SF2446, NEW BENZO[*a*]NAPHTHACENE QUINONE ANTIBIOTICS

II. THE STRUCTURAL ELUCIDATION

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Structures of new antibiotics SF2446A1, A2, A3, B1, B2 and B3 have been deduced by means of spectral analyses and chemical studies. The structure of SF2446A1 which is a main product of fermentation and has the strongest antimicrobial activity, has been proposed to be 11-(2,4-di-*O*-methyl-β-L-rhamnopyranosyl)amino-5,6,6a,14a-tetrahydro-1,6,8,14a-tetrahydroxy-6a-methoxy-2-methoxycarbonyl-3-methyl-benzo[*a*]naphthacene-7,9,12,14-tetraone. All of antibiotics have a novel benzo[*a*]naphthacene quinone skeleton and SF2446A1, A2, B1 and B2 have an *N*-glycosidic linkage with 2,4-di-*O*-methyl-L-rhamnose.

In the preceding paper[1], we have reported on the new antibiotics SF2446A1, A2, A3, B1, B2 and B3 (**A1, A2, A3, B1, B2** and **B3**) active against mycoplasmas and Gram-positive bacteria. The structural determination of antibiotics SF2446 was executed by spectroscopic analyses and chemical studies. All structures of antibiotics SF2446 contain a benzo[*a*]naphthacene quinone skeleton and **A1, A2, B1** and **B2** have an additional *N*-glycosyl sugar. Natural quinones with this ring system were reported previously, for example, G-2N, G-2A[2] and KS-619-1[3,4], but quinone antibiotics having an *N*-glycosyl sugar from microorganisms have not been reported. In this paper, we report the structural elucidation of antibiotics SF2446.

Structures of A1, A2 and A3

Antibiotics SF2446 were shown to contain an aromatic conjugated system and quinone and/or semiquinone carbonyls by their UV and IR spectra[1]. The molecular formulae of **A1, A2** and **A3** were determined to be $C_{34}H_{35}NO_{15}$, $C_{34}H_{35}NO_{15}$ and $C_{26}H_{21}NO_{11}$, respectively, from the elemental analyses, field desorption mass spectrometry (FD-MS)[1], ^1H and ^{13}C NMR spectra (Tables 1 and 2). The isomerization reactions of **A1** to **A2** and **A2** to **A1** occurred under mild acidic condition. Methanolyses of **A1** and **A2** in 1 N HCl - MeOH under reflux gave the same aglycone and methylglycoside. Also the difference of chemical shifts of their sugar moieties was clearly observed in their ^{13}C NMR spectra. Accordingly, it was deduced that **A1** and **A2** were stereoisomers having the same *N*-glycoside. The spectral data of the aglycone were in good agreement with those of **A3**[1] (Tables 1 and 2). In the methanolyses of **A1** and **A2**, an additional aglycone ($C_{27}H_{22}O_{12}$, FD-MS *m/z* 538 (M^+)) was obtained. The structure of the methylglycoside was determined to be methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside by ^1H and ^{13}C NMR spectra and an observation of nuclear Overhauser effect (NOE) between 3-H and 5-H of the sugar. By comparison of the optical rotation of the glycoside ($[\alpha]_D^{20}$ −51.3° (*c* 1, $CHCl_3$)) with that of synthetic methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside ($[\alpha]_D^{22}$ −56° (*c* 1.5, $CHCl_3$))[5], the absolute configuration of the sugar was deduced the L-configuration. Furthermore, NOE's were observed among 1′-H, 3′-H and 5′-H of **A1** and also between 3′-H and 5′-H of **A2** in their ^1H NMR spectra. Therefore, the modes of sugar linkage of **A1** and **A2** are regarded as β and α, respectively.

Table 1. ^{1}H NMR chemical shifts of SF2446.

