[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Isocycloheximide

A. J. LEMIN AND J. H. FORD

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Isocycloheximide has been shown to be a stereoisomer of cycloheximide.

Increasing interest in antibiotics as potential anti-tumor and pesticidal agents has resulted in the discovery of a number of new cycloheximide analogues and isomers.[1-5]

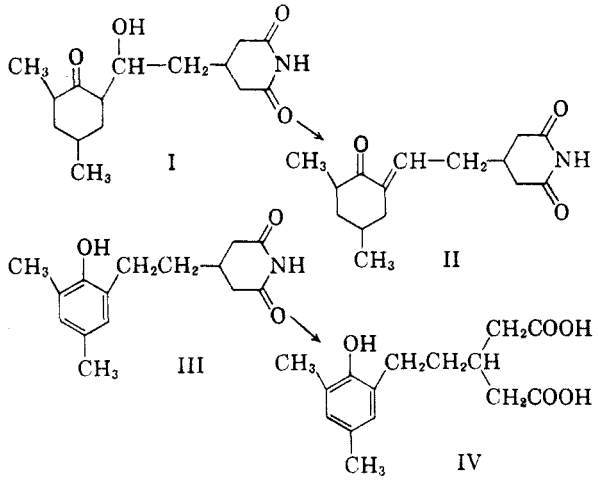
During the work-up of pooled mother liquors from the crystallization of cycloheximide[6] a compound with a sharp melting point lower than that of cycloheximide was isolated. The empirical formula, $C_{15}H_{23}NO_4$, was the same as that of cycloheximide and the infrared absorption spectrum was almost identical with that of cycloheximide. Although the new substance was apparently closely related to cycloheximide, the fact that they were different was confirmed by the preparation of an acetate from the new compound which was shown to differ from cycloheximide acetate. Since cycloheximide has four centers of asymmetry it was suspected that the new compound was a stereo-isomer and it was provisionally named isocycloheximide.

Isocycloheximide was found to have about 30 per cent of the activity of cycloheximide by *Saccharomyces pastorianus* bioassay[7] and about 30 per cent of the toxicity when determined intravenously in mice.[8]

Proof that isocycloheximide was stereoisomeric with cycloheximide and some indication of the centers involved were obtained as follows. When cycloheximide was chromatographed on acetic acid–deactivated alumina, a small amount of isocycloheximide was formed. Shaking cycloheximide with acid-deactivated alumina also produced isocycloheximide. The separation of isocycloheximide from cycloheximide proved to be difficult and hence the product was identified as the acetate.

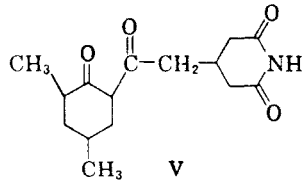
Dehydration of both cycloheximide (I) and isocycloheximide (I) by treatment with pyridine hydrochloride gave the same anhydrocycloheximide[6b] (II) indicating that isocycloheximide and cycloheximide have the same glutarimide and cyclohexanone structures.

Further evidence for the arrangement of carbons and the ketonic oxygen was afforded by bromination experiments. Bromination of cycloheximide with one molar equivalent of bromine followed by spontaneous dehydrobromination and dehydration led to the production of a phenol (III).



Bromination-dehydrobromination of isocycloheximide gave the same phenol, which on hydrolysis gave the glutaric acid (IV).

The secondary hydroxyl group is not involved in the isomerization of cycloheximide to isocycloheximide. This is shown by the fact that the isomerization of dehydrocycloheximide[6b] (V) gives the same product, isodehydrocycloheximide, that is obtained by the oxidation of isocycloheximide with chromic acid.



Rigorous proof of the stereochemical relationship between isocycloheximide and cycloheximide is lacking. However, since carbon-4 is distant from activating centers, it appears that this center retains its configuration[9] during the isomerizations.

