

acetone and in  $\text{NaHCO}_3$  solution, and insoluble in water and petroleum ether.

**Catalytic Reduction of (VII)**—To a solution of 3 g. of (VII) in 20 cc. glacial  $\text{AcOH}$ , 0.5 g. of  $\text{PdCl}_2$  dissolved in 5 cc. of conc.  $\text{HCl}$  was added and the mixture was shaken in  $\text{H}_2$  stream at a room temperature for 4 hrs. About 1 mole of  $\text{H}_2$  was absorbed. The filtrate from the reaction mixture was distilled under a reduced pressure and the residue was extracted with acetone. A crystalline hydrochloride of *N*- $\beta$ -chloroethylglycine separated when the acetone extract was added with ether. It was purified by dissolving it in benzene and adding petroleum ether into the solution. m.p.  $53\text{--}54^\circ$ . *Anal.* Calcd. for  $\text{C}_4\text{H}_9\text{O}_2\text{NCl}_2$ : C, 27.59; H, 5.21; N, 8.05. Found: C, 27.75; H, 5.09; N, 8.02.

**N-Bis( $\beta$ -chloroethyl)taurine N-Oxide (III)**—Six grams of 30%  $\text{H}_2\text{O}_2$  was added with caution into 6 g. of  $\text{Ac}_2\text{O}$ . Into this mixture, 5 g. of *N*-bis( $\beta$ -chloroethyl)taurine was added in portions and the mixture was kept below  $30^\circ$  for 5 hrs. under stirring. The reaction mixture was filtered if necessary, and the filtrate was evaporated by aspiration under  $40^\circ$ . The crystalline residue obtained here was washed with 20 cc.  $\text{EtOH}$  and recrystallized from a large quantity of hot  $\text{EtOH}$  as colorless scales, m.p.  $160^\circ$  (decomp.). Yield, 2 g. It did not form a hydrochloride. It was readily soluble in water but less soluble in  $\text{EtOH}$ . *Anal.* Calcd. for  $\text{C}_6\text{H}_{13}\text{O}_4\text{NCl}_2\text{S}$ : C, 27.07; H, 4.92; N, 5.27. Found: C, 27.35; H, 4.57; N, 5.58.

### Summary

*N*-Oxides of *N*-bis( $\beta$ -chloroethyl)-glycine, -alanine, -taurine, and their derivatives were prepared and their chemical properties, toxicity, and effect upon the Yoshida sarcoma of rats were examined. Contrary to our expectations, inhibitory action of these *N*-oxides against neoplastic growth was found to be inferior than that of *N*-bis( $\beta$ -chloroethyl)-amino acids.

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## 66. Hirotaka Otomasu: Studies on Phenazines. VII\*. Nitration of Phenazine and its Derivatives.

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There are only few reports on the nitration of phenazine and its derivatives. Claus<sup>1)</sup> nitrated phenazine by heating it with a mixture of sulfuric and nitric acids, and claimed to have obtained 2-nitrophenazine. Kehrmann<sup>2)</sup> carried out the same reaction but under a rather mild conditions, and obtained 1,3-dinitrophenazine.

On nitration of phenazine mono-*N*-oxide, Wohl<sup>3)</sup> reported the formation of a dinitro compound but he did not determine the positions of the nitro groups.

In this paper, nitration of phenazine and its derivatives under a fixed conditions, i.e. sulfuric acid and potassium nitrate at  $0^\circ$ , is reported.

Phenazine (I) was nitrated with concentrated sulfuric acid and potassium nitrate at  $0^\circ$ , but the reaction did not proceed, recovering the starting material. When reaction temperature was raised to  $60^\circ$ , 1,3-dinitrophenazine (II) was obtained.

From phenazine 5-*N*-oxide (III), two mono-nitro compounds, m.p.  $204^\circ$  and  $213^\circ$ , were produced in a good yield. The nitro compound of m.p.  $204^\circ$ , the main product, was determined to be 3-nitrophenazine 5-*N*-oxide (IV), as 1- and 3-positions of phenazine 5-

