Vancosamine. A Novel Branched Chain Amino-sugar from the Antibiotic Vancomycin

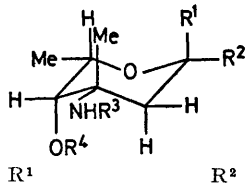
By ROGER M. SMITH, A. W. JOHNSON,* and R. D. GUTHRIE

(*School of Molecular Sciences, University of Sussex, Falmer, Brighton* BN1 9QJ)

Summary Acid hydrolysis of the antibiotic vancomycin from *Streptomyces orientalis* yields the 3-amino-2,3,6-trideoxy-3-*C*-methyl-L-*lyxo*-hexopyranose, vancosamine, the structure of which is assigned on the basis of chemical and spectroscopic studies, particularly n.m.r. spectroscopy.

WE report the structure and stereochemistry of the branched chain amino-sugar vancosamine (**1**), $C_7H_{15}NO_3$, which was obtained by acid hydrolysis of vancomycin, an antibiotic derived from *Streptomyces orientalis*.[1] It has previously been shown that acid hydrolysis of vancomycin yielded glucose[2] and an amino fragment, which was also isolated by acid hydrolyses of the related antibiotics ristomycin, ristocetin, and actinoidin.[3] The amine fragment was assigned the impossible formula $C_{12}H_{25}N_2O_5$, and it is probably related to or even identical with, the amino-sugar isolated in the present work.

Hydrolysis of vancomycin with 2N-hydrochloric acid, and separation of the basic compounds using an Amberlite IR-120(H^+) ion exchange resin yielded a mixture of amines.[3]



	R^1	R^2	R^3	R^4
(**1**)	OH,H		H	H
(**2**)	OMe	H	H	H
(**3**)	OMe	H	Bz	Bz
(**4**)	H	OMe	Bz	Bz
(**5**)	OEt	H	Bz	Bz
(**6**)	H	OEt	Bz	Bz
(**7**)	OEt	H	Bz	H
(**8**)	OMe	H	Bes	Bes
(**9**)	H	OMe	Bes	Bes
(**10**)	OMe	H	Bes	Ac

Bes = $PhSO_2$

After treatment with methanolic hydrogen chloride, separation of the mixture by t.l.c. (3% NH_4OH–Bu^nOH sat. H_2O) yielded methyl-α-L-vancosaminide (**2**). The n.m.r. spectrum (C_5D_5N) contained peaks at τ 7·94 (s, 3-Me) and 8·60 (d, J 6·5 Hz, 5-Me), 5·23 (d, J 4·5 Hz, 1-H), 7·44 (dd, J 4·5, 13·5 Hz, 2*ax*-H), 7·72 (d, J 13·5 Hz, 2*eq*-H), 5·97 (s, 4-H), 5·92 (*q*, J 6·5 Hz, 5-H), and 6·78 (s, OMe), in agreement with the suggested structure. The c.d. spectrum† of (**2**) in 'Cupra A' contained a negative absorption at about 600 nm demonstrating a negative chirality between the *cis*-hydroxy- and amino-groups and hence the L-configuration for the amino-sugar.[4]

When the mixture of basic compounds was acetylated using Ac_2O–MeOH, and the product treated with methanolic hydrogen chloride and finally acylated with benzenesulphonyl chloride in pyridine, the acetyl ester (**10**) was obtained. Thus the initially formed *N*-acetyl group has migrated to the adjacent *cis*-hydroxy-function under the acidic conditions.[5]

Benzoylation of vancomycin, followed by methanolysis yielded methyl dibenzoyl-α-L-vancosaminide (**3**), m.p. 168—169°,‡ $[\alpha]_D^{22}$ — 191° (*c* 0·11, MeOH), λ_{max} (MeOH) 227, 270 (infl.) nm (log ϵ 4·44, 3·25), ν_{max} ($CHCl_3$) 3400, 1725, 1705, 1670 cm^{-1}, τ ($CDCl_3$) 5·13 (d, J 4·5 Hz, 1-H), and a gum, methyl dibenzoyl-β-L-vancosaminide (**4**), $[\alpha]_D^{22}$ —64° (*c* 0·14, MeOH), λ_{max} (MeOH) 228, 270 (infl.) nm (log ϵ 4·31, 3·15), ν_{max} ($CHCl_3$) 3400, 1725, 1705, 1668 cm^{-1}, τ ($CDCl_3$) 5·36 (dd, J 9·5, 2·0 Hz, 1-H).

Irradiation of the C-3 methyl group resonance (τ 8·12) of the dibenzoyl compound (**3**) caused a 7% NOE (nuclear Overhauser effect), enhancement of the axial C-5 proton signal (τ 5·71, qd, J 6·5, 1 Hz), and no detectable enhancement of the C-4 signal (τ 4·89). Furthermore, on irradiation of the C-3 N*H* resonance (τ 3·37) there was no observable change in the C-5 proton signal and a small (2—3%) change in the C-4 proton signal, thus confirming the *cis*-diaxial configuration of the C-5 proton and C-3 methyl group.

Benzenesulphonylation of vancomycin followed by methanolysis yielded the corresponding derivatives, methyl

† C.d. spectra were kindly determined by Dr. P. M. Scopes, Westfield College, London, and by Dr. A. J. McCaffery of this School.

