

Communications to the editor

THE PREPARATION OF GARAMINE,
A NOVEL PSEUDODISACCHARIDE
FROM SISOMICIN

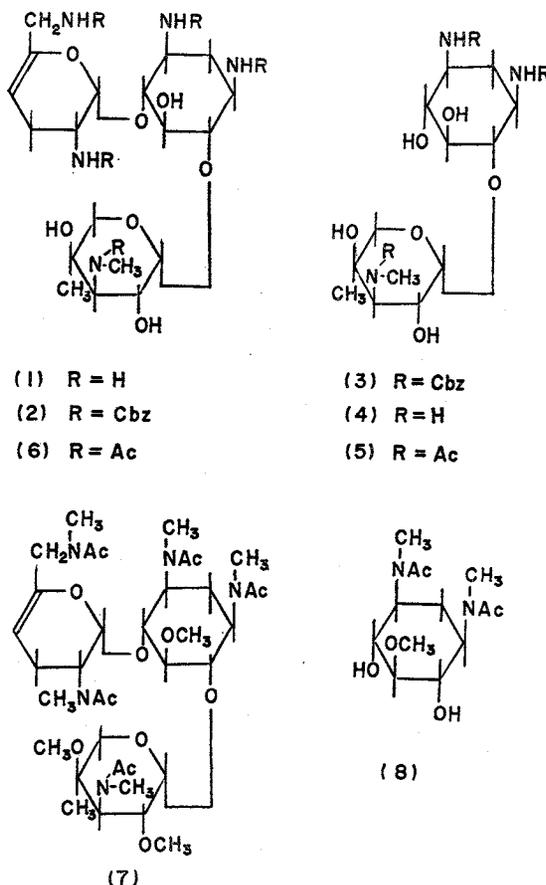
Sir:

Recent studies in these laboratories have established the gross structure¹ of sisomicin (1), a novel unsaturated aminoglycoside antibiotic produced by *Micromonospora inyoensis* and have demonstrated that the unsaturated sugar was attached to the 4-position of 2-deoxy-streptamine.² Evidence for the position of attachment of the garosamine is given below.

Sisomicin (1) was converted to the penta-N-carbobenzoxy derivative (2)* which was found to be highly labile towards even mild acidic reagents. A solution of 2 in tetrahydrofuran on treatment with Amberlite IR 120 (H⁺) ion-exchange resin at 25°C effected a smooth conversion to N-carbobenzoxygarosamine (3) in 71% yield. The conversion could also be effected by adjusting the pH to 1 by means of a few drops of concentrated sulphuric acid, or by treating the solution with *m*-chloroperbenzoic acid, and aqueous sodium bicarbonate, or by treatment with hypobromous acid, and barium carbonate. Hydrogenation of the N-carbobenzoxygarosamine (3) over 10% palladium on carbon gave a novel disaccharide which was named garamine (4), $[\alpha]_D^{25} + 135.4^\circ$ (H₂O), δ (D₂O) 1.19 (3H, S, 4'-CH₃), 2.51 (3H, S, 3'-NCH₃), 2.57 (1H, d, $J_{2'/3'} = 10.5$ Hz, H_{2'}), 3.30 (1H, d, $J_{5'/a, 5'/e} = 12.5$ Hz, H_{5'/a}), 3.79 (1H, dd, $J_{2'/3'} = 10.5$ Hz, $J_{1', 2'} = 4$ Hz, H_{2'}), 4.03 (1H, d, $J_{5'/a, 5'/e} = 12.5$ Hz, H_{5'/e}), 5.06 ppm. (1H, d, $J_{1', 2'} = 4$ Hz, H_{1'}). The mass spectrum of 4 showed an M⁺+1 ion at *m/e* 322 with prominent fragment ions at *m/e* 191, 173, 163, 145, and 160³ consistent with the proposed structure. Methanolysis of garamine (4) using 6N hydrochloric acid in methanol at reflux temperatures, gave 2-deoxystreptamine**, and methyl garosaminide** lending chemical support to the structure of garamine (4). The c. d. spectrum*** of a cuprammonium A complex of

N-acetylgarosamine (5) exhibited a positive extremum at 290 *mμ* and a negative extremum at 550 *mμ* consistent with a positive dihedral angle for the glycol,⁴ clearly establishing that the garosamine was located at the 6-position of 2-deoxystreptamine in sisomicin.

Further confirmation for the location of the unsaturated sugar at the 4-position and of garosamine at the 6-position in sisomicin was obtained by the following series of reactions. Sisomicin (1) was converted to the N-acetyl derivative (6) by treatment with acetic anhydride in methanol. The N-acetate (6) was markedly more stable towards acid hydrolysis to garamine than was the corresponding N-carbobenzoxy



* All compounds gave satisfactory microanalytical, and spectral data.

** Identical in all respects with authentic samples.

*** The authors are indebted to Dr. R. D. GUTHRIE, University of Sussex, Brighton, England, for running the Cupra A c. d. spectrum.

derivative (2). Permethylation of the N-acetate (6) using sodium hydride, and methyl iodide in dimethylformamide gave the derivative (7). The mass spectrum of 7 showed a molecular ion at m/e 755 in agreement with a permethylated structure, with characteristic fragment ions at m/e 239, 230, 509, 500, 317, 299, 289 and 271.⁸⁾ Hydrolysis of 7 with 6 N hydrochloric acid on a steam bath gave the methylated deoxystreptamine derivative (8) which was identical (m. s., t.l.c., g.l.c. of the TMS ether) with an authentic sample.⁵⁾ The formation of a 5-methylated deoxystreptamine by the above process clearly confirmed by chemical means that the sugars were glycosidically attached to the 4- and 6-positions in deoxystreptamine in sisomicin. The total structure, and absolute stereochemistry of sisomicin* may now be represented by the structure 1.

The authors express their thanks to Dr. M. YUDIS and his colleagues for providing spectral and analytical services.

M. KUGELMAN
A.K. MALLAMS
H.F. VERNAY

Research Division, Schering Corporation
Bloomfield, New Jersey, 07003, U.S.A.

(Received January 29, 1973)

References

- 1) COOPER, D.J.; R.S. JARET & H. REIMANN: Structure of sisomicin, a novel unsaturated aminoglycoside antibiotic from *Micromonospora inyoensis*. Chem. Comm. 1971: 285 (1971)
- 2) REIMANN, H.; R.S. JARET & D.J. COOPER: Sisomicin: Stereochemistry and attachment of the unsaturated sugar moiety. Chem. Comm. 1971: 924 (1971)
- 3) DANIELS, P.J.L.; M. KUGELMAN, A.K. MALLAMS, R.W. TKACH, H.F. VERNAY, J. WEINSTEIN & A. YEHASKEL: Mass spectral studies on aminocyclitol antibiotics. Chem. Comm. 1971: 1629 (1971)
- 4) BUKHARI, S. T. K.; R. D. GUTHRIE, A. I. SCOTT & A. D. WRIXON: Circular dichroism of cuprammonium complexes of diols and of amino-alcohols. Chem. Comm. 1968: 1580 (1968)
- 5) COOPER, D.J.; P.J.L. DANIELS, M.D. YUDIS, H.M. MARIGLIANO, R.D. GUTHRIE & S.T.K. BUKHARI: The gentamicin antibiotics. III. The gross structures of the gentamicin C components. J. Chem. Soc. (C) 1971: 3126 (1971)

* The sugar linkages were also determined in dihydrosisomicin by H. REIMANN and R.S. JARET, unpublished results.