

A Column Procedure for the Esterification of Organic Acids with Diazomethane at the Microgram Level

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A simple procedure is described in which micro- and submicrogram amounts of organic acids can be converted to methyl esters with diazomethane. The gas, generated in only micromole amounts, contacts the acids which have been applied to a column of Celite contained in a capillary. The resultant esters are then eluted for chromatographic analysis. The entire procedure can be done in less than 5 min.

Although a variety of reagents are available for forming methyl esters of organic acids, none rival diazomethane in simplicity, speed, and mildness of conditions. Use of reagents other than diazomethane is prompted by the toxic and explosive nature of the gas and perhaps also to its preparation and relatively short storage life. Several procedures have been described designed to reduce some of these shortcomings by working with milligram quantities of organic acids and reduced levels of diazomethane (1,2).

The need for an efficient esterification procedure for microgram and submicrogram amounts of organic acids became apparent in our work on trace constituents isolated from milkfat. We have therefore developed a simple esterification technique in which the acids are applied to a microcolumn of Celite contained in a melting point capillary and briefly exposed to diazomethane vapor generated only in micromole quantities.

SOLUTIONS AND APPARATUS

Solution 1

One gram of *N*-methyl-*N*-nitroso-*p*-toluene-sulfonamide (Diazald, Aldrich Chemical Co., Milwaukee, Wis.) and 2 ml of 2-(2-ethoxyethoxy) ethanol (Chromatoquality, Matheson, Coleman, and Bell, East Rutherford, NJ) were dissolved in 3 ml of redistilled (from KOH pellets) diethyl ether. The solution was stored at room temperature in a test tube fitted with a Teflon-lined screw cap and was stable for at least 4 mo.

Solution 2

A 50% aqueous solution of potassium hydroxide.

Apparatus

Melting point capillaries 100 mm long, 1.5–2.0 mm o.d. (Fisher Scientific Co., Silver Spring, Md.) cut approximately in half; Celite 545 (Johns-Manville Co., Baltimore, Md.) was used as received. One milliliter narrow mouth bottles (Arthur H. Thomas, Philadelphia, Pa.) with rubber stopper sleeves (septums) 7/32 in. length and 15/64 in. top diam (SGA Scientific Inc., Bloomfield, NJ).

EXPERIMENTAL PROCEDURE

A microcolumn is prepared in a fashion similar to that described for hydrogenation at the microgram level (3). A cut capillary is dabbed into Celite 545 until a column of the powder approximately 2.5 cm in length is retained. The Celite is pressed into a compact column about 1.5 cm in length by using the ends of two paper clips or other suitable tampers. Two microliters of diethyl ether containing 0.5–5 μg of the acid(s) is applied to the column and the sides of the capillary are washed down with two 2- μl aliquots of ether from a clean syringe. The capillary is inserted (wetted end down) through a rubber septum until about 2 cm of the capillary passes through the septum. A stirring bar 5–6 mm long is cut from a paper clip or other suitable wire and placed in a 1-ml serum bottle. Ten microliters each of solutions 1 and 2 are transferred to the vial, the septum inserted immediately and the solution stirred magnetically for 2 min. The capillary is then removed and the methyl ester(s) eluted by injecting 10–15 μl of CH_2Cl_2 , CCl_4 , diethyl ether, or CS_2 into either end of the capillary. The first 5–6 μl (5–6 mm) of solvent emerging from the column contains all of the ester(s) and is removed from the capillary with a hypodermic syringe for chromatographic analysis.

The efficiency of esterification was monitored by gas-liquid chromatography (glc). A 4 ft \times 1/8 in. silanized stainless steel column packed with 7.5% ethylene glycol adipate and 2% H_3PO_4 on 90–100 mesh Anakrom ABS was used. Other glc conditions were: Instrument, Hewlett Packard 5750; Detector, FID; helium flow rate, 40 psi; injection port temperature, 230°C; column temperature, 55–195°C programmed at 6°C/min.

Retention time of authentic methyl esters was used as evidence that the expected product had been obtained. When no authentic methyl ester was available, a mass spectrum was obtained on the peak for molecular

weight determination and detection of the methyl ester function. The LKB-9000 gas chromatograph-mass spectrometer was employed.

