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Short Communication

Preparation of methyl esters of fatty acids with trimethylsulphonium hydroxide -an appraisal

A. H. El-Hamdy[☆] and W. W. Christie
Hannah Research Institute, Ayr, Scotland KA6 5HL (UK)

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ABSTRACT

The rates of reaction of the new basic transesterification catalyst, trimethylsulphonium hydroxide (TMSH), have been compared with an established sodium methoxide-catalysed procedure. Although the latter is more rapid, the difference is only of practical importance when cholesterol esters are transesterified. Some losses of polyunsaturated components from fish oils were observed when methods were employed in which TMSH was injected directly into the heated injection port of the gas chromatograph, as had been recommended.

INTRODUCTION

Preparation of methyl ester derivatives of fatty acids for gas chromatographic (GC) analysis is one of the fundamental reactions in lipid chemistry [1–5]. Trimethylsulphonium hydroxide (TMSH) was introduced in 1979 as a mild methylating agent for acidic organic molecules [6]. It can be used in two ways, i.e. to methylate free acids by pyrolysis of the salt in the heated injection port of a gas chromatograph [6,7], or to effect base-catalysed transesterification of lipids [8–12]. As the only by-products of these reactions are dimethylsulphide and methanol, the excess reagent itself decomposing to these compounds, methyl esters can be prepared for chromatographic analysis in simple one-pot procedures with little or no work-up.

TMSH has been used to methylate bacterial fatty acids [11,121, which include monoenoic, branched-chain and cyclopropane components, and a limited range of animal and vegetable fats and oils [8–11]. It is evident from the published papers that the reagent gives as clean GC traces with stable baselines as any other procedure. However, there is no information on the rate of the reaction with different lipid classes. Nor has there been any quantitative comparison with data obtained by alternative procedures for polyunsaturated fatty acid components, which might be most sensitive to alteration. We have now addressed these problems.

EXPERIMENTAL

Reagents

Trimethylsulphonium iodide was obtained from Aldrich (Gillingham, UK). It was converted to an 0.2 A4 solution of the hydroxide by two different methods, *i.e.* by reaction with silver oxide in methanol [7,8], and by reaction with an ion-exchange resin [10,12].

***Correspondence** to:* W. W. Christie, The Scottish Crop Research Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK (present address).
☆ Present address: AI-Fateh University, Tripoli, Libya.

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All other solvents and reagents were "reagent" or "HPLC" grade and were from FSA Scientific Apparatus (Loughborough, UK). Synthetic lipid standards were from Sigma (Poole, UK). Cod liver oil was from a local pharmacy.

Methylation

Timed reaction studies were carried out at room temperature by dissolving the lipid (0.1 to 3 mg), with methyl nonadecanoate (0.1 to 0.5 mg) added as internal standard, in sodium-dried diethyl ether (0.5 ml) and methyl acetate (20 μl), before 0.2 *M* TMSH in methanol (100 μl) was added. The reaction was stopped at specified time intervals by adding acetic acid (5 μl); the solvent was removed on a rotary evaporator, and the sample was re-dissolved in hexane for GC analysis. In some experiments, the reaction was not stopped by the addition of acid.

For comparison purposes, an established base-catalysed transesterification procedure was used with conditions as above except for 1 *M* sodium methoxide in dry methanol as catalyst (20 μl) [13].

Free fatty acids, prepared by hydrolysis of cod-liver oil [5], were converted to methyl esters with TMSH in a pyrolysis-methylation reaction. TMSH solution (0.1 ml) was added to the free acids (1 mg) in methyl-tert.-butyl ether (0.5 ml) and an aliquot (1 μl) was injected directly into the heated injection port of the gas chromatograph. For comparison purposes, 1% sulphuric acid in methanol was used for methylation prior to injection [5].

Gas chromatography

GC analyses of fatty acid methyl esters were performed on a Carlo Erba Model 4130 capillary gas chromatograph (Erba Science, Swindon, UK), equipped with a split/splitless injector maintained at 260°C. A fused-silica capillary column (25 m × 0.22 mm I.D., film thickness 0.2 μm) coated with Carbowax 20M (Chrompack UK, London, UK) was used. The oven temperature was programmed for three min isothermally at 175°C, then to 205°C at a rate of 4°C/min and held at the final temperature for 20 min more. Hydrogen was the carrier gas. Fatty acids methyl esters were identified by reference to standards and were quantified by electronic integration.

RESULTS AND DISCUSSION

Two methods have been described for preparation of the TMSH reagent. One involving the use of an ion-exchange resin [10,12] was tried first and gave excellent preliminary results. However, in attempting to do timed experiments to measure the rate of reaction, it was observed that the products were free fatty acids which were only converted to methyl esters when injected into the gas chromatograph, *i.e.* reaction proceeded entirely via the pyrolytic mechanism, since the reagent was effecting hydrolysis rather than transesterification. This is presumed to be because water was retained by the ion-exchange resin during conversion to the correct salt form, and this passed into the reagent. When the reagent was prepared under anhydrous conditions with dry methanol and silver oxide [7,8], methyl esters were prepared directly by transesterification. The latter procedure was adopted in all the experiments described below. The reagent prepared in this way is an equimolar mixture of the hydroxide and methoxide.