(1) T. Okuda, M. Suzuki, Y. Egawa, and K. Astimo, *Chem. and Pharm. Bull. (Japan)*, **6**, 328 (1958).

(2) K. V. Rao and W. P. Cullen, 134th Meeting ACS, Chicago, Ill., September 1958.

(3) A. J. Lemin, G. A. Boyack, W. C. Haskett, M. F. Murray, W. T. Sokolski, A. Steinhards, and G. Swank, 132nd Meeting, ACS, New York, N. Y., September 1957.

(4) R. Paul, *Bull. Soc. Chim. France*, 1316 (1955).

(5) R. R. Herr, 6th Ann. Symposium on Antibiotics, Washington, D. C., October 1958.

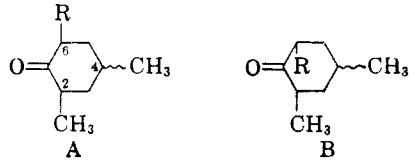
(6) (a) J. H. Ford and B. E. Leach, *J. Am. Chem. Soc.*, **70**, 1223 (1948). (b) E. C. Kornfeld, R. G. Jones, and T. V. Parke, *J. Am. Chem. Soc.*, **71**, 150 (1949).

(7) A. J. Whiffen, *J. Bact.*, **56**, 283 (1948).

(8) O. F. Swoap, unpublished data.

(9) The absolute configuration at carbon-4 is known: C. Djerassi, E. J. Eisenbraun, and J. Osiecke, *J. Am. Chem. Soc.*, **80**, 1261 (1958).

Further since two isomeric dehydrocycloheximides can be obtained, at least one of the remaining asymmetric centers (2 or 6) must be inverted. Since the same anhydrocycloheximide is obtained from both isomers, it is possible that the only center involved in the isomerizations is at carbon 6. This provisional hypothesis leads to configurations A and B for cycloheximide and isocycloheximide, isomerization being from 2,6-*trans* to 2,6-*cis* systems.



A somewhat similar change in the configuration of the 19-methyl group in the ketone obtained by the oxidation of taraxastene is known. This epimerization was also carried out by alumina treatment of the ketone.[10]

EXPERIMENTAL

Isocycloheximide. (a) *From the mother liquors from cycloheximide crystallizations.* The mother liquors from several batch crystallizations of cycloheximide (a total of 30 l. of amyl acetate solution) which had been allowed to stand at 5–10° for up to 18 months were evaporated under reduced pressure. Water was added to the resulting brown sticky mass and the residual amyl acetate removed by vacuum distillation as the water azeotrope. The aqueous concentrate was extracted with chloroform and the extract was treated with 3 kg. of carbon to effect a partial decolorization. The decolorized solution was concentrated under reduced pressure to a thick sirup and 25 l. of isopropanol was added. Most of the remaining chloroform was removed by concentrating the solution under vacuum to a volume of 20 l. After three months of storage at 5–10° the solution gave 1218 g. of crude isocycloheximide, m.p. 100–102°, $[\alpha]_D^{25}$ +36° (c = 10, CH_3OH).

Anal. Calcd. for $C_{15}H_{23}NO_4$: C, 64.02; H, 8.24. Found: C, 64.06; H, 8.04.

An infrared absorption spectrum in chloroform solution was found to have functional group bands identical with those of cycloheximide but there were slight differences in the low cm.$^{-1}$ regions.

Chromatography of 5.0 g. of the crude isocycloheximide on a mixture of 50 g. of activated carbon (Darco G-60) and 50 g. of kieselguhr (Celite 545) and elution with acetone-water mixtures gave the following solid fractions: with 1.2 l. of 30% acetone-water, 1.61 g., m.p. 75–83°, $[\alpha]_D^{25}$ +29.5° (c = 1.0, CH_3OH), with 400 cc. of 40% acetone-water, 0.43 g., m.p. 74–85° $[\alpha]_D^{25}$ +32° (c = 1.0 CH_3OH), with 400 cc. of 80% acetone-water, 0.72 g. m.p. 95–99°, $[\alpha]_D^{25}$ +32° (c = 1.0, CH_3OH).

