Alkali-Isomerization Reactions of Unsaturated Fatty Acids

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ONE of the major problems in lipid chemistry and biochemistry has been the complete analysis of fatty acid composition of lipid materials. Prior to 1940, the fatty acid composition of fats and other lipid materials could be determined only in the most simple cases, and then only by rather tedious methods.

The composition of mixtures containing only saturated fatty acids and such common unsaturated fatty acids as oleic, linoleic, and linolenic could be ascertained with reasonable accuracy, but the quantitative analysis of more complex mixtures containing fatty acids of longer and shorter chain lengths, and fatty acids with 4, 5, and 6 double bonds, was quite impossible. Analysis of the fatty acid composition of the simpler fats and oils involved lead salt separations, and iodine value and thiocyanogen value determinations.

A more simple and useful method of fatty acid analysis became possible as a result of the development in the early 1940's of the ultraviolet spectrophotometer as a common laboratory instrument, and the discovery that unsaturated fatty acids, by suitable chemical treatment, could be made to absorb at certain characteristic wavelengths in the ultraviolet region. At first, this method could be applied only to relatively simple mixtures, but in recent years, it has been extended so that now, in some cases, it may be used for the analysis of certain mixtures containing fatty acids with as many as six double bonds.

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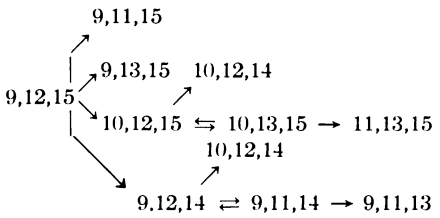
The common polyunsaturated fatty acids found in animal and vegetable tissues are characterized by methylene-interrupted unsaturation; that is, the double bond systems are separated by single methylene groups. In addition, in their natural state, most of the common polyunsaturated fatty acids have their double bonds almost entirely in the *cis* configuration. Structures of this type do not show any appreciable absorption in the near-ultraviolet range. However, it has been found that various reagents can shift the positions of protons along the carbon chain, a so-called prototropic shift, so that double bonds are moved into conjugated positions.

In the methods of analysis most commonly used at present, this isomerization is accomplished by means of strong alkali. The fatty acids to be isomerized are heated, at 180° C for a given period, with potassium hydroxide in ethylene glycol or glycerol, after which the isomerized mixture is diluted appropriately with solvents and its absorption in the ultraviolet region is determined.

When linoleic acid (two double bonds) is alkali-isomerized, one or the other of the double bonds tends to move predominantly toward the remaining double bond. The reason for this is that the conjugated diene thus produced is a relatively stable structure, thermodynamically. Thus, a mixture of 9,11- and 10,12-octadecadienoates is produced from normal linoleic acid. In addition, because the *trans* configuration is more stable than the *cis*, those double bonds that have moved are found predominantly in the *trans* configuration.

The conjugated dienes formed from linoleate by alkali-isomerization absorb ultraviolet light strongly in the region of 233 mμ. Conjugated trienes, however, absorb strongly at 268 mμ, and conjugated tetraenes at 315 mμ.

Normal linolenic acid is *cis*, *cis*, *cis*-9,12,15-octadecatrienoic acid. When this acid is alkali-isomerized, many isomers can be formed. The possible transitions are illustrated in the following diagram, and, under the conditions usually employed, the final product consists mainly of a mixture of all of these forms.



Thus, under normal conditions, the alkali-isomerization of linolenic acid results in the production of a mixture of conjugated dienes and trienes. Consequently, the product absorbs strongly at both 233 and 268 mμ. Similarly, the alkali-isomerization of arachidonic acid leads to a mixture of conjugated dienes, trienes, and tetraenes, absorbing at 233, 268, and 315 mμ, respectively.

The alkali-isomerization-spectrophotometric method of analysis is an empirical procedure, and it is necessary to establish spectral constants for the various pure polyunsaturated fatty acids by alkali-isomerizing under the conditions that subsequently will be employed in analysis. Measurement of ultraviolet absorption at the various wavelengths then establishes constants which may be employed in calculating the original composition of alkali-isomerized mixtures of polyunsaturated fatty acids.

A Note on Increasing the Accuracy of the Micro Alkaline Isomerization Technic for Determination of Unsaturated Fatty Acids

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THE alkaline isomerization technic has been used successfully for the analysis of vegetable oils. This method has been made more sensitive by increasing the concentration of the KOH used for isomerization[1] and has been used for determinations of small quantities of unsaturated fatty acids.

Two inherent difficulties of the original method in its adaptation for micro analysis were: *first*, the lack of sensitivity, especially in the area for tetraenoic acids, and, *second*, the high absorption in the ultraviolet by the reagent, especially at 233 mμ wavelength The first problem was satisfactorily solved by the increased KOH concentration, but the second problem was further complicated, since the alkali increased the absorption by the reagent.

Usually in macro analysis 100 mg of fatty acids are isomerized with 5 ml of the reagent. In micro analysis, 1 mg of fatty acids is usually isomerized with 1 ml of the reagent, a twenty fold difference in the proportion of reagent to fatty acids. In some micro-analyses of fatty acids the absorption by the fat may be less than 10 per cent of the total absorption!

The following simple procedure has been introduced to reduce the absorption due to the reagent blank. After cooling the isomeriza-

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