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Deuterium Substitution in an Electron Spin Resonance Study of Radiation-induced Free Radicals^{1a}BY ICHIRO MIYAGAWA^{1b} AND WALTER GORDY

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Effects of deuterium substitution on the electron spin resonance of X-irradiated organic compounds were investigated. The compounds range from carboxylic acids, amino acids and amides to proteins. Substitution of deuterium for the hydrogens of polar groups—hydroxyl, carboxyl and amino—showed that the hydrogens of these groups generally do not contribute, or give only a slight contribution, to the hyperfine structure of the resonance pattern. Exceptions were found, however, notably formic acid and glycine. Most often the hydrogens bonded to carbon gave the hyperfine structure of the e.s.r. patterns. In some instances the additional information provided by deuterium substitution allowed a fairly definite identification of the free radicals.

Introduction

Electron spin resonance patterns exhibiting proton hyperfine structure have been observed for a number of X-irradiated organic substances containing NH, NH₂ or OH as well as hydrocarbon groups.²⁻⁵ In many of these it was not certain whether the hydrogens giving rise to the hyperfine structure were bonded to C, O or N. In most of them, the hydrogens attached to N or O can be replaced by deuterium simply by repeated treatments with D₂O, whereas the hydrogens attached to carbon are not replaced by this method. Thus one can readily substitute D for H in carboxylic, amine or amino groups without replacing the hydrogens attached to the carbons. In this way one can learn whether the hydrogens bonded to carbons interact with the electron spin of the free radical.

The proton and deuteron have different spins, $1/2$ and 1, respectively, also different magnetic moments, 2.79255 and 0.857354, respectively. For these reasons the structures which they impart to the electron spin resonance patterns are qualitatively different. A single interacting proton gives a doublet, whereas a deuteron substituted for it would give a triplet with components of equal intensity and with only about one sixth the spacing of the doublet. In general, the total spread of the hydrogen hyperfine structure of a given free radical will be about three times that of its completely deuterated counterpart.

Experimental Method

The direct exchange was done by 99.5% heavy water, and the treatment was repeated three times successively. For protein, each solution was kept at 35° for 48 hr., because a slow exchange has been observed with insulin.⁶ For HCOOD, the drying was done by anhydrous copper sulfate. The DCOOD and D₂CCOOD samples were prepared by thermal decomposition from deuterated oxalic acid (COOD)₂ and deuterated malonic acid D₂C(COOD)₂, respectively. The DCOOH sample was prepared by exchanging DCOOD with water. The CH₃COOD sample

was obtained by dropping acetyl chloride into heavy water. The purity was determined in some of the compounds by infrared analysis. The purity, which is shown in parentheses after each chemical formula, is: HCOOD (97%), DCOOH (95%), DCOOD (95%), CH₃COOD (88%) and DC₂COOD (88%).

Most of the measurements were done at 23 kMc./sec., but some were done also at 9 and 34 kMc./sec. The spectrometer was the same as that employed in the previous studies.²⁻⁵ The magnetic field was modulated at 45 c./sec., and the first derivative of the actual absorption curve was recorded on curved coordinate paper. The magnetic field increases from left to right in all the absorption curves shown.

The irradiation was given by a 50 kc. X-ray machine. Dosages of a few million roentgens were employed.

Formic and Acetic Acids.—When X-irradiated and observed at 77°K, both formic and acetic acid were earlier found to give doublets,^{2b} but that of formic acid was measured to be of about 12 gauss separation while that of acetic acid was found to be of the order of 25 gauss. Both patterns underwent changes when the samples were allowed to warm. It was suggested that the doublet first observed might arise from an interaction with the hydroxyl hydrogen.^{2b} Deuterium substitution provides a critical test of this assumption.

In the present work observations were made on both partially and completely deuterated samples of formic acid. The sample of HCOOD, X-irradiated and observed at 77°K showed a doublet of somewhat wider spacing than the 12 gauss originally observed for HCOOH but with a new central component (see Fig. 1). A remeasurement of the HCOOH with low amplitude modulation revealed that the doublet originally reported is probably a superposition of two incompletely resolved doublets, one of the order of 12 gauss and the other of the order of 18 gauss separation. The 12-gauss doublet collapses into a central component in HCOOD, but the outer doublet of 18 gauss appears to remain unchanged. This central component actually should be a triplet with component spacings of about 2 gauss, but such a small splitting would not be resolvable. Thus the deuterium substitution indicates that the 12-gauss doublet arises from the carboxyl H and the wider doublet from the CH hydrogen. Furthermore, the two doublets evidently arise from different radicals, for they do not have the same relative intensities under all conditions. The structure of the resonance for DCOOH should be a superposition of a closely spaced triplet and a 12-gauss doublet and is not expected to be resolvable.

(1) (a) This research was supported by the Office of Ordnance Research, Department of the Army, and by the United States Air Force through the Air Force Office of Scientific Research and Development Command. Reproduction in whole or in part is permitted for any purpose of the United States Government. (b) The Institute for Solid State Physics, The University of Tokyo, Tokyo, Japan.

(2) (a) W. Gordy, W. B. Ard and H. Shields, *Proc. Natl. Acad. Sci.*, **41**, 983 (1955); (b) W. Gordy, W. B. Ard and H. Shields, *ibid.*, **41**, 996 (1955).

(3) G. McCormick and W. Gordy, *J. Phys. Chem.*, **62**, 783 (1958).

(4) H. Shields and W. Gordy, *ibid.*, **62**, 789 (1958).

(5) C. F. Luck and W. Gordy, *THIS JOURNAL*, **78**, 3240 (1956).

(6) K. Linderstrom-Lang, Symposium on Peptide Chemistry, No. 2 the Chemical Society, London, 1 (1955).

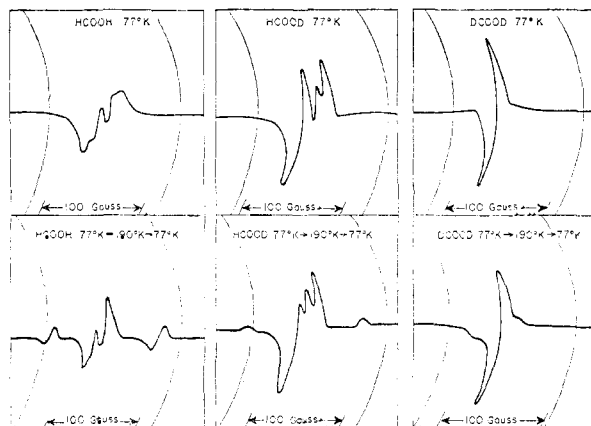


Fig. 1.—Comparison of the e.s.r. patterns (first derivative curves) at 9 kMc./sec. of various isotopic species of X-irradiated formic acid. The top curves at 77°K. were obtained immediately after irradiation at that temperature; the lower curves were obtained after the samples irradiated at 77°K. were warmed to 190°K. and again cooled to 77°K.

An unresolved resonance (not shown) of about 12 gauss in width was observed for this form. Likewise, the resonance for the completely deuterated formic acid, DCOOD, is a single line (Fig. 1) as would be expected, since its closely spaced hyperfine structure would not be resolvable.

In the earlier work it was found that when HCOOH irradiated at 77°K. was warmed to Dry Ice temperature, 190°K., it gave a new doublet of 135 gauss separation.² It was suggested that this doublet might arise from the free radical HCO formed as a secondary radical while the sample warmed. The present studies on deuterated samples are favorable to this interpretation in that they show that the structures arise from the CH hydrogen rather than from the carboxyl hydrogen. The 135-gauss spacing is unchanged when HCOOH is altered to HCOOD. See the weak outer components in the 190°K. curves for HCOOH and HCOOD in Fig. 1. A corresponding triplet with total spread of 40 gauss would be expected for the DCO free radical produced from the DCOOH or DCOOD. The shoulders which appear on the curves for DCOOD when it is warmed to Dry Ice temperature (see Fig. 1) could be the outer components of the triplet. The central component would be obscured by the stronger resonance which is superimposed.

Thus, in irradiated formic acid, doublets from three different free radicals seem to have been observed. The deuterium substitution proves that two of them, the 135-gauss doublet and the 18-gauss doublet, arise from the CH proton. As earlier proposed, the 135-gauss doublet may arise from the HCO free radical.

We suggest that the 18-gauss doublet arises from a radical of the general form HCRR' which may conceivably have one of the similar chemical forms I, II or III

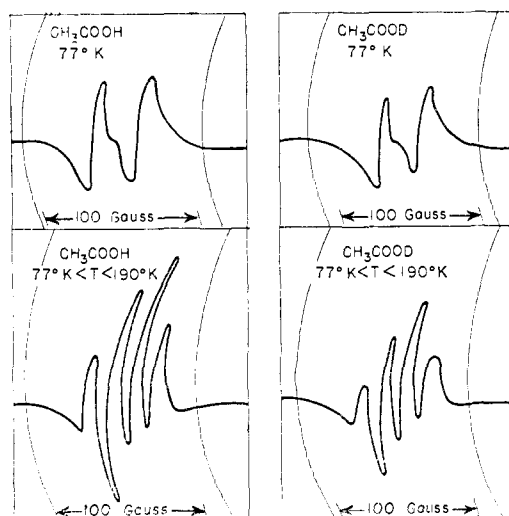
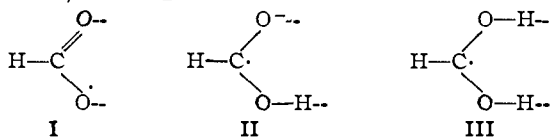


Fig. 2.—Comparison of the e.s.r. patterns (first derivative curves) at 9 kMc./sec. of X-irradiated CH₃COOH and CH₃COOD. The top curves were obtained at 77°K. immediately after irradiation at that temperature; the lower curves were obtained after the same samples had warmed to temperatures between 77° and 190°K.

any one of which might be produced readily by the irradiation from the formic acid dimer. The dotted lines in the structures indicate hydrogen bonds to neighboring molecules. There are two equivalent structures of form I in which the double bond and the unpaired electron are exchanged between the two oxygens. In form I the unpaired electron is mainly on the oxygen atoms, and the spin density on the carbon is very small, probably too small to allow significant interaction with the CH hydrogen. In forms II and III the electron spin density is concentrated mainly on the carbon, and the coupling to the CH hydrogen should be near that of the hydrogen in the free methyl radical,^{7,8} which is about 25 gauss. The measured doublet splitting of 18 gauss is in satisfactory agreement. The reduction in the coupling below that for the methyl radical indicates that about 30% of the spin density is concentrated on the oxygens.

From the e.s.r. patterns we cannot distinguish between II and III, although III is to be preferred because it is electrically neutral. In II, however, the negative oxygen might be largely neutralized by the partially ionic hydrogens which form hydrogen bridges to it.

The deuterium substitution indicates that the formic acid doublet of 12 gauss separation results from the splitting of the carboxylic hydrogen, and it may arise from the free radical, O=C—O—H. However, the additional information provided by deuterium substitution is still insufficient to provide unquestionable identification of the chemical formulas of the radicals.

The doublet found for acetic acid when X-irradiated and observed at 77°K. is unchanged when deuterium is substituted for the hydrogen bonded to oxygen (Fig. 2). It is apparently the same as

(7) W. Gordy and C. G. McCormick, *THIS JOURNAL*, **76**, 3243 (1956).

(8) B. Smaller and M. S. Matheson, *J. Chem. Phys.*, **28**, 1169 (1958).

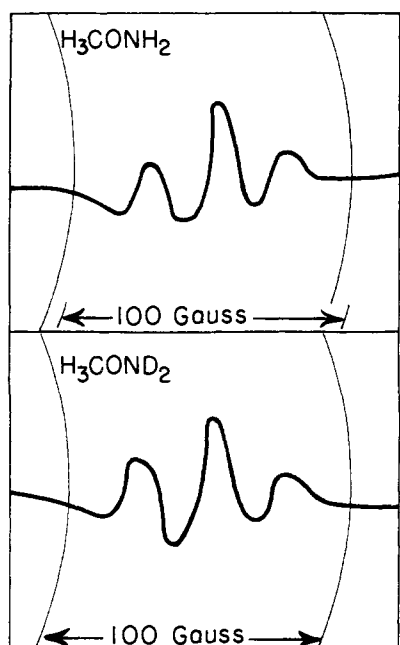
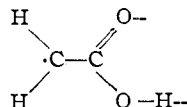


Fig. 3.—Comparison of the e.s.r. patterns (first derivative curves) at 23 kMc./sec. of X-irradiated H_3CONH_2 and H_3COND_2 at room temperature.

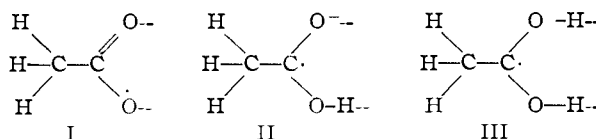
that previously reported^{2b} for CH_3COOH . The doublet spacing observed here is about 30 gauss. When the sample is warmed above 77°K . the doublet of CH_3COOD changes to a closely spaced quartet such as was observed for CH_3COOH (Fig. 2). The pattern changes further to a triplet^{2b} with total spread of about 50 gauss when warmed further to 190°K . and recooled to 77°K .

The triplet pattern must arise from equivalent CH_2 proton coupling, and it probably arises from the free radical similar to that proposed for ir-



radiated acetamide below. As expected for this type of radical the observed splitting per proton, 25 gauss, is like that for the free methyl radical.^{7,8}

An interesting feature of these results is the doublet observed for CH_3COOD at 77°K . This doublet splitting cannot be due to D and must arise from a hydrogen of the CH_3 group. One might postulate that a hydrogen of the CH_3 group breaks off and becomes attached to something else, to form a radical with a single coupling proton. However, this does not seem probable, nor would it explain the formation of the quartet when the sample is warmed. Originally, the quartet was attributed to the radical CH_3CO_2 , and the present observation of the quartet for CH_3COOD is in agreement with this interpretation. We seem to require a free radical which can be produced by irradiation at 77°K . of either CH_3COOD or CH_3COOH , which at that temperature gives a doublet and which when warmed somewhat above 77°K . gives a closely spaced quartet. Plausible free radicals which meet these requirements are I, II or III analogous to those proposed for irradiated



formic acid. Such radicals could conceivably be formed directly from the hydrogen-bonded acetic acid dimer. The dotted lines indicate hydrogen bonds to neighboring molecules.

The coupling to the CH_3 hydrogens in each of the above forms would occur through hyperconjugation. However, this method of coupling would seemingly be more effective for II and III than for I, in which the spin density would be concentrated mainly on the two oxygens. For forms II and III the averaged coupling per proton of the CH_3 group should be of the same order but somewhat less than that for the CH_3 group of the free ethyl radical, 25 gauss.^{7,8} The averaged coupling for the CH_3 group in irradiated acetic acid is 15 gauss, or only 60% as much. This reduction indicates concentration of spin density on the oxygens of the order of 40%. The difference from the lesser amount of 30% found for formic acid is probably not of significance.

If the CH_3 group were to rotate rapidly about the CC axis, the above free radicals should have equal coupling of all three hydrogens. If, however, this group were to cease rotation at 77°K . and if one of the protons were to come to rest in a plane perpendicular to the CCO_2 plane, only this proton should have significant coupling, because the odd electron would migrate primarily in the π molecular orbital, which is perpendicular to the CCO_2 plane. The coupling of the CH proton, which occurs through hyperconjugation, varies approximately as the square of the cosine of the dihedral angle between the CH bond and the symmetry plane of the π orbital.⁹ Thus the coupling, A , of each proton can be expressed as

$$A = A_0 \cos^2 \theta$$

where A_0 is the coupling of the proton for which the CH bond is in the plane of symmetry of the π bond and therefore perpendicular to the CCO_2 plane. We assume that the proton which gives the 30 gauss doublet at 77°K . is in the π plane, with $\theta = 0$. We thus obtain from the observed doublet splitting a value for A_0 of 30 gauss. The other two CH bonds would lie outside the π plane by 60° and thus should give couplings of only 7.5 gauss. This splitting is of the order of the line width and hence would not be resolved.

When the CH_3 group begins to rotate relative to the COO group, all three hydrogen nuclei will couple equally to the electron spin. We can estimate the coupling expected for each by averaging the coupling, A , over the complete cycle. Thus

$$A_{\text{av.}} = \frac{\int_0^{2\pi} A_0 \cos^2 \theta d\theta}{\int_0^{2\pi} d\theta} = \frac{A_0}{2}$$

With $A_0 = 30$ gauss, as determined by the doublet splitting at 77°K ., we obtain $A_{\text{av.}} = 15$ gauss for each of the three protons. One would thus ex-

(9) C. Heller and H. M. McConnell, *J. Chem. Phys.*, **42**, 1535 (1960).

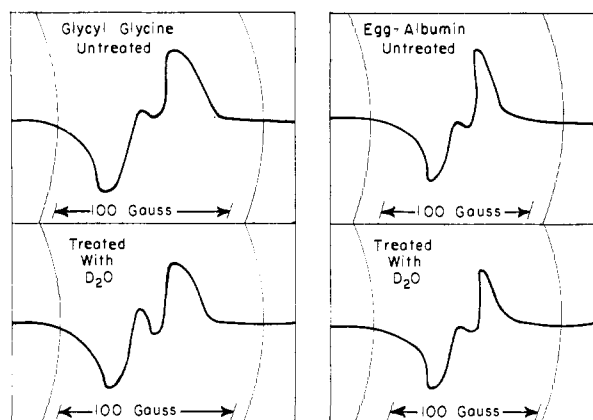
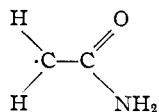


Fig. 4.—Comparison of e.s.r. patterns (first derivative curves) at 23 kMc./sec. of X-irradiated glycylglycine and egg albumin with samples which had been treated with D_2O . The irradiation and observations were made at room temperature.

pect a quartet with component spacing of 15 gauss, or a total spread of 45 gauss at the temperature at which the CH_3 rotation occurs. This is in good agreement with the observed pattern for $190^\circ K$. The consistency of the results for 77 and $190^\circ K$. provides convincing evidence for a free radical having the general form CH_3CRR' , which includes I, II or III. The specific structure, however, cannot be ascertained from these results.

Acetamide.—Acetamide, CH_3CONH_2 , irradiated and observed at room temperature, gives a triplet e.s.r. pattern.⁵ This pattern is unchanged when D is substituted for H in the amino group. Compare the resonances for CH_3CONH_2 and for CH_3COND_2 in Fig. 3. Therefore the triplet splitting arises from the H atoms bonded to carbon rather than from the NH_2 hydrogens. The component spacing in the triplet, 24 gauss, corresponds to that found in the free methyl radical.⁷ Hence, the probable form of the radical is



in which the NH_2 nuclei would not likely give a resolvable splitting. This radical would be bound by the usual hydrogen bridges to the amino and carbonyl groups of neighboring molecules.

Glycine and Alanine.—The absorption curve of glycine consists of five lines, in which the central three lines are much stronger than the outer two lines, as observed previously.^{2a} When glycine was deuterated to D_2NCH_2COOD , the outer weak doublet disappeared and a new absorption appeared, the spread of which is about 50 gauss. It is conclusive that coupling occurs between the unpaired electron and the hydrogen of the COOH group or the hydrogens of the NH_2 group, in addition to the CH_2 coupling. After this result was obtained, a single crystal of deuterated glycine was prepared and studied.¹⁰ More specific information was obtained thereby, but the particular form of the free radical, or radi-

(10) M. Katayama and W. Gordy, *Bull. Am. Phys. Soc., Series 2*, **4**, 253 (1960).

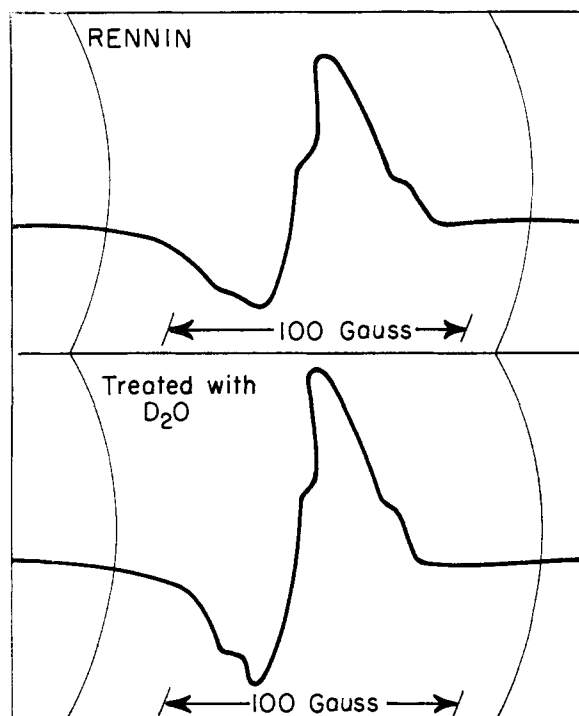


Fig. 5.—Comparison of the e.s.r. pattern (first derivative curve) at 23 kMc./sec. of X-irradiated rennin, untreated and treated with D_2O .

cal, is still uncertain. Ghosh and Whiffen¹¹ report evidence for N^{14} coupling from their results on a single crystal of normal glycine. Because of these more detailed studies on single crystals now available, we shall not elaborate our results further.

The first studies^{2a} of X-irradiated alanine in the powder form revealed a quintet pattern. Later detailed studies of a single crystal¹² showed the free radical to be CH_3CHR , where the R group has no nuclei giving resolvable splitting of the e.s.r. pattern and must presumably be NH_2 or $COOH$. In agreement with this interpretation, the powdered, D_2O -treated samples in the present studies gave a quintet pattern similar to that of the normal alanine, but the components appeared to be somewhat sharper in the deuterated samples. This is evidence that the hydrogens of the R group have sufficient coupling to broaden the lines.

Glycylglycine and Certain Proteins.—Earlier it was found^{2a} that a number of proteins when subjected to ionizing irradiation at room temperature give a common doublet resonance of about 20 gauss spacing and with a g factor of about 2.0036. The constituent amino acids of these proteins after similar irradiation gave varied patterns, all different from the protein doublet. Such a doublet was found, however, to be characteristic of glycylglycine, (glycyl)₂-glycine and (glycyl)₃-glycine, as well as of acetylglycine.

Originally,^{2a} the doublet resonance common to glycylglycine and certain proteins was thought to arise from an unpaired electron spin concentrated on an oxygen and interacting through dipole-dipole coupling to a proton of the hydrogen bridge.

(11) D. K. Ghosh and D. H. Whiffen, *Mol. Phys.*, **2**, 285 (1959).

(12) I. Miyagawa and W. Gordy, *J. Chem. Phys.*, **32**, 255 (1960).

Since deuterium readily can be substituted for the bridging hydrogens, we have sought to test this hypothesis by repeated treatment of the samples with D₂O.

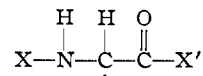
Powdered samples of glycylglycine and (glycyl)₂glycine have been treated with D₂O and then X-irradiated. Their resulting spin resonance patterns were found to be the same as those for the untreated samples. Comparative curves for glycylglycine are shown in Fig. 4. Similar comparisons were made of D₂O-treated and untreated samples of certain proteins—egg albumin, ovalbumin and pepsin—each of which upon irradiation at room temperature gives the characteristic doublet. Each was found to give the same doublet pattern after treatment with D₂O. The results are illustrated by the curves for egg albumin shown in Fig. 4. From these results we conclude that the characteristic doublet does not arise from the bridging NH hydrogen in the protein but from a hydrogen bonded to a CH hydrogen, which would not be replaced by the D₂O treatment.

It is interesting that rennin does not give the doublet resonance upon irradiation at room temperature but an incompletely resolved quartet. This pattern is not changed by treatment of the sample with D₂O (see Fig. 5). Thus the quartet structure must arise from protons bonded to carbon.

