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A Crystalline Imidazolidine Derivative of Streptomycin

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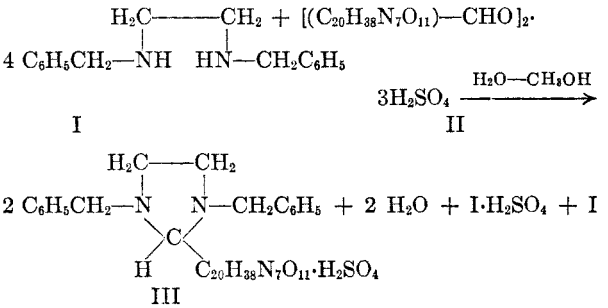
The C-formyl group of streptomycin sulfate reacts with an excess of N,N′-dibenzylethylenediamine to produce crystalline 1,3-dibenzyl-2-streptomycylimidazolidine sulfate in a high yield.

Streptomycin sulfate can be regenerated nearly quantitatively by treatment of the imidazolidine with benzaldehyde and sulfuric acid.

In the course of determining the most satisfactory conditions for the preparation of N,N′-dibenzylethylenediamine (I) by the reductive alkylation of ethylenediamine with benzaldehyde, a crystalline by-product was isolated and identified[1] as 1,3-dibenzyl-2-phenylimidazolidine (IV).[2] Our attention was thus focused upon the studies of van Alphen,[2] Lob,[3] and Rameau[4] wherein a variety of aldehydes had been found to undergo facile condensation with N,N′-disubstituted ethylenediamines to form the corresponding 1,2,3-trisubstituted imidazolidines (tetrahydroimidazoles). It was reported that acetone and other ketones had failed to undergo the condensation with I, even under drastic conditions,[2,3] suggesting specificity for the formyl function. In general the aldehyde derivatives of I were claimed to be stable in alkaline solution, but to be readily hydrolyzed in the presence of dilute mineral acids with regeneration of the original aldehyde.

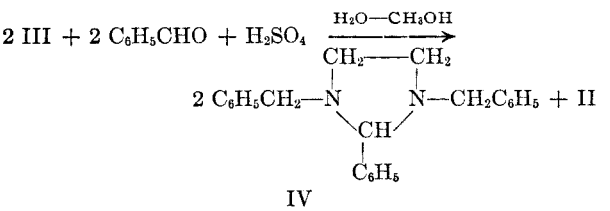
Although aldose derivatives of I had not been disclosed, the preparation of imidazolidines incorporating the aldehyde group of streptomycin was undertaken. The reaction of streptomycin sulfate (II) with I proceeded smoothly to yield crystalline 1,3-dibenzyl-2-streptomycylimidazolidine sulfate (III).[5] Subsequent to the inception of this work, Billman, Ho, and Caswell[6] reported that N,N′-bis-(*p*-methoxybenzyl)ethylenediamine is a satisfactory reagent for the characterization of aldehydes.



The primary problem encountered in the preparation of III was the slight solubility of streptomycin sulfate in most organic solvents. However, a mixture of five volumes of water to six of methanol was found to give mutual solubility of the reactants. Inasmuch as the diamine (I) alone was not very soluble in these proportions of methanol and water, the observed solubility of the mixture of reactants was apparently caused by the rapid onset of the condensation. Heating the reaction mixture to 45–50° for short periods gave marked acceleration of the reaction rate without deleterious effects. An excess of the diamine (I) served as an acid-binding agent, as shown in the equation. A ratio of four moles of the diamine (I) to one of streptomycin sulfate afforded optimum yields of III.

The imidazolidine (III) was obtained as a white, crystalline solid containing two molecules of water. Removal of the water of hydration *in vacuo* over phosphorus pentoxide did not destroy the crystalline structure. Its bio-potency against *Bacillus subtilis* was excellent, approaching the theoretical value based on the streptomycin content. As in the case of other imidazolidines it was very sensitive to dilute mineral acid, being readily hydrolyzed to streptomycin and the diamine (I) at room temperature. Titration studies indicated that only one molecule of sulfuric acid was present in III, and this was confirmed by elemental analyses. The material was very insoluble in the common organic solvents. At 25° its methanol solubility was 0.2 mg./ml., and it was soluble in water only to the extent of 5.5 mg./ml. (*p*H 8.8–8.9).

The regeneration of streptomycin sulfate from the imidazolidine (III) was effected either by acid hydrolysis or aldehyde interchange,[7] yielding ma-



(1) Unpublished work of C. T. Holdrege.

(2) van Alphen, *Rec. trav. chim.*, **54**, 93 (1935).

(3) Lob, *Rec. trav. chim.*, **55**, 860 (1936).

(4) Rameau, *Rec. trav. chim.*, **57**, 194 (1938).

(5) In this nomenclature it is understood that the term "streptomycyl" represents the streptomycin molecule minus the aldehyde group.

(6) Billman, Ho, and Caswell, *J. Org. Chem.*, **17**, 1375 (1952).

(7) Johnson, U. S. Patent 2,717,893 (1955).

terial of high purity in either case. The latter method was found to give the better results.

