Glutarimide Antibiotics. IX. The Stereochemistry of the Dihydrocycloheximides and the Configuration of the Hydroxyl Group of Cycloheximide

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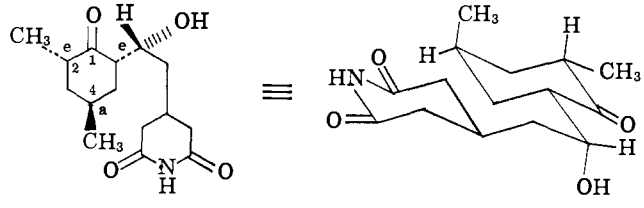
The structures of the dihydrocycloheximides have been reinvestigated both chemically and by means of n.m.r. spectroscopy. This has resulted in new stereochemical assignments being made to the hydroxyl groups of these compounds and to clarification of their chemistry. By a simple procedure the configuration of the hydroxyl group in cycloheximide has been shown to be (R), and not (S) as originally designated. Application of the method to isocycloheximide and naramycin-B shows that they also have their respective hydroxyl groups in the (R) configuration.

I. Introduction

Chronologically the total synthesis[1] of cycloheximide (I) was accomplished prior to the correct determination of the configuration of its hydroxyl group.[2] Nevertheless, we have chosen to report the full details of the latter problem first, because in the light of this knowledge, certain stereochemical facets of the synthesis of I will be illuminated.

As it has transpired, elucidation of the configuration of the hydroxyl group in question rests completely on knowing the stereochemical orientation of the ring hydroxyl in the dihydrocycloheximides and their



I

isomers. Thus, of necessity, this last problem must be dealt with first. It already had received some attention from Okuda,[3] but as will become apparent from this article these results have needed considerable revision. When we began our investigations, Okuda had assigned an equatorial orientation to the 1-hydroxyl group of the dihydrocycloheximide (II), m.p. 131–132°, first obtained by Kornfeld, *et al.*,[4] from I by hydrogenation in acetic acid using a platinum catalyst. This assignment was based on the fact that hindered ketones tend to give equatorial alcohols under such conditions.[5] However, models suggest that in the vicinity of the ketone the lower face of I is more hindered than the upper and that an axial cyclohexanol might be expected in accordance with the von Auwers–Skita hydrogenation rule.[6]

Reductions of cycloheximide acetate (III) using the above catalytic conditions and by sodium borohydride or lithium tri(*t*-butoxy)aluminum hydride were also examined by Okuda.[3] The former procedure led to a dihydrocycloheximide acetate (IV), m.p. 165°, as noted by Kornfeld,[4] the hydroxyl of which was thought to be on the ring and which was assigned an axial orientation. On the other hand, the complex metal hydrides afforded V, m.p. 177–178°, isomeric with IV, which was considered to have the ring hydroxyl group equatorial. In contrast reduction of cycloheximide tosylate (VI) was found to give only one alcohol (VII) no matter what method of reduction was employed. Since VII also could be prepared by the monotosylation of diol II, its ring hydroxyl was necessarily assigned an equatorial disposition.

With these assignments made and finding that the borate ester of II differed little in optical rotation from the parent diol, Okuda then logically assigned the (*S*) configuration to side-chain asymmetric center of I. Heavy support for this conclusion was adduced from an infrared study of the hydrogen bonding differences between the two supposedly stereoisomeric monoacetates IV and V.

II. Stereochemistry of the Ring Hydroxyl Groups

A. Dihydrocycloheximides. Our suspicions concerning the correctness of the above analysis were aroused when we found that *chromic acid oxidation of IV did not regenerate cycloheximide acetate.* Instead a new keto acetate (VIII), m.p. 150°, was isolated, in excellent yield. A rescrutiny of the reaction mixture obtained from the catalytic hydrogenation of III then revealed that while IV predominated, V was also present as a minor product. Contrary also to the claims of Okuda, complex metal hydride reduction of III afforded, rather than a single compound, a mixture of IV and V with the latter in slight excess in this case. Most revealing, however, was the observation that II, IV, and V all gave the same diacetate (IX) when treated with acetic anhydride–pyridine, under mild conditions. The only rational explanation of the above facts lies in the following conclusions. (a) All of the above reductions have the same stereochemical consequence. (b) In the case of cycloheximide acetate, a greater or lesser amount of acetyl group transfer[7] from the side-chain hydroxyl group to the ring

(1) F. Johnson, N. A. Starkovsky, A. C. Paton, and A. A. Carlson, *J. Am. Chem. Soc.*, 86, 118 (1964).

(2) A brief summary of this work has been published: N. A. Starkovsky and F. Johnson, *Tetrahedron Letters*, 919 (1964).

(3) T. Okuda, *Chem. Pharm. Bull.* (Tokyo), 7, 259 (1959); *ibid.* 7, 671 (1959).

(4) E. C. Kornfeld, R. G. Jones, and T. V. Parke, *J. Am. Chem. Soc.*, 71, 150 (1949).

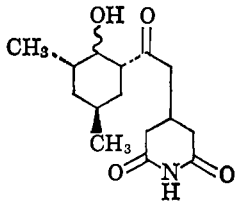
(5) (a) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953); (b) W. G. Dauben, E. J. Blanz, J. Jiu, and R. A. Micheli, *J. Am. Chem. Soc.*, 78, 3752 (1956).

(6) Reference 5a, footnote 23. See also J. H. Brewster, *J. Am. Chem. Soc.*, 76, 6361 (1954); R. J. Wicker, *J. Chem. Soc.*, 2165 (1956).

(7) This transfer which appears to be both acid and base catalyzed was to return to haunt us during our synthetic work on I.

