Polyketomycin, a New Antibiotic from *Streptomyces* sp. MK277-AF1

II. Structure Determination

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A new antibiotic, polyketomycin, was isolated from the culture broth of *Streptomyces* sp. MK277-AF1. The structure was determined by various NMR spectroscopies, X-ray crystallographic analysis and degradation experiments.

In the course of our screening program for novel antibiotics, we have found polyketomycin (**1**) from the culture broth of *Streptomyces* sp. MK277-AF1. In the preceding paper, the taxonomy of the producing strain, production, isolation, pysico-chemical properties and biological activities of **1** are reported[1]. In this paper, we describe the structure determination of **1** (Fig. 1).

Results and Discussion

Structure Elucidation

Polyketomycin (**1**) was obtained as an orange powder. The UV spectrum of **1** showed absorption maxima at 208, 243, 282 and 445 nm. The visible absorption band exhibited characteristic bathochromic shifts in alkaline solution. This UV spectrum was very similar to that of dutomycin[2]. The IR spectrum of **1** indicated the presence of hydroxyl, ester and quinone groups at 3430, 1680 and 1635 cm^{-1}, respectively.

The molecular formula of **1** was deduced as $C_{44}H_{48}O_{18}$ (MW 864) from the FAB-MS peaks at *m/z* 865 $(M+H)^+$ and *m/z* 864 (M^-), and from HRFAB-MS [found *m/z* 864.2831 (M^-), calcd. *m/z* 864.2841 for $C_{44}H_{48}O_{18}$], which was supported by the ^1H and ^{13}C NMR spectra of **1** (Table 1). The ^{13}C NMR, DEPT and HMQC spectra of **1** revealed the presence of twenty-two sp^3 carbons consisting of seven methyl, a methoxy, four methylene, seven methine and three quaternary carbons. In addition, **1** contained twenty-two sp^2 carbons consisting of three methine and nineteen quaternary carbons. The ^1H NMR spectrum indicated the presence of six exchangeable hydroxyl groups in addition to the protons described above.

The four partial structures (Fig. 2a, b, c and d) were established by analysis of the ^1H-^1H COSY, HMQC and HMBC spectra. The UV spectra of **1** resembled closely similar to those of dutomycin, and comparison of the NMR data of **1** including HMBC with those of the aglycone in dutomycin[2] suggested that the structure of the aglycone moiety in **1** was depicted as shown in Fig. 2a. The ^1H and ^{13}C NMR spectra of **1** indicated the presence of two sugar moieties (b and c), because the characteristic pairs of signals at δ_H 4.71 (1′-H) and δ_C 102.03 (C-1′), and δ_H 5.06 (1″-H) and δ_C 100.06 (C-1″) were assignable to the anomeric signals. The ^1H-^1H COSY data showed connectivity from the 1′-H to the

Fig. 1. Structure of polyketomycin (**1**).

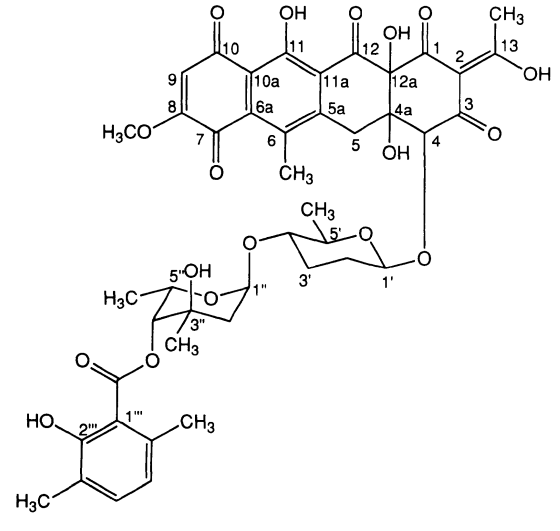
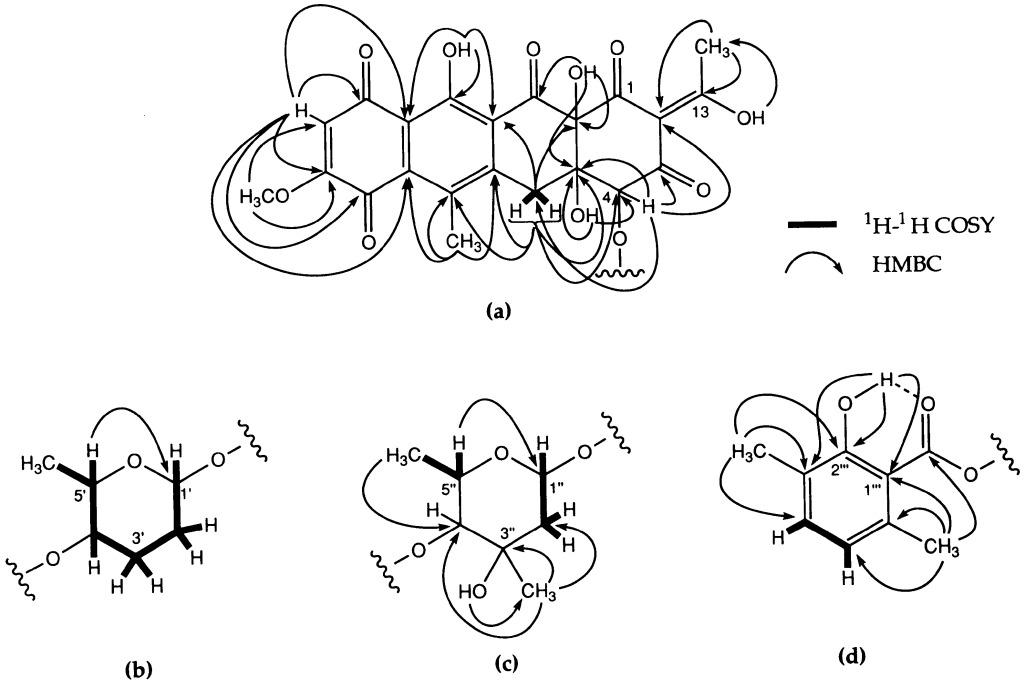


