NEW ANTIVIRAL ANTIBIOTICS, CYCLOVIRACINS B_1 AND B_2

II. STRUCTURE DETERMINATION

MITSUAKI TSUNAKAWA, CHIKAKO KOTAKE, TETSURO YAMASAKI,
TOSHIO MORIYAMA, MASATAKA KONISHI
and TOSHIKAZU OKI

Bristol-Myers Squibb Research Institute,
2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

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The structures of novel antiviral antibiotics, cycloviracins B_1 and B_2 have been determined by means of chemical and spectroscopic methods including 2D NMR correlation spectroscopy. The antibiotics are unique macrocyclic diesters consisting of two D-glucoses, three 2-*O*-methyl-D-glucoses and two (C_{24} and C_{26}) hydroxy fatty acids.

Cycloviracins B_1 and B_2 are new antiviral antibiotics isolated from the fermentation broth of *Kibdelosporangium albatum* No. R 761-7 (ATCC 55061). Their production, isolation, physico-chemical and biological properties are reported in the preceding paper[1]. We report their structure determination in this paper.

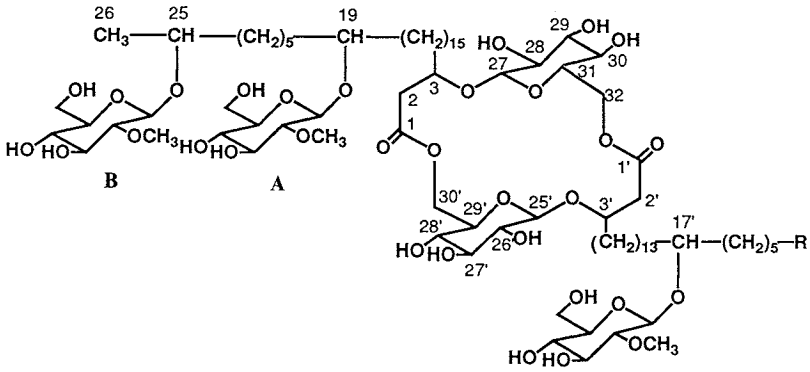
General Structural Characteristics

Cycloviracins B_1 (**1a**) and B_2 (**1b**) are neutral, white amorphous powders. The molecular formulae of **1a** and **1b** were determined to be $C_{83}H_{152}O_{33}$ and $C_{83}H_{150}O_{33}$, respectively, based on the elemental analysis and negative FAB-MS. The IR spectrum of **1a** exhibited absorption bands at 3410, 1740 and $1100\sim1000\,cm^{-1}$ and that of **1b** was similar to **1a** with an additional band at $1720\,cm^{-1}$. The ^1H NMR spectrum of **1a** (Table 1) revealed the presence of five anomeric protons (δ 5.23$\sim$4.82), three *O*-methyls (δ 3.82$\times$2 and 3.77), two *C*-methyls (δ 1.34 and 1.22) and more than thirty methylenes (δ 1.1$\sim$1.9). The spectrum of **1b** (Table 1) was similar to that of **1a** except the presence of a singlet methyl (δ 2.03) and a low-field triplet methylene (δ 2.36) but lack of one of the two doublet methyls (δ 1.34) in **1a**. The spectral data suggested that **1a** and **1b** were composed of five sugars and long alkyl chain(s) (possibly fatty acids) with their structural difference being in only that the $-CHOHCH_3$ moiety of **1a** was $-COCH_3$ in **1b**. The ^{13}C NMR spectra of **1a** and **1b** (Table 2) supported the above assumption.

Degradation Studies (Chart 1)

Upon alkaline hydrolysis, **1a** afforded the hydrolysis products **2** ($C_{32}H_{62}O_{10}$), **3** ($C_{41}H_{78}O_{15}$) and **4** ($C_6H_{12}O_6$), while **1b** gave **5** ($C_{32}H_{60}O_{10}$) in addition to **3** and **4**. **4** was identified as an anomeric mixture of D-glucose by the spectral data[2,3] and optical rotation. Compound **3** showed two anomeric protons (δ 4.85 and 4.82), three *O*-methyls (δ 3.82, 3.77 and 3.41) and one *C*-methyl (δ 1.22) in the ^1H NMR and gave two products, **6** and **7** upon acid hydrolysis. The ^1H NMR spectrum of **2** exhibited one anomeric proton (δ 4.83), two *O*-methyls (δ 3.79 and 3.40) and one *C*-methyl (δ 1.32), while the spectrum of **5** showed that the doublet methyl (δ 1.32) of **2** was replaced by a singlet methyl (δ 2.03) reflecting the structural difference of **1a** and **1b** as discussed earlier. Upon acid methanolysis, **2** afforded **7** ($C_8H_{16}O_6$)

Fig. 1. Structures of cycloviracins B_1 (**1a**) and B_2 (**1b**).



