Identification of a 3-(2-Piperidyl)pyridinium Derivative ('Anabilysine') as a Cross-linking Entity in a Glutaraldehyde-treated Protein

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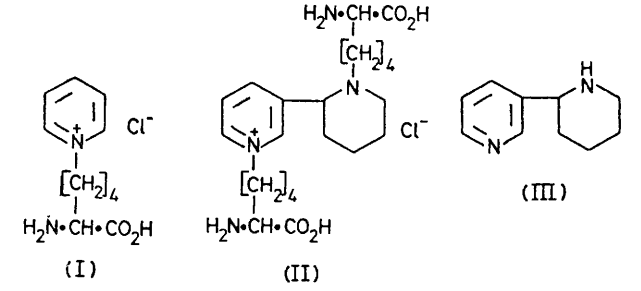
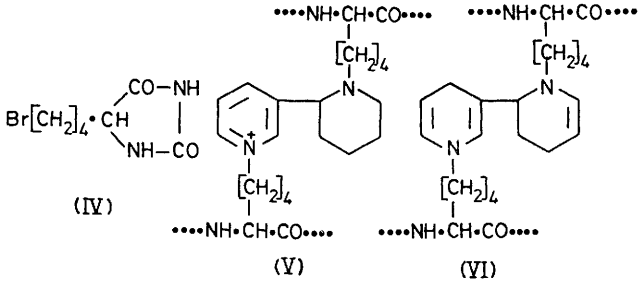
Summary The 3-(2-piperidyl)pyridinium derivative (II), 'anabilysine,' has been isolated from acid hydrolysates of glutaraldehyde-treated ovalbumin and its structure confirmed by comparison with a diastereoisomer synthesised from anabasine.

IN a previous communication[1] we reported the isolation of 1-(5-amino-5-carboxypentyl)pyridinium chloride (I), from acid hydrolysates of glutaraldehyde-treated ovalbumin. A second pyridinium derivative has now been obtained from such hydrolysates, after performic acid oxidation to convert any pendant aldehyde-groups into carboxy and so prevent further condensation, and purified by successive chromatography on Dowex 50 WX8, Sephadex G25, and CM-52 cellulose. This material (λ_{max} 263 nm, inflection at 269 nm, in H_2O at pH 5) showed in its 100 MHz

^{1}H n.m.r. spectrum three signals [δ(CF_3·CO_2D) 9·24 (s), 9·00 (br. d), and 8·3 (br. t); relative areas 1 : 2 : 1] strongly indicative of a 1,3-disubstituted pyridinium compound. A chromatographically and spectroscopically indistinguishable product was obtained more readily by the acid hydrolysis of the product obtained from α-acetyl-lysine, attached to 3-aminopropyltriethoxysilane-treated glass beads, and glutaraldehyde. This compound we have named 'anabilysine,' since it is derived from two lysine residues and contains the anabasine skeleton (see below).





The ^{1}H n.m.r. spectrum and the formation of both a mono- and a di-*N*-benzyloxycarbonyl derivative by reaction with benzyl chloroformate were in accord with structure (II) for anabilysine. The ^{13}C noise-decoupled n.m.r. spectrum in D_2O gave strong support to this structure, showing separate peaks for 17 of the 22 carbon atoms in (II); the two carbonyl carbons and the two lysine α-carbons each gave only one peak and the six (β-, γ-, and δ-) lysine side-chain carbons two doublets (at −26·5 and −27·5 p.p.m. from MeOH) and a singlet (at −27·8 p.p.m.).

Structure (II) was confirmed by synthesis. Reaction of anabasine (III) and 5-(4-bromobutyl)hydantoin (IV) in refluxing methanol, followed by acid hydrolysis, gave a product with a satisfactory elemental analysis, chromatographically indistinguishable from anabilysine and differing spectroscopically only in that the lysine side-chain carbons in this mixture of diastereoisomerides gave four singlets (at −26·1, −26·3, −27·1, and −27·5 p.p.m.) in place of the two doublets (at −26·5 and −27·5 p.p.m.) referred to above.

Anabilysine is the first cross-linking entity to be isolated from glutaraldehyde-treated proteins and its isolation affords *prima facie* evidence for the presence of cross-linkages of type (V), which could easily arise by internal oxidation–reduction of the isomeric cross-linkages (VI) derived from two lysine side-chains and two molecules of glutaraldehyde. It is not claimed that cross-linkages of type (V) are the only ones present in glutaraldehyde-treated proteins. As in the case of elastin, where desmosine and isodesmosine cross-linkages are accompanied by others involving reduced pyridine residues,[2] it is likely that they are accompanied by others, *e.g.* (VI), based on the same 2,3′-bipyridyl skeleton in different oxidation states. Furthermore, the intensity of absorption at *ca.* 265 nm in glutaraldehyde-treated proteins is considerably higher than would result if every modified lysine side-chain were converted into a pyridinium residue; the discrepancy would be fully accounted for by the presence of some of the more intensely absorbing 2,3′-bipyridinium analogue of (V).

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[1] P. M. Hardy, G. J. Hughes, and H. N. Rydon, *J.C.S. Chem. Comm.*, 1976, 157.
[2] M. A. Paz, P. M. Gallop, O. O. Blumenfeld, E. Henson, and S. Seifter, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 289.