An Electron Spin Resonance Study of the Relative Stabilities of Free[1] Radicals Trapped in Irradiated Methanol at 77°K.

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Free radicals produced by ultraviolet irradiation in a methanol matrix at 77°K. exhibit half lives which range from ten minutes to a week. Observations of radical concentrations under varying conditions of temperature and isotopic substitution indicate that, while there is some decay by recombination, most of the radicals decay by hydrogen abstraction from the parent molecule that forms the matrix. The activation energy for this process has been determined for several radicals. A prominent asymmetric resonance which develops during the decay of the photolytically produced e.s.r. spectrum has been assigned to the methoxy radical.

Introduction

Previous work has established the identity of a number of free radicals which become trapped in a frozen methanol matrix during ultraviolet irradiation.[2] In the present study, electron spin resonance techniques are employed to determine the effects of time, temperature, and isotopic substitution on free radical concentrations. An evaluation of the factors influencing the relative stabilities of the radicals has provided a fairly clear picture of the modes of decay of the radicals produced by photolysis, and has permitted the study of the methoxy radical, which appears as an end product of free radical reactions occurring in the solid phase at low temperature.

Experimental

A Varian Associates Model V 4500 ESR spectrometer employing 100 kc. modulation was used to record the first derivative of the absorption spectrum. The liquid nitrogen dewars and the variable temperature dewar also were supplied by Varian. The magnetic field was monitored with a Harvey-Wells Electronic Inc. Model G-501 gaussmeter. The g-values of the spectra were determined by comparison with DPPH for which the g-value is accurately known to be 2.0036. Estimates of radical concentrations were made by comparison with a weighed sample of DPPH. It is known that the integrated absorption curve is proportional to the height x (the peak-to-peak distance),[3] where the peak-to-peak distance is measured from the maximum to the minimum of the first derivative curve. The signal intensity is therefore proportional to the signal height, and the half lives of the various radicals were determined from plots of the logarithm of signal height *versus* time.

All irradiations were made with a low pressure mercury lamp, with Vycor filter employed to eliminate the 1849 Å. mercury line. (Use of the filter necessitated a 30-minute irradiation period to provide convenient concentrations of radicals, in contrast to a 15-minute irradiation period which was satisfactory using unfiltered light.) Irradiations with and without the filter showed no other difference, with one exception. Unfiltered light, used to irradiate CD_3OH, produced the septet of the CD_3 radical, as well as a broad line identified with the CD_3O radical. The CDO radical also appeared. However, if the filter was used, the singlet of the methoxy radical was not present as an irradiation product, but appeared later, as will be described.

Samples of liquid methanol were usually placed in silica tubes at room temperature, frozen in a liquid nitrogen Dewar, and irradiated for 30 minutes before being placed in a second Dewar situated in the microwave cavity. This arrangement permitted a high intensity of actinic light to reach the sample and resulted in greatly increased concentrations of radicals over those previously reported.[2]

Several variations on this procedure were employed in order to check such possible effects as stabilization of radicals on the glass surface and radical reactions involving oxygen dissolved in the sample.

A deposit of alcohol, frozen on a central support, was suspended in the liquid nitrogen dewar during irradiation, and then suspended in a dewar in the e.s.r. cavity. A sample prepared in this way produced spectra which could be interpreted without question of additional signals from the glass or surface reactions

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(2) P. J. Sullivan and W. S. Koski, *J. Am. Chem. Soc.*, **84**, 1 (1962).

(3) F. J. Adrian, E. L. Cochran and V. A. Bowers, *J. Chem. Phys.*, **36**, 1661 (1962).

at the glass contact. It was found that this treatment made no change in the e.s.r. spectra.

Another sample of alcohol was carefully outgassed to remove dissolved oxygen. Nitrogen was bubbled through the alcohol heated to the boiling point; the alcohol was then placed on a vacuum line and resublimed several times, after each sublimation the system was pumped to a pressure of 10^{-5} mm. This sample was irradiated and the e.s.r. spectrum recorded as usual. Both the initial spectrum and the free radical reactions taking place during warm-up were the same as those in samples which were not outgassed.

For the purpose of the following discussion, it will be helpful to enumerate the radicals present in large quantity immediately after irradiation, under the conditions described above. In CH_3OH and CH_3OD, these were the formyl radical, CHO, and the methyl radical, CH_3. Smaller quantities of the methanol radicals, CH_2OH and CH_2OD, were also present. In CD_3OH, the only radical present in large quantity was CD_3, with the smaller concentrations of CDO. The deuterated alcohols were made by Volk Radiochemical Co. of Chicago.

