[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING OF THE UNIVERSITY OF PENNSYLVANIA]

Reactions of Phenanthraquinone with Aromatic Aldehydes and Ammonia in Alkaline Media

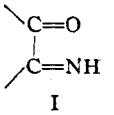
BY EDGAR A. STECK[1] AND ALLAN R. DAY

The investigations of Japp and co-workers[2] showed that phenanthraquinone reacts with aromatic aldehydes in the presence of ammonia to form 2-substituted phenanthroxazoles, 2-substituted phenanthrimidazoles or both. Their work indicates that the formation of oxazole or imidazole is largely determined by the nature of the substituents present in the aromatic aldehyde.[3] The two reactions, leading to imidazole formation and oxazole formation, are not mutually exclusive. For example, it has been reported that the reaction of phenanthraquinone with benzaldehyde and ammonia yields only the corresponding oxazole. Repetition of this reaction, during the present investigation, has shown that small amounts of 2-phenylphenanthrimidazole are also formed.

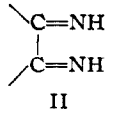
It has been noted in the present work that when sodium hydroxide is added to Japp's reaction mixture imidazole formation becomes the predominant reaction in every case examined. Most of the reactions were carried out in the presence of cupric acetate. The latter facilitates the separation of the imidazoles through the formation of highly insoluble copper derivatives, but exerts no influence on the extent of imidazole formation (see Experimental).

The addition of an organic base, such as piperidine or triethylamine, to Japp's reaction mixture does not produce a similar alteration in the course of the reaction. The possibility that sodium hydroxide hydrolyzes the oxazole and subsequent action of ammonia converts the hydrolysis product into the corresponding imidazole has been eliminated by previous work.[4]

In spite of intensive study, the specific role played by the sodium hydroxide has not been established experimentally. Certain conclusions, however, are justified.[5] Phenanthroquinonimine (I) and phenanthraquinone di-imine (II) represent the necessary initial intermediates for the formation of phenanthroxazoles and phenanthrimidazoles, respectively, from phenanthraquinone. Since phenanthrimidazole formation predominates under the influence of sodium hydroxide, it may be assumed that the presence of a strong base is necessary to catalyze the aldol type reaction between the second carbonyl group and ammonia.



I



II

Experimental

Phenanthraquinone.—This compound was prepared according to Graebe[6] and purified through its bisulfite addition product.[7] The product was finally recrystallized from 50% acetic acid, yield 55–62%, m. p. 208–209.5°.

Phenanthraquinonimine.—The quinonimine was prepared by the method of Pschorr,[8] yield 73–76%, m. p. 158.5–160°.

Reaction of Phenanthraquinone with Benzaldehyde and Aqueous Ammonia.—The directions of Japp and Wilcock[2] were followed without modification. The crude product melted at 202–203°. It was refluxed with an excess of cupric acetate in aqueous alcoholic solution. This treatment converted the 2-phenylphenanthrimidazole into its highly insoluble copper salt. The mixture was cooled, filtered and the residue extracted with hot alcohol to remove the 2-phenylphenanthroxazole, yield 67%, m. p. 204.5–205°. The copper salt of the imidazole, which remained after the extraction, was suspended in hot alcohol and decomposed with hydrogen sulfide. The copper sulfide was removed and the imidazole recovered by evaporating the filtrate and recrystallizing the residue from pyridine and water, yield 3%, m. p. 312–313°. Mixed melting point with an authentic sample was 312–313°.

Reaction of Phenanthraquinonimine with Hydrobenzamide.—The procedure of Kreps and Day[9] was used. The crude reaction product was treated as above and separated into two fractions: 2-phenylphenanthroxazole, yield 93%, m. p. 204.5–205°; and 2-phenylphenanthrimidazole, yield 2%, m. p. 312–313°.

Reaction of Phenanthraquinone with Benzaldehyde, Aqueous Ammonia and Cupric Acetate.—The Japp and Wilcock method was modified by the addition of one equivalent of cupric acetate; yield of 2-phenylphenanthroxazole 62–65%, yield of 2-phenylphenanthrimidazole 3–6%.

Reaction of Phenanthraquinone with Benzaldehyde, Cupric Acetate, Ammonium Hydroxide and Sodium Hydroxide.—To a suspension of 5 g. (0.024 mole) of phenanthraquinone in 100 cc. of alcohol was added 3 g. (0.028 mole) of benzaldehyde, 2.6 g. (0.025 mole) of cupric acetate in 150 cc. of 28% aqueous ammonia and 3 cc. of 40% sodium hydroxide. The mixture was heated for thirty minutes on a steam-bath, cooled and filtered. The solid was extracted with hot alcohol to remove 2-phenylphenanthroxazole, yield 3%, m. p. 204–205°. The residue was suspended in hot alcohol and decomposed with hydrogen sulfide. The crude 2-phenylphenanthrimidazole, obtained by evaporating the filtrate from the copper sulfide, was recrystallized from pyridine and water, yield 65%, m. p. 312–313°.

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(2) Japp and Streatfield, *J. Chem. Soc.*, **41**, 146 (1882); Japp and Wilcock, *ibid.*, **39**, 225 (1881).

(3) The specific effects of substituents is now being studied as a separate investigation in this Laboratory.

(4) Jaffe and Day, *J. Org. Chem.*, **8**, 43 (1943).

(5) See McCoy and Day [THIS JOURNAL, **65**, 2159 (1943)] for mechanisms involved in oxazole and imidazole formation.

(6) Graebe, *Ann.*, **167**, 140 (1873).

