in.) of sodium silico-aluminate (400 g) (*3*). The column had been previously washed with 10 percent aqueous acetone (250 ml) to remove any free alkali and then with petroleum ether (500 ml) to remove acetone. The chromatogram was developed with petroleum ether to give first a nearly colorless filtrate (500 ml, 0.11 g), followed by a yellow-colored filtrate (1000 ml). The latter fraction gave, after removal of solvent by distillation under reduced pressure, a concentrate of neoretinene b (1.2 g, E(1 percent, 1 cm) (254, 375 mμ) = 445, 1115.

When this concentrate, in petroleum ether (2 ml), was cooled to −18°C, crystals were obtained (0.6 g) that consisted of neoretinene b mixed with some neovitamin-A aldehyde [neoretinene a (*1*)]. The orange prisms of neoretinene b were separated manually from the yellow needles of neovitamin-A aldehyde and were recrystallized from petroleum ether.

The neoretinene b thus prepared had mp 63.5° to 64.4°C and E(1 percent, 1 cm) (255, 376 mμ) = 595, 857 (ethanol, Cary instrument, Fig. 1). The ratio of the absorption at the so-called "cis" peak at 254 mμ to that at the main absorption maximum (376 mμ) was 0.69. Wald kindly arranged for a test on a sample of the crystals by incubation with opsin and confirmed its identification as neoretinene b.

Hubbard and Wald (*1*) have described the concentration of neoretinene b and the preparation of some crystalline material, but the amount was reported to be too small to determine its extinction coefficient. It was found to have an absorption maximum at 377.5 mμ (ethanol) and an extinction coefficient estimated to be between 900 and 1000. Its "cis" peak ratio was about 0.5. By private communication, Wald has indicated that other highly purified, although noncrystalline, preparations have subsequently been made in his laboratory.

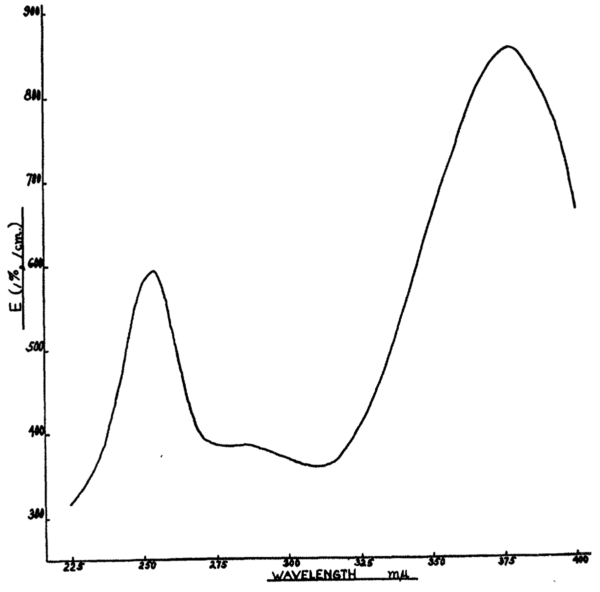

Fig. 1. Spectrophotometric curve of neoretinene b.

Neoretinene b was reduced with both lithium aluminum hydride and sodium borohydride to give the corresponding vitamin A. The absorption curve of a noncrystalline preparation, made by reduction of the aldehyde with lithium aluminum hydride, had a rather broad maximum with E(1 percent, 1 cm) (322 mμ) = 940 (ethanol) and a subsidiary maximum at 233 mμ having E(1 percent, 1 cm) = 270. From the data on the corresponding vitamin-A aldehyde isomers, it was expected that the extinction coefficient of this vitamin-A isomer [temporarily called neovitamin Ab (*1*)] would be substantially lower than that of all-trans vitamin A E(1 percent, 1 cm) (325 mμ) = 1830. Crystallization experiments on the isomer are in progress.

A preliminary bioassay by our biochemistry department, using the rat liver-storage method, has indicated that neoretinene b has about half the biological potency of all-trans vitamin-A aldehyde. Thus, this bioassay method does not reflect the biological importance that neoretinene b is considered to have in the formation of the visual pigment rhodopsin. The bioassay work will be described in a separate publication from these laboratories.

