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.

(Received July 11, 1953)

---

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

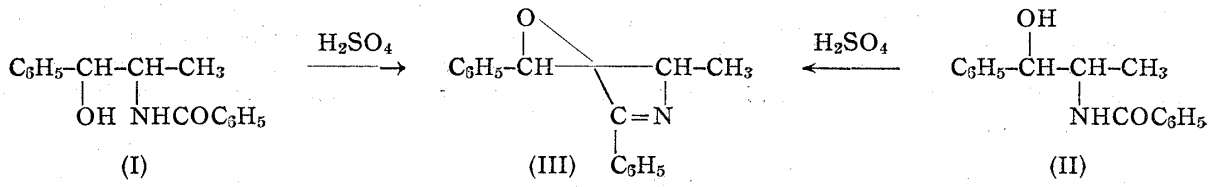
* Katakasu, Fukuoka (田口胤三, 小島正治).

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).

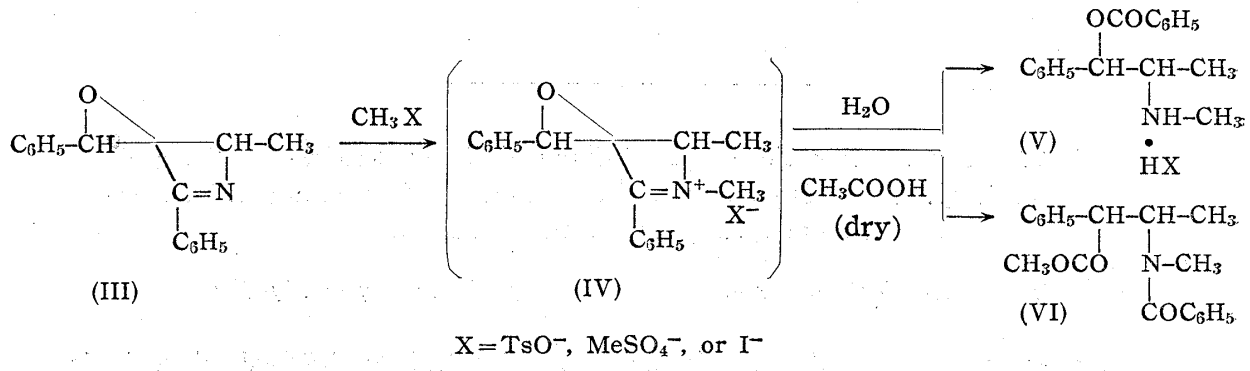
steric results to those 2-aminoalcohols, suggesting the existence of a common mechanism. Accordingly the formation of N-methyloxazolinium salt as the first intermediate is probably pictured but no direct proof has been obtained. Perhaps this compound is so unstable that its isolation is impossible or difficult.

When a basic oxazoline is treated with methylating agents under dry conditions, the corresponding N-methyloxazolinium salt is expected to be formed, even if it cannot be purified and identified. From results of this treatment, some chemical properties of N-methyloxazolinium salt will be made clear and a new general method of N-monomethylation may be established in consequence of addition of water or acetic acid accompanied with its rapid ring opening.

For these purposes the action of methylating agents on *dl-trans*-2,5-diphenyl-4-methyloxazoline (III) was studied. The oxazoline (III) has been obtained by action of concentrated sulfuric acid both on N-benzoyl-*dl*-norephedrine (I) and N-benzoyl-*dl*-ψ-norephedrine (II).[5]



As the methylating agents, methyl tosylate, dimethyl sulfate, and methyl iodide were used and heated with the oxazoline (III) under anhydrous condition. In all cases, the resulting substance is thought to be *dl-trans*-2,5-diphenyl-3,4-dimethyloxazolinium salt (IV), but being very sensitive to moisture and rapidly adding one mole of water to form a salt of O-benzoyl-*dl*-ψ-ephedrine (V) with retention, it could not be purified. As soon as the former was formed it was separated, avoiding moisture and immediately boiled in dry acetic acid-sodium acetate to give N-benzoyl-O-acetyl-*dl*-ephedrine (IV) with inversion.



X=TsO$^-$, $MeSO_4^-$, or I^-

In this reaction the N-monomethylation was complete. Above facts give ground for supposing the oxazolinium salt (IV) formation to be as shown. An application is as follows: When general 2-aminoalcohols are treated similarly as these examples, their N-monomethylation may be accomplished and if they are interconvertible at C with a hydroxyl group, the resultant products may be stereochemically controlled with retention or inversion in the course of procedure.

In connection with these experiments, the oxazoline (III) was treated with dry acetic acid. As a result O-acetyl-N-benzoyl-*dl*-norephedrine was obtained with inversion of the configuration. Besides, some salts of O-benzoyl-*dl*-ψ-ephedrine were prepared as samples for identification.

5) N. Nagai, S. Kanao: Ann., 470, 157(1929).

