ture of 395 mg. of (III), 2.4 g. of HCOOH, and 1.2 g. of $CO(NH_2)_2$ was heated in $N_2$ stream at 110~120° for 2 hr., at 120~180° for 1 hr., and at 180° for 3 hr. After cool, the mixture was diluted with water and extracted with benzene. The benzene solution was washed consecutively with 4% NaOH, 5% HCl, and $H_2O$, and evaporation of the solvent left 130 mg. of a product. This was adsorbed on 2.6 g. of alumina which was eluted with 180 cc. of benzene and 50 cc. of a mixture of 0.5% EtOH and benzene, from which 64 mg. of a powdery product was obtained. This was recrystallized from $Et_2O$ to 13.2 mg. of white needles, m.p. 196°. *Anal.* Calcd. for $C_{20}H_{27}O_4N$ : C, 69.54; H, 7.88; N, 4.06. Found : C, 69.51; H, 7.66; N, 4.08. IR $\lambda_{max}^{KBr}$ $\mu$ : 3.04 (NH), 6.03 (NH-CO), 6.45 (NH). UV $\lambda_{max}^{EtOH}$ m$\mu$ (log $\varepsilon$) : 220 (4.44), 254 (4.14). IR $\lambda_{max}^{CHCl_3}$ $\mu$ : 2.90, 3.34, 3.42, 3.51, 5.92, 6.25, 6.40, 6.72, 6.81, 6.86, 6.97, 7.11, 7.16, 7.32, 7.40, 7.43, 7.57, 7.72, 7.81, 7.92, 8.10, 8.39, 8.82, 9.09, 9.70, 10.04, 10.22, 10.41, 10.65, 10.84, 11.92.

The authors express their deep gratitude to Prof. K. Tsuda of the University of Tokyo and Mr. M. Matsui, Director of this Laboratory, for their unfailing guidance and encouragement throughout the course of this work. They are indebted to Dr. S. Ikuma of this laboratory for technical help and to the members of this laboratory who undertook elemental analysis and measurement of infrared and ultraviolet spectra.

## Summary

Demethoxydeoxyhexahydrocolchlicine (XX), one of the decomposition products of colchicine was synthesized. 1-O-Methylpyrogallol was derived to 3-methoxyl-4-hydroxy-8,9,10,11-tetrahydrobenzo[*b*]cyclohepta[*d*]pyran-6-one (IV) and to methyl 2-[6-(2-methoxycarbonylethyl)-2,3,4-trimethoxyphenyl]-1-cycloheptenecarboxylate (XIV) via the 1-formyl compound (VIII) and 1-(2-carboxyethyl) compound (XII) of (VI), and Dieckmann condensation of (XIV) gave 1,2,3-trimethoxy-5,6,10,12-hexahydrobenzo[*a*]heptalen-7(8*H*)-one, which was also obtained by dehydrative condensation of the free acid (XIII) of (XIV). (III) was derived to its oxime which was reduced and acetylated to *dl*-7-acetamido-1,2,3-trimethoxy-5,6,7,8,9,10,11,12-octahydrobenzo[*a*]heptalene (XIX) whose double bond was rearranged to form the racemic compound of (XX). The position of the double bond in (XX) was determined. *dl*-N-formyldeacetyldemethoxydeoxyhexahydrocolchicine (XXI) was obtained in one step from (III) by the Leuckart reaction.

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*UDC 547.94 : 582.572.2*

## 46. **Takahiro Nakamura** : Studies on the Total Synthesis of *dl*-Colchiceine. III.[1]

(*Takamine Laboratory, Sankyo Co., Lte.*[*1])

As reported in the preceding paper,[1] *dl*-demethoxydeoxyhexahydrocolchicine (I) was synthesized as an intermediate for a total synthesis of colchicine. The levorotatory compound of (I) had been obtained from colchicine by the method of Rapoport and others.[2] In order to introduce further double bond into the C-ring of (I) to form the tropilidene ring, the levorotatory compound of (I) was submitted to dehydrogenation reaction with selenium dioxide and mercuric acetate but the reaction did not materialize. Dryden and

*1 Nishi-Shinagawa, Shinagawa-ku, Tokyo (中村隆洋).

1) Part II : This Bulletin, **10**, 291 (1962).