Proton	A1 (ppm, m)	A2 (ppm, m)	A3 (ppm, m)	B1 (ppm, m)	B2 (ppm, m)	B3 (ppm, m)
1-OH	12.08 s	11.85 s	12.10 s	12.04 s	12.06 s	12.06 s
4-H	6.54 br s	6.49 br s	6.54 br s	6.49 br s	6.50 br s	6.51 br s
5-H	3.33 br d (J=19.4), 3.55 dd (J=19.4, 7.0)	3.35 br d (J=19.1), 3.54 dd (J=19.1, 6.3)	3.34 br d (J=19.5), 3.57 dd (J=19.5, 6.6)	3.08 ddd (J=19.2, 8.7, 2.1), 3.14 ddd (J=19.2, 9.7, 7.4)	3.08 ddd (J=18.8, 8.6, 2.0), 3.12 ddd (J=18.8, 9.4, 7.4)	3.08 ddd (J=19.3, 8.8, 2.0), 3.15 ddd (J=19.3, 9.8, 7.4)
6-H	4.96 br dd (J=10.2, 7.0)	4.97 dd (J=10.6, 6.3)	4.97 dd (J=9.8, 6.6)	2.24 ddd (J=12.6, 9.7, 8.7), 2.76 ddd (J=12.6, 7.4, 2.1)	2.26 ddd (J=12.3, 9.4, 8.6), 2.77 ddd (J=12.3, 7.4, 2.0)	2.24 ddd (J=12.9, 9.8, 8.8), 2.76 ddd (J=12.9, 7.4, 2.0)
6-OH	4.66 d (J=10.2)	4.92 d (J=10.6)	4.68 br d (J=9.8)			
6a-OCH_3	3.41 s	3.42 s	3.40 s	3.23 s	3.25 s	3.23 s
8-OH	14.18 s	14.26 s	14.32 s	14.15 s	14.19 s	14.25 s
10-H	5.87 s	6.19 s	5.95 s	5.86 s	6.19 s	5.94 s
11-NH	6.86 d (J=9.0)	6.84 d (J=5.7)		6.84 d (J=8.8)	6.40 d (J=5.9)	
11-NH_2			5.58 br			5.43 br
13-H	8.21 s	8.47 s	8.20 s	8.21 s	8.34 s	8.22 s
14a-OH	5.22 br s	5.96 br s	5.24 br s	4.71 s	4.86 s	4.70 s
15-OCH_3	3.85 s	3.48 s	3.85 s	3.82 s	3.80 s	3.84 s
16-H	2.40 br s	2.32 br s	2.40 br s	2.33 br s	2.37 br s	2.38 br s
1′-H	4.68 dd (J=9.0, 1.3)	5.25 dd (J=5.7, 1.8)		4.69 dd (J=8.8, 1.3)	5.19 dd (J=5.9, 2.0)	
2′-H	3.69 dd (J=2.8, 1.3)	3.87 dd (J=3.3, 1.8)		3.70 dd (J=3.1, 1.3)	3.68 dd (J=3.5, 2.0)	
2′-OCH_3	3.80 s	3.60 s		3.80 s	3.56 s	
3′-H	3.72 ddd (J=9.2, 4.4, 2.8)	4.39 br ddd (J=8.6, 7.4, 3.3)		3.75 br ddd (J=9.2, 4.0, 3.1)	4.02 ddd (J=8.6, 7.4, 3.5)	
3′-OH	2.51 d (J=4.4)	3.03 br d (J=7.4)		2.63 br d (J=4.0)	2.65 d (J=7.4)	
4′-H	3.13 dd (J=9.5, 9.2)	3.14 dd (J=9.0, 8.6)		3.14 dd (J=9.2, 9.2)	3.10 dd (J=8.6, 8.6)	
4′-OCH_3	3.61 s	3.57 s		3.61 s	3.55 s	
5′-H	3.34 dq (J=6.2, 9.5)	3.60 dq (J=9.0, 6.3)		3.34 dq (J=9.2, 6.2)	3.50 dq (J=8.6, 6.3)	
6′-H	1.36 d (J=6.2)	1.22 d (J=6.3)		1.35 d (J=6.2)	1.27 d (J=6.3)	

δ: ppm from TMS (0 ppm) in $CDCl_3$, m: multiplicity.
Coupling constants (Hz) are in parentheses.

Table 2. ^{13}C NMR chemical shifts of SF2446.

