acid[18] was refluxed on a steam bath for 30 min. and then was evaporated to dryness. Upon dissolving the residue in ether and adding ether saturated with hydrogen chloride, S-benzoyl-L-cysteinylglycine ethyl ester hydrochloride separated as an oil. The supernatant liquor was decanted and the hydrochloride was washed several times with ether, each washing being followed by decantation. The remaining solvent was removed by evaporation. In the meantime, a solution containing 0.63 g. (0.003 mole) of carbobenzoxyglycine, 25 ml. of chloroform, and 0.42 ml. of triethylamine was cooled to $-5°$ and 0.39 ml. of isobutyl chloroformate was added. The solution of the anhydride was allowed to stand at $-5°$ for 15 min. and was added to the dipeptide hydrochloride, followed by the dropwise addition of 0.42 ml. of triethylamine. After being allowed to stand at room temperature overnight, the solution was diluted with chloroform and washed successively with 1 *N* hydrochloric acid, potassium hydrogen carbonate solution, and water, dried over sodium sulfate, and evaporated to dryness. The residue was crystallized from acetone–petroleum ether; the yield was 0.73 g. (49%), m.p. 105–108°, $[\alpha]^{18}D$ $-30.8°$ (*c* 2, dimethylformamide).

Anal. Found: C, 57.77; H, 5.56; S, 6.22.

N-Carbobenzoxyglycyl-S-acetyl-L-cysteinylglycine Ethyl Ester (VIIb). To a solution of 0.10 ml. of thioacetic acid in 1 ml. of ethyl acetate, 0.175 ml. of triethylamine and 0.135 g. (0.00025 mole) of O-tosyltripeptide VI were added. The solution was allowed to stand at 20° for 24 hr. It was diluted with ethyl acetate and washed with cold 1 *N* sulfuric acid, water, potassium hydrogen carbonate solution, and water, dried over sodium sulfate, and evaporated to dryness. Crystallization of the residue from acetone–petroleum ether yielded 0.091 g. (83%) of VIIb, m.p. 90–95°. After recrystallization from acetone–petroleum ether (recovery 80%), the melting point was 92–95°, $[\alpha]^{19}D$ $-28.6°$ (*c* 1.5, dimethylformamide), R_f 0.15.

Anal. Calcd. for $C_{19}H_{25}N_3O_7S$: C, 51.93; H, 5.73; N, 9.56; S, 7.29. Found: C, 51.92; H, 5.98; N, 9.66; S, 6.89.

Acknowledgments. The authors wish to thank Dr. H. Mantzos for the microanalyses and Dr. G. C. Stelakatos for help in preparing the manuscript.

(18) F. Weygand and W. Steglich, *Z. Naturforsch.*, **14b**, 472 (1959).

Glutarimide Antibiotics. VII. The Synthesis of *dl*-Neocycloheximide and the Determination of the Cyclohexanone Ring Stereochemistry of Cycloheximide, Its Isomers,[1] and Inactone

Francis Johnson, N. A. Starkovsky, and William D. Gurowitz

Contribution from The Dow Chemical Company, Eastern Research Laboratory, Framingham, Massachusetts. *Received March 6, 1965*

The Nielsen condensation of dl-cis-2,4-dimethylcyclohexanone with 3-glutarimidylacetaldehyde has been examined as a synthetic approach to compounds having the gross structure of cycloheximide (I). The sole crystalline product from the reaction is a new isomer of I and has been named dl-neocycloheximide. A combination of chemical methods and n.m.r. spectroscopy has been used to elucidate completely the cyclohexanone ring stereochemistry of cycloheximide and its stereochemical isomers naramycin-B, isocycloheximide, and neocycloheximide. The skeletal structure of inactone has been confirmed and its stereochemistry has been elucidated. Comments on the stereochemistry of E-73 and streptovitacin-A are noted.

Introduction

The glutarimide antibiotics constitute a truly fascinating group of mold products. Their diverse spectrum of biological activity alone serves to place them in a unique category insofar as naturally occurring organic compounds are concerned. To date twelve distinct substances belonging to this class have been isolated, the best known of these being cycloheximide[2] (I). This particular compound not only is a highly effective fungicide,[2,3] having excellent systemic activity against tomato late blight, cherry leaf spot rust, and white pine blister rust, but is also the most potent rodent repellent known.[4] It also shows toxicity toward algae,[5] protozoa,[6] higher plants,[7] and animals.[8] In addition it has marked antitumor activity[9] but suffers from being somewhat too toxic to the host to be used in this respect. Recently it has been shown that one of

(1) A preliminary account of this work has appeared: F. Johnson, W. D. Gurowitz, and N. A. Starkovsky, *Tetrahedron Letters*, 1167, 1173 (1962).

(2) A. J. Whiffen, J. N. Bohonas, and R. L. Emerson, *J. Bacteriol.*, **52**, 610 (1946); B. E. Leach, J. H. Ford, and A. J. Whiffen, *J. Am. Chem. Soc.*, **69**, 474 (1947).

(3) J. R. Vaughn, *Phytopathology*, **41**, 36 (1951); T. T. McLure, *ibid.*, **42**, 14 (1952); H. D. Wells and B. P. Robinson, *ibid.*, **44**, 509 (1954); D. Cation, *Am. Fruit Grower*, **74**, 29 (1954); J. M. Hamilton and M. Szkolnik, *Proc. N. Y. State Hort. Soc.*, 58 (1955).

(4) J. F. Welch, *J. Agr. Food Chem.*, **2**, 142 (1954).

(5) C. Palmer and T. E. Maloney, *Ohio J. Sci.*, **55**, 1 (1955).

(6) J. B. Loefer and T. S. Matney, *Physiol. Zool.*, **25**, 272 (1952).

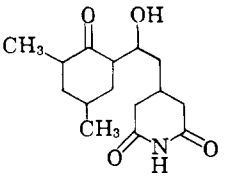
(7) J. H. Ford, W. Klomparens, and C. L. Hamner, *Plant Disease Reptr.*, **42**, 680 (1958).

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

(9) J. C. Bateman and C. T. Klopp, *Proc. Am. Assoc. Cancer Res.*, 1, 3 (1953); H. C. Reilly, C. C. Stock, S. M. Buckley, and D. A. Clark, *Cancer Res.*, **13**, 684 (1953); B. Sokoloff and F. Homburger, "Progress in Experimental Tumor Research," Vol. 1, J. B. Lippincott Co., Philadelphia, Pa., 1960, p. 360.

the modes of action of I is that of inhibiting protein synthesis.[10]

Undoubtedly this broad biological activity coupled with the ostensible simplicity of its molecular structure have been responsible in recent years for the heightened chemical interest in this compound. From the organic viewpoint the question of synthesizing I poses an interesting challenge. However, in 1958 when we began our studies only the gross structure (Ia) of I had been elucidated[11] and no stereochemical studies

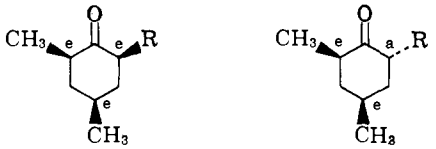


Ia

had been made. Djerassi, *et al.*,[12] however, had succeeded in showing that the 4-methyl group of I has the (*S*) configuration, and one somewhat premature attempt[13] also had been made to synthesize I.

