Summary

The synthesis of some C/D *cis*-15-ketopregnane derivatives from hecogenin is described. Hecogenin was degraded to II which in turn was converted to V *via* III. Catalytic hydrogenation of V affords the five reduction products, VIII to XIII. VIII was dehydrogenated with selenium but Jacobs' hydrocarbon was not obtained.

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108. Ikuo Suzuki, Toshiaki Nakashima, and Natsuko Nagasawa : Studies on Cinnolines. IV.*1 On Nitration of Cinnoline 2-Oxide.

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In a previous communication,[1] it was reported that cinnoline 2-oxide gave 5-, 6-, and 8-nitrocinnoline 2-oxides on warming with a mixture of nitric and sulfuric acids, and gave 5-nitrocinnoline 2-oxide by treatment with benzoyl nitrate. The present paper describes a detail of the experiments for nitration of cinnoline 2-oxide and deals with the relationship between the product ratio of nitro compounds, the concentration of sulfuric acid, and the reaction temperature.

Morley[2] obtained 5-nitrocinnoline and 8-nitrocinnoline in approximately equal proportions by nitration of cinnoline using fuming nitric acid and concentrated sulfuric acid at 20° for one hour. Ochiai and Ikehara[3] reported that isoquinoline N-oxide gave on warming with potassium nitrate and sulfuric acid at 60° for 3 hours 5-nitroisoquinoline N-oxide in 90% yield and 8-nitroisoquinoline N-oxide in 2% yield.

When cinnoline 2-oxide (I) was warmed with a mixture of nitric and sulfuric acids at 90° for 2.5 hours, three kinds of mononitro cinnoline 2-oxide were produced : yellow needles (II), m.p. 215~217° (in 3% yield), pale yellow needles (III), m.p. 212~213° (in 15% yield), pale yellow needles (IV), m.p. 228° (decomp.) (in 23% yield). In this reaction, I was recovered in 31% yield. When this reaction was carried out at 70° for 3 hours, II, III and IV were obtained in 1.1, 15, and 13% yields with 42% recovery of I.

Catalytic hydrogenation of III and IV over Adams platinum catalyst gave monoaminocinnolines and monoaminocinnoline 2-oxides. The former compounds were found to be identical with 6-[4] and 8-aminocinnoline[5] (VI and VII), obtained by hydrogenation of 6-[2] and 8-nitrocinnoline[6] over palladium-charcoal, by comparing their spectra and by determining the mixed melting point, respectively, hence the structures of III and IV