Recrystallization of the highest melting fraction gave isocycloheximide identical with the sample obtained by recrystallizating the crude preparation.

A 200-tube counter-current distribution of the crude product using a two phase system consisting of benzene (10 parts), methanol (5 parts), and water (1 part) gave only one peak consisting of isocycloheximide identical with the sample obtained by recrystallizing the crude product.

(b) *By isomerizing cycloheximide using deactivated alumina.* Alumina, (Brockman Grade 1, Fisher Scientific Company, 80–200 mesh, 457 g.) was deactivated by suspending in 600 cc. of dry benzene, adding 27.42 cc. of 10% acetic acid and shaking the mixture for 15 hr. Cycloheximide (20 g.) was then added and the mixture shaken. At intervals of 1 hr., about 20 g. of the alumina was removed, filtered, and the solid extracted with 50 cc. of chloroform. Evaporation of the chloroform left a white solid the specific rotation of which was measured (CH_3OH). After 6.5 hr. shaking at room temperature the sample melted at 77–84°, and the rotation had reached a maximum of $[\alpha]_D^{25}$ +26° (CH_3OH), (70% conversion as calculated from the rotation).

The isomerization product was identified by a counter-current distribution of 2.75 g. of the isomerization mixture between benzene (10 parts), methanol (5 parts), and water (1 part) using ten funnels each containing 200 cc. of each phase. Funnel 8 contained 0.62 g. of impure isocycloheximide, m.p. 87–93° identified by a comparison of its infrared absorption spectrum with that of the authentic isocycloheximide. Funnel 7 contained 0.49 g. of solid, m.p. 83–90°, which on acetylation (acetic anhydride–pyridine) gave isocycloheximide acetate, m.p. 156–159° $[\alpha]_D^{25}$ +51° (c = 1.0 CH_3OH). A mixture melting point with isocycloheximide acetate prepared from authentic isocycloheximide was not depressed. The infrared absorption spectra of the two samples were identical.

Anal. Calcd. for $C_{17}H_{25}NO_5$: C, 63.15; H, 7.79; N, 4.33. Found: C, 62.94; H, 7.80; N, 4.43.

Dehydration of cycloheximide and isocycloheximide. A solution of 28 g. of cycloheximide in 100 cc. of pyridine containing 20 cc. of concentrated hydrochloric acid was refluxed for 7 hr. Addition of water (100 cc.) and cooling gave a white crystalline precipitate, m.p. 132–133° (27.5 g.) undepressed on admixture with anhydrocycloheximide, and having an infrared absorption spectrum identical with that of anhydrocycloheximide.[6b]

A similar reaction using isocycloheximide gave the same product identified by melting point, mixture melting point, and infrared absorption spectra comparisons.

3-[2-(2-Hydroxy-3,5-xylyl) ethyl] glutarimide. To a solution of 25 g. of cycloheximide in 500 cc. of chloroform was added a solution of 15 g. of bromine in 150 cc. of chloroform. The mixture was allowed to stand at room temperature until colorless, and then washed with 1% pyridine solution, 5% hydrochloric acid, and with water until the washings were neutral. Evaporation of the solvent under reduced pressure gave 22.3 g. of a brown oil which crystallized from ether, m.p. 148–155°.

Anal. Calcd. for $C_{15}H_{19}NO_3$: C, 69.0: H, 7.38; N, 5.37. Found: C, 69.19; H, 7.25; N, 5.29.