Experimental[6]

dl-trans-**2,5-Diphenyl-4-methyloxazoline (III)**—Prepared by the use of Nagai's procedure from N-benzoyl-*dl*-ψ-norephedrine: b.p_5 155~160°; picrate, m.p. 140~141°.

Formation of N-benzoyl-*dl*-norephedrine from *dl-trans*-2,5-diphenyl-4-methyloxazoline with inversion—To 0.4 g. of the oxazoline (III) was added the solution obtained by refluxing 5 g. of glacial acetic acid and 0.5 g. of acetic anhydride mixture in an oil bath for 3 hours. After cooling and pouring into 150 cc. of water the resulting precipitate was filtered, weighed (0.4 g.), and melted at 139~141°. Recrystallization from benzene gave O-acetyl-N-benzoyl-*dl*-norephedrine as colorless needles, m.p. 142~143° (Fodor; m.p. 143~144°) *Anal.* Calcd. for $C_{18}H_{19}O_3N$: N, 4.71. Found: N, 4.53.

On refluxing a sample of the material in 10% sodium hydroxide, an oily product appeared which solidified. After filtering and recrystallization from benzene, it melted at 142~143° and was identical with N-benzoyl-*dl*-norephedrine and not with O-acetyl-N-benzoyl-*dl*-norephedrine (m.p. 142~143°) by admixture.

Salt formations of O-benzoyl-*dl*-ψ-ephedrine picrate—Prepared by the application of sodium picrate on O-benzoyl-*dl*-φ-ephedrine hydrochloride; m.p. 155~156°. *Anal.* Calcd. for $C_{23}H_{22}O_9N_4$: N, 11.24. Found. N, 11.04.

Tosylate—One g. of N-benzoyl-*dl*-φ-ephedrine and 1 g. of *p*-toluenesulfonic acid in 1 cc. of water were dissolved by heating and after cooling the resulting precipitate was recrystallized from benzene, giving colorless needles, m.p. 86°. *Anal.* Calcd. for $C_{27}H_{27}O_5NS$: N, 3.17. Found: N, 2.97.

On treatment with sodium picrate, the tosylate was converted to the picrate which was identical with the picrate of O-benzoyl-*dl*-φ-ephedrine.

Hydriodide—On warming a mixture of 0.5 g. of N-benzoyl-*dl*-ephedrine, 0.5 cc. of 30% hydriodic acid, and 0.5 cc. of water, a viscous liquid resulted and solidified when a small amount of ethanol was added. After filtering, recrystallization from ethanol gave colorless needles, m.p. 189~191°. *Anal.* Calcd. for $C_{17}H_{19}NO_2 \cdot HI$: N, 3.52. Found: N, 3.43.

Application of sodium picrate on the hydriodide formed the picrate which was identical with the picrate of O-benzoyl-*dl*- -ephedrine.

Formation of N-benzoyl-*dl*-ψ-ephedrine from *dl-trans*-2,5-diphenyl-4-methyloxazoline with retention—a) A mixture of 1 g. of *dl-trans*-2,5-diphenyl-4-methyloxazoline and 0.7 g. of methyl tosylate was heated in an oil bath until a transparent vitreous mass resulted. The temperature of the oil bath was allowed to rise to 180°. After cooling, the resulting substance did not crystallize but on exposure to air, crystallized through a viscous oil. Recrystallization from benzene or ethyl acetate gave 0.8 g. of colorless needles, m.p. 85~86°. An admixture with an authentic sample of O-benzoyl-*dl*-φ-ephedrine tosylate did not depress the melting point. Also the picrate derived from the former was identical with the picrate of the latter. An aqueous suspension of the tosylate was made alkaline with aqueous ammonia to form an oily product which rapidly solidified. After filtering and recrystallization from benzene-petroleum ether, it melted at 114~115°. Yield, quantitative. A mixed m.p. with a sample of N-benzoyl-*dl*-φ-ephedrine showed no depression. *Anal.* Calcd. for $C_{17}H_{19}NO_2$: N, 5.20. Found: N, 4.95.

b) A solution containing 0.7 g. of the oxazoline (III) and 0.45 g. of dimethyl sulfate in 2 cc. of anhydrous benzene was heated in a sealed tube at 100° for 2.5 hours and formed two layers. After cooling and removing the upper benzene-layer by decantation, the crystallization of the residue was attempted without success. To one portion of the material dissolved in dilute ethanol was added an aqueous solution of sodium picrate and the remainder of the material dissolved in water was made alkaline with sodium bicarbonate. In both cases the resulting precipitates were treated similarly as in the case (a) and proved to be the same as the picrate of O-benzoyl-*dl*-φ-ephedrine and N-benzoyl-*dl*-φ-ephedrine, respectively.

c) A solution containing 0.7 g. of the oxazoline (III) and 0.5 g. of methyl iodide in 2 cc. of anhydrous benzene was heated at 100° in a sealed tube for 2.5 hours, forming two layers. After cooling and removing benzene (the upper layer) by decantation, the residue solidified on adding ether. The remaining solid was very hygroscopic and failed to crystallize. On standing in air it changed to a viscous oil, to which was added a small amount of ethanol, resulting in the appearance of crystals. After filtering and recrystallizing from ethanol, it melted at 189~191°, weighed 0.3 g., and was identical with O-benzoyl-*dl*-φ-ephedrine hydriodide by admixture. The hydriodide was derived to the picrate of O-benzoyl-*dl*-φ-ephedrine and N-benzoyl-*dl*-φ-ephedrine by treating exactly as described in (a) and (b).

