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RADIATION-INDUCED REACTIONS OF AMINO ACIDS AND PEPTIDES*

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March 1971

ABSTRACT

Reaction mechanisms in the radiolysis of amino acids, peptides and related compounds are reviewed. Information on aqueous and solid state systems is included. In aqueous solutions the reactions induced by attack of OH, H and e^-_{aq} on these several classes of biochemical compounds are formulated along with a description of the effects of oxygen and other second solutes. Evidence for the role of excited-molecule reactions in the radiolysis of peptides in concentrated aqueous solutions is presented. Specific roles of ionization and excitation in the radiolysis of amino acids and peptides in the solid state are formulated.

During the past few years there has been a very marked increase in the amount of work being done on the radiation chemistry of bio-organic compounds both in aqueous solution and in the solid state. We cannot, of course, review this entire field in the space alloted us here. However, one specific area of such research that our group at Berkeley has been particularly interested in is the chemistry of N-C bond cleavage in the radiolysis of amino acids, peptides, and related compounds. A variety of reaction modes have been established at our laboratory and elsewhere and these studies are the subject of the present review.

The radiolytic deamination of the simpler α-amino acids glycine and alanine in oxygen-free solutions was observed some twenty years ago by Stein and Weiss[45] and by Dale and co-workers.[12] These early X-ray studies were limited primarily to the determination of ammonia yields which for both glycine and alanine approach $G(NH_3) \simeq 5$ at the higher solute concentrations. Subsequent work by Sharpless et al.[43] and by Weeks and Garrison[48] established the over-all reaction stoichiometry. Major product yields in the γ-radiolysis of glycine and alanine in oxygen-free solution are summarized in Table I.

Chemistry in these aqueous systems is initiated by the radiation-induced decomposition of water[3,11,23]

$$H_2O \xrightarrow{\sim\sim} H_2O_2,\ H_2,\ OH,\ H,\ e^-_{aq},\ H^+ \qquad (1)$$

where $G_{OH} = 2.74$, $G_{e^-_{aq}} = 2.76$, $G_H = 0.55$, $G_{H_2} = 0.40$, $G_{H_2O_2} = 1.00$.[5] The magnitude of the $G(NH_3)$ valves observed in Table I and the fact that both fatty acid and keto acid are observed as major organic products indicate that deamination by both e^-_{aq} and OH occurs. To separately evaluate the processes

chemically, it is convenient to add a second solute which preferentially scavenges OH (and H) but at the same time is relatively unreactive toward e^-_{aq}. Formate ion is such a scavenger in that the rate constants[22] for the reactions

$$OH + HCOO^- \longrightarrow H_2O + COO^- \quad (2)$$

$$H + HCOO^- \longrightarrow H_2 + COO^- \quad (3)$$

are $1 \times 10^9\ M^{-1}\ sec^{-1}$ and $3 \times 10^8\ M^{-1}\ sec^{-1}$ respectively, whereas the rate of

$$e^-_{aq} + HCOO \longrightarrow HCO_2^= \quad (4)$$

is $< 10^6\ M^{-1}\ sec^{-1}$. The effect of added formate on $G(NH_3)$ from oxygen-free solutions of glycine and alanine is shown in Fig. 1.[46] It is seen that $G(NH_3)$ in both cases drops rapidly with increasing formate concentration and then levels off and becomes essentially independent of the concentration of the radical scavenger. The keto acid yield drops essentially to zero with the drop in $G(NH_3)$ while the fatty acid yield is unaffected by formate ion even at high concentrations as shown in Fig. 2. If now, increasing concentrations of an electron scavenger such as chloracetate ion

$$e^- + ClCH_2COO^- \longrightarrow Cl^- + CH_2COO^- \quad (5)$$

are added at the higher formate concentrations, then the yield of fatty acid decreases to zero. The evidence is then that a major fraction of the hydrated electrons react with these amino acids according to the stoichiometry

$$e^- + NH_3^+CHRCOO^- \longrightarrow NH_3 + \dot{C}HRCOO^- \qquad (6)$$

We see in Table I that $G(H_2)$ from neutral glycine solutions is appreciably greater than the sum $G_H + G_{H_2}$. This is accounted for in terms of the branching reaction[46]

$$e^- + NH_3^+CHRCOO^- \longrightarrow NH_2CHRCOO^- + H \qquad (7)$$

Reaction 7 is analogous to the conversion of e^-_{aq} to H by NH_4^+ as observed by Jortner et al.[27] Both OH and H react with glycine and alanine at the α-carbon position[46]

$$OH + NH_3^+CHRCOO^- \longrightarrow NH_3\dot{C}RCOO^- + H_2O \qquad (8)$$

$$H + NH_3^+CHRCOO^- \longrightarrow NH_3\dot{C}RCOO^- + H_2 \qquad (9)$$

Subsequent reactions include

$$NH_3^+\dot{C}RCOO^- + \dot{C}RCOO^- \longrightarrow NH_2^+{=}CRCOO^- + RCHCOO^- \qquad (10)$$

$$2\ NH_3^+\dot{C}RCOO^- \longrightarrow NH_2^+{=}CRCOO^- + NH_4^+CRCOO^- \qquad (11)$$

$$H_2O + NH_2^+{=}CRCOO^- \longrightarrow NH_4^+ + RCOCOO^- \qquad (12)$$

A small fraction of the $NH_3^+\dot{C}RCOO^-$ and $\dot{C}RCOO^-$ radicals undergo dimerization to yield diaminosuccinic acid, aspartic acid, and succinic acid in low yield.[48] If the yield of the branching reaction is adjusted to conform to the observed hydrogen yields, then the above reaction scheme accounts both qualitatively and

quantitatively for the radiation chemistry of glycine and alanine in aqueous solution. Recent studies in which the radical products of reaction 6 are observed directly by means of optical spectroscopy[33] and esr spectroscopy[32,41,42] clearly substantiate the formulations derived in the chemical studies.

One might assume on the basis of the above formulations that reductive cleavage of the N-C bond represents a characteristic radiation-chemical reaction of amines generally. However, Riesz[36] has found no evidence for such reaction in the radiolysis of oxygen-free solutions of methyl ammonium ion and we have found that reductive deamination is unimportant in the radiolysis of β-amino acids in aqueous solution.[46] Apparently, the C=O function must be α to the N-C linkage. We have speculated on the possibility that e^-_{aq} adds to the C=O function of the α-amino acids and that dissociation of the reduced intermediate then ensues[46,50]

$$e^-_{aq} + NH_3^+CHRCOO^- \longrightarrow NH_3^+CHRC\begin{matrix} O^- \\ O^- \end{matrix} \longrightarrow NH_3 + \dot{C}HRCOO^- \qquad (13)$$

The recent esr studies by Sevilla[41] are in support of this formulation.

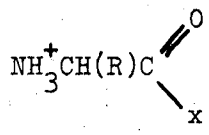
The simple oligo peptide derivatives of glycine and alanine also undergo reductive deamination by e^-_{aq}. It is of interest here to compare the ammonia yields from glycine and glycylglycine in oxygen-free solution under γ-rays. We have found[50] as shown in Fig. 3 that ammonia is produced as a major product in the γ-radiolysis of glycylglycine but the value is lower than with glycine. However, there is relatively little effect of added formate on $G(NH_3)$ from glycylglycine as shown in Fig. 4. Apparently almost all of the free ammonia from glycylglycine is produced via reductive deamination. In agreement with

this we find that addition of the electron scavenger, chloracetate ion, reduces $G(NH_3)$ essentially to zero as shown in Fig. 5. The reason that OH attack in this system does not yield ammonia is because such attack does not occur at the carbon position α to the NH_3^+ group but rather at the carbon position α to the peptide bond.[50]

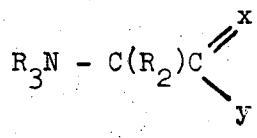
$$OH + NH_3^+CH_2CONHCH_2COO^- \longrightarrow H_2O + NH_3^+CH_2CONH\dot{C}HCOO^- \qquad (14)$$

The chemistry of the peptide radicals, $RCONH\dot{C}R_2$, is treated in a following section.

Reductive deamination is a general and characteristic reaction of electrons with compounds containing the grouping[50]



where x represents O^-, OH, OR, NHR, etc. Studies of Clay and Kabi[9] indicate that e^-_{aq} reacts with benzyldimethylamine cation and benzyltrimethyl ammonium ion to yield dimethylamine and trimethylamine respectively. This suggests that the reactive grouping in the general case corresponds to[50]



Many examples of the reductive cleavage of the N-C bond have recently been studied by Neta and Fessenden[32] and by Sevilla[41,42] using esr spectroscopy to observe directly the free radical intermediates.

The introduction of oxygen at sufficiently high relative concentrations results in a blocking of the reductive deamination reaction since the reducing species are preferentially scavenged to yield the hydroperoxy radical

$$e^-_{aq} + O_2 \longrightarrow O_2^- \tag{15}$$

$$O_2^- + H_2O \rightleftharpoons HO_2 + OH^- \tag{16}$$

The OH reaction is not inhibited by oxygen and in the case of glycine the α-carbon radicals formed via OH attack (reaction 8) react in turn with oxygen to yield ammonia and keto acid[16,47]

$$NH_3^+\dot{C}HCOO^- + O_2 + H_2O \longrightarrow NH_4^+ + HCOCOO^- + HO_2 \tag{17}$$

The intermediate processes involved in the over-all reaction 17 remain to be established. In any case, the major product stoichiometry in oxygenated solutions of glycine is approximated by $G(NH_3) \simeq G(\text{carbonyl}) \simeq G_{OH}$.

Although the OH reaction is localized at the α-carbon position in the case of glycine, it is clear that with the more complex amino acids other competing loci are available for reaction. For example, increasing the length of the aliphatic side-chain increases the number of C-H bonds susceptible to OH attack. Hence, the relative importance of oxidative deamination would be expected to decrease. This effect is shown in Fig. 6 where $G(NH_3)$ for a number of aliphatic amino acids is plotted as a function of the number of C-H bonds in the amino acid residue. Liebster and Kopoldova[28] have made extensive studies of the effects of side-chain substitution on the deamination yield.