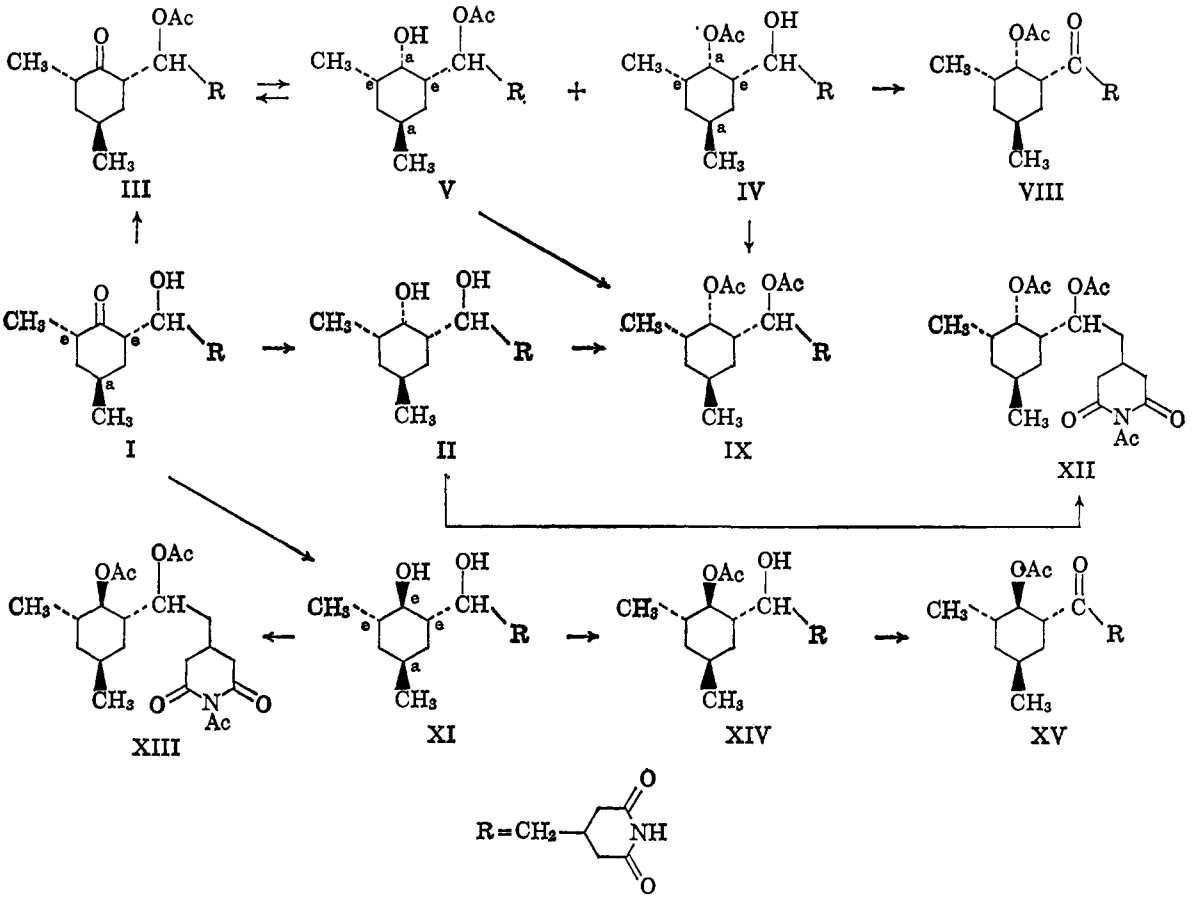
hydroxyl occurs, depending on the reducing agent used.[8]

That V, not IV, is in actual fact dihydrocycloheximide acetate is reinforced by its chromic acid oxidation to cycloheximide acetate in excellent yield. Consequently, both IV and VIII must be regarded as belonging to the ψ-cycloheximide (X) series.[9] Addi-



X

Chart I



tional chemical evidence for this conclusion lies in the fact that VIII does not afford 2,4-dimethylcyclohexanone when treated with base. By contrast cycloheximide acetate, in common with its parent alcohol[4] I, undergoes such a retroaldol reaction with ease.

In an attempt to obtain the missing cyclohexanol isomeric with II, cycloheximide (I) was reduced with diphenyltin dihydride, a neutral reducing agent whose use was designed to avoid the possibility of isomerizing the base-sensitive I, prior to reduction. This procedure afforded a quantitative yield of a mixture of diols which was resolved by column chromatography into II (80% crude yield) and a new diol (XI),[10] m.p. 163°. The latter, on refluxing with acetic anhydride containing pyridine, afforded an O,O,N-triacetyl derivative XII, different from that (XIII) prepared from II. Monoacetylation of XI led to an hydroxy acetate (XIV) which again must belong to the ψ-cycloheximide series since oxidation with chromic acid did not afford cycloheximide acetate, but a new keto acetate[11] (XV), m.p. 123°.

With the above derivatives of known gross structure (Chart I) in hand, it now became feasible with the aid of an n.m.r. analysis to make stereochemical assignments to their ring hydroxyl or acetoxyl functions. This was made possible by the observations of Lemieux, *et al.*,[12] who found that axial proton signals occur at higher field than those of the corresponding equatorial protons. In addition, and more useful to us, was their

(8) The two dihydroactidionic acids obtained from IV and V by basic hydrolysis followed by acidification were claimed by Okuda[8] to be distinct substances and to show melting point depression phenomena when mixed. Although we have not examined this point experimentally, in the light of the above argument it seems more likely that they are identical especially since both have m.p. 173–175°.

(9) M. Suzuki, *Chem. Pharm. Bull.* (Tokyo), 8, 788 (1960).

(10) This diol is identical with the product, m.p. 158°, obtained by Suzuki[9] by the reduction of cycloheximide with lithium aluminum hydride or lithium tri(*t*-butoxy)aluminum hydride. He had tentatively suggested it to be a diol derived from the cycloheximide isomer, naramycin-B. We were able to reproduce his work only with the latter reducing agent, but even then the yields of XI varied capriciously.

(11) Both VIII and XV have been reported previously by Suzuki[9] who obtained them by partial oxidation of the diols II and XI followed by acetylation. Suzuki obtained VIII as a hemihydrate, whereas we did not, so that a comparison of the physical data is meaningless. However, in the case of XV, the reported melting point, 119.5–120.5°, agrees favorably with our own.

(12) R. U. Lemieux, R. K. Kullig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, 80, 6098 (1958). See also A. H. Lewin and S. Winstein, *ibid.*, 84, 2464 (1962); Y. Kawazoa, Y. Sato, T. Okamato, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), 11, 328 (1963); H. Boothe and N. Franklin, *Chem. Ind.* (London), 954 (1963); ref. 17; N. S. Bhacca and D. H. Williams, "Applications of N.M.R. Spectroscopy in Organic Chemistry," Holden-Day Inc., San Francisco, Calif., 1964, p. 51.

Table I

Compd.	Spectra in $CDCl_3$: Methyl signals	Spectra in $CDCl_3$: CHOR on ring	Spectra in $CDCl_3$: CHOR on side chain	Spectra in pyridine: Methyl signals	Spectra in pyridine: CHOR on ring	Spectra in pyridine: CHOR on side chain
Cycloheximide (I)	58.9 (6.1); 73.6 (6.7)	...	244b	59.1 (6.1); 68.7 (6.6)	...	265b
Cycloheximide acetate (III)	59.2 (6.0); 75.7 (6.6)	...	319b	59.4 (6.4); 70.6 (6.6)	...	347b
Cycloheximide chloroacetate[a]	57.0 (6.5); 73.6 (7.0)	...	328b	56.5 (6.3); 67.0 (6.7)	...	341b
Cycloheximide tosylate (VI)	56.2 (6.3); 72.2 (6.9)	...	301b	57.5 (6.2); 68.3 (6.7)	...	323b
Dihydrocycloheximide acetate (V)	55.8 (5.8); 58.9 (6.9)	219s	319b	65.5 (5.3); 62.2 (6.8)	234s	328b
Dihydrocycloheximide (II)	... ...[b]	242s	228b	60 (5 ~ 6); 63 (6 ~ 7)	233s	246b
Dihydrocycloheximide diacetate (IX)	47.5 (5.7); 59.0 (6.8)	302s	282b	47.6 (5.7); 56.3 (6.8)	310s	301b
N-Acetyldihydrocycloheximide diacetate (XII)	49.4 (5.9); 60.9 (7.0)	306s	286b	48.3 (6.0); 58.4 (7.0)	312s	...[c]
Dihydro-ψ-cycloheximide acetate (IV)	46.8 (6.0); 58.8 (6.8)	305s	202b	49.6 (6.0); 59.3 (6.7)	318s	215b
ψ-Cycloheximide acetate (VIII)	50.2 (6.0); 58.4 (6.9)	320s	...	50.9 (5.9); 58.2 (7.2)	...[c]	...[c]
Dihydrocycloheximide (XI)	... ...[b]	207b	238b	70.7 (6.2); 60.4 (6.8)	209b	268b
Dihydro-ψ-cycloheximide acetate (XIV)	51.8 (6.0); 58.9 (7.1)	261b	211b	52.5 (6.1); 58.0 (7.0)	310b	249b
ψ-Cycloheximide acetate (XV)	50.8 (5.7); 61.7 (7.0)	298b	...	50.3 (5.7); 56.5 (7.0)	...[c]	...[c]
N-Acetyldihydrocycloheximide diacetate[d] (XIII)	49.0 (5.7); 60.4 (6.8)	300b	262b	... ...	...	...
Acetonide[e] (XXIII)	57.2 (6.8); 52.6 (5.9)	226s	238b	56.0 (6.8); 56.5 (5.9)	222s	242b
Acetonide[e] (XXVIII)	57.7 (6.7); 51.6 (5.3)	<190	214b	62.6 (7.0); 48.7 (5.6)	<190	225b