RESULTS AND DISCUSSION

The following acids were investigated: benzoic, caprylic, *trans*-cinnamic, 1-cyclohexene-1-carboxylic, 3-cyclohexene-1-carboxylic, 4-cyclooctene-1-carboxylic, 4,7,10,13,16,19-docosahexaenoic, elaidic, 2-furoic, glutaric, 9(10)-hydroxyundecanoic, lauric, lignoceric, linoleic, 2-methylhexanoic, 4-methylvaleric, oleic, 11-oxopalmitic, phenylacetic, phenylpropionic, palmitic, petroselenic, sebacic, sorbic, stearic, tridecanoic, trimethylacetic, and trimethylbenzoic. All acids were completely converted to methyl esters with the exception of trimethylacetic and 1-cyclohexene-1-carboxylic which were estimated to be converted to the extent of approximately 95%. The dicarboxylic acids, glutaric and sebacic gave the dimethyl ester

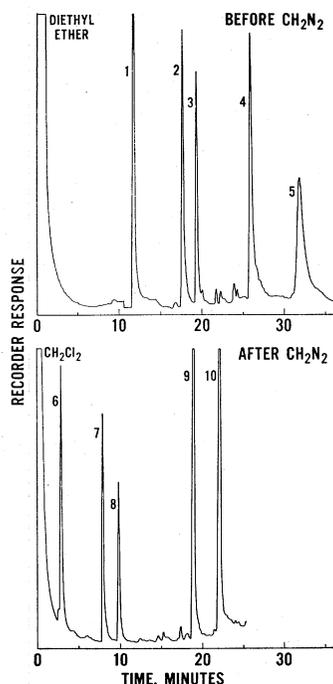


FIG. 1. Gas-liquid chromatograms of a mixture of acids before (top) treatment with diazomethane vapor and of the resultant esters after (bottom) exposure to the reagent. (1) 3-cyclohexene-1-carboxylic acid; (2) phenylacetic acid; (3) 2,4,6-trimethylbenzoic acid; (4) linoleic acid; (5) 11-oxopalmitic acid; (6) methyl-3-cyclohexene-1-carboxylate; (7) methylphenylacetate; (8) methyl 2,4,6-trimethylbenzoate; (9) methyl linoleate; (10) methyl 11-oxopalmitate.

exclusively. It could not be ascertained whether any acid escaped esterification as both acids will not emerge in a reasonable length of time under the glc conditions used. With phenylpropionic acid, the methyl ester was readily obtained but the free acid apparently decarboxylated in the gas chromatograph giving phenylacetylene which emerged under the solvent peak.

Acids applied to the column in a mixture are quantitatively methylated as judged by the absence of unreacted acid after treatment with diazomethane. This is illustrated in Fig. 1.

Successful methylation was also achieved when 1 μg of an acid dissolved in 100 μl of ether was applied to a column in 10- μl aliquots, evaporating the ether in a stream of air or N_2 between applications.

Approximately 7 μmoles (294 μg) of diazomethane can be theoretically generated from 10 μl of solution 1. This is an adequate amount to completely esterify 5 μg of an acid under the prescribed conditions. With 10 μg of an acid on the column 80–90% esterification occurs. Accordingly, if it is desired to completely esterify more than 5 μg of acid(s) the volumes of solutions 1 and 2 must be increased.

Using the appearance of extraneous peaks as a criterion for side reactions, none of the acids investigated gave any suggestion that such reactions had occurred. However, if the reaction time was extended from 2 min to 5 min, a small peak, with a retention time of about 6 min was evident. This peak also appeared in a blank run, indicating that it emanated from the reagents.

REFERENCES

1. ROPER, R., AND MA, T. S. (1957) *Microchem. J.* **1**, 245.
2. SCHLENK, H., AND GELLERMAN, J. L. (1960) *Anal. Chem.* **32**, 1412.
3. SCHWARTZ, D. P., BREWINGTON, C. R., AND WEIHRAUCH, J. L. (1972) *Microchem. J.* **17**, 677.