B A C

	R
Cycloviracin B_1 (**1a**)	$-\overset{23'\ 24'}{CHCH_3}$ OH
Cycloviracin B_2 (**1b**)	$-\overset{23'\ 24'}{C}-CH_3$ O

Table 1. ^1H NMR data of cycloviracins B_1(**1a**) and B_2 (**1b**) (400 MHz, pyridine-d_5).

Cycloviracin B_1 (**1a**)		Cycloviracin B_2 (**1b**)	
δ 5.23	(2H, br-d, J=9.4)	δ 5.24	(2H, br-d, J=9.8)
4.89	(1H, d, J=7.7)	4.90	(1H, d, J=7.7)
4.85	(1H, d, J=7.7)	4.85	(1H, d, J=7.7)
4.82	(1H, d, J=7.7)	4.82	(1H, d, J=8.1)
4.4~4.6	(8H, m)	4.4~4.6	(8H, m)
4.34	(4H, m)	4.34	(4H, m)
4.15	(10H, m)	4.15	(10H, m)
3.9~4.1	(8H, m)	3.9~4.1	(7H, m)
3.86	(3H, m)	3.86	(3H, m)
3.82	(6H, s)	3.82	(6H, s)
3.77	(3H, s)	3.77	(3H, s)
3.62	(2H, dd, J=4.7, 15.4)	3.62	(2H, dd, J=4.3, 15.4)
3.42	(3H, q-like, J=8.7)	3.42	(3H, q-like, J=8.6)
2.79	(2H, dd, J=9.4, 15.4)	2.79	(2H, dd, J=9.0, 15.4)
1.1~1.9	(76H, m)	2.36	(2H, t, J=7.3)
1.34	(3H, d, J=6.4)	2.03	(3H, s)
1.22	(3H, d, J=6.4)	1.1~1.9	(74H, m)
		1.22	(3H, d, J=6.0)

δ in ppm downfield from TMS, using pyridine-d_5 (7.55 ppm) as an internal standard.

and **8** ($C_{26}H_{52}O_5$), while **5** yielded **7** and **9** ($C_{26}H_{50}O_5$). Compound **7** was further separated by a silica gel chromatography to **7a** and **7b** which were identified as methyl α- and β-2-*O*-methyl-D-glucopyranoside, respectively, by a comparison with the authentic samples[4,5].

Structures of **6**, **8** and **9**

Compound **6** ($C_{28}H_{56}O_5$) showed an ester carbonyl band at 1740 cm^{-1} in the IR spectrum. The ^1H

Table 2. ^{13}C NMR data of cycloviracins B_1 (**1a**), B_2 (**1b**) and the degradation products **3**, **5**, **6**, **10** and **11** (100 MHz, pyridine-d_5).

Carbon*	1a	1b	3	5	6	10	11
1,1′	171.7	171.7	161.6	161.4	172.2	172.4	172.3
2,2′	42.4	42.4	40.2	43.4	40.2	41.7	41.6
3,3′	78.9	78.9	78.3	78.6	78.0	77.4	77.3
19	79.3	79.3	79.2		70.9		79.2
25	74.2	74.2	74.1		67.0		74.1
26	19.5	19.5	19.5		24.3		19.5
27,25′	106.4	106.4				104.9	104.8
28,26′	75.1	75.1				75.2	75.2
29,27′	78.3	78.3				78.4	78.4
30,28′	72.1	72.1				71.9	71.8
31,29′	74.5	74.5				78.1	78.0
32,30′	65.3	65.4				63.0	62.9
17′	79.3	79.2		79.2		79.2	
22′	40.1	43.4		43.4		43.4	
23′	67.0	208.1		208.1		208.0	
24′	24.3	29.6		29.6		29.6	
1,1′-CO_2CH_3					51.4	51.4	51.3
3,3′-OCH_3			56.6	56.6	56.7		
1A	103.1	103.1	103.0				103.0
2A	85.3	85.3	85.2				85.2
3A	77.7	77.7	77.7				77.7
4A	71.8	71.8	71.8				71.7
5A	78.0	78.0	78.0				78.0
6A	62.9	62.9	62.8				62.8
2A-OCH_3	60.7	60.8	60.7				60.7
1B	101.8	101.8	101.7				101.7
2B	85.0	85.1	85.0				85.0
3B	77.6	77.6	77.5				77.5
4B	71.7	71.7	71.7				71.7
5B	78.0	78.0	77.9				77.9
6B	62.8	62.8	62.7				62.7
2B-OCH_3	60.6	60.6	60.6				60.6
1C	103.1	103.1		103.1		103.1	
2C	85.3	85.3		85.3		85.3	
3C	77.7	77.7		77.7		77.7	
4C	71.8	71.8		71.8		71.7	
5C	78.1	78.1		78.0		78.0	
6C	62.9	62.9		62.9		62.9	
2C-OCH_3	60.7	60.8		60.8		60.8	

