

According to the results of biological assay, 1 I.U. corresponds to 4.3~5.5  $\gamma$  of nitrogen. With samples of low potency, however, the present method gave larger nitrogen values against the international unit of the samples. It may be that biological assay gives lower values than actual ones with increase of protein-like impurities.

More data are necessary for ascertaining the accuracy of the present method, but the authors' belief is that insulin preparations with at least 15 I.U. could be determined by the present chemical method.

The authors express their thanks to the Shimizu Pharmaceutical Company for their donation of various samples of insulin preparations.

### Summary

When subjected to paper electrophoresis under the present conditions (buffer, Theorell buffer solution of pH 9~11; ionic strength, 0.07; electric pressure, 300 v; time, 5 hours) insulin migrated towards the anode by ca. 4 cm., forming a clear band. The insulin band was located by duplicate method, cut out, and eluted with water. The eluate was decomposed by the micro-Kjeldahl method and nitrogen in the product determined by azotometry. From the comparison of the results with those of biological assay it was concluded that the present chemical method is applicable for determining the potency of insulin preparations.

(Received October 4, 1954)

## 2. Tanezo Taguchi and Masaharu Kojima : Studies in Stereochemistry. IV.<sup>1)</sup>

Alkanolamines. (4). Regular *dl*-2,5-Diphenyl-4-methyloxazoline :

The Formation and Action of Methyl Tosylate.

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Of the two forms of *dl*-2,5-diphenyl-4-methyloxazoline, the  $\psi$ -form (VII) has been obtained<sup>2)</sup> but not the regular form (VI). It has been found<sup>2)</sup> that the action of concentrated sulfuric acid on *dl*- $\psi$ -N-benzoylnorephedrine does not give the regular *dl*-oxazoline but the *dl*- $\psi$ -oxazoline with retention. Therefore, the formation of the regular *dl*-oxazoline (VI) was studied and it was found to be accomplished by two methods. In addition, the regular *dl*-oxazoline (VI) was treated with methyl tosylate in order to see the feasibility of this method for N-monoalkylation of  $\beta$ -aminoalcohols, reported in the previous papers.<sup>1,3,4)</sup>

The formation of the regular *dl*-oxazoline (VI) was successfully attained by two methods, one being based on the reaction between *dl*-norephedrine and benzimidooethyl ether hydrochloride,<sup>5)</sup> and the other, the boiling of *dl*- $\psi$ -1-phenyl-1-chloro-2-benzoylaminopropane (IV) in absolute ethanol containing anhydrous sodium carbonate. *dl*- $\psi$ -1-Phenyl-1-chloro-2-benzoylaminopropane (IV) is prepared by the benzylation of *dl*- $\psi$ -1-phenyl-1-chloro-2-aminopropane (III) which is obtained without the accompaniment of the other racemic isomer on the treatment of either *dl*-norephedrine (I) or *dl*- $\psi$ -nor-

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1) Part III : J. Pharm. Soc. Japan, **74**, 1293(1954).

