# Synthesis and characterization of 1- and 2-monoglycerides of anteiso fatty acids

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ABSTRACT The branched-chain fatty acids D-(+)-12-methyltetradecanoic acid ($C_{15}$ anteiso) and D-(+)-14-methylhexadecanoic acid ($C_{17}$ anteiso) were isolated from the lipids of *Listeria monocytogenes* and their 1- and 2-monoglycerides were prepared. Reaction intermediates and products were purified without isomerization by column chromatography. Thin-layer chromatography on Florisil impregnated with boric acid and nuclear magnetic resonance were used in characterizing the 1- and 2-monoglycerides. The value of the latter method for analyzing glyceride structure is discussed.

KEY WORDS bacterial lipids · anteiso fatty acids · 1- and 2-monoglycerides · synthesis · chromatography · column · thin-layer · silica gel–boric acid · gas–liquid · nuclear magnetic resonance · isomerization · glyceride structure · monocytosis

THE SYNTHESIS OF monoglycerides of anteiso fatty acids was undertaken as part of a research program aimed at identifying the monocytosis-producing agent in *Listeria monocytogenes*. This bacterium was isolated by Murray in 1924 following an infection in a laboratory rabbit colony (1, 2) and the characteristic monocytosis which it produces in infected animals was described by Murray, Webb and Swann (3). It was later shown by Stanley that a monocytosis-producing agent could be extracted from *L. monocytogenes* with chloroform (4) and this was confirmed by Murray and associates (1, 5). More recently, separation of chloroform extracts of *L. monocytogenes* in our laboratory by column chromatography provided evidence that active material was eluted in the monoglyceride fraction,[1] and analysis of this fraction by gas–liquid chromatography showed peaks corresponding to monoglycerides of the two main fatty acids of *L. monocytogenes*, the branched-chain $C_{15}$ and $C_{17}$ anteiso acids. These fatty acids were therefore isolated from the bacterial lipids and their monoglycerides were prepared for biological testing.

Abbreviations: TLC, thin-layer chromatography; NMR, nuclear magnetic resonance.

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[1] K. K. Carroll, J. H. Cutts, and E. G. D. Murray, unpublished observations.

## EXPERIMENTAL METHODS AND RESULTS

### *Fatty Acids*

The lipids of *L. monocytogenes* were extracted by suspending 50 g of lyophilized bacteria in 400 ml of chloroform–methanol (2:1), adding 100 g of glass powder (200 mesh—Fisher Scientific Co., Toronto, Ontario) and 250 g of 4 mm glass beads, and shaking overnight in a mechanical shaker to disrupt the bacteria and facilitate extraction. The mixture was filtered through coarse filter paper and the bacteria were reextracted twice more with 500 ml portions of chloroform–methanol (2:1). The combined filtrates were taken to dryness and the residues taken up in chloroform, leaving behind a certain amount of insoluble material. The chloroform-soluble extract represented 5–7% of the original dry weight of bacteria. The *L. monocytogenes* used for these experiments was grown in tryptose broth culture at the Karolinska Institute, Stockholm, through the cooperation of Prof. C.-G. Hedén and Dr. B. Holmström.

Methyl esters of the mixed fatty acids of *L. monocytogenes* were prepared by dissolving the lipids in reagent grade methanol, adding 10% (v/v) of acetyl chloride slowly, and then refluxing the mixture for 2 hr (6). The cooled reaction mixture was extracted with Skellysolve B to remove the methyl esters and the extract was washed with water and dried over sodium sulfate. Analysis of the methyl esters by gas–liquid chromatography showed that the $C_{15}$ and $C_{17}$ anteiso acids were the major constituents, with lesser amounts of $C_{14}$ and $C_{16}$ normal and iso acids

