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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UDC 615.779.931-011

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.

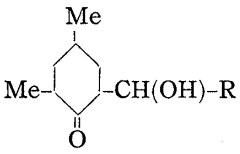
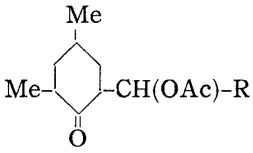
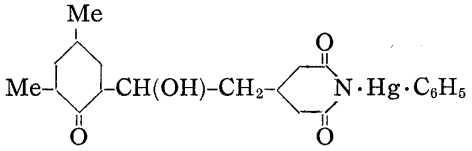
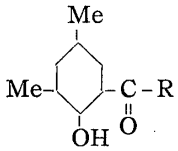
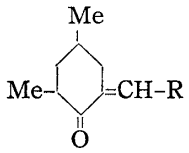
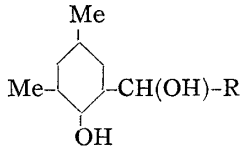
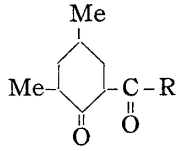
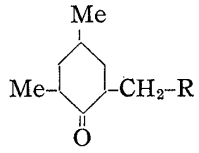
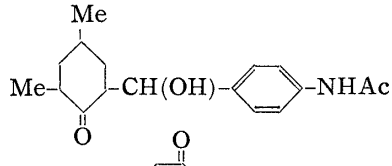
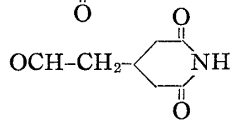
Recently it was found that several resorcinols give a specific coloration when heated with cycloheximide in the presence of hydrochloric acid. This finding made it possible to determine cycloheximide spectrophotometrically by a simpler procedure.

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).

TABLE I. Susceptibility of Cycloheximide and its Related Compounds to the Colorimetry

Substance	Plane Structure[a]	Relative Value (%)[b] Present Method	Forist's Method
Cycloheximide		100	100
Naramycin-B		98.5	99.0
Isocycloheximide		94.6	—
Cycloheximide Acetate		94.5	—
Isocycloheximide Acetate		96.0	—
N-Phenylmercuricyclo-heximide[1]		97.7	104.0
ψ-Cycloheximide[10]		7.5	—
Anhydrocycloheximide		93.8	101.0
Dihydrocycloheximide[3]		0.7	109.0
Dehydrocycloheximide[3]		2.4	—
Deoxycycloheximide[11]		2.1	—
Acetamido-ketol-N[7]		87.7[c]	—
Glutarimide-β-acetaldehyde[12]		—	133.5

a) R=-CH_2-CH<$^{CH_2-CO}_{CH_2-CO}$>NH

b) Absorbancies in the present method were measured at 400 mμ after heating the mixed solution of 1 ml. o fsample solution, 2 ml. of 10% resorcinol and 2 ml. of conc. HCl for 30 min. Relative values illustratedin the Table were calculated from the following equation :

$$\text{Relative value } (\%) = \frac{\text{Molar extinction coefficient shown by test sample}}{\text{Molar extinction coefficient shown by cycloheximide}} \times 100$$

c) This compound showed its λ_{max} at 408 mμ in colored solution.

spectra of the colored solution being illustrated in Fig. 1.*3 Considering the intensity of absorption, solubility of the colored product in the solution and the cost of the reagens, resorcinol was chosen in the foregoing experiment.

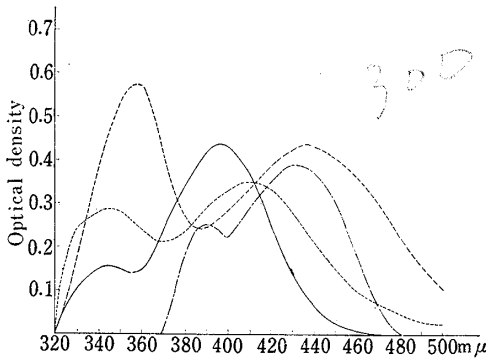


Fig. 1. Absorption Spectra shown by Cycloheximide when heated with Various Resorcinols in Hydrochloric Acid

——— Resorcinol
..... Orcinol
---------- Phloroglucinol
—·—·— Naphthoresorcinol

One ml. of cycloheximide solution (100 mcg./ml.), 2 ml. of 0.2*M*. resorcinols solution and 2 ml. of conc. HCl were mixed, heated for 30 min. in boiling water bath, cooled, readjusted to 5 ml. and measured in 1 cm. cell vs. a reagent blank similarly prepared. Distilled water was used as a solvent, but in case of naphthoresorcinol EtOH was used.

