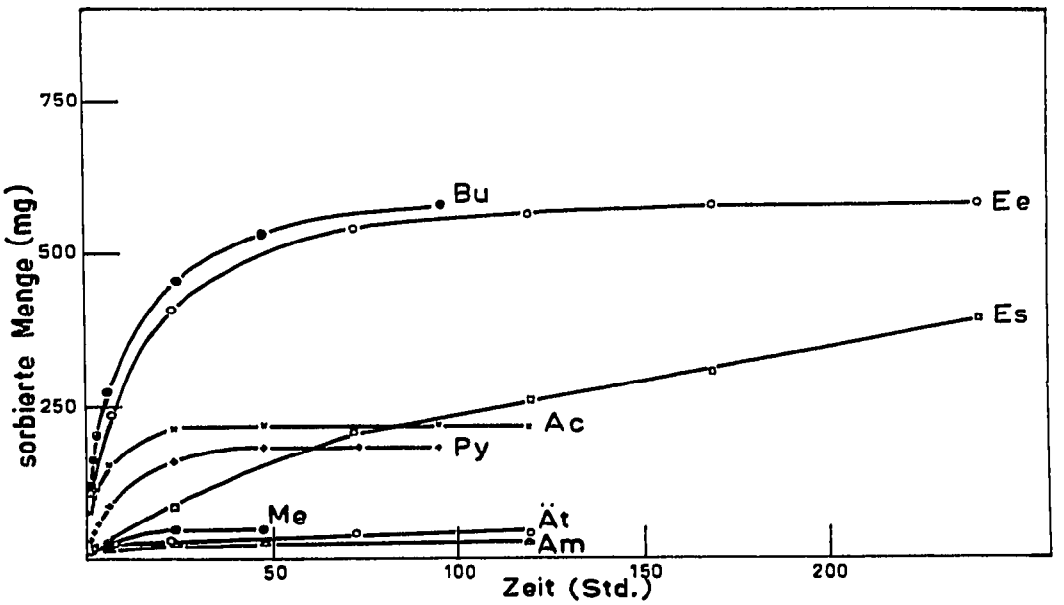

Fig. 3. Sorption von Dämpfen an Siliconstopfen. Bezeichnungen wie bei Fig. 2.

körpern zwar Gummistopfen verwendet, die Fehler aber mit Hilfe der Blindwerte durch eine relativ umständliche Berechnung korrigiert. Dabei erwies es sich als günstig, dass die in Fig. 1 gezeigten Kurven im doppelt logarithmisch geteilten Netz Geraden sind.

Wurde der Kopfraum über Flüssigkeiten analysiert, so wurden keine Abnahmen der Aromastoffgehalte im Gasraum beobachtet, wahrscheinlich deshalb, weil die Aromastoffe aus der Flüssigkeit schnell genug nachgeliefert wurden.

Universitäts-Institut für Lebensmittelchemie,
Frankfurt a.M. (B.R.D.)

H. G. MAIER

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Artefacts in amino acid analysis

Ninhydrin-positive products of carbohydrate hydrolysis

Amino acids analysed using standard procedures for amino acid analysers[1,2] often can be identified solely on the basis of the time of their elution from the resin column. This criterion has met general acceptance for the common amino acids in protein hydrolysates, but is not satisfactory for many components of physiological fluids because of the presence of a number of artefacts and unknown compounds.

Many of the hydrolysates analysed in this laboratory contain polysaccharide and lignin derivatives. A number of ninhydrin-positive substances appeared between cysteic and aspartic acids at the beginning of analyses. This paper describes experiments which indicate that some of the peaks were artefacts derived from 'caramelisation' of carbohydrates during hydrolysis.

