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118. **Tomoharu Okuda** : Studies on Streptomyces Antibiotic, Cycloheximide. IV.[1] Some Observations on Stereochemical Configurations of Naramycin-A (Cycloheximide) and Naramycin-B.

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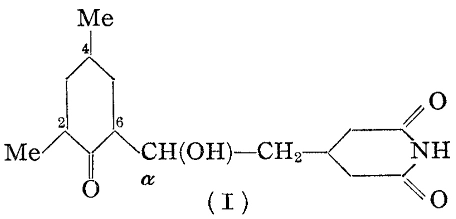
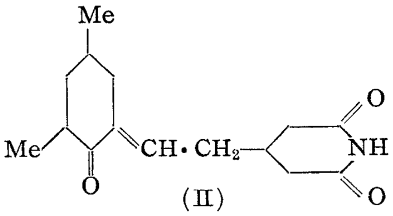
It was reported in the previous papers[2,3] that a Streptomyces (*Streptomyces naraensis nov. sp.*) produced two antiyeast antibiotics named Naramycin-A and -B, and that Naramycin-A was identical with cycloheximide (Actidione) discovered by Leach, *et al.*[4] and Naramycin-B was a stereoisomer of Naramycin-A.

In the present paper, some configurational observations concerning these antibiotics, whose plane structure is shown as (I), are described.



(I)



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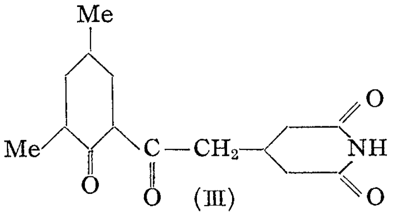

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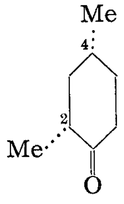

(IV)

I. Absolute Configuration of the Asymmetric Carbon at 4-Position

Naramycin-A and -B produce the same anhydro compound (anhydrocycloheximide) (II) by dehydration with phosphorus pentoxide, the same oxidized product (dehydrocycloheximide) (III) by oxidation with chromium trioxide, and the same *cis-d*-2,4-dimethylcyclohexanone (IV) by means of retro-aldolization with sodium hydroxide. These experimental data lead to the conclusion that the absolute configuration of C-4 (asymmetric carbon at 4-position) of Naramycin-A and -B is identical. Eisenbraun, *et al.*[5] reported that *cis-d*-2,4-dimethylcyclohexanone belongs to (2R:4R)-series after Cahn's new conven-

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1) Preliminary Communication : T. Okuda, M. Suzuki, Y. Egawa, K. Ashino : This Bulletin, **6**, 328(1958); T. Okuda : *Ibid.*, **7**, 137(1959).

2) Part I. T. Okuda, K. Ashino, Y. Egawa, M. Suzuki : *Ibid.* **6**, 711(1958).

3) Part II. T. Okuda, M. Suzuki, Y. Egawa : *Ibid.*, **7**, 27(1959).

4) F. E. Leach, J. H. Ford, A. F. Whiffen : J. Am. Chem. Soc., **69**, 474(1947).

5) E. J. Eisenbraun, J. Osiecki, C. Djerassi : *Ibid.*, **80**, 1261(1958).

tion.6) Consequently, it is certain that the absolute configuration of C–4 belongs to (S)–series in Naramycin–A and –B.

II. Absolute Configuration of the Asymmetric Carbon at *α*-Position

It would be difficult to determine the absolute configuration of C–4 chemically, but it is possible by adapting Freudenberg's rule of shift7) to optical rotation values of Naramycins and their O–acylates. As illustrated in Table I, the fact that there are fairly regular differences towards the same direction (positive) between $[\alpha]_D$ values of acylated Naramycin–A's and Naramycin–A as compared with acylated Naramycin–B's and Naramycin–B suggests that the configuration of C–α may be the same.

