Investigation of the Stereochemistry of Cycloheximide

By HOWARD J. SCHAEFFER and VIJAY K. JAIN

Cycloheximide has been converted by a series of reactions into a dideoxycycloheximide which was found to be optically active. This observation establishes that the methyl groups in dideoxycycloheximide are oriented in a *trans* manner. The significance of this result and an interpretation of the stereochemistry of certain other products are presented.

CYCLOHEXIMIDE, a glutarimide antibiotic which was isolated from *Streptomyces griseus*, exhibits antifungal and antitumor activity. Since the initial discovery of cycloheximide (1), several reports have appeared which describe the isolation of certain stereoisomers of this antibiotic. Okuda and co-workers have isolated naramycin A, naramycin B, and α-epi-isocycloheximide from *Streptomyces naraenses novo;* it has been established that naramycin A is identical with cycloheximide and that naramycin B and α-epi-isocycloheximide are stereoisomers of cycloheximide (2–4). Later, Lemin and Ford described the isolation of isocycloheximide from *S. griseus* and showed that isocycloheximide could be formed by the isomerization of cycloheximide with acid-deactivated alumina (5). Thus, there are four known antibiotics which are stereoisomeric to each other.

We became interested in determining the stereochemistry of cycloheximide and have reported certain preliminary results of our study (see *footnote*); this paper gives the complete details of this work and discusses the stereochemistry of certain degradation products of cycloheximide.

The method which we selected for the determination of the stereochemistry of the methyl groups involved the removal of the keto and hydroxyl groups of cycloheximide to form a dideoxy compound. Since the dideoxy compound would have methyl groups at the 1,3-positions and the glutarimide side chain at the 5-position, it could exist in a D (or L) form or in a *meso*-configuration. Therefore, the determination of the optical activity of the dideoxy compound would determine the relative configuration of the methyl groups because the compound with *cis*-methyl groups has a plane of symmetry and must be optically inactive, whereas the compound with *trans*-methyl groups does not possess a plane of symmetry and would be optically active. To prepare dideoxycycloheximide (VIII), cycloheximide (I) was dehydrated with phosphorus pentoxide in benzene solution and gave anhydrocycloheximide (II) (6). Hydrogenation of anhydrocycloheximide (II) with a palladium-on-charcoal catalyst gave deoxycycloheximide (III, m.p. 114–115°) in a 75% yield. Further hydrogenation of III with a platinum catalyst gave in good yield dihydrodeoxycycloheximide (VI) which upon treatment with thionyl chloride gave the corresponding chloro derivative (VII). When VII was allowed to react with zinc and acetic acid, the chlorine atom was removed, and the desired dideoxycycloheximide (VIII) was obtained. The structure of VIII was established by elemental analysis and by its infrared spectrum. Furthermore, the N.M.R. spectrum[1] of VIII revealed that it was free of absorption in the olefinic hydrogen region, thereby eliminating the possibility of contamination by the dehydrohalogenation product. Determination of the optical rotation of VIII gave $[\alpha]_D^{26}$ −19.0°.[2] For the reasons previously discussed, this observation establishes that the methyl groups of VIII are *trans*. This result leads to the conclusion that the methyl groups in cycloheximide are also *trans*. An identical stereochemical assignment for cycloheximide has been made by Lawes, who showed that pyrolysis of I gave (+)-*trans*-2,4-dimethylcyclohexanone (7).

Since it has been established that the methyl groups in VIII are *trans*, it is logical to assign a *trans* configuration to the methyl groups in III, VI, and VII. To prove that an isomerization had not occurred during the preparation of VI by catalytic hydrogenation of III, dihydrodeoxycycloheximide (VI) was oxidized with chromium trioxide in acetic acid, and a good yield of III was obtained. In addition, an attempt was made to isomerize III with *p*-toluenesulfonic acid, but only unchanged starting material was recovered. Because it is difficult to

---

Received April 19, 1963, from the School of Pharmacy, Department of Medicinal Chemistry, State University of New York at Buffalo, Buffalo.

Accepted for publication June 7, 1963.

