(7) **2-Diethylmalonyl-6-aminobenzothiazole**—A solution of sodium diethyl malonate, prepared from 2.0 g. of diethyl malonate and 0.15 g. of sodium in 50 cc. of xylene was heated with 0.9 g. of 2-chloro-6-aminobenzothiazole at the boiling point of xylene for 2 hours. An oily substance separated on adding water to the solution, and was extracted with a large amount of ether. After drying with sodium carbonate, ether was removed, and the residue was recrystallized from a mixture of benzene and benzine(b.p. 80~100°). It melted at 136° and weighed 0.5 g. (34%). *Anal.* Calcd. for $C_{14}H_{16}O_4N_2S$: N, 9.09. Found: N, 9.28.

(8) **Reaction between 2-chloro-6-nitrobenzothiazole and ethyl acetoacetate**—A solution of sodium ethyl acetoacetate, prepared from 2.0 g. of ethyl acetoacetate and 0.3 g. of sodium in 200 cc. of toluene, was heated with 2.0 g. of 6-nitro-2-chlorobenzothiazole for 1 hour at 100°. Toluene was removed and on adding water to the residue an amorphous substance separated. After air-drying it weighed 2.6 g. It was recrystallized from glacial acetic acid to afford a yellow amorphous substance and weighed 2.0 g. Yield, 70%. *Anal.* Calcd. for $C_{13}H_{12}O_5N_2S$: C, 49.60; H, 3.89; N, 9.09. Found: C, 49.73; H, 4.03; N, 8.80.

(9) 2-Bromobenzothiazole was employed instead of 2-chlorobenzothiazole. The procedures similar to the case of 2-chlorobenzothiazole were carried out.

Summary

Malonic ester synthesis and related reactions in heterogenous aromatic series were carried out, involving 2-halobenzothiazole, diethyl malonate, and ethyl acetoacetate from which following new compounds were prepared. 2-Diethylmalonylbenzothiazole, m.p. 148°, ethyl α-(6-nitro-2-benzothiazolyl)-acetoacetate, m.p. 215~217°(decomp.), and 6-amino-2-diethylmalonylbenzothiazole, m.p. 211°.

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76. Takeo Ueda, Isao Nakata, and Shin-ya Ito: Studies on Electrolytic Reduction of Streptomycin.

(*Pharmaceutical Institute, Keio-Gijuku University**)

Dihydrostreptomycin has been prepared by hydrogenation of streptomycin, especially by means of catalytic reduction1). Although several methods of reduction with chemical reducing agents have been proposed, none has been taken up for the preparation of dihydrostreptomycin2). In view of the importance of this drug, we were prompted to try another method, *viz.*, electrolytic reduction of streptomycin. Though a similar method has already been published in the patent proposed by E. R. Squibb & Sons3), the methods described there were somewhat ambiguous and some differences were observed between the claims made therein and the results obtained by us. This paper describes the important factors found for the electrolytic reducion of streptomycin.

Streptomycin is considered structurally as a kind of a glycoside, and therefore, its reduction might be referred to that of aldose, since dihydrostreptomycin is obtained by the reduction of the formyl group in the streptose portion of streptomycin to alcoholic group. On electrolytic reduction of aldoses, it has been shown by many authors4) that aldoses should be electrolyzed in alkaline catholyte to increase molecules susceptible to reduction, and with cathode possessing higher hydrogen overvoltage to approach the vulger reduction potential of aldose to the electrode potential.

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* Shinano-machi, Shinjuku-ku, Tokyo (上田武雄, 中田 公, 伊藤信也).

1) U. S. Pat. 2,498,574.

2) Japan. Pat. Appl. No. 3885/1952.

3) Japan. Pat. Appl. No. 1034/1952.

4) S. M. Ca nter, Q. P. Peniston: J. Am. Chem. Soc., 62, 2113(1940), *et seq.*

It was, however, actually somewhat troublesome to prepare polyalcohols by electrolytic reduction of the corresponding aldoses under such limited conditions, and similar difficulties might be assumed in the case of electrolytic reduction of streptomycin. Moreover, it was considered that the behavior of streptomycin against reduction might differ from that of common aldoses because of the difference of formyl group in the streptose portion from that in aldoses.

In taking such into consideration, this work was carried out to find the most favorable conditions for the electrolytic reduction of streptomycin.