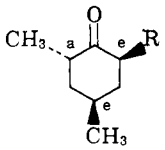
During the past 5 years several attempts have been made to deduce the fine structure of I and its naturally occurring stereoisomers naramycin-B[14] (II) and isocycloheximide[15] (III).

The earliest attempt was that of Okuda[16] using an O.R.D. method. Of the four stereoisomers (Ib–e) possible, Ib was tentatively assigned to naramycin-B



Ib

Ic



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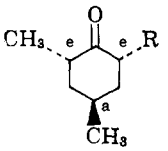
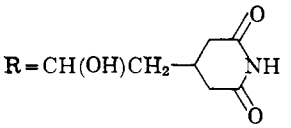


Ie



and Ic to cycloheximide, although it was thought that the latter existed as the 2-axial, 4-axial, 6-equatorial conformer. In a later publication[17] in which the O.R.D. curves of isocycloheximide (III) and naramycin-B were studied it was concluded that the former should be represented by Ib and the latter by Id. In the interim Lawes[18] showed by pyrolysis experiments that the methyl groups in cycloheximide are *trans* oriented and he advanced arguments[19] which supported Id for this compound. That the methyl groups in I are *trans* oriented was also shown quite independently by Schaeffer and Jain[20] although the evidence which formed the basis for this conclusion is now in doubt.[21] Subsequently, in a refined analysis of the optical rotatory dispersion data, Okuda and Suzuki[22] concluded that cycloheximide has structure Ie rather than Ic. The Japanese workers have followed this up by applying Lawes' pyrolysis procedure to naramycin-B and isocycloheximide.[23] As expected, the former affords *trans*-2,4-dimethylcyclohexanone and the latter yields the corresponding *cis* isomer.

One of the difficulties implicit in O.R.D. work is the inaccessibility to analysis of substituents in the 4-position of a cyclohexanone ring. This together with the fact that little previous stereochemical work had been done on simple 2,4,6-trisubstituted cyclohexanones undoubtedly accounts for most of the trouble encountered in determining the stereochemistry of I and its isomers. In addition O.R.D. analysis cannot be applied to racemic compounds and, as we expected to be working with this type of material, we sought an alternative approach for the determination of the stereochemistry of these substances.

We would now like to report in full[1] unequivocal methods which lead to the same stereochemical conclusions that were finally obtained by the Japanese workers, but which have the advantage of being applicable to the racemic as well as to the optically active series of compounds. We were aided in this, quite fortuitously, by some early synthetic work, which will be described first.

Synthetic Aspects

The initial optical rotatory dispersion analysis[16] led us to believe that cycloheximide could be synthesized directly *via* some form of aldol condensation between *cis*-2,4-dimethylcyclohexanone (IV) and 3-glutarimidylacetaldehyde (V).[24] However because of the well-established sensitivity of I to base it seemed that the condensation would have to be carried out under neutral or only slightly basic conditions. First, therefore, we examined the Schöpf method[25] using 6-methyl-2-carboxycyclohexanone (VI) as a model. When an aqueous solution of I, buffered to pH 7.12, was treated with V, there was obtained, in addition to some irresolvable glass, trace amounts of crystalline

(10) D. Kerridge, *J. Gen. Microbiol.*, **19**, 497 (1958); C. J. Shepherd, *ibid.*, **18** *iv* (1958); E. J. Hewitt and M. M. R. K. Afridi, *Nature*, **183**, 57 (1959); B. W. Coursen and H. D. Sisler, *Am. J. Botany*, **47**, 541 (1960); M. R. Siegel and H. D. Sisler, *Nature*, **200**, 675 (1963); *Biochim. Biophys. Acta*, **87**, 70, 83 (1964).

(11) E. C. Kornfeld and R. G. Jones, *Science*, **108**, 437 (1948); E. C. Kornfeld, R. G. Jones, and T. V. Parke, *J. Am. Chem. Soc.*, 71, 150 (1949).

(12) E. J. Eisenbraun, J. Osiecki, and C. Djerassi, *ibid.*, **80**, 1261 (1958).

(13) M. A. Acitelli, Ph.D. Thesis, Cornell University, 1957.

(14) T. Okuda, M. Suzuki, Y. Egawa, and K. Ashino, *Chem. Pharm. Bull.*, 7, 27 (1959).

(15) A. J. Lemin and J. H. Ford, *J. Org. Chem.*, **25**, 344 (1960).

(16) T. Okuda, *Chem. Pharm. Bull.* (Tokyo), **7**, 137 (1959); *ibid.*, 7, 659 (1959). See also T. Okuda, M. Suzuki, Y. Egawa, and K. Ashino, *ibid.*, **6**, 711 (1958).

(17) T. Okuda, M. Suzuki, and Y. Egawa, *ibid.*, **8**, 335 (1960).

(18) B. C. Lawes, *J. Am. Chem. Soc.*, **84**, 239 (1962); Abstracts, 139th National Meeting of the American Chemical Society, St. Louis, Mo., March 1961, p. 33N.

(19) Recently J. Wolinsky and D. Chan, *J. Am. Chem. Soc.*, **85**, 937, (1963), have pointed out that Lawes' conclusions are not necessarily justified by the reasoning involved.

(20) H. J. Schaeffer and V. K. Jain, *J. Pharm. Sci.*, **50**, 1048 (1961).

(21) N. A. Starkovsky, F. Johnson, and A. A. Carlson, *Tetrahedron Letters*, 1015 (1964).

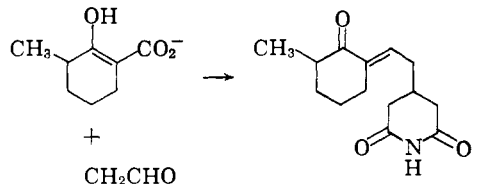
(22) T. Okuda and M. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **9**, 1014 (1961).

(23) T. Okuda, M. Suzuki, T. Furamai, and H. Takahashi, *ibid.*, **10**, 639 (1962); 11, 730 (1963).

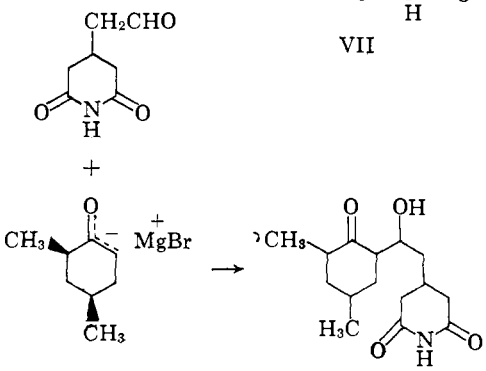
(24) D. D. Phillips, M. A. Acitelli, and J. Meinwald, *J. Am. Chem. Soc.*, **79**, 3517 (1957).

