The Electron Spin Resonance Spectra of Disulfides Irradiated with Ultraviolet Light

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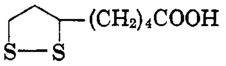
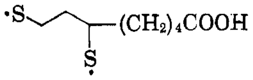
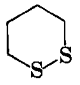
The electron spin resonance (e.s.r.) spectra obtained by irradiating a series of disulfides with ultraviolet light at their absorption maximum are presented.

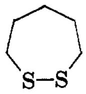
The reports of Calvin and co-workers[2-4] indicated strongly that lipoic acid (I) produces a dithyl radical (II) during the photosynthetic process. The involvement of this dithiyl radical (II) in other enzymatic systems has been alluded to by several groups.[5-7]


I


II

In support of the postulation of the above investigators, the e.s.r. spectrum of the dithiyl radical (II) has been obtained from lipoic acid (I) (λ_{max} 334 mμ) by photolysis using monochromatic light (334 mμ), filtered light (λ >300 mμ), and unfiltered ultraviolet light. *o*-Dithiane (III) (λ_{max} 286 mμ), 1,2-dithiepane (IV) (λ_{max} 259 mμ), and methyl disulfide (V) (λ_{max} 250 mμ) also give thiyl radicals when monochromatically photolyzed at their ultraviolet absorption maximum and photolyzed with unfiltered ultraviolet light.[8]


III


IV

$CH_3-S-S-CH_3$
V

The spectra shown in Figure 1 were obtained after low-intensity irradiation at the λ_{max} of the corresponding disulfides. The arrows indicate the areas in the spectra which have a signal to background noise ratio of less than 1. A ponderal effect seems evident in a comparison of spectral intensity and the ratio of total mass to the mass of the disulfide linkage.

The spectra obtained from the photolysis of a 2 *M* solution of lipoic acid (I) using a high-intensity ultraviolet lamp and removing light with wave lengths of less than 300 mμ are shown in Figure 2. The intensity and shape of the curves are related to the amount and duration of irradiation.

The concentration of the disulfide also effects the shape and intensity of the curves. A dilute solution of lipoic acid (I), after high intensity irradiation, produced curve A, Figure 3. A dilute solution of methyl disulfide gave spectrum B, Figure 3.

In an attempt to obtain better resolved spectra, the four disulfides were photolyzed with unfiltered ultraviolet light and the e.s.r. spectra were produced at slightly higher klystron power. The latter causes some artificial broadening as can be observed from the corresponding "shift" in *g* values of the thiyl radical spectra shown in Figures 4 and 5 at 50 db., compared with the *g* values of the thiyl radical spectra shown in Figures 6 and 7 at 45-db. attenuation.

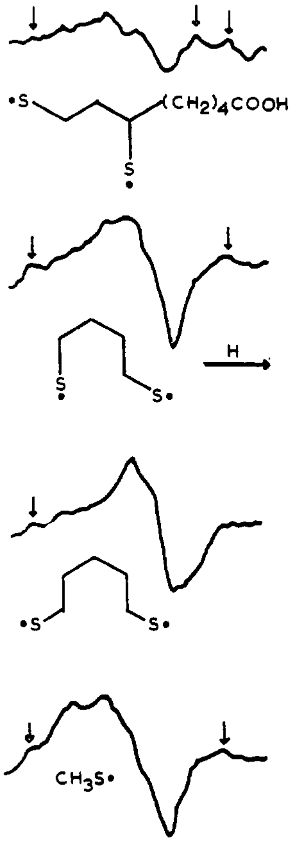


Figure 1.

On examination of the spectra it appears that, under the conditions of the photolyses, only S–S homolytic cleavage occurs. Hyperfine structure was not observed in the e.s.r. spectra even at high signal detection levels. The latter should be present if C–S bond fission had taken place.[9] Similarly, evidence of hydrogen abstraction is not present, nor was a triplet state observed.

From the above observations and a *g* value[10] similar to one reported by Truby and co-workers,[11] as well as

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[1] National Institutes of Health Predoctoral Fellow, 1963–1964.

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[4] M. Calvin and J. A. Barltrop, *J. Am. Chem. Soc.*, **74**, 6153 (1952).

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[6] A. Gluharty and D. R. Sanadi, *Proc. Natl. Acad. Sci. U. S.*, **46**, 608 (1960).

