CHROM. 4667

Preparation of volatile derivatives of amino acids on a solid support followed by direct injection into the gas chromatography column

In gas chromatography the test material is usually dissolved in a suitable solvent and an aliquot injected on to the column by means of a microsyringe. Because of the problems encountered, which become greater as the volume of the liquid injected increases, only a small portion of the total sample is usually used. The dilution of the test material by solvent often necessitates working at a high sensitivity with a high detector "noise" level. Furthermore, the solvent often interferes with those sample peaks having short retention times. In order to avoid these drawbacks, we have tried to find a system whereby a derivatization reaction can take place on a solid support, and is followed by direct injection on to the column. SAROFF[1] first proposed such a method for amino acids (see BLAU[2]). In this paper we present a method for preparing the N-trifluoroacetyl amino acid methyl ester derivatives[3,4] on a platinum support.

Apparatus

Gas chromatograph, Pye Series 104, Model 24, fitted with two flame ionization detectors (W. G. Pye Ltd., P.O. Box 60, Cambridge, Great Britain). Speedomax W, 1 mV recorder (Leeds and Northrup Ltd., Birmingham, Great Britain). Glass column, 3.2 m × 2.5 mm I.D., packed with Diatoport S, 80–100 mesh coated with 2.5% (w/w) of stationary phase (XE60–QF–1-MS200, 100 cSt in the proportions 46:27:27 (w/w) respectively[3]).

The apparatus for carrying out the derivatization was made from Rotaflo stopcock units (Scientific Supplies Co. Ltd., Vine Hill, London, Great Britain) as shown in Fig. 1.

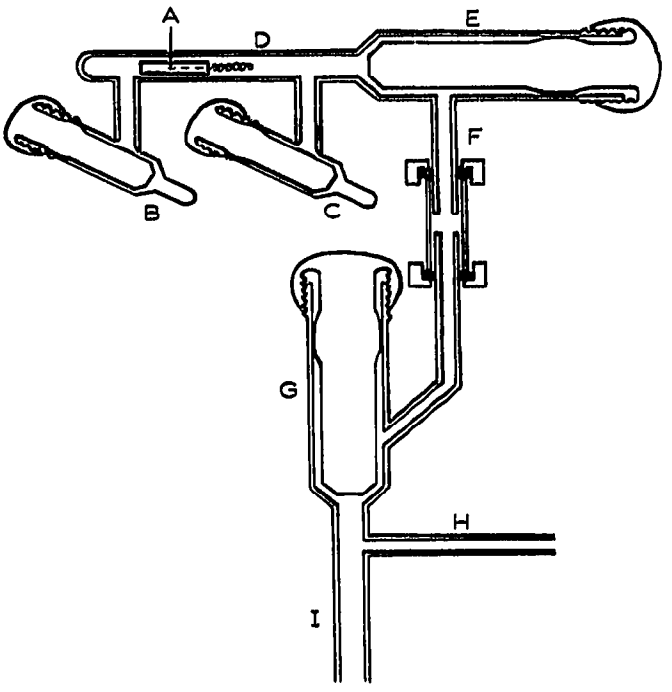
An aqueous solution containing 2.5 mμmole of each amino acid was evaporated on to a small ball of platinum wire. This was made by winding (50 cm × 0.05 mm diameter) loosely on to a 0.5 cm length of thick platinum wire. Depending on the method of winding the liquid-holding capacity of the ball was 10–20 μl. To prevent the ball from contacting surrounding surfaces the protruding thick wire was inserted into a small block of teflon, A in Fig. 1.

Derivatization procedure

About 50 μl of dry methanolic HCl (4 mmole/ml) was placed in vessel B (see Fig. 1) and the stopcock closed. About 50 μl of freshly distilled trifluoroacetic (TFA) anhydride was placed in C and the stopcock closed. The piece of teflon with the projecting platinum was introduced into the body of the apparatus (D) by unscrewing the stopcock E. The outlet F was connected to an oil pump and the reaction vessel evacuated (1–10 torr.). Stopcock E was then closed to the side arm F.

Esterification. Stopcock B was opened and the apparatus was placed in an oven at 70° for 1 h. B was then closed and excess methanolic HCl removed by the pump through the side arm F with stopcock E open. After 10 min E was again closed with vacuum conditions in D.