It must be emphasized that the experimental conditions described here differ in several ways from those previously reported,[2] and that the present study will be concerned only with radicals whose concentrations are sufficiently large to permit observation over a reasonably long time period.

Results

I. The Methyl Radicals, CH_3 and CD_3.—The methyl quartet in a CH_3OH matrix decayed with a 10–15 minute half life at 77°K., while CD_3 in CD_3OH appeared to be considerably more stable, having a half life of 2–3 days. The small oxygen-containing radical, CHO, decayed even more slowly, with a 6–8 day half life at 77°K. It was in an attempt to evaluate the factors determining these differences that the following observations were made.

At initial concentrations in the range of 10^{16} to 10^{17} spins per cc., the methyl radical could be observed for more than an hour at 77°K., despite the rapid decay of the signal (see Fig. 1). The temperature of a sample of irradiated CH_3OH could be raised about 10° without any marked change in the rate of disappearance of the quartet, indicating a decay process with very low activation energy. Above 90°K., the rate of decay became very rapid, but this effect was originally thought due to some change occurring during warm-up in the rapidly frozen matrix. While the methyl radical decayed, the over-all concentrations of radicals in CH_3-OH decreased, and no new signals developed. It was concluded that the predominant mode of decay of CH_3 was radical recombination. This could be reconciled with apparent first-order kinetics by assuming that the methyl radicals, diffusing through the matrix, recombine in large part with other radicals present in relatively large amounts in the matrix.

A problem was immediately evident. If the CD_3 radical also decayed by radical recombination, the half life might show some increase due to a small secondary isotope and, probably, second-order kinetics since the concentrations of radicals other than CD_3 were small in that case, but an increase in half life from 10 minutes to 2 days seemed wholly unreasonable. On the other hand, if the decay process were hydrogen abstraction
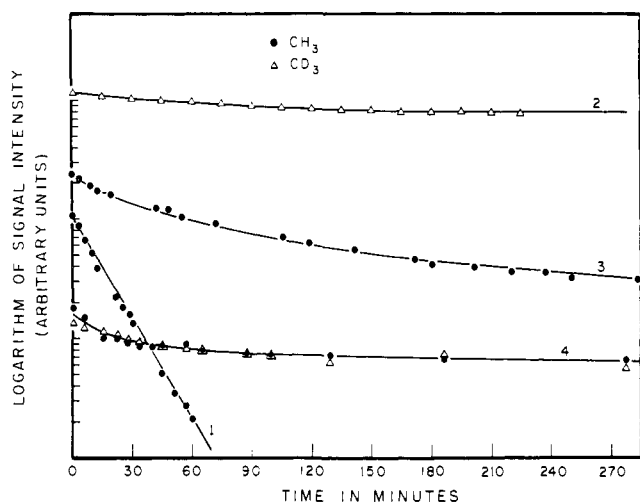
Fig. 1.—Curve 1 shows the decay of CH_3 radicals produced by irradiation in a CH_3OH matrix at 77°K. Curve 2 is for CD_3 in CD_3OH under the same conditions. Curve 3 is for CH_3 radicals in methyl acetate. Curve 4 shows the decay of both CH_3 and CD_3 radicals produced in a mixture of 10% CH_3OH in CD_3OH. All are at 77°K.

Fig. 2.—E.s.r. spectra of radicals in CH_3OH, CD_3OH, C_2H_5-$OOCH_3$, and a mixture of 10% CH_3OH in CD_3OH immediately after irradiation. The more prominent radicals are marked in the line reconstructions: (A) CH_3OH, (B) CD_3OH, (C) methyl acetate, (D) 10% CH_3OH in CD_3OH. (The two central lines of the CH_3 spectrum are hidden under the lines of the CD_3 spectrum.) All samples were irradiated for 30 minutes at 77°K. and recorded immediately.

in the CH_3OH matrix, and deuterium abstraction in the CD_3OH matrix, a primary isotope effect could explain a stabilization factor of 100 or more at 77°K. It will be seen, however, that the conclusion that CH_3 in CH_3-OH decayed by radical recombination was considered in the light of further evidence to be essentially correct, and that, unexpectedly, the crucial factor in the different initial behavior of CH_3 and CD_3 was the concentrations of other radicals in the matrix.

