for determination of the mass spectra of several compounds.

References

Bruce, M. T., and Zwar, J. A. (1966), *Proc. Roy Soc., Ser. B 165*, 245.

Burrows, W. J., Armstrong, D. J., Kaminek, M., Skoog, F., Bock, R. M., Hecht, S. M., Dammann, L. G., Leonard, N. J., and Occolowitz, J. (1970) *Biochemistry 9*, 1867, and references therein.

Burrows, W. J., Skoog, F., and Leonard, N. J. (1971), *Biochemistry 10*, 2189.

Dyson, W. H., Chen, C. M., Alam, S. N., Hall, R. H., Hong, C. I., and Chheda, G. B. (1970), *Science 170*, 328.

Fox, J. E. (1969), Conference on Cytokinins, University of Wisconsin, Madison, Wisconsin, April.

Fox, J. E., Sood, C. K., Buckwalter, B., and McChesney, J. D. (1971), *Plant Physiol. 47*, 275.

Fox, J. E., Sood, C. K., and McChesney, J. D. (1969), *Plant Physiol. Suppl. 44* (6), 2.

Hall, R. H. (1970), *Progr. Nucl. Acid Res. Mol Biol. 10*, 57.

Hecht, S. M., Bock, R. M., Schmitz, R. Y., Skoog, F., and Leonard, N. J. (1971), *Proc. Nat. Acad. Sci. U. S.* (in press).

Hecht, S. M., Leonard, N. J., Occolowitz, J., Burrows, W. J., Armstrong, D. J., Skoog, F., Bock, R. M., Gillam, I., and Tener, G. M. (1969), *Biochem. Biophys. Res. Commun. 35*, 205.

Hecht, S. M., Leonard, N. J., Schmitz, R. Y., and Skoog, F. (1970), *Phytochemistry 9*, 1173.

Kende, H., and Tavares, J. (1968), *Plant Physiol. 43*, 1244.

Koshimizu, K., Kusaki, T., Mitzui, T., and Matsubara, S. (1967), *Tetrahedron Lett.*, 1317.

Leonard, N. J., Achmatowicz, S., Loeppky, R. N., Carraway, K. L., Grimm, W. A. H., Szweykowska, A., Hamzi, H. Q., and Skoog, F. (1966), *Proc. Nat. Acad. Sci. U. S. 56*, 709.

Leonard, N. J., and Grimm, W. A. H. (1967), *Biochemistry 6*, 3625.

Leonard, N. J., Hecht, S. M., Skoog, F., and Schmitz, R. Y. (1969), *Proc. Nat. Acad. Sci. U. S. 63*, 175.

Letham, D. S. (1966a), *Life Sci. 5*, 1999.

Letham, D. S. (1966b), *Phytochemistry 5*, 269.

Matsubara, S., Koshimizu, K., and Nakahira, R. (1968), *Sci. Rep. Kyoto Pref. Univ. 19A*, 19.

McDonald, J. J., Leonard, N. J., Schmitz, R. Y., and Skoog, F. (1971), *Phytochemistry 10*, 1429.

Robins, R. K., Furcht, F. W., Grauer, A. D., and Jones, J. W. (1956b), *J. Amer. Chem. Soc. 78*, 2418.

Robins, R. K., Holum, L. B., and Furcht, F. W. (1956a), *J. Org. Chem. 21*, 833.

Schmitz, R. Y., and Skoog, F. (1970), *Plant Physiol. 45*, 537.

Skoog, F., and Armstrong, D. J. (1970), *Ann. Rev. Plant Physiol. 21*, 359.

Skoog, F., Hamzi, H. Q., Szweykowska, A. M., Leonard, N. J., Carraway, K. L., Fujii, T., Helgeson, J. P., and Loeppky, R. N. (1967), *Phytochemistry 6*, 1169.

Suhadolnik, R. J. (1970), Nucleoside Antibiotics, New York, N. Y., Wiley, pp 364–385.

