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120. Tomoharu Okuda : Studies on Streptomyces Antibiotic, Cycloheximide. VI.[1,2] The Absolute Configuration of Naramycin-A (Cycloheximide) and its Isomeric Naramycin-B.

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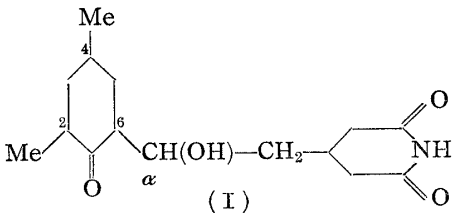
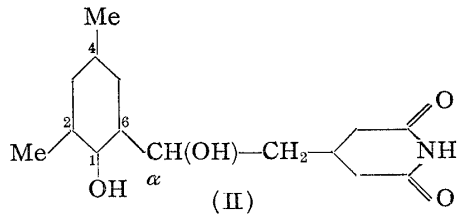
As reported in Part IV[3] of this series, Naramycin-A (identified with cycloheximide) was assumed to have (4S:6S)-configuration and its stereoisomeric Naramycin-B to have (4S:6R)-configuration. The present paper is a report on the absolute configuration of the asymmetric carbon at α-position which is the same in both antibiotics.

In compounds like Naramycins and Dihydronaramycins which have two bulky moieties such as cyclohexyl and dioxopiperidinyl group in the molecule, the whole molecule tends to stabilize itself by locating these moieties linearly as far as possible and the free rotation between 6- and α-carbons is thought to be restricted to some extent.



Plane structure of Naramycins



Plane structure of Dihydronaramycins

Based on the above assumption, the possible structures depictable for Naramycins are limited to four formulae illustrated in Chart 1, in which C-4 (asymmetric carbon at 4-position) belongs to (S)-series and 6-alkyl substituent orients equatorially to the cyclohexanone ring. The configurations of C-2 are out of consideration now. In these formulae, substituents at C-6 and C-α should be in staggered conformation. Therefore, if one looks along the C-6$\sim$C-α bond from C-6 and C-2$\sim$C-1 bond from C-2, substituents at C-6, C-α, C-1, and C-2 would possess their spatial positions as shown in the right column in Chart 1.

In cyclohexanone, projection angle between the carbonyl group and adjacent equatorial bond is 15°, and that between carbonyl group and axial bond is 105°, so that it is evident from the right column in Chart 1 that the carbonyl group is situated rather closely to equatorial position than to axial position. Thus, the spatial relationship between the carbonyl group and α-hydroxyl group in the formulae (A) and (C) is closer than that in the formulae (B) and (D). It should be noted that the spatial relationship between the carbonyl and α-hydroxyl groups is different when the configuration of C-6 is different, even if the configuration of C-α is the same (formula (A) against (D), or formula (B) aganist (C)).

As reported previously,[3] Naramycins have the same configuration at C-α, but in their IR spectra Naramycin-A showed ν_{OH} absorption of intramolecular hydrogen bonding, whereas Naramycin-B showed that of an intermolecular one. In other words, Naramycin-A has rather an unsymmetrical structure, distance between the carbonyl and α-hydroxyl groups being closer, whereas Naramycin-B has a more symmetrical structure, the distance being greater. This phenomenon is also explainable from the

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1) Preliminary Communication. T. Okuda : This Bulletin, **7**, 259(1959).
2) Part V. T. Okuda : *Ibid.*, **7**, 666(1959).
3) Part IV. *Idem.* : *Ibid.*, **7**, 659(1959).

