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Identification of the Aglycon Part of Vineomycin A_1 with Aquayamycin

Vineomycin A_1 (formerly OS-4742 A_1) produced by *Streptomyces matensis* subsp. *vineus*, is an antibacterial and antitumor antibiotic. The aglycon part obtained by mild hydrolysis turned out to be identical with aquayamycin, and its ^{13}C-NMR assignment was also determined.

Keywords——vineomycin A_1; OS-4742 A_1; *Streptomyces matensis* subsp. *vineus*; antibiotic; aquayamycin; P-1894B; ^{13}C-NMR

Vineomycin A_1 (formerly OS-4742 A_1, **1**) is a component of new antibiotics, vineomycins (A_1, A_2, B_1 and B_2), which are produced by *Streptomyces matensis* subsp. *vineus*.[1] Ōmura *et al.* have reported that it is active against Gram-positive bacteria and Sarcoma 180 solid tumor on mice and has a quinone-type chromophore and sugar moieties.[1] During the investigation of the structure and biosynthesis of the antibiotic, we realized that a chromophoric aglycon obtained by mild acid hydrolysis turned out to be identical with aquayamycin.[2] Described in the present paper is the identification together with the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) assignment of the aglycon of **1**.

In order to obtain a chromophoric aglycon, **1** was treated under mild conditions (0.6 N HCl, room temperature) providing a reddish-orange product. After purification by preparative thin-layer chromatography (TLC) ($CHCl_3$–MeOH, 5: 1), recrystallization from a benzene–hexane mixture gave red plates (**2**): $C_{25}H_{26}O_{10}$, *Anal.* Found C; 61.88, H; 5.58%. Calcd C; 61.72, H; 5.39%, mp 175—180° (dec.), MS (FD); *m/z* 486 (M^+) 478 ($M^+ - H_2O$) 450 ($M^+ - 2H_2O$), $[\alpha]_D^{20}$ +130° (c=1, dioxane).

The infrared absorption (ν_{max}^{KBr} cm^{-1}; 1635, 1615) and the ultraviolet spectrum [λ_{max}^{EtOH} nm (log ε); 219 (4.30), 318 (3.61), 419 (3.65)] of **2** indicated the presence of a quinone group, which was supported by signals at 184.3 ppm (C-12) and 190.4 ppm (C-7) in the ^{13}C-NMR spectrum, the latter carbonyl group is hydrogen-bonded to the phenolic hydroxyl group.[3] A non-protonated carbon signal at 116.0 ppm (C-7a) was characteristic of the C-2 carbon of an enol form of 1,3-diketone system[4] and a phenolic carbon signal at 159.5 ppm (C-8) was also observed. In addition, an aromatic AB spin system [δ_H 7.44 ppm (H-11) and 7.73 ppm (H-10), J=8 Hz] was observed in the ^{1}H-NMR spectrum of **2**. These data suggest a 2,3,6- or 2,3,8-trisubstituted

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5-hydroxyl-1,4-naphthoquinone skeleton.[3,5]

The carbonyl carbon signal at 208.0 ppm (C-1) which appeared as a triplet (J_{C-H}=6 Hz) in the undecoupled spectrum and its infrared absorption at 1730 cm^{-1} imply a non-conjugated carbonyl group in a six-membered ring.[6] In the ^{1}H-NMR spectrum of **2**, two pairs of AB type methylene doublets (δ_H 2.00 ppm and 2.04 ppm, J=15 Hz; 2.65 ppm and 2.82 ppm, J=13 Hz) were observed, and the latter pair was attributable to α-position (H-2α and H-2β, respectively) to the carbonyl group by their chemical shifts,[7] while the former was attributed to γ-position (H-4β and H-4α, respectively) because a W-type long range coupling was observed between H-2α and H-4α. These data imply the presence of 2,3,5-trisubstituted cyclohexanone skeleton.

