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THE ABSOLUTE CONFIGURATION OF P-1894B (VINEOMYCIN A_1), A POTENT PROLYL HYDROXYLASE INHIBITOR

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The absolute configuration of P-1894B (vineomycin A_1 (I)), a potent prolyl hydroxylase inhibitor, has been established as I from the absolute configuration of two sugar moieties (II, VI) obtained by chemical degradation of I.

KEYWORDS —— P-1894B; vineomycin A_1; Streptomyces albogriseolus subsp. No. 1894; enzyme inhibitor; prolyl hydroxylase inhibitor; absolute configuration; methyl α-L-rhodinoside; methyl 2,3,6-trideoxy-α-L-glycero-hexopyranosid-4-ulose; methyl α-L-amicetoside; aquayamycin

P-1894B (I) is a potent prolyl hydroxylase inhibitor produced by Streptomyces albogriseolus subsp. No. 1894. Details of the production, isolation, identification and biological properties of I have been reported.[1,2] Recently, Imamura et al.[3] reported that the antitumor antibiotic vineomycin A_1 is identical with I and the aglycone part of I is identical with aquayamycin. Although the relative configuration of I has previously been determined by X-ray crystallographic analysis,[4] the absolute configuration was not known.

In order to establish the absolute configuration of I, we isolated and identified the sugar and aglycone parts of I. This paper deals with the structural elucidation of I by chemical degradation (Chart 1) and spectral analysis.

On acid hydrolysis in 0.3N aq.H_2SO_4, I liberated compounds II and III. The compound II was determined to be L-rhodinose(2,3,6-trideoxy-L-threo-hexopyranose)[5] obtained from aclacinomycin N_1,[6] by comparing its $[\alpha]_D$ value, elemental analysis, mass spectrum (MS), infrared (IR) absorption spectrum and Rf values on co-thin-layer chromatography using several developing solvents.

Compound III: orange-red needles, mp 200-203°C (dec.), Anal. Calcd for $C_{25}H_{26}O_{10}$: C, 61.72; H, 5.39; O, 32.89. Found: C, 60.30; H, 5.66; O, 32.55, field-desorption mass (FD-MS) m/z: 486 (M^+), 468 (M^+-H_2O), 450 (M^+-2H_2O), $[\alpha]_D^{20}$+149° (dioxane). The IR, ultraviolet (UV), proton magnetic resonance (^{1}H-NMR) and carbon magnetic resonance (^{13}C-NMR) spectra of III were essentially identical with those of aquayamycin.[3,7]