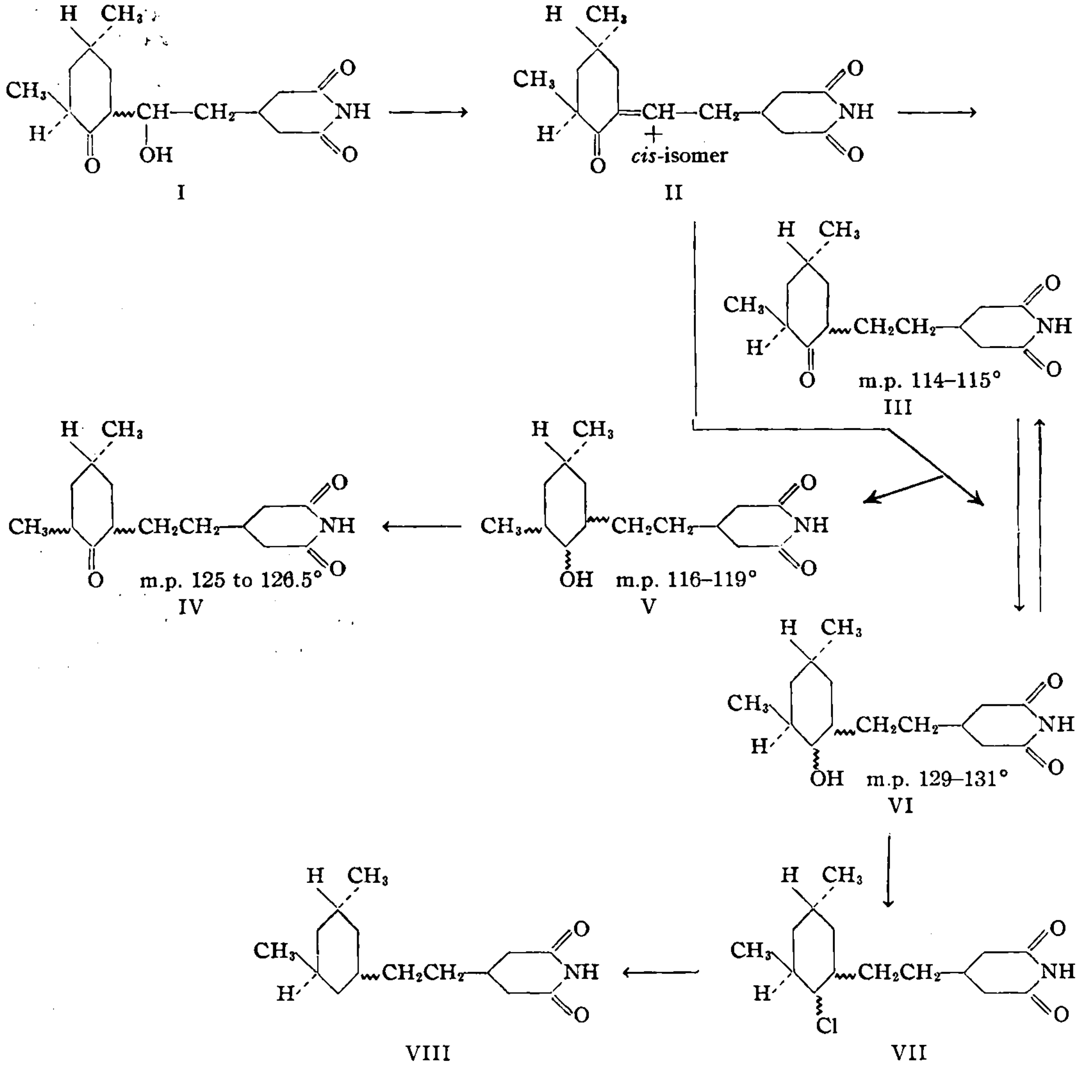
This investigation was supported in part by research career program award CA-K6-18718 and research grant CY-4812 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

Abstracted from the Doctor of Philosophy dissertation submitted by V. K. Jain.

Preliminary report: Schaeffer, H. J., and Jain, V. K., THIS JOURNAL, 50, 1048(1961).

[1] The authors are indebted to Professor E. Wenkert who determined and interpreted the N.M.R. spectra.

[2] This value was erroneously reported in the communication as $[\alpha]_D^{26}$ −9.07°.



visualize an isomerization occurring in the subsequent steps, we believe the assignment of *trans* methyl groups in III, VI, and VII is most probably justified.

It was also found that VI could be prepared directly by hydrogenation of anhydrocycloheximide with a platinum catalyst. However, in this reaction, two dihydrodeoxycycloheximides (V and VI) were obtained. The new dihydrodeoxycycloheximide (V) on oxidation with chromium trioxide in acetic acid gave a new deoxycycloheximide (IV). Okuda has made a similar observation concerning the hydrogenation of III but has reported that on oxidation V gave III and VI gave IV. In addition, on the basis of optical rotatory dispersion studies on III, he has assigned the *cis* configuration to the methyl groups in III and V (8). These results are in direct contrast to our findings.

