ON THE "MINOR BASIC AMINO ACID RESIDUES" OF AMPHOMYCIN

Sir:

In a recent communication[1] from this laboratory it was reported that in addition to L-*threo*- and D-*erythro*-α, β-diaminobutyric acids some yet unidentified basic amino acids also occur in the acid hydrolysates of amphomycin[2]. These minor constituents were provisionally designated according to their relative elution times (in minutes counting from the ammonia peak) on the short column of the SPACKMAN-STEIN-MOORE amino acid analysis system[3] as -11, $+5$ and $+10$ compounds. Subsequently, the somewhat surprising observation was made that if hydrolysis with constant boiling hydrochloric acid at 110°C is extended beyond the usual 16 hours, the amount of these basic components gradually decreases and they are virtually absent after about 70 hours of hydrolysis. A concomitant increase in the values of valine and of *erythro*-α,β-diaminobutyric acid indicated that the "minor components" are peptides of these two amino acids.

Hindered hydrolysis of peptide bonds between amino acids with large nonpolar side chains is well known (cf. *e. g.* ref. 4), yet a bond between valine and a diamino acid *a priori* did not seem to belong to this category. As a tentative explanation we propose that protonation of a free amino group hinders the formation of a second positive charge on an amide bond if this bond belongs to an amino group on a vicinal carbon atom. Consequently the rate of acid catalyzed hydrolysis appreciably decreases.

Attempts to isolate the minor components, that is the dipeptides of L-valine and *erythro*-D-α, β-diaminobutyric acid were only partially successful. The $+10$ species was obtained by ion-exchange chromatography but still contaminated by free D-*erythro*-α,β-diaminobutyric acid. On prolonged hydrolysis valine and *erythro*-α, β-diaminobutyric acid were liberated from this dipeptide. Deamination with N_2O_3 followed by acid hydrolysis led to the disappearence* of valine, which, therefore, must be N-terminal. Since the $+10$ component is the one which emerges first on brief hydrolysis of amphomycin and is most dominant in the hydrolysates and because deamination of the antibiotic followed by hydrolysis yields threonine (albeit only about 20~25 % of the calculated amount), it was tentatively concluded that the $+10$ peak corresponds to α-(L-valyl)-D-*erythro*-α, β-diaminobutyric acid.

Acylation of D-*erythro*-α, β-diaminobutyric acid in aqueous pyridine at pH 9 with tert. butyloxycarbonyl-L-valine *p*-nitrophenyl ester followed by removal of the protecting group with trifluoroacetic acid yielded a mixture which on the short column of the amino acid analyzer[3] gave three peaks corresponding to unchanged *erythro*-α, β-diaminobutyric acid and to the $+5$ and $+10$ components, the $+5$ compound being present in the largest amount. In conjunction with the observation that during hydrolysis the $+5$ species emerges gradually after the $+10$ compound has already appeared and that "hydrolysis" of the $+10$ material also gives rise to the $+5$ species, it was assumed that $+5$ is β-(L-valyl)-D-*erythro*-α,β-diaminobutyric acid, and forms through an N→N shift. So far no clue was found for the nature of the -11 component.

From these observations it follows that no basic amino acids other than the two α, β-diaminobutyric acids occur in amphomycin. Yet deamination followed by hydrolysis results in the formation of not only threonine but also of a small amount of an amino acid which in amino acid analysis[3] emerges five minutes later than threonine. Comparison of the elution pattern with that of a hydrolysate to which some α-methylserine was added revealed it as the amino acid present in the hydrolysates of deaminated amphomycin indicating that an α-methyl-α, β-diaminopropionic acid residue with its β-amino group free and its α-amino

* The α, β-diaminobutyric acid yielded no ninhydrin-positive product in this reaction. Perhaps through the participation of a carbonium ion at its β-carbon atom the valyl residue was displaced from the α-amino group which in turn was deaminated.

group participating in a peptide bond might occur in some minor members of the antibiotic family. However, a thorough examination of the basic amino acid fractions by nmr spectra failed to reveal α-methyl-α,β-diaminopropionic acid; these spectra demonstrated the two isomeric α,β-diaminobutyric acids as the only basic constituent of amphomycin.

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