GLC of Trimethylsilyl Derivatives of Pantothenyl Alcohol and Pantothenates

A. R. PROSSER and A. J. SHEPPARD

Abstract □ The trimethylsilyl ester-ethers of pantothenic acid, calcium pantothenate, and sodium pantothenate and the trimethylsilyl ethers of pantothenyl alcohol were prepared readily and quantitatively by using a mixture of bis(trimethylsilyl)acetamide, trimethylchlorosilane, trimethylsilylimidazole, and dimethyl sulfoxide. GLC column packings of 5% SE-30 and 3% OV-1 were demonstrated to yield symmetrical, resolved peaks for the trimethylsilyl derivatives. Quantitatively, 25 ng. of the trimethylsilyl derivatives, based on the starting materials, was effectively measured with the flame-ionization detector; GLC responses were linear as the quantity of the compound increased. The structures and molecular weights of the derivatives were confirmed by NMR and mass spectrometry.

Keyphrases □ Pantothenyl alcohol, pantothenates—analysis □ Trimethylsilyl esters, pantothenyl alcohol, pantothenates—synthesis, determination □ GLC—analysis □ NMR spectroscopy—structure

The naturally occurring isomer of pantothenic acid is the *d*(+)-enantiomorph. Synthetic pantothenic acid is a racemic mixture and, since only the *d*(+)-isomer is biologically active, this fact must be taken under consideration if the *dl* mixture is to be used as a source of pantothenic acid. Synthetic *d*-pantothenic acid as the calcium or sodium salt is widely used in pharmaceutical products; the calcium salt is used more often in solid products because of its ease of handling. Aqueous dispersions of combined oil- and water-soluble vitamins, in which calcium ions must be avoided to prevent precipitation of other components, contain the sodium salt.

Pantothenyl alcohol, referred to commercially as panthenol, is now widely used as a source of pantothenate activity in pharmaceutical vitamin products because it is more stable than the pantothenate salts, especially in liquid multivitamin products that must be slightly acid in order to preserve the thiamin content. Panthenol itself has no pantothenate activity; in fact, it is an inhibitor of many lactic acid-producing bacteria. The inhibitory property of panthenol was reported early in the microbiological work on pantothenic acid (1); it was made the basis of the microbiological assay for panthenol (2) and was recommended as the preferred method. Panthenol is quantitatively converted to pantothenic acid in the animal body (3) and is equivalent to pantothenic acid in man (4, 5).

As an extension of the work of this laboratory on the GLC of water-soluble vitamins (6–10) and the work of others (11–19), the behavior of the trimethylsilyl derivatives of the pantothenates and panthenol was investigated. Although a number of methods have long been available for the identification and determination of the pantothenates and panthenol (19–26), GLC has been successfully used for the analysis of ethyl pantothenate diacetate and panthenol triacetate (9, 10) and should be readily adaptable to the analysis of the diether monoester trimethylsilyl derivative of the pantothenates and the triether trimethylsilyl derivative of panthenol. This report describes a one-step procedure for the rapid and

Table I—Summary of NMR Spectrum of Tritrimethylsilyl Pantothenic Acid

Proton(s)	Band Center, p.p.m.[a]	Description	Number of Protons
$-OSi(CH_3)_3$	0–0.2	Three singlets	27
$-C(CH_3)_2-C-$	0.60, 0.72	Two singlets	6
$-C-CH_2-C(=O)-$	2.33	Triplet (J = 6 Hz.)	2
$-O-CH_2-C-$	3.13	*ab* Pattern (J = 9.5 Hz.)	2
$-N-CH_2-C-$	3.25	Triplet (J = 6 Hz.)	2
$-C(-O-)-CH-C(=O)-$	3.83	Singlet	1
$-C(=O)-NH-C-$	(Below 5.5)	(Broad)	1

[a] Referred to tetramethylsilane.

