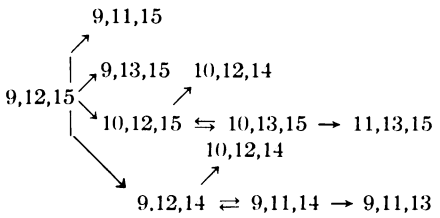
Normal linolenic acid is *cis*, *cis*, *cis*-9,12,15-octadecatrienoic acid. When this acid is alkali-isomerized, many isomers can be formed. The possible transitions are illustrated in the following diagram, and, under the conditions usually employed, the final product consists mainly of a mixture of 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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tion tubes following the isomerization, about 2 ml water is added and sufficient hydrochloric acid to make the solution acid. Petroleum ether is then added, the tubes are shaken and the petroleum ether fraction is transferred. This is repeated several times to assure quantitative transfer of the fatty acids. The petroleum ether fraction is then evaporated to dryness under partial vacuum and the fatty acids are dissolved in sufficient methanol for the spectrophotometric reading.

REFERENCE

1. HERB, S. F. and RIEMENSCHNEIDER, R. W.: Spectrophotometric micromethod for determining polyunsaturated fatty acids. *Anal. Chem.* 25: 953, 1953.