2) H. Rapoport, A. R. Williams, J. E. Campion, D. E. Pack : J. Am. Chem. Soc., **76**, 3693 (1954).

others[3] had obtained tropilidene by the application of two moles of N-bromosuccinimide and dehydrobromination of the intermediate bromo compound without its isolation. Accordingly, two moles of N-bromosuccinimide was applied to the levorotatory (I). The mixture colored red at first by liberation of bromine within 5 minutes but the solution was discologed by the end of the reaction. Dehydrobromination with collidine afforded levorotatory compound (II) of m.p. 207°, whose analytical values and formation of levorotatory (I) by absorption of 2 moles of hydrogen on catalytic hydrogenation over palladiumcarbon indicated the presence of three double bonds. Rapoport and others[2,4] had shows that (I) resists reduction either over platinum dioxide or in acetic acid to leave the double bond at 12~12a. If there are three double bonds in the levorotatory (II), it should absorb 2 moles of hydrogen. Since the application of 3 moles of N-bromosuccinimide to colchicine for 10~15 minutes fails to cause any reaction, bromine is not considered to enter the A- or B-ring of (I) within 5 minutes of the application of N-bromosuccinimide. Consequently, bromination must have taken place in the C-ring of (I) and all three double bonds should be in the C-ring of (II) which must have formed a tropilidene ring. In the ultraviolet spectrum of cycloheptene, absorption maximum of over log $\varepsilon$ 3.0 in intensity is in a shorter wave-length range than 200 mμ, while those of cycloheptadiene and tropilidene are at 248 mμ (log $\varepsilon$ 3.87) and 266 mμ (log $\varepsilon$ 3.62), the maximum shifting to a longer wave-length side by ca. 18 mμ with increasing number of the conjugated double bond. The compound (I) with one double bond in the C-ring has absorption maximum at 255 mμ (log $\varepsilon$ 4.10), while (II) has the maximum at 284 mμ (log $\varepsilon$ 3.97), which proves that the C-ring had formed a tropilidene ring. If a double bond had been formed at 7-position in the B-ring, where the acetamido group is attached, the amide-CO absorption in the infrared spectrum should have shifted to a shorter wave-length side by its conjugation effect.

Jones discussed the infrared spectra of N-substituted amides[5] and stated that N-alkylamides showed absorption at 5.95~5.97 μ and N-arylamides at 5.88 μ, the absorption of CO shifting to a shorther wave-length region by the conjugation effect of a double bond. Since the absorption for amide-CO appears at 6.12 μ in (II) and at 6.10 μ in (I), there is no great difference and it may be concluded that there is no double bond at C-7 in the B-ring.

Further, the absorptions for CH at 3.34 and 3.43 μ in the infrared spectrum (in chloroform solution) of (II) differ from those of (I) but identical with those of the compounds having a tropilidene ring, such as colchicine, colchiceine, and colchiceinamide, which also suggest that (II) is a tropilidene type. All these evidences show that (II) is *l*-1,2,3-trimethoxy-7-acetamido-5,6,7,9(or 5,6,7,7a)-tetrahydrobenzo[*a*]heptalene.

Some crystals of m.p. 204° were obtained, besides (II), from collidine treatment of the reaction product of (I) with N-bromosuccinimide. Analytical values of this substance corresponded to the molecular formula of $C_{21}H_{26}O_4NBr$ and it was considered to be a diene monobromide but its further treatment with collidine failed to cause dehydrobromination. Its ultraviolet spectrum showed maximum absorption at 270 mμ (log $\varepsilon$ 3.50) and the double bond was assumed to be conjugated.

In order to change the tropilidene ring in (II) to a tropone ring, oxidation of (II) with selenium dioxide or chromium trioxide and pyridine was attempted[6] but failed.

3) H. L. Dryden, Jr., B. E. Burgert: *Ibid.*, **77**, 5633 (1955).
4) H. Rapoport, A. R. Williams, J. E. Campion, D. E. Pack: *Ibid.*, **77**, 2392 (1955).
5) "Chemical Applications of Spectroscopy," Ed. W. West, 526. A. Weissberger: "Technique of Organic Chemistry," Vol. IX, 525 (1956). Interscience Publishers Inc., New York.
6) G. Sunagawa, N. Soma, H. Nakao: Paper presented at the Kwanto Local Meeting of the Pharmaceutical Society of Japan, 1959; T. Mukai, T. Tezuka, K. Osaka: Paper presented at the 12 th Annual Meeting of the Chemical Society of Japan, Kyoto, 1959.

