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THE STRUCTURES OF NOVEL NUCLEOSIDE ANTIBIOTICS, MIHARAMYCIN A AND MIHARAMYCIN B

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Summary: Based on ^{13}C- and ^{1}H-NMR spectral analysis the structures of miharamycins A and B have been determined to be novel 9-substituted 2-aminopurine nucleoside antibiotics as shown in Fig. 3.

Miharamycins A and B are antibiotics produced by Streptomyces miharaensis SF-489 and active against rice blast disease[1]. Although these compounds were isolated some 15 years ago, their structures have remained unclear, because chemical manipulations to prepare suitable derivatives for structural elucidation were unsuccessful. We wish to report herein the structural studies of these novel nucleoside antibiotics, miharamycins A and B accomplished based on ^{1}H- and ^{13}C-NMR spectral analysis.

The physicochemical properties of miharamycin A (I) and B (II) are as follows; miharamycin A dihydrochloride, colorless crystals, $C_{20}H_{30}N_{10}O_9.2HCl$, SIMS: m/z 555 $(M+1)^+$, Anal. found, C;40.07, H;6.02, N;21.59, Cl;9.12%. calcd., C;38.28, H;5.14, N;22.32, Cl;11.30%, mp. 210-214° C (dec.), $[\alpha]_D^{24}$ -59° (c 1, H_2O), UV λmax (H_2O) 217.5nm(ε22900), 244nm(ε6580) and 307nm(ε6770). The IR spectrum of I (KBr) showed the presence of -OH , -NH and amide functions (νmax 3341, 1660, 1620, 1585, 1090 and 1050 cm^{-1}), miharamycin B hydrochloride, colorless crystals, $C_{20}H_{30}N_{10}O_8.HCl$, SIMS: m/z 539 $(M+1)^+$, Anal. found, C;39.89, H;5.97, N;22.08, Cl;4.98%. calcd., C;41.78, H;5.44, N;24.36, Cl;6.17%, mp. 215-218° C (dec.), $[\alpha]_D^{20}$ -63° (c 1, H_2O), UV λmax (H_2O) 218nm(ε21200) 244nm(ε6100) and 307nm(ε6320), IR νmax(KBr) 3341, 1659, 1621, 1585, 1089, 1063 cm^{-1}.

I contains one more oxygen atom than II. Both the compounds gave positive ninhydrin reaction. Because satisfactory elemental analytical results could not be obtained due to paucity of the samples and strong basic nature of I and II, their molecular formulae have been determined based on ^{13}C-NMR, ^{1}H-NMR and mass spectral data and considerations of partial structures (vide infra).

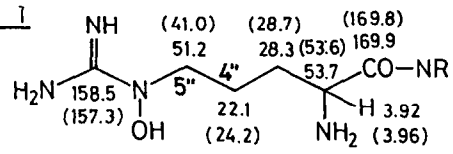
The 100 MHz ^{13}C-NMR spectra[2] of I and II taken in D_2O revealed the following 20 carbons; in I, 4 X CH_2 (δ_C 22.1, 28.3, 51.2 and 76.8), 7 X CH (53.7, 56.1,

66.6, 74.4, 76.9, 78.1 and 78.8), 1 X -C- (81.3), 2 X CH= (142.5 and 150.3), 4 X -C= (126.6, 152.3, 158.5 and 160.1), 1 X -CONH and 1 X -COOH (169.9 and 174.8); in II, 4 X CH_2 (δ_C 24.2, 28.7, 41.0 and 76.9), 7 X CH (53.6, 56.2, 66.7, 74.5, 76.7, 78.4 and 78.5), 1 X -C- (81.4), 2 X CH= (142.8 and 150.5), 4 X -C= (127.1, 153.1, 157.3 and 160.5), 1 X -CONH and 1 X -COOH (169.8 and 174.5).

