Special Task Group for Preparation of Methyl Esters

This Task Group was established to devise and recommend a method for the preparation of methyl esters from either the free acids or from the triglycerides, which would be rapid, reliable, simple and widely applicable. The method was to be designed especially for the preparation of methyl esters as required for analyses by either infrared spectrophotometry or by gas chromatography. The first collaborative investigations of this group with soybean oil fatty acids showed that the methanol:acid and the methanol:BF_3 procedures were preferred by a majority of the collaborators and were most promising (3). As their second collaborative effort, the Task Group obtained a triglyceride sample containing approximately 25% elaidate and 40% ricinoleate, and prepared mixed fatty acids. Portions of each of these materials were sent to collaborating laboratories for preparation of methyl esters from the fatty acids by the Methanol:sulfuric acid and by the methanol:BF_3 methods and from triglycerides by the methanol:sulfuric acid procedure. These collaborative tests, in which seven laboratories participated, were completed and analyzed early during the past year. Results are given in Table III. From a yield standpoint, both procedures give essentially complete recovery of methyl esters. The hydroxyl values and the content of ricinoleate calculated from them and from GLC analysis indicate little or no loss of hydroxyl groups during esterification of either acids or triglycerides. Similar conclusions can be derived from the results of *trans* determination. Both methods appear equally satisfactory, and can be used to prepare methyl esters from fatty acids containing hydroxyl groups and isolated *trans* unsaturation. However, the methanol:H_2SO_4 procedure can be used to prepare esters directly from triglycerides. Because of this wider applicability, and because the Uniform Methods Committee has requested that we limit our recommendation to a single method, the Task Group voted to devote a major portion of its future efforts to further study of this method.

A draft of a Tentative Method for the preparation of methyl esters by the methanol:H_2SO_4 procedures has been prepared. This draft was discussed at the Spectroscopy Subcommittee Meeting on Monday, April 20, in New Orleans, La. The draft will be sent to all members of the Task Group for their comments, changes, and corrections before it is submitted to the Instrumental Techniques Committee with recommendation for referral to the Uniform Methods Committee for inclusion as a Tentative Method of the Society.

A third collaborative effort of this Task Group consisted of a test of the methanol:sulfuric acid procedure when employed to prepare methyl esters from glycerides and fatty acids containing isolated *trans* unsaturation, conjugated diene and hydroxyl groups. Results of this collaborative test, in which six laboratories participated are given in Table IV. This method appears to have little, if any effect on *trans* unsaturation or on conjugated diene content. The hydroxyl determination of these samples were, however, very inconsistent, and further collaborative effort is being planned to ascertain the reason for this inconsistency.

ROBERT T. O'CONNOR, Chairman
E. M. SALLEE, Subcommittee Chairman
ROBERT R. ALLEN, Subcommittee Chairman
W. T. COLEMAN, Subcommittee Chairman
J. R. CHIPAULT, Subcommittee Chairman

ACKNOWLEDGMENT

Anderson, Clayton & Company, and the Hormel Institute, University of Minnesota, contributed to the very highly purified new primary standards.

REFERENCES

1. Sallee, E. M., Allen, R. R., Coleman, W. T., Chipault, J. R., and O'Connor, R. T., JAOCS *41*, 158–160 (1964).
2. See JAOCS *39*, No. 10 p. 40, Oct. 1962.
3. O'Connor, R. T., and J. R. Chipault, JAOCS *40* (No. 3) 14, 32–34 (1963).

• *Letter to the Editor*

Mean First Double Bond Distance in Natural Fat Fatty Acids and Its Influence on Azelaoglyceride Analysis

THE MEAN POSITION of the first double bond (mean first double bond distance, MFDB) in the unsaturated fatty acids in a natural fat determines the miscellar molecular weight (MMW) of the dibasic acids remaining in the azelaoglycerides when the fat is oxidized and is of essential importance in azelaoglyceride analysis. Prior to 1955 it was the custom to assume that the MFDB distance in the common vegetable and animal fats of palmitic-stearic-oleic-linoleic type was nine carbon atoms corresponding to azelaic acid and this assumption was made use of in the original gravimetric azelaoglyceride analysis technique (1).

The following evidence, however, rendered the above assumption untenable:

A. Examination by an improved lead salt procedure showed that the common vegetable and animal fats contained a minimum of 2–8% of solid iso-oleic acids, based upon the amount of unsaturated acids present (2). Since vegetable fats as well as lard did not show presence of *trans* isomers by IR spectrophotometry, the iso-oleic acids in these would be *cis*-positional isomers (2).

B. Various proportions of $\Delta^{8:9}$ *cis*-octadecenoic acids were detected in purified oleic acid from various sources (3–5); however, purification will usually tend to remove isomers occurring in smaller proportions.

C. More recently evidence obtained by recalculating earlier data on ripening niger seed (5) and on "after-ripening" linseed (7) has indicated that the more unsaturated acids in vegetable fats are produced by a process of desaturation of the less unsaturated and in the case of linseed this was further confirmed by the new technique of χ "extended after-ripening" (7). It is hence quite possible that polyethenoid acids in natural fats also may not contain the first double bond exclusively in the $\Delta^{9:10}$ position since they may possibly be derived from all the monoethenoid acids.

