Summary

2,3-Ethylenedioxy-N-methylmorphinan and 3,4-ethylenedioxy-N-methylmorphinan, synthesized earlier, were submitted to the Hofmann degradations and by syntheses of their degradation products their structures were confirmed as 2,3- and 3,4-ethylenedioxyphenanthrene.

(Received September 5, 1959)

UDC 615.779.931-011

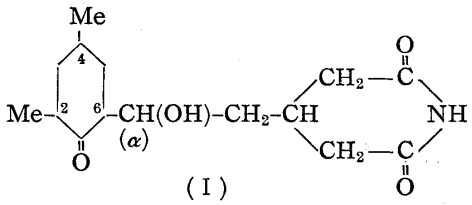
61. Tomoharu Okuda, Makoto Suzuki, and Yoshiyuki Egawa : Studies on Streptomyces Antibiotic, Cycloheximide. VII. On the Configuration of Naramycin-B and Isocycloheximide.

(*Tokyo Research Laboratory, Tanabe Seiyaku Co., Ltd.**1)

In the previous papers the authors reported the isolation, physicochemical characteristics, and absolute configuration of antifungal antibiotic Naramycin-B which is isomeric to cycloheximide and produced as a by-product of cycloheximide in the fermentation broth of *Streptomyces naraensis* nov. sp.[1~3] As referred in Part II of this series, several isomers of cycloheximide are known, among which isocycloheximide was reported by Hamilton, *et al.*[4] and Lemin, *et al.*[5] on its agricultural use and thought to be derived as described in the British and U.S. Patents[6] from cycloheximide by aging the latter in the solution or by isomerization in the presence of acid-deactivated alumina.

Recently, the authors had an opportunity to compare Naramycin-B directly with isocycloheximide in compliance with the request made by Dr. Alan J. Lemin, The Upjohn Co., U.S.A., who kindly offered the samples of isocycloheximide and its acetate.*2

In the present paper will be described the differences observed in comparing the two antibiotics and their absolute configurations deduced therefrom. Plane structure of these antibiotics is illustrated as (I).



(I)

Comparison of the two antibiotics was made mainly by physicochemical methods, viz. 1) determination of mixed melting point, 2) infrared spectra, 3) ultraviolet spectra,

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*1 Toda-machi, Kita-adachi-gun, Saitama-ken (奥田朝晴, 鈴木真言, 頴川吉之).

*2 Melting point and optical rotation of isocycloheximide and its acetate were given as m.p. 98~100°, $[\alpha]_D^{24}$ +26°(MeOH), and m.p. 162~165°, $[\alpha]_D^{24}$ +51°(MeOH), respectively.

1) T. Okuka, M. Suzuki, Y. Egawa, K. Ashino : This Bulletin, **7**, 27(1959).
2) T. Okuda : *Ibid.*, **7**, 137(1959). 3) *Idem* : *Ibid.*, **7**, 259(1959).
4) J.M. Hamilon, M. Szkolnik, E. Sondheimer : Science, **123**, 1175(1956).
5) A.J. Lemin, G.A. Boyack, W.C. Haskett, A. Steinhards, G. Swank : Abstract of Papers, 132nd Meeting of the American Chemical Society, 24A(1957).
6) Brit. Pat. 799,731 (Aug. 13, 1958); U.S. Pat. 2,903,457 and 2,903,458 (Sept. 8, 1959) (The Upjohn Company).

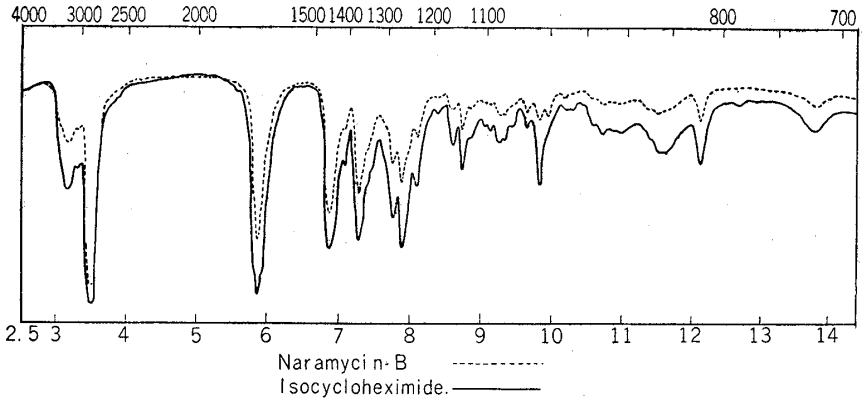
4) optical rotation measurement, and 5) measurement of optical rotatory dispersion curves.

