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128. Makoto Suzuki: Studies on Streptomyces Antibiotic, Cycloheximide. XI.[1] Preparation and Chemical Structure of the Oxidation Products (ψ-Cycloheximides) from Dihydrocycloheximides.

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The present paper concerns with the preparation and chemical structure of ψ-cycloheximides, the oxidation product of dihydrocycloheximides, whose microbiological activities were reported in the preceding paper.[1]

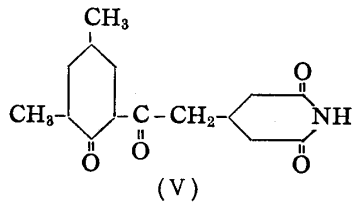
In one of the papers of this series, one of the collaborators (T. Okuda) suggested that dihydrocycloheximide which was obtained by catalytic reduction of cycloheximide in acid solution using platinum dioxide as a catalyst had an equatorial hydroxyl group on cyclohexane ring.[2] According to Klyne[3] an equatorial hydroxyl group is less susceptible to oxidation than an axial one, thus one could expect that mild oxidation would produce new oxidation products, in which β-hydroxy-ketone moiety is different from that in cycloheximide. This is the reason why the present experiments were carried out.

I. Preparation and Chemical Characteristics of ψ-cycloheximides

Two dihydrocycloheximides, e.g. α- and δ-dihydrocycloheximide (II and III), were used as the starting material in this experiment. The former was the product of catalytic reduction of cycloheximide (I) in acid medium and identical with dihydroactidione named by Kornfeld, *et al.*,[4] of which absolute configuration has been assumed by T. Okuda.[2] The latter δ-isomer was the one newly derived from cycloheximide by reduction with lithium aluminium hydride or lithium aluminium tris(*tert*-butoxide) in this laboratory, of which absolute configuration remained unclarified.

The mild oxidation procedure described by Bowden, *et al.*[5] and recommended by Djerassi, *et al.*[6] in their experiments on oxidation of unsaturated alcohols was adopted with a slight modification. They used the so-called 8*N* chromic acid solution (CrO_3–H_2SO_4) in acetone and succeeded in selective oxidation of the hydroxyl group, leaving the ethylenic or acetylenic linkage intact.

When α-dihydrocycloheximide dissolved in 15 volumes of acetone was oxidized with 2 moles of 8*N* aqueous solution of chromium trioxide, keeping the reaction temperature at 20°, a new oxidation product named ψ-cycloheximide-I (IV), m.p. 135~136.5°, $[\alpha]_D^{19}$ −2°, was obtained in 35% yield, 6.5% of α-dihydrocycloheximide being recovered. When the reaction temperature was not controlled and allowed to rise up to 40°, the products were contaminated with undesirable dehydrocycloheximide (β-diketone)[4] (V). It must be



(V)

*1 Toda-machi, Kita-adachi-gun, Saitama-ken (鈴木真言).

1) Part X. M. Suzuki: Yakugaku Zasshi, **80**, 1217(1960).
2) Part VI. T. Okuda: This Bulletin, **7**, 671(1959).
3) W. Klyne: "Progress in Stereochemistry," **1**, 63(1954). Butterworths Scientific Publications, Ltd., London.
4) E. C. Kornfeld, R. G. Jones, T. V. Parke: J. Am. Chem. Soc., **71**, 150(1949).
5) K. Bowden, J. M. Heilbron, E. R. H. Jones, B. C. L. Weedon: J. Chem. Soc., **1946**, 39.
6) C. Djerassi, R. R. Engle, A. Bowers: J. Org. Chem., **21**, 1547(1956).

added that oxidation of dihydrocycloheximide according to Bowden's original procedure did not give ψ-cycloheximide but produced dehydrocycloheximide (V).

By a similar oxidation procedure as above, δ-dihydrocycloheximide (III) gave another stereoisomeric oxidation product, named ψ-cycloheximide-II (VI), m.p. 115~116°, $[\alpha]_D^{17}$ +23.2°, in 42% yield.

These ψ-cycloheximides showed the same analytical data as cycloheximide, corresponding to $C_{15}H_{23}O_4N$ or its hemihydrate, and gave monoacetates by acetylation with acetic anhydride in pyridine. Therefore, it was certain that these ψ-cycloheximides were isomeric and also with cycloheximide itself. Remarkable differences from cycloheximide, however, were noticed in that these products gave no distinct odor of 2,4-dimethylcyclohexanone by alkaline hydrolysis, nor dehydrated products by the agency of phosphorus pentoxide in benzene, and they also gave no corresponding semicarbazones nor oximes. Infrared spectra of these substances showed that carbonyl group was undoubtedly present in the molecule, because new $\nu_{C=O}$ band was observed beside those due to glutarimide moiety present in the starting dihydrocycloheximides (new $\nu_{C=O\ (ketone)}^{Nujol}$ 1709 and 1692 cm^{-1} for ψ-cycloheximide-I and II, respectively). Therefore, it was thought that these substances had a carbonyl group of weak reactivity.

Both ψ-cycloheximides showed negative ferric chloride color reaction and positive (pink) Legal's color reaction[7] with alkaline solution of nitroprusside, the latter reaction suggesting the presence of an active methylene group (-CO-CH_2-) in the molecule.

Optical rotatory dispersion (RD) curves of ψ-cycloheximides and their acetates in methanol are illustrated in Fig. 1. It is to be noted that ψ-cycloheximide-I and its acetate showed the negative Cotton-effect curves as cycloheximide, whereas ψ-cycloheximide-II and its acetate showed positive curves, and that ψ-cycloheximide-I showed decrease in the positive value of its $[M]$ on acetylation. Considering from the above physical properties, especially from their RD curves, the configuration of hydroxyl group in ψ-cycloheximide-I is different from that of cycloheximide, and thus ψ-cycloheximide-I was assumed as one of the position isomers of cycloheximide as was illustrated with (IV), because, if the oxidation had left the C-α hydroxyl group of the starting α-dihydrocycloheximide intact, the above phenomenon would not have been observed.