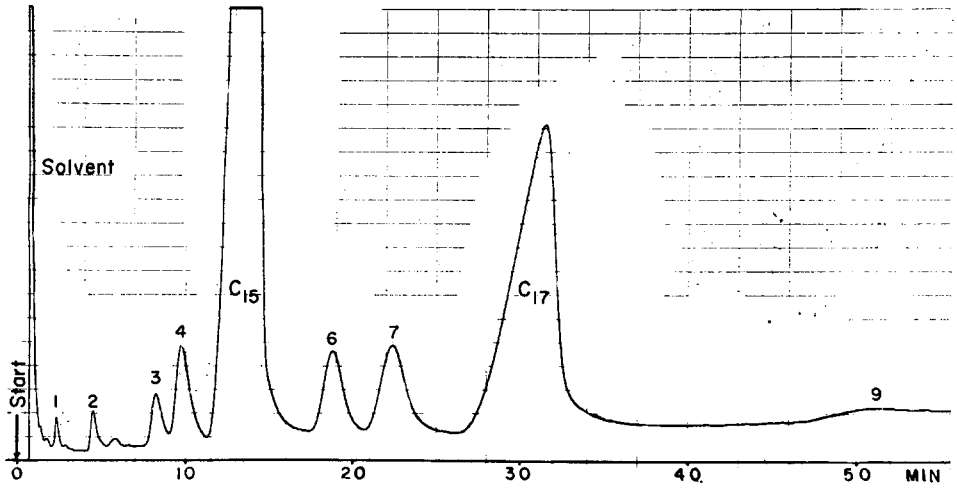


FIG. 1. Gas–liquid chromatogram of fatty acid methyl esters from lipids of *L. monocytogenes* run on a Barber Colman Model 5340 with thermal conductivity detector. Column 5 ft × 3/8 inch, packed with Dow Corning silicone oil no. 710, 5% on Chromosorb W. Temperature, 180°C. The two major components were identified as $C_{15}$ and $C_{17}$ anteiso acids as described in the text. Peaks 4 and 7 were identified as myristic and palmitic acids and peaks 3 and 6 as $C_{14}$ and $C_{16}$ iso acids on the basis of retention times.

and trace amounts of a few other fatty acids (Fig. 1).

The mixture of fatty acid methyl esters (800 mg) was separated by liquid–liquid partition chromatography on a column (3.5 × 70 cm) containing 200 g of Celite treated with dimethylsilane and impregnated with heptane (7). The moving phase consisted of acetonitrile—methanol (85:15 v/v) equilibrated with heptane. Fractions (15 ml) were collected and the bulk of fatty acid esters was eluted between tubes 45 and 75. Tubes 49–57 inclusive contained $C_{15}$ anteiso methyl ester of at least 98% purity as judged by gas chromatography, while tubes 65–75 contained $C_{17}$ anteiso ester of similar high purity. Tubes 58–64 contained mixtures of these esters and the $C_{16}$ iso and normal esters, while tubes 45–48 contained $C_{14}$ esters in addition to $C_{15}$ anteiso ester.

Hydrolysis of the purified methyl esters with methanolic 0.5 N NaOH followed by recrystallization of the fatty acids from petroleum ether (35–60°C) gave D-(+)-12-methyltetradecanoic acid (mp 22–23°C), $[\alpha]_D^{25} = +5.0°$(in acetone); and D-(+)-14-methylhexadecanoic acid (mp 36–37°C), $[\alpha]_D^{25} = +4.6°$(in acetone), amide (mp 89.2–89.7°C). These data correspond closely to published values for $C_{15}$ and $C_{17}$ anteiso acids (8–10) and the melting points of the acids were not depressed by mixing with authentic samples kindly provided by Dr. A. W. Weitkamp.

### *Preparation of 1-Monoglycerides*

A mixture of 488 mg (2 mmoles) of D-(+)-12-methyltetradecanoic acid ($C_{15}$ anteiso acid) and 3 ml of redistilled thionyl chloride was refluxed for 2 hr with exclusion of moisture. The excess thionyl chloride was then removed under vacuum with heating and the acyl chloride, dissolved in 4 ml of chloroform (alcohol-free and dry), was added to a mixture of 317 mg (20% excess) of redistilled DL-1,2-isopropylidene glycerol (Aldrich Chemical Co. Inc., Milwaukee, Wis.) and 1 ml of dry pyridine with cooling to 0–5°C. Reaction was allowed to proceed for 45 hr at room temperature, the chloroform was removed under vacuum, and the residue was washed three times with cold water to remove most of the pyridine. The washed residue was taken up in petroleum ether and the soluble material (773 mg) was chromatographed on a column (1.0 × 32 cm) of Florisil with 6% added water (11), with 50-ml portions of hexane–ether (100:0, 97:3, and 85:15) as eluents. The 1-(12′-methyltetradecanoyl)-2,3-isopropylidene glycerol (1-$C_{15}$-acyl-isopropylidene glycerol) was eluted with 85:15 hexane–ether, giving 572 mg (80% of theory) of purified oily product.

Analysis: $C_{21}H_{40}O_4$; calculated: C, 70.78; H, 11.28
found: C, 70.74; H, 11.51

This was then converted to the monoglyceride by removal of the isopropylidene group with hydrochloric acid in ether (12). The product was recrystallized three times from ether–petroleum ether in the cold giving 1-(12′-methyltetradecanoyl)-glycerol (1-$C_{15}$-monoglyceride) in 47% yield (299 mg), mp 47–49°C.