Heavily overlapped methylene carbons are not included in this table.

* Carbon numbers and alphabetical suffixes are the same as in Fig. 1.

Chart 1.

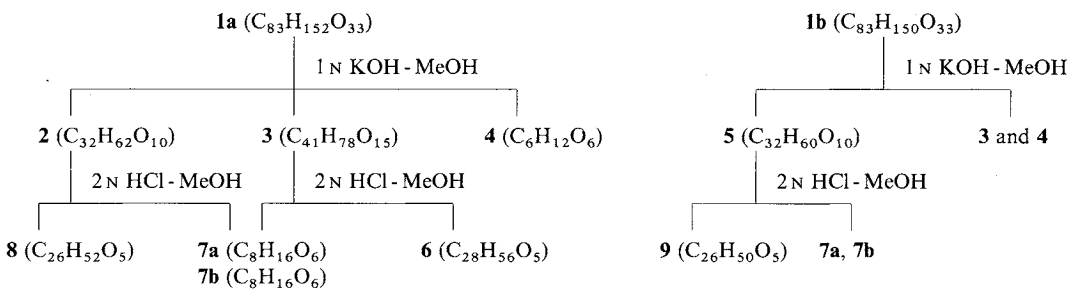
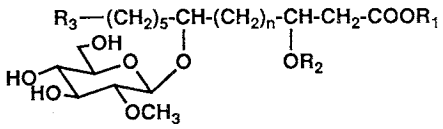
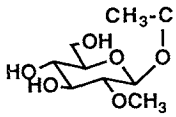
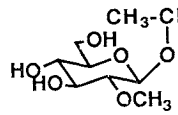


Fig. 2. Structures of the degradation products **2**, **3**, **5**, **10** and **11**.

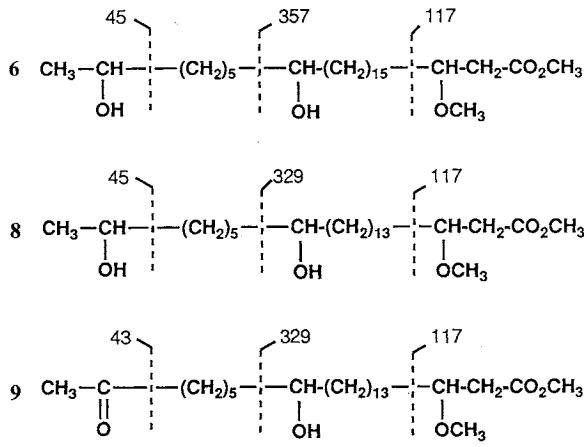


	R_1	R_2	R_3	n
2	H	CH_3	CH_3–CH(OH)–	13
3	H	CH_3		15
5	H	CH_3	CH_3–CO–	13
10	CH_3	β-D-glucopyranosyl	CH_3–CO–	13
11	CH_3	β-D-glucopyranosyl		15