(25) C. Schöpf and K. Thierfelder, *Ann.*, **518**, 127 (1935).

product whose ultraviolet (λ_{max} 2410 Å. (ϵ 6840)) and infrared spectra indicated it to be VII.[26] Both the yield and nature of the product were disappointing and, rather than pursue the reaction, we turned to an



VII

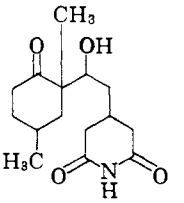


VIII

alternate method which seemed to hold promise, the Nielsen condensation.[27] When the magnesiobromide salt of racemic IV (formed *in situ* from IV and N-methylanilinomagnesium bromide) was treated with V in a benzene–tetrahydrofuran medium, there could be isolated from the glassy reaction product[28] in 4–12% yield a highly crystalline material, m.p. 195–196°, which we have named *dl*-neocycloheximide (VIII). Elemental and infrared analysis indicated it to be isomeric with cycloheximide. The insolubility of VIII prevented a direct solution infrared spectral comparison with cycloheximide and its isomers. However its more soluble acetate, prepared in the usual way, gave a spectrum which was different from any of the known stereoisomeric acetates.

Stereochemical Aspects

In an attempt to correlate VIII with a known compound it was oxidized using the Jones reagent.[31] Surprisingly the product obtained in 95% yield proved to be a 1,3-diketone (IX), m.p. 100–101°, and not the expected keto–enol. This material proved unusually stable for it could be recrystallized unchanged from hot ethyl acetate. It did, however, slowly (2 hr.) develop a purple color with ferric chloride solution, thus proving it to have the gross structure assigned to it rather than a *gem*[29,30] structure (X). This was confirmed by treatment of VIII with pyridine hydrochloride or, better, iodine in boiling toluene which led to *dl*-anhydroisocycloheximide[26,32] identified by comparison with an authentic specimen. Heating IX for a short period at 100° effected its quantitative conversion



X

to the enolic, *dl*-dehydroisocycloheximide (XI[15]) which gave an immediate color with ferric chloride solution. Reconversion of XI to a diketonic form by crystallization from a polar solvent could not be accomplished.

It is also proved possible to oxidize isocycloheximide to a 1,3-diketone (XII)[32] by carrying out the Jones oxidation at 0°. However this material was relatively unstable by comparison with IX, for attempted crystallization from hot ether–methylene chloride effected its conversion to XI. It also gave a fully developed ferric chloride color within 30 min. and its solution infrared spectrum in chloroform was different from that of IX.

Although a sufficient amount of another isomer, α-epiisocycloheximide,[30] was not available to us for oxidation, we have recently succeeded in synthesizing the racemic compound (these results will be reported later). One of the easily available intermediates, a dihydro derivative (*i.e.*, a diol isomeric with dihydrocycloheximide), was oxidized at 0° and afforded the racemic form of XII, as was evident from the identity of their solution infrared spectra. Thus isocycloheximide and α-epiisocycloheximide differ only in the configuration of the hydroxyl group in the side chain as claimed by the Japanese workers.[30]

Disregarding for the moment the absolute configuration of the hydroxyl group, neocycloheximide must differ then from isocycloheximide and α-epiisocycloheximide in the orientation of its hydroxyethylglutarimide side chain.

Oxidation of cycloheximide likewise afforded a 1,3-diketone (XIII) whose behavior resembled that of XII rather than IX. It gave a ferric chloride test within 30 min. and could not be recrystallized from hot solvents without its isomerizing to the enolic dehydrocycloheximide XIV. Needless to say the solution infrared spectrum of XIII was different from that of IX or XII.

The difference in iron chelation behavior between IX and XII or XIII is best ascribed to the former having an axial side chain at the 6-position whereas the latter two compounds have this group equatorially oriented. Justification for these assignments can be found by considering the transition states for enolization of the

(26) Parallel results were obtained by B. C. Lawes, *J. Am. Chem. Soc.*, **82**, 6412 (1960), when he condensed V with *cis*-2,4-dimethyl-6-formylcyclohexanone in a mildly basic medium and obtained anhydroisocycloheximide.

(27) A. T. Nielsen, C. Gibbons, and C. A. Zimmerman, *ibid.*, **73**, 4696 (1951).

(28) This same condensation has been examined by T. Okuda and his co-workers.[29] They were able to obtain a crystalline material only after acetylation of the glassy product. This we at first surmised[1] was identical with the acetate of VIII because of the closeness of their melting points. Subsequent work, however, has shown that their product is indeed isocycloheximide acetate as first claimed. It is interesting to note that when optically active IV was used in this condensation[30] both isocycloheximide and α-epiisocycloheximide were obtained in low yield. No optically active compound corresponding to V was isolated.

(29) T. Okuda, M. Suzuki, and Y. Egawa, *Yakugaku Kenkyu*, **33**, 371 (1961); Y. Egawa, M. Suzuki, and T. Okuda, *Chem. Pharm. Bull.* (Tokyo), **11**, 589 (1963).

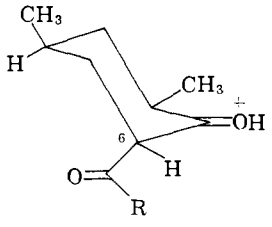
(30) T. Okuda, M. Suzuki, and Y. Egawa, *J. Antibiotics* (Tokyo), **14**, 158 (1961); M. Suzuki, Y. Egawa, and T. Okuda, *Chem. Pharm. Bull.* (Tokyo), **11**, 582 (1963).

(31) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, **39** (1946).

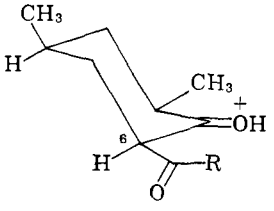
(32) Recently it has been shown by N. A. Starkovsky, F. Johnson, and A. A. Carlson, *Tetrahedron Letters*, 1015 (1964), that the material called anhydrocycloheximide in the literature to date has *cis*-oriented methyl groups. Thus it is better called anhydroisocycloheximide, as will transpire from the ensuing arguments in this article.

diketone forms and making use of the finding by Corey and Sneen[33] that in a rigid, cyclic system a hydrogen atom adjacent to a ketone is removed at a faster rate when it is in an axial as opposed to an equatorial position in an enolization process. Two assumptions are first necessary concerning the enolization process in question: first, that the more stable conformers of the diketones are actually the forms undergoing enolization. This is not unreasonable since the less stable conformers of all of these ketones must have at least one strong 1,3-diaxial repulsive interaction, and therefore constitute forms which over-all must have much higher transition energies[34] in the enolization process than their more stable conformers. Second, it must be presumed that enolization concerns only the ring ketone. Again this seems plausible since the stability of endocyclic *vs.* exocyclic double bonds is well documented[35] and presumably this must also apply to a partially formed bond, *i.e.*, the transition state.

Consider now the loss of the equatorial 6-proton from IX, protonated on the ring carbonyl. The bond-breaking process in this case will not be facilitated by conjugation with the π-bond of the ring ketone because



IX



XII

the latter system is at right angles to the orbital of this proton. Thus the transition state is unassisted in this case. On the other hand in the corresponding case of XII, the orbital of the C–H bond that is breaking is essentially in the same plane as the π-system of the ring ketone, and the transition state closely resembles the starting material.