Let us turn our attention now to some of the radiation chemical properties of the simpler α-amino acids in the solid state. Dale[12] in his early studies observed that the X-radiolysis of solid glycine <u>in vacuo</u> produces free ammonia with $G(NH_3) \simeq 5$. This is a remarkably high yield for the production of heavy fragments in the solid state and it seems quite unlikely that ammonia production involves a homolytic cleavage of the N-C bond via

$$NH_3^+CH(R)COO^- \xrightarrow{\sim} \dot{N}H_3^+ + \dot{C}H(R)COO^- \qquad (18)$$

since caging effects in the solid state would lead to a preferential recombination of the radical pair formed in Eq. (18). The finding that the hydrated electron, e^-_{aq}, reacts with the α-amino acids via reductive deamination prompted the suggestion[15] that reaction of secondary electrons in solid α-amino acid systems may also lead to deamination. Ionic processes in the solid state would then be represented by

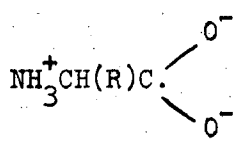
$$NH_3^+CH(R)COO^- \xrightarrow{\sim} NH_3^+\dot{C}(R)COO^- + H^+ + e^- \qquad (19)$$

$$e^- + NH_3^+CH(R)COO^- \longrightarrow NH_3 + \dot{C}H(R)COO^- \qquad (20)$$

The subsequent reactions of the radicals $NH_3^+\dot{C}(R)COO^-$ and $\dot{C}H(R)COO^-$ may occur via steps 10,11 in part in the solid and are completed along with reaction 12 on dissolution of the irradiated solid. This scheme leads to the stoichiometry $G(NH_3) \simeq G(\text{keto acid}) + G(\text{fatty acid}) \simeq 5$; $G(\text{keto acid}) \simeq G(\text{fatty acid}) \simeq 2.5$. Soon after this proposal was made it was shown by Meshitsuka <u>et al.</u>[30] that glyoxylic acid and acetic acid are indeed produced as major products along with ammonia in the γ-radiolysis of solid glycine. Data are summarized in Table II.

The simple oligopeptide derivatives of glycine and alanine also undergo reductive deamination on radiolysis in the solid state. Glycylglycine, for example, yields $G(NH_3) = G(\text{acetylglycine}) \simeq 3$.[4]

Recent results obtained by esr methods provide convincing physical evidence of the importance of reaction 20 in the radiolysis of the simpler α-amino acids in the solid state:[6,44] on irradiation at 77°K the initially observed radical has the electron located at the carboxyl group



and on warming to intermediate temperatures the initial radical dissociates to yield

$$NH_3 + \cdot CH(R)COO^-$$

The radiation chemistry of cysteine in aqueous solution is confined to the sulfur moiety; deamination is not observed.[1,13] A fairly detailed study of the effects of γ-rays on solid cysteine has recently been made.[34] Yields of major products are summarized in Table III. We see that ammonia is among the major products. The fact that carbonyl products are not liberated in any appreciable yield indicates that little of the observed ammonia, $G(NH_3) = 1.8$, arises through the formation of labile imino derivatives of the type $NH_2^+=C(CH_2SH)COO^-$. Apparently reaction akin to steps 10,11 of the "glycine" mechanism do not contribute to the radiation chemistry of solid cysteine.

The low carbonyl yields also show that the over-all stoichiometries*

$$RSH \xrightarrow{\sim} H_2 + NH_2^+{=}C(CH_2SH)COO^- \quad (21)$$

$$RSH \xrightarrow{\sim} H_2S + NH_2^+{=}C(CH_3)COO^- \quad (22)$$

are unimportant since the organic products of reaction 21,22 are imino acids and hydrolyze to yield carbonyl products (and ammonia) on dissolution of the solid.

The following reaction scheme appears to satisfy the experimental requirements of this system

$$RSH \xrightarrow{\sim} R\dot{S} + H^+ + e^- \quad (23)$$

$$RSH \xrightarrow{\sim} R\dot{S} + H \quad (24)$$

followed by

$$H + RSH \longrightarrow H_2 + R\dot{S} \quad (25)$$

$$\longrightarrow H_2S + NH_3^+CH(\dot{C}H_2)COO^- \quad (26)$$

$$e^- + RSH \longrightarrow NH_3 + \dot{C}H(CH_2SH)COO^- \quad (27)$$

$$e^- + RSH \longrightarrow H_2 + NH_2CH(CH_2\dot{S})COO^- \quad (28)$$

$$\longrightarrow H_2S + NH_2CH(\dot{C}H_2)COO^- \quad (29)$$

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*For purposes of simplicity we use the symbol RSH to represent the cysteine zwitterion $NH_3^+CH(CH_2SH)COO^-$.

and by the radical removal steps

$$RSH + \dot{C}H(CH_2SH)COO^- \longrightarrow R\dot{S} + CH_2(CH_2SH)COO^- \quad (30)$$

$$RSH + NH_2CH(\dot{C}H_2)COO^- \longrightarrow R\dot{S} + NH_2CH(CH_3)COO^- \quad (31)$$

$$2R\dot{S} \longrightarrow RSSR \text{ (cystine)} \quad (32)$$

We need not consider all of the detailed arguments for this particular mechanism. The main point here is that ammonia liberation from cysteine appears to be produced almost exclusively through the dissociation capture of e^- via step 27. The liberation of ammonia in this reaction yields the $\dot{C}H(CH_2SH)COO^-$ radical which abstracts H from cysteine to give β-mercaptopropionic acid which is a major component of the "NH_2-free" product fraction of Table III.

It is assumed that deamination via reaction 27 involves the addition of e^- to the C=O linkage of cysteine followed by dissociation of the N-C bond as in the reaction of e^- with glycine. Some support for this argument is to be found in the results obtained in the γ-radiolysis of solid cystamine. This compound which does not contain the C=O group as a trapping center for e^- does not yield ammonia in any appreciable yield as shown in Table IV.

The SH group of cysteine represents a competing trapping center for e^-. In fact, as we have noted, e^-_{aq} in aqueous solutions of cysteine reacts exclusively through dissociative attachment to yield $HS^-(H_2S)$.[1,13] On the other hand, recent studies of the radiation chemistry of thiophenol[29] and ethyl mercaptan[31] in the pure liquid state indicate that dissociative capture reactions of the type

$$RSH + e^- \longrightarrow R + HS^- \quad (33)$$

$$RSH + e^- \longrightarrow RS^- + H \quad (34)$$

do not occur in these relatively non-polar liquids. The evidence is that reactions 33,34 become exothermic only if solvation energies in a polar medium can be utilized in the over-all energetics. It is not clear that such factors are involved in reactions of e^- in a polar solid such as cysteine.

However, simple dissociative attachment of e^- need not necessarily be involved in steps 28,29. An alternate explanation is that e^- is captured by the sulfhydryl group to give RSH^- and that chemistry then ensues as a consequence of proton transfer from an adjacent NH_3^+ group. Some evidence for such concerted action is to be found in the fact that $G(H_2) + G(H_2S)$ from N-acetylcysteine is markedly lower than the corresponding values for cysteine as seen in Table IV.

Let us consider now some aspects of the radiation chemistry of peptides. The N-acylamino acids represent the simplest models for such study. Radiolysis of simple peptides such as N-acetylglycine and N-acetylalanine in dilute O_2-free solution does not lead to N-C bond cleavage in any appreciable yield in the absence of other solutes. However, in oxygenated solution the peptide chain is degraded with formation of amide-like products.[16,17]

The production of amide ammonia in the γ-radiolysis of N-acetylalanine in oxygenated solution is shown in Fig. 7 as a function of peptide concentration. The ammonia yield increases abruptly with increasing solute concentration and levels off at $G(NH_3) = 2.9 = G_{OH}$. In these dilute solutions the reducing species H and e^-_{aq} which are formed in the radiation-induced step 1 are

scavenged via

$$O_2 + e^-_{aq}(H) \longrightarrow O_2^-(HO_2) \tag{35}$$

where the products of reaction 35 are related by the equilibrium[10] $HO_2 \rightleftharpoons H^+ + O_2^-$. The OH radicals attack the peptide at the carbon position α to the NH function[2]

$$OH + RCONHCHR_2 \longrightarrow H_2O + RCONH\dot{C}R_2 \tag{36}$$

The peptide radicals $RCONH\dot{C}R_2$ are then scavenged by oxygen

$$O_2 + RCONH\dot{C}R_2 \longrightarrow RCONHC(\dot{O}_2)R_2 \tag{37}$$

In earlier work[16] it was suggested that the simplest scheme for subsequent chemistry involves

$$HO_2 + RCONHC(\dot{O}_2)R_2 \longrightarrow RCONHC(OOH)R_2 + O_2 \tag{38}$$

$$H_2O + RCONHC(OOH)R_2 \longrightarrow RCONHC(OH)R_2 + H_2O_2 \tag{39}$$

where the dehydropeptide derivative $RCONHC(OH)R_2$ is labile and yields ammonia and carbonyl on mild hydrolysis.

$$RCONHC(OH)R_2 \longrightarrow RCONH_2 + R_2CO \tag{40}$$

$$H_2O + RCONH_2 \longrightarrow RCOOH + NH_3 \tag{41}$$

If degradation of the peptide chain does occur predominantly through the scheme formulated in Eqs. (38-41), then it is clear that the ammonia and carbonyl

yields should be in the relationship $G(NH_3) = G(R_2CO) = 2.9 = G_{OH}$. However, the combined yield of carbonyl products, pyruvic acid and acetaldehyde, is quite low with $G(R_2CO) \simeq 0.4$ as seen in Fig. 7.

