclopropane ring.

Mass Spectra. A comparison of the mass spectra of the synthetic methyl *cis*-3,4-methylene dodecanotes with the metabolic degradation product of the CMO acids is shown in Figure 8. The spectrum for the

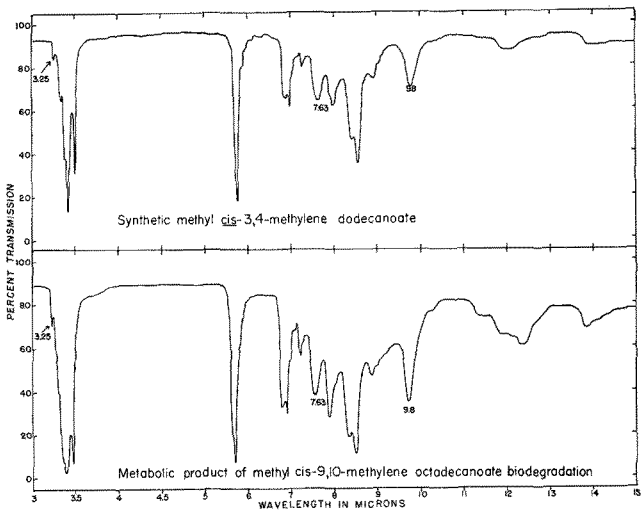

FIG. 5. Comparison of the IR spectra of synthetic methyl *cis*-3,4-methylene dodecanoate and the metabolic product of the CMO ester isolated from rat body fat.

synthetic isomers is identical with the metabolic product isolated from the rat adipose tissue. The mass spectrum of the synthetic methyl *trans*-3,4-methylene dodecanoate was identical with TMO metabolic product, and as expected, identical with the spectra shown in Figure 8 for the *cis* isomer. The characteristic fragmentation pattern is very similar to that described earlier for the CMO and TMO esters. The increased relative intensities of ion peaks m/e 74, previously described (26) and m/e 59, due to the cleavage between carbon No. 1 and 2, are probably due to the influence of the cyclopropane ring adjacent to the α-carbon. The double cleavage of the cyclopropane ring (postulated earlier) is supported by the relative intensities of ion peaks m/e 87 and 101. The lack of equal intensities of the two ions can be explained on the basis of the position of the propane ring in the molecule.

GLC Analysis. GLC analysis of mixtures of each of the synthetic methyl *cis*- and *trans*-3,4-methylene dodecanoates and the corresponding biodegradation products of the CMO and TMO esters gave only one peak at several temperatures on polar and nonpolar phases. It should be noted that these cyclopropane esters have the same order of elution as the CMO

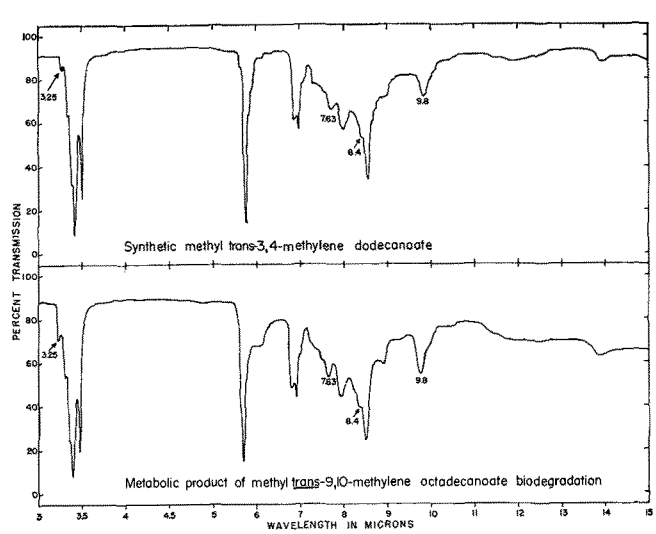

FIG. 6. Comparison of the IR spectra of synthetic methyl *trans*-3,4-methylene dodecanoate and the metabolic product of the TMO ester isolated from rat body fat.