Table II—Summary of NMR Spectrum of Tritrimethylsilyl Panthenol

Proton(s)	Band Center, p.p.m.[a]	Description	Number of Protons
$-OSi-(CH_3)_3$	0.0, 0.04, 0.05	Three singlets	27
$-C(CH_3)_2-C-$	0.79, 0.84	Two singlets	6
$-C-CH_2-C-$	1.63	Quintet with fine structure (J = 6 Hz.)	2
$-O-CH_2-C-$	3.24	*ab* Pattern (J = 9 Hz.)	2
$-C-CH_2-O-$	3.22	Triplet (J = 6 Hz.)	2
$-N-CH_2-C-$	3.59	Triplet (J = 6 Hz.)	2
$-C(-O-)(-CH-)-C(=O)-$	3.94	Singlet	1
$-C(=O)-NH-C-$	6.81	(Broad)	1

[a] Referred to tetramethylsilane.

quantitative preparation of the trimethylsilyl derivatives of pantothenic acid, calcium pantothenate, sodium pantothenate, and pantothenyl alcohol. The structure confirmation and GLC determination of the trimethylsilyl derivatives of the pantothenates and panthenol are presented.

EXPERIMENTAL

Instrumentation—A model 5000 Barber-Colman gas chromatograph[1], fitted with a high temperature hydrogen flame-ionization detector (FID) and 5-mv., 2-sec., 27.9-cm. (11-in.) strip chart recorder, was used for the analysis of the trimethylsilyl derivatives.

Materials—The following were used: *d*-pantothenyl alcohol[2], *d*-pantothenic acid calcium salt[2], *dl*-pantothenyl alcohol[2], *dl*-pantothenic acid[3], *d*-pantothenic acid sodium salt[4], bis(trimethylsilyl)acetamide[5], bis(trimethylsilyl)trifluoroacetamide[5], trimethylchlorosilane[6], trimethylsilylimidazole[6], pyridoxine (pyridoxol) hydrochloride USP reference standard[7], dimethyl sulfoxide[8], and hexane (*n*-hexane, pure grade)[9]. All other reagents used were ACS reagent grade and required no further purification.

Preparation of Trimethylsilyl Derivatives—The diether monoester trimethylsilyl derivative of pantothenic acid was prepared from *dl*-pantothenic acid, *d*- and *dl*-calcium pantothenate, and *d*-sodium pantothenate. The triether trimethylsilyl derivative of panthenol was prepared from *d*- and *dl*-pantothenyl alcohol.

GLC Standards—Standard solutions of the trimethylsilyl derivatives were prepared as follows. A 50-ml. volumetric flask, with a 24/40 stopper and stirring bar, was charged with 146.9 mg. of *d*-sodium pantothenate and 125.0 mg. of *d*-pantothenyl alcohol. To these compounds was added 5 ml. of a 4:2:2 mixture of bis(trimethylsilyl)acetamide – trimethylsilylimidazole – trimethylchlorosilane or bis(trimethylsilyl)trifluoroacetamide–trimethylsilylimidazole–trimethylchlorosilane. The reaction mixture was stirred for 10 min. at room temperature, and 0.2 ml. of dimethyl sulfoxide was added. The mixture was stirred for another 5 min. and then diluted to volume with benzene. The concentration of each derivative, based on the starting material, was equivalent to 2.5 mg./ml. of panthenol.

Calibration Standards—Aliquots of 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 ml. were taken from the GLC standard solution and diluted to 10 ml. with benzene. After dilution, a standardized injection of 2 μl. contained 0.25, 0.50, 0.75, 1.00, 1.50, and 2.00 mcg., respectively, of each trimethylsilyl derivative.

Spectroscopy Standards—The trimethylsilyl derivatives for the spectroscopic studies were prepared individually in the manner described for GLC standards, except that dimethyl sulfoxide was omitted, *n*-hexane was used in place of benzene, and the mixture was heated at 60° for 10 min. The reaction solvents were removed with a stream of nitrogen; the oily residue was taken up in *n*-hexane, heated for 5 min. at 60°, cooled, and filtered to remove the NaCl. The *n*-hexane was then removed with nitrogen, and the oily residue was placed in a sealable ampul (color break) for spectroscopic analysis.

GLC—Two column packings were evaluated for their usefulness to resolve the trimethylsilyl derivatives of panthenol and the pantothenates:

Column 1—244 × 0.4 cm. i.d. packed with a liquid phase of 5% SE-30 (w/w) on 100–120-mesh Gas Chrom Q. Operating conditions for the separation of the trimethylsilyl derivatives were: column, 185°; detector, 270°; flash heater, 270°; and carrier gas, 80 ml./min. (40 p.s.i.).

