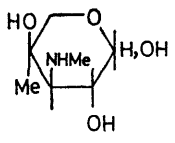
The Gentamicin Antibiotics. Part III.[1a] The Gross Structures of the Gentamicin C Components

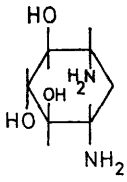
By **David J. Copper,**† **Peter J. L. Daniels,* Milton D. Yudis, Henrietta M. Marigliano,** and (in part) **R. D. Guthrie** ‡ and (Miss) **S. T. K. Bukhari,**‡ Schering Corporation, Bloomfield, New Jersey 07003, U.S.A.

Methanolysis of the gentamicin C components gave methyl garosaminide and the corresponding gentamines, isolated as crystalline tetra-*N*-acetates. The absolute stereochemistry of the 2-deoxystreptamine portion of these tetra-*N*-acetates was determined by a circular dichroism method. Methylation of the penta-*N*-acetylgentamicin C components followed by acid hydrolysis and reacetylation gave, in each case, *NN'*-diacetyl-2-deoxy-*NN'*,5-*O*-trimethylstreptamine, establishing the gentamicins to be 4,6-disubstituted 2-deoxystreptamine derivatives.

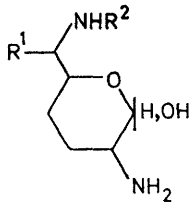
PRECEDING publications [1] have established the gentamicin C components to be pseudotrisaccharides containing garosamine (1) and a purpurosamine (3) glycosidically linked to 2-deoxystreptamine (2). The positions of these linkages and the absolute stereochemistry around the 2-deoxystreptamine unit are the subject of this paper.



(1)



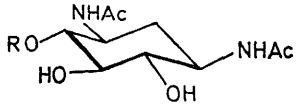
(2)



(3)

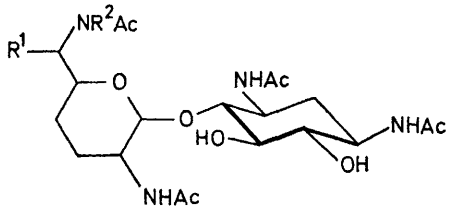
From gentamicin
C_1 $R^1 = R^2 = Me$
C_2 $R^1 = Me$, $R^2 = H$
C_{1a} $R^1 = R^2 = H$

Methanolysis of the purified gentamicin C components gave methyl garosaminide and the three gentamines, designated gentamines C_1, C_2, and C_{1a} corresponding to gentamicins C_1, C_2, and C_{1a}, respectively; *N*-acetylation of the gentamines with acetic anhydride in methanol gave tetra-*N*-acetylgentamines, obtained as crystalline solids. A periodate test [2] of these *N*-acetyl derivatives demonstrated the presence of vicinal diol groupings and periodate titration of tetra-*N*-acetylgentamines C_1 and C_2 resulted in rapid uptake of 1 equiv., after which oxidation ceased. The c.d. of the cuprammonium complexes of the tetra-*N*-acetylgentamines was measured in Cupra A solution.[3] All three compounds showed a positive c.d.



(4)

effect at 570 nm and a negative effect at 280 nm, characteristic of the formation of a δ-chelate and indicating a negative dihedral angle for the vicinal diol grouping [3] as in (4). The positions of attachment of the purpurosamine units and the absolute configuration of the 2-deoxystreptamine portions were thus established and the structures of the tetra-*N*-acetylgentamines are as depicted in (5).



(5)

per-*N*-acetylgentamines
C_1 $R^1 = R^2 = Me$
C_2 $R^1 = Me$, $R^2 = H$
C_{1a} $R^1 = R^2 = H$

N-Acetylation of the gentamicin C components afforded penta-*N*-acetyl derivatives as hydrated amorphous solids. Methylation of penta-*N*-acetylgentamicin C_1 with methyl iodide and silver oxide in dimethylformamide according to a published [4] procedure gave the penta-*N*-acetyl-per-*NO*-methyl derivative (6) in good yield. Methylation of the *N*-acetyl derivatives of C_2 and C_{1a} with methyl iodide and sodium hydride in dimethylformamide gave quantitative yields of the permethylated compounds (6) § and (7), isolated as amorphous monohydrates. Hydrolyses of these materials from all three C gentamicins gave *NN'*-diacetyl-2-deoxy-*NN'*,5-*O*-trimethylstreptamine (8) in yields of 20, 72, and 68% for gentamicins C_1, C_2, and C_{1a}, respectively.

