Vancosamine: the Structure and Configuration of a Novel Amino-sugar from Vancomycin

By **A. W. Johnson,* Roger M. Smith,** and (in part) **R. D. Guthrie,** School of Molecular Sciences, University of Sussex, Falmer, Brighton BN1 9QJ

An amino-sugar, vancosamine, derived from the antibiotic vancomycin, is shown to be 3-amino-2,3,6-trideoxy-3-*C*-methyl-L-*lyxo*-hexopyranose (1). A number of acylated derivatives have been prepared and their physical properties, particularly c.d. and n.m.r. spectra, have been compared and evaluated.

VANCOMYCIN [1] is a broad spectrum antibiotic produced by *Streptomyces orientalis* and is considered to be related to the peptide antibiotics actinoidin, ristomycin, and ristocetin.[2] Lomakina and her co-workers [3] have reported that as well as differing sugars, all four antibiotics yielded a common amino-sugar on mild acid hydrolysis, and this was assigned the formula $C_{12}H_{25}N_2O_5$ (*sic*) on the basis of elemental analysis and molecular weight determinations. It was found to contain two *C*-methyl groups, one amino-group and one aldehyde function. It is probable that this compound is similar to, or identical with, the compound reported in the present work and that the earlier workers were misled by an erroneous molecular weight determination, possibly caused by complex formation.

Previous chemical studies [1,4] of the acid hydrolysis of vancomycin have shown that it yields *N*-methyl-D-leucine, L-aspartic acid, D-glucose, and ' vancomycin acid ' (13) (see later). In the present work, two aliphatic acids were formed on prolonged acid hydrolysis of vancomycin; after methylation of the acids, the esters were separated and identified as methyl 4-oxopentanoate (methyl levulinate) and the methyl ester of ' vancomycin acid.' The i.r. spectrum of the latter compound contained bands at 1735 (ester carbonyl) and 1711 (ketone carbonyl) cm^{-1}, and the mass spectrum showed peaks at *m/e* 158 (M^+), 129(M^+ — Et), and 127(M^+ — 31). 4-Oxopentanoic acid is formed readily by mild acid treatment of D-glucose,[5] by way of 5-hydroxymethylfurfural, and thus the acid (13) can be postulated to have been formed from a 3-*C*-methyl-6-deoxy-sugar by a similar route (*cf.* ref. 6).

When vancomycin was hydrolysed for 10 min at 100° in 2N-hydrochloric acid (conditions similar to those used by Lomakina *et al.*[3]) a precipitate formed rapidly, and after cooling the solution the precipitate was separated. The filtrate was neutralised and run through a column of Amberlite IR-120 (H^+) ion-exchange resin, and the eluate, by trimethylsilylation and examination by g.l.c., was found to contain mainly α- and β-glucose. The column was then eluted with dilute ammonium hydroxide to yield an amine fraction (A) [7] which corresponded to the amino-sugar reported earlier.[3] Attempts to acylate fraction (A) were generally unsuccessful or gave low yields. Trimethylsilylation did not yield a volatile product and it was concluded that in neutral or basic solution intermolecular condensation was occurring, probably between the aldehyde and amino-functions. Attempted acetylation of fraction (A) by various methods gave low yields; the only product isolated was *NOO'*-triacetyl-β-L-vancosamine (4). The n.m.r. spectrum (see Table) contained a signal at τ 4·15 (dd, *J* 10·0 and 2·5 Hz) assignable to a C-1 axial proton. The signal associated with the methyl of the *N*-acetyl group was at τ 8·13; thus it was not possible to assign it confidently to an axial (τ 8·07—8·14) or to an equatorial (τ 8·15—8·20) group.[8] However it appeared at significantly higher field than the axial

[1] 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. 606.
[2] Review: M. G. Brazhnikova, N. N. Lomakina, F. Sztaricskai, M. Puksás, S. Makleit, and R. Bognár, *Kém. Kozlemén*, 1967, **27**, 143.
[3] N. N. Lomakina, I. A. Spiridonova, R. Bognár, M. Puksás, and F. Sztaricskai, *Antibiotiki*, 1968, **13**, 975.
[4] F. J. Marshall, *J. Medicin. Chem.*, 1965, **8**, 18.
[5] F. H. Newth, *Adv. Carbohydrate Chem.*, 1951, **6**, 83; E.F.L.J. Anet, *ibid.*, 1964, **19**, 181.
[6] M. M. Runde, E. W. Scott, and J. R. Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 1284.
[7] Preliminary communication, A. W. Johnson, R. M. Smith, and R. D. Guthrie, *Chem. Comm.*, 1972, 361.
[8] F. W. Lichtenthaler and P. Emig, *Tetrahedron Letters*, 1967, 577; *Carbohydrate Res.*, 1968, **7**, 121; F. W. Lichtenthaler and H. Zinke, *J. Org. Chem.*, in the press.

N-acetyl signal (τ 8·05) in the spectrum of the evernitrose (14) [9] reduction product, which also contains the 3-amino-3-deoxy-3-*C*-methyl system.

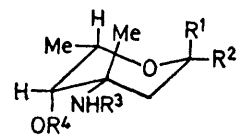
Subsequently it was found that treatment of fraction (A) with methanolic hydrogen chloride yielded a mixture of mainly two compounds. Separation by t.l.c. yielded methyl α-L-vancosaminide (2), $C_8H_{15}NO_3$, as its acetate salt, and an unidentified compound, apparently unrelated to vancosamine. No β-anomer (3) was observed, although treatment of the glycoside (2) with methanolic hydrogen chloride apparently caused rapid equilibration. Neutralisation of the acetate salt of (2) gave the free glycoside as a viscous gum for which a satisfactory elemental analysis was not obtained. The n.m.r. spectrum of the glycoside (2) (see Table) in [$^2H_5$]pyridine was the only example observed among all the vancosamine derivatives where the signal of the C-2 axial proton appeared at higher field than that of the C-2 equatorial proton. In the *N*-acylated derivatives the axial proton was presumably shielded by the adjacent carbonyl group.