TABLE I. $[\alpha]_D$ Values of Naramycins and their Acylates

Compound	Series	$[\alpha]_D$ (in MeOH)	$[M]_D$
Naramycin	A$^{c)}$	−0.73 (c=10)$^{a)}$	−2.1
	B	+50.2 (c=2)$^{a)}$	+141.0
Acetate	A$^{d)}$	+24.56 (c=2)$^{a)}$	+80.0
	B	+62.15 (c=2)$^{a)}$	+201.0
Benzoate	A	+10.0 (c=1)$^{b)}$	+38.5
	B	+54.6 (c=1)$^{b)}$	+210.0
3,4-Dichlorobenzoate	A	+13.4 (c=1)$^{b)}$	+61.0
	B	+53.2 (c=1)$^{b)}$	+242.0

a) Measured at 12.5°.
b) Measured at 11°.
c) Reported : $[\alpha]^{25}_{D}$ −3.0(c=10, MeOH),8) +6.8°(c=2, H_2O),8) $[\alpha]^{29}_{D}$ −3.38°(c=9.47, EtOH).9)
d) Reported : $[\alpha]^{25}_{D}$ +22°(c=2.3, MeOH).8)

III. Conformation of the Substituent at 6-Position

Infrared absorption spectra of Naramycin–A and –B were measured in Nujol mulls and also in diluted carbon tetrachloride solution below 0.005 molar concentration where intermolecular hydrogen bonding would be negligible.10)

As illustrated in Table II, Naramycin–A shows the intramolecularly associating ν_{OH} band as well as intermolecularly associating band while Naramycin–B shows only the intermolecularly associating one in solid state. In diluted solution both antibiotics exhibit the ν_{OH} band due to intramolecular hydrogen–bonded hydroxyl group. It is to be noted that neither antibiotic shows any stretching band due to free hydroxyl group in diluted solution. Comparing the infrared spectra of Naramycin–A and –B with those of two diastereoisomeric 2–(*α*–hydroxy–*p*–halobenzyl)cyclohexanones given by Huitric and Kumler,11) it is suggested that a long substituent at 6–position in Naramycin–A and –B must orient equatorially to cyclohexanone ring, because, in the case of axial orientation, there should not be an intramolecular hydrogen bonding.

TABLE II. Infrared Spectra of Naramycins (cm^{-1})

		Naramycin–A	Naramycin–B
Nujol	OH	3509, 3390∼3333 (w)	near 3226
	C=O	1709	1689
CCl_4*	OH	3540∼3534	3534
	C=O	1711	1711

* Concentration : 0.02%.

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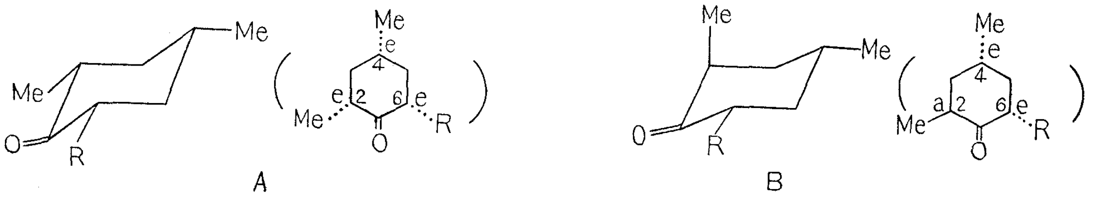
6) R.S. Cahn, C.K. Ingold, V. Prelog : Experientia, **12**, 81(1956).
7) K. Freudenberg : Ber., **66**, 177(1933).
8) J.H. Ford, B.E. Leach : J. Am. Chem. Soc., **70**, 1223(1948).
9) E.C. Kornfeld, R.G. Jones, T.V. Parke : *Ibid.*, **71**, 150(1949).
10) c.f. R.N. Jones, C. Sandorfy : "Chemical Applications of Spectroscopy," 422(1956), W. West.
11) A.C. Huitric, W.D. Kumler : J. Am. Chem. Soc., **78**, 1147(1956).

IV. Configuration of the Asymmetric Carbon at 6-Position. 1) Optical Rotatory Dispersion Curve of Naramycins

Above observations suggest that the structure of Naramycins must be one of four possible structures shown in Chart 1.