Isolation, Identification, and Specific Localization of Di-2-ethylhexyl Phthalate in Bovine Heart Muscle Mitochondria*

Darius J. Nazir,† Aurora P. Alcaraz, Barbara A. Bierl, Morton Beroza, and Padmanabhan P. Nair‡

ABSTRACT: A component associated with the triglycerides of beef heart mitochondria has been shown to be identical with di-2-ethylhexyl phthalate. Studies on the intracellular distribution of di-2-ethylhexyl phthalate in heart muscle show that this compound is associated with mitochondrial fractions.

During the course of our studies on the fatty acids of several lipid classes from subcellular fractions of heart muscle, we observed an aberrant peak associated with the fatty acids of mitochondrial triglycerides. This component, which was originally suspected to be a highly unsaturated fatty acid, at times formed approximately 60% of the total esterified fraction derived from the triglycerides of mitochondria. The specific localization of this component prompted us to investigate its structure and distribution. This paper describes the isolation and characterization of di-2-ethylhexyl phthalate (DEHP)[1] in heart muscle mitochondria. The detection of DEHP is unique in that (a) this compound has not been reported previously in mammalian tissues; (b) it has been detected in heart muscle mitochondria from several other species and is not limited to the ruminants.

Experimental Section

Subcellular Fractionation. Beef hearts were obtained fresh from the abattoir and transported to the cold room packed in Dry Ice. All general procedures were carried out at the

* From the Biochemistry Research Division, Department of Medicine, Sinai Hospital of Baltimore, Inc., Baltimore, Maryland 21215, and from the Entomology Research Division, U. S. Department of Agriculture, Beltsville, Maryland. *Received June 14, 1971.* This work was supported by Grant HE-05283 and General Research Support Grant 5SO-1-FR-05478 from the National Institutes of Health, U. S. Public Health Service. A preliminary report of this work was presented at the 51st Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Ill., April 18, 1967.

† Present address: Division of Medical Research, University of Saskatchewan, Saskatoon, Can.

‡ To whom to address correspondence.

[1] Abbreviation used is: DEHP, di-2-ethylhexyl phthalate.

temperature of the cold room (5°). The muscle was minced in a meat grinder and gently washed with 0.25 M sucrose containing 0.001 M EDTA (pH 7.0) and then homogenized in a Sorvall Omni-Mixer at top speed for 20 sec with the same sucrose solution (2.5 ml/g of tissue).

Light and heavy mitochondrial fractions were isolated as described by Crane *et al.* (1956) and Green and Ziegler (1963). After removal of the mitochondrial fractions, the supernatant was further separated into "microsomal" and "cytoplasmic" fractions (Nazir *et al.*, 1967). All subcellular particulate fractions were essentially homogenous by electron microscopy. Protein concentration was measured by the Lowry modification of the Folin–Ciocalteau procedure (Layne, 1957).

Extraction and Fractionation of Lipids. Following Folch extraction with 20 volumes of chloroform–methanol (2:1, v/v) (Fisher certified) and washing (Folch *et al.*, 1957; Nazir *et al.*, 1967), the lipids were separated by a modified silicic acid chromatographic procedure (Hirsch and Ahrens, 1958; Wheeldon *et al.*, 1965; Berry *et al.*, 1965).

The purity of each fraction was established by methods described in a previous publication from this laboratory (Wheeldon *et al.*, 1965). The triglyceride fraction (4% ether–hexane) derived from the total lipids of heavy mitochondria was used for the identification of di-2-ethylhexyl phthalate.

Fisher certified reagents were used throughout and the solvents were distilled before use. Control runs with solvents alone were made to confirm the absence of impurities which would interfere with final gas–liquid chromatographic studies. The fatty acids in lipid fractions were esterified (Wheeldon *et al.*, 1965) with methanolic HCl in the presence of dimethoxypropane following a modified procedure (Berry *et al.*, 1965).

