Notes

The Gentamicin Antibiotics. 8. Structure of Gentamicin A_2

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This communication deals with the elucidation of the structure of gentamicin A_2. The isolation of A_2 is published in the accompanying paper.[1a] The mass spectrum of A_2 indicated a molecular weight of 455 and elemental analysis was in good agreement with the composition $C_{17}H_{33}N_3O_{11}\cdot 2H_2O$. The mass spectrum also indicated ions characteristic of the deoxystreptamine moiety (*m/e* 191, 173, 163, and 145), a monoaminomonodeoxyhexosyldeoxystreptamine (*m/e* 352, 334, 324, 306, and 162), and a pentosyldeoxystreptamine unit (*m/e* 323, 305, 295, 277, and 133) in the molecule.[1-3]

Hydrolysis of gentamicin A_2[1b] with 6 *N* hydrochloric acid at 100° for 1 hr followed by analysis of the hydrolysate by paper chromatography using 1-butanol–pyridine–water–acetic acid (6:4:3:1) as the developer confirmed the presence of deoxystreptamine. The R_f values of the monosaccharides produced agreed well with those of glucosamine and xylose on paper chromatography using an ethyl acetate–pyridine–water (8:2:1) system as developer.[11]

The ^1H NMR spectrum of A_2 contained signals readily recognizable as arising from the deoxystreptamine and glucosamine portions of the molecule [δ 1.19 (H-2_{ax}, $J_{2ax,eq}$ = 12.5 Hz, $J_{1,2ax} = J_{2ax,3}$ = 12.5 Hz), 1.95 (H-2_{eq}, $J_{1,2eq} = J_{2eq,3}$ = 3.5 Hz), 2.77 (H-2′, $J_{2',3'}$ = 10 Hz), 5.23 ppm (H-1′, $J_{1',2'}$ = 3.5 Hz)].[1a,2] A second anomeric proton doublet appeared at δ 5.02 ppm with a splitting of 2.5 Hz.

N-Acetylation of A_2 afforded tri-*N*-acetylgentamicin A_2[1c] in quantitative yield, which confirmed the presence of three amino groups in the molecule [δ 1.51 (H-2_{ax}, $J_{2ax,2eq}$ = 12.0 Hz), 2.0, 2.02, and 2.06, (*N*-acetyls), 5.09 (H-1″, $J_{1'',2''}$ = 3.0 Hz), 5.38 (H-1′, $J_{1',2'}$ = 3.5 Hz)]. The chemical shifts and spin couplings of the signals due to the anomeric hydrogens in A_2 and its tri-*N*-acetyl derivative indicate these protons to be equatorially oriented and cis to the neighboring hydrogens.

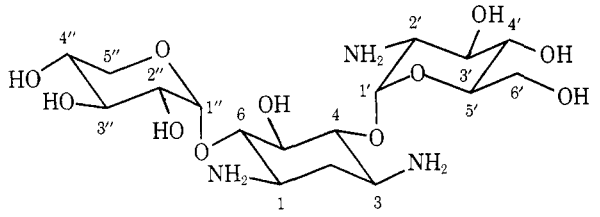
The proton noise decoupled ^{13}C NMR spectra of gentamicin A_2 measured at basic and acidic pD's[4-8] led to the recognition of the paromamine moiety in the molecule and established the configuration and ring size of the pentose unit and its position of attachment to paromamine.[1a] In Table I, the ^{13}C chemical shifts of gentamicins A_2, A, paromamine, and methyl α-D-xylopyranoside[9] are compared. The presence of the paromamine moiety in A_2 is clear from a comparison of the chemical shifts of carbon atoms 1–6 and 1′–6′ of A_2 with those of the corresponding carbons in A and paromamine. The close agreement (Table I) between the chemical shifts of carbon atoms 2–5 of methyl α-D-xylopyranoside and the corresponding atoms 2″–5″ of A_2 confirms the configuration and ring size of the pentose unit as α-xylopyranoid. The resonance position of C-1″ of A_2 (δ 101.4 ppm) corresponds well with that of A (δ 100.8 ppm) and differs by only 1 ppm from that of methyl α-D-xylopyranoside (δ 100.4 ppm). The linkage position of the α-xylopyranosyl unit to deoxystreptamine was established as 6-O by inspection of the chemical shifts of C-6 in A_2 and A. These atoms resonate at δ 87.9 ppm. On acidification, the C-6 resonance of A_2 is shielded by 4.1 ppm and the C-5 resonance experiences virtually no shift. This phenomenon[5] is also observed in the case of A where the magnitude of the shielding of C-6 is 3.7 ppm (Table I).[10] Furthermore, as shown in the accompanying paper,[1a] the magnitudes of the deshielding of C-6 and of shielding of C-5 on conversion of paromamine to a 6-O-linked pseudotrisaccharide is diagnostic for establishing the position of linkage of the second monosaccharide unit. Thus, $\delta_{C\text{-}6}$ (A_2) − $\delta_{C\text{-}6}$ (Par) = 9.6