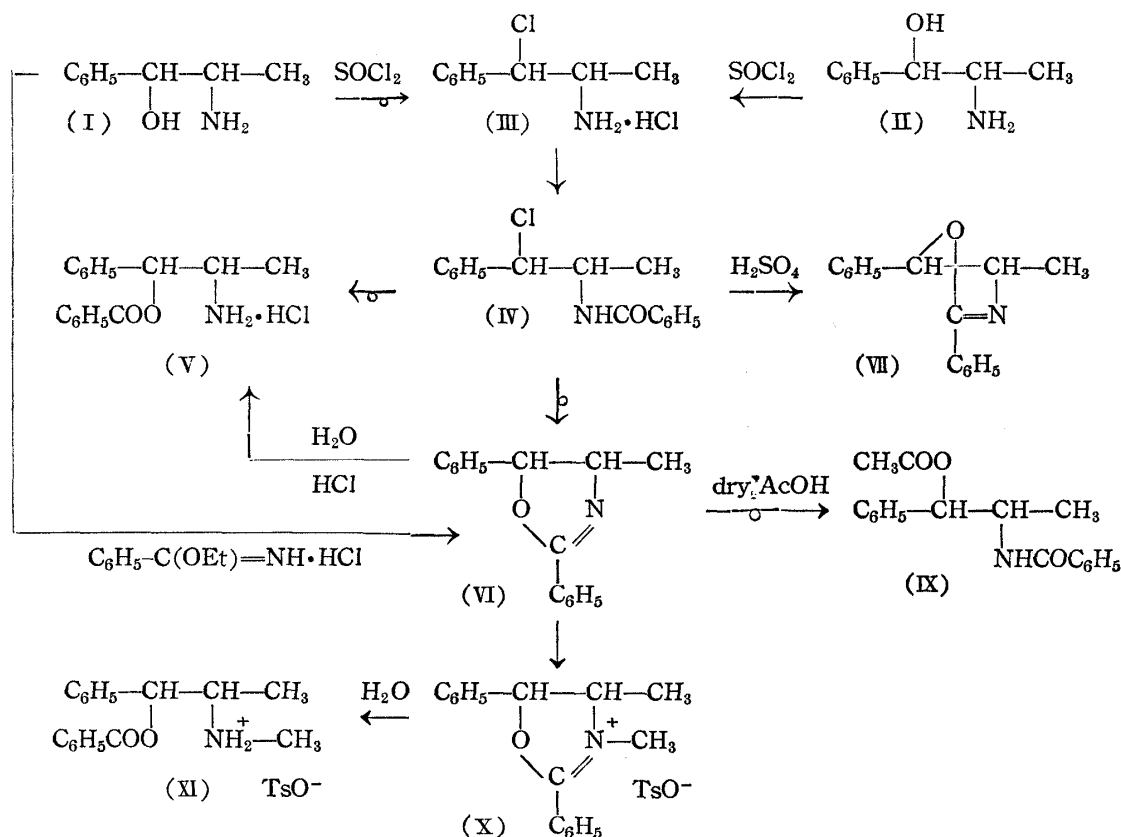
2) N. Nagai, S. Kanao : Ann., **470**, 157(1929).

3) T. Taguchi, M. Kojima : This Bulletin, **1**, 325(1953).

4) T. Taguchi, M. Kojima : J. Pharm. Soc. Japan, **74**, 1133(1954).

5) At the Annual Meeting of the Pharmaceutical Society of Japan (1954) it was reported that the reaction was unsuccessful but later prolongation of reaction time allowed successful completion.

ephedrine (II) with thionyl chloride. This result is quite analogous to the findings on the same treatment of *dl*-ephedrine or *dl*- $\psi$ -ephedrine.<sup>6)</sup> Therefore,  $\psi$ -configuration can be assigned for the *dl*-1-phenyl-1-chloro-2-aminopropane (III) in accordance with the case



of *dl*-ephedrines. Moreover, the fact that *dl*-1-phenyl-1-chloro-2-benzoylamino-3-phenylpropane gives regular *dl*-form of O-benzoylnorephedrine hydrochloride (V) on boiling in water has provided further evidence for the foregoing assignment. The oxazoline (VI) reacts with aqueous hydrochloric acid yielding regular *dl*-1-phenyl-1-benzoyloxy-2-aminopropane hydrochloride (V) and with dry acetic acid yielding *dl*- $\psi$ -1-phenyl-1-acetoxy-2-benzoylamino-3-phenylpropane (IX). In the light of the stereochemical knowledge of today, these results affirm the assignment of regular form for the oxazoline (VI). Further, *dl*- $\psi$ -1-phenyl-1-chloro-2-benzoylamino-3-phenylpropane (IV) was treated with concentrated sulfuric acid and gave *dl*- $\psi$ -2,5-diphenyl-4-methyloxazoline (VII) with even number of inversions.