Additional evidence for the axial nature of the large side chain in neocycloheximide comes from a study of the hydroxyl infrared absorption bands of I, III, and VIII at ~0.65% concentration in acetonitrile. Both of the former compounds show a band at 2.84 whereas neocycloheximide exhibits absorption at 2.79 μ. Thus in VIII there is little intramolecular hydrogen bonding between the hydroxyl and ketone groups as could be expected from their relative positions, whereas moderate hydrogen bonding of this type occurs in both I and III.

The fact that neocycloheximide is derived from *cis*-2,4-dimethylcyclohexanone (III) supports the assignment of *cis*-methyl groups in VIII and thus in III also. Corroborative evidence for this comes from the work of Lemin and Ford[15] who showed that under equilibrating conditions XIV affords a 90% yield of the enolic dehydroisocycloheximide (XI). As a corollary cycloheximide must have *trans* methyl groups.

(33) E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, **78**, 6269 (1956).

(34) Conversely, of course, if enolization of IX can take place only when its large side chain is equatorially oriented, thus requiring a less favorable conformational state for the cyclohexanone ring, this would account for the slower rate of its enolization *vis à vis* XII or XIII.

(35) H. C. Brown, J. H. Brewster, and H. Schechter, *J. Am. Chem. Soc.*, **76**, 467 (1954).

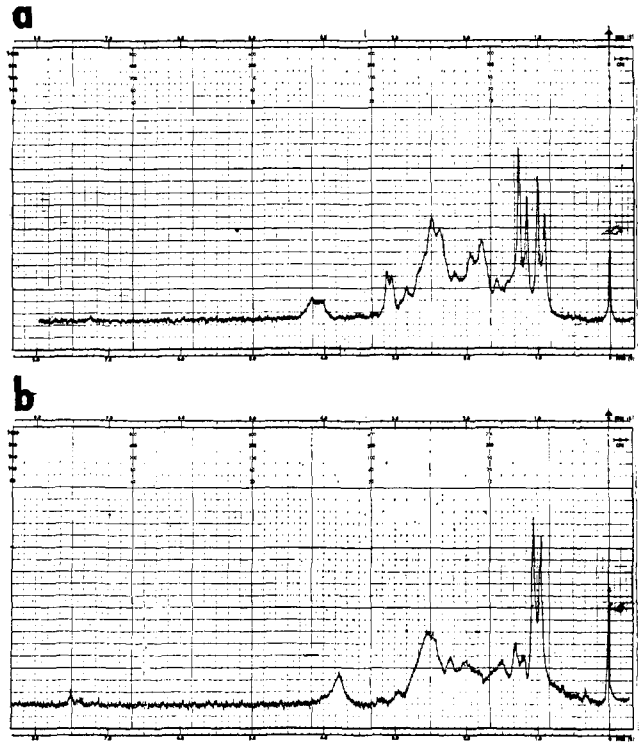


Figure 1. N.m.r. spectra: a, cycloheximide; b, isocycloheximide.

Thus far we can conclude that neocycloheximide has structure Ic, isocycloheximide has structure Ib, and cycloheximide has structure Id or Ie. At this point it did not appear feasible to pursue the problem any further chemically, and we turned to a study of their n.m.r. spectra in the hope that the stereochemical assignments could be completed. Unfortunately no simple correlation between spectra[36] and stereochemistry was immediately forthcoming. All that could be observed was that the spectra of cycloheximide (Figure 1a) and naramycin-B had two distinct doublets, one at higher field (~60 c.p.s.) and one at lower field (~70 c.p.s.), whereas those of isocycloheximide (Figure 1b), *dl*-α-epiisocycloheximide; and neocycloheximide showed only superimposed doublets (~58 c.p.s.). In view of this we decided to examine the n.m.r. spectra of the *cis* and *trans* forms of the comparably rigid 4-*t*-butyl-2-methylcyclohexanone and 2-*t*-butyl-4-methylcyclohexanone[37] to determine if the individual methyl group doublets had any special characteristics that could be applied to the spectra of I and its isomers. This proved successful when the following three parameters were measured: (a) the position of the methyl doublets (recorded as the middle of the doublet which is, to a first approximation, the center of gravity) in deuteriochloroform, (b) the coupling constant J in c.p.s., and (c) the direction and magnitude of the displacement of the peaks occurring on substitution of

(36) Spectra were determined in deuteriochloroform and peaks measured downfield from TMS taken at 0 c.p.s.

(37) These ketones were resolved into their pure isomeric forms by g.l.c. using tris(cyanoethyl)glycerol according to methods previously described.[38]

(38) N. L. Allinger and H. M. Blatter, *J. Am. Chem. Soc.*, **83**, 994 (1961). See also C. Beard, C. Djerassi, J. Sicher, F. Sipos, and M. Tichy, *Tetrahedron*, **19**, 919 (1963).

Table I. N.m.r. Spectra of Model Ketones

Cyclohexanone	Me group stereochemistry	Positions of Me peaks, c.p.s.[a] In $CDCl_3$	In pyridine
2-Methyl	Largely equatorial	60.6 (6.2)	60.3 (6.3)
4-Methyl	Largely equatorial	60.3 (5.5)	51.5 (5.4)
trans-4-*t*-Bu-2-Me	Axial	69.3 (7.2)	64.6 (7.2)
cis-4-*t*-Bu-2-Me	Equatorial	61.1 (6.3)	61.8 (6.3)
trans-2-*t*-Bu-4-Me	Axial	66.8 (6.3)	60.8 (6.3)
cis-2-*t*-Bu-4-Me	Equatorial	60.1 (5.6)	52.6 (5.6)

[a] *J* values are given in parentheses.

Table II. N.m.r. Spectra of Cycloheximide and Related Compounds[39]

Compd.	Position of proton signals, c.p.s.[a] In deuteriochloroform: Methyl doublets 2-CH_3	4-CH_3	C*H*OR[a]	In pyridine: Methyl doublets 2-CH_3	4-CH_3	C*H*OR
Cycloheximide	58.9 (6.1)	73.6 (6.7)	244	59.1 (6.1)	68.7 (6.6)	265
Cycloheximide acetate	59.2 (6.0)	75.7 (6.6)	319	59.4 (6.4)	70.6 (6.6)	347
Cycloheximide chloroacetate	57.0 (6.4)	73.6 (7.0)	328	56.5 (6.3)	67.0 (6.7)	341
Cycloheximide tosylate	56.2 (6.3)	72.2 (6.9)	301	57.5 (6.2)	68.3 (6.7)	323
N-Methylcycloheximide acetate	58.3 (6.4)	74.8 (6.8)	317	59.3 (6.2)	71.0 (6.6)	346
Dehydrocycloheximide (diketo form)	59.0 (6.3)	72.5 (7.0)		...	...	
Isocycloheximide (III)	59	59		58.3 (6.4)	52.5 (5.8)	254
Isocycloheximide acetate	60.6 (6.3)	58.2 (5.9)	317	58.4 (6.2)	52.1 (5.9)	
dl-α-Epiisocycloheximide	60.2 (6.3)	60.7 (5.8)	252			
dl-α-Epiisocycloheximide acetate	58.8	58.8	324	57.6 (6.5)	50.3 (5.8)	346
Dehydroisocycloheximide (diketo form)	60.6	60.6		...	...	
Naramycin-B (II)	72.7 (7.4)	59.0 (5.9)	223	64.4 (7.1)	52.1 (5.8)	
Naramycin-B acetate	71.4 (7.2)	60.9 (6.0)	321	63.8 (7.2)	53.0 (5.8)	347
Neocycloheximide (IV)	...	...		63.8 (6.4)	50.3 (6.0)	261
Neocycloheximide acetate	57	57	319	61.4 (6.3)	48.4 (5.8)	
E-73 (V)	61.8 (5.8)	108.7 (singlet)	253	61.4 (5.8)	111.6 (singlet)	270
Streptovitacin A	...	...	...	65.6 (6.1)	105.0 (singlet)	...

