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Letter to the Editor

Preparation of pyrrolidides from fatty acids via trimethylsilyl esters for gas chromatographic–mass spectrometric analysis

Sir,

Pyrrolidides have been recognized as useful derivatives of fatty acids for their characterisation by gas chromatography–mass spectrometry (GC–MS) with electron impact ionization[1–4]. This functional group directs the fragmentation of a carbon chain in such a way that structural features such as the location of branchings or of one or two double bonds can be recognized much more readily than with the acid itself or its esters[2].

Later, other derivatives, such as β-picolines[5], dimethyloxazolines[6] and triazolopyridines[7], were found which show the same type of fragmentation even more strongly and now even the double bonds in polyunsaturated acids can be located. The formation of the derivatives requires activation of the acid in a first step, either to the acid chloride[5], to an activated ester[6,7] or at least to a methyl ester in the case of the pyrrolidide[1].

However, from an experimental point of view, all these derivatives suffer from the serious shortcoming that, in contrast to esterification with diazomethane or trimethylsilylation with a commercial reagent, their preparation from the acids is not sufficiently simple for everyday use in the laboratory. It therefore appeared worthwhile to study the preparation of such fragmentation-directing derivatives on a microscale with the aim of developing a procedure comparable in simplicity to esterification or silylation and preferably also without the need for work-up before GC–MS analysis.

Considering the nucleophilic power of pyrrolidine, which is sufficient to convert various esters into pyrrolidides under mild conditions, we reasoned that trimethylsilyl (TMS) esters would be converted analogously. This would allow a simple experimental set-up because the preparation of the TMS ester and subsequent conversion to the amide could be carried out in one procedure. The technique would resemble very closely that of the commonly used silylation procedure, except that the tertiary base pyridine would be replaced with pyrrolidine.

Fig. 1 shows the results of a kinetic study on the reaction of stearic acid with three commercially available silylating reagents and pyrrolidine. It turned out that the best reagent is trimethylsilylimidazole (TSIM), which produces a quantitative yield of the pyrrolidide in less than 6 h at room temperature. As is common practice in silylation derivatization for GC–MS analysis, the liquid reagent is employed in large excess and serves as the solvent of the reaction mixture. With regard to the amount of

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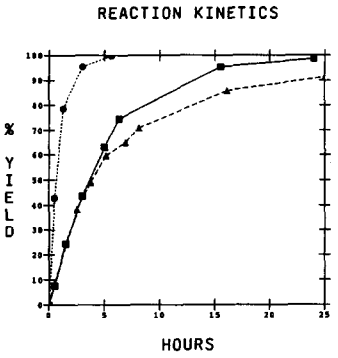

Fig. 1. Yield of stearylpyrrolidide from stearic acid. Experimental conditions: 1 mg of stearic acid, 100 μl of pyrrolidine, 100 μl of trimethylsilyl reagent. ● = Trimethylsilylimidazole; ■ = hexamethyldisilazane; ▲ = bistrimethylsilyltrifluoroacetamide. All experiments at room temperature.

pyrrolidine, it is essential to use an excess over the silylating reagent because the latter reacts with the acid and also with the pyrrolidine, but only *free* pyrrolidine appears to be able to attack the TMS ester under mild conditions. We usually employ TSIM and pyrrolidine in 1:1 volume ratio, which corresponds to a 1:2 molar ratio. The total volume is usually 200 μl. The reaction mixture is used directly for GC–MS analysis.

If alcohol groups are present in the sample, it is found that they are silylated under the conditions employed, but silylation often appears incomplete in GC–MS analysis. This is presumably due to desilylation in the injector of the gas chromatograph by the free pyrrolidine present in the reaction mixture. In such instances an additional silylation step has to be performed after the completion of the pyrrolidide formation. In this step sufficient reagent, now preferably bistrimethylsilylacetamide is added to convert the free pyrrolidine into its TMS derivative.

Ester groups present in the sample are split by the TSIM–pyrrolidine reagent under the conditions employed, resulting in a pyrrolidide and a TMS ether as expected. This reaction proceeds more slowly and heating is usually required to complete it in a practicable time. Studies are under way to find the optimum conditions for this reaction which may be analytically useful in itself.

Unfortunately, a comparatively simple procedure has not been found for the conversion of acids to the other derivatives mentioned above[5–7]. The nucleophilicity of these reagents appears to be too weak for an attack on TMS esters under mild conditions.

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F. Hoffmann-La Roche Ltd.,
Department ZFE/Phy,
CH-4002 Basle (Switzerland)

WALTER VETTER
WILLI WALTHER

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