Further study[20] of the oxidation products from N-acetylalanine reveals that the principal nitrogen-free organic compounds produced in this system are acetic acid and carbon dioxide. Yield data are summarized in Table V. To account for the production of these oxidation products in the observed yield it has been proposed that the peptide peroxy radicals $RCONHC(\dot{O}_2)R_2$ are removed not through reaction 38 but rather via

$$2\ RCONHC(\dot{O}_2)R_2 \longrightarrow 2\ RCONHC(\dot{O})R_2 + O_2 \qquad (42)$$

In the case of N-acetylalanine reaction 42 is followed by

$$O_2 + RCONHC(O)(R)-C(OH)=O \longrightarrow RCONHCOR + CO_2 + HO_2 \qquad (43)$$

to yield the diacetamide derivative which is labile on mild hydrolysis

$$H_2O + RCONHCOR \longrightarrow RCONH_2 + RCOOH \qquad (44)$$

The HO_2 radicals formed in reactions 35 and 43 are removed via

$$2\ HO_2 \longrightarrow H_2O_2 + O_2 \qquad (45)$$

The sequence of reactions 1,35-37 followed by reactions 42-45 yield an over-all stoichiometry in good agreement with the N-acetylalanine data of Table V.

The similarity in the nature of the oxidation products derived from N-acetylalanine and polyalanine as observed in Table V indicates that the

scheme of reactions 1,35-37 and 42-45 also applies to the radiolytic oxidation of the polypeptide main-chain. With polyalanine (molecular weight 2000) we must assume that OH removal through reaction 36 occurs at random along the chain. The peroxy radicals $RCONHC(\dot{O}_2)R_2$ so formed are then removed as shown in reaction 42 to yield the alkoxy radical, $RCONHC(\dot{O})R_2$. We envisage the next step, i.e. the analogue of reaction 43, as involving the enol form of the adjacent peptide linkage

$$RCONH\underset{\underset{R}{|}}{\overset{\overset{\dot{O}}{|}}{C}}-\overset{\overset{OH}{|}}{C}=N-R + O_2 \longrightarrow RCONHCOR + O=C=N-R + HO_2 \qquad (46)$$

where

$$O=C=N-R + H_2O \longrightarrow CO_2 + RNH_2 \qquad (47)$$

follows essentially instantaneously.

Additional support for the argument that the OH radical attacks the peptide main-chain at the α-carbon position as formulated in Eq. (36) is to be found in studies in which FeIII is used in place of oxygen as the oxidizing solute.[2] Heavy metal ions such as FeIII and CuII oxidize organic free radicals in aqueous solution by electron transfer and by ligand transfer. Such reactions in the case of the peptide radical $RCONH\dot{C}R_2$ would correspond to

$$FeIII + RCONH\dot{C}R_2 \longrightarrow RCON=CR_2 + FeII + H^+ \qquad (48)$$

$$H_2O + FeIII + RCONH\dot{C}R_2 \longrightarrow RCONHC(OH)R_2 + FeII + H^+ \qquad (49)$$

The organic products of reactions 48 and 49 are dehydropeptide derivatives which readily decompose on mild hydrolysis

$$2\ H_2O + RCON{=}CR_2 \longrightarrow RCOOH + NH_3 + R_2CO \qquad (50)$$

$$H_2O + RCONHC(OH)R_2 \longrightarrow RCOOH + NH_3 + R_2CO \qquad (51)$$

The formation of ammonia and pyruvic acid in 0.1M N-acetylalanine solution containing FeIII is shown in Fig. 8. At the higher FeIII concentrations the reducing species e^-_{aq} and H are preferentially scavenged via

$$e^-_{aq} + FeIII \longrightarrow FeII \qquad (52)$$

$$H + FeIII \longrightarrow FeII + H^+ \qquad (53)$$

Under these conditions the yield for peptide oxidation via reaction 36 followed by reactions 48-49 is in accord with

$-G(\text{peptide}) = G(NH_3) = G(RCOCOOH) \simeq 3.2 = G_{OH} + G_{H_2O_2}$. Hydrogen peroxide formed in the radiation-induced step 1 reacts rapidly with FeII to give an additional yield of OH radicals

$$FeII + H_2O_2 \longrightarrow OH + OH^- + FeIII \qquad (54)$$

The maximum in the yield curve in Fig. 8 is attributed to the onset of the reaction

$$H + RCONHCHR_2 \longrightarrow RCONH\dot{C}R_2 + H_2 \qquad (55)$$

in competition with reaction 53 at the lower FeIII concentrations. We conclude then from these findings that both H and OH attack the peptide chain at the α position to yield the radicals $RCONH\dot{C}R_2$ which are then quantitatively oxidized by FeIII of shown in Eqs. (48,49).

Quite recently the pulse radiolysis technique has been successfully employed by Hayon and co-workers[24] in recording the absorption spectra of a number of peptide radicals of the type $RCONH\dot{C}R_2$.

Now, in the absence of oxidizing solutes such as O_2 and FeIII, the electron produced in reaction 1 adds to the peptide bond[25,26]

$$e^-_{aq} + RCONHCHR_2 + H_2O \longrightarrow R\dot{C}(OH)NHCHR_2 + OH^- \qquad (56)$$

There is, however, relatively little net reduction of the peptide linkage. The major path for removal of the reduced radical $R\dot{C}(OH)NHCHR_2$ appears to be the back reaction

$$R\dot{C}(OH)NHCHR_2 + RCONH\dot{C}R_2 \longrightarrow 2\ RCONHCHR_2 \qquad (57)$$

where $RCONH\dot{C}R_2$ represents the product of OH attack. It has been found[25,26] that certain labile organic compounds are effective at low concentrations in blocking the back-reaction 57 by virtue of the H-atom transfer reactions

$$R\dot{C}(OH)NHCHR_2 + RSH \longrightarrow RCH(OH)NHCHR_2 + RS \qquad (58)$$

$$RCH(OH)NHCHR_2 \longrightarrow RCHO + R_2CHNH_2$$

As noted in Table VI, acetaldehyde appears as a major product with $G(CH_3CHO) \simeq 2.5$ in solutions of N-ethylacetamide and acetamide containing the thiol, cysteine.

It is to be noted that the thiols, because of their marked reactivity, are ordinarily found to act as protective agents in the radiolysis of aqueous systems. Of interest, from both the chemical and biological standpoint, is the finding that RSH at low concentrations induces a very striking enhancement in the radiolytic lability of the peptide and amide linkages.

As outlined above, the back-reaction 57 represents the major path for radical removal in the radiolysis of simple peptides in oxygen-free solution. A small fraction of the $RCONH\dot{C}R_2$ radicals undergo further oxidation of the type[16]

$$2\ RCONH\dot{C}R_2 \longrightarrow RCON{=}CHR_2 + RCONHCHR_2 \qquad (59)$$

$$H_2O_2 + RCONH\dot{C}R_2 \longrightarrow RCONHC(OH)R_2 + OH \qquad (60)$$

where the H_2O_2 of reaction 60 is derived from the radiation-induced step 1. The oxidized products of reactions 59,60 are labile and readily decompose on mild hydrolysis as indicated in Eqs. (50,51) to give $G(NH_3) \simeq G(R_2CO) \simeq 0.4$.

Although the above reactions provide an explanation of the radiation chemistry of the simpler N-acetyl amino acids in dilute solution, other processes become of major importance at higher solute concentrations.[38,39] In the case of N-acetylalanine there is a very marked increase in the amide ammonia yield as the solute concentration is increased above 0.1M as shown in Fig. 9. We also see that the carbonyl yield is essentially independent of solute concentration over the range studied. Propionic acid is the principle concomitant product associated with the enhancement in the amide yield from N-acetylalanine.

The production of amide and fatty acid at the higher solute concentrations does not appear to be related in any significant way to the reactions of

OH, H, e^-_{aq}. We have found[38] that addition of second-solutes at concentrations sufficient to quantitatively scavenge the products of water radiolysis has relatively little effect on the amide yield in concentrated aqueous solution. The evidence is that a new reaction mode sets in at concentrations above 0.1M and that such reaction is of the form

$$e^- + RCONHCHR_2 \longrightarrow RCONHCHR_2^* + e \qquad (61)$$

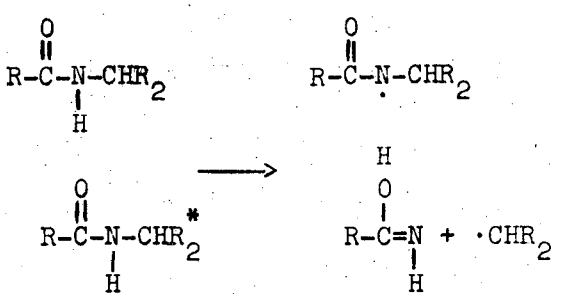
$$RCONHCHR_2^* + RCONHCHR_2 \longrightarrow RCONH\dot{C}R_2 + RCONH_2 + \dot{C}HR_2 \qquad (62)$$

Platzman[35] proposed some years ago that in the γ-radiolysis of concentrated aqueous solutions the solute may undergo electronic excitation through interaction with low-energy electrons as formulated in Eq. (61).

Now, aromatic compounds are, of course, known to be effective scavengers of excited states providing the energy level of the aromatic compound is lower than that of the excited species.[49] We find experimentally[38,39] that naphthalene sulfonic acid, benzoic acid and benzaldehyde are remarkably effective in quenching the formation of amide ammonia in 2M N-acetyalanine. Phenol and benzene sulfonic acid, on the other hand, are without effect even at the higher concentrations. Typical data are summarized in Fig. 10.