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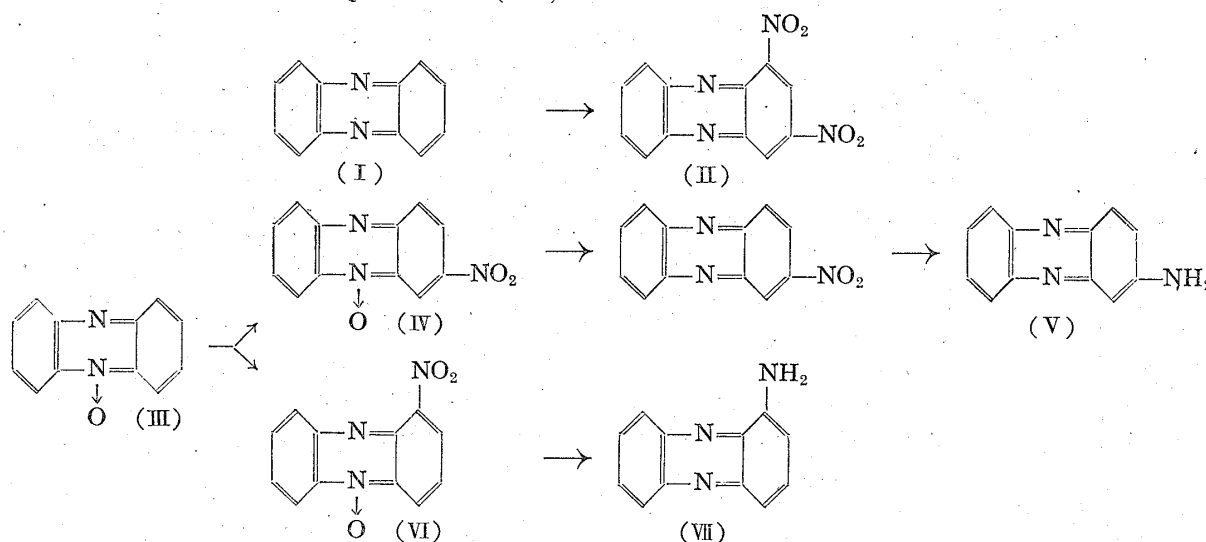
1) A. Claus; Ber., 8, 39 (1875).

2) F. Kehrmann, E. Havas: Ber., 46, 347 (1913).

3) A. Wohl: Ber., 36, 4139 (1903).

N-oxide were activated by N-oxide against electrophilic reagent and the reduction product of (IV) was identical with 2-aminophenazine (V)<sup>4</sup>.

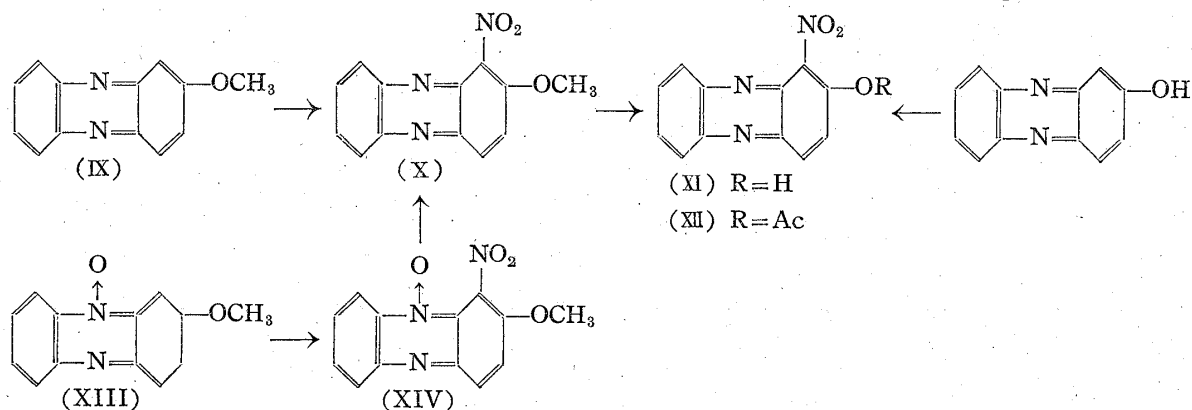
The other nitrophenazine N-oxide, m.p. 213°, was assumed to be 1-nitrophenazine 5-N-oxide (VI), and actually, the reduction product of this nitro compound was found to be identical with 1-aminophenazine (VII)<sup>5</sup>.



Nitration of phenazine 5,10-di-N-oxide did not proceed smoothly, the reaction product being a small amount of 3-nitrophenazine 5-N-oxide.