Several comments concerning the stereochemistry of anhydrocycloheximide (II) are necessary. It is now apparent that anhydrocycloheximide, whether obtained by synthesis (9) or by dehydration of cycloheximide (6), naramycin B (3) or isocycloheximide (5) must be a mixture of stereoisomers. For example, in the synthesis of anhydrocycloheximide (9), the 2,4-dimethylcyclohexanone which was employed was a mixture of *cis* and *trans* compounds in the ratio of 9:1. The initial product which was obtained in an analytically pure form had a m.p. of 130–133° and $[\alpha]_D^{25}$ −12.7°. These data are in excellent agreement for II which is obtained from the antibiotics. Lawes further states (9) that exhaustive recrystallizations of the synthetic anhydrocycloheximide or that prepared by dehydration of cycloheximide afforded a single isomer of cycloheximide with a m.p. 139–141° and $[\alpha]_D^{25}$ −33.2°. This pure isomer of anhydrocycloheximide may have either *cis* or *trans* methyl groups.

A number of attempts were made to convert dihydrodeoxycycloheximide (V) into a dideoxycycloheximide so that information could be obtained concerning the stereochemistry of this series of products. When an attempt was made to replace the hydroxyl function of V by a bromo or chloro group, the reaction proceeded with dehydration and the desired product was not obtained. Therefore, at the present time, we prefer to leave the stereo chemistry of IV and V unassigned.

EXPERIMENTAL[3]

Preparation of Deoxycycloheximide (III).—A solution of anhydrocycloheximide (6) (7.52 Gm, 28.6 mmoles) in 200 ml. of absolute ethanol was added to 750 mg. of 5% palladium-on-charcoal catalyst. The mixture was hydrogenated at room temperature and at an initial pressure of 48.8 p.s.i. Reduction was completed after 24.8 mmoles of hydrogen had been absorbed. After filtration, the solvent was removed *in vacuo*, and the semisolid residue on crystallization from methanol and water gave 5.76 Gm. (75.9%) of deoxycycloheximide (III), m.p. 114–115°, $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3200 and 3100 (NH); 1710 and 1700 (C=O).

Anal.[4]—Calcd. for $C_{15}H_{23}NO_3$: C, 67.89; H, 8.74. Found: C, 67.90; H, 9.03.

Hydrogenation of Anhydrocycloheximide with a Platinum Catalyst.—A solution of 7.50 Gm. (28.4 mmoles) of anhydrocycloheximide (6) in 200 ml. of glacial acetic acid was added to a pre-reduced platinum oxide catalyst (1.50 Gm.). The mixture was hydrogenated at room temperature at an initial pressure of 59.0 p.s.i. Reduction was complete after 54.2 mmoles of hydrogen had been absorbed. The catalyst was removed by filtration; the filtrate was concentrated *in vacuo* to a semisolid residue. The fractional crystallization of the residue from benzene and hexane gave two products: dihydrocycloheximide (VI), 1.82 Gm. (24.1%), m.p. 124–126°, and isodihydrodeoxycycloheximide (V), yield, 2.44 Gm. (32.2%), m.p. 112°. One recrystallization of VI from ethanol and water gave the pure product 129–131°. $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3500 (OH); 3200 and 3100 (NH); 1710 and 1690 (C=O). Recrystallization of V from benzene and hexane gave the pure sample, m.p. 115–116°. $\bar{\nu}$ cm.$^{-1}$ (KBr); 3500 (OH); 3200 and 3100 (NH); 1710 and 1690 (C=O). The reported melting point for VI is 130–131° and for V 117–118° (8).

Hydrogenation of Deoxycycloheximide.—A solution of 4.78 Gm. (18.0 mmoles) of deoxycycloheximide (III) in 200 ml. of glacial acetic acid was added to 500 mg. of pre-reduced platinum oxide catalyst. The mixture was hydrogenated at room temperature until the absorption of hydrogen stopped. After filtration, the solvent was removed *in vacuo;* the semisolid residue on crystallization from ethanol and water gave 3.60 Gm. (75.0%) of dihydrodeoxycycloheximide (VI), m.p. 123–125°. One recrystallization of the crude product from ethanol and water gave the pure sample, m.p. 128–131°, mixed m.p. 130–132° with an authentic sample of dihydrodeoxycycloheximide; $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3500 (OH); 3200 and 3100 (NH); 1710 and 1690 (C=O).

