Glutarimide Antibiotics. XII. A Stereochemical Investigation of the Dehydration Products of Cycloheximide[1]

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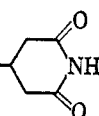
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The dehydration of cycloheximide using any of the previously published methods is shown to be accompanied by isomerization. The products, the anhydrocycloheximides (II and XI), belong to the isocycloheximide series in that they have *cis*-related methyl groups. Reduction of either of these anhydrocycloheximides leads to the same saturated ketone except that in the latter case the product is racemic. A rationale for this result is presented. The alcohols derived from these ketones have been characterized and have been shown to regenerate the parent ketone on oxidation. A number of the conflicting literature reports surrounding these compounds have been reconciled.

The stereochemical features of the dehydration products of cycloheximide (I) and those of some closely related, reduced compounds have been in serious doubt for a number of years. Conflicting literature claims and some surprising results that have been reported in this area led us to reinvestigate the problem.

Background.—The dehydration of cycloheximide (I) was first carried out by Kornfeld, Jones, and Parke[2] using phosphorus pentoxide in boiling benzene. They assigned structure II to their product, mp 134–135°, without stereochemical comment since details of the fine structure of I were not available at that time.
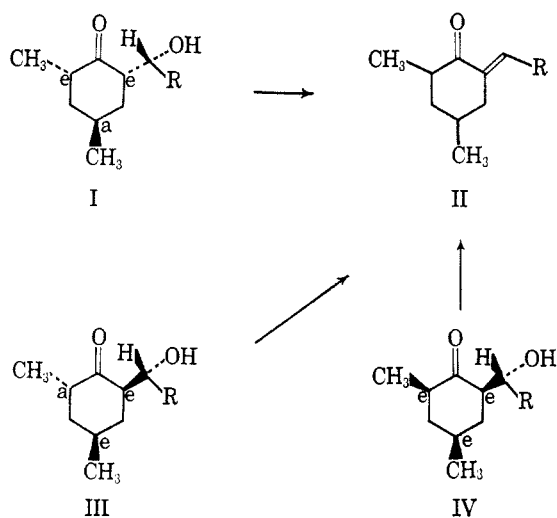
In these and other formulas, R=—CH₂—

Subsequently an identical substance was obtained by Japanese workers[3] from naramycin-B (III) using Kornfeld's conditions,[2] or by means of boron trifluoride in acetic acid. Shortly thereafter Lemin and Ford[4] reported that yet another isomer of I, isocycloheximide (IV), afforded II when refluxed with aqueous pyridine hydrochloride. Spectroscopic evidence was presented to prove identity of their product with that from I.

The first suggestion that II might have *cis* methyl groups was put forward by Okuda.[5] He reduced II to the saturated ketone V and measured the latter's ORD
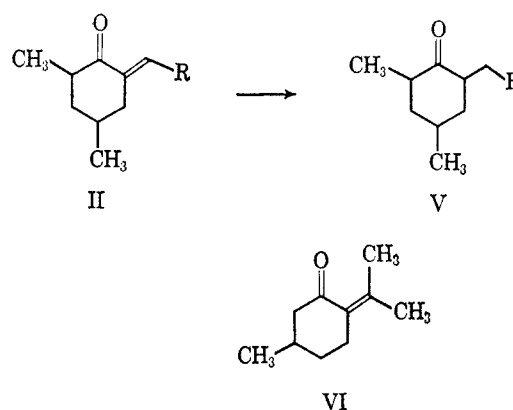
curve. From this and some further reduction experiments he concluded that all substituents on the cyclohexanone ring of V were equatorially oriented. In support of the equatorial position assigned to the large side chain, the analogous reduction of pulegone (VI) to menthone (2-*eq*-isopropyl-5-methylcyclohexanone) was cited. This argument was weakened, however, by the assignment of *trans* methyl groups to one of the alcohols obtained by complete reduction (catalytic) of the α,β-unsaturated ketone system of II (see Chart I).

CHART I

(1) A partial account of this investigation has already been published [N. A. Starkovsky, F. Johnson, and A. A. Carlson, *Tetrahedron Letters*, 1015 (1964)].

(2) E. C. Kornfeld, R. G. Jones, and T. V. Parke, *J. Am. Chem. Soc.*, **71**, 150 (1949).
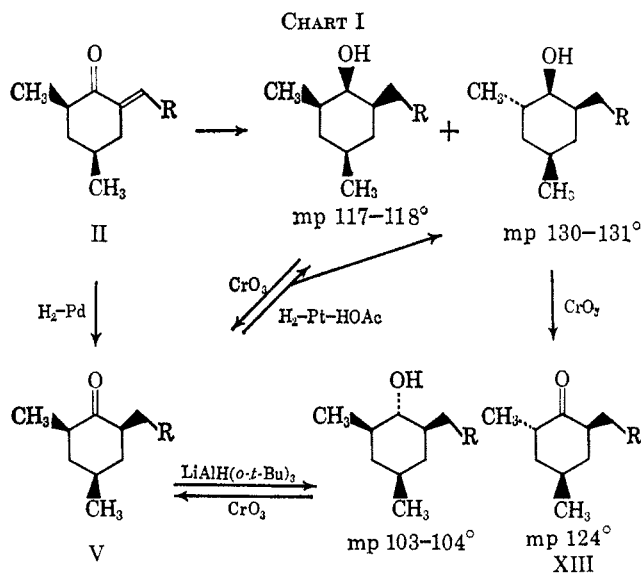
(3) T. Okuda, M. Suzuki, Y. Egawa, and K. Ashino, *Chem. Pharm. Bull.* (Tokyo), **7**, 27 (1959).

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

(5) T. Okuda, *Chem. Pharm. Bull.* (Tokyo), **7**, 666 (1959).