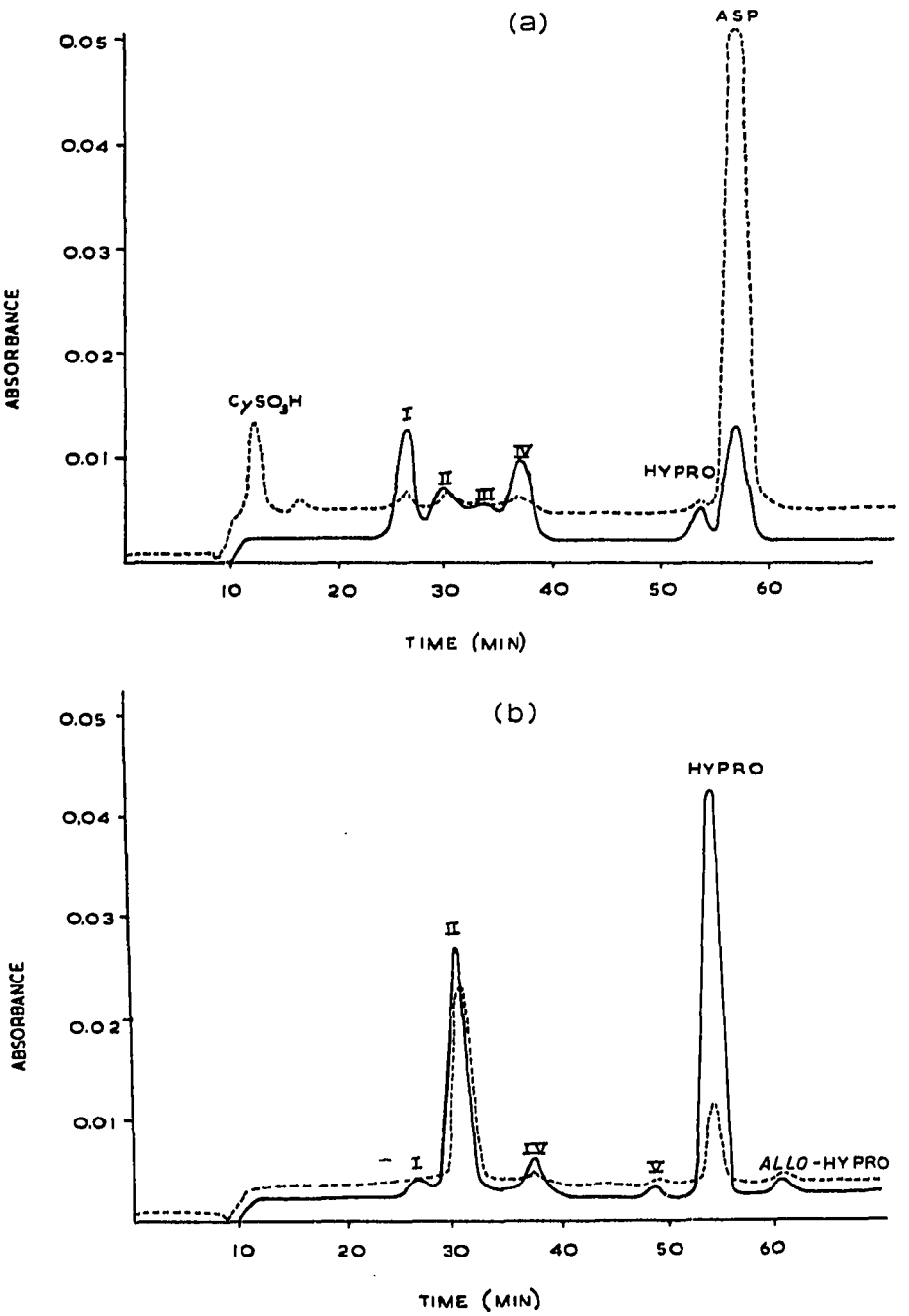

Fig. 1. Analysis of hydrolysates containing hydroxyproline and carbohydrates. (a) Part of the amino acid analysis of hydrolysed seed coats. (b) Complete analysis of a standard mixture of hypro and sucrose (see text). Peaks I, II, IV and V are derived from carbohydrate hydrolysis. ——, Absorbance at 440 nm; - - - - -, absorbance at 570 nm.

Materials and methods

Dried pea (*Pisum sativum*, L.) seed coats, which contain polysaccharide, lignin and protein[3], were hydrolysed in 6 *N* HCl at 105° for 20 h *in vacuo*. Test solutions containing 2 μmole 4-hydroxyproline (hypro) and 0.2 μmole sucrose either together or separately were hydrolysed in 0.2 ml 6 *N* HCl under the same conditions. The hydrolysates were made to a final volume of 5 ml with citrate buffer pH 2.2 (ref. 4), and 1.0 ml was analysed using a Beckman 120C amino acid analyser by the recommended 4-h procedure with expanded recorder range card.

Results and discussion

Fig. 1a shows the first part of the elution pattern obtained during amino acid analyses of hydrolysed pea seed coat material. Peaks I–IV were characterised by their high absorbance at 440 nm relative to that at 570 nm. Peak II was further characterised as having almost equal absorbance at the two wavelengths.

Fig. 1b shows the elution pattern obtained by amino acid analysis of an hydrolysate containing hypro and sucrose. Peaks I, II and IV correspond exactly with those in Fig. 1a; peak III is absent and there is an additional peak (V). The absorbance pattern of peak II was the same in both natural and synthetic hydrolysates.

None of the peaks I–V were present in hydrolysates of hypro. There were no peaks in the positions of hypro or *allo*-hypro when sucrose hydrolysates were analysed. All hydrolysates of seed coat or sucrose appeared pale yellow before analysis but there was no absorbance at 440 nm without reaction with ninhydrin. It is likely that the yellow colour was due to 'caramelised' products of carbohydrate hydrolysis. No attempt has been made to characterise these artefacts further. Their existence serves to emphasise the need for care in identification of ninhydrin-positive components, particularly in view of the fact that the elution positions of 3-hydroxyproline and 3,4-dihydroxyproline[5] are in this part of the chromatogram.

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Department of Botany, The University of British Columbia, Vancouver 8, B. C. (Canada) I. E. P. TAYLOR

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