We are grateful to R. H. Delaney for technical assistance during the early work on this problem and to A. P. Besancon and assistants of these laboratories for the ultraviolet spectrographic measurements.

References and Notes

1. R. Hubbard and G. Wald, *J. Gen. Physiol.* **36**, 269 (1952).
2. Communication No. 202 from the Research Laboratories, Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N.Y.
3. Doucil from Philadelphia Quartz Co.

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The Radical Dissociation of Aryldisulfides

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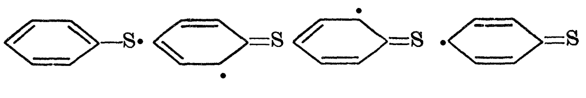
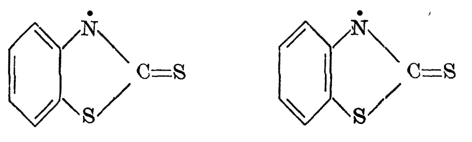
A considerable time ago (*1–3*) I investigated the problem of the dissociation of simple aryldisulfides, such as phenyldisulfide, into radicals with monovalent sulfur. In these studies I concluded that the investigated aryldisulfides do not undergo a ***reversible*** dissociation into *long-life* radicals. Later, Schönberg, Rupp, and Gumlich (*4*) reached the contrary conclusion, based partly on a different interpretation of my experiments and partly on new experimental data furnished by Schönberg and his associates. Schönberg's views have been shared by several subsequent authors (*5–7*). Considering the theoretical importance of the problem indicated, a review of existing data has been made and additional experimental evidence has been obtained supporting a position that differs from that of Schönberg and his followers.

1) We do not know all the factors that cause the formation and relative stability of long-life organic

radicals. However, from what we do know, we must conclude that the existence of long-life aromatic sulfenyl radicals, such as $C_6H_5S\cdot$, is very improbable. There is no steric influence and the resonance possibilities are restricted to only four canonical structures:



2) Unfortunately, we know nothing about the paramagnetic susceptibility of aryldisulfides. Cutforth and Selwood (*8*) have found that 2,2′-dithiobisbenzothiazole is paramagnetic in toluene solution above 100°C. They assume that the free radicals are formed through dissociation at the sulfur-sulfur bond but state that the magnetic results do not prove such dissociation; excitation to a triplet state would yield comparable results. At any rate, this compound is not an aryldisulfide and any radicals formed by dissociation of the S—S link have more possible canonical structures than aryl S· radicals have, two of them with bivalent nitrogen (*9*):



This disulfide shows a surprisingly weak thermochromism.

3) The so-called "long-life" organic radicals have only a short life-span at elevated temperature where they decompose and generally disproportionate. However, phenyldisulfide for which Schönberg, Rupp, and Gumlich (*4*) postulated a radical dissociation at 100°C can be distilled at 190° to 192°C (15 mm) (*10*) without decomposition. This boiling point shows the proper relationship to the boiling point of phenylmonosulfide, 157° to 158°C (16 mm) (*11*). The disulfide can be heated in benzene solution in the dark at 200°C for 6 hr without decomposition (*3*). At a sufficiently high temperature any organic compound will break down, and it was to be expected that then phenyldisulfide would dissociate at its weakest link—that is, the S—S bond, with formation of *short-life* radicals. Schönberg and Mustafa (*12*) found that phenyldisulfide decomposes and disproportionates at 270°C. However, this high temperature formation of short-life radicals furnishes no support for the assumption of the existence of long-life radicals at a temperature 170° lower.

4) Schönberg, Hauptmann, their coworkers, and I have all described various reactions in which the S—S bond of aromatic disulfides is split by reactants such as triphenylmethyl (*1*), diphenyldiazomethane (*13*), triphenylphosphine (*14*), metals (*1, 4, 15*), and sodium ketyls (*4*). However, this circumstantial evidence of the presence of sulfur radicals is of dubious value. As a matter of fact, in one specific case (*3*) I furnished experimental proof that the reaction with the metal proceeds in the absence of such preformed radicals. On the other hand, it is quite possible, and in some cases probable, that aromatic disulfides will form short-life radicals under the impact of other radicals or metals.