Preparation of Dihydrocycloheximide Chloride (VII).—A solution of thionyl chloride (6 ml.) in *p*-dioxane (30 ml.) was cooled in an ice bath for 15 minutes; a solution of dihydrodeoxycycloheximide (VI), (1.58 Gm., 5.87 mmoles) in 30 ml. of *p*-dioxane was added. The reaction mixture was allowed to stand overnight at room temperature, concentrated *in vacuo* and the semisolid residue on crystallization from methanol and water gave 1.05 Gm. (62.5%) of dihydrodeoxycycloheximide chloride (VII), m.p. 131–132°. Three recrystallizations of the crude product from methanol and water gave the analytical sample, m.p. 136–138°. $[\alpha]^{25}_{D} = -6.38$ (4.23%) in methanol; $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3300 and 3200 (NH); 1750 and 1700 (C=O).

Anal.—Calcd. for $C_{15}H_{24}NO_2Cl$: C, 63.03; H, 8.46; N, 4.90; Cl, 12.40. Found: C, 63.29; H, 8.29; N, 5.05; Cl, 12.14.

Preparation of Dideoxycycloheximide (VIII).—Zinc dust (0.6 Gm.) was added to a solution of dihydrodeoxycycloheximide chloride (0.362 Gm., 1.26 mmoles) in 6 ml. of glacial acetic acid, water (1.8 ml.), and the reaction mixture was stirred for 1.5 hours at 80°. After filtration, water was added to the filtrate which caused crystallization of the crude product; yield, 0.251 Gm. (79.4%) of dideoxycycloheximide (VIII), m.p. 135–137°. Two recrystallizations from acetic acid and water gave the analytical sample, m.p. 135–137°; $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3290 and 3180 (NH); 1740 and 1690 (C=O). $[\alpha]^{26}_{D} = -19.0$ ($c = 0.63$ methanol).

Anal.—Calcd. for $C_{15}H_{25}NO_2$: C, 71.67; H, 10.02; N, 5.57. Found: C, 71.90; H, 9.84; N, 5.76.

Oxidation of Dihydrodeoxycycloheximide (VI).—To a solution of 96 mg. (0.36 mmoles) of dihydrodeoxycycloheximide (VI), in 2.5 ml. of glacial acetic acid was added a solution of chromium trioxide (32 mg.) in 0.4 ml. of water. The reaction solution was heated on steam bath for 10 minutes and then was allowed to stand for 3.5 hours at room temperature. After dilution with water (10 ml.), the solution was extracted with chloroform (4 × 15 ml.); the organic extract was washed with a saturated solution of sodium bicarbonate (3 × 20 ml.), water (25 ml.), dried with anhydrous magnesium sulfate, and then filtered. The volatile materials were removed *in vacuo* from the filtrate, and the residue on crystallization from methanol and water gave 66 mg. (70%) of deoxycycloheximide (III), m.p. 114.5 to 115.5°, mixed m.p. 112.5 to 115°, with the authentic sample of deoxycycloheximide (III); $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3200 and 3100 (NH); 1730, 1700, and 1680 (C=O). The deoxycycloheximide was further identified by thin-layer chromatography on silica gel when compared with the authentic sample of deoxycycloheximide (R_f 0.17 chloroform containing 1% methanol).

Oxidation of Isodihydrodeoxycycloheximide (V).—A solution of chromium trioxide (140 mg.) in 2 ml. of water was added to a solution of 314 mg. (1.17 mmoles) of isodihydrodeoxycycloheximide (V) in 7.5 ml. of glacial acetic acid. The reaction mixture was heated on steam bath for 10 minutes, then was allowed to stand at room temperature for 3 hours. After dilution with water (40 ml.), the solution was extracted with chloroform (4 × 3 ml.); the organic extract was washed with a saturated solution of sodium bicarbonate (3 × 30 ml.), water (30 ml.), dried with anhydrous magnesium sulfate, then filtered. The volatile materials were removed *in vacuo* from the filtrate, and the semisolid residue on crystallization from methanol and water gave 150 mg. (48.4%) of isodeoxycycloheximide (IV), m.p. 120–122°. Two recrystallizations of the crude product from methanol and water gave the analytical sample, m.p. 125 to 126.5°; $\bar{\nu}$ in cm.$^{-1}$ (KBr); 3200 and 3100 (NH); 1730, 1700, and 1680 (C=O).

---

[3] The infrared spectra were determined on a Perkin-Elmer model 137 spectrophotometer; the ultraviolet spectra were determined on a Perkin-Elmer model 4000A spectrophotometer. The melting points were determined on a Kofer Heizbank and are corrected.

[4] The analyses reported in this paper were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

Anal.—Calcd. for $C_{15}H_{23}NO_3$: C, 67.89; H, 8.74; N, 5.27. Found: C, 68.09; H, 8.92; N, 5.62.

