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Absolute Stereochemistry of Immunosuppressive Macrolide Brasilinolide A and Its New Congener Brasilinolide C

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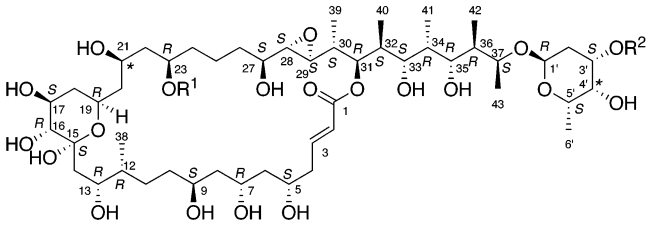
Received December 4, 2003

Brasilinolide A (**2**) is a 32-membered polyhydroxyl macrolide with immunosuppressive activity isolated from a pathogenic actinomycete, *Nocardia brasiliensis* IFM0406. The absolute configurations at 26 chiral centers of **2** and its new congener, brasilinolide C (**1**), were determined on the basis of the spectral data of **1** and degradation products derived from **1** and **2**.

Introduction

Many macrolides with unique structures as well as various biological activities have been isolated from terrestrial actinomycetes.[1] Polyhydroxy macrolides such as notonesomycin A[2] or liposidolide A[3] are unique compounds having a number of chiral centers with a tetrahydropyran ring, an epoxide, and a malonyl or a fatty acid ester. The gross structures and partial relative stereochemistry of these macrolides have been elucidated mainly by means of 2D NMR data, whereas the absolute configurations remain unsolved. During our search for bioactive substances from pathogenic actinomycetes of genus *Nocardia*,[4] we have previously isolated a 32-membered macrolide, brasilinolide A[5] (**2**), with a sugar moiety and a malonyl group as well as a tricyclic metabolite, brasilicardin A,[6] containing a rhamnose, an *N*-acetylglucosamine, and an amino acid moiety from the actinomycete *Nocardia brasiliensis* IFM-0406. Both compounds exhibited potent immunosuppressive activity in mouse mixed lymphocyte assay. More recently, we have isolated a new brasilinolide congener, brasilinolide C (**1**), from the same strain and determined the absolute configurations at 26 chiral centers of **1** on the basis of the spectral data of **1** and its degradation products.



1 (21*S*,4′*S*): $R^1 = R^2 = H$
2 (21*R*,4′*R*): $R^1 = COCH_2CO_2H$, $R^2 = COC_4H_9$-*n*

Furthermore, the absolute stereochemistry of **2** was elucidated by the chemical correlation between **1** and **2**. Here we describe the isolation of **1** and the absolute stereochemistry of **1** and **2**.

Results and Discussion

Isolation and Structure of 1. The supernatant of the fermentation broth (80 L) of *N. brasiliensis* IFM-0406 was subjected to a Diaion HP-20 column (50% MeOH(aq) → MeOH), in which fractions eluted with MeOH were separated on silica gel and C_{18} columns and followed by C_{18} HPLC (MeOH/H_2O and then MeOH/H_2O/CF_3CO_2H) to afford **1** (13.4 mg/L of medium) as a colorless solid together with **2**[5] (0.6 mg/L).

The molecular formula of **1** was established to be $C_{49}H_{88}O_{20}$ by HRFABMS data [*m*/*z* 1019.5760 $(M + Na)^+$, Δ −0.7 mmu]. ^1H and ^{13}C NMR spectra suggested the presence of an ester carbonyl, 2 sp^2 methines, a quaternary carbon due to hemiketal, 25 sp^3 methines including 20 oxymethines, 13 sp^2 methylenes, and 7 methyl carbons, which were similar to those of **2**[5] except for the absence of a malonyl group and a pentanoyl group. Detailed analysis of 2D NMR data including the $^1H-^1H$ COSY, TOCSY, HSQC, HSQC-TOCSY, HMBC, and ROESY spectra revealed gross structures of aglycon and sugar moieties of **1** (Figure 1). The $^1H-^1H$ COSY, TOCSY,

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10.1021/jo035773v CCC: $27.50
Published on Web 01/24/2004

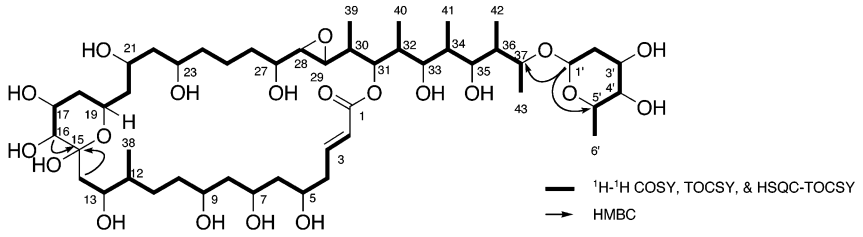

FIGURE 1. Selected 2D NMR correlations for **1**.