The use of ketonic solvents in the preparation of III was obviated when, contrary to the experience of Lob[5] but in accord with the findings of Billman and co-workers,[6] it was found that acetone reacts with N,N′-dibenzylethylenediamine. The product, 1,3-dibenzyl-2,2-dimethylimidazolidine, is a colorless, crystalline compound of m.p. 78–79°. The scope of this reaction of N,N′-dibenzylethylenediamine with ketones has not been determined.

EXPERIMENTAL

1,3-Dibenzyl-2-streptomycylimidazolidine sulfate (III). A solution of 324 g. (1.40 moles) of N,N′-dibenzylethylenediamine (I) in 2000 ml. of methanol was added rapidly to a stirred solution of 491 g. (0.67 mole) of streptomycin sulfate in 1675 ml. of water at room temperature. The resultant clear solution was heated rapidly to 45° and maintained at 45–50° for 30 minutes. After a few minutes of heating the solution clouded and within ten minutes a copious precipitation of a white, crystalline solid had begun. At the end of the half-hour heating period the mixture was cooled to 5° and filtered. The product was washed with 1350 ml. of chilled methanol and air-dried for a day at room temperature. Further drying *in vacuo* over P_2O_5 for two days at 25° gave a constant weight of 571 g. (90.5%); m.p. 238–244° (dec.).[8] The melting range was highly dependent upon the rate of heating. Titration studies and analytical data indicated that only one molecule of sulfuric acid was present in the compound. The material was so insoluble in the common organic solvents that no satisfactory method of recrystallization was found.

Anal. Calc'd for $C_{37}H_{57}N_9O_{11}\cdot H_2SO_4\cdot 2H_2O$: C, 47.4; H, 6.76; S, 3.42; H_2O, 3.74. Found: C, 47.2; H, 6.71; S, 3.37; H_2O, 3.84.

A bio-assay of 1,3-dibenzyl-2-streptomycylimidazolidine sulfate dihydrate against *Bacillus subtilis* gave a value of 603 u/mg. (theory—620 u/mg.). The water solubility was 3400 u/ml. (5.5 mg./ml.) at 25°.

A 1.5-g. sample of the product (III) was heated for three hours on the steam-bath in 25 ml. of 6 *N* HCl and the mixture was cooled and filtered. When dry the collected solid weighed 0.5 g., representing a 96% recovery of N,N′-dibenzylethylenediamine (I) as the dihydrochloride, m.p. 305–306° (dec.) after one recrystallization from acetone-water. A mixture melting point determination of this derivative with an authentic sample of N,N′-dibenzylethylenediamine dihydrochloride showed no depression.

Regeneration of streptomycin sulfate from 1,3-dibenzyl-2-streptomycylimidazolidine sulfate (III). *A. By acid hydrolysis.* Compound III (18.76 g., 0.02 mole) and 6 *N* sulfuric acid (10 ml., 0.06 equivalent) were added alternately in several portions to 20 ml. of agitated water. The solid dissolved rapidly and solution was nearly complete before N,N′-dibenzylethylenediamine sulfate began to crystallize. After agitation for 30 minutes the crystalline precipitate was removed by filtration and washed with 20 ml. of water in three portions. The dried cake amounted to 5.75 g. (85%). The filtrate contained streptomycin sulfate and the remainder of the N,N′-dibenzylethylenediamine sulfate. The latter could be removed as the free base by toluene extraction at *p*H 8. The resulting aqueous solution was suitable for the precipitation of high quality streptomycin sulfate by addition to methanol, or for reduction to a solution of dihydrostreptomycin sulfate which could be crystallized subsequently by addition of methanol.

B. By aldehyde interchange.[7] To a suspension of III (9.38 g., 0.01 mole) in 25 ml. of methanol was added 25 ml. of water containing 0.01 equivalent of sulfuric acid. Benzaldehyde (1.1 ml., 0.011 mole) was added immediately and the mixture was agitated vigorously. After most of the III was dissolved a new solid began to separate. Agitation was continued for 20 minutes, after which time the precipitate was removed by filtration and washed with 5 ml. of water. The dried cake of 1,3-dibenzyl-2-phenylimidazolidine (IV) weighed 3.26 g. (99.5%). The filtrate contained essentially pure streptomycin sulfate suitable for further processing. Small amounts of N,N′-dibenzylethylenediamine (I) or benzaldehyde remaining could be removed by the extraction procedure mentioned in Part *A*.

1,3-Dibenzyl-2,2-dimethylimidazolidine. A solution of 4.8 g. (0.02 mole) of N,N′-dibenzylethylenediamine (I) in 25 ml. of acetone was refluxed for one hour, diluted with 25 ml. of freshly-boiled water, seeded and kept at 5–10° for five days. The crystalline product was collected and dried *in vacuo* overnight at room temperature over phosphorus pentoxide, 5.3 g. (95%) of product was obtained. Recrystallization from 15 ml. of absolute alcohol yielded 3.8 g. of large colorless crystals, m.p. 78–79°.

Anal. Calc'd for $C_{19}H_{24}N_2$: C, 81.4; H, 8.63. Found: C, 81.6; H, 8.45.

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(8) Melting points are uncorrected. We are indebted to R. M. Downing for the microanalyses.