[a] See ref. 1. [b] The methyl doublets were not sufficiently well resolved for inclusion. [c] Concentration too low to permit accurate determination of position of absorption. [d] Insufficient material available for determination of the spectrum in pyridine. [e] The ketal methyl groups of XXIII absorbed at 79.8 and 81.6 c.p.s. in $CDCl_3$ and at 82.5 and 85.5 c.p.s. in pyridine whereas the absorptions for the corresponding methyls of XXVIII had peaks at 82.4 and 87.0 or both at 86.6 c.p.s., in pyridine.

finding that in a system of fixed conformation the spin–spin coupling constant between neighboring hydrogens in axial orientations is about two to three times larger than that for neighboring hydrogens in other orientations. In cycloheximide and its derivatives both the 2- and 6-positions of the cyclohexane ring have axial hydrogen atoms,[13] and the ring itself is conformationally rigid.[14] Thus we can expect that in these compounds the peaks representing the C*H*OR ring protons should be sharp (s) when the OR function is axial and broad (b) when it is equatorial, because the magnetic environments of both the 2- and 6-protons are roughly the same. The peak positions are listed in Table I measured in both chloroform and pyridine,[15] methyl group absorptions also being included. In our compounds, as in the *cis*- and *trans*-4-*t*-butylcyclohexanols and their acetates reported by Lemieux,[12] the ring C*H*OR protons showed little resolution. The peaks could, however, be divided quite cleanly into two types, one having a half-height width of around 7, the other approximately 15 c.p.s., which we have designated sharp and broad, respectively. Before considering some individual compounds, it is well to note that because the side-chain C*H*OR proton is coupled with three adjacent hydrogens, two of which are not conformationally fixed, it can be expected to have a broad absorption peak regardless of its configuration. This is illustrated by the data of Table I for cycloheximide and its esters, all of which show such a peak. The position of the latter, of course, depends on the functionality of the OR group. Significantly in a number of cases examined its half-height breadth was considerably larger than 15 c.p.s.

Looking now at the data for the first dihydrocycloheximide (II), it can be seen that this compound displays both a broad and a sharp peak in its spectrum[16] as does its monoacetate (V). However, in the latter it is the broad peak which is shifted downfield so that this peak must be due to the side-chain C*H*OR proton. On the other hand, in the spectrum of the related monoacetate (IV), it is the sharp peak which is shifted downfield since in this compound the ring oxygen bears the acetyl group. Thus it can only be concluded, contrary to the Japanese work, that II and its derivatives have axial cyclohexyl oxygen functions as depicted in Chart I. This is borne out by the data for the di- and triacetylated derivatives IX and XII, respectively, and for ψ-cycloheximide acetate (VIII) which only shows a single sharp peak at 320 c.p.s.

The second dihydrocycloheximide (XI) shows two broad bands for C*H*OR absorption as does the triacetylated derivative (XIII), but, significantly, the related ψ-cycloheximide acetate (XV) exhibits only one broad C*H*OR peak at 298 c.p.s. Thus in these compounds, the ring-oxygen function must be equatorially oriented. As mentioned above, the broad peaks can be dif-

(13) Part VII: F. Johnson, N. A. Starkovsky, and W. D. Gurowitz, *J. Am. Chem. Soc.*, 87, 3492 (1965).

(14) The possibility that the dihydrocycloheximides exist in the alternate chair form of I seems unlikely since this would involve severe diaxial interaction of the groups at the 2- and 6-positions.

(15) Measurements were made in pyridine, sometimes because of solubility problems and sometimes because in chloroform solution, the hydroxyl proton peak overlapped slightly with the C*H*OR proton absorption. Changing to pyridine solution clearly indicated which peak was due to the latter, by promoting rapid exchange of the OH proton.

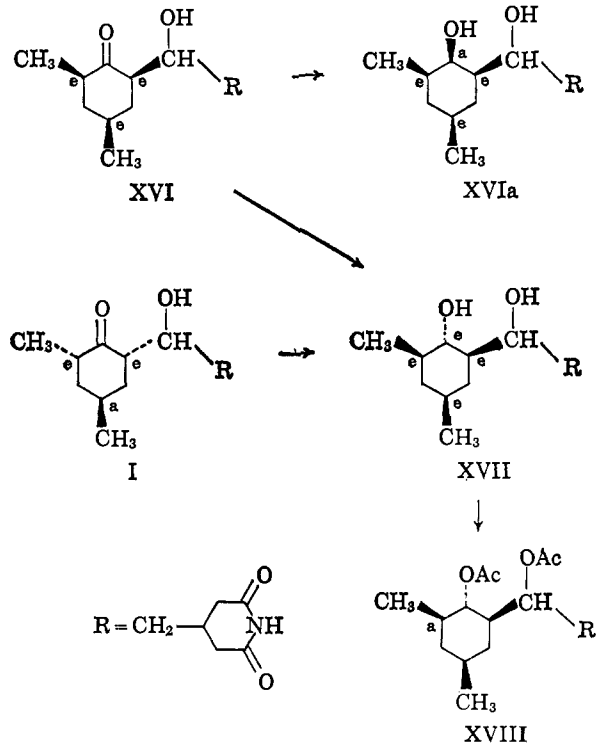
(16) All measurements recorded in this paper were made downfield from TMS (taken at 0 c.p.s.) as an internal standard (at 60 Mc.).

ferentiated on the basis of their relative half-height widths. Corroborative evidence for such differentiation of the broad peaks comes from comparing the shifts of the C*H*OR peaks between XI and its acetate (XIV) with those between II and its acetate (IV). On the basis of the above assignment, the peak due to the ring C*H*OH proton of XI undergoes a shift downfield of 54 c.p.s. whereas the side-chain C*H*OH proton peak moves 27 c.p.s. upfield. In the case of II the ring C*H*OH proton peak moves 63 c.p.s. downfield and again the side-chain C*H*OH proton shifts upfield by 26 c.p.s. In pyridine the corresponding shifts between XI and XIV are 101 c.p.s. downfield and 19 c.p.s. upfield whereas for II and IV, the values are 87 c.p.s. downfield and 31 c.p.s. upfield.