Gas–Liquid Chromtography of Methyl Esters. The composition of methylated fatty acids was determined by quantitative gas–liquid chromatography using argon β ionization detectors containing 20 mCi of ^{90}Sr sources (Barber-Colman Model 10 and Electronic Instruments for Research Model AU-8).

Gas-Chrom P, 80-100 mesh (Applied Science Laboratories, Inc.), previously acid washed and deactivated with 2% (v/v) dimethyldichlorosilane (Nair and Turner, 1963) was used for coating with either diethylene glycol succinate (200 ml of an 8% solution in acetone/15 g of support) or Apiezon L (AEI (Manchester) Ltd. from Apiezon Products Ltd., 200 ml of a 4% solution in chloroform/15 g of support). Siliconized, U-shaped glass columns, 8 ft × 4 mm i.d. and 6 ft × 5 mm i.d., were generally used. The diethylene glycol succinate and Apiezon L columns were operated at temperatures of 180 and 200° and outlet flow rates of 75 and 300 ml per min, respectively, with a detector polarizing voltage of 1000.

Quantitation of Di-2-ethylhexyl Phthalate. The fraction eluted with 4% ether in hexane from the silicic acid column was evaporated to a small volume under partial vacuum, and aliquots of 5–10 μl were analyzed by gas–liquid chromatography on a two-component SE52/XE60 column as described by Nair *et al.* (1966) at a temperature of 200° and outlet flow rate of 250 ml/min. Peak areas obtained by triangulation were compared with these obtained with authentic DEHP.

Microhydrogenation and Microozonization of Di-2-ethylhexyl Phthalate Isolated by Preparative Gas-Liquid Chromatography. DEHP was isolated by preparative gas–liquid chromatography (10 ft × 8 mm diethylene glycol succinate column operated at a temperature of 200° and an outlet flow rate of 750 ml/min) and about 2 μg of the substance was subjected to microhydrogenation (Beroza and Sarmiento, 1966). Another aliquot (5 μg) was examined by the microozonization procedure of Beroza and Bierl (1966).

TABLE I: Retention Time Data on the Unidentified Component from Bovine Heart Muscle Mitochondrial Triglyceride Relative to Methyl Octadecanoate.[a]

Stationary Phases	Unknown Compound	DEHP[b]
Diethylene glycol succinate[c]	13.6	13.5
Apiezon L[d]	4.4	4.4
SE52–XE60 (2:1, v/v)[e]	4.6	4.6

[a] Methyl octadecanoate = 1.00. [b] DEHP = di-2-ethylhexyl phthalate. [c] Column temperature: 180°; outlet flow rate; 75 ml/min. [d] Column temperature: 200°; outlet flow rate; 300 ml/min. [e] Column temperature: 200°; outlet flow rate; 250 ml/min. Experimental details are given in the text.

Saponification. Approximately 5 mg of the unknown compound were saponified with 30% aqueous KOH for 2 hr. The nonsaponifiable portion was extracted with ether five times. The ether extract was washed with distilled water to neutrality and dried over anhydrous $MgSO_4$. The residue left upon removal of the solvent was redissolved in a minimum volume of hexane, and aliquots from this solution were chromatographed on diethylene glycol succinate (50° and 40 ml/min), Apiezon L (55° and 30 ml/min), and SE52/XE60 (65° and 70 ml/min) columns.

The aqueous layer that remained after extraction of the nonsaponifiables was acidified to pH 1.0 with HCl to release the free acid. This solution was adjusted to about pH 5.0 with 1 N KOH, was lyophilized overnight, and the residue was extracted with tetrahydrofuran. The residue obtained after removal of tetrahydrofuran was methylated and chromatographed on diethylene glycol succinate (175° and 70 ml/min), Apiezon L (175° and 120 ml/min), and SE52/XE60 (175° and 50 ml/min) columns.

