of the reference acid, a conclusion which is contrary to the observed facts.)

We suggest that the origin of this behavior is steric in nature—the observed weakening of the base occurring with the first member of the series in which the alkyl group of the base can interfere with the acid (H^+ or BMe_3). It is obvious that the point in the series at which the weakening is observed will depend upon the size of the reference acid. In the case of a large acid, such as trimethylboron, such interference occurs in a base having a shorter chain than in the case where the acid is the small proton. It would be expected that an even larger acid, *e. g.*, triethylboron or tri-*n*-propylboron, would lead to a shift of the irregularity to methylamine, or even to ammonia. This prediction is now being tested.

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RECEIVED APRIL 19, 1944

THE RELATIONSHIP OF STRUCTURE TO ACTIVITY OF SULFANILAMIDE TYPE COMPOUNDS

Sir:

Kumler and Daniels[1] have suggested that the resonating form of the *p*-amino group with a separation of charge is a fundamental factor for the bacteriostatic activity of sulfanilamide type compounds. The experimental evidence for this suggestion is "that sulfanilamide has a higher (ultraviolet) extinction coefficient in base than in water."[2]

Because of the theoretical importance placed on this single observation, and since previous studies in this Laboratory[3] were not in agreement with it, we have repeated the work using a more suitable instrument (Beckman Spectrophotometer Model DU).[4] The data obtained for sulfanilamide are recorded in Fig. 1 and Table I. The additive function $-\log_{10}T$ was used in Fig. 1 to demonstrate the reliability of the method of correcting for solvent absorption.

TABLE I

ULTRAVIOLET SPECTRAL DATA ON SULFANILAMIDE

Form	Wave length of peak absorption, Å.	Extinction coefficient
Molecular (1 *N* NaCl)	2585	16,380 ± 49[a]
Ionic (1 *N* NaOH)	2505	16,150 ± 48
Difference	80	230 ± 97
Per cent. difference		1.42 ± 0.6

[a] The limits of error are those encountered in quadruplicate determinations.

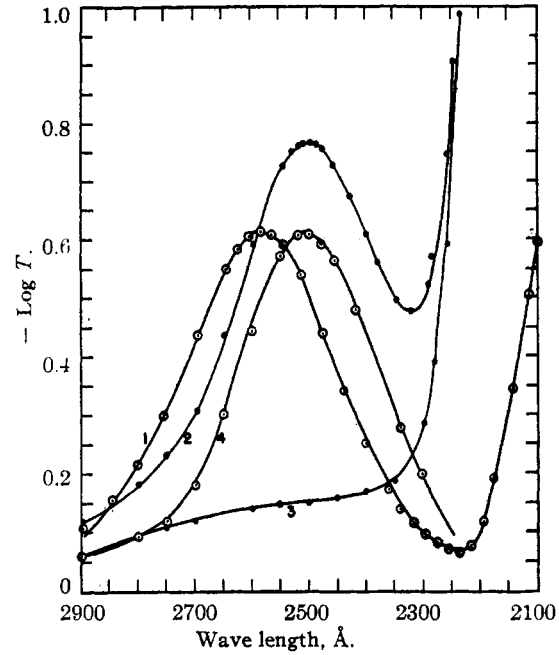

Fig. 1.—Log_{10} of per cent. light transmitted by a 1-cm. sample of: 1, 3.78×10^{-5} *M* sulfanilamide in 1 *N* NaCl, 1 *N* NaCl blank; 2, 3.78×10^{-5} *M* sulfanilamide in 1 *N* NaOH, water blank; 3, 1 *N* NaOH, water blank; 4, 3.78×10^{-5} *M* sulfanilamide in 1 *N* NaOH, solid line obtained by subtracting curve 3 from 2, and points -⊙- from measurements with 1 *N* NaOH blank.

These results do not confirm those of Kumler and Strait, and there appears to be no adequate experimental basis for the assumption that the resonating form with a separation of charge contributes more in a sulfonamide ion. Moreover, the lack of correlation between the base constants and bacteriostatic activity of sulfanilamide type compounds in general[5] does not support the hypothesis that the base weakening resonance form is a fundamental factor for activity.

(1) Kumler and Daniels, THIS JOURNAL, **65**, 2190 (1943).

(2) Kumler and Strait, *ibid.*, **65**, 2349 (1943).

(3) E. J. Robinson and D. Richardson, personal communication.

(4) This instrument was made available to us through the courtesy of Dr. V. du Vigneaud and Dr. W. Summerson, Department of Biochemistry, Cornell University Medical School, New York, N. Y.

(5) Bell and Roblin, THIS JOURNAL, **64**, 2905 (1942).

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STAMFORD, CONN. RICHARD O. ROBLIN, JR.

RECEIVED MARCH 15, 1944

SYNTHETIC THIOPHANE DERIVATIVES

Sir:

Karrer and Schmid[1] have recently described the synthesis of thiophanone-3 and of 2-methylthiophanone-3. Results essentially in agreement with those of the Swiss investigators have been obtained in this Laboratory.

(1) Karrer and Schmid, *Helv. Chim. Acta*, **27**, 116, 124 (1944).

