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SYNTHESIS OF RADIOACTIVE STILBAMIDINE DIISETHIONATE

(STILBENE-4,4'-DICARBOXAMIDINE-C¹⁴ DIISETHIONATE)

by

James C. Reid

September 29, 1949

Berkeley, California

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ABSTRACT

Stilbamidine diisethionate (Stilbene-4,4⁰-dicarboxamide diisethionate) labeled with Carbon 14 in the amidine groups has been prepared from isotopic sodium cyanide. The method employed consists of the conversion of 4,4⁰-dibromostilbene to dinitrilostilbene followed by the conversion of the dinitrile to the diamidine.

* Present address: National Cancer Institute, National Institutes of Health, Bethesda 14, Maryland.

** The work described in this paper was sponsored by the Atomic Energy Commission.

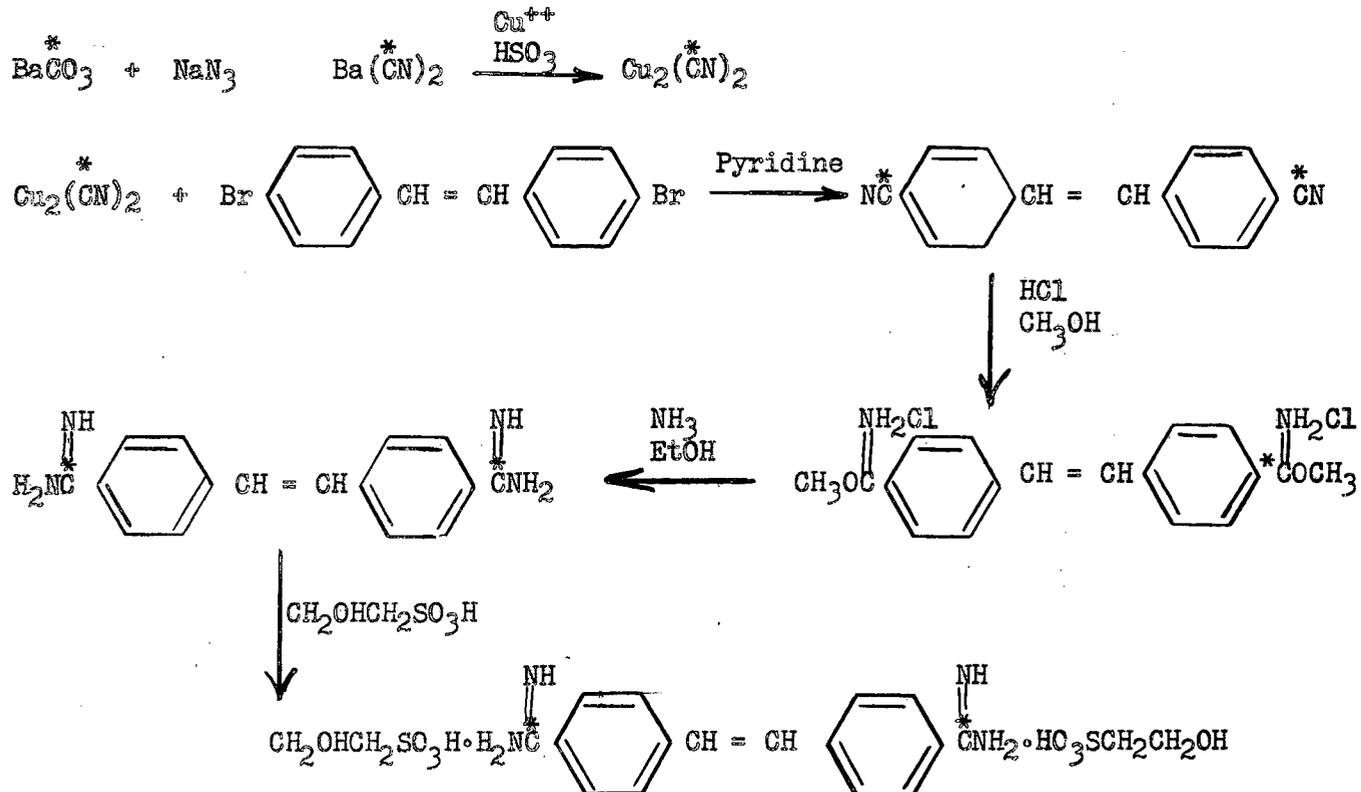
SYNTHESIS OF RADIOACTIVE STILBAMIDINE DIISETHIONATE
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Stilbamidine has been labeled in this laboratory in order to study its metabolism by tracer technique. The compound was prepared as the salt of isethionic acid which, because of its easy solubility, is the form in which the drug is ordinarily administered. The reaction sequence is given below.



