A Marked Effect of Conformation in the Radiolysis of Poly-α-L-glutamic Acid in Aqueous Solution
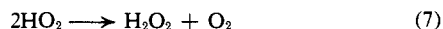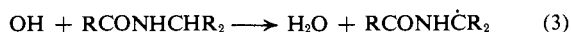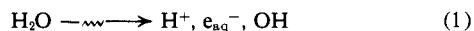
Sir:

Compounds containing the peptide bond undergo chemical degradation at the N–C linkage on irradiation in aqueous solution under oxygen. The schematics of the radiation-induced reactions are given by[1]

$$H_2O \xrightarrow{\quad\rightsquigarrow\quad} H^+, e_{aq}^-, OH \qquad (1)$$

$$e_{aq}^- + O_2 + H_2O \underset{\longrightarrow}{\overset{\longleftarrow}{\quad}} HO_2 + OH^- \qquad (2)$$

$$OH + RCONHCHR_2 \longrightarrow H_2O + RCONH\dot{C}R_2 \qquad (3)$$

$$O_2 + RCONH\dot{C}R_2 \longrightarrow RCONHC(\dot{O}_2)R_2 \qquad (4)$$

$$RCONHC(\dot{O}_2)R_2 \longrightarrow RCON{=}CR_2 + HO_2 \qquad (5)$$

$$H_2O + RCONHC(\dot{O}_2)R_2 \longrightarrow RCONH_2 + R_2CO + HO_2 \qquad (6)$$

$$2HO_2 \longrightarrow H_2O_2 + O_2 \qquad (7)$$

The dehydropeptides $RCON{=}CR_2$ as a class are readily hydrolyzed in dilute mineral acid under the

$$RCON{=}CR_2 + 2H_2O \longrightarrow RCOOH + NH_3 + R_2CO \qquad (8)$$

conditions conventionally employed to liberate ammonia from organic amides.[1,2] Hence, regardless of the relative rates of reactions 5 and 6, each RCO-NH$\dot{C}$R$_2$ radical yields one molecule of ammonia on hydrolysis of the irradiated system. The over-all stoichiometry of steps 1 to 7 plus the hydrolysis steps is given by[3]

$$RCONHCHR_2 + O_2 + 2H_2O \longrightarrow$$
$$RCOOH + NH_3 + R_2CO + H_2O_2 \qquad (9)$$
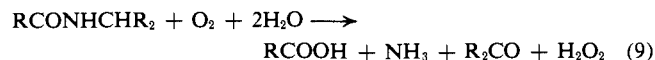
We report here a study of the γ-ray induced oxidation of poly-α-L-glutamic acid (PGA) over the pH range 3.5 to 9. Dilute solutions of PGA (Pilot Chemicals, Lot G-9, mol. wt. 140,000) were purified by prolonged dialysis, adjusted to the desired pH value through addition of NaOH or H_2SO_4, and irradiated under oxygen at 1 atm. in sealed Pyrex tubes at a γ-ray dose rate of 1.2×10^{18} e.v./g./min. The tubes were rotated at intervals to prevent depletion of oxygen in the liquid phase. A series of control runs established the time and speed of rotation required to ensure the presence of excess oxygen under all conditions of pH. (PGA below pH 4 is metastable and precipitation occurs if the solution is agitated too vigorously.) Following irradiation, the solutions were made 2 *N* in H_2SO_4, hydrolyzed under nitrogen for 3 hr. at 100°, and assayed for ammonia[4] and carbonyl products.[1,5] α-Ketoglutaric and pyruvic acids were identified as the major carbonyl products. Acetaldehyde and glyoxylic acid were detected in low yield. The validity of the analytical procedures was established through analysis of known mixtures of the four carbonyl products.

(1) (a) W. M. Garrison, M. E. Jayko, and W. Bennett-Corniea, *Radiation Res.*, 16, 483 (1962); (b) W. M. Garrison and B. M. Weeks, *ibid.*, 17, 341 (1962).

(2) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, N. Y., 1961, p. 843.