(Carboethoxymethyl)-(β-carboethoxyethyl) sulfide, obtained by the reaction of thioglycolic ester with acrylic ester in the presence of a small amount of piperidine, was cyclized by means of sodium metal in benzene suspension. The product, thiophan-3-on-4-carboxylic ester,[2] $C_7H_{10}O_3S$, boiled at 96° at 4 mm. and gave a phenylhydrazone, $C_{13}H_{16}N_2O_2S$, m. p. 100–101°[3] from aqueous ethanol (Calcd.: C, 59.1; H, 6.1; N, 10.6. Found: C, 59.1; H, 6.0; N, 10.7), and a semicarbazone, $C_8H_{13}N_3O_3S$, m. p. 176° (Calcd.: N, 18.2. Found: N, 18.1). The ester, on acid hydrolysis, gave thiophanone-3, C_4H_6OS, b. p. 83–85° at 29 mm., darkens on standing, semicarbazone, $C_5H_9N_3OS$, m. p. 196° dec., dinitrophenylhydrazone, $C_{10}H_{10}N_4O_4S$, m. p. 179° dec. (Calcd.: N, 19.8. Found: N, 19.7).

2-Methylthiophan-3-on-4-carboxylic ester, $C_8H_{12}O_3S$, b. p. 93–95° at 4.5 mm., was obtained by cyclization of (α-carboethoxyethyl)(β-carboethoxyethyl) sulfide. On hydrolysis it gave 2-methylthiophanone-3, C_5H_8OS, b. p. 82° at 28 mm., unstable in air, semicarbazone, $C_6H_{11}N_3OS$, m. p. 185–186°, dinitrophenylhydrazone, $C_{11}H_{12}N_4O_4S$, m. p. 161–162° (Calcd.: C, 44.7; H, 4.1. Found: C, 44.6; H, 4.2).

Circumstances made necessary the termination of our studies on synthetic thiophanes in August, 1942. It is intended to resume these unfinished investigations after the war.

(2) The assigned formula is based on analogy; see Prill and McElvain, THIS JOURNAL, **55**, 1235 (1933).

(3) Karrer and Schmid (ref. 1, p. 127) obtained two phenylhydrazones, m. p. 141.5–142.5° and m. p. 167°; possible explanations to account for this discrepancy cannot be investigated at this time.

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HARRY COHEN

RECEIVED APRIL 21, 1944

THE NATURE OF THE LUCIFERIN–LUCIFERASE SYSTEM

Sir:

In the luminescent system extracted from *Cypridina*, the substrate, luciferin, is dialyzable, while the enzyme, luciferase, is not.[1] Reduced luciferin undergoes reversible dark oxidation by oxygen or ferricyanide, and apparently irreversible luminescent oxidation by luciferase plus oxygen. Like dihydrocoenzyme, with an absorption maximum at 3400 Å., considerably purified, reduced luciferin solutions have an absorption peak near 3200 Å. On adding oxygen this absorption diminishes, while a new peak at 4300 Å. appears, shifting quickly to 4700 Å., then disappearing.[2] In presence of oxygen, aqueous luciferin is unstable. The absorption at 4700 Å. resembles that of certain flavoproteins, and practically coincides with the luminescence maximum at 4750 Å. The energy of this luminescent transition corresponds to 59,430 calories as compared to 57,340 calories available by direct oxidation of two hydrogen atoms on glucose, the substrate known to increase tremendously the luminescence of washed luminous bacteria.

We have now observed that a concentrated solution of luciferase, after prolonged dialysis with several hundred volumes of distilled water, and storage for some eighteen months in a refrigerator, will luminesce with oxygen, following reduction by (1) $Na_2S_2O_4$, (2) reduced Coenzyme I solution (partially reduced by pure hydrogen and platinized platinum, then added anaerobically to luciferase), (3) reduced riboflavin (similarly treated), (4) washed cells of *E. coli* plus glucose, and (5) growing culture of *E. coli*.[3] A portion of the luciferase solution gave luminescence repeatedly on successive additions of an excess of hydrosulfite then oxygen, but finally ceased. Luminescence continuous for some hours resulted when hydrogen, plus slight oxygen impurity, was passed through some luciferase solution containing platinized asbestos. Oxidized coenzyme, oxidized riboflavin, ferrocyanide, or glucose without bacteria, as expected, did not reduce the luciferase.

A consideration of the above facts indicates that the luminescent system consists of a pyridine nucleotide plus flavoprotein. According to the absorption spectrum, "luciferin" apparently contains both Coenzyme (I or II) and a flavine prosthetic group, the former component providing a reductant, and the latter, after loose combination with its specific protein, comprising molecules excitable by oxidation. Some of the excited molecules radiate and are not destroyed, but others, failing to radiate are destroyed by their absorbed energy. This phenomenon causes the "irreversible reaction" of luminescence. Such destruction of non-radiating excited molecules is responsible for the familiar degradation of riboflavin in solution in the light, and also phthalhydrazides during luminescent oxidation.

Thus, in luminous bacteria, light emission presumably occurs when flavoprotein, reduced by hydrogen from suitable substrates (*e. g.*, glucose) via the dehydrogenase–coenzyme system, is oxidized directly by oxygen. The effects of cyanide on oxygen consumption and luminescence, respectively, indicate that most of the hydrogen proceeds step-wise, by electron transfer, through the cytochrome–heme system to oxygen. With chlorophyll substituted for the related heme molecule, the same system of catalysts operating in the reverse direction would lead to photosynthesis. In luminescence two hydrogens are oxidized for the quantum emitted, while in photosynthesis single hydrogens are made available.

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RECEIVED APRIL 17, 1944

(1) Harvey, "Living Light," Princeton University Press, Princeton, N. J., 1941.

(2) Chase, *J. Biol. Chem.*, **150**, 433 (1943).

(3) Doctors Chase, Schlenk and Kunitz kindly supplied experimental materials.