Table I
^{13}C Chemical Shifts of Gentamicin A and A_2, Paromamine, and Methyl α-D-Xylopyranoside[a]

	Gentamicin A		Gentamicin A_2		Paromamine		Methyl α-D-
Carbon atom	pD 8.5	2.0	pD 8.5	2.0	pD 8.5	2.0	xylopyranoside[b]
1	51.5	50.3	51.4	50.8	51.1	50.7	
2	36.5	28.5	36.2	29.5	36.7	29.3	
3	50.3	49.6	50.2	49.9	50.3	49.9	
4	88.6	80.9	88.1	80.9	88.8	81.4	
5	75.1	74.5	75.0	74.4	76.8	75.6	
6	87.9	84.2	87.9	83.8	78.3	74.5	
1′	101.7	97.7	101.5	97.3	102.0	97.9	
2′	56.2	54.8	56.1	55.1	56.1	55.1	
3′	74.7	69.8	74.5	69.8	74.6	70.1	
4′	70.9	70.3	70.8	70.2	70.8	70.4	
5′	73.8	74.5	73.8	73.6	73.8	73.3	
6′	61.6	61.5	61.6	61.3	61.6	61.3	
1″	100.8	101.4	101.4	101.8			100.4
2″	70.9	67.1	72.5	72.4			72.2
3″	62.8	61.3	74.0	74.3			74.1
4″	68.7	64.3	70.2	69.7			70.2
5″	63.0	63.3	62.5	63.4			61.8

[a] Chemical shifts are in parts per million downfield from Me_4Si for solutions in D_2O. [b] Values obtained in our laboratory are in excellent agreement with the published ones.[9]

ppm and $\delta_{C\text{-}5}$ (A_2) − $\delta_{C\text{-}5}$ (Par) = −1.5 ppm in the bases and at pD 2, these are 9.3 and −1.2 ppm, respectively. These values are in excellent agreement with the values reported for structurally related compounds.[1a] Therefore, it can be concluded that in A_2 the α-xylopyranosyl unit is located at the 6-position of paromamine. The absolute configuration of xylose in A_2 has been shown by Nagabhushan and Daniels[10] to be D by a novel application of ^{13}C NMR spectroscopy. The structure of gentamicin A_2 is therefore as shown below.



gentamicin A_2

Registry No.—Gentamicin A_2, 55715-66-7.

References and Notes

(1) (a) T. L. Nagabhushan, W. N. Turner, P. J. L. Daniels, and J. B. Morton, *J. Org. Chem.*, preceding paper in this issue. (b) $[\alpha]^{26}D$ +141° (*c* 0.4, water). (c) Satisfactory elemental analysis was obtained for this compound. $[\alpha]^{26}D$ +138° (*c* 0.4, water).
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(3) P. J. L. Daniels, M. Kugelman, A. K. Mallams, R. W. Tkach, H. F. Vernay, J. Weinstein, and A. Yehaskel, *Chem. Commun.*, 1629 (1971).
(4) J. B. Morton, R. C. Long, P. J. L. Daniels, R. W. Tkach, and J. H. Goldstein, *J. Am. Chem. Soc.*, **95**, 7464 (1973).
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(9) A. S. Perlin, B. Casu, and H. J. Koch, *Can. J. Chem.*, **48**, 2596 (1970).
(10) T. L. Nagabhushan and P. J. L. Daniels, *Tetrahedron Lett.*, **10**, 747 (1975).
(11) The presence of xylose can best be demonstrated by hydrolyzing tri-*N*-acetylgentamicin A_2 with 1 *N* hydrochloric acid at 100° for 1.5 hr.

Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances. XXXIII. The Ochrolifuanines and Emetine[1]

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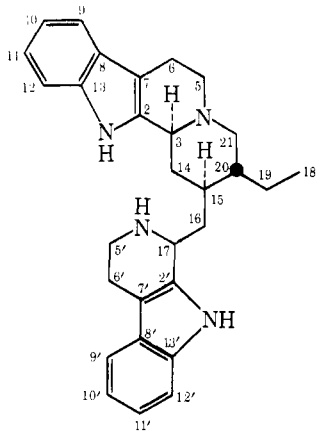
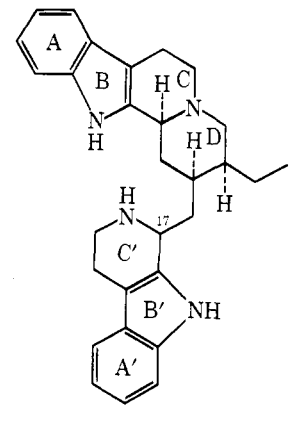
Recently two new alkaloids were isolated from *Ochrosia lifuana* Guillaumin and shown to possess structures **1a** and **1b**.[2,3] Their synthesis and that of two of their stereoisomers soon followed.[3] In view of the success in the use of ^{13}C NMR spectroscopy as an analytical tool for the differentiation of stereoisomers among yohimboid, ajmalicinoid, and corynanthoid alkaloids,[4,5] a ^{13}C NMR analysis of ochrolifuanine A (**1a**), ochrolifuanine B (**1b**), and the synthetic isomers **2a** and **2b** was undertaken. The ^{13}C NMR data[5] on models **3**, **4**, **5a**, and **5b** were used in this connection.



1a, 17β-H
b, 17α-H



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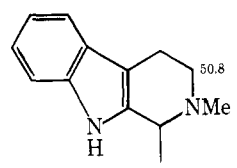
b, 17α-H



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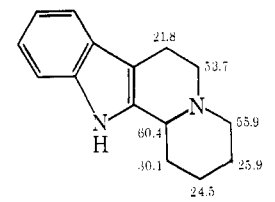

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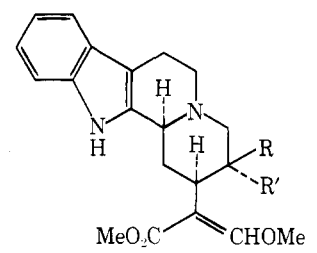

5a, R = H; R′ = Et
b, R = Et; R′ = H

The chemical shift information obtained from proton noise-decoupled and single-frequency off-resonance decoupled spectra of isomers **1a**, **1b**, **2a**, and **2b** as well as the data depicted on **3** and **4** and outlined in Table I for substances **5a** and **5b** permitted shift assignment for all carbons of the four substances under study except for two of their methylenes as well as two of their methines. Differentiation of the methylenes, C(14) and C(16), is based on the known C(14) shift of 37.2 ppm in yohimbane.[4] Ochrolifuanine A (**1a**) and ochrolifuanine B (**1b**) must have their C(14) more shielded owing to an added acyclic γ effect, while compounds **2a** and **2b** possess an even more shielded C(14) in view of the addition of another γ effect as a consequence of the axiality of their ethyl group. The distinction of the methines C(15) and C(20) of **1a** and **1b** is based on the idea of the shift similarity noted for the equivalent carbons in model **4** being retained in a case in which both carbons are equatorially ethylated and C(15) being shielded by the C(17) substituents. The same shift order can be expected for **2a** and **2b**. The total shift assignment portrayed in Table I was confirmed by a lanthanide shift study of **2b** with Yb(DPM)$_3$. The shift agent coordinates almost exclusively at the site of the secondary amine.

It is noteworthy that the C(5), C(6), C(5′), and C(6′) shifts are constant in the four substances **1a**, **1b**, **2a**, and **2b** and that there is a distinct difference between the C(5) and C(5′) shifts and the C(6) and C(6′) resonances. The constancy of the C(3) shift reflects the identity of the *trans*-quinolizidine conformation in the four compounds.[5] The dissimilarity of the C(17) shifts is a result of differences of rotamer populations of the equatorial C(15) side chain