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STRUCTURAL INVESTIGATION OF THE ANTIBIOTIC SPORAVIRIDIN XIII[1)]
THE TOTAL STRUCTURES OF N-ACTYLSPORAVIRIDINS

Ikumi Kimura, Yasushi Ota, Reiko Kimura, Tetsuya Ito, Yoko Yamada, Yukio Kimura, Yumiko Sato, Hideyuki Watanabe, Yuji Mori, Ken-ichi Harada and Makoto Suzuki*

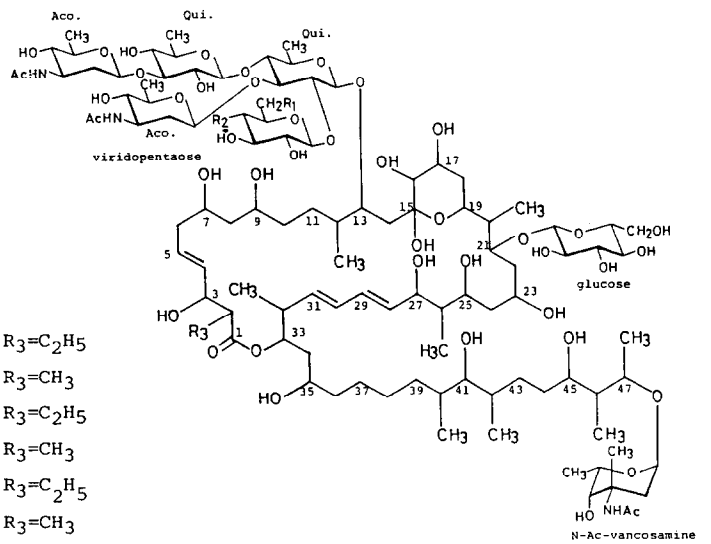
Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan

Takashi Iwashita

Suntory Institute for Bioorganic Research, Shimamoto-cho Mishima-gun, Osaka 618,Japan

Summary: Based on a variety of degradative reactions and spectroscopic analyses of the four pseudoaglycones, which were obtained by treatment of N-acetylsporaviridins (N-Ac-SVD) with DBU, the total structures of N-Ac-SVD were deduced as shown in Fig.1.

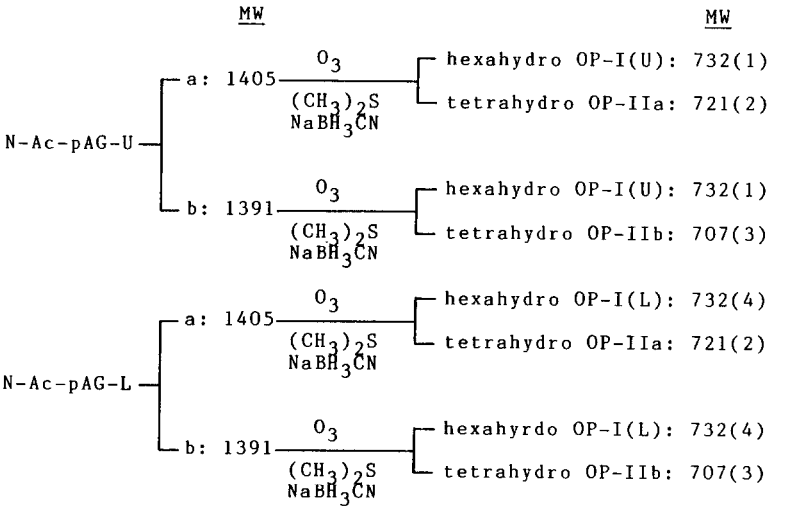
In the preceding paper, we reported the specific glycosidic bond cleavage of N-Ac-SVD and the physico-chemical properties of the four pseudoaglycones, N-Ac-pAG-Ua, -Ub, -La and -Lb[1)]. This paper describes the structures of their ozonolysis products and the total structures of N-Ac-SVD (Fig. 1).