The rates of reaction of TMSH and sodium methoxide with cod liver oil triacylglycerols, dipalmitoylphosphatidylcholine and cholesteryl palmitate were determined by stopping the reaction at timed intervals by the addition of acetic acid, and measuring the amounts of methyl esters relative to the internal standard by CC. The results are shown in Fig. 1. It is evident that reaction was somewhat more rapid with sodium methoxide as catalyst in each instance, but with triacylglycerols and phospholipids the reaction was essentially complete in about 5 min. The difference would not be of practical importance with these lipids.

It is well established that cholesterol esters are transesterified only slowly by most reagents (note the difference of time scale in Fig. 1) [5,13]. Cholesterol esters were transesterified completely in 1 to 2 h with the sodium methoxide method, but the reaction was still far from complete after 5 h with TMSH. In practice, complete esterification should be possible with the latter and cholesterol esters if a higher concentration of reagent and higher temperatures were to be employed.

The analysis of a fish oil, because of its high content of polyunsaturated fatty acids, is a more rigorous test of a method than is application to depot

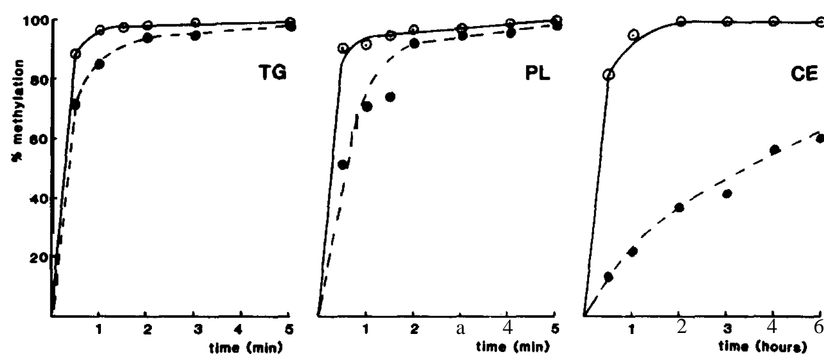

Fig. 1. Rates of transesterilication of lipid classes with sodium methoxide (0) and trimethylsulphonium hydroxide (TMSH) (0). TG = Triacylglycerols (cod liver oil); PL = phospholipids (dipalmitoylphosphatidylcholine); CE = cholesterol esters (cholesteryl palmitate).

fats or vegetable oils. Fatty acid compositions of cod liver oil, determined by GC after various methylation procedures were applied, are shown in Table I. To simplify matters, only the data for 20:5(*n*− 3) and 22:6(*n*− 3) fatty acids are listed, since these are the components at greatest risk from an over-vigorous reaction. Sodium methoxide-catalysed transesterification [5] is a well-established and safe method against which others can be judged. TMSH used under similar conditions, *i.e.* in which the reaction was stopped with acetic acid so no TMSH was injected into the GC column, gave results that were not significantly different. However, when the medium was not acidified and an aliquot of the reaction mixture was injected directly onto the column, as has been recommended [8–12], a loss of approximately 5% of the polyunsaturated components was observed. Somewhat greater losses were obtained when free acids were methylated with TMSH in a pyrolysis-methylation procedure [6,7] or with methanolic sulphuric acid [5], although part of these losses may be because of the more extensive manipulations involved because of the initial hydrolysis step.

TABLE I

CONTENT OF 20:5(*n*− 3) AND 22:6(*n*−3) IN COD LIVER OIL AS DETERMINED BY GC FOLLOWING TRANSESTERIFICATION WITH TMSH, OR BY ALTERNATIVE METHODS

Results are means and standard deviations of three analyses.

No.	Method	Fatty acid (%, w/w)	
		20:5(*n*− 3)	22:6(*n*− 3)
1	Sodium methoxide transesterification [13]	10.58 ± 0.059	9.75 ± 0.197
2	TMSH; same conditions as in 1	10.36 ± 0.078	9.48 ± 0.108
3	As 2 but acetic acid not added to stop reaction (and solvents not evaporated) [8–12]	10.05 ± 0.035	9.04 ± 0.116
4	Pyrolysis-methylation of free acids with TMSH [6,7]	9.07 ± 0.047	7.70 ± 0.425
5	Methanol-l % sulphuric acid [5]	8.42 ± 0.215	7.13 ± 0.309

In practice with TMSH, it may not always be necessary to stop the reaction by adding acid, especially for samples that do not contain significant amounts of polyunsaturated fatty acids. TMSH should pyrolyse to give volatile products only, and no deleterious effects on the column were observed in this study. However, there remains a possibility that column life might be shortened over a period of several months if any unchanged reagent survived the pyrolysis. It seems to be a sensible precaution (when long-chain fatty acids only are present) to employ the simple expedient of stopping the reaction with acetic acid, evaporating excess solvents and re-dissolving in hexane for analysis, when TMSH is used. Minimal losses of polyunsaturated fatty acids should then occur, even on prolonged storage, and column life may be lengthened. Under these conditions, the reaction is still a simple one-pot one. TMSH is easy to prepare under safe conditions in small quantities, and can be recommended for more general use.

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