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GLUTARIMIDE ANTIBIOTICS. PART III.[1] THE DETERMINATION OF THE STEREOCHEMISTRY OF THE METHYL GROUPS OF CYCLOHEXIMIDE ISOMERS BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY.

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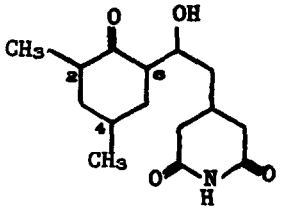
In recent years, attempts have been made to establish the configuration of the antibiotic cycloheximide (I) and its known isomers.[2-6] Differing opinions have been expressed by Lawes[4] and Okuda, et al.[2,3,6]



I

We wish to report on the correlation between the n.m.r. spectra of model cyclohexanone derivatives and compounds belonging to the cycloheximide group of antibiotics. This correlation gives a clean-cut answer to the stereochemical problems involved in determining the configurations of the groups on the cyclohexanone ring of I.

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1. Part II preceding article.
2. T. Okuda, Chem. and Pharm. Bull. (Japan) 7, 659 (1959).
3. T. Okuda, M. Suzuki and Y. Egawa, ibid. 8, 335 (1960).
4. B. C. Lawes, J. Am. Chem. Soc. 84, 239 (1962).
5. H. J. Schaeffer and V. K. Jain, J. Pharm. Sci. 1048 (1961).
6. T. Okuda and M. Suzuki, Chem. and Pharm. Bull. (Japan) 9, 1014 (1961).

Our method is based on the properties of the prominent methyl doublets in the n.m.r. spectra[7] of the cycloheximide isomers. Three parameters were measured: a) the position of the methyl doublets (recorded as the middle of the doublet which we have assumed is the centre of gravity) expressed in c.p.s., b) the coupling constant J in c.p.s. and c) the displacement of the peaks in c.p.s. occurring upon substitution of pyridine for deuterochloroform.[8]

Table I presents data pertaining to the isomeric 2- and 4-methylcyclohexanones. The methyl peaks of these two compounds occur at almost the same location when spectra are run in deuterochloroform but the J values differ, the 2-methyl isomer having the larger splitting constant. Finally, in the case of the latter compound the methyl peak remains sensibly in at the same place upon change of solvent from deuterochloroform to pyridine whereas it is displaced upfield by 8.8 c.p.s. in the case of the 4-isomer.

Although these results already indicate a marked difference between 2- and 4-methyl groups, the values obtained only represent average positions between the axial and equatorial conformers because of the interchange possible in unrestricted rings.[9] This

7. Spectra were recorded with a 60 Mc Varian instrument using deuterochloroform and pyridine as solvents. Tetramethylsilane at +10.00 was used as an internal standard.
8. Whenever possible, 10% solutions were used. However, differences in the solubility of the substances examined did not always permit the spectra to be determined at the same concentrations. Therefore, because of solution effects among others, the figures obtained are reliable only to 1 c.p.s. in expressing the positions of the peaks and to 0.1 c.p.s. in expressing J values.
9. The energy difference between 2a-methylcyclohexanone and its 2e-conformer has been estimated[10] to be about 1.6 Kcal/mole essentially the same value as that between the 2a- and 2e-conformers of 2-methylcyclohexane[11] indicating 2-methylcyclohexanone to exist, at room temperature, 95% in the equatorial state. To a first approximation 4-methylcyclohexanone probably has the same composition.

TABLE I

Cyclohexanone	Me group Stereochemistry	Positions of Me peaks (in c.p.s.)* in $CDCl_3$	in pyridine
2-methyl	largely equat.[9]	-60.6 (6.2)	-60.3 (6.3)
4-methyl	largely equat.[9]	-60.3 (5.5)	-51.5 (5.4)
trans-4-tert-Bu-2-Me	axial	-69.3 (7.2)	-64.6 (7.2)
cis-4-tert-Bu-2-Me	equat.	-61.1 (6.3)	-61.8 (6.3)
trans-2-tert-Bu-4-Me	axial	-66.8 (6.3)	-60.8 (6.3)
cis-2-tert-Bu-4-Me	equat.	-60.1 (5.6)	-52.6 (5.6)

*J values are given in brackets.

prompted us to prepare 4-tert-butyl-2-methylcyclohexanone and 2-tert-butyl-4-methylcyclohexanone in both their cis and trans forms.[12] From the n.m.r. spectra of these ketones the following facts stand out: a) axial methyl peaks occur at lower field than equatorial peaks when the spectra are taken in deuterochloroform, b) the J value for a 2a-methyl is the largest of the four isomers and the J value of the 4e-methyl is the lowest, c) change of solvent from deuterochloroform to pyridine leads to a marked displacement to higher field of all peaks except that of a 2e-methyl group which moves slightly to lower field. The 4e-methyl group peak suffers the strongest displacement, viz. + 7.5 c.p.s.