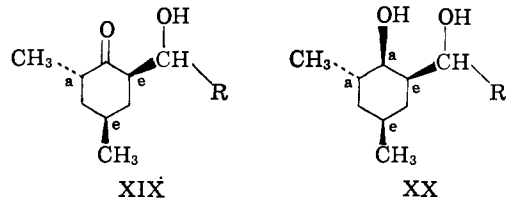
The absorption shifts for the two series compare quite well. On acetylation, the downfield shifts of the ring C*H*OR proton peaks are to be expected, but the marked upfield shifts of the corresponding side-chain hydrogen peaks are somewhat surprising. Similar behavior is to be noted for the ring C*H*OR proton peak of II when its side-chain hydroxyl is acetylated. The assignments of the ring C*H*OH protons in II and XI also find some support, albeit limited, in the extensive work of Eliel, *et al.*,[17] who found that, in general, equatorial protons of this type absorb in the range 198–250 c.p.s., whereas the corresponding axial protons have peaks in the range 145–225 c.p.s.

The methyl group absorptions of this group of compounds have been assigned by analogy with those of the related cyclohexanone compounds. In the latter the axial methyl groups are always coupled to a larger degree with the adjacent ring proton than are equatorial methyl groups.[13] Extension of this finding to compounds II to XIII permits the assignment of the methyl doublets as shown in Table I. However, that such an extension can in fact be made by examining model cyclohexanols, having conformational rigidity, has not been confirmed as yet.

B. Dihydro Derivatives of Isocycloheximide and Naramycin-B.[18] The reduction of isocycloheximide (XVI) has not been reported previously. When this was carried out catalytically, using a platinum catalyst in acetic acid, a diol (XVI), m.p. 132–133°, was obtained in good yield. On the other hand, reduction of XVa or cycloheximide with aluminum isopropoxide in benzene solution led, in moderate yield, to a new diol (XVII), m.p. 176–177°, which afforded only an amorphous diacetate (XVIII). The n.m.r. spectra of these compounds are summarized in Table II. The method of preparation of XVIa would strongly suggest that its ring hydroxyl group should be axially oriented and this is confirmed by the fact that its n.m.r. spectrum shows both sharp and broad C*H*OH proton peaks. The corresponding peaks shown by XVII and XVIII are both broad, indicating their ring oxygen functions to be equatorially oriented, as could be anticipated from the type of reduction used to prepare XVII. The catalytic reduction of naramycin-B (XIX) was carried out under the same conditions as those used for



isocycloheximide. This led to a crystalline diol (XX) which showed two C*H*OH proton peaks (Table II) in its n.m.r. spectrum, one sharp and one broad. Thus by analogy with previous work, XX must have an axial ring hydroxyl group. Unfortunately, lack of



XIX prevented examination of its reduction by complex metal hydrides.

Table II

Compd.[a]	Spectra in $CDCl_3$: C*H*OR on ring	Spectra in $CDCl_3$: C*H*OR on side chain	Spectra in pyridine: C*H*OR on ring	Spectra in pyridine: C*H*OR on side chain
Dihydroisocycloheximide (XVIa)	237s	221b	254s	234b
Dihydroisocycloheximide (XVII)[b]	...	...	231b	246b
Dihydroisocycloheximide diacetate (XVIII)	297b	278b	305b	282b
Dihydro Naramycin-B (XX)[c]	222s	233b	...	...

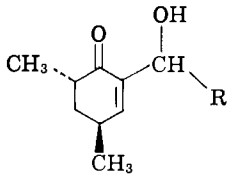
[a] The methyl doublets of these compounds were not sufficiently distinct for inclusion in the table. [b] Insoluble in chloroform. [c] Insufficient material was available for a pyridine solution spectrum.

Completion of the stereochemical assignments to the ring-hydroxyl groups of the diols II, XI, XVIa, XVII, and XX now sets the stage for the determination of configuration of the hydroxyl group of cycloheximide, its isomers, and that of inactone (XXI), which has been related to I,[13] by inference. The procedure is outlined

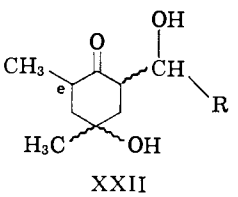
(17) E. L. Eliel, M. H. Gianni, T. H. Williams, and J. B. Stothers, *Tetrahedron Letters*, 741 (1962).

(18) Both isocycloheximide and naramycin-B, besides being natural products, can be derived from cycloheximide by base-catalyzed isomerization: T. Okuda, M. Suzuki, T. Furamai, and H. Takahashi, *Chem. Pharm Bull.* (Tokyo), **10**, 639 (1962); *ibid.*, **11**, 730 (1953).

below and should be applicable to related antibiotics such as streptovitacin-A (XXII).[19]



XXI

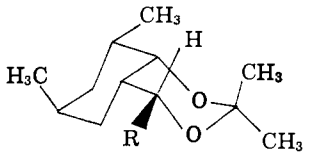


XXII

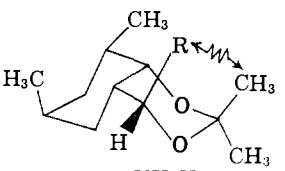
III. The Configuration of the Side-Chain Hydroxyl Group

The method used for solving this problem can be put in the form of a proposition, namely that *the determination of the configuration of the hydroxyl group of I rests solely on knowing which dihydrocycloheximide (II or XI) will form an acetonide and which will not, under comparable conditions.* Proof of this can be derived unequivocally from the following stereochemical arguments.

Since diol II has an axial ring-hydroxyl group, the two theoretically possible acetonides are XXIII and XXIV, depending on the relative position of the side-chain hydroxyl group. If the latter has the (*R*) configuration, then acetonide XXIII formation should be possible

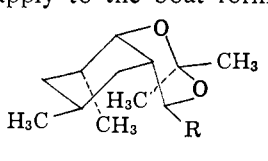


XXIII



XXIV

because here the 3-glutarimidomethyl group (R) necessarily assumes an equatorial orientation in the ketal ring. Thus in this case there is little steric resistance to ketal formation. On the other hand, if the side-chain hydroxyl has the opposite configuration (*S*), the ketal to be expected would be XXIV. In this latter molecule, there is a large, nonbonded 1,3-interaction between the R group, which now is necessarily axial, and the axial methyl group of the isopropylidene function. Thus if XXIV could be expected to form at all, its rate of formation could be expected to be very slow by comparison with that of XXIII. The possibility that ketal formation in this case could occur *via* the other chair conformer of diol II seems remote since this form itself contains a 1,3-diaxial interaction involving the 2- and 6-groups of the cyclohexane ring. Even so, the ketal that would arise from such a conformer would have structure XXV, wherein the mutual interference of the methyl groups would be so great that it would undoubtedly prohibit the formation of such a molecule under normal conditions. Similar considerations apply to the boat form of either ring.