By contrast Lawes[6] was able to effect a total synthesis of II by combining the aldehydes VII and VIII. Because VII had been prepared from *d-cis*-2,4-dimethylcyclohexanone, this suggested that II also should have

VII   VIII

II
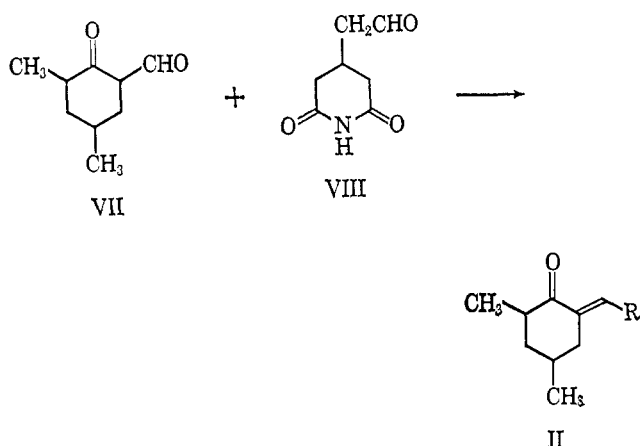
cis-related methyl groups. However, the poor yield of pure II, obtained only after exhaustive recrystallization of the total crude product mitigated against this conclusion. Lawes also found that, whereas crude II had mp 130–133°, $[\alpha]^{25}$D $-12.7°$, the highly purified product obtained synthetically or from I, had mp 139–141°, $[\alpha]^{25}$D $-33°$, thus suggesting all previously prepared samples of II to be quite impure.

The problem took on added interest with the report by Schaeffer and Jain[7] that catalytic reduction of II to the unsaturated alcohol followed by deoxygenation afforded the dideoxycycloheximide IX. The latter was

II   IX
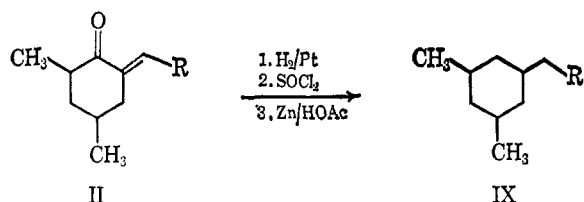
found to be optically active ($[\alpha]^{26}$D $-19°$), thus providing evidence for the *trans* relationship of the methyl groups in cycloheximide I. If the methyl groups had been *cis* related, *compound IX would have been a meso form.* By inference then all intermediates between I and IX, including II, necessarily had to have *trans*-oriented methyl groups.

In the light of the true stereochemical picture subsequently developed[8,9] for cycloheximide and its isomers (as depicted in I, III, and IV) it seemed strange that the most thermodynamically stable isomer, IV (*i.e.*, with all ring substituents equatorial) should give rise, under equilibrating conditions, to a preponderence of a compound having *trans*-related methyl groups and which for all intents and purposes must be the thermodynamically less stable isomer of II. Further complications then arose on two fronts.

(6) B. C. Lawes, *J. Am. Chem. Soc.*, **82**, 6413 (1960).

(7) H. J. Schaeffer and V. K. Jain, *J. Pharm. Sci.*, **50**, 1048 (1961); **53**, 144 (1964).

(8) T. Okuda and M. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **9**, 1014 (1961); T. Okuda, M. Suzuki, T. Furumai, and T. Takahashi, *ibid.*, **11**, 730 (1963).

(9) F. Johnson, W. D. Gurowitz, and N. A. Starkovsky, *Tetrahedron Letters*, 1173 (1962); *J. Am. Chem. Soc.*, **87**, 3492 (1965).

A.—Schaeffer and Jain[10] reported that the refluxing of cycloheximide tosylate (X) in dimethylformamide led to a new dehydration product, *epi*-anhydrocycloheximide which was assigned structure XI.[11] The methyl groups of this compound were assumed to be *cis* related since catalytic reduction led to a saturated ketone XII, mp 151–152°, ostensibly different from V (which of course they regarded as having *trans* methyl groups). Deoxygenation of XII by procedures similar to those used for II then afforded *an optically inactive* dideoxycycloheximide, allegedly showing this series of compounds to have *cis* related methyl groups.

X

XI   XII
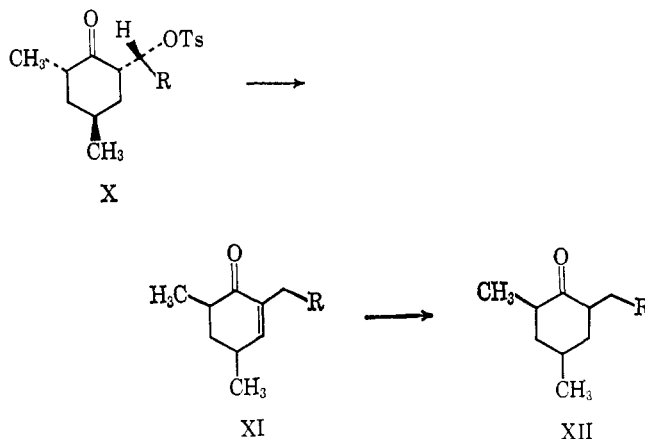
The picture thus appeared complete except for one unusual fact; *viz.*, acid isomerization experiments[7,10] with V or XII had shown them both to be very stable and to undergo no interconversion.

B.—The results obtained earlier by Okuda[5] in reduction studies on II and V were not consistent with those obtained by Schaeffer and Jain.[7] The results presented by Okuda,[5] in the belief that II had *cis* related methyl groups, are shown in Chart I. Except in the case of V, good physical data in support of these structures was not provided.

Schaeffer and Jain[7] also obtained two alcohols having melting points of 115–116° and 129–131° by the Pt-catalyzed reduction of II in acetic acid. However, in direct contrast with Okuda's results, they found that the former alcohol gave a ketone, mp 125°, on oxidation, whereas the latter gave V. They assigned *trans* methyl groups to the higher melting alcohol but did not commit themselves to stereoformulas for the lower melting alcohol or the ketone derived from it.

To further complicate matters, another alcohol, mp 140–141°, had been obtained[10] from XII by catalytic (Pt) hydrogenation in acetic acid. It regenerated XII on chromic acid oxidation.

Inspection of the findings presented above did not lead to any picture that would permit a direct rational correlation of the data. On the contrary the situation with its conflicting and unusual results could only be described as chaotic.

Reinvestigation.—We initiated our research in this area by electing (a) to investigate the acid-catalyzed dehydration of cycloheximide and (b) to re-examine the stereochemistry of deoxycycloheximide V and

(10) H. J. Schaeffer and V. K. Jain, *J. Pharm. Sci.*, **52**, 639 (1963).