The reason that certain aromatic compounds are effective quenchers and others are not becomes evident on examination of the energy-level diagram given in Fig. 11. Data for the singlet and triplet levels of the aromatic compounds are taken from Calvert and Pitts.[8] The value of the singlet level of N-acetylalanine is from the work of Saidel.[40] We observe first that although all of the aromatic solutes studied here have singlet levels below the peptide

upper singlet, not all are effective quenchers as we have noted. The correlation appears when the triplet levels are considered. Those compounds that are effective quenchers viz., benzaldehyde, naphthalene sulfonic acid, and benzoic acid have the lower triplet levels as compared to phenol and benzene sulfonic acid. The change from quenching to nonquenching occurs between benzoic acid (27,200 cm^{-1}) and phenol (28,500 cm^{-1}). In other words, the energy of the excited state, $RCONHCHR_2^*$, in the case of N-acetylalanine, would then be between these two values at $\sim$ 28,000 $cm^{-1} \simeq$ 80 kcal $\simeq$ 3.5 eV. We envisage the chemistry of the excited state to be of the form



(63)

where $RCO\dot{N}CHR_2$ rearranges essentially instantaneously to yield the long-lived radicals, $RCONH\dot{C}R_2$.

Main-chain degradation occurs also in the radiolysis of peptides in the solid state.[18,19] This is manifested in the formation of labile amide-like products which are readily degraded on mild hydrolysis to yield free ammonia. The 100 eV yield for radiolytic degradation of the peptide bond as measured in terms of $G(NH_3)$ after hydrolysis is given in Table VII for a variety of aliphatic, aromatic, and sulfur containing α-amino acids in the N-acetyl form. In the case of the aliphatic series we see that the length of the side chain has relatively little effect on the yield for main-chain degradation. The effect

of the aromatic groups of acetylphenylalanine and acetyltyrosine is to quench in part the yields of those reactions that lead to formation of amide ammonia. The sulfur moiety of methionine, on the other hand, appears to be relatively ineffective in quenching such reactions.

In inquiring into the nature of the radiolytic processes that lead to degradation of the peptide chain, we have completed a detailed study of the reaction products formed in the γ-radiolysis of simple peptide derivatives of alanine, viz. N-acetyl-DL-alanine and poly-DL-alanine. The data are summarized in Table VIII. The major organic products in the order of decreasing yield are propionic acid, acetaldehyde, pyruvic acid, and lactic acid.

The formation of propionic acid as the principal organic product of these peptide derivatives of alanine implies that main-chain cleavage is involved as the major decomposition mode. We tentatively define the stoichiometry of this cleavage in terms of

$$RCONHCHR_2 \longrightarrow RCO\dot{N}H + \dot{C}HR_2 \tag{64}$$

$$RCO\dot{N}H + RCONHCHR_2 \longrightarrow RCONH_2 + RCONH\dot{C}R_2 \tag{65}$$

$$\dot{C}HR_2 + RCONHCHR_2 \longrightarrow CH_2R_2 + RCONH\dot{C}R_2 \tag{66}$$

where the radicals $RCONH\dot{C}R_2$ are long-lived and correspond to the radical species observed at room temperature by esr measurements[7] and by the use of tritiated radical scavengers.[37]

The formulation of Eqs. (64-66) is intended only to convey the nature of the over-all stoichiometry. Ionic and/or excited species are presumably

involved as actual intermediates, since caging effects in the solid phase would lead to preferential recombination of the radical pair of Eq. (64).

We have already noted that excited-molecule intermediates appear to be involved in the production of amide and fatty acid in the radiolysis of peptides in concentrated aqueous solution. Reaction similar to those given in Eqs. (61-63) could also occur in the solid state and would satisfy the stoichiometric requirements of Eqs. (64-66). Arguments supporting the role of excitation processes in the radiolysis of solid peptides have been given.[18]

The production of lactic acid, pyruvic acid, and acetaldehyde shown in Table VIII is formulated in terms of the stoichiometric relationships

$$RCONHCHR_2 \longrightarrow R\overset{\overset{NH}{\|}}{C}OCHR_2 \qquad (67)$$

$$RCONHCHR_2 \longrightarrow RCON{=}CR_2 + H_2(2H) \qquad (68)$$

$$RCONHCHR_2 \longrightarrow RCON{=}CHR + RH \qquad (69)$$

The radiation-induced N-O shift represented by Eq. (67) leads to formation of the labile imino ester, which species is readily hydrolyzed to yield ammonia and the hydroxyacid, lactic acid

$$R\overset{\overset{N}{\|}}{C}OCHR_2 + H_2O \longrightarrow RCOOH + NH_3 + RCHOHR \qquad (70)$$

The unsaturated products (dehydropeptides) of steps 68,69 are labile, as we have noted in Eqs. 50,51, and yield keto acid and aldehyde respectively on mild hydrolysis.

The production of amide and fatty acids as major products in the radiolysis of the above systems clearly implies that main-chain fragmentation occurs concomitantly. Friedberg and co-workers[21] report that polyamino acids such as polyglutamic acid and polylysine and also gelatin show lower intrinsic viscosities and lower number-average molecular weight after radiolysis in the solid state; G values for main-chain degradation of 1.8, 4.1, and 1.4, respectively, were calculated. Globular proteins, on the other hand, do not appear to degrade appreciably on radiolysis as solids.[14] Friedberg and co-workers[21] have suggested that the secondary and tertiary structure of the globular proteins favor fragment recombination.

FOOTNOTES AND REFERENCES

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Table I. Yields of major products in the γ-radiolysis of oxygen-free solutions of glycine and alanine, 1M, pH6.4 (Refs. 43,46).

Product	Yield, G	
	Glycine	Alanine
Ammonia	4.3	4.3
Keto acid	2.1	1.6
Fatty acid	1.2	1.0
Aldehyde	0.5	0.5
Hydrogen	2.0	1.3

Table II. Product yields in the γ-radiolysis of solid glycine, evacuated (Ref. 30).

Product	Yield (G)
Ammonia	4.8
Acetic acid	2.3
Glyoxylic acid	2.5
Hydrogen	0.2
Methylamine	0.2
Carbon dioxide	0.2

Table III. Product yields in the γ-radiolysis of solid cysteine (Ref. 34).

Product	G
Hydrogen	3.1 ± 0.1
Hydrogen sulfide	1.5 ± 0.1
Ammonia	1.8 ± 0.1
Cystine	5.0 ± 0.5
"NH_2-free" fraction	1.0 ± 0.1
Pyruvic acid	$\leqslant$ 0.1
Total carbonyl	$\leqslant$ 0.1

Table IV. Comparative product yields in the γ-radiolysis of cysteine and related compounds in the solid state (Ref. 34).

	G		
	H_2	H_2S	NH_3
Cysteine $NH_3^+CH(CH_2SH)COO^-$	3.1	1.5	1.8
Cystamine $(NH_3^+CHCH_2SH)Cl^-$	5.6	1.2	< 0.1
N-Acetylcysteine $CH_3CONHCH(CH_2SH)COOH$	0.5	0.9	< 0.1
S-Methylcysteine $NH_3^+CH(CH_2SCH_3)COO^-$	~ 0.2	...	5.1
Glycine $NH_3^+CH_2COO^-$	~ 0.2		5.2
Alanine $NH_3^+CH(CH_3)COO^-$	~ 0.2		5.4

Table V. Product yields in the γ-radiolysis of N-acetylalanine and polyalanine in oxygenated solution (Ref. 20).

Product	Yield (G)[a]	
	0.05M N-acetylalanine	0.5% polyalanine
NH_3	2.9	3.9
CH_3COOH	3.0	~ 3.9
CO_2	2.0	2.4
H_2O_2	2.2	2.2
ROOH[b]	0.5	---
$CH_3COCOOH$	~ 0.2	1.2
CH_3CHO	~ 0.2	0.4

[a]Product yields are independent of dose up to ~ 2×10^{19} e⁻/ml.

[b]Unspecified.

Table VI. Effect of cysteine (RSH) on the γ-ray induced reduction of acetamide and N-ethylacetamide in oxygen-free solution (Ref. 25,26).

Amide Solution (M pH7)[a]	(RSH), M	$G(CH_3CHO)$
acetamide	none	~ 0.1
acetamide	4×10^{-4}	2.4[b]
N-ethylacetamide	none	< .05
N-ethylacetamide	4×10^{-4}	2.8[c]

[a]Since the rate constant for reaction of cysteine with e^-_{aq} corresponds to $k = 2 \times 10^{10}\ M^{-1}\ sec^{-1}$ it was necessary in this series of experiments to increase the amide concentration to 1 M to insure the preferential scavenging of e^-_{aq} by RCONHCHR. Concentrations of RSH much below 4×10^{-4} M are experimentally impracticable because of excessive depletion of the mercaptan during radiolysis.

[b]This yield is dose dependent and the value 2.4 represents the extrapolated yield at zero dose.

[c]At dosages below 2.5×10^{18} eV/gm.

Table VII. γ-ray induced degradation of solid N-acetylamino acids, $CH_3CONHCH(R)COOH$ (Ref. 18).

N-acetyl Derivative[a]	(R)	$G(NH_3)$[b]
glycine	$-H$	2.68
alanine	$-CH_3$	3.4
α-aminobutyric acid	$-CH_2CH_3$	2.7
leucine	$-CH_2CH(CH_3)_2$	3.2
glutamic acid	$-CH_2CH_2COOH$	2.3
phenylalanine	$-CH_2(C_6H_5)$	0.8
tyrosine	$-CH_2(C_6H_4OH)$	1.6
methionine	$-CH_2CH_2SCH_3$	2.3

[a]N-acetyl-DL-amino acids were used with the exception of N-acetyl-L-glutamic acid.

[b]After hydrolysis.

Table VIII. Product yields in the γ-radiolysis of N-acetyl-DL-alanine and poly-DL-alanine (Ref. 18).

Product	G	
	N-acetylalanine	Polyalanine
ammonia (total)	3.4	3.6
amide	2.8	3.1
free	0.6	0.5
propionic acid	1.4	1.8
pyruvic acid	0.4	~ 1
acetaldehyde	0.8	~ 0.4
lactic acid	⩽ 0.2	---
acrylic acid	trace	---
hydrogen	0.40	0.45

FIGURE CAPTIONS

Fig. 1. Ammonia yields from 1.0 M alanine (0) and 1.0 M glycine (●) as a function of sodium formate concentration in oxygen-free solution at pH 6.4 under γ-radiolysis (Ref. 46).