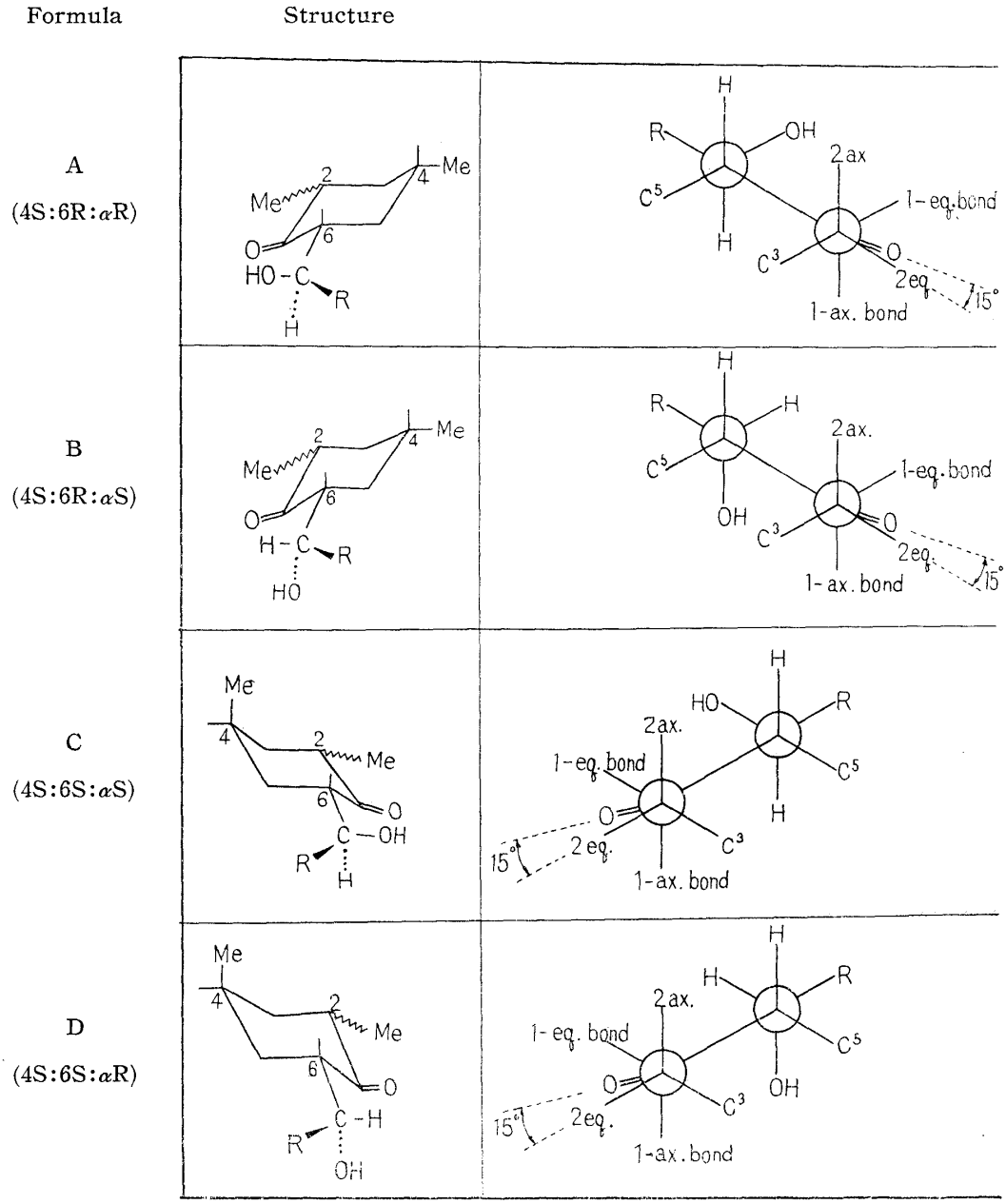
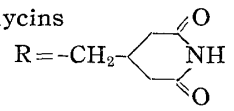


Chart 1. Possible Structure for Naramycins



above facts. From these and other findings that Naramycin-A and -B have (6S)- and (6R)-configuration, respectively, it is predictable that the configuration of C-α belongs to (S)-series.

As described above, direction of the carbonyl group is closer to equatorial, so that a similar spatial relation would exist between 1- and α-hydroxyl groups in dihydrocycloheximides. These relationships are summarized in Table I.

Table I suggests that the absolute configuration of C-α may be assumed if the absolute configuration of C-6 and orientation of 1-hydroxyl group were confirmed and, further, if the spatial relationship between 1- and α-hydroxyl groups were made clear. Attempts to determine the absolute configuration of C-α were made with these intentions.

Table I. Spatial Correlation between α- and 1-Hydroxyl Groups in Dihydrogenated Cycloheximides

Case No.	Absolute configuration at 6-carbon	Absolute configuration at α-carbon	Spatial relation between α- and 1-hydroxyl groups	
			1-OH (eq.)	1-OH (ax.)
1	S	S	*cis*-like[a]	*trans*-like[b]
2	S	R	*trans*-like	*cis*-like
3	R	S	*trans*-like	*cis*-like
4	R	R	*cis*-like	*trans*-like

[a] Spatially both OH groups exist very nearly without rotation between α- and 6-carbons.
[b] Both OH groups come near only after rotation between α- and 6-carbons.

Unfortunately, the usual conformational studies were not applicable to Naramycins because they were readily decomposed by alkali and dehydrated by mineral acid even when cooled, and also by organic acid under warm condition. The epimerization carried out in acetic acid at room temperature was unsuccessful, because the activity of Naramycins for microörganisms remained unchanged even after 48 hours. Usual solvolysis was also unsuccessful.

Consequently, the following experiments, mainly based on reduction procedure, had to be followed to elucidate the configuration of C-α. It is regretful that the reductions with sodium-alcohol or by sodium-moist ether, which were said to give equatorial hydroxyl stereospecifically, were unfavorable for Naramycins owing to their sensitivity to these reducing agents. Experiments were possible only by applying the other reducing agents which were used for stereochemical investigations and from the result obtained elucidation of the configuration of newly produced hydroxyl group in dihydrocycloheximide was made.

