even though maintained at low temperature. For this reason the procedure for measuring the cresolase activity of tyrosinase has been modified to eliminate the fifteen to twenty minute temperature equilibration period prior to initiating the reaction by mixing the enzyme and substrate. The *p*-cresol reaction is characterized by a lag period that varies in length with a number of factors including the purity of the enzyme.[6] During this lag period the enzyme does not appear to be seriously inactivated. The presence of substrate and protective protein gelatin prevents the type of inactivation shown in Table I which occurs when the enzyme stands alone in highly diluted solution. The modified procedure, described below, utilizes the lag period as the temperature equilibration period prior to closing the manometers.

Experimental

The procedure developed for measurement of the cresolase activity is as follows: The cold stock solution of enzyme was diluted with ice-cold water to the proper volume for activity measurement and 1.00-ml. aliquots were added immediately to reaction mixtures already prepared from 2.0 ml. of McIlvaine buffer *p*H 7.0, 1.0 ml. of *p*-cresol solution (4 mg. per ml.), 1.0 ml. of gelatin solution (5 mg. per ml.) and enough water to make the final reaction volume 8.0 ml. (including the enzyme solution). The enzyme was added last and directly to this reaction mixture just prior to placing the respirometer flasks on the manometers (Warburg type). The flasks with attached manometers were then placed in a 25.00 ± 0.01° thermostat, and the shaker was started immediately (120 oscillations per minute). The reaction was allowed to proceed for five to ten minutes with the manometers open until temperature equilibrium was established. During this time, little oxygen was absorbed because of the lag period of the reaction. After closing the manometers, readings were taken at five-minute intervals throughout the linear course of oxygen uptake (usually twenty to thirty minutes). The units of activity were calculated from the rate of oxygen uptake during this linear period, using an uptake of 10 cu. mm. per minute as equal to one unit of cresolase activity. In accordance with the experience of previous workers in this Laboratory, it was found desirable to dilute the enzyme so that the reaction flask contained between 0.5 and 2.5 units. All activity measurements were made in triplicate using a fourth flask containing everything except enzyme as a barometric control.

(6) J. M. Nelson and C. R. Dawson, *Adv. Enzymology*, **4**, 99 (1944).

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The Conductance of Tetraethylammonium Sulfamate in Liquid Hydrogen Sulfide[1]

BY EDGAR E. LINEKEN[2]

The original purpose of this investigation was to find the effect of varying the number of alkyl groups substituted for the hydrogen atoms of the ammonium ion on the conductance of substituted ammonium sulfamates in liquid hydrogen sulfide. This effect for substituted ammonium chlorides has been reported by Lineken and Wilkinson.[3] The conductance of a partially substituted ammonium sulfamate might be expected to be much lower than that of completely substituted ammonium sulfamate because of the probable attraction of the amino group present in the sulfamate ion for an unsubstituted hydrogen in the ammonium ion (in addition to the well-known attraction of an unsubstituted proton of an ammonium ion for the negative ion of a salt when dissolved in a liquid of low dielectric constant).

The conductance of tetraethylammonium sulfamate was found to be comparable in magnitude with the conductance of tetraethylammonium chloride. The solubility of trimethyl- and *n*-propylammonium sulfamates and of ammonium sulfamate each appeared to be nil and the conductance of liquid hydrogen sulfide did not increase upon agitation with either of these compounds. Thus the effect of incomplete substitution was to reduce greatly the solubility of the compounds and the purpose mentioned above was not realized.

Experimental

Sulfamates.—These were all prepared from sulfamic acid prepared from urea according to the method of Baumgarten.[4] The partially substituted ammonium sulfamates and ammonium sulfamate were prepared by the methods described by Butler and Audrieth[5] for those compounds. Tetraethylammonium sulfamate was prepared by adding sulfamic acid to an excess of the hydroxide (10% aqueous solution). The salt was recovered by evaporation and dried with dry air under reduced pressure. After washing with anhydrous ether, the drying was repeated. The resulting product was not sufficiently pure and it was then recrystallized from amyl acetate, washed with anhydrous ether, and dried at 110°.

