our procedure yields a nitrite:ester ratio appreciably lower than the value predicted by Hay.

If we apply the same general considerations to reactions involving the chloroglyceryl dinitrate and ethylglyceryl trinitrate and calculate ratios, we obtain an appreciably lower value in nitrite yield in the case of ethylglyceryl trinitrate. This suggests that the presence of an ethyl group has increased the stability of the molecule to oxidation-reduction or has favored reactions not resulting in nitrite formation. In the case of chloroglyceryl dinitrate having no nitrate group on carbon-1, an even higher degree of stability is indicated.

It appears that in each of the three nitrate esters one nitrate group is readily reduced and a second nitrate is reduced more slowly (or less completely). In spite of this apparent lack of stoichiometry, the highly consistent results obtained from repeated trials indicate that with reasonable care in the control of experimental conditions employing standard recovery curves, a satisfactory analytical procedure can be devised.

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Investigation of the Stereochemistry of Cycloheximide and Its Degradation Products

By HOWARD J. SCHAEFFER and VIJAY K. JAIN

Cycloheximide tosylate has been shown to undergo elimination of *p*-toluenesulfonic acid to give a new anhydrocycloheximide. By a series of reactions this new anhydrocycloheximide has been converted into a dideoxycycloheximide which was shown to be optically inactive. This observation establishes the cis-orientation of the methyl groups in the new dideoxycycloheximide. The series of reactions employed and the stereochemistry of the products obtained are discussed.

THE DETERMINATION of the stereochemistry of the glutarimide antibiotics has been under investigation recently in a number of laboratories (1–4, 7). On the basis of these studies, it has been demonstrated both by chemical degradation (7) and by thermal degradation (1) that the two methyl groups in cycloheximide are oriented in a *trans* manner. We have now observed certain other degradative and isomerization reactions on cycloheximide which bear on its stereochemistry.

Treatment of cycloheximide (I) with *p*-toluenesulfonyl chloride in pyridine gave a good yield of the corresponding tosylate (II). When an attempt was made to displace the tosylate group in II with iodide ion using acetone as the solvent, only unchanged starting material was recovered. Therefore, II was treated with sodium iodide in dimethylformamide; the product isolated from this reaction was not the corresponding iodide, but rather an iodine-free compound which exhibited ultraviolet absorption at 235 mμ (ϵ, 9480). On the basis of its elemental analysis, infrared and ultraviolet spectra, and its further reactions, this compound has been shown to be *epi*-anhydrocycloheximide (III).[1] Subsequently, it was learned that this interesting elimination and rearrangement reaction occurred merely by heating II in dimethylformamide. However, if the reaction is carried out in the presence of one equivalent of sodium bicarbonate, elimination of *p*-toluenesulfonic acid occurs with the formation of anhydrocycloheximide (IV)—identical with anhydrocycloheximide prepared by known procedures (5).

Catalytic hydrogenation of *epi*-anhydrocycloheximide (III) with a palladium-on-charcoal catalyst gave *epi*-deoxycycloheximide (V) which upon further hydrogenation with a platinum catalyst gave *epi*-dihydrodeoxycycloheximide (VI). Compound VI could also be prepared by the direct hydrogenation of III with a platinum catalyst. When VI was allowed to react with phosphorus tribromide in dioxane solution, the corresponding bromo compound VII was obtained which after reaction with zinc and acetic acid

