*p*-**Ethylaminobenzamide.**—A mixture of 6.8 g. (0.05 mole) of *p*-aminobenzamide, 7.8 g. (0.05 mole) of ethyl iodide and 5 g. of sodium bicarbonate in 75 cc. of 35% aqueous alcohol was refluxed on the steam-bath for eight hours. Most of the solvent was distilled off, water added, and the solid filtered off. The product was dissolved in 70 cc. of dilute hydrochloric acid, cooled in ice water and to it, with stirring, was added a solution of 3 g. of sodium nitrite in 15 cc. of water. The nitroso derivative was filtered off, washed with water and dried; crude yield, 5.5 g. It was dissolved in 180 cc. of hot absolute alcohol and filtered with charcoal. Dry hydrogen chloride was bubbled into the cooled and well stirred filtrate at a rapid rate until all the solid went into solution. The clear solution was stirred at room temperature for four and one-half hours, filtered with charcoal, and evaporated almost to dryness. Water was added to dissolve the hydrochloride which separated and the solution was made alkaline with ammonium hydroxide. On standing, the product separated out. It was recrystallized from hot water.

*p*-**Propylaminobenzamide.**—A mixture of 10.8 g. of *p*-aminobenzamide, 60 g. of zinc dust, 100 cc. of glacial acetic acid, and 200 cc. of absolute alcohol was refluxed on a steam-bath with stirring. Five grams of freshly distilled propionaldehyde was added over a period of one hour and refluxing continued for one hour longer. The solid was filtered off and the filtrate was steam distilled to remove the alcohol and acetic acid. The residue (about 750 cc.) was cooled and the solid which separated was filtered off and recrystallized from dilute alcohol. Compound IX was prepared by a similar procedure.

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# COMMUNICATIONS TO THE EDITOR

## THE NATURE OF CYPRIDINA LUCIFERIN

*Sir:*

Johnson and Eyring have recently stated[1] that "'luciferin' apparently contains both coenzyme (I or II) and a flavin prosthetic group." Ball and Ramsdell[2] are led to "suspect that flavin-adenine dinucleotide may play some role in the luminescent mechanisms of the firefly." Since *Cypridina* luciferin is oxidizable[3] reversibly[4] this latter suggestion is a plausible possibility. The more definite conclusion of Johnson and Eyring[1] is *a priori* a very attractive one because of the fundamental importance of the coenzymes and flavins in cellular oxidations and the occurrence of flavins in a number of oxidases. Unfortunately, a reëxamination of the experimental material on luciferin does not entirely confirm their conclusion.

In regard to the absorption spectrum of *Cypridina* luciferin, comparison should be made with the flavins rather than the flavoproteins. Oxidized riboflavin has maxima at about 3600 and 4500 Å.[5] while reduced riboflavin is colorless. Reduced luciferin concentrates show a maximum at about 4300 Å.[6] and hence, since this is probably due to luciferin, it is a colored compound. This band disappears after brief aeration and a new band appears at about 4700 Å. The 4700 Å. band disappears after prolonged exposure of the solution to air while a band at about 3600 Å. appears.

Johnson and Eyring report[1] that luminescence occurs after treatment of "luciferase" solution with $Na_2S_2O_4$, reduced coenzymes or riboflavin. Such an experiment is difficult to interpret if the observed luminescence was faint. It is easily possible for the partially dark adapted eye to see one millionth part of the light emitted by the luciferin from a small amount of *Cypridina*.[7] In any case if reduced riboflavin is to be regarded as a substrate in the luminescent reaction identical with or analogous to luciferin, the amount of light emitted should be large and should be related to the amount of substrate oxidized.

Indirect studies on the oxidation–reduction potential of luciferin[4] place it near toluhydroquinone and hydroquinone.[8] The oxidation reduction potential of riboflavin is 0.4 or 0.5 v. lower than this at *p*H 7.0, while that of coenzyme is still lower. It has been reported that luciferin concentrates contain no nitrogen,[9] although the sensitivity of the method in relation to the amount of partially purified luciferin was not stated. In unpublished experiments of Dr. M. Kunitz, attempts to measure coenzyme I in luciferin preparations were unsuccessful. Here the sensitivity was such that the luciferin sample could hardly have contained as much as 2% of coenzyme.

The available data, therefore, although they may not exclude the conclusion of Johnson and Eyring, certainly give it little support.

(1) Johnson and Eyring, THIS JOURNAL, **66**, 848 (1944).
(2) Ball and Ramsdell, *ibid.*, **66**, 1419 (1944).
(3) Harvey, *J. Gen. P.*, **1**, 133 (1918).
(4) Anderson, *J. Cell. & Comp. Physiol.*, **8**, 261 (1936).
(5) Warburg and Christian, *Biochem. Z.*, **298**, 150 (1938).
(6) Chase, *J. Biol. Chem.*, **150**, 433 (1943).
(7) Harvey, *Science*, **57**, 501 (1923).
(8) This suggests the desirability of investigating luciferin as a possible link between flavins and oxygen.
(9) Chakravorty and Ballentine, THIS JOURNAL, **63**, 2030 (1941).