Table 1. ^{13}C and ^{1}H NMR assignments of polyketomycin (**1**) in $CDCl_3$.

Position	δ_C (ppm)	δ_H (ppm)	Position	δ_C (ppm)	δ_H (ppm)
1	192.4**		1'	102.03	4.71 dd (1.6. 9.0)
2	110.54		2'	30.09	1.67 m
3	195.66				2.15 m
4	73.34	4.48 br s	3'	29.32	1.57 m
4a	75.51				2.22 m
4a-OH		2.92 br	4'	80.03	3.16 dt (4.4, 10.0)
5	34.92	3.06 d (17.6)*	5'	74.33	3.05 m
		3.79 d (17.6)	6'	17.27	0.61 d (6.0)
5a	150.64		1"	100.06	5.06 br d (3.8)
6	132.69		2"	37.09	1.71 br d (14.6)
6-CH_3	16.67	2.59 s			1.98 br dd (3.8, 14.6)
6a	132.32		3"	68.66	
7	181.36		3"-CH_3	25.72	1.12 s
8	161.17		3"-OH		3.94 br s
8-OCH_3	56.95	3.93 s	4"	75.63	5.09 br s
9	108.68	6.12 s	5"	62.42	4.49 br q (6.0)
10	190.26		6"	16.8	1.15 d (6.0)
10a	113.72		1"'	110.86	
11	161.98		1"'-CO	171.37	
11-OH		14.19 s	2"'	161.75	
11a	123.34		2"'-OH		11.63 s
12	190.48**		3"'	124.66	
12a	80.97		3"'-CH_3	15.88	2.23 s
12a-OH		4.96	4"'	135.48	7.18 d (7.6)
13	201.09		5"'	122.29	6.65 d (7.6)
13-CH_3	26.68	2.72 s	6"'	138.4	
13-OH		18.09 br	6"'-CH_3	24.56	2.54 s

Chemical shifts in ppm from TMS as an internal standard.
* The coupling constants (Hz) are in parentheses.
** These assignments are exchangeable.

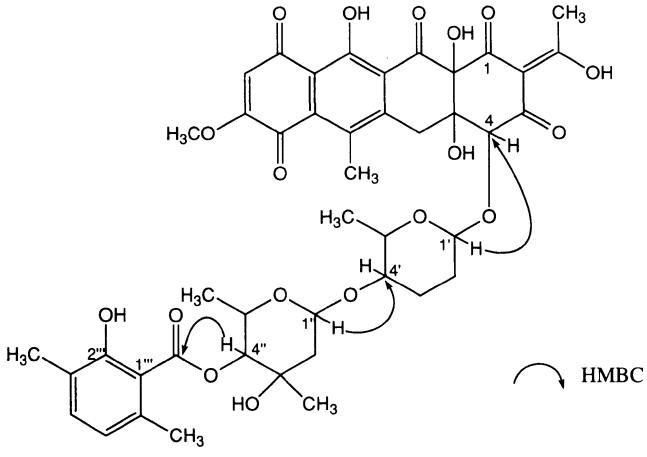
Fig. 2. Partial structures of **1**.



