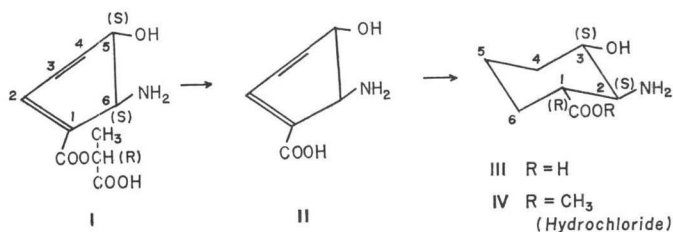


THE ABSOLUTE STRUCTURE OF  
 ORYZOXYMYCIN

Sir:

Oryzoxymycin<sup>1</sup> (I) is an antibiotic produced by *Streptomyces venezuelae* var. *oryzoxymyceticus*, possessing an inhibitory activity against *Xanthomonas oryzae*. It is easily converted to an inactive crystalline dimerization product. In a previous paper<sup>2</sup>, we reported the structure of I as D-2-[(+)-6-amino-*trans*-5-hydroxy-1,3-cyclohexadiene-1-carboxyloxy]-propionic acid. In this communication, the absolute configuration of I determined by the application of a copper complex method<sup>3</sup> is presented.

As previously reported<sup>2</sup>, hydrolysis of I with 2 N sodium hydroxide at 60°C for 5 hours gave D-lactic acid and (+)-6-amino-*trans*-5-hydroxy-1,3-cyclohexadiene-1-carboxylic acid (II). The latter compound was formerly isolated from a



culture filtrate of a mutant of *Streptomyces aureofaciens* by McCORMICK *et al.*<sup>4</sup> Catalytic hydrogenation of II in water with ADAMS platinum oxide under atmospheric pressure afforded 2-amino-3-hydroxycyclohexane-1-carboxylic acid (III) as colorless crystals, mp 276°C (dec),  $[\alpha]_D^{25} -32^\circ$  (c 1, water) (mp 276°C (dec),  $[\alpha]_D^{25} -34.6^\circ$  (c 0.5, water) in literature<sup>4</sup>). The III was also prepared by alkaline hydrolysis of tetrahydro-oryzoxymycin<sup>2</sup>.

Esterification of III with 10% hydrogen chloride in methanol at room temperature for 21 hours afforded 2-amino-3-hydroxy-1-methoxycarbonylcyclohexane hydrochloride (IV) as colorless needles in a 75% yield, mp 195°C (dec),  $[\alpha]_D^{25} -12^\circ$  (c 1, water).

*Anal.* Calcd. for C<sub>8</sub>H<sub>15</sub>NO<sub>3</sub>·HCl:

C 45.82, H 7.69, N 6.68, Cl 16.90.

Found: C 45.67, H 7.15, N 6.40, Cl 14.80.

Chemical shifts and coupling constants on

Table 1. Chemical shifts and coupling constants for (1*R*, 2*S*, 3*S*)-2-amino-3-hydroxy-1-methoxycarbonylcyclohexane (IV)

	Chemical shifts ( $\delta$ )	Coupling constants (Hz)	
		J <sub>1,2</sub>	J <sub>2,3</sub>
1-H	3.16 (d, t)	10.5	
2-H	3.70 (t)	10.5	11.0
3-H	4.08 (d, t)		11.0
Methoxy-H <sub>3</sub>	4.20 (s)		

the pmr spectrum (100 Mz) of IV in D<sub>2</sub>O using tetramethylsilane as the external reference ( $\delta=0$ ) are shown in Table 1. The values of J<sub>1,2</sub> and J<sub>2,3</sub> showed that three vicinal substituents on the cyclohexane ring are all equatorial, that is, in *trans-trans* relation. By the application of the TACu method\* established by S. UME-

ZAWA *et al.*<sup>3</sup>, IV showed negative contribution ( $\Delta[M]_{436(TACu)} -1010^\circ$ ) and thus the absolute configuration of IV was determined to be 1*R*; 2*S*; 3*S*. The aqueous solution (2 ml) of IV (4.2 mg) with tetraamminecopper (II) sulfate (78.0 mg) showed pH 9.5, but IV was not hydrolyzed to III during the measurement of optical rotation, which was confirmed by the detection of IV (Rf 0.66) on thin-layer chromatography of Silica gel G (E. Merck) using butanol-acetic acid-water (2:1:1 in volume) (III: Rf 0.56).

From the data described above, the absolute structure of I must be 2(*R*)-[(5*S*, 6*S*)-6-amino-5-hydroxy-1,3-cyclohexadiene-1-carboxyloxy]-propionic acid.

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\* Tetraamminecopper (II) sulfate (TACu) forms a complex with vicinal amino and hydroxyl groups when they have ~60° dihedral angle, showing approximately  $\Delta[M] \pm 900^\circ$  at 436 nm<sup>3</sup>.

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#### References

- 1) HASHIMOTO, T.; S. KONDO, T. TAKITA, M. HAMADA, T. TAKEUCHI, Y. OKAMI & H. UMEZAWA: Oryzoxymycin, a new antibiotic. *J. Antibiotics* 21: 653~658, 1968
- 2) HASHIMOTO, T.; S. TAKAHASHI, H. NAGAWA, T. TAKITA, K. MAEDA & H. UMEZAWA: The structure of oryzoxymycin and its dimerization product. *J. Antibiotics* 25: 350~355, 1972
- 3) UMEZAWA, S.; T. TSUCHIYA & K. TATSUTA: Studies on aminosugars. XI. Configurational studies of aminosugar glycosides and aminocyclitols by a copper complex method. *Bull. Chem. Soc. Jap.* 39: 1235~1243, 1966
- 4) McCORMICK, J. R. D.; J. REICHENTHAL, U. HIRSCH & N. O. SJOLANDER: Biosynthesis of the tetracyclines. III. A new amino acid from *Streptomyces aureofaciens*: (+)-*trans*-2,3-dihydro-3-hydroxyanthranilic acid. *J. Amer. Chem. Soc.* 84: 3711~3714, 1962