In order to obtain crystalline derivatives of the amino-sugar, vancomycin was treated with benzoyl chloride in pyridine and the crude product was methanolysed. The resulting benzoyl derivatives were separated by chromatography to give partially benzoylated methyl glucosides * thought to be methyl 3,4-di-*O*-benzoyl-α-D-glucoside and methyl 3,4,6-tri-*O*-benzoyl-α- and -β-D-glucosides, as well as methyl *NO*-dibenzoyl-α- and -β-L-vancosaminides [(5) and (6)]. The formation of these partially substituted derivatives of D-glucose suggested that the vancosamine was probably linked to the aglycone through the 2-position of D-glucose and that a second labile group was present on the 6-position, as the 2- and 6-positions in glucose are normally the most reactive towards benzoylation.[10] As only the α-anomer (5) of methyl *NO*-dibenzoyl-L-vancosaminide was crystalline, the reaction sequence was repeated using benzenesulphonyl chloride, which gave crystalline methyl α- and β-*NO*-bisphenylsulphonyl-L-vancosaminide [(10) and (11)]. It was found later that if the crude sugar fraction from an acid hydrolysis of vancomycin was evaporated in presence of ethanol, and the product was benzoylated, the crystalline ethyl α- and β-*NO*-dibenzoyl-L-vancosaminides (7) and (8) could be readily isolated. The optical rotations of all six *NO*-diacyl compounds were measured, and a comparison of the values for each anomeric pair showed that in each case the α-anomer had a more negative rotation than the β-form. Thus, if Hudson's isorotation rules [11] for carbohydrates apply for amino-sugar derivatives, then vancosamine (1) has the L-configuration.

N.m.r. spectra of derivatives of vancosamine [a]

Compound	C(1)H [b]	C(2)*ax*H [b]	C(2)*eq*H [b]	C(4)H	C(5)H	C(6)H	C(1)OCH	C(3)Me	C(3)NH	Other signals
(2) [c]	5·23 d (4·5)	7·44 dd (4·5, 3·5)	7·72 d (13·5)	5·97 s	5·92 q (6·5)	8·60 d (6·5)	6·78	7·94		
(2) [d]	6·33 d (4)	7·6 m		4·04 s	5·40 q (6·5)	8·30 d (6·5)	6·20	7·97		
(4)	4·15 dd (10·0, 2·5)	*ca.* 8·0	7·58 dm (12·5)	5·13br s	6·00 qd (6·5, 1·5)	8·81 d (6·5)		8·36	4·31 [e]	7·91, 7·82 (OAc) 8·13 (NAc)
(5)	5·13 d (4·5)	7·94 dd (14·0, 4·5)	7·22 dm (14·0)	4·89 s	5·71 qd (6·5, 1·0)	8·73 d (6·5)	6·64	8·12	3·37	1·9—2·0 (*o*-aromatic H) 2·4—2·7 (*m*,*p*-aromatic H)
(6)	5·36 dd (9·5, 2·0)	8·10 dd [f] (12·5, 9·5)	7·32 dm (12·5)	4·81 s	5·94 qd (6·5, 1·0)	8·72 d (6·5)	6·44 s	8·20	3·53	1·9—2·0 (*o*-aromatic H) 2·4—2·7 (*m*,*p*-aromatic H)
(7)	4·99 d (4·5)	7·81 dd (14·0, 4·5)	7·20 d (14·0)	4·8 s	5·66 q (6·5)	8·76 d (6·5)	6·39 [g]	8·06	3·3	1·9—2·0 (*o*-aromatic H) 2·4—2·7 (*m*,*p*-aromatic H) 8·75 t (7·0) (CH_2·CH_3)
(8)	5·28 d (9·5, 2·0)	8·06 dd (13·0, 9·5)	7·36 dm (13·0)	4·81 s	5·96 q (6·5)	8·73 d (6·5)	6·22 [h]	8·20 s	3·54	1·9—2·0 (*o*-aromatic H) 2·4—2·7 (*m*,*p*-aromatic H) 8·75 t (7·0) (CH_2·CH_3)
(9)	5·20 d (4·5)	8·12 dd (14·0, 4·5)	7·54 d (14·0)	6·54 d (8·5)	5·83 q (6·5)	8·74 d (6·5)	6·32 [j]	8·22	3·20	1·9—2·0 (*o*-aromatic H) 2·4—2·7 (*m*,*p*-aromatic H) 8·80 t (7·0) (CH_2·CH_3) 7·12 d (8·5) (OH) [k]
(10	5·42 d (4·0)	8·16 dd (14·0, 4·0)	7·83 d (14·0)	5·55 s	5·94 q (6·5)	8·87 d (6·5)	6·80	8·50	4·50 [k]	1·9—2·0 (*o*-aromatic H) 2·4—2·6 (*m*,*p*-aromatic H)
(11)	5·66 dd (9·5, 2·5)	8·40 dd (13·5, 9·5)	7·76 dm (13·5)	5·62 s	6·16 qm (6·5)	8·83 d (6·5)	6·62	8·50	4·17	1·9—2·0 (*o*-aromatic H) 2·4—2·6(*m*,*p*-aromatic H)
(12)	5·34 t (3·0)	8·06 d (2H) (3·0)		5·29 s	6·00 q (6·5)	8·92 d (6·5)	6·76	8·52	5·06 [k]	1·9—2·0 (*o*-aromatic H) 2·4—2·6 (*m*,*p*-aromatic H)

[a] Measured at 100 MHz for $CDCl_3$ solutions unless otherwise stated; τ values (standard Me_4Si); coupling constants (Hz) in parentheses. [b] C(1)H, C(2)*ax*H, and C(2)*eq*H formed an ABX system. [c] Solvent [$^2H_5$]pyridine. [d] In D_2O (Me_4Si external standard). [e] Exchanges slowly with D_2O. [f] Irradiation at frequency of C(1)H signal causes collapse. [g] CH_3·CH_3, AB portion of ABX_3 system; J_{AB} 9·5 Hz, $J_{AX} = J_{BX}$ 7·0 Hz. [h] CH_3·CH_3, AB portion of ABX_3 system; J_{AB} 10·0 Hz, $J_{AB} = J_{BX}$ 7·0 Hz. [j] On adding D_2O becomes singlet. [j] CH_3·CH_3, AB portion of ABX_3 system; J_{AB} 9·5 Hz, $J_{AB} = J_{BX}$ = 7·0 Hz, δ_{AB} 24·8 Hz. [k] Exchanges rapidly with D_2O.