Analysis: $C_{18}H_{36}O_4$; calculated: C, 68.35; H, 11.48
found: C, 68.53; H, 11.62

In a repeat of this synthesis using the same amount of

starting material, the 1-acyl isopropylidene glycerol was split with boric acid in trimethyl borate (13, 14). The acyl isopropylidene glycerol (588 mg, 1.65 mmoles) was dissolved in 4 ml of trimethyl borate (Aldrich Chemical Co. Inc., Milwaukee, Wis.), 205 mg of boric acid (3.3 mmoles) was added, and the mixture was heated at 90°C with exclusion of moisture until the boric acid dissolved (approximately 10 min). The trimethyl borate was then removed under vacuum at 90°C and the residue was kept under vacuum at this temperature for a further 10 min. After cooling, the reaction mixture was dissolved in ether and washed four times with water to split the borate complex.[2] The product in this case was purified by chromatography on a column (1.0 × 33 cm) of acid-treated Florisil (15), with 50-ml portions of hexane–ether (100:0, 97:3, 85:15, 50:50 and 0:100) as eluents. A trace of unreacted acyl isopropylidene glycerol was eluted with 85:15 hexane–ether and the monoglyceride with ether. This gave 408 mg (64% of theory) of product, mp 47–49°C.

The D-(+)-14-methylhexadecanoic acid ($C_{17}$ anteiso acid) (544 mg, 2 mmoles), treated as described above, was converted to 1-(14′-methylhexadecanoyl)-2,3-isopropylidene glycerol of mp 22–24°C, in 78% yield.

Analysis: $C_{23}H_{44}O_4$; calculated: C, 71.90; H, 11.52
found: C, 71.97; H, 11.62

This was converted by treatment with boric acid to 1-(14′-methylhexadecanoyl)-glycerol (1-$C_{17}$-monoglyceride) in 62% yield, mp 55–57°C.

Analysis: $C_{20}H_{40}O_4$; calculated: C, 69.75; H, 11.71
found: C, 69.63; H, 11.88

1-Monopalmitin (mp 75–76°C) was also prepared by the same route as a model compound for comparative studies.

These monoglycerides were prepared from D-anteiso fatty acids and DL-isopropylidene glycerol and are therefore in each case mixtures of two diastereomers which were not resolved by chromatography on either Florisil or alumina (Woelm).

### *Preparation of 2-Monoglycerides*

1,3-Benzylidene glycerol was prepared by standard methods (14, 16, 17) from glycerol, benzaldehyde and a catalytic amount of *p*-toluenesulfonic acid. The product initially melted at 62–63°C but on standing at room temperature this slowly increased to about 80°C as noted by earlier workers (17).

The $C_{15}$ anteiso acid (488 mg, 2 mmoles) was converted to the acyl chloride and this was reacted with 432 mg (20% excess) of 1,3-benzylidene glycerol in the presence of chloroform and pyridine as described above for the preparation of 1-monoglyceride (14, 18). The product (890 mg of petroleum ether-soluble material) was purified by chromatography on a Florisil column giving 672 mg (84% yield) of 2-(12′-methyltetradecanoyl)-1,3-benzylidene glycerol which crystallized in star forms (mp 32–33°C).

Analysis: $C_{25}H_{40}O_4$; calculated: C, 74.35; H, 9.97
found: C, 74.46; H, 10.21

This product contained a small amount of a conformational isomer melting at 44–45°C which was eluted from the Florisil column with Skellysolve B–ether 85:15 slightly ahead of the lower melting isomer. Similar isomers have been separated previously by chromatography on alumina (19) and evidence has been presented that the main product originates from a *cis*-1,3-benzylidene glycerol (mp 63°C) and exists as an equilibrium mixture of *cis* forms with the acyl group in axial and the benzyl group in equatorial position in the 6-membered ring and vice versa. The minor product, on the other hand, originates from a *trans*-1,3-benzylidene glycerol (mp 83°C) and exists in a *trans* form with both acyl and benzyl groups in equatorial position (17, 19, 20).

The benzylidene group was removed from the acyl benzylidene glycerol by treatment with boric acid and trimethyl borate as described above for removal of the isopropylidene group (14, 21). The ether solution containing the final product was taken to dryness below 30°C and the 2-monoglyceride was dried in vacuo instead of using sodium sulfate, which caused partial isomerization of 2- to 1-monoglyceride. Since the 2-monoglyceride isomerized more rapidly than the 1-monoglyceride (14), it could not be purified by chromatography on acid-treated Florisil but was chromatographed without appreciable isomerization on a column of acid-treated Florisil (or silicic acid) impregnated with 10% (w/w) of boric acid,[3] using the same eluting solvents as for 1-monoglycerides. The over-all yield was about 60% of theoretical and one recrystallization from Skellysolve B–ether 3:1 gave D-(+)-2-(12′-methyltetradecanoyl)-glycerol (2-$C_{15}$-monoglyceride) melting at 41–42°C, $[\alpha]_D^{26}$ = + 4.76° (in chloroform).