As illustrated in Table I, the depression of melting point was not so evident among these antibiotics, whereas remarkable mixed melting point depression was observed when Naramycin-B acetate or isocycloheximide acetate was admixed with cycloheximide acetate. Infrared spectra of the two antibiotics were quite similar to each other except for some subtle absorption bands (Fig. 1), and infrared spectrum of isocycloheximide in Nujol and in chloroform showed that the substituent at an asymmetric carbon at 6-position of this antibiotic orients equatorially to cyclohexanone ring as does that of Naramycin-B. Ultraviolet spectra also showed considerable resemblance to each other (Fig. 2). Therefore, these antibiotics seem to be quite similar in nature but it seems still inappropriate

Table I. Determinations of Mixed Melting Point[a)]

Compound	m.p. (°C)	Mixed with	m.p. (°C)	Mixed m.p. (°C)
Naramycin-B	101 ~102	Isocycloheximide	98~99	96.5~ 97.5
Naramycin-B	99 ~100	Isocycloheximide	98~99	94 ~ 95.5
Naramycin-B acetate	155.5[b)]	Isocycloheximide acetate	161~162	154 ~156
Naramycin-B acetate	155.5	Isocycloheximide acetate[c)]	154~155	152 ~152.5
Naramycin-B acetate	150.5~152	Naramycin-A acetate	147~147.5	128.5~131.5
Isocycloheximide acetate	161 ~162	Naramycin-A acetate	147~147.5	130 ~132.5

a) Melting points were measured in H_2SO_4 bath and are uncorrected.
b) On further recrystallization of previously reported Naramycin-B acetate (m.p. 150.5~152°), melting point was raised to this degree.
c) This acetate was newly derived from isocycloheximide, further purification being impossible because of the lack of the material.



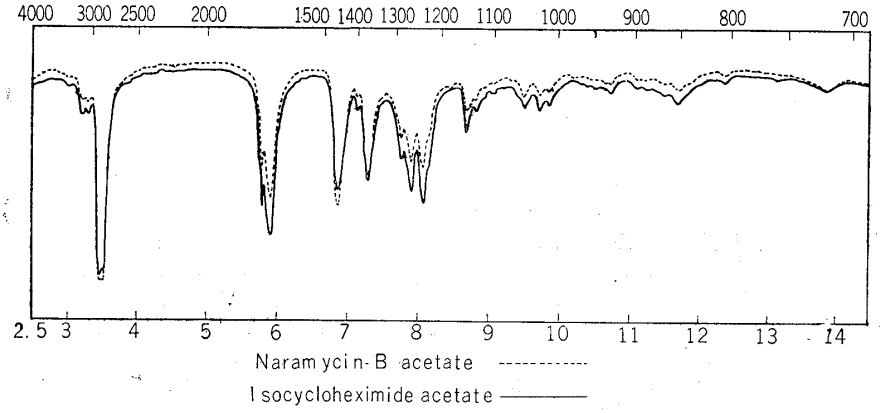


Fig. 1. Infrared Spectra of Naramycin-B, Isocycloheximide, and their Acetates (in Nujol mull)