N-Ac-SVD-A_1: R_1=H, R_2=OH, R_3=C_2H_5
N-Ac-SVD-A_2: R_1=H, R_2=OH, R_3=CH_3
N-Ac-SVD-B_1: R_1=H, R_2=NHAc, R_3=C_2H_5
N-Ac-SVD-B_2: R_1=H, R_2=NHAc, R_3=CH_3
N-Ac-SVD-C_1: R_1=OH, R_2=OH, R_3=C_2H_5
N-Ac-SVD-C_2: R_1=OH, R_2=OH, R_3=CH_3

Fig. 1

Ozonolysis of N-Ac-pAG-Ua was carried out in methanol at -78℃ followed by decomposition of the ozonide with $(CH_3)_2S$. Further reduction of the ozonolysis products with $NaBH_3CN$ afforded hexahydro OP-I(U) 1 and tetrahydro OP-IIa 2 (Scheme 1). Compound 1 gave two molecular ion species, $(M+Na)^+$ ion at m/z 755 and $(M+H)^+$ ion at m/z 733 by secondary ion mass spectrometry (SIMS), demonstrating that the molecular weight of 1 is 732. The 25 MHz ^{13}C-NMR spectrum of 1



Scheme 1

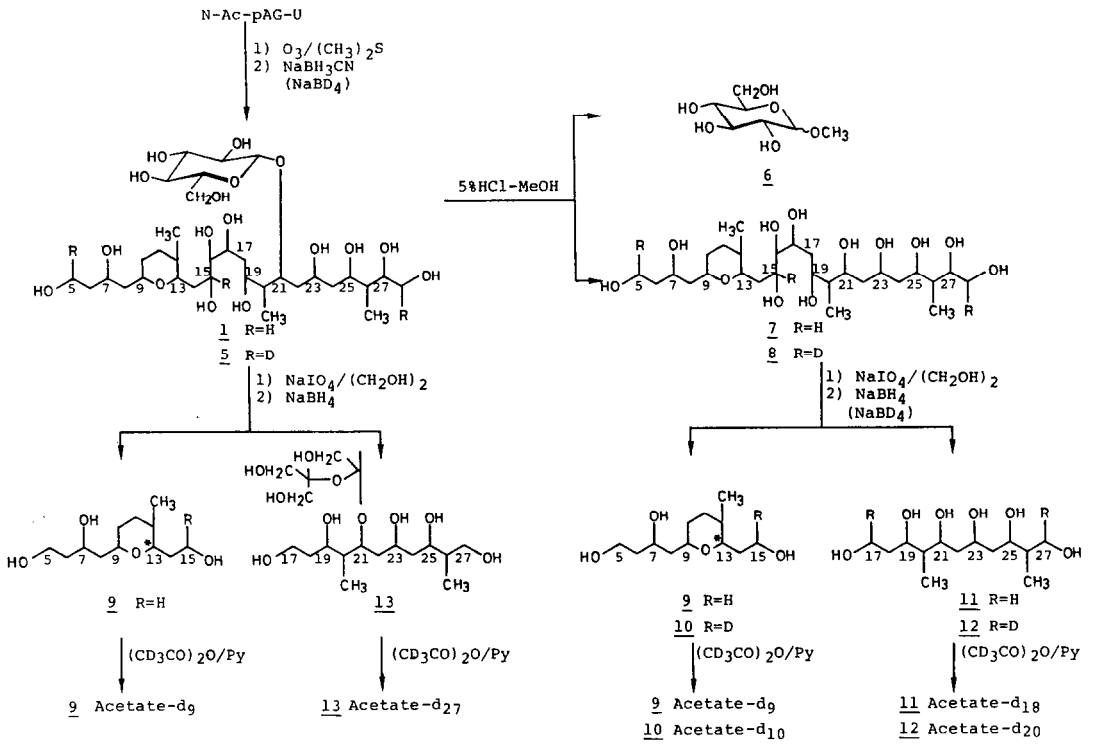
in CD_3OD indicated the presence of 33 carbon atoms and among them the signal with lowest chemical shift (104.5ppm) was assignable to the anomeric carbon of β-D-glucose. Therefore, the molecular formula of 1 was found to be $C_{33}H_{64}O_{17}$ including glucose unit from the ^{13}C-NMR and SIMS spectral analyses.

Compound 2 showed the $(M+Na)^+$ and $(M+H)^+$ ions at m/z 744 and m/z 722, respectively in its SIMS spectrum. The ^{13}C-NMR spectrum of 2 showed 37 signals , in which the anomeric carbon of N-acetyl-α-L-vancosamine appeared at 98.5ppm. The ^{13}C-NMR and IR spectra revealed the presence of an ester bond (175.9ppm and 1705cm^{-1}, respectively). From these results the molecular formula of 2 was established to be $C_{37}H_{71}NO_{12}$ (MW 721).