(3) There is evidence (ref. 1) that certain peptides yield organic products in addition to the indicated carbonyl; however, the main point here is that each RCONH$\dot{C}$(O$_2$)R$_2$ radical ultimately yields one molecule of ammonia regardless of the path of degradation.

(4) E. J. Conway and A. Berne, *Biochem. J.*, 27, 419 (1933).

(5) (a) T. E. Friedemann and G. E. Haugen, *J. Biol. Chem.*, 147, 415 (1943); (b) G. R. A. Johnson and G. Scholes, *Analyst*, 79, 217 (1954).

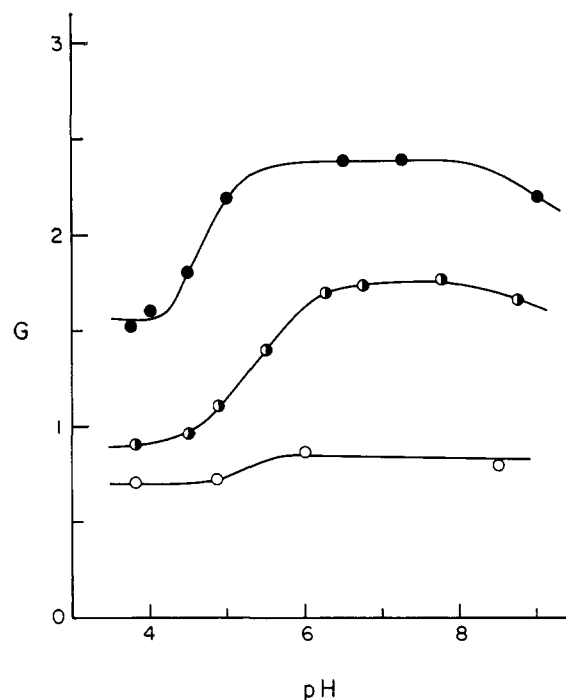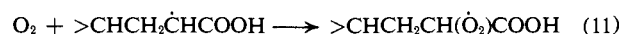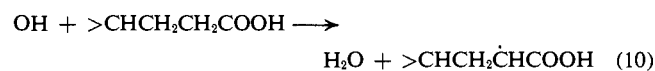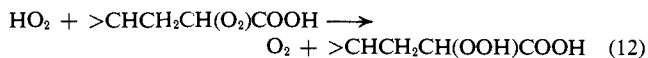Figure 1. Effect of pH on the yield of ammonia (●), total α-keto acids (◑), and α-ketoglutaric acid (○) in the γ-radiolysis of 0.15% poly-α-L-glutamic acid: G = molecules/100 e.v.

Product yields from 0.15% PGA solutions over the pH range 3.5 to 9 are summarized in Figure 1. It is seen that the yield of ammonia and the combined yield of α-keto acids increase abruptly to their maximum values with increasing pH over the narrow range pH $\simeq$ 4.5 to pH $\simeq$ 6. That this effect is not a result of incomplete scavenging of OH radicals at pH <6 is shown by the fact that product yields at both pH 4 and 7 are independent of the PGA concentration from 0.15% down to at least 0.015%. Nor does it appear that the sharp break in the pH–yield curves is directly related to changes in hydrogen ion concentration or degree of ionization of side-chain carboxyl groups, *per se*. This is shown by results obtained with N-acetylglutamic α-methyl ester, a radiation-chemical model for the single-residue segment of the PGA chain; ammonia and carbonyl yields from 0.05 *M* solutions of this low molecular weight peptide derivative of glutamic acid are essentially independent of pH over the entire range pH 3 to 8, with $G(NH_3) \simeq G({>}C{=}O) \simeq 2$.
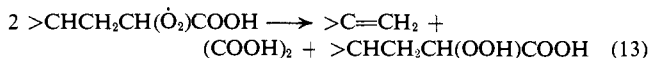
The data of Figure 1 show that G(pyruvic) increases sharply with $G(NH_3)$ with increasing pH from 4.5 to 6, whereas the yield of α-ketoglutaric is essentially pH independent in accord with the schematics of eq. 5 and 6. The evidence is that pyruvic acid (plus ammonia) arises from a parallel reaction involving OH attack at a locus other than the peptide chain. After a detailed consideration of the possible chemical consequences of OH attack at each of the various C–H bonds of the glutamic acid residue, we conclude that pyruvic acid is produced as a consequence of OH attack at the C–H bond α to the side-chain carboxyl group. Consider the reactions