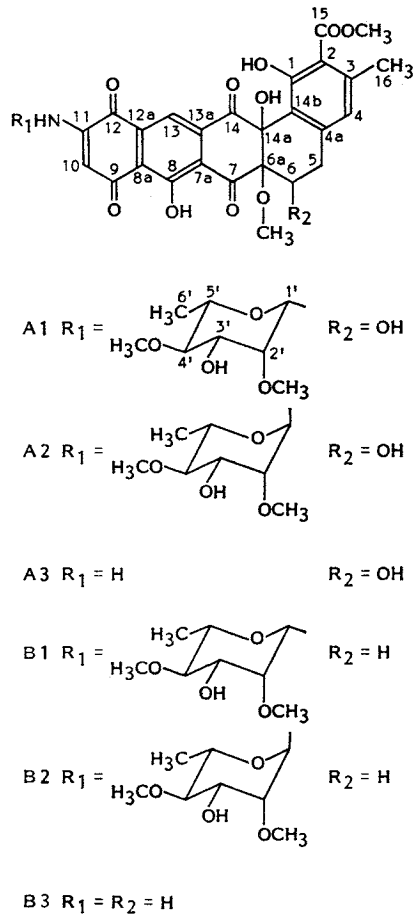
Carbon	A1 (ppm, m)	A2 (ppm, m)	A3 (ppm, m)	B1 (ppm ,m)	B2 (ppm, m)	B3 (ppm, m)
C-1	160.1 s	160.1 s	160.1 s	160.2 s	160.2 s	160.3 s
C-2	109.9 s	109.7 s	109.9 s	109.4 s	109.5 s	109.5 s
C-3	143.3 s	143.1 s	143.3 s	142.4 s	142.4 s	142.4 s
C-4	124.3 d	124.1 d	124.3 d	124.1 d	124.2 d	124.2 d
C-4a	142.8 s	142.7 s	142.7 s	145.1 s	145.0 s	145.1 s
C-5	38.1 t	38.1 t	38.0 t	26.7 t	26.7 t	26.8 t
C-6	62.8 d	62.8 d	62.8 d	18.4 t	18.5 t	18.5 t
C-6a	84.6 s	84.6 s	84.6 s	87.1 s	87.2 s	87.1 s
6a-OCH_3	52.6 q	52.6 q	52.6 q	52.3 q	52.3 q	52.3 q
C-7	190.0 s	190.2 s	190.0 s	190.3 s	190.2 s	190.3 s
C-7a	123.9 s	124.0 s	123.9 s	124.0 s	124.1 s	124.1 s
C-8	162.4 s	162.5 s	162.3 s	162.4 s	162.5 s	162.4 s
C-8a	118.7 s	118.7 s	118.8 s	118.4 s	118.4 s	118.6 s
C-9	188.7 s	188.8 s	188.7 s	188.7 s	188.7 s	188.8 s
C-10	104.2 d	105.3 d	104.3 d	104.2 d	105.5 d	104.5 d
C-11	147.2 s	147.3 s	149.0 s	147.1 s	147.3 s	148.9 s
C-12	179.3 s	179.5 s	179.7 s	179.4 s	179.6 s	179.8 s
C-12a	133.9*s	133.9*s	133.9*s	133.7*s	133.7*s	133.7*s
C-13	116.3 d	116.7 d	116.2 d	116.4 d	116.8 d	116.4 d
C-13a	140.1*s	140.5*s	140.0*s	140.9*s	141.2*s	140.9*s
C-14	196.3 s	196.5 s	196.3 s	197.7 s	198.1 s	197.9 s
C-14a	78.8 s	79.1 s	78.8 s	77.9 s	78.1 s	78.0 s
C-14b	119.8 s	120.0 s	119.7 s	120.9 s	120.9 s	120.9 s
C-15	172.1 s	171.9 s	172.1 s	172.1 s	172.1 s	172.2 s
15-OCH_3	52.3 q	52.2 q	52.3 q	52.2 q	52.2 q	52.3 q
C-16	23.9 q	23.9 q	23.9 q	23.8 q	23.9 q	23.9 q
C-1′	79.3 d	77.5 d		79.2 d	77.5 d	
C-2′	79.6 d	79.8 d		79.6 d	79.8 d	
2′-OCH_3	62.5 q	58.9 q		62.5 q	58.9 q	
C-3′	75.2 d	70.9 d		75.2 d	70.8 d	
C-4′	82.8 d	83.2 d		82.8 d	83.0 d	
4′-OCH_3	61.2 q	60.8 q		61.2 q	60.7 q	
C-5′	73.4 d	68.6 d		73.3 d	68.7 d	
C-6′	18.1 q	17.8 q		18.0 q	17.7 q	

δ: ppm from TMS in $CDCl_3$.
m: Multiplicity.
* Assignments interchangeable.