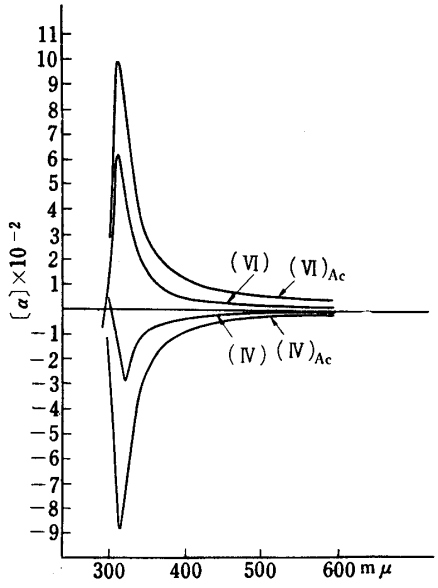


Fig. 1. Rotatory Dispersion Curves of ψ-Cycloheximides

(IV) ψ-Cycloheximide-I
(IV)Ac ψ-Cycloheximide-I acetate
(VI) ψ-Cycloheximide-II
(VI)Ac ψ-Cycloheximide-II acetate

7) E. Legal: Jahresber. über Fortschr. Chem. (1883) 1648. cf. M. Ishidate, M. Akatsuka: Kagaku-no-Ryoiki, **3**, 600(1949). Nankodo, Tokyo. Cycloheximide showed negative (pale yellow) Legal's color reaction.

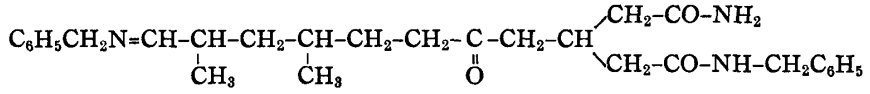
Chemical studies on ψ-cycloheximides described in the next section will give definite evidences on this matter.

II. Assignment of Chemical Structure of ψ-Cycloheximides

Detailed studies on the structure of ψ-cycloheximides were made mainly by using ψ-cycloheximide-I, because the absolute configuration of α-dihydrocycloheximide, the starting material for ψ-cycloheximide-I, has already been established and the antimicrobial activity of ψ-cycloheximide-I was stronger than that of ψ-cycloheximide-II as reported previously.[1]

ψ-Cycloheximide-I (IV) reverted slowly into its starting α-dihydrocycloheximide (II) on catalytic reduction over Adams platinum dioxide in glacial acetic acid (yield, 65%), whereas on similar treatment in dehydrated ethanol it gave another dihydrocycloheximide, named β-dihydrocycloheximide (VII), m.p. 150~151°, $[\alpha]_D^{28}$ −19.3°. The latter is probably the C-α epimer of the former. In both cases of reduction, absorption of hydrogen was so slow that the presence of some steric interference around the carbonyl group in ψ-cycloheximide-I was assumed probably due to the strong intramolecular hydrogen bonding between carbonyl and hydroxyl groups (infrared spectrum of (IV) : cm^{-1} Nujol mull, ν_{OH} 3401, 3356 (shoulder), $\nu_{C=O}$ 1709; in $CHCl_3$ (0.5%), ν_{OH} 3436, $\nu_{C=O}$ 1712).

As described by Kornfeld, *et al.*,[4] cycloheximide, when treated with benzylamine, was cleaved into two fragments by retroaldolization to give *cis-d*-2,4-dimethylcyclohexanone (VIII) and a benzylamine degradation product (IX), m.p. 176° (reported[4] 180~182°(corr.)). α-Dihydrocycloheximide (II) was not cleaved into smaller fragments by a similar treatment, but gave a kind of amido-benzylamide (X), m.p. 116~118°, $[\alpha]_D^{22.5}$ +11.0°, containing C-15 unit of the whole molecule. Thus, it was inferred that a compound having the structure of β-ketol type as cycloheximide underwent a cleavage of C-C junctures (retroaldolization), whereas a compound not having β-ketol structure retained the original C-C



(XI)

bondings. An attempt to obtain the anticipated compound (XI) through benzylamine degradation of ψ-cycloheximide-I failed to give the desired product but a kind of amido-benzylamide (XII), m.p. 152~155°, $[\alpha]_D^{25.3}$ +14.7°, was produced, in which β-ketol structure was retained as evidenced from its analytical data and also from its infrared spectrum. The presence of a carbonyl group in (XII) was also certain from the fact that (XII) gave the same dihydrogenated product (XIII), m.p. 158~161°, $[\alpha]_D^{25.6}$ +33.3°, on catalytic reduction with platinum dioxide, both in glacial acetic acid and in ethanol. This dihydrogenated product (XIII) was not identical but isomeric with the above benzylamine degradation product (X) from α-dihydrocycloheximide. These facts suggested that ψ—cycloheximide-I had a carbonyl group of weak reactivity and had a structure of β-ketol type different from that present in cycloheximide.

Degradation studies were also attempted using liquid ammonia instead of benzylamine, which, if successful, does not produce further asymmetric center. Cycloheximide gave by the action of liquid ammonia *cis-d*-2,4-dimethylcyclohexanone (VIII) and another degradation product of m.p. 266°. The structure of this degradation product was not clarified but this compound seemed to come from C_7-fragment in cycloheximide and had two acid-amide moieties from its infrared spectrum. On degradation with liquid ammonia, α-dihydrocycloheximide (II) gave the diamide (XIV), described by Kornfeld, *et al.*,[4] of m.p. 174~174.5° (reported[4] 174~177° (corr.)), $[\alpha]_D^{27.5}$ +7.1°, and β-dihydrocycloheximide (VII) gave another isomer (XV), m.p. 168~169°, $[\alpha]_D^{24.5}$ +13.6°, in a fair yield. By similar procedures,