In reduction experiments, yield and uniformity of the product were always kept in mind. Only when no other product was found in the reaction product, the yield of the crude product was regarded as the yield of a sole product in the reaction. Experiments are outlined below.*2

Naramycin-A gave dihydrocycloheximide (Ⅲ) reported by Kornfeld, *et al.*[4] by catalytic reduction in acetic acid using platinum oxide catalyst and gave dihydrocycloheximide-boric acid complex (Ⅳ) by the action of sodium borohydride in hydr. methanol. (Ⅳ) was also derived from (Ⅲ) in theoretical yield by recrystallizing (Ⅲ) in 30% acetone containing 5% of boric acid. Dihydrocycloheximide (Ⅲ) and its boric acid complex (Ⅳ) gave the same dihydroactidionic acid (Ⅴ) on alkaline hydrolysis and gave the same product ($C_{18}H_{30}O_5NB$) (Ⅵ) by the action of BF_3-ether complex in acetone.

By the action of tosyl chloride in pyridine under ice-cooling, (Ⅲ) gave monotosyldihydrocycloheximide (Ⅶ) (yield, 76%). The same tosylate was also derived from tosylcycloheximide*3 (Ⅷ) by means of following 4 kinds of reduction procedures; (1) catalytic hydrogenation in AcOH using PtO_2 catalyst (yield, av. 60%); (2) reduction with $NaBH_4$ in hydr. MeOH (yield, 72%); (3) reduction with $LiAlH_4$ in tetrahydrofuran (yield, 73%); and (4) reduction with $LiAlH(tert\text{-}BuO)_3$ in tetrahydrofuran (yield, 79%).

By catalytic reduction of acetylcycloheximide in acetic acid using platinum oxide catalyst, monoacetyldihydrocycloheximide (Ⅹ) (IR : ν_{OH} 3390~3320 cm^{-1}, ν_{NH} 3226 cm^{-1}(Nujol)) reported by Kornfeld, *et al.*[4] was obtained (yield, 67%). This acetate (Ⅹ) was not identical with monoacetyldihydrocycloheximide (Ⅺ) (IR : ν_{OH} 3509 cm^{-1}, ν_{NH} 3175 cm^{-1}(Nujol))

*2 All m.p.s are not corrected and all [α] values were measured in dehyd. MeOH.
*3 Mesylcycloheximide was also prepared, but this compound was more unstable than tosylcycloheximide and was not used for further investigations.
4) E. C. Kornfeld, R. G. Jones, T. V. Parke : J. Am. Chem. Soc., **71**, 150(1949).
5) D. H. R. Barton : J. Chem. Soc., **1953**, 1027.