The difference in radical concentrations between CH_3OH and CD_3OH may be seen in Fig. 2-A and 2-B. An isotope effect in irradiation, which favors the rupture of a bond to hydrogen rather than to deuterium, drastically affects the relative concentrations of radicals in the two otherwise similar compounds. In ordinary methanol, the CHO radical usually was present in concentrations an order of magnitude greater than those of the methyl radical, while in CD_3OH, there was little tendency for deuterium to be removed during irradiation and consequently relatively little production of CDO.

The decay curve for CD_3 in CD_3OH had a very slight initial curvature, then straightened out to show apparent first-order kinetics (Fig. 1). This was interpreted to mean that during the time interval marked by the curved portion, some decay by recombination occurred, but that after a reduction in radical concentration, the only important decay process was hydrogen abstraction from the matrix, showing pseudo first-order kinetics. This conclusion was supported by measurements of the decay rate at several temperatures which yielded an activation energy for decay of about 6 kcal./mole. In addition, a large methoxy radical signal developed during the decay of the CD_3 septet.

It was considered desirable to study the methyl radicals under a variety of conditions, and two other relevant experiments were performed. Irradiation of methyl acetate produced the methyl quartet, with only small quantities of the CHO radical and an underlying triplet (Fig. 2-C). The decay curve for CH_3 in this matrix is included in Fig. 1. The straight line portion of the curve corresponds to a half life of about two hours. This curve was also interpreted to indicate some decay by recombination, after which hydrogen abstraction becomes the important decay process. The underlying triplet increased in size as the methyl signal decayed. An activation energy of 4–5 kcal./mole was found for hydrogen abstraction by CH_3 in
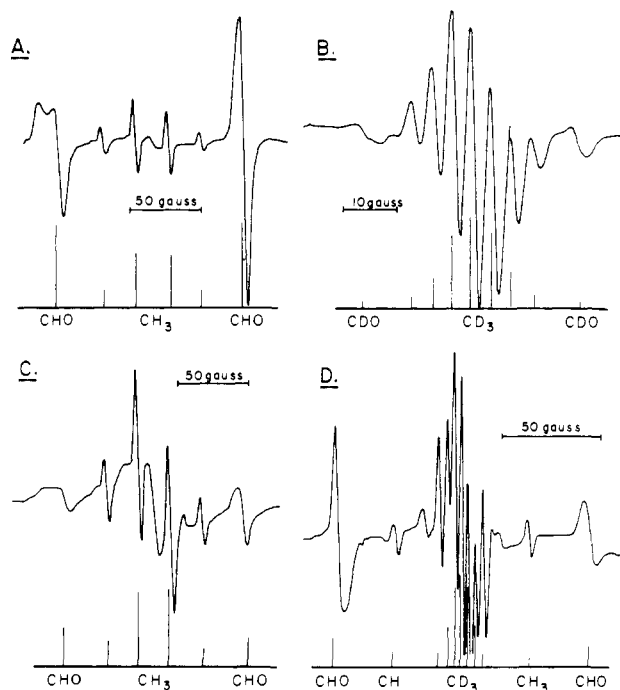
methyl acetate. If a similar rate of decay by hydrogen abstraction is assumed for CH_3 in a methanol matrix, occurring simultaneously with the radical recombination reaction, then the rapid disappearance of the radical above 90°K. may be ascribed to the rate of decay by hydrogen abstraction becoming more rapid than the diffusion-controlled rate of decay for radical recombination.

To obtain a more direct comparison of the behavior of CH_3 and CD_3, a mixture of about 10% CH_3OH in CD_3OH was irradiated. The signals of both methyl radicals were thus obtained in a matrix containing moderate concentrations of CHO and other radicals (Fig. 2-D). The decay curves in this case show the curvature thought to be associated with the radical recombination process, then straighten out to show a half life of about 8 hours (see Fig. 1). This seems reasonable for decay by hydrogen abstraction in a matrix containing more than 50% deuterium. The important result is that the decay of CH_3 and CD_3 is very similar when both radicals are present in the same matrix.