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Experimental

Isethionic Acid: - Barium isothionate was prepared from diethyl sulfate and fuming sulfuric acid (3). An aqueous solution of the salt was exactly neutralized with sulfuric acid to prepare free isethionic acid. After removal of barium sulfate, the solvent was evaporated in vacuum at room temperature and the white, highly deliquescent residue was dried in high vacuum.

Sodium Cyanide-C¹⁴: - Isotopic sodium cyanide was prepared as an aqueous solution containing excess sodium hydroxide by reduction of radioactive barium carbonate with sodium azide (4). The yield was 60%.

Cuprous Cyanide-C¹⁴: - Radioactive cuprous cyanide was prepared from cupric sulfate and isotopic sodium cyanide in the presence of bisulfite. Bisulfite serves to reduce the cupric ion (5); without it, the cupric ion is reduced at the expense of cyanide, half of which is lost as cyanogen. The reaction vessel was a 40 ml. conical Pyrex centrifuge tube fitted with a three-hole rubber stopper. Through the respective holes were inserted a sealed stirrer, the stem of a small separatory funnel and a gas outlet tube. The outlet tube was connected to an absorber containing alkaline permanganate to catch any cyanogen which might be formed. Into the centrifuge tube was placed a solution of 1.50 g. (6.00 mmoles) of cupric sulfate in 10 ml. of water acidified with three drops of 6M sulfuric acid. The separatory funnel received 15 ml. of a solution containing 3.85 mmoles of sodium cyanide and 1.00 g. (3.97 mmoles) of sodium sulfite heptahydrate. The solution was about 0.5M in sodium hydroxide. A drop of phenolphthalein was added to the solution, followed

(3) A. A. Goldberg, J. Chem. Soc., 1942, p. 716.

(4) A. W. Adamson, J. Am. Chem. Soc., 69, 2564 (1947).

(5) H. J. Barker, J. Chem. Soc., 1943, p. 79.

by enough 6M sulfuric acid to discharge the pink color. The cyanide solution was added dropwise to the cupric sulfate while the mixture was stirred vigorously. A white precipitate of cuprous cyanide formed rapidly, and after fifteen minutes a gentle current of air was drawn through the apparatus to carry any gaseous products into the permanganate. The cuprous cyanide was collected by centrifugation, washed and vacuum dried. The yield was 0.350 g., 100%. The yield in this step ordinarily ran around 80%. The product was not removed from the centrifuge tube because the physical properties of the substance make it difficult to transfer quantitatively. The tube was used as the reaction vessel in the next step.

4,4'-Dicyano-¹⁴C-stilbene: - The synthesis from this point on follows a previously reported method (6). To the cuprous cyanide prepared in the manner described above was added 665 mg. (1.95 mmoles) of 4,4-dibromostilbene and the solids were mixed with a porcelain spatula. One milliliter of dry C.P. pyridine was then stirred in and the mixture was refluxed one and one-half hours (bath temperature 190-210°). The mixture was cooled somewhat, 2 ml. more pyridine was added and the mixture was refluxed five minutes. It was then cooled almost to the point of solidification and 9 ml. of concentrated hydrochloric acid was poured in. Lumps were broken up by thorough stirring and the mixture was centrifuged. After three washes with 2 ml. portions of concentrated hydrochloric acid followed by three 2 ml. portions of water, a clean yellow product was obtained melting at 265-272°. The yield was 471 mg., 104%. This material was used in the next step without further purification.