Fig. 2. Product yields from 1.0 M alanine as a function of sodium formate concentration in oxygen-free solution at pH 6.4 under γ-radiolysis (Ref. 46).

Fig. 3. Effect of glycylglycine and glycine concentrations on ammonia yields in oxygen-free solution at pH 6.5 under γ-radiolysis (Ref. 50).

Fig. 4. Effect of formate concentration on ammonia yields in the γ-radiolysis of 1.0 M glycine (0) and 0.2 M glycylglycine (●) in oxygen-free solution at pH 6.5 (Ref. 50).

Fig. 5. Effect of chloracetate concentration on ammonia (●) and chloride ion (▲) yields in the γ-radiolysis of 0.2 M glycylglycine, oxygen-free, pH 6.5 (Ref. 50).

Fig. 6. Ammonia yields in the γ-radiolysis of an homologous series of α-amino acids in oxygenated solution, pH 6, glycine (0), alanine (◐), α-aminobutyric acid (◐) norvaline (◐), norleucine (◐) (Ref. 16).

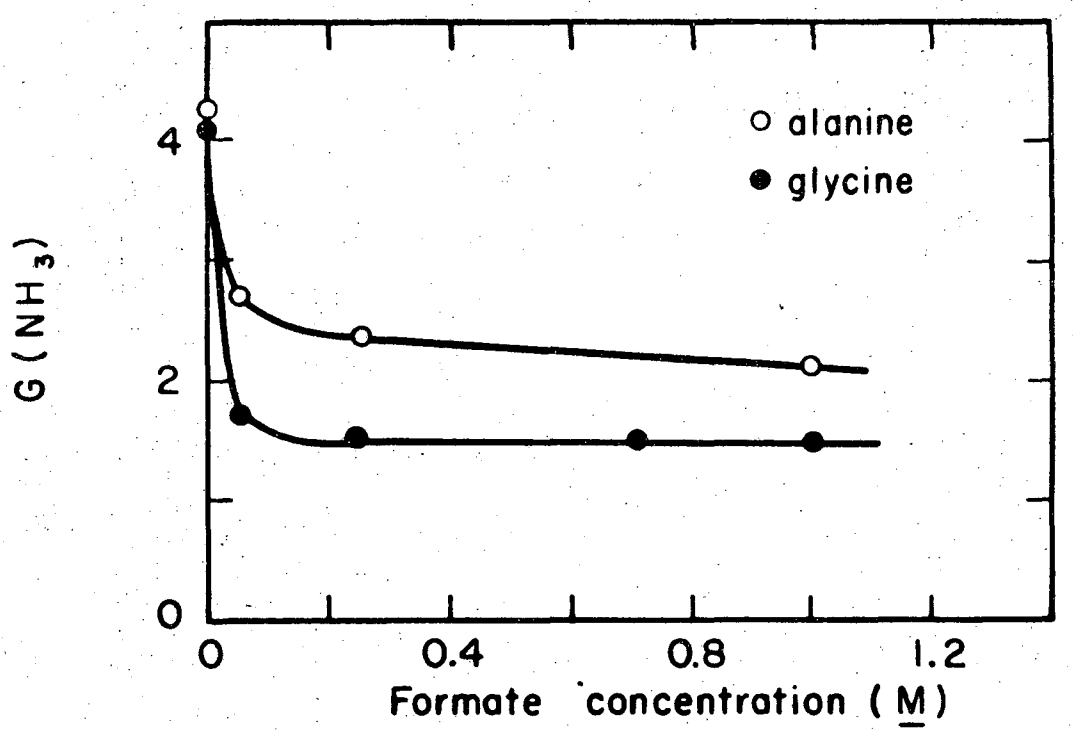
Fig. 7. Effect of solute concentration in the γ-radiolysis of N-acetylalanine in oxygenated solution: $G(NH_3)$, pH 7 (0); pH 3 (●); $G(CH_3COCOOH + CH_3CHO)$, pH 3 (Δ) (Ref. 20).

Fig. 8. Effect of FeIII concentration on the yields of ammonia (●) and pyruvic acid (▲) from 0.1 M acetylalanine and on ammonia (0) and glyoxylic acid (Δ) from 0.1 M acetylglycine (Ref. 2).

Fig. 9. Ammonia (0), propionic acid (□) and carbonyl (Δ) yields as a function of N-acetylalanine concentration in oxygen-free solution at pH 7 under γ-radiolysis (Ref. 39).

Fig. 10. Effects of excitation scavengers on ammonia yields in oxygen-free 2M N-acetylalanine solutions under γ-radiolysis; benzene sulfonic acid (O) phenol (●) benzoic acid (◐) naphthalene sulfonic acid (◐) (Ref. 39).

Fig. 11. Singlet (—) and triplet (---) energy levels of excitation scavengers used in Ref. 39. The line (···) represents the energy level of $RCONHCHR_2^*$.



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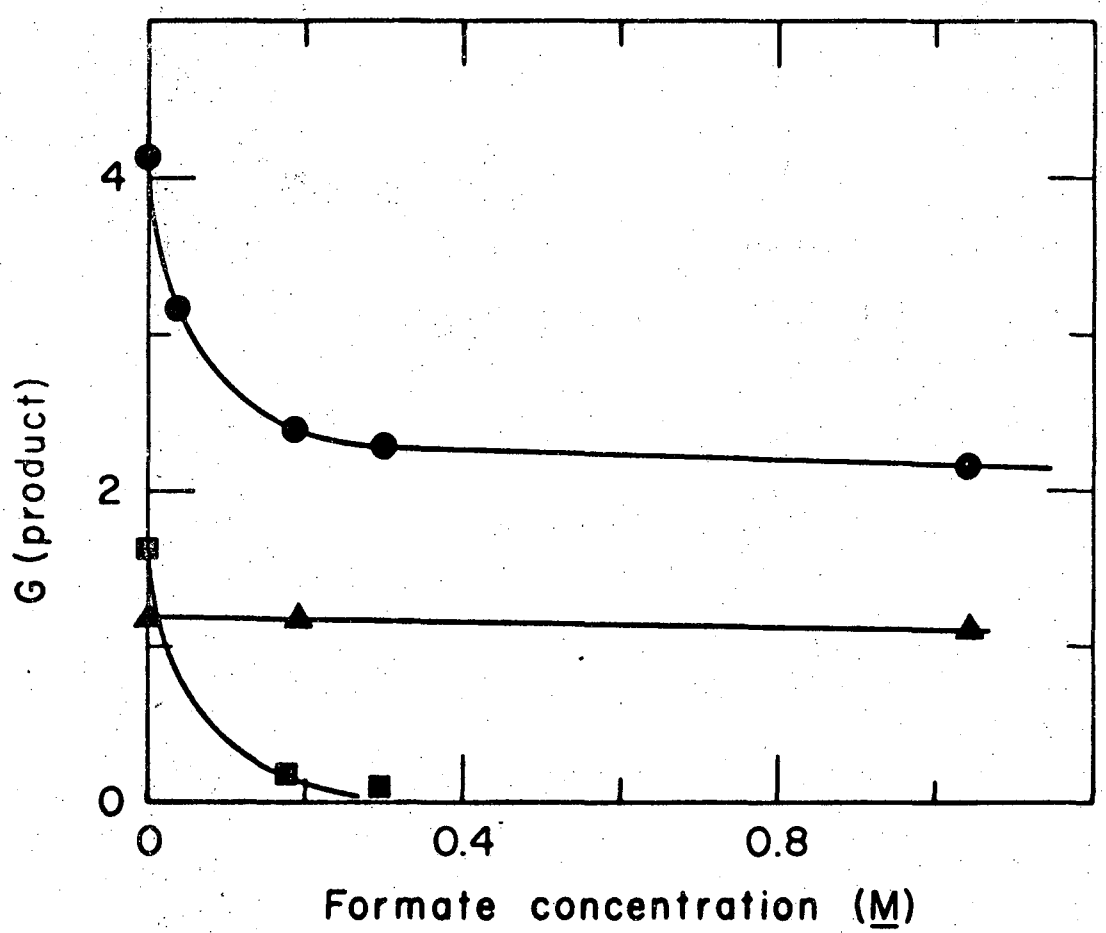