The UV spectra of I and II are almost completely identical each other and indicate the presence of 9-substituted 2-aminopurine[3] as a chromophore. This partial structure is also supported by the ^{13}C-NMR spectral data of I (C-2 160.1, C-4 152.3, C-5 126.6, C-6 150.3 and C-8 142.5) which agree well with those of 2-aminopurine-9-β-D-riboside[4].

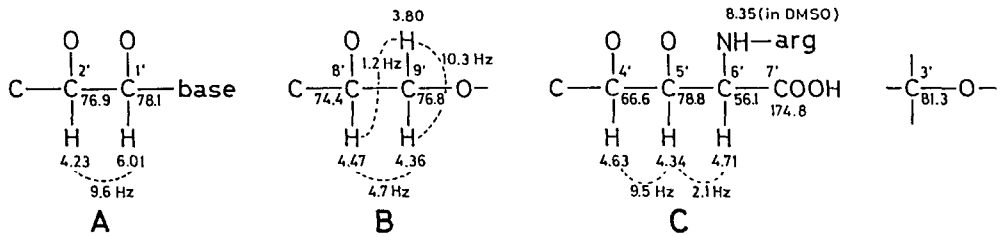
Whereas II was positive to Sakaguchi reaction, I was negative suggesting the presence of a monosubstituted guanidine in II and a disubstituted guanidine in I. In agreement with these results, amino acid analyses detected arginine and an unknown basic amino acid in the acid hydrolysates of II and I, respectively. The stereochemistry of the arginine was determined to be L based on its optical rotation ($[\alpha]_D^{20}$=+10.3° (c 0.5, in 1N HCl)). In the ^{13}C-NMR spectra of II and I, carbon resonances due to these amino acid moieties are assigned as shown in Fig. 1. Downfield shift of C-5" by 10.2 ppm and upfield shift of C-4" by 2.1 ppm in I are reasonably explained by a substituent on N_5" in I. Taking into account of the difference of the molecular formulae between I and II, this substituent must be a hydroxy group to give N_5-hydroxyarginine. The ^{13}C-NMR spectral data of this amino acid residue are in good agreement with literature values[5]. The amino acid structure in I is further supported by 400 MHz ^{1}H-NMR[2] spectral data as follows and spin decoupling experiments: δ_H, H-2" 3.92(1H, t, J=6.2 Hz), H-3" 1.80 (2H, m), H-4" 1.47 and 1.62 (2H, m) and H-5" 3.30 (2H, m).

Fig. 1



^{13}C-NMR spectral data of N_5-hydroxyarginine and arginine (in parentheses) moieties

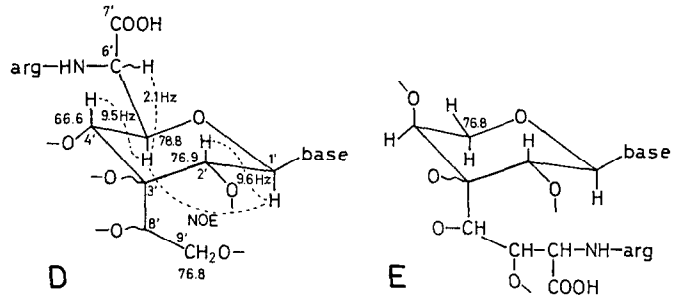
The remaining moiety $C_9H_{13}NO_7$ common to I and II is assigned to the sugar residue which contains a free carboxylic acid (δ_C 174.8, pKa' 3.0 in I). The ^{1}H-NMR and ^{13}C-NMR spectral analysis of I revealed the following partial structures.