‡ All crystalline compounds had concordant elemental analyses and spectra.

dibenzenesulphonyl-α-L-vancosaminide (**8**), m.p. 132—133°, $[\alpha]_D^{24}$ — 109° (*c* 0·34, MeOH) and methyl dibenzenesulphonyl-β-L-vancosaminide (**9**), m.p. 151—154°, $[\alpha]_D^{24}$ — 6·5° (*c* 0·31, MeOH). Acid hydrolysis of vancomycin followed by evaporation of the crude sugar fraction with added ethanol, and benzoylation of the product yielded ethyl dibenzoyl-α-L-vancosaminide (**5**), m.p. 131—133°, $[\alpha]_D^{25}$ — 179° (*c* 0·27, MeOH) and ethyl dibenzoyl-β-L-vancosaminide (**6**), m.p. 97—99°, $[\alpha]_D^{25}$ — 82° (*c* 0·24, MeOH). The optical rotations of the α-methyl anomers of each of these diacyl derivatives was more negative than the β-

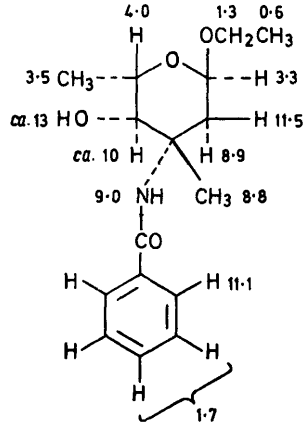


FIGURE. *Extrapolated chemical shifts* (*p.p.m.*) *for addition of equimolar* $Eu^{III}(fod)_3$ *to the benzamide* (**7**).

isomer confirming the assignment of the L-configuration to (**1**).[6] The c.d. spectrum† of both (**5**) and (**6**) contained negative Davydov bands at 237 nm in agreement with a negative chirality of the two *cis*-benzoyl groups.[7] The positions of the *ortho*-aromatic proton signals in all the dibenzoyl compounds were displaced downfield to τ 1·9 indicating that there was an interaction between the *cis*-benzoyl groups.

Partial alkaline hydrolysis of the dibenzoyl compound (**5**), τ 4·86 (s, 4-H), yielded the *N*-benzoyl derivative (**7**), τ 6·54 (d, *J* 8·5 Hz, 4-H) and 7·12 (d, *J* 8·5 Hz, 4-OH, exchanged with D_2O). The n.m.r. spectrum of this monohydroxy-compound was studied using the contact shift reagent $Eu^{III}(fod)_3$ and the extrapolated shifts (p.p.m.) for an equimolar solution are shown in the Figure. The lanthanide ion was apparently co-ordinated between the secondary hydroxy-group and the *cis*-amide oxygen atom.[8] The contact shift of the *ortho*-aromatic signals was particularly marked, whereas the *meta*- and *para*-proton signals were barely affected.[9]

Previous workers had isolated the so-called 'vancomycin acid' Et·CO·CHMe·CH_2·CO_2H from prolonged acid treatment of vancomycin[10] and in the present work, laevulinic acid has also been isolated. These γ-keto-acids can be regarded as being formed *via* furans from vancosamine and glucose, respectively.[11]

Vancosamine can thus be assigned the 3-amino-2,3,6-trideoxy-3-*C*-methyl-L-*lyxo*-hexopyranose structure (**1**) and is the first naturally occurring branched chain amino-sugar with geminal methyl and amino groups to be reported. The ^{13}C n.m.r. spectrum of (**8**) fully supports the suggested configuration.[12]

This work was carried out during the tenure of an Imperial Chemical Industries, Limited Research Fellowship (R.M.S.). The authors thank Eli Lilly and Company, Indianapolis, Indiana, U.S.A., for the supply of vancomycin and the determination of NOE spectra.

Note added in proof: The same structure (**1**) has been proposed for the amino-sugar from vancomycin by Williams and his co-workers[13] but without assigning the absolut configuration.

(*Received, 26th January* 1972; *Com.* 115.)

[1] R. C. Pittenger and R. B. Brigham, *Antibiotics and Chemotherapy*, 1956, **6**, 642; M. H. McCormick, W. M. Stark, G. E. Pittenger, R. C. Pittenger, and J. M. McGuire, Antibiotics Annual 1955—1956, Medical Encyclopedia Inc., New York, 1956, p. 606.
[2] H. M. Higgins, W. H. Harrison, G. M. Wild, H. R. Bungay, and M. H. McCormick, Antibiotics Annual 1957—1958, Medical Encyclopedia Inc., New York, 1958, p. 906.
[3] N. N. Lomakina, I. A. Spiridonova, R. Bognar, M. Puskas, and F. Sztaricskai, *Antibiotiki*, 1968, **13**, 975.
[4] S. T. K. Bukhari, R. D. Guthrie, A. I. Scott, and A. D. Wrixon, *Tetrahedron*, 1970, **26**, 3653.
[5] G. Fodor and L. Otvos, *Chem. Ber.*, 1956, **89**, 701.
[6] C. S. Hudson, *J. Amer. Chem. Soc.*, 1909, **31**, 66.
[7] N. Harada, K. Nakanishi, and S. Tatsuoka, *J. Amer. Chem. Soc.*, 1969, **91**, 5896; N. Harada, H. Sato, and K. Nakanishi, *Chem. Comm.*, 1970, 1691.
[8] L. R. Isbrandt and M. T. Rogers, *Chem. Comm.*, 1971, 1378.
[9] *Cf.* N. S. Bhacca and J. D. Wander, *Chem. Comm.*, 1971, 1505.
[10] F. J. Marshall, *J. Medicin. Chem.*, 1965, **8**, 18.
[11] *Cf.* F. H. Newth, *Adv. Carbohydrate Chem.*, 1951, **6**, 83.
[12] Dr. G. Lukacs, personal communication.
[13] W. D. Weringa, D. H. Williams, Feeney, J. P. Brown, and R. W. King, *J. Chem. Soc., Perkin I*, 1972, 443.