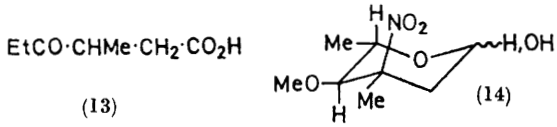


	R1	R2	R3	R4
(1)	H, OH		H	H
(2)	OMe	H	H	H
(3)	H	OMe	H	H
(4)	H	OAc	Ac	Ac
(5)	OMe	H	Bz	Bz
(6)	H	OMe	Bz	Bz
(7)	OEt	H	Bz	Bz
(8)	H	OEt	Bz	Bz
(9)	OEt	H	Bz	H
(10)	OMe	H	$PhSO_2$	$PhSO_2$
(11)	H	OMe	$PhSO_2$	$PhSO_2$
(12)	H, OMe		$PhSO_2$	Ac

* As these compounds have not been described previously, the assigned structures are based on n.m.r. spectra. Further work is necessary to confirm the given structures.

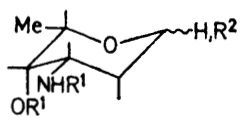
[9] A. K. Ganguly, O. Z. Sarre, and H. Riemann, *J. Amer. Chem. Soc.*, 1968, **90**, 7129.
[10] J. M. Williams and A. C. Richardson, *Tetrahedron*, 1967, **23**, 1369.
[11] C. S. Hudson, *J. Amer. Chem. Soc.*, 1909, **31**, 66.

As found for 6-deoxyhexoses,[12] the C-5 methyl group signal appeared at slightly higher field in the n.m.r. spectra of the α-*NO*-diacyl anomers compared with the β-*NO*-diacyl anomers. In all the spectra, the

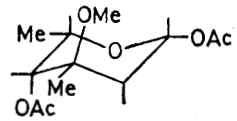


(13) (14)

C-1 and C-2 protons appeared as an ABX system, which permitted a ready distinction of the α-forms ($J_{1.2ax}$ 4, $J_{1.2eq}$ 0 Hz) and β-forms ($J_{1.2ax}$ 9·5, $J_{1.2eq}$ 2 Hz), and these values were similar to those of known compounds, *e.g.* methyl *NO*-diacetyl-α-L-daunosaminide (15) ($J_{1.2}$ 2 Hz)[13] and 1,4-di-*O*-acetyl-β-L-arcanose (17) ($J_{1.2ax}$ 9, $J_{1.2eq}$ 3 Hz).[14]



(15) R^1 = Ac, R^2 = α-OMe
(16) R^1 = H, R^2 = OH



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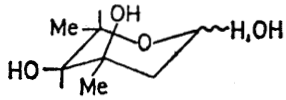

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The small coupling between the C-4 and C-5 protons of the *NO*-diacylvancosaminides indicated (*cf.* ref. 15) that they had a *cis*-relationship similar to that existing in methyl *NO*-diacetyldaunosaminide (15) ($J_{4.5}$ *ca.* 1 Hz)[13] and unlike the *trans*-diaxial structure found for L-mycarose (18) ($J_{4.5}$ 10 Hz).[16] Frequently, the C-2 equatorial proton signals were broadened by unresolved fine coupling indicating that a long-range interaction with the C-4 proton was present, as this proton was in the appropriate W configuration, *cf.* 1,4-di-*O*-acetyl-arcanose (17) ($J_{2.4}$ *ca.* 1 Hz).[17] The low-field signal assigned to the imino-proton was rapidly lost on addition of deuterium oxide to the phenylsulphonyl derivative, but the exchange was very slow with the benzoyl or the acetyl compound. In the spectra of the ethyl glycosides (7) and (8) the signals of the methylene group appeared as a complex AB pattern of quartets ($J_{AX} = J_{BX} = 7$ Hz) due partly to its proximity to the chiral centre and partly to restricted rotation of the ethyl group. None of the n.m.r. spectra permitted a ready assignment of the C-3 configuration. Attempts were made to relate the stereochemistry of the C-3 and C-4 phenylsulphonyl groups by cyclisation to an aziridine[18] but no reaction was observed. Unsuccessful attempts were also made to eliminate the C-4 phenylsulphonyloxy-group[19] as the first step of a degradative scheme.

The c.d. spectrum of methyl α-L-vancosaminide (2) in Cupra A showed a negative Cotton effect at 600 nm, indicating a negative chirality of the two *cis*-functions.[20] As well as supporting the assignment of the amino-group to an equatorial position (diaxial systems do not form a complex[20]), the chirality confirmed the assignment of the L-configuration to the sugar. The c.d. spectra of the ethyl *NO*-dibenzoyl compounds (7) and (8) in methanol contained a negative band at 237 nm and a positive band at 222 nm; the sign of the longer wavelength band indicated that the two aromatic systems had a negative chirality, in complete agreement with the copper complexing. This is the first time that the c.d. band of a benzoate-benzamide system has been reported. Nakanishi and his co-workers[21] have shown that the Davydov splitting is caused by an interaction of the π–π^* transition dipoles of a di-*O*-benzoyl system, and that it can also be observed when other aromatic systems interact with a benzoate chromophore. An interaction between the aromatic rings was also apparent in the n.m.r. spectra of the *NO*-dibenzoyl compounds, in which the *ortho*-proton signals apparently had been displaced by *ca.* 0·4 p.p.m. On mild alkaline hydrolysis, the *NO*-dibenzoyl compound (7) was converted into the hydroxy-benzamide (9), the c.d. spectrum of which contained no strong band above 220 nm.

Further n.m.r. spectroscopic evidence was obtained by a fuller examination of the *NO*-dibenzoyl derivative (5). Irradiation at the frequency of the C-3 methyl proton signal (τ 8·12) resulted in a 7% NOE (nuclear Overhauser effect) enhancement of the C-5 proton signal (τ 5·71), and no observable change in the C-4 proton signal (τ 4·89). In a complementary experiment the irradiation at the frequency of the C-3 imino-proton signal at τ 3·37 caused no change in the C-5 proton signal, but the C-4 proton signal showed a small but reproducible 2—3% enhancement. Thus the C-3 methyl group is in an axial configuration close to the C-5 proton. This result has been confirmed[22] by examination of the ^{13}C n.m.r. spectrum ($CDCl_3$) of the *NO*-bisphenylsulphonyl derivative (10).