Carbon Skeleton Chromatography. Carbon skeleton chromatography of the unknown compound was carried out as described by Beroza (1962) and Beroza and Sarmiento (1963). The catalyst used was 1% palladium on Gas-Chrom P at a temperature of 300°. A 15 ft × 5 mm o.d. copper column packed with 5% squalane on 60–80 mesh Chromosorb W at a temperature of 60° and an outlet flow rate of 30 cc of hydrogen/min was used for the separation of fragments obtained from the parent compound.

Results

During the course of our studies on the fatty acids of lipid classes from subcellular fractions of heart muscle homogenate, we consistently noticed an unidentified peak in the gas chromatograms of fatty acid methyl esters derived from the triglyceride fraction of heavy mitochondria. The retention values characteristic of this compound, obtained by gas–liquid chromatography on diethylene glycol succinate, Apiezon L, and SE52/XE60 columns, are presented in Table I. The compound was subsequently isolated in pure form by preparative gas–liquid chromatography on diethylene glycol succinate.

TABLE II: Retention Time Data for Several Closely Related Alcohols Relative to Isoamyl Alcohol on Three Different Stationary Phases.

Stationary Phases	Relative Retention Time[a]							
	1-Butanol	Isobutyl Alcohol	Isoamyl Alcohol	4-Heptanol	2-Ethyl 1-hexanol	1-Octanol	2-Octanol	Unknown
Diethylene glycol succinate	0.73	0.51	1.00[b]	1.58	4.77	7.15	3.42	4.77
Apiezon L			1.00[c]	5.49	14.82	20.75	12.60	14.82
SE52–XE60			1.00[d]	3.19	8.72	12.45	6.82	8.72

[a] Isoamyl alcohol = 1.00. [b] Isoamyl alcohol = 4.3 min. [c] Isoamyl alcohol = 1.7 min. [d] Isoamyl alcohol = 2.0 min. Experimental conditions are described in the text. Unknown sample represents the alcohol moiety of the compound isolated from bovine heart muscle mitochondria.

The unknown compound was shown to be unaltered both on microhydrogenation combined with gas–liquid Chromatography and on microozonization (Beroza and Sarmiento, 1966; Beroza and Bierl, 1966). These observations indicated the absence of aliphatic double bonds so that the compound could not be an unsaturated fatty acid as was at one time suspected.

In carbon skeleton chromatography (Beroza, 1962; Beroza and Sarmiento (1963) multiple bonds are saturated and functional groups containing oxygen are stripped from compounds giving as products the parent hydrocarbon and/or the next lower homolog, which are identified by their retention times. In the present study, the unknown compound, subjected to carbon skeleton chromatography, gave peaks with retention times corresponding to *n*-heptane (11.9 min), a branched-chain hydrocarbon (3-methylheptane which is 2-ethylhexane, 20.0 min), and a weak peak for benzene (9.5 min). The breakdown pattern and relative proportions in which each of the hydrocarbons were obtained suggested a disubstituted benzene ring with a 2-ethylhexyl side chain (Figure 1). Authentic DEHP (Matheson Coleman & Bell) gave products with retention times identical with those obtained with the unknown compound.

The infrared spectrum of the isolated compound (0.5-mm micro-KBr pellet containing 25 μg of DEHP in 10 mg of KBr) showed a band at 13.5 μ indicating the presence of an ortho-disubstituted benzene ring. Absorption bands at 7.9, 8.9, and 9.4 μ were attributed to carbon–oxygen absorption, characteristic of phthalates. Other absorption bands were present at 5.8 μ for the ester carbonyl, at 6.9 μ for the aliphatic C–H, and at 7.3 μ for the methyl groups. The ratios indicated that the alcohol moiety was longer than propyl and possibly branched. Data from carbon skeleton chromatography and the infrared spectrum were consistent with the structural features of DEHP. Identical results were obtained when authentic DEHP was subjected to carbon skeleton chromatography and infrared spectroscopy (Sadtler standard ir spectrum no. 28).