Analysis: $C_{18}H_{36}O_4$; calculated: C, 68.35; H, 11.48
found: C, 68.50; H, 11.48

The $C_{17}$ anteiso acid was similarly converted to a *cis*-

[2] The reaction is extremely sensitive to moisture and may fail if moisture is not completely excluded. If this happens, a white precipitate forms during the washing of the ether solution with water and the acyl isopropylidene glycerol is recovered almost unchanged from the ether solution.

[3] The boric acid was dissolved in methanol in a round bottom flask, the acid-treated Florisil added, and the methanol evaporated on a rotary evaporator. The impregnated Florisil was activated for 1 hr at 110°C.

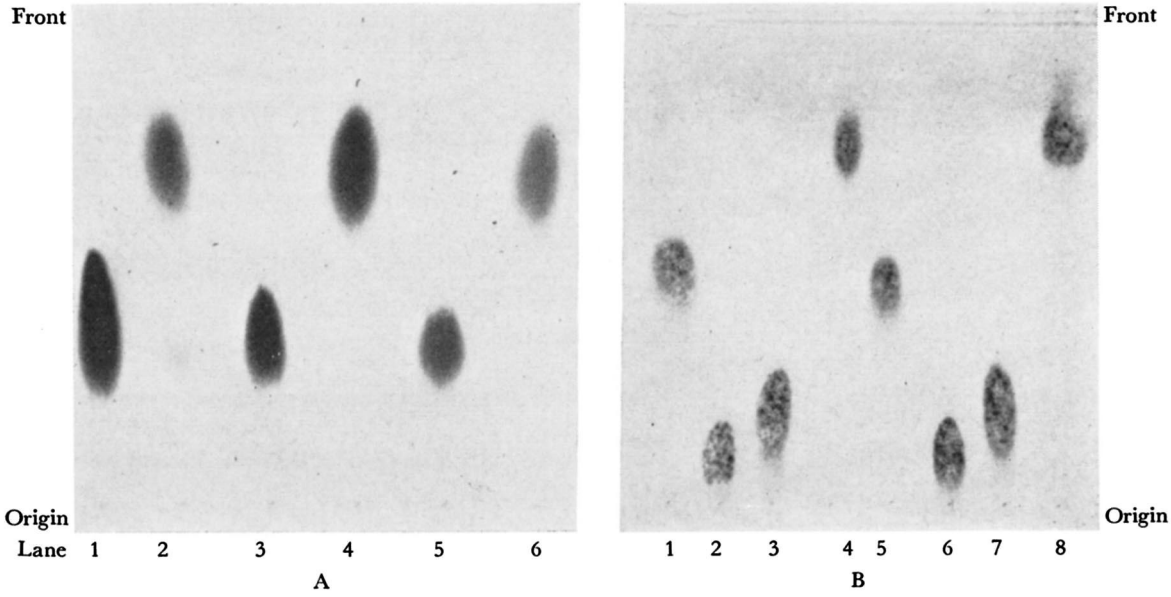


FIG. 2A. Thin-layer chromatogram of 1- and 2-monoglycerides on Silica Gel G impregnated with 10% (w/w) boric acid. Developing solvent: commercial chloroform–methanol (98:2, v/v). Spots detected by spraying with 50% sulfuric acid and charring.
Lanes 1 and 2: 1- and 2-$C_{15}$-monoglycerides respectively
Lanes 3 and 4: 1- and 2-$C_{17}$-monoglycerides respectively
Lanes 5 and 6: 1- and 2-monopalmitins respectively
FIG. 2B. Same adsorbent as for 2A. Developing solvent: chloroform–acetone (88:12, v/v).
Lane 1: $C_{15}$-anteiso acid
Lane 2: 1-$C_{15}$-monoglyceride
Lane 3: 2-$C_{15}$-monoglyceride
Lane 4: 2-$C_{15}$-acyl-1,3-benzylidene glycerol (*cis*)
Lanes 5–8: the corresponding derivatives of the $C_{17}$-anteiso acid

benzylidene glycerol derivative in 86% yield, mp 41–42°C.

Analysis: $C_{23}H_{44}O_4$; calculated: C, 74.95; H, 10.25
found: C, 74.93; H, 10.33

This in turn gave D-(+)-2-(14′-methylhexadecanoyl)-glycerol (2-$C_{17}$-monoglyceride) in 62% yield with mp 48–49°C, $[\alpha]_D^{26} = +4.38°$ (in chloroform).

Analysis: $C_{20}H_{40}O_4$; calculated: C, 69.75; H, 11.71
found: C, 69.60; H, 11.66

The *trans*-benzylidene glycerol derivative mp 50–51°C was also isolated as described above. 2-Monopalmitin (mp 67–68°C) was prepared by the same route for comparative purposes.