[a] In all the acetate compounds listed (except E-73), $COCH_3$ peaks occur within the range of 118–125 c.p.s. in CDCl *and* pyridine. *J* values in c.p.s. given between brackets. In some cases overlap of peaks prevented accurate determination of splitting constants. Neocycloheximide was practically insoluble in deuteriochloroform as was streptovitacin-A.

pyridine for deuteriochloroform.[39,40] Table I presents data pertaining to the four isomeric ketones in question and to 2- and 4-methylcyclohexanone. Several features of this table are significant: (a) axial methyl groups occur at lower field than equatorial groups irrespective of their being at the 2- or 4-position; (b) the *J* value of a 2-axial-methyl group is the largest and that of a 4-equatorial-methyl group the smallest; (c) change of solvent from deuteriochloroform to pyridine leads to a marked displacement to higher field of all doublets except that of a 2-equatorial-methyl group which if anything suffers a slight displacement to a lower field. Although of secondary importance it is worth noting that the 4-equatorial-methyl group doublet undergoes the largest displacement (~7 c.p.s. upfield). The data for the simple methylcyclohexanones are in remarkably good agreement with the disubstituted cases, especially when the differences (small but significant) in rigidity of the systems are considered.

(39) Whenever possible 10% solutions were used. However, differences in the solubility of the substances examined did not always permit the spectra to be determined at this concentration. Therefore because of solution effects among others, the figures obtained are reliable only to ±1 c.p.s. in expressing the positions of the peaks and to ±0.1 c.p.s. in expressing *J* values.

(40) The energy difference between 2-axial-methylcyclohexanone and its 2-equatorial conformer has been estimated[38] to be about 1.6 kcal./mole, indicating 95% of 2-methylcyclohexanone to exist as the 2-equatorial conformer at room temperature. To a first approximation 4-methylcyclohexanone can be assumed to have the same composition.

Table II shows data pertaining to cycloheximide and its isomers together with a number of their derivatives.

Inspection of the *J* values of this table shows that they can be placed roughly in four groups, *viz.*, with values of ~7.3, ~6.8, ~6.3, and ~5.9 c.p.s. By correlation with Table I the highest and lowest values are assigned a 2-axial- and a 4-equatorial-methyl group, respectively.

With this information at hand it is now possible to deduce the stereochemistry of I and its isomers.

Cycloheximide. In this case all compounds have a low-field methyl doublet, indicative of an axial position, which is moved (~5 c.p.s.) to higher field in pyridine solution. On the other hand the methyl doublet at higher field if anything moves (~1 c.p.s.) to slightly lower field when the medium is changed from deuteriochloroform to pyridine. This behavior, judged in the light of Table I, can only be indicative of a molecule containing both a 4-axial- and a 2-equatorial-methyl group. This is confirmed by the fact that both doublets have intermediate *J* values (6.3–6.8 c.p.s.). Thus cycloheximide must be represented by Ie.

Isocycloheximide and α-Epiisocycloheximide. All compounds in this class display a doublet, occurring at relatively high field, which is displaced appreciably (~7 c.p.s.) to even higher field when pyridine is substituted for deuteriochloroform as the solvent. Again

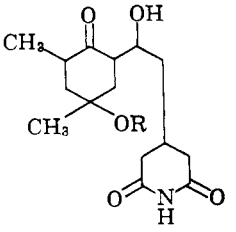
this doublet has the smallest of the splitting constants of all the compounds of Table II. These data permit this methyl group to be assigned as 4-equatorial. The other doublet behaves in much the same way as the high-field doublet of cycloheximide and is characteristic of a 2-equatorial-methyl group. Therefore all compounds in this category are represented by Ib, a conclusion which reinforces the chemical evidence obtained earlier.

Naramycin-B. Both compounds display one doublet which from the point of view of the three criteria used (chemical shift, displacement, and J value) is identical with the 4-methyl doublet of isocycloheximide (*i.e.*, indicative of a 4-equatorial-methyl group). On the other hand the other peak occurring in deuteriochloroform at low field (~72 c.p.s.) having the largest J value (~7.2 c.p.s.) and being strongly displaced upfield by change of solvent must be due to a 2-axial-methyl group. Therefore naramycin-B is represented by Id.

Neocycloheximide. Arguments here are similar to those made for isocycloheximide and thus corroborate the deductions made above on chemical evidence that neocycloheximide has the 2,4-equatorial orientation for its methyl groups, *i.e.*, that it is represented by Ic.[41] It is, however, worth noting that the shifts the doublets undergo on solvent change are much greater in this case than in the other examples discussed above. This must reflect the changes in solvent orientation around the molecules caused by the large axial hydroxyethylglutarimide group.

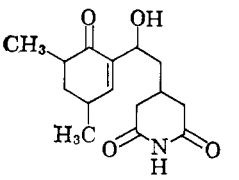
The assignments thus deduced for isocycloheximide and its isomers confirm the final conclusions arrived at by Okuda.[22] They also are completely in line with both thermal degradation[18,23] and isomerization experiments[23] performed with these compounds.

E-73 and Streptovitacin-A. The gross structure of E-73 has been deduced to be XV (R = Ac) by Rao[42] but no stereochemical work has been reported. Streptovitacin[43] has been shown to be the parent alcohol (XV, R = H) of E-73. Again no stereochemical comment was recorded. In these compounds the 2-methyl group doublets occur at high field and have J values in keeping with equatorial methyl groups. Change of solvent from deuteriochloroform to pyridine effects no appreciable displacement of the doublet, so that the latter can with some confidence be assigned to a 2-equatorial-methyl group. Nothing can be said of the side chain or the 4-methyl group orientations, but the biological activities of XV (R = OAc) and XV (R = OH) would lead one to regard them as 4-equatorial-acetoxy and 4-equatorial-hydroxycycloheximide, respectively, especially since naramycin-B, isocycloheximide,[44] and neocycloheximide[44] have little or no activity to speak of.



XV

Inactone. This glutarimide derivative was first isolated by Preud'homme and Dubost[45] from cultures of *Streptomyces griseus.* Its structure XVI was assigned by Paul and Tchelitcheff[46] on the basis of elemental, ultraviolet, and infrared analyses and the fact that it did not give a dimethylcyclohexanone on alkaline degradation. Its dihydro reduction products however did give 2,4-dimethylcyclohexanone and methanetriacetic acid under these conditions. Two tetrahydro



XVI

derivatives were also obtained, but neither these nor the dihydro compounds they reported appeared to correspond to known compounds. For this reason we elected to reinvestigate the structure of inactone.