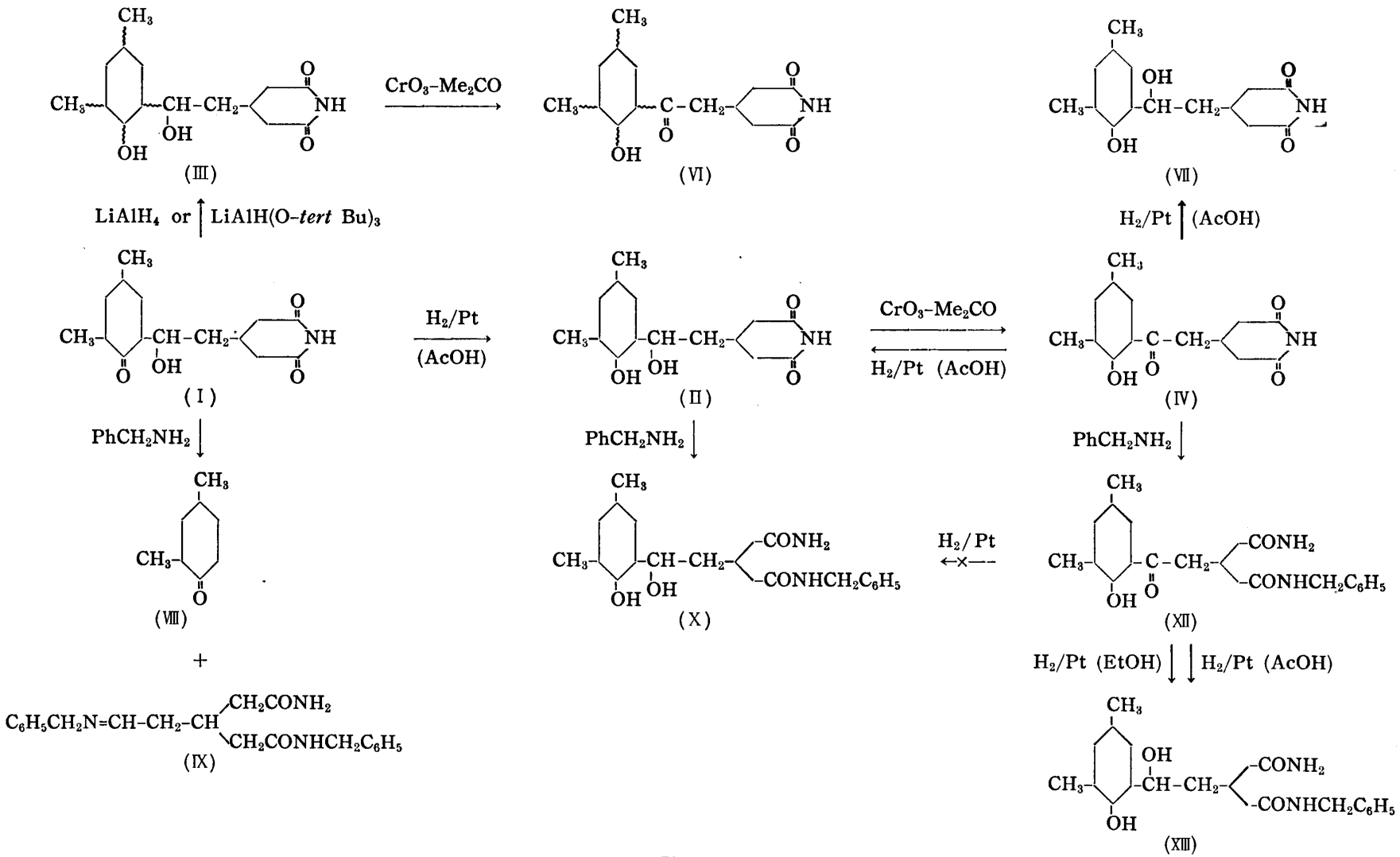


Chart 1.

ψ-cycloheximide-I (IV) gave a kind of degradation product (XVI), m.p. 172°, $[\alpha]_D^{22}$ ca. 0°, which retained the original β-ketol structure as evidenced from its RD curve of anomalous type (Fig. 2) with the same sign (negative) of Cotton effect as that of parent ψ-cycloheximide-I, because, in case carbonyl function disappeared as in α-dihydrocycloheximide and its diamides (XIV), any distinct Cotton effect would not be observed in their RD curves (Fig. 2). As seen above, the behaviors of cycloheximide, ψ-cycloheximide-I, and dihydrocycloheximides towards liquid ammonia were similar to those towards benzylamine.

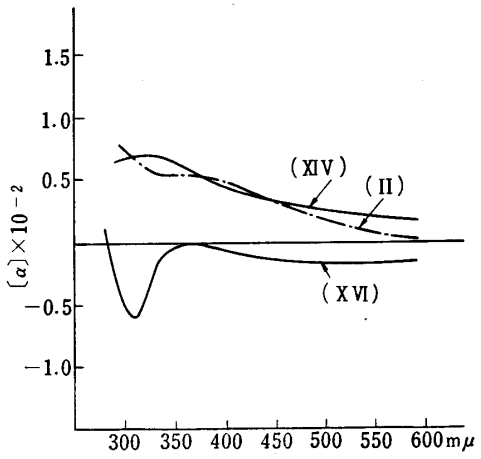


Fig. 2. Rotatory Dispersion Curves of Degradation Products from Liquid Ammonia

(II) α-Dihydrocycloheximide
(XIV) α-Dihydrocycloheximide diamide
(XVI) ψ-Cycloheximide-I diamide