(11) An excellent discussion of the mechanism of isomerization of systems of type II to those represented by XI, is presented in a recent article by A. Hassner and T. C. Mead, *Tetrahedron*, **20**, 2201 (1964).

the latter's relationship to *epi*-deoxycycloheximide (XII).

Dehydration of cycloheximide by any of the published procedures[2-4,10,12] leads to crude material that melts anywhere from 118–121°[2] to 132–133°[12] in yields up to 60%.[10] We have found that the best yields (88%), of a product, mp 132–133°, result from treating I with acetic acid containing a trace of sulfuric acid, at 25° for 2 hr.

Recrystallization of material isolated from any of these reaction procedures led to the same pure anhydrocycloheximide II, mp 139–140°, $[\alpha]^{25}D$ $-26°$ (*cf.* Lawes who found $[\alpha]^{25}D$ $-33°$, a value we were unable to reproduce). Attempts to isolate a second pure product from the mother liquors of these crystallizations met with failure.

When the dehydration of I was carried out according to Rao[12] and the initially formed precipitate allowed to redissolve, *epi*-anhydrocycloheximide (XI)[11] could be isolated in poor yield. The same result could be obtained by allowing I to stand in 50% hydriodic acid at temperatures below 0°. Interestingly when I or II was added to the latter reagent at room temperature good yields of the known saturated ketone V could be isolated. Probably this reduction occurs by the addition of hydrogen iodide to the double bond of the α,β-unsaturated ketone followed by reductive deiodination of the β-iodo ketone, again by hydrogen iodide. No further experimental work was done however to test this contention.

The nmr spectra of II and XI did not yield any stereochemical information except to show that the former probably has the vinylic hydrogen *syn* to the ketone. In known *syn*-2-ethylidenecyclohexanones[13] this proton absorbs around 396 cps, but in the *anti* isomers, at approximately 335 cps. II shows absorption at 383 cps (multiplet). The nmr spectrum of XI shows a peak at 390 cps consistent with what is to be expected for such a cyclohexenone. By comparison the spectra of carvone[14] and inactone[15] (XIV) display peaks at 405 and 415 cps, respectively.

XIV
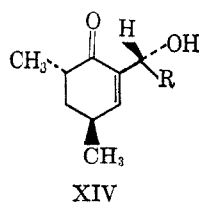
Infrared and ultraviolet spectra support the gross structures assigned to II and XI. In particular, II shows an ethylenic band at 6.15 μ indicative of a cisoid α,β-unsaturated ketone of this type,[16] whereas in XI the double-bond absorption is masked by the ketone band just below 6 μ as it is in the corresponding compounds, 4,6-dimethyl-2-cyclohexenone[17] and inactone (XIV).

For further stereochemical information we turned to an examination of the two ketones V and XII. The

(12) K. V. Rao, *J. Am. Chem. Soc.*, **82**, 1129 (1960).
(13) J. E. Dubois and N. Dubois, *Compt. Rend.*, 715 (1963).
(14) Varian High Resolution N.M.R. Spectra Catalog, Varian Associates, Palo Alto, Calif., 1962, Spectrum No. 271.
(15) R. Paul and S. Tchelitcheff, *Compt. Rend.*, 1316 (1955).
(16) *Cf.* pulegone which shows absorption in the infrared spectrum at 6.15–6.20 μ.
(17) Unpublished results of F. Johnson and D. F. Moakley.

former was obtained in excellent yield according to the published procedure[5]—hydrogenation of II over a palladium-on-charcoal catalyst. Under similar conditions XI afforded a gummy solid (*cf.* ref 10) which on chromatography led to XII identical in physical properties with that described by Schaeffer and Jain.[10] A small amount of a second compound was also isolated. This we were unable to purify completely (mp 140–147°) but believe it to be the known[18] α-deoxyactiphenol XV

XI

XII   XV
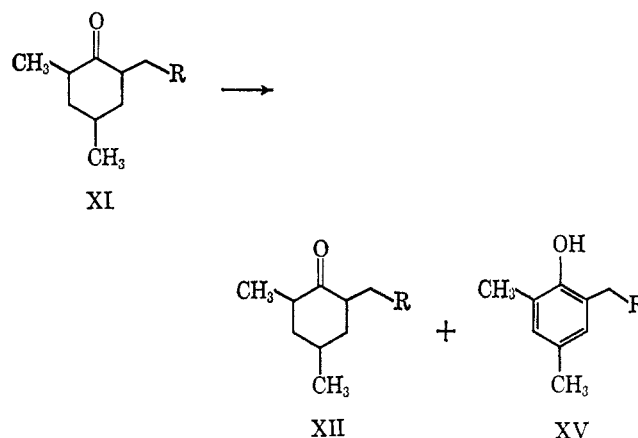
(mp 147–148°). Their ultraviolet spectra correspond and the nmr spectrum of our sample was characterized by two overlapping peaks at 133 cps equivalent to six protons (2-methyl groups on a phenyl ring), one peak at 322 cps (OH proton), and a signal at 405 cps (equivalent to two protons) typical of aromatic hydrogen. Such disproportionations of cyclohexenones under these conditions are well documented.

The stereochemistry of the methyl groups of V and XII was tackled by the nmr technique that we had used[9] with the parent keto alcohols I, III, and IV. The pertinent data together with the corresponding spectral features of suitable derivatives of the latter substances are listed in Table I.

TABLE I

| | Position of the methyl peaks[a] | | | |
| | In deuteriochloroform | | In pyridine | |
Compound	2-Me	4-Me	2-Me	4-Me
Isocyclohexi-mide acetate	60.6 (6.3)	58.2 (5.9)	58.4 (6.2)	52.1 (5.9)
dl-α-Epiisocy-cloheximide acetate	58.8	58.8	57.6 (6.5)	50.3 (5.8)
Cycloheximide acetate	58.2 (6.3)	75.6 (6.7)	59.4 (6.4)	70.6 (6.6)
Naramycin-B acetate	71.4 (7.2)	60.9 (6.0)	63.8 (7.2)	53.0 (5.8)
Ketone V	58.0 (6.1)	57.7 (5.8)	59.8 (6.2)	50.6 (5.8)
Ketone XII	58.2 (6.1)	58.1 (5.9)	59.3 (6.1)	51.0 (5.9)

[a] The positions of the doublets were measured in cycles per second downfield from tetramethylsilane (TMS) taken at 0 cps; *J* values in cycles per second are noted in brackets.