The structural analysis of the aglycone moiety of **A2** was mainly performed by a long range ^1H-^{13}C shift correlation spectrometry (^1H-^{13}C COSY) and long range selective proton decoupling (LSPD) experiments as explained in detail below. The 1′-H anomeric proton at 5.25 ppm was coupled to the 11-NH proton at 6.84 ppm which was further coupled to the three carbons at 105.3 (C-10), 147.3 (C-11) and 179.5 ppm (C-12). The 10-H proton at 6.19 ppm was coupled to the four carbons at 118.7 (C-8a), 188.8 (C-9), 147.3 and 179.5 ppm. The aromatic quaternary carbon at 118.7 ppm was further coupled to the two protons at 14.26 (8-OH) and 8.47 ppm (13-H). The former hydrogen-bonded phenolic hydroxyl proton was also coupled to the two carbons at 124.0 (C-7a) and 162.5 ppm (C-8) and the latter aromatic proton was coupled to the carbon at 124.0 ppm and the two carbonyl carbons at 179.5 and 196.5 ppm (C-14). Accordingly, the C-9 and C-12 were regarded as a pair of quinone carbonyl carbons and the 13-H proton was located at the *peri* position to the carbonyl carbons C-12 and C-14.

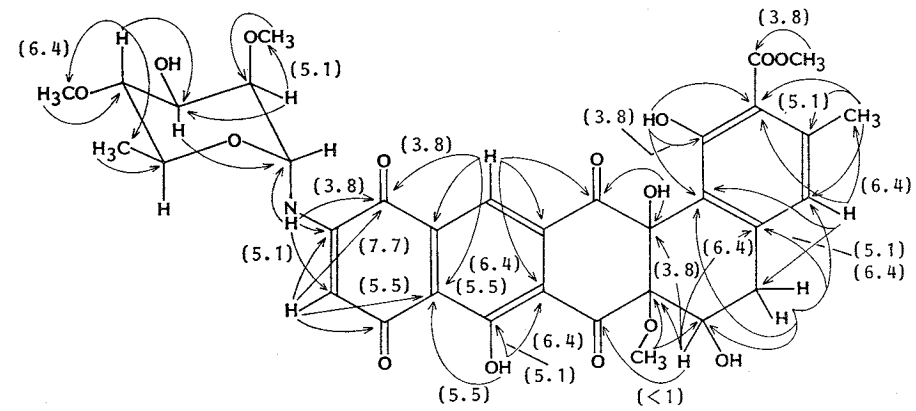
The partial structure of C-7a to C-14 including the sugar moiety was deduced as shown in Fig. 1. In addition, by long range ^{1}H-^{1}H shift correlation spectrometry (long range ^{1}H-^{1}H COSY), it was clarified that the 4-H proton at 6.49 ppm was coupled to the aromatic methyl protons at 2.32 ppm (16-H) and the fixed methylene protons at 3.35 and 3.54 ppm (5-H). The 4-H proton was also coupled to the two aromatic quaternary carbons at 109.7 (C-2) and 120.0 ppm (C-14b) which were further coupled to the 1-OH proton at 11.85 ppm. It was deduced that the C-2 carbon at 109.7 ppm, which was coupled to the 1-OH, 4-H and 16-H protons, was connected to a previously unassigned functional group, the methoxycarbonyl group. The highfield resonance of the C-2 carbon was explained by formulating it as the center carbon in the enolized β-keto ester-like system. These results indicated that the aromatic methyl group, methoxycarbonyl group and phenolic hydroxyl group were located at the *ortho*, *meta* and *para* position to the C-4 carbon at 124.1 ppm, respectively. Furthermore, the C-5 carbon at benzylic position bonded to a carbinol carbon at 62.8 ppm (C-6) and the 6-H proton was coupled to the four carbons at 142.7 (C-4a), 84.6 (C-6a), 190.2 (C-7) and 79.1 ppm (C-14a). By low power irradiation at 3.42 (6a-OCH_3) and 5.96 ppm (14a-OH), enhancement of the signals at 62.8 and 84.6 ppm, and 79.1 and 196.5 ppm was observed, respectively. From these results, the methoxy group at 3.42 ppm and hydroxyl group at 5.96 ppm were shown to be attached to the C-6a and C-14a carbons, respectively. The data of a long range ^{1}H-^{13}C COSY and LSPD experiments were summarized in Fig. 2.