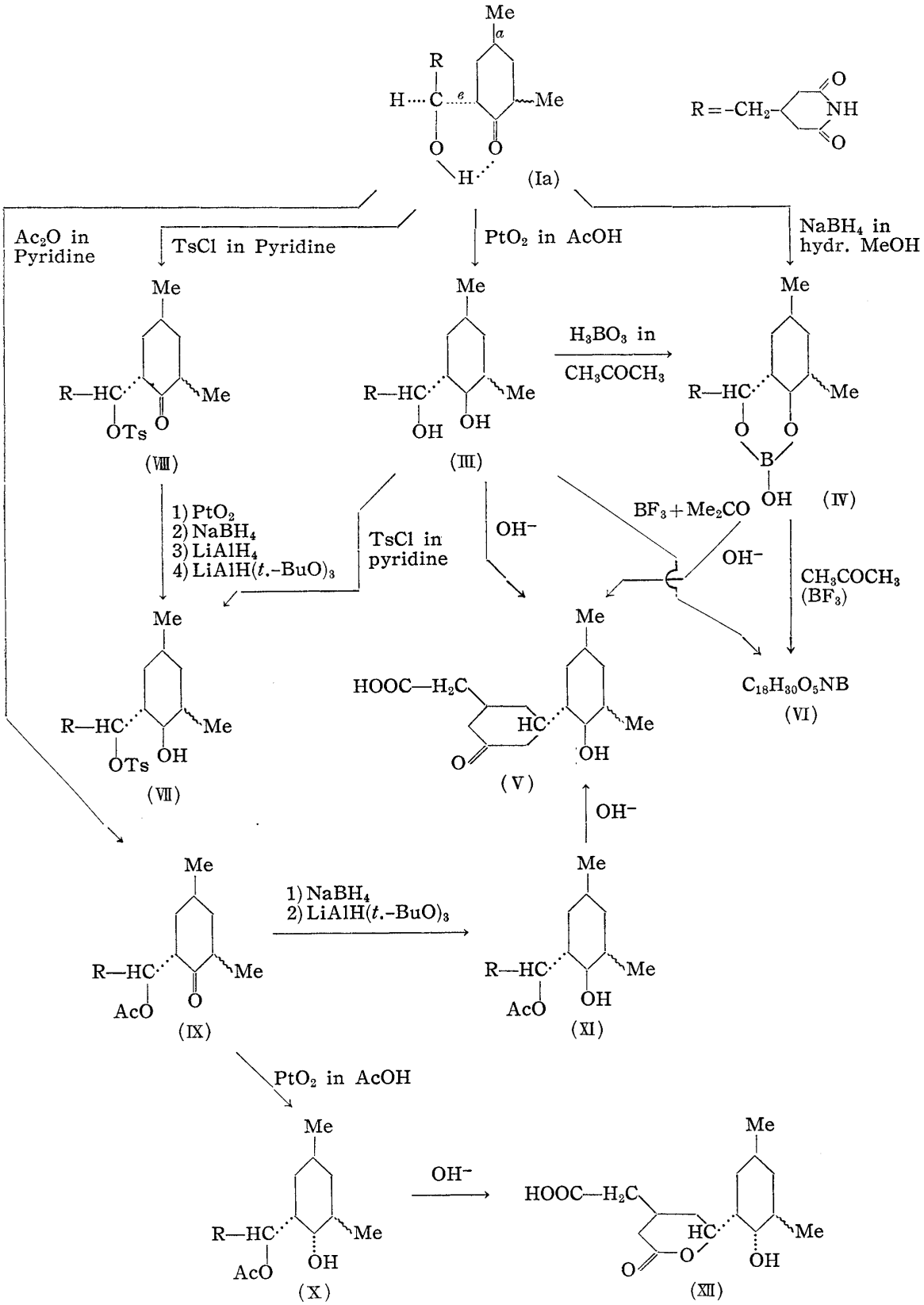


Chart 2.

which was obtained from acetylcycloheximide by the action of $NaBH_4$ (yield, 20%) or $LiAlH(tert\text{-}BuO)_3$ (yield, 86%). On alkaline hydrolysis, (XI) gave the above-mentioned dihydroactidionic acid (V), whereas (X) gave an isomeric dihydroactidionic acid (XII). The above experiments are summarized in Chart 2. Throughout the reactions, it was observed that Naramycin-A tended to give a single product on hydrogenation, while Naramycin-B gave a mixture of reaction products, details of which will be reported elsewhere.

Stereochemical consideration on the above reactions are given below.

1) Naramycin-A and its tosylate gave a kind of hydrogenated compound as a sole product, in which the orientation of the substituents was similar to that in monoacetyldihydrocycloheximide (XI) obtained by the reduction of acetylcycloheximide with $NaBH_4$ or $LiAlH(tert\text{-}BuO)_3$, but different from those of monoacetyldihydrocycloheximide (X) obtained by reduction of acetylcycloheximide with PtO_2 in acid medium. Isomerism between the two monoacetyldihydrocycloheximides is presumably due to the difference of the orientation of their 1-hydroxyl group, because only in the case of catalytic reduction of acetate, it is difficult to imagine that the isomerization of substituents on the cyclohexanone ring took place. The difference between the IR spectra of the two monoacetyldihydrocycloheximides is too great to account for the conformational difference of substituents except the 1-hydroxyl group.

2) It is to be noted that only in the case of reduction of acetylcycloheximide with PtO_2 and $NaBH_4$ as well as $LiAlH(tert\text{-}BuO)_3$ gave different kinds of hydrogenated products. This should be ascribed to the different kind of stereochemical surroundings in acetylcycloheximide, compared with those in cycloheximide and its tosylate, and this difference is also thought to cause the depressed yield on the reduction of acetylcycloheximide with $NaBH_4$ compared with that with $LiAlH(tert\text{-}BuO)_3$, notwithstanding the fact that the reduction of tosylcycloheximide was effected in similar yields both with $NaBH_4$ and with $LiAlH(tert\text{-}BuO)_3$.

3) It has been reported by Barton, *et al.*[5] and by Dauben, *et al.*[6] that catalytic reduction with PtO_2 in acid medium tends to give an axial hydroxyl compound in unhindered cyclohexanone and an equatorial one in hindered ketone.

4) Hückel, *et al.*[7] reported as a result of their extensive examinations that $LiAlH_4$-reduction was not always stereospecific, but his conclusions do not contradict the findings of Dauben, *et al.*[6] that reduction with $LiAlH_4$ should be "product development controlled" rather than "steric approach controlled," and gives equatorially hydroxylated compounds from normal cyclohexanones and axially hydroxylated ones especially from hindered ketones.

5) Wheeler and Mateos[8] reported that $LiAlH(tert\text{-}BuO)_3$ should be more "product development controlled" than $LiAlH_4$ and that, from their experiments on 3-oxosteroids, when an equatorially hydroxylated compound is expectable by $LiAlH_4$-reduction, $LiAlH(tert\text{-}BuO)_3$ gives greater amount of equatorial isomer and less of an axial isomer.

6) Dauben, *et al.*[6] reported that $NaBH_4$, compared with $LiAlH_4$, is more "steric approach controlled" rather than "product development controlled."