$$OH + {>}CHCH_2CH_2COOH \longrightarrow$$
$$H_2O + {>}CHCH_2\dot{C}HCOOH \qquad (10)$$

$$O_2 + {>}CHCH_2\dot{C}HCOOH \longrightarrow {>}CHCH_2CH(\dot{O}_2)COOH \qquad (11)$$

where $>$CHCH$_2$CH$_2$COOH represents the side chain of the glutamic acid residue. Peroxy radicals of the type formed in reaction 11 are relatively long-lived and we suggest they are subsequently removed through the competing reactions

$$HO_2 + >CHCH_2CH(O_2)COOH \longrightarrow$$
$$O_2 + >CHCH_2CH(OOH)COOH \quad (12)$$

and

$$2 >CHCH_2CH(\dot{O}_2)COOH \longrightarrow >C=CH_2 +$$
$$(COOH)_2 + >CHCH_3CH(OOH)COOH \quad (13)$$

Degradation reactions akin to reaction 13 have been described by Russell[6] and by Durup, et al.[7] It is seen that the product $>$C=CH$_2$ in the above nomenclature corresponds to the acrylic acid derivative RCONHC-(=CH$_2$)R (a tautomeric form of the dehydropeptide, RCON=C(CH$_3$)R), which on hydrolysis yields ammonia and pyruvic acid. Also, we conclude from the results obtained with N-acetylglutamic α-methyl ester that the relative rates of reactions 12 and 13 are essentially independent of the degree of ionization of the reacting species over the pH range 3 to 8.

Now, to explain the pronounced effect of pH on G (NH$_3$) and G(pyruvic) from PGA we note first that a unique characteristic of the radiation chemistry of a macromolecular substance in aqueous solution is that each molecule undergoes reaction with a relatively large number of OH radicals even at the lowest practicable dosages. For example, with a 0.15% solution of PGA, a γ-ray dose of 3×10^{18} e.v./g. produces but one OH per 100 glutamic acid residues but at the same time this corresponds to about 20 OH radicals per PGA molecule (mol. wt. 140,000). However, since PGA above pH 6 has the random coil configuration,[8] the various segments of the macromolecule are free to interact both intermolecularly and intramolecularly, and we find at pH $>$6 no essential differences between the macromolecule and the low molecular weight model from the standpoint of product yields. But, as the pH of the solution is decreased, PGA undergoes a coil $\rightarrow$ helix transition over the pH range 6 to 4.5 which as we have noted is the significant pH range of Figure 1. With PGA in the helix form, the peroxy radicals are frozen in a fixed spatial arrangement and it is obvious that the probability of reaction 13 is greatly reduced; hence reaction 12 is favored and as a result G(NH$_3$) and G(pyruvic) decrease as seen in Figure 1.[9]

(6) G. A. Russell, Chem. Ind. (London), 49, 1483 (1956).
(7) M. Durup, J. Durup, F. Kuffer, and M. Magat, Proc. 2nd Intern. Conf. Peaceful Uses At. Energy, Geneva, 29, 143 (1958).
(8) See, for example, J. Applequist and J. L. Breslow, J. Am. Chem. Soc., 85, 2869 (1963).
(9) This work was done under the auspices of the U. S. Atomic Energy Commission.

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## Tetrafluoroethylene Complexes of Transition Metals

Sir:

Since the first preparation of perfluorotetramethylene-iron tetracarbonyl,[1] there have been numerous attempts

(1) K. F. Watterson and G. Wilkinson, Chem. Ind. (London), 991

to make transition metal complexes of tetrafluoroethylene (TFE).[2] We wish to report the isolation of the first simple TFE complexes, compounds of rhodium or iridium, from which the fluoroolefin can be displaced under mild conditions.

