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Isomerization Study of Cycloheximides and Thermal Degradation of Naramycin-B. Chemical Proof to the Proposed Absolute Configuration of Cycloheximides*[1]

Lemin and Ford[1] reported on the isomerization of cycloheximide (Naramycin-A) into isocycloheximide by ageing the former in a solution or by treating the former with acid deactivated alumina. Recently the present writers found that cycloheximide isomerizes smoothly under basic environments as well. For example, when the benzene solution of cycloheximide (10 g.) was heated in the presence of sodium methoxide (1 g.) at 60~80° for about 6 to 10 hours, an isomerization product was recovered from the reaction mixture. Among the basic catalysts tested, sodium methoxide and potassium carbonate were found most adequate. Isolation of an isomerization product from the above reaction mixture was achieved by partition chromatography on diatomaceous earth (Celite Analytical Filter-Aid (John-Manville Co.)) using ethyl acetate-cyclohexane-McIlvain phosphate buffer (pH. 5.0) (1:9:1) solvent system. The isomerization product thus obtained was identical with isocycloheximide.[1,2]

Partition chromatogaphy described above made possible to prepare pure Naramycin-B readily. When the mixture of cycloheximide isomers was chromatographed on this system, isocycloheximide was eluted first, followed by Naramycin-B and finally cycloheximide was eluted. When anhydrocycloheximide co-existed in the mixture, this compound preceded isocycloheximide. During this procedure any evidence of isomerization or dehydration was not noticed. Naramycin-B purified by this procedure was purer than that reported previously[3] and its physicochemical properties should be revised as follows.

Naramycin-B : Colorless thin prisms, m.p. 112~113°
$[\alpha]_D^{21.8}$ +55.8° (c=5.0, MeOH)

Naramycin-B acetate : Colorless prisms, m.p. 155.5°
$[\alpha]_D^{21.8}$ +74.5° (c=2.0, MeOH)

Infrared spectra of pure cycloheximides in 1100~1000 cm^{-1} region (Fig. 1) are of use to find out the presence of isomer (s) in a crystalline compound.

In the course of isomerization of cycloheximide described above, when the heating

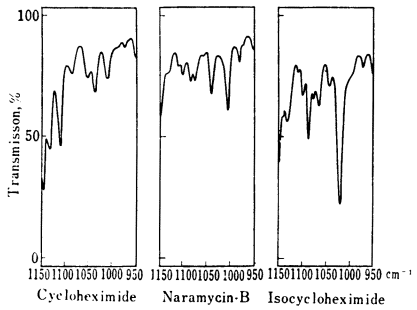

Fig. 1. Infrared Spectrum of Cycloheximides (1150~950 cm^{-1} Region in Nujol)

*[1] Preliminary Report of "Studies on Streptomyces Antibiotic, Cycloheximide (Part XVIII)."

1) A. J. Lemin, J. H. Ford : J. Org. Chem., **25**, 344 (1960); U. S. Patents : No. 2,903,457 and 2,903,458 (1961).
2) T. Okuda, M. Suzuki, Y. Egawa : J. Antibiotics, **14A**, 158 (1961).
3) T. Okuda, M. Suzuki, Y. Egawa, K. Ashino : This Bulletin, **7**, 27 (1959); T. Okuda, M. Suzuki, Y. Egawa, K. Kotera : Yakugaku Kenkyu, **33**, 530 (1961).

of cycloheximide solution was restricted only for 1 to 2 hours, Naramycin-B was recovered from the reaction mixture together with starting cycloheximide and isocycloheximide. Isomerization studies under the cited condition were carried out on Naramycin-B, isocycloheximide and α-epiisocycloheximide*[2] respectively, and it was found that Naramycin-B turned into isocycloheximide as in the case of cycloheximide, whereas isocycloheximide and α-epiisocycloheximide were recovered respectively as they were. Thus, it was deduced that, among the four isomers, isocycloheximide and α-epiisocycloheximide are most stable and Naramycin-B is more stable than cycloheximide.

Lawes[4] proved chemically the *trans*-relationship of two methyl groups at C-2 and C-4 in cycloheximide based upon his finding that cycloheximide gave (2S:4R)-2,4-dimethylcyclohexanone by thermal degradation.*[3] As a result of thermal degradation of Naramycin-B conducted by similar procedure as that of Lawes', it was ascertained that Naramycin-B did give *trans*-2,4-dimethylcyclohexanone ($[\alpha]_D^{22}$ +58.2° (c=5.0, MeOH); Yield, 47%), which was identical with the ketone obtained from cycloheximide itself.

These isomerization and pyrolysis studies gave strong chemical supports as to the absolute configurations of cycloheximides presented in Part XVII,[5] especially on the identity of the absolute configuration of C-α position in cycloheximide, Naramycin-B and isocycloheximide, and on the *trans*-relationship of two methyl groups at 2 and 4 positions of Naramycin-B. Relative stability of cycloheximides presumed from the proposed configurations was also consistent with the present findings.

Details of this paper will be published later.

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*2 A compound referred to as A_2 in J. Antibiotics, **14A**, 158 (1961).
*3 By a completely different procedure, Schaeffer and Jain obtained the same conclusion as to the relationship of two methyl groups of cycloheximide. (H.J. Schaeffer, V.K. Jain: J. Pharm. Sci., **50**, 1048 (1961)).

4) B.C. Lawes: J. Am. Chem. Soc., **84**, 239 (1962).
5) T. Okuda, M. Suzuki: This Bulletin, **9**, 1014 (1961).