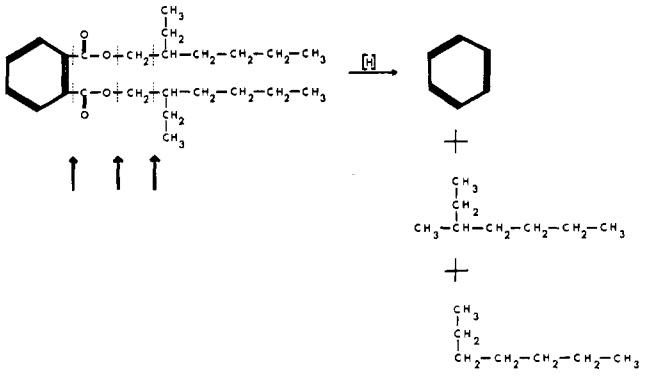

FIGURE 1: Diagramatic representation of structural subunits derived from di-2-ethylhexyl phthalate upon carbon skeleton chromatography.

Following drastic alkaline hydrolysis, the alcohol portion, upon gas chromatography on diethylene glycol succinate, Apiezon L, and SE52–XE60 columns, revealed the presence of a single component having retention times identical with those of authentic 2-ethyl 1-hexanol (Table II). When the acidic portion was methylated and subjected to gas chromatography, only one component having the same retention characteristics as those of dimethyl phthalate was detected (Table III).

Elemental Analysis for Di-2-ethylhexyl Phthalate. Anal. Calcd for $C_{24}H_{38}O_4$: C, 73.85; H, 9.74; O, 16.41. Found (unknown compound): C, 73.64; H, 9.64; O, 16.70.

TABLE III: Relative Retention Time Data of *o*-, *m*-, and *p*-Dimethyl Esters of Benzenedicarboxylic Acids on Three Different Stationary Phases.

Stationary Phases	Relative Retention Time[a]			
	Ortho	Meta	Para	Unknown
Diethylene glycol succinate	1.21	1.10	1.00[b]	1.21
Apiezon L	0.63	1.00	1.00[c]	0.63
SE52–XE60	0.92	1.07	1.00[d]	0.92

[a] Dimethyl terephthalate = 1.00. [b] Dimethyl terephthalate = 11.7 min. [c] Dimethyl terephthalate = 3.8 min. [d] Dimethyl terephthalate = 2.5 min. Experimental conditions are described in the text. Unknown sample represents the methylated acid moiety of the compound isolated from bovine heart muscle mitochondria.

TABLE IV: Distribution of Di-2-ethylhexyl Phthalate (DEHP) among Subcellular Fractions from Bovine Heart Muscle.

Subcellular Fraction	Protein[a] (%)	DEHP (mg/100 g of Muscle)	DEHP[b] (mg/100 g of Total Lipid)	DEHP[c] (mg/100 g of Total Triglyceride)
Nuclear	73.4	Trace[d]		
Heavy mitochondrial	0.2	13.5	304.1	1752.6
Light mitochondrial	0.3	0.013	0.293	1.689
Microsomal	0.8	0.015	0.338	1.948
Cytoplasmic	25.3	0.033	0.743	4.283

[a] Mean of three determinations. [b] Represents the DEHP in 100 g of total lipid from whole heart homogenate, distributed among subcellular fractions. Values were computed from total lipid content of whole heart muscle (4.44 g/100 g of muscle). [c] Represents the DEHP in 100 g of total triglyceride from whole heart homogenate, distributed among subcellular fractions. Values were computed from total triglyceride content of whole heart muscle (0.77 g/100 g of muscle). [d] DEHP was found only in traces and could not be quantitatively determined.

Mass Spectrometry. The mass spectrum of the isolated compound obtained on a Consolidated Electrodynamics Corp. Model 21-110B mass specrotrometer was identical with that of an authentic sample of DEHP. The molecular formula established by peak matching the molecular ion $C_{24}H_{38}O_4^+$ was found to be 390.2765 compared to a theoretical value of 390.2770. The *m/e* values of fragments with highest relative abundance corresponded to rearrangement ions of phthalic anhydride (149; M − 241), the diacid fragment (167; M − 223) (15), and the one derived by the loss of a 2-ethylhexyl group from the parent molecular ion (279; M − 111).