This product contained traces of bromine. However, on refluxing 5.0 g. of the solid with 25 cc. of γ-collidine for 1 hr. and work up by dilution with water, extraction with chloroform, washing the chloroform with dilute hydrochloric acid and with water, followed by removal of the solvent, 3.63 g. of the product, 3-[2-(2-hydroxy-3,5-xylyl)ethyl]-glutarimide, m.p. 153–157° was obtained. This sample was bromine free. Recrystallization from methanol gave the analytical sample, m.p. 155–157° $[\alpha]_D^{25}$ ±0° (c = 1.0 CH_3OH).

Anal. Calcd. for $C_{15}H_{19}NO_3$: C, 69.0; H, 7.38; N, 5.37. Found: C, 69.28; H, 7.52; N, 5.38.

The ultraviolet absorption spectrum had a maximum at 278 mμ, ϵ = 2050, and the compound was freely soluble in dilute sodium hydroxide solution.

Bromination of isocycloheximide. Bromination of 1.0 g. of isocycloheximide in 20 cc. of chloroform using 0.57 g. of bromine in 5.7 cc. of chloroform and working up as described for the bromination of cycloheximide gave 3-[2-(2-hydroxy-3,5-xylyl)ethyl]glutarimide m.p. 155–158°, undepressed on admixture with the sample obtained from cycloheximide.

Anal. Calcd. for $C_{15}H_{19}NO_3$: C, 69.0; H, 7.38; N, 5.37. Found: C, 69.03; H, 7.00: N, 5.43.

3-[2-(2-Hydroxy-3,5,-xylyl)ethyl]glutaric acid. A solution of 1.0 g. of 3-[2-(2-hydroxy-3,5-xylyl)ethyl]glutarimide in

(10) T. R. Ames, J. L. Beton, A. Bowers, T. G. Halsall, and E. R. H. Jones, *J. Chem. Soc.*, 1907 (1954).

20 cc. of 10% aqueous potassium hydroxide solution was refluxed for 1 hr. Cooling, addition of water, and extraction with ether removed neutral products. Acidification of the aqueous solution, extraction with ether and washing the ether with water until the washings were neutral and evaporation of the solvent gave 3-[2-(2-hydroxy-3,5-xylyl)ethyl]-glutaric acid, m.p. 131–132.5°.

Anal. Calcd. for $C_{15}H_{20}O_5$: C, 64.3; H, 7.55. Found: C, 64.25; H, 7.56.

Dehydroisocycloheximide. (a) *From isocycloheximide.* A solution of 1.0 g. of isocycloheximide in 20 cc. of acetone was oxidized by the addition of a chromic acid–sulfuric acid mixture.[11] When the mixture retained a brown color for 5 min., 20 cc. of water was added. The mixture was extracted with ether, the ether solution washed with water until the washings were neutral and the extract dried over anhydrous sodium sulfate. Distillation of the solvent and crystallization of the oily residue from aqueous acetone gave 0.81 g. of dehydroisocycloheximide, m.p. 152–154°, $[\alpha]_D^{25}$ −20° (c = 1.0 CH_3OH).

Anal. Calcd. for $C_{15}H_{21}NO_4$: C, 64.49; H, 7.58; N, 5.05. Found: C, 64.67; H, 7.30; N, 5.05.

(11) R. Curtis, *J. Chem. Soc.*, 461 (1953).

When a solution of dehydroisocycloheximide in aqueous acetone was added to an excess of aqueous copper acetate a green precipitate of the copper complex formed immediately.

(b) *From dehydrocycloheximide.* To a solution of 1 g. of dehydrocycloheximide[6b] in 20 cc. of pyridine was added 6 cc. of concentrated hydrochloric acid and the mixture was refluxed for 3 hr. Addition of 20 cc. of water to the hot solution followed by cooling gave 0.9 g. of dehydroisocycloheximide, m.p. 151–154°, $[\alpha]_D^{25}$ −23° (c = 1.0 CH_3OH) identified by a mixture melting point (undepressed) and by comparison of the appropriate infrared absorption spectra.

Anal. Calcd. for $C_{15}H_{21}NO_4$: N, 5.05. Found: N, 4.99.