*1 Part III : This Bulletin, **13**, 713 (1965).
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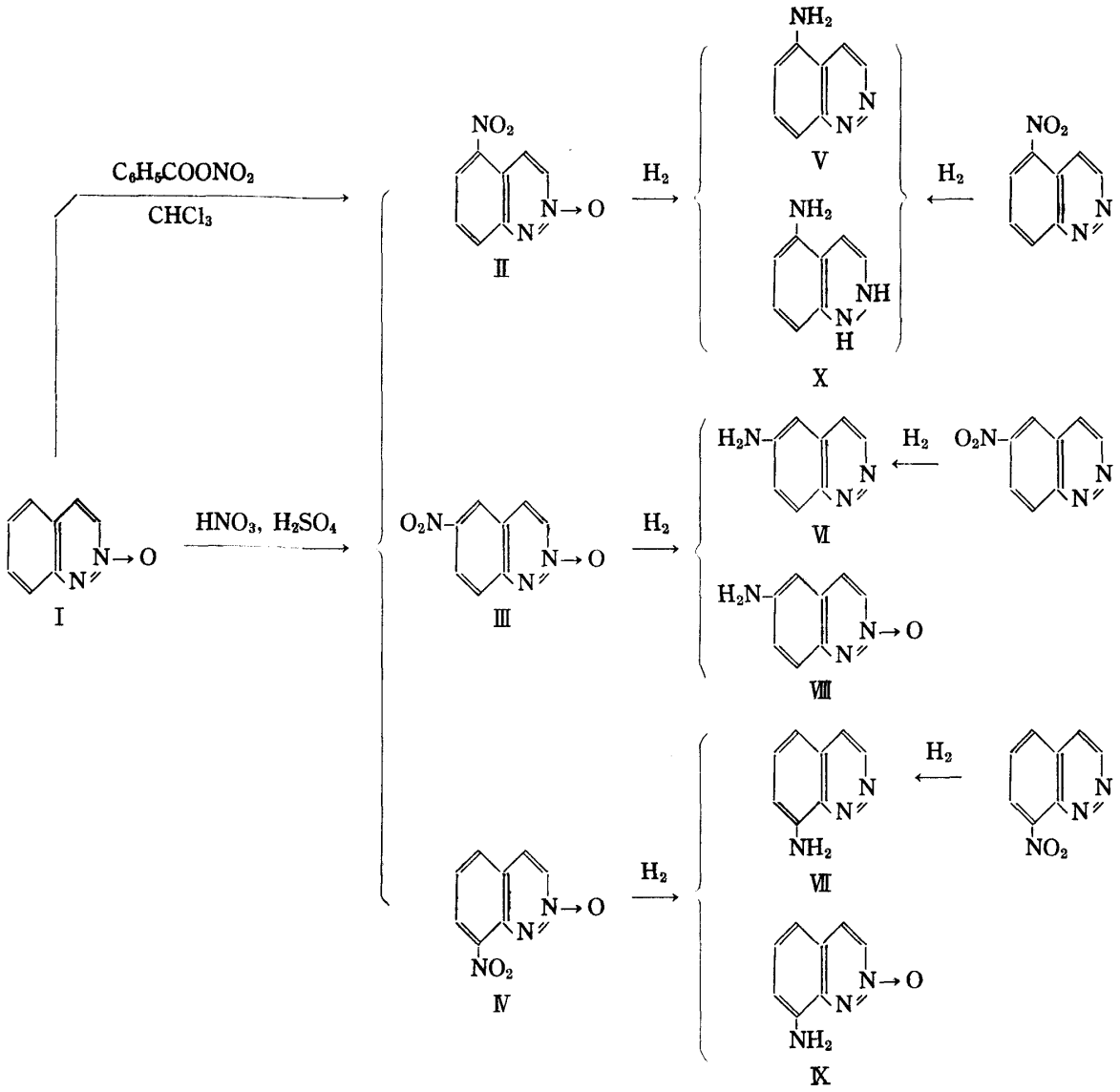
1) I. Suzuki, T. Nakashima, N. Nagasawa : This Bulletin, **11**, 1326 (1962).
2) J. S. Morley : J. Chem. Soc., **1951**, 1971.
3) E. Ochiai, M. Ikehara : Yakugaku Zasshi, **73**, 666 (1953).
4) A. R. Osborn, K. Schofield : J. Chem. Soc., **1955**, 2100.
5) E. J. Alford, *et al.* : *Ibid.*, **1952**, 3009.
6) E. J. Alford, K. Schofield : *Ibid.*, **1953**, 609.



were confirmed to be 6- and 8-nitrocinnoline 2-oxide. The latter compounds also were obtained by catalytic hydrogenation of Ⅲ and Ⅳ over palladium-charcoal, and this fact indicats that they are 6- and 8-aminocinnoline 2-oxides (Ⅷ and Ⅸ), respectively.

Catalytic hydrogenation of Ⅱ over Adams platinum catalyst gave monoaminocinnoline in 5% yield, yellowish green needles, m.p. 157~159°, and also yellow scales, m.p. 137° (decomp.) in 36% yield. The former compound was found to be identical with 5-aminocinnoline[4] (Ⅴ), obtained by hydrogenation of 5-nitrocinnoline[5] over palladium-charcoal, by infrared spectra and mixed melting point. The latter compound gave analytical data consistent with monoaminodihydrocinnoline, and was found to be identical with a by-product obtained by catalytic hydrogenation of 5-nitrocinnoline over palladium-charcoal. Hence the structure of Ⅱ was confirmed to be 5-nitrocinnoline 2-oxide, and dihydro compound is presumed as 5-amino 1,2- or 1,4-dihydrocinnoline (Ⅹ).*[3,7]

*3 Dihydrocinnoline had been assigned the 1,2-dihydro-structure, but in 1963 Besford[7] established the hydrazone structure, that is, 1,4-dihydro-structure, by NMR. 5-Amino-dihydrocinnoline may be described with 1,4-dihydro-structure.

7) L. S. Besford, *et al.*: J. Chem. Soc., **1963**, 2867.

The Table I shows the compounds synthesized and their analytical data.

Table I.