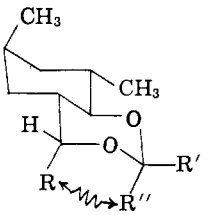


XXV

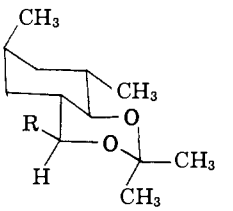
On the basis of the above arguments, the fact, that *experimentally, diol II does give an acetonide under very mild conditions* is then compelling evidence that the side-chain asymmetric center of cycloheximide has the (*R*) configuration[20] as shown in I.[21]

It is pertinent to note that this acetonide (XXIII) can be hydrolyzed back under acidic conditions to diol II in quantitative yield. Thus its formation is not accompanied by any isomerization such as was observed during the formation of isopropylidene derivatives of the flavan-3,4-diols.[22]

A logical deduction from this assignment is that the epimeric diol (diol XI) should not give an acetonide or, at best, should form an acetonide very slowly. This follows because the expected acetonide should have structure XXVI (R′ = R″ = CH_3) where once again the 1,3-diaxial interaction discussed above is



XXVI



XXVII

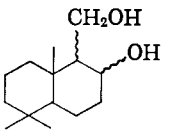
present. Experimentally it did not prove possible to prepare an acetonide from XI. Under all conditions tried, only starting material was recovered, thus confirming the arguments and the (*R*) assignment made above. As might be anticipated, diol XI rather slowly does give a benzylidene derivative (XXVI, R′ = C_6H_5; R″ = H) since the nonbonded steric interaction arising only from the axial R group and a hydrogen atom (the phenyl group is undoubtedly equatorial) is much less in this case.

In the light of the above comments, the acetonide XXVII could now be expected to form from a molecule having the (*S*) configuration in the side chain. Unfortunately derivatives of this series having such a mirror-image side chain were not available for examination. Nevertheless, in the diols XVIa and XVII from isocycloheximide we had available the alternative and next best possibility, namely two compounds whose substituents at the 1,2- and 6-positions of their cyclohexanone rings are the mirror images of diols II and XI, respectively. If we assume for the moment that the side-chain asymmetric center of isocycloheximide (and naramycin-B) has the (*R*) configuration,[18] it is possible to predict from the foregoing results that diol XVII, in which the ring hydroxyl is equatorial, should

(19) R. R. Herr, *J. Am. Chem. Soc.*, **81**, 2595 (1959).

(20) Although this represents a reversal of the assignment made by T. Okuda,[3] his data concerning the borate ester of II are still valid and add to our argument, since we have also reassigned the opposite configuration to the ring hydroxyl group.

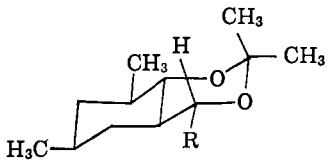
(21) An interesting and related application of the formation of acetonides from 1,3-diols has been reported by Stadler, *et al.*, *Helv. Chim. Acta*, **40**, 1373 (1957). They studied the ability of the four isomers of the 1,3-diol i to form acetonides under standard conditions. They obtained high yields only in the absence of appreciable nonbonded interactions. The latter, when present, were of the interannular type (represented for example by XXV above) and not of the intraannular kind with which, principally, we are dealing here.



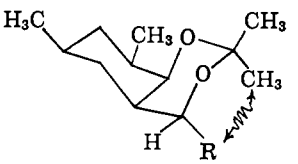
i

(22) S. Fujise, S. Hiskida, T. Onuma, K. Adachi, Y. Fujise, and T. Munekata, *Bull. Chem. Soc. Japan*, **35**, 1245 (1962).

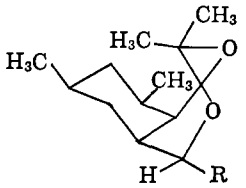
form an acetonide (XXVIII) whereas XVIa, in which the ring hydroxyl is axial, should not. In the latter case this would again be a result of the steric interaction shown in the expected product XXIX. The experimental findings were completely in accord with these predictions, XVII affording an isopropylidene derivative, whereas XVIa did not. It should be noted that



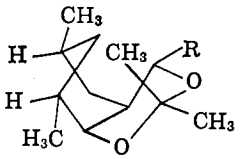
XXVIII



XXIX



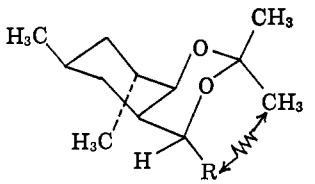
XXX



XXXI

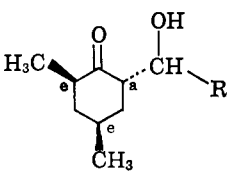
other possible conformations of the acetonide XXIX, such as XXX and XXXI, are more hindered internally and are even less likely to exist than XXIX.

In the case of the diol XX derived from naramycin-B in which the ring hydroxyl is axially oriented, attempted formation of an acetonide was unsuccessful. Once again the expected compound XXXII would have a severe 1,3-diaxial interaction in the ketal ring.



XXXII

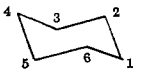
The results obtained then with the diols of isocycloheximide and naramycin-B conclusively demonstrate that their side-chain asymmetric centers have the (*R*) configuration in common with cycloheximide and its derivatives. Implicit in the argument also is confirmation of what has always been assumed, that is, that base-catalyzed isomerization of I does not affect the side-chain asymmetric center.[23] Apart from α-epi-



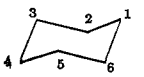
XXXIII

isocycloheximide, the only isomer in which the relative configuration of the side-chain hydroxyl group remains unknown is neocycloheximide[24] (XXXIII). In this compound the side chain is axially oriented[12] and it is obvious from models that no matter what the relative orientations of the hydroxyl group might be in the dihydroneocycloheximides, no acetonide formation could be expected, as is borne out by experimental findings.[25]

On the basis of the above work, it is possible to predict which other diols of this series will or will not form



normal



iso

acetonides. If we consider the absolute configurational conformation of the cyclohexanone ring of cycloheximide as normal and that of isocycloheximide and naramycin-B as iso as seen above, the results and predictions concerning acetonide formation can be summarized as shown in Table III. (These data obviously also apply to the mirror images of the compounds listed.)

Confirmation of some of these predictions and the use of this method to determine the relative orientation of the side-chain hydroxyl groups of a number of synthetic diols will be included in future publications.

Experimental Section

Melting points are uncorrected. N.m.r. spectra were recorded with an A-60 Varian spectrometer and infrared spectra were obtained as Nujol mulls (unless otherwise stated) with a Baird Model 4-55 recording spectrometer.