On the other hand, ozonolysis of N-Ac-pAG-Ub gave rise to degradation products 1 and 3 using the same manner as N-Ac-pAG-Ua. The SIMS spectrum of 3 gave the $(M+Na)^+$ and $(M+H)^+$ ions at m/z 730 and 708, respectively, which are smaller by 14 mass units than those of 2. Comparison of the ^{13}C-NMR data of 3 with those of 2 revealed that 3 has one less methylene units than 3.

Ozonolysis of N-Ac-pAG-La and -Lb afforded 4 and 2, and 4 and 3, respectively, as shown in Scheme 1. The SIMS spectrum of 4 showed the $(M+H)^+$ ion at m/z 733, indicating that 4 has the same molecular weight as 1. However 4 was found to be different from 1 by comparison of their ^{13}C-NMR spectra and TLC behavior. These results suggest that structural differences between N-Ac-pAG-U and -L are found in the OP-I moiety. The structure of 1 was determined based on their spectral data of further degradation products as shown in Scheme 2.

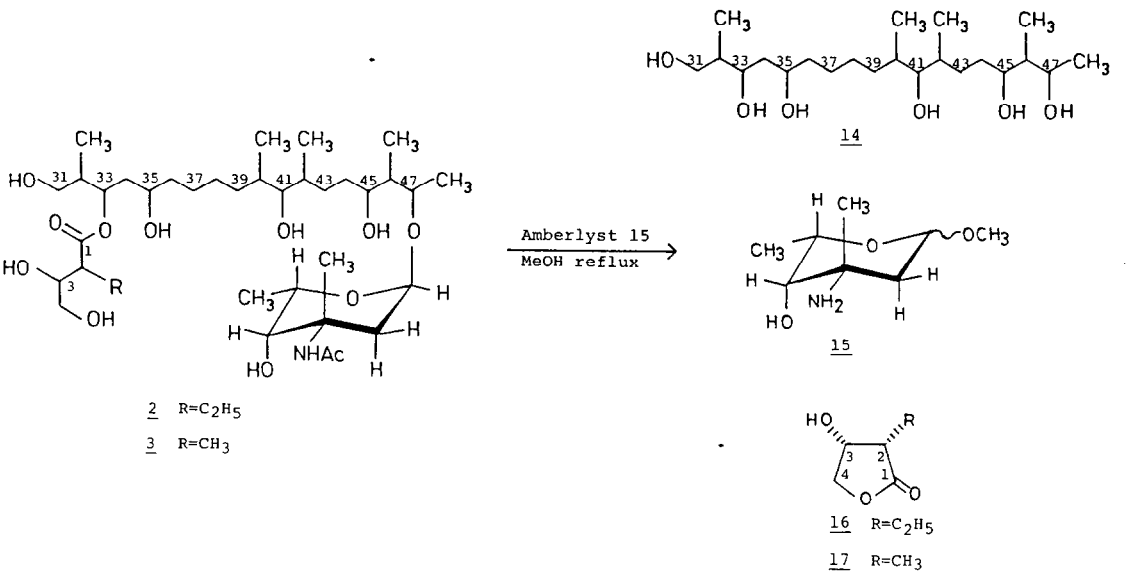
Treatment of 1 with 5% HCl-MeOH followed by purification by Sephadex LH-20 column chromatography gave 6 and 7. Compound 6 was identified as methyl D-glucoside by spectroscopic analyses. The molecular formula of 7, $C_{27}H_{54}O_{12}$, was established by its SIMS ($(M+H)^+$ at m/z 591) and ^{13}C-NMR spectra. Subsequently compound 7 was oxidized with $NaIO_4$ in MeOH-H_2O (1:1) at room temperature followed by $NaBH_4$ reduction to afford 9(U) and 11 with loss of $C_2H_5O_2$ unit. The molecular formulae of 9(U) and 11 were established as $C_{12}H_{24}O_4$ and $C_{13}H_{28}O_6$, respectively by their ^{13}C-NMR and chemical ionization (CI) mass spectra. Structures of 9(U) and 11 were determined by 2D-NMR spectroscopy of their perdeuteroacetylated compounds. In order to clarify the position of 1,2- glycol system, reduction of the periodate oxidation products of 7 with $NaBD_4$