Since only a small sample (140 mg.) of XVI was available, we first studied its mass spectrum (Figure 2a) and for comparison examined the mass spectra of cycloheximide (Figure 2b) and streptimidone[47,48] (Figure 2c). In the latter two spectra the dominant mode of decomposition takes the anticipated retro-aldol course leading to major peaks at *m/e* 126 and 138, respectively. On the other hand the mass spectrum of XVI shows a major fragment at *m/e* 152. This observation in itself is sufficient to confirm the position of the double bond in XVI, because allylic cleavage[49] to give such a fragment not only would be expected, but could only come from a structure such as XVI. Supporting evidence for this conclusion comes from the absence of a peak at *m/e* 124 (retroaldol) in the spectrum of inactone. Having confirmed the structure of XVI, we turned our attention to its stereochemistry. This we approached at first as we had done with cycloheximide and its isomers, namely, by the preparation of the appropriate model compounds *cis-* and *trans-*4,6-dimethylcyclohex-2-enones.[50] However, the n.m.r. spectra of these materials and that of inactone showed little correlation using the parameters mentioned earlier.

(41) It is of course recognized that neocycloheximide, because it is racemic, is a mixture of Ic and its mirror image.

(42) K. V. Rao, *J. Am. Chem. Soc.*, **82**, 1129 (1960).

(43) R. R. Herr, *ibid.*, **81**, 2595 (1959).

(44) Both naramycin-B and isocycloheximide have been reported[15] to have approximately 30% of the activity of cycloheximide against *Saccharomyces pastorianus.* However, samples of naramycin-B supplied to us by T. Okuda could be shown to contain 25–30% of cycloheximide by careful integration of the "impurity" peaks in its n.m.r. spectrum. Thus such activity can be ascribed largely to the cycloheximide content. Again, when isocycloheximide was carefully purified it showed only low level activity (5–8% that of I) against the above organism. Thus it must be assumed that the original testing was done with a sample contaminated with cycloheximide. Biological testing of these materials was done in collaboration with Drs. M. Siegel and H. Sisler of the University of Maryland.

(45) J. Preud'homme and M. Dubost, "Communication au Congres Internationale de Chemie Organic," Zurich, 1955.

(46) R. Paul and S. Tchelitcheff, *Bull. soc. chim. France*, 1316 (1955).

(47) R. P. Frohardt, H. W. Dion, Z. L. Jakubowski, A. Ryder, J. C. French, and Q. R. Bartz, *J. Am. Chem. Soc.*, **81**, 5500 (1959); P. W. K. Woo, H. W. Dion, and Q. R. Bartz, *ibid.*, **83**, 3085 (1961).

(48) E. E. van Tamelen and V. Haarstad, *ibid.*, **82**, 2974 (1960).

(49) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 83.

(50) The details of the preparation of these compounds will be reported separately.

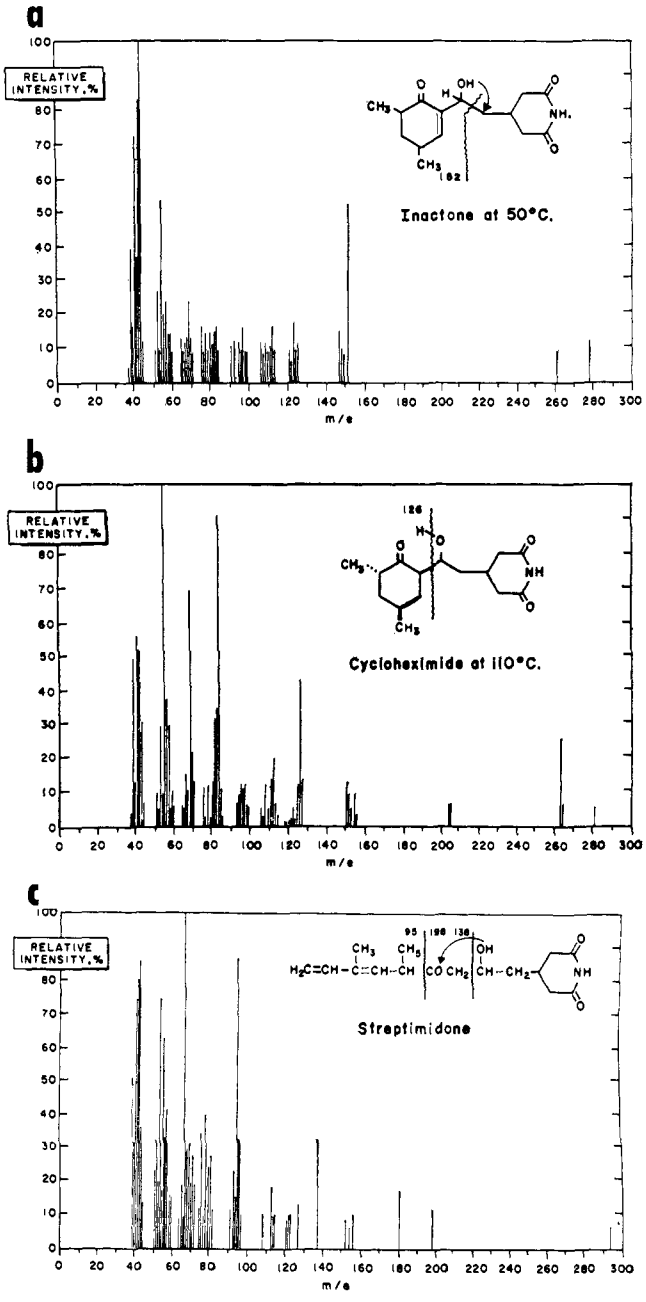
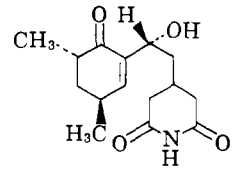


Figure 2. Mass spectra: a, inactone; b, cycloheximide; and c, streptimidone.

This perhaps is due to the differences in rigidity of the systems involved. At this point the dihydro derivatives of XVI seemed the best alternative stereochemical approach. A close inspection of the published infrared spectra of the three reduction products, α-, β-, and γ-isoactidiones, isolated by the French workers, suggested that the β- and γ-compounds may be impure forms of the α-isomer. Moreover the spectrum of the latter compound was almost identical with the spectra of naramycin-B published by the Japanese workers,[14,23] especially in the crucial 9–10 μ region.