Attempted Isomerization of Deoxycycloheximide (III).—A solution 0.10 Gm. (0.38 mmoles) of deoxycycloheximide (III) and 80 mg. (0.41 mmoles) of *p*-toluenesulfonic acid was refluxed for 18 hours. The reaction mixture was allowed to cool to room temperature, then poured onto ice. After the ice had melted, the mixture was filtered, and the filtrate was extracted with chloroform (4 × 30 ml.). The organic layer was washed with saturated solution of sodium bicarbonate (2 × 25 ml.), water (25 ml.), and dried with anhydrous magnesium sulfate. After filtration the volatile materials were removed *in vacuo;* the semisolid residue on crystallization from methanol and water gave 0.05 Gm. (50%) of deoxycycloheximide, m.p. 110–112°; $\bar{\nu}$ in cm.$^{-1}$ (KBr), 3200 and 3100 (NH); 1730, 1700, and 1680 (C=O). One recrystallization of the crude material from methanol and water gave a pure sample of deoxycycloheximide, m.p. 114–115°, mixed m.p. 112–114°, with an authentic sample of deoxycycloheximide.

REFERENCES

(1) Leach, B. E., Ford, J. H., and Whiffen, A. J., *J. Am. Chem. Soc.*, **69**, 474(1947).
(2) Okuda, T., Suzuki, M., Egawa, Y., and Ashino, K., *Chem. Pharm. Bull. Tokyo*, **6**, 328(1958).
(3) *Ibid.*, **7**, 27(1959).
(4) Okuda, T., and Suzuki, M., *ibid.*, **9**, 1014(1961).
(5) Lemin, A. J., and Ford, J. H., *J. Org. Chem.*, **25**, 344 (1960).
(6) Kornfeld, E. C., Jones, R. G., and Parke, T. V., *J. Am. Chem. Soc.*, **71**, 150(1949).
(7) Lawes, B. C., *ibid.*, **84**, 239(1962).
(8) Okuda, T., *Chem. Pharm. Bull. Tokyo*, **7**, 666(1959).
(9) Lawes, B. C., *J. Am. Chem. Soc.*, **82**, 6413(1960).

Segregation Kinetics of Particulate Solids Systems I

Influence of Particle Size and Particle Size Distribution

By JAMES L. OLSEN† and EDWARD G. RIPPIE

Rates of segregation of initially randomly distributed mixtures of steel spheres of various sizes have been studied. Extent of segregation was determined by sampling the entire system and expressed as the standard deviation of the sample compositions from the mean composition of the system. The several idealized systems studied showed apparent first-order kinetics, and rate constants were determined as a function of particle size and particle size distribution. Segregation in these studies proceeded to an equilibrium state in which mixing and unmixing effects were balanced.

MIXTURES OF SOLIDS are involved in many processes of industrial and technical importance. The glass, ceramic, paint, powder metallurgy, cement, and pharmaceutical industries are among those in which control of systems of solid particles is essential for the production of an acceptable end product. Such general characteristics as particle size distribution, "average shape," pore size, tendency to flow, and uniformity of composition are of prime importance (1).

Since the manufacture of tablets is generally accomplished by the compression of granulated or powdered solids, uniformity of dosage depends to a large extent on the uniformity of mixing of the granulation. The problem of maintaining a uniform mixture is particularly acute where many powdered ingredients having different size distributions, densities, and shapes are to be combined in a single tablet.

Solids mixing as a unit operation has received most attention in the literature in connection with specific types of mixers. Rate equations allowing approximate calculations of the time of mixing necessary to achieve a complete mix have been derived by several workers (2–5). Again, these studies primarily reflect the character of a specific mixer rather than the nature of the mechanisms of mixing and unmixing in general. It is thought, however, that mixing proceeds to a state wherein processes (convection, shear, and diffusion) leading to a random mix balance the tendency to segregate (5). Mixers are adjusted so that this segregation effect is minimized; however, subsequent handling of the mix may render it unsatis-

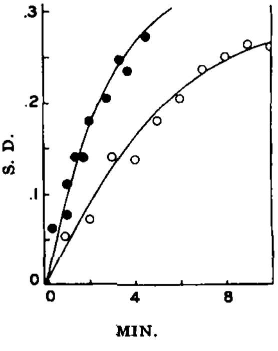


Fig. 1.—Plot of the standard deviation of a binary system *vs.* time of agitation. Solid points are at a 1:1 weight ratio of 8/32 *vs.* 3/32 in. steel balls. Open points are at a 1:1 weight ratio of 6/32 *vs.* 3/32 in. steel balls.

Received May 6, 1963, from the Department of Pharmaceutical Technology, College of Pharmacy, University of Minnesota, Minneapolis.
Accepted for publication June 7, 1963.

† Present address: Clark-Cleveland, Inc., Binghamton, N. Y.