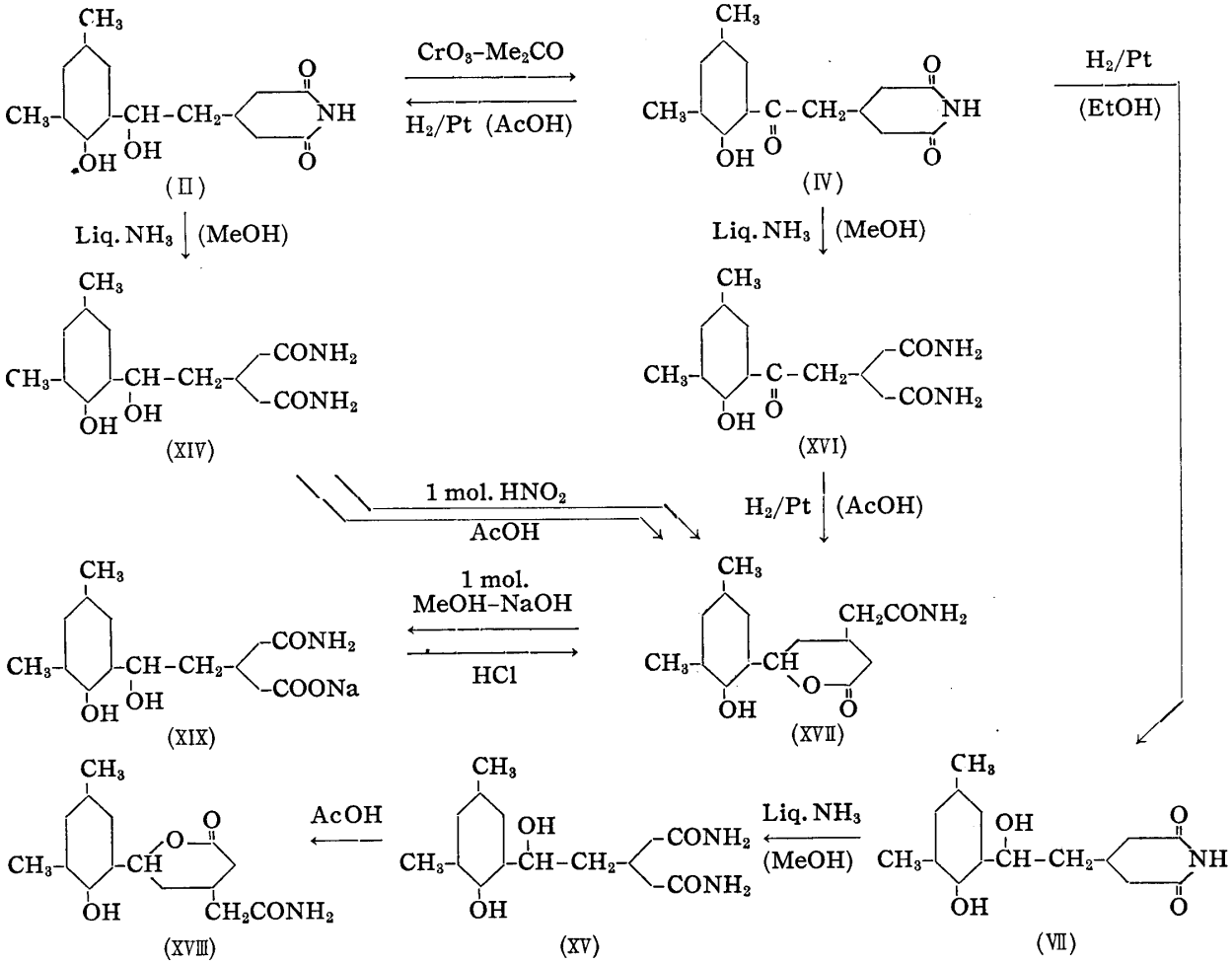


Chart 2.

ψ-Cycloheximide-I diamide (XVI) on reduction with platinum dioxide in dehydrated ethanol, gave β-dihydrocycloheximide diamide (XV) mentioned above, whereas, on reduction with the same catalyst in glacial acetic acid, (XVI) did not form the dihydrogenation product but a product of m.p. 188~189°, $[\alpha]_{320}^{26.7}$ −14.7°, which corresponded to $C_{15}H_{25}O_4N$ (XVII), containing one less ammonia unit than dihydrocycloheximide diamide, $C_{15}H_{28}O_4N_2$. This compound was certain to have a lactone ring in the molecule, because (XVII) gave sodium monocarboxylate (XIX) on hydrolysis with 1.1 moles of methanolic sodium hydroxide and the latter (XIX), on treatment with hydrochloric acid, returned to the starting (XVII). This lactonic compound (XVII) was also derived from α-dihydrocycloheximide diamide (XIV) by treating with glacial acetic acid and in a similar procedure, β-dihydrocycloheximide diamide (XV) was converted into the corresponding β-lactonic acid amide (XVIII), m.p. 183~184°, $[\alpha]_{320}^{27}$ −33.0°. Lactonization of α-dihydrocycloheximide diamide (XIV) was also effected by treating with 1 mole of nitrous acid.

Considering from the above lactonization experiments and also from the fact that ψ-cycloheximide-I diamide (XVI) remained intact on treatment with glacial acetic acid, it was assumed that the lactonization of (XVI) to (XVII) occurred after the reduction of carbonyl group in (XVI) to (XIV) in acid medium, followed by hydrolysis of the diamide (XIV), and that the hydroxyl group which lactonized was the one not at C-1 position but at C-α position in α-dihydrocycloheximide diamide.

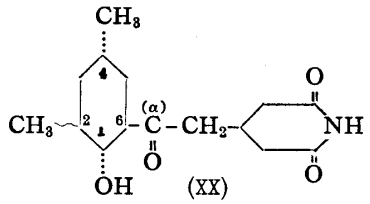
These considerations, therefore, led to the conclusion that the carbonyl group of ψ-cycloheximide-I was present in C-α position in the molecule. The above reaction sequences are summarized in Charts 1 and 2.

As illustrated in Table I, a similar tendency of $[\alpha]$ value differences at 320 mμ was noticed between α- and β-isomers of dihydrocycloheximide diamide and of dihydrocycloheximide lactonic acid amide, respectively derived from α- and β-dihydrocycloheximides which are epimeric to each other probably at their C-α position.

Table I. Differences of $[\alpha]$ Values at 320 mμ between α- and β-Series of Liquid Ammonia Degradation Products of Dihydrocycloheximides

	α-Series		β-Series		$\Delta[\alpha]_{320}:(\alpha-\beta)$
	Diamide (XIV)	+72.0°	Diamide (XV)	+47.0°	+25.0°
	Lactone (XVII)	−14.7°	Lactone (XVIII)	−33.0°	+18.3°
$\Delta[\alpha]_{320}$ (Lactone−diamide)	(XVII)-(XIV)	−86.7°	(XVIII)-(XV)	−80.0°	

Different chemical behavior and color reaction of ψ-cycloheximides from those of cycloheximide and also a sequence of chemical reactions, especially lactonization of ψ-cycloheximide and its derivatives, gave good support for the proposed structure for ψ-cycloheximide, in which the carbonyl group is present in but outside (C-α position) of cyclohexane ring. Therefore, the absolute configuration of ψ-cycloheximide-I would be formulated as (XX), in which asymmetric centers belong to (1R:4S:6R)-series according to the presentation reported in Part VI of this series.[2]



(XX)

The absolute configuration of ψ-cycloheximide-II derived from δ-dihydrocycloheximide remains unsolved because of the obscurity of the configuration of the latter. However, there are many bases for elucidation of the configurations of ψ-cycloheximide-II and its

starting δ-dihydrocycloheximide, such as (1) ψ-cycloheximide-II exhibited positive Cotton-effect curve, (2) ψ-cycloheximide-II acetate showed larger dextrorotatory value than the parent ψ-cycloheximide-II, whereas ψ-cycloheximide-I acetate showed opposite phenomenon, and (3) the C-1 hydroxyl group in ψ-cycloheximide-II resisted mild oxidation as that of ψ-cycloheximide-I. These facts lead to the consideration that the configuration of δ-dihydrocycloheximide might belong to that of Naramycin-B series, especially with regard to its C-6 position.

Experimental

(All m.p.s are not corrected)

α-Dihydrocycloheximide (II)—Prepared by the procedure described by Kornfeld, *et al.*[4] Colorless prisms, m.p. 131~132° (from 30% Me_2CO), $[\alpha]_D^{17}$ +13.2° (c=1, MeOH). RD in MeOH (c=0.1, Zr*[2]): $[\alpha]_{589}^{26.3}$ +3°, $[\alpha]_{400}$ +50°, $[\alpha]_{320}$ +55°, $[\alpha]_{300}$ +84°. IR (in Nujol) cm^{-1}: ν_{OH} 3484, 3390, ν_{NH} 3192, $\nu_{C=O(imide)}$ 1739, 1689.