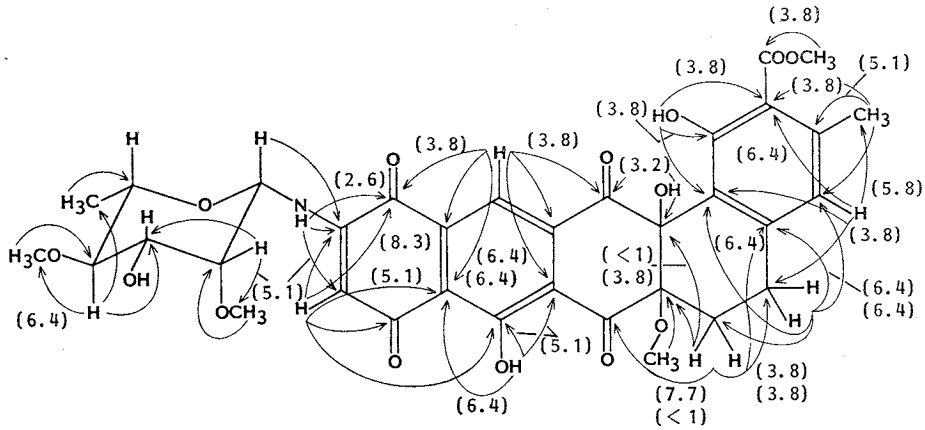
Fig. 1. The structures of SF2446.
The carbon numbers apply to the assignments of NMR.



On comparison of the ^{13}C NMR spectrum of **A1** with that of pentaacetyl **A1**, it is apparent that the C-14 carbon signal of pentaacetyl **A1** occurs at 10.1 ppm higher field from its position in the spectrum of **A1**. This evidence presumably indicated the presence of a hydrogen-bond between the C-14 carbonyl group and the 1-OH proton in **A1**. Accordingly, the remaining two bonds were assigned for C-7 to C-7a and C-14a to C-14b. From these results, the structure of **A2** was determined to be 11-(2,4-di-*O*-methyl-α-L-rhamnopyranosyl)amino-5,6,6a,14a-tetrahydro-1,6,8,14a-tetrahydroxy-6a-methoxy-2-methoxycarbonyl-3-methyl-benzo[*a*]naphthacene-7,9,12,14-tetraone. **A1** was the β-anomer of **A2**, and **A3** was the aglycone of **A1** and **A2**. The structure of the aglycone obtained as a minor product from the methanolysis was shown to be 11-deamino-11-methoxy-**A3** by FD-MS, ^{1}H and ^{13}C NMR spectra.

Fig. 2. The summary of long range ^{1}H-^{13}C COSY and LSPD experiments of SF2446A2 and SF2446B1.



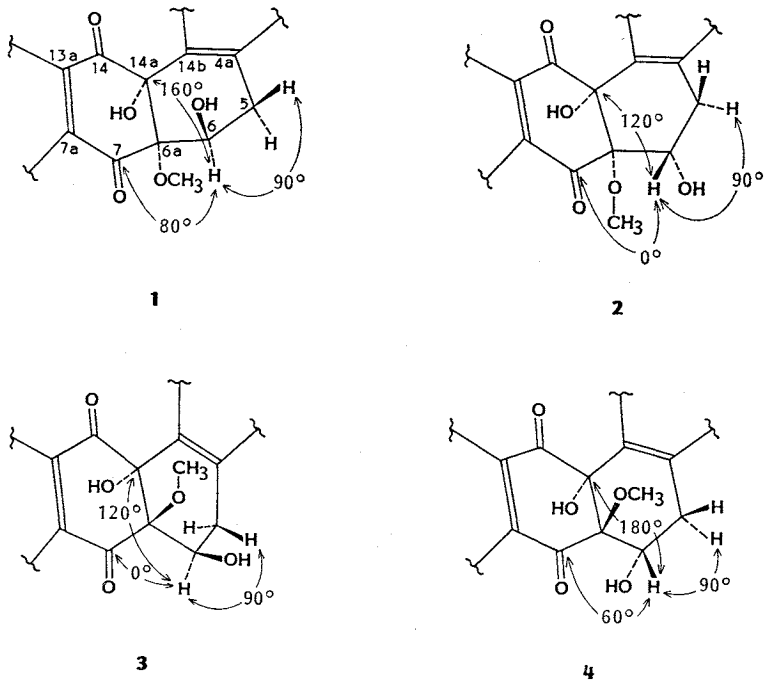


Arrows indicate the carbon coupled to the proton or enhancement of the carbon signal by irradiation of the proton, and the values in the parentheses represent the coupling constants (Hz).