Nozoe and others[7] obtained tropone and tropilidene by application of alkali to the tropylium salt and acid treatment of ditropyl ether so formed to effect disproportionation. Dreiding and others[8] also obtained tropone by heating ditropyl ether over acid-treated silica gel. Therefore, the tropylium salt was prepared by the method of Kursanov and others[9] for the preparation of tropylium cation. This tropylium salt was derived to an ether by treatment with alkali and further treated with conc. hydrochloric acid to form the levorotatory tropone compound (III). (III) is soluble in dilute hydrochloric acid and is a weakly basic substance that can be extracted from hydrochloric acid solution with dichloroethane. It forms a reddish orange precipitate with 2,4-dinitrophenylhydrazine, showing that it is a ketone compound. Ultraviolet spectrum of (III) shows absorption maximum at 343 mμ, while that of tropone has the maximum at 310 mμ and those of 2-phenyltropone, 3-phenyltropone, 4-phenyltropone, and colchicide respectively at 230, 320; 222, 275; 233, 325; and 328 mμ. It is clear, therefore, that the C-ring in (III) has formed a tropone ring.

In the case of a substituted tropilidene, position of the ketone group is somewhat doubtful. Nozoe and others[7] stated that 3- and 4-phenyltropones are obtained from phenyltropilidene. Considering the structure of (III), its troponation is likely to occur at 9-, 10-, and 11-positions, and 9-, 10-, and 11-tropone compounds should have been formed. Consequently, (III) may be a mixture of two or three of these compounds. As will be shown later, 1-colchiceinamide was obtained from (III) and this fact shows that this mixture contains the 9-tropone compound, i.e. colchicide. This levorotatory (III) came as a powder and could not be induced to crystallize.

(III) was then aminated by the hydrazine method of Nozoe and others[10] and basic portion of its product yielded a crystalline levorotatory compound (IV), which was identified with 1-colchiceinamide from infrared and ultraviolet spectra and by admixture. A yellow crystalline powder, m.p. ca. 160°, was obtained from the mother liquor left after removal of (IV) and its ultraviolet absorption spectrum was similar to those of colchiceinamide and isocolchiceinamide but its structure is still unknown.

Since the cycloheptene C-ring in (I) was successfully derived to tropilidene, tropone, and aminotropone ring, attempt was made to derive the C-ring of racemic (I) into a tropolone ring. The racemic (I) was bolied with 2 moles of N-bromosuccinimide for 5 minutes and dehydrobrominated with collidine to racemic (II), m.p. 140°. The infrared absorption bands at 3.34 and 3.43 μ in this racemic compound (II) differ from those of racemic (I), but are identical with those of the tropilidene ring in colchicines.

This racemic (II) was treated with phosphorus pentachloride, decomposed with alkali to change the tropylium salt to an ether, and treated with hydrochloric acid to obtain the racemic tropone compound (III), which remained a brown powder and did not crystallize. Amination of this racemic (III) with hydrazine gave a sparingly soluble, bright yellow crystals, m.p. 196°, which retained one mole of water of crystallization even after drying in high vacuum at 130° for a long period of time. The infrared absorption spectrum of this substance in chloroform solution agreed with that of colchiceinamide in the region above 3 μ, although the transmittance at around 2.9 μ differed. Since its ultraviolet absorption spectrum agreed well with that of colchiceinamide, it may be considered that the yellow crystals of m.p. 196° are that of *dl*-colchiceinamide with one mole of water of crystallization.

7) T. Ikemi, T. Nozoe, H. Sugiyama : Chem. & Ind.(London), **1960**, 932.
8) A. S. Dreiding, *et al.* : Helv. Chim. Acta, **43**, 457 (1960).
9) D. N. Kursanov, M. E. Vol'pin : Doklady Akad. Nauk. S. S. S. R., **113**, 339 (1957).
10) T. Nozoe, T. Mukai, T. Minegishi, T. Fujisawa : Sci. Repts. Tohoku Univ., Ser. A, **37**, 388 (1953); T. Nozoe, T. Mukai, K. Takase : *Ibid.*, **39**, 164 (1956).