TABLE I. The ^{1}H-NMR Data for the Pyran Ring of **2**

Proton[a]	δ_H, ppm (multiplicity)	Coupling constant, Hz (coupled proton)
H-2′	4.77 (d)	11(H-3′α)
H-3′α	1.34 (m)	
H-3′β	2.36(ddd)	1.5(H-2′), 13(H-3′α), 5.5(H-4′)
H-4′	3.67 (m)	11(H-3′α), 5(H-3′β), 9(H-5′)
H-5′	3.01 (t)	9(H-4′), 9(H-6′)
H-6′	3.40 (dq)	9(H-5′), 6(H-7′)
H-7′	1.35 (d)	6(H-6′)

a) The proton numbering follows the aquayamycin numbering as shown in Fig. 1.

The ^{1}H-NMR spectrum of **2** indicates the presence of 2-substituted 4,5-dihydroxy-6-methyltetrahydropyran ring, as shown in Table I. All the spin-couplings were confirmed by decoupling experiments at 400 MHz. These results together with the additional data, *i.e.*, one singlet methyl group [1.23 ppm (H-13)] and one pair of AB type olefinic proton doublets [6.36 ppm (H-5) and 6.79 ppm (H-6), J=10 Hz] in the ^{1}H-NMR and three non-protonated oxygen-bearing sp^3 carbon signals (78.4 ppm, 79.4 ppm and 82.8 ppm) in ^{13}C-NMR, suggest probable identity of **2** with aquayamycin. This was in fact confirmed by 400 MHz ^{1}H-NMR spectrum of **2**, which was completely superimposed with that of aquayamycin. Futhermore, **2** showed identical mobility with aquayamycin on co-TLC using several developing solvents.

TABLE II. ^{13}C-NMR Assignment of **2**.

Carbon No.[a]	δ_C, ppm[b] (multiplicity)[c]	Carbon No.[a]	δ_C, ppm[b] (multiplicity)[c]
1	208.0 (s)	4a	79.4 (s)
2	54.1 (t)	6a	141.1 (s)
3	78.4 (s)	7a	116.0 (s)
4	45.5 (t)	11a	132.8 (s)
5	147.0 (d)	12a	140.6 (s)
6	119.0 (d)	12b	82.8 (s)
7	190.4 (s)	2′	73.2 (d)
8	159.5 (s)	3′	41.7 (t)
9	140.0 (s)	4′	74.4 (d)
10	135.0 (d)	5′	79.6 (d)
11	120.9 (d)	6′	78.4 (d)
12	184.3 (s)	7′	19.4 (q)
13	31.0 (q)		

a) The carbon numbering follows the aquayamycin numbering as shown in Fig. 1.
b) Chemical shifts are given downfield from internal Me_4Si for CD_3OD solution.
c) Multiplicities in the off-resonance decoupling spectrum.

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An assignment of the ^{13}C-NMR spectrum (Table II) was made from the characteristic chemical shifts and their multiplicities, as well as using selective proton decoupling and long range selective proton decoupling experiments.[4b]

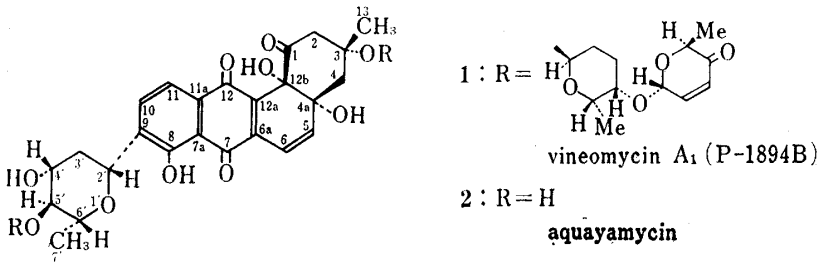

Fig. 1. Structures of Vineomycin A_1 (P-1894B) (**1**) and Aquayamycin (**2**)

The X-ray structure of P-1894B, a collagen proline hydroxylase inhibitor produced by *Streptomyces albogriseolus* subsp. No. 1894, was recently reported[8] and the Takeda group suggested identity of P-1894B with **1**. Our data (^{1}H- and ^{13}C-NMR, IR and UV) of **1** so far obtained are completely compatible with the reported structure of P-1894B. As a result, the relative stereochemistry of aquayamycin was determined as shown in Fig. 1.

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