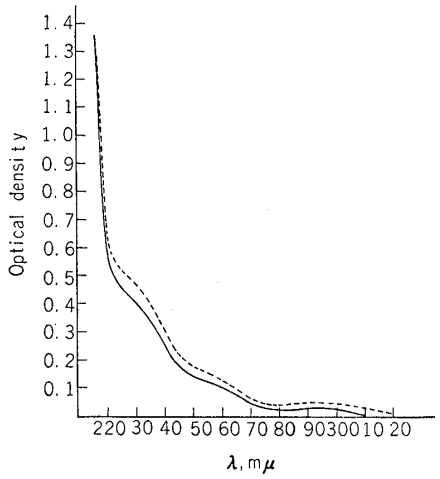


Fig. 2. Ultraviolet Spectra of Naramycin-B and Isocycloheximide

—— Naramycin-B 500 γ/cc. (MeOH), 23°
------ Isocycloheximide 500 γ/cc. (MeOH), 24°

to discuss the identity of Naramycin-B and isocycloheximide only from the above data.

Measurements of optical rotation and rotatory dispersion curves (R.D. curves) played an important rôle for clarifying the situation. As shown in Table II, both antibiotics showed dextrorotation and increased positive values on changing them into acetates. The tendency to increase dextrorotation was greater in isocycloheximide than in Naramycin-B. It is to be noted that Naramycin-B exhibited a considerably higher optical rotation value in methanol than in chloroform, whereas isocycloheximide showed no remarkable change of rotational value in methanol or in chloroform.

Table II. Optical Rotation of Naramycin-B, Isocycloheximide, and their Acetates

Compound	m.p. (°C)	$[\alpha]_D$ Value
Naramycin-B	109~110	+50.2° (c=2, MeOH; 12.5°)
Naramycin-B	101~102	+50.0° (c=1, MeOH; 24.5°) +30.4° (c=1, $CHCl_3$; 24.5°)
Isocycloheximide	98~99	+26.1° (c=2, MeOH; 24.0°) +23° (c=0.1, MeOH; 25.7°) +22° (c=0.1, $CHCl_3$; 22.8°)
Naramycin-B acetate	150.5~152	+62.2° (c=2, MeOH; 15°)
Isocycloheximide acetate	161~162	+51.0° (c=1, MeOH; 24°)

R.D. curves of both antibiotics*3 and their acetates were measured using zirconium or mercury lamp as a source of light (Figs. 3 and 4). Isocycloheximide exhibitited a positive Cotton-effect curve as does Naramycin-B,*4 but following differences were observed. Naramycin-B and its acetate showed the peak at 315 mμ, while isocycloheximide and its acetate did at 310 mμ. Moreover, the height at the peak shown by Naramycin-B and its acetate was about three times as high as that of isocycloheximide and its acetate. Optical rotation value of the isocycloheximide supplied to the authors was lower than that described in the patent[6] (maximum value cited, +36°), and a very pure sample of isocycloheximide may show higher peak in the R.D. curve, but never higher than that of isocycloheximide acetate which has higher melting point than Naramycin-B acetate and is thought to be sufficiently pure.

*3 In the measurement of R.D. curve of Naramycin-B, a sample showing similar melting point (m.p. 101~102°) as isocycloheximide was chosen for comparison. R.D. curve of purest Naramycin-B was already shown in a previous paper.[2]

*4 Isocycloheximide, measured in dioxane, showed the same shape of a positive Cotton-effect curve as in methanol.

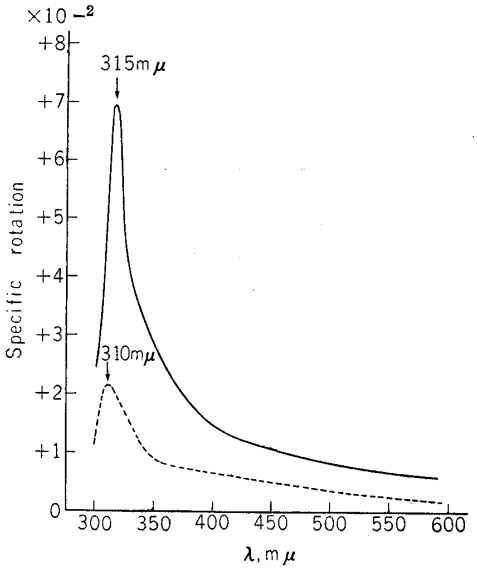


Fig. 3. R. D. Curves of Naramycin-B and Isocycloheximide (Zirconium lamp)
—— Naramycin-B
Temp. 22.8° (c=0.10, MeOH)
------ Isocycloheximide
Temp. 21.6° (c=0.10, MeOH)