If the MFDB distance is different from the assumed nine carbon atoms then the original calculations of

the azelaoglyceride composition (1) would be in error and in order to ascertain the extent of error usually involved, the dibasic acids remaining in the IAG (azelaoglycerides giving insoluble magnesium salts) (1) in a number of fats were isolated, using published procedures (8,9). The IAG was hydrolyzed with alcoholic potash and the higher saturated acids separated according to the improved Bertram separation (10). This procedure has been shown to produce quantitative separation of all higher saturated acids (11) and hence the filtrates would contain only dibasic acids. The filtrates were combined, concentrated, and acidified with sulphuric acid, saturated with sodium chloride or sulphate and extracted 6–8 times with liberal quantities of sulphuric ether. The extracts were united and worked up in the usual way and MMW of the isolated acids determined by titration.

In a number of instances the MMW of the dibasic acids was 188: e.g., *Mangifera indica, Mimusops elengi* and *Myristica beddomei.* Since vegetable fats generally contain some *cis*-iso oleic acids (1) this would be due to the occurrence of isomers having higher and lower MFDB distances and indicates a minimum of three dibasic acids. More frequently the MMW of the dibasic acids ranged from 190–200, e.g. *Artocarpus hirsuta*-200, *Bauhinia variegata*-200, *Cassia tora*-196, *Cassia fistula*-196, *Adenanthera pavonina*-194, *Strychnos potatorum*-194, *Cassia occidentalis*-192, and *Clitorea ternatea*-192. Mean shift of double bond by one carbon atom would require change in MMW of 14 units only and hence the above results indicate the presence of appreciable amounts of isomers not having the first double bond in the $\Delta^{9:10}$ position.

When azelaoglycerides are analyzed gravimetrically (1) the MMW of the dibasic acids is important and the number of homologs is not. The former is of the order of 188–196 except in two rare instances and at 196 the proportions of monoazelains calculated on the assumed value of 188 will be only 2.3%, on monoazelain basis, less than that required on the value of 196: this is within the range of experimental error (1) or deviation due to compositeness of sample (12) when the disaturated glyceride content is less than ca. 50%. We have used a correction factor for this variation (8) after it was discovered that the assumed value of 188 may not always be found.

The fact that azelaic acid is usually not the only dibasic acid occurring in the azelaoglycerides from the common fats makes the analysis of the azelaoglycerides by GLC techniques more complex than has been realized (13) since quantitative interpretations of gas-liquid chromatograms will be conclusive only after proof of resolution of azelaoglycerides is obtained by identification of requisite number of individual or carbon number peaks. A second homologous dibasic acid will produce one more homolog for each monoazelain, two more for each diazelain and three more triazelains: altogether eight individual compounds rather than three if only one saturated acid is present. The presence of homologous dibasic acids will also give rise to the problem of isomeric azelaoglycerides having different saturated and dibasic acids. Hence, we cannot be sure that resolution of azelaoglycerides has been obtained until the number of the latter actually present is known. It has not yet been shown with certainty that gas-liquid chromatograms of mixed acid esters produce resolution of all positional isomers. Lard fatty acids did not show any such separation (14) whereas isomeric monoethenoid acids would undoubtedly be present in the same (2). Further even if the resolution is obtained it will not, as yet, give any idea of the nature of the dibasic acids present in the azelaoglycerides. The number and nature of the dibasic acids in the azelaoglycerides must be determined first by proper oxidative techniques for each fat and the possible number of azelaoglycerides computed theoretically. Until the resolution obtained agrees with the number of peaks thus computed it will be doubtful whether adequate resolution has taken place on the column. This is particularly important in the present case (13) since the results have not been calculated on the basis of peak area-percentage relationships in spite of the fact that this relationship has been found to be valid for medium and long chain trisaturated glycerides (15).

A. R. S. KARTHA
Division of Chemistry
Indian Agricultural Research Institute
New Delhi, India

REFERENCES

1. Kartha, A. R. S., JAOCS *30*, 280 (1953).
2. Narayanan, R., and A. R. S. Kartha, J. Sci. Ind. Res. (India) *15B*, 368 (1956).
3. Allen, R. R., and A. A. Kiess, JAOCS *32*, 400 (1955).
4. Benton, F. L., A. A. Kiess and H. J. Harwood, JAOCS *36*, 457 (1959).
5. Feuge, R. O., and E. R. Cousins, JAOCS *37*, 267 (1960).
6. Narayanan, R., and A. R. S. Kartha, J. Sci. Ind. Res. (India) *18C*, 41 (1959).
7. Kartha, A. R. S., J. Sci. Ind. Res. (India) *20B*, 443 (1961).
8. Narayanan, R., "The Component Acids and Glyceride Structures of Some Natural Fats," Ph.D. Thesis, University of Delhi, 1958.
9. Kartha, A. R. S., and R. Narayanan, Ind. J. Chem. (in press).
10. Kartha, A. R. S., J. Sci. Ind. Res. (India) *13A*, 72 (1954).
11. Sethi, A. S., and A. R. S. Kartha, J. Sci. Ind. Res. (India) *15B*, 103 (1956).
12. Kartha, A. R. S., J. Sci. Food Agric. *14*, 515 (1963).
13. Youngs, C. G., and M. R. Subbaram, JAOCS *41*, 218 (1964).
14. Herb, S. F., P. Magidman and R. W. Riemenschneider, JAOCS *37*, 127 (1960).
15. McCarthy, M. J., A. Kuksis and J. M. Beveridge, Can. J. Biochem. Physiol. *40*, 1693 (1962).

[Received September 21, 1964—Accepted February 1, 1965]