From the above facts, the following considerations would be possible. Considering the facts stated in foregoing (4), (5), and (6) that the $LiAlH_4$ and $LiAlH(tert\text{-}BuO)_3$ could reduce acetyl- and tosyl-cycloheximides in similar yields, whereas $NaBH_4$ could reduce tosylate in fair yield but acetate in poor yield, suggests that $LiAlH_4$ and $LiAlH(tert\text{-}BuO)_3$

6) W. G. Dauben, G. J. Fonden, D. S. Noyce: J. Am. Chem. Soc., **78**, 2579(1956); W. G. Dauben, E. J. Blanz, J. Jiu, R. A. Micheli: *Ibid.*, **78**, 3752(1956).
7) W. Hückel, M. Maier, E. Jordan, W. Seeger: Ann. **616**, 46(1958).
8) O. H. Wheeler, J. L. Mateos: Chem. & Ind. (London), **1957**, 395.

behaved as if both acylates were unhindered ketones, so as to be "product development controlled," while $NaBH_4$ behaved as if some hindrance did exist in acetylcycloheximide, so as to be "steric approach controlled." This fact suggests that the 1-hydroxyl group of monotosyldihydrocycloheximide has equatorial orientation.

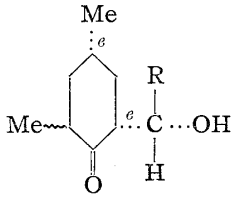
In the reduction of monocyclic ketones, $LiAlH_4$ and PtO_2 often give the same hydrogenated product, but more frequently they give isomeric compounds. In the latter case, reduction with PtO_2 usually gives an axially hydroxylated isomer and $LiAlH_4$, an equatorial one. Thus, the fact that PtO_2 and $LiAlH(tert\text{-}BuO)_3$ gave different hydrogenated compounds in the reduction of acetylcycloheximide suggests that both reducing agents acted as against unhindered ketone. It may therefore be assumed from the foregoing facts outlined in (3), (4), and (5) that, of the two isomers obtained in the reduction of acetylcycloheximide, monoacetyldihydrocycloheximide (X) formed by PtO_2 reduction has axial hydroxyl group at 1-position while (XI) formed by $LiAlH(tert\text{-}BuO)_3$ has an equatorial hydroxyl. This also supports the previous assumption.

The IR spectra of monoacetyldihydrocycloheximides (X) and (XI) suggest that (X) has a symmetrical structure and (XI) is not symmetrical. This phenomenon is quite similar to that observed in the IR spectra of Naramycin-A and -B, so that it is more profitable to decide that (XI) has equtorial 1-hydroxyl group and (X), the axial one. This observation suggests that 1-hydroxyl and α-acetoxyl groups present in monoacetyldihydrocycloheximide (XI), or 1- and α-hydroxyl groups in dihydrocycloheximide (III) are in close proximity. The fact that the formation of boric acid-complex from dihydrocycloheximide was effected quantitatively without any marked change in optical rotatory values suggests that borate ring formation caused no peculiar change of spatial relationships and supports the fact that 1- and α-hydroxyl groups in dihydrocycloheximide are situated quite close to each other.

It ts interesting that monotosyldihydrocycloheximide (VII) exhibits $\nu_{C\text{-}OH}$ band at 1040 cm^{-1} in its IR spectrum (Nujol) and the previously reported dihydrodeoxycycloheximide[2] having equatorial 1-hydroxyl group, at 1058 cm^{-1}. In many hydroxylated steroids, a compound which has equatorial hydroxyl group is said to show $\nu_{C\text{-}OH}$ band at 1050 cm^{-1} and one having axial group, at 1000 cm^{-1}.

Consequently, it is concluded that case No. 1 in Table I would correspond to dihydrocycloheximide (III). Therefore, the absolute configuration of C-α in Naramycin-A belongs to (S)-series. Thus, Naramycin-A is formulated as (Ia) or as formula (C) in Chart 1.

From the hitherto reported informations it is deducible that Naramycin-B has (4S: 6R:αS)-configuration and would be formulated as (XIII) or as formula (B) in Chart 1.



(XIII)

The orientation of 2-methyl group primarily belongs to (R)-series, but this 2-methyl group has a chance to epimerize itself owing to the appearance of 2-alkyl ketone effect or 1,3-diaxial repulsion. Therefore, this matter cannot be decided hastely. Further isomers, if any, would give the clue to this problem.

The author wishes to express his sincere appreciation to Professor S. Sugasawa of the University of Tokyo for his kind guidances, to Dr. S. Yamada, the Director of this Laboratory, for his interest and encouragement in this work, and to Messrs. M. Suzuki and Y. Egawa of this Laboratory for their enthusiastic collaboration and stimulating discussions throughout this work. The author thanks Mrs. F. Hisamichi, and Messrs. T. Yoda and T. Kōno for carrying out the microanalyses, and Mr. K. Kotera for his help in infrared analysis. The author is sincerely grateful to NIKKEN Chemicals Co., Ltd. for their kind supply of crude Naramycins.

