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DEHYDROPEPTIDE PRODUCTION IN THE RADIOLYSIS OF SOLID PEPTIDES: A RADIOLYTIC SYNTHESIS OF PYRAZINE DERIVATIVES FROM CYCLIC DIPEPTIDES

Mathilde Kland-English and Warren M. Garrison

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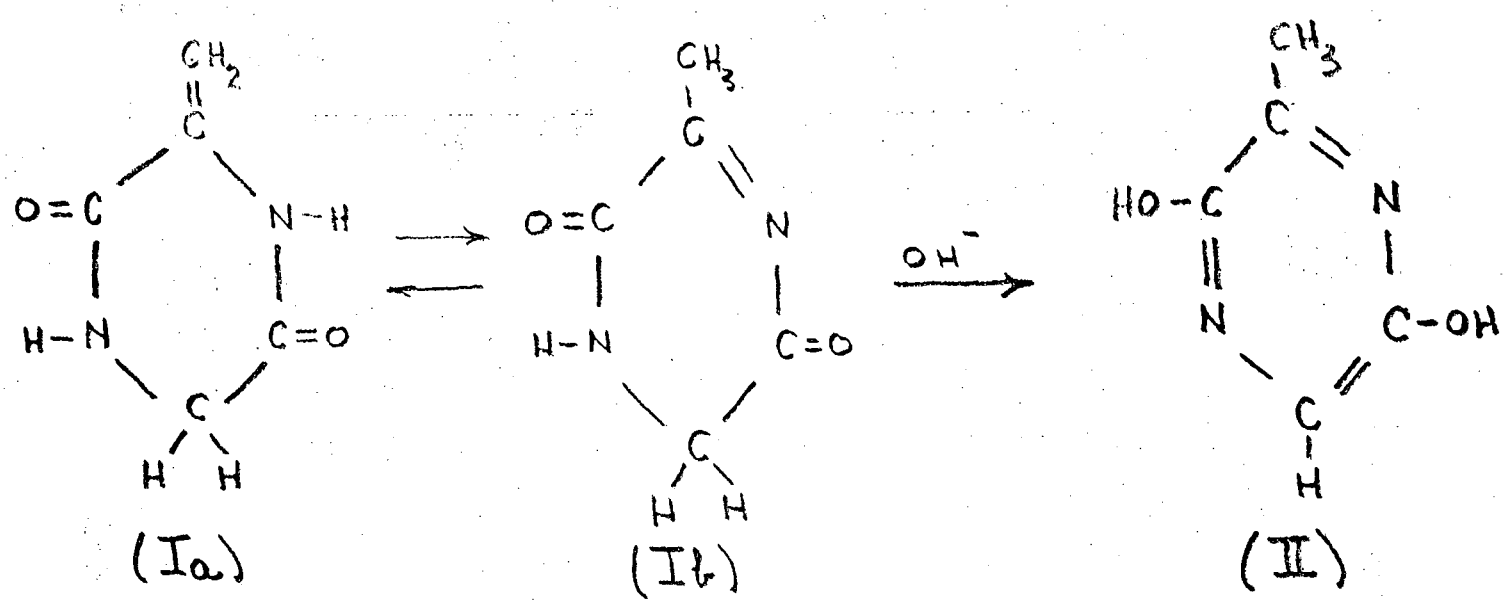
Among the chemical changes observed on radiolysis of compounds containing the peptide bond are those attributed[1-3] to the production of unsaturated linkages which may be represented in terms of the tautomeric structures, $\sim CON{=}C(CH_2R)\sim \rightleftarrows \sim CONH\text{-}C({=}CHR)\sim$. Compounds containing this type of chemical function have been designated dehydropeptides; such compounds are stable in water yet are readily hydrolyzed to yield ammonia and a carbonyl product on heating in dilute mineral acid, $\sim CON{=}C(CH_2R) + 2H_2O \rightarrow \sim COOH + NH_3 + RCH_2CO\sim$.[4] The experimental evidence for production of dehydropeptides in the radiolysis of proteins and simpler peptide sy tems has recently been reviewed.[5] Identification has been based primarily on studies of the formation of ammonia and carbonyl function during hydrolysis of the irradiated peptide system. For example, the hydrolytic production of ammonia and pyruvic acid from γ-irradiated acetylalanine (solid, evacuated) has been found to parallel very closely the hydrolytic degradation of authentic acetyldehydroalanine.

