Double Bond Migration in Methyl Eleostearate During Gas-Liquid Chromatographic Analysis

DURING RECENT work in our laboratory concerned with identification of unknown materials collected from gas-liquid chromatographic (GLC) columns, it was desirable to know what effect the conditions used during collection would have on the double bond positions in methyl β-eleostearate.

Morris, et al. (3) have concluded that GLC analyses on both metal and glass columns produce a considerable amount of *cis, trans* isomerization in methyl eleostearate (both α and β). However, no work was reported in the literature on bond migration during GLC analysis.

Preparation of Methyl β-Eleostearate. β-Eleostearic acid (*trans, trans, trans*-9, 11, 13-octadecatrienoic acid) was prepared and purified essentially as described by Hoffmann, et al. (2); [m.p. 70.5C, reported: 71-72C (1)]. UV analysis indicated the product contained 98.4% β-eleostearic acid [ε for β-eleostearic acid = 61,000; λ max. 268 mμ (1)]. The methyl ester was prepared by refluxing the acid 1 hr with 4% hydrochloric acid in methanol. UV analysis of the ester in absolute ethanol showed 88% conjugated triene calculated as methyl β-eleostearate.

Oxidation of Methyl β-Eleostearate. A small portion of this methyl β-eleostearate was subjected to periodate-permanganate oxidation essentially as described by von Rudloff (4) except that the reactants (in 60% tertiary butyl alcohol) were stirred magnetically at room temp for 16 hr. The resulting cleavage products were recovered and methylated by refluxing 1 hr with 1% sulfuric acid in methanol. The esters of monobasic acids were removed from the mixture *in vacuo* at 40C. Recovery of dibasic acid esters was 89% of theory, based on starting wt of methyl eleostearate. GLC analyses of these esters showed that they contained 93.2% methyl nonanedioate and a number of other components, none of which were present in amt larger than 1 %.

GLC Collection of Methyl Eleostearate and Subsequent Oxidation. A sample of the same batch of methyl β-eleostearate was run through a GLC column and collected at the exit to determine the effect of this treatment on the positions of unsaturation. The column and conditions used were as follows: Column, 20% LAC-2-R 446 on 60–80 mesh Celite, glass U-type 200 x 0.6 cm I.D. operated at 195C; helium flow rate, 97 ml/min at inlet pressure of 40 psi. Other parameters were: injection port, 268C; detector bath (thermal conductivity), 275C; fraction collector, 278C; and detector current, 200 ma.

The ester was trapped in a small glass U-tube containing ether-extracted cotton saturated with absolute methanol. The trap was cooled in an ice-water bath during collection. The collected ester was washed from the trap with ethyl ether and the solvent was removed *in vacuo* below room temp. Approximately 70% of the material injected into the column was recovered. This percentage compared favorably with a value of 75% methyl oleate recovered by the same technique. The recovered ester contained 81% conjugated triene calculated as methyl β-eleostearate, and no UV max was present in the conjugated diene region.

The collected methyl eleostearate (0.083 g) was cleaved oxidatively, and the products were recovered and identified like the previous sample except the monobasic acids were determined by direct GLC analysis of a small portion of the recovered product before methylation. Comparison of this chart with one obtained by analysis of a known mixture of monobasic acids indicated the products were butanoic, pentanoic, and hexanoic acids in the approximate ratio of 1:2:1, and propanoic and heptanoic acids in much smaller amt. The dibasic acids did not interfere in this analysis.

The remaining oxidation mixture was esterified. The monobasic portion was removed as before and the residue (0.052 g) was analyzed by GLC. The following composition (in area percent) was obtained for the dibasic acid portion: heptanedioate, 6.2; octanedioate, 18.5; nonanedioate, 43.2; decanedioate, 25.3; and undecanedioate, 6.8.

These results show that double bond migration does occur during GLC analysis of methyl eleostearate along with *cis,trans* isomerization (3). The value of 81% conjugated triene in the collected ester indicates that the conjugated triene grouping has been partially shifted as a unit in one direction or the other. This conclusion is supported by the absence of conjugated diene absorption in the UV spectrum and by the relative percentages of monobasic and dibasic acids in the oxidation products of the collected ester.

These results emphasize that preparative GLC should be used cautiously since misleading chemical data may result if the material is unstable to GLC conditions.

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