6′-H (δ_H 0.61). In the HMBC spectrum, the anomeric carbon C-1′ was coupled to the 5′-H (δ_H 3.05), which indicated the presence of a 2,3,6-trideoxy sugar unit (Fig. 2b). The hydroxyl proton at δ_H 3.94 (3″-OH) showed a cross peak to a carbon at δ_C 25.72 (3″-CH_3). Methyl proton δ_H 1.12 (3″-CH_3) was coupled to a quaternary carbon C-3″ (δ_C 68.66) and also showed connectivity to C-2″ (δ_C 37.09) and C-4″ (δ_C 75.63). Moreover, C-4″ was coupled to the 6″-H (δ_H 1.15). The 5″-H (δ_H 4.49) showed coupling to an anomeric carbon C-1″. These observations suggested the presence of a 2,6-dideoxy sugar unit (Fig. 2c). The phenolic hydroxyl group at δ_H 11.63 (2‴-OH) showed coupling to C-1‴ (δ_C 110.86), C-2‴ (δ_C 161.75) and C-3‴ (δ_C 124.66). The chemical shift of C-2‴ suggested a hydroxyl group was attached to it. The chemical shift of the 2‴-OH showed it to chelate with the carbonyl carbon at δ_C 171.37 (1‴-CO). In the HMBC spectrum, a cross peak between the methyl proton δ_H 2.54 (6‴-CH_3) and the 1‴-CO confirmed connectivity to C-1‴ and 1‴-CO. The above evidence revealed the presence of a 3,6-dimethylsalicyloyl moiety (Fig. 2d).

The connectivities among the four partial structures (**a**～**d**) were demonstrated by the HMBC spectrum (Fig. 3). The anomeric proton 1′-H was coupling to C-4 (δ_C 73.34), which showed the glycosidic bond between C-4 and C-1′. Similarly, the other anomeric proton 1″-H was coupling to C-4′ (δ_C 80.03), which showed the glycosidic bond between C-4′ and C-1″. The chemical shift of the carbonyl carbon 1‴-CO (δ_C 171.37) suggested that an oxygen atom was attached to it. The 1‴-CO was coupled to the 4″-H (δ_H 5.09), which showed the presence of an ester bond between C-4″ and the 1‴-CO. The anomeric configurations of the sugar moieties **b** and **c** were found to be β and α, respectively, because the vicinal coupling constants of the doublet of doublets of 1′-H (δ_H 4.71) and the broad doublet of 1″-H (δ_H 5.06) were 1.6 and 9.0 Hz, and <1 Hz and 3.8 Hz, respectively. All signals in the ^1H and ^{13}C NMR spectra were thus been assigned, and all correlations found in the various NMR spectroscopies were in perfect agreement with the proposed structure (Fig. 1).

Fig. 3. Connectivities among partial structures (**a**, **b**, **c** and **d**) by HMBC.



Degradation Studies

The structure and the stereochemistry of degradation products (**2**～**7**), obtained from **1**, were determined by analysis of the various NMR spectra, the comparison of their optical rotations with those of the published data and X-ray crystallographic analysis.

Methanolysis of **1** with 0.01 N HCl-MeOH gave **2** (aglycone), **3a** (α-glycoside), **3b** (β-glycoside), **4a** (α-glycoside), **4b** (β-glycoside), **5a** (α-glycoside) and **5b** (β-glycoside) as shown in Fig. 4. The compound **3a** was further converted by methanolysis (0.1 N HCl-MeOH) to **4a**, **5a** and **5b**. These compounds were related to the sugar moieties of axenomycin B[3]. The methyl glycoside **4a** was found to be methyl α-D-amicetoside (methyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside), because the coupling constant between 4-H (δ_H 3.28) and 5-H (δ_H 3.52) was 9.2 Hz and the observed optical rotation of **4a** was $[\alpha]_D^{24}$ +120° (*c* 0.13, H_2O) (Lit[4]. $[\alpha]_D^{20}$ +130° (*c* 1.0, H_2O)). On the other hand, **5a** and **5b** were found to be α- and β-anomers by the analysis of ^1H NMR, respectively. The relative stereochemistry of **5a** (α-anomer) was determined by NOE difference experiments. NOEs were observed between the 1-OCH_3 (δ_H 3.43) and the 3-OH (δ_H 4.32), and the 1-OCH_3 (δ_H 3.43) and the 5-H (δ_H 4.44), respectively. **5a** was saponified in 0.2 N KOH to give **6** (methyl glycoside) and **7**. The ^1H and ^{13}C NMR spectra and optical rotation of **6** were in agreement with those of methyl α-L-axenoside (methyl 2,6-dideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranoside) ; $[\alpha]_D^{24}$ −132° (*c* 0.04, $CHCl_3$) (Lit[5]. $[\alpha]_D^{22}$ −148° (*c* 0.1, $CHCl_3$)). The structure of **7** was determined to be 3,6-dimethylsalicylic acid by the analysis of the NMR spectra.

The aglycone, **2** was crystallized from a $CHCl_3$-MeOH solution to give red prismatic crystals. The relative stereochemistry of **2** was established by X-ray analysis. Fig. 5 indicates the relative orientation of the hydroxyl groups at C-5 and C-18 is *cis*.

These degradation studies were consistent with the structure of **1** which was elucidated from the NMR data. The structure of polyketomycin is similar to the structure

Fig. 4. Degradation products of **1**.