Column 2—244 × 0.4 cm. i.d. packed with a liquid phase of 3% OV-1 (w/w) on 110–120-mesh Anakrom ABS. Operating conditions for the trimethylsilyl derivative separation were: column, 200°; detector, 280°; flash heater, 280°; and carrier gas, 80 ml./min. (40 p.s.i.).

All columns were U-shaped Pyrex glass and were preconditioned for 24 hr. at 295° with a carrier gas flow rate of 60 ml./min. Nitrogen was used as the carrier gas throughout the study. Compressed air and hydrogen flow rates used with the FID were 460 and 36 ml./min., respectively. Peak areas were determined by triangulation (peak width at half-height × peak height = peak area).

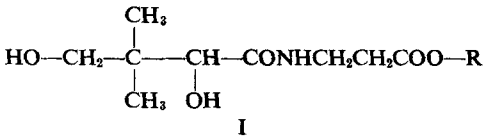
Efficiency of Derivative Preparation—During the preparation of the GLC standards, equal aliquots of the reaction mixture were taken for GLC analysis every 2 min. for 10 min. after addition of the dimethyl sulfoxide. The trimethylsilyl ether derivatives were

[1] Barber-Colman, Rockford, Ill.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] K & K Laboratories, Inc., Plain View, N. Y.
[4] Mann Research Laboratories, Division of Becton Dickinson & Co., New York, N. Y.
[5] Supelco, Inc., Bellefonte, Pa.
[6] Applied Science Inc., State College, Pa.
[7] USP Reference Standards, Bethesda, Md.
[8] Crown Zellerbach Corp., Chemical Products Division, Camas, Wash.
[9] Phillips Petroleum Co., Special Products Division, Bartlesville, Okla.

completely formed at 14 min., as indicated by constant peak height. Pantothenic acid and panthenol can also be converted to their trimethylsilyl derivatives by using a 4:1 mixture of bis(trimethylsilyl)acetamide–trimethylsilylimidazole and diluting with *n*-hexane or benzene. Trimethylchlorosilane was required to convert calcium and sodium salts of pantothenic acid to its trimethylsilyl ester derivative. The dimethyl sulfoxide was added as a solvent and catalyst.

RESULTS AND DISCUSSION

Compounds involved and their identifications are as follows:



I

Compound I—Pantothenic acid, R = H, mol. wt.: 219.23; calcium pantothenate, R = Ca/2, mol. wt.: 476.53/2; and sodium pantothenate, R = Na, mol. wt.: 241.21.

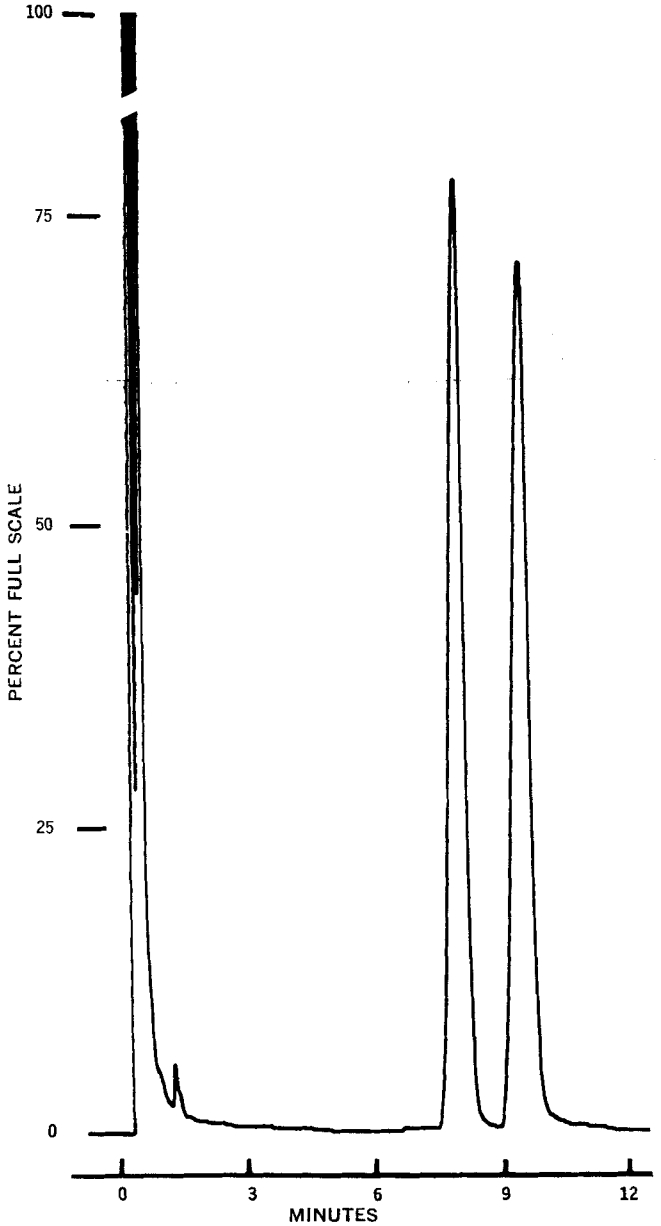
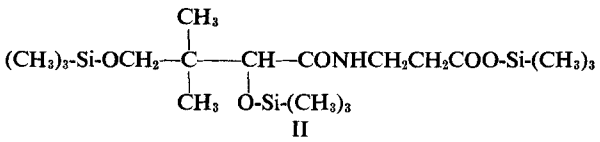