Conversion of the Nitrile to the Imido-ester: - The dicyanostilbene was transferred to a 15 x 150 mm. Pyrex ignition tube, which was then constricted near the mouth. Water introduced by the glassworking lamp was removed by attaching the tube to a high

(6) S. Banche, H. J. Barber and A. M. Woolman, J. Chem. Soc., 1943, p. 1.

vacuum line for a few minutes. To the contents of the tube were added 5 ml. of C.P. chloroform and 0.75 mg. of C.P. methanol. The mixture was saturated at 0° with dry hydrogen chloride and the tube was sealed. It was then rotated mechanically for sixty hours at room temperature. The tube was opened, two volumes of ether was added and the reaction mixture was transferred to a centrifuge tube, which was capped to exclude moisture. After being washed twice by centrifugation with dry ether, the yellow imido-ester dihydrochloride was dried in high vacuum. It weighed 590 mg. (7%). The product was used in the next step without further purification.

Stilbamidine Diisethionate: - The imido-ester was transferred to a 15 x 150 mm.

Pyrex ignition tube and the tube was constricted near the open end. The constriction was placed far enough below the mouth of the tube that moisture from the burner could not enter the tube. Into the tube was introduced 10 ml. of a 10% solution of anhydrous ammonia in anhydrous ethanol and the tube was sealed. It was then heated thirty-six hours at 60°. The tube was opened and the diamidine (free base) was collected by centrifugation. After being washed by centrifugation with two 5 ml. portions of dry ether and dried in a high vacuum, the white product weighed 410 mg. (96%).

Purification was effected by conversion of the diamidine to the hydrochloride, followed by reprecipitation of the free base. To accomplish this the amidine still contained in the centrifuge tube in which it was collected was thoroughly moistened with 0.5 ml. of concentrated hydrochloric acid, and the mixture was diluted with 5 ml. of water. The tube was warmed to 60° and the mixture was centrifuged. The supernatant solution was pipeted into a 125 ml. flask, where it deposited yellow crystals of the dihydrochloride on cooling. The residue in the centrifuge tube was extracted repeatedly with water and the supernatant solution in each case was pipeted into the flask.

As each new portion was added to the contents of the flask, more amidine dihydrochloride separated, thrown down by the salting-out effect of the relatively high hydrochloric acid concentration of the solution in the flask. When this criterion showed that nothing more was being dissolved from the residue in the centrifuge tube, this residue was treated with 5 ml. of 6M hydrochloric acid and then with water again. This repetition of the extraction procedure produced a small additional crop of hydrochloride.

The stilbamidine dihydrochloride was then precipitated more completely from the solution in the 125 ml. flask by making the solution, which had a total volume of about 80 ml., 2M in hydrochloric acid. The yellow dihydrochloride was collected by filtration. After being sucked as dry as possible, the hydrochloride was dissolved in 8 ml. of warm water and the solution was treated with charcoal and filtered. The nearly colorless filtrate was chilled in ice and the free amidine was precipitated with 6M ammonia. The white product was collected by centrifugation and washed once with 1 ml. of cold water. It was then dissolved without delay in a solution of 0.63 g. of isethionic acid in 2 ml. of water. A slight residue was removed by centrifugation and stilbamidine diisethionate was precipitated from the supernatant solution by adding twenty volumes of acetone. The precipitate was collected by centrifugation and redissolved in 2 ml. of water. After centrifugation to remove a slight turbidity, the diisethionate was again precipitated with acetone, filtered and dried at 60° in vacuum. The pale yellow product weighed 278 mg. (34% based on imido-ester dihydrochloride) and melted with decomposition and evolution of gas at 308-312° uncor.

Analysis

Calcd. for $C_{20}H_{28}O_3N_4S_2$: C, 46.50; H, 5.46; N, 10.85; S, 12.4.

Found: C, 47.41; H, 5.30; N, 10.17; S, 12.97.

The overall yield based on sodium cyanide was 19%.

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