NMR spectrum exhibited three *O*-methines (δ 3.6~4.2), two *O*-methyls (δ 3.63 and 3.33), one methylene adjacent to a carbonyl group (δ 2.70 and 2.47), twenty other methylenes and one *C*-methyl. The ^{13}C NMR (Table 2) showed the presence of an ester carbonyl in addition to the above assigned groups. Thus, **6** was determined to be methyl ester of a straight chain C_{26}- acid having one *O*-methyl and two hydroxy groups. In fact, **6** gave a di-*O*-acetate (*m/z* 556, M^+) by treatment with acetic anhydride in pyridine. In the ^1H NMR spectrum of diacetyl-**6**, two *O*-methine protons were observed at lower field (δ 4.86×2), and one of them was identified to be adjacent to the terminal methyl (δ 1.19) by ^1H-^1H COSY spectrum. The COSY experiment demonstrated that the *O*-methyl-bearing methine proton (δ 3.62) was connected to the methylene (δ 2.53 and 2.41) adjacent to the ester carbonyl group. Thus, the partial structures of $-CH(OAc)CH_3$ and $-CH(OMe)CH_2CO_2Me$ was established for diacetyl-**6**. The positions of the *O*-methyl and hydroxyl groups were unequivocally established by the EI-MS (Fig. 3). **6** showed prominent fragment ion peaks at *m/z* 357, 325, 293, 275, 117, 75 and 45 in addition to *m/z* 436 $(M-2H_2O)^+$ and its acetate gave the corresponding 42 mass units higher ions (*m/z* 399 and 87). It is well known that *m/z* 75 and 117 are the key fragment ions observed in the mass spectra of 3-*O*-methyl fatty acid methyl esters[6]. The ions at *m/z* 357 and 45 together with *m/z* 75 indicated that the OCH_3 and two hydroxyl groups were located at C-3, and C-19 and 25, respectively. Thus, **6** is methyl 3-methoxy-19,25-dihydroxyhexacosanoate (Fig. 3). The molecular formula ($C_{26}H_{52}O_5$) of **8** and its very similar physico-chemical properties to those of **6** suggested that **8** is a C_2 unit shorter acid analog of **6**. Diagnostic fragment ions were observed at *m/z* 408 $(M-2H_2O)^+$, 329, 297, 265, 247, 117, 75 and 45 in the EI-MS establishing **8** to be methyl 3-methoxy-17,23-dihydroxytetracosanoate (Fig. 3).

The IR spectrum of **9** ($C_{26}H_{50}O_5$) showed a ketone carbonyl band at 1710 cm^{-1} in addition to an ester band at 1740 cm^{-1}. The EI-MS spectrum of **9** exhibited the fragment ions *m/z* 424 $(M-H_2O)^+$, 329, 297, 265, 247, 117, 75 and 43. The characteristic ion of **9** at *m/z* 43 instead of *m/z* 45 of **6** and **8** confirmed the assumption that the carbon terminal of **9** is CH_3CO–. Thus, **9** was determined to be methyl 3-methoxy-17-hydroxy-23-oxotetracosanoate (Fig. 3).

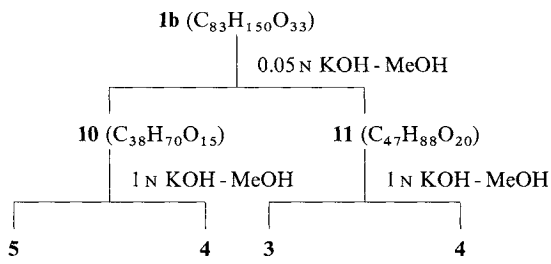
Fig. 3. Structures and mass fragmentations of **6**, **8** and **9**.



Structures of **2**, **3** and **5**

Compound **3** was assigned to have a molecular formula of $C_{41}H_{78}O_{15}$ by negative HRFAB-MS, and showed a strong IR absorption at around 1680 cm^{-1}. Its ^{13}C NMR was indicative of one acid carbon (δ 161.6), two anomeric carbons (δ 103.0 and 101.7) and three OCH_3 (δ 60.7, 60.6 and 56.6) suggesting that **3** was a free acid of **6** containing two 2-*O*-methyl-D-glucose. The sugars were determined to attach to C-19 and C-25 hydroxyls based on the observed glycosidation shift[7] ($\Delta\delta_C$: +8.3 at C-19 and +7.1 at C-25) between **3** and **6** (Table 2). The large coupling constant (J=7.7 Hz) of two anomeric protons allowed to assign β-configuration of the sugars. Thus, **3** was 3-methoxy-19,25-di-(2-*O*-methyl-β-D-glucopyranosyloxy)hexacosanoic acid (Fig. 2).

Chart 2.



The molecular formulae of **2** and **5** were determined to be $C_{32}H_{62}O_{10}$ and $C_{32}H_{60}O_{10}$, respectively, by negative HRFAB-MS. The IR band at 1680 cm^{-1} and the carbon signals (Table 2) suggested that **5** was a free acid of **9** containing one 2-*O*-methyl-D-glucose at C-17. The splitting of the anomeric proton (J=8.1 Hz) was indicative of β-configuration of the sugar. Similarly, **2** was determined to be the free acid of **8** having 2-*O*-methyl-D-glucose at C-17. Thus, **2** and **5** are 3-methoxy-17-(2-*O*-methyl-β-D-glucopyranosyloxy)-23-hydroxytetracosanoic acid and its 23-oxo analog, respectively (Fig. 2).