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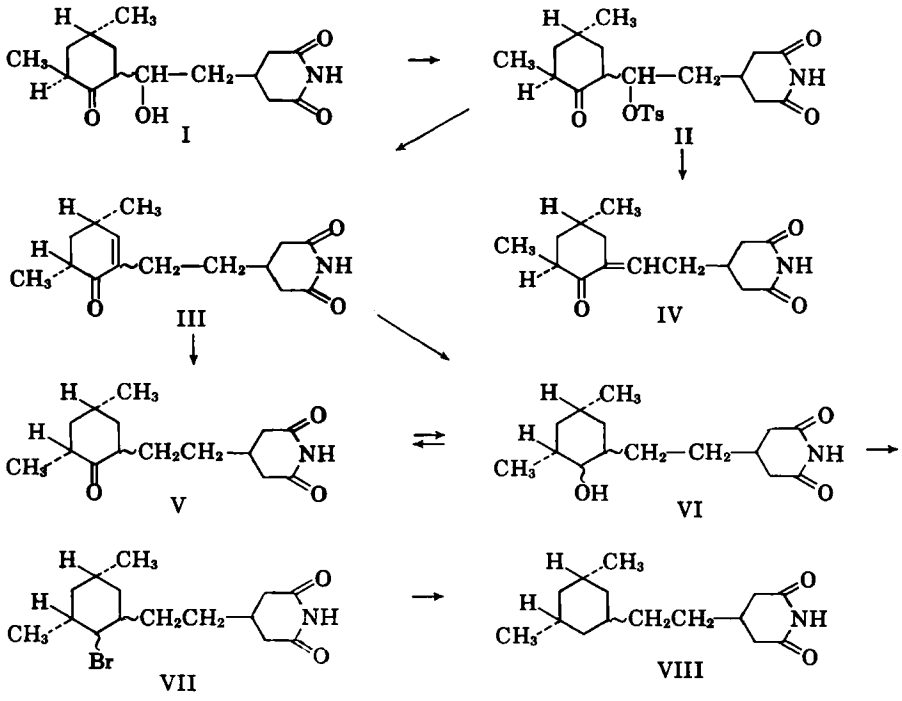
[1] In order to distinguish the degradation products which we have prepared in this research from the degradation products which have previously been prepared (5, 7), we have used the prefix *epi* for our new compounds. In each case, the *epi* compounds have been shown to be different from the previously prepared degradation products by comparing the infrared and ultraviolet spectra, melting points and mixture melting points, and by thin-layer chromatography.



gave *epi*-dideoxycycloheximide (VIII). An attempt to determine the optical activity of VIII revealed that it was optically inactive. This observation establishes that the methyl groups in VIII are *cis* since only the *cis*-compound possesses a plane of symmetry and therefore must be optically inactive.

Since it is known that the methyl groups in I (1, 7), and therefore in II, are *trans*, it is now apparent that during the preparation of III from II—in addition to the rearrangement of the double bond from *exo* to *endo*—an isomerization of the methyl group alpha to the ketone occurred to give the thermodynamically stable *cis* isomer. When III is hydrogenated with a palladium-on-charcoal catalyst, a new *epi*-deoxycycloheximide (V) is obtained. Hydrogenation of V with a platinum catalyst gave a new *epi*-dihydrodeoxycycloheximide (VI). Oxidation of VI with chromium trioxide in glacial acetic acid gave a good yield of V. An attempted isomerization of V with *p*-toluenesulfonic acid in dimethylformamide resulted in the recovery (in good yield) of unchanged starting material. Therefore, these results make it improbable that isomerization occurred during the hydrogenation of III. Since it is difficult to visualize an isomerization occurring in subsequent reactions, the assignment of the *cis*-methyl groups in III, V, VI, and VII is most probably justified.

By a series of reactions similar to those used in this work, anhydrocycloheximide (IV) was converted into a dideoxycycloheximide which was optically active (7). The observation that the dideoxycycloheximide was optically active established that the methyl groups were *trans;* therefore the methyl groups in cycloheximide (I) were also *trans*. Because the compounds obtained in this investigation are stereoisomeric with the compounds obtained in the previous series, we believe that our assignment of the *trans* configuration of methyl groups in the previous series is supported by the present work.

EXPERIMENTAL[2]

Cycloheximide Tosylate (II).—A solution of cycloheximide (3.96 Gm., 14.1 mmoles) in pyridine (15 ml.) was cooled in an ice bath for 30 minutes and *p*-toluenesulfonyl chloride (2.86 Gm., 15.4 mmoles) was added. The reaction mixture was allowed to stand overnight at room temperature and then was poured onto ice. After the ice had melted, the crystals were collected by filtration, washed with water, and then dried *in vacuo* at room temperature. Yield, 4.45 Gm. (71.7%) of cycloheximide tosylate, m.p. 115°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3200 and 3100 (NH), 1710 and 1700 (C = 0), 1600 (phenyl). One recrystallization of the crude material from benzene gave the analytical sample, m.p. 114°. The compound decomposed on standing at room temperature. This compound has recently been prepared by Okuda, who reported a m.p. of 100.5 to 101.5° (6).