Reductions of Cycloheximide Acetate (III). Preparation of IV and V. A. H_2–Pt. A solution of cycloheximide acetate (3.0 g.) in acetic acid (100 ml.) was added to a prereduced platinum catalyst (from 0.5 g. of PtO_2) in acetic acid (50 ml.). The mixture was stirred with hydrogen at room temperature and pressure until gas absorption ceased (16 hr.). The catalyst was removed by filtration and the solvent was evaporated at reduced pressure. The residual colorless glass was taken up in methylene chloride and the solution was washed with mild aqueous base and water and dried over anhydrous magnesium sulfate. The methylene chloride solution was then concentrated on a steam bath with repeated addition of ether until crystallization commenced. The initial crude product (2.6 g.), m.p. 150–155°, was repeatedly crystallized from methylene chloride–ether and afforded the pure hydroxyacetate IV (1.05 g.), m.p. 168–169.5°, $[\alpha]^{26}D$ +19° (*c* 2.0, CH_3OH) (lit.[3] m.p. 165°, $[\alpha]^{19}D$ +29° (*c* 1.0, CH_3OH)). Its infrared spectrum showed bands at 2.86 (OH), 2.94 and 3.06 (NH), 5.78, 5.82, 5.91, 7.81, 7.97, 8.70, 9.26, 9.42, 9.60, 9.74, 10.18, 11.42, and 11.97 μ.

The mother liquors from the first crystallization above were evaporated to low bulk and afforded a crude white solid (0.5 g.). Crystallization of this material from methylene chloride gave a mixture of rosette-shaped and small, cubic crystals. The former were hand picked from the liquid and twice recrystallized from the same solvent pair. This gave the pure isomeric hydroxyacetate V (75 mg.), m.p. 177–179°, which did not depress the melting point of a specimen prepared according to method B below.

B. LiAlH(t-OBu)$_3$. A solution of cycloheximide acetate (0.5 g.) in dry tetrahydrofuran (10 ml.) was

(23) This center would of course be involved if the formation of isocycloheximide and naramycin-B from cycloheximide were to proceed *via* consecutive retroaldol and aldol reactions.

(24) For the purposes of simplification, we have chosen to deal with only one of the enantiomers of this compound although in reality the substance is racemic.

(25) This work will be reported in a later publication.

Table III

Series	Orientation of cyclohexane ring substituents: 2-CH_3	4-CH_3	−OH	Side chain	Config. of asymmetric center	Acetonide formation	Parent hydroxy ketone
Normal	Eq.	Ax.	Ax.	Eq.	(*R*)	Possible (XXIII)	Cycloheximide
	Eq.	Ax.	Eq.	Eq.	(*R*)	Impossible (XXVI)	
	Eq.	Ax.	Ax.	Eq.	(*S*)	Impossible (XXIV)	α-Epicycloheximide
	Eq.	Ax.	Eq.	Eq.	(*S*)	Possible (XXVII)	
Iso	Ax.	Eq.	Ax.	Eq.	(*R*)	Impossible (XXXII)	Naramycin-B
	Ax.	Eq.	Eq.	Eq.	(*R*)	Possible	
	Ax.	Eq.	Ax.	Eq.	(*S*)	Possible	α-Epinaramycin-B
	Ax.	Eq.	Eq.	Eq.	(*S*)	Impossible	
	Eq.	Eq.	Ax.	Eq.	(*R*)	Impossible (XXIX)	Isocycloheximide
	Eq.	Eq.	Eq.	Eq.	(*R*)	Possible (XXVIII)	
	Eq.	Eq.	Ax.	Eq.	(*S*)	Possible	α-Epiisocycloheximide
	Eq.	Eq.	Eq.	Eq.	(*S*)	Impossible	
	Eq.	Eq.	Ax.	Ax.	(*R*)	Impossible	Neocycloheximide and α-epineocycloheximide
	Eq.	Eq.	Eq.	Ax.	(*R*)	Impossible	
	Eq.	Eq.	Ax.	Ax.	(*S*)	Impossible	
	Eq.	Eq.	Eq.	Ax.	(*S*)	Impossible	

added to $LiAlH(t\text{-}OBu)_3$ (0.5 g.) in the same solvent (10 ml.) at 0 to −5° with stirring during 15 min. After stirring for 2 hr. at this temperature, water (10 ml.) was added, followed by 20% acetic acid (10 ml.). The solvents were then removed at less than 40° under reduced pressure. The glassy residue was treated with water and methylene chloride and the organic layer was removed and worked up as in A above. The product was crystallized from ether and gave white, needle-shaped crystals, m.p. 150–155° (0.1 g.). Repeated crystallization from methylene chloride–ether mixtures gave the pure hydroxyacetate IV (25 mg.), m.p. 169–170°, $[\alpha]^{26}D$ +19.3° (*c* 1.5, CH_3OH), which did not depress the melting point of a specimen prepared according to procedure A above.

The mother liquors from above were concentrated and afforded 0.3 g. of crystalline material. Recrystallization of this material from aqueous methanol then gave pure V, m.p. 177–179°, $[\alpha]^{26}D$ +31.7° (*c* 2.4, CH_3OH) (lit.[3] m.p. 177–178°, $[\alpha]^{19}D$ +34.3° (*c* 2.0, CH_3OH)). The compound exhibited bands in its infrared spectrum at 2.83 (OH), 3.11 and 3.22 (NH), 5.78, 5.81, 5.91, 7.74, 7.90, 8.01, 8.12, 8.60, 9.45, 9.65, 9.74, 10.13, and 10.80 μ.

Oxidation of V to Cycloheximide Acetate (III). A sample (0.5 g.) of V was stirred with a cold 2% solution (50 ml.) of chromium trioxide in 96% acetic acid until the mixture was homogeneous. After 3 hr. at 25° the solution was diluted with water and extracted with methylene chloride. Evaporation of the solvent afforded cycloheximide acetate (0.34 g.), m.p. 151–152°, $[\alpha]^{26}D$ +21° (*c* 3.6, methanol), identical with the product obtained by the direct acetylation of cycloheximide (lit.[4,26] m.p. 148–149° and 150–152°, $[\alpha]^{25}D$ +22° (methanol)).

ψ-Cycloheximide Acetate (VIII). Oxidation of IV (0.5 g.) under conditions identical with those described for the preparation of III above afforded VIII which crystallized from methylene chloride–ether as colorless needles (0.32 g.), m.p. 150–151°, $[\alpha]^{25}D$ −25° (*c* 3.5, methanol). Its infrared spectrum showed bands at 3.02, 3.23, 5.74, 5.82–5.90, 7.75, 7.90, 8.04, 8.10, 8.66, 9.75–9.86, and 11.86 μ.

(26) J. H. Ford and B. E. Leach, *J. Am. Chem. Soc.*, 70, 1223 (1948).

Anal. Calcd. for $C_{17}H_{25}NO_5$: C, 63.1; H, 7.8; N, 4.3. Found: C, 63.3; H, 7.6; N, 4.3.

Dihydrocycloheximide Diacetate (IX). A solution of II in acetic anhydride containing 3 drops of pyridine was allowed to stand at room temperature for 2 days. After evaporation of the solvents under vacuum, the gummy residue was repeatedly crystallized from ether–petroleum ether (b.p. 30–60°) giving colorless needles of IX, m.p. 80–90° (effervescing), becoming fluid at 90–93°. The analytical sample was dried at 80° and obtained as a glass which became mobile at 90–93°, $[\alpha]D$ +13.0° (*c* 3.9, chloroform). Its infrared spectrum showed bands at 3.12 and 3.22 (NH), 5.75 and 5.86 (C=O), 7.90, 8.05, 8.69, 9.41, 9.57, 9.76, 10.22, 10.64, and 11.39 μ. In chloroform solution (10 mg./ml.) the characteristic bands occurred at 2.96 (NH), 5.75–5.85 (C=O), 7.27, 8.00–8.35, 8.29, and 9.75 μ. Its n.m.r. spectrum showed the presence of only two acetyl methyl singlets at 123 and 125 c.p.s.