The fully methylated structures (6) and (7) were substantiated by their ^1H n.m.r. spectra. These were complex owing to the numerous possibilities for amide rotamers, but integration indicated the correct number of methyl groups. The mass spectra of these compounds gave relatively abundant molecular ions or $(M + 1)^+$ peaks at *m/e* 771 and 772 and *m/e* 757 and 758. The base peak in each case was at *m/e* 230, corresponding

† *Present address:* Smith, Kline, and French Laboratories, Philadelphia, Pennsylvania.

‡ School of Molecular Sciences, University of Sussex, Brighton BN1 9QJ.

§ Methylation of *N*-acetylgentamicins C_1 and C_2 affords the same product (6), since the parent antibiotics differ only in the presence of an extra *N*-methyl group in C_1.[1,5]

[1] (a) Part II, D. J. Cooper, M. D. Yudis, H. M. Marigliano, and T. Traubel, *J. Chem. Soc.* (*C*), 1971, 2876; (*b*) D. J. Cooper, M. D. Yudis, R. D. Guthrie, and A. M. Prior, *J. Chem. Soc.* (*C*), 1971, 960.

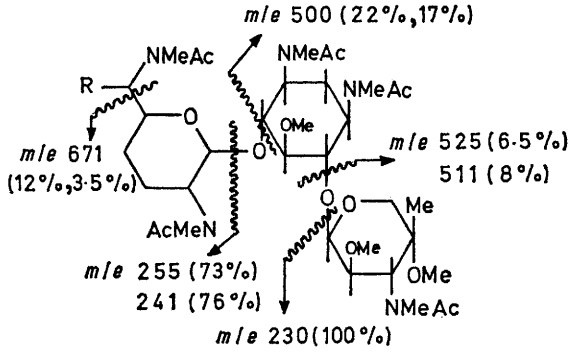
[2] F. Feigl, 'Spot Tests in Organic Analysis,' Elsevier, London, 1960, p. 128.

[3] S. T. K. Bukhari, R. D. Guthrie, A. I. Scott, and A. D. Wrixon, *Tetrahedron*, 1970, **26**, 3653.

[4] R. U. Lemieux and R. J. Cushley, *Canad. J. Chem.*, 1963, **41**, 858.

[5] P. J. L. Daniels and R. T. Tkach, unpublished work.

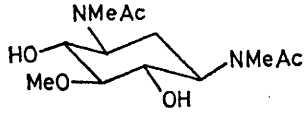
to the fully methylated garosamine fragment, with substantial peaks at *m/e* 255 (73%) and 241 (76%), corresponding to the purpurosamine units. Remaining substantial fragments are shown in structures (6) and (7).*



(6) R = Me
(7) R = H

Mass spectral cleavages of per-*N*-acetyl-per-*NO*-methylgentamicins. Peaks and percentages are given for compound (6), then for (7).

The methylated product (6) from gentamicin C_1, prepared by use of silver oxide, gave a mass spectrum identical to that of the sample prepared from gentamicin C_2, with the exception that peaks due to a product containing one less methyl group were also present. Detailed examination of the mass spectrum revealed that these were due to non-specific incomplete methylation, and this result must account for the lower yield of the 2-deoxystreptamine derivative (8) from gentamicin C_1.

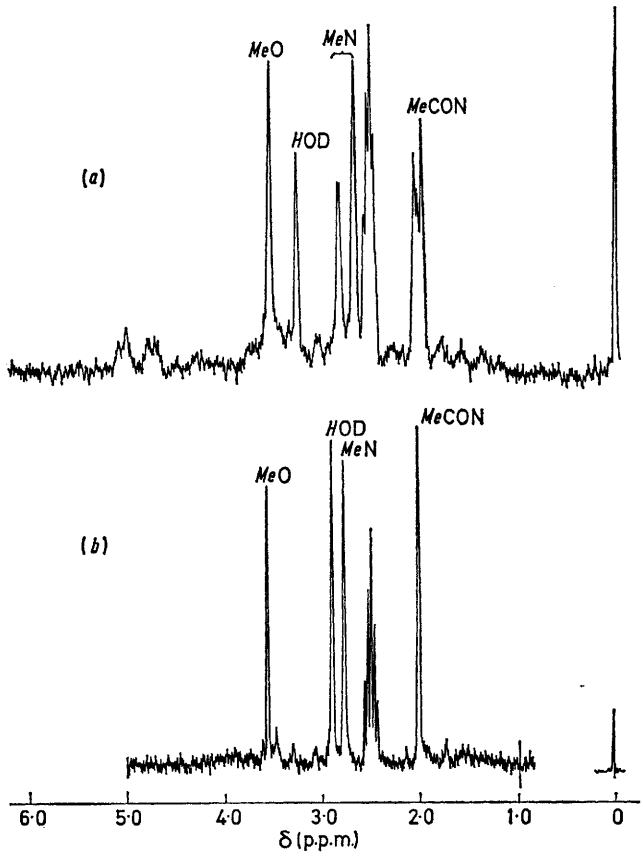


(8)