Hydrolysis of racemic (IV) with alkali[11] gave crystalline (V) of m.p. 167°, which was identical with *dl*-colchiceine[12] by admixture and from infrared and ultraviolet spectral data. Saponification of (V) produced bright yellow microcrystals (VI), m.p. 246° (decomp.), which showed no depression on admixture with *dl*-deacetylcolchiceine.[11] Optical resolution of (VI) to *l*-colchiceine had already been made by Corrodi and others,[12] and, therefore, total synthesis of colchicine has finally been accomplished by the method different from those of Eschenmoser and others[13] or of van Tamelen and others.[14]

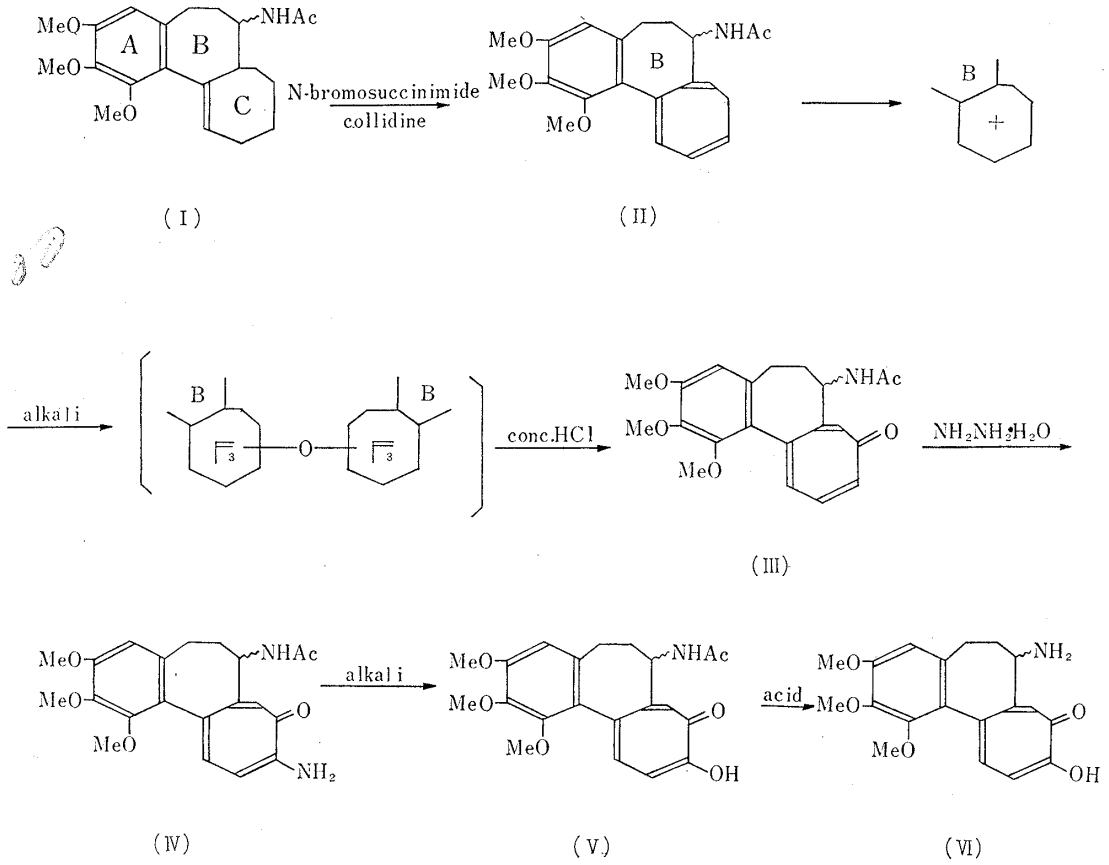


Chart 1.