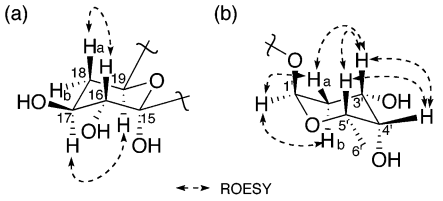

FIGURE 2. ROESY correlations and relative stereochemistry for the (a) C-15−C-19 and (b) C-1′−C-6′ portions in **1**. *J* (Hz) (H/H): H-16/H-17, 9.3; H-18a/H-19, 10.6; H-18b/H-19, <2; H-1′/H-2′a, <1; H-1′/H-2′b, 3.2.

and HSQC-TOCSY spectra indicated three partial structural units of H-2 to H_2-14, H-16 to H_3-43, and H-1′ to H_3-6′. HMBC correlations for H_2-14 (δ_H 1.99 and 1.83) and H-16 (δ_H 3.39) to a hemiketal carbon at C-15 (δ_C 100.53) revealed the presence of a tetrahydropyran ring (C-15−C-19). The relative stereochemistry of the tetrahydropyran ring was deduced from ROESY correlations and ^{1}H−^{1}H coupling constants as shown in Figure 2. The existence of a *trans*-epoxide at C-28−C-29 was indicated by a small *J*(H-28,H-29) value (1.9 Hz). The HMBC correlation for H-1′ to C-5′ suggested the presence of a sugar moiety (C-1′−C-6′), which was elucidated to be 2-deoxyfucopyranose by ROESY correlations and ^{1}H−^{1}H couplings as shown in Figure 2. The HMBC correlation for H-1′ to C-37 indicated that the sugar was connected to C-37. The ester linkage between C-1 and C-31 was deduced from HMBC correlations for H-2 and H-31 to C-1. Thus, the gross structure of **1** was elucidated to be a deacyl form of **2**, that is, lacking both 23-*O*-malonyl and 3′-*O*-pentanoyl groups.

Absolute Stereochemistry of 2-Deoxy-α-L-fucopyranose (4) and the Structure of Aglycon 3. To obtain the sugar moiety of **1**, it was subjected to hydrolysis followed by C_{18} HPLC separation to afford a macrocyclic aglycon (**3**) and **4**, the latter of which was converted into methyl 2-deoxy-α-L-fucopyranoside (**5**) by treatment with hydrogen chloride in methanol (Scheme 1). To apply the dibenzoate rule[8] for **5**, the 3,4-bis-*O*-benzoate **6** was prepared. The CD spectrum of **6** showed the negative first and positive second Cotton effects [λ_{ext} 237 ($\Delta\epsilon$ −20.5) and 222 (+7.6) nm], thus indicating 3*S* and 4*S* configurations for **5**. Therefore, the sugar moiety in **1** was determined to be 2-deoxy-α-L-fucopyranoside,[9] and the absolute configurations at C-1′, C-3′, C-4′, and C-5′ were assigned as *R*, *S*, *S*, and *S*, respectively.

The molecular formula of the macrocyclic aglycon **3** was revealed to be $C_{43}H_{78}O_{17}$ by HRFABMS data [*m*/*z* 867.5356 $(M + H)^+$, Δ −1.8 mmu]. ^{1}H and ^{13}C NMR data (Table 1) of C-1−C-26 and C-38 in **3** were close to those of the corresponding portion in **1**, while ^{1}H and ^{13}C chemical shifts for the C-27−C-37 and C-39−C-43 portions in **3** differed from those of **1**. Chemical shifts for H-28 (δ_H 3.61) and H-29 (δ_H 3.74) in **3** were at lower field than those of **1**, suggesting that the epoxide ring in **1** was opened for **3**. The HMBC correlation for H-33/C-29 suggested that a tetrahydropyran ring was formed between C-29 and C-33 through O-29. Thus, the gross structure of the aglycon was assigned as **3**. The relative stereochemistry of C-28−C-29 was suggested to be *erythro*, since the tetrahydrofuran ring might be produced by an S_N2 addition of the oxygen atom at C-33 to the *trans*-epoxide.

