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October 1969

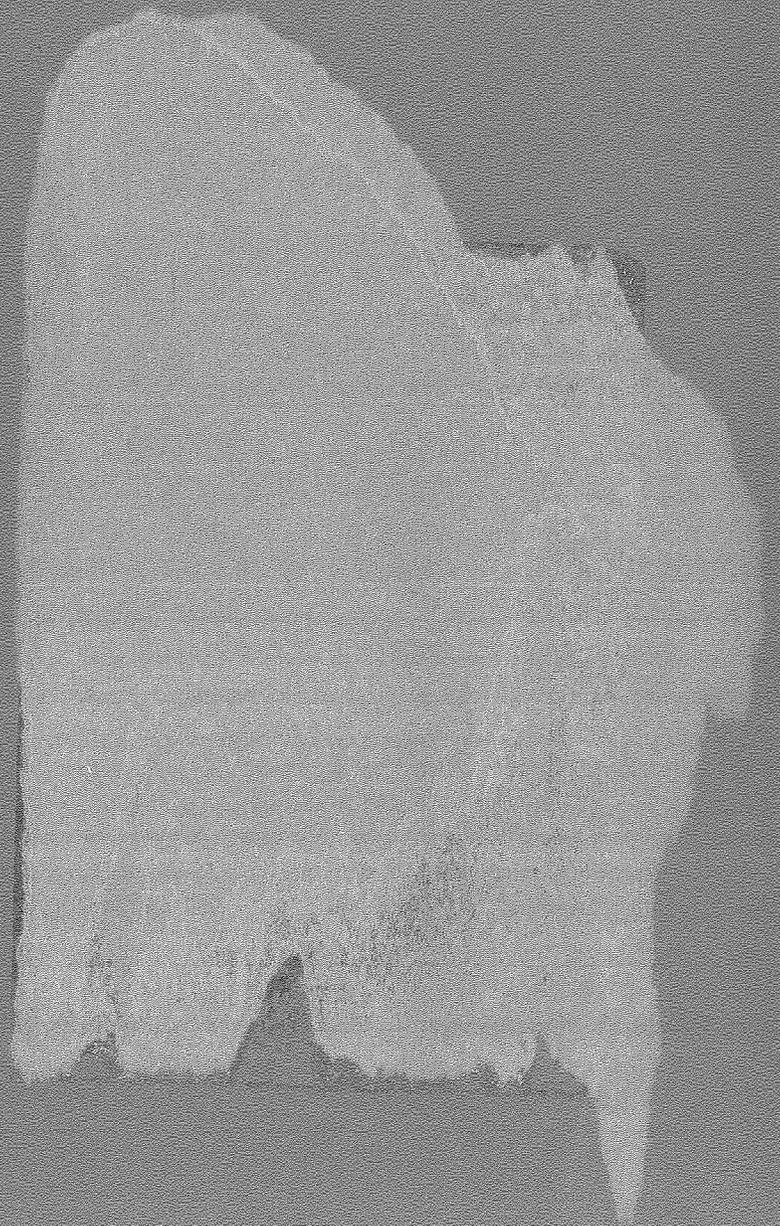
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KINETICS FOR FREE-RADICAL CONVERSION PROCESSES
IN IRRADIATED DL-VALINE*

Paul Karl Horan,[†] Thormod Henriksen,[‡] and Wallace Snipes[†]

Donner Laboratory and Lawrence Radiation Laboratory
University of California, Berkeley, California 94720

October 1969

ABSTRACT

The kinetics for two free-radical conversions in irradiated DL-valine has been studied by use of electron spin resonance (ESR) spectroscopy. In DL-valine three different radicals are observed. At 77° K only one of these radicals R(1) is formed, which upon subsequent warming converts into the intermediate radical R(2) with an efficiency of approximately unity. The reaction, studied in the temperature range 110° to 125° K, was found to be first-order with an activation energy of 7.6 ± 0.2 kcal/mole. Upon further warming, a third radical R(3) is formed. This formation consists of two separate processes, each proceeding by first-order kinetics. The activation energies, measured in the temperature range 200 to 260° K, are 6.6 ± 0.3 kcal/mole and 8.3 ± 0.5 kcal/mole, with the smaller activation energy process occurring at slightly lower temperatures.

Short title: Free Radical Conversion in Valine.

*This work was supported by AEC Contract W-7405-eng-48, NASA's Bioscience Program, and AEC Contract AT(30-1)-3799.

[†]Present address: Department of Biophysics, The Pennsylvania State University, University Park, Pennsylvania 16802.

[‡]Present address: Norsk Hydro's Institute for Cancer Research, Montebello, Norway.

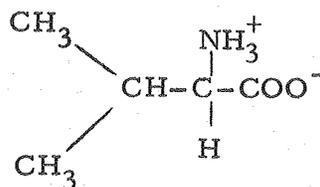
It was found that the concentration of R(3), as observed after warming to 300° K, was significantly greater than the initial concentration of R(1). This increase in total radical concentration is associated with the production of R(3) by the process having an activation energy of 6.6 kcal/mole. Possible mechanisms for the conversions are discussed in light of the known structures of R(2) and R(3).

INTRODUCTION

A primary purpose of many electron spin resonance (ESR) investigations of irradiated compounds of biological importance is to gain some insight into the mechanisms whereby ionizing radiation causes biological damage. One aspect of these studies has been the identification and characterization of free-radical intermediates in the radiation damaging process. Low-temperature experiments have in certain cases allowed the trapping of the initial radical-ions, which are extremely short-lived at room temperature.¹⁻⁴ The stable radicals at room temperature are usually neutral species resulting from breakage of covalent bonds. Although the identity of the radicals is known for many compounds, for irradiation both at low temperatures and at room temperature, little is known about the detailed mechanisms for radical formation. The radicals formed at low temperature generally convert, upon warming, to the room-temperature radicals, but the nature of these conversion processes is not understood. Furthermore, there have been very few studies of the kinetics for these free-radical conversions.