Lead coated with lead dioxide was employed as an anodic material, and diluted sulfuric acid, as the anolyte. In order to find the most active cathodic material, several kinds of metals, unreactive with streptomycin, were examined as to their electrolytic properties. The catholyte was composed of an aqueous solution of streptomycin salt and various inorganic salt, pH being adjusted by the addition of acid or alkali, to find the most appropriate form of additive inorganic salt and the most favorite hydrogen ion concentration of the catholyte. The anolyte and the catholyte were separated from each other with a porcelain diaphragm. Current efficiency and the end point of electrolysis were determined with a gas coulometer inserted in the circuit.

After the electrolysis was completed, dihydrostreptomycin produced was separated as a new complex salt with 3-phenylazo-4-hydroxynaphthalenesulfonic acid, from the catholyte, followed by the liberation of dihydrostreptomycin from the complex salt with hydrochloric or sulfuric acid.

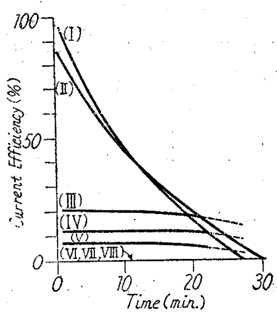

Fig. 1.

Inorganic salt added: Na_2SO_4(pH 6).
Cathodic materials:
I-Amalgamated Pb
II-Pb
III-Hg
IV-Alloy of Pb with Sb
V-Amalgamated Ni
VI-Ni
VII-Pt
VIII-Platinized Pt

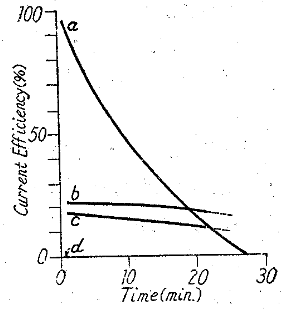

Fig. 2.

Cathodic material: Amalgamated Pb.
Inorganic salt added:
a-Na_2SO_4 (pH 6).
b-Na_2SO_4 (pH 3, with H_2SO_4).
c-NaH_2PO_4+NaOH (pH 6)
d-$Na_2B_4O_7$+H_3BO_3+NaCl (pH 7).

The results obtained are shown in Figs. 1 and 2. It was made evident that amalgamated lead was the most active cathodic material among the metals examined, sodium sulfate the most appropriate additive salt, and pH 6 the most favorite hydrogen ion concentration of the catholyte.

Discussions and Conclusion

Although there were many problems to be solved regarding electrolytic reduction of streptomycin the present experiments were carried out mainly for the choice of cathodic materials, pH of the catholyte, and salt to be added to the catholytes. The concentration of streptomycin was limited by shortage of the sample, and cathodic current density, current concentration, bath voltage, and bath temperature were readily decided by preliminary tests referring to the reports on the electrolytic reduction of aldoses.

The observations with several cathodic material as described in the experimental part show that streptomycin could be reduced, more or less, with all the metals except nickel, platinum, and platinized platinum, but most favorably with two cathodic materials, amalgamated lead and lead. The fact that the higher hydrogen overvoltage of the cathodic material gave better results agrees with findings in the electrolytic reduction of aldoses. However, mercury itself, even though of the highest overvoltage, was not so favorable as anticipated because mercury perhaps lowered the current efficiency due to its rapid staining of the surface. Thus, it was concluded that the most preferable cathodic material for the reduction was amalgamated lead, followed by lead, and the other metals were practically of no use.

Among inorganic salts to be added to the catholyte, sodium sulfate was found to be the most preferable in concentration where the current density was not so lowered, and the addition of phosphate and borate possessing buffer effect resulted in the lowering of current efficiency. This fact also agrees with the case of the electrolytic reduction of aldoses.

As for the hydrogen ion concentration of the catholyte, it was concluded that with even the most preferable cathodic material, the pH of the catholyte should be kept above 6 during the electrolysis and the lowering of pH resulted in the decrease of the current efficiency. Streptomycin, unstable in alkaline medium, could be reduced without much loss, even when the pH of the catholyte was increased up to 9, if the bath temperature was kept below 20°. For the purpose of avoiding alkaline oxidation, it might be better to keep pH of the catholyte around 6 by adding an acid to the catholyte during the electrolysis.

The specification of the patent did not show clearly the most favorable conditions necessary for the electrolytic reduction of streptomycin and claimed the process with less favorable cathodic materials such as platinum, alloy of antimony and lead, etc. Our findings did not coincide with these claims of the patent.

According to our observations with the recovery of dihydrostreptomycin from its solutions, it was made clear that 3-phenylazo-4-hydroxynaphthalenesulfonic acid could be used in place of the known azo dyestuffs to separate dihydrostreptomycin as a complex salt from the catholyte as well as other kinds of solutions containing dihydrostreptomycin. The reagent was preferable to neutral red, methyl orange, etc., since the solubility of the complex salt of dihydrostreptomycin with the reagent was less than those with other known azo dyestuffs.