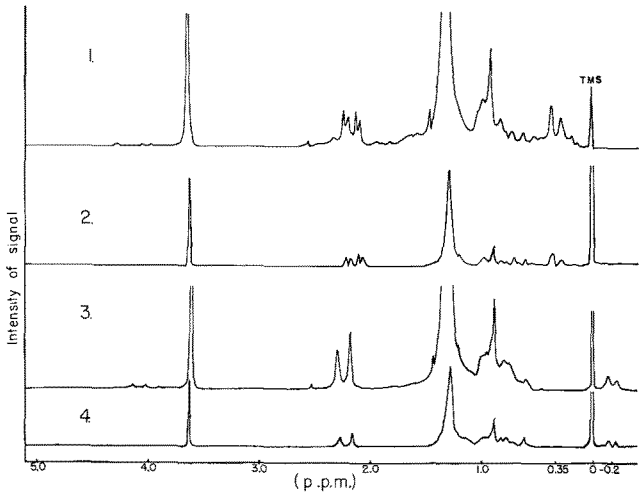

FIG. 7. Comparison of proton resonance spectra of methyl *cis*- and *trans*-3,4-methylene dodecanoate with the metabolic degradation products of the CMO and TMO esters. (1) Synthetic methyl *trans*-3,4-methylene dodecanoate. (2) Metabolic product of methyl *trans*-9,10-methylene octadecanoate biodegradation. (3) Synthetic methyl *cis*-3,4-methylene dodecanoate. (4) Metabolic product of methyl *cis*-9,10-methylene octadecanoate biodegradation.

and TMO esters.

Melting Points. The melting points obtained for the synthetic geometrical isomers were in close agreement with those obtained for the isolated biodegradation products of the CMO and TMO acids. Again, as was the case with the CMO and TMO acids, the *cis* isomer had a higher melting point than the *trans*. It is interesting to note that in order to solidify both acids the temperature had to be lowered to approximately −79C, yet the *cis* isomer melted above zero. These acids have remained liquids in a freezer at −20C for approximately 2 months.

Elemental Analyses. Elemental analysis data obtained for each of the isomeric synthetic esters and the biodegradation products were in close agreement

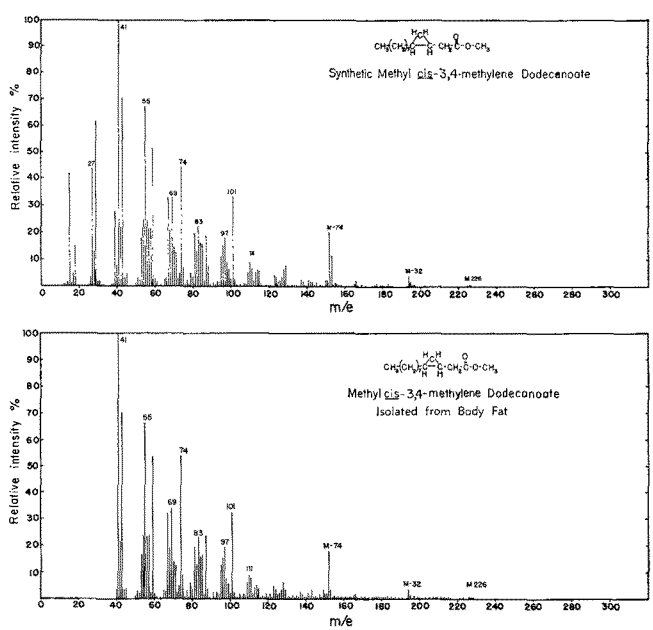

FIG. 8. Comparison of the mass spectra of synthetic *cis* cyclopropane fatty ester with the CMO metabolic degradation product isolated from rat adipose tissue. The mass spectrum obtained for the *trans* isomer was identical with that of the TMO degradation product which was also identical with the spectra shown above.

with one another and the theoretical values.

Optical Rotatory Dispersion Analyses. Preferential degradation of the racemic CMO and TMO esters would have rendered either of the biodegradation products optically active. Optical rotatory dispersion curves of each of the esters indicated no optical activity at the concentration used. These data, in addition to those obtained for the CMO and TMO esters, indicate that either member of a racemic mixture of either geometrical cyclopropane isomers can be metabolized equally well by the rat.

Biochemical Significance

It has been demonstrated unequivocally that the biodegradation products of racemic methyl *cis*- and *trans*-9,10-methylene octadecanoate are racemic methyl *cis*- and *trans*-3,4-methylene dodecanoate, respectively. The metabolic products are apparently the result of the inabilities of the beta oxidation enzyme system to continue down the fatty acid chain. Normal fatty acid metabolism appeared unaffected by the low level of the cyclopropane fatty acids fed. From these data, a number of conclusions can be drawn about the metabolism of cyclopropane fatty acids. First, even when consumed at low levels they can be found to accumulate in the adipose tissue but probably do not affect normal fatty acid metabolism as do cyclopropene fatty acids. Second, the acids are presumably catabolized by beta oxidation down to the ring, depending on the number of carbon atoms between the ring and the carboxyl groups, where the enzyme system is apparently unable to catabolize the rest of the chain. This is supported by the fact that they accumulate in the adipose tissue. This conclusion is similar to that drawn from the classical work of Knoop, but was arrived at by using fatty acids that occur in nature. Third and last, both members of a racemic mixture of either the *cis* or *trans* isomer appears to be metabolized equally well.

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