## Experimental*2

***dl*-7-Acetamido-1,2,3-trimethoxy-5,6,7,9(or 5,6,7,7a)-tetrahydrobenzo[*a*]heptalene (II)**——A mixture of 1.44 g. of *dl*-1,2,3-trimethoxy-7-acetamido-5,6,7,7a,8,9,10,11-octahydrobenzo[*a*]heptalene*3 (I) and 1.59 g. of N-bromosuccinimide in 200 cc. of $CCl_4$ was boiled for 5 min., cooled, and filtered. Evaporation of $CCl_4$ from the filtrate at a temperature below 40° left 2.66 g. of yellow powder. This powder was boiled with 20 cc. of collidine in $N_2$ atmosphere, at 160~180°, for 2 hr., cooled, and diluted with benzene. This solution was washed with dil. HCl and $H_2O$, the solvent was evaporated at below 40°, and 1.37 g. of powder was obtained. The powder was adsorbed on 70 g. of alumina, which was washed with 5 L. of benzene and eluted with 800 cc. of a mixture of 0.5% EtOH and

*2 All m.p.s are uncorrected.
*3 Compound (XX) in Part II.[1]

11) R. M. Horowitz, G. E. Ullyot: J. Am. Chem. Soc., **74**, 587 (1952); Fr. Santavy: Chem. Listy, **46**, 280 (1952).
12) H. Corrodi, E. Hardegger: Helv. Chim. Acta, **40**, 193 (1957).
13) J. Schreiber, W. Leingruber, M. Persaro, P. Schudel, A. Eschenmoser: Angew. Chem., **71**, 637 (1959).
14) E. E. van Tamelen, J. A. Spencer, Jr., D. S. Allen, Jr., R. L. Orris: J. Am. Chem. Soc., **81**, 6341 (1959).

benzene, and 1.05 g. of orange powder was obtained. This product still contained bromine and was therefore further dehydrobrominated by boiling 1.05 g. of it with 4 g. of Zn dust and 4 g. AcOK in 20 cc. of EtOH for 2 hr. The mixture was cooled, diluted with benzene, and filtered. The filtrate was washed with water and the solvent was evaporated to leave 850 mg. of a powder. The powder was adsorbed on 17 g. of alumina, which was washed with 1.19 L. of benzene and eluted with 170 cc. of a mixture of 0.5% EtOH and benzene. The residue from the eluate was recrystallized from $Et_2O$ to 580 mg. of white prisms, m.p. 140°. *Anal.* Calcd. for $C_{21}H_{25}O_4N$: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.86; H, 7.22; N, 3.66. IR $\lambda_{max}^{CH}{}^{3}$ μ: 2.85, 2.96 (NH), 5.99 (NH-CO). UV: $\lambda_{max}^{EtOH}$ 284 mμ (log ε 3.86).

In a similar manner, 500 mg. of levorotatory compound (I) was treated and levorotatory (II), was obtained as colorless prisms (from AcOEt), m.p. 207°. Yield, 80 mg. *Anal.* Calcd. for $C_{21}H_{25}O_4N$: C, 70.96; H, 7.09; N, 3.94. Found: C, 71.03; H, 6.91; N, 4.23. IR $\lambda_{max}^{Nujol}$ μ: 3.02 (NH), 6.12 (NH-CO). UV: $\lambda_{max}^{EtOH}$ 284 mμ (log ε 3.97). $[\alpha]_D^{27}$ −198°(c=0.80, EtOH).

During the purification of levorotatory (II) through alumina chromatography, 229 mg. of an oily substance was obtained from the initial effluent of benzene washing. This oily substance was recrystallized from $Et_2O$ to 113 mg. of white needles, m.p. 184°. This was chromatographed through 8 g. of alumina and second fraction of 80-cc. eluate afforded 40 mg. of white needles, m.p. 204°, as recrystallized from $Et_2O$. *Anal.* Calcd. for $C_{21}H_{16}O_4NBr$: C, 57.67; H, 5.97; N, 3.21, Br, 18.27. Found: C, 57.89; H, 6.00; N, 2.84; Br, 17.92. UV: $\lambda_{max}^{EtOH}$ 270 mμ (log ε 3.50).

***dl*-7-Acetamido-1,2,3-trimethoxy-6,7-dihydrobenzo[*a*]heptalen-9(5*H*)-one (III)**——A solution of 2 g. of racemic (II) dissolved in 300 cc. of $CCl_4$ was added dropwise into a solution of 10 g. of $PCl_5$ dissolved in 100 cc. of $CCl_4$ with stirring by which gel-like, reddish orange precipitate formed. After standing the mixture for 24 hr. at room temperature, the mixture was poured into 100 cc. of ice water containing 12 g. of NaOH, the solution was adjusted to pH 6.0 with $NaHCO_3$ solution, and the aqueous phase was extracted with $CH_2Cl_2$. The organic solve ntsolutions were combined, washed with water, and the solvent was evaporated, leaving 2 g. of a dark brown oil. This oily residue was dissolved in 200 cc. of conc. HCl, allowed to stand at room temperature for 3 days, and diluted with 10 volumes of $H_2O$. The solution was filtered, the dil. HCl solution was washed with benzene, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with dil. HCl solution, the solvent was evaporated, and 140 mg. of reddish brown powder was obtained. UV: $\lambda_{max}^{EtOH}$ 316~330 mμ (log ε 3.69).