[2] 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 Kofler Heizbank and are corrected.

Anal.[3]—Calcd. for $C_{22}H_{29}NO_6S$: C, 60.66; H, 6.71; N, 3.21; S, 7.36. Found: C, 60.19; H, 6.34; N, 3.13; S, 6.90.

***epi*-Anhydrocycloheximide (III).**—A solution of cycloheximide tosylate (3.6 Gm., 8.4 mmoles) in 12 ml. dimethylformamide was heated under reflux for 40 minutes. The reaction mixture was allowed to cool to room temperature and then poured onto ice. After the ice had melted, the crystals were collected by filtration, and washed with water. Yield, 1.3 Gm. (59%) of *epi*-anhydrocycloheximide, m.p. 83–84°. On recrystallization from ethanol and water, the crude material gave the analytical sample, m.p. 85–86°. $\lambda_{max.}^{EtOH}$ ($\epsilon \times 10^{-3}$) 235 mμ (9.48), $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3200 and 3100 (NH), 1740 and 1690 (C = 0).

Anal.—Calcd. for $C_{15}H_{21}NO_3$: C, 68.41; H, 8.04; N, 5.32; Found: C, 67.96; H, 7.91; N, 5.38.

Anhydrocycloheximide (IV).—A mixture of cycloheximide tosylate (1.0 Gm., 2.3 mmoles) and sodium bicarbonate (0.4 Gm., 4.6 mmoles) in dimethylformamide (15 ml.) was heated under reflux for 20 minutes. The reaction mixture was allowed to cool to room temperature and then poured onto ice. After the ice had melted, the crystals were collected by filtration and washed with water. Yield, 0.36 Gm. (60%) of anhydrocycloheximide, m.p. 125–127°. On recrystallization from benzene and hexane, the crude material gave the pure sample, m.p. 132–134°; mixed m.p. with authentic sample of anhydrocycloheximide 130 to 133.5° (5). $\lambda_{max.}^{EtOH}$ 240 mμ (ϵ = 8140); $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3200 and 3100 (NH), 1710 and 1690 (C = 0), 1610 (C = C).

***epi*-Deoxycycloheximide (V).**—A solution of 0.20 Gm. (0.76 mmole) of *epi*-anhydrocycloheximide in 200 ml. of absolute alcohol was added to 150 mg. of 5% palladium-on-charcoal catalyst which had been wetted with a few drops of water. The mixture was hydrogenated at room temperature and at an initial pressure of 40.7 p.s.i. until no more hydrogen was absorbed. The solution was filtered, and the solvent was removed *in vacuo*. The residue was crystallized from ethanol and water and gave 0.13 Gm. (65%) of *epi*-deoxycycloheximide, m.p. 151–152°. Two recrystallizations of the crude product from ethanol and water gave the analytical sample, m.p. 151–152°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3100 and 3200 (NH), 1690, 1700, and 1730 (C = 0). $[\alpha]_D^{24}$ = −4.1 (c = 4.8 $CHCl_3$).

Anal.—Calcd. for $C_{15}H_{23}NO_3$: C, 67.89; H, 8.74; N, 5.27. Found: C, 67.93; H, 8.63; N, 5.34.