### *TLC of Monoglycerides and Intermediates*

1- and 2-monoglycerides are not separated by TLC on Silica Gel G (22) but can be separated by chromatography on hydroxylapatite (23, 24) or on Silica Gel impregnated with 10% (w/w) boric acid (25). The latter method was more satisfactory in our hands (Fig. 2). The best separation of 1- and 2-monoglycerides was achieved by developing the chromatograms with chloroform–methanol 98:2 (Fig. 2A), a solvent mixture used by Morris for separation of *threo*- and *erythro*-glycols (26). Another solvent mixture, chloroform–acetone 88:12 (25), was useful for following reactions during the preparation of 1- and 2-monoglycerides (Fig. 2B). In this system, the 2,3-isopropylidene derivatives of 1-monoglycerides run at $R_f$ 0.70, the *cis*-1,3-benzylidene derivatives of 2-monoglycerides at $R_f$ 0.75 (Fig. 2B, 4 and 8) and *trans*-1,3-benzylidene derivatives at $R_f$ 0.81.

### *Oxidation of Monoglycerides by Periodic Acid*

The monoglycerides were oxidized with periodic acid and the liberated formaldehyde was determined colorimetrically with chromotropic acid according to the method of Karnovsky and Brumm (27). A sample of 1-monopalmitin kindly provided by Dr. F. H. Mattson was used as standard. This sample showed no evidence of 2-monopalmitin on thin-layer chromatography. The results indicated that the 1-monoglycerides had the following degree of purity: $C_{15}$ monoglyceride, 98.3%; $C_{17}$ monoglyceride, 98.8%; and our monopalmitin, 99.3%. Results for the 2-monoglycerides indicated the presence of the following amounts of 1-isomer: $C_{15}$ monoglyceride, 4.6%; $C_{17}$ monoglyceride, 3.4%; and monopalmitin, 3.2%.

### *GLC of Monoglycerides and Intermediates*

Chromatography of 1-monoglycerides on a 6 ft × 1/8 inch column of 3% SE-30 (silicone rubber gum, General Electric) on siliconized Gas-Chrom P at 200°C gave the following retention times: $C_{15}$ monoglyceride, 9 min; monopalmitin, 14.3 min; and $C_{17}$ monoglyceride, 18.2 min. The 2-monoglycerides had the same retention time as their 1-isomers. Under the same conditions the isopropylidene and benzylidene intermediates had the following retention times: 1-$C_{15}$-acyl-2,3-isopropylidene glycerol, 5.9 min; 1-$C_{17}$-acyl-2,3-isopropylidene glycerol, 11.8 min; *cis*-2-$C_{15}$-acyl-1,3-benzylidene glycerol, 37.5 min; *trans*-2-$C_{15}$-acyl-1,3-benzylidene glycerol, 50.7 min; *cis*-2-$C_{17}$-acyl-1,3-benzylidene glycerol, 71.8 min; and *trans*-2-$C_{17}$-acyl-1,3-benzylidene glycerol, 98.8 min.

### *Analysis of Glyceride Structure by NMR*

Nuclear magnetic resonance spectra of the monoglycerides prepared during this study are shown in Fig. 3. All spectra were obtained with a Varian A-60 spectrometer and 10–16% solutions (w/v) in $CCl_4$ (spectra A, B, C, D) or $CDCl_3$ (spectra E and F). Peak positions are given relative to internal tetramethylsilane. The amount of hydrogen in each peak was determined by integration.

Beginning with the standard tetramethylsilane peak at 0 ppm (Fig. 3), the next peak at 0.7–1.0 ppm is due to protons on methyl groups. The $C_{15}$ and $C_{17}$ anteiso acids (spectra A,B,C,D) have two methyl groups whose protons are not completely equivalent and do not have exactly the same chemical shift and splitting, while palmitic acid (spectra E and F) has only one methyl group. The large peak at 1.1–1.4 ppm represents all other hydrogens in the fatty acid chain except the methylene group next to the carbonyl group, which is affected by the higher electron density of the carbonyl group and resonates at a lower field (2.1–2.4 ppm) (28).

For distinguishing 1- and 2-monoglycerides, peaks due to protons attached to the glycerol carbons are of primary importance. The spectra of 1-monoglycerides (A, C, and E) have a peak at 3.5–3.7 ppm due to the two protons attached to the 3-carbon of glycerol. The two protons on the 1-carbon are affected by the high electron density of the ester group and appear at a lower field (4.0–4.2 ppm). The 2-carbon proton always gives rise to a broad peak at 3.7–4.0 ppm, between the peaks due to protons on the 1- and 3-carbons.