The n.m.r. spectrum of compound (9) contained signals at τ 6·54 (d, *J* 8·5 Hz), assignable to the C-4 proton and at τ 7·12 (d, 8·5 Hz, exchanged with D_2O), assignable to the C-4 hydroxy-proton. The *ortho*-aromatic proton

[12] H. B. Sinclair and R. T. Sleeter, *Tetrahedron Letters*, 1970, 933.
[13] F. Arcamone, G. Cassinelli, P. Orezzi, G. Franceschi, and R. Mondelli, *J. Amer. Chem. Soc.*, 1964, **86**, 5335.
[14] G. B. Howarth, W. A. Szarek, and J. K. N. Jones, *J. Org. Chem.*, 1969, **34**, 476.
[15] P. L. Durette and D. Horton, *Org. Magnetic Resonance*, 1971, **3**, 417; an explanation on the basis of electronegativity effects is also possible: *cf.* D. H. Williams and N. S. Bhacca, *J. Amer. Chem. Soc.*, 1964, **86**, 2742.
[16] W. Hofheinz, H. Grisebach, and H. Friebolin, *Tetrahedron*, 1962, **18**, 1265.
[17] G. Roncari and W. Keller-Schierlein, *Helv. Chim. Acta*, 1966, **49**, 705.
[18] B. R. Baker and T. L. Hullar, *J. Org. Chem.*, 1965, **20**, 4049.
[19] H. R. Nace, *J. Amer. Chem. Soc.*, 1959, **81**, 5428.
[20] S. T, K. Bukhari, R. D. Guthrie, A. I. Scott, and A. D. Wrixon, *Tetrahedron*, 1970, **26**, 3653.
[21] K. Nakanishi and N. Harada, *J. Amer. Chem. Soc.*, 1969, **91**, 3989; N. Harada, K. Nakanishi, and S. Tatsuoka, *ibid.*, p. 5896.
[22] G. Lukacs and R. M. Smith, *Bull. Soc. chim. France*, in the press.

signals now appeared at the usual position of τ 2·3. The n.m.r. spectrum of (9) in carbon tetrachloride was examined in the presence of increasing amounts of $Eu(fod)_3$ [23] up to 0·15 mol. equiv. A series of linear shifts relative to added lanthanide was observed, and the values extrapolated to 1 mol. equiv. are shown in the Figure. The large shift of the *ortho*-aromatic signals agreed with the assignment of the amide to an equatorial position and suggested that, in the complex, the europium was chelated between the hydroxy- and the amide carbonyl oxygen atoms.[23] The surprisingly small shift of the *meta*- and *para*-proton signals was presumed to be a consequence of the angular dependence of the influence of the europium. A similar distinction was found in 1-phenyl-*c*-4-t-butylcyclohexan-*r*-1-ol, the *meta*- and *para*-proton signals of which were barely shifted compared with the *ortho*-proton signals.[24]

Attempts to prepare *N*-acetylvancosaminides were generally unsuccessful. However, when the fraction

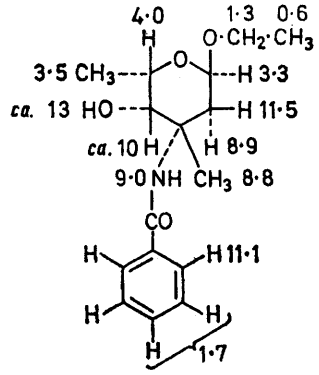
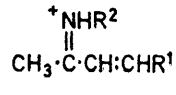


FIGURE Extrapolated chemical shifts (p.p.m.) for addition of $Eu(fod)_3$ (1 mol. equiv.) to the benzamide (9)

(A) (see before) was first treated with acetic anhydride in methanol and then hydrogen chloride to form the glycoside, and the product was finally treated with benzenesulphonyl chloride in pyridine, a small yield of a homogeneous product was obtained. The i.r. spectrum contained a band at 1740 but no band at 1680 cm^{-1}, and in the n.m.r. spectrum the imino-proton signal was rapidly removed by deuterium oxide exchange. Thus the product was not the expected *N*-acetyl-benzenesulphonate but the *O*-acetyl-*N*-phenylsulphonyl derivative (12) which has apparently been formed by an $N \longrightarrow O$ migration, probably during the methanolysis step.[25]

The mass spectra of the vanocosamine derivatives all contained prominent peaks assignable to the ions (19) formed from C-1, C-2, and C-3 of the original compound. This peak was present at *m/e* 204 [$C_{12}H_{14}NO_2$ (19; R^1 = OMe, R^2 = Bz)] and 218 (19; R^1 = OEt, R^2 = Bz) in the spectra of the methyl [(5) and (6)] and ethyl [(7)—(9)] benzoyl derivatives of vancosamine respectively, and at *m/e* 240 [$C_{11}H_{14}NO_3S$ (19; R^1 = OMe, R^2 = $PhSO_2$)] in those of the methyl bis- and monophenylsulphonyl compounds [(10)—(12)], further confirming the rearranged structure of the last compound. The ion (19) was also observed as a peak at *m/e* 100 (19; R^1 = OMe, R^2 = H) in the mass spectrum of the methyl glycoside (2); a peak at *m/e* 73 corresponded to the rest of the molecule.



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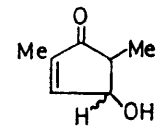

(20)