In a similar manner, the levorotatory (II), m.p. 207°, afforded levorotatory (III), UV: $\lambda_{max}^{EtOH}$ 343 mμ (log ε 3.76).

***dl*-7-Acetamido-10-amido-1,2,3-trimethoxy-6,7,-dihydrobenzo[*a*]heptalen-9(5*H*)-one (IV)**——A solution of 140 mg. of racemic (III) dissolved in 5 cc. of dehyd. EtOH and added with 0.2 cc. of $NH_2NH_2 \cdot H_2O$ was boiled for 7.5 hr., EtOH was evaporated, and the residue was dissolved in $CHCl_3$. $CHCl_3$ solution was extracted with dil. HCl solution which was washed with $CHCl_3$ and basified with NaOH. The alkaline solution was extracted with $CHCl_3$, the extract was washed with $H_2O$, and the solvent was evaporated to leave 15 mg. of a yellow powder. Its recrystallization from hydrous MeOH gave 10 mg. of bright yellow needles, m.p. 196°. This product contained 1 mole of water of crystallization which could not be liberated even by drying at 130° for 10 hr. in high vacuum. *Anal.* Calcd. for $C_{21}H_{24}O_5N_2 \cdot H_2O$: C, 62.67; H, 6.51; N, 6.96. Found: C, 62.94; H, 6.70; N, 6.81. IR $\lambda_{max}^{CHCl_3}$ μ: 2.77 ($H_2O$), 2.85, 2.93, 3.03 (NH, $NH_2$), 6.00, 6.26, 6.45 (CONH, $NH_2$, CO). UV $\lambda_{max}^{EtOH}$ mμ (log ε): 245 (4.96), 354 (4.54), 370 (4.46), 400 (4.20).

The same treatment of 1.5 g. of levorotatory (III) gave 15 mg. of levorotatory (IV) as yellow prisms (from AcOEt + petr. ether) m.p. 254°, undepressed on admixture with colchiceinamide,[1] m.p. 254°. *Anal.* Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.66; H, 6.22; N, 7.38. IR $\lambda_{max}^{Nujol}$ μ: 2.83, 2.99, 3.11, 6.01, 6.24, 6.44, 6.65, 6.75, 7.08, 7.15, 7.42, 7.58, 7.71, 7.82, 8.06, 8.36, 8.75, 9.08, 9.52, 9.92, 10.15, 10.37, 10.82, 11.12, 11.55, 11.65, 11.85, 12.03, 12.54, 12.78, 13.08, 13.30, 13.47, 13.70. UV $\lambda_{max}^{EtOH}$ mμ (log ε): 246 (4.51), 354 (4.32), 370 (4.27), 400 (4.07). $[\alpha]_D^{27}$ −140°(c=1.01, $CHCl_3$).

***dl*-7-Acetamido-10-hydroxy-1,2,3-trimethoxy-6,7-dihydrobenzo[*a*]heptalen-9(5*H*)-one(*dl*-Colchiceine)(V)**——A mixture of 40 mg. of racemic (IV) and 2% NaOH was heated on a steam bath for 1 hr. and the clear solution was cooled. This was washed with benzene, filtered, and the filtrate was acidified. The yellow powder that separated was collected by filtration, washed with water, and recrystallized from hydr. MeOH to 30 mg. of prisms, m.p. 167°, undepressed on admixture with *dl*-colchiceine, m.p. 167°, obtained by the method of Corrodi and others.[11] *Anal.* Calcd. for $C_{21}H_{23}O_6N \cdot H_2O$: C, 62.53; H, 6.25; N, 3.47. Found: C, 62.31; H, 6.09; N, 3.54. IR $\lambda_{max}^{CHC}{}^{3}$ μ: 2.86, 3.04, 3.15, 3.34, 3.42, 3.54, 5.95, 6.20, 6.44, 6.71, 6.87, 6.98, 7.10, 7.39, 7.55, 7.84, 7.94, 8.10, 8.35, 8.51, 8.76, 9.11, 9.53, 9.96, 10.17, 10.50, 10.78, 10.94, 11.06, 11.64, 11.83. UV $\lambda_{max}^{EtOH}$ mμ (log ε): 243 (4.55), 349(4.28), 405 (3.32).