Experimental

All m.p.s are not corrected and all $[\alpha]$ values were measured in dehydrated MeOH.

Dihydrocycloheximide (III)—According to the method described by Kornfeld, *et al.*,[4] 500 mg. of Naramycin-A was reduced at atmospheric pressure in 8 cc. of glacial AcOH using 150 mg. of PtO_2 catalyst. Reduction was complete after 1.05 moles of H_2 had been absorbed (about 40 min.). The solution was filtered and AcOH was removed *in vacuo*. The residual syrup was treated with a small amount of water. The product was recrystallized from dehyd. benzene to 410 mg. of pure dihydrocycloheximide (yield, 82.5%) as colorless prisms, m.p. 131.5~132°; $[\alpha]_D^{17}$ +13.2°(c=1.0). *Anal.* Calcd. for $C_{15}H_{25}O_4N$: C, 63.58; H, 8.89; N, 4.94. Found: C, 63.45; H, 8.79; N, 4.92.

Dihydrocycloheximide-Boric Acid Complex (IV)—A solution of 2.81 g. of Naramycin-A dissolved in 25 cc. of MeOH was ice-cooled (−5°) and added with 0.45 g. of $NaBH_4$ dissolved in 5 cc. of MeOH and 8.4 cc. of water, after which the mixture was kept at 3~5° for 1 hr. with stirring. The solution was slightly acidified with 20% AcOH and the solvent was removed *in vacuo*. The residue was recrystallized from 50% acetone to white crystals, m.p. 167~169° (yield, 81%). These crystals were recrystallized further from 70% acetone to fine white needles, m.p. 174~175°; $[\alpha]_D^{17}$ +13.6°(c=1.0). *Anal.* Calcd. for $C_{15}H_{24}O_5NB$: C, 58.25; H, 7.76; N, 4.53; B, 3.51. Found: C, 58.54; H, 7.73; N, 5.01; B, 3.23.

Dihydrocycloheximide-boric acid complex (IV) was also obtained by recrystallization of 285 mg. of dihydrocycloheximide from 30% acetone containing 5% HBO_3. m.p. 174.5~175°; $[\alpha]_D^{17}$ +14.0°(c=1.0). Yield, 280 mg. This product showed no m.p. depression on admixture with the above product. *Anal.* Found: C, 58.48; H, 7.70; N, 4.86; B, 3.32.

(VI), $C_{18}H_{30}O_5NB$—To the solution of dihydrocycloheximide (200 mg.) in 4 cc. of dehyd. acetone, 0.4 cc. of BF_3-ether complex (BF_3 47.3%) was added and the mixture was kept at room temperature overnight. 0.4 cc. of water was added to the reaction mixture to decompose an excess of the reagent and the solvent was removed *in vacuo*. 170 mg. of crystalline residue was recrystallized from 30% acetone (yield, 50%) to colorless prisms, m.p. 137~138°; $[\alpha]_D^{25}$ +2.9°(c=0.5).

This product was also obtained by similarly treating (IV) with BF_3-ether complex (yield, 51%). m.p. 137~138°; $[\alpha]_D^{25}$ +2.4°(c=0.5). This product showed no m.p. depression on admixture with the above product. *Anal.* Calcd. for $C_{18}H_{30}O_5NB$: C, 61.53; H, 8.55; N, 3.99. Found: C, 61.91; H, 8.61; N, 4.15.

From the analytical data this product seemed to be a compound in which dihydrocycloheximide-boric acid complex was associated with 1 mole of acetone unit.

Tosylcycloheximide (VIII) and Mesylcycloheximide—175 mg. of tosyl chloride was added to the solution of 200 mg. of Naramycin-A dissolved in 1.5 cc. of dehyd. pyridine and the mixture was kept for 30 hr. in a refrigerator after which the excess of pyridine was removed *in vacuo*. Water was added to the residue and 240 mg. of white solid was obtained (yield, 66.5%). By repeated recrystallization from MeOH pure tosylcycloheximide was obtained as colorless prisms, m.p. 100.5~101.5°. *Anal.* Calcd. for $C_{22}H_{29}O_6NS$: C, 60.69; H, 6.67; N, 3.22. Found: C, 60.64; H, 6.68; N, 3.13.

This tosylate was slightly unstable at ordinary temperature and turned brown within 2 days.

By similarly treating Naramycin-A with mesyl chloride, mesylcycloheximide was obtained as colorless prisms, m.p. 112~112.5°(decomp.). *Anal.* Calcd. for $C_{16}H_{25}O_6NS$: C, 53.48; H, 6.96; N, 3.90. Found: C, 53.52; H, 6.98; N, 4.24.

The mesylate was more labile than the tosylate.