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- (1) H. Box and H. Freund, J. Chem. Phys. 41, 2571 (1964).
 - (2) H. Box, H. Freund, and E. Budzinski, J. Chem. Phys. 46, 4470 (1967).
 - (3) H. Box, H. Freund, and K. Lilga, J. Chem. Phys. 42, 1471 (1965).
 - (4) J. Herak and V. Galogaza, J. Chem. Phys. 50, 3101 (1969).

In the work reported here, the kinetics for the free-radical conversions in DL-valine (I),



(I)

irradiated at 77° K, were studied at various temperatures. DL-valine was chosen because the identity of two radicals, formed at different stages of warming, has been determined previously.^{2,5} Activation energies were measured for three different first-order processes, and an estimate of the conversion efficiencies was obtained.

EXPERIMENTAL PROCEDURE

Samples of DL-valine, obtained commercially, were sealed in evacuated quartz tubes and irradiated with 6.5-MeV electrons from a linear accelerator to a dose of 5 MR. The irradiations were carried out at 77° K, with one end of the ESR tube shielded. Following irradiation, the sample was shaken to the shielded end of the tube and then transferred to the ESR spectrometer (Varian 4502, X-band).

Temperature control was achieved with the Varian Variable Temperature Accessory. An iron-constantan thermocouple inside an ESR tube was placed in the cavity at the sample position. When the desired temperature was reached, the thermocouple was removed,

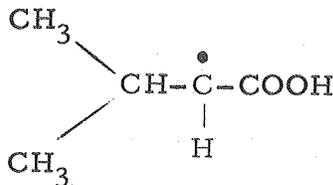
(5) H. Shields, P. Hamrick, and D. DeLaigle, J. Chem. Phys. 46, 3649 (1967).

the sample was inserted, and the conversion at that temperature was measured. Following this, the temperature was again measured with the thermocouple in the cavity. The temperature before and after each experiment varied by no more than $\pm 1.0^\circ \text{K}$. The relative free-radical concentration before and after various heat treatments was obtained by double integration of first-derivative ESR spectra. Pitch serves as a reference substance in these experiments and a Varian dual cavity operating in the TE_{104} mode was used.

RADICAL SPECIES

Box, Freund, and Budzinski² in a single-crystal study of DL-valine suggested that the ESR spectrum observed after irradiation at 77°K was due to the presence of radical ions. Although it is not known whether the low-temperature spectrum is due to more than one type of radical, the data presented below suggest that only one species is present following irradiation at 77°K . In this paper, the radicals induced at 77°K will be referred to collectively as R(1), and their polycrystalline spectrum is shown in Fig. 1 (upper curve). The spectrum is a broad singlet with some evidence for additional structure in the wings.

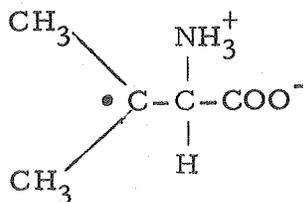
The spectrum in Fig. 1 labeled R(2) is due to a radical that has been identified² as



R(2)

This spectrum appears, concurrently with the disappearance of the R(1) spectrum, when the irradiated sample is warmed to temperatures above 110° K. The hyperfine couplings in R(2) produce a spectrum with lines at magnetic field positions where R(1) has no appreciable absorption. By measuring the height h_2 of one of these lines (see Fig. 1), the increase in concentration of R(2) could be studied at various temperatures.

A third radical R(3) appears upon further warming to temperatures above 200° K. This radical is identical to the one formed when DL-valine is irradiated at room temperature, and has the structure^{2, 5}



R(3)

Its spectrum has hyperfine lines at field positions where neither R(1) nor R(2) absorbs (Fig. 1). In order to monitor the production of both R(2) and R(3), portions of the spectra around h_2 and h_3 (see Fig. 1) were measured at increased spectrometer sensitivity. These portions were swept every 30 seconds or every minute at various temperatures.

CONVERSION OF R(1) TO R(2)

Figure 2 shows the increase in concentration of R(2) with time at 118° K. A slight lag is seen in the conversion near the origin, due to the time required for temperature equilibrium to be reached.

Following the rapid rise in the first 10 min, a leveling is observed, with the concentration of R(2) being constant after about 40 min. The conversion of R(1) to R(2) can be adequately described by a first-order process with a rate constant k that is temperature dependent. The appropriate differential equations

$$\frac{dR(1)}{dt} = -kR(1) \quad (1)$$

and

$$\frac{dR(2)}{dt} = kR(1) \quad (2)$$

can be solved to give

$$R(1) = R(1)_0 \exp(-kt) \quad (3)$$

and

$$R(2) = R(1)_0 [1 - \exp(-kt)], \quad (4)$$

where $R(1)_0$ is the initial concentration of R(1). Double integration of first-derivative spectra before and after the conversion showed that the total radical concentration remained constant. Thus $R(2)_\infty$, the final concentration of R(2), equals $R(1)_0$, and Eq. (4) can be written in the form

$$\log \left[1 - \frac{R(2)}{R(2)_\infty} \right] = -kt. \quad (5)$$

Data for two different temperatures are plotted according to Eq. (5) in Fig. 3. These straight-line plots confirm that the conversion is first-order, and from their slopes the rate constants can be determined.

The temperature-dependent rate constants were found to obey the Arrhenius equation

$$k(T) = A_0 \exp(-E_a/RT). \quad (6)$$

The activation energy obtained from this plot (Fig. 4) is $E_a = 7.6 \pm 0.2$ kcal/mole. The estimated error is based on least-squares error analysis.