II. The Oxygen-containing Radicals, CHO, CH_2OH, and CH_2OD.—The formyl and methanol radicals decayed at about the same rate, with a half life of 6–8 days at 77°K. The decay of these radicals may be observed in Fig. 3, where a sample of CH_3OD has been chosen to illustrate typical results. In the sequence shown, the temperature has been raised to hasten the decay of the less stable radicals, but the same reactions take place, though more slowly, at liquid nitrogen temperature. Figure 3-A shows the spectrum of CH_3-OD immediately after irradiation. Comparison with Fig. 2-A indicates that the isotope effect in irradiation has resulted in a smaller concentration of formyl radicals in the case where production of CHO involves the removal of the deuteron at the hydroxyl position.

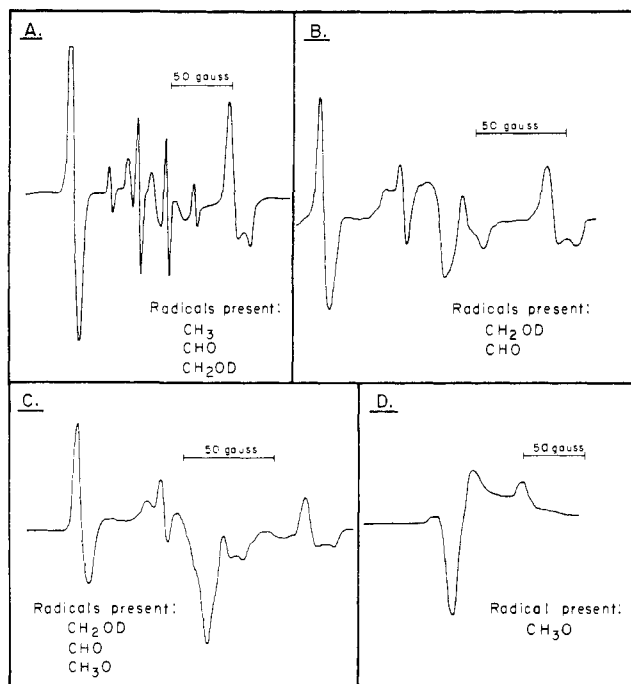In Fig. 3-B, the quartet has completely disappeared, leaving the formyl lines and the distorted triplet of the

Fig. 3.—Decay of radicals in CH₃OD: (A) CH₃OD irradiated for 30 minutes at 77°K. and recorded immediately; (B) same as A, 30 minutes after irradiation, T is 97°K.; (C) same as A, 1 hour after irradiation, T is 120°K.
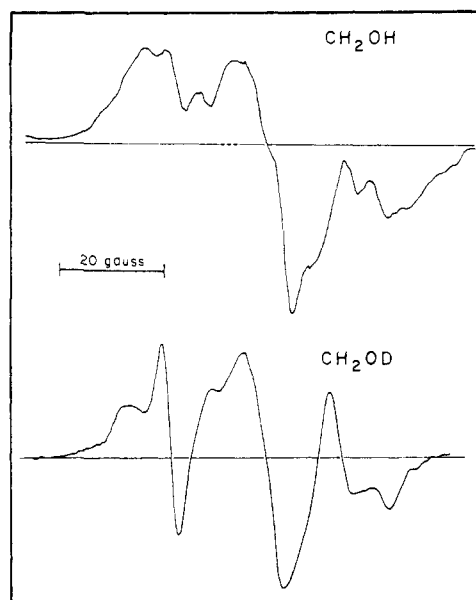

Fig. 4.—Comparison of e.s.r. spectra of CH₂OH and CH₂OD.

CH₂OD radical. Here the magnetic field has been swept more slowly and the amplitude of the signal increased slightly. The most marked change, apart from the disappearance of the methyl radical, is the decrease in size in the signal of the formyl radical. At 77°K. also, there is a relatively rapid decrease in the formyl signal during the decay of the quartet, after which the decay is quite slow. In Fig. 3-C, a single line has begun to distort the center of the spectrum. A reaction taking place in the solid phase results in the appearance of the methoxy radical as an end product. Apparently the CHO and CH₂OD radicals are partially immobilized in the matrix by hydrogen bonding; and after the complete decay of the methyl radicals they can only decay by hydrogen abstraction from the matrix. In Fig. 3-D, only the asymmetric singlet of the methoxy radical remains. In Fig. 3-A and 3-D,
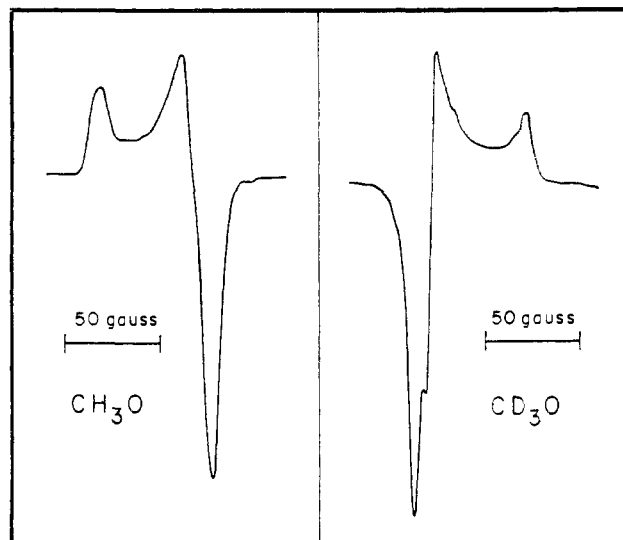