It was difficult to establish the stereochemistry from spectroscopic data such as ^{1}H−^{1}H and ^{1}H−^{13}C coupling constants or ROESY data for the aglycon **3**, since the ^{1}H and ^{13}C NMR data were too complicated. To determine the absolute stereochemistry of 22 stereogenic centers in **3**, the following strategy was planned: (1) fragmentation through oxidative degradation, (2) determination of relative and absolute configurations of degradation products, (3) determination of absolute configurations of parts not included in the degradation products.

Oxidative Degradation of Aglycon 3 in 1. For cleavage between C-15 and C-17 and between C-27 and C-28 in the aglycon **3**, oxidative degradation of **3** using sodium periodate was carried out as follows. Reduction of the hemiketal at C-15 with sodium borohydride and then methanolysis of the ester linkage at C-1 with sodium methoxide were followed by oxidation with sodium periodate to afford a mixture of degradation products, which was treated with sodium borohydride and then pivaloyl chloride to give three segments, the 15-*O*-pivaloyl ester of the C-1−C-15 segment (**7**), the 17,27-bis-*O*-pivaloyl ester of the C-17−C-27 segment (**8**), and the 28-*O*-pivaloyl ester of the C-28−C-37 segment (**9**) (Scheme 1). The gross structures of **7**−**9** were assigned by analysis of ^{1}H−^{1}H COSY and HMQC spectra as well as HRFABMS data. Since these degradation products contained three or four hydroxyl groups adjacent to chiral centers, the relative and absolute stereochemistries were determined using these hydroxyl groups.

Absolute Configurations at C-5, C-7, C-9, and C-13 in 1. Since the 15-*O*-pivaloyl ester of the C-1−C-15 segment (**7**) contained a 1,3,5-triol portion, acetonization was performed to obtain its 5,7-isopropylidene derivative

(7) Tanaka, Y.; Komaki, H.; Yazawa, K.; Mikami, Y.; Nemoto, A.; Tojyo, T.; Kadowaki, K.; Shigemori, H.; Kobayashi, J. *J. Antibiot.* **1997**, *50*, 1036−1041.

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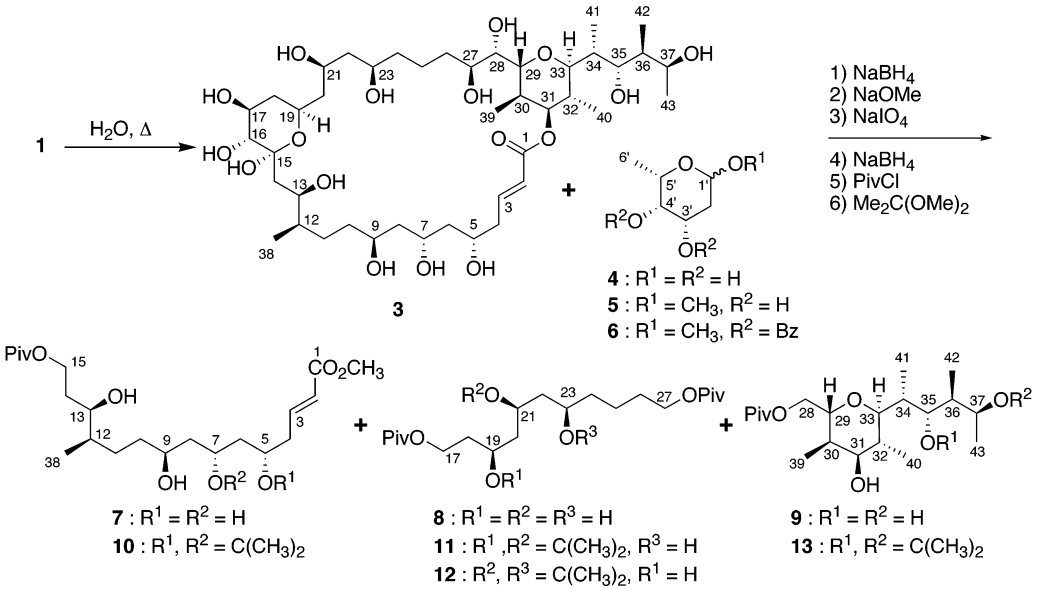
(9) The $[\alpha]_D$ value [−140° (*c* 0.1, H_2O)] of **5** corresponded well with that of methyl 2-deoxy-α-L-fucopyranoside [−160° (*c* 0.19, H_2O)]: Korytnyk, W.; Sufrin, J. R.; Bernacki, R. J. *Carbohydr. Res.* **1982**, *103*, 170−175.

SCHEