373. *The Free Radicals Formed by the Action of γ-Rays on Solid Glycine.*

By D. K. Ghosh and D. H. Whiffen.

Electron resonance spectra of γ-irradiated glycine and its deuterated derivatives are presented and their subsequent changes with time and heating are followed. $\dot{N}H_2$ appears to be the dominant radical product and there is evidence for at least one unidentified radical as well as the radical $^+H_3N\cdot\dot{C}H\cdot CO_2^-$ postulated in an earlier publication.

There is currently considerable interest in the products from the irradiation damage of biological materials and consequently of the simpler compounds from which they are built. One such compound is glycine, $^+H_3N\cdot CH_2\cdot CO_2^-$, and in an earlier communication [1] it was shown by interpretation of part of the electron resonance spectrum that one product from the action of γ-rays on glycine was the radical $^+H_3N\cdot\dot{C}H\cdot CO_2^-$. Evidence was obtained in the earlier work for the presence of a second species of radical, but its chemical nature was not suggested. Uebersfeld and Erb [2] also state that two radicals are present and suggest $R\cdot\dot{C}H_2$ for the dominant species. This suggestion has been repeated by Symons [3] who believes the structure to be $\dot{C}H_2\cdot CO_2^-$, whereas Gordy, Ard, and Shields [4] favoured $\dot{C}H_2\cdot NH_3^+$. Studies were undertaken with deuterated glycine to confirm these suggestions, but in the event the results suggest that the dominant radical is probably $\dot{N}H_2$.

Spectra and Discussion

Dominant Radical: probably $\dot{N}H_2$.—It is apparent from Figs. 1*a* and 2 that the radical which dominates the spectrum of freshly irradiated glycine gives three peaks of relative intensity 1 : 2 : 1 both for the polycrystalline material and for the single crystal. This finding is in agreement with earlier workers [4,5,6] on polycrystalline material. Such a pattern can only come from two hydrogen atoms. In all orientations of a single crystal the 1 : 2 : 1 intensity pattern remains, which shows that the two hydrogen atoms are fully equivalent. The plausible chemical structures for the radical do not possess such symmetry for a fixed radical orientation and it must be presumed that the equivalence is a consequence of rotational motion in the crystal.

Earlier workers [2,3,4] have presumed the hydrogens to belong to a $-\dot{C}H_2$ radical group, but the spectra of the deuterated species (Fig. 1) provide overwhelming evidence against this view. The corresponding radicals derived from $^+H_3N\cdot CD_2\cdot CO_2^-$ show a very similar triplet with identical spacing (compare Figs. 1*a* and *b*) whereas the radicals from $^+D_3N\cdot CH_2\cdot CO_2^-$ (Fig. 1*c*), like those from $^+D_3N\cdot CD_2\cdot CO_2^-$ (Fig. 1*d*) show only a single unresolved line. This is to be expected when hydrogen is replaced by deuterium, since the splitting due to the deuterium is only about one-seventh of that due to protium and could not be resolved in polycrystalline samples. Since it is unlikely that a deep-seated rearrangement has occurred, this is evidence that the two hydrogen atoms were originally attached to the nitrogen. It suggests that the radicals are either $^+H_2\dot{N}\cdot CH_2\cdot CO_2^-$ or $\dot{N}H_2$. Although the former radical seems inherently the more likely, there are difficulties in this attribution. By comparison with other electron resonance results,[3] it is extremely probable that such a radical would show strong coupling to three, if not to all four, hydrogen

---

[1] Ghosh and Whiffen, *Mol. Phys.*, 1959, **2**, 285.
[2] Uebersfeld and Erb, *Compt. rend.*, 1956, **242**, 478.
[3] Symons, *J.*, 1959, 277.
[4] Gordy, Ard, and Shields, *Proc. Nat. Acad. Sci., U.S.A.*, 1955, **41**, 983.
[5] Uebersfeld, *Ann. Phys.*, 1956, **1**, 395.
[6] Ehrenberg, Ehrenberg, and Zimmer, *Acta Chem. Scand.*, 1957, **11**, 199.

atoms. From the line widths of the spectra of Figs. 1*a* and 2 which show $\Delta H_{m.s.} \leqslant 26$ Mc./s. the coupling of the H(C) atoms must be less than 13 Mc./s. each. This is so small as to suggest quite strongly that the H(C) atoms are not part of the radical.