CONVERSION OF R(2) TO R(3)

Figure 5 shows the increase in concentration of R(3) with time at 227° K. Although the initial portion of the curve shows a rapid rise in R(3) concentration, the overall shape of the curve is different from those for conversion of R(1) to R(2). The R(3) concentration levels off much more gradually, indicating a fast and a slow process for production of R(3) at this temperature. Figure 6 shows an attempt to plot the data according to Eq. (5). It is apparent from this plot that a single, first-order process will not explain the results. However, two first-order processes with different rate constants can adequately describe the formation of R(3). Thus, the results after approximately 15 min at 227° K can be fitted by a straight line in this semilogarithmic plot. By subtracting the projected line (i. e., the slow process) from the data at short times the differences are fitted nicely by another straight line (the inset in Fig. 6). The two first-order processes are referred to as the slow and the fast processes, a description which may be limited to the temperature range used for these experiments (200° to 260° K).

From plots such as that in Fig. 6, the rate constants for the slow process at various temperatures were determined. These are plotted according to the Arrhenius Eq. (6) in Fig. 7. The activation energy for the slow process is 8.3 ± 0.5 kcal/mole.

A somewhat different method was used to determine the activation energy for the fast process, because extremely long times are required to obtain the value of $R(3)_{\infty}$ at temperatures below 227° K. Samples of identical weight were irradiated with identical doses, and

the initial rate of increase of R(3) was measured at different temperatures. Although this does not give the absolute values for the rate constants, it gives the relative values at different temperatures, and this is sufficient to determine E_a for the fast process. In Fig. 7, the rate constants for the fast process have been normalized to the value at 227° K, which was determined from the data of Fig. 6. The activation energy for the fast process is 6.6 ± 0.3 kcal/mole. It can be seen that the two Arrhenius plots of Fig. 7 will cross at some higher temperature, so the designations of fast and slow to the processes described here are of course limited to temperatures below that point.

From double integration of first-derivative spectra, it was found that the total radical concentration does not remain constant in the temperature range 200 to 260° K. In five separate experiments, the total radical concentration in samples that had been warmed to 240° K for 5 min was approximately 1.5 times that for the same samples at 77° K. All measurements were made at 77° K to ensure equal spectrometer sensitivities. This increase in total radical concentration is associated with the conversion of R(2) to R(3) rather than the conversion of R(1) to R(2), since no increase in concentration was observed in samples warmed to 131° K for 5 min. Furthermore, the increase was found to be associated with the fast process for the production of R(3), by the following experiment. The total radical concentration was measured as a function of time at 227° K, and it was found that the only net increase occurred in the first 5 min. The kinetics for the slow and fast processes at 227° K (Fig. 6) show that the fast process is 80% complete after 5 min whereas the slow process is only about 12% complete. If

the increase were associated with the slow process, the concentration should rise over a period of half an hour, and this was not found to be the case. This probably means that the slow process is a true conversion of R(2) into R(3), whereas the fast process is a production of R(3) by some other mechanism.