Scheme 2

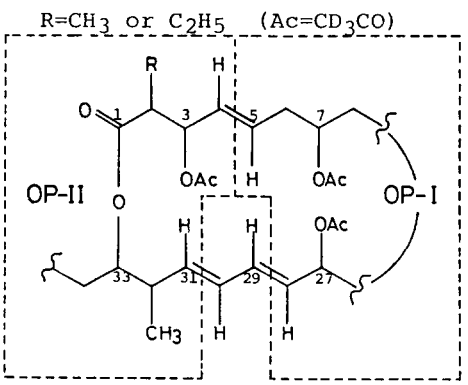
instead of $NaBH_4$ was carried out to give 10(U) and 12. The ^{1}H-NMR spectra of 10(U) and 12 showed incorporations of one deuterium atom at C-15 in 10(U), and two deuterium atoms at C-17 and -27 in 12, suggesting that the 1,2-glycol units were presented at C-15 and -16, C-16 and C-17, and C-27 and -28. Among them the presence of the consecutive triol system at C-15, -16 and -17 was also supported by 2D-NMR spectrum of the perdeuteroacetylated compound of 7. On the other hand, the $NaIO_4$ oxidation of 1 followed by $NaBH_4$ reduction gave 9(U) and 13. Compound 13 showed the $(M+H)^+$ ion at m/z 414 in its SIMS spectrum. The location of D-glucose was determined as C-21 in 1 by 2D-NMR spectra of perdeuteroacetylated compounds of 13. And the position of the hemiketal was defined as C-15 from the fact that a deuterium atom was incorporated at this position in 5 . In the same manner of 1 , hexahydro OP-I(L)4 gave 11 and 13 as common products. However, 9(L) was found to be different from 9(U) by comparison of the ^{1}H- and ^{13}C-NMR spectra and so 9(U) and 9(L) found to be an epimeric pair with respect to C-13.

On the other hand, methanolysis of 2 with Amberlyst 15 in methanol gave 14, 15 and 16. Analogously, 3 gave 14, 15 and 17 (Scheme 3). Compound 14 showed the $(M+H)^+$ ion at m/z 407 in CI (i-C_4H_{10}) mass spectrum. The molecular formula of 14 was assigned as $C_{22}H_{46}O_6$ by CIMS and ^{13}C-NMR spectroscopy. The structure of 14 was determined by its high resolusion (HR) MS and 2D-NMR spectra of its perdeuteroacetylated compound. Compound 15 was identified as methyl L-vancosaminide. Compounds 16 and 17 showed the $(M+H)^+$ ions at m/z 131 and 117, respectively by CI (i-C_4H_{10}) mass spectra. This result suggests that the structural difference between 2 and 3 is present in 16 and 17. IR spectra of 16 and 17 indicated the presence of γ-lactone (1770cm $^{-1}$). Structures of 16 and 17 were confirmed as 2-ethyl-3-hydroxybutyrolactone and 2-methyl-3-hydroxybutyrolactone, respectively, by their ^{1}H- and ^{13}C-NMR spectra. The absolute configurations of both compounds were determined to be 2S,3R by comparing their optical rotations with



Scheme 3

those of synthetic compounds. Spin decoupling experiments of perdeuteroacetylated compounds of 2 and 3 showed that N-Ac-vancosamine is attached to C-47 of the aglycone with α configulation. The location of the ester bond was deduced to be at C-33 on the basis of the following argument. Ozonolysis of the pseudoaglycone followed by treatment with $(CH_3)_2S$ and purification by silica gel chromatography gave the α, β-unsaturated aldehyde. ($C_{31}H_{57}NO_8$, UV: λ_{max}^{EtOH} 230nm)



Scheme 4

The structures of ozonolysis products of N-Ac-pAG were thus elucidated to be 1 and 4 as OP-I and 2 and 3 as OP-II, respectivery. The connection of each ozonolysis product for the total structure was mainly performed by 2D-NMR spectra of the perdeuteroacetyl pseudoaglycones (Scheme 4).

In this way, the total structures of N-Ac-SVD were deduced as shown in Fig. 1, which are characterized by a 34-membered polyhydroxylactone with an intramolecular hemiketal (C-15, -19), a viridopentaose (C-13), a D-glucose (C-21) and an N-Ac-vancosamine (C-47). To the best of our knowledge, these are the largest among the macrolide antibiotics reported so far from the viewpoint of molecular weight[2-4].

References.

1). I. Kimura, K. Yamamoto, K.-I. Harada and M. Suzuki, Tetrahedron Lett. preceding paper in this issue.

2). K. Fukushima, T. Arai, S. Iwasaki, M. Namikoshi and S.Okuda, J. Antibiotics, 35, 1480(1982).

3). K. Takesako and T. Beppu, J. Antibiotics, 37, 1170(1984).

4). S. Omura, Macrolide Antibiotics-Chemistry, Biology and Practice, ed. S. Omura, 509(1984) Academic Press.

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