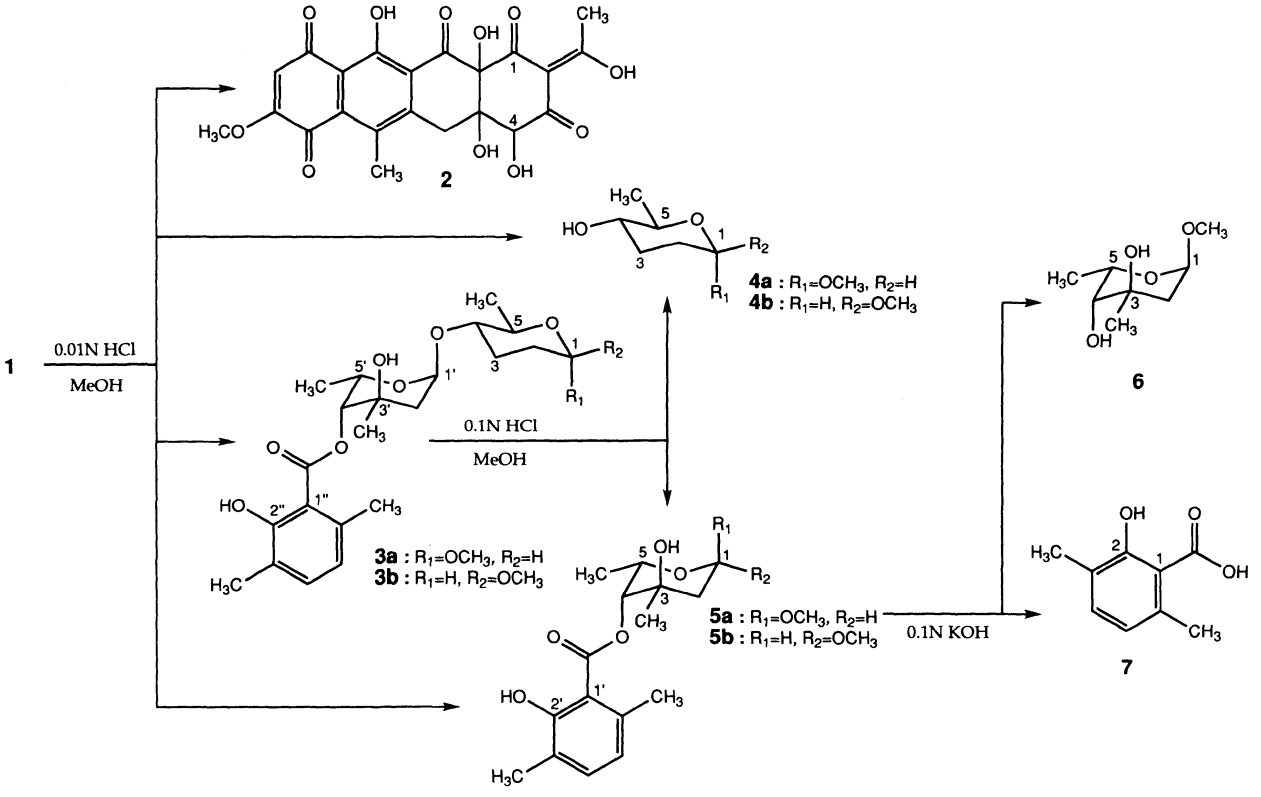
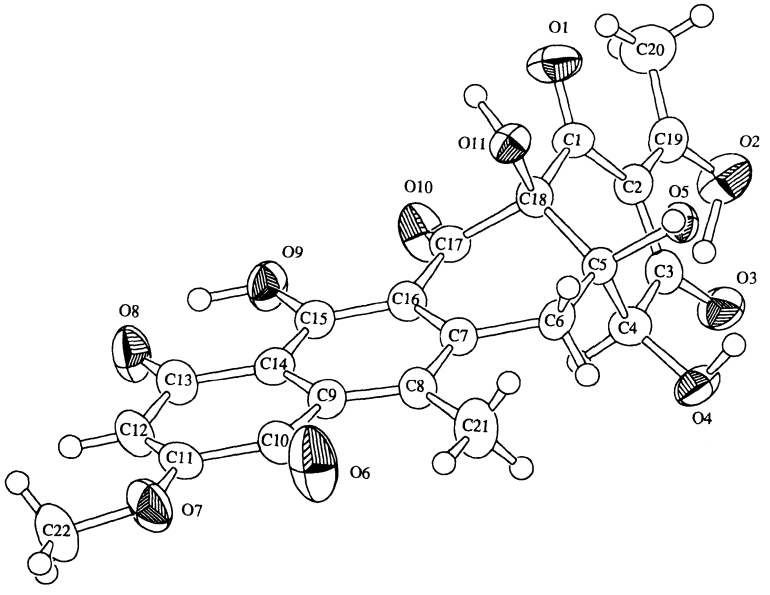


Fig. 5. Molecular structure of **2**.



of dutomycin. The difference between polyketomycin and dutomycin is ascribed to the dimethylsalicyloyl moiety. Thus, the relative structure of polyketomycin (**1**) was determined to be 4-[*O*-2,6-dideoxy-4-*O*-(2-hydroxy-3,6-dimethylbenzoyl)-3-*C*-methyl-α-L-*xylo*-hexopyranosyl-(1→4)-2,3,6-trideoxy-β-D-*erythro*-hexopyranosyloxy]-1,2,3,4,4a,5,7,10,12,12a-decahydro-4a,11,12a-trihydroxy-2-(1-hydroxymethylidene)-8-methoxy-6-methylnaphthacene-1,3,7,10,12-pentaone.