Structures of **10** and **11**

In order to elucidate the presumed ester linkages between D-glucose, **2** (or **5**) and **3**, the mild alkaline hydrolysis (0.05 N KOH-MeOH) was carried out for **1b** (Chart 2) to yield two hydrolysis products **10** ($C_{38}H_{70}O_{15}$) and **11** ($C_{47}H_{88}O_{20}$). **10** showed an ester and a ketone by the IR spectrum (1710 and 1730 cm^{-1}) and ^{13}C NMR (δ 208.0 and 172.4). Two anomeric (δ 104.9 and 103.1), two OCH_3 (δ 60.8 and 51.4) and one C–CH_3 (δ 29.6) carbons were also identified by the ^{13}C NMR (Table 2). Upon further hydrolysis with 1 N KOH-MeOH, **10** afforded **5** and **4**. This result together with the spectral data indicated that **10** was methyl 3-(β-D-glucopyranosyloxy)-17-(2-*O*-methyl-β-D-glucopyranosyloxy)-23-oxoteracosano-

ate (Fig. 2). **11** showed only an ester carbonyl in the IR spectrum (1735 cm^{-1}) and gave **3** and **4** by alkaline hydrolysis. These facts indicated that **11** was methyl 3-(β-D-glucopyranosyloxy)-19,25-di-(2-*O*-methyl-β-D-glucopyranosyloxy)hexacosanoate (Fig. 2). All the anomeric protons in **10** and **11** showed large coupling constants ($J=7\sim8$ Hz) allowing to assign β-configuration of the sugars.

Structures of **1a** and **1b**

The ^{13}C NMR data of **1a**, **1b** and the degradation products **3**, **5**, **6**, **10** and **11** were summarized in Table 2. The assignments were made on the basis of the ^{1}H-^{1}H, ^{1}H-^{13}C and ^{13}C-^{1}H long range COSY NMR spectra. Taking into consideration of the molecular formulae of **10** ($C_{38}H_{70}O_{15}$), **11** ($C_{47}H_{88}O_{20}$) and **1b** ($C_{83}H_{150}O_{33}$) and their ^{13}C NMR data, it is reasonable that **1b** was cleavaged only at two ester bonds (C-1 and C-1′) to give **10** and **11** by alkaline methanol. Most of the carbon signals of **10** and **11** corresponded well to those of **1b** with the exception of C-5 and C-6 signals of the D-glucose moieties. The chemical shifts of C-29′ and C-31, and C-30′ and C-32 in **1b** were observed at δ 74.5 and 65.4, respectively, while the corresponding signals of **10** and **11** were δ 78.1 and 63.0, and δ 78.0 and 62.9, respectively. These differences could be ascribed to β-upfield shift ($\Delta\delta$ −3.6 and −3.5 at C-29′ and C-31, respectively) and α-down field shift ($\Delta\delta$ +2.4 and +2.5, at C-30′ and C-32, respectively) by acylation at C-6 hydroxyls of the two D-glucoses in **1b**[8].

Accordingly, a macrocyclic diester structure as depicted in Fig. 1 was established to **1b**, and **1a** was its 23′-hydroxy analog.

Discussion

The structures of cycloviracins B_1 and B_2 have been established by a combination of chemical degradation and spectral analysis of the products. They are macrocyclic dilactones composed of two D-glucose, three 2-*O*-methyl-D-glucose and two polyhydroxylated fatty acids. There have been reported two antibiotics rhamnolipids[9] and glykenins[10] consisting of sugars and fatty acids. These antibiotics are straight chain glycosylated fatty acids or acyl fatty acids and have inhibitory activity against Gram-positive bacteria and viruses. Cycloviracins B_1 and B_2 distinctly differ from these known antibiotics in that they form macrocyclic diester structures. Both cycloviracins B_1 and B_2 were strongly active against herpes simplex virus type 1[1] but the hydrolysis products lacked the activity except that compound **11** showed a reduced activity against the virus.

Experimental

IR spectra were determined on a Jasco IRA-1 spectrometer and optical rotations on a Jasco DLP-400 automatic polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Varian FT-80A or Jeol JMN-GX 400 spectrometer. EI-MS and FAB-MS were measured on a Jeol JMS-AX 505H mass spectrometer. Thin layer chromatography (TLC) was performed on silica gel plates (Kiesel gel 60 F_{254}, Merck).