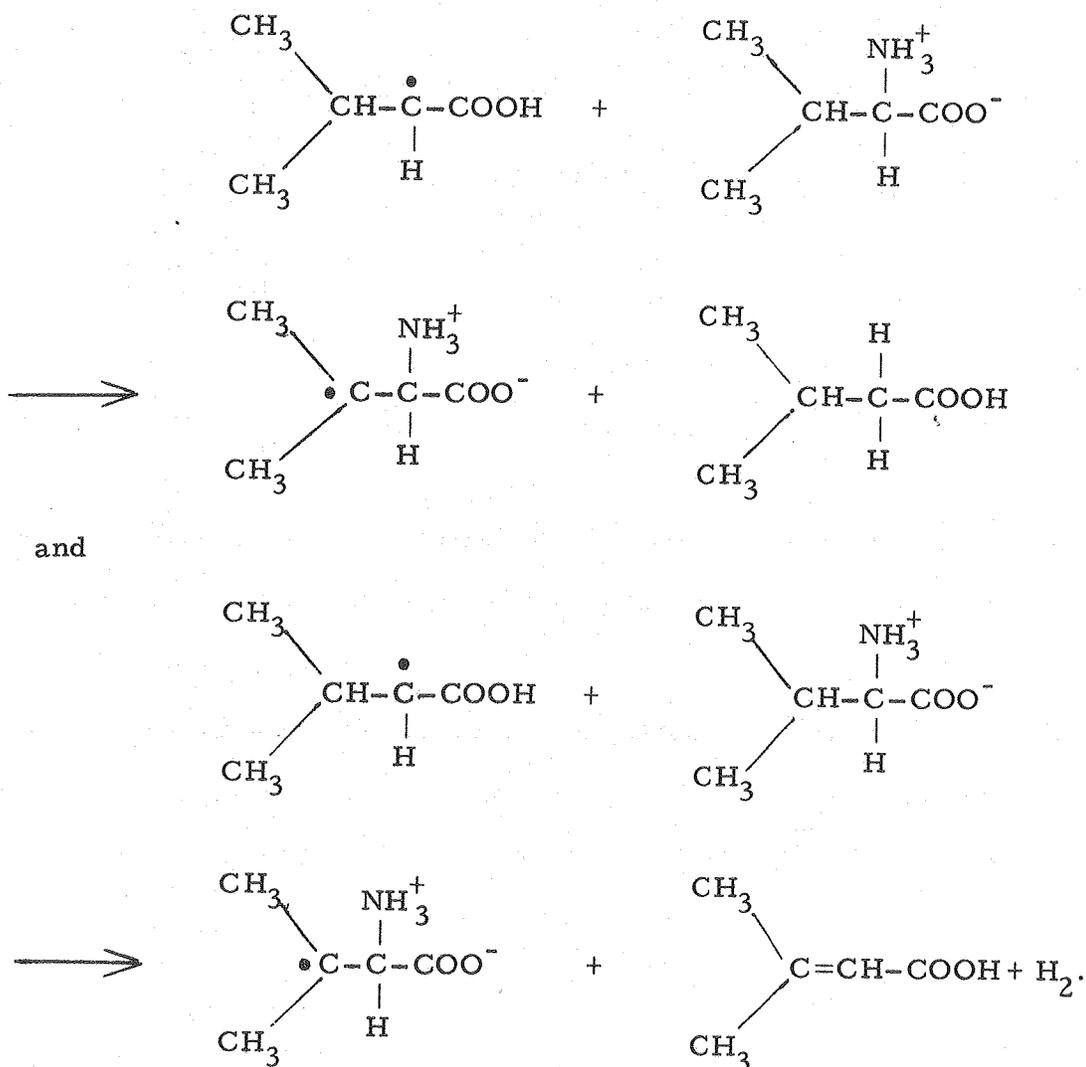
DISCUSSION OF MECHANISMS

The following discussion of possible mechanisms for free-radical conversion is brief, since it must of necessity be largely speculation. The radical R(1) converts into R(2) with very simple kinetics. This probably means that R(1) is a single species, since identical kinetics for more than one species would be only fortuitous. If only one radical species is present at 77° K, this is itself interesting. As ionizations occur randomly during irradiation, giving rise to a variety of ionic species, the observation of a single species at this temperature suggests that some electronic rearrangements have occurred in the molecular structure.

The simplicity of the kinetics and the unit efficiency would be consistent with the conversion of R(1) into R(2) being intramolecular. If R(1) is a negative ion of the original valine molecule, spontaneous liberation of NH₃ would give a radical similar to R(2), provided that valine is in the zwitterionic form. This conversion need not necessarily involve interactions with neighboring molecules.

Based on the structures of R(2) and R(3), it is reasonable to assume that the conversion of R(2) to R(3) is intermolecular in nature, since R(3) has the NH₃⁺ group intact. Two possibilities for inter-

molecular processes that would produce R(3) at the expense of R(2) are



The latter process is consistent with some experiments by Sommermeyer et al.,⁶ who found molecular hydrogen when valine was irradiated in the solid state.

No adequate explanation can be given for the net increase in radical concentration that takes place with the production of R(3) by

(6) K. Sommermeyer, J. Stegle, and G. H. Schnepel, *Atompraxis* 13, 20 (1967).

the fast process. One possibility is that other radical species or charge carriers exist at temperatures below 200° K which have absorptions too broad to be detectable on derivative ESR tracings. If these radicals converted into R(3), the total radical concentration would appear to increase. Another possibility is that the fast process is in fact a production of R(3) at the expense of R(2), but that more than one R(3) is produced for each R(2) lost. A specific mechanism for such a limited chain reaction is difficult to visualize, however.

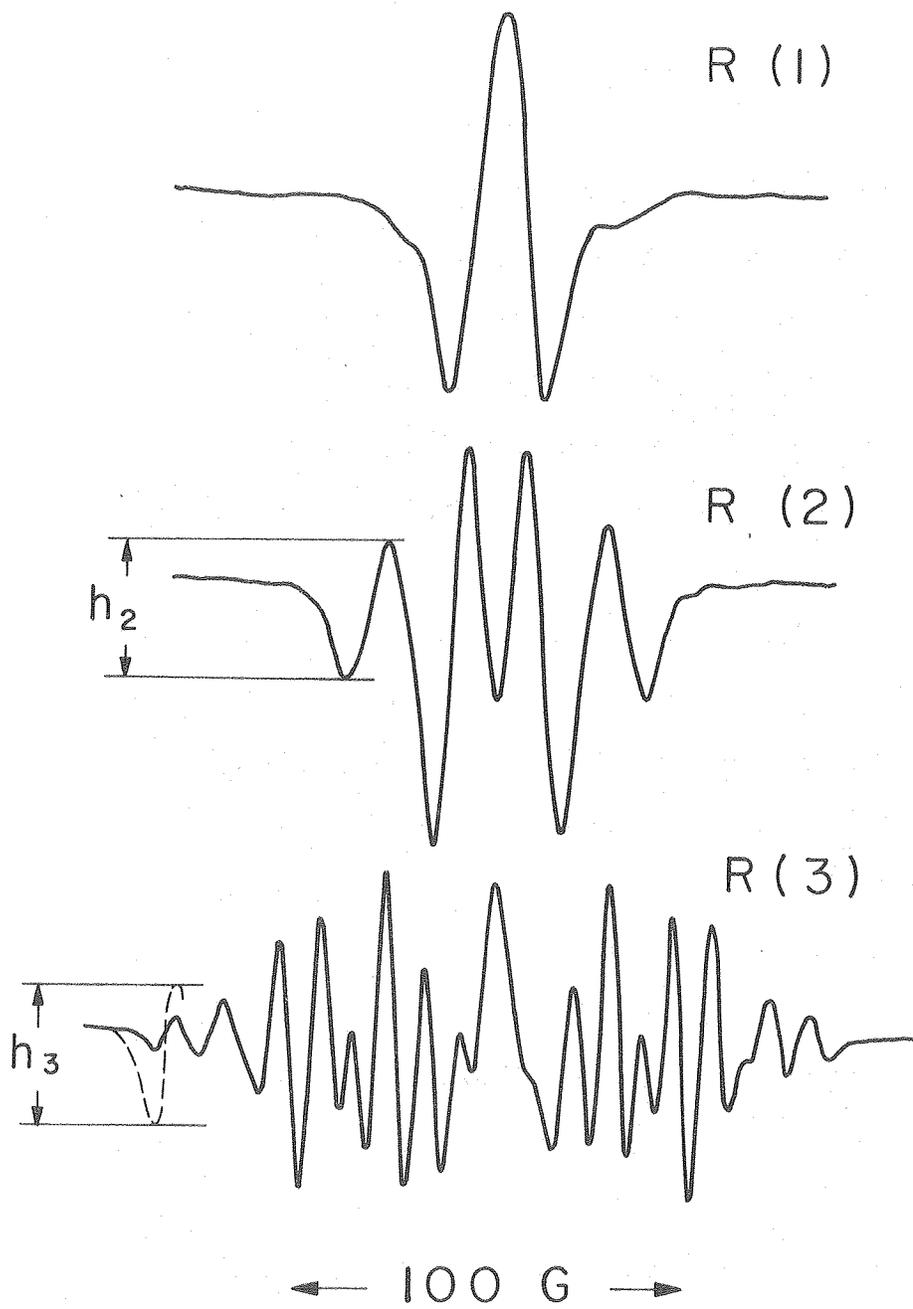
All the radical processes reported here for DL-valine have activation energies in the range of 5 to 10 kcal/mole. These values are much smaller than the value of 22.6 kcal/mole reported previously⁷ for thermal decay of R(3) in the temperature range near 400° K. This difference is interesting, because conversion reactions may be quite localized in the crystal whereas decay reactions must of necessity involve the migration of unpaired electrons over much larger distances.