By prolonged treatment of vancomycin with 0·05N-hydrochloric acid at 100° in presence of Amberlite IR-120(H^+) ion-exchange resin,[26] a non-polar oil was obtained. The u.v. spectrum [$\lambda_{max.}$ 224 nm] and i.r. spectrum [$\nu_{max.}$ 3620, 1720, and 1645 cm^{-1}] suggested the presence of a hydroxycyclopentenone (*cf.* 2-methylcyclopentenone,[27] $\lambda_{max.}$ 226 nm, $\nu_{max.}$ 1711 and 1642 cm^{-1}). The mass spectrum of the oil contained peaks at *m/e* 126 ($C_7H_{10}O_2$), 111 ($C_6H_7O_2$), and 108 and, surprisingly, was identical with the spectrum of fraction (A) (see before). Moreover, the spectrum of the product obtained from fraction (A) by treatment with hexamethyldisilazane and trimethylsilyl chloride contained peaks at *m/e* 198, 183, and 108, thus suggesting that the compound $C_7H_{10}O_2$ contained one hydroxy-group. The n.m.r. spectrum of the oil suggested the presence of two components in a 3 : 1 ratio, but these could not be resolved by g.l.c. Interpretation of the signals assigned to the major component (see Experimental section) suggested that it was the isomer of structure (20), in which the C-4 and C-5 substituents are *trans*. The chemical shifts agree well with those of the protons of 2,5,5-trimethylcyclopentenone [28] [τ 8·2 (2-Me) and 2·8—3·0 (3-H)] and the *trans*-coupling ($J_{4,5}$ 2·5 Hz) with the values found for cyclopentenone ($J_{4,5\text{-}trans}$ 2·2; $J_{4,5\text{-}cis}$ 7·2 Hz).[29] The spectrum of the minor component was similar and corresponded to the *cis*-isomer of (20). An attempt was made to separate the isomers as their 2,4-dinitrophenylhydrazones but although only one product was detected by t.l.c., the n.m.r. spectrum suggested that a mixture again was present. In order to prevent the apparent rapid equilibration of the C-4 hydroxy-group, a 3,5-dinitrobenzoate of the oil was prepared. The product contained two components which were separated by t.l.c., and the major derivative was shown to be the *trans*-isomer by n.m.r. spectroscopy. These cyclopentenones

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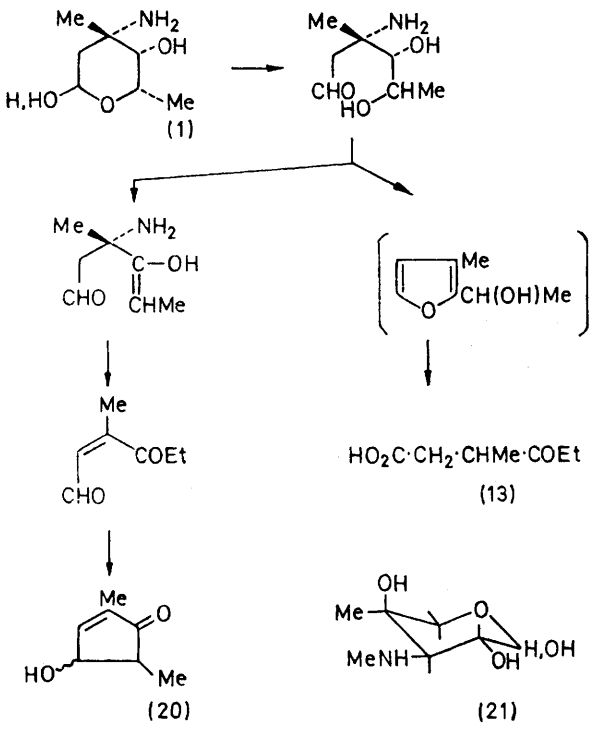
(20) are thought to be formed by a route similar in the initial stages to that proposed for the formation of 'vancomycin acid' (13),[6] but instead of formation of a furan, an intramolecular aldol reaction has occurred leading to structure (20).

The results of these studies agree with the assignment of the structure (1), 3-amino-2,3,6-trideoxy-3-*C*-methyl-L-*lyxo*-hexopyranose, to the amino-sugar vancosamine. It thus becomes the first naturally occurring amino-sugar to be found containing methyl and amino-groups on the same carbon atom, although many similar synthetic compounds are known, principally as products of nitroethane condensation reactions.[30]



In spite of its novelty in the foregoing respect, vancosamine falls into the general class of 2,6-dideoxy-3-*C*-methylhexoses of which several have been found in nature, generally, but not always, with the L-configuration. Those discovered so far have contained a hydroxy-group {mycarose[17] [*cf.* (18)], olivomycose,[31] and evermicose[32]}, a methoxy-group {arcanose[14] [*cf.* (17)], and cladinose[33]} or a nitro-group, as in evernitrose (14).[9]

Only two other naturally occurring branched-chain amino-sugars are known, neither carrying an additional substituent at the carbon atom carrying the amino-group, namely garosamine (21)[34,35] and sibirosamine.[36] There is an interesting structural relationship between vancosamine, daunosamine (16),[13] and the *NN*-dimethyl derivative of the latter, rhodosamine;[37] vancosamine is 3-*C*-methyldaunosamine.

Since this manuscript was prepared, Williams *et al.*[38] have described the isolation of the amino-sugar from vancomycin. These authors have assigned the same structure to the amino-sugar as ourselves although they reported only one derivative of vancosamine, the *NOO'*-triacetyl compound (4); this was obtained as a crystalline solid, m.p. 70—71°. The absolute configuration of vancosamine was not established.

EXPERIMENTAL

I.r. spectra were measured on a Perkin-Elmer 257 and u.v. spectra on a Unicam SP 6000 instrument. N.m.r. spectra were measured on a Varian HA-100 instrument for solutions in deuteriochloroform, unless otherwise stated. Optical rotations were determined using a 2 cm cell on a Perkin-Elmer 141 instrument. Mass spectra were measured either on a Perkin-Elmer Hitachi R.M.U. 6-E or an A.E.I. MS9 instrument. Microanalyses were carried out by Mr. A. Olney. T.l.c. was carried out on Kieselgel GF_{254} and light petroleum refers to the fraction of b.p. 60—80°.

Vancomycin.—Vancomycin sulphate and free base were supplied by Eli Lilly and Co. and were used without further purification.

Acid Hydrolysis of Vancomycin. The Amino-fraction (A) *and Methyl* α-L-*Vancosaminide* (2).—A solution of vancomycin sulphate (5 g) in 2N-hydrochloric acid was heated at 100° on a water-bath for 20 min; after 5 min a precipitate had formed. The mixture was then cooled (ice) and the precipitate, aglucovancomycin, separated. The filtrate was evaporated to dryness and a solution of the residue in water was filtered through a column of Amberlite IRA-400 (OH^-) resin (20 g). The basic filtrate was run through a column of Amberlite IR-120 (H^+) (30 g) resin to give a neutral filtrate consisting mainly of glucose (silylation and g.l.c.). The column was rinsed with water and then the basic fraction (462 mg) was obtained by elution with aqueous 3% ammonium hydroxide. Sometimes this was freed from brown tarry material by filtering through cellulose in butan-1-ol saturated with water, but the n.m.r. spectrum (D_2O) was unchanged by this step.