NN'-Diacetyl-2-deoxy-*NN'*,5-*O*-trimethylstreptamine (8) was obtained as a crystalline solid whose gross structure was confirmed by microanalytical data and mass spectrum (*m/e* 288). In agreement with its symmetrical structure it showed no optical activity and gave a negative periodate test for vicinal glycols; it showed no c.d. in cuprammonium solution. The ^{1}H n.m.r. spectrum was unexpectedly complex at ambient temperature [see Figure (*a*)]. The *N*-methyl groups gave two sharp singlets of equal intensity at δ 2·83 and 2·86 and a broader band at δ 2·69 p.p.m.; three signals were observed for the acetyl methyl groups and a singlet for the *O*-methyl group. At 120°, however, the spectrum simplified [Figure (*b*)], and sharp single resonances were observed for each of these functional groups, at δ 2·77, 2·01, and 3·55 p.p.m., respectively. The multiplicities observed at ambient temperature can be explained on the basis that compound (8) exists as an approximately statistical mixture of rotational isomers (9a, b, and c).

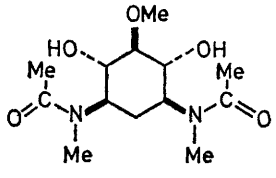
* Detail work on the mass spectra of aminoglycosides will be reported later.

In (9a) both *N*-methyl substituents are *cis* to the acetyl carbonyl group and the absorption is shifted significantly

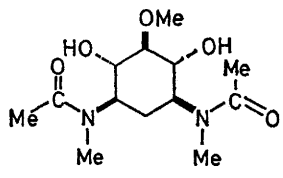


^{1}H N.m.r. spectra [$(CD_3)_2SO$; D_2O added; 60 MHz] of compound (8) (*a*) at ambient temperature, (*b*) at 120°

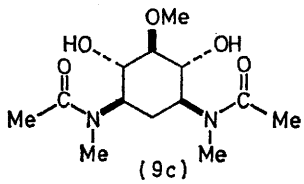
(*ca.* 10 Hz) upfield relative to that of the *N*-methyl groups of (9c), which are *trans* to the acetyl carbonyl group.[6] In (9b) one of the *N*-methyl groups is *cis* and the other



(9a)



(9b)

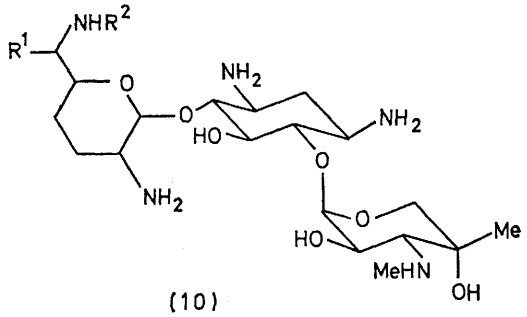


(9c)

trans, contributing an upfield and a downfield signal. A similar situation has been reported in the n.m.r. spectra of some 1,2-diphosphoranes.[7]

[6] H. Paulsen and K. Todt, *Chem. Ber.*, 1967, **100**, 3385.
[7] M. A. Shaw, J. C. Tebby, R. S. Ward, and D. H. Williams, *J. Chem. Soc.* (*C*), 1967, 2442.

The evidence presented establishes the points of linkage of garosamine and the purpurosamines to 2-deoxystreptamine as C-6 and C-4, respectively. The ^1H n.m.r. spectra of the gentamicin C components (CD_3OD; 60 MHz) have been described;[1a] the anomeric protons give doublets (J *ca.* 3·5 Hz) in the region δ 5·0—5·2 p.p.m. Since the hydrogen atom at C-2 of garosamine is axial, the linkage of this unit to 2-deoxystreptamine must be axial and the structures of the gentamicin C components are as shown in (10).