Structures of **B1**, **B2** and **B3**

The summary of a long range ^{1}H-^{13}C COSY and LSPD experiments of **B1** was shown in Fig. 2. The long range coupling pattern of **B1** strongly resembled that of **A2**. **B1** easily isomerized to **B2** by treatment with acidic methanol and methanolysis of **B1** or **B2**[1] gave the aglycone (**B3**) and the same methylglycoside derived from **A1** and **A2**. The ^{1}H and ^{13}C NMR spectra of **B1** showed that the difference of **B1** from **A1** was only at the C-6 position. An oxymethine carbon signal at 62.8 ppm in the ^{13}C NMR spectrum of **A1** appeared as a methylene carbon signal at 18.4 ppm in that of **B1**. And also, the molecular formula of **B1**, which was established by elemental analysis, FD-MS, ^{1}H and ^{13}C NMR spectra, was less one oxygen atom in comparison with that of **A1**. Accordingly, the structure of **B1** was determined to be 11-(2,4-di-*O*-methyl-*β*-L-rhamnopyranosyl)amino-5,6,6a,14a-tetrahydro-1,8,14a-trihydroxy-6a-methoxy-2-methoxycarbonyl-3-methyl-benzo[*a*]naphthacene-7,9,12,14-tetraone. **B2** was the *α*-anomer of **B1**, and **B3** was the aglycone of **B1** and **B2**.

Fig. 3. The relative stereochemistry of SF2446A1, A2 and A3.



Relative Stereochemistry of C-6, C-6a and C-14a

In the ^{1}H and ^{13}C NMR spectra of **A2**, the $^{3}J_{HH}$ coupling constants between 5-H/6-H were 6.3 and 0 Hz and the $^{3}J_{CH}$ values between C-7/6-H and between C-14a/6-H were <1 and 3.8 Hz, respectively. Vicinal C-H couplings $^{3}J_{CH}$ have a similar relationship to the dihedral angle as to $^{3}J_{HH}$[6]. When a dihedral angle between 5-H_α and 6-H_β, or 5-H_β and 6-H_α is fixed at 90 degrees as in the case where $^{3}J_{HH}=0$ Hz, four possible structures (not counting enantiomers) are proposed as shown together with approximate dihedral angles between 6-H and C-7, and 6-H and C-14a in Fig. 3. In these structures, only structure **1** is satisfied by the $^{3}J_{CH}$ values above. Furthermore, in the ^{13}C NMR spectrum of **B1**, the 6-H proton at 2.76 ppm was coupled to the C-7 ($J<1$ Hz) and C-14a ($J=3.8$ Hz) carbons as was the case in the ^{13}C NMR spectrum of **A2**. In addition, another 6-H proton at 2.24 ppm was coupled to the C-7 ($J=7.7$ Hz) and C-14a ($J<1$ Hz) carbons. This fact further supported structure **1**. From these results, the stereochemistry of C-6, C-6a and C-14a was deduced to be (6*R*, 6a*S*, 14a*R*) or (6*S*, 6a*R*, 14a*S*).

Experimental

General

UV and IR spectra were recorded on a Shimadzu UV-260 spectrophotometer and a Hitachi 260-10 IR spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM-GX400 spectrometer with TMS as an internal standard in $CDCl_3$. MP's were determined with a Yanaco MP-S3 micro mp apparatus and are uncorrected. Mass spectra were recorded with a Hitachi M-80B mass spectrometer. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter.

Conversion of **A1** into **A2** and **A2** into **A1**

A solution of **A1** (10.5 mg) in 1 N HCl - MeOH (2.2 ml) was kept for 2 hours at room temp and

then the reaction mixture was concentrated to dryness. The crude powder was purified by preparative TLC ($CHCl_3$ - MeOH, 20 : 1) to give a dark red powder (4.1 mg, 39%) which was identical with natural **A2** by ^1H NMR, UV and FD-MS spectra and Rf value on TLC, and recovered **A1** (4.5 mg, 43%).

Treatment of **A2** (12.0 mg) in the same condition gave **A1** (4.2 mg, 35%) and recovered **A2** (5.7 mg, 48%).