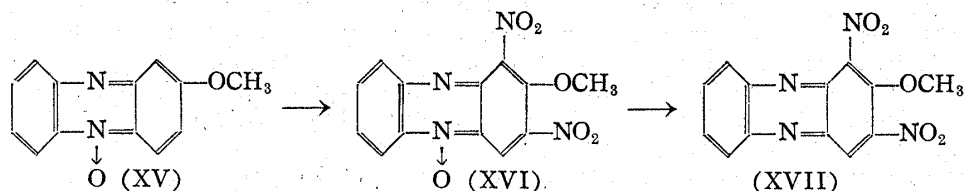
Nitration of the two isomers of 2-methoxyphenazine mono-N-oxide<sup>6</sup>) was then carried out. Hegedüs<sup>7</sup>) carried out the nitration of 2-methoxyphenazine (IX) and obtained 1-nitro-2-methoxyphenazine (X), m.p. 233°. Further, he prepared 1-nitro-2-hydroxyphenazine (XI), 1-nitro-2-methoxyphenazine (X), and 1-nitro-2-acetoxyphenazine (XII) starting from 2-hydroxyphenazine.

When 2-methoxyphenazine 10-N-oxide (XIII) was nitrated, a mononitro derivative (XIV) was produced in a good yield. This was deoxygenated by heating with dimethylaniline and acetic anhydride to form 1-nitro-2-methoxyphenazine (X), which coincided well with that prepared by Hegedüs<sup>7</sup>). 1-Nitro-2-hydroxyphenazine and 1-nitro-2-acetoxyphenazine were also prepared to certify the position of the nitro group.

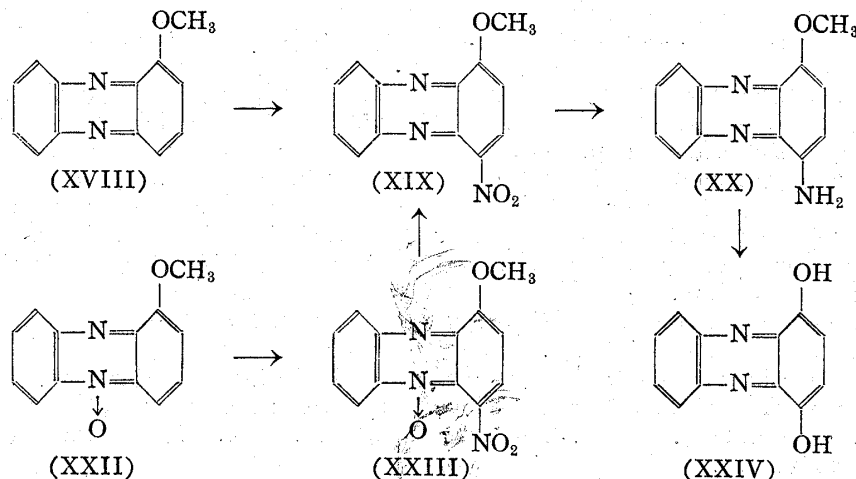


- 4) O. Fischer, E. Hepp : Ber., 22, 355 (1889); A. Wohl, M. Lange : Ber., 43, 2186 (1910); I. Pachter, M. C. Kloetzel : J. Am. Chem. Soc., 74, 971 (1951).
- 5) F. Kehrmann, P. Prunier : Helv. Chim. Acta, 7, 984 (1924); B. Hegedüs : *Ibid.*, 33, 766 (1950); L. Birkofer, A. Widmann : Chem. Ber., 86, 1295 (1953); A. Albert, H. Duewell : C. A., 41, 4498 (1947).
- 6) Positions of oxides were discussed in the previous papers (J. Pharm. Soc. Japan, 72, 1128 (1952); 73, 23 (1953)).
- 7) B. Hegedüs : C. A. 41, 6262 (1947).

In the case of nitrating 2-methoxyphenazine 5-N-oxide (XV), the reaction product was dinitro compound. As in 2-methoxyphenazine 5-N-oxide, 1- and 3-positions of phenazine nucleus were activated by the N-oxide and methoxyl groups and it was assumed that the dinitro compound produced must be 1,3-dinitro-2-methoxyphenazine 5-N-oxide (XVI). This was deoxygenated to form 1,3-dinitro-2-methoxyphenazine (XVII).