As shown in Table I, this colorimetric determination is applicable not only for cycloheximide but also for its stereoisomeres (naramycin-B[5] isocycloheximide[6]) and related synthetic compounds.[7] Among the derivatives of cycloheximide, its acylate, N-phenylmercuricycloheximide[1] salt[1] and anhydrocycloheximide[8] were sensitive to this colorimetry. From these observations it was concluded that the coloration is principally ascribed to the α,β-unsaturated ketone moiety existing in anhydrocycloheximide (more probably anhydrocycloheximide acid[8]) or the like. Thus, this color reaction is typical for the compounds which give rise to an α,β-unsaturated ketone moiety when heated in hydrochloric acid.

After the pre-examination of the effects of the concentration of reagents and of heating time upon absorbancy, as illustrated in Fig. 2, 3, and 4 it was found that adequate concentration of reagents in final solution was over 0.2*M* for resorcinol and over 5*N* for hydrochloric acid and the heating for over 20 minutes was necessary. It wasalso found that the color developed on heating was quite stable at least for 8 hours at room temperature as shown in Table II.

Table II. Stability of Coloration at Room Temperature (20°)

Lapse	Relative Absorbancy[a] (%)	Lapse	Relative Absorbancy[a] (%)
0 time	100	2 hr.	100
10 min.	100	4 hr.	100
40 min.	100	8 hr.	99.1
1 hr.	100	48 hr.[b]	98.8

a) Relative absorbancy $(\%)=\dfrac{\text{Absorbancy when re-measured}}{\text{Initial absorbancy}}\times 100$

b) Kept in refrigerator.

*3 Several phenols including these reagents were well known to be used for the detection and colorimetric determination of sugars,[9] but phenols like α-naphthol, thymol, pyrogallol and pyrocatechol gave no coloration with cycloheximide at all.

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7) M. Suzuki : This Bulletin, 8, 706 (1960).
8) K. V. Rao : J. Am. Chem. Soc., **82**, 1129 (1960).
9) cf. T. Momose, Y. Ohkura : This Bulletin, **4**, 209 (1956).
10) M. Suzuki : This Bulletin, 8, 778 (1960).
11) T. Okuda : *Ibid.*, **7**, 666 (1959).
12) D. D. Phillips, M. A. Acitelli, J. Meinwald : J. Am. Chem. Soc., **79**, 3571 (1957).

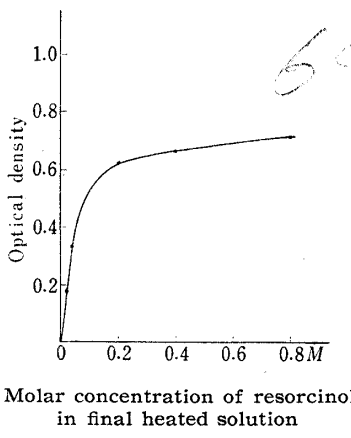

Fig. 2. Effect of Concentration of Resorcinol Solution upon Absorbancy

One ml. of cycloheximide solution (100 mcg./ml.), 2 ml. of various concentrations of resorcinol solution and 2 ml. of conc. HCl were mixed, heated for 20 min., cooled, readjusted to 5 ml., and measured at 400 mμ vs. a reagent blank similarly prepared.

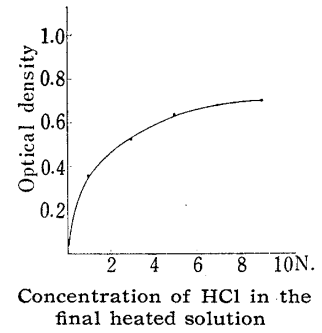

Fig. 3. Effect of Concentration of Hydrochloric Acid upon Absorbancy

One ml. of cycloheximide solution (100 mcg./ml.), 2 ml. of 10% resorcinol solution and 2 ml. of various concentrations of HCl were mixed and treated as described in Fig. 2.