[7] J. W. Newton, *Nature*, **195**, 349 (1962).

[8] An investigation of the nature of the disulfide transition state in the ultraviolet is currently in progress.

[9] W. E. Haines, G. L. Cook, and J. S. Ball, *J. Am. Chem. Soc.*, **78**, 5213 (1956).

[10] A *g* value of 2.026 was obtained for lipoic acid (I) at the Varian Associates E.s.r. Applications Laboratory by approximating the first derivative crossover point and using diphenylpicryl hydrazyl (DPPH) as the reference.

[11] F. K. Truby, C. MacCallum, and H. E. Hesse, *J. Chem. Phys.*, **37**, 2777 (1962).

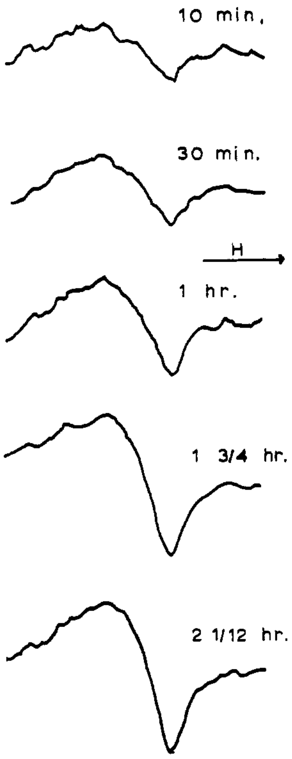

Figure 2.

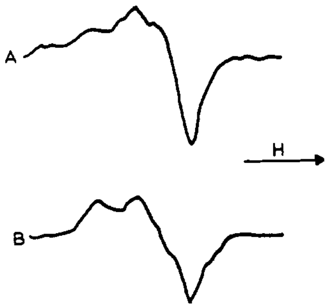

Figure 3.

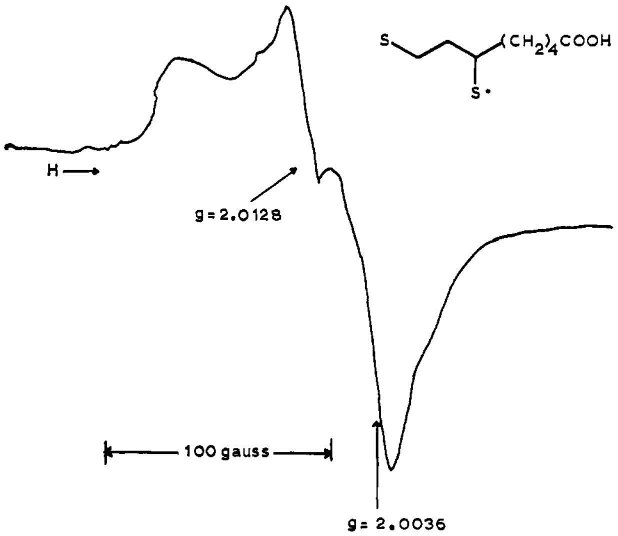

Figure 4.—E.s.r. spectrum of the lipoic acid dithiyl radical at 50 db. and 9155.8 Mc.

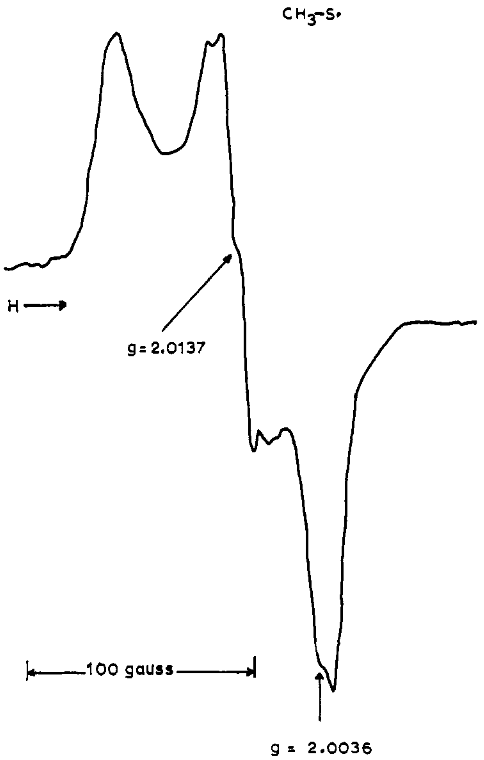

Figure 5.—E.s.r. spectrum of the methyl thiyl radical at 50 db. and 9157.0 Mc.