The spectra of the 2-monoglycerides (B, D, and F) present a quite different picture. Peaks due to the protons on the 1- and 3-carbons, which have no adjacent ester group, are found together as a doublet at a higher field (3.6–3.8 ppm), in nearly the same position as the peak due to protons on the 3-carbon of 1-monoglycerides. The proton on the 2-carbon is, however, influenced by the presence of the ester group and its peak is found at a lower field (4.6–5.0 ppm). While the protons in 1-monoglycerides are all different, the 2-monoglycerides give spectra of type $AX_4$, which means that protons in 1- and 3-positions (X) are equivalent and appear as a doublet split by the 1-proton from position 2 (A). The latter appears as a quintet split by the four protons at the 1- and 3-positions. The coupling constant between the A and X protons is $J_{AX} = 5.1$ cycles/sec (29).

The hydroxyl protons are of less value in these analyses because they are easily exchanged and may be influenced by solvents, temperature, concentration, and hydrogen bonding (30). In monogycerides, peaks due to hydroxyl protons sometimes coincide with those produced by protons attached directly to the glycerol carbons (see spectra A and C), but these can be readily distinguished by exchange with $D_2O$, which eliminates peaks from hydroxyl protons. Hydroxyl protons can also be detected by altering temperature or concentration. At higher temperatures or lower concentrations, these peaks are displaced to higher fields and the reverse occurs at lower temperatures or higher concentrations (31).

The *cis*- and *trans*-1,3-benzylidene glycerol derivatives of $C_{15}$ and $C_{17}$ anteiso acids could be easily recognized by NMR. They gave the same type of spectra as reported for similar compounds by other investigators (20).

## DISCUSSION

The syntheses of monoglycerides described above were based on well-established methods (12–14, 21, 32). In our experience, boric acid in trimethyl borate was preferable to hydrochloric acid in ether for removal of blocking groups because the latter gave rise to isomerization of the monoglycerides (14). This was serious, however, only in the case of 2-monoglycerides. Some relatively new chromatographic techniques were utilized for purification and identification of the products, and thin-layer chromatography on Silica Gel impregnated with boric acid (25) was found to be particularly useful for monitoring the reactions and for distinguishing between 1- and 2-monoglycerides. It is worth noting that 1-monoglycerides could be chromatographed on Florisil or acid-treated Florisil columns without significant isomerization provided the material did not remain too long in contact with the adsorbent. Isomerization of 2-monoglycerides occurred on these columns but could be prevented by using acid-treated Florisil impregnated with boric acid. An attempt to separate 1- and 2-monoglycerides on the acid-treated Florisil–boric acid column gave a partial separation, with the 2-monoglyceride emerging slightly ahead of the 1-monoglyceride. 1- and 2-Monoglycerides of each individual fatty acid had identical retention times