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## COLORIMETRIC TESTS FOR DDT AND RELATED COMPOUNDS

*Sir:*

The revolutionary development of the insecticide DDT,[1] the major portion of the technical product being 2,2-bis(*p*-chlorophenyl)-1,1,1-tri-

(1) Annand, *J. Econ. Entomol.*, **37**, 125 (1944); Froelicher, *Soap and Sanit. Chem.*, **20** (7), 115 (1944).

chloroethane, has made a sensitive colorimetric test essential for its detection and determination in such fields of study as spray residues, pharmacology, solubility in water, and vapor pressure. We wish to report the development of a very sensitive color test for this and related compounds based on nitration to polynitro derivatives and the production of intense colors upon addition of methanolic sodium methylate to a benzene solution of the nitration products.

Several pure chromogenic compounds were prepared by nitration with concentrated sulfuric acid–fuming nitric acid (1:1 by volume) at 100° for 1 hour and recrystallization from acetone–ethanol: Tetranitro-*p,p'*-DDT, or 2,2-bis-(4-chloro-3,5-dinitrophenyl)-1,1,1-trichloroethane, m. p. 223.5–224.5° (cor.). *Anal.* Calcd. for $C_{14}H_5Cl_5N_4O_8$: C, 31.46; H, 0.94. Found: C, 31.66; H 1.07.[2] Nitration products of DDD, or 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane, and of the ortho, para isomer of DDT, or 2-*o*-chlorophenyl-2-*p*-chlorophenyl-1,1,1-trichloroethane, prepared in the same manner, m. p. 224.5–225.5° (cor.) and 229.5–230° (cor.), respectively.

Spectrophotometric curves were obtained[3] in the range 400–750 mμ on a mixture of one volume of a benzene solution of each of the nitrated compounds with two volumes of sodium methylate reagent (5.0 g. sodium per 100 ml. methanol solution) at a final concentration of 0.020 g. of compound per liter. A General Electric recording spectrophotometer was used with 1-cm. absorption cells. Data taken from the curves are given in Table I.

TABLE I

SPECTROPHOTOMETRIC DATA

| Nitrated derivative from | Wave length mμ | Specific extinction Liters/g./cm. |
|---|---|---|
| *p,p'*-DDT | 600 max. | 48.0 |
| | 443 min. | 5.9 |
| *p,p'*-DDD | 598 max. | 38.7 |
| | 442 min. | 4.7 |
| *o,p'*-DDT | 590 max. | 25.0 |
| | 558 min. | 22.7 |
| | 511 max. | 27.8 |
| | 426 min. | 5.6 |

The curves were obtained within 10 minutes after adding the reagent to minimize the effect of the slow fading of the colors. The derivatives of *p,p'*-DDT and *p,p'*-DDD give blue colors with one absorption maximum and one minimum in the range studied, while the derivative of the ortho, para isomer of DDT gives a violet red color with two maxima and two minima.

The spectrophotometric curves indicate the feasibility of determining both the total and relative amounts of *p,p'*-DDT and *o,p'*-DDT in mixtures of the two, and the high extinction values obtained illustrate the sensitivity that can be expected in the detection of DDT. While a number of interferences have been found, some would not be expected to occur or be used with DDT; others can be removed or detected in some manner. A colorimetric analytical method for the determination of DDT making use of these reactions has been developed, and a description is being prepared.

The chemical reactions of these polynitro compounds and the nitration of degradation products of DDT (such as the dehydrochlorinated derivative) are being further investigated. More complete spectrophotometric data and a theory which accounts for the colors and absorption spectra produced by sodium methylate will be elaborated upon in forthcoming papers.

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(2) Analysis by W. F. Barthel.

(3) Grateful acknowledgment is made to Sylvan H. Newberger, of the U. S. Food and Drug Administration, for coöperation in securing the spectrophotometric data.

## THE STRUCTURE OF ETHYL β-IONYLIDENEACETATE[1]

*Sir:*

Since ethyl β-ionylideneacetate, prepared from β-ionone by the Reformatsky reaction, is an important intermediate in the synthesis of compounds related to vitamin A, its "β" structure must be definitely established. The "β" structure proposed by Karrer, *et al.*,[2] has been questioned recently by Sobotka and co-workers[3] on the basis of pyrolysis and absorption spectra studies.

These workers obtained α-ionone by heating the barium salt derived from the ester obtained from the Reformatsky reaction on β-ionone, indicating that rearrangement from β to α form had occurred during either the Reformatsky reaction or the heat treatment of the barium salt. Since the absorption spectrum of the ethyl β-ionylideneacetate does not conform to that anticipated for a compound containing three olefin bonds conjugated with a carbethoxy group, they have concluded that a rearrangement from β to α structure occurs during the Reformatsky reaction. They believe that stereoisomerism accounts for the differences in the products prepared from the α- and β-ionones.

Recent work in this laboratory[4] on the ionylideneacetones prepared from the ionylideneacetates and cyanoacetates seems to justify the assignment of the "β" structure to the ester obtained from β-ionone. We have now obtained additional evidence for the "β" structure from ozonolysis of the ester in question. In duplicate

(1) This work was made possible by a research grant from Sharp and Dohme, Inc.

(2) Karrer, Salomon, Morf and Walker, *Helv. Chim. Acta*, **15**, 878 (1932).

(3) (a) Sobotka, Bloch and Glick, THIS JOURNAL, **65**, 1961 (1943); (b) Sobotka and Bloch, *Chem. Rev.*, **34**, 435 (1944).

(4) Young, Andrews and Cristol, THIS JOURNAL, **66**, 520 (1944).