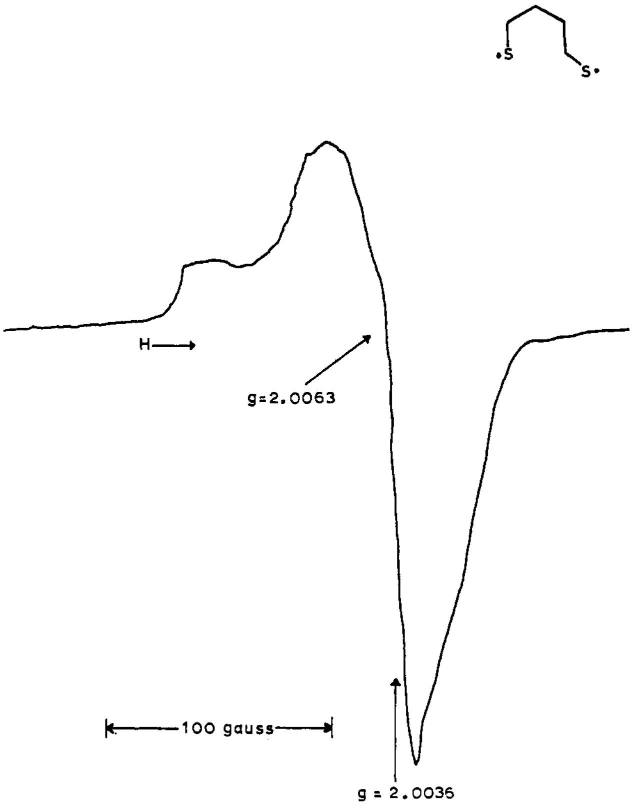

Figure 6.—E.s.r. spectrum of the *o*-dithiane dithiyl radical at 45 db. and 9152.8 Mc.

very broad characteristic "sulfur patterns,"[11-13] the conclusion is that the resonance is produced by thiyl radicals with the unpaired spins localized at the sulfur atoms.

(12) T. Hendricksen, *J. Chem. Phys.*, **38**, 1926 (1963).

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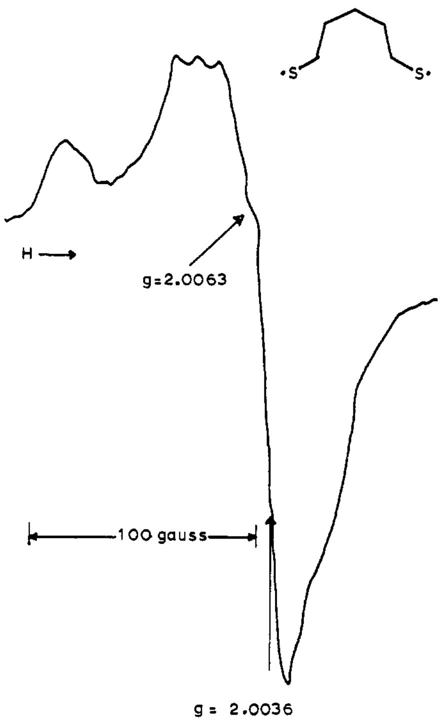

Figure 7.—E.s.r. spectrum of the 1,2-dithiepane dithiyl radical at 45 db. and 9155.0 Mc.

Experimental Section[14]

o-Dithiane (IV).—The method of Calvin and co-workers[2] was used to prepare o-dithiane (IV). The disulfide was sublimed twice at room temperature to give a 30% yield of a white solid, m.p. 32–33°, $\lambda_{\max}^{\mathrm{EtOH}}$ 286 mμ (ε 300) [lit.[2] m.p. 32–33°, $\lambda_{\max}^{\mathrm{EtOH}}$ 286 mμ (ε 294.5)].

1,2-Dithiepane (V).—The method of Calvin and co-workers[2] was used to prepare 1,2-dithiepane (V). On distillation 1.58 g. (5.4%) of an oil, b.p. 40–41° at 1.5 mm. [lit. b.p. 57–60° at 5 mm.,[15] $\lambda_{\max}^{\mathrm{EtOH}}$ 256 mμ (ε 435),[15] 259 mμ (ε 467)[2]], was obtained.