The amino-fraction (A) (136 mg) was dissolved in methanolic 1·5N-hydrogen chloride (10 ml), and after 20 min at room temperature the solution was evaporated to dryness. The residue was fractionated twice by t.l.c. on Kieselgel H [3% HOAc–n-BuOH(sat.H_2O)]. Only the least polar component (82 mg), R_F 0·4, could be related to vancosamine. This fraction was dissolved in butan-1-ol saturated with water, and the solution was

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neutralised with ammonium hydroxide and evaporated. A solution of the residue in butan-1-ol saturated with water was filtered through cellulose CF1 to give, as a gum homogeneous by t.l.c., *methyl* α-L-*vancosaminide* (*methyl* 3-*amino*-2,3,6-*trideoxy*-3-C-*methyl*-α-L-lyxo-*hexopyranoside*) (2), $[\alpha]_D^{15}$ −118° (*c* 0·09 in MeOH), $\nu_{max.}$ ($CHCl_3$) 3700, 3600, and 1600 cm^{-1}; *m/e* 175·12079 ($C_8H_{17}NO_3$ requires 175·12083), 144, 118, 100, 86, and 73. The c.d. spectrum solution in Cupra A contained a clear negative band at about 600 nm.

Triacetyl-β-L-*vancosamine* (3-*Acetamido*-OO′-*diacetyl*-2,3,6-*trideoxy*-3-C-*methyl*-β-L-lyxo-*hexopyranoside*) (4).—(*a*) An aqueous solution of vancomycin (8·9 g) was heated under reflux with Amberlite ion-exchange resin IR-120 (H^+) (40 g). The resin was separated and eluted with aqueous 10% ammonium hydroxide to give a mixture of bases (1·4 g). The mixture was acetylated with acetic anhydride in pyridine; the product, in ethyl acetate, was chromatographed on alumina and the eluate (116 mg) was separated by t.l.c. (EtOAc) to yield *triacetylvancosamine* (4) (23 mg), R_F 0·3; $\nu_{max.}$ (film) 3300, 1745, and 1655 cm^{-1}; *m/e* 244 (M^+ −43), 230, 227, 170, and 128.

(*b*) The triacetate could be obtained also by acetylation of the amine fraction (A) but the yields were low (g.l.c. purity > 95%).

Methyl NO-*Dibenzoylvancosaminides* (*Methyl* 3-*Benzamido*-O-*benzoyl*-2,3,6-*trideoxy*-3-C-*methyl*-L-lyxo-*hexopyranosides*) (5) *and* (6)].—A solution of vancomycin sulphate (5·5 g) in pyridine (25 ml) and benzoyl chloride (10 ml) was kept at room temperature overnight. Methanol (50 ml) was added and the solution evaporated to dryness. The residue was washed with ether to remove methyl benzoate, leaving a white powder, that was dissolved in methanolic 1·5N-hydrogen chloride and heated under reflux for 7 h. The solvent was evaporated off and the residue partitioned between water and ethyl acetate. The organic fraction yielded a yellow oil (6·4 g) an ethereal solution of which was filtered through a silica gel column to give a colourless oil (1·89 g). This oil was chromatographed on neutral alumina; ether eluted methyl benzoate (1·1 g), and ethyl acetate eluted a mixture of compounds (197 mg.). T.l.c. of the mixture (ether–light petroleum) gave two main fractions. The less polar fraction (65 mg), R_F 0·6, crystallised from ether–light petroleum to give white needles of the α-*anomer* (5) (31 mg), m.p. 168—169°, $[\alpha]_D^{22}$ −191° (*c* 0·1 in MeOH); $\lambda_{max.}$ (MeOH) 227 and 270infl nm (log ε 4·44 and 3·25); $\nu_{max.}$ ($CHCl_3$) 3400 (NH), 1725, 1705 ($PhCO_2$), and 1670 (PhCO·NH) cm^{-1}; $\nu_{max.}$ (film) 3320 (NH), 1729, and 1642 cm^{-1}; *m/e* 383 (M^+), 351, 204·10304 ($C_{12}H_{14}NO_2$ requires 204·10245), 160, and 105 (Found: C, 68·9; H, 6·5; N, 3·65. $C_{22}H_{25}NO_5$ requires C, 68·9; H, 6·5; N, 3·65%).

Separation of the more polar fraction (82 mg), R_F 0·5, by t.l.c. (ether) yielded a mixture of partially benzoylated glucosides (40 mg.), R_F 0·9, and as a gum, the β-*anomer* (6) (36 mg), R_F 0·8, $[\alpha]_D^{22}$ −64° (*c* 0·14 in MeOH); $\lambda_{max.}$ (MeOH) 228 and 270infl nm (log ε 4·31 and 3·15); $\nu_{max.}$ ($CHCl_3$) 3400 (NH), 1725 ($PhCO_2$), 1705, and 1668 (PhCO·NH·) cm^{-1}; *m/e* 383·17246 (M^+) ($C_{22}H_{25}NO_5$ requires 383·17326), 351, 337, 257, 252, 204, and 105.

Ethyl NO-*Dibenzoylvancosaminides* [(7) *and* (8)].—A solution of vancomycin sulphate (5 g) in 2N-hydrochloric acid (50 ml) was heated at 100° for 8 min. A precipitate rapidly formed and after cooling the solution was filtered. The aqueous filtrate was evaporated and the residue dissolved in absolute ethanol; the solution was evaporated giving a gum (1·6 g). A solution of this gum in pyridine (20 ml) and benzoyl chloride (5 ml) was kept at room temperature overnight. The mixture was poured into water and extracted with ethyl acetate to give, after drying and evaporation, a gum (4·4 g). This was dissolved in ether and filtered through alumina (Spence type H); material from the eluate (1·7 g) was chromatographed on silica gel. Elution with chloroform yielded a mixture (840 mg) that was separated by t.l.c. (ether–light petroleum) into two fractions. The major fraction (451 mg) in chloroform was filtered through silica gel and fractionated by t.l.c. (chloroform) to give glucose derivatives (138 mg) and the crude α-anomer (168 mg), R_F 0·4. Repeated recrystallisation from ether–light petroleum gave needles of the α-*anomer* (7) (20 mg), m.p. 131—133°, $[\alpha]_D^{25}$ −179° (*c* 0·27 in MeOH); c.d. $\lambda_{max.}$ (MeOH) 222 and 237 nm ($\Delta\varepsilon$ + 3·2 and −6·8); $\lambda_{max.}$ 227 and 270infl nm (log ε 4·42 and 3·30); $\nu_{max.}$ (Nujol) 3390, 3350 (NH), 1720, 1705 (PhCO·O), and 1660 (PhCO·NH) cm^{-1}; *m/e* 397 (M^+), 352, 351 (M^+ − EtOH), 218, 160, and 105 (Found: C, 69·3; H, 6·85; N, 3·6. $C_{23}H_{27}NO_5$ requires C, 69·5; H, 6·85; N, 3·5%).