Nuclear Magnetic Resonance Spectra. The nuclear magnetic resonance spectrum of the isolated compound was identical with that of a reference sample of DEHP.

Separation of Diesters of Phthalic Acid by Gas–Liquid Chromatography. The separation of several diesters of phthalic acid with 5% SE30 on Chromosorb W (6 ft × 6 mm o.d. stainless steel column) at 225° with a flow rate of 30 cc/min of nitrogen is represented in Figure 2. Although Perkins (1967) was unable to distinguish between di-2-ethylhexyl phthalate and di-*n*-octyl phthalate, our results show that these compounds could be easily distinguished on a nonpolar column.

Subcellular Distribution in Heart Muscle Homogenate. The triglyceride fraction of total lipids obtained from the various subcellular fractions of bovine heart muscle homogenate was subjected to gas chromatography on a two-component mixed-phase column (SE52–XE60) to quantitate DEHP. The heavy mitochondrial fraction accounted for almost all of the total DEHP in the cell. Quantitation of DEHP in the nuclear fraction was rendered inaccurate on account of extremely low levels of DEHP in relation to other lipids in this fraction (Table IV).

Di-2-ethylhexyl Phthalate from Heart Muscle Mitochondria of Other Species. DEHP was quantitated in mitochondria from heart muscle of rat, rabbit, and dog (Table V). The results indicated that DEHP is not confined to heart muscle mitochondria of ruminants.

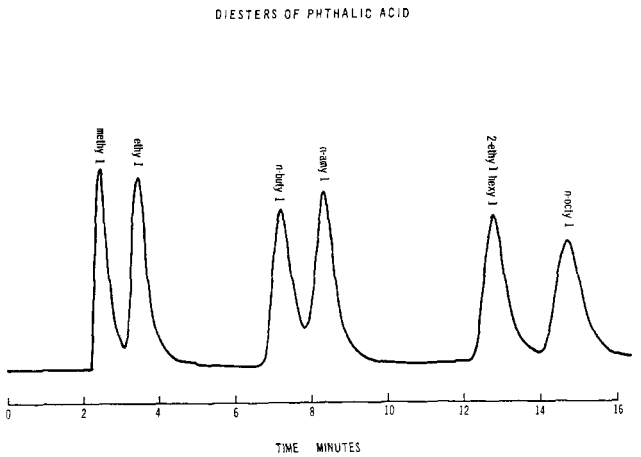

FIGURE 2: Gas–liquid chromatographic separation of diesters of phthalic acid on a 5% SE30 column. Experimental details are given in the text.

Discussion

Since di-2-ethylhexyl phthalate is a compound widely used as a plasticizer in the production of synthetic resins, flexible films, and tubing, and as an additive in vacuum pump oils, these materials were scrupulously avoided in the processing of the samples. In certain experiments, subcellular fractionation up to the stage of the postmitochondrial supernatant was performed in stainless steel containers in a Sorvall RC-2B refrigerated centrifuge. Moreover, control experiments witout tissue (solvents and other reagents) were essentially negative, showing that DEHP did not originate as a contaminant from laboratory reagents or equipment. When the lipids of each of the five subcellular fractions of beef heart muscle (nuclear, heavy and light mitochondrial, microsomal, and cytoplasmic) were separated into fourteen fractions by silicic acid chromatography, none of the fractions revealed the presence of DEHP, except the triglyceride fraction of heavy mitochondria (4% ether in hexane) in which DEHP formed up to 60% of the total ester fraction. In our experiments, mitochondrial triglycerides constituted over 20% of the total lipids in this subcellular fraction (Nazir *et al.*, 1967).

Johnson and Roots (1964) implicated anhydrous methanolic HCl as being a source of such artifacts as esters of

TABLE V: Di-2-ethylhexyl Phthalate in Heart Muscle Mitochondria from Other Species.