δ-Dihydrocycloheximide (III)—To a solution of 0.5 g. of $LiAlH_4$ dissolved in 25 cc. of dehyd. tetrahydrofuran, 0.3 g. of *tert*-BuOH was added dropwise at room temperature and the mixture was stirred for 1 hr. to produce the solution of $LiAlH(tert\text{-}BuO)_3$.[8] A solution of 2.81 g. of cycloheximide dissolved in 15 cc. of dehyd. tetrahydrofuran was added dropwise into the above solution under ice cooling (−5° to 0°). The mixture was stirred for 1 hr. at 3° to 5° and a small amount of H_2O was added to decompose an excess of reducing agent. The solution was slightly acidified with 20% AcOH, added with 25 cc. of Et_2O to separate inorganic substances, filtered, and concentrated *in vacuo* to a syrup.

The oily syrup was dried, treated with benzene to effect solidification, and 1.86 g. of crude product (m.p. 147~152°) was recrystallized repeatedly from AcOEt-benzene mixture to 1.78 g. of colorless fine needles, m.p. 157~158° (yield, 63%). $[\alpha]_D^{30}$ ca. 0° (c=1, MeOH). From the remaining benzene solution and also from AcOEt-benzene solution, 0.65 g. of the product was further obtained. *Anal.* Calcd. for $C_{15}H_{25}O_4N$: C, 63.58; H, 8.89; N, 4.94. Found: C, 63.12; H, 8.94; N, 5.35. IR (in Nujol) cm^{-1}: ν_{OH} 3448~3390, ν_{NH} 3195, $\nu_{C=O(imide)}$ 1730, 1701.

The same product of m.p. 157~158° was also obtained in 48% yield by treating 2.81 g. of cycloheximide dissolved in 10 cc. of tetrahydrofuran with 0.4 g. of $LiAlH_4$ dissolved in 20 cc. of the same solvent, at 0° for 1.5 hr.

ψ-Cycloheximide-I (IV)—To a solution of 1 g. of α-dihydrocycloheximide in 16 cc. of dehyd. Me_2CO, a solution of 650 mg. of CrO_3 dissolved in 1 cc. of H_2O was added dropwise, keeping the temperature at 20~23°, and kept for 3 hr. at room temperature. The reaction mixture was added with 30 cc. of H_2O and extracted repeatedly with AcOEt. The combined extract was washed with satd. NaCl solution and H_2O, dried over anhyd. Na_2SO_4, and concentrated *in vacuo*. 600 mg. of the residual syrup was redissolved in AcOEt, poured onto acid-activated alumina, and eluted with the same solvent, followed by 10% MeOH-AcOEt.

AcOEt fraction was concentrated *in vacuo* to a syrup, added with benzene-Et_2O mixture, and kept over night in a refrigerator. Oily material turned to a crude solid (m.p. 124~126°) by this treatment and gave on recrystallization from 50% MeOH 300 mg. of fine colorless prisms, m.p. 135~136°, $[\alpha]_D^{19}$ −2.0° (c=1, MeOH) (yield, 33%). *Anal.* Calcd. for $C_{15}H_{23}O_4N \cdot \frac{1}{2}H_2O$: C, 62.07; H, 8.28; N, 4.90. Found: C, 62.56; H, 8.44; N, 5.10. IR (in Nujol) cm^{-1}: ν_{OH} 3401, ν_{NH} 3356, $\nu_{C=O(ketone)}$ 1709, $\nu_{C=O(imide)}$ 1736, 1664. (in $CHCl_3$), (c=0.5%, 1 mm. cell): ν_{OH} 3436, ν_{NH} 3344, $\nu_{C=O(ketone)}$ 1712, $\nu_{C=O(imide)}$ 1733, 1689. RD in MeOH (c=0.1, Zr): $[\alpha]_{589}^{25.5}$ −2°, $[\alpha]_{400}$ −40°, $[\alpha]_{330}$ −181°, $[\alpha]_{320}$ −267°, $[\alpha]_{317.5}$ −287° (trough), $[\alpha]_{310}$ −178°, $[\alpha]_{300}$ +14°, $[\alpha]_{290}$ +39° (Xe).

From the 10% MeOH-AcOEt fraction, 65 mg. of the starting α-dihydrocycloheximide was recovered.

In another experiment, it was found that when the reaction temperature was not controlled and allowed to rise up to 40°, the desired ψ-cycloheximide-I was contaminated with dehydrocycloheximide with positive $FeCl_3$ color reaction. Though it was possible to separate dihydro- and dehydro-cycloheximide from ψ-cycloheximide-I by fractional chromatography on alumina, the yield of ψ-cycloheximide was less than before.

ψ-Cycloheximide-II (VI)—To a solution of 500 mg. of δ-dihydrocycloheximide (III) dissolved in 8 cc. of Me_2CO, 8*N* aq. solution of CrO_3 (325 mg.) was added dropwise at 20° and the mixture was allowed to stand for 2 hr. at room temperature. A reaction mixture was added with 10 cc. of H_2O and ex-

*2 Zr: Zirconium lamp used as a source of light. Xe: Xenone lamp used as a source of light.

8) H. C. Brown, R. F. MacForlin: J. Am. Chem. Soc., **78**, 252(1956).

tracted repeatedly with AcOEt. The extract was washed, dried, and concentrated *in vacuo* to pale yellowish oily syrup, which was dissolved in AcOEt, poured onto 10 volumes of acid-activated alumina, and eluted with the same solvent. The eluate of AcOEt-fraction was concentrated to a colorless syrup, which, on treatment with Et_2O, soon solidified to 252 mg. of crude crystals, m.p. 108~111°. Crude product was recrystallized from benzene followed by 30% MeOH to 210 mg. of small leaflet crystals of m.p. 115~116°(yield, 42%). $[\alpha]_D^{17}$ +23.2°(c=1, MeOH). *Anal.* Calcd. for $C_{15}H_{23}O_4N$; C, 64.03; H, 8.24; N, 4.98. Found: C, 63.73; H, 7.99; N, 4.72. IR (in Nujol) cm^{-1}: ν_{OH} 3333, ν_{NH} 3165, $\nu_{C=O(ketone)}$ 1692, $\nu_{C=O(imide)}$ 1727, 1678. RD in MeOH (c=0.1, Zr): $[\alpha]_{589}^{25.4}$ +11°, $[\alpha]_{400}$ +40°, $[\alpha]_{320}$ +433°, $[\alpha]_{310}$ +619°(peak), $[\alpha]_{300}$ +275°, $[\alpha]_{280}$ −90 (Xe).

100 mg. of the starting δ-dihydrocycloheximide was recovered from the eluate of 10% MeOH-AcOEt fraction which followed the AcOEt-fraction on alumina chromatography.