As discussed above, it may be expected that our findings in the electrolytic reduction of streptomycin could be applied to the preparation of dihydrostreptomycin on a larger scale.

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Experimental

Anode—A sheet of lead having total surface area of 80 cm^2, coated with lead dioxide by preliminary electrolytic oxidation.

Cathode—A sheet of amalgamated lead, lead, platinum, platinized platinum, nickel, amalgamated nickel, and alloy of lead with antimony, each having total surface area of 80 cm^2 and mercury. Amalgamated lead was made from lead treated as a cathode in a solution of mercury salt and then plated with mercury. Amalgamated nickel was also made by the same method as for amalgamated lead.

Anolyte—5∼10% Sulfuric acid.

Catholyte—A solution of 1 g. of streptomycin sesquisulfate (T.U. 750 mg.) in 50 cc. of 10%

sodium sulfate or in buffer of phosphate or borate; pH being adjusted by the addition of acid or alkali.

Current density—0.5 A/dm^2.

Bath voltage—4~6 Volts.

In a 300-cc. beaker were concentrically placed the anode, 150 cc. of the anolyte, and a 70-cc. porceline cup, in which were placed the cathode, a thermometer, and the catholyte. The cup was stoppered tightly with bored stopper, from the hole of which unreacted hydrogen gas was let out. The cell was connected with an ammeter, a voltmeter, a suitable regulating resistance, and a gas coulometer in the circuit, and then electrolyzed. The current efficiency with each unit time was calculated from the difference between the volume of hydrogen generated from the gas coulometer and that of unreacted hydrogen from the cathode chamber. The reaction was completed when 33 cc. of hydrogen was absorbed at 17°. Current efficiencies are shown in Figs. 1 and 2.

The reaction mixture was filtered, acidified with diluted hydrochloric acid, where the pH decreased to 4.8, and filtered again. In order to prove that streptomycin was reduced, a little portion of the filtrate was allowed to stand for 24 hours with equimolecular amount of hydroxylamine at 25°, the pH being kept around 4, and then its activity tested (T.U. 670 mg.). A solution of 1.12 g. of sodium 3-phenylazo-4-hydroxynaphthalenesulfonate was added to the remaining filtrate, allowed to stand overnight, and the precipitate formed was collected by filtration and washed with water. To a suspension of the precipitate in 50 cc. of water diluted hydrochloric acid was added, with efficient stirring, where the pH was kept below 2, and filtered. The filtrate was bone-blacked, filtered, concentrated to 5 cc. of its volume in a frozen state, and precipitated with acetone (T.U. 570 mg.).

Summary

Experiments were carried out to find the most favorable conditions for the electrolytic reduction of streptomycin, and it was found that amalgamated lead was the most favorable metal among those examined for the cathodic material, sodium sulfate, the most preferable among the inorganic salts to be added to the catholyte, and that the hydrogen ion concentration should be kept above 6 during the electrolysis. At the same time it was found that 3-phenylazo-4-hydroxynaphthalenesulfonic acid could be used in place of the known azo dyestuffs to separate dihydrostreptomycin from the catholyte.

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77. **Tanezo Taguchi and Masaharu Kojima:** Studies in Stereochemistry. I. Alkanolamines. (1). Action of Methylating Agents on *dl-trans*-2,5-Diphenyl-4-methyloxazoline.

(*Pharmaceutical Institute, Medical Faculty, University of Kyushu**)

In the interconversion of diastereoisomeric 2-aminoalcohols, McCasland and Carter[1] reported that normal steric results were altered by the "acyl participation" and proposed its mechanism. The most important of the postulated intermediates is oxazoline which stands between inversion and retention. Winstein[2] later proved the formation of the corresponding oxazoline in the course of detosylation of N-acyl-O-tosyl-*dl-trans*-2-aminocyclohexanol.

Also in the interconversion of diastereoisomeric 2-methylaminoalcohols such as ephedrine, it has been reported by several authors[3,4] that the acyl participation gave analogous

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* Katakasu, Fukuoka (田口胤三, 小島正治).

[1] G. E. McCasland, R. K. Clark, Jr., H. E. Carter: J. Am. Chem. Soc., **71**, 637(1949).

[2] S. Winstein, R. Boschan: *Ibid.*, **72**, 4669(1950).

[3] K. Tanaka: J. Pharm. Soc. Japan, **70**, 216(1949).

[4] S. Ikuma: *Ibid.*, **72**, 953(1952).