Figure 1—*Chromatogram of 1.00 mcg./μl. of panthenol as the panthenol tritrimethylsilyl derivative (8.0 min.) and 1.18 mcg./μl. of sodium pantothenate as the pantothenic acid tritrimethylsilyl derivative (9.5 min.) in* n-*hexane on a 5% SE-30 column at 185°.*

Table III—Response of Pantothenates and Pantothenol Trimethylsilyl Derivatives[a]

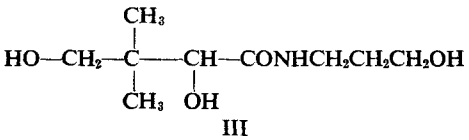
Starting Material, mcg.	Peak Area, mm.[2b] Tritrimethylsilyl Pantothenic Acid	Tritrimethylsilyl Panthenol
0.25	2.7 ± 0.1	9.3 ± 0.2
0.50	12.1 ± 0.5	19.5 ± 0.7
0.75	22.0 ± 0.8	30.3 ± 1.2
1.00	35.0 ± 1.4	42.5 ± 1.7
1.50	55.5 ± 1.6	65.0 ± 2.2
2.00	77.5 ± 1.9	86.0 ± 2.4

[a] 10× attenuation, electrometer output 1 × 10^{-10} amp., hydrogen flame detector, and a 5% SE-30 column. [b] Mean of six analyses ± standard deviation.



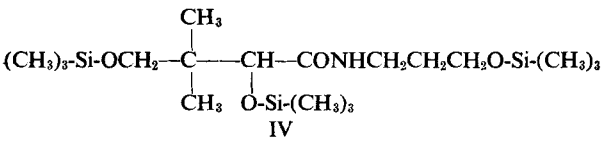
II

Compound II—Tritrimethylsilyl pantothenic acid, mol. wt.: 435.79.



III

Compound III—Panthenol, mol. wt.: 205.26.



IV

Compound IV—Tritrimethylsilyl panthenol, mol. wt.: 421.82.

After conditions of silylation were found that produced only one reaction product per compound in quantitative yield, NMR and mass spectrometry were used to confirm that the trimethylsilyl derivatives (II and IV) were actually formed from the parent compounds (I and III).

NMR Spectroscopy—The spectra of chloroform-*d* solutions of II and IV were determined at 60 and 100 MHz.[10]; internal $CHCl_3$ served as the reference standard. The spectra were compatible with the proposed structures of II and IV; they are summarized in Tables I and II.

Mass Spectrometry—An aliquot of the individually prepared spectrometry standard was prepared for mass spectrometry by breaking open the ampul and removing the contents. The oily brown liquid was subjected to mass spectroscopic analysis. The mass spectrum of IV exhibited a molecular ion of low relative abundance at *m/e* 421. An intense ion produced by the loss of a methyl group, commonly present in trimethylsilyl derivatives and often used to confirm their molecular weights, was observed at *m/e* 406. Characteristic product ions at *m/e* 277 and *m/e* 247 involved cleavage of the bonds on either side of the secondary carbon bearing the silyl ether group, with a hydrogen migration in the case of the ion at *m/e* 277. In both instances, the positive charge remained with the fragment containing the silyl ether group. These two ions substantiated the structural assignments. Product ions, attributed more specifically to the silyl ether group and the alkyl portion of the molecule, were observed at *m/e* 73, 89, 103, 117, and 131.