Table I shows the calculated and determined sulfur values for these compounds and the melting points of some of them. Sulfur was determined as barium sulfate after converting the sulfamate to sulfate with nitrite in acid solution.[5] The purity of *n*-propylammonium sulfamate was not checked because a test showed that it appeared not to dissolve in the solvent and, if a trace did dissolve, it did not conduct. The other compounds which behaved similarly were analyzed before being so tested.

TABLE I

Compound, sulfamate	Sulfur, % Calcd.	Found	M. p.,[a] °C.
Ammonium	28.09	28.14, 27.90	.....
Trimethylammonium	20.51	20.27	150–152
Tetraethylammonium	14.17	14.17	152

[a] These melting points were determined with an uncalibrated thermometer. Sharpness of melting, as a criterion of purity, rather than the exact melting point, was of interest.

Solvent and Method of Measurement of Conductance.—Hydrogen sulfide was prepared, purified and liquefied as previously described.[3] The equipment and method used for conductance measurements were as outlined by

(1) The work described in this paper was done at the University of Vermont, Burlington, Vermont.

(2) Present address: Calco Chemical Division, American Cyanamid Company, Bound Brook, New Jersey.

(3) E. E. Lineken and J. A. Wilkinson, THIS JOURNAL, **62**, 251–256 (1940).

(4) P. Baumgarten, *Ber.*, **69**, 1929–1937 (1936).

(5) M. Josetta Butler and L. F. Audrieth, THIS JOURNAL, **61**, 914–915 (1939).

Lineken.[6] Qualitative tests for conducting power were made in some instances with small volumes of hydrogen sulfide in test tubes using unstandardized dip type electrodes.

Conductance Data.—The results of conductance measurements on solutions of tetraethylammonium sulfamate in liquid hydrogen sulfide are shown in Table II and in Fig. 1.

TABLE II

CONDUCTANCE OF TETRAETHYLAMMONIUM SULFAMATE IN LIQUID HYDROGEN SULFIDE[a]

C	$k \times 10^6$	Λ
0.00016	0.4833	3.0
.000789	1.107	1.40
.001421	1.593	1.121
.003749	3.217	0.8581
.005949	4.558	0.7662

[a] C is concentration in moles per liter. k is the specific conductance of the solute. (The specific conductance of the solvent was 11×10^{-10} ohms^{-1} cm.$^{-1}$.) Λ is the molar conductance.

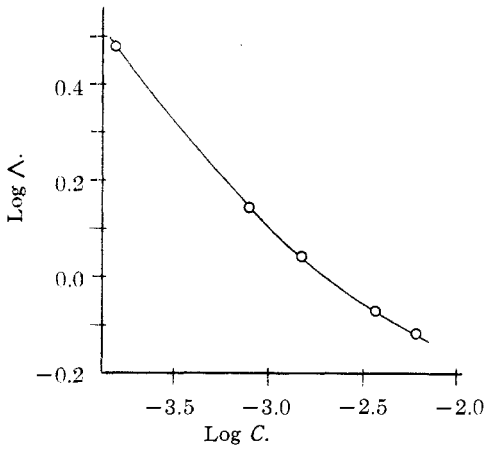

Fig. 1.

The specific conductance of the solute in a solution containing excess solid and presumably saturated, because the resistance of the solution was not changed by further agitation, was 4.79×10^{-6} ohm^{-1} cm.$^{-1}$.

The resistances were measured at about 1000 cycles per second. The resistance of the most dilute solution varied considerably less than 0.1% with changes in frequency (470 to 2640 cycles per second). It was not considered necessary to examine each solution for possible frequency effects because the specific conductance range involved was small.

(6) Lineken, THIS JOURNAL, **68**, 1966 (1946).