***epi*-Dihydrodeoxycycloheximide (VI).**—A solution of 7.39 Gm. (28.0 mmoles) of *epi*-anhydrocycloheximide in 200 ml. of glacial acetic acid was added to a pre-reduced platinum dioxide catalyst (1.50 Gm.). The mixture was hydrogenated at room temperature at 59.8 p.s.i. Reduction was complete after 55.2 mmoles of hydrogen had been absorbed. After filtration, the solvent was removed *in vacuo*, and the semisolid residue on crystallization from benzene and hexane gave 2.22 Gm. (29.6%) of *epi*-dihydrodeoxycycloheximide, m.p. 137–138°. Two recrystallizations from methanol and water gave the analytical sample, m.p. 140–141°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3500 (OH), 3240 and 3110 (NH), 1690 and 1710 (C = 0). $[\alpha]_D^{23}$ = −0.309 (c = 3.2 acetone).

Anal.—Calcd. for $C_{15}H_{25}NO_3$: C, 67.38; H, 9.42; N, 5.24. Found: C, 67.16; H, 9.54; N, 5.15.

Hydrogenation of *epi*-Deoxycycloheximide.—A solution of 0.21 Gm. (0.80 mmole) of *epi*-deoxycycloheximide in 200 ml. glacial acetic acid was added to 75 mg. of pre-reduced platinum dioxide catalyst. The mixture was hydrogenated at room temperature until the absorption of hydrogen stopped. After filtration, the solvent was removed *in vacuo*, and the semisolid residue on crystallization from methanol and water gave 0.15 Gm. (71.3%) of *epi*-dihydrodeoxycycloheximide, m.p. 140.5 to 142°, mixed m.p. with an authentic sample of *epi*-dihydrodeoxycycloheximide 140.5–141.5°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3500 (OH); 3200 and 3100 (NH), 1690 and 1710 (C = 0).

Dihydrodeoxycycloheximide Bromide (VII).—A solution of *epi*-dihydrodeoxycycloheximide (0.28 Gm., 1.1 mmole) in *p*-dioxane (5 ml.) was cooled in an ice bath for 15 minutes; a solution of phosphorus tribromide in *p*-dioxane (5 ml.) was added. The reaction mixture was allowed to stand overnight at room temperature and then concentrated *in vacuo*. The colorless residue after crystallization from benzene and hexane gave 0.10 Gm. (27%) of *epi*-dihydrodeoxycycloheximide bromide, m.p. 142–144°. On recrystallization from benzene and hexane, the crude material gave the analytical sample, m.p. 151 to 151.5°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3100 and 3200 (NH), 1690, and 1740 (C = 0).

Anal.—Calcd. for $C_{15}H_{24}NO_2Br$: C, 54.54; H, 7.32; N, 4.24; Br, 24.19. Found: C, 54.33: H, 7.15; N, 4.25; Br, 24.09.

***epi*-Dideoxycycloheximide (VIII).**—Zinc dust (0.85 Gm.) was added to a solution of *epi*-dihydrodeoxycycloheximide bromide (0.58 Gm., 1.75 mmoles) in 10.6 ml. of glacial acetic acid; the reaction mixture was stirred for 2 hours at 80°. After filtration, water was added to the filtrate which caused crystallization of the crude product. Yield, 0.34 Gm. (77.2%) of *epi*-dideoxycycloheximide, m.p. 120°. Two recrystallizations from methanol and water gave the analytical sample, m.p. 124–126°. $\bar{\nu}$ in cm.$^{-1}$ (KBr): 3290 and 3180 (NH); 1740 and 1690 (C = 0). $[\alpha]_D^{24}$ = 0.0 (c = 1.496 acetone).

Anal.—Calcd. for $C_{15}H_{25}NO_2$: C, 71.67; H, 10.02; N, 5.57. Found: C, 71.51; H, 9.85; N, 5.69.