Compound	m. p. a)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
II	215~217°	$C_8H_5O_3N_3$	50.26	2.64	21.99	50.48	2.78	21.56
III	212~213°	〃	50.26	2.64	21.99	50.21	2.87	21.89
IV	228°	〃	50.26	2.64	21.99	50.13	2.39	22.17
V	157~159°	$C_8H_7N_3$	66.19	4.86	28.95	65.67	4.93	29.31
VI	201~202°	〃	66.19	4.86	28.95	66.33	4.92	28.96
VII	91~ 92°	〃	66.19	4.86	28.95	66.37	4.78	29.25
VIII	247°	$C_8H_7ON_3$	59.62	4.38	26.07	59.68	4.74	26.41
IX	212°	〃	59.62	4.38	26.07	59.61	4.64	25.58
X	137°	$C_8H_9N_3$	65.28	6.16	28.55	65.23	6.39	28.60

a) All melting points were uncorrected.

On the other hand the nitration of cinnoline 2-oxide with benzoyl nitrate in chloroform gave 5-nitrocinnoline 2-oxide in 1.5% yield with 91% recovery of I.

Results and Discussion

As above mentioned, the nitration cinnoline 2-oxide is somewhat different from nitration of cinnoline and isoquinoline N-oxide, that is,cinnoline 2-oxide afforded 6-nitrocinnoline 2-oxide accompanied with 5- and 8-nitrocinnoline 2-oxide with mixed acid, and gave 5-nitrocinnoline 2-oxide with benzoyl nitrate in small yield. In views of these facts, it seems reasonable to assume that the orienting effect of the 2-oxide function extended an effect to the benzene ring of cinnoline as shown in Chart 1.

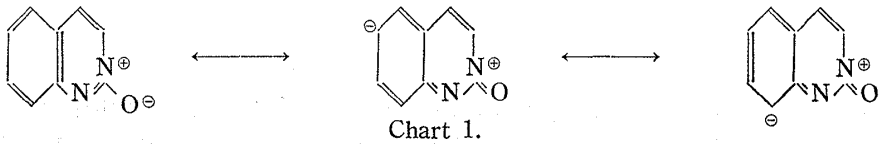


Chart 1.

Ochiai and Okamoto[8] reported that the polar effect of the N-oxide function showed strikingly temperature-dependence. Recently, Hamana and Nagayoshi[9] have found that the orienting effect of N-oxide of 6-substituted quinoline derivatives depended upon the concentration of sulfuric acid rather than the reaction temperature. The following investigation was undertaken to examine the influence of the concentration of sulfuric acid and the reaction temperature on nitration of cinnoline 2-oxide.

The nitration was carried out using potassium nitrate and varying the concentration of sulfuric acid and the reaction temperature as summarized in Table II. Produced nitro compounds were separated by preparative thin-layer chromatography.

The increase of the concentration of sulfuric acid and raising the reaction temperature increase the overall yield of nitro compounds as shown in Table II.*4

*4 The results obtained from nitration using nitric acid are not inconsistent with these data, because in nitration with mixed acid, the medium is diluted with nitric acid (about 60%).

8) E. Ochiai, T. Okamoto: Yakugaku Zasshi, **70**, 384 (1950).

9) M. Hamana, T. Nagayoshi: This Bulletin, **14**, 319 (1966).

Table II. Reaction Condition and Yields (%) of Nitrocinnoline 2-Oxides

Temp. & Time	Compound	Concentration of H_2SO_4 75%	80%	85%	90%	95%
110°	II	2.6	3.7	8	17	20
2 hr.	III	15	21	25	17	6.3
	IV	12	18	37	58	66
	II+III+IV	30	43	70	92	92
	recovery	56	42	20	2.3	3.1
80°	II	1.2	3.1	10	18	21
2 hr.	III	5.6	13	23	13	5.0
	IV	4.3	14	41	59	67
	II+III+IV	11	30	74	90	93
	recovery	87	66	21	6.0	0.8
60°	II	0.7	1.4	7.1	14	21
2 hr.	III	0.8	4.6	14	13	4.7
	IV	0.6	6.0	30	53	68
	II+III+IV	2.1	12	51	80	94
	recovery	96	84	46	14	1.3
25°	II	<0.1	0.6	7.4	18	21
4 days	III	<0.1	1.2	9	9	4.2
	IV	0.5	2.6	28	67	72
	II+III+IV	0.6	4.4	44	94	97
	recovery	98	94	56	2.1	1.1

100 mg. of I was treated with 100 mg. of KNO_3 in 0.6 ml. of sulfuric acid.

