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The determination of tryptophan oxidation products using an amino acid analyzer

Studies on the oxidative modification of tryptophan and tryptophan residues in proteins have necessitated the development of a rapid method for the separation and estimation of a variety of oxidation products. This is of particular interest because such modifications have been suggested as a route to the non-enzymatic cleavage of tryptophyl peptide bonds[1–3].

We wish to report the results obtained using a 10-cm column of Aminex A5 resin in conjunction with a Spinco Model 120 B amino acid analyzer, according to the principle described by SPACKMAN, STEIN AND MOORE[4].

Materials and methods

Samples of 3a-hydroxy-2,3,3a,8a-tetrahydropyrroloindole-2-carboxylic acid[5] and 4-(2-amino-2-carboxyethyl)quinazoline[6] were prepared by Dr. W. E. SAVIGE of this laboratory. Oxindolyl-3-alanine[7] and dioxindolyl-3-alanine[8] were prepared by published methods. γ-(*o*-Aminophenyl)homoserine was prepared by the sodium borohydride reduction of kynurenine. All other amino acids were obtained from normal commercial sources.

Separation of these materials was achieved by pumping the column at 55° for 20 min with pH 3.05 sodium citrate buffer and then changing to pH 4.25 buffer. Our

TABLE I

SUMMARY OF COMPOUNDS ELUTED FROM AN AMINO ACID ANALYZER

Compound	*Elution time (min)*	*Colour factor (Trp = 1)*
Taurine	12.5	1.14
3a-Hydroxy-2,3,3a,8a-tetrahydropyrroloindole-2-carboxylic acid	31	0.55[a]
	52	0.71[a]
Norleucine	60	1.22
Dioxindolyl-3-alanine	63	1.02
Tyr–Phe	65	–
4-(2-Amino-2-carboxyethyl)-quinazoline	76	0.84
Oxindolyl-3-alanine	81	0.54
5-Hydroxytryptophan	84	1.00
3-Hydroxykynurenine	86	1.2
Kynurenine	86	1.18
Ammonia	90	0.95
γ-(*o*-Aminophenyl)homoserine	102	0.96
	108	0.96
Tryptophan	105	1.0
Ornithine	137	–
Lys–His	150	–

[a] Calculated from peak at 570 nm although peak at 440 nm is higher.

experience with this separation and with separating nine products, derived from various oxidations of tryptophan, is summarized in Table I. The time required for an analysis to tryptophan is 110 min. These conditions were found to be suitable for rapid, semi-quantitative assays for products resulting from various oxidative treatments of tryptophan, even though many derivatives were found to elute close together.

Using longer columns, after *e.g.* BERRIDGE *et al.*[9], and/or longer elution times with the lower pH buffer all of the above products, with the exception of 3-hydroxykynurenine and kynurenine, could be separated from one another but the time taken for a complete analysis was greatly increased. Moreover this system could often be successfully applied to protein hydrolysates as the region between the Tyr–Phe peak and the Lys–His peak was large enough to contain the more prominent tryptophan oxidation products.