The cyclic dipeptides, such as glycine anhydride and alanine anhydride also show an analogous post-irradiation production of ammonia and α-keto acid during acid hydrolysis.[6] We note too that the dehydropeptide derivatives of these cyclic compounds represent isomeric forms of the 2,5-dihydroxypyrazines. Quite recently, Sorkin[7] has given evidence that the dehydro derivative of glycylalanine anhydride, that is, 3-methylene-2,5-diketopiperazine (prepared

by the classical chemical method of Bergman and Miekely[8]), undergoes isomerization in alkaline solution to give 3-methyl-2,5-dihydroxypyrazine. We report here analogous evidence for the formation of pyrazine derivatives through γ-radiolysis of the amino acid anhydrides followed by dissolution of the irradiated solids in alkaline solution.

Glycylalanine anhydride was synthesized after the method of Fischer and Scheibler.[9] Glycine anhydride and alanine anhydride were obtained from the Nutritional Biochemicals Corp. The anhydrides were recrystallized three times from water, dried, and ground to a microcrystalline powder. Samples were evacuated on the vacuum line in pyrex ampoules for a minimum of 48 hours. After irradiation (Co^{60} γ-rays, 1.7×10^{18} ev/gm/min), the ampoules were opened to the atmosphere and the contents were dissolved to a concentration of 0.1 M in 0.02 N sodium hydroxide under nitrogen aeration. Complete dissolution required two to three minutes. Changes in the absorption spectra of the oxygen-free solutions were followed differentially (against a 0.1 M solution of unirradiated anhydride) over the range 300 mμ to 400 mμ.

Solutions of irradiated glycylalanine anhydride (3-methyl-2,5-diketopiperazine) show a rapid increase in optical density with time over the 300-400 mμ range with λ_{max} = 370 mμ. The spectrum is completely developed after about two hours. Authentic 3-methylene-2,5-diketopiperazine[8] was found to exhibit almost identical spectrophotometric properties. The fully developed spectra obtained with irradiated glycylalanine anhydride and authentic 3-methylene-2,5-diketopiperazine in oxygen-free .02 N sodium hydroxide solution are shown in Fig. 1. Appearance of the absorption maximum at 370 mμ is attributed to the formation of the dihydroxypyrazine nucleus (II) via the isomerization reaction:



The absorption spectrum of an authentic dihydroxypyrazine (3,6-dimethyl) prepared after Karmas and Spoerri[10] is included in Fig. 1 for purposes of comparison. It is noted that the dihydroxypyrazines in alkaline solution are rapidly destroyed on exposure to air.

Isolation of 3-methylene-2,5-diketopiperazine (Ia,Ib) from irradiated glycylalanine anhydride has been accomplished. A methanolic extract of the irradiated solid was transferred to filter paper and chromatographed in parallel with authentic 3-methylene-2,5-diketopiperazine; butanol saturated with water was used as the developing liquid. The authentic methylene derivative is readily detected at $R_f \simeq 0.4$ under ultraviolet illumination; the irradiated glycylalanine anhydride also gives a strongly absorbing component at this R_f value. The two areas were separately eluted and examined spectrophotometrically in oxygen-free 0.02 N sodium hydroxide. The characteristic growth in the 300 to 400 mμ region was observed in both cases and the final curves were essentially identical to those given in Fig. 1. The yield of 3-methylene-2,5-diketopiperazine from glycylalanine anhydride at the lowest dosage studied (5×10^{20} ev/gm) corresponds to a G value of ~ 0.55.

The results obtained with alanine anhydride appear to be somewhat more complicated. In this case the development of the absorption curve is accompanied by a gradual shift in λ_{max} from 370 mμ to 360 mμ. However, the spectra obtained within 15 minutes of the time of dissolution coincide with that given by authentic 2,5-dihydroxy-3,6-dimenthylpyrazine.