The electron resonance spectrum of the $\dot{N}H_2$ radical has been observed by Foner, Cochran, Bowers, and Jen [7] at 4·2° K in the products trapped after an electric discharge in ammonia and argon. Their hydrogen coupling of 67·03 Mc./s. may be compared with the isotropic coupling of the radical from glycine, 54 ± 5 Mc./s. However, at 4·2° K there is a strong coupling to the N atom of 28·90 Mc./s. which was not observed in the present experiments although it would have greatly modified the spectra of Fig. 2 had it been present. This discrepancy could be resolved if the relaxation time for the nitrogen nucleus were short compared to the reciprocal of the coupling constant. Under this inequality the electron–nitrogen coupling would not affect the observed spectrum. This disappearance of the coupling is analogous to the more familiar case which arises with the double nuclear resonance technique where one nucleus is " stirred " by a high-intensity radio-frequency field. The nuclear quadrupole coupling of the nitrogen to the framework of the radical might provide a mechanism for greatly reducing the relaxation time for rotating radicals. This might be inoperative at 4·2° K if the radicals were only rotating slowly in spherical cavities in the argon matrix. That the radicals are not fixed in the argon matrix is indicated by the narrow lines, without appreciable line broadening due to anisotropic couplings, which are shown in the figure of ref. 7.

FIG. 1. *The derivative spectra of freshly irradiated polycrystalline samples.* (*a*) $^+H_3N{\cdot}CH_2{\cdot}CO_2^-$; (*b*) $^+H_3N{\cdot}CD_2{\cdot}CO_2^-$; (*c*) $^+D_3N{\cdot}CH_2{\cdot}CO_2^-$; (*d*) $^+D_3N{\cdot}CD_2{\cdot}CO_2^-$.

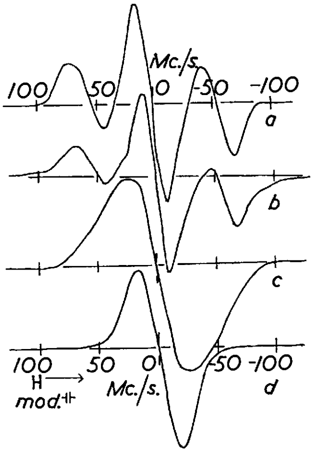
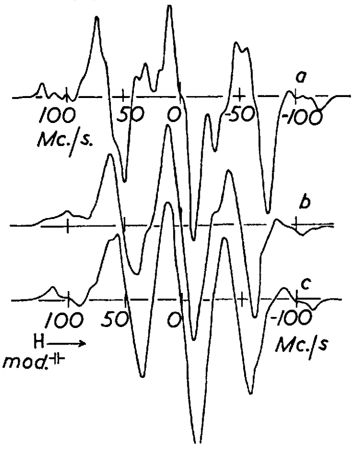


FIG. 2. *The derivative spectra of a freshly irradiated single crystal of* $^+H_3N{\cdot}CH_2{\cdot}CO_2^-$. *Magnetic field along the directions* (*a*) (1,0,0), (*b*) (0,1,0), (*c*) (0,0,1).



Because of underlying spectra due to other radicals accurate measurements on the freshly irradiated single crystal are not possible. Approximate measurements suggest that the coupling tensor of the hydrogen atoms has cylindrical symmetry about the (1,0,0) direction.* With the magnetic field along (1,0,0) as Fig. 2*a* the coupling constant is 62 Mc./s. for each hydrogen atom and in the perpendicular plane, as Figs. 2*b* and *c*, it is 50 Mc./s.

The lack of spherical symmetry shows that NH_2 rotates about only one axis and that this lies along (1,0,0) in the crystal. It is known [8,1] that the C–N bonds of the $^+H_3N{\cdot}CH_2{\cdot}CO_2^-$ molecules lie close to this direction. Nuclear resonance data [9] suggest that

---

* For definition of the co-ordinate system see ref. 1.

[7] Foner, Cochran, Bowers, and Jen, *Phys. Rev. Letters*, 1958, **1**, 91.

[8] Albrecht and Corey, *J. Amer. Chem. Soc.*, 1939, **61**, 1087.

[9] Kromhaut and Moulton, *J. Chem. Phys.*, 1955, **23**, 1673.

the NH_3^+ groups of glycine rotate at room temperature as does the NH_3^+ group of the $^+H_3N{\cdot}\dot{C}H{\cdot}CO_2^-$ radical.[1]