ψ-Cycloheximide-I Acetate (IV)$_{Ac}$—To a solution of 300 mg. of ψ-cycloheximide-I (IV) dissolved in 1.5 cc. of Ac_2O, 1.5 cc. of dehyd. pyridine was added dropwise at room temperature and kept standing over night. The solvent was removed *in vacuo*, the residual syrup was treated with Et_2O-hexane mixture to form a solid, which gave on recrystallization from Et_2O-hexane mixture 70 mg. of pure ψ-cycloheximide-I acetate (IV)$_{Ac}$ as colorless prisms, m.p. 115~116°, $[\alpha]_D^{26.3}$ −17.2° (c=0.5, MeOH, Zr) (yield, 20%). *Anal.* Calcd. for $C_{17}H_{25}O_5N \cdot \frac{1}{2}H_2O$: C, 61.45; H, 7.83, N, 4.25. Found: C, 61.65; H, 8.08; N, 4.34. IR (in Nujol) cm^{-1}: ν_{NH} 3145, $\nu_{C=O(ester)}$ 1733, $\nu_{C=O(ketone)}$ 1689, $\nu_{C=O(imide)}$ 1653, $\nu_{C-O(ester)}$ 1235. RD in MeOH (c=0.1, Zr): $[\alpha]_{589}^{21.2}$ −23°, $[\alpha]_{350}$ −233°, $[\alpha]_{320}$ −705°, $[\alpha]_{312.5}$ −872°, $[\alpha]_{310}$ −877°(trough), $[\alpha]_{300}$ −308°.

ψ-Cycloheximide-II Acetate (VI)$_{Ac}$—Twenty mg. of ψ-cycloheximide-II was acetylated as above with 0.1 cc. of Ac_2O dissolved in 0.1 cc. of pyridine. Fine colorless prisms (from Et_2O), m.p. 119.5~120.5°(yield, 70%). RD in MeOH (c=0.1, Zr): $[\alpha]_{589}^{21.2}$ +40°, $[\alpha]_{350}$ +261°, $[\alpha]_{320}$ +783°, $[\alpha]_{310}$ +997° (peak), $[\alpha]_{300}$ +424°.

Reduction of ψ-Cycloheximide-I

1) **Catalytic Reduction in Acid Solution**—Two hundred mg. of ψ-cycloheximide-I was reduced at atmospheric pressure in 12 cc. of glacial AcOH using 50 mg. of PtO_2 catalyst. Absorption of H_2 was very slow and after about 5 hr., reduction was completed. The solution was filtered and AcOH was removed *in vacuo*. The residual syrup was treated with a small amount of H_2O and 150 mg. of crude product was recrystallized from 30% Me_2CO to 130 mg. of pure colorless prisms, m.p. 130~131°(yield, 65%). The product was identified with α-dihydrocycloheximide.

2) **Catalytic Reduction in Neutral Solution; Preparation of β-Dihydrocycloheximide (VII)**—One g. of ψ-cycloheximide-I was reduced at atmospheric pressure in 40 cc. of dehyd. EtOH using 300 mg. of PtO_2 catalyst. Reduction ceased after 0.5 mole of H_2 had been absorbed (about 9.5 hr.). The solution was treated as above. The crude product was fractionally recrystallized from dehyd. Me_2CO to 200 mg. of colorless prisms, m.p. 150~151°, $[\alpha]_D^{28}$ −19.3° (c=2, MeOH), $[\alpha]_{300}^{25.2}$ −100°(c=0.1, MeOH) (yield, 20%). This product was not identical with the above α-dihydrocycloheximide but showed the same analytical data as the latter. Thus, the former seemed to be an isomer of the latter and was named β-dihydrocycloheximide. *Anal.* Calcd. for $C_{15}H_{25}O_4N$: C, 63.58; H, 8.89; N, 4.49. Found: C, 63.84; H, 9.10; N, 4.88.

Benzylamine Degradation of Cycloheximide (IX)—One g. of cycloheximide was treated with 2.5 cc. of freshly distilled benzylamine according to the procedure described by Kornfeld, *et al.*[4] and 250 mg. of pure degradation product (IX) was obtained as colorless needles, m.p. 176° (reported[4] 180~182°(corr.)). *Anal.* Calcd. for $C_{21}H_{25}O_2N_3$: C, 71.77; H, 7.17; N, 11.96. Found: C, 71.43; H, 7.19; N, 11.51.

Benzylamine Degradation of ψ-Cycloheximide-I: Formation of ψ-Cycloheximide-I Amido-benzylamide (XII)—One g. of ψ-cycloheximide-I and 2.5 cc. of freshly distilled benzylamine was warmed on a steam-bath for 2.5 hr. in N_2 atmosphere. The mixture was cooled, added with Et_2O-hexane mixture, and allowed to stand in a refrigerator over night. The lower syrupy layer was separated from the upper solvent by decantation and dried in a desiccator. The remaining syrup was treated with dehyd. Et_2O and 650 mg. of white precipitate gave, by recrystallization from MeOH-Et_2O (1:3) mixture, 400 mg. of pure degradation product (XII) as pale yellowish prisms, m.p. 152~155°, $[\alpha]_D^{25.3}$ +14.7°, $[\alpha]_{320}^{25.3}$ +23.3°(c=0.3, MeOH, Zr) (yield, 30%). *Anal.* Calcd. for $C_{22}H_{32}O_4N_2$: C, 68.04; H, 8.25; N, 7.22. Found: C, 68.04; H, 8.51; N, 7.11. IR (in Nujol) cm^{-1}: ν_{OH} 3333, ν_{NH} 3268, 3021, $\nu_{C=O(ketone)}$ 1701 (weak), $\nu_{C=O(amide)}$ 1658 (doublet), δ_{NH} 1638.

Benzylamine Degradation of α-Dihydrocycloheximide; α-Dihydrocycloheximide Amido-benzylamide (X)—Six hundred mg. of α-dihydrocycloheximide (II) was treated with 1.5 cc. of benzylamine as described above. 300 mg. of pure degradation product (X) was obtained as colorless prisms, m.p. 116~118°, $[\alpha]_D^{25.5}$ +11.0°, $[\alpha]_{320}^{25.5}$ +21.0° (c=0.3, MeOH, Zr) (yield, 35%). *Anal.* Calcd. for $C_{22}H_{34}O_4N_2$: C, 67.75; H, 8.79; N, 7.18. Found: C, 67.71; H, 8.35; N, 6.77. IR (in Nujol) cm^{-1}: ν_{OH} 3350, ν_{NH} 3268, 3165, $\nu_{C=O(amide)}$ 1661, 1639, δ_{NH} 1618.