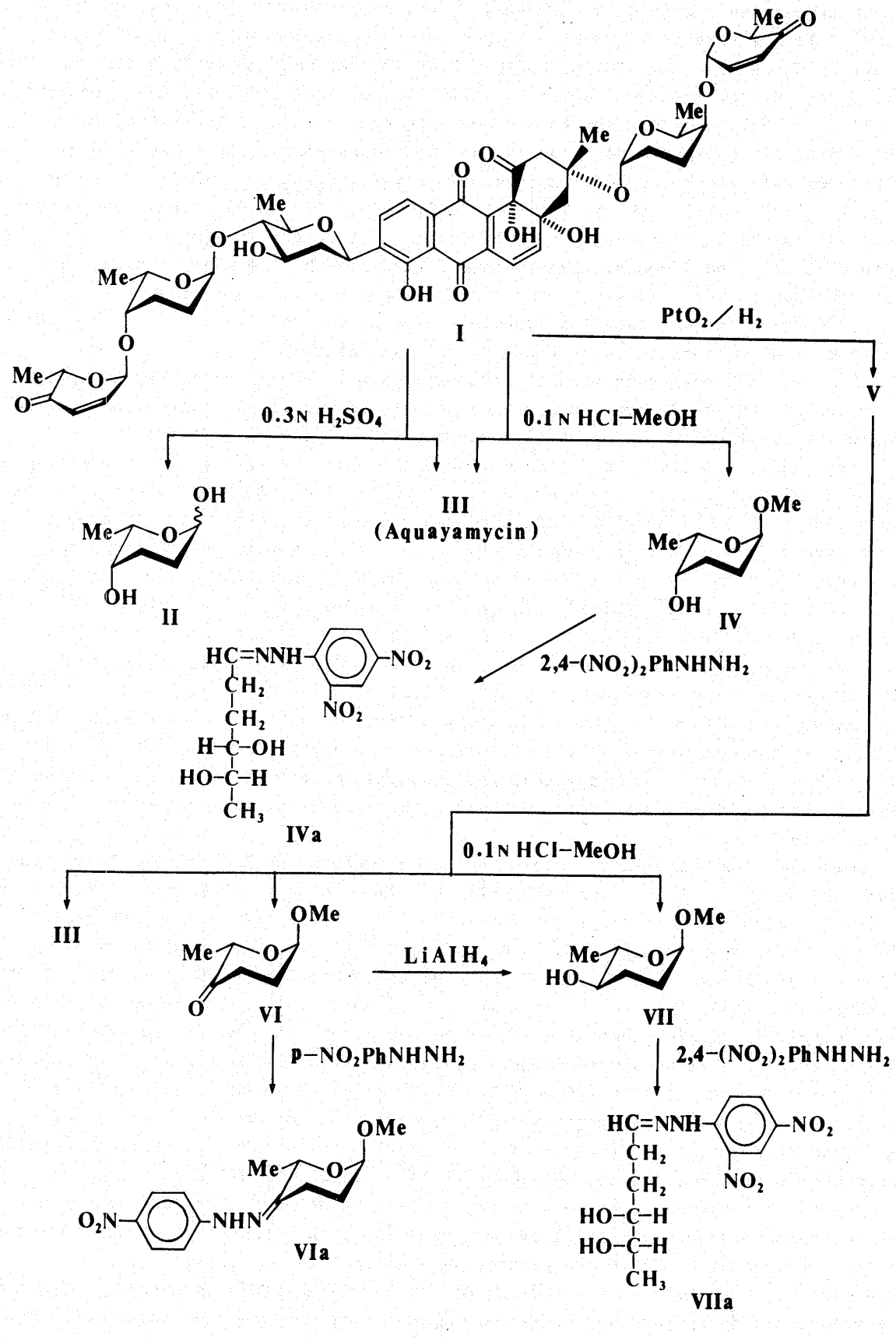

Chart 1

On methanolysis with 0.1N HCl-MeOH, I gave compound (IV) [$C_7H_{14}O_3$: MS m/z: 146 (M^+), 115 (M^+-OMe); $[\alpha]_D^{20}$ -85.23° (acetone); ^{1}H-NMR ($CDCl_3$) δ : 1.20 (3H, d, J=6 Hz, 6-CH_3), 1.50-2.10 (4H, m, 2,3-H), 3.38 (3H, s, OMe), 3.40-3.60 (1H, m, 4-H), 3.96 (1H, dq, J=2 and 6 Hz, 5-H), 4.70 (1H, dd, J=1 and 3 Hz, 1-H)] together with III. All the chemical shifts and coupling constants of IV were confirmed by spin decoupling experiments, and IV was identified as (4S,5S)-2,3,6-trideoxy-L-threo-hexose(2,4-dinitrophenyl)hydrazone[8] (IVa) [$C_{12}H_{16}N_4O_6$; mp 116°C; MS m/z: 312 (M^+); $[\alpha]_D^{20}$ -18.1° (pyr.); IR (KBr) 3400, 3300, 1620 cm^{-1}]. Thus, compound IV was determined to be methyl α-L-rhodinoside.[8] From the foregoing, the sugar, a component of I, was demonstrated to be α-L-rhodinose by the fact that the two sugar components II and IV were respectively recovered by two different kinds of acid hydrolysis, and the fact that physico-chemical data of 2,4-dinitrophenylhydrazone from IV were identical with those in the literature.[8]

Catalytic hydrogenation with 5% PtO_2 of I gave V as a mixture. Methanolysis of V with 0.1N HCl-MeOH gave a mixture of methylated sugars and aglycones. Chromatographic purification of the methanolysates furnished compounds III, VI, and VII.