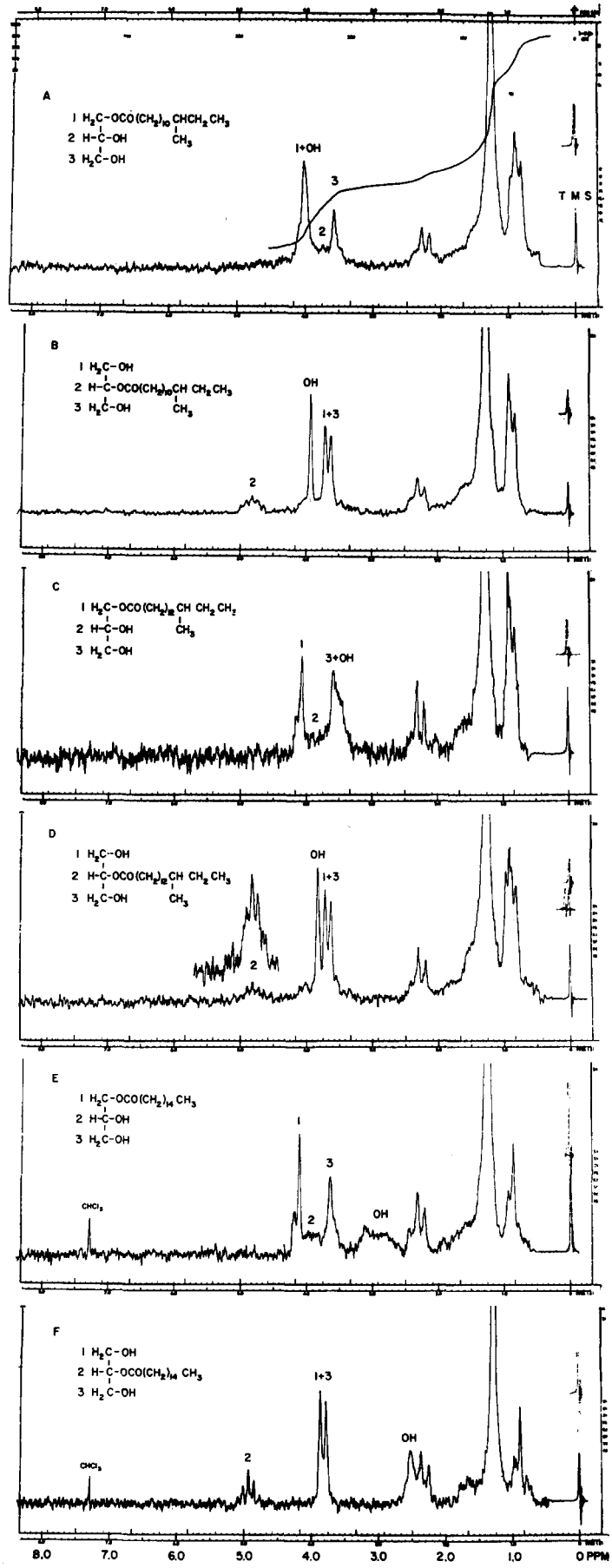


FIG. 3. NMR spectra of 1- and 2-monoglycerides of $C_{15}$ anteiso acid, $C_{17}$ anteiso acid, and palmitic acid at 60 megacycles/sec. TMS, tetramethylsilane.

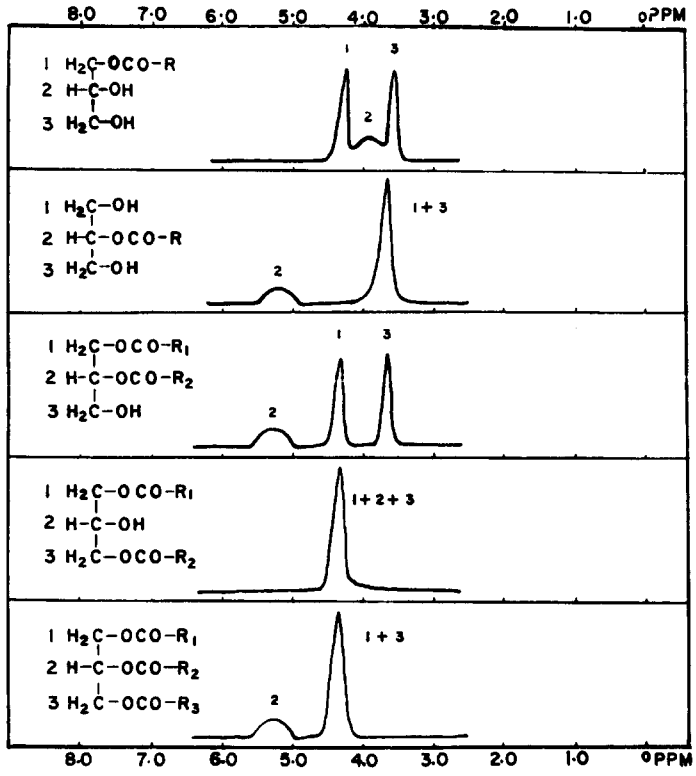


FIG. 4. Diagram showing approximate positions of peaks due to protons attached to glycerol carbons in NMR spectra of mono-, di-, and triglycerides.

on gas–liquid chromatograms, but a recent paper by Wood, Raju, and Reiser indicates that 1- and 2-monoglycerides may be separated by gas–liquid chromatography of their trimethylsilyl derivatives (33).

TLC of newly synthesized 1- and 2-monoglycerides indicated that no significant isomerization occurred during synthesis and purification. After 1-monoglycerides had been stored for 3 months at room temperature in crystalline form, the $C_{15}$ and $C_{17}$ monoglycerides showed small amounts of the 2-isomer when analyzed by TLC but the monopalmitin was unchanged. After only one week at room temperature, the 2-$C_{15}$- and 2-$C_{17}$-monoglycerides showed similar isomerization to 1-monoglycerides but no change was observed with 2-monopalmitin. In each case the $C_{15}$ monoglyceride isomerized faster than the $C_{17}$ monoglyceride.

Periodic acid oxidation always indicated a greater degree of isomerization than TLC but evidence was obtained that some isomerization may occur during the oxidation procedure. If the oxidation of 2-monoglycerides was prolonged from 1 hr, as in the normal procedure, to 3 hr, the analyses showed a significant increase in the amount of 1-monoglyceride present. Further, experiments with different dilutions of monoglycerides showed that TLC should be capable of detecting isomerization at a level of 1 or 2%, yet freshly prepared monoglycerides showed only one spot on TLC whereas the oxidation method indicated 3–4% isomerization in some cases.

The structures of 1- and 2-monoglycerides were readily distinguished by NMR spectroscopy, a technique well established in organic chemical analysis (28, 31), and the method may also be used to distinguish 1,2-diglycerides, 1,3-diglycerides, and triglycerides (29, 34). Fig. 4 illustrates the general pattern of peaks produced by protons attached to glycerol carbons of mono-, di-, and triglycerides. This diagram indicates only approximate positions of the peaks and not their proper splitting. The chemical shift of protons attached to glycerol carbons may be altered slightly by the chemical structure of the fatty acids attached to the glycerol, but the patterns should in general resemble those depicted in Fig. 4. In glycerides containing unsaturated fatty acids, double-bond protons will give rise to peaks in approximately the same range of the spectrum as the proton on the 2-carbon of 2-monoglycerides, 1,2-diglycerides, and triglycerides, and the intensity of this peak will be increased to an extent determined by the number of double-bond protons.