Fig. 5.—The methoxy radicals.

the machine conditions were exactly the same. Comparing the signals in 3-A and 3-D, it appears that after some pairing of spins during radical recombination the unpaired spins lost by hydrogen abstraction show up as unpaired spins in the methoxy radical. An activation energy of 6 kcal./mole was found for the decay of the formyl radical by hydrogen abstraction.

The methanol radicals are shown in detail in Fig. 4. Comparison of the spectra indicates that the triplet of the CH₂OH radical has an additional splitting due to the hydroxyl hydrogen, which is measured as about 4 gauss. In CH₂OD, the influence of the deuteron is too small to be observable. The lower spectrum exhibits an asymmetry which is thought to arise from the same causes as the asymmetry of the formyl doublet.[3] Both g-value anisotropy and magnetic dipole anisotropy are present, and these effects add on one of the outer components of the triplet, and subtract on the other. The center component probably shows g-value anisotropy only, a fact which may explain the difficulty in observing the corresponding deuterated radical, CD₂OH. For this radical, the line-to-line splitting is appreciably less than the line width due to g-value anisotropy.

III. The Methoxy Radicals, CH₃O and CD₃O.— The methoxy radicals were relatively stable at 77°K. Samples stored for more than a week revealed little or no weakening of the methoxy signal. The temperature had to be raised to 150°K. before the disappearance of the signal became rapid.

CH₃O and CD₃O show an interesting variation in their spectra (Fig. 5). The CH₃O radical appears to have the two g-values characteristic of a radical of axial symmetry and might lead to some doubt as to the spatial orientation of the orbital containing the unpaired electron. The spectrum of the CD₃O radical, however, has the seven points of inflection corresponding to three g-values,[4] and proves that the radical is not, magnetically speaking, axially symmetric.

The observed g-value shifts compared to the free electron g-value are qualitatively in good agreement with values predicted by McConnell and Robertson.[5] Assuming a Z axis along the C–O bond, an X axis perpendicular to the plane containing the C–O bond and the orbital of the unpaired electron, and a Y axis approximately parallel to the orbital of the unpaired electron, the experimental g-values may be assigned as follows. A large positive shift in the g-value, g_x =

(4) F. K. Kneubühl, *J. Chem. Phys.*, **33**, 1074 (1960).

(5) H. M. McConnell and R. E. Robertson. *J. Phys. Chem.*, **61**, 1018 (1957).

2.034, occurring when the magnetic field lies along the Z axis, arises from the promotion of one of the non-bonding electrons into the half-filled orbital of the unpaired electron. A small positive g-value change, $g_x = 2.007$, probably results from the promotion of an electron from the C–O bond into the half-filled orbital. The undeviated g-value, $g_y = 2.002$, corresponds to a magnetic field parallel to the orbital of the unpaired electron.

Conclusion

The results of the stability studies may be summarized briefly. Methyl radicals, which apparently diffuse through the matrix, disappear both by recombination and by hydrogen abstraction. If over-all radical concentrations are very large, the recombination reaction predominates; otherwise the hydrogen abstraction is more important. Small radicals, such as CHO and CH_2OH, are apparently immobilized to a considerable extent in the matrix by hydrogen bonding, and after the complete disappearance of the methyl radicals, decay by hydrogen abstraction from the matrix. The methoxy radical appears as an end product of this reaction.

