

the ratio of catecholase to cresolase units was found to approach the value of 2 to 1 for a series of high cresolase preparations.

Facts obtained in the study of high cresolase preparations such as: (1) the constant ratio between the cresolase and catecholase activities; (2) the limit of cresolase activity when based on the amount of catecholase activity; (3) the dependency of both activities on the copper content; (4) the ratio of the two activities resembling closely that in the fresh water extract of the plant, all point to the conclusion that tyrosinase as it occurs in the common mushroom is one enzyme complex rather than two separate independent factors.

#### Summary

1. A method is described for preparing tyro-

sinase from the common mushroom, *Psalliota campestris*, high in cresolase activity.

2. In the preparations high in cresolase activity the latter is proportional to copper, and to catecholase activity, both activities being dependent on the same copper content.

3. The Adams and Nelson method of determining catecholase activity does not apply in preparations high in cresolase activity.

4. In high cresolase preparations the volume of oxygen uptake corresponding to the inactivation of an amount of catecholase containing  $\gamma/350$  of copper, was found to be 150 c. mm. instead of 100 c. mm. as found by Ludwig and Nelson in the case of high catecholase preparations.

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## The Reaction of 2-Chloro-5-nitropyridine and Thiourea

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In a recent paper<sup>1</sup> a description of a method of preparation of 5,5'-dinitro-2,2'-dipyridylsulfide (I) is included. A better method of obtaining this compound has now been found, using the reaction of 2-chloro-5-nitropyridine and thiourea in aqueous solution. This new procedure results in a better yield and a high degree of purity for compound I.

When these two reagents are allowed to react in absolute alcohol, an addition product (II) results which can be decomposed with sodium carbonate to form 2-thiol-5-nitropyridine.<sup>2</sup>

That certain halogen compounds and thiourea can produce either thiols or sulfides has been demonstrated by others. Depending upon the conditions of the experiment, 2-chlorobenzothiazole and thiourea react in aqueous solution<sup>3</sup> to give predominantly either the sulfide or thiol; in this case no addition compound could be isolated. Also, the reaction of 4-chloroquinoline and thiourea<sup>4</sup> in alcoholic solution gives an addition compound which can be decomposed by sodium carbonate to yield either the sulfide or the thiol.

Inasmuch as an addition compound could be

isolated in this present investigation, several reactions involving compound II were studied in order to determine a possible mechanism for the formation of sulfide (I) in aqueous solution. (a) It was found that upon heating the addition compound (II) in aqueous solution, it decomposed slowly to form the sulfide. At the end of one hour, 20% of I was formed, at the end of three and one-half hours, 60%. The decomposition was complete after eight hours. (b) When compound II was allowed to react with 2-chloro-5-nitropyridine (III) more sulfide was obtained than could be expected if only compound II had decomposed. (c) With compound II and 2-thiol-5-nitropyridine (IV) under the same conditions, as much sulfide was formed in one hour as in (b) in four hours. (d) In the preparation of the addition compound, if any water was present in the alcohol, the reaction did not proceed as expected, but rather the sulfide was obtained.

From the experimental data, it seemed reasonable to assume that the addition compound, in aqueous solution, decomposed to form some thiol (IV) which then reacted with II to yield the sulfide. The reaction of II and III could be explained on this basis since some thiourea would be expected to be liberated from the reaction of the

(1) THIS JOURNAL, **62**, 173 (1940).

(2) Rath and Binz, *Ann.*, **487**, 105 (1931).

(3) Watt, *J. Org. Chem.*, **4**, 436 (1939).

(4) Rosenhauer, Hoffman and Heuser, *Ber.*, **62**, 2730 (1929).

addition compound (II) and 2-thiol-5-nitropyridine (IV). The chloro compound (III) could then react with thiourea to form the addition compound. Further, III can react with IV to give a 40% yield of sulfide (I) in four hours.

The fact that unsymmetrical sulfides can be prepared by the interaction of II with certain halogen compounds was illustrated by the use of chloroacetic acid. This reaction yielded both the sulfide (I) and 5-nitro-2-pyridylthioglycolic acid. The latter also was prepared from the reaction of 2-thiol-5-nitropyridine and chloroacetic acid, in aqueous solution. If the same assumption is made, namely, that compound II decomposes to form 2-thiol-5-nitropyridine, then the formation of 5-nitro-2-pyridylthioglycolic acid from chloroacetic acid and II is easily explained.