Solutions prepared from irradiated glycine anhydride show the characteristic dihydroxyprazine spectrum and on standing there is no detectable shift in λ_{max} from 370 mμ to shorter wavelengths. However, glycine anhydride differs from both glycylalanine and alanine anhydrides in that the absorption spectrum shown in Fig. 1 is fully developed within the two to three minute period required for dissolution. This behavior may be interpreted as evidence for rapid isomerization of dehydroglycine anhydride which must necessarily have only the endocyclic form as given by structure Ib.

Finally we should like to comment on the fact that all three solid anhydrides develop a yellow coloration which is visually detectable at radiation dosages as low as $\sim 5 \times 10^{19}$ ev/gm. The effect is most pronounced in the case of glycine anhydride. Coloration is not appreciably diminished by exposure to oxygen and remains visible in water extracts of the irradiated solids. This coloration then, cannot be ascribed to an optical absorption by free radicals or ionic transients. It is noted that the authentic dihydroxypyrazines are yellow in the solid state and, although we have made only preliminary spectrophotometric studies of the irradiated solid anhydrides, the available evidence suggests that isomerization of dehydropeptides occurs to some extent in the solid state. A mechanism for the radiation-induced isomerization of the ~CO-NH~ linkage in solid peptide systems has recently been described.[5] A detailed report of the various aspects of the radiation chemistry of the cyclic dipeptides will be forthcoming.

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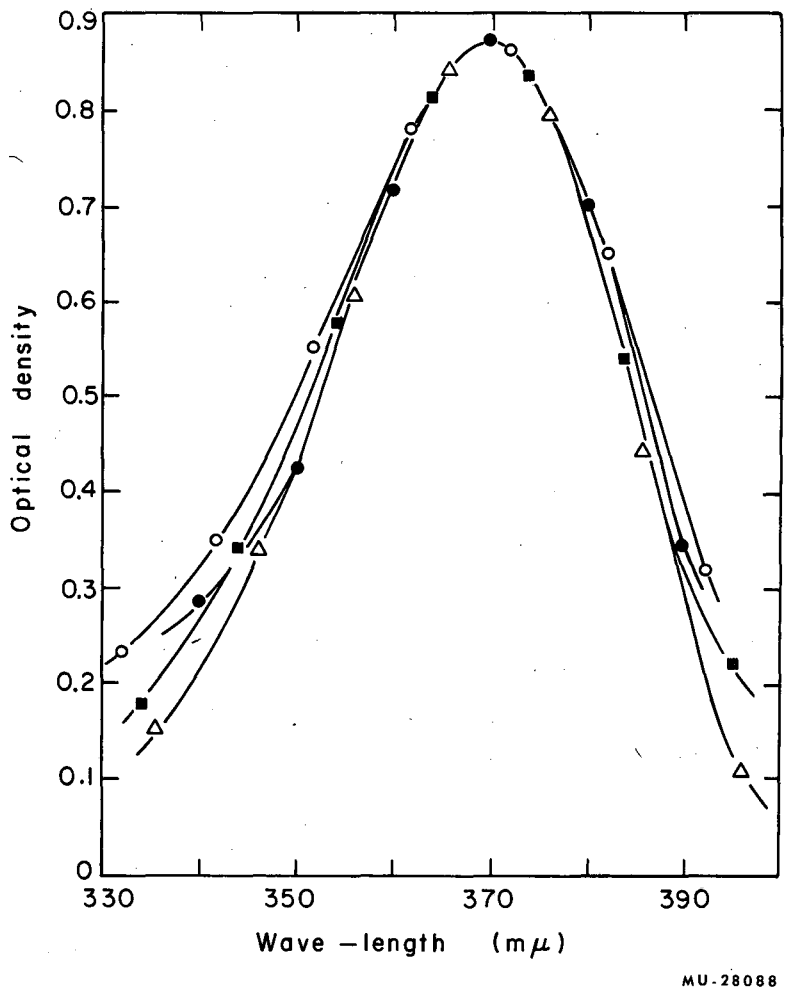

Fig. 1. Absorption spectra of irradiated cyclic dipeptides and authenic dehydropeptides in .02N sodium hyroxide O, irradiated glycine anhydride; □ authenic 3-methylene-2,5-diketopiperazine; ● authentic 2,5-dihydroxy-3,6-dimethylpyrazine.

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