(7) Courtot, *Ann. chim.*, [10] **14**, 69 (1930).

(8) Pschorr, *Ber.*, **35**, 2739 1(902).

(9) Kreps and Day, *J. Org. Chem.*, **6**, 140 (1941).

Reaction of Phenanthraquinone with Benzaldehyde, Ammonium Hydroxide and Sodium Hydroxide.—The reactants were mixed in the same proportions as used in the above experiment (omitting the cupric acetate) and heated for two hours. Most of the product separated on cooling and the remainder was obtained by concentrating the filtrate. The 2-phenylphenanthrimidazole and 2-phenylphenanthroxazole were separated by the cupric acetate method; yield of imidazole was 70–73%, m. p. 312–313°; yield of oxazole 3–7%, m. p. 204–205°.

Reaction of Phenanthraquinone with Benzaldehyde and Ammonia in the Presence of Piperidine.—Phenanthraquinone (2.08 g., 0.01 mole) was suspended in 50 cc. of alcohol and 100 cc. of 28% aqueous ammonia added. A solution of 1.3 g. (0.012 mole) of benzaldehyde and 1.4 g. (0.016 mole) of piperidine in 10 cc. of alcohol was then added and the mixture heated on a steam-bath for one hour. The mixture was cooled and the solid removed by filtration. The crude product was treated as described previously. The sole detectable product was 2-phenylphenanthroxazole, yield 68%, m. p. 204.5–205°.

Reaction of Phenanthraquinone with Salicylaldehyde, Cupric Acetate, Ammonium Hydroxide and Sodium Hydroxide.—See the corresponding reaction with benzaldehyde for the procedure followed. The precipitate which formed during the course of the reaction was a mixture of the copper salts of the corresponding imidazole and oxazole. This behavior was not surprising in view of the phenolic nature of the hydroxyl group present. The copper salts were suspended in hot alcohol and decomposed with hydrogen sulfide. The filtrate from the copper sulfide was concentrated to obtain the mixture of oxazole and imidazole. Several recrystallizations from aqueous pyridine were required to separate the more soluble oxazole from the imidazole. Yield of 2-(2′-hydroxyphenyl)-phenanthroxazole was 9.5%, m. p. 246–247°[10]; yield of 2-(2′-hydroxyphenyl)-phenanthrimidazole, 54%, m. p. 287–288°. Mixed melting points with authentic samples showed no depression.

Reaction of Phenanthraquinone with *m*-Nitrobenzaldehyde, Cupric Acetate, Ammonium Hydroxide and Sodium Hydroxide.—The procedure for the corresponding reaction with benzaldehyde was used in this case without modification. The crude 2-(3′-nitrophenyl)-phenanthrimidazole was recrystallized from pyridine and water, yield 77.8%, m. p. 271–272° [11] No oxazole was isolated from this reaction.

Summary

1. A method for the preparation of phenanthrimidazoles from phenanthraquinone, aromatic aldehydes and ammonium hydroxide in the presence of sodium hydroxide has been developed. The probable role of the sodium hydroxide has been discussed.

2. A method for the separation of most phenanthrimidazoles from phenanthroxazoles through the use of cupric acetate has been described.

(10) Stein and Day, THIS JOURNAL, **64**, 2567 (1942).

(11) Steck and Day, *ibid.*, **65**, 452 (1943).

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Streptomyces Antibiotics. VI. Isolation of Streptothricin

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Methods have been found for the purification and isolation of streptothricin from the culture broths of *Streptomyces lavendulae*.

Crystalline salts of streptothricin and streptomycin have been described.[3] The preparation of streptothricin helianthate[3] and other procedures for the purification of streptothricin are described herein.

Streptothricin concentrates were first prepared by Waksman and Woodruff[4] from the culture broths of *Streptomyces lavendulae*. After treatment with Norite-A, the adsorbate was eluted with dilute acid and the eluate was neutralized and evaporated to a concentrate which was used for biological studies. The adsorbate was also eluted with acidified alcohol, and the eluate was neutralized and treated with ether to give a precipitate which was used for biological tests.[5]

Fried and Wintersteiner[6] described the crystalline reineckate of streptothricin which was stated to have been obtained by the following sequence of steps: charcoal adsorption, elution with mineral acid, precipitation with phosphotungstic acid, conversion of regenerated bases to crude picrate, chromatography of picrate, reineckate.

Although our methods for obtaining streptothricin had certain steps which were modifications of those described above, other steps were different. Thus, the following sequence of steps has been used satisfactorily: charcoal adsorption, elution with formic acid solutions, precipitation with picric acid and direct conversion to hydrochlorides, chromatography of hydrochlorides, helianthate.

After a study of elution procedures, it was found that the streptothricin could be eluted satisfactorily from the charcoal adsorbate by a solution of formic acid in methyl alcohol–water. The eluate was then partially concentrated *in vacuo*. Addition of acetone caused the formation of a precipitate which showed 40-80 units/mg. activity. The recovery of activity as the formate from the broth was 30–60%.

Further purification was effected by treating the crude formate or hydrochloride with picric acid in water. The somewhat selective separation of streptothricin picrate resulted in a con-

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(2) U. S. Army.

(3) Kuehl, Peck, Walti and Folkers, *Science*, **102**, 34 (1945).

(4) Waksman and Woodruff, *Proc. Soc. Exp. Biol. Med.*, **49**, 207 (1942).

(5) Waksman, *J. Bact.*, **46**, 299 (1943).

(6) Fried and Wintersteiner, *Science*, **101**, 613 (1945).