5) The thermochromism of aryldisulfides (*1*) which plays such an important part in Schönberg's arguments and particularly in Mustafa's and Kamel's recent paper (*7*) must be considered. The phenomenon that compounds reversibly change their absorption and color with rising and falling temperature is a very common one and may have various causes, sometimes trivial ones. Although it is correct to state that a reversible dissociation into long-life colored radicals is accompanied by thermochromism, the reverse conclusion would be untenable. Colored aryldisulfides also show their thermochromism in the crystalline state (*1*) where a dissociation equilibrium is highly improbable. Frequently thermochromism is the precursor of a dissociation occurring at a higher temperature (*2, 16*). I have shown (*2*) that the mercury salts of benzenethiol and of 4-dimethylamino-benzene-thiol are thermochromic in solid and dissolved state; in this case the dissociation into the disulfide plus mercury manifests itself by the appearance of the metal at a temperature above the range within which thermochromism is conspicuous.

6) Piccard (*17*) has shown that a reversible dissociation into colored radicals can be demonstrated by the fact that *Beer's law* is not obeyed. Schönberg, Rupp, and Gumlich (*4*) stated that solutions of phenyldisulfide in ethylene bromide, in anisole, and in naphthalene do not obey this law at 100°C. The alleged invalidity of Beer's law has been regarded as an unequivocal proof of the existence of long-life sulfur radicals. However, I had previously found (*1*) that xylene solutions of phenyldisulfide of approximately the same concentrations as used by Schönberg, Rupp, and Gumlich do obey Beer's law at 130°C. When recent experiments by Kharasch, Nudenberg, and Meltzer (*18*) made the existence of long-life benzene-sulfenyl radicals at 100°C very improbable, the validity of Beer's law was reinvestigated with modern spectrophotometric equipment (*19*). Anisole solutions of phenyldisulfide in a concentration range even greater than that used by Schönberg, Rupp, and Gumlich were used, and it was found that within the limits of error Beer's law is valid at 100°C. With this result, the only strong experimental support for Schönberg's theory becomes void.

For maintaining the solution in the cell at an elevated constant temperature, we used an instrument described by Kienle, Royer, and McCleary (*20*). (Although it was originally designed for a different purpose, this instrument proved to be very well suited for this investigation.) A comparatively large volume of solution is heated, with thermostatic control (±0.5°) in a separate chamber and is circulated at great speed (complete circulation, 7 to 12 sec) through the cell.

The measurements were carried out with the modified (*21*) Hardy-type (*22*) recording spectrophotometer in the range of 4000 to 7000 A. The molar absorptivity ε (molar extinction coefficient) was calculated according to the equation

$$\varepsilon = \log_{10}(1/T)/bc,$$

where T is the transmittance (the ratio of the radiant power transmitted through the solution to the radiant power transmitted through the solvent), b is the length of the cell path (0.75 cm), and c is the concentration in moles per liter. We chose the wavelength of about 4100 A, at which Schönberg, Rupp, and Gumlich reported the greatest deviation from Beer's law. (Table 1.)

The limits of error in determining ε are estimated to be about $\pm$ 10 percent. The average value for ε at 100°C was 6, while Rupp reported a very considerable increase on dilution in an even smaller concentration range than the one used in the present work. The absorption curves were straight lines and did not show the peak characteristic of free organic radicals.

7) The formation and the reactions of short-life radicals of the type RS have been extensively investigated during the past 15 years. Disulfides form such radicals under the influence of irradiation, peroxides, and/or alkyl radicals. They are exceedingly reactive and initiate chain reactions, resulting in the "abnormal" addition of thiols to double bonds, polymerizations, redistributions of RS groups, and so forth. In investigating the formation of short-life radicals from disulfides, Kharasch, Nudenberg, and Meltzer (*18*) have made an important contribution to the problem of long-life sulfenyl radicals. They investigated under which conditions disulfides form short-life radicals which initiate the addition of mercaptans to double bonds and the polymerization of butadiene-styrene. They found that the disulfides, among them phenyldisulfide, had no effect in the dark. Even at 90°C, phenyldisulfide did not catalyze the addition of lauryl mercaptan to styrene, but irradiation greatly increased the rate of this addition. The emulsion copolymerization of butadiene-styrene at 50°C was not catalyzed by p-anisyldisulfide in the dark but was very much so upon irradiation. The obvious conclusion from these experiments is that up to at least 90°C phenyldisulfide does not form long-life radicals, but it does form short-life radicals on irradiation. If phenyldisulfide would reversibly dissociate at 100°C to such an extent that Beer's law would not hold, as Schönberg maintained, the C_6H_5S radicals should initiate Kharasch's reactions in the dark.