Catalytic Reduction of ψ-Cycloheximide-I Amido-benzylamide (XII); Formation of Dihydro-

cycloheximide Amido-benzylamide (XIII)—One hundred mg. of (XII) was reduced at atmospheric pressure in 12 cc. of EtOH using 20 mg. of PtO_2 catalyst. The reduction was complete after 1.1 moles of H_2 had been absorbed (about 2 hr.). The filtered solution was concentrated *in vacuo* to oily syrup, which, on treatment with Et_2O, turned to a solid. The crude product was recrystallized from hydr. EtOH to 65 mg. of colorless prisms of (XIII), m.p. 158~160°, $[\alpha]_D^{25.6}$ +33.3° (c=0.3, MeOH, Zr), $[\alpha]_{320}^{25.6}$ +45.3° (c=0.3, MeOH, Zr) (yield, 65%). *Anal.* Calcd. for $C_{22}H_{34}O_4N_2$: C, 67.75; H, 8.79; N, 7.18. Found: C, 67.58; H, 8.93; N, 7.01.

The same product, m.p. 158~161°, was also obtained by the catalytic reduction of (XII) in glacial AcOH using the same catalyst (yield, 29%).

Degradation of Cycloheximide by Liquid Ammonia—To a solution of 1 g. of cycloheximide dissolved in 6 cc. of MeOH, 20 cc. of liquid ammonia dissolved in 10 cc. of MeOH was added, and allowed to stand for 30 min. under dry-ice cooling and for 5 hr. at room temperature during which excess of NH_3 was evaporated. The remaining methanolic solution was concentrated to a syrup, which was dissolved in Et_2O and fractionated chromatographically on acid-treated alumina.

From the first eluate of Et_2O, *cis-d*-2,4-dimethylcyclohexanone was recovered and identified as its semicarbazone (160 mg.), m.p. 195~196° (decomp.) (authentic sample, m.p. 198~199° (decomp.)). *Anal.* Calcd. for $C_9H_{17}ON_3$: C, 59.02; H, 9.29; N, 22.95. Found: C, 58.97; H, 9.08; N, 22.75.

From the subsequent eluate of MeOH, 100 mg. of pale yellowish prisms of m.p. 266~268° (decomp.) were obtained. From the following analytical data this product seemed to come from C_6 or C_7 unit of cycloheximide molecule. *Anal.* Found: C, 47.80; H, 7.40; N, 19.00. IR (in Nujol) cm^{-1}: ν_{NH_2} 3367, 3289, $\nu_{>NH(?)}$ 3155, $\nu_{C=O(amide)}$ 1669, 1656, δ_{NH_2} 1621, $\delta_{>NH}$ 1590, ν_{C-N} 1414, 1399.

Degradation of α-Dihydrocycloheximide by Liquid Ammonia; Formation of α-Dihydrocycloheximide Diamide (XIV)—One g. of α-dihydrocycloheximide dissolved in 6 cc. of MeOH was treated with 20 cc. of liquid NH_3 in 10 cc. of MeOH and 700 mg. of crude degradation product, m.p. 168~169°, was obtained. Recrystallization from 75% dioxane gave 650 mg. of pure α-dihydrocycloheximide diamide (XIV) as colorless prisms, m.p. 174~174.5°, $[\alpha]_D^{27.5}$ +7.1° (c=2, MeOH), $[\alpha]_{320}^{27.5}$ +72.0° (c=0.3, MeOH, Zr) (yield, 61%). *Anal.* Calcd. for $C_{15}H_{28}O_4N_2$: C, 59.97; H, 9.34; N, 9.23. Found: C, 59.89; H, 9.39; N, 9.60. IR (in Nujol) cm^{-1}: ν_{OH} 3378, 3322 (sh.), ν_{NH} 3205, $\nu_{C=O(amide)}$ 1672, 1656, δ_{NH_2} 1626. RD in MeOH (c=0.1, Zr); $[\alpha]_{589}^{27.4}$ +19.3°, $[\alpha]_{400}$ +41°, $[\alpha]_{320}$ +72°, $[\alpha]_{305}$ +66°.

Degradation of β-Dihydrocycloheximide by Liquid Ammonia; Formation of β-Dihydrocycloheximide Diamide (XV)—A solution of 55 mg. of β-dihydrocycloheximide (VII) dissolved in 0.33 cc. of MeOH was treated with 1.1 cc. of liquid NH_3 dissolved in 0.55 cc. of MeOH and 35 mg. of crude product thus obtained was recrystallized from MeOH-Et_2O to 30 mg. of pure β-dihydrocycloheximide diamide (XV) as colorless prisms, m.p. 169°, $[\alpha]_D^{27.5}$ +13.6° (c=0.1, MeOH, Zr), $[\alpha]_{320}^{27.5}$ +33.0° (c=0.1, MeOH, Zr) (yield, 52%). *Anal.* Calcd. for $C_{15}H_{28}O_4N_2$: C, 59.97; H, 9.34; N, 9.23. Found: C, 59.90; H, 9.29; N, 9.08. IR (in Nujol) cm^{-1}: ν_{OH} 3367, ν_{NH} 3287 (sh.), 3165, $\nu_{C=O(amide)}$ 1669, 1661, δ_{NH_2} 1621.

This product was identical with the dihydrogenation product of ψ-cycloheximide-I diamide mentioned below.

Degradation of φ-Cycloheximide-I by Liquid Ammonia; Formation of φ-Cycloheximide-I Diamide (XVI)—To a solution of 500 mg. of ψ-cycloheximide-I (IV) in 3 cc. of MeOH, a solution of 10 cc. of liquid NH_3 in 5 cc. of MeOH was added under dry-ice cooling and allowed to stand for 30 min. under dry-ice cooling and for 5 hr. at room temperature to remove excess of NH_3. The remaining solution was concentrated *in vacuo* to a syrup. The residue was recrystallized from EtOH-Et_2O mixture to 202 mg. of pure ψ-cycloheximide-I diamide as colorless prisms, m.p. 172°, $[\alpha]_D^{22}$ ca. 0° (c=2, MeOH) (yield, 38%). *Anal.* Calcd. for $C_{15}H_{26}O_4N_2$: C, 60.40; H, 8.72; N, 9.40. Found: C, 60.18; H, 9.15; N, 9.34. IR (in Nujol) cm^{-1}: ν_{OH} 3378, ν_{NH} 3165, 3300, $\nu_{C=O(ketone)}$ 1695, $\nu_{C=O(amide)}$ 1669, 1658, δ_{NH_2} 1623. RD in MeOH (c=0.3, Zr); $[\alpha]_{589}^{28.2}$ −6.7°, $[\alpha]_{400}$ −7°, $[\alpha]_{320}$ −45°, $[\alpha]_{310}$ −61° (trough), $[\alpha]_{300}$ −49°, $[\alpha]_{280}$ +4° (Xe).

