Synthesis and Stability of *N*-Nitrosodipeptides

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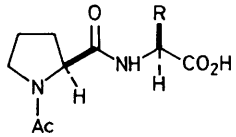
The synthesis, characterization, and chemical properties of the *N*-nitroso derivatives of some *N*-(*N*-acetylprolyl)-peptides are described.

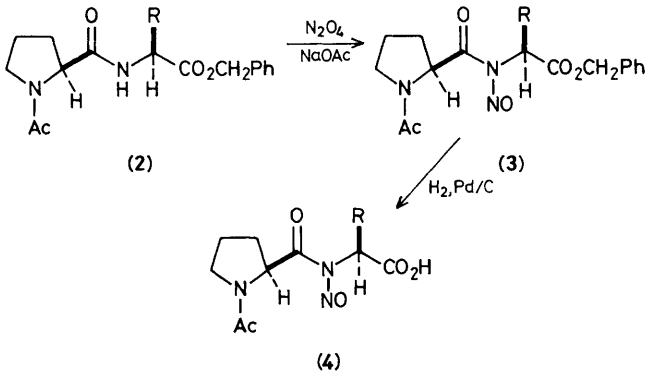
The formation of *N*-nitroso compounds in the stomach has been cited as a potential factor in human cancer,[1] and recent measurements show that human gastric aspirates contain small amounts of these substances.[2,3] With one exception,[4] their structure is unknown, but they may well derive from proteins and peptides, the commonest dietary *N*-compounds. It is known that terminal primary amino groups of proteins and peptides react with nitrous acid to give diazo-products.[5,6] Evidence for the nitrosation of the peptide *N*-atom to give an *N*-nitroso product, however, has proven elusive. Bonnett and his colleagues found only tentative ^{15}N n.m.r. evidence for *N*-nitrosodipeptides,[7] but they were able to isolate the *N*-nitroso derivatives of some *N*-acetyl-α-amino acid esters.[5,8] Chow and Polo[9] also prepared some *N*-acyl-*N*-nitroso-α-amino acids in solution, which apparently decomposed rapidly in the presence of base.

We now report the first synthesis, isolation, and characterization of authentic *N*-nitrosopeptides together with a resumé of their stability and chemical properties. To avoid concurrent deamination of a primary amino group, the initial studies have concerned *N*-acetylprolylpeptides (**1a**—**c**). These compounds are suitable models for proteins.

The synthesis is summarised in Scheme 1 and exemplified by the procedure for *N*-(*N*-acetyl-L-prolyl)-*N*-nitrosoglycine (**4a**). Thus, benzyl *N*-(*N*-acetyl-L-prolyl)glycinate (**2a**) in CH_2Cl_2 was treated with gaseous N_2O_4 following White's procedure[10] to give the *N*-nitroso derivative (**3a**) in quantitative yield as a yellow oil with the expected u.v., i.r. and ^1H n.m.r. spectra. Hydrogenolysis of (**3a**) in EtOH at ambient temperature and pressure over 5% Pd on charcoal produced a yellow solid in 43% yield, which on recrystallization from ethyl acetate gave *N*-(*N*-acetyl-L-prolyl)-*N*-nitrosoglycine, (**4a**),† m.p. 110 °C; $\lambda_{max.}$ (EtOH) 238, 389, 403, and 426 nm (log ε 3.76, 1.79, 1.97, and 1.98); $\nu_{max.}$ (Nujol) 3200—2300,



(**1**)
a; R = H, **b**; R = Me, **c**; R = CH_2Ph



(**2**) (**3**) (**4**)