Anal. Calcd. for $C_{19}H_{29}NO_6$: C, 62.1; H, 8.0; N, 3.8. Found: C, 62.0; H, 7.7; N, 4.1.

IX, exhibiting properties identical with those described above, was obtained on acetylation of IV or V.

N-Acetyldihydrocycloheximide Diacetate (XII). A solution of II (0.6 g.) in acetic anhydride (2 ml.) and pyridine (0.5 ml.) was heated for 16 hr. on a steam bath. The excess organic liquids were removed *in vacuo* and the residue was taken up in methylene chloride and chromatographed over silica gel (15 g.). Elution with methylene chloride containing 10% ethyl acetate afforded the product XII as a colorless glassy oil (66 mg.) which refused to crystallize. Its infrared spectrum showed no absorption in the 2.5–3.4-μ region but had bands at 5.58, 5.72, 5.78, and 5.90 μ indicative of N- and O-acetyl functions and an imide group. Its n.m.r. spectrum was also indicative of a triacetyl compound having three singlet peaks for acetyl hydrogen at 124, 126, and 146 c.p.s.

N-Acetyldihydrocycloheximide Diacetate (XIII). A mixture of XI (0.2 g.), anhydrous sodium acetate (1.25 g.), and acetic anhydride (20 ml.) was heated on the steam bath for 16 hr. Isolation of the product, as in the case of XII above, afforded a crude material (0.22 g.) which was dissolved in methylene chloride and

chromatographed over silica gel (8 g.). After washing the column with the latter solvent, the product was eluted with methylene chloride containing 10% ethyl acetate. Crystallization from ether–petroleum ether (b.p. 30–60°) gave pure XIII as colorless needles (82 mg.), m.p. 119–120°, $[\alpha]^{24}D$ +60.9° (*c* 1.0, $CHCl_3$). Its infrared spectrum showed carbonyl absorption at 5.63, 5.78, and 5.93 and other bands at 7.86, 7.91, 8.05, 8.45, 8.65, 9.26, 9.47, 9.60, 9.75, 10.52, 10.77, and 11.26 μ. Acetyl hydrogen absorption occurred at 124, 126, and 148 c.p.s. in the n.m.r. spectrum.

Anal. Calcd. for $C_{21}H_{31}NO_7$: C, 62.2; H, 7.6; N, 3.4. Found: C, 62.5; H, 7.3; N, 3.5.

Dihydrocycloheximide (*XI*). *A.* A solution of cycloheximide (5.2 g.) in dry tetrahydrofuran (40 ml.) was slowly added at 0°, with efficient stirring, under nitrogen, to a solution of diphenyltin dihydride (19.8 g.) in dry ether (40 ml.). The mixture was stirred overnight, then filtered, and concentrated under reduced pressure to a viscous gum (26 g.). The latter was dissolved in methylene chloride (100 ml.), stirred for 30 min. to convert the diphenyltin dihydride to the dichloride, and then chromatographed over silica gel (100 g.). The first fractions eluted with methylene chloride were largely diphenyltin dichloride. Later fractions eluted with 75% ethyl acetate–methylene chloride (1 l.) afforded crude II (4.0 g.) which after crystallization from methylene chloride ether gave the pure compound (2.2 g.), m.p. 131–132°, identified spectrally and by a mixture melting point determination with an authentic sample.

Further elution of the column with ethyl acetate (200 ml.) and ethyl acetate containing 10% methanol (300 ml.) led to crude XI (0.82 g.). Two recrystallizations from methylene chloride–ether yielded the pure material (0.33 g.), m.p. 166–167°, $[\alpha]^{26}D$ 0° (*c* 1.0, CH_3OH). Its infrared spectrum showed bands at 2.91 and 2.97 (OH), 3.06 (NH), 5.77, 5.82–5.89, 7.76, 7.92, 8.06, 8.63, 9.30, 9.39, 9.50, 9.59, 9.68, 10.11, 11.41, and 11.68 μ.

Anal. Calcd. for $C_{15}H_{25}NO_4$: C, 63.6; H, 8.9; N, 4.9. Found: C, 63.4; H, 8.9; N, 5.2.

B. A solution of lithium aluminum hydride (0.5 g.) in dry freshly distilled tetrahydrofuran was treated with *t*-butyl alcohol (0.7 g.) and, after allowing the mixture to stand at room temperature for 1 hr., with cycloheximide (2.80 g.) in tetrahydrofuran (20 ml.). During the latter addition the temperature was maintained below 0° and the mixture was stirred efficiently. After 1 additional hr. at 0° an excess of cold 1% acetic acid was added followed by a few milliliters of cold, 10% hydrochloric acid. After working up in the usual way (dilution with ice–water and extraction with methylene chloride), an oily residue was obtained, which after two crystallizations from methylene chloride–ether gave XI (~0.8 g.), m.p. 166–167°. This did not depress the melting point of a specimen prepared according to method A above, and had the same infrared spectrum.

Dihydro-ψ-cycloheximide Acetate (*XIV*). A solution of the diol XI (0.4 g.) in dry pyridine (5 ml.) was treated at 0°, dropwise, while stirring, with acetyl chloride (0.15 ml.) in methylene chloride (5 ml.). After stirring at 0° for 2 hr., the solution was poured into a mixture of ice and water, then extracted with methylene chloride. Evaporation of the washed extract afforded a gum which crystallized from methylene chloride–ether as needles (0.3 g.), m.p. 184°. The mother liquors afforded three further crops of the same material (total 54 mg.) when carefully fractionated. Its infrared spectrum showed characteristic peaks at 2.84 (OH), 3.14 and 3.28 (NH), 5.81, 5.89, 7.69, 7.86, 8.70, and 9.55 μ, and its n.m.r. spectrum in deuteriochloroform showed only one peak due to the acetyl hydrogens at 129 c.p.s.

Anal. Calcd. for $C_{17}H_{27}NO_5$: C, 63.1; H, 8.3; N, 4.3. Found: C, 63.0; H, 8.2; N, 4.5.

ψ-Cycloheximide Acetate (*XV*). This was prepared from XIV (0.1 g.) by the method described above for the oxidation of V to cycloheximide acetate. The crude product was crystallized from ether–petroleum ether (b.p. 30–60°) and afforded XV (58 mg.), m.p. 123° (lit.[9] m.p. 119.5–120.5°). It showed characteristic absorption in the infrared spectrum having bands at 3.14 and 3.22 (NH), 5.77, 5.86, 7.60, 7.86, 8.09, 8.65, 8.91, 9.18, 9.60, 9.73, and 11.23 μ.

Anal. Calcd. for $C_{17}H_{25}NO_5$: C, 63.1; H, 7.8; N, 4.3. Found: C, 63.0; H, 7.8; N, 4.5.