Methanolyses of **A1** and **A2**

A solution of **A2** (85.0 mg) in 1 N HCl - MeOH (12 ml) was refluxed for 15 hours and then poured into water (100 ml) and extracted with $CHCl_3$ (100 ml×2). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified by preparative TLC ($CHCl_3$ - MeOH, 15 : 1) followed by a column chromatography on silica gel ($CHCl_3$ - MeOH, 30 : 1) to afford the aglycone (32.8 mg, 51%) which was identical with **A3** from the ^1H and ^{13}C NMR, UV and FD-MS spectral data, the methylglycoside (15.7 mg, 63%) and 11-deamino-11-methoxy-**A3** (12.3 mg, 19%).

In a similar manner, methanolysis of **A1** (22.0 mg) yielded **A3** (8.3 mg, 50.3%), the methylglycoside (3.5 mg, 53.8%) and 11-deamino-11-methoxy-**A3** (4.8 mg, 28.2%).

Methylglycoside: $[\alpha]_D^{20}$ −51.3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.72 (br d, J=1.5 Hz, 1-H), 3.35 (s, 1-OCH_3), 3.46 (dd, J=1.5 and 3.8 Hz, 2-H), 3.49 (s, 2-OCH_3), 3.80 (ddd, J=3.8, 9.0 and 9.2 Hz, 3-H), 2.39 (d, J=9.0 Hz, 3-OH), 2.96 (dd, J=9.2 and 9.5 Hz, 4-H), 3.58 (s, 4-OCH_3), 3.55 (dq, J=6.2 and 9.5 Hz, 5-H), 1.31 (d, J=6.2 Hz, 6-H); ^{13}C NMR ($CDCl_3$) δ 97.2 (d, C-1), 54.8 (q, 1-OCH_3), 80.6 (d, C-2), 58.9 (q, 2-OCH_3), 71.3 (d, C-3), 83.8 (d, C-4), 60.8 (q, 4-OCH_3), 67.0 (d, C-5), 17.8 (q, C-6).

11-Deamino-11-methoxy-**A3**: FD-MS *m/z* 538 (M^+); mp 183~185°C; ^1H NMR ($CDCl_3$) δ 12.09 (s, 1-OH), 6.54 (br s, 4-H), 3.34 (br d, J=19.5 Hz, 5-H), 3.55 (dd, J=6.6 and 19.5 Hz, 5-H), 4.96 (dd, J=6.6 and 10.2 Hz, 6-H), 4.66 (br d, J=10.2 Hz, 6-OH), 3.40 (s, 6a-OCH_3), 13.47 (s, 8-OH), 6.16 (s, 10-H), *3.94 (s, 11-OCH_3), 8.28 (s, 13-H), 5.21 (br s, 14a-OH), *3.85 (s, 15-OCH_3), 2.40 (br s, 16-H), (*: Interchangeable assignments); ^{13}C NMR ($CDCl_3$) δ 160.1 (s, C-1), 110.0 (s, C-2), 143.4 (s, C-3), 124.3 (d, C-4), 142.7 (s, C-4a), 38.0 (t, C-5), 62.7 (d, C-6), 84.6 (s, C-6a), 52.6 (q, 6a-OCH_3), 190.5 (s, C-7), 123.3 (s, C-7a), 162.2 (s, C-8), 118.3 (s, C-8a), 189.6 (s, C-9), 110.2 (d, C-10), 161.1 (s, C-11), 57.0 (q, 11-OCH_3), 177.9 (s, C-12), *134.8 (s, C-12a), 116.8 (d, C-13), *140.8 (s, C-13a), 196.0 (s, C-14), 78.9 (s, C-14a), 119.7 (s, 14b), 172.0 (s, C-15), 52.4 (q, 15-OCH_3), 23.9 (q, C-16), (*: Interchangeable assignments); IR (KBr) cm^{-1} 3430, 2950, 1720, 1685, 1655, 1625, 1455, 1415, 1380, 1350, 1310, 1260, 1215, 1160, 1120, 995, 925, 875; UV λ_{max}^{MeOH} nm (ε) 217 (23,500), 240 (22,400), 282 (sh, 6,500), 310 (sh, 4,300), 425 (3,200); $\lambda_{max}^{HCl\text{-}MeOH}$ nm (ε) 219 (21,100), 242 (22,700), 282 (sh, 6,200), 310 (sh, 4,300), 427 (3,600); $\lambda_{max}^{NaOH\text{-}MeOH}$ nm (ε) 214 (59,800), 230 (sh, 22,100), 275 (sh, 9,200), 290 (sh, 8,100), 340 (sh, 3,200), 564 (3,800).