1st Group : (4:6)-*cis*-Structure



2nd Group : (4:6)-*trans*-Structure

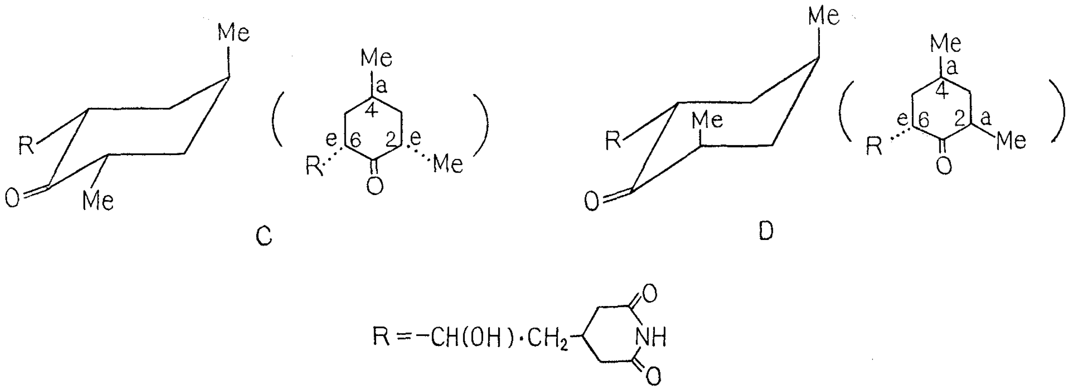


Chart 1. Possible Structures for Naramycins

It is evident that all of these possible structures have 6-equatorially substituted α-hydroxy-β-(2,6-dioxo-4-piperidinyl)ethyl group and have C-4 belonging to (S)-series. The structures (A) and (B) have (4:6)-*cis* configuration, their C-6 belonging to (R)-series, and the structures (C) and (D) have (4:6)-*trans* configuration, their C-6 belonging to (S)-series. Any one of these structures would be possible for Naramycin-A and -B.

Rotatory dispersion (R.D.) curves of Naramycin-A and -B play important roles in deciding which of these four structures corresponds to Naramycin-A and which to Naramycin-B. R.D. curves of Naramycins were measured through the courtesy of Prof. C. Djerassi of the Wayne State University, U.S.A. As illustrated in Fig. 1, Naramycin-A exhibited a single, negative Cotton-effect curve similar to (2R:4R)-2,4-dimethylcyclohexanone,[*2] while Naramycin-B showed a single, positive Cotton-effect curve.

The R.D. curve of a compound which has more than two asymmetric centers generally represents the sum of all partial rotations due to individual asymmetric centers in the molecule. For example, the R.D. curve of (2R:4R)-2,4-dimethylcyclohexanone is regarded as the single negative Cotton-effect curve due to C-2 superimposed on the plain negative curve due to C-4. Thus, the R.D. curves of Naramycins represent the sum of all partial rotations due to asymmetric carbons at 2-, 4-, 6-, and α-positions.

Djerassi, *et al.*[12,13] observed that a carbonyl compound, e.g. (−)-4-methylcyclohept-

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[*2] Full chart of R.D. curve of this compound was kindly supplied from Prof. C. Djerassi (cf. Footnote 5).

12) Private Communication from Prof. C. Djerassi (cf. C. Djerassi, G.W. Krakower : J. Am. Chem. Soc., **81**, 237(1959)).

13) Private Communication from Prof. C. Djerassi (cf. C. Djerassi, L.E. Geller : J. Am. Chem. Soc., **81**, 2789(1959)).

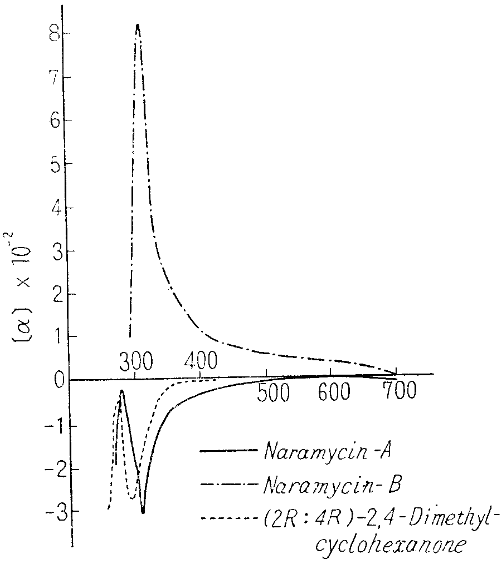

Fig. 1.
R. D. curves of Naramycins

anone and (+)-homoterpenyl methyl ketone, which has asymmetric carbon separated from a carbonyl function by two carbon atoms, does not give a Cotton-effect curve, but exhibits a plain dispersion curve, which is called "Distance Factor." Thus, the partial rotation due to C-4 in Naramycins is indifferent to the Cotton effect owing to the "Distance Factor" similar to the partial rotation due to C-4 in (2R:4R)-2,4-dimethylcyclohexanone.