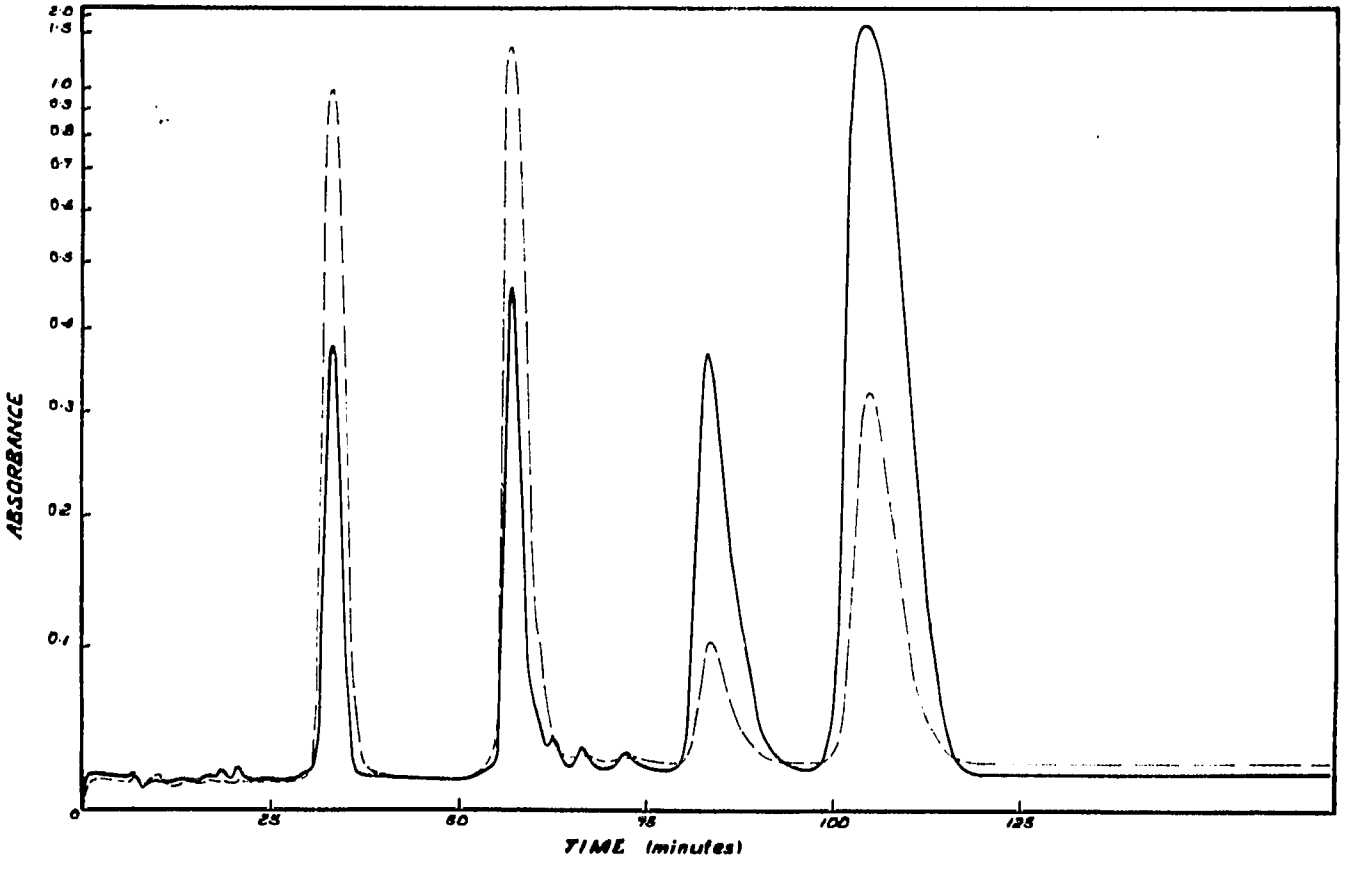

Fig. 1. Amino acid analysis of the products resulting from the peracetic acid oxidation of tryptophan[5], showing the two isomers of 3a-hydroxy-2,3,3a,8a-tetrahydropyrroloindole-2-carboxylic acid and tryptophan with the peak of an unknown amino acid between them. The continuous line represents optical density at 570 nm and the broken line represents optical density at 440 nm.

An example of the degree of separation expected is shown by the analysis performed on the mixture of products resulting from the oxidation of tryptophan with peracetic acid[5] (Fig. 1). The two isomers of 3a-hydroxy-2,3,3a,8a-tetrahydropyrrolo-

indole-2-carboxylic acid are clearly separated from unreacted tryptophan and an unknown oxidation product.

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1 A. PREVIERO, M. A. C. PREVIERO AND P. JOLLES, *Biochem. Biophys. Res. Commun.*, 22 (1966) 17.
2 A. PREVIERO, M. A. C. PREVIERO AND P. JOLLES, *Biochim. Biophys. Acta*, 124 (1966) 400.
3 M. MORISHITA AND F. SAKIYAMA, *Bull. Chem. Soc. Jap.*, 43 (1970) 524.
4 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1185, 1190.
5 W. E. SAVIGE, *Aust. J. Chem.*, in press.
6 W. E. SAVIGE, *Aust. J. Chem.*, 24 (1971) 1285.
7 T. WIELAND, O. WEIBERG AND W. DILGER, *Ann. Chem.*, 592 (1955) 69.
8 P. L. JULIAN, E. E. DAILEY, H. C. PRINTY, H. L. COHEN AND S. HAMASHIGE, *J. Amer. Chem. Soc.*, 78 (1956) 3503.
9 B. J. BERRIDGE, W. R. CHAO AND J. H. PETERS, *Anal. Biochem.*, 41 (1971) 256.

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