Monotosyldihydrocycloheximide (VII)—i) Tosylation of dihydrocycloheximide (III): 200 mg. of dihydrocycloheximide (III) was dissolved in 1.5 cc. of dehyd. pyridine and tosylated with 175 mg. (1.2 moles) of tosyl chloride in a refrigerator for 48 hr. The solvent was removed *in vacuo*, water was added to the residue, and the residue was recrystallized from 80% MeOH to 363 mg. of monotosyldihydrocycloheximide (yield, 76%) as colorless needles, m.p. 140.5~141.5°; $[\alpha]_D^{25}$ +48.5°(c=1.0). IR (Nujol) ν_{C-O}: 1040 cm^{-1}. *Anal.* Calcd. for $C_{22}H_{31}O_6NS$: C, 60.27; H, 7.08; N, 3.20; S, 7.30. Found: C, 60.22; H, 7.11; N, 3.32; S, 7.30.

ii) Reduction of tosylcycloheximide (VIII): a) Catalytic reduction with PtO_2 catalyst in acid medium: 1.5 g. of tosylcycloheximide (VIII) was reduced at atmospheric pressure in 65 cc. of glacial

AcOH using 500 mg. of PtO_2 catalyst at 16°. Reduction was complete after 1.12 moles of H_2 had been absorbed (about 3 hr.). The solution was filtered and the solvent was removed *in vacuo*. The residual syrup was dissolved in hot 80% MeOH. First crystals crystallized out on cooling and second crop of crystals came out on further standing. By evaporating the mother liquor to 1/2 volume, third crop of crystals was obtained.

The first crystal (360 mg., m.p. 137~138°) was recrystallized from 80% MeOH to give the pure material (m.p. 140~140.5°). The second crystal (750 mg., m.p. 129~131°) gave three crystals melting at 140~141° (410 mg.), 99.5~100.5° (65 mg.) and 126~129° (ca. 90 mg.) on further fractional recrystallization from 80% MeOH. The third crystals (100 mg., m.p. 97~99°) and the crystals melting at 99.5~100.5° fractionized from the second crystals agreed with starting tosylcycloheximide (Ⅷ).

The crystals melting at 140~141° (total, 770 mg.) was the aimed monotosyldihydrocycloheximide and this was the sole product of the reaction. The yield was 58% of the theory on discounting the starting material. On this reaction 170 mg. of the starting material was recovered (recovery, ca. 11%).

On the experiment at 24°, the yield of monotosyldihydrocycloheximide was 62% and the recovery of the starting material was ca. 5%. This product showed no m.p. depression on admixture with the product described in paragraph i).

b) Reduction with $NaBH_4$—A solution of $NaBH_4$ (105 mg.) in 5 cc. of MeOH and 3.3 cc. of water added dropwise to the solution of 1.09 g. of tosylcycloheximide (Ⅷ) in 17 cc. of MeOH and 7 cc. of pyridine at −5° to 0°. After completion of the addition, the solvent became slightly opaque. 1 cc. of pyridine was added to the mixture and kept at 3~5° for 1.5 hr., after which the solution was acidified with 20% AcOH to decompose an excess of the reagent. The solvent was removed *in vacuo* and water was added to the residue. The product was recrystallized from 50% acetone to give 790 mg. of hydrogenated product (m.p. 136~138°; yield, 72%). The product was recrystallized further from 80% MeOH to colorless needles, m.p. 139.5~140.5°. The product showed no m.p. depression on admixture with the product mentioned in paragraph a). Other products were not detected in the mother liquor.

c) Reduction with $LiAlH_4$—1.31 g. of tosylcycloheximide in dehyd. tetrahydrofuran (10 cc.) was added dropwise to the solution of $LiAlH_4$ (0.12 g.) in the same solvent (7 cc.) at −5° to 0°, and allowed to stand for 1.5 hr. at 3~5°, after which water and then 20% AcOH were added to the reaction mixture to decompose excess of the reducing agent and to acidify the mixture. On adding 10 cc. of ether to the solvent inorganic substance precipitated out. The solution was filtered and the solvent was removed *in vacuo*. On treating the residual syrup with hydr. MeOH, 960 mg. of crude product was obtained (yield, 73%). This product was recrystallized from 80% MeOH to colorless needles, m.p. 139.5~140.5°. Other components were not found in the mother liquor. The product was in accord with monotosyldihydrocycloheximide mentioned above.

d) Reduction with $LiAlH(tert\text{-}BuO)_3$—To the solution of $LiAlH(tert\text{-}BuO)_3$ which was prepared from $LiAlH_4$ (120 mg.) and *tert*-BuOH (670 mg.) in dehyd. tetrahydrofuran (7 cc.),[9] tosyl cycloheximide (1.31 g.) in 10 cc. of dehyd. tetrahydrofuran was added dropwise at −5° to 0°. The mixture was kept at 3~5° for 1.5 hr., after which small amount of water was added to decompose an excess of reducing agent. After being acidified with 20% AcOH, 10 cc. of ether was added to the mixture by which an inorganic substance precipitated out. The solution was filtered and evaporated *in vacuo*. Hydr. MeOH was added to the residual syrup to give the solid product (1.04 g., m.p. 136~138°, yield, 79%). The product was recrystallized from 80% MeOH to colorless needles, m.p. 139.5~140.5°. This product was identical with the product described above. Another product was not found from the mother liquor.