8) The present correct nomenclature of radicals RS is *sulfenyl* radicals (*23*) (derived from RSOH, sulfenic acids). Other names used by various authors, such as *thiol radicals, thiyls, mercaptide radicals, thioalkyls* and *thioaryls*, and *mercaptyls*, are awkward and incorrect.

Table 1. Phenyldisulfide in anisole.

c (10^{-2} mole/lit)	ε, Kodama, 100°C	ε, Rupp, 100°C (*4*)
0.6	5.6	
1.25		4.1
1.3	5.9	
2.5	6.4	3.2
10.0	6.4	1.6

In conclusion the experimental and theoretical evidence available at present speaks strongly against the dissociation of aryldisulfides into long-life sulfenyl radicals.

References and Notes

1. H. Lecher, *Ber.* **48**, 524 (1915).
2. ———, *Ber.* **48**, 1425 (1915).
3. ———, *Ber.* **53**, 577 (1920).
4. A. Schönberg, E. Rupp, and W. Gumlich, *Ber.* **66**, 1932 (1933).
5. A. Schönberg, A. Mustafa, and W. Askar, *Science* **109**, 522 (1949).
6. D. A. Shirley and P. A. Roussel, *Science* **113**, 208 (1951).
7. A. Mustafa and M. Kamel, *Science* **118**, 411 (1953).
8. H. G. Cutforth and P. W. Selwood, *J. Am. Chem. Soc.* **70**, 278 (1948).
9. C. G. Moore, *J. Chem. Soc.* **1952**, 4232.
10. F. Krafft and W. Vorster, *Ber.* **26**, 2813 (1893).
11. W. Dilthey *et al., J. prakt. Chem.* [2] **124**, 81 (1930).
12. A. Schönberg and A. Mustafa, *J. Chem. Soc.* **1949**, 889.
13. A. Schönberg, O. Schütz, and J. Peter, *Ber.* **62**, 440 (1929).
14. A. Schönberg, *Ber.* **68**, 163 (1935) ; A. Schönberg and M. Z. Barakat, *J. Chem. Soc.* **1949**, 892.
15. H. Hauptmann and B. Wladislaw, *J. Am. Chem. Soc.* **72**, 707, 710 (1950) ; H. Hauptmann *et al., Ann.* **576**, 45 (1952).
16. W. Biltz, *Z. anorg. Chem.* **127**, 181 (1923).
17. J. F. Piccard, *Ann.* **381**, 347 (1911).
18. M. S. Kharasch, W. Nudenberg, and T. H. Meltzer, *J. Org. Chem.* **18**, 1233 (1953).
19. These experiments were performed by S. P. Kodama of the photometric laboratory of American Cyanamid Co.
20. R. H. Kienle, G. L. Royer, and H. R. McCleary, *Textile Research J.* **16**, 616 (1946).
21. R. H. Kienle and E. I. Stearns, *Instruments* **20**, 1057 (1947).
22. A. C. Hardy, *J. Opt. Soc. Amer.* **25**, 305 (1935) ; J. L. Michaelson, *ibid.* **28**, 365 (1938).
23. *Chemical Abstracts* **39**, 5892, 5898 (1945).

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The Isolation and Identification of "Bound" Morphine

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Gross and Thompson (*1*), for the dog, and Oberst (*2, 3*), for the human being, found that morphine was excreted in the urine, not only in the free form, but also in a combined form. Because other substances with either a phenolic or alcoholic hydroxyl group are excreted as glucuronides, it was suggested that morphine might also be excreted in this form. When the idea was tested (*3*) by measuring the concentration of glucuronic acid in hydrolyzed urine after increasing doses of morphine, the concentration was found to increase proportionally. Thus, it was concluded that probably morphine was "bound" with glucuronic acid.

Since the time of appearance of the afore-mentioned references (*1–3*), no additional specific identification of "bound" morphine has been made. This paper (*4*) reports on the isolation and identification of the conjugated morphine.

An 11-kg dog was anesthetized and infused with 4 g