Compound VI: colourless unstable liquid, MS m/z: 144 (M^+), 113 (M^+-OMe), $[\alpha]_D^{20}$ -149.9° ($CHCl_3$). The IR spectrum ν_{Max}^{KBr} cm^{-1}: 1725, 1130, 1060 and positive p-anisaldehyde-H_2SO_4 test of VI indicated the presence of ketosugar. Treatment of the ketone VI with p-nitrophenylhydrazine gave the crystalline p-nitrophenylhydrazone (VIa) [mp 158-159°C; high-resolution MS m/z: 279.1235 (Calcd for $C_{13}H_{17}N_3O_4$: 279.1219); $[\alpha]_D^{20}$ -198.7° ($CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ : 1.47 (3H, d, J=6 Hz, 6-CH_3), 1.72-2.20 (2H, m, 2-H), 2.47 (2H, m, 3-H), 3.46 (3H, s, OMe), 4.54 (1H, q, J=6 Hz, 5-H), 4.82 (1H, t, J=4.5 Hz, 1-H), 7.05 (2H, d, J=9 Hz, Aryl 2,6-H), 7.56 (1H, br s, NH, disappeared in D_2O), 8.14 (2H, d, J=9 Hz, Aryl 3,5-H); IR (KBr) 3330, 1600, 760, 700 cm^{-1}]. Compound VIa was identical with synthetic methyl 2,3,6-trideoxy-α-D-glycero-hexopyranosid-4-ulose(p-nitrophenyl)hydrazone,[9] $[\alpha]_D^{20}$ +347° ($CHCl_3$) in mp, IR and ^{1}H-NMR spectra, but opposite in optical rotation. Consequently, the structure of VI was elucidated as methyl (5S)-2,3,6-trideoxy-α-L-glycero-hexopyranosid-4-ulose.

Compound VII: colourless liquid, Anal. Calcd for $C_7H_{14}O_3$: C, 57.53; H, 9.59. Found: C, 57.40; H, 9.75, high-resolution MS m/z: 146.0956 (Calcd for $C_7H_{14}O_3$: 146.0943), $[\alpha]_D^{23}$ -115° ($CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ : 1.27 (3H, d, J=6 Hz, 6-CH_3), 1.54 (1H, br s, 4-OH, disappeared in D_2O), 1.6-2.0 (4H, m, 2,3-H), 3.2-3.4 (1H, m, 4-H), 3.36 (3H, s, OMe), 3.58 (1H, dq, J=6 and 9 Hz, 5-H), 4.62 (1H, br s, 1-H); IR (KBr) 3400, 1130, 1050 cm^{-1}. Treatment of VII with 2,4-dinitrophenylhydrazine gave crystalline 2,3,6-trideoxy-L-erythro-hexose(2,4-dinitrophenyl)hydrazone (VIIa) [mp 159-160°C; high-resolution MS m/z: 312.1063 (Calcd for $C_{12}H_{16}N_4O_6$: 312.1070); $[\alpha]_D^{23}$ +7.85° (pyr.); IR (KBr) 3400, 3300, 1605 cm^{-1}], which was identified with L-amicetose 2,4-dinitrophenylhydrazone[10] by comparison of mp, $[\alpha]_D$ value, MS, ^{1}H-NMR and IR spectra. By the reduction of VI with $LiAlH_4$, VII was also obtained stereospecifically as reported by Albano et al.[9] In the ^{1}H-NMR spectrum of VII, the coupling constants of the methine proton 4-H through 5-H (J=9 Hz) indicated that these protons were all axial and the small coupling constant (J=2 Hz) between 1-H and 2-H indicated that these protons were equatorial-equatorial and equatorial-axial. Therefore, the structure of VII was established as methyl α-L-amicetoside(methyl 2,3,6-trideoxy-α-L-erythro-hexopyranoside)[10] and thus it was evidenced

that VII was derived from the terminal sugar moiety (aculose)[11] of I by the catalytic hydrogenation.

Taking into account the reaction mechanism together with the physico-chemical data of compounds VI, VII, and VIIa, the sugar, a component of I, should be α-L-aculose(2,3,6-trideoxy-α-L-glycero-hex-2-enopyranos-4-ulose).[11]

As a result, the absolute configuration of P-1894B was established as I from the absolute configuration of the two chemically identified sugars (II, VI).

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