Fig. 1 illustrates the relation of the product ratio*5 of the 5-, 6- and 8-nitrocinnoline 2-oxide to the concentration of sulfuric acid at several temperatures.

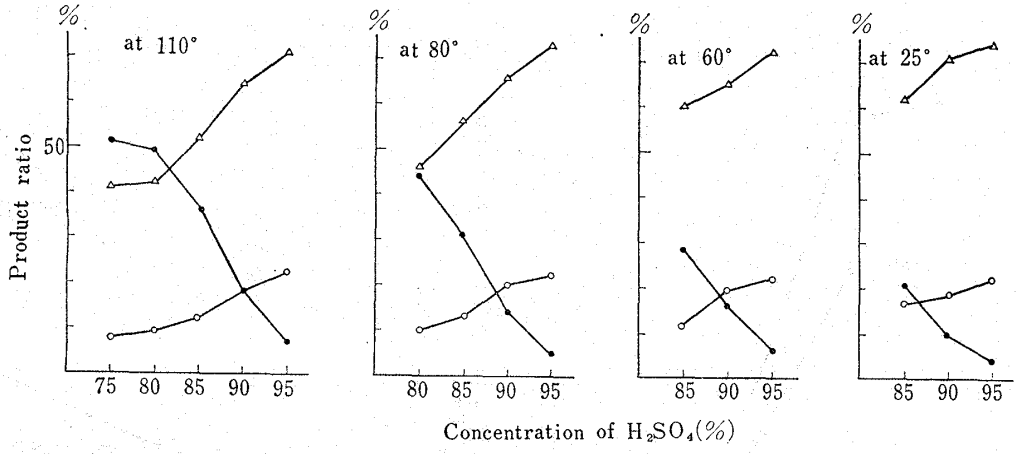


Fig. 1. Relation between the Product Ratio of each Nitro Compound and the Concentration of Sulfuric Acid at Several Temperatures (Cases below 27% total yield were omitted.)

5-NO_2 Cinnoline 2→0 ○—○ 6-NO_2 Cinnoline 2→0 ●—● 8-NO_2 Cinnoline 2→0 △—△

The relationship between the reaction temperature and the product ratio of each nitro compound was observed as shown in Fig. 2.

*5 Product ratio is defined : The ratio of yield of each nitro compound to the yield of the whole nitro compounds, expressed in percentage.

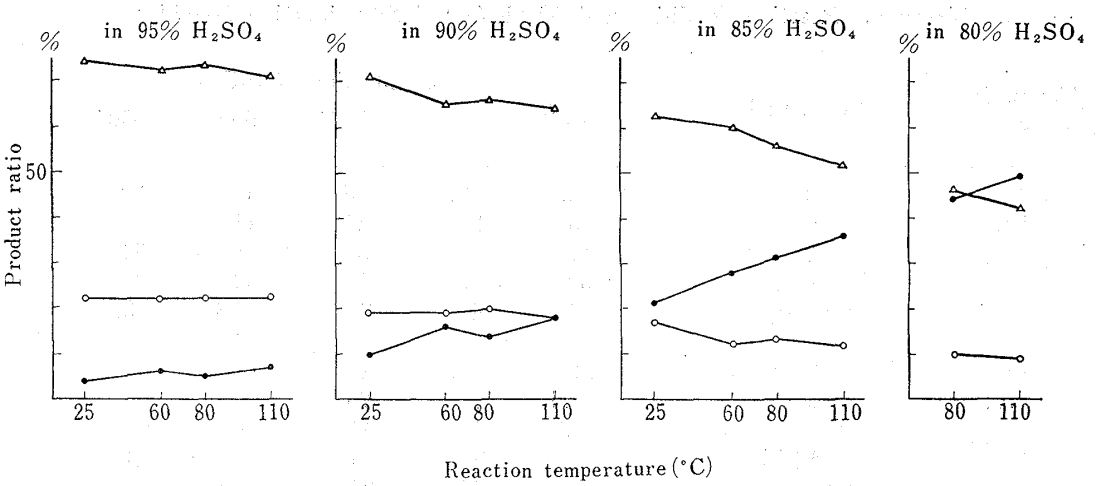


Fig. 2. Relation between the Product Ratio and the Reaction Temperatures (Cases below 27% total yield were omitted.)