The minor band (193 mg) was repeatedly chromatographed (t.l.c.; ether) to yield the impure β-anomer (57 mg). Crystallisation from ether–light petroleum gave needles of the β-*anomer* (8) (15 mg), m.p. 97—99°, $[\alpha]_D^{25}$ −82° (*c* 0·24 in MeOH); c.d. $\lambda_{max.}$ (MeOH) 222 and 237 nm ($\Delta\varepsilon$ +2·8 and −4·0); $\lambda_{max.}$ (MeOH) 228 and 270infl nm (log ε 4·19 and 2·97); $\nu_{max.}$ (Nujol) 3320 (NH), 1730 (PhCO·O), 1640 (PhCO·NH), and 1560 cm^{-1}; *m/e* 397 (M^+), 352, 351 (M^+ − EtOH), 218, and 105 (Found: C, 69·5; H, 7·1; N, 3·5%).

Ethyl N-*Benzoyl*-α-L-*vancosaminide* (*Ethyl* 3-*Benzamido*-2,3,6-*trideoxy*-3-C-*methyl*-α-L-lyxo-*hexopyranoside*) (9).—A solution of the *NO*-dibenzoyl derivative (8) (133 mg) in methanol (10 ml) and N-sodium hydroxide (10 ml) was kept at room temperature for 1 h, diluted with water (150 ml), and extracted with ether (3 × 50 ml). The extract was washed, dried and evaporated to give a gum (84 mg). Fractionation of the gum by t.l.c. (ether) yielded, as a gum, the major component, the N-*benzoyl derivative* (9) (68 mg), R_F 0·5, $[\alpha]_D^{25}$ −137° (*c* 0·10 in MeOH), no dichroism observed; $\lambda_{max.}$ (MeOH) 225 and 265infl nm (log ε 4·43 and 3·30); $\nu_{max.}$ ($CHCl_3$) 3570 (OH) 3420 (NH), 1665 (PhCO·NH), and 1585 cm^{-1}; *m/e* 293 (M^+), 275, 248, 219, 218, 190, and 105 (Found: C, 65·3; H, 7·95; N, 4·75. $C_{16}H_{23}NO_4$ requires C, 65·5; H, 7·9; N, 4·8%).

Methyl NO-*Bisphenylsulphonylvancosaminides* (*Methyl* 2,3,6-*Trideoxy*-3-C-*methyl*-4-O-*phenylsulphonyl*-3-*phenylsulphonylamino*-L-lyxo-*hexopyranosides*) [(10) *and* (11)].—A solution of vancomycin sulphate (5·4 g) in pyridine (40 ml) was treated with benzenesulphonyl chloride (10 ml) at 0°. After 3 h more benzenesulphonyl chloride (10 ml) and pyridine (10 ml) were added at room temperature, and the mixture was left overnight. The solution was diluted with methanol and evaporated to leave a solid which was repeatedly washed with ether. The residue was dissolved in methanol, the solution evaporated, and the solid residue neutralised with aqueous 5% sodium hydrogen carbonate (400 ml.) and extracted with chloroform (4 × 100 ml). The extract was evaporated, leaving a solid (9·4 g), which was dissolved in methanolic 3N-hydrogen chloride. The solution was heated under reflux for 7 h. The solvent was evaporated off and the residue

dissolved in water and extracted with ethyl acetate; the extract was washed, dried, and evaporated to give an oil (5·2 g). A solution of the oil in ethyl acetate (250 ml) and ether (50 ml) was washed with water (100 ml) and aqueous 5% sodium hydrogen carbonate and then extracted with N-sodium hydroxide (2 × 100 ml). The alkaline solution was acidified and extracted with ether; evaporation of the extract yielded a mixture (319 mg). This was redissolved in ether and filtered through silica gel; the eluate was separated into two main components by t.l.c. (ether). More of the product was obtained by column chromatography of the alkali-insoluble material followed by t.l.c. separation as before. The less polar components from each source were combined (162 mg; R_F 0·7) and crystallised from ether–light petroleum and then from ethyl acetate–light petroleum to give colourless crystals of the *α-anomer* (10) (76 mg), m.p. 132—133° (decomp.), $[\alpha]_D^{24}$ −109° (*c* 0·34 in MeOH); $\lambda_{max.}$ (MeOH) 220, 260, 266, and 273 nm (log ε 4·24, 3·11, 3·24, and 3·17); $\nu_{max.}$ (Nujol) 3340 (NH), 1450, and 1330 cm^{-1}; *m/e* 455 (M^+), 313, 240·06780 ($C_{11}H_{14}NO_3S$ requires 240·06943), 141, and 117 (Found: C, 53·0; H, 5·75; N, 2·85. $C_{20}H_{25}NO_7S_2$ requires C, 52·75; H, 5·55; N, 3·05%).

The combined more polar fraction (80 mg; R_F 0·6), after crystallisation from ethyl acetate–light petroleum yielded white needles of the *β-anomer* (11) (26 mg), m.p. 151—154° (decomp.), $[\alpha]_D^{24}$ −6·5° (*c* 0·31 in MeOH); $\lambda_{max.}$ (MeOH) 220, 253, 259, 266, and 273 nm (log ε 4·23, 2·88, 3·11, 3·23, and 3·11); $\nu_{max.}$ (Nujol) 3310 (NH) and 1460 cm^{-1}; *m/e* 455 (M^+), 314, 240, 140, and 117 (Found: C, 52·75; H, 5·6; N, 2·9%).