Species	DEHP[a] (μg)
Rat	129
Rabbit	118
Dog	36

[a] Expressed as micrograms of DEHP in mitochondria derived from 100 g of original heart muscle.

carboxylic acids which were formed during methanolysis and appeared on their chromatograms. These artifacts were assumed to have originated either as contaminants from methanol or were formed by the oxidative breakdown of methanol followed by condensation reactions. However, carefully controlled experiments conducted in this laboratory have failed to substantiate the claims of Johnson and Roots (1964). Since DEHP was quantitatively recovered by direct gas–liquid chromatography of mitochondrial "triglycerides" on an SE52–XE60 column without prior transesterification the possibility of DEHP arising as an artifact of methanolysis was also ruled out. On the other hand, under our conditions of esterification, DEHP was not attacked, as evidenced by its appearance together with fatty acid methyl esters.

To our knowledge, the localization of DEHP in mitochondria of higher animals has not been previously reported. At this time, there is no firm evidence to show whether DEHP is a biosynthetic constituent of heart muscle mitochondria, or whether it arises from dietary sources and becomes specifically localized in heart muscle mitochondria. Although terephthalic acid, the positional isomer of phthalic acid would be rapidly eliminated from the animal body following hydrolysis (Hoshi and Kuretani, 1965; Hoshi *et al.*, 1966) recent evidence indicates that DEHP is not attacked by nonspecific esterases (Jaeger and Rubin, 1970).

That the specific localization of DEHP in bovine heart muscle mitochondria is not confined to the ruminants has been shown by its presence in other species including the dog, the rat, and the rabbit. However, from preliminary work, we have not been able to demonstrate the presence of similar amounts of DEHP in tissues which are not related to the cardiovascular system.

The specific localization of DEHP in heart muscle mitochondria is significant in that this substance is likely to influence the bioenergetics of the myocardial cell. Future studies will have to be directed toward determining the action of DEHP on the pathophysiology of mitochondrial metabolism.

References

Beroza, M. (1962), *Anal. Chem. 34*, 1801.
Beroza, M., and Bierl, B. A. (1966), *Anal. Chem. 38*, 1976.
Beroza, M., and Sarmiento, R. (1963), *Anal. Chem. 35*, 1353.
Beroza, M., and Sarmiento, R. (1966), *Anal. Chem. 38*, 1042.
Berry, J. F., Cevallos, W. H., and Wade, R. R. (1965), *J. Amer. Oil Chem. Soc. 42*, 492.
Crane, F. L., Glenn, J. L., and Green, D. E. (1956), *Biochim. Biophys. Acta 22*, 475.
Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957), *J. Biol. Chem. 226*, 497.
Green, D. E., and Ziegler, D. M. (1963), *Methods Enzymol. 6*, 418.
Hirsch, J., and Ahrens, E. H., Jr. (1958), *J. Biol. Chem. 233*, 311.
Hoshi, A., and Kuretani, K. (1965), *Yakugaku Zasshi 85*, 905.
Hoshi, A., Takagi, J., Yanai, R., and Kuretani, K. (1966), *Yakugaku Zasshi 86*, 963.
Jaeger, R., and Rubin, R. (1970), *Science 170*, 460.
Johnson, P. V., and Roots, B. J. (1964), *J. Lipid Res. 5*, 477.
Layne, E. (1957), *Methods Enzymol. 3*, 447.
Nair, P. P., Sarlos, I., and Machiz, J. (1966), *Arch. Biochem. Biophys. 114*, 488.
Nair, P. P., and Turner, D. A. (1963), *J. Amer. Oil Chem. Soc. 40*, 352.
Nazir, D. J., Alcaraz, A., and Nair, P. P. (1967), *Can. J. Biochem. 45*, 1725
Perkins, E. G. (1967), *J. Amer. Oil Chem. Soc. 44*, 197.
Wheeldon, L. W., Schumert, Z., and Turner, D. A. (1965), *J. Lipid Res. 6*, 481.