5-NO_2 Cinnoline 2→0 ○—○ 6-NO_2 Cinnoline 2→0 ●—● 8-NO_2 Cinnoline 2→0 △—△

As it can be seen in these tables and figures, the product ratios of 5- and 8-nitrocinnoline 2-oxide decrease and the product ratio of 6-nitrocinnoline 2-oxide increases with decreasing the concentration of sulfuric acid used. On the other hand, the effect of the reaction temperature is highly dependent on the product ratio of nitro compounds with decreasing the concentration of sulfuric acid: the product ratio of 6-nitro compound increases as the reaction temperature increases, and in contrast, the higher the reaction temperature is, the lower the product ratios of 5- and 8-nitro compounds are, excluding as 95% sulfuric acid was used.

As indicated in Chart 1, the orienting effect of the 2-N-oxide function was shown at 6- and 8-positions, and electronic effect of cinnoline ring itself was shown at 5- and 8-positions, namely, the both effects are cumulative at 8-position.

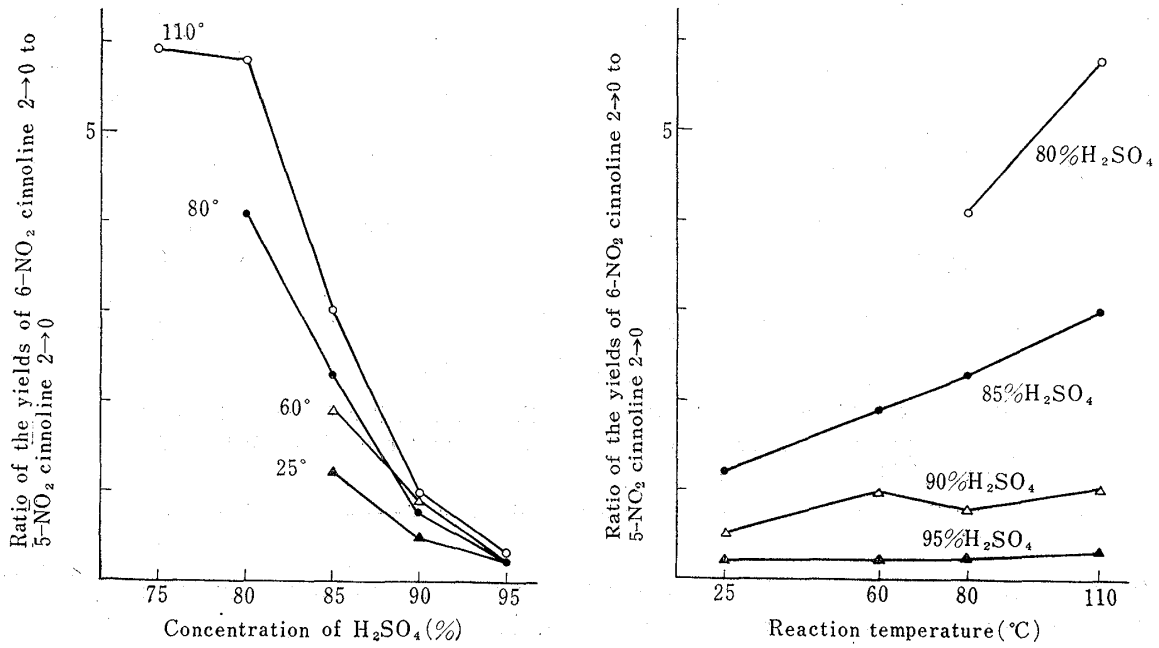


Fig. 3. Relation between the Ratio of the Yields of 6-NO_2 Cinnoline N→O to 5-NO_2 Cinnoline N→O, and the Concentration of Acid or the Reaction Temperatures (Cases below 27% total yield were omitted.)

In order to examine the relationship between the effect of 2-N-oxide and nucleophilic activity of cinnoline ring, the correlation between the ratio of the yield of 6-nitro compound to the yield of 5-nitro compound, and the concentration of acid and the reaction temperature was illustrated in Fig. 3.

The result obtained Fig. 3., suggested that the positions taken by the nitro group on nitration of cinnoline 2-oxide are dependent upon the concentration of sulfuric acid and the reaction temperature. Therefore, it may be concluded that the orienting effect of cinnoline 2-oxide becomes apparent in relatively lower concentration of sulfuric acid, and in lower concentration of the acid is also affected by the reaction temperature.