Monoacetyldihydrocycloheximides (X and XI)—a) Catalytic Reduction of Acetylcycloheximide (Ⅸ): 1.08 g. of acetylcycloheximide, which had been prepared from Naramycin-A according to the procedure described by Ford and Leach,[10] was reduced at atmospheric pressure in 40 cc. of glacial AcOH using 300 mg. of PtO_2 as a catalyst at 24°. Reduction was completed after a theoretical amount (75 cc.) of H_2 had been absorbed (about 2 hr.). The solution was filtered and then AcOH was removed *in vacuo*. The residue was recrystallized from 50% MeOH to 650 mg. of colorless prisms (yield, 67% on discounting the starting material). The product was further recrystallized from 50% MeOH to colorless prisms, m.p. 165°; $[\alpha]_D^{19}$ +29.0°(c=1.0). This product was in accord with the monoacetyldihydrocycloheximide (X) described by Kornfeld, *et al.*[4] On further investigation of the mother liquor, other product except the starting acetylcycloheximide (ca. 100 mg.) was not obtained. *Anal.* Calcd. for $C_{17}H_{27}O_5N$: C, 62.75; H, 8.37; N, 4.31. Found: C, 62.38; H, 8.38; N, 4.64.

b) Reduction with $NaBH_4$: Acetylcycloheximide (1.08 g.) was reduced with $NaBH_4$ in a similar way described for the reduction of tosylcycloheximide. By treating the reaction product with 30% acet-

9) H. C. Brown, R. F, McFarlin: J. Am. Chem. Soc., **78**, 252(1956).
10) J. H. Ford, B. E. Leach: *Ibid.*, **70**, 1223(1948).

one, 208 mg. of crystals were obtained (yield, 20%). m.p. 177~178°; $[\alpha]_D^{19}$ +34.3°(c=2.0). *Anal.* Calcd, for $C_{17}H_{27}O_5N$: C, 62.75; H, 8.37; N, 4.31. Found : C, 62.71; H, 8.15; N, 4.31. This was different from the product described in the above paragraph and was identical with the product described below. Another isomer was expectable from the acetone mother liquor, but nothing was obtained as a solid product.

c) Reduction with LiAlH(*tert*-BuO)$_3$: 1.62 g. of acetylcycloheximide (IX) was reduced with LiAlH-(*tert*-BuO)$_3$ by the same procedure used for the reduction of tosylcycloheximide. 1.4 g. of crude product (m.p. 167~169°) was obtained (yield, 86%). The product was recrystallized from 50% MeOH to colorless needles, m.p. 177~178°, and showed no m.p. depression on admixture with the product described in the paragraph b). From the mother liquor other isomers were not detected.

Dihydroactidionic Acid (V)—Dihydrocycloheximide (III), dihydrocycloheximide-H_3BO_3 complex (IV), and monoacetyldihydrocycloheximide (XI) were hydrolyzed with 20% NaOH according to the manner described by Kornfeld, *et al.*[4] and the same dihydroactidionic acid (V) was obtained (yield, 87%, 83%, and 72%, respectively). Colorless scaly crystals, m.p. 174~175°. *Anal.* Calcd. for $C_{15}H_{24}O_5$ (for the dihydroactidionic acid derived from dihydrocycloheximide (III)) : C, 63.36; H, 8,51. Found : C, 63.06; H, 8.67.

Isomer of Dihydroactidionic Acid (XIII)—Monoacetyldihydrocycloheximide (X) derived from acetylcycloheximide by catalytic reduction with PtO_2 was hydrolyzed with 20% NaOH in a similar way described by Kornfeld, *et al.*[4] The crude product was recrystallized from 30% EtOH to colorless prisms, m.p. 173~173.5° (yield, 71%). The m.p. of this was depressed remarkably on admixture with the above dihydroactidionic acid (V) (mixed m.p. 159~163°). From the analytical data, this product seemed to be an isomer of (V). *Anal.* Calcd. for $C_{15}H_{24}O_5$: C, 63.36; H, 8.51. Found : C, 63.69; H, 8.55.

Summary

The stereochemical considerations of dihydrogenated cycloheximide and its acylates were made and it was found that α-hydroxyl group in cycloheximide is situated quite closely to equatorial 1-hydroxyl group which is obtainable by reducing the carbonyl group in the molecule. This finding, together with previously reported information, leads to the conclusion that Naramycin-A should have (4S:6S:αS)-configuration, and its isomeric Naramycin-B should have (4S:6R:αS)-configuration.

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