Experimental

General

NMR spectra were obtained on a JEOL JNM-A500 spectrometer at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Chemical shifts are given in ppm using TMS as an internal standard. UV absorption spectra were measured with a Hitachi U-3210 spectrophotometer. IR absorption spectra were recorded with a HORIBA FT-210 spectrometer. FAB-MS and HRFAB-MS were measured with a JEOL JMS-SX 102 spectrometer. APCI (atomspheric pressure chemical ionization)-MS were measured with a HITACHI M-1200H mass spectrometer. Optical rotations were taken by a Perkin-Elmer 241 polarimeter.

Methanolysis of **1**

A suspension of **1** (280 mg) in 0.01 N HCl-MeOH (15 ml) was stirred at room temperature for 24 hours. The reaction mixture was filtered and the precipitate was washed with MeOH. The precipitate was dried under reduced pressure to give aglycone **2** (119.6 mg) as a red powder. The filtrate was concentrated to a small volume after neutralization with 0.1 M $NaHCO_3$. The concentrate was mixed with $CHCl_3$ (100 ml) and H_2O (100 ml). The $CHCl_3$ layer was concentrated and the residue was purified by silica gel column chromatography (Wakogel C-200, 18 g; *n*-hexane - EtOAc, 3 : 1) to give **5a** (11.9 mg) as a colorless syrup and a crude mixture of compounds containing **3a**, **3b**, **4a**, **4b** and **5b**. The crude compounds were further purified by silica gel TLC (Merck Kieselgel $60F_{254}$, *n*-hexane - EtOAc, 3 : 1) to give **3a** (9.4 mg), **3b** (1.7 mg), **4a** (2.7 mg), **4b** (<0.1 mg) and **5b** (9.6 mg) as colorless syrups. Methanolysis (0.1 N HCl-MeOH) of **3a** gave **4a**, **5a** and **5b**, which were identical with the samples obtained from **1**.

2: APCI-MS *m/z* 459 $(M+H)^+$, *m/z* 457 $(M-H)^-$; HRFAB-MS *m/z* 459.0918 $(M+H)^+$ (calcd *m/z* 459.0927 for $C_{22}H_{19}O_{11}$): mp >200°C: UV λ_{max} (1,4-dioxane) nm (log ε) 240.8 (4.60), 282.8 (4.40), 444.4 (3.88), λ_{max} (1,4-dioxane-HCl) nm (log ε) 240.8 (4.60), 284.0 (4.39), 443.6 (3.88), λ_{max} (1,4-dioxane-NaOH) nm (log ε) 236.4 (4.35), 267.2 (4.39), 565.6 (3.90); ^{1}H NMR ($CDCl_3$-CD_3OD (1 : 1)) δ 6.20 (1H, s), 4.25 (1H, br), 3.96 (3H, s, 8-OCH_3), 3.76 (1H, d, *J*=18.0 Hz, 5-H), 3.20 (1H, d, *J*=18.0 Hz, 5-H), 2.68 (3H, s), 2.65 (3H, s).