Now the regular *dl*-oxazoline (VI) was reacted with methyl tosylate. Though the resulting oily product was presumed to be regular *dl*-N-methyloxazolinium tosylate (X) it was very sensitive to moisture and rapidly added one mole of water to convert to *dl*-O-benzoyl-ephedrine tosylate (XI) in a good yield. Thus, *dl*-norephedrine or *dl*- $\psi$ -norephedrine was converted to *dl*-ephedrine via the regular *dl*-oxazoline by the application of the new N-monoalkylation of  $\beta$ -aminoalcohols.

The authors are indebted to the Ministry of Education for the Grant in Aid for Scientific Research and to Mr. T. Hattori and Miss T. Kawano for the microanalyses.

### Experimental<sup>7)</sup>

***dl*- $\psi$ -1-Phenyl-1-chloro-2-aminopropane Hydrochloride (III)**—a) To a solution of 6.3 g. of *dl*- $\psi$ -norephedrine (II) in 15 cc. of  $\text{CHCl}_3$ , a solution of 15 g. of  $\text{SOCl}_2$  in 10 cc. of  $\text{CHCl}_3$  was added

6) K. Tanaka: J. Pharm. Soc. Japan, **70**, 212(1950).

7) All melting points are uncorrected.

under cooling in an ice-water bath and the mixture was allowed to stand overnight at a room temperature. Resulting colorless crystals were collected by filtration; yield, 4.1 g., m.p. 197~199°(decomp.). After the mother liquor was concentrated at a reduced pressure, a residual gummy product was allowed to stand with addition of ether to induce crystallization. After filtration and washing with EtOH, colorless crystals weighed 2.4 g., m.p. as above. Total yield, 6.5 g. (75.6%). Crystallization from EtOH gave colorless needles, m.p. 200~201°(decomp.). *Anal.* Calcd. for  $C_9H_{13}NCl_2$ : N, 6.79. Found: N, 6.86.

b) *dl*-Norephedrine (I) was worked up exactly as described above and gave colorless needles of m.p. 200~201°(decomp.) which were identical with *dl*- $\psi$ -1-phenyl-1-chloro-2-aminopropane hydrochloride by admixture. Yield, 85.7%.

*dl*- $\psi$ -1-Phenyl-1-chloro-2-benzoylaminopropane (IV)—Treatment of 3.8 g. of (III), 3 g. of  $BzCl$ , and 10 cc. of ether by the Schotten-Baumann procedure gave 4.9 g. of a solid, m.p. 129~130°, which crystallized from dehyd. EtOH as colorless microneedles, m.p. 131~132°. *Anal.* Calcd. for  $C_{16}H_{18}ONCl$ : N, 5.12. Found: N, 5.33.

*dl*-O-Benzoylnorephedrine Hydrochloride (V)—A solution of 1 g. of (IV) in 11 cc. of EtOH and 3 cc. of water was refluxed for 2 hrs., evaporated to dryness under a reduced pressure, and left 1.1 g. of crystals. Recrystallization from EtOH gave colorless needles, m.p. 221°(decomp.). *Anal.* Calcd. for  $C_{16}H_{18}O_2NCl$ : N, 4.80. Found: N, 4.93. Picrate: Yellow needles from ethanol, m.p. 188~189°(decomp.). *Anal.* Calcd. for  $C_{22}H_{20}O_9N_4$ : N, 11.57. Found: N, 11.61.

*dl*-N-Benzoylnorephedrine—On allowing *dl*-O-benzoylnorephedrine to stand in aq. 5% NaOH at room temperature for 24 hrs., (V) changed to an oily product which later crystallized. Collection by filtration and recrystallization from benzene showed m.p. 143~144°. A mixed m.p. with an authentic sample of *dl*-N-benzoylnorephedrine was undepressed.