The parameters that can be used to analyze this data and the validity of their use have been discussed previously.[9] At this point it is sufficient to say that in the nmr spectrum (a) in deuteriochloroform solution, axial methyl groups in this series absorb around 70 cps, whereas their equatorial counterparts absorb about 60 cps no matter whether they are at the 2 or 4 position of the cyclohexanone ring; (b) the *J* value of a 2-*ax*-

(18) K. V. Rao, *J. Org. Chem.*, **25**, 661 (1960).

CHART II

methyl group is the largest and that of a 4-eq-methyl group the smallest observed for this type of ketone; (c) a change of solvent from deuteriochloroform to pyridine leads to a marked displacement upfield of all methyl doublets except that of a 2-eq-methyl group. The latter stays put or moves slightly downfield.

In each case under discussion the doublets are at ~58 cps and are superimposed. A change of solvent from deuteriochloroform to pyridine causes one of the doublets to shift upfield, whereas the other shifts but slightly downfield. Again none of the doublets has a J value in the region of 7 cps, i.e., there are no 2-ax-methyl groups, but in each case one doublet has the lowest J value (~5.8 cps) that is observed in this series. The latter must then be associated with a 4-eq-methyl.

In the light of this analysis the only sensible conclusion is that the methyl groups in both V and XII are cis oriented. Confirmation of this lies in the very close resemblance of their spectra to the acetates of isocycloheximide and α-epiisocycloheximide, both of which have cis-related methyl groups.

We at first thought that V and XII might differ in the orientation of their large side chain. However all attempts by us to interconvert V and XII by base treatment, which included heating at 100° for 18 hr with solid potassium carbonate, failed, starting materials being recovered in each case, in a pure state.[19] The difficulty was resolved when it was found that *the solution spectra of these two ketones were identical.* The only rational explanation of this phenomenon is that *one of these two compounds is the racemic form of the other.* That the dl form is XII, is supported by the

(19) Dr. T. Okuda (Tanabe Seiyaku Co., Ltd., Tokyo) has informed us that he also has been unable to isomerize V.

fact that all optical rotations quoted by Schaeffer and Jain[10] for derivatives of XII are essentially zero. The optical rotations (sodium D line) of XII and significantly, of epi-anhydrocycloheximide (XI) which were not reported by these authors,[10] we have found to be zero. In addition the optical rotatory dispersion curves of XI and XII showed no departure from the abscissa whereas V gave a real curve exhibiting a weak positive Cotton effect very similar to that of isocycloheximide.[20]

Thus we conclude that all compounds of the so-called epi series reported by Schaeffer and Jain[10] are racemic, have cis-oriented methyl groups, and in reality belong to the isocycloheximide series. A plausible mechanism for the racemization which occurs during the formation of XI can be visualized as shown in Chart II, through an $\alpha\beta \rightleftharpoons \beta\gamma$ double-bond equilibration,[21] an equilibration which is undoubtedly catalyzed by the p-toluene-sulfonic acid liberated from X.

Finally we have prepared XI from totally synthetic dl-α-epiisocycloheximide[22,23] (XVI, R' = H) by boiling its tosylate (XVI, R' = Ts; prepared as shown below) in dimethylformamide. The product was identical in all respects with that prepared from X. Since we have now shown unequivocally that V has cis methyl groups, we must conclude that all of its derivatives, including the dideoxycycloheximide IX, have cis methyl groups.

(20) T. Okuda, M. Suzuki, and Y. Egawa, Chem. Pharm. Bull. (Tokyo), **8**, 335 (1960).
(21) If we invoke such a mechanism there appears to be no reason why partial migration of the double bond to the Δ² position could not take place as shown below.
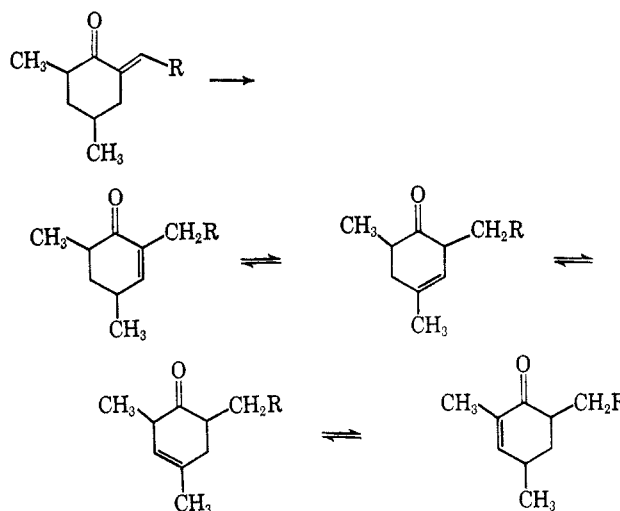
Indeed we have recently found[16] that 4,6-dimethyl-2-cyclohexenone undergoes isomerization largely to 2,4-dimethyl-2-cyclohexenone on prolonged treatment with hydrochloric acid. Although we did not pursue this line of questioning much further, the nmr spectra of all our samples of XI show a peak (intensity being sample dependent) at 105 cps highly characteristic of a methyl group on a double bond. Thus we believe that XI is in reality a mixture of the Δ² and Δ⁵ isomers despite the fact that we treat it in this paper as a single entity. The only reaction of significance that we have carried out with XI is catalytic reduction. Here if catalyst approach is axially oriented, the same product would be obtained from each isomer, a conclusion that we feel is supported by the experimental results, since a largely homogeneous product is obtained from this reaction. Insofar as the nmr of XI is concerned (vide infra) it could be expected that both the Δ² and Δ⁵ components would show vinylic proton absorption at the same position since one could expect these protons to be magnetically equivalent.

(22) F. Johnson and A. A. Carlson, Tetrahedron Letters, **885** (1965); F. Johnson, A. A. Carlson, and N. A. Starkovsky, J. Org. Chem., **31**, 1327 (1966).
(23) It should be noted that the use of the term epi in connection with this substance bears no relationship to its use in designating substances derived from XI. It is merely an unfortunate coincidence that previous authors have used the term to describe completely unconnected phenomena.