The data for the simple methylcyclohexanones are in remarkably good agreement with that of the more highly substituted ketones especially when the differences in rigidity of the two ring systems are considered.

Table II shows data pertaining to the cycloheximide isomers. A consideration of the J values shows that four different values

10. N. L. Allinger and H. M. Blatter, J. Am. Chem. Soc. 83, 994 (1961).
11. E. L. Eliel and M. Rerick, ibid. 82, 1367 (1960).
12. These ketones were prepared according to the method of Allinger and Blatter.[10] The constants of the cis and trans forms of the latter ketone (previously unknown) will be published in a fuller paper at a later date.

occur within a margin of error of ± 0.1 c.p.s. viz. 7.2; 6.7; 6.3 and 5.9 c.p.s. By correlation with Table I the highest value is indicative of a 2a-methyl group and the lowest of a 4e-methyl group. Compounds in Group A all display a peak with J = 5.9 occurring at relatively high field and displaced to about -52.5 ± 0.6 c.p.s. in pyridine. These facts allow us to assign this peak to a 4e-methyl group. The other peak also occurring at relatively high field, showing only a slight displacement on change of solvent and having J = 6.3 c.p.s. can belong only to a 2e methyl group. Therefore, compounds in group A are all 2e,4e-compounds.

TABLE II

	Compound	Position of the methyl peaks* in $CDCl_3$ 2 Me	4 Me	in pyridine 2 Me	4 Me
A	Isocycloheximide	59.0†	59.0†	57.9(6.2)	51.9(6.0)
A	Isocycloheximide acetate	60.6(6.3)	58.2(5.9)	57.4(6.2)	53.1(5.9)
A	α-Epi-isocycloheximide acetate	58.3†	58.3†	58.9(6.4)	52.0(5.8)
B	Naramycin B	72.7(7.3)	59.0(5.9)	64.4(7.1)	52.1(5.8)
B	Naramycin B acetate	71.4(7.2)	60.9(6.0)	63.8(7.2)	53.0(5.8)
C	Cycloheximide	58.2(6.3)	75.6(6.7)	61.0(6.3)	70.0(6.8)
C	Cycloheximide acetate	58.2(6.3)	75.6(6.7)	59.4(6.4)	70.6(6.6)
D	Neocycloheximide acetate	57.0†	57.0†	61.4(6.3)	48.4(5.8)

* J values given between brackets

† J values not given because of uncertainty in measurements in almost superimposed doublets.

A similar argument permits the same assignment to be made to neocycloheximide (D of Table II), thus corroborating the deductions made concerning its structure in the previous article.[1] Compounds of the B group display one peak which from

the point of view of the three criteria used (J-value, position and displacement) is identical to the 4-methyl peak of isocycloheximide. On the other hand, the other peak occurring at low field (-72.1 ± 0.7 c.p.s.) and having J = 7.2 c.p.s., and being strongly displaced upfield on change of solvent must be due to a 2a-methyl group. Thus, compounds in the Naramycin B series have the 2a,4e-configuration.

Cycloheximide and its acetate (group C) are similar to isocycloheximide in what concerns the peak with J = 6.3 c.p.s. indicating a 2e-methyl group. The other peak with J = 6.7 c.p.s. occurring at low field is in sharp contrast to the second peak of isocycloheximide and corresponds in accordance with the three criteria to a 4a-methyl group. It follows that cycloheximide has the 2e,4a-configuration.

These assignments are in agreement with the chemistry of cycloheximide and its isomers. Thermal degradation of both cycloheximide[4] and naramycin B[13] gives rise to trans-2,4-dimethylcyclohexanone. In addition, they support the conclusions of T. Okuda based on optical rotatory dispersion measurements but are not in agreement with the postulations of Lawes.[4]

The above studies have been extended to a variety of compounds in this area and have proved particularly valuable for assignments of configuration in cases of racemic compounds and to cyclohexanones having a methyl substituent in the 4-position. Both of these classes of compounds are unsuited to study by optical rotatory dispersion methods. A full report will be published in the near future.

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13. T. Okuda - Private communication.