DL-valine is the first compound, to our knowledge, for which the kinetics of the various radical reactions have been studied in detail from the low-temperature radical stage to the decay of the final radical. Other compounds may be expected to have processes, some similar and some different, analogous to those observed in DL-valine. Further studies of the activation energies involved in different compounds may shed some light on the mechanism for radiation damage.

(7) P. Horan, W. Taylor, G. K. Strother, and W. Snipes, *Biophysical J.* 8, 164 (1968).

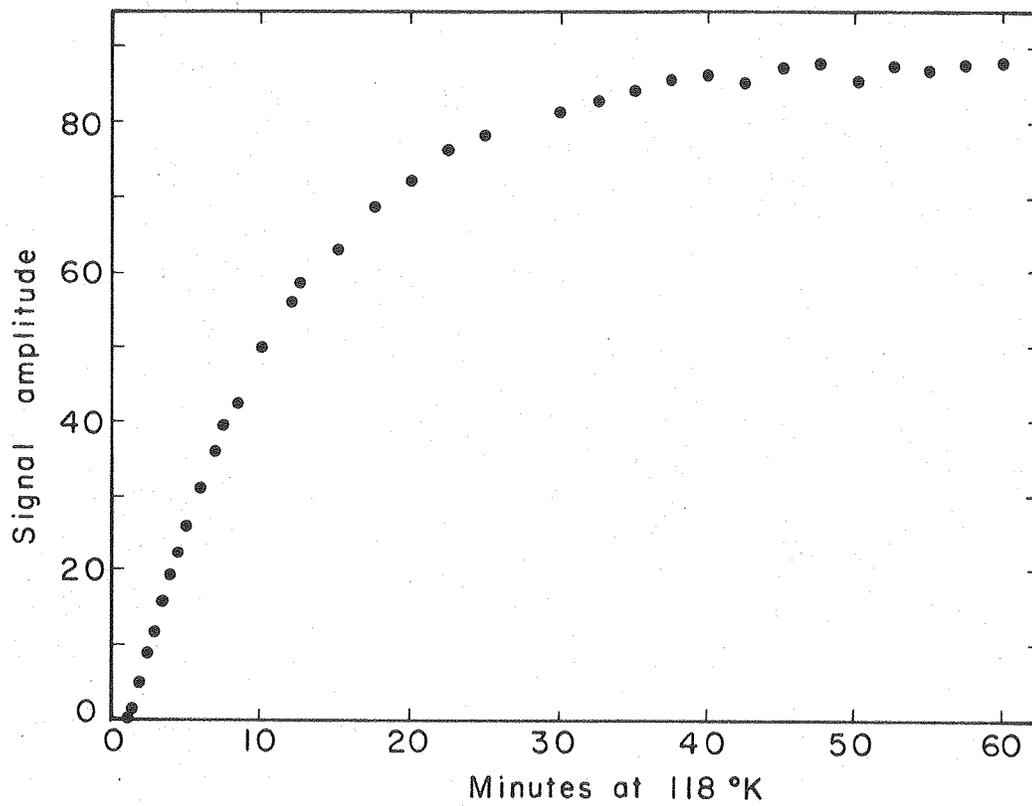
FIGURE CAPTIONS

- Fig. 1. Second-derivative ESR spectra of irradiated DL-valine. The upper spectrum was taken following irradiation at 77° K with no heat treatment. The middle spectrum was taken at 77° K after the sample had been warmed to 163° K for 5 min. The lower spectrum is for 5 min heat treatment at 295° K. The line heights h_2 and h_3 were used to monitor the concentration of R(2) and R(3) during heat treatments.
- Fig. 2. Formation of radical R(2) at 118° K. The sample was irradiated at 77° K (dose 5 MR) and then transferred to the Variable Temperature Accessory which was kept at the desired temperature. The signal amplitude h_2 vs time from the transfer was measured.
- Fig. 3. Plot of data according to Eq. (5) for two temperatures, in the conversion of R(1) to R(2).
- Fig. 4. Arrhenius plot of the rate constant for conversion of R(1) to R(2). The line corresponds to an activation energy of 7.6 kcal/mole.
- Fig. 5. Signal amplitude h_3 vs time for DL-valine at 227° K. Otherwise as for Fig. 2.
- Fig. 6. Plot of data at 227° K according to Eq. (5). For times up to 5 min, the values of the straight line were subtracted from the data points to give the inset plot.
- Fig. 7. Arrhenius plots of the rate constants for the two processes that lead to production of R(3). The lines correspond to activation energies of 8.3 kcal/mole for the slow process and 6.6 kcal/mole for the fast process.