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KALAMAZOO, MICH.

[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

Cyclic and Acyclic Amides of *cis*-β-(*p*-Bromobenzoyl)-β-methylacrylic Acid

ROBERT E. LUTZ, CARROLL T. CLARK,[2,3] AND JOSEPH P. FEIFER

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Assigned structures of cyclic and acyclic *cis*-β-bromobenzoyl-β-methylacrylic "amides" II–IV, of the cyclic pseudo "acid chloride" V, and of the cyclic and acyclic esters VII–VIII, have been confirmed by ultraviolet and infrared absorption studies. The γ-hydroxylactams show relative pK_a' values of 11.7–11.8, the *cis* acid (the γ-hydroxylactone, Xa) 6.4, the *trans* acid 4.4, and the γ-anilinolactone IIIa 8.4. The anions of the γ-hydroxylactams are cyclic XIII whereas the anions of the acid XIa and the γ-anilinolactone XVI are acyclic. Diazomethane converts the γ-anilinolactone to the acyclic (*cis*) anil-ester XVIII. The cyclic pseudo acid chloride is shown to undergo attack at the chloride group by alcohol and by aromatic amines, but reacts at the lactone carbonyl group with the more basic aliphatic secondary amines.

This paper elaborates upon earlier studies[4,5] of ring-chain tautomerism of *cis*-β-(*p*-bromobenzoyl)-β-methylacrylic acid (Xa), its "acid chloride" which is believed to have the γ-chlorolactone structure V, and its three types of "amides,"[5] the acyclic (normal or true) amides II, the γ-aminolactones III, and the γ-hydroxylactams IV. Examples of all three of the "amide" types had been obtained by reactions between ammonia or amines and the "acid chloride" V. Ammonia and methylamine produced γ-hydroxylactams IVa and IVb; aniline and methylaniline gave γ-aminolactones IIIa and IIIb; and dimethylamine gave the normal (true) *cis* amide IIa. Stereoisomerization by irradiation of the *trans* tertiary amides, the dimethylamide (Id) and the methylanilide (Ie), gave the normal *cis* amides (IIa and IIb), but the *trans* primary amide (Ia) and two *trans* secondary amides (the methylamide Ib and the anilide Ic) went beyond stereoisomerization with subsequent cyclization to the γ-hydroxylactams IVa–c.

The cyclic structure for the acid chloride V had been assigned because of the low rate of alcoholysis and hydrolysis,[6] and because the ester produced by alcoholysis was cyclic (VII). However, this is not sound evidence because the rate of alcoholysis of an acyl chloride group in an acyclic structure of type VI would depend on the steric hindrance involved in this configuration and on its concentra-

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(2) (a) Present address: University of Georgia, Athens, Ga. (b) This paper is based on a dissertation by C.T.C., University of Virginia, 1958. (c) The work was reported at the Atlantic City A.C.S. Meeting, September 1959, abstr. p. 14p. (*Cf.* also ref. 3.)

(3) R. E. Lutz and C. T. Clark, *J. Org. Chem.*, in press.

(4) (a) R. E. Lutz, P. S. Bailey, C-K. Dien, and J. W. Rinker, *J. Am. Chem. Soc.*, **75**, 5039 (1953), and references cited therein; (b) J. W. Rinker, Dissertation, University of Virginia, 1955.

(5) (a) R. E. Lutz and F. B. Hill, *J. Org. Chem.*, **6**, 175 (1940); (b) F. B. Hill, Dissertation, University of Virginia, 1940; (c) See references cited in (a) and (b). (d) *Cf.* also polarographic studies by S. Wawzonek, H. A. Laitinen, and S. J. Kwiatkowski, *J. Am. Chem. Soc.*, **66**, 827 (1944).

(6) R. E. Lutz and R. J. Tayler, *J. Am. Chem. Soc.*, **55**, 1168 (1933).