Subsequently, 1-methoxyphenazine (XVIII) and its 5-N-oxide (XXII)<sup>6)</sup> were nitrated by the same method. From (XXII), mononitro compound was obtained, which on deoxygenation with dimethylaniline and acetic anhydride, yielded the same nitrophenazine as that from 1-methoxyphenazine. The nitrophenazine was reduced to the aminophenazine (XX) which on heating with 25% hydrochloric acid in a sealed tube produced 1,4-dihydroxyphenazine (XXIV) previously prepared by Yosioka and Otomasu<sup>8)</sup>. Therefore, in these cases, 4-position of phenazine nucleus was substituted by the nitro group.



Lastly, Sandmeyer reaction of 2-aminophenazine was carried out and 2-chlorophenazine<sup>9)</sup> was obtained in a moderate yield.

The author is grateful to Prof. Dr. Ishidate for his encouragement and to Prof. Dr. Ochiai for his kind advices. His thanks are also due to Mr. Kimura for microanalyses.

### Experimental

**Nitration of Phenazine: 1,3-Dinitrophenazine (II)**—Phenazine (1.5 g.) was dissolved in conc.  $H_2SO_4$  (6 cc.) and cooled to  $0^\circ$ . To this solution, powdered  $KNO_3$  (1.5 g.) was added in portions with stirring and the temperature was maintained at  $60^\circ$  for 3 hours. The solution was poured into ice water, whereupon yellowish precipitate separated. This was recrystallized from acetic anhydride to give yellow needles that darkened at about  $225^\circ$  with sintering. *Anal.* Calcd. for  $C_{12}H_6O_4N_4$ : C, 53.33; H, 2.22; N, 20.78. Found: C, 53.85; H, 2.25; N, 20.22.

When the reaction was carried out at  $0^\circ$ , no reaction product was obtained, recovering the original substance.

**Nitration of Phenazine 5-N-Oxide (III)**—Phenazine 5-N-oxide (4.5 g.), conc.  $H_2SO_4$  (45 cc.), and potassium nitrate (2.4 g.) were treated as described above at  $0^\circ$ . The crude crystalline product was dissolved in benzene and chromatographed on alumina. The eluate was concentrated until crystals deposited. 3.8 g. of 3-nitrophenazine 5-N-oxide (IV) was obtained as orange red needles, m.p.  $204^\circ$

8) I. Yosioka, H. Otomasu: This Bulletin, 2, 53 (1954).

9) H. McCombie, H. A. Scarborough, W. A. Waters: J. Chem. Soc., 1928, 353.

(from ethanol). *Anal.* Calcd. for  $C_{12}H_7O_3N_3$ : C, 59.75; H, 2.90; N, 17.42. Found: C, 59.90; H, 2.95; N, 17.43.

From the benzene mother liquor, 0.3 g. 1-nitrophenazine 5-N-oxide (VI) as yellow needles, m.p. 213° (from ethanol), was obtained. *Anal.* Calcd. for  $C_{12}H_7O_3N_3$ : C, 59.75; H, 2.90; N, 17.42. Found: C, 59.98; H, 2.58; N, 17.68.

**2-Nitrophenazine**—A mixture of 3-nitrophenazine 5-N-oxide (0.5 g.), dimethylaniline (5 cc.), and acetic anhydride (5 cc.) was gently refluxed for 2 hours. After the reaction, dimethylaniline and acetic anhydride were distilled off under a reduced pressure and the residue was purified by chromatography on alumina. It gave yellow needles, m.p. 225° (from toluene). Yield, 0.4 g. *Anal.* Calcd. for  $C_{12}H_7O_2N_3$ : C, 64.00; H, 3.11; N, 18.66. Found: C, 63.78; H, 3.23; N, 18.45.

**2-Aminophenazine (V)**—2-Nitrophenazine (0.3 g.) was reduced with palladium oxide in methanol. It gave red needles, m.p. 283° (from xylene). Yield, 0.24 g.

The same substance was obtained in 89% yield by the catalytic reduction of 3-nitrophenazine 5-N-oxide. For this substance, Fischer, *et al.*<sup>4)</sup> recorded m.p. 265°, Wohl, *et al.*<sup>4)</sup>, m.p. 283°, and Pachter, *et al.*<sup>4)</sup>, m.p. 265~267°. *Anal.* Calcd. for  $C_{12}H_9N_3$ : N, 21.53. Found: N, 21.80.

**1-Aminophenazine (VII)**—1-Nitrophenazine 5-N-oxide, m.p. 213°, (0.2 g.) was reduced catalytically. Red needles of m.p. 175~176° (from ligroine) was obtained. Yield, 0.12 g. For this substance, Kehrmann, *et al.*<sup>5)</sup> recorded m.p. 170~172°, Hegedüs<sup>5)</sup>, m.p. 179~181°, and Birkofer, *et al.*, m.p. 176°. *Anal.* Calcd. for  $C_{12}H_9N_3$ : N, 21.53. Found: N, 21.40.