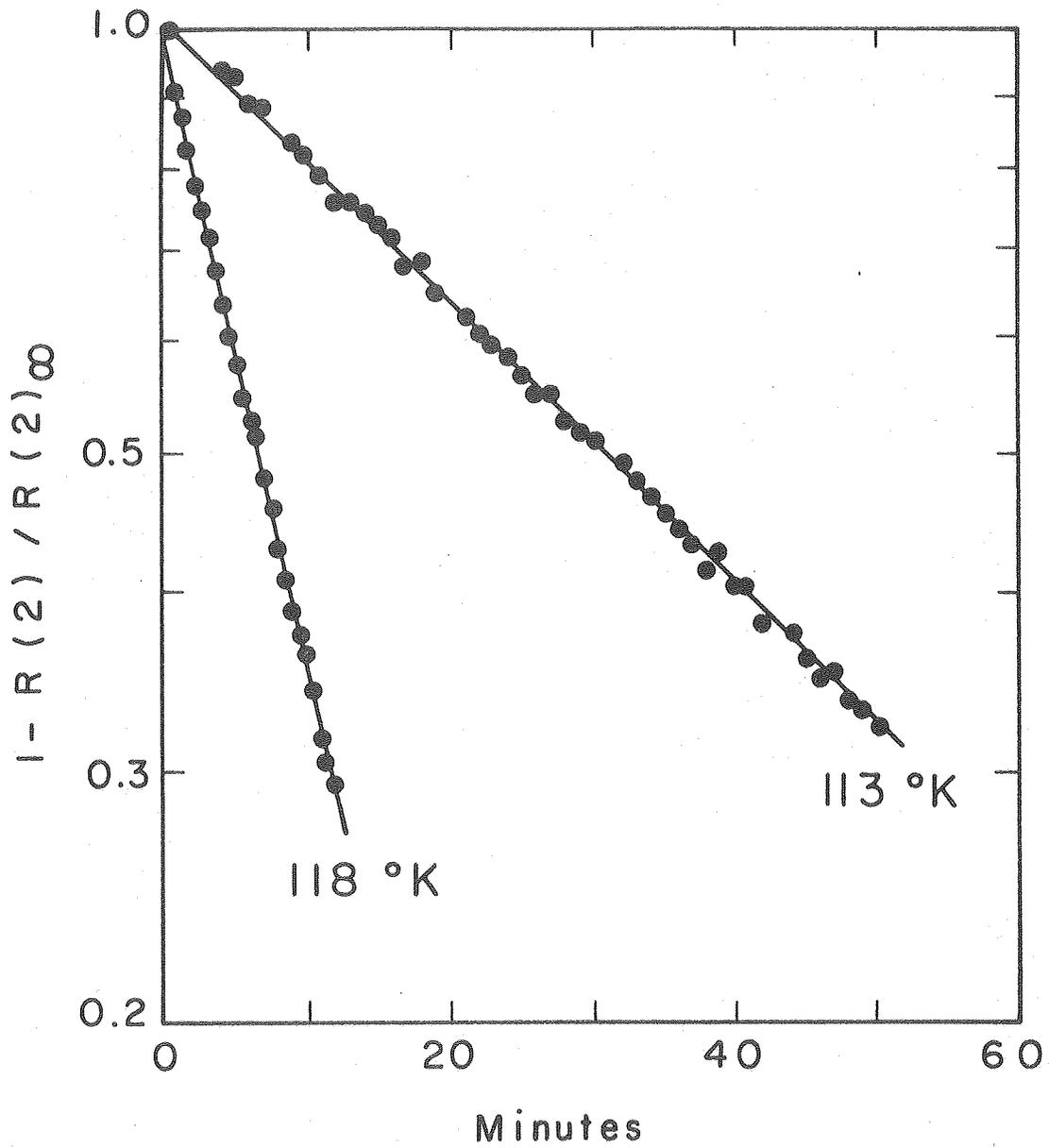
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Fig. 1



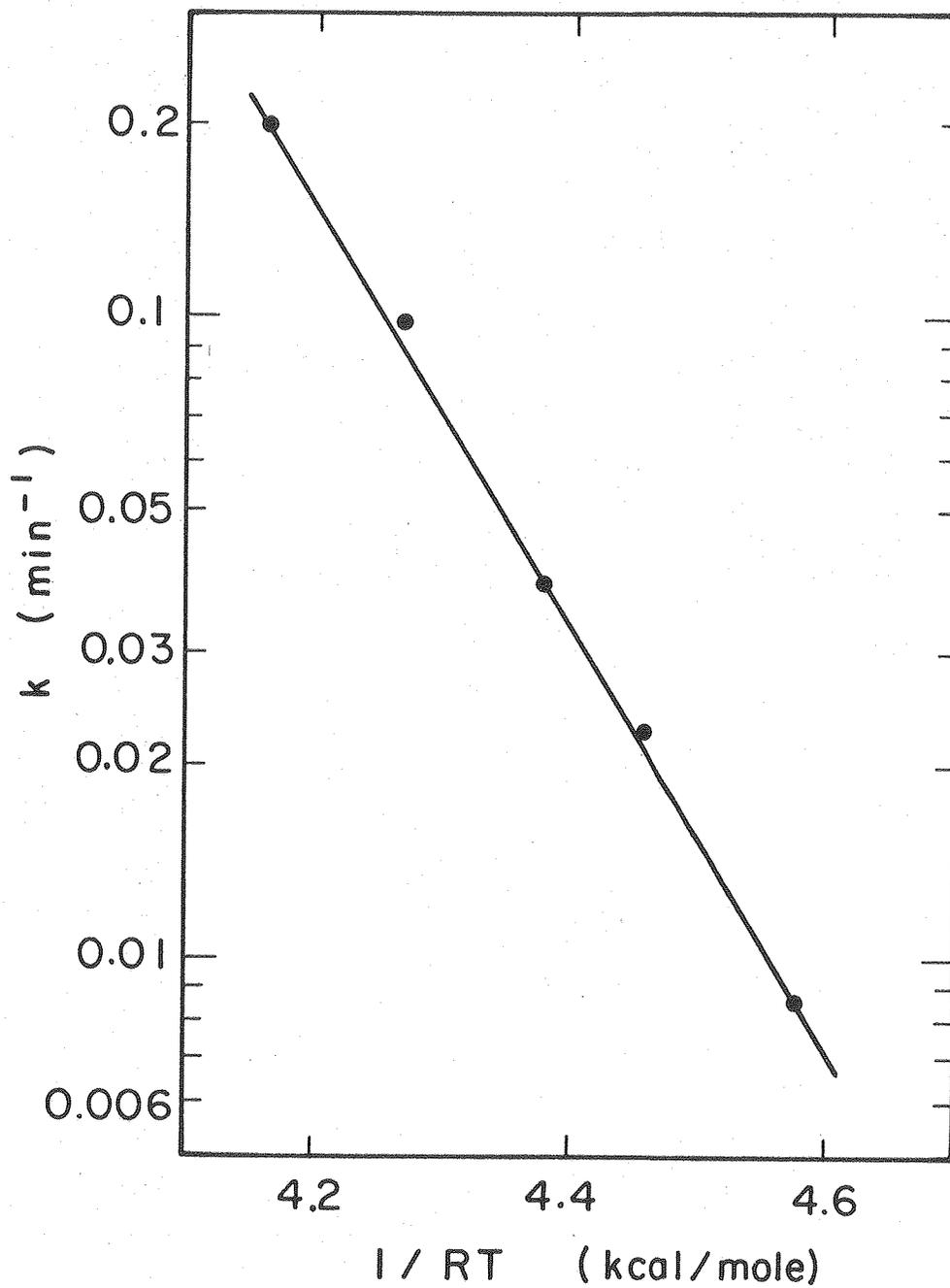
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Fig. 2