(10)

gentamicin { C_1 $R^1 = R^2 = Me$; C_2 $R^1 = Me$, $R^2 = H$; C_{1a} $R^1 = R^2 = H$

In a footnote to a recent paper[8] it was noted that periodate oxidation of gentamicin C, followed by vigorous acid hydrolysis, afforded 2-deoxystreptamine. This result was interpreted as proof of 4,6-linkage of the sugars to 2-deoxystreptamine, since in a 4,5-linked structure this system would not be expected to survive. We confirmed this finding in our early work; however we also noted that gentamicin C will readily react with much larger amounts of periodate than is consistent with simple cleavage of the vicinal amino-alcohol groupings, and in the antibiotic neomycin, which possesses sugars 4,5-linked to 2-deoxystreptamine, a small but easily detectable yield of 2-deoxystreptamine could be demonstrated after periodiate oxidation and acid hydrolysis. We therefore feel that evidence of linkage obtained in this manner should be interpreted with great caution.

EXPERIMENTAL

N.m.r. spectra were determined with a Varian A60A spectrometer at 60 MHz. Mass spectra were determined with an Atlas CH5 spectrometer, with use of a direct inlet system. M.p.s were taken with a Kofler hot-stage apparatus. T.l.c. was performed on silica gel plates (4 × 8 in) and compounds were located with iodine vapour and by sulphuric acid charring. Column chromatography utilized chromatographic grade silica gel (J. T. Baker and Co.). C.d. spectra were measured with a JASCO/UV/ORD/5 instrument fitted with a c.d. attachment.

Per-N-acetylgentamines.—The parent gentamicin was methanolysed and the corresponding gentamine was isolated as described earlier.[1b] The gentamine (2·0 g) was dissolved in methanol (50 ml), acetic anhydride (3·5 ml) was added, and the solution was set aside for 24 h at room temperature. It was then evaporated to dryness *in vacuo*; the syrupy residue was dissolved in methanol (20 ml) and induced to crystallize by the addition of ether to incipient turbidity. Recrystallization to constant m.p. was achieved in the same manner, yielding the per-*N*-acetylgentamine C components (5) as needles (see Table). All three compounds gave weak molecular ions in their mass spectra [*m/e* 486 (C_1), 472 (C_2), and 458 (C_{1a})] as well as more intense $(M + 1)^+$ ions; significant peaks corresponding to diacetylpurpurosamine ions were observed at *m/e* 241 (21%), 227 (22%), and 213 (9·5%) from tetra-*N*-acetylgentamines C_1, C_2, and C_{1a}, respectively. All three compounds gave a positive periodate test for vicinal glycol groups.[2] Tetra-*N*-acetylgentamines C_1 and C_2 were oxidized with sodium periodate and the uptake was determined by the method of Gardell.[9] In both cases 1 equiv. was consumed in 5 h with no further uptake after 24 h.

Per-N-acetyl-per-NO-methylgentamicin C_1 (6).—(a) *From per-N-acetylgentamicin* C_1. Per-*N*-acetylgentamicin C_1 (3·5 g) was dissolved in dimethylformamide (100 ml) containing suspended freshly prepared silver oxide (30 g). Methyl iodide (20 ml) was added with stirring and cooling and the suspension was stirred at room temperature for 24 h. More methyl iodide (20 ml) was added and stirring was continued for a further 72 h. The suspension was filtered and the residue was washed with dimethylformamide (2 × 50 ml). The combined filtrate and washings were evaporated to dryness *in vacuo* and the residue was extracted with boiling chloroform (2 × 200 ml). The filtered extract was washed with water, dried ($MgSO_4$), and evaporated *in vacuo* affording compound (6) as a foam (2·4 g), m.p. 165—170°, $[\alpha]_D^{26}$ +165° (*c* 0·3 in $CHCl_3$), *m/e* 771 ($M^{+\cdot}$) and 757 ($M^{+\cdot}$ for compound with one less methyl group); other degradations as already described (Found: C, 57·2; H, 8·15; N, 8·7. $C_{37}H_{65}N_5O_{12}$ requires C, 57·6; H, 8·5; N, 9·05%).