Low-Intensity Irradiation of Lipoic Acid (I), **Methyl Disulfide** (III), ***o*-Dithiane** (IV), **and 1,2-Dithepane** (V).—Monochromatic photolysis was performed outside the e.s.r. cavity utilizing a 2 *M* solution of the designated disulfide at 77°K. for 24 hr. The V-4546 liquid nitrogen dewar used in these photolyses was fitted with a reflector on the side opposite to the light source and the cell was rotated 30° after each addition of liquid nitrogen (approximately 1 hr.). A Bausch and Lomb No. 33-86-01 grating monochromator with an air-cooled deuterium arc lamp and fused quartz condenser system (no. 33-86-35) using an entrance slit width of 1.34 mm. and an exit slit width of 0.75 mm. was used for these irradiations.

High-Intensity Photolysis of Methyl Disulfide (III) **and Lipoic Acid** (I).—A 1×10^{-3} *M* solution of methyl disulfide (III) and a 2×10^{-2} *M* solution of lipoic acid (I) were photolyzed outside of the e.s.r. cavity, as described in the above procedure, for 1.5 hr. utilizing the high-intensity Hanovia lamp. No reflector was placed opposite the light source side of the dewar.

Filtered High-Intensity Photolysis of Lipoic Acid (I).—A 2 *M* solution of lipoic acid (I) was photolyzed as described in the above procedures for 2 hr. and 5 min. with a high-intensity Hanovia lamp fitted with a 1.9–2.1 mm. Corning C.S. No. 0-53 filter to filter all wave lengths shorter than 300 mμ. Spectra were recorded at 10 min., 30 min., 1 hr., 1.75 hr., and 2 hr. and 5 min. after irradiation had commenced.

Unfiltered High-Intensity Photolysis of Lipoic Acid (I), ***o*-Dithiane** (III), **1,2-Dithiepane** (IV), **and Methyl Disulfide** (V).—A 1×10^{-5} *M* solution of o-dithiane (III) and 1×10^{-3} *M* solutions of lipoic acid (I) and 1,2-dithiepane were photolyzed for 30 min. with the JES-UV-1 ultraviolet irradiation unit no. UV-116. The 1×10^{-3} *M* solution of methyl disulfide (V) was photolyzed with the same lamp for 60 min.

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(14) Melting points were obtained in a Thomas–Hoover Unimelt and are corrected. Ultraviolet spectra were recorded utilizing a Beckman DB spectrophotometer. Methyl disulfide was purchased from Eastman Organic Chemicals Co., and lipoic acid from the Aldrich Chemical Co. These compounds were used without further purification. E.s.r. spectra in Figures 1–3 were obtained with a Varian Associates V-4500 spectrophotometer using a 6-in. magnet, 100-kc. field modulation, Fieldial, and V-4546 liquid nitrogen accessory. The modulation amplitude was chosen to avoid artificial broadening. All of these spectra presented are the first derivative of the absorption integral over the range of 3.2 ± 0.125 kgauss. The e.s.r. cells were made of Engelhard signal-free quartz tubing (10-in. lengths, 4-mm. o.d., 3-mm. i.d.). Degassing of the samples was accomplished between 1×10^{-5} and 0.5×10^{-5} mm. Chlorobenzene was used as the solvent for all samples and under the conditions specified gave no e.s.r. signal. High-intensity irradiation was accomplished with a Hanovia high-pressure quartz mercury vapor lamp, no. 5-654A-10, fitted with a no. 7490 Alzak aluminum reflector hood assembly, to which was attached a light-tight, beam-concentrating reflector shield. The spectra reported in Figures 4–7 were recorded using a JEOLCO JES-3BX No. 3141 e.s.r. instrument. DPPH was used as the standard marker at $g = 2.0036$ for the g-value measurements and the field scale was measured by Mn^{2+} and field calibrator. A JES-UCD-2X insertion-type dewar no. 481X was employed to obtain the liquid nitrogen temperature (77°K.). The JES-UV-1 ultraviolet irradiation unit no. UV-116 lamp was used for the unfiltered irradiations. A 100-kc. field modulation unit was also used in conjunction with the e.s.r. instrument.

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