No molecular ion was observed in the mass spectrum of II. However, an intense ion at *m/e* 420 (M-15) supported 435 as the molecular weight of the compound. Furthermore, product ions at *m/e* 291 and *m/e* 247, occurring by processes described for IV, were consistent with the postulated structure.

[10] Varian A-60 and HA-100 NMR spectrometers.

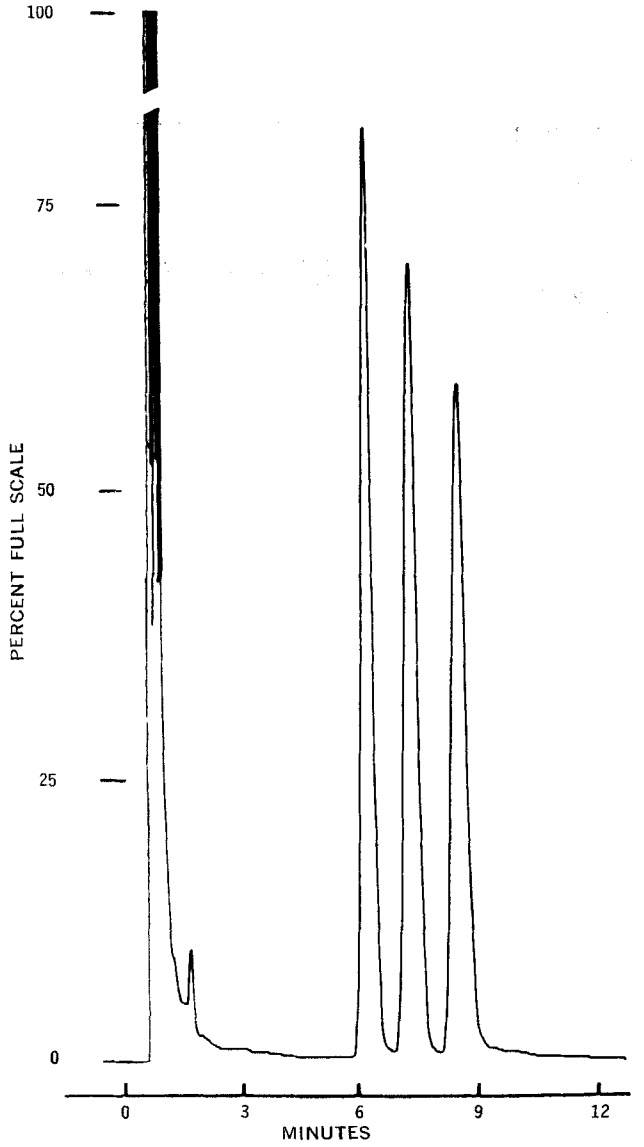


Figure 2—*Chromatogram of 1.00 mcg./μl. of pyridoxol as the pyridoxol tritrimethylsilyl ether (6.3 min.), 1.00 mcg./μl. of panthenol as the panthenol tritrimethylsilyl ether (7.5 min.), and 1.16 mcg./μl. of calcium pantothenate as the pantothenic ditrimethylsilyl ether monotrimethylsilyl ester (8.8 min.) in benzene on a 3% OV-1 column at 200°.*

The postulated structures shown for the two tritrimethylsilyl derivatives give the molecular weights expected.

Effect of Immobile Phases—The elution peaks of II and IV gave distinct peaks on a 5% SE-30 column operated at 185° and 80 ml./min. (Fig. 1). The 3% OV-1 column, operated at 200° and 80 ml./min., gave distinct elution peaks of trimethylsilyl pyridoxol, II, and IV (Fig. 2). The tritrimethylsilyl derivative of pyridoxol (6.3 min. retention time) was used as a reference standard (1.00) to calculate the relative retention times for II (1.2) and IV (1.4) and may be used as an internal standard for samples not containing pyridoxol.

Effect of Quantity Injected—Response data for II and IV, which were determined on a 5% SE-30 column, are given in Table III. These data show that the response was linear as the quantity of compound was increased.

Compound II, used for these data, was prepared from sodium pantothenate. However, the calcium and sodium pantothenate and pantothenic acid can be used interchangeably for obtaining these data when calculated on an equal mole basis.

