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Note

A procedure for boron trifluoride-catalyzed esterification suitable for use in gas chromatographic analysis

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Treatment with an alcohol and a Lewis acid is the commonly used esterification procedure in the gas chromatographic analysis of organic acids in biological materials[1]. When methanol is used as the alcohol[2,3], difficulties are encountered owing to the volatility of the corresponding esters, *i.e.*, losses during the concentration step and interference with solvents in the gas chromatographic analysis, while propanol[4] and butanol[5] produce more stable and less volatile esters.

Boron trifluoride has been shown[6] to be the most efficient catalyst as it produces pure esters in high yields in the shortest time. However, the reaction mixture containing boron trifluoride cannot be injected directly into the gas chromatographic column because the boron trifluoride may alter the packing and produce secondary reactions[1]. Therefore, at the end of the esterification reaction, the alcohol–boron trifluoride reagent is usually decomposed by adding water or a salt solution, and the esters are then extracted into an organic solvent. By this procedure, the recoveries of minimal amounts of acids do not seem to be quantitative; in fact, in the extraction step, the alcohol may assist in transferring the ester between water and the organic solvent.

In this paper, we describe an esterification procedure in which the extraction step is no longer required. We have applied this method to fumaric, benzoic and stearic acids, as models, employing isopropanol–boron trifluoride and *n*-butanol–boron trifluoride as reagents; before the gas chromatography stage, the boron trifluoride is completely neutralized by adding an organic base (triethylamine or pyridine), thus forming a well known complex[7] that is insoluble in non-polar solvents. The gas chromatographic analysis of isopropyl and *n*-butyl esters gives better recoveries than those obtained by employing the water extraction procedure.

EXPERIMENTAL

Reagents

Boron trifluoride was purchased from J. T. Baker, Phillipsburgh, N.J., U.S.A. All solvents and other compounds were obtained from Carlo Erba, Milan, Italy. Isopropanol and *n*-butanol were refluxed over calcium chloride and re-distilled before use.

The alcohol–boron trifluoride reagent was prepared by bubbling boron tri-

fluoride gas into the alcohol at 0° until a concentration of 2 mequiv./ml was attained. The boron trifluoride content was determined by adding a known excess of pyridine and titrating the base with 0.1 *N* hydrochloric acid to pH 3.

Apparatus

A Fractovap GV gas chromatograph (Carlo Erba) equipped with a flame ionization detector was used. The instrument contained two U-shaped columns (2 m × 2.5 mm): one column was packed with 3% OV-17 on Gas-Chrom Q, 100–120 mesh (Applied Science Labs., State College, Pa., U.S.A.) and the other with 2% neopentyl glycol succinate (NPGS) (LAC 767) on Gas-Chrom P, 100–120 mesh (Carlo Erba). Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min; the flow-rates of hydrogen and air were 33 and 400 ml/min, respectively. The injector and detector temperatures were 200° and 250°, respectively. The column temperature was kept at 80° for 1 min after the injection, then programmed at 20°/min to 210° and kept at 210° for 5 min.

Procedure

Two equal aliquots of each acid (25–100 μg) were placed in two screw-capped stoppered 3-ml tubes. Alcohol–boron trifluoride reagent (0.15 ml) was added and the reaction was carried out at 100° for 20 min. To one sample, anhydrous diethyl ether (0.2 ml), *n*-pentane (0.2 ml) and a mixture (1 :1, v/v) of *n*-pentane and triethylamine (or pyridine) (0.15 ml) were added with shaking in an ice-bath (the neutralization is exothermic) and the suspension was centrifuged. The second sample was added with 1 ml of water and extracted three times with 0.4 ml of chloroform for 2 min. The combined extracts were transferred into a tube containing a few grains of anhydrous sodium sulphate. After adding to both samples 50 μg of phenanthrene (as internal standard) in diethyl ether (0.1 ml), 1 μl of each supernatant was injected into the gas chromatograph.

Quantitative analysis

Peak areas were calculated as peak height times the width at half height and corrected against the peak area of the internal standard (phenanthrene). The corrected peak areas of the esters from the two neutralization methods were compared. The values obtained by the decomposition with water are reported as a percentage of those obtained by our method. For the calibration graphs, 5, 10, 20 and 30 μg of each acid were placed in different 1-ml tubes and made to react with the alcohol–boron trifluoride reagent (100 μl) as previously described. In order to precipitate boron trifluoride, 40 μl each of diethyl ether and *n*-pentane and 50 μl of a mixture of *n*-pentane + pyridine or triethylamine were used; 10 μg of phenanthrene in diethyl ether (40 μl) were finally added.

RESULTS AND DISCUSSION

The acids examined were chosen from those of biological interest but with different chemical features (one dicarboxylic, one aromatic and one fatty acid) in order to verify the suitability of the method. For the same purpose, two different alcohols, isopropanol and butanol, were used.

The neutralization of boron trifluoride by the addition of an organic base in a non-polar solvent permits the complex base–boron trifluoride to be precipitated and a supernatant containing the esters without boron trifluoride to be obtained; this solution can be injected directly into the gas chromatographic columns.

