Photochemistry of Aliphatic Dipeptides in Aqueous Solution

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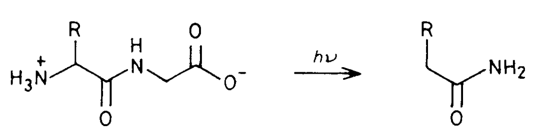
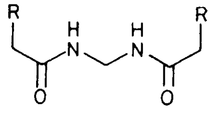
U.v. irradiation of aqueous solutions of glycylglycine or alanylglycine in the absence of oxygen leads to quantitative N-terminal deamination, and the formation of acetamide or propionamide, respectively, in high chemical yield.

The photochemistry of small peptides has been a popular model in the investigation of the effect of u.v. radiation on proteins.[1] Although most attention has been given to dipeptides containing aromatic groups, residues with saturated side chains are more numerous in proteins and this, together with the possible involvement of peptide bonds following absorption elsewhere,[2] indicates the potential importance of photochemical studies with simple aliphatic peptides. There have been several reports of such studies, but most of the conclusions are based on the spectroscopic observation of transient species[3] or on otherwise wholly qualitative data.[4] We have isolated and obtained chemical yields of products from the photolysis of glycylglycine and alanylglycine in degassed aqueous solution, and the results suggest that a major photochemical pathway that may be widely applicable to peptides and proteins has previously escaped detection.

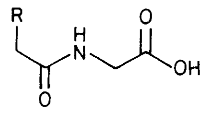
In a typical experiment a 0.03 M dipeptide solution was irradiated under nitrogen using a 400 W medium-pressure



(1)



(2)

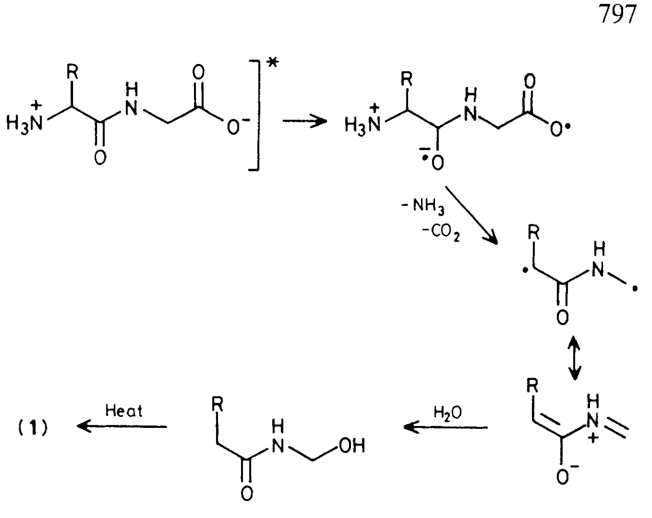


(3)

mercury arc with a quartz water-cooling jacket, until 50% of the peptide had reacted. The formation of amide (**1**, R = H or Me) was monitored by g.c. (packing: Chromosorb 101; temp. 215 °C) and h.p.l.c. (packing: Spherisorb 5µm ODS-2; eluant: 7×10^{-4} M sodium octanesulphonate in 5×10^{-3} M KH_2PO_4, pH 2.6 with H_3PO_4). Amide yields obtained using g.c. were consistently higher than those from h.p.l.c. measurements suggesting the presence of a thermally labile intermediate. Photolysed solutions were therefore heated under reflux for 1.5 h (under these conditions, the extent of hydrolysis of the remaining dipeptide is very small) prior to further work-up, which involved continuous liquid–liquid extraction with dichloromethane, followed by silica gel column chromatography of the organic layer and ion exchange chromatography [Dowex 50W-X8(H^+)] of the aqueous layer. In the case of glycylglycine, the silica gel column chromatography was preceded by removal of crystalline acetamide from the solvent-free residue by trituration with cold dichloromethane.

Yields of amide from glycylglycine and alanylglycine after 50% conversion were 53 and 47%, respectively. These high values are especially noteworthy in the light of previous reports which do not include primary amides among the products observed in the photolysis of aliphatic dipeptides.[3—7] The yields suggest, moreover, that a particularly favourable pathway is available for photo-initiated degradation, involving N-terminal deamination and NH–CH_2 bond cleavage. The former was confirmed by an increase in the pH of solutions by 1.8 units over the course of the irradiation, and by strong positive reactions when they were treated with Nessler's reagent. Colorimetric analysis[8] in the case of glycylglycine after 13% conversion showed the yield of ammonia to be 97%. Carbon dioxide was also observed by g.c. and mass spectroscopy to be a major component of the volatile products,[6] while bis(amido)methanes (**2**) and *N*-acylglycines (**3**) were found among the minor products isolated from the organic and aqueous fractions, respectively.

Although some photolytic terminal deamination of aliphatic dipeptides has been reported previously,[4a,5a] Johns found no evidence for it in the solid state,[6,7] and flash photolysis[3a] and recent spin trapping e.s.r. studies[5b] of photolysed aqueous solutions in the absence and presence of oxygen, respectively, detected only decarboxylation radicals at low pH and, in the e.s.r. study, secondary deamination radicals at high pH. Terminal deamination has been established, however, as the initial step in the reaction of electrons with aliphatic oligopeptides in aqueous solution,[9] so the relatively specific photodegradations we have observed could well be initiated by an analogous process involving photoinduced intramolecular electron transfer from carboxylate to peptide carbonyl (Scheme 1). Such mechanisms are of considerable interest in the context of peptide chain mediated charge migration discussed by several authors in recent years.[10]



Scheme 1

There has been frequent emphasis in reports of peptide and protein photochemistry on the importance of α-carbon radicals,[1] but most of the evidence arises from observing these radicals either directly, or *via* spin-trapping, using e.s.r. spectroscopy.[11] It is difficult to explain the present results in terms of α-carbon radicals, and it seems likely that an important process not involving these intermediates has escaped detection.

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