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Note

Gas–liquid chromatography of Schiff bases of amino acid methyl esters

Extensive research in recent years[1–13], notably by GEHRKE *et al.*, has culminated in methods for the separation and quantitation of volatile derivatives of twenty common amino acids by gas–liquid chromatography (GLC). The two preferred derivatives are either the N-trifluoroacetyl *n*-butyl esters[11] or the trimethylsilyl esters[12]. A modification by Moss *et al.*[13] uses N-heptafluorobutyryl *n*-propyl esters. Derivatisation requires reactions in sealed or tightly capped tubes at 150°.

This paper reports results on the volatile derivatives formed by the addition of benzaldehyde or 2,4-pentanedione to amino acid methyl esters under very mild conditions.

Materials and methods

Gas chromatographic analyses were made using a Beckman Model GC45 dual-column instrument equipped with flame ionisation detectors. Samples were analyzed on 6 ft. × 2 mm I.D. glass columns packed with SP400 (Beckman). The injection port temperature was 250° and the detector temperature 300°. The column was maintained at 100° for 5 min and then temperature programmed to 280° in 16 min. The electrometer range was 1 K with an attenuation of 4 coupled to a 1-mV recorder. This range resulted in a full-scale deflection of 2×10^{-8} A. Helium flow-rate was 80 ml/min.

Amino acids were obtained from Calbiochem (Los Angeles, Calif.). Solutions of each in 0.1 *N* HCl contained 5 nmoles/μl. For derivatisation, 50-μl aliquots were taken to dryness in 1-dram stoppered vials (Kimble No. 60975.L) either by a stream of nitrogen or in a desiccator over NaOH pellets. The dry residues were then treated with 0.25 ml of methanolic thionyl chloride prepared by the addition of 10 ml of thionyl chloride (Eastman Organic) to dry ice–acetone cooled methanol (reagent grade, 0.1% water) and making up to 100 ml with methanol. The stoppered vials were incubated at 40° for 1 h and then blown down to dryness with nitrogen. The residues were then mixed with 50 μl of a solution of 2.5 ml of bicarbonate-washed benzaldehyde (J. T. Baker Chem. Co.) and 2.5 ml of pyridine made up to 25 ml with methanol, and a 1-μl aliquot injected into the gas chromatograph.

For pentanedione derivatisation, 50 μl of a solution of 2.5 ml 2,4-pentanedione (Matheson, Coleman and Bell) and 2.5 ml of pyridine made up to 25 ml with methanol were used. The stoppered tubes were kept at 80° for 10 min before injection of a 1-μl aliquot into the gas chromatograph.

Results and discussion

In Table I the elution temperatures of the peaks from the derivatised amino acids are collected. Those peaks of at least 20% of full-scale deflection and sharp are regarded as evidence of a significant yield of derivative whilst those of less than

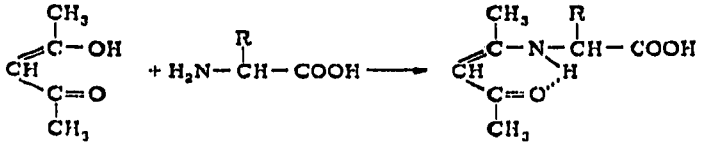
TABLE I

ELUTION TEMPERATURES OF DERIVATISED AMINO ACIDS

Amino acid	*Elution temperature* (°C)	
	Benzaldehyde derivatives	*Pentanedione derivatives*
Ala	130	140
Gly	130	143
Val	155	150
Leu	170	160
Ile	170	160
Ser	170	165
Thr	170	not made
Pro	negative	175
Hypro	negative	not made
Asp	190	not made
Cys	190 (and 140)	(180)
CysH	190 (and 140)	(180)
Glu	208	180
Met	210	180
Phe	220	210
His	(235)	negative
Tyr	250	not made
Arg	(181)	negative
Lys	270	255
Try	negative	255

20% (bracketed) are regarded as being due to poor derivatisation or to artifacts.

It is not surprising that the benzaldehyde results with Pro and Hypro were negative and this would augur more in favor of the use of pentanedione as the reagent of choice. The pentanedione Schiff bases are present in the form of enamines stabilized by a hydrogen bond[14]



and presumably the amino acids Pro and Hypro will also form enamines with pentanedione, whereas this is impossible with benzaldehyde.

Reaction of the amino acid esters with benzaldehyde is very rapid. Thus a solution of leucine methyl ester and the benzaldehyde reagent was kept at room temperature for 1 h and then treated with an excess of vanillin. On GLC analysis, no vanillin derivative (which elutes about 30° higher) was observed.

Also, the benzaldehyde or vanillin derivative of leucine methyl ester can be prepared "on column" by injecting the ester followed by a shot of the reagent. With pentanedione, this cannot be done, and derivatisation at room temperature is slower than with benzaldehyde. The arbitrary period of 10 min at 80° gives

sizable peaks. Derivatisation conditions for arginine and histidine and conditions for the complete separation of the remaining eighteen derivatives remain to be done. Their stability (at least one week at 20°) and their ease of preparation may provide a useful additional method for not only amino acid esters but also for other compounds containing basic primary and secondary amino groups.

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1 C. W. GEHRKE, R. W. ZUMWALT AND L. L. WALL, *J. Chromatogr.*, 37 (1968) 398.
2 C. W. GEHRKE AND D. L. STALLINGS, *Separ. Sci.*, 2 (1967) 101.
3 D. ROACH AND C. W. GEHRKE, *J. Chromatogr.*, 44 (1969) 269.
4 D. ROACH AND C. W. GEHRKE, *J. Chromatogr.*, 43 (1969) 303.
5 D. ROACH, C. W. GEHRKE AND R. W. ZUMWALT, *J. Chromatogr.*, 43 (1969) 311.
6 M. STEFANOVIC AND B. L. WALKER, *Anal. Chem.*, 39 (1967) 710.
7 W. J. MCBRIDE, JR. AND J. D. KLINGMAN, *Anal. Biochem.*, 25 (1968) 109.
8 A. DARBRE AND K. BLAU, *J. Chromatogr.*, 29 (1967) 49.
9 A. DARBRE AND A. ISLAM, *Biochem. J.*, 106 (1968) 923.
10 J. R. COULTER AND C. S. HANN, *J. Chromatogr.*, 36 (1968) 42.
11 C. W. GEHRKE, K. KUO AND R. W. ZUMWALT, *J. Chromatogr.*, 57 (1971) 209.
12 C. W. GEHRKE AND K. LEIMER, *J. Chromatogr.*, 57 (1971) 219.
13 C. W. MOSS AND M. A. LAMBERT, *J. Chromatogr.*, 60 (1971) 134.
14 M. BODANSZKY AND M. ONDETTI, *Peptide Synthesis*, Wiley, New York, 1966, p. 39.

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