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CHEMICAL EVIDENCE FOR MAIN-CHAIN SCISSION AS A MAJOR DECOMPOSITION MODE IN THE RADIOLYSIS OF SOLID PEPTIDES

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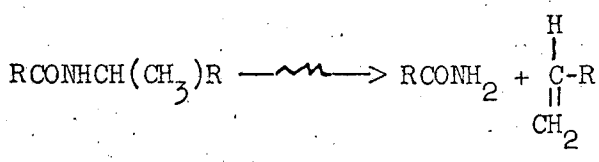
We find that a major chemical effect of ionizing radiation on peptide derivatives of the aliphatic α-amino acids is the formation of labile "amide-like" products which are readily degraded to yield ammonia on mild hydrolysis. The N-acetyl derivatives of alanine, valine and leucine, for example, give $G(NH_3)$ values of 3.8, 3.0 and 2.7 (molecules/100 ev) respectively on irradiation with Co^{60} γ-rays in vacuo; the irradiated solids were dissolved in water in the absence of oxygen and hydrolyzed for 90 minutes in 2 N HCl to effect a quantitative liberation of ammonia which was then assayed after the method of Conway.[1]

A detailed study of other reaction products from N-acetylalanine has been made to obtain evidence as to the mechanism of the radiation-induced degradation. Organic acids were assayed by gas-phase chromatography[2] and by partition chromatography.[3,4] In certain runs, the acetylalanine was "spiked" with $CH_3CONHCH(CH_3)C^{14}OOH$ to facilitate product identification; the radiometric techniques have been described.[4] Carbonyl products were determined as the 2, 4-dinitrophenylhydrazone derivatives.[4,5] A colorimetric method[6] was used to set a limit on the yield of lactic acid. Gaseous products were pumped-off following

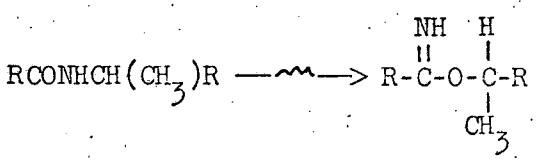
complete dissolution of an irradiated sample in degassed water on the vacuum line. Gases were analyzed by mass spectrometry. A Varian EPR spectrometer, model E-3, was used to measure the production of spin centers in the irradiated solids.

The evidence is that the observed radiolytic degradation of acetylalanine cannot be represented simply in terms of the rearrangement



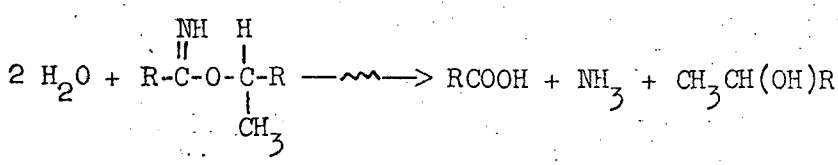
(1)

since we find G(acrylic acid) < 0.2. The possibility that the N, O shift



(2)

occurs in high yield and leads to ammonia formation through subsequent hydrolysis of the labile imino ester



(3)

is negated by the fact that G(lactic acid) $\leq$ 0.2. Similarly, we conclude that the production of dehydropeptide via

$$RCONHCH(CH_3)R \rightsquigarrow R\text{-}\overset{\overset{O}{\|}}{C}\text{-}N{=}\underset{\underset{CH_3}{|}}{C}\text{-}R + H_2(2H) \qquad (4)$$

is relatively unimportant in view of the fact that we find G(pyruvic acid) $\simeq$ 0.4; the dehydropeptides are hydrolyzed quantitatively to yield carbonyl and ammonia

$$2\ H_2O + R\text{-}\overset{\overset{O}{\|}}{C}\text{-}N{=}\underset{\underset{CH_3}{|}}{C}\text{-}R \longrightarrow RCOOH + NH_3 + CH_3COR \qquad (5)$$

under the conditions of hydrolysis employed here.[7] Additional evidence that the dehydrogenation reaction 4 occurs in low yield is given by the gas-yield data which show $G(H_2) = 0.4$.[8]

We do find that propionic acid is produced as a major product, G(propionic acid) $\simeq$ 1.2. It is clear that main-chain scission occurs and the simplest formulation in accord with present observations is given by:

$$RCONHCH(CH_3)R \rightsquigarrow RC\dot{O}NH + \dot{C}H(CH_3)R \qquad (6)$$

followed by the abstraction reactions

$$RC\dot{O}NH + RCONHCH(CH_3)R \longrightarrow RCONH_2 + RCONH\dot{C}(CH_3)R \qquad (7)$$

$$\dot{C}H(CH_3)R + RCONHCH(CH_3)R \longrightarrow CH_2(CH_3)R + RCONH\dot{C}(CH_3)R \qquad (8)$$

to give amide, fatty acid and the long-lived α-carbon radicals which have been observed in irradiated peptides at room temperatures through EPR spectroscopy.[9,10] On dissolution in water (oxygen-free), the α-carbon radicals, $RCONH\dot{C}(CH_3)R$, undergo dimerization to yield the α, α-diaminosuccinic acid derivative.[11] The formulation of reactions 6, 7, 8 is intended only to convey the nature of the overall reaction stoichiometry.[12] Ionic or "hot" radical intermediates presumably are involved since cage effects in the condensed medium would lead to preferential radical-recombination.

We estimate from EPR measurements that the yield of the long-lived α-carbon radicals is roughly G ~ 3. This value and the propionic acid yield value of G=1.2 are somewhat lower than would be predicted from the reaction sequence 6, 7, 8 on the basis of G(amide)~3.8. The apparent discrepancy may be understood if a fraction of the $\dot{C}H(CH_3)R$ radicals are removed through radical-radical interactions in the spur-regions of high radical concentration. That the "amide" type of radiolytic degradation of the peptide chain is not confined to the N-acyl-amino acid configuration is shown by the fact that the G values for ammonia and propionic acid from poly-DL-alanine are almost identical with those obtained with N-acetylalanine.

It has been suggested that the long-lived α-carbon radicals are produced in peptide radiolysis through side-chain cleavage,[13,14] e.g.,

$$RCONHCH(CH_3)R \longrightarrow RCONH\dot{C}(CH_3)R + H \qquad (9)$$

followed by

$$H + RCONHCH(CH_3)R \longrightarrow RCONH\dot{C}(CH_3)R + H_2 \qquad (10)$$

where H formed in reaction 9 may have excess kinetic energy. However the low $G(H_2)$ values observed in the γ-radiolysis of simple peptides, polypeptides and protein,[8] does not support the concept that reaction 10 represents a major source of α-carbon radicals. On the other hand, reaction 9 followed by

$$H + RCONHCH(CH_3)R \longrightarrow RCONH_2 + \dot{C}H(CH_3)R \qquad (11)$$

(or the equivalent heterolytic steps involving e^- and H^+) would satisfy the requirement of main-chain scission. Note that the stoichiometry of reactions 9, 11 followed by 8 is equivalent to that given by the reaction sequence 6, 7, 8.

Detailed reports of these studies will appear in forthcoming publications.

Acknowledgement is due to Miss Sibyl A. Cole for assistance in some of the measurements.

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(12) The propionic acid yield from acetylalanine is directly proportional to dosage up to 10^{21} ev/gm and the dose-yield plot extrapolates through the origin. We conclude from this that propionic acid is not produced through the reduction by e^- or H of acrylic acid formed in reactions akin to 1. However we cannot rule out the possibility that acrylic acid "steady-states"

at a concentration level below that detectable by our present analytical techniques.

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