XVI
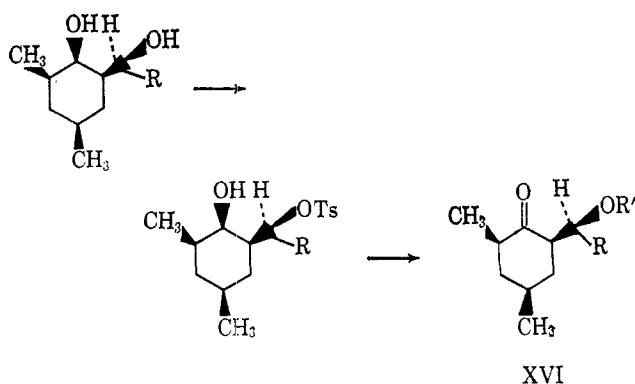
On this basis IX should be optically inactive and except for experimental error or the adventitious presence of some highly levorotatory impurity, we are at a lack to account for the $[\alpha]^{26}{}_D$ of $-19°$ reported[7] for this compound. A logical conclusion also would be that the methyl groups of II have *cis* stereochemistry. However there still remains the possibility that II does indeed have *trans* methyl groups but that during, or prior to, reduction to V the catalyst causes isomerization at the 2-methyl group *via* an enolic intermediate.

To preclude this likelihood, II was reduced in ether with diphenyltin dihydride, a neutral chemical reducing agent calculated to avoid any enolizing process. This afforded an oily unsaturated alcohol XVII [vinyl proton absorption at 316.5 cps; CHOH proton at 211 cps ($J = 8$ cps)] which when further reduced with hydrogen in the presence of a platinum catalyst gave a single crystalline alcohol XVIII, mp 112–113° (the stereochemistry assigned to the hydroxyl group of XVII and of XVIII is discussed below). Oxidation of this


II XVII

XVIII
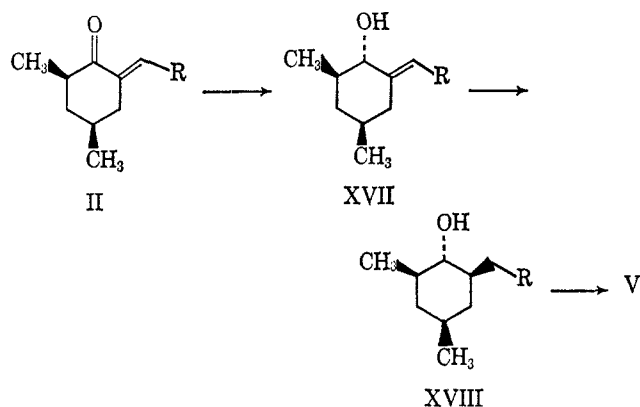
alcohol using Jones conditions[24] then yielded V. Inescapably the conclusion is reached that II has *cis* methyl groups and that dehydration of I under any of the conditions so far reported is accompanied by isomerization[11] at C-2. In an attempt to prepare an anhydrocycloheximide with *trans* methyl groups we pyrolyzed cycloheximide acetate at 180° but again II was obtained. Even the oily ethyl cycloheximide carbonate, prepared in the usual way, afforded II on pyrolysis at 250°. With these results, experiments in this direction were abandoned.

The nature of the reduction products of V represents the last remaining problem in this area. In our hands reduction of V with lithium aluminum tri(*t*-butoxy)hydride afforded a mixture of alcohols, mp 99–101° (*cf.*

(24) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 38 (1946).

Okuda[5]) in 88% yield. Careful chromatography resolved this mixture into its two components, the minor product, an alcohol (XIX) of mp 133–150.5°, and the second, and major product, an easily recognized substance, the previously obtained XVIII. When V was reduced with hydrogen and a platinum catalyst the same mixture of alcohols was obtained, although XIX constituted the bulk of the product. In this case XVIII was not isolated in a pure state but its presence could be recognized spectroscopically. That XIX has
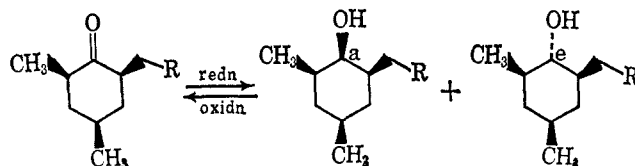
an axial and XVIII an equatorial hydroxyl group is evident from nmr data. The spectrum of XIX displays a relatively sharp peak at 215 cps ($W_H \approx 8$ cps) for a >CHOH proton. Both the shape and position are those to be expected for an equatorial proton of this type.[25,26] On the other hand in the spectrum of XVIII there is no peak visible at a field lower than 190 cps. We believe that the >CHOH proton in this molecule has a broad absorption at around 190 cps, but is partially masked by absorption due to other protons in the molecule. Again if the hydroxyl is equatorial, the axial proton in question would be expected to absorb in the range 145–225 cps.[26] These assignments are in agreement with the order of elution of the alcohols on chromatography, the axial alcohol having precedence.

As might be expected, XIX on chromic acid oxidation regenerated V, as had XVIII (*vide supra*), thus affording a completely consistent picture. Needless to say catalytic hydrogenation of XII afforded the racemic form of XIX[27] an easily crystallized material, which when oxidized by chromic acid led back to XII as previously reported by Schaeffer and Jain.[10]

It now seems very likely that of these two alcohols only XIX had been obtained in a pure state previously.[7] The material of mp 103–104° obtained by Okuda[5] from the complex metal hydride reduction of V is almost certainly a mixture of XIX and XVIII since they are impossible to separate by crystallization. The same remarks apply to the alcohol,[5] mp 117–118°, obtained from the catalytic reduction of V. We have found that appropriate mixtures of XIX and XVIII crystallize well together and have reasonably sharp melting points that vary from 102–103° to 128–131° depending on their composition.

The nature of the alcohol of mp 116–119°[7] and that of mp 130–131°,[5] obtained ostensibly from the catalytic reduction of II, is a matter for speculation. Each alcohol on oxidation gives a ketone of mp 125° which may or may not be different. We have never encountered a ketone of this melting point in our studies.