Oxidation of *epi*-Dihydrodeoxycycloheximide.—To a solution of 0.21 Gm. (0.78 mmoles) of *epi*-dihydrodeoxycycloheximide in 5.1 ml. of glacial acetic acid was added a solution of chromic acid (0.69 Gm.) in 0.75 ml. of water. The reaction solution was heated on steam bath for 10 minutes, then was allowed to stand for 3 hours at room temperature. After dilution with 20 ml. of water, the solution was extracted with chloroform (four 20-ml. portions). The organic extract was washed with a saturated solution of sodium bicarbonate (three 25-ml. portions), water (30 ml.), dried with anhydrous magnesium sulfate, and then filtered. The volatile materials were removed *in vacuo*, and the semisolid residue on crystallization from ethanol and water gave 0.13 Gm. (66.7%) of *epi*-deoxy-

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

cycloheximide, m.p. 151–153°; mixed m.p. 150 to 151.5° with an authentic sample of *epi*-deoxycycloheximide. $\tilde{\nu}$ in cm.$^{-1}$ (KBr): 3200 and 3100 (NH), 1730, 1700, and 1680 (C = 0). The *epi*-deoxycycloheximide was further identified by thin-layer chromatography on silica gel when compared with the authentic sample of *epi*-deoxycycloheximide (R_f = 0.15, in chloroform containing 1% methanol).

Attempted Isomerization of *epi*-Deoxycycloheximide.—A solution of 0.10 Gm. (0.38 mmole) of *epi*-deoxycycloheximide and *p*-toluenesulfonic acid (0.08 Gm., 0.41 mmole) in 2.0 ml. of dimethylformamide was refluxed for 40 minutes. The reaction mixture was allowed to cool to room temperature and then poured onto ice. After the ice had melted, the crystals were collected by filtration and dried *in vacuo;* yield, 0.07 Gm. of *epi*-deoxycycloheximide, m.p. 140°. On recrystallization from ethanol and water, the crude material gave the pure sample, m.p. 149.5 to 152°; mixed m.p. 150–152° with the authentic sample of *epi*-deoxycycloheximide. $\tilde{\nu}$ in cm.$^{-1}$ (KBr): 3200 and 3100 (NH), 1730, 1700, and 1680 (C = 0). The *epi*-deoxycycloheximide was further identified by thin-layer chromatography on silica gel when compared with the authentic sample of *epi*-deoxycycloheximide (R_f = 0.15 chloroform containing 1% methanol).

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Photometric Determination of Pentylenetetrazol in Pharmaceutical Preparations

By ROBERT A. DAOUST

Pentylenetetrazol is quantitatively precipitated from aqueous solution by cuprous chloride reagent. The pentylenetetrazol–cuprous chloride complex is dissolved in concentrated nitric acid; the solution is adjusted to pH 4 with acetate buffer and sodium hydroxide solution, and the deep blue color of the copper complex developed with tetraethylenepentamine is determined photometrically at 580 mμ. The method can be applied to samples containing from 2–10 mg. pentylenetetrazol and furnishes a rapid means of determining small amounts of the drug in the presence of other ingredients in pharmaceutical preparations.

PENTYLENETETRAZOL, a white, odorless, crystalline drug with a slightly pungent bitter taste is applied extensively as a central nervous system stimulant in the treatment of schizophrenia in shock therapy of psychoses and as an analeptic in narcotic poisoning.

This paper describes a method for the photometric determination of pentylenetetrazol in pharmaceutical liquids and tablets; the method can be performed rapidly and simply on small amounts of samples suitable for pharmaceutical control work. A gravimetric method which is determined by salting the drug from its aqueous solution with ammonium sulfate, followed by extraction with carbon tetrachloride has been officially adopted by "The National Formulary" (1, 2). Another gravimetric method involving precipitation with phosphotungstic acid is favored by some workers (3, 4). Numerous procedures have been reported for the assay of pentylenetetrazol: refractometric (5, 6), indirect polarographic (7), complexometric (8–10), colorimetric (11, 12), and volumetric and potentiometric methods (12).

The principle of the method proposed involves the precipitation of pentylenetetrazol from its aqueous solution with an excess of cuprous chloride reagent to give an insoluble, silver-white, crystalline, double salt complex which is then dissolved in concentrated nitric acid. The acid decomposes the pentylenetetrazol–cuprous chloride complex into its components and simultaneously oxidizes the cuprous salt to the cupric form. The acid solution is adjusted to pH 4 with acetate buffer and sodium hydroxide solution, and the absorbance of the deep blue color of the copper complex developed with tetraethylenepentamine

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