water, the solution was treated with charcoal, filtered and acidulated with hydrochloric acid to a *p*H of 2. The bright-yellow precipitate was collected, washed with water and dried at 80°; yield 7.0 g., 85.3%; m. p. 219–221°. A sample was purified by dissolving in sodium bicarbonate solution and reprecipitating with hydrochloric acid; m. p. 220–222°.

Anal. Calcd. for $C_{17}H_{11}O_2N_3$: C, 70.57; H, 3.83. Found: C, 70.49; H, 3.97.

2-Amino-dibenzo[f,h]quinoxaline.—Eight grams of crude, melted 2-amino-dibenzo[f,h]quinoxaline-3-carboxylic acid was held at 250° until the frothing ceased. The mass was cooled, powdered, and extracted in a Soxhlet with acetone. The acetone was distilled and the residue triturated with ether; yield 4.28 g., 63.7%; m. p. 238–241°. A pure sample was prepared by agitating the crude with 10% sodium hydroxide and extracting with a mixture of two volumes of alcohol and eight volumes of ether, and concentrating the extracts *in vacuo* to a small volume, chilling, collecting the crystals, etc.; yield 85%, m. p. 249°.

Anal. Calcd. for $C_{16}H_{11}N_3$: C, 78.35; H, 4.51; N, 17.14. Found: C, 78.20; H, 4.62; N, 17.39.

2-Sulfanilamido-dibenzo[f,h]quinoxaline.—2-Amino-dibenzo[f,h]quinoxaline (4.7 g.) was condensed with acetylsulfanilyl chloride (5.3 g.) in anhydrous pyridine. The reaction product was isolated, deacetylated and purified in the usual manner; yield 4.54 g., 59%, m. p. 258–260°.

Anal. Calcd. for $C_{22}H_{16}N_4O_2S$: C, 65.97; H, 4.03; N, 14.00. Found: C, 66.22; H, 4.38; N, 13.79.

2-N⁴-Acetylsulfanilamido-dibenzo[f,h]quinoxaline.—This was obtained in a pure form by acetylating 2-sulfanilamido-dibenzo[f,h]quinoxaline with acetic anhydride; m. p. 265–267°.

Anal. Calcd. for $C_{24}H_{18}N_4O_3S$: C, 65.12; H, 4.12; N, 12.67. Found: C, 64.69; H, 4.38; N, 12.55.

THE RESEARCH LABORATORIES
MERCK & CO., INC. JOHN WEIJLARD
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RECEIVED MARCH 24, 1945

COMMUNICATIONS TO THE EDITOR

PARAMAGNETISM OF THE PHOSPHORESCENT STATE

Sir:

Lewis and Kasha[1] have shown that the phosphorescent state is the triplet state. Hence the phosphorescent state must be paramagnetic. We have undertaken to measure this paramagnetism, using an apparatus similar to that of Theorell,[2] except that the two suspending filaments (extremely fine glass threads, 140 cm. long) and the horizontal suspended bar (of very thin-walled glass tubing) were all contained in a framework of 2-cm. glass tubing, which guarded against draughts, and also permitted the apparatus to be filled with any gas at any pressure. The capillary tip of the horizontal bar which held the phosphorescent sample, centered in the magnetic field, was observed in a microscope which read the position to about 2 μ. With a total suspended weight of 0.14 g., a horizontal displacement of 1 μ is produced by a force of 0.1 microgram.

When the long thin phosphor was centered in the magnetic field the light from a high pressure mercury arc was thrown from below upon one-half of the sample. With no field, illumination produced no observable deflection.

In the magnetic field, the illuminated part, because of the triplet state molecules, should move inward. In our first experiments, while a small movement in the expected direction was observed at the moment when the illumination began, this was followed almost immediately by a much larger deflection in the reverse direction. This remarkable phenomenon, which we are studying further, was found only when the gas surrounding the sample contained oxygen. When the surrounding gas was argon or carbon dioxide only a movement in the expected direction occurred.

Our phosphors were of fluorescein dissolved in boric acid glass, and of such concentration and thickness as to absorb practically all of the incoming light. Hence the number of paramagnetic molecules formed per cm.^{2} of illuminated sample should depend only upon the intensity of the light. We used two long thin rectangular samples: one, a solid slab of the boric acid solution, the other of similar material coarsely ground and attached to a ribbon of cellophane by a thin coating of rubber cement.