Trifluoroacetylation. Stopcock C was opened and the apparatus kept at 30° for



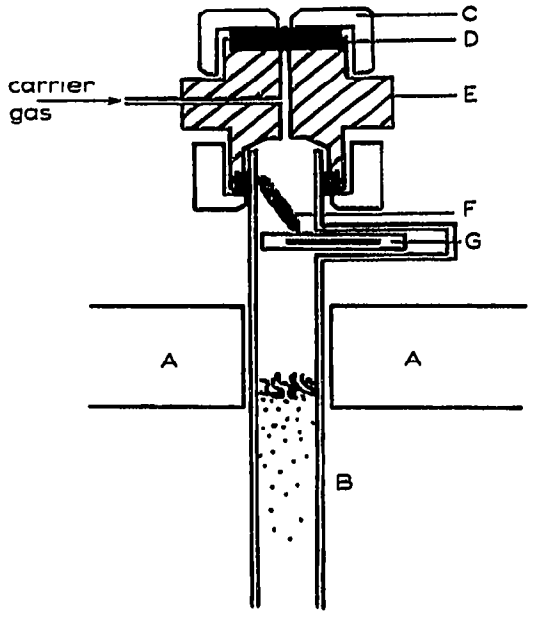


Fig. 1. Apparatus for derivatization of amino acids on a solid support. A = block of teflon supporting platinum wire; B and C = Rotaflo stopcock TF2/13 or TF2/C1/13, D = reaction vessel; E = Rotaflo stopcock TF6/18; F = side tube; G = Rotaflo stopcock TF6/18; H = carrier gas inlet, I = tube leading to inlet heater zone of gas chromatography column.

Fig. 2. Diagram of the injection head for solid injection. A = inlet heater zone; B = glass column, C = knurled nut, D = septum for conventional injection by syringe; E = inlet injection block with carrier gas inlet; F = platinum ball, G = glass covered magnet.

10 min. These conditions were satisfactory for all the amino acids, alanine to phenylalanine[3]. It was, however, necessary to maintain the apparatus at 80° for 60 min in order to obtain good derivatization of lysine, tyrosine and arginine. At the end of the reaction period stopcock C was closed.

Sample injection

Method 1. The apparatus was connected to the arm of the Rotaflo unit (G) shown in Fig. 1 by means of two back-nuts tightening on to a centre-piece at F. After opening stopcock E, the platinum ball was made to fall into tube F by tapping slightly; E was then closed. When stopcock G was unscrewed momentarily the platinum fell into the inlet heater zone of the gas chromatography column. The carrier gas flow through tube H was unaffected during this process. The Rotaflo unit (G) may be attached to the chromatography column by fittings as shown at F, or a permanent glass-to-glass junction can be made. About 8 platinum balls may be dropped in before it becomes necessary to open the column at G to extract these.

Method 2. This simpler method is shown in Fig. 2. The gas flow through the column was interrupted when the knurled nut (C) was unscrewed and the platinum was allowed to fall on to the glass-covered soft steel magnet (G). When the baseline on the recorder was re-established, sideways movement of the magnet allowed the platinum to drop into the inlet heater zone (A) of the column (B). It took about 10–15 sec to open, drop in the platinum, and reclose. One criticism of this method is that the

carrier gas is flowing past the sample in the cold and thus some of the more volatile compounds may be carried on to the column before the platinum is allowed to drop into the heated zone. In practice we found that peak shapes and HETP values were equivalent to those obtained by the conventional method of injection by syringe.

Discussion

The method of preparing the derivatives gave good peaks for all the amino acids shown in the separation, alanine to phenylalanine[3]. Methionine gave a peak height lower than expected and this may be due to an oxidative effect catalyzed by platinum. The temperature of trifluoroacetylation could not be greater than 30°, otherwise losses of the more volatile derivatives occurred due to evaporation in the reaction compartment. Some of the less volatile amino acids[3] presented some problems because of the need for more drastic trifluoroacetylating conditions. Arginine gave a high yield only when a mixture of equal volumes of TFA acid and TFA anhydride were used and some condensation was allowed to occur on the platinum before standing at 80° for 1 h. Under these conditions tryptophan gave a low yield.

The system is of use for other compounds where it is possible to carry out the derivatization in the vapour phase[5,6]. Theoretically, the total sample applied to the platinum need only be that which produces a sufficient quantity of derivative to give a response with the detector.

The support of this work by the Science Research Council and the Medical Research Council of Great Britain by grants awarded to A.D. is gratefully acknowledged. B.T. expresses his gratitude to the S.R.C. for financial support as a research assistant.

Our thanks are due to Professor H. R. V. ARNSTEIN for his interest and encouragement.

Université Catholique de Louvain, B. TEUWISSEN
Laboratoire de Radiochimie,
Cliniques St-Pierre, Louvain (Belgium)

Biochemistry Department, A. DARBRE
King's College, Strand,
London, W.C. 2 (Great Britain)

1 H. A. SAROFF, *4th Intern. Sympos. Gas Chromatog., Paris, 1965.*
2 K. BLAU, in H. A. SZYMANSKI (Editor), *Biomedical Applications of Gas Chromatography*, Vol. 2, Plenum Press, New York, 1968, p. 1.
3 A. DARBRE AND A. ISLAM, *Biochem. J.*, 106 (1968) 923.
4 A. ISLAM AND A. DARBRE, *J. Chromatog.*, 43 (1969) 11
5 J. K. NORYMBERSKI AND A. RIONDEL, *Experientia*, 23 (1967) 318.
6 M. P. VAN DAMME AND P. OSINSKI, personal communication.

Received February 11th, 1970