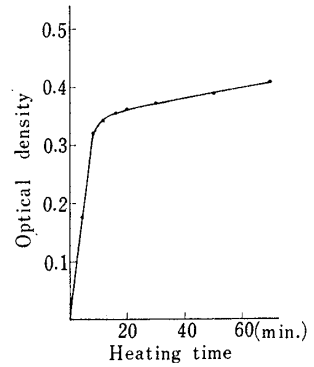

Fig. 4. Effect of Heating time upon Absorbancy

One ml. of cycloheximide solution (100 mcg./ml.), 2 ml. of 10% resorcinol solution and 2 ml. of conc. HCl were mixed and treated as described in Fig. 2, when the heating time was varied.

From the foregoing fundamental experiments, the following procedure was recommendable for the practice.

Reagents——10% Resorcinol aqueous solution : 10 g. of resorcinol, reagent grade, in 100 ml. of distilled water.

Concentrated hydrochloric acid (ca. 35%).

Cycloheximide standard solution : 20 to 100 mcg./ml. aqueous solution.

Procedure——A sample of 2 to 5 mg. of the material to be analyzed is accurately weighed into a 25-ml. volumetric flask, dissolved in distilled water (or in small amounts of alcohol) and diluted to 25 ml. with distilled water.

A 1-ml. aliquot of the sample solution is transferred to a graduated test tube, added with 2 ml. of 10% resorcinol solution and 2 ml. of hydrochloric acid, and immersed in a boiling water bath for 30 minutes, when a yellow color appears. The volume of the cooled solution is readjusted to 5 ml. with distilled water and the absorbancy of the resulting solution is measured at 400 mμ in a 1-cm. cell vs. a reagent blank similarly prepared.

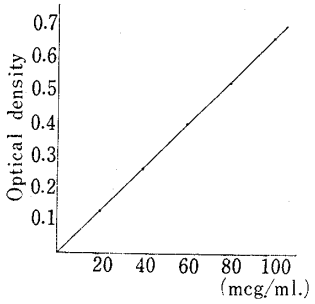

Fig. 5. Standard Curve for Spectrophotometric Determination of Cycloheximide

One ml. of various concentration of Cycloheximide solution, 2 ml. of 10% resorcinol and 2 ml. of conc.HCl were mixed, heated for 30 min. in boiling water bath, cooled, readjusted to 5 ml., and measured at 400 mμ vs. a reagent blank similarly prepared.

The percent cycloheximide in the sample is obtained by calculating from the standard curve (Fig. 5) similarly prepared using 1 ml. of 20 to 100 mcg./ml. cycloheximide standard solution, standard curve being expressed by the following equation.

$$\text{Optical density} = -0.004 + 0.00665 \times \text{concentration (mcg./ml.)}$$

(Standard error of estimate : 0.004)

It must be added that this colorimetric procedure is interferred in the cases when the cycloheximide preparation contains the contaminants like carbohydrates which are sensitive to this color reagent.

The authors express their gratitude to Dr. K. Abe, the Superintendant of this Laboratory, for his grant of publication. The authors' thanks are also indebted to Dr. A. Kiyomoto, Dr. M. Suzuki and Mr. Y. Egawa, for their instructive suggestions.

Summary

A new and simple procedure is described for the spectrophotometric determination of cycloheximide and its related compounds, using resorcinol and hydrochloric acid as colorimetric reagents.

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48. Mitsuji Sano : Studies on Nucleosides and Nucleotides. III.*1 Synthesis of Glycosyl-2-thiouracils from Glycosylthioureas.

(*Research Laboratory, Daiichi Seiyaku Co., Ltd.**2)

A new method for synthesis of glycosyl-2-thiothymine from glycosylthiourea was established and reported in Part II of this series.*1 Later, this method was extended to the synthesis of glycosyl-2-thiouracil, and 1-(β-D-glucopyranosyl)-2-thiouracil*3 and 1-(β-D-ribofuranosyl)-2-thiouracil (2-thiouridine) were successfully synthesized.

Before carrying out the present synthesis, preliminary experiment was carried out for preparation of 1-methyl-2-thiouracil from N-methylthiourea and, 3-ethoxyacryloyl

*1 Part II : This Bulletin, **9**, 709 (1961).
*2 Hirakawa-bashi, Sumida-ku, Tokyo (佐野光司).
*3 Nomenclature of uracils used in the present paper followed that used in the Chemical Abstracts.