3a: APCI-MS *m/z* 456 $(M+NH_4)^+$, *m/z* 437 $(M-H)^-$; HRFAB-MS *m/z* 437.2192 $(M-H)^-$ (calcd *m/z* 437.2175 for $C_{23}H_{33}O_8$); UV λ_{max} (MeOH) nm (log ε) 212.0 (4.45), 250.0 (4.05), 321.4 (3.71); ^{1}H NMR ($CDCl_3$) δ 4.62 (1H, br d, *J*=3.0 Hz, 1-H), 3.36 (3H, s, 1-OCH_3), 1.75 (1H, m, 2-Hax), 1.86 (1H, m, 2-Heq), 1.89 (1H, m, 3-Hax), 1.98 (1H, m, 3-Heq), 3.27 (1H, dt, *J*=4.8, 9.2 Hz, 4-H), 3.68 (1H, qd, *J*=6.0, 9.2 Hz, 5-H), 1.23 (3H, d, *J*=6.0 Hz, 6-H), 5.15 (1H, br d, *J*=3.8 Hz, 1′-H), 1.82 (1H, br d, *J*=14.2 Hz, 2′-Hax), 2.02 (1H, dd, *J*=3.8, 14.2 Hz, 2′-Heq), 4.21 (1H, br s, 3′-OH), 1.16 (3H, s, 3′-CH_3), 5.13 (1H, br s, 4′-H), 4.57 (1H, br q, *J*=6.4 Hz, 5′-H), 1.15 (3H, d, *J*=6.4 Hz, 6′-H), 11.61 (1H, s, 2″-OH), 2.23 (3H, s, 3″-CH_3), 7.18 (1H, d, *J*=7.6 Hz, 4″-H), 6.64 (1H, d, *J*=7.6 Hz, 5″-H), 2.55 (3H, s, 6″-CH_3); ^{13}C NMR ($CDCl_3$) δ 97.21 (C-1), 54.51 (1-OCH_3), 29.31 (C-2), 26.11 (C-3), 81.10 (C-4), 67.25 (C-5), 18.10 (C-6), 100.02 (C-1′), 37.26 (C-2′), 68.71 (C-3′), 25.75 (3′-CH_3), 75.86 (C-4′), 62.32 (C-5′), 16.78 (C-6′), 110.93 (C-1″), 171.37 (1″-CO), 161.71 (C-2″), 124.62 (C-3″), 15.87 (3″-CH_3), 135.39 (C-4″), 122.22 (C-5″), 138.40 (C-6″), 24.56 (6″-CH_3); $[\alpha]_D^{24}$ −35.89° (*c* 0.69, MeOH).

3b: APCI-MS *m/z* 456 $(M+NH_4)^+$, *m/z* 437 $(M-H)^-$; HRFAB-MS *m/z* 437.2181 $(M-H)^-$ (calcd *m/z* 437.2175 for $C_{23}H_{33}O_8$); UV λ_{max} (MeOH) nm (log ε) 212.6 (4.34), 250.2 (3.94), 321.2 (3.60); ^{1}H NMR ($CDCl_3$) δ 4.37 (1H, dd, *J*=2.0, 9.0 Hz, 1-H), 3.48 (3H, s, 1-OCH_3), 1.60 (1H, m, 2-Hax), 1.91 (1H, m, 2-Heq), 1.60 (1H, m, 3-Hax), 2.20 (1H, m, 3-Heq), 3.26 (1H, ddd, *J*=4.8, 9.0, 10.0 Hz, 4-H), 3.41 (1H, qd, *J*=6.0, 9.0 Hz, 5-H), 1.29 (3H, d, *J*=6.0 Hz, 6-H), 5.14 (1H, br d, *J*=3.8 Hz, 1′-H), 1.82 (1H, br d, *J*=14.4 Hz, 2′-Hax), 2.03 (1H, dd, *J*=3.8, 14.4 Hz, 2′-Heq), 4.16 (1H, br s, 3′-OH), 1.17 (3H, s, 3′-CH_3), 5.12 (1H, br s, 4′-H), 4.53 (1H, br q, *J*=6.4 Hz, 5′-H), 1.16 (3H, d, *J*=6.4 Hz, 6′-H), 11.65 (1H, s, 2″-OH), 2.23 (3H, s, 3″-CH_3), 7.18 (1H, d, *J*=7.6 Hz, 4″-H), 6.64 (1H, d, *J*=7.6 Hz, 5″-H), 2.55 (3H, s, 6″-CH_3); ^{13}C NMR ($CDCl_3$) δ 102.33 (C-1), 56.34 (1-OCH_3), 30.38 (C-2), 29.51 (C-3), 80.47 (C-4), 74.15 (C-5), 18.24 (C-6), 100.06 (C-1′), 37.24 (C-2′), 68.77 (C-3′), 25.76 (3′-CH_3), 75.78 (C-4′), 62.38 (C-5′), 16.81 (C-6′), 110.91 (C-1″), 171.41 (1″-CO), 161.75 (C-2″), 124.65 (C-3″), 15.89 (3″-CH_3), 135.46 (C-4″), 122.28 (C-5″), 138.43 (C-6″), 24.59 (6″-CH_3); $[\alpha]_D^{24}$ −101.23° (*c* 0.16, MeOH).

Methyl 2,3,6-trideoxy-α-D-*erythro*-hexopyranoside (Methyl α-D-amicetoside) (**4a**): APCI-MS *m/z* 164 $(M+NH_4)^+$; ^{1}H NMR ($CDCl_3$) δ 4.63 (1H, br d, *J*=2.8 Hz, 1-H), 3.36 (3H, s, 1-OCH_3), 1.73 (1H, m, 2-Hax), 1.83 (1H, m, 2-Heq), 1.75 (1H, m, 3-Hax), 1.87 (1H, m, 3-Heq), 3.28 (1H, dt, *J*=4.8, 9.2 Hz, 4-H), 3.52 (1H, qd, *J*=6.0, 9.2 Hz, 5-H), 1.28 (3H, d, *J*=6.0 Hz, 6-H); ^{13}C NMR ($CDCl_3$) δ 97.32 (C-1), 54.45 (1-OCH_3), 29.56 (C-2), 27.63 (C-3), 72.12 (C-4), 69.27 (C-5), 17.91 (C-6); $[\alpha]_D^{27}$ +119.9° (*c* 0.13, H_2O) (Lit[4]. $[\alpha]_D^{20}$ +130°

(*c* 1.0, H_2O)).