2-Methyl group in Naramycins affects the Cotton effect, but when another asymmetric carbon(s) is (are) present at the same time, the effect of this methyl group is not as strong as to surpass the effect of another asymmetric center, even if the conformation of 2-methyl group is equatorial or axial. This is illustrated in many compounds, for example, 17a-α- and -β-methyl-17-oxo-D-homosteroid[14] exhibits a negative Cotton-effect curve like its parent substance, and C-4-epimeric tetrahydrosantonins[15] and 4α- and 4β-methylcholestan-3-ones[15] exhibit positive Cotton-effect curves as their parent nor compounds. Therefore, it is assumed that the conformational difference of 2-methyl groups in the two Naramycins, if any, may cause the amplitude variation but never a change of the sign of Cotton effect in the R. D. curves of Naramycins.

Consequently, it seemed inevitable to conclude that the difference in signs of Cotton effect in Naramycins results from the configurational difference between C-6 to which long substituents including α-asymmetric center are attached. However, the configuration of C-α is the same in the two Naramycins and it must be due to the difference of the absolute configuration of C-6 that the actual alternation of the sign of Cotton effect exists. This assumption is strongly supported by the fact[15] that 2α(*eq.*)-methoxymethyl-*trans*-9-methyl-1-decalone gives a single, positive Cotton-effect curve, as does its parent 2-unsubstituted decalone, and the 2β(*ax.*)-isomer produces an inversion of the sign of Cotton effect.

From the above considerations it is seen that one of the Naramycins must have (6R)-(4:6)-*cis*-configuration and the other (6S)-(4:6)-*trans*-configuration.

2) Absolute Configuration of an Asymmetric Carbon at 6-Position in Naramycins.

It is supposed that the partial contributions of C-αs for the R. D. curves of Naramycins would be similar, because their absolute configurations are the same as described above. Therefore the following consideration makes it possible to assume the absolute configuration of C-6 in Naramycin-A and -B.

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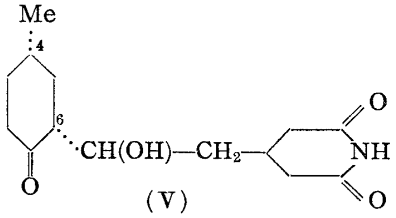
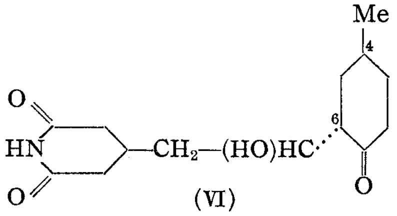
[14] C. Djerassi, R. Riniker, B. Riniker: J. Am. Chem. Soc., 78, 6362(1956).
[15] C. Djerassi, O. Halpern, H. Halpern, R. Riniker: *Ibid.*, 80, 4001(1958).

In the structures designated for the Naramycins which are illustrated in Chart 1, the problem is the sign of the Cotton effect which may be expected from (4:6)-*cis*-structures (formulae A and B), and from (4:6)-*trans*-structures (formulae C and D). As mentioned above, partial contribution of 2-methyl group is not so strong as to surpass that of 6-substituent. Therefore it would be better to regard an imaginative 2-nor-4:6-*cis*-cycloheximide (V) as the parent compound for the structures A and B, and 2-nor-4:6-*trans*-cycloheximide (VI) for the structures C and D, than to regard (2R:4R)-2,4-dimethylcyclohexanone (IV) as the parent compound for Naramycins.



(V)



(VI)

It is expectable that (V) which has a structure antipodal to (IV) exhibits a positive Cotton-effect curve and that (VI) which has similar surroundings as (IV) at 2(or 6)-position of a carbonyl function gives a negative Cotton-effect curve similar to (IV). Therefore, Naramycin-A and -B which respectively exhibited negative and positive Cotton-effect curve have the structure in which methyl group was introduced into 2-position of 2-nor-(4:6)-*trans*- and 2-nor-(4:6)-*cis*-cycloheximide, respectively.