The melting points (120–121.5° and 132–134°) quoted for the samples of anhydrocycloheximide used in ex-

(25) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, 80, 6098 (1958); F. Johnson, N. A. Starkovsky, and A. A. Carlson, *ibid.*, 87, 4612 (1965).

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

(27) We did not search the mother liquors for the racemic form of XVIII but undoubtedly it is present in small amount.

perimental work by these authors[5,7] leads one to question their purity. From a stereochemical point of view it could be expected that under the equilibrating conditions used for the preparation of II, about 20% of the total crude material would have *trans* methyl groups. There is thus the distinct possibility that the anomalous alcohols and the ketone(s), mp 125°, derived from them, arise from this component of crude II and do have *trans* methyl groups. Our failure to isolate an anhydrocycloheximide with *trans* methyl groups[28] from the mother liquors of II, has precluded a rigorous examination of this thesis.

Ancillary Note.—Apart from *l*-cycloheximide all of the compounds described in this paper have the alkyl groups of their cyclohexane ring, equatorially oriented. Thus, they belong to the isocycloheximide series and their names should bear the term iso. We did not attempt to introduce this terminology earlier since to do so would have further complicated matters. The two sets of established nomenclature, both inaccurate, necessarily had to be used in the discussion section. However, in the Experimental Section the correct name is used for each individual compound according to the series to which it belongs and the name or names used previously are given parenthetically.

Experimental Section

Melting points are uncorrected. Nmr 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.

l-**Anhydroisocycloheximide** (II) was prepared according to established methods[2-4,10,12] or by one of the following procedures.

A.—A solution of cycloheximide (0.5 g) in benzene (5 ml) was added to a suspension of powdered aluminum chloride (1.0 g) in cold benzene (5 ml). The mixture was stirred at 25° overnight then decomposed by the addition of iced water. Evaporation of the organic phase afforded II (0.34 g), mp 130–133°. Its infrared spectrum showed characteristic bands at 5.80, 6.15, 7.75, 7.81, 8.04, and 8.70 μ; infrared, λ_{max}^{EtOH} 240 mμ (ϵ 9100).

B.—Cycloheximide (0.5 g) was dissolved in cold glacial acetic acid (20 ml) containing 2 drops of concentrated sulfuric acid. After 2 hr at 25° the mixture was diluted with cold water and extracted with methylene chloride. Evaporation of the solvent led to II (0.44 g), mp 132–133°, which was raised to 139–140°, $[\alpha]^{26}D$ −26° (c 5.0, CHCl$_3$), by four recrystallizations from aqueous acetone.

Monotosylation of *dl*-**Dihydro-α-epiisocycloheximide.**—*dl*-Dihydro-α-epiisocycloheximide[22] (1.0 g) in pyridine (7 ml) was treated slowly with *p*-toluenesulfonyl chloride (0.8 g) in methylene chloride (5 ml) at room temperature.

After standing at room temperature for 24 hr the mixture was taken to dryness at reduced pressure and at a temperature <40°. The residue was processed in the usual way, ether being used as the extraction solvent. The product which crystallized from ether had mp 143–145° (1.0 g). One further crystallization afforded the pure monotosylate, mp 147–148°.

Anal. Calcd for C$_{22}$H$_{31}$NO$_6$S: C, 60.3; H, 7.1; N, 3.2; S, 7.3. Found: C, 60.4; H, 7.2; N, 3.3; S, 7.5.

Infrared showed significant bands at 2.78, 3.15, 3.24, 5.83, 5.90, 6.25, 7.37, 7.46, 7.74, 7.83, 8.39, 8.46, 8.60, 9.11, 10.77, 10.92, 11.07, 12.29, 12.72, 14.04, and 15.14 μ.

dl-**α-Epiisocycloheximide Tosylate (XVI, R = Ts).**—The monotosylate (0.38 g) was dissolved in acetone (15 ml) and water (5 ml) and the solution cooled to ~5°. During 10 min a solution (5.5 ml) of chromium trioxide (0.73 g) and sulfuric acid (0.58 ml)

in water, was added. After 5 hr at room temperature, isopropyl alcohol (3 ml) was added and 1 hr later the reaction mixture was taken to small bulk under reduced pressure at <20°. The residue was triturated with water (30 ml) and methylene chloride (180 ml). The organic extract was separated, dried over magnesium sulfate, and taken to dryness. The residue crystallized from ether–methylene chloride as hard white crystals, mp 126–127° (0.312 g). Further crystallization did not improve the melting point.

Anal. Calcd for C$_{22}$H$_{29}$NO$_6$S: C, 60.7; H, 6.7; N, 3.2; S, 7.4. Found: C, 60.7, H, 6.5; N, 3.2; S, 7.2.

Infrared showed bands at 3.14, 3.24, 5.79–5.91, 6.25, 7.45, 8.39, 8.49, 8.61, 8.86, 10.26, 11.00, 11.22, 12.11, 13.00, 14.61, and 15.10 μ.

dl-epi-**Anhydrocycloheximide (XI). A. By the Action of Acids on I.**—1-Cycloheximide (8 g.) was dissolved in 6N HCl (100 ml) and the mixture held at 85° until the initially formed precipitate of II had redissolved (~15 min). The solution was diluted with water and extracted several times with methylene chloride. Evaporation of this extract gave a colorless oil (5.5 g) which was dissolved in a mixture of methylene chloride and ethyl acetate (1:1) and percolated through a column of silica gel (100 g). The eluates afforded a crystalline solid (4 g) which was recrystallized from methylene chloride–ether solution and afforded XI (1.8 g), mp 86–88°, $[\alpha]^{25}D$ 0° (c 2.0, CHCl$_3$) (lit.[10] mp 83–84°). Its infrared spectrum showed bands at 3.12 and 3.23 (NH), 5.84, 5.91, 5.98, 7.81, 7.90, 7.96, and 8.65 μ. It was identical with that of a specimen prepared according to Schaeffer and Jain[10] from cycloheximide tosylate.

XI could also be obtained by treating I (0.5 g) with 5 ml of 50% hydriodic acid at −5° for 1 hr, or with 20 ml of 48% hydrobromic acid at 25° for 1 hr, followed by using the isolation procedure described above.