Methyl 2,3,6-trideoxy-β-D-*erythro*-hexopyranoside (Methyl β-D-amicetoside) (**4b**): APCI-MS *m/z* 164 $(M+NH_4)^+$; ^1H NMR ($CDCl_3$) δ 4.37 (1H, dd, *J*=2.0, 9.2 Hz, 1-H), 3.48 (3H, s, 1-OCH_3), 1.58 (1H, m, 2-Hax), 1.89 (1H, m, 2-Heq), 1.49 (1H, m, 3-Hax), 2.07 (1H, m, 3-Heq), 3.30 (1H, m, 4-H), 3.30 (1H, m, 5-H), 1.33 (3H, d, *J*=6.0 Hz, 6-H); ^{13}C NMR ($CDCl_3$) δ 102.52 (C-1), 56.31 (1-OCH_3), 30.57 (C-2), 31.02(C-3), 76.77 (C-4), 71.66 (C-5), 18.05 (C-6).

5a: APCI-MS *m/z* 342 $(M+NH_4)^+$, *m/z* 323 $(M-H)^-$; HRFAB-MS *m/z* 323.1492 $(M-H)^-$ (calcd *m/z* 323.1495 for $C_{17}H_{23}O_6$); UV λ_{max} (MeOH) nm (log ε) 212.6 (4.41), 250.2 (4.01), 320.6 (3.67); ^1H NMR ($CDCl_3$) δ 4.93 (1H, br d, *J*=3.8 Hz, 1-H), 3.43 (3H, s, 1-OCH_3), 1.83 (1H, td, *J*=1.2, 14.4 Hz, 2-Hax), 2.03 (1H, dd, *J*=3.8, 14.4 Hz, 2-Heq), 4.32 (1H, br s, 3-OH), 1.16 (3H, s, 3-CH_3), 5.13 (1H, br, 4-H), 4.44 (1H, br q, *J*=6.4 Hz, 5-H), 1.58 (3H, d, *J*=6.4 Hz, 6-H), 11.68 (1H, s, 2'-OH), 2.23 (3H, s, 3'-CH_3), 7.18 (1H, d, *J*=7.8 Hz, 4'-H), 6.65 (1H, d, *J*=7.8 Hz, 5'-H), 2.57 (3H, s, 6'-CH_3); ^{13}C NMR ($CDCl_3$) δ 99.0 (C-1), 55.28 (1-OCH_3), 36.73 (C-2), 68.86 (C-3), 25.83 (3-CH_3), 75.88 (C-4), 61.79 (C-5), 16.89 (C-6), 110.97 (C-1'), 171.45 (1'-CO), 161.72 (C-2'), 124.60 (C-3'), 15.89 (3'-CH_3), 135.39 (C-4'), 122.25 (C-5'), 138.49 (C-6'), 24.61 (6'-CH_3); $[\alpha]_D^{24}$ −152.23° (*c* 0.76, MeOH).

5b: APCI-MS *m/z* 342 $(M+NH_4)^+$, *m/z* 323 $(M-H)^-$; HRFAB-MS *m/z* 323.1520 $(M-H)^-$ (calcd *m/z* 323.1495 for $C_{17}H_{23}O_6$); UV λ_{max} (MeOH) nm (log ε) 212.6 (4.45), 250.4 (4.05), 322.2 (3.71); ^1H NMR ($CDCl_3$) δ 4.73 (1H, dd, *J*=3.0, 9.0 Hz, 1-H), 3.53 (3H, s, 1-OCH_3), 1.74 (1H, dd, *J*=9.0, 13.5 Hz, 2-Hax), 1.79 (1H, ddd, *J*=1.5, 3.0, 13.5 Hz, 2-Heq), 1.27 (3H, s, 3-CH_3), 4.98 (1H, t, *J*=1.5 Hz, 4-H), 4.25 (1H, dq, *J*=1.5, 6.4 Hz, 5-H), 1.19 (3H, d, *J*=6.4 Hz, 6-H), 11.64 (1H, s, 2'-OH), 2.23 (3H, s, 3'-CH_3), 7.18 (1H, d, *J*=7.8 Hz, 4'-H), 6.65 (1H, d, *J*=7.8 Hz, 5'-H), 2.60 (3H, s, 6'-CH_3); ^{13}C NMR ($CDCl_3$) δ 99.90 (C-1), 56.45 (1-OCH_3), 40.08 (C-2), 71.51 (C-3), 27.48 (3-CH_3), 75.26 (C-4), 68.04 (C-5), 16.83 (C-6), 110.82 (C-1'), 171.58 (1'-CO), 161.65 (C-2'), 124.40 (C-3'), 15.85 (3'-CH_3), 135.52 (C-4'), 122.36 (C-5'), 139.00 (C-6'), 25.05 (6'-CH_3); $[\alpha]_D^{24}$ −24.74° (*c* 0.87, MeOH).

