Location of Olefinic Links in Long-chain Esters by Methoxymercuration–Demercuration followed by Gas Chromatography–Mass Spectrometry

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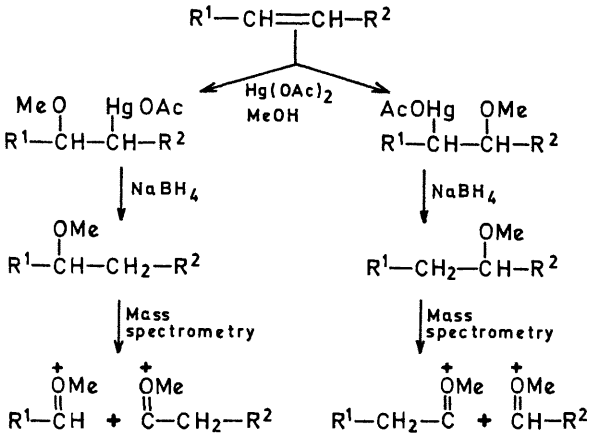
Summary The position of double bonds in mono- and poly-enoic long-chain esters may be conveniently determined by reduction of their methoxymercuration products with sodium borohydride followed by combined gas chromatography–mass spectrometry of the resulting methoxylated esters.

METHOXYMERCURI-ADDUCTS of long-chain unsaturated esters have been used for several years as a means of separating such esters from those containing no olefinic links.[1] Recently, a procedure for the reductive demercuration of hydroxymercuri-adducts with sodium borohydride has been described.[2] The application of this reductive method to methoxymercuri-derivatives of long-chain esters should lead to methoxy-derivatives intrinsically suitable for combined gas chromatographic–mass spectrometric

(g.c.–m.s.) determination of the position of double bonds in the parent esters:



A successful procedure has been devised as follows. The material under investigation is dissolved in methanol and kept in the dark at room temperature with a slight excess of mercuric acetate for 24 hr. Excess solid sodium borohydride is added followed by a few drops of acetic acid, and the mixture is evaporated to dryness, partitioned between ether and water, and the crude methoxy-compounds obtained from the ethereal layer. The methoxy-derivatives are isolated by t.l.c. and then subjected to g.c. and m.s. either combined or in successive steps. The two isomeric methoxy-compounds formed from any one double bond in the esters investigated so far are not separated by gas chromatography on packed columns of polyethylene glycol succinate or silicone gum rubber (SE 30); these isomeric derivatives, therefore, may be treated as a single component for gas chromatographic purposes.

The procedure described above has been applied to methyl oleate, elaidate, linoleate, and linolenate and also to a naturally-occurring ester mixture from the lipids of *Lactobacillus casei*. The position of the double bonds in methyl oleate and elaidate is revealed by the intense ions in the mass spectra of their methoxylated derivatives at *m/e* 157, 171, 201, and 215 which represent the fragments $CH_3.(CH_2)_n.CH.\overset{+}{O}Me$ ($n = 7,8$) and $Me\overset{+}{O}.CH.(CH_2)_m.CO_2Me$ ($m = 7,8$) respectively. Combined g.c.–m.s. of the methoxylated esters from *L. casei* revealed the presence of octadec-11-enoic and hexadec-9-enoic esters in the ratio 6·5 : 1; a mass spectrum of the unresolved mixture was also satisfactory in indicating the structure of both esters. The cyclopropane (lactobacillic) acid methyl esters present in esters of *L. casei* were found to be unattacked by the $Hg(OAc)_2$–MeOH reagent.

Depending on the reaction conditions, di- or poly-unsaturated esters can give rise to completely or partially methoxylated products which may be isolated by t.l.c. or g.c. The mass spectra of the derivatives of such esters exhibit the expected fragmentations, namely cleavages adjacent to >CH–OMe groups. Certain derivatives have more simple spectra than others. For example, the spectrum of the unsaturated monomethoxy-derivatives of linoleate is dominated by two very intense peaks at *m/e* 129 and 215 representing cleavage adjacent to a >CH–OMe group allylic with respect to the remaining double bond. Enhanced allylic cleavages were also observed in the spectra of the uncompletely methoxylated derivatives of linolenate but were not as pronounced as those in the spectrum of the linoleate derivative. By adjustment of the quantity of mercuric acetate and the time of reaction it is possible to obtain in each case a high proportion of a preferred derivative, thus simplifying the mass spectral analysis.

The procedure outlined above compares favourably with other methods[3] developed recently for location of unsaturation in long-chain compounds by g.c.–m.s. especially in respect of the simplicity of the reaction procedure, the volatility and stability of the derivatives and the general applicability to mono- and poly-unsaturated compounds.

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