Methyl O-Acetyl-N-phenylsulphonyl-α-L-vancosaminide (*Methyl 4-O-Acetyl-2,3,6-trideoxy-3-C-methyl-3-phenylsulphonylamino-α-L-lyxo-hexopyranoside*) (12).—A solution of the amine fraction (A) (279 mg) in methanol (20 ml) and acetic anhydride (3 ml) was kept at room temperature overnight, then evaporated to dryness. The residue was dissolved in dilute methanolic hydrogen chloride and the solution was kept at room temperature for 3 days. The solvent was evaporated off and the residue was treated with pyridine (20 ml) and benzenesulphonyl chloride (5 ml) and set aside overnight at room temperature. The solution was then diluted with water (200 ml) and extracted with ether to give, after drying and evaporation, an oil (731 mg). A solution of the oil in chloroform was filtered through alumina (Spence type H) and then an ethereal solution was filtered through silica gel. The eluate (213 mg) was evaporated and the product separated by t.l.c. (ether) to yield the bisphenylsulphonyl derivative (10) (37 mg) and crude acetyl derivative (81 mg). Repeated purification by t.l.c. (chloroform) yielded, as a homogeneous oil, the *acetyl phenylsulphonyl derivative* (12) (49 mg), $[\alpha]_D^{23}$ −125° (*c* 0·12 in MeOH); $\lambda_{max.}$ (MeOH) 223, 260, 266, and 272 nm (log ε 3·93, 2·85, 2·93, and 2·85); $\nu_{max.}$ ($CHCl_3$) 3480 (NH) and 1740 ($MeCO_2$) cm^{-1}; *m/e* 357 (M^+), 240, and 141 (Found: C, 53·95; H, 6·55; N, 3·6. $C_{16}H_{23}NO_6S$ requires C, 53·8; H, 6·5; N, 3·9%).

4-Hydroxy-2,5-dimethylcyclopent-2-enones (20).—A solution of vancomycin (10 g) in 0·05N-hydrochloric acid (500 ml) was mixed with Amberlite IR-120 (H^+) resin (100 g) and heated under reflux for 22 h. The resin was separated and the aqueous solution was evaporated to dryness. The residue was dissolved in water (50 ml) and the solution extracted with ethyl acetate (3 × 50 ml). The combined extract was dried and evaporated to give an oil (212 mg). A solution of the oil in ether was filtered through alumina (Spence type H) to give a colourless oil (117 mg), which was chromatographed on silica gel to give a mixture of cyclopentenones (20) (114 mg), $\lambda_{max.}$ (MeOH) 224 nm (log ε 3·96); $\nu_{max.}$ (CCl_4) 3620 (OH), 1720 (C=O), and 1645w (C=C) cm^{-1}; $\nu_{max.}$ (film) 3400 (OH), 1708 (C=O), and 1640 (C=C) cm^{-1}; *m/e* 126 (M^+), 124, 111, 109, 108, and 95 (the peak at *m/e* 124 due to the enedione formed by oxidation in the spectrometer); high resolution *m/e* 126·0669 (Calc. for $C_7H_{10}O_2$: 126·0680) and 111·0448 (Calc. for $C_6H_7O_2$: 111·0446). G.l.c. analysis revealed the presence of two components in a 3 : 1 ratio and the n.m.r. spectra showed two sets of signals with a 3 : 1 ratio: major component (4-OH and 5-Me *trans*), τ 8·78 (d, *J* 7·5 Hz, 5-Me), 8·20 (t, *J* 1·5 Hz, 2-Me), 7·72 (dq, *J* 2·5 and 7·5 Hz, 5-H), 5·54 (sextet, *J* 2 Hz, 4-H), and 2·58 (qd, *J* 1·25 and 2·5 Hz, 3-H); minor component (4-OH and 5-Me *cis*), τ 8·82 (d, *J* 7 Hz, 5-Me), 8·20 (t, *J* 1·5 Hz, 2-Me), 7·32 (qd, *J* 7·5 and 13 Hz, 5-H), 5·12 (ddd, *J* 6, 3, and 2 Hz, 4-H), and 3·04 (q, *J* 2 Hz, 3-H). In both spectra the hydroxy-proton appeared as a broad singlet at τ *ca.* 6·6, removed by addition of D_2O.

A solution of the hydroxy-ketones (20) (14 mg) and 3,5-dinitrobenzoyl chloride (58 mg) in pyridine (2 ml) was kept overnight at room temperature. The solution was diluted with water (10 ml) and extracted with ether; the extract was washed, dried, and evaporated to yield a mixture (20 mg). Separation on t.l.c. (ether–light petroleum) gave a minor component (5·0 mg), R_F 0·4, and a major component (16·3 mg), R_F 0·5. which on rechromatography yielded the *trans*-3,5-dinitrobenzoate as a colourless solid (11 mg), m.p. 74—84°, u.v. end absorption; $\nu_{max.}$ (Nujol) 1730 (ester), 1709 (C=O), and 1630 (C=C) cm^{-1}; *m/e* 320 (M^+), 292, 195 [$C_6H_4(NO_2)_2CO$], and 108; τ ($CDCl_3$) 8·64 (d, *J* 7 Hz, 5-Me), 8·12 (d, *J* 1 Hz, 2-Me), 7·50 (dq, *J* 7 and 2·5 Hz, 5-Me), 4·32 (q, *J* 2 Hz 4-H), 2·85 (dq, *J* 2 and 1 Hz, 3-H), and 0·95 and 0·82 (aromatic H). Irradiation at the frequency of the band at τ 7·50 affected the band at τ 4·32; irradiation at the frequency of the band at τ 4·32 caused the band at τ 7·50 to collapse to a quartet; irradiation at the frequency of the band at τ 2·85 caused no change in the spectrum.

Treatment of the hydroxy-ketones (20) (14 mg) with 2,4-dinitrophenylhydrazine hydrochloride in 2N-hydrochloric acid yielded a major fraction (21 mg), which on repeated recrystallisation from ether–light petroleum yielded crystals (homogeneous by t.l.c.) of a mixture of two epimeric 2,4-dinitrophenylhydrazones (2·3 mg), m.p. 190—200°; $\lambda_{max.}$ 371 nm; $\nu_{max.}$ (Nujol) 3300, 1630, and 1592 cm^{-1}; *m/e* 306 (M^+), 290 ($M^+ - 17$), 289 ($M^+ - 18$), 271 ($M^+ - 35$), 124, and 109 (Found: C, 50·2; H, 4·7; N, 17·2. Calc. for $C_{13}H_{14}N_4O_5$: C, 51·0; H, 4·6; N, 18·3%).

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