Experimental

Nitration of Cinnoline 2-Oxide (I)——a) With mixed acid at 90°: To a mixture of 3.00 g. of I and 4 ml. of conc. H_2SO_4, 1.8 ml. (1.2 mol.) of 60% HNO_3 was added dropwise at 60°, and the mixture was warmed at 90° for 2.5 hr. After cooling the reaction mixture was poured into 50 ml. of ice-water, the precipitates were collected, washed with water, and dried in a desiccator. The precipitates were dissolved in $CHCl_3$, passed through a column of Florisil, and eluted with $CHCl_3$. The solvent was evaporated from the initial fraction of the eluate, and the residue (m.p. 221° (decomp.), 0.89 g., 23%) was recrystallized from EtOH to give pale yellow needles, Ⅳ, m.p. 228° (decomp.).

The solvent was evaporated from the following fraction of eluate, and the residue (m.p. 208~211°, 0.59 g., 15%) was recrystallized from EtOH to give pale yellow needles, Ⅲ, m.p. 212~213°.

The initial filtrate and washing were extracted with $CHCl_3$, the $CHCl_3$ solution was washed with aqueous $NaHCO_3$, and dried over anhyd. Na_2SO_4. The $CHCl_3$ solution was concentrated to a small volume, passed through a column of Al_2O_3, and eluted with $CHCl_3$. The solvent was evaporated from the initial fraction of the eluate, and the residue (m.p. 212~215°, 0.12 g., 3%) was recrystallized from EtOH to give yellow needles, Ⅱ, m.p. 215~217°.

The solvent was evaporated from the following fraction of eluate, and gave crystals (0.94 g., 31%). The crystals were identical with starting material by comparison of their IR spectra.

b) With mixed acid at 70°: To a mixture of 2.90 g. of I and 4 ml. of conc. H_2SO_4, 1.8 ml. (1.2 mol.) of 60% HNO_3 was added dropwise at 60°, and the mixture was warmed at 70° for 3 hr. By treating the reaction mixture as described in (a), 41 mg. (1.1%) of Ⅱ, 0.57 g. (15%) of Ⅲ, 0.49 g. (13%) of Ⅳ and 1.21 g. (42%) of starting material were obtained, respectively.

c) With H_2SO_4-KNO_3: The concentration of H_2SO_4, the reaction temperature and the reaction time are given in Table Ⅱ. A mixture of 100 mg. of I, 100 mg. (1.4 mol.) of KNO_3 and 0.6 ml. of H_2SO_4 was reacted under condition in Table Ⅱ. After cooling the reaction mixture was poured into ice-water, extracted with $CHCl_3$ solution was washed with aqueous $NaHCO_3$, dried over anhyd. Na_2SO_4, and concentrated to a small volume.

The $CHCl_3$ solution containing I, Ⅱ, Ⅲ, and Ⅳ was separated by preparative thin-layer chromatography, using a 20×20 cm. plate was coated with silica gel G in 0.5 mm. The separated bands containing each compound could be seen under ultraviolet light.

Firstly, the mixture was developed with $CHCl_3$ using multiple developing method. The two bands were revealed under ultraviolet light, scraped off the plate, and were extracted from silica gel G with acetone, respectively. The mixture of I, Ⅱ, and Ⅲ was obtained from upper band, and Ⅳ was obtained from lower band.

Next, the mixture of I, Ⅱ, and Ⅲ was developed with benzene-acetone (9:1) by the same method. The mixture of Ⅱ and Ⅲ was obtained upper band, and starting material was obtained from lower band. Finally, the mixture of Ⅱ and Ⅲ was developed with $CHCl_3$ by the same method. Ⅱ and Ⅲ were separated into two bands, and obtained by extraction from the silica gel G with acetone. The yields of these compounds were shown in Table Ⅱ.

d) With BzCl-$AgNO_3$: To a solution of 2.50 g. of I in 50 ml. of $CHCl_3$, 2.5 g. (1.0 mol.) of BzCl was added at 0°, and next 3.5 g. (1.2 mol.) of finely powdered $AgNO_3$ was added in small portions keeping below −10° under stirring. Stirring was continued for 4 hr. at the same temperature, and the mixture was allowed to stand for a week at room temperature. After removal of the AgCl, the filtrate was passed through of column of Al_2O_3, and eluted with $CHCl_3$. The solvent was evaporated from the initial fraction of the eluate, and the residue (m.p. 210~214°, 0.07 g., 1.5%) was recrystallized from EtOH to give yellow needles, m.p. 215~217°. No melting point depression was observed on admixture with Ⅱ obtained in (a), and the IR spectra of the two samples were identical.