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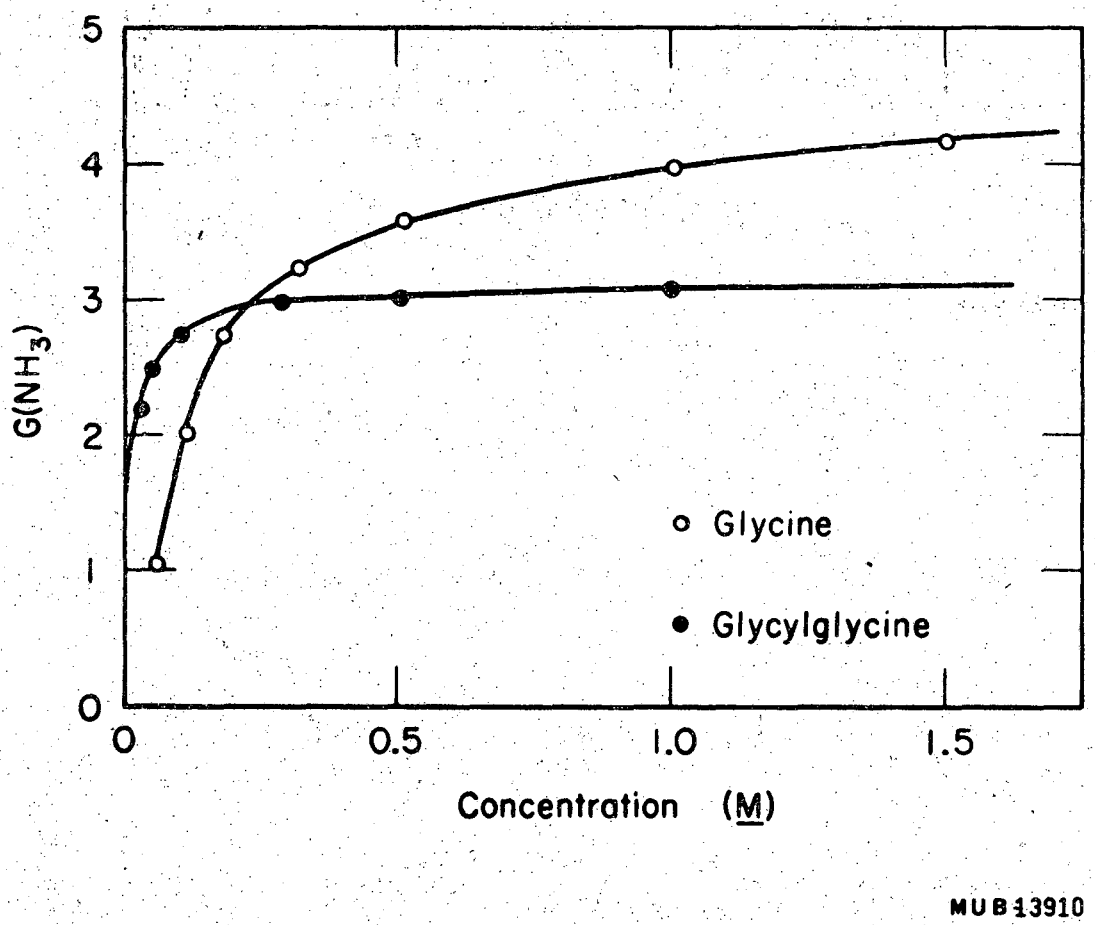
Fig. 2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4
3
2
1
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Concentration (M)
Glycine
Glycylglycine
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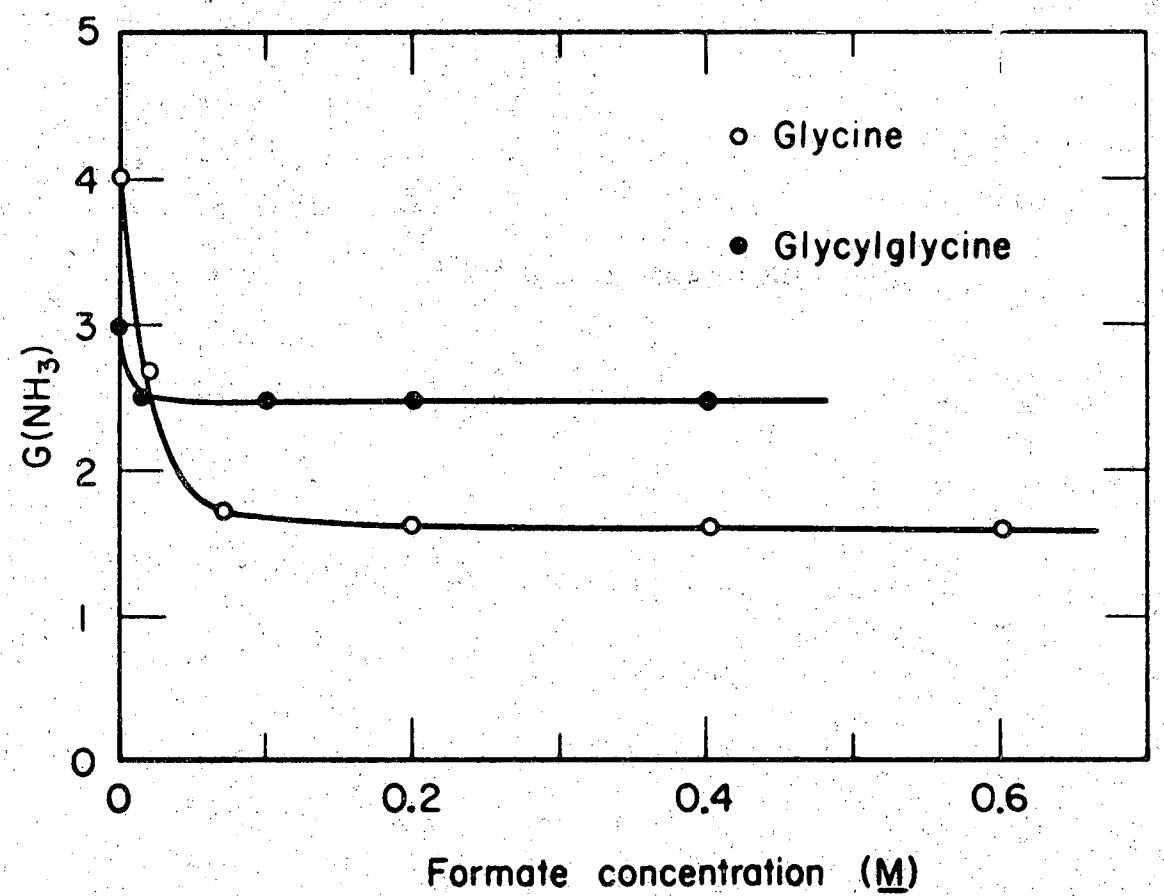
Fig. 3



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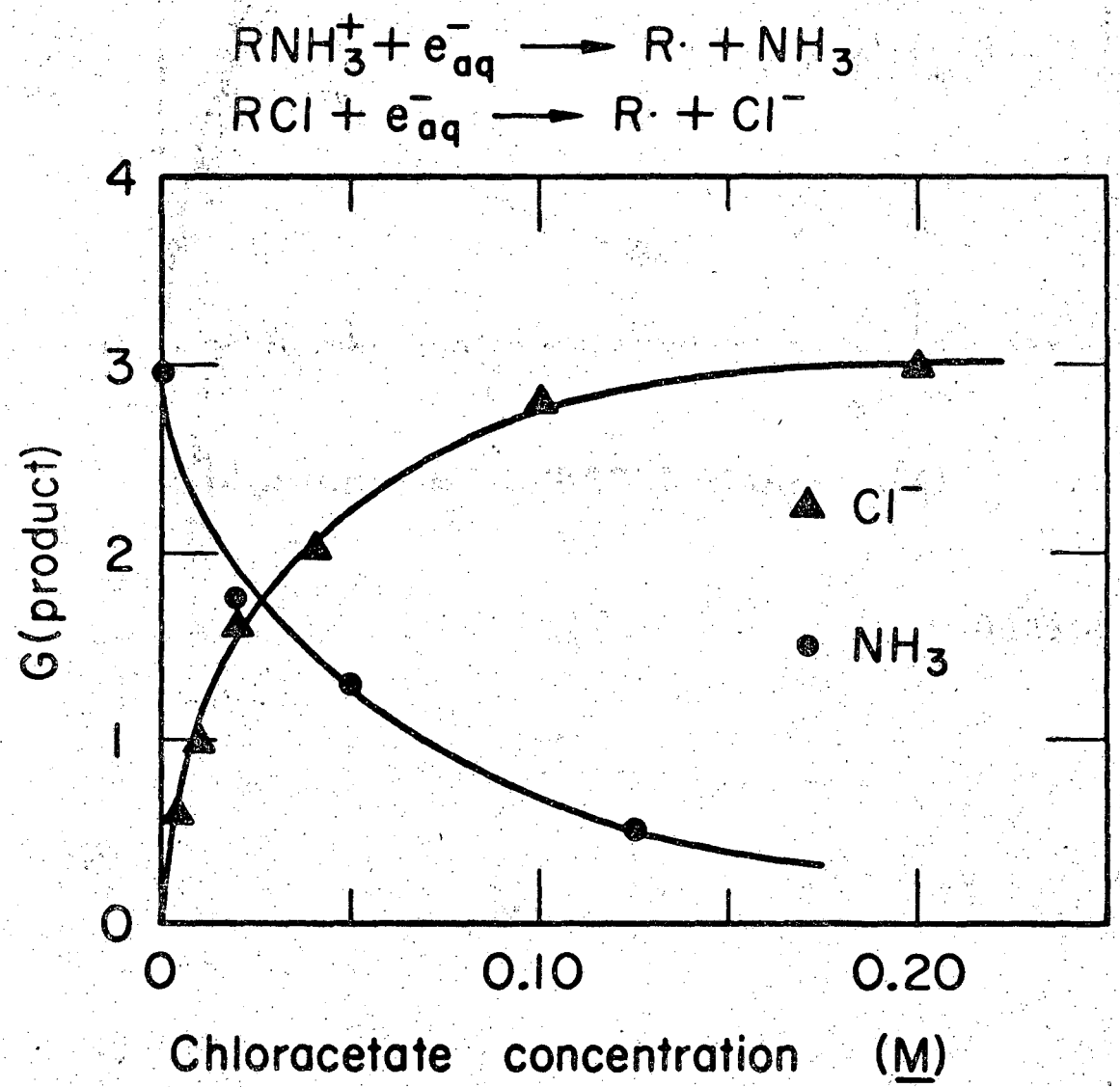