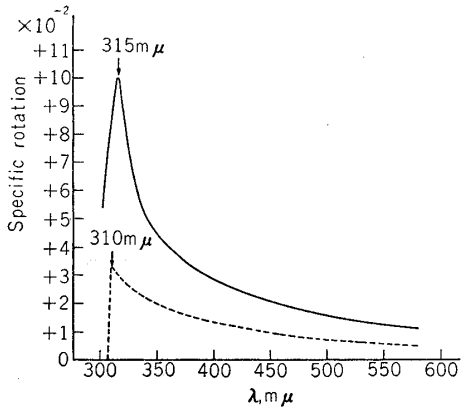
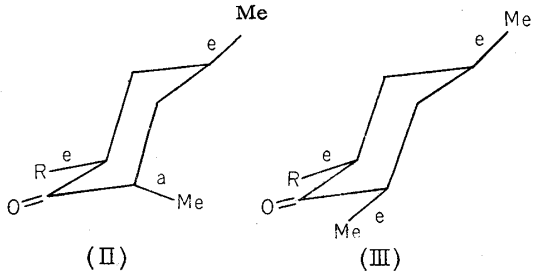
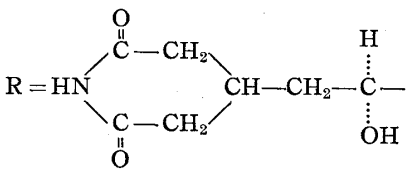


Fig. 4. R. D. Curves of Naramycin-B Acetate and Isocycloheximide Acetate (Mercury lamp)
—— Naramycin-B acetate
Temp. 25.3° (c=0.10, MeOH)
------ Isocycloheximide acetate
Temp. 24.5° (c=0.10, MeOH)

From these optical data and also from the foregoing physicochemical data, it is concluded that Naramycin-B is quite similar but not equal to isocycloheximide.

By adaptation of the rule of shift to the difference of optical rotation value observed in isocycloheximide, cycloheximide, and their acetates, it becomes possible to consider that isocycloheximide has the same configuration as cycloheximide at the asymmetricca r-bon in α-position.*5 This consideration and also the fact that isocycloheximide exhibits a positive Cotton-effect curve as does Naramycin-B and has an equatorial substituent at its 6-position suggest, according to the interpretations previously presented,[2] that isocycloheximide must have the same configuration as Naramycin-B in its 4-, 6-, and α-asymmetric carbons, the absolute configuration being (4S:6R:αS)-configuration. Therefore, it is deduced that the configurational difference between Naramycin-B and isocycloheximide is in the asymmetric carbons at 2-position. Thus, the two antibiotics may be illustrated as (II) or (III), respectively.





*5 If the isocycloheximide supplied to the authors was derived from cycloheximide according to the procedures described in the British Patent, it means that the correctness of adapting the rule of shift is chemically proved. On this problem, the correctness was justified by experiments with optically active β-hydroxy-ketones, details of which will be published by one of the authors (M. Suzuki) in succeeding papers. Naramycin-B seems to be derivable from Naramycin-A (cycloheximide) under certain conditions.

The remarkable differences in height observed in R.D. curves of Naramycin-B and isocycloheximide, and their acetates give definite ground for discussing their stereochemical features. It was previously stated[2] that, if the remaining stereochemical surroundings are the same, the configuration of methyl group adjacent to carbonyl function plays an important rôle in the amplitude variation of the R.D. curves and that the contribution to the sign of Cotton effect caused by alkyl group situated axially at a carbon atom adjacent to carbonyl function would be similar to that caused by the axial halogen group which makes definite contribution to the partial rotation, according to Djerassi's "axial haloketone rule."[7] These findings*[6] make it appropriate to conclude that Naramycin-B which shows higher peak than isocycloheximide in their R.D. curves must have an axially oriented 2-methyl group as depicted in (II) and isocycloheximide must have an equatorial one, corresponding to formula (III). Thus, Naramycin-B and isocycloheximide respectively belong to (2S:4S:6R:αS)- and (2R:4S:6R:αS)-series.