Alkaline Hydrolysis of **1a** and **1b**

Upon a small scale of alkaline hydrolysis (1 N KOH - MeOH, 43°C, 1.5 hours), **1a** and **1b** were found to afford degradation products **2**, **3** and **4** and **3**, **4** and **5**, respectively by TLC. In order to isolate the products, a large scale degradation was carried out. A solution of the cycloviracin mixture (**1a** : **1b** = 1 : 1, 170 mg) in 1 N KOH - MeOH (35 ml) was left standing at 43°C for 1.5 hours. The reaction mixture was diluted with water, adjusted to pH 7 with 6 N HCl and evaporated. The aqueous solution was then extracted with ethyl acetate at pH 2.5. The extract was evaporated to give a residue (130 mg) which was purified by a silica gel column chromatography (Wakogel C-200, 50 ml) eluted with CH_2Cl_2 - MeOH (25 : 1, 15 : 1 and 9 : 1). Each fractions were monitored by TLC (SiO_2 : CH_2Cl_2 - MeOH 3 : 1, H_2SO_4 detection). The eluate

containing **5** (Rf 0.5) was concentrated to give a pale-yellow oil (17 mg) of **5**. Similarly, the eluates containing **2** (Rf 0.37) and **3** (Rf 0.24) afforded a pale yellow oil (14 mg) of **2** and a white sticky solid (43 mg) of **3**, respectively.

The water layer after ethyl acetate above extraction was concentrated and chromatographed on columns of Amberlite CG-50 (NH_4^+) with water and Sephadex LH-20 with 50% MeOH to give a white sticky solid (23 mg) of **4**.

2: (−) HRFAB-MS, $(M-H)^-$, *m/z* calcd: 605.4265, found: 605.4260, ^{1}H NMR (400 MHz, pyridine-d_5) δ 4.83 (1H, d, J=7.7 Hz), 4.48 (1H, dd, J=2.6 and 11.5 Hz), 4.32 (1H, dd, J=5.1 and 11.5 Hz), 4.12 (2H, m), 3.95 (3H, m), 3.82 (1H, m), 3.79 (3H, s), 3.40 (3H, s and 1H, m), 2.86 (1H, dd, J=6.8 and 15.0 Hz), 2.67 (1H, dd, J=5.5 and 15.0 Hz), 1.32 (3H, d, J=6.0 Hz), 1.2∼1.8 (36H, m).

3: (−) HRFAB-MS, $(M-H)^-$, *m/z* calcd: 809.5262, found: 809.5266, IR (neat) cm^{-1} 3400, 2920, 2850, 1680 (br), 1100∼1000, ^{1}H NMR (400 MHz, pyridine-d_5) δ 4.85 (1H, d, J=7.7 Hz), 4.82 (1H, d, J=7.7 Hz), 4.51 (2H, dd, J=2.6 and 11.3 Hz), 4.34 (2H, dd, J=5.9 and 11.3 Hz), 4.14 (4H, m), 4.03 (1H, q, J=6.2 Hz), 3.94 (2H, m), 3.85 (2H, m), 3.82 (3H, s), 3.77 (3H, s), 3.43 (1H, t, J=9.2 Hz), 3.41 (3H, s), 3.40 (1H, t, J=9.2 Hz), 2.87 (1H, dd, J=7.0 and 15.0 Hz), 2.69 (1H, dd, J=5.5 and 15.0 Hz), 1.2∼1.8 (40H, m), 1.22 (3H, d, J=6.2 Hz), ^{13}C NMR see Table 2.

4: FAB-MS *m/z* 179 $(M-H)^-$, $[\alpha]_D^{25}$ +45° (*c* 0.5, H_2O), **4** was identical with D-glucopyranose from the spectral data and by comparison with an authentic sample.

5: (−) HRFAB-MS, $(M-H)^-$, *m/z* calcd: 603.4108, found: 603.4101, IR (neat) cm^{-1} 3400, 2920, 2800, 1680 (br), 1100∼1000. ^{1}H NMR (400 MHz, pyridine-d_5) δ 4.84 (1H, d, J=8.1 Hz), 4.51 (1H, dd, J=2.2 and 11.7 Hz), 4.35 (1H, dd, J=5.1 and 11.7 Hz), 4.18 (1H, t, J=8.8 Hz), 4.14 (1H, t, J=8.9 Hz), 3.94 (2H, m), 3.87 (1H, ddd, J=2.2, 5.1 and 8.8 Hz), 3.81 (3H, s), 3.43 (1H, t, J=8.1 Hz), 3.41 (3H, s), 2.87 (1H, dd, J=7.0 and 15.0 Hz), 2.69 (1H, dd, J=5.5 and 15.0 Hz), 2.36 (2H, t, J=7.3 Hz), 2.03 (3H, s), 1.2∼1.8 (34H, m), ^{13}C NMR see Table 2.