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Fig. 5

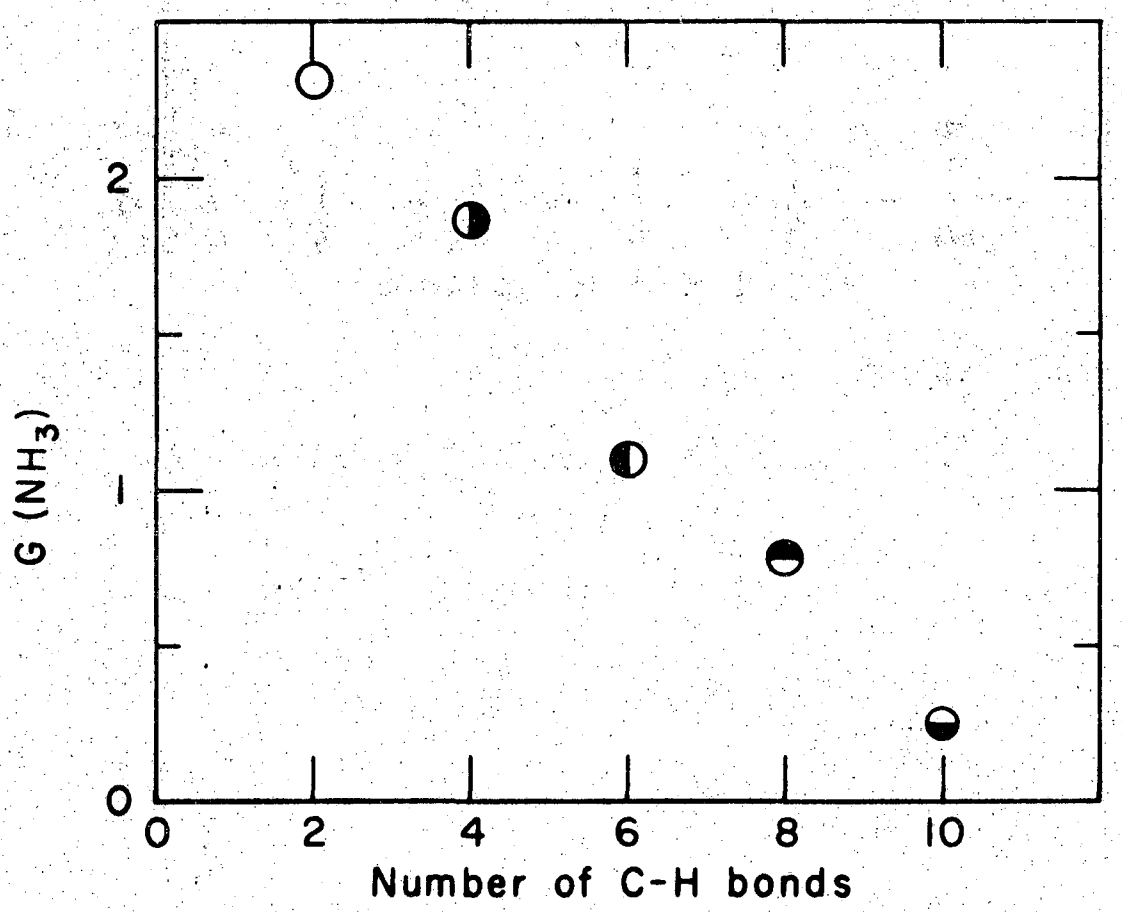

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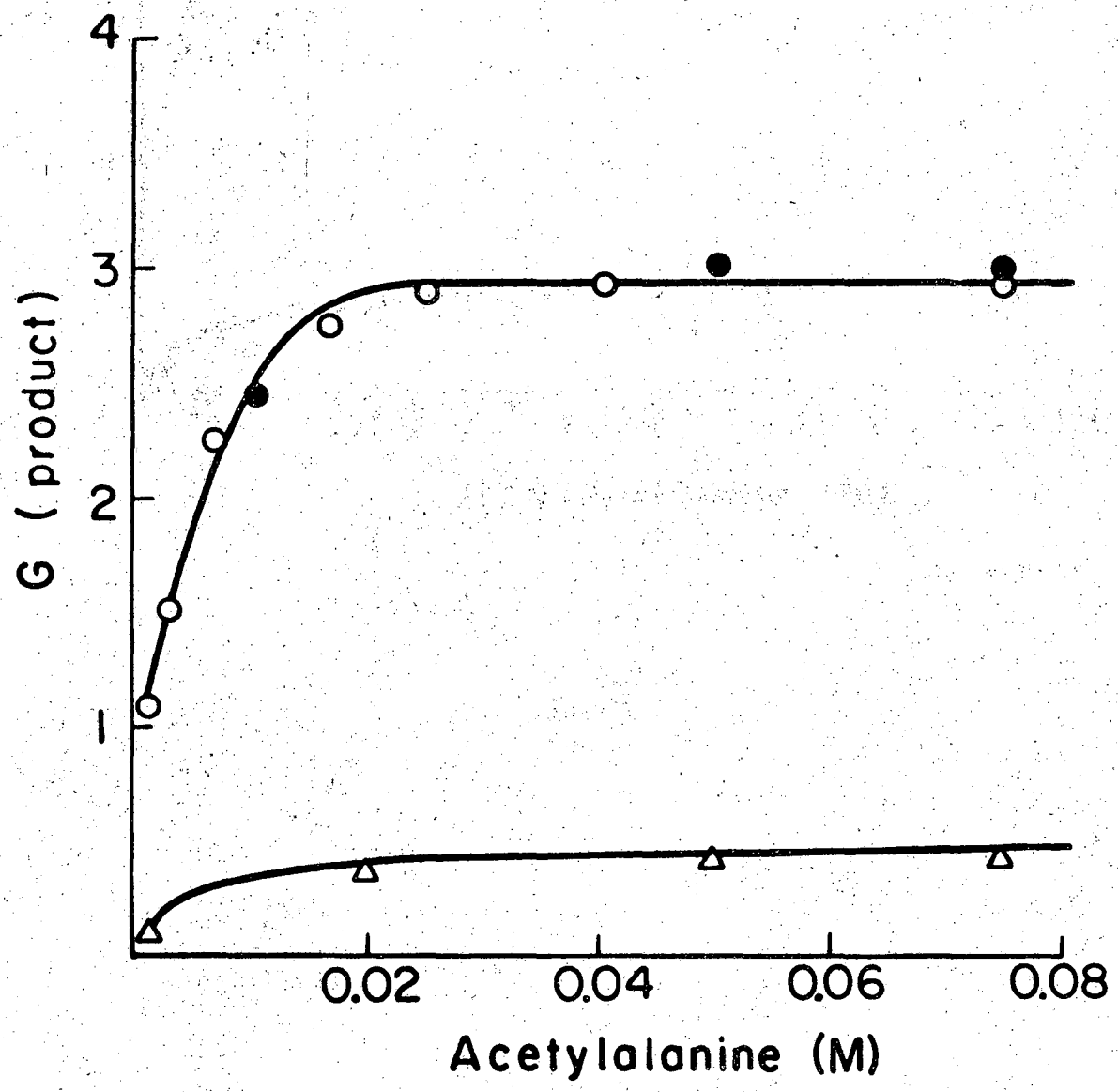
Fig. 6



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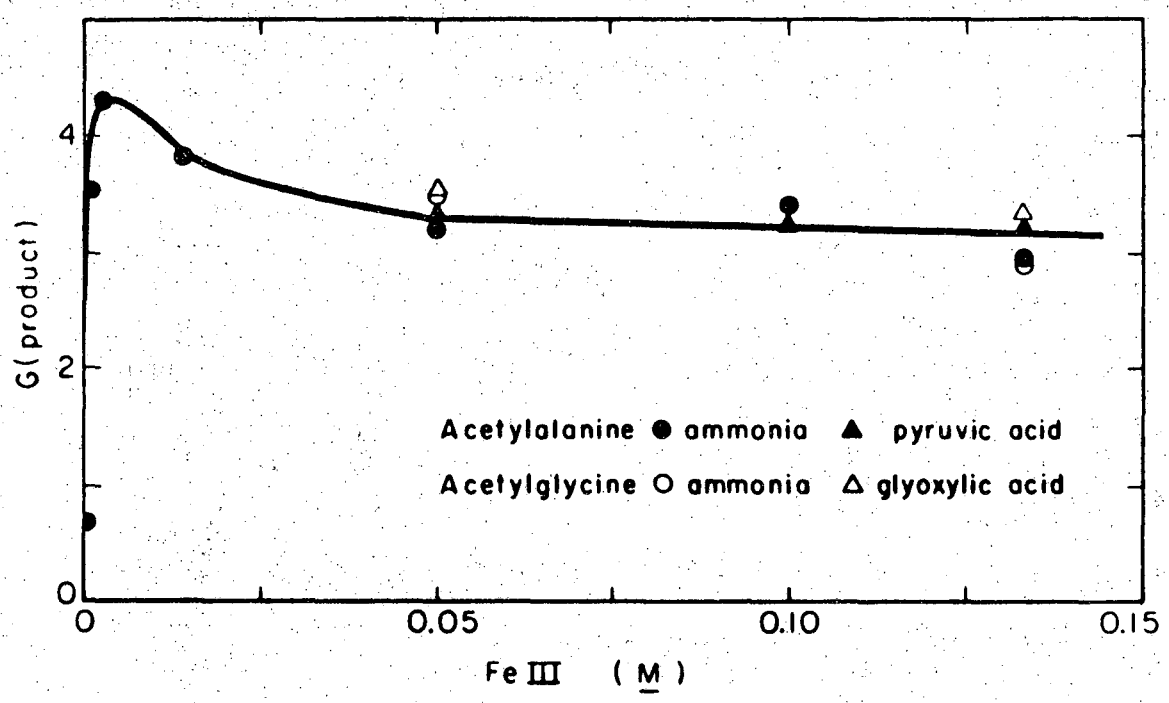