The rhodium complexes are obtained by displacing ethylene from 2,4-pentanedionatobis(ethylene)rhodium-(I)[3] or μ-dichlorotetraethylenedirhodium(I)[4] with tetrafluoroethylene at room temperature and atmospheric pressure. The products (1 and 2) retain one ethylene ligand per metal atom which is not displaced by continued exposure to TFE. Ethylene but not TFE is
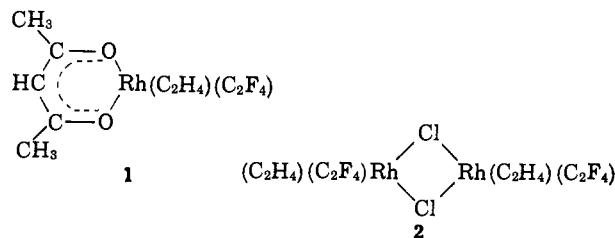
displaced from 1 or 2 by phosphines, amines, nitriles, or cyanide ion. Both ethylene and TFE are displaced by 1,5-cyclooctadiene or carbon monoxide.

The iridium complex 3 is prepared by the addition of TFE to chlorocarbonylbis(triphenylphosphine)iridium(I).[5] It readily evolves TFE on standing in benzene
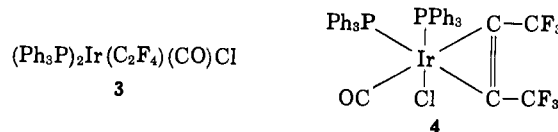
solution at 25 or at 100° under vacuum. Its hexafluoro-2-butyne analog 4 is even more remarkable in that it evolves butyne reversibly in the solid state.

The TFE complexes of rhodium (1 and 2), in contrast to the σ-bonded fluoroolefin complexes obtained earlier,[1,2,6] show chemical properties similar to those of conventional olefin complexes based on σ-donor–π-acceptor bonds.[7] The facile displacement of TFE by the chelating diolefin, 1,5-cyclooctadiene, is reminiscent of conventional olefin-exchange reactions. Contrary to this chemical evidence, however, the spectra of the iridium complexes 3 and 4 suggest extensive σ-bonding. Thus, the bonding of hexafluorobutyne in 4 may approach that of a [C$_4$F$_6$]$^{2-}$ ion coordinated to iridium(III) by two σ-bonds, analogous to that of the oxygen ligand in (Ph$_3$P)$_2$(CO)IrCl(O$_2$).[8] The infrared spectrum of 4 shows a C=C stretching vibration at 1770 cm.$^{-1}$ like that in (Ph$_3$P)$_2$Pt(CF$_3$CCCF$_3$)[6a] and intermediate between the C=C frequencies in[9] 5 and 6.

Ethylenebis(triphenylphosphine)nickel[10] undergoes ethylene displacement when it is treated with TFE, but

(1959); ibid., 1358 (1960); H. H. Hoehn, G. Wilkinson, et al., J. Chem. Soc., 2738 (1961).
(2) This topic has been reviewed by P. M. Treichel and F. G. A. Stone in "Advances in Organometallic Chemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1964, p. 143.
(3) R. Cramer, J. Am. Chem. Soc., 86, 217 (1964).
(4) R. Cramer, Inorg. Chem., 1, 722 (1962).
(5) L. Vaska and J. W. DiLuzio, J. Am. Chem. Soc., 83, 2784 (1961).
(6) (a) J. L. Boston, S. O. Grim, and G. Wilkinson, J. Chem. Soc., 3468 (1963); (b) M. R. Churchill and R. Mason, Proc. Chem. Soc., 365 (1963).
(7) M. J. S. Dewar, Bull. soc. chim. France, 18, C79, (1951); J. Chatt and L. A. Duncanson, J. Chem. Soc., 2939 (1953).
(8) L. Vaska, Science, 140, 809 (1963).
(9) W. Mahler, J. Am. Chem. Soc., 84, 4600 (1962).
(10) G. Wilke and G. Hermann, Angew. Chem., 74, 693 (1962).