The characteristic chemical shift of the proton H-1' at δ_H 6.01 and coupling constant ($J_{1',2'}$=9.6 Hz) showed C-1' (δ_C 78.1) to be an anomeric carbon and trans diaxial relationship for H-1' and H-2' in a pyranose ring system. The chemical

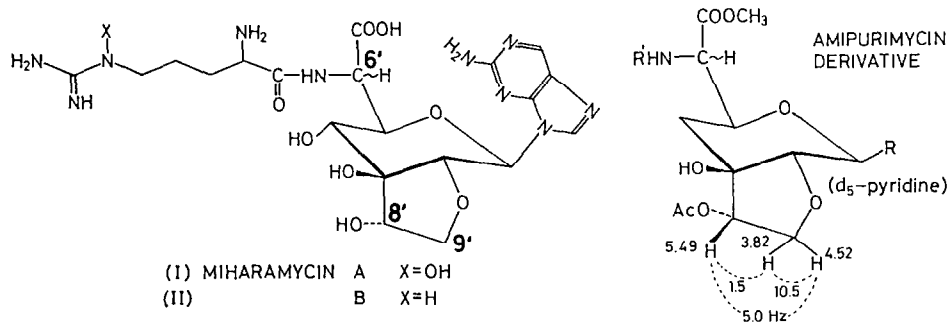
shift of C-6' in partial structure C (δ_C 56.1 and δ_H 4.71) can be compared with those of the acylated α-carbons in amino acids suggesting the substituent on C-6' to be a carboxylic acid. This relationship was confirmed by long range selective proton decoupling (LSPD)[6] irradiating H-5' or H-6' whereupon the triplet (δ_C 174.8, $^2J_{C-H}=^3J_{C-H}$=ca. 4.5 Hz) of the carboxylic acid collapsed to a broad singlet. Since the sugar moiety contains only one quaternary carbon (-C-O-, δ_C 81.3), A, B and C must be combined via this carbon to give pyranose D or E in Fig. 2.

Fig. 2



The structure E is readily excluded, since notwithstanding the expected upfield shift[7] by the bulky substituent at C-3' in E, the chemical shift of the oxymethylene carbon (δ_C 76.8) is too low as compared with the corresponding carbon in α-D-lyxopyranose[8] (δ_C 64.2) or α-D-arabinopyranose[9] (δ_C 66.40). On the other hand, the structure D is supported by the strong NOE observed between H-1' and H-5'. Comparison of the chemical shift of C-9' (δ_C 76.8) with those of the hydroxymethyl carbons in polyols (δ_C 64-67)[10] suggests C-9' to be part of an ether structure. Since the molecular formula requires an additional unsaturation and since alkylation of C-2' but not of C-4' is strongly supported by the downfield shift[11] of the C-2' signal (δ_C C-2' 76.9 vs. C-4' 66.6), C-2' and C-9' are connected through an oxygen only in the stereochemically possible manner as shown in Fig. 3. The magnitude of the coupling constant ($J_{4',5'}$=9.5 Hz) indicates trans diaxial relationship of H-4' and H-5'. Thus the structures of I and II have been determined as shown in Fig. 3. Miharamycins are closely related to a

Fig. 3



cyclic derivative of amipurimycin obtained by treatment of N-acetyl amipurimycin methyl ester with mesyl chloride by Goto et al[12]. The large downfield shift of C-9' of amipurimycin on alkylation (δ_C 61.9 → 73.7)[13] agrees well with the proposed structure of I. Similarity of the coupling constants and chemical shifts of protons in these five membered ether rings not only supports the structures of I and II but also determines the stereochemistry of C-8'. The absolute configuration of I including the stereochemistry at C-6' remains to be established.

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References and Footnotes

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13) The detailed NMR spectral information on the cyclic derivative of amipurimycin was kindly given by Professor T. Goto. The relevant data are as follows; δ_H in d_5-pyridine at 100°C, 6.55 (H-1', d, J=9.5 Hz), 5.49 (H-8', dd, J=5 and 1.5 Hz), 5.10 (H-6', m, J=8 and 4 Hz), 4.96 (H-5', m), 4.52 (H-9'a, dd, J=10.5 and 5 Hz), 4.51 (H-2', d, J=9.5 Hz) and 3.82 (H-9'b, dd, J=10.5 and 1.5 Hz), δ_C in d_5-pyridine, 79.4, 79.1, 78.8, 77.6, 76.2 (C-1', 2', 3', 5', 8') and 73.7 (C-9').

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