Acetylation of **A1**

To a solution of **A1** (30.5 mg) in anhydrous pyridine (2 ml) was added acetic anhydride (0.2 ml) at room temp. After standing for 2 hours, the solution was treated with 0.5 ml of MeOH and stirred for 30 minutes and then concentrated to dryness. The residue was dissolved in $CHCl_3$ (50 ml) and washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude powder was purified by preparative TLC ($CHCl_3$ - MeOH, 50 : 1) to give pentaacetyl **A1** as a reddish orange powder (31.8 mg, 80%).

Pentaacetyl **A1**: FD-MS *m/z* 907 (M^+), 217 (sugar fragment ion); ^1H NMR ($CDCl_3$) δ *2.11 (s, 1-$OCOCH_3$), 6.89 (br s, 4-H), 2.97 (br dd, J=4.2 and 17.0 Hz, 5-H), 3.74 (br dd, J=9.5 and 17.0 Hz, 5-H), 6.17 (dd, J=4.2 and 9.5 Hz, 6-H), 2.20 (s, 6-$OCOCH_3$), 3.09 (br s, 6a-OCH_3), *2.40 (br s, 8-$OCOCH_3$), 5.90 (br s, 10-H), 6.52 (d, J=9.0 Hz, 11-NH), 8.80 (s, 13-H), *2.17 (s, 14a-$OCOCH_3$), 3.66 (s, 15-OCH_3), 2.33 (br s, 16-H), 4.73 (dd, J=1.3 and 9.0 Hz, 1′-H), 3.82 (dd, J=1.3 and 3.0 Hz, 2′-H), 3.71 (s, 2′-OCH_3), 4.95 (dd, J=3.0 and 10.0 Hz, 3′-H), 2.20 (s, 3′-$OCOCH_3$), 3.28 (dd, J=9.0 and 10.0 Hz, 4′-H), 3.52 (s, 4′-OCH_3), 3.40 (dq, J=6.2 and 9.0 Hz, 5′-H), 1.33 (d, J=6.2 Hz, 6′-H), (*: Interchangeable assignments); ^{13}C NMR ($CDCl_3$) δ *145.5 (s, C-1), *121.6 (s, C-2), 141.4 (s, C-3), 128.5 (d, C-4), 138.0 (s, C-4a), 35.6 (t, C-5), 61.2 (d, C-6), 87.5 (s, C-6a), 54.6 (q, 6a-OCH_3), 189.3 (s,

C-7), 128.0 (s, C-7a), *149.3 (s, C-8), 127.4 (s, C-8a), 180.3 (s, C-9), 107.7 (d, C-10), 145.0 (s, C-11), 179.1 (s, C-12), *135.4 (s, C-12a), 123.0 (d, C-13), *138.4 (s, C-13a), 186.2 (s, C-14), 77.2 (s, C-14a), *125.4 (s, C-14b), 165.5 (s, C-15), 52.0 (q, 15-OCH_3), 21.5 (q, C-16), 78.8 (d, C-1′), 78.5 (d, C-2′), 62.1 (q, 2′-OCH_3), 76.5 (d, C-3′), 79.6 (d, C-4′), 61.0 (q, 4′-OCH_3), 73.2 (d, C-5′), 17.9 (q, C-6′), (166.4, 167.5, 168.7, 169.8, 170.0, 20.5, 21.2×2, 21.3, 21.4 (pentaacetyl groups)), (*: Interchangeable assignments).

Conversion of **B1** into **B2** and **B2** into **B1**

A solution of **B1** (15.2 mg) in 1 N HCl - MeOH (2.4 ml) was kept for 1 hour at 40°C. After removal of the solvents, the residual powder was purified by preparative TLC (hexane - acetone, 1 : 1) to afford a dark red powder (5.9 mg, 39%) of **B2** and recovered **B1** (7.8 mg, 51%).

Similarly, treatment of **B2** (10.8 mg) by 1 N HCl - MeOH at 40°C for 1 hour afforded **B1** (4.0 mg, 37%) and unchanged **B2** (4.9 mg, 45%).

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