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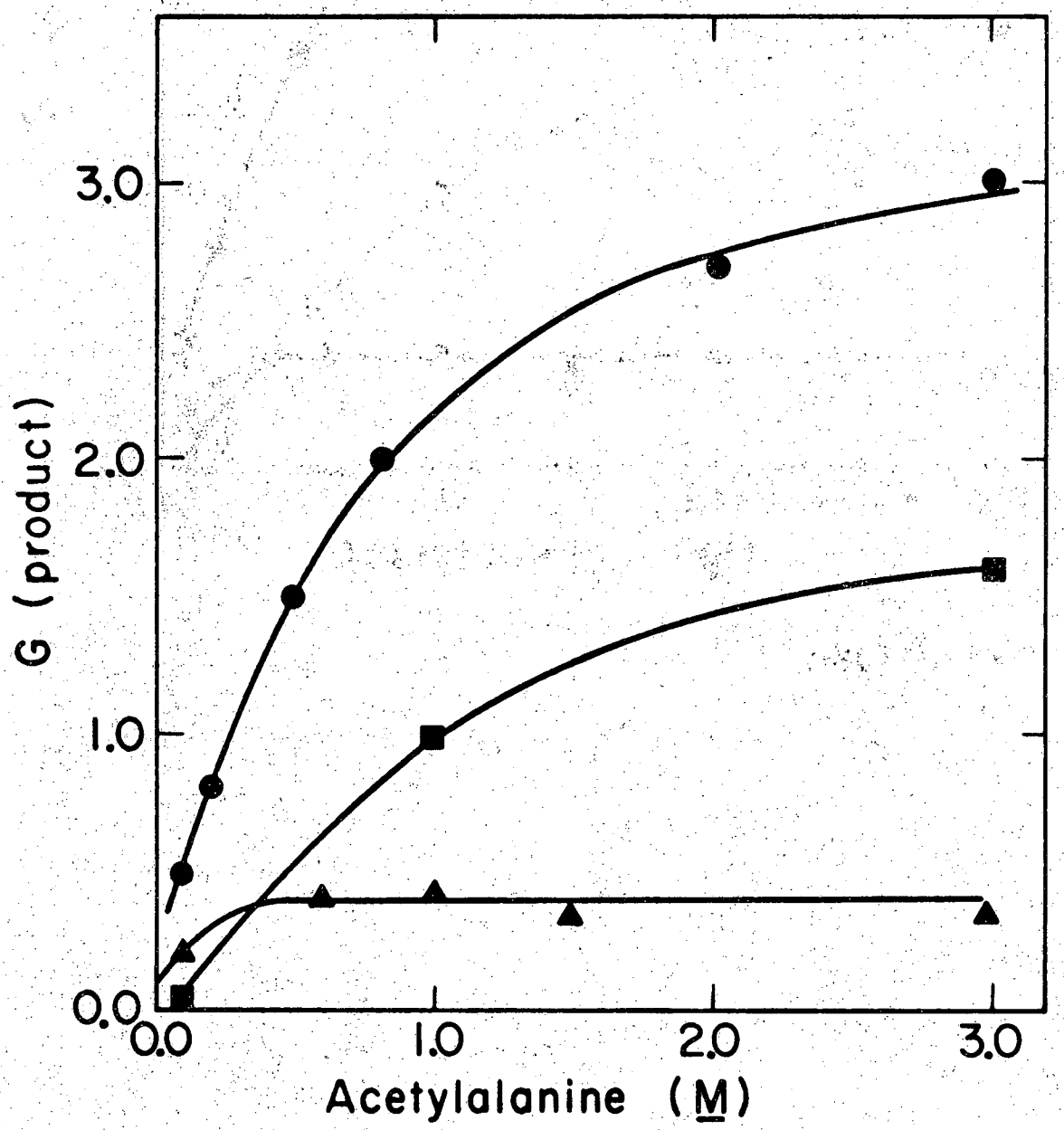

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Fig. 9

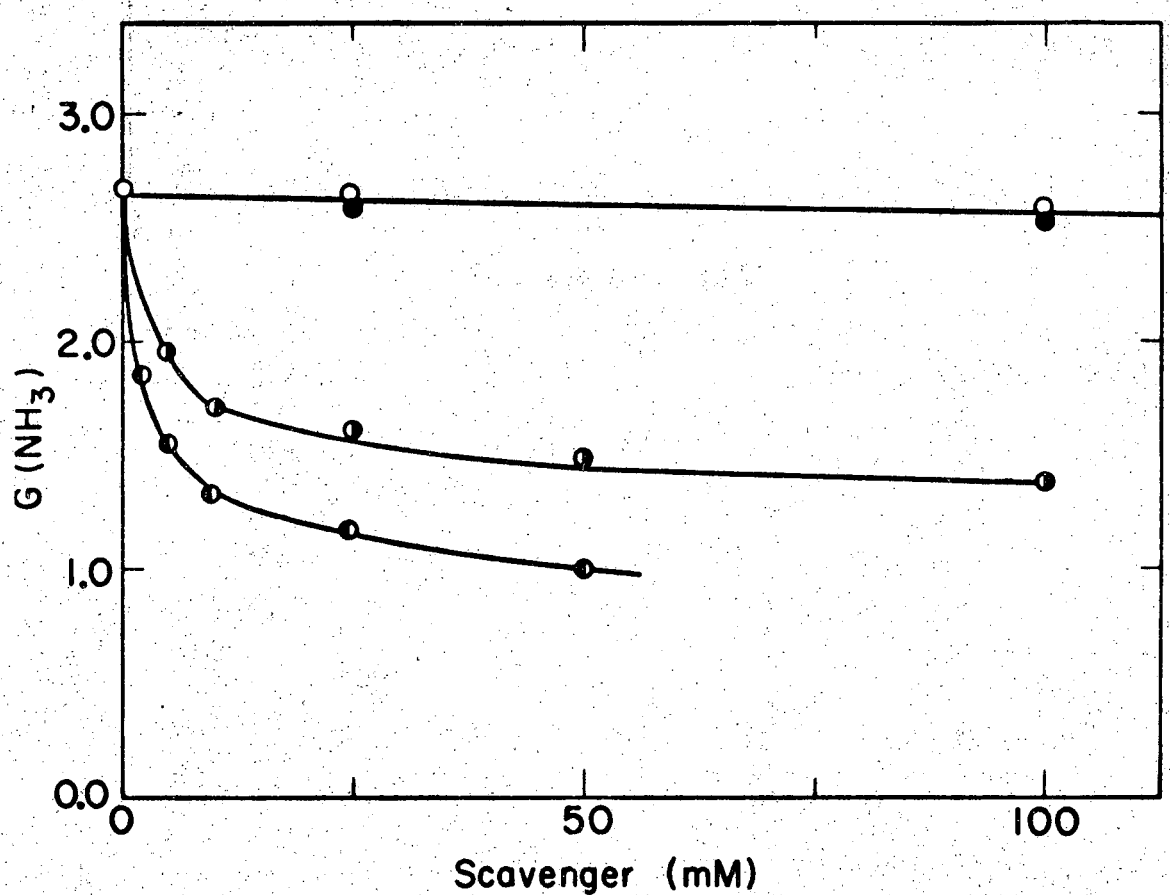

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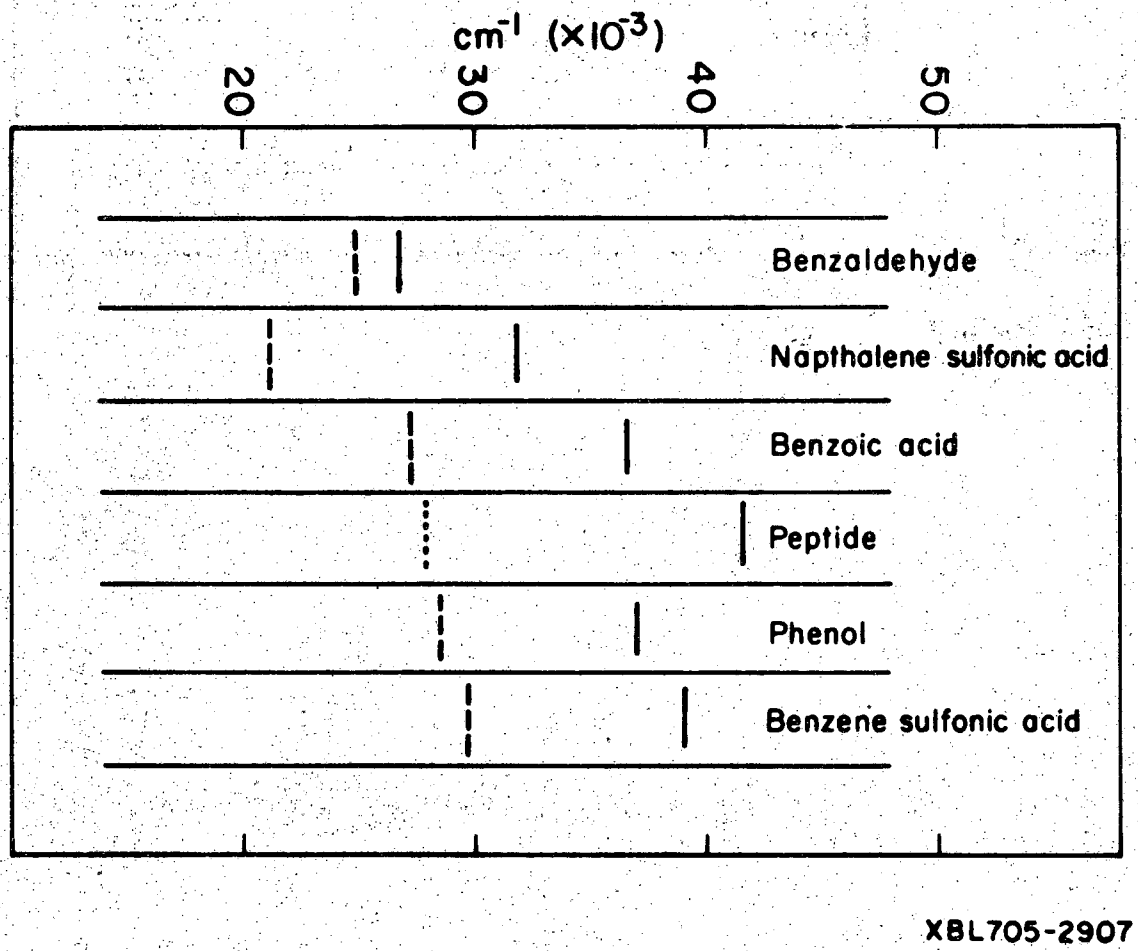
Fig. 10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20
30
40
50
Benzaldehyde
Napthalene sulfonic acid
Benzoic acid
Peptide
Phenol
Benzene sulfonic acid
XBL705-2907

Fig. 11

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