Measurements and analysis of the orientation-dependence of the doublet resonance in single crystals of untreated acetylglycine¹³ and of the orientation-dependence of the doublet resonance in silk strands and chicken feather quill¹⁴ indicate

(13) I. Miyagawa, Y. Kurita and W. Gordy, *J. Chem. Phys.*, **33**, 1599 (1960).

that this characteristic resonance arises from a free radical of the form



in which the electron spin is concentrated mainly on the α -carbon of the peptide chain. Here X and X' represent the remaining parts of the structure which do not measurably affect the resonance. The doublet splitting results, therefore, from the CH hydrogen, in agreement with the results reported here. Actually, our preliminary results on deuterium-substitution in glycylglycine and certain proteins were obtained before those of the orientation studies and were of assistance in the interpretation of the orientation effects.

A preliminary account¹⁵ of these studies was given earlier at a meeting of the American Physical Society. A similar study of deuterium substitution in the dicarboxylic series, HO₂C(CH₂)_nCO₂H, has been made by Box and Freund.¹⁶ We have also obtained results on these compounds, which will not be described since they indicate the same form of radicals as reported by Box and Freund, by Grant, Ward and Whiffen¹⁷ and by Heller and McConnell¹⁸ on single crystals.

(14) W. Gordy and H. Shields, *Proc. Natl. Acad. Sci. U.S.A.*, **46**, 1124 (1960).

(15) W. Gordy and I. Miyagawa, *Bull. Am. Phys. Soc. Series 2*, **4**, 227 (1960).

(16) H. C. Box and H. G. Freund, *ibid.*, **3**, 262 (1958).

(17) P. M. Grant, R. B. Ward and D. H. Whiffen, *J. Chem. Soc. (London)*, 4635 (1958).

(18) C. Heller and H. C. McConnell, *Bull. Am. Phys. Soc. Series 2*, **4**, 252 (1960).

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Radiolysis of Pentane in the Adsorbed State¹

BY J. W. SUTHERLAND AND A. O. ALLEN

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Pentane adsorbed on synthetic zeolites and on silica gel was exposed to γ -rays and yields of the various products were studied in detail as a function of pentane content. Radiolysis in the zeolite systems shows a number of peculiar features which depend on the nature of the positive ions present in the zeolite. Radiolysis of pentane adsorbed on silica gel is inhibited by the presence of small amounts of adsorbed salts. Skeletal isomerization is prominent in radiolysis of pentane on silica gel when the pentane content is low but is decreased by the presence of additional pentane or by lowering the temperature of irradiation. The results are discussed in terms of the behavior of excited electrons in solids.

Caffrey and Allen² found that the yields of products in the radiolysis of pentane by gamma rays were profoundly affected when the pentane was adsorbed on a chemically inert mineral solid of high surface area. Such effects are reminiscent of the effects of catalysts in directing reactions of organic compounds. Surface sensitization of radiolysis differs fundamentally from catalysis in that the reactions induced under radiation can as well move away from thermodynamic chemical equilibrium as toward it.

(1) Research performed under the auspices of the U. S. Atomic Energy Commission. A preliminary account of this work was presented at the Conference on the Uses of Large Radiation Sources in Industry, Warsaw, September, 1959, and will be published in the Proceedings of this Conference.

(2) J. M. Caffrey, Jr., and A. O. Allen, *J. Phys. Chem.*, **62**, 33 (1958).

The present work extends this study to a more detailed investigation of the silica gel-pentane system and to the radiolysis of pentane held in the lattice of synthetic zeolites or "molecular sieves." The zeolites have an open crystalline structure consisting essentially of a series of cages, the largest of which are connected together to form long tunnels through the material that can be penetrated by foreign molecules. Sodium ions, distributed through the cavities, can be replaced by other positive ions simply by immersing the substances in a salt solution. The zeolites therefore provide a large homogeneous adsorbing surface, the chemical nature of which is altered easily, and they seem particularly well suited to the systematic study of the effect of surface variables on radiolysis.