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The Interaction of 6-Chloro-2-methoxy-9-phenoxyacridine and Ethylene Diamine

BY R. L. MCKEE[1] AND R. W. BOST

Recently it has been reported[2] that the reaction between 9-chloroacridine and an excess of ethylenediamine forms N,N′-bis-(9-acridyl)-ethylenediamine rather than the expected 9-(β-aminoethylamino)-acridine.

In confirmation of this report, we wish to record the similar formation of N,N′-bis-(6-chloro-2-methoxy-9-acridyl)-ethylenediamine.

Experimental

A mixture of 5.5 g. (0.016 mole) of 6-chloro-2-methoxy-9-phenoxyacridine,[3] 10 g. of phenol and 2.0 g. (0.033 mole) of ethylenediamine (Eastman Kodak Co., 95–100% dried over potassium hydroxide and distilled) was warmed on a steam-bath for two hours. Concentrated hydrochloric acid (10 cc.) was added, followed by 50 cc. of ether. The precipitate was filtered and washed with ether. The solid was ground thoroughly under concentrated ammonium hydroxide, filtered, washed with water and dried. The product was rather difficultly soluble in alcohol or dioxane and was recrystallized from absolute alcohol containing 20% of pyridine. The yield was 3.5 g. (81.5% of the theoretical) of fine yellow crystals melting with decomposition at 184–186°.

Anal. Calcd. for $C_{30}H_{24}Cl_2N_4O_2$: N, 10.34; Cl, 13.05. Found: N, 10.35, 10.39; Cl, 13.19.

(1) Wm. S. Merrell Co., Postdoctoral Fellow.

(2) Albert and Gledhill, *J. Soc. Chem. Ind.*, **64**, 169 (1945).

(3) Drozdov, *J. Gen. Chem., U. S. S. R.*, **7**, 1668 (1937); through *C. A.*, **32**, 160 (1938).

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RECEIVED SEPTEMBER 23, 1946

The Reaction between 4-Methoxybenzaldehyde and 2,3-Dimethylquinoxaline

BY R. L. MCKEE,[1] MARTHA K. MCKEE AND R. W. BOST

The reaction between 2,3-dimethylquinoxaline and a number of aromatic aldehydes has been studied by Bennett and Willis.[2] However, these authors carried out the condensations according to a standardized procedure to determine the effect of structure on reactivity, thereby frequently leaving the fate of the major portions of the reactants unascertained; 2,3-dimethylquinoxaline and *p*-methoxybenzaldehyde thus reacted to form 2-*p*-methoxystyrylquinoxaline (10%) and 2,3-di-*p*-methoxystyrylquinoxaline (10%).

A somewhat more exhaustive examination of this reaction is here reported. It is of interest that no 2′-*p*-methoxyphenyl-2,3-trimethylenequinoxaline could be found.

Experimental

2,3-Dimethylquinoxaline (15.8 g., 0.10 mole) and 27.2 g. (0.20 mole) of *p*-anisaldehyde were refluxed together in 43 cc. of acetic anhydride for thirteen hours, after which 35 cc. of solvent was removed by distillation. Water (20 cc.) was added to the residue, followed by a dropwise addition of acetone until the solution was homogeneous at its boiling point. Crystallization was allowed to proceed for thirty-six hours in the icebox, after which the solid was filtered and washed with a little ether. The brilliant yellow product weighed 8.5 g. (22%) and consisted of 2,3-di-*p*-methoxystyrylquinoxaline (m. p. 167–169°).

The filtrate from this product was steam distilled while still acidic, resulting in the recovery (by ether extraction of the distillate) of 11.0 g. of anisaldehyde. The residue in the still was made slightly alkaline (sodium hydroxide) and again steam distilled, thus yielding 1.0 g. of 2,3-dimethylquinoxaline (m. p. 102–104°). The still residue was extracted with ether, and the ether layer was dried over potassium carbonate and allowed to concentrate slowly. No crystallization occurred, and the oil was sub-

(1) Wm. S. Merrell Co., Postdoctoral Fellow.

(2) Bennett and Willis, *J. Chem. Soc.*, 1960 (1928).