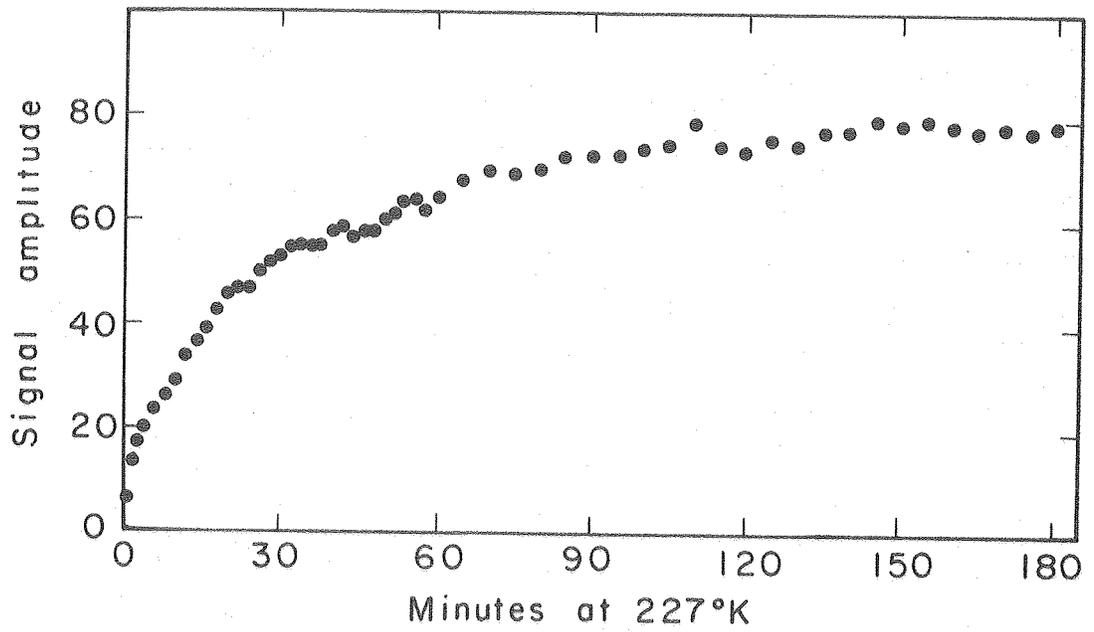
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Fig. 3



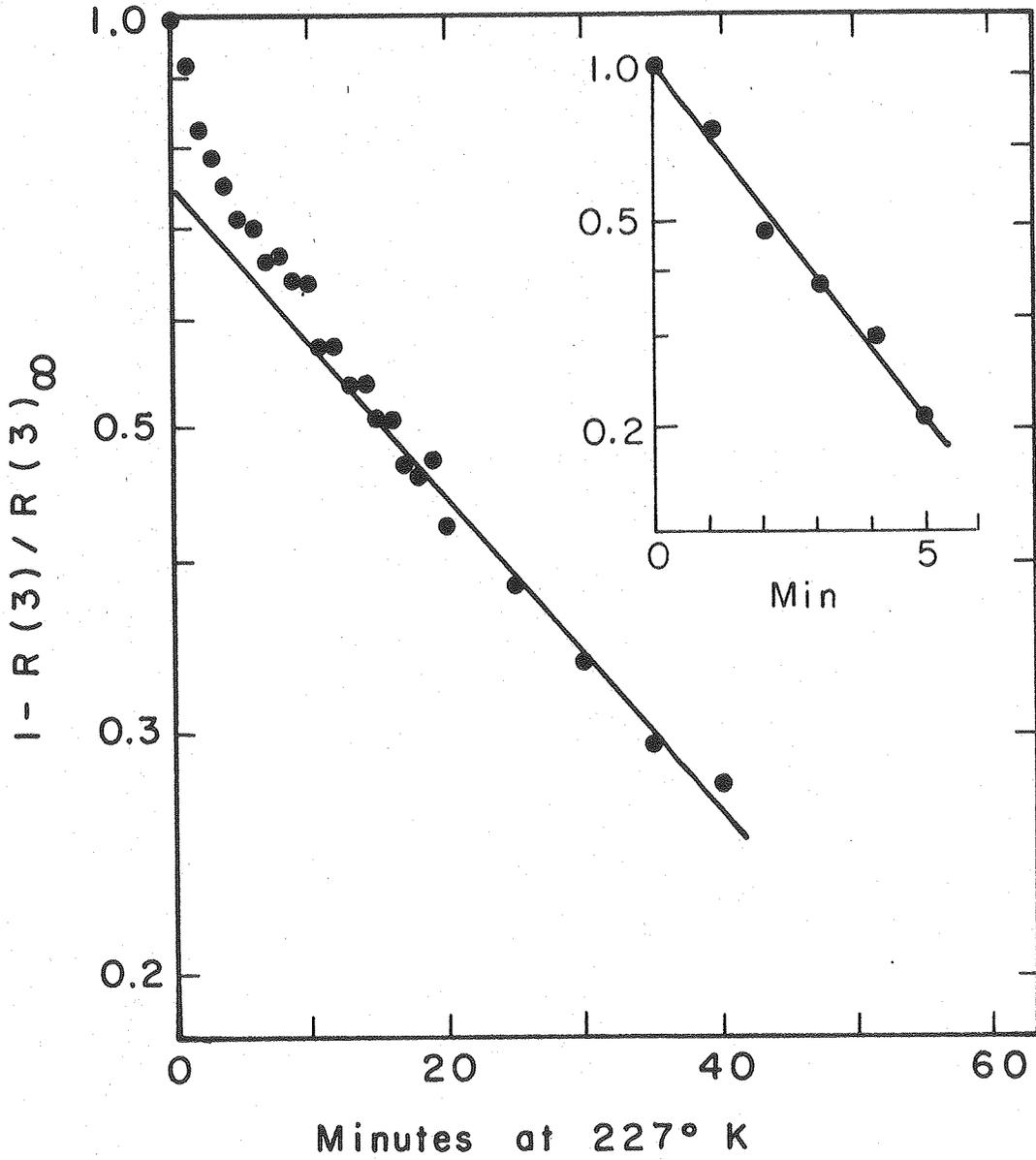
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Fig. 4