The solvent was evaporated from the following fraction of eluate, and gave crystals (2.27 g. 91%). The crystals were identical with starting material by comparison of their IR spectra.

Catalytic Hydrogenation of 5-Nitrocinnoline——A mixture of 70 mg. of 5-nitrocinnoline[5] and 30 ml. of EtOH was hydrogenated in the presence of Pd-C prepared from 0.2 g. of charcoal and 10 ml. of 1% $PdCl_2$ solution, until 30 ml. (3 mol.) of H_2 was absorbed. After removal of the catalyst, the filtrate was distilled off under reduced pressure to dryness. The residue was dissolved in $CHCl_3$, passed through a column of Al_2O_3, and eluted with $CHCl_3$. The solvent was evaporated from the initial fraction of the eluate, and the residue (m.p. 132° (decomp.), 22 mg., 37%) was recrystallized from benzene to give yellow scales, X, m.p. 137° (decomp.).

The solvent was evaporated from the following fraction of the eluate, and the residue (m.p. 156~159°, 16 mg., 27%) was recrystallized from $CHCl_3$ to give yellowish green needles, 5-aminocinnoline[4] (V), m.p. 157~159° (lit., m.p. 160~161°).

Catalytic Hydrogenation of 5-Nitrocinnoline 2-Oxide (II)——A mixture of 100 mg. of II and 30 ml. of EtOH was hydrogenated in the presence of Pt catalyst prepared from 50 mg. of PtO_2, until 51 ml. (4 mol.) of H_2 was absorbed. By treating in the same manner described in 5-nitrocinnoline, 28 mg. (36%) of X and 4 mg. (5%) of V were obtained, and they were identical with X and V by the comparison of their IR spectra.

Catalytic Hydrogenation of 6-Nitrocinnoline——A mixture of 24 mg. of 6-nitrocinnoline[2] and 30 ml. of EtOH was hydrogenated in the presence of Pd-C prepared from 0.1 g. of charcoal and 4 ml. of 1% $PdCl_2$ solution, until 10 ml. (3 mol.) of H_2 was absorbed. After removal of the catalyst, the filtrate was distilled off under reduced pressure to dryness. The purification of the residue over Al_2O_3 with $CHCl_3$ gave 8 mg. (40%) of 6-aminocinnoline[4] (VI), m.p. 199~200° (lit., m.p. 203~204°).

Catalytic Hydrogenation of 6-Nitrocinnoline 2-Oxide (III)——a) With Pd-C Catalyst: A mixture of 100 mg. of III and 30 ml. of EtOH was hydrogenated in the presence of Pd-C prepared from 0.1 g. of charcoal and 5 ml. of 1% $PdCl_2$ solution, until 37 ml. (3 mol.) of H_2 was absorbed. After removal of the catalyst, the filtrate was concentrated to a small volume. Yellow needles, VIII (58 mg.), m.p. 247° (decomp.) were obtained in 70% yield.

b) With Pt Catalyst: A mixture of 100 mg. of III and 30 ml. of EtOH was hydrogenated in the presence of Pt catalyst prepared from 50 mg. of PtO_2, until 51 ml. (4 mol.) of H_2 was absorbed. After removal of the catalyst, the filtrate was distilled off under reduced pressure to dryness. The residue was extracted with $CHCl_3$, and $CHCl_3$ insoluble substance was 30 mg. (35%) of VIII, m.p. 244° (decomp.). The substance were indentical with VIII obtained in (a) by comparison of IR spectra.

The $CHCl_3$ extracted solution was passed through a column of Al_2O_3, and eluted with $CHCl_3$. The solvent was evaporated and the residue (m.p. 200~201°, 42 mg., 55%) was recrystallized from benzene-EtOH to give yellow scales, VI, 201~202°. No melting point depression was observed on admixture with 6-aminocinnoline obtained by reduction of 6-nitrocinnoline, and IR spectra of the two samples were identical.