Regular *dl*-2,5-Diphenyl-4-methyloxazoline (VI)—a) A mixture of 2 g. of (IV) in 20 cc. of dehyd. EtOH and 0.8 g. of anhyd.  $Na_2CO_3$  was refluxed for 15 hrs. After cooling, filtering off undissolved substance, and evaporating EtOH, the residue was taken up in ether, dried over anhyd.  $Na_2SO_4$ , and ether removed. The residue was a colorless oil, b.p. 175~177°, and weighed 1.2 g. Picrate: Yellow needles from abs. EtOH, m.p. 153°. *Anal.* Calcd. for  $C_{22}H_{18}O_8N_4$ : C, 56.62; H, 3.89; N, 12.01. Found: C, 56.87; H, 3.99; N, 12.09.

When the picrate was recrystallized from 80% aq. EtOH, it converted into *dl*-O-benzoylnorephedrine picrate, m.p. 188~189°. Treatment of the oxazoline base with 10% HCl yielded *dl*-O-benzoylnorephedrine hydrochloride (V), m.p. 221°(decomp.).

b) A solution of 0.9 g. of *dl*-norephedrine (I) in 5 cc. of dehyd. EtOH and 1.35 g. of benzimidodethyl ether in 10 cc. of dehyd. EtOH was allowed to stand for 2 days at a room temperature. After filtration of the resulting  $NH_4Cl$ , the filtrate was evaporated to dryness under a reduced pressure. Distillation of the residue gave a small amount of the first fraction of b.p. 110° and 0.85 g. of a second fraction of b.p. 175~180°. The picrate of the latter fraction melted at 152~153° and was identical with the picrate obtained under (a).

*dl*- $\psi$ -O-Acetyl-N-benzoylnorephedrine (IX)—A mixture of 0.7 g. of (VI) and dehyd. HOAc (prepared by refluxing 5 cc. of HOAc with 0.2 cc. of  $Ac_2O$ ) was refluxed for 5 hrs. with exclusion of moisture. After cooling, the reaction mixture was poured into 50 cc. of water,  $NaHCO_3$  was added for neutralization, and the separated oil was extracted with ether. On allowing to stand, colorless needles, m.p. 128~130°, separated from the ether solution. Yield, 0.3 g. Recrystallization from benzene gave colorless needles, m.p. 130~131°, which was identical with an authentic sample prepared according to the direction of Fodor.<sup>8)</sup>

*dl*- $\psi$ -N-Benzoylnorephedrine—A suspension of (IX) in 3 cc. of 10% NaOH was refluxed for 30 mins. The undissolved crystals disappeared gradually to turn into an oily substance which solidified on cooling. Recrystallization from benzene gave colorless needles of m.p. 127~128°, which showed no depression by admixture with an authentic sample of *dl*- $\psi$ -N-benzoylnorephedrine, m.p. 127~128°.

*dl*- $\psi$ -2,5-Diphenyl-4-methyloxazoline (VII)—To 0.1 g. of (IV) was added conc.  $H_2SO_4$  until in solution. After the evolution of HCl ceased, the solution was poured onto ice, neutralized with  $K_2CO_3$ , and an oil that separated was taken up in ether. The ether solution was treated with a saturated ether solution of picric acid, causing precipitation of yellow crystals, m.p. 140~141°. Recrystallized from dehyd. EtOH, it was identified with the picrate of *dl*- $\psi$ -oxazoline<sup>2)</sup> by admixture.

*dl*-O-Benzoylphedrine Tosylate (XI)—A mixture of 0.7 g. of (VI) and 0.6 g. of methyl *p*-toluenesulfonate was heated in an oil bath until a transparent vitreous mass resulted. After cooling, a small amount of 80% aq. EtOH was added and allowed to stand, by which the mixture solidified. Recrystallization from EtOAc gave colorless needles, m.p. 149~151°; yield, 0.8 g., which showed no depression by admixture with *dl*-O-benzoylphedrine tosylate.<sup>4)</sup> Picrate: m.p. 199~200°.

*dl*-N-Benzoylphedrine—On treatment with NaOH, (XI) was converted to *dl*-N-benzoylphedrine, m.p. and mixed m.p. 107~108°; yield, quantitative.