The magnetic force at 20,000 gauss, per cm. width, was 4.1×10^{-3} mg. for the first sample, and 4.7×10^{-3} mg. for the second, each being a mean of 10 and 20 experiments, respectively; but neither of the two means is accurate to much better than 10%. The illumination was through a filter of concentrated aqueous copper sulfate. Experiments with screens showed that the displacement is proportional to the intensity of this blue light.

Assuming the magnetic moment of triplet state fluorescein to be that of O_2, and from the curve (Fig. 6) of Lewis, Lipkin and Magel,[3] showing the percentage of phosphorescent molecules as a function of light intensity, our numerical results correspond to those calculated for 20% of their

(1) Lewis and Kasha, THIS JOURNAL, **66**, 2100 (1944); **67**, 994 (1945).

(2) Theorell, *Arkiv. Kemi. Mineral. Geol.*, **16A**, No. 1 (1943).

(3) Lewis, Magel and Lipkin, THIS JOURNAL, **62**, 2973 (1940).

highest intensity, which is about what we would guess for our set-up.

CHEMICAL LABORATORIES OF THE
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RECEIVED JUNE 16, 1945

THE ALLERGENIC PRINCIPLES OF POISON IVY. IV. ON THE MECHANISM OF THE ENZYMATIC OXIDATION OF CATECHOLS[1]

Sir:

Our study of the immunochemistry of the alkylcatechols present in *Rhus toxicodendron* has led us to examine the mechanism of the enzymatic oxidation of these and related substances. The oxidation of catechol itself by tyrosinase has been extensively investigated, and the resulting data have been interpreted in terms of a sequence of reactions by Dawson and Nelson.[2]

When such a sequence of reactions involves chromophoric molecules, in the ideal case the observed absorption D at any wave length λ and at any time t during the process may be expressed as

$$D_{\text{obs.}, \lambda, t} = l\,[\epsilon_{\lambda A}(A) + \epsilon_{\lambda B}(B) + \epsilon_{\lambda C}(C) + \cdots \epsilon_{\lambda N}(N)]$$

where l is the length of the cell used, (A), (B), (C), and . . . (N) are the molar concentrations of those components of the sequence which are present in sufficient concentration at time t to contribute a measurable increment to the total absorption, and where $\epsilon_{\lambda A}$, $\epsilon_{\lambda B}$, $\epsilon_{\lambda C}$ . . . and $\epsilon_{\lambda N}$ are the molecular extinction coefficients at λ of those components. If each such component and its molecular extinction coefficients be known, the kinetics of the sequence may be determined by repeatedly scanning a range of absorbing wave lengths during the process. If they be unknown, hypothetical mechanisms may be tested by comparing the spectrophotometric kinetics of the total process with those initiated by postulated intermediates.

By scanning the 220–400 mμ absorption region of *catechol* at intervals of four minutes during its enzymatic oxidation[3] it is now found that the postulated formation of hydroxy-*p*-quinone[2] is not detectable. Under conditions optimal for this process[2] the initial maximum absorption of catechol at 275.5 mμ disappeared rapidly with simultaneous development of a new maximum at 380 mμ, that characteristic of the initial absorption of *o-benzoquinone*. Scattering curves then appeared throughout the observed range of both spectra, with inflexions at 260–290 mμ. *Hydroxy-p-quinone* under similar conditions but in the absence of enzyme displayed an intense initial absorption at 260 mμ; this degraded quickly with the formation of a new maximum at 340 mμ. The latter was relatively stable; only in sixteen hours did a final spectrum with maximum at 290 mμ appear. Significantly, in the presence of tyrosinase the initial maximum absorption of *hydroxyhydroquinone* at 287 mμ disappeared rapidly with formation of successive maxima at 260 mμ, 335–340 mμ, and at 285–287 mμ.

On the basis of this evidence, of that in the cited review, and of unpublished analyses of the polymeric oxidation products of catechol, it is probable that the initial product of the enzymatic oxidation of catechol, *o*-benzoquinone, participates in a polymerization to a phenolic chain susceptible to further enzymatic oxidation. Kinetic spectrophotometry of the autoxidation, oxidation in the presence of unsaturated fatty esters, and oxidation in the presence of tyrosinase of 3-*n*-pentadecylcatechol (hydrourushiol) has similarly established 3-*n*-pentadecyl-*o*-benzoquinone as the initial oxidation product in each case. The technique is being extended to related problems.

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(1) For the third paper in this series, see Mason, THIS JOURNAL, **67**, 418 (1945).

(2) Dawson and Nelson, *Advances in Enzymology*, **IV**, 99-152 (1944).

(3) The purified mushroom tyrosinase was generously contributed by Professor Charles R. Dawson.