Catalytic Hydrogenation of 8-Nitrocinnoline——Thirty milligrams of 8-nitrocinnoline[6] was hydrogenated and purified as for 6-nitrocinnoline. It gave 7 mg. (28%) of 8-aminocinnoline[5] (VII), m.p. 88~90° (lit., for $C_8H_7N_3 \cdot 1/2H_2O$, m.p. 89~92°).

Catalytic Hydrogenation of 8-Nitrocinnoline 2-Oxide (IV)——a) With Pd-C Catalyst: One hundred milligrams of IV was hydrogenated and separated as for III. It gave 45 mg. (53%) of yellow scales, IX, m.p. 212° (decomp.).

b) With Pt Catalyst: One hundred milligrams of IV was hydrogenated and treated as for III. The residue was dissolved in $CHCl_3$, passed through a column of Al_2O_3, and eluted with $CHCl_3$. The solvent was evaporated from the initial fraction of the eluate, and the residue (m.p. 88~91°, 37 mg., 49%) was recrystallized from petroleum ether to give yellow needles, VII, m.p. 91~92°. No melting point depression was observed on admixture with 8-aminocinnoline (VII) obtained by reduction of 8-nitrocinnoline, and IR spectra of the two samples were identical.

The solvent was evaporated from the following fraction of the eluate, and the residue was 28 mg. (33%) of IX, m.p. 210° (decomp.). The substance was identical with IX obtained in (a) by comparison of IR spectra.

The authors express their gratitudes to Prof. E. Ochiai, the Director of Itsuu Laboratory, for his helpful advices, and to Prof. M. Hamana of Kyushu University for valuable discussions. They are grateful to Dr. T. Itai for the encouragement and helpful advices. They are indebted to members of microanalytical center of University of Tokyo for the analysis data.

Summary

Cinnoline 2-oxide (I) gave 5-, 6- and 8-nitrocinnoline 2-oxides (II, III, and IV) on warming with nitric and sulfuric acids or potassium nitrate in sulfuric acid. The nitration of I with benzoyl nitrate afforded II. 5-, 6- and 8-Aminocinnolines (V, VI, VII), 6- and 8-aminocinnoline 2-oxides (VIII and IX), and 5-aminodihydrocinnoline (X) were synthesized for the determination of the structures of II, III, and IV.

The effect of 2-N-oxide of I in this reaction was affected by the concentration of sulfuric acid and the reaction temperature.

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109. Goro Tsukamoto, Kiyoshi Harada, and Isamu Utsumi : Studies on Thiamine Disulfide. XVI.*1 Geometrical Isomers of Thiaminic Acids.*2

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A number of thiol-type thiamine derivatives are in current clinical use as easily absorbable, long-acting vitamin B_1 preparations. With the development of the massive administration therapy, the curative efficacy has been intensive and the wide indications have been established so far.[1] These thiamine derivatives show the same therapeutic effect despite of their different chemical structure when administered at the large dose which is considerably over the physiological requirement of the vitamin.

In this situation, the therapeutic effect of these derivatives has been considered to be due to their pharmacological one. But no one has yet found the reasonable pharmacological ground for this point. Under the circumstances, the following consideration was made for this problem and led us to carry out the present investigation. These derivatives would be metabolized to a substance which has the pharmacological efficacy, and the metabolite probably would be directly formed from thiamine hydrochloride with difficulty as compared with the formation from the thiol-type thiamine derivatives. Meantime, it is well known that cysteic acid, hypotaurine and taurine appear in the course of metabolism of cysteine and cystine[2~4].

From these points, the authors have supposed that SH group of the various thiamine derivatives would be oxidized to SO_3H to produce 2-(2-methyl-4-amino-5-pyrimidyl)methylformamido-5-hydroxy-2-pentene-3-sulfonic acid (hereinafter referred to IIb and called thiaminic acid) which had not been reported in any literatures. The oxidation of various thiol-type thiamine derivatives was thus attempted and gave thiaminic acid (IIb) or its O-acyl derivatives such as IIa.[5,6] In this investigation, symmetrical disulfide type thiamine, O-benzoylthiamine disulfide (I), was oxidized to give 2-(2-methyl-4-amino-5-pyrimidyl)methylformamido-5-benzoyloxy-2-pentene-3-sulfonic acid (IIa) (hereinafter referred to O-benzoylthiaminic acid) with the yield of 50~60% under the optimum oxidizing condition by hydrogen peroxide. This low yield implied that there

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