B. By Isomerization of 1-Anhydroisocycloheximide (II).—*l*-Anhydroisocycloheximide (II, 1.0 g) was added to 6 N HCl (15 ml) and the mixture kept at 82° for 30 min. Isolation of the product by the procedure described above afforded XI (0.27 g), mp 83–85°, as heavy cubic crystals.

C. From *dl*-**α-Epiisocycloheximide Tosylate (XVI, R′ = Ts).** —*dl*-α-Epiisocycloheximide tosylate (0.155 g) was refluxed in dimethylformamide (1.5 ml) for 40 min. The mixture was cooled and poured into water and ice; the product (85 mg) was isolated by extraction with methylene chloride (three 20-ml portions). XI purification was effected by chromatography over silica gel (3 g) using methylene chloride containing increasing amounts of ethyl acetate as the eluent. Methylene chloride containing 7 to 10% ethyl acetate eluted the desired material (49 mg) which was crystallized twice from ether–petroleum ether (bp 30–60°) to give XI, mp and mmp 80–83°. The infrared spectrum (Nujol mull) was identical with that of a specimen prepared by procedure A or B.

Two-Stage Reduction of *l*-**Anhydroisocycloheximide to Alcohol XVIII.**—*l*-Anhydroisocycloheximide (II, 0.5 g) was suspended in dry ether (50 ml) and diphenyltin dihydride (2.0 g) added in one portion. The mixture was stirred for 3 days but the anhydroisocycloheximide dissolved completely during the first 6 hr. Thereafter the suspended tin compounds were removed by filtration and the filtrate evaporated to dryness. The residue was dissolved in methylene chloride and stirred for 1 hr to destroy unreacted diphenyltin dihydride. The solution was then percolated through a column of silica gel (30 g). Washing the column with methylene chloride (300 ml) removed the diphenyltin dichloride and subsequent elution with ethyl acetate (200 ml) afforded a viscous oil (0.49 g). The infrared spectrum showed bands at 2.81 (OH), 3.11, 3.23, 5.76, 5.94, 7.76, 7.87, 8.65, 9.45, 11.45 (broad), and 13.84 μ (broad). All attempts to crystallize this material proved fruitless.

The oil (0.42 g) was dissolved in ethyl acetate (25 ml) and hydrogenated over platinum (from 0.22 g of PtO$_2$) at room temperature and pressure. Hydrogen absorption ceased after 1 hr and the catalyst and solvent were then removed in the usual way. The residual gum (0.4 g) was crystallized from ether–petroleum ether (bp 30–60°) and yielded a highly crystalline product, mp 105–107° (0.18 g). Recrystallization gave the pure alcohol XVIII, mp 111–112°, $[\alpha]^{24}D$ +19.0° (c 1.5, CHCl$_3$). Its infrared spectrum showed bands at 2.81, 3.14, 3.23, 5.80, 5.95, 7.81, 7.90, 8.12, and 9.49 μ, whereas in chloroform solution bands appeared at 2.80, 2.96, 5.85, 6.86, 7.30, 8.02 (broad), 8.72, and 9.80 μ.

Anal. Calcd for C$_{15}$H$_{25}$NO$_3$: C, 67.4; H, 9.4; N, 5.2. Found: C, 67.4; H, 9.5; N, 5.0.

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(28) This material must be present however at least to the extent of 15–18% since a consideration of the isomerizational equilibria of such a dimethylcyclohexanone derivative would lead one to conclude that the two forms, *cis* and *trans*, should differ in energy by about 0.9 kcal/mole. More rigorous methods of separation or a different experimental approach undoubtedly would make the *trans* isomer available.

The total mother liquors from the second reduction above were combined and taken to dryness. The resulting gum (0.24 g) was oxidized in acetone (10 ml) by means of an aqueous chromium trioxide solution (3 ml, 1 N in oxygen) containing sulfuric acid (2.8 ml/100 ml of solution). Isolation in the usual way gave a glassy product (0.22 g) which crystallized from ether to give the saturated ketone V (0.11 g) having mp 109–110° after one further crystallization from the same solvent.

d-Deoxyisocycloheximide (V).—This was prepared according to known procedures[5,10] or as follows. *l*-Cycloheximide or *l*-anhydroisocycloheximide (0.3 g) was added to 8 ml of 50% hydriodic acid and the solution was allowed to stand at room temperature for 1 hr, during the course of which time iodine separated. The mixture was poured into an excess of cold water, then extracted with methylene chloride. Evaporation of the solvent yielded a gum which separated from ether as colorless crystals of V, mp 104–109°. Recrystallization from aqueous acetone gave pure V, mp 111–112°, $[\alpha]^{26}D$ +0.5° (*c* 6.5, MeOH) [lit.[5] mp 109.5–110.5°, $[\alpha]^{29}D$ +1.2° (*c* 1.0, MeOH)]. V was characterized by infrared bands at 3.11, 3.22, 5.77, 5.83, 5.92, 7.90, 8.01, 8.72, 8.85, 10.13, and 11.30–11.40 μ. It gave these ORD data ν in mμ ([α], deg): 625 (4.56), 555 (6.75), 500 (10.25), 454 (19.8), 410 (23.8), 385 (36.5), 370 (48.3), 357 (65), 345 (88.9), 333 (117.6), 323 (141.2), 312 (314), 303 (219), 294 (7.36), 286 (−22.8), 278 (−350), 270 (−397) (*c* 1.056, CH₃OH).