Formation of N-benzoyl-*dl*-ephedrine from *dl-trans*-2,5-diphenyl-4-methyloxazoline with

6) All melting points are uncorrected. The authors are indebted to Mr. T. Hattori for the microanalyses.

inversion—a) A solution containing 0.6 g. of the oxazoline (III) and methyl tosylate in 4 cc. of dry toluene was refluxed in an oil bath for 2 hours and two layers formed. After cooling and removing the toluene layer by decantation, a solution prepared by refluxing 5 g. of glacial acetic acid, 0.5 g. of acetic anhydride, and 0.5 g. of fused sodium acetate for 10 minutes was immediately added to the residue. The mixture was refluxed in an oil bath for 3 hours with exclusion of moisture, cooled, and poured into 150 cc. of water, causing precipitation of crystals, m.p. 125~128°. After filtering and recrystallization from benzene-petroleum ether, 0.5 g. of colorless needles, m.p. 130~131°, were obtained. *Anal.* Calcd. for $C_{19}H_{21}O_3N$ (O-acetyl-N-benzoyl-*dl*-ephedrine): N, 4.22. Found: N, 4.25.

A small piece of the crystal in 10% sodium hydroxide was heated in a boiling water bath, for 45 min., the resulting oil product solidifying soon after cooling. After filtering, washing with water, and recrystallizing from benzene-petroleum ether, it melted at 107° and was proved the same as N-benzoyl-*dl*-ephedrine and not with N-benzoyl-*dl*-ψ-ephedrine by admixture.

b) A solution containing 0.7 g. of the oxazoline (III) and 0.45 g. of dimethyl sulfate in 3 cc. of anhydrous benzene was refluxed on a water bath for 40 minutes avoiding moisture. Subsequent treatments were conducted after the manner of (a) and 0.7 g. of O-acetyl-N-benzoyl-*dl*-ephedrine was obtained, from which N-benzoyl-*dl*-ephedrine was formed.

Summary

When the methylating agents are applied to oxazolines, derived from 2-aminoalcohols, N-methyloxazolinium salts are formed. The products seem to be so unstable that addition of one mole of water causes conversion into 2-methylaminoalcohols. If they are resoluble, retention and inversion of configuration simultaneously with the ring opening, may be controlled by some reaction conditions. Based on these suppositions, *dl-trans*-2,5-diphenyl-4-methyloxazoline was reacted with methylating agents and subsequently treated in the presence of either water or dry acetic acid-sodium acetate. Consequently, by the former treatment N-methylation and retention of configuration occured, yielding *dl*-ψ-ephedrine derivatives. On the contrary, by the latter treatment, N-monomethylation and inversion of configuration resulted, forming *dl*-ephedrine derivatives. Thus *dl*-norephedrine and *dl*-ψ-norephedrine can be converted to either *dl*-ephedrine or *dl*-ψ-ephedrine, for the *dl-trans*-oxazoline is derived from either *dl*-norephedrine or *dl*-ψ-norephedrine and N-mono methylation is complete.

(Received July 14, 1953)

---

78. **Minoru Kawashima and Shizuko Fujii:** Biochemical Studies on Acidomycin. II.* Increase of Urinary Biotin Excretion of Acidomycin-Treated Rabbits.

(*Research Laboratory, Takeda Pharmaceutical Industries, Ltd.***)

The authors recently reported that intramuscular injection of 200 γ per g. body weight of acidomycin sodium into rabbits increased an anti-acidomycin factor in their urine, and that the factor seemed to be biotin from the result of inhibition analyses with *Lactobacillus caseii, Lactobacillus arabinosus 17-5*, and *Mycobacterium tuberculosis typus avium*, and partition chromatographic analyses of urine samples[1].

Stokes, *et al.*[2] stated that some kind of lactic acid bacteria appear to require for their growth an exogenous supply of both biotin and aspartic acid if the biotin is limited to

* "Studies on Acidomycin VI[1]" is designated as "Biochemical Studies on Acidomycin. I."

** 4-chome, Juso-Nishino-cho, Higashi-yodogawa-ku, Osaka (川島 実, 藤井シズ子).

1) M. Kawashima, *et al.*: This Bulletin., 1, 94(1953).
2) J. L. Stokes: J. Bact., 54, 219(1947).