Reduction of φ-Cycloheximide-I Diamide (XVI)

1) **Catalytic Reduction in Ethanol; Preparation of β-Dihydrocycloheximide (XV)**—Seventy mg. of ψ-cycloheximide-I diamide was reduced at atmospheric pressure in 9 cc. of EtOH using 20 mg. of PtO_2 catalyst. Reduction was complete after 1.05 moles of H_2 had been absorbed (about 5.5 hr.). The filtered solution was concentrated *in vacuo* and the residual syrup solidified with an addition of Et_2O. The product (50 mg., m.p. 167.5~168°) was recrystallized from MeOH-Et_2O mixture to 47 mg. of pure sample as colorless prisms, m.p. 168°, $[\alpha]_D^{24.5}$ +13.6° (c=1, MeOH), $[\alpha]_{320}^{25.3}$ +29.0° (c=0.1, MeOH, Zr) (yield, 67%).

This product was identical with β-dihydrocycloheximide diamide mentioned above, no depression being observed on admixture of samples (mixed m.p. 167.5~168°).

2) **Catalytic Reduction in Acetic Acid**—Seventy mg. of ψ-cycloheximide-I diamide (XVI) was reduced at atmospheric pressure in 9 cc. of glacial AcOH using 20 mg. of PtO_2 catalyst. Reduction was complete after 1.05 moles of H_2 had been absorbed (about 2 hr.). The filtered solution was concentrated *in vacuo* and the product (58 mg., m.p. 178.5~179°) was recrystallized from 30% EtOH to 50 mg. of color-

less needles, m.p. 188~188.5°, $[\alpha]_{320}^{26.7}$ −13.0° (c=0.5, MeOH, Zr) (yield, 75%). This product was found to be the same as α-dihydrocycloheximide lactonic acid amide (XVII) described below. *Anal.* Calcd. for $C_{15}H_{25}O_4N$: C, 63.66; H, 8.90; N, 4.95. Found : C, 63.88; H, 9.22; N, 5.03. IR (in Nujol) cm^{-1} : ν_{OH} 3390, 3425 (sh.), ν_{NH} 3300, 3195 (sh.), $\nu_{C=O(lactone)}$ 1706 (associated), $\nu_{C=O(amide)}$ 1664, δ_{NH_2} 1631, $\nu_{C-O(lactone)}$ 1267.

α-Dihydrocycloheximide Lactonic Acid Amide (XVII)—A solution of 70 mg. of α-dihydrocycloheximide diamide (XIV) dissolved in 9 cc. of glacial AcOH was allowed to stand at room temperature over night. The solvent was removed *in vacuo*, the residual syrup solidified on treatment with Et_2O and was recrystallized from 50% EtOH as colorless needles (45 mg.), m.p. 188~188.5°, $[\alpha]_{320}^{26.7}$ −14.7° (c=0.5. MeOH, Zr) (yield, 69%). No depression was observed on admixture with the above-mentioned hydrogenation product from ψ-cycloheximide-I diamide. *Anal.* Calcd. for $C_{15}H_{25}O_4N$: N, 4.95. Found: N, 4.89.

α-Dihydrocycloheximide diamide remained intact on treatment with 10% AcOH but, when $NaNO_2$ was present simultaneously, turned to the lactonic acid amide via Na salt of amido-carboxylic acid (XIX) as described below.

To a solution of 90 mg. of α-dihydrocycloheximide diamide dissolved in 0.9 cc. of 10% AcOH and 1.5 cc. of EtOH, a solution of 21 mg. of $NaNO_2$ in 0.2 cc. of water was added dropwise under ice cooling and the mixture was allowed to stand for 2 hr. under continuous cooling. The solvent was removed *in vacuo* to give 76 mg. of crude (XIX), m.p. 215° (darkened). (XIX) thus obtained was redissolved in a small amount of H_2O and acidified with 20% HCl. 45 mg. of colorless prisms, m.p. 185.5~186°, precipitated, which showed no mixed m.p. depression with the above α-lactonic acid amide (XVII). *Anal.* Calcd. for $C_{15}H_{25}O_4N$: N, 4.95. Found : N, 5.39.

Hydrolysis of α-Dihydrocycloheximide Lactonic Acid Amide (XVII) and Relactonization of the Product—A solution of 100 mg. of α-lactonic acid amide (XVII) in 0.5 cc. of MeOH was added with a solution of 21 mg. (1.1 moles) of NaOMe in 1 cc. of MeOH containing 1 drop of H_2O and allowed to stand at room temperature for 2 hr. The solution was concentrated *in vacuo*, the residual Na salt of amido-carboxylic acid (XIX) was redissolved in a small amount of H_2O, and acidified with 1 drop of 20% HCl. White crystalline precipitate, m.p. 185~185.5°, was collected and recrystallized from 50% EtOH to 80 mg. of colorless needles, m.p. 188~188.5° (yield, 80%).

This product showed no depression on admixture with the starting α-lactonic acid amide (XVII). *Anal.* Calcd. for $C_{15}H_{25}O_4N$: C, 63.66; H, 8.90; N, 4.95. Found : C, 63.88; H, 9.18; N, 4.82.

β-Dihydrocycloheximide Lactonic Acid Amide (XVIII)—A solution of 35 mg. of β-dihydrocycloheximide diamide (XV), prepared from β-dihydrocycloheximide, dissolved in 4.5 cc. of glacial AcOH was allowed to stand at room temperature over night. The solvent was removed *in vacuo*, the residue solidified on addition of Et_2O, and 23 mg. of crude product was recrystallized from 50% EtOH to 20 mg. of pure β-dihydrocycloheximide lactonic acid amide as colorless needles, m.p. 183~184°, $[\alpha]_D^{27.0}$ −9.3° (c=0.3, MeOH, Zr), $[\alpha]_{320}^{27.0}$ −33.0° (c=0.3, MeOH, Zr). *Anal.* Calcd. for $C_{15}H_{25}O_4N$: C, 63.66; H, 8.90; N, 4.95. Found : C, 63.48; H, 8.99; N, 4.77.

β-Dihydrocycloheximide diamide prepared from ψ-cycloheximide-I diamide by catalytic reduction in EtOH gave the same product, m.p. 183~184°, $[\alpha]_{320}^{24.4}$ −46.3° (c=0.3, MeOH, Zr), by the some procedures.

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Summary

New position isomers of cycloheximide named ψ-cycloheximides were prepared from dihydrocycloheximides by mild oxidation and (IV) was assigned to their structures.

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