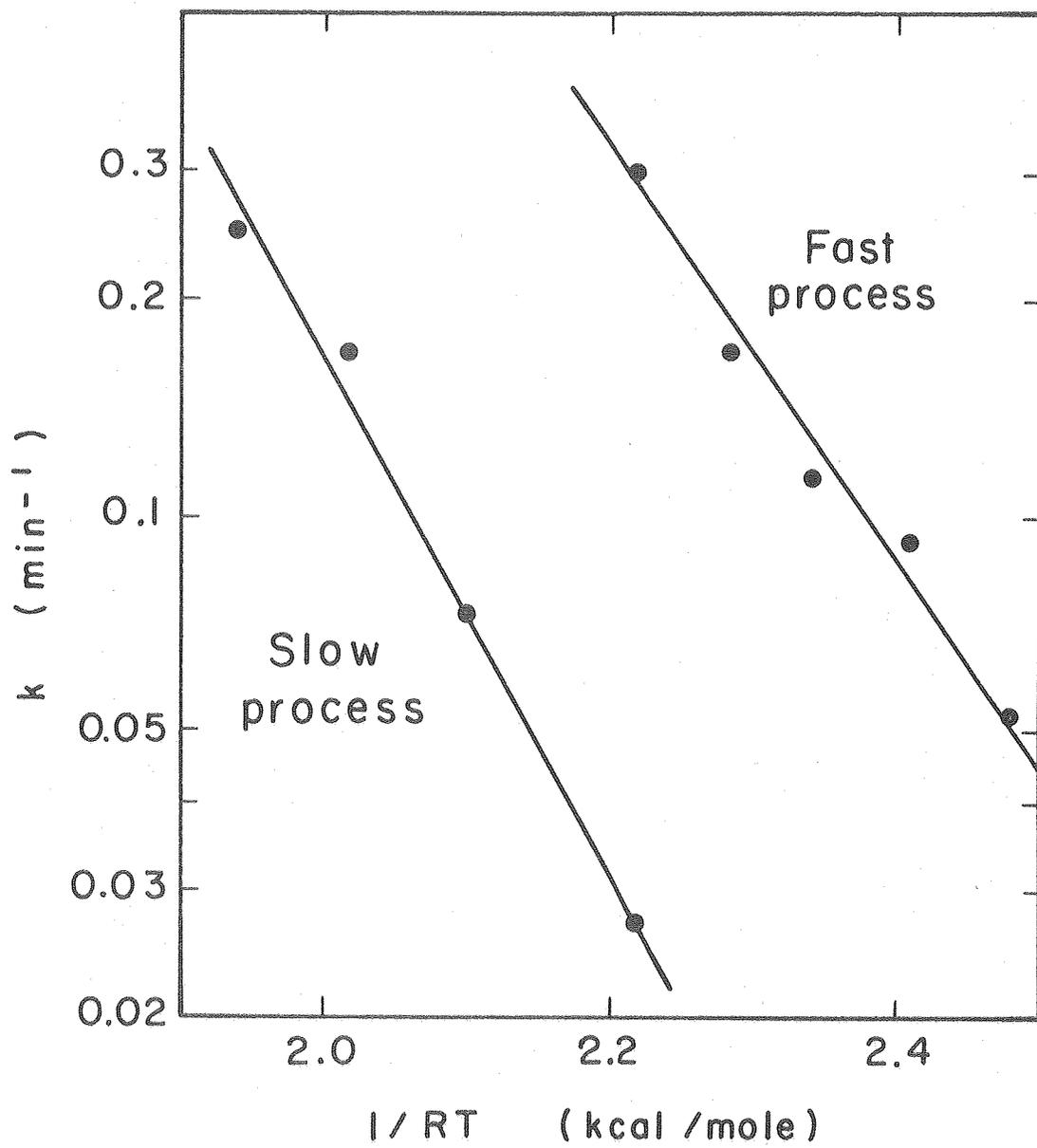
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Fig. 5



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Fig. 6



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Fig. 7

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