Anal. Calcd for $C_{15}H_{23}NO_3$: C, 67.8; H, 8.7; N, 5.3. Found: C, 67.6; H, 8.6; N, 5.2.

dl-Deoxyisocycloheximide (*epi*-Deoxycycloheximide, XII) and α-Deoxyactiphenol (XV).—*dl-epi*-Anhydroisocycloheximide (1.0 g) in absolute alcohol (125 ml) was hydrogenated over a 10% palladium-on-charcoal catalyst at 25° under atmospheric pressure. Hydrogen absorption (80 ml) ceased after 10 min. The product, isolated in the usual way, was a gummy solid which was dissolved in methylene chloride and chromatographed over silica gel (30 g). Elution of the column with methylene chloride containing 15% ethyl acetate afforded a white solid (0.5 g.) Recrystallization of this material gave pure XII, mp 148–150° $[\alpha]^{26}D$ 0° (*c* 2.0, CHCl₃) (lit.[10] mp 151–152°). Its main infrared bands are located at 3.10, 3.21, 5.75, 5.82, 5.91, 7.88, 7.97, 8.70, and 8.82 μ. In 1% chloroform solution, the spectrum was identical with the chloroform solution of V having bands at 2.95 (NH), 5.85 (broad) (C=O), 6.84, 8.05, 8.70, and 8.82 μ. Its nmr spectrum was also identical with that of V.

Further elution of the column with methylene chloride containing 20% ethyl acetate gave a colorless solid which was recrystallized from methylene chloride–ether to give XV, mp 140–147°, λ_{max} at 282 mμ (ϵ 1630) [lit.[18] mp 147–148°, λ_{max} at 280 mμ (ϵ 2000)]. Its infrared spectrum showed bands at 2.90 (OH), 3.12 and 3.24 (NH), 5.90–5.95, 7.90, 8.30, 8.61, 8.69, 11.38, and 11.50–11.74 μ.

Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.9; H, 7.3; N, 5.4. Found: C, 68.7; H, 7.9; N, 5.3.

Reduction of *d*-Deoxyisocycloheximide (V). A. With LiAl-(*t*-BuO)₃H.—Lithium aluminum hydride (0.228 g) was added to dry tetrahydrofuran (30 ml) under nitrogen and dry *t*-butyl alcohol (1.33 g) was added slowly. The mixture was stirred at ice-bath temperatures for 30 min before a solution of V (0.5 g) in tetrahydrofuran (20 ml) was added over 15 min. After a further 2 hr of stirring, water (10 ml) was added followed by dilute 20% acetic acid (10 ml). The precipitate was removed by filtration and the tetrahydrofuran removed from the filtrate under reduced pressure. The residual liquid was extracted with methylene chloride (four 25-ml portions) the extracts washed with water, sodium bicarbonate solution, and water, and finally dried over anhydrous sodium sulfate. Removal of the solvent afforded a crystalline, white solid (0.439 g), mp 99–101°.

The material was then dissolved in methylene chloride and chromatographed over silica gel (25 g). Elution of the column with 50-ml portions (total 800 ml) of methylene chloride con-

taining up to 19% ethyl acetate did not afford any material. Methylene chloride containing 20% ethyl acetate (250 ml) initially eluted crystalline material of mp 125–129° (46 mg). Recrystallization of this compound gave the pure alcohol XIX, mp 129.5–130.5°, $[\alpha]^{24}D$ −0.94 (*c* 2, CHCl₃). Its infrared spectrum showed bands at 2.83, 3.10, 3.25, 5.78, 5.96, 7.09, 7.90, 8.05, 8.19, and 10.11 μ.

Anal. Calcd for $C_{15}H_{25}O_3N$: C, 67.4; H, 9.4; N, 5.2. Found: C, 67.2; H, 9.3; N, 5.1.

Further elution of the column with this solvent combination (600 ml) gave mixtures of materials (184 mg) of variable melting point. Elution with methylene chloride containing 30–50% ethyl acetate then gave material (134 mg) of mp 107–110°. Recrystallization of this product from ether–petroleum ether (bp 30–60°) gave the pure alcohol XVIII, mp 112–113°, $[\alpha]^{24}D$ +19.75° (*c* 2.0, CHCl₃), spectroscopically identical with that obtained previously.

B. With Hydrogen and a Platinum Catalyst.—A solution of V (0.6 g, mp 111–112°) in acetic acid (50 ml) was stirred under hydrogen with platinum oxide (0.3 g) at room temperature and pressure until gas absorption ceased (1 hr). Removal of the platinum catalyst and the solvent by standard procedures afforded a colorless glass (0.58 g) which was chromatographed over silica gel (20 g). The column was developed with methylene chloride containing up to 15% ethyl acetate. The same solvent pair (450 ml) in a 4:1 ratio then eluted material (0.327 g) of mp 128–130° which after one crystallization from methylene chloride–ether yielded the pure alcohol XIX, mp 130–130.5° (0.2 g), identical spectroscopically with that obtained by procedure A.

Further elution of the column by the same solvents gave materials having progressively lower melting points. The lowest melting fractions (mp 115–121°) were combined (70 mg) and recrystallized from ether–petroleum ether (bp 30–60°) to give plates, mp 117–118°. The infrared spectrum of the material, however, showed it to be a mixture of the alcohols XIX and XVIII. No further attempt was made to separate them.

Reduction of *dl*-Deoxyisocycloheximide (*epi*-Deoxycycloheximide, XII).—A solution of XII (0.1 g) in acetic acid (20 ml) was reduced in the presence of a platinum catalyst (0.1 g PtO₂) as described for B above. The product crystallized from methylene chloride–ether giving *dl*-XIX as white crystals, mp 141–142°, (lit.[10] mp 140.5–142°). Its main infrared bands occurred at 2.93, 3.16, 3.27, 5.82, 5.91, 7.70, 7.81, 8.13, 8.51, 8.67, 10.06, 10.56, and 11.25 μ and in chloroform solution at 2.80, 2.96, 5.86, 6.86, 7.31, 8.00 (broad), and 8.71 μ. The latter spectrum was identical with a chloroform solution spectrum of *l* XIX.

Oxidation of Alcohols XVIII and XIX.—A solution of XIX (50 mg, mp 130–130.5°) in acetone (3 ml) was oxidized at room temperature by means of the dropwise addition of a solution (2.5 ml) of chromium trioxide (66.6 g/l.) in aqueous 1.1 N sulfuric acid. After standing overnight isopropyl alcohol (1 ml) was added and 1 hr later the reaction mixture was worked up in the usual way. The product crystallized from ether to give *d*-deoxyisocycloheximide (V), mp 111–112.5° (40 mg), spectroscopically identical with an authentic sample.

When a similar oxidation was performed with XVIII (20 mg), the yield of crude product, mp 108–112°, was quantitative. A further recrystallization gave the pure material again identical in all respects with V.