### Experimental

**Reaction of 2-Chloro-5-nitropyridine (III) and Thiourea in an Aqueous Medium.**—Twenty-five grams of the chloro compound (III), 12.5 g. of thiourea and 425 cc. of water were heated on the steam-bath with vigorous stirring. Several times during the reaction the chloro compound, which deposited on the sides of the flask, was washed back into solution with hot water. At the end of five hours, the hot reaction mixture was filtered and the 5,5'-dinitro-2,2'-dipyridylsulfide (I) which had separated from solution during the reaction was filtered and dried; yield 19 g. (87%); m. p. 133–135°. After one crystallization from alcohol, the sulfide melted at 135–136.5° alone or when mixed with a sample of I prepared from 2-chloro-5-nitropyridine and sodium sulfide.

*Anal.* Calcd. for  $C_{10}H_8N_4O_4S$ : N, 20.14. Found: N, 20.17.

**Reaction of III and Thiourea in Absolute Alcohol.**—Compound III (6 g.) was added to a warm solution of thiourea (3 g.) in absolute alcohol (60 cc.). The mixture was refluxed for one hour and then filtered: yield 7.3 g. (83%). The 2-chloro-5-nitropyridine–thiourea addition compound (II) crystallized from large amounts of absolute alcohol in small light yellow prisms which melted with decomposition at 187–190°.

*Anal.* Calcd. for  $C_8H_7N_2O_2SCl$ : N, 23.88. Found: N, 24.10.

**Decomposition of II in Water.**—Four 0.5-g. samples of II were dissolved in water (10 cc.) and heated on the steam-bath. At the end of certain time intervals, a sample was removed and cooled with water. The sulfide (I) which had precipitated was collected by filtration and dried. The products obtained had similar melting points, ranging from 133–136°. The amounts of I formed were as follows: one-half hour, 9.1%; one hour, 21.9%; two hours,

46%; three and one-half hours, 60%. With a sample of II (2 g.) in water (50 cc.), heating for eight hours gave the theoretical yield of I.

The addition of sodium carbonate to a solution of II in water yielded 2-thiol-5-nitropyridine (IV).

**Reaction of II and III.**—Compound III (0.68 g.) was added to a solution of II (1 g.) in water (30 cc.) and heated on the steam-bath with stirring. After four hours the reaction mixture was filtered and the sulfide (I) collected: yield 0.69 g., m. p. 133–135°.

**Reaction of II and IV.**—Compound IV (0.67 g.) was added to a solution of II (1 g.) in water (30 cc.) and heated on the steam-bath. At the end of fifteen minutes, some sulfide (I) began to separate from solution. After one hour the reaction mixture was filtered, yield 0.65 g.

**Reaction of II and Chloroacetic Acid.**—A solution of II (3 g.) and chloroacetic acid (1.2 g.) in water (50 cc.) was heated on the steam-bath for two and one-half hours. The mixture was filtered, and the sulfide which was formed was collected. On cooling, a light yellow precipitate separated from the filtrate, yield 0.29 g. After recrystallizing several times from hot water, the new product was dried in the oven, m. p. 125–128°. Mixed melting points with a sample of 5-nitro-2-pyridylthioglycolic acid prepared by the action of chloroacetic acid on 2-thiol-5-nitropyridine (IV) gave no depression.

**5-Nitro-2-pyridylthioglycolic Acid.**—A solution of chloroacetic acid (1 g.) and compound IV (1.7 g.) in water (50 cc.) was refluxed for one hour. The reaction mixture changed from an orange to a light yellow color. The solution was allowed to cool, and the precipitate which formed was filtered, yield 2.06 g. (89%). The melting point of the crude material extended over a wide range. After several recrystallizations from water, the compound was obtained in the form of light yellow needles. The product was dried in a drying pistol at 90°, m. p. 127–129°. The compound is soluble in base but insoluble in cold water.

*Anal.* Calcd. for  $C_7H_8N_2O_4S$ : N, 13.08. Found: N, 13.14.

### Summary

An improved method of synthesis of 5,5'-dinitro-2,2'-dipyridylsulfide by the reaction of 2-chloro-5-nitropyridine and thiourea in aqueous solution, is described.

The reaction of 2-chloro-5-nitropyridine and thiourea in absolute alcohol yielded an addition compound which could be decomposed to give either 2-thiol-5-nitropyridine or 5,5'-dinitro-2,2'-dipyridylsulfide.

A mechanism is proposed for the formation of 5,5'-dinitro-2,2'-dipyridylsulfide in aqueous solution.

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