As the solvent for the precipitation, *n*-pentane seemed to be ideal because of its volatility and non-polarity, but, as it is not soluble in the alcohol–boron trifluoride reagent, the added organic base agglomerates the reaction mixture. Hence the prior addition of diethyl ether to the reaction mixture is necessary in order to make the solution homogeneous during precipitation. The precipitates obtained with triethylamine were transparent and gel-like, while those obtained with pyridine were white, flaky and easily separated. Therefore, pyridine was the base of choice except for the esters that gave peaks which interfered with the pyridine tailed peak. Both stationary phases used (NPGS and OV-17) were found to be efficient; the retention times of the isopropyl and *n*-butyl esters analyzed are reported in Table I.

TABLE I

RETENTION TIMES RELATIVE TO PHENANTHRENE (1.00) OF ISOPROPYL AND *n*-BUTYL ESTERS ANALYZED ON OV-17 AND NPGS

Compound	*OV-17*	*NPGS*
Isopropyl fumarate	0.46	0.37
Isopropyl benzoate	0.53	0.34
Isopropyl stearate	1.27	0.89
n-Butyl fumarate	0.59	0.80
n-Butyl benzoate	0.69	0.62
n-Butyl stearate	1.69	1.33

In order to compare our method of neutralization of boron trifluoride with that used previously, we studied the corresponding recoveries by gas chromatographic analysis. The analysis was carried out on NPGS, precipitating boron trifluoride with triethylamine for isopropyl esters and with pyridine for *n*-butyl esters. The results are reported in Tables II and III.

TABLE II

RECOVERY OF ISOPROPYL ESTERS OBTAINED BY THE TWO DIFFERENT METHODS OF BORON TRIFLUORIDE NEUTRALIZATION

Results given are percentages of isopropyl esters recovered after neutralization with water relative to the same ester recovered after neutralization with triethylamine. Single values are means from five independent experiments, and the ranges are given in parentheses.

Acid	*Amount of acid esterified (μg)*			
	25	*50*	*75*	*100*
Fumaric	70 (63–75)	84 (81–88)	86 (82–91)	98 (92–102)
Benzoic	71 (65–76)	79 (75–82)	88 (83–92)	96 (90–99)
Stearic	76 (69–81)	85 (80–88)	87 (81–92)	99 (96–103)

TABLE III

RECOVERY OF *n*-BUTYL ESTERS OBTAINED BY THE TWO DIFFERENT METHODS OF BORON TRIFLUORIDE NEUTRALIZATION

Results given are percentages of *n*-butyl esters recovered after neutralization with water relative to the same ester recovered after neutralization with pyridine. Single values are means from five independent experiments, and the ranges are given in parentheses.

Acid	*Amount of acid esterified (μg)*			
	25	*50*	*75*	*100*
Fumaric	83 (78–87)	86 (81–89)	92 (87–95)	101 (96–105)
Benzoic	78 (73–82)	91 (87–94)	94 (90–98)	100 (94–103)
Stearic	80 (74–84)	82 (78–86)	88 (83–92)	101 (96–104)

It was found that the results obtained on OV-17 and using both bases for both esters were comparable with those reported earlier. With large amounts of acids (1–10 mg), the recoveries proved to be similar using both methods. With small amounts (below 100 μg), our method gave better recoveries. The calibration graphs for esters of fumaric, benzoic and stearic acids (Fig. 1) show a linear response up to 50 μg.

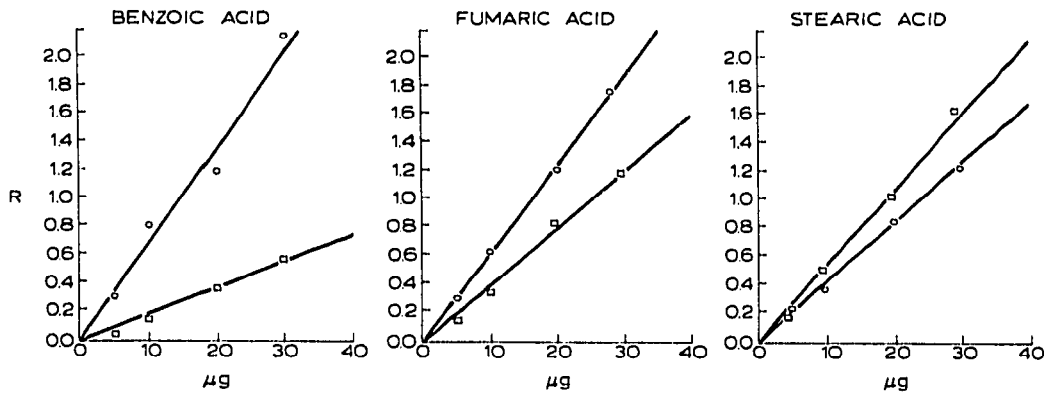

Fig. 1. Calibration graphs for the isopropyl (□) and *n*-butyl (○) esters analyzed. On the abscissa are recorded the amounts of acid and on the ordinate the ratios (R) between areas of esters and area of phenanthrene (10 μg).

We can therefore state that the method described here, in comparison with the usual techniques, simplifies the esterification procedure and improves the product recoveries.

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