8) G. Fodor, V. Bruckner, J. Kiss, G. Óhegy: J. Chem. Soc., 1948, 342.

### Summary

Of the two forms of *dl*-2,5-diphenyl-4-methyloxazolines, the  $\psi$ -form has been obtained, but not the regular form. Therefore, the formation of the latter was studied and was successfully obtained by two ways, the one being based on the reaction between *dl*-norephedrine and benzimidoethyl ether hydrochloride, and the other on the treatment of *dl*- $\psi$ -1-phenyl-1-chloro-2-benzoylaminopropane with boiling absolute ethanol containing anhydrous sodium carbonate. Further, the regular oxazoline was reacted with methyl tosylate and yielded *dl*-O-benzoylphenedrine tosylate. The purpose of the reaction was to find an example of the new method of N-monoalkylation of  $\beta$ -amino-alcohols, which was reported in the previous papers.<sup>1,3,4)</sup>

(Received October 25, 1954)

### 3. Masao Tomita and Yasuo Inubushi : Studies on the Alkaloids of Menispermaceous Plants. CXXII.<sup>1)</sup> Structure of Trilobine and Isotrilobine. (12).<sup>2)</sup>

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Until recently, it was considered that trilobine stood in the same structural relation<sup>3)</sup> to isotrilobine as oxyacanthine (I, R=H) did to berbamine (II, R=H), which indicated that if (III) was assumed to represent trilobine, then (IV) would correspond to isotrilobine, and *vice versa*. However, this ambiguity was removed by the recent work of the same authors<sup>4)</sup> who succeeded in deriving some of the trilobine-isotrilobine type bases from the oxyacanthine-berbamine series. In a series of these studies, it was found that in spite of the fact that O-methylanhydrodemethyloxyacanthine<sup>2)</sup> of the trilobine type derived from oxyacanthine possesses a structure represented by (III), it did not agree with any of trilobine or isotrilobine, and unexpectedly, O-methylanhydrodemethyloxyacanthine methyl methine, trilobine methyl methine, and isotrilobine methyl methine were all found to be identical. This fact suggested that trilobine and isotrilobine must have an identical structure (III) and that they are stereoisomers of each other, the stereochemical arrangements about the two asymmetric centers differing from those of oxyacanthine.

On the other hand, by a previous work by Tomita and his co-workers of applying the sodium-liquid ammonia fission to the oxyacanthine-berbamine type bases and by determining the constitutions and the specific rotations of the bisected bases of the coclaurine type thereby obtained, the chemical structure of the original bases, as well as the configurations of the two asymmetric centers in them, have been elucidated. As regards the stereochemical configurations of the two asymmetric centers of some of the oxyacanthine series including O-methyloxyacanthine,<sup>5)</sup> O-methylrepandine,<sup>6)</sup> cepharanthine,<sup>7)</sup> and epistephanine,<sup>1)</sup> it was considered for a time by comparison of the values of

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- 1) Part CXXI. M. Tomita, E. Fujita : This Bulletin, **2**, 378(1954).
- 2) Part (11). Y. Inubushi, M. Kozuka : This Bulletin, **2**, 215(1954).
- 3) M. Tomita : Fortschr. Chem. org. Naturstoffe, **9**, 203(1952).
- 4) M. Tomita, Y. Inubushi, M. Kozuka : This Bulletin, **1**, 360, 368(1953); Y. Inubushi : *Ibid.*, **2**, 1(1954); M. Tomita, Y. Inubushi : *Ibid.*, **2**, 6(1954); Y. Inubushi : *Ibid.*, **2**, 11, 215(1954).
- 5) E. Fujita : J. Pharm. Soc. Japan, **72**, 213, 217(1952).
- 6) E. Fujita, T. Saijoh : *Ibid.*, **72**, 1232(1952).
- 7) M. Tomita, Y. Sasaki : This Bulletin, **1**, 105(1953); *ibid.*, **2**, 89, 375(1954).