In our hands reduction of inactone over a rhodium-on-alumina catalyst led to a crystalline product, m.p. 82–92°, whose n.m.r. spectrum showed it to be essentially a mixture[51] of naramycin-B and cycloheximide, with the former predominating. Integration of the methyl doublets suggested the presence of approximately 30% of cycloheximide whereas biological assay[44] gave a slightly lower value of around 28% cycloheximide. Thus it appears that the α- β-, and γ-isoactidiones are, in reality, mixtures of cycloheximide and naramycin-B. These results lead us to conclude that inactone has *trans*-oriented methyl groups. Since *dl*-cycloheximide[52] has only 50% of the biological activity[44] of *l*-cycloheximide, one can safely assume that *d*-cycloheximide is inactive. As a direct consequence, and because it gives rise to some *l*-cycloheximide on reduction, inactone must belong to the series with the same absolute configuration and can be represented fully as XVIa with the side-chain hydroxyl in the (*R*) configuration.[53]



XVIa

Experimental

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 spectrophotometer.

dl-Neocycloheximide (VIII). N-Methylaniline (22.8 g.) in dry benzene (70 ml.) was added dropwise at 5°, during 10 min., to ethylmagnesium bromide solution (152 ml., 1.62 *N*) contained in a three-necked flask that was protected with a drying tube and through which a gentle stream of nitrogen was passing. There was then added *dl-cis*-2,4-dimethylcyclohexanone[54] (29.75 g.) in dry benzene (35 ml.) over a 20-min. period, the same temperature being maintained. Finally 3-glutarimidylacetaldehyde (25.5 g.) in dry tetrahydrofuran (450 ml.) was introduced over 1 hr. The reaction mixture was stirred for an additional hour, then allowed to stand at 0–5° for 16 hr. Cold acetic acid (120 ml.) was added and the mixture was diluted with 1 l. of ice water. Extraction was accomplished with methylene chloride (five 200-ml. portions) and the combined extracts were washed with water, sodium hydrogen carbonate solution, and once again with water, and finally dried over anhydrous sodium sulfate. The methylene chloride solution was concentrated to 200 ml., then passed through a 5 × 20 cm. column of silica gel. Elution of the column was accomplished by methylene chloride (1 l.), methylene chloride–ethyl acetate (19:1, 1 l.), and methylene chloride–ethyl acetate (1:1, 2 l.). The first two fractions were discarded and the third, on evaporation, afforded an oil (5 g.) which crystallized from methylene chloride to give crude

(51) All attempts to separate naramycin-B from cycloheximide have failed. Even the best samples of the former compound obtained by the Japanese workers after very laborious efforts do not appear[44] to be free of cycloheximide. Our attempts at separation of these two substances were equally abortive.

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

(53) N. A. Starkovsky and F. Johnson, *Tetrahedron Letters*, 919 (1964).

(54) The large-scale preparation of this ketone was carried out by W. B. Trapp and H. E. Hennis of the Midland Division of The Dow Chemical Co., and we gratefully acknowledge their assistance.

neocycloheximide as a white solid, m.p. 185–190° (1.77 g., 4.7%). A further recrystallization from methanol raised its melting point to 195–196°. VIII is very sparingly soluble in methylene chloride, chloroform, and ether and moderately soluble in boiling water, hot methanol, and acetone. Its infrared spectrum showed bands at 2.90 (OH), 3.10 and 3.24 (NH), 5.84 and 5.95 (C=O), and 7.75, 7.91, 8.18, 8.64, 9.42, 9.72, 10.50, and 11.87 μ.

Anal. Calcd. for $C_{15}H_{23}NO_4$: C, 64.0; H, 8.2; N, 5.0. Found: C, 63.8; H, 8.3; N, 5.0.

When a rigorous chromatography (400 fractions) was performed on the crude product of this reaction, it was possible to raise the yield of VIII to approximately 12%.

dl-Neocycloheximide Acetate. *dl*-Neocycloheximide (100 mg.) dissolved in a mixture of pyridine (3 ml.) and acetic anhydride (2 ml.) was allowed to stand at room temperature for 24 hr. Isolation of the product in the usual way led to the acetate, m.p. 130–131° (84 mg.), rising to 132° on recrystallization from very dilute acetone. This compound showed infrared bands at 3.12 and 3.23 (NH), 5.75, 5.83, and 5.90 (C=O), and 7.81, 7.91, 8.12, 8.61, 9.49, and 9.71 μ.

Anal. Calcd. for $C_{17}H_{25}NO_5$: C, 63.1; H, 7.8; N, 4.3. Found: C, 63.0; H, 7.7; N, 4.2.

dl-Anhydroisocycloheximide. *dl*-Neocycloheximide (100 mg.) in toluene (60 ml.) containing iodine (200 mg.) was refluxed for 1 hr. The cooled reaction mixture was washed with sodium hydrogen sulfite solution and water, then dried and evaporated to dryness. The residue on crystallization from ether–petroleum ether (b.p. 30–60°) afforded *dl*-anhydroisocycloheximide, m.p. 115–117° (lit.[25] m.p. 118–120°) (38 mg.), which did not depress the melting point of an authentic sample, m.p. 116°, prepared from *dl*-cycloheximide acetate.

d-Isocycloheximide. *l*-Cycloheximide (8.0 g.) in dry benzene (40 ml.) was refluxed with powdered lithium aluminum hydride (2.65 g.) for 17 hr. The solution was filtered, washed with water, and dried over anhydrous magnesium sulfate, and the benzene was removed under reduced pressure. The residue was crystallized first from methylene chloride–ether, then water, and furnished essentially pure *d*-isocycloheximide (3.0 g.), m.p. 101–103°, $[\alpha]^{25}D$ +32° (*c* 1.7, $CHCl_3$) (lit.[23] m.p. 98–100°, $[\alpha]^{25}D$ +33°).

Oxidation of dl-Neocycloheximide. *dl*-Neocycloheximide (225 mg.) in acetone (28 ml.; distilled from $KMnO_4$) was treated at room temperature with an aqueous solution of chromium trioxide (10.5 ml., 2 *N* in oxygen and 1 *N* in H_2SO_4). After standing overnight, the solution was diluted with water (100 ml.) and then extracted with methylene chloride. The extract was washed with water and dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The residual solid, m.p. 89–94° (222 mg.), was recrystallized from methylene chloride and ethyl acetate or ether to give the pure dione IX, m.p. 100–101°, resolidifying at 110° and remelting at 135–138°. Its infrared spectrum showed bands at 3.13 and 3.24 (NH), 5.83, 5.91, and 5.96 (C=O), and 7.74, 7.92, 8.65, 8.91, 9.46, and 9.70 μ, but had no absorption in the 6.0–6.5-μ region characteristic of the enolic forms of 1,3-diones. In chloroform solution its infrared spectrum exhibits bands at 9.44 and 9.65 μ which distinguish it from the diketones derived from cycloheximide and isocycloheximide.

Anal. Calcd. for $C_{15}H_{21}NO_4$: C, 64.5; H, 7.6; N, 5.0. Found: C, 64.4; H, 7.6; N, 5.0.