To elucidate the configuration of Naramycin, the following consideration is also possible. It was shown by Prof. C. Djerassi[16] that a substituent introduced equatorially into α-carbons of a cyclohexanone does not affect the original shape of R. D. curve. The sign of Cotton effect shown by Naramycin-A is similar to that of (2R:4R)-2,4-dimethylcyclohexanone, so that it is assumed that Naramycin-A is a compound in which α-hydroxy-β-(2,6-dioxo-4-piperidinyl)ethyl group is introduced equatorially into 6-position of (2R:4R)-2,4-dimethylcyclohexanone, whereas Naramycin-B is originally a compound having axially introduced long alkyl substituent and actually stabilized itself in its ring-inverted form. The question is, what form, diaxial or diequatorial form of (2R:4R)-2,4-dimethylcyclohexanone, should be regarded as its original form. A close investigation of the R. D. curves of cyclohexanones having an alkyl substituent at 2-position makes it possible to think that 2-alkyl group behaves as if it were an axial halogen

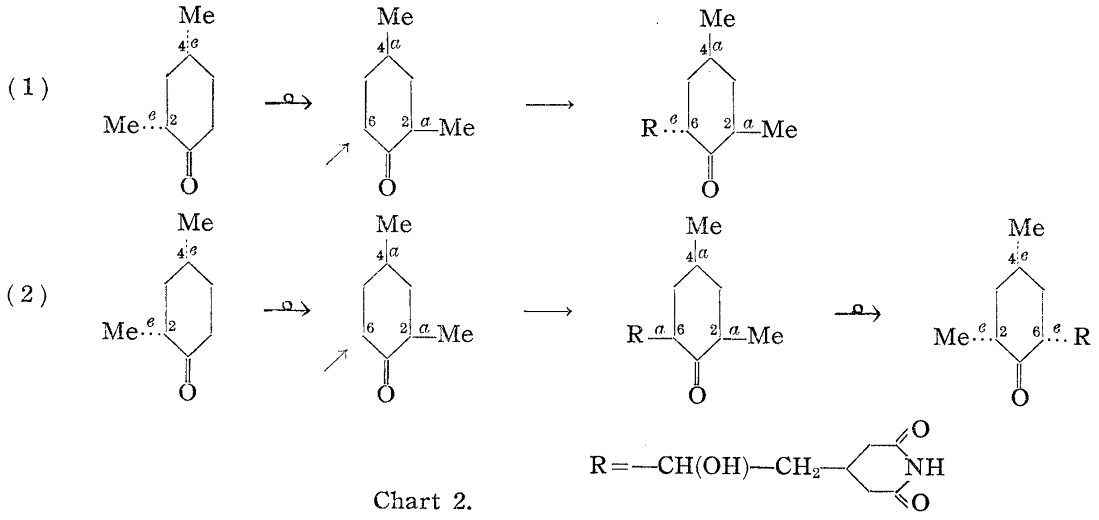


Chart 2.

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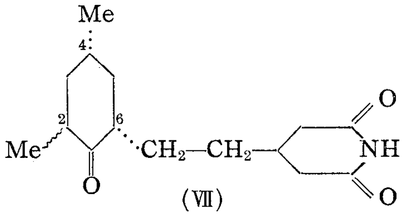
[16] Private Communication from Prof. C. Djerassi.

atom and exhibits the sign of Cotton effect which would be expected according to the "axial haloketone dispersion rule."[17] Therefore, in discussing the R. D. curve of (2R: 4R)-dimethylcyclohexanone, 2a,4a,-form should be regarded as its original form. Consequently, Naramycin-A would be represented as (1) in Chart 2 and has a 4:6-*trans*-configuration. Thus, its absolute configuration of C-6 belongs to (S)-series. On the other hand, Naramycin-B would be represented as (2) and has 4:6-*cis*-configuration having (6R)-structure.

The configuration of 2-methyl group primarily belongs to (R)-series, but this 2-methyl group has a chance to epimerize itself and should not be determined hastily.

(4:6)-*cis*-Deoxycycloheximide (VII) was synthesized and its R. D. curve measured. This compound exhibited a positive sign of Cotton effect as was expected. Details will be reported in the succeeding paper. The different appearance of infrared spectra of Naramycins discussed in Section III can be explained by assuming Naramycin-A and -B to have a different configuration at 6-position, even if the cofiguration of α-carbon is the same. Details will also be discussed in forthcoming papers.



(VII)

The author expresses his deep gratitude to Professor C. Djerassi of the Wayne State University, U. S. A., for the measurement of rotatory dispersion curves and for his instructive and pertinent suggestions through private communications. The author appreciates the kind guidance of Prof. S. Sugasawa of the University of Tokyo for his good offices in introducing the author to Professor C. Djerassi. He is also grateful to Dr. S. Yamada, the Director of this Laboratory, for his encouragement, to Messrs. M. Suzuki and Y. Egawa for their enthusiastic collaboration throughout the course of this work, to Mr. K. Kotera for infrared analysis, and to Mrs. F. Hisamichi and Mr. T. Yoda for elemental analysis. Thanks are also due to NIKKEN Chemicals Co. Ltd. for their supply of crude Naramycins.