Dihydroisocycloheximide (*XVI*). Isocycloheximide (1.0 g., m.p. 97–99°) in acetic acid (40 ml.) was hydrogenated over a platinum catalyst (from 0.4 g. of PtO_2) at room temperature and pressure until hydrogen uptake ceased (12 hr.). The acetic acid was removed under reduced pressure and the residue was crystallized from chloroform and then water. The purified product (0.5 g.) had m.p. 132–133°, $[\alpha]^{24}D$ +19.1° (*c* 1.0, $CHCl_3$). Its infrared spectrum in chloroform solution showed bands at 2.89, 2.95, 5.84, 8.40, 8.71, 9.67, 10.10, 10.54, 10.84, and 11.39 μ.

Anal. Calcd. for $C_{15}H_{25}NO_4$: C, 63.6; H, 8.9; N, 4.9. Found: C, 63.6; H, 8.9; N, 5.0.

Dihydroisocycloheximide (*XVII*). *l*-Cycloheximide (4.0 g., m.p. 114–116°) was refluxed for 2 hr. with aluminum isopropoxide (6.12 g.) in dry benzene (25 ml.). The reaction mixture was cooled and poured into a mixture of ice–water (100 ml.) and methylene chloride. After filtration, the organic layer was separated and washed with cold 1 *N* hydrochloric acid, saturated sodium bicarbonate solution, then water. The organic phase was dried over anhydrous magnesium sulfate and evaporated to dryness. Crystallization of the resulting gummy solid from methylene chloride–ether afforded the diol (1.05 g.) as colorless needles, m.p. 176–177°, $[\alpha]^{24}D$ +77.4° (*c* 1.0, CH_3OH). Its chloroform solution spectrum showed bands at 2.87, 2.97, 5.85, 8.86, 8.73, 9.32, 9.75, 10.27, 10.47, 10.88, and 11.21 μ.

Anal. Calcd. for $C_{15}H_{25}NO_4$: C, 63.6; H, 8.9; N, 4.9. Found: C, 63.5; H, 8.7; N, 5.1.

Substitution of isopropyl alcohol for benzene in the above reaction afforded XVII in 33% yield. When isocycloheximide was used in place of cycloheximide similar yields of XVII were obtained.

Dihydroisocycloheximide Diacetate (*XVIII*). Diol XVII (0.5 g.) was allowed to stand overnight with pyridine (2 ml.) and acetic anhydride (2 ml.). The mixture was poured into water and stirred for 30 min. Extraction of this liquid with methylene chloride followed by the usual isolation procedures afforded a

frothy solid (0.45) which defied crystallization. It was dissolved in methylene chloride and adsorbed on a column of silica gel (15 g.). The column was washed with methylene chloride (50 ml.) and the diacetate was eluted with 100 ml. of the same solvent containing 15% ethyl acetate. Evaporation of the solvent led to an amorphous solid, m.p. *ca.* 100°, whose n.m.r. spectrum was characteristic of a pure diacetate. Its infrared spectrum showed bands at 3.10, 3.22, 5.77, 5.90, 8.08, 8.66, 9.70, 10.13, 10.65, and 11.19 μ.

Anal. Calcd. for $C_{19}H_{29}NO_6$: C, 62.1; H, 8.0; N, 3.8. Found: C, 61.9; H, 7.8; N, 3.8.

Dihydronaramycin-B (XX). Naramycin-B[27] (0.1 g., m.p. 110–112°, $[\alpha]^{24}D$ +54°) was reduced catalytically under the conditions described above for the preparation of XVI. The crude glassy material (75 mg.) thus obtained was dissolved in methylene chloride and chromatographed over silica gel (4 g.). Elution of the column with methylene chloride containing 25% ethyl acetate (100 ml.) afforded only an oily substance (12 mg.) which was discarded. Elution with ethyl acetate (50 ml.), however, gave a crystalline product (48 mg.) which was further purified by recrystallization from ether–petroleum ether (b.p. 30–60°) mixtures. The pure compound had m.p. 164–165°, $[\alpha]^{24}D$ +16° (*c* 0.7, chloroform). Its chloroform solution showed bands in the infrared spectrum at 2.95, 3.06, 3.13, 3.25, 5.80, 5.94, 7.72, 7.86, 8.60, 8.79, 9.30, 9.60, 11.80, and 12.00 μ.

Anal. Calcd. for $C_{15}H_{25}NO_4$: C, 63.6; H, 8.9; N, 4.9. Found: C, 63.7; H, 8.9; N, 4.8.

Preparation of Acetonides. Both of the following methods were applied to the diols under discussion.

A. The diol (0.2 g.) was refluxed in acetone (20 ml.) in the presence of anhydrous copper sulfate for 16 hr. After filtration the solution was evaporated to dryness and the residue was crystallized from an appropriate solvent.

(27) This sample, which contained about 30% cycloheximide, was as pure as we or other workers have been able to obtain.

B. The diol (0.2 g.) in acetone (20 ml.) was treated at 0° with a cold solution of sulfuric acid (1 g.) in acetone (20 ml.). After 2 hr. at room temperature the mixture was poured into excess sodium bicarbonate solution. The latter was extracted with methylene chloride and the extract was dried and evaporated under reduced pressure. The residue was then crystallized from a suitable solvent.

In this way diol II afforded acetonide XXIII which crystallized from petroleum ether (b.p. 30–60°) in almost quantitative yield, m.p. 141.5°, $[\alpha]^{26}D$ 0° (*c* 2.0, $CHCl_3$). Its infrared spectrum showed absorption at 3.10, 3.2, 5.76, 5.85, 5.92, 7.06, 7.91, 8.30, 8.50, and 8.63 μ.

Anal. Calcd. for $C_{18}H_{29}NO_4$: C, 66.9; H, 9.0; N, 4.3. Found: C, 67.0; H, 9.2; N, 4.6.

Similarly dihydroisocycloheximide (XVII) afforded an excellent yield of the acetonide XXVIII which crystallized from aqueous acetone, m.p. 126°. Its infrared absorption spectrum showed peaks at 3.12, 3.23, 5.79, 5.90, 7.92, 8.30, 8.50, and 8.70 μ.

Anal. Found: C, 67.2; H, 8.8; N, 4.2.

The diols XI, XVI, and XX were recovered in good yield when subjected to either of the above conditions.

Benzylidene Derivative (XXVI, R′ = C_6H_5; R′ = H). A solution of diol XI (0.12 g.) in ether (3 ml.) was treated with benzaldehyde (0.4 g.) and a trace (14 mg.) of *p*-toluenesulfonic acid. After standing for 2 days at room temperature the crop of crystals which had deposited was separated (0.1 g.). Two recrystallizations from methylene chloride–ether afforded the pure benzylidene compound as thick, flat plates, m.p. 201–202°. Its infrared spectrum showed significant bands at 3.07, 3.20, 5.76–5.89, 7.85, 7.96, 8.03, 8.71, 9.07, 10.02, 12.20, 13.35, 13.35, 13.94, and 14.24 μ.

Anal. Calcd. for $C_{22}H_{29}NO_4$: C, 71.1; H, 7.9; N, 3.8. Found: C, 70.9; H, 7.8; N, 3.9.

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