The presence of methyl radicals in the photolysis of methanol with 2500 Å. radiation deserves comment since their presence is unexpected because of the low absorption in this spectral region. There may be some possibility of the CH_3 radicals being produced by residual 1849 Å. light but this appears to be unlikely. In view of the role that trace amounts of impurities have played in radiation chemistry it is reasonable to suspect such a cause. However, irradiation of alcohol samples to which small amounts of suspected impurities such as aldehydes, ketones, etc., have been added failed to produce sufficiently high concentrations of methyl radicals to account for the observations reported here. There is also the possibility that the methyl radicals arise from some secondary process. In fact in the approach used in this study it is not feasible to isolate the primary photolytic effect from secondary processes such as successive photolysis or reactions of hot primary radicals with other fragments or the matrix.

It may also be noted that the assignment of the resonance shown in Fig. 5 to the methoxy radical is not completely unambiguous since a similarly shaped resonance would be expected for an appropriate peroxy radical. Experiments with carefully deoxygenated samples, however, show that, if the radical is of the peroxy type, it does not result from reaction with dissolved oxygen.

Hydration of Deoxyribonucleic Acid. II. An Infrared Study[1]

By Michael Falk,[2] Karl A. Hartman, Jr., and R. C. Lord

The infrared spectrum of deoxyribonucleic acid (DNA) has been studied in the region of 4000 to 400 cm.$^{-1}$ as a function of relative humidity. From frequency and intensity changes of infrared bands it is concluded that the $PO_2^-Na^+$ groups of DNA become hydrated in the range of 0 to 65% relative humidity, while the hydration of the bases begins above this range. The strength of hydrogen bonding is greatest for the first water molecules to adsorb and decreases thereafter, approaching the strength of hydrogen bonding of liquid water.

Introduction

Deoxyribonucleic acid (DNA) is a large polymer molecule composed of many molecular subunits (purine and pyrimidine bases, deoxyribose, and diesterified phosphate groups) and gives rise to a complex infrared spectrum. A detailed assignment of every feature in the spectrum is not possible at present, but partial assignment of the more prominent bands is useful.

By comparison with the spectra of the constituent molecules many bands in the spectrum of DNA have been assigned to specific modes of vibration of particular groups of atoms. The first infrared studies of DNA were made by Blout and Fields[3] and by Blout and Lenormant,[4] who made many assignments. The infrared spectrum of DNA has been examined in detail by Sutherland and Tsuboi,[5] who were the first to report the changes in the spectrum of solid DNA films occurring as a function of relative humidity. Bradbury, Price and Wilkinson reported studies of DNA[6] as well as of nucleoprotamine[7] and nucleohistone[8] and were the first

to present plots of the dichroic ratio of several infrared bands as a function of the water content of DNA. They also reported a plot of the frequency of the band due to the antisymmetric stretching vibration of the PO_2^- group of DNA against the water content of the specimen. Several new assignments were made by Tsuboi,[9] who also listed the changes in the spectrum of solid DNA upon dehydration and showed that some of the changes are similar to those which occur when DNA is denatured in solution by heating,[9] by treatment with deoxyribonuclease,[9] or by treatment with formamide.[10]

In the present study we have investigated the infrared spectra of solid films of lithium and sodium salts of DNA (NaDNA and LiDNA) in both deuterated and undeuterated form, in the range 4000 to 400 cm.$^{-1}$. Both polarized and non-polarized spectra were recorded as a function of relative humidity (r.h.) with special care taken to attain equilibrium. As we are largely in agreement with the frequencies and assignments reported by previous investigators, only the *changes* in the infrared spectrum of DNA brought about by hydration and dehydration will be described, particularly those changes which provide information about the ranges of relative humidity in which various molecular subgroups

(1) This work was supported by Grant No. A-2262(C3) from the National Institutes of Health, Public Health Service.

(2) Atlantic Regional Laboratory, National Research Council (Canada), Halifax, Canada.

(3) E. R. Blout and M. Fields, *Science*, **107**, 252 (1948); *J. Biol. Chem.*, **178**, 335 (1949); *J. Am. Chem. Soc.*, **72**, 479 (1950).

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(7) E. M. Bradbury, W. C. Price and G. R. Wilkinson, *ibid.*, **4**, 39 (1962).

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(10) Y. Kyogoku, M. Tsuboi, T. Shimanouchi and I. Watanabe, *J. Mol. Biol.*, **3**, 741 (1961).