Experimental

R. D. Curves of Naramycin-A and -B—R. D. Curves of Naramycins were measured through the courtesy of Prof. C. Djerassi of the Wayne State University, U. S. A.

R. D. of Naramycin-A in MeOH (c=0.16) : $[\alpha]^{30}_{700}$ −15°, $[\alpha]_{589}$ −8°, $[\alpha]_{400}$ −38°, $[\alpha]_{350}$ −80°, $[\alpha]_{325}$ −180°, $[\alpha]_{312.5}$ −310°(trough), $[\alpha]_{295}$ −107°, $[\alpha]_{282.5}$ not measured.

According to the private communication from Prof. C. Djerassi, R. D. curve of Naramycin-A agreed well with that of authentic Actidione. The latter exhibited a peak at 282.5 mμ ($[\alpha]_{282.5}$ −20° (c=0.106, MeOH)).

R.D. of Naramycin-B in MeOH (c=0.084) : $[\alpha]^{28}_{700}$ ca. 0°, $[\alpha]_{589}$ +40°, $[\alpha]_{400}$ +112.5°, $[\alpha]_{350}$ +250°, $[\alpha]_{325}$ +610°, $[\alpha]_{312.5}$ +815°(peak), $[\alpha]_{295}$ +125°.

Benzoyl- and 3,4-Dichlorobenzoyl-naramycins—A solution of 1 g. of Naramycin-A dissolved in 7.5 cc. of dehyd. pyridine was added to 0.6 g. of BzCl under ice-cooling. After being kept in the refrigator over night, the solvent was distilled off under a reduced pressure and the oily residue solidified on addition of cold water. The product was collected, dried (yield, 73%), and recrystallized from 5 volumes of MeOH to white prisms, m.p. 162.5~163°. *Anal.* Calcd. for $C_{22}H_{27}O_5N$: C, 68.57; H, 7.01; N, 3.64. Found : C, 68.41; H, 6.99; N, 3.63.

By similar procedures, Naramycin-B gave Benzoylnaramycin-B (yield, 66%). The crude benzoate was recrystallized from *iso*-PrOH to white needles, m.p. 159.5~160.5°. *Anal.* Calcd. for $C_{22}H_{27}O_5N$: C, 68.57; H, 7.01; N, 3.64. Found : C, 68.98; H, 6.97; N, 3.45.

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[17] C. Djerassi, W. Klyne : J. Am. Chem. Soc., **79**, 1509(1957); C. Djerassi, J. Osiecki, R. Riniker, B. Riniker : *Ibid.*, **80**, 1216(1958).

Benzoylation of Naramycin-A with 3,4-dichlorobenzoyl chloride as with BzCl gave crude 3,4-dichlorobenzoylnaramycin-A (yield, 48%) which was recrystallized from 10 volumes of MeOH to fine white needles, m.p. 175~176°. *Anal.* Calcd. for $C_{22}H_{25}O_5NCl_2$: C, 58.15; H, 5.51; N, 3.08. Found: C, 58.31; H, 5.43; N, 3.52.

By a similar procedure 3,4-dichlorobenzoylnaramycin-B was obtained from Naramycin-B (yield, 47%) as colorless plates (from dehyd. MeOH), m.p. 146.5~147.5°. *Anal.* Calcd. for $C_{22}H_{25}O_5NCl_2 \cdot \frac{1}{2}H_2O$: C, 57.02; H, 5.61; N, 3.02. Found : C, 57.19; H, 5.51; N, 3.23.

Summary

The configuration of two isomeric antibiotics, Naramycin-A (cycloheximide) and Naramycin-B was elucidated as follows, the plane structure being depicted as (I) :

1) Asymmetric carbon at α-position in both antibiotics has the same configuration.

2) Absolute configuration of asymmetric carbons at 4-position is the same in both antibiotics and belongs to (S)-series.

3) Both antibiotics have an equatorially oriented 6-[α-hydroxy-β-(2,6-dioxo-4-piperidinyl)ethyl] group, but the configuration of asymmetric carbon at 6-position is different in the two antibiotics.

4) Assuming that the partial rotation due to α-carbon in both antibiotics is the same, because their absolute configuration is the same, Naramycin-A has (4S:6S)-structure and Naramycin-B has (4S:6R)-structure.

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