When a sample of IX was heated for 2 hr. in a drying pistol over refluxing toluene and then recrystallized from ethanol–petroleum ether (b.p. 30–60°), dehydroisocycloheximide, m.p. 146–147.5°, was obtained. Its identity was established by comparison with an authentic specimen prepared according to Ford and Lemin.[15]

Oxidation of l-Cycloheximide. *l*-Cycloheximide (1.0 g.) in acetone (115 ml.) was oxidized as above at 0–5° using the same oxidizing solution (43 ml.). During the work-up procedure temperatures were kept at <15°. The methylene chloride extract on evaporation at 0–5° left a colorless glass (0.58 g.) which crystallized when scratched in ether to give the diketonic form XIII of dehydrocycloheximide (0.42 g.) as silvery white plates m.p., 97–99°. One further recrystallization, accomplished at room temperature by dissolving the product in a little methylene chloride and adding ether, afforded pure XIII, m.p. 99–101°, resolidifying, then remelting 175–177°, $[\alpha]^{23}D$ −40.8° (*c* 1.0, $CHCl_3$). Its infrared spectrum showed bands at 5.8–5.95, 7.75, 7.95, 8.65, 8.65, and 9.4 μ, but did not show absorption in the 6.2–6.3-μ region. In chloroform solution (2.5%) no significant bands between 8.8 and 12.0 μ could be observed.

Anal. Calcd. for $C_{15}H_{21}NO_4$: C, 64.5; H, 7.6; N, 5.0. Found: C, 64.3; H, 7.5; N, 5.0.

Attempted crystallization of XIII from hot solvents such as ethyl acetate led almost quantitatively to the enolic form (XIV) of dehydrocycloheximide, m.p. 177–178° (lit.[11] m.p. 177–180°), $[\alpha]^{25}D$ −28.4° (*c* 0.94, $CHCl_3$). Solid XIII could be kept indefinitely at −70° but storage at −10° led to XIV in 3 weeks while at room temperature isomerization was complete after several days.

Oxidation of d-Isocycloheximide. *d*-Isocycloheximide (80 mg.) in acetone (9 ml.) was oxidized as described above using 3.6 ml. of the oxidizing solution, the reaction being kept at 0–5°. The product, isolated in exactly the same way as described for XIII, was a colorless glass which crystallized from ether to give XII (53 mg.) as a crystalline powder (43 mg.), m.p. 104–106°, resolidifying at 106°, then remelting at 143°, $[\alpha]^{25}D$ −6.3° (*c* 4.3, $CHCl_3$). Further purification of this material proved difficult and analytical data were obtained for the more easily handled *dl* form. When recrystallized from hot solvents or heated at 110°, XII afforded dehydroisocycloheximide in its enolic form XI, m.p. 148–149°, $[\alpha]^{25}D$ −22° (*c* 1.0, CH_3OH), (lit.[15] m.p. 151–154°, $[\alpha]^{25}D$ −23°).

The *dl* form of XII was obtained from the oxidation of *dl*-dihydro-α-epiisocycloheximide (0.244 g.) in acetone (20 ml.) at 0° using a solution of chromium trioxide (0.4 g.) and sulfuric acid (0.25 g.) in water (10 ml.). After 1.3 hr., the reaction mixture was worked up as described above. The methylene chloride extract after drying over anhydrous magnesium sulfate afforded a colorless glass (0.12 g.) which crystallized readily from ether to give *dl*-XII as small plates, m.p. 120–123° (80 mg.). Room temperature recrystallization

from methylene chloride–ether led to the pure compound, m.p. 121–123°, resolidifying and remelting at 146°.

The infrared spectrum of this dione in chloroform solution was identical with that obtained from *d*-isocycloheximide above. No absorption was apparent in the 6.0–6.5-μ region but a strong band at 8.86 μ distinguished it from the diones derived from neocycloheximide and cycloheximide.

Anal. Calcd. for $C_{15}H_{21}NO_4$: C, 64.5; H, 7.6; N, 5.0. Found: C, 64.6; H, 7.6; N, 4.9.

The stability properties of XII were very similar to those of XIII.

When specimens (2 mg.) of XII and IX were separately dissolved in methanol (0.5 ml.) and 2 drops of 1% ferric chloride solution was added, the solution containing XII developed a purple color within 30 min. At this stage the solution of IX was only pale brown, but it reached the same shade of purple after 2 hr.

N-Methylcycloheximide Acetate. *l*-Cycloheximide acetate was dissolved in dry acetone (40 ml.) and refluxed overnight with potassium carbonate (2 g.) and methyl iodide (2 ml.). After filtration, the solution was evaporated to dryness and the colorless residue was crystallized from ether–methylene chloride to give the title compound as colorless crystals, m.p. 140–141°, $[\alpha]^{25}D$ +27° (*c* 2.0, methanol) (lit.[11] for this compound prepared by another route, m.p. 138.5–140°, $[\alpha]^{25.5}D$ +25.9°). The compound was soluble in ether and petroleum ether (b.p. 30–60°).

Anal. Calcd. for $C_{18}H_{27}NO_5$: C, 64.1; H, 8.2; N, 4.2. Found: C, 64.3; H, 8.0; N, 4.2.

Cycloheximide Chloroacetate. *l*-Cycloheximide (1.0 g.) in methylene chloride (10 ml.) containing pyridine (1.0 g.) was cooled in an ice bath and a solution of chloroacetyl chloride (0.523 g.) in methylene chloride (10 ml.) then was added dropwise, with good stirring, during 10 min. Stirring at +5° was continued for 2.5 hr. and the solution then was washed with hydrochloric acid (1 *N*, two 10-ml. portions) and water (two 5-ml. portions), and finally dried over anhydrous sodium sulfate. Removal of the solvent left a glass (1.2 g.) which crystallized from methylene chloride–ether to give cycloheximide chloroacetate, m.p. 133–135° (1.0 g.). A single recrystallization from the same solvents afforded the pure compound, m.p. 135.5–136.5° (0.85 g.), $[\alpha]^{26}$ +7° (*c* 1.0, $CHCl_3$).

Anal. Calcd. for $C_{17}H_{24}ClNO_5$: C, 57.0; H, 6.7; Cl, 9.9; N, 3.9. Found: C, 57.2; H, 6.8; Cl, 10.2; N, 4.1.

Hydrogenation of Inactone. Inactone (90 mg.) in ethyl acetate (15 ml.) was hydrogenated at room temperature and pressure in the presence of a 5% rhodium-on-alumina catalyst (56 mg.). Hydrogen (7.4 ml., 65%) was absorbed during 1 hr. and the catalyst then was replaced. Hydrogenation was continued until gas absorption ceased. The latter reached only 85% of theory. Filtration followed by evaporation of the solvent led to a glass (80 mg.). This did not crystallize well and was chromatographed over silica gel (2 g.). Elution of the column with methylene chloride (50 ml.) afforded only traces of gum, and the same solvent containing 15% ethyl acetate (ten 25-ml. portions) led to a crystalline solid (52 mg.), m.p. 82–92°. The infrared spectrum of this material showed broad absorption in the 2.8–3.2-μ region, typical of naramycin-B. Weak bands at 8.51, 8.65, 9.20, 9.30, 9.65, 9.90, and 12.10 μ were also characteristic of this substance. Impurity peaks at 10.8 and 11.10 μ indicated the presence of some cycloheximide. The n.m.r. spectrum of this material showed it to be largely naramycin-B contaminated with cycloheximide, and integration of the methyl group doublets indicated the latter to be present to the extent of 25–32%, a result confirmed by biological assay.[44]

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