(b) *From per-N-acetylgentamicin* C_2. Per-*N*-acetylgentamicin C_2 (2·2 g) in dry dimethylformamide (6 ml) was added to a stirred suspension of sodium hydride (0·96 g) in the same solvent (15 ml). The mixture was stirred for 1 h at room temperature, then cooled in a water-bath, and methyl iodide (6 g) was added slowly. Stirring was continued at room temperature for 24 h, more methyl iodide (3 g) was added, and stirring was then continued for a further 60 h. Methanol was added, insoluble material was filtered off, and the filtrate was evaporated *in vacuo*. The residue was dissolved in water; the aqueous solution was extracted five times with chloroform and the combined extracts were dried (Na_2SO_4) and evaporated affording *compound* (6) as an amorphous foam (2·6 g.). After being dried

Per-*N*-acetylgentamine	Yield * (%)	M.p.	Calc. (%) C	H	N	Found (%) C	H	N	$[\alpha]_D^{26}$ (*c* 0·3 in H_2O)
C_1, $C_{22}H_{38}N_4O_8$	27	251—254·5°	54·3	7·85	11·5	54·3	7·9	11·35	+115°
C_2, $C_{21}H_{36}N_4O_8,H_2O$	44	263—265·5	51·4	7·8	11·4	51·2	7·45	11·2	+128
C_{1a}, $C_{20}N_{34}N_4O_8$	25	284·5—286	52·4	7·45	12·2	52·3	7·55	11·9	+95

* After two recrystallizations.

[8] H. Maehr and C. P. Schaffner, *J. Amer. Chem. Soc.*, 1970, **92**, 1697.

[9] S. Gardell, *Methods of Biochemical Analysis*, 1958, **3**, 289.

at 96° and 0·035 mmHg for 16 h the sample had $[\alpha]_D^{26}$ +162° (*c* 0·3 in MeOH), *m/e* 771 (2%, $M^{+\cdot}$), 698 (5·5%, loss of MeCO·NHMe), 671 (12%), 525 (6·5), 500 (22), 255 (73), and 230 (100) (Found: C, 56·55; H, 8·65; N, 9·0. $C_{37}H_{65}N_5O_{12},H_2O$ requires C, 56·25; H, 8·55; N, 8·85%).

Per-N-*acetyl-per*-NO-*methylgentamicin* C_{1a} (7).—Method (b) applied to per-*N*-acetylgentamicin C_{1a} (5·0 g) afforded the *compound* (7) (5·95 g) as an amorphous foam. A sample dried at 100° and 0·035 mmHg for 17 h had $[\alpha]_D^{26}$ +146° (*c* 0·3 in MeOH), *m/e* 758 [1·5%, $(M + 1)^+$], 757 (0·15%, $M^{+\cdot}$), 684 (1·5%, loss of MeCO·NHMe), 671 (3·5%), 511 (8), 500 (17), 241 (76), and 230 (100) (Found: C, 55·6; H, 8·3; N, 9·3. $C_{36}H_{63}N_5O_{12},H_2O$ requires C, 55·7; H, 8·45; N, 9·05%).

NN′-*Diacetyl*-2-*deoxy*-NN′,5-O-*trimethylstreptamine* (8).—(a) *From per*-N-*acetyl-per*-NO-*methylgentamicin* C_{1a} (7). Compound (7) (4·95 g) was warmed on a steam-bath with 6N-hydrochloric acid (100 ml) for 2 h. The solution was evaporated to dryness *in vacuo*; the residue was dissolved in water (100 ml) and treated with Amberlite IR 401S resin (OH^- form) until strongly basic. The resin was filtered off, the filtrate was evaporated to dryness *in vacuo*, and the residue was dissolved in methanol (100 ml) and treated with acetic anhydride (10 ml). After 1 h at room temperature the solution was evaporated *in vacuo*; the residue was dissolved in the lower phase of chloroform–methanol–concentrated ammonia (2 : 1 : 1) and applied to a column of silica gel (60 g). Elution with the same solvent system afforded *compound* (8) as a white crystalline solid (1·36 g, 72%), m.p. 221—222·5° (from ethyl acetate–methanol) $[\alpha]_D^{26}$ +0° (*c* 0·9 in MeOH), $\nu_{max.}$ (Nujol) 3378 and 3300 (OH), and 1661 and 1618 (amide) cm^{-1}, *m/e* 288 ($M^{+\cdot}$) (Found: C, 54·35; H, 8·5; N, 9·95. $C_{13}H_{24}N_2O_5$ requires C, 54·15; H, 8·4; N, 9·7%).

(b) *From per*-N-*acetyl-per*-NO-*methylgentamicins* C_1 *and* C_2. By an identical procedure compound (6) from gentamicins C_1 and C_2 afforded *NN′*-diacetyl-2-deoxy-*NN′*, 5-*O*-trimethylstreptamine (8) in yields of 20 and 68%, respectively. The product in each case was identified by m.p., mixed m.p., t.l.c., and n.m.r. and i.r. spectra.

We thank J. Weinstein and A. Yehaskel for their assistance.

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