Preparation of Compounds **6** and **7**

To a solution of **5a** (8.4 mg) in MeOH (0.4 ml), 1 N KOH (0.1 ml) was added and stirred at room temperature for 16 hours. To the reaction mixture, $CHCl_3$ (50 ml) and H_2O (50 ml) was added. The $CHCl_3$ layer was concentrated and the residue was purified by silica gel column chromatography (Wakogel C-200, 1.5 g; *n*-hexane - EtOAc, 3 : 1) to give **6** (0.6 mg) as a colorless syrup. The H_2O layer was adjusted to pH 2.0 with 1 N HCl and extracted with $CHCl_3$ (50 ml). The extract was washed with H_2O (50 ml) and concentrated to dryness to give **7** (2.2 mg) as a white powder.

Methyl 2,6-dideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranoside (Methyl α-L-axenoside) (**6**): APCI-MS *m/z* 177 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 4.81 (1H, br d, *J*=4.0 Hz, 1-H), 3.38 (3H, s, 1-OCH_3), 1.67 (1H, br d, *J*=14.6 Hz, 2-Hax), 1.92 (1H, dd, *J*=4.0, 14.6 Hz, 2-Heq), 4.01 (1H, br s, 3-OH), 1.25 (3H, s, 3-CH_3), 3.14 (1H, br d, *J*=7.6 Hz, 4-H), 1.68 (1H, br d, *J*=7.6 Hz, 4-OH), 4.31 (1H, br q, *J*=6.4 Hz, 5-H), 1.27 (3H, d, *J*=6.4 Hz, 6-H); ^{13}C NMR ($CDCl_3$) δ 99.07 (C-1), 55.21 (1-OCH_3), 35.47 (C-2), 70.17 (C-3), 26.05 (3-CH_3), 74.63 (C-4), 62.52 (C-5), 16.70 (C-6); $[\alpha]_D^{24}$ −132.0° (*c* 0.04, $CHCl_3$) (Lit[5]. $[\alpha]_D^{22}$ −148° (*c* 0.100, $CHCl_3$).

3,6-Dimethylsalicylic acid (**7**): APCI-MS *m/z* 165 $(M-H)^-$; UV λ_{max} (MeOH) nm (log ε) 209.0 (4.67), 245.0 (4.01), 311.2 (3.78), λ_{max} (MeOH-HCl) nm (log ε) 210.8 (4.62), 246.2 (4.09), 316.0 (3.82), λ_{max} (MeOH-NaOH) nm (log ε) 244.2 (sh., 3.89), 304.4 (3.79): ^1H NMR ($CDCl_3$) δ 2.23 (3H, s, 3-CH_3), 7.20 (1H, d, *J*=7.5 Hz, 4-H), 6.66 (1H, d, *J*=7.5 Hz, 5-H), 2.58 (3H, s, 6-CH_3); ^{13}C NMR ($CDCl_3$) δ 110.25 (C-1), 175.53 (1-CO), 162.05 (C-2), 124.44 (C-3), 15.81 (3-CH_3), 136.11 (C-4), 122.28 (C-5), 139.84 (C-6), 23.98 (6-CH_3).

X-Ray Crystallography

Crystals of **2** were obtained from a $CHCl_3$-MeOH solution. A red prismatic crystal having the approximate dimensions of 0.04 × 0.05 × 0.30 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated CuKα radiation and a rotating anode generator. Crystal data are shown in Table 2. Of the 4959 reflections which were collected, 2874 were unique (R_{int}=0.026). No decay correction was applied. The structure was solved by direct methods (SHELXS 86)[6] and expanded using Fourier techniques (DIRDIF-94)[7]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 2206 observed reflections ($I > 1.5\sigma(I)$) and 592 variable parameter and converged with unweighted and weighted agreement factors of R=0.039 and R_w=0.049†. The maximum and

† The atomic parameters, bond lengths and angles have been sent to the Cambridge Crystallographic Data Centre.

Table 2. Crystal data of **2**.

Formula	$C_{22}H_{18}O_{11}$
Formula weight	458.38
Crystal system	triclinic
Space group	P1
Lattice Parameters	
a	10.167(3) Å
b	14.115(4) Å
c	7.428(1) Å
α	104.61(2)°
β	101.42(2)°
γ	103.82(2)°
V	963.1(5) Å^3
Z	2
D_{calc}	1.581 g/cm^3
μ(CuKα)	11.11cm^{-1}

minimum peaks on the final difference Fourier map corresponded to 0.21 and $-0.17e^-/Å^3$, respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

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