Scheme 1

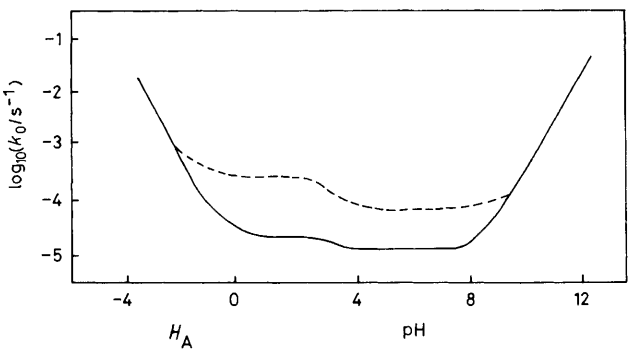
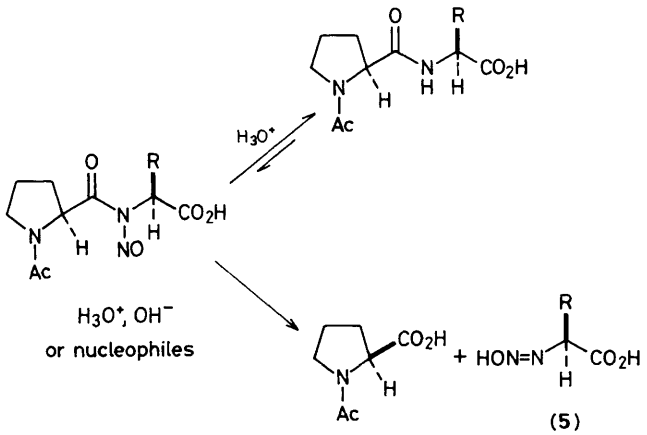


Figure 1. Rate–pH profile for the decomposition of (**4a**) (solid line) and (**4b**) (dotted line) in aqueous media at 25 °C.

† Satisfactory elemental analyses were obtained.

1740, 1590, and 1510 cm^{-1}; δ_H [60 MHz, $(CD_3)_2CO$]2.10 (3H, s), 1.7—2.4 (4H, m), 3.5—3.9 (2H, m), 4.6 (2H, s), and 5.5—5.9 (1H, br. m). the L-alanine derivatives (**4b**), m.p. 109 °C, and (**4c**), m.p. 177 °C, were prepared similarly and showed spectroscopic properties consistent with the proposed structures.

The stabilities of compounds (**4a—c**) in aqueous media were monitored spectrophotometrically at λ 238 or 405 nm. The reactions showed excellent first-order behaviour over 3—4 half-lives and pseudo-first-order rate coefficients {rate = k_o[(**4**)]} were determined from the integrated rate expression. The variation of k_o with pH (or H_A where appropriate) for (**4a**) and (**4b**) at 25 °C is summarised in Figure 1. Rapid decomposition is apparent only in strong acid or alkali. In strong acid, denitrosation and hydrolytic deamination proceed concurrently (Scheme 2) as observed previously for *N*-nitrosamides.[11] Denitrosation is the more strongly acid-catalysed pathway which becomes predominant at high acidity. Above pH 1, however, only deamination is important, and this pathway is catalysed by both HO^- (Figure 1) and added nucleophiles (*e.g.* thiols or amines). The diazotic acid [(**5**), Scheme 2] is a metastable intermediate and its formation was demonstrated by alkylation (by $-CHRCO_2H$) of both 4-(*p*-nitrobenzyl)pyridine and benzenethiol. Over the pH range 1—8 at 25 °C, decomposition occurs slowly with $t_{\frac{1}{2}}$ *ca.* 10 h for (**4a**) and 3 h for (**4b**). At pH *ca.* 3.5 an inflexion related to



Scheme 2

ionization of the terminal carboxylic acid is apparent (Figure 1). In aqueous solution the free acid is more labile than the carboxylate ion, and there is no evidence of the rapid intramolecular decomposition of the carboxylate ion reported by Chow and Polo[9] for *N*-acyl-*N*-nitroso-α-amino acids in organic solvents.

The results suggest that *N*-nitrosopeptides are sufficiently stable for their absorption through the stomach and the duodenum. On decomposition, they generate an alkylating agent (**5**) which is characteristic of many chemical carcinogens.[12]

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