Among the two epimeric cyclic compounds having the α-methyl group to carbonyl function, whose R.D. curves were reported by Djerassi, *et al.*, some of the epimers display similar kind of Cotton-effect curve with the peak or trough at a wavelength different by 5~10 mμ from each other.*[7] Furthermore, it has been reported by Mazur and Sondheimer[8] that an epimer which has an axial α-methyl group causes remarkable change in optical rotation when measured in methanol and chloroform, whereas the equatorial one exhibits similar values in both solvents. It is interesting that these experimental findings are not contradictory to the above observation.

It must be added, on the stability order of these antibiotics, that Naramycin-B which has 2a,4e,6e-conformation would not be necessarily more labile than isocycloheximide which has 2e,4e,6e-conformation owing to the appearance of 2-alkyl-ketone effect,[9] referring to the findings reported by Cornubert and his co-workers[10] that *trans*-2,6-dialkylcyclohexanones are as stable as *cis*-isomers. Further discussions on this problem will be made later when the situations have been made clear.

Experimental

Rotatory Dispersion Curves of Naramycin-B, Isocycloheximide, and their Acetates—Naramycin-B (m.p. 101~102°): R.D. in MeOH (c=0.1) (zirconium lamp): $[\alpha]_{589}^{22.8}$ +56°, $[\alpha]_{400}$ +146°, $[\alpha]_{350}$ +281°, $[\alpha]_{320}$ +640°, $[\alpha]_{315}$ +690° (peak), $[\alpha]_{310}$ +458.4°.
Isocycloheximide (m.p. 98~99°): R.D. in MeOH (c=0.1) (zirconium lamp): $[\alpha]_{589}^{21.6}$ +19°, $[\alpha]_{400}$ +66°, $[\alpha]_{350}$ +87°, $[\alpha]_{315}$ +206°, $[\alpha]_{310}$ +216° (peak), $[\alpha]_{307.5}$ +192°, $[\alpha]_{300}$ +114°.
Naramycin-B acetate (m.p. 155.5°): R.D. in MeOH (c=0.1) (mercury lamp): $[\alpha]_{579}^{23.3}$ +107°, $[\alpha]_{366.4}$ +381°, $[\alpha]_{315}$ +994° (peak), $[\alpha]_{310}$ +896°.
Isocycloheximide acetate (m.p. 161~162°): R.D. in MeOH (c=0.1) (mercury lamp): $[\alpha]_{579}^{25.3}$ +54°, $[\alpha]_{366.4}$ +191°, $[\alpha]_{315}$ +300°, $[\alpha]_{310}$ +325° (peak), $[\alpha]_{308}$ +54°, $[\alpha]_{305}$ −3°.

The authors express their deep gratitude to Dr. S. Sugasawa, Professor Emeritus of the University of Tokyo, for his kind guidance and encouragement, and to Professor C. Djerassi of the Wayne State University, U.S.A., for the kind measurement of rotatory dispersion curves and for his instructive suggestions. The authors are also grateful to Professor Y. Hirata of the University

*6 These findings are more properly explained by the "octant rule" of Prof. C. Djerassi which is to be discussed in his coming papers. (Added in proof) The "octant rule" established by Prof. Djerassi was discussed in his recent publications (Record of Chemical Progress, **20**, 101 (1959); "Optical Rotatory Dispersion Applications to Organic Chemistry," 178 (1960), McGraw-Hill Book Co., New York).

*7 This phenomenon is notable in epimers of 4-methylcholestan-3-one, 17*a*-methyl-17-oxo-D-homosteroid, and 30-nortaraxastan-20-one (C. Djerassi, *et al.*: J. Am. Chem. Soc., **78**, 6362(1956) (Fig. 8); *ibid.*, **80**, 4001(1958) (Fig. 5).