**Nitration of Phenazine Di-N-oxide**—Phenazine di-N-oxide was nitrated by the same procedure as described above. Small amount of 3-nitrophenazine 5-N-oxide was obtained.

**1-Nitro-2-methoxyphenazine 10-N-Oxide (XIV)**—2-Methoxyphenazine 10-N-oxide (XIII) (3.4 g.) was nitrated in conc.  $H_2SO_4$  and  $KNO_3$  at 0°. It gave orange needles of m.p. 243° (decomp.) (from benzene). Yield, 98%. *Anal.* Calcd. for  $C_{13}H_9O_4N_3$ : C, 57.56; H, 3.32; N, 15.50. Found: C, 57.71; H, 3.24; N, 15.79.

**1-Nitro-2-methoxyphenazine (X)**—1-Nitro-2-methoxyphenazine 10-N-oxide (0.5 g.) was deoxygenated by heating with dimethylaniline and acetic anhydride. Yellow needles, m.p. 238° (from toluene). Yield, 0.4 g. Hegedüs<sup>7)</sup> recorded m.p. 233°. *Anal.* Calcd. for  $C_{13}H_9O_3N_3$ : N, 16.47. Found: N, 16.67.

**1-Nitro-2-hydroxyphenazine (XI)**—1-Nitro-2-methoxyphenazine (0.2 g.) was refluxed with hydrobromic acid (4 cc.) and glacial acetic acid (2 cc.) for 1 hour. 0.12 g. of yellowish microneedles, m.p. 223° (from benzene), was obtained. *Anal.* Calcd. for  $C_{12}H_7O_3N_3$ : N, 17.43. Found: N, 17.02. The same substance was produced from 1-nitro-2-methoxyphenazine 10-N-oxide, but in this case reaction must be continued for 5 hours.

**1-Nitro-2-acetoxyphenazine (XII)**—Colorless needles, m.p. 198° (from ligroine). *Anal.* Calcd. for  $C_{14}H_9O_4N_3$ : N, 14.84. Found: N, 14.74.

**1,3-Dinitro-2-methoxyphenazine 5-N-Oxide (XVI)**—Nitration conditions for 2-methoxyphenazine 5-N-oxide (XV) were the same as described above. It gave orange long plates, m.p. 268° (decomp.) (from benzene). Yield, 90%. *Anal.* Calcd. for  $C_{13}H_8O_6N_4$ : C, 49.37; H, 2.53; N, 17.72. Found: C, 49.51; H, 3.00; N, 17.23.

**1,3-Dinitro-2-methoxyphenazine (XVII)**—Yellow needles, m.p. 264~266° (from toluene). *Anal.* Calcd. for  $C_{13}H_8O_5N_4$ : C, 52.00; H, 2.67; N, 18.67. Found: C, 52.37; H, 2.31; N, 18.86.

**1-Methoxy-4-nitrophenazine (XIX)**—1-Methoxyphenazine (XVIII) was nitrated by the usual method. Yield, 98%. It gave yellow needles, m.p. 224° (from acetic anhydride). *Anal.* Calcd. for  $C_{13}H_9O_3N_3$ : C, 61.17; H, 3.53; N, 16.47. Found: C, 61.25; H, 3.74; N, 16.20.

**1-Methoxy-4-nitrophenazine 5-N-Oxide (XXIII)**—This compound was obtained by the nitration of 1-methoxyphenazine 5-N-oxide (XXII). Orange yellow needles, m.p. 223° (decomp.) (from acetic anhydride). Yield, 95%. *Anal.* Calcd. for  $C_{13}H_9O_4N_3$ : C, 57.56; H, 3.32; N, 15.50. Found: C, 57.11; H, 3.34; N, 15.25.

**Deoxygenation of 1-Methoxy-4-nitrophenazine 5-N-Oxide**—1-Methoxy-4-nitrophenazine 5-N-oxide was deoxygenated by heating with dimethylaniline and acetic anhydride. It gave yellow needles of m.p. 224° (from acetic anhydride), which was found to be identical with 1-methoxy-4-nitrophenazine (XIX).