***dl*-7-Amino-10-hydroxy-1,2,3-trimethoxy-6,7-dihydrobenzo[*a*] heptalen-9(5*H*)-one (*dl*-Deacetylcolchiceine)(VI)**——A solution of 100 mg. of (V) dissolved in 2 cc. of EtOH and added with 2 cc. of conc. HCl was boiled for 24 hr. The solvent was evaporated, and the residue was mixed with water.

This was filtered, the filtrate was washed with $Et_2O$, and adjusted to pH 7.0 with $K_2CO_3$. This was extracted with $CHCl_3$, which was washed with $H_2O$ and the solvent was evaporated. The yellow powder residue was recrystallized from a mixture of 0.4 cc. of $CHCl_3$ and 0.6 cc. of EtOH to yellow microcrystals, m.p. 234°(decomp.). Repeated recrystallization from the same mixture afforded 70 mg. of bright yellow microcrystals, m.p. 245~246°(decomp.). Undepressed on admixture with *dl*-deacetylcolchiceine, m.p. 246°(decomp.), obtained by the method of Corrodi and others.[11]

The author expresses his deep gratitude to Prof. K. Tsuda of the University of Tokyo, Mr. M. Matsui, the Director of this laboratory, and Dr. G. Sunagawa, the Assistant Director, for their unfailing guidance and encouragement throughout the course of the present work. The author is indebted to Prof. T. Nozoe of the Tohoku University for valuable advices, to Dr. S. Ikuma of this laboratory for technical assistance, and to the members of this laboratory who undertook elemental analysis and measurement of infrared and ultraviolet spectra.

## Summary

*dl*-Demethoxydeoxy-hexahydrocolchicine (I) was derived to *dl*-colchiceine (V) by changing the C-ring in (I) to a tropilidene ring (II) and to the tropone compound (III), its amination to form *dl*-colchiceinamide (IV), and its saponification. Thus, the total synthesis of racemic colchiceine (V) from 1-O-methylpyrogallol was successfully concluded. Partial synthesis of colchiceinamide (IV) from the levorotatory (I) was also carried out.

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## 47. Masaru Takeshita, Hiroko Takahashi, and Tomoharu Okuda*1 : Studies on Streptomyces Antibiotic, Cycloheximide. XIII.[1] New Spectrophotometric Determination of Cycloheximide.

(*Tokyo Research Laboratory, Tanabe Seiyaku Co., Ltd.**2)

Many reports have been published on the spectrophotometric determination of antibiotics. Among these, the one reported by Forist and Theal[2] is the only paper which is concerned with the colorimetric determination of antibiotic cycloheximide (actidione,[3] naramycin-A[4]). This procedure, indeed, has much advantages on accuracy, precision and reduction of working hours as compared with the microbiological assay usually adopted, but the inconvenience due to the instability of the coloration is inevitable.

Recently it was found that several resorcinols give a specific coloration when heated with cycloheximide in the presence of hydrochloric acid. This finding made it possible to determine cycloheximide spectrophotometrically by a simpler procedure.

All of resorcinols tested (resorcinol, orcinol, phloroglucinol and naphthoresorcinol) gave a yellow coloration when heated with cycloheximide in hydrochloric acid, absorption

*1 Inquiries should be to.
*2 Toda-machi, Kita-adachi-gun, Saitama-ken (竹下 勝, 高橋裕子, 奥田朝晴).

1) Part XII. T. Okuda, Y. Takanashi, K. Ashino, M. Tsuruoka : This Bulletin, **9**, 515 (1961).
2) A. A. Forist, S. Theal : Anal. Chem., **31**, 1042 (1959).
3) E. C. Kornfeld, R. G. Jones, T. V. Parke : J. Am. Chem. Soc., **71**, 150 (1949).
4) T. Okuda, K. Ashino, Y. Egawa, M. Suzuki : This Bulletin, **6**, 711 (1958).