Acid Methanolysis of **2**, **3** and **5**

A solution of **3** (40 mg) in 2 N HCl-MeOH (4 ml) was heated to reflux for 2 hours and evaporated. The residue was diluted with water and then extracted with ether. Evaporation of the ethereal extract afforded a white powder (19 mg) of **6**. Concentration of the water layer gave a light-brown solid (**7**, 24 mg) which was applied on a column of silica gel (40 ml) eluted with EtOAc-MeOH-H_2O (10 : 0.5 : 0.05). The first anthrone positive fractions were pooled and concentrated to give a white sticky solid (3.0 mg) of **7b** and the second gave a solid (7.8 mg) of **7a**. By a similar treatment, **2** (4 mg) afforded white powders of **8** (2 mg), **7a** and **7b**, and **5** (12 mg) afforded **9** (6 mg) and **7a** and **7b** mixture.

6: (+) HRFAB-MS, $(M+H)^+$, *m/z* calcd: 473.4206, found: 473.4209, IR (KBr) cm^{-1} 3340, 2920, 2850, 1740, 1470, 1130, 725, ^{1}H NMR (80 MHz, pyridine-d_5) δ 3.6∼4.2 (3H, m), 3.63 (3H, s), 3.33 (3H, s), 2.70 (1H, dd, J=7 and 15 Hz), 2.47 (1H, dd, J=6 and 15 Hz), 1.1∼1.9 (43H, m). ^{13}C NMR see Table 2.

7a: FAB-MS *m/z* 209 $(M+H)^+$, $[\alpha]_D^{25}$ +138° (*c* 0.4, H_2O), ^{1}H NMR (400 MHz, D_2O) δ 5.06 (1H, d, J=3.7 Hz), 3.88 (1H, dd, J=2.6 and 12.5 Hz), 3.77 (1H, dd, J=5.5 and 12.5 Hz), 3.69 (1H, t-like, J=9.5 Hz), 3.62 (1H, m), 3.49 (3H, s), 3.44 (3H, s), 3.43 (1H, t-like, J=9.5 Hz), 3.30 (1H, dd, J=3.7 and 9.9 Hz).

7b: FAB-MS *m/z* 209 $(M+H)^+$, $[\alpha]_D^{25}$ −15° (*c* 0.2, H_2O), ^{1}H NMR (400 MHz, D_2O) δ 4.44 (1H, d, J=8.1 Hz), 3.93 (1H, dd, J=2.2 and 12.5 Hz), 3.73 (1H, dd, J=5.9 and 12.5 Hz), 3.59 (6H, s), 3.55 (1H, t-like, J=9.2 Hz), 3.45 (1H, m), 3.40 (1H, t-like, J=9.2 Hz), 3.03 (1H, dd, J=8.1 and 9.5 Hz). **7a** and **7b** were identified as methyl α and β-2-*O*-methyl-D-glucopyranoside respectively by their spectral data. **7b** and its acetate were identical with their authentic samples by TLC.

8: (+) HRFAB-MS, $(M+H)^+$, *m/z* calcd: 445.3893, found: 445.3906, IR (KBr) cm^{-1} 3350, 2920, 2850, 1740, 1470, 1130, 720.

9: (+) HRFAB-MS, $(M+H)^+$, *m/z* calcd: 443.3737, found: 443.3728, IR (KBr) cm^{-1} 3330, 3240, 2920, 2850, 1740, 1710, 1470, 1100, 725, ^{1}H NMR (80 MHz, pyridine-d_5) δ 3.6∼3.9 (2H, m), 3.65 (3H, s), 3.35 (3H, s), 2.71 (1H, dd, J=7 and 15 Hz), 2.51 (1H, dd, J=6 and 15 Hz), 2.35 (2H, t, J=7 Hz), 2.04 (3H, s), 1.2∼1.8 (34H, m).