**1-Methoxy-4-aminophenazine (XX)**—1-Methoxy-4-nitrophenazine was reduced catalytically with palladium oxide in methanol. Dark violet plates, m.p. 214° (from benzene). *Anal.* Calcd. for  $C_{13}H_{11}ON_3$ : C, 69.33; H, 4.89; N, 18.67. Found: C, 69.54; H, 4.72; N, 18.86.

**1-Methoxy-4-acetaminophenazine**—Orange red needles, m.p. 231° (from ethanol). *Anal.* Calcd. for  $C_{15}H_{13}O_2N_2$ : C, 67.42; H, 4.87; N, 15.73. Found: C, 67.40; H, 4.82; N, 15.49.

**1,4-Dihydroxyphenazine (XXIV)**—1-Methoxy-4-aminophenazine (1.2 g.) was heated with 25% HCl (20 cc.) in a rocking bomb for 8 hours at 180°. The reaction mixture was basified with dil. NaOH and filtered. The filtrate was neutralized with acetic acid, and the brown crystals that precipitated was recrystallized from chloroform to deep red needles, m.p. 232~234°, not depressed on admixture with the authentic specimen of 1,4-dihydroxyphenazine. *Anal.* Calcd. for  $C_{12}H_8O_2N_2$ : N, 13.21. Found: N, 13.07.

**2-Chlorophenazine**—To the solution of 2-aminophenazine (0.2 g.) in conc. HCl (4 cc.), sodium nitrite (0.18 g. in 2 cc. water) was added and diazotized at 0°. To the mixture, freshly prepared cuprous chloride (0.4 g.) was added in portions. After standing for 20 minutes at room temperature, the reaction mixture was warmed on a water bath at 60°. This was neutralized with ammonia water, the precipitate that separated was dissolved in benzene, and chromatographed on alumina. It gave pale yellow flat needles, m.p. 135~136° (from ligroine), not depressed on admixture with the authentic specimen of 2-chlorophenazine. Pachter, *et al.*<sup>4)</sup> and McCombie, *et al.*<sup>9)</sup> recorded m.p. 138~139°. Yield, 0.14 g. *Anal.* Calcd. for C<sub>12</sub>H<sub>7</sub>N<sub>2</sub>Cl: N, 13.05. Found: N, 13.17.

### Summary

- 1) Phenazine and some of its derivatives were nitrated under the same conditions and the position of the nitro groups substituted was determined.
- 2) Phenazine was not nitrated easily at 0°, but when the reaction temperature was raised to 60°, formed 1,3-dinitrophenazine.
- 3) Phenazine mono-N-oxide was nitrated to form 3-nitrophenazine 5-N-oxide in a good yield, and 1-nitro compound as a by product.
- 4) Phenazine di-N-oxide was not nitrated smoothly and only a small amount of 3-nitrophenazine 5-N-oxide was obtained.
- 5) 2-Methoxyphenazine 10-N-oxide was nitrated to form 1-nitro derivative.
- 6) From 2-methoxyphenazine 5-N-oxide, 1,3-dinitro-2-methoxyphenazine 5-N-oxide was obtained.
- 7) 1-Methoxyphenazine and its 5-N-oxide were substituted both at 1-position by the nitro group.

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### 67. Toru Masuda and Mitsuko Asai: Application of Chromatography. XX<sup>1)</sup>. Quantitative Determination of Cocarboxylase Preparations.

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In general, the synthesis of pure cocarboxylase (TDP) in various methods is attended with some difficulty. From the practical point of view, however, less pure preparations will do, so long as they have a constant purity and have no unwanted activities. A method, therefore, is needed which can determine exactly the purity of the preparations.

The purity of cocarboxylase preparations has hitherto been determined by measuring their biochemical cocarboxylase activity. Since, however, the method is conducted using apocarboxylase isolated, for example, from yeast, the values always fluctuated depending on the purity of the apocarboxylase used. Such circumstances necessitated the authors to find a chemical method which can be carried out easily and which gives exact values.

The crude TDP may be contaminated by monophosphate (TMP), triphosphate (TTP), or by free thiamine in view of the mode of its preparation.

The molecule of thiamine essentially has a positive electric charge. If phosphoric acid combines with the molecule to form an ester, the positive electric charge ought to decrease in proportion to the increase of phosphoric acid radical. Different esters, therefore, would

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1) Satoru Kuwada: Application of Chromatography. XX. Parts I~XIX by S. Kuwada and his co-workers were published in Vitamins, 1950~1954 (in Japanese).