7) C. Djerassi, W. Klyne: J. Am. Chem. Soc., **79**, 1506(1957); C. Djerassi, J. Osiecki, R. Riniker, B. Riniker: *Ibid.*, **80**, 1216(1958); C. Djerassi, I. Fornaguera, O. Mancera: *Ibid.*, **81**, 2383(1959).
8) Y. Mazur, F. Sondheimer: J. Am. Chem. Soc., **80**, 5220(1958).
9) W. Klyne: Experientia, **12**, 119(1956).
10) R. Cornubert, *et al.*: Bull. soc. chim. France, **1950**, 631, 636; **1954**, 367.

of Nagoya and to Drs. Y. Mashiko and A. Kato of the Government Chemical and Industrial Research Institute, Tokyo, for their generous favor in taking the rotatory dispersion curves. Authors' thanks are due to Dr. K. Abe, the Director of this Laboratory, for his encouragement, to Mr. K. Kotera for infrared analysis, and to Mrs. F. Hisamichi, and Messrs. T. Yoda and T. Kōno for elementary analysis.

Summary

Physicochemical comparisons were made on Naramycin-B and isocycloheximide, which are stereoisomers of cycloheximide, and it was confirmed that the two antibiotics are similar but not quite equal to each other, and that, mainly from the interpretation of their rotatory dispersion curves, the absolute configurations of Naramycin-B and isocycloheximide respectively belong to (2S:4S:6R:αS)- and (2R:4S:6R:αS)-series.

(Received September 9, 1959)

UDC 577.164.11

62. Shizuka Kasahara: Action of Cyanogen Bromide on Thiamine. III.[1] Synthesis of 2-Methyl-6-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-5,6-dihydropyrimido[4,5-*d*]pyrimidine (Neocyanothiamine).

(*Osaka Branch, National Hygienic Laboratory*[*1])

The first paper of this series[2] reported that the sodium salt (II) of thiol-type thiamine was converted into N-(1-methyl-2-thiocyanato-4-hydroxy-1-butenyl)-N-[(2-methyl-4-amino-5-pyrimidinyl)methyl]formamide (cyanothiamine) (IV) by the action of cyanogen bromide.

Zima, *et al.*[3] obtained the sodium salt of thiamine, the so-called yellow salt, by the action of three equivalents of sodium ethoxide on thiamine hydrochloride (I) and presumed this salt to possess the chemical structure formulated as (III). However, presence of a cyclized thiol-type thiamine (III) has not been confirmed, nor has the derivative of (III) been obtained as yet. If thiamine changes into the sodium salt (III) as reported by Zima, *et al.*, thiocyanato derivative of (III) may be formed by the action of cyanogen bromide on the same salt.

Addition of three equivalents of sodium ethoxide to the dehydrated ethanol suspension of thiamine hydrochloride (I) afforded a yellow solution, the action of one equivalent of cyanogen bromide on which gave white needles, m.p. 152.5° (decomp.) (substance A). The elemental analyses of substance A gave the formula $C_{13}H_{15}ON_5S$.

The ultraviolet absorption spectrum of substance A in chloroform was quite similar to that of 2,8a-dimethyl-6,7,5a,8a-tetrahydrofuro[2,3-*h*]thiochromine (VIII),[4] as shown in Table I.

The infrared absorption spectrum of substance A showed absorption peaks at 2169 (SCN), 3356 (ν_{OH}), and 1051 cm^{-1} ($\nu_{C\text{-}O}$) and resembled that of thiochrome (VIII) except for the peak at 2169 cm^{-1} (SCN). Accordingly, substance A does not have a primary amino group

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*1 6 Hōensaka-cho, Higashi-ku, Osaka (笠原 閑).

1) Part II : Yakugaku Zasshi, **79**, 963(1959).
2) Part I : *Ibid.*, **77**, 1133(1957).
3) O. Zima, R. R. Williams : Ber., **73**, 941(1940).
4) H. Hirano : Yakugaku Zasshi, **74**, 59(1954).