This explanation for the absence from the spectra of coupling to the nitrogen nucleus is supported by Fig. 3*a* which shows the spectrum of freshly irradiated polycrystalline glycine measured at 90° K. It is clear that the spectrum is considerably wider than that in Fig. 1*a*, indicating that a further coupling must be taken into account. Attempts to fit this spectrum with the coupling parameters of Foner, Cochran, Bowers, and Jen[7] were not successful, but the synthetic derivative curve of Fig. 3*b* is obtained for the nine lines to be expected with a hydrogen coupling of 60 Mc./s. each and a nitrogen coupling of 39 Mc./s. and a line width $\Delta H_{m.s.}$ of 36 Mc./s. This reproduces the main features of the spectrum but not its asymmetry consequent on a small anisotropic *g* variation. If the radical is really $\dot{N}H_2$ and this analysis is essentially correct,* there is a large variation of the nitrogen coupling with environment. It is 29 Mc./s. in an argon matrix and 39 Mc./s. in the glycine crystal. This coupling is known to be very sensitive to electronic wave function since the value[7] for $\dot{N}D_2$ is 33 Mc./s. in an argon matrix.

FIG. 3. (*a*) *The derivative spectrum of freshly irradiated polycrystalline* $^+H_3N{\cdot}CH_2{\cdot}CO_2^-$ *measured near* 90° K; (*b*) *synthetic derivative spectrum for couplings* N = 39 Mc/s., H = 60 Mc/s. *each*, $\Delta H_{m.s.}$ = 36 Mc/s. *with Gaussian lines at the positions indicated.*

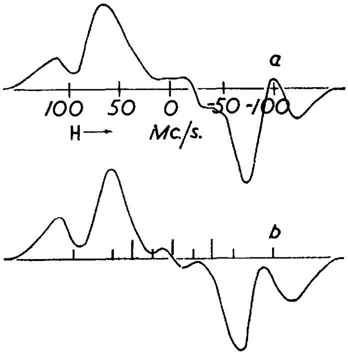
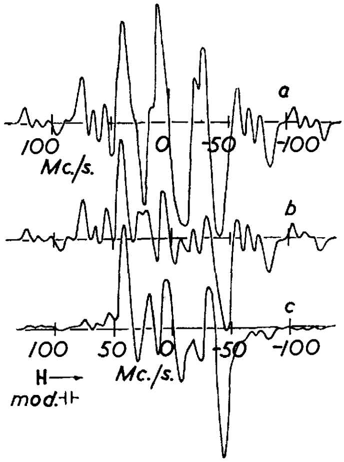


FIG. 4. *The derivative spectra for an irradiated single crystal with the magnetic field along* (1,0,0) (*a*) *after storage at room temperature for* 7 *days,* (*b*) *after brief* (*ca.* 30 *min.*) *heating at* 160° C, (*c*) *after prolonged* (*ca.* 6 *hours*) *heating at* 160°C.



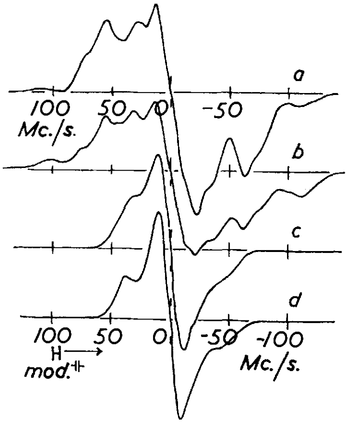


FIG. 5. *Derivative spectra of irradiated polycrystalline samples heated at* 160° C *for* 45 *min.*
(*a*) $^+H_3N{\cdot}CH_2{\cdot}CO_2^-$; (*b*) $^+H_3N{\cdot}CD_2{\cdot}CO_2^-$; (*c*) $^+D_3N{\cdot}CH_2{\cdot}CO_2^-$; (*d*) $^+D_3N{\cdot}CD_2{\cdot}CO_2^-$.

---

* *Note added in proof.* Servant, Augoyard, and Ngoc Chau (*Compt. rend.*, 1959, **249**, 71) find a different spectrum again at 77° K. These changes deserve fuller study.

The reason for the greater line width in Fig. 1*c* than in 1*d* when both should be attributed to $\dot{N}D_2$ radicals is not clear and is tentatively attributed to the presence of $\dot{N}HD$ radicals.

The Radical $^+H_3N\cdot\dot{C}H\cdot CO_2^-$.—There is nothing in the present work which affects the qualitative conclusions about the radical $^+H_3N\cdot\dot{C}H\cdot CO_2^-$ given previously.[1] As explained earlier, this radical has a spectrum of wider extent and its features can be most plainly seen in the region over 100 Mc./s. from the centre of the pattern. These are more prominent in Figs. 4*a* and *b* than in either 2*a* or 4*c*, and the radical is intermediate in its stability to heat between the $\dot{N}H_2$ radical and the third radical discussed below.