Acetylation of **6**, **8** and **9** in acetic anhydride in pyridine gave diacetyl-**6**, diacetyl-**8** and monoacetyl-**9**, respectively. Diacetyl-**6**: EI-MS *m/z* 556 (M^+), 496 $(M-AcOH)^+$, 436 $(M-2AcOH)^+$, 357, 325, 293, 275, 117 and 75. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.86 (2H, septet-like, J=6.4 Hz), 3.69 (3H, s), 3.62 (1H,

quintet-like, $J=6.1$ Hz), 3.34 (3H, s), 2.53 (1H, dd, $J=7.3$ and 15.0 Hz), 2.41 (1H, dd, $J=5.1$ and 15.0 Hz), 2.03 (3H, s), 2.02 (3H, s), 1.6~1.4 (*ca.* 12H, m), 1.3~1.2 (*ca.* 34H, m), 1.19 (3H, d, $J=6.4$ Hz).

Diacetyl-**8**: EI-MS *m/z* 528 (M^+), 468 $(M-AcOH)^+$, 408 $(M-2AcOH)^+$, 329, 297, 265, 247, 117 and 75.

Monoacetyl-**9**: EI-MS *m/z* 484 (M^+), 424 $(M-AcOH)^+$, 329, 297, 265, 247, 117, 75.

Mild Alkaline Hydrolysis of **1b**

A solution of **1b** (170 mg) in 0.05 N KOH-MeOH (34 ml) was kept at 42°C for 40 minutes. The reaction mixture was adjusted to pH 7, evaporated and extracted with *n*-butanol at pH 2.0. The extract was concentrated *in vacuo* to dryness (170 mg) which was chromatographed on a silica gel column (50 ml) eluted with CH_2Cl_2-MeOH (20:1, 10:1, 7:1 and 3.1). The eluates were checked by TLC (SiO_2: CH_2Cl_2-MeOH 3:1). Evaporation of the first anthrone positive eluate (Rf 0.43) gave a residue which was purified by a column of Sephadex LH-20 (100 ml) with 90% MeOH to yield a white sticky solid (40 mg) of **10**. The second (Rf 0.18) were worked up in a similar way to obtain a white, hygroscopic powder (70 mg) of **11**.

10: FAB-MS *m/z* 765 $(M-H)^-$, $[\alpha]_D^{25}$ −27° (*c* 0.7, MeOH), IR (neat) cm^{-1} 3400, 2920, 2850, 1730, 1710, 1100~1000, ^1H NMR (400 MHz, pyridine-d_5) δ 4.96 (1H, d, $J=7.7$ Hz), 4.84 (1H, d, $J=8.1$ Hz), 4.51 (1H, dd, $J=2.6$ and 11.5 Hz), 4.49 (1H, dd, $J=2.6$ and 11.5 Hz), 4.48 (1H, m), 4.35 (1H, dd, $J=5.1$ and 11.5 Hz), 4.31 (1H, dd, $J=5.6$ and 11.5 Hz), 4.17 (4H, m), 3.97 (1H, t-like, $J=8.5$ Hz), 3.92 (2H, m), 3.86 (1H, m), 3.81 (3H, s), 3.63 (3H, s), 3.43 (1H, t, $J=8.3$ Hz), 3.07 (1H, dd, $J=6.4$ and 15.4 Hz), 2.70 (1H, dd, $J=6.4$ and 15.4 Hz), 2.36 (2H, t, $J=7.7$ Hz), 2.03 (3H, s), 1.2~1.8 (34H, m), ^{13}C NMR see Table 2.

11: FAB-MS *m/z* 971 $(M-H)^-$, $[\alpha]_D^{24}$ −27° (*c* 0.5, MeOH), IR (KBr) cm^{-1} 3420, 2930, 2850, 1735, 1100~1000. ^1H NMR (400 MHz, pyridine-d_5) δ 4.95 (1H, d, $J=7.7$ Hz), 4.85 (1H, d, $J=7.7$ Hz), 4.81 (1H, d, $J=7.7$ Hz), 4.50 (4H, m), 4.34 (3H, m), 4.15 (6H, m), 3.86~4.06 (6H, m), 3.81 (3H, s), 3.77 (3H, s), 3.63 (3H, s), 3.41 (2H, q, $J=8.4$ Hz), 3.06 (1H, dd, $J=6.2$ and 15.4 Hz), 2.70 (1H, dd, $J=6.2$ and 15.4 Hz), 1.16~1.82 (40H, m), 1.22 (3H, d, $J=6.2$ Hz), ^{13}C NMR see Table 2.

Upon treatment with 1 N KOH-MeOH at 43°C for 1 hour, **10** afforded **5**, methyl ester of **5** and **4**, and **11** yielded **3**, methyl ester of **3** and **4** by a similar treatment.

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