Bioassays of the monoglycerides of $C_{15}$ and $C_{17}$ anteiso acids carried out by Dr. J. H. Cutts of the Cancer Research Laboratory, University of Western Ontario, failed to show evidence of monocyte-producing activity. It is

planned to publish details of these assays in a separate communication.

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## References

1. Murray, E. G. D. *Trans. Roy. Soc. Can. Sect. V, Ser. III, 47:* **15**, 1953.
2. Seeliger, H. P. R. *Listeriosis*. S. Karger, New York, 1961.
3. Murray, E. G. D., R. A. Webb, and M. B. R. Swann. *J. Pathol. Bacteriol.* **29:** 407, 1926.
4. Stanley, N. F. *Australian J. Expt. Biol. Med. Sci.* **27:** 123, 1949.
5. Beaulieu, M. *Can. J. Microbiol.* **9:** 473, 1963.
6. Carroll, K. K. *Can. J. Biochem. Physiol.* **40:** 1115, 1962.
7. Privett, O. S., and E. C. Nickell. *J. Am. Oil Chemists' Soc.* **40:** 189, 1963.
8. Weitkamp, A. W. *J. Am. Chem. Soc.* **67:** 447, 1945.
9. Velick, S. F., and J. English, Jr. *J. Biol. Chem.* **160:** 473, 1945.
10. Kaneda, T. *J. Biol. Chem.* **238:** 1222, 1963.
11. Carroll, K. K. *J. Lipid Res.* **2:** 135, 1961.
12. Baer, E., and H. O. L. Fischer. *J. Am. Chem. Soc.* **67:** 2031, 1945.
13. Hartman, L. *J. Chem. Soc.* **no vol:** 4134, 1959.
14. Mattson, F. H., and R. A. Volpenheim. *J. Lipid Res.* **3:** 281, 1962.
15. Carroll, K. K. *J. Am. Oil Chemists' Soc.* **40:** 413, 1963.
16. Hibbert, H., and N. M. Carter. *J. Am. Chem. Soc.* **51:** 1601, 1929.
17. Verkade, P. E., and J. D. van Roon. *Rec. Trav. Chim.* **61:** 831, 1942.
18. Stimmel, B. F., and C. G. King. *J. Am. Chem. Soc.* **56:** 1724, 1934.
19. Dobinson, B., and A. B. Foster. *J. Chem. Soc.* **no vol:** 2338, 1961.
20. Baggett, N., B. Dobinson, A. B. Foster, J. Homer, and L. F. Thomas. *Chem. Ind. (London)* **no vol:** 106, 1961.
21. Martin, J. B. *J. Am. Chem. Soc.* **75:** 5482, 1953.
22. Privett, O. S., and M. L. Blank. *J. Lipid Res.* **2:** 37, 1961.
23. Hofmann, A. F. *J. Lipid Res.* **3:** 391, 1962.
24. Hofmann, A. F. In *New Biochemical Separations*, edited by A. T. James and L. J. Morris. D. Van Nostrand Co., New York, 1964, pp. 283–294.
25. Thomas, A. E. III, J. E. Scharoun, and H. Ralston. *J. Am. Oil Chemists' Soc.* **42:** 789, 1965.
26. Morris, L. J. In *New Biochemical Separations*, edited by A. T. James and L. J. Morris. D. Van Nostrand Co., New York, 1964, pp. 295–319.
27. Karnovsky, M. L., and A. F. Brumm. *J. Biol. Chem.* **216:** 689, 1955.
28. Jackman, L. M. *Nuclear Magnetic Resonance Spectroscopy*. Pergamon Press, London, 1959.
29. Chapman, D. *J. Chem. Soc.* **no vol:** 131, 1963.
30. Pimentel, G. C., and A. L. McClellan. *The Hydrogen Bond*. Freeman and Co., London, 1960.
31. Pople, J. A., W. G. Schneider, and H. J. Bernstein. *High-resolution Nuclear Magnetic Resonance*. McGraw-Hill Co., New York, 1959.
32. Fischer, E., M. Bergmann, and H. Bärwind. *Ber. Deut. Chem. Ges.* **53:** 1589, 1920.
33. Wood, R. D., P. K. Raju, and R. Reiser. *J. Am. Oil Chemists' Soc.* **42:** 161, 1965.
34. Hopkins, C. Y. *J. Am. Oil Chemists' Soc.* **38:** 664, 1961.