Sensitivity—The GLC procedure for the analyses of II and IV is very sensitive. Using the FID, an attenuator setting of 1, an electrometer output of 1×10^{-10} amp., and a 0–1-mv. recorder, 2–4 ng. of I and III as Compounds II and IV was detectable. However, quantitative measurement at these low levels is difficult because of the low signal-to-noise ratio.

CONCLUSIONS

A rapid and accurate silylation procedure has been presented which will yield derivatives of all of the pantothenates (calcium pantothenate, pantothenic acid, and sodium pantothenate) and panthenol. Studies are in progress to apply the GLC procedure to pharmaceuticals containing I and III. This procedure may also find use as a monitoring device for determining pantothenic acid and its derivatives in the formation or degradation of coenzyme A.

REFERENCES

(1) E. E. Snell and W. J. Shive, *J. Biol. Chem.*, **158,** 551(1945).
(2) O. D. Bird and L. McCready, *Anal. Chem.*, **30,** 2045(1958).
(3) H. Pfaltz, *Z. Vitaminforsch.*, **13,** 236(1943).
(4) E. Burlet, *ibid.*, **14,** 318(1944).
(5) S. H. Rubin, J. M. Cooperman, M. E. Moore, and J. Scheiner, *J. Nutr.*, **35,** 499(1948).
(6) A. R. Prosser and A. J. Sheppard, *Fed. Proc.*, **25,** 669(1966).
(7) A. R. Prosser, A. J. Sheppard, and D. A. Libby, *J. Ass. Offic. Anal. Chem.*, **50,** 1348(1967).
(8) A. R. Prosser and A. J. Sheppard, *J. Pharm. Sci.*, **57,** 1004 (1968).
(9) A. R. Prosser and A. J. Sheppard, *Fed. Proc.*, **27,** 256(1968).
(10) A. R. Prosser and A. J. Sheppard, *J. Pharm. Sci.*, **58,** 718 (1969).
(11) W. Korytnyk, G. Fricke, and B. Paul, *Anal. Biochem.*, **17,** 66(1966).
(12) L. T. Sennello, F. A. Kummerow, and C. J. Argoudelis, *J. Heterocycl. Chem.*, **4,** 295(1967).
(13) L. T. Sennello and C. J. Argoudelis, *Anal. Chem.*, **41,** 171(1969).
(14) M. Vecchi and K. Kaiser, *J. Chromatogr.*, **26,** 22(1967).
(15) T. Imanari and Z. Tamura, *Chem. Pharm. Bull.*, **15,** 896 (1967).
(16) Y. Ohnishi, Z. Horii, and M. Makita, *Yakugaku Zasshi*, **87,** 747(1967).
(17) B. E. Haskell, *Fed. Proc.*, **27** (2), 1899(1968).
(18) G. Horning, E. A. Boucher, and A. M. Moss, *J. Gas Chromatogr.*, **5,** 297(1967).
(19) J. V. Wisniewski, *Facts Method Sci. Res.*, **7**(2), 4(1966).
(20) M. Schmall and E. G. Wollish, *Anal. Chem.*, **29,** 1509(1957).
(21) T. Panalaks and J. A. Campbell, *ibid.*, **33,** 1038(1961).
(22) *Ibid.*, **34,** 64(1962).
(23) R. G. Panier and J. A. Close, *J. Pharm. Sci.*, **53,** 108(1964).
(24) J. Vachek, *Pharmazie*, **21,** 222(1966).
(25) W. D. Hubbard, M. E. Hintz, D. A. Libby, and R. P. Sutor, Jr., *J. Ass. Offic. Agr. Chem.*, **48,** 1217(1965).
(26) A. F. Zappala and C. A. Simpson, *J. Pharm. Sci.*, **50,** 845 (1961).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 12, 1970, from the *Division of Nutrition, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, DC 20204*

Accepted for publication February 19, 1971.

The authors are indebted to Dr. Ernest Lustig and Mrs. Elizabeth A. Hansen, Division of Food Chemistry and Technology, Food and Drug Administration, for furnishing and interpreting the NMR data. The authors also thank Mr. Joseph N. Damico and Mr. Robert P. Barron, Division of Food Chemistry and Technology, for furnishing and interpreting the mass spectra data.