The Most Heat-stable Radical.—The spectra shown in Figs. 4*c* and 5*a* cannot be attributed to either of the radicals discussed above and it is clear that a third radical species is present after prolonged heating. Comparison of Fig. 5*a* with Fig. 5*b* and of these two with Figs. 5*c* and 5*d* shows that the dominant coupling is to hydrogen atoms originally attached to the nitrogen atoms.

The spectra from a single crystal which has been strongly heated are very complex (*e.g.*, Fig. 4*c*) and very dependent on the magnetic-field direction. A complete set of measurements was made but these have so far defied interpretation. Many lines appear to have non-integral intensity ratios and full interpretation must need the extended theory required for the $\dot{C}H(OH)\cdot CO_2H$ radical.[10] No suggestion is therefore made about the chemical structure of this stable radical.

Quantitative estimation of radical concentrations requires a double integration of the derivative curve and is very difficult to perform accurately. In one experiment a sample showing the spectrum of Fig. 1*a* was heated until it showed the spectrum of Fig. 5*a*. A comparison of the intensities obtained by double integration indicated that the final radical concentration was over half the original; a confirmatory result was obtained with $^+H_3N\cdot CD_2\cdot CO_2^-$. The very tentative conclusion may be made that the more stable radicals are formed by chemical reaction of the dominant radical rather than directly in the irradiation process.

Conclusions.—In view of the uncertainties which remain it would be premature to postulate detailed mechanisms for the various chemical reactions. The present indications are that irradiation of solid glycine by γ-rays at room temperature leads to the predominant formation of a radical species believed to be $\dot{N}H_2$ and that these are free to rotate in the crystal at room temperature. When kept at room temperature for several days, these radicals partially disappear and this disappearance is accelerated by heat. At this stage the radical $^+H_3N\cdot\dot{C}H\cdot CO_2^-$ with its wider spread of spectrum appears prominently, but on prolonged heating it too disappears to leave a third, unidentified radical. There is slight evidence that the later radicals are formed by reaction of the $\cdot NH_2$ radicals rather than in the original radiation process.

EXPERIMENTAL

Materials.—Glycine was commercial material recrystallised from water. $^+H_3N\cdot CD_2\cdot CO_2^-$ was supplied by Merck of Canada and stated to be of 98% isotopic purity. $^+D_3N\cdot CH_2\cdot CO_2^-$ was obtained by repeated dissolution and recovery of normal glycine from D_2O (99·98%); and $^+D_3N\cdot CD_2\cdot CO_2^-$ from a similar treatment of $^+H_3N\cdot CD_2\cdot CO_2^-$. The infrared spectra of these materials were in accord with qualitative expectations for the species involved. In particular the relative simplicity of the spectra suggested that the species were isotopically pure. The N-deuterated materials were handled in a dry box. Irradiation from a ^{60}Co γ-ray source was carried out at room temperature on evacuated samples for 10—20 hr. Samples kept thereafter at liquid-air temperatures showed no change of spectrum in contrast to those kept at room temperature or heated. Heated samples were held at 160° c *in vacuo* for varying times. The same set of spectral changes occurred on each occasion but the time required for any degree

---

[10] Atherton and Whiffen, *Mol. Phys.*, in the press.

of change was not reproducible. It appeared to be a function of crystal size, thickness of glass tube, sample density, etc., and the variations are tentatively ascribed to variation in thermal conductivity. Details of the spectra are given in the Figures and their legends. All spectra were centred near $g = 2{\cdot}00$.

Spectrometer.—The spectrometer operating at 9200 Mc./s. with 490 c./s. field modulation has already been described.[11] During the period occupied by the present work the spectrometer was converted to 125 kc./s. modulation,[12] but this change had no effect on the spectra beyond an improvement of better than a factor of 10 in the signal-to-noise ratio. An improved field sweep calibration using a nuclear resonance probe has shown the earlier calibration to be in error. Consequently the numerical couplings quoted earlier [1] should be reduced by 12%, as should the spin densities on the hydrogen and nitrogen atoms. All spectra, except that of Fig. 3*a*, were measured at room temperature.

We thank Mr. B. P. Goddard for his technical assistance in maintaining the spectrometer and in the preparation of deuterated materials, and Mr. D. Pooley for his assistance with the conversion to 125 kc./s. modulation. We acknowledge a Fellowship from the University Appeals Fund granted to D. K. G.

THE CHEMISTRY DEPARTMENT, THE UNIVERSITY,
BIRMINGHAM, 15.

[*Received, August* 19*th*, 1959.]

[11] Abraham, Ovenall, and Whiffen, *Trans. Faraday Soc.*, 1958, **54**, 1128.

[12] Ingram, " Free Radicals," Butterworths, London, 1958.