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Discussion

Diazomethane as a highly selective fatty acid methylating reagent for use in gas chromatographic analysis

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Sir,

The confusion displayed by a recent article [1] about the use of diazomethane as a GC derivatizing reagent of fatty acids (FA) in biological samples should not be left to stand unchallenged. Confused are a report on the unwise use of diazomethane directly on a plasma–methanol mixture [2] with the use of this reagent under proven conditions on lipid extracts [3]. The latter reaction is usually performed in diethyl ether with some methanol in a catalytic role, at room temperature or below, and has been shown to be highly rugged [4,5], not at all sensitive to "small variations in reaction conditions" [1]. In an attempt to reconstitute the awareness among some chromatographers of the qualities of diazomethane the salient points of the mentioned studies [3–5] are presented and enhanced with a selection of relevant syntheses.

Reactions of diazomethane in a plasma–methanol medium should not only lead to a higher pH due to the consumption of acids [6] but also by reaction with negative ions like chloride [5,7]. That this increase of strong bases can lead to solvolysis of ester lipids should have been known [6–11]. Solvolysis rates are highly pH-dependent. Catalysis by diazomethane had been invoked in Refs. [8,9], but this was not proven. Also, synthetic chemists consider esters to be inert toward diazomethane [12–15], so esterification is often used to protect carboxyl groups in syntheses involving this reagent. The workup of serum without hydrolysis of lipids has been discussed elsewhere [16].

Not only is solvolysis easily and completely avoidable [3–5], but other deleterious reactions (not mentioned in Ref. [1]) can be eliminated as well. Reviews [17] and [18] should be consulted for general information on scope and mechanisms of reactions. Non-activated double bonds, like those of FA, do not react [4,12,19,20]. Activated (electron poor) double bonds give addition reactions [10,11,13,21], but usually cannot compete with the carboxyl group, or can be prevented from competing by cooling [22–24]. The extremely fast reaction of methylene with olefins is, undeservedly, another point of confusion. It is not formed under the low light conditions used for methylation of FA and, therefore, can not interfere in this context.

No reports were found on reactions of diazomethane with cyclopropanes. Other functional groups are not present in common FA, arachidonoid chemistry [23,25] is a different matter. Here, the published [26] functional group reaction sequence, $-CO_2H \geq -COBr \geq -COCl \geq -OH \geq -COCH$, should be of interest. The methylation of leukotriene A_4, which made TLC and characterization of this unstable epoxide acid possible [25], is a particularly

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instructive example of the utility of diazomethane. It would be a considerable loss to falsely place it on the level of reagents which really can not be used in the presence of ester lipids: methyliodide, dimethylformamide dimethylacetal [4] or acetyl chloride/methanol [27].

In this laboratory the synthesis of the reagent has, however, not been highly reliable until it was noted that cooling the outer vessel of the MNNG diazomethane generator [17] eliminated the problem. The cooling is done with a bromobenzene melt which is just below the boiling point (−23°C) of diazomethane. The entraining ether should not touch the inner reactant container whose content should not freeze. Freezing stops generation of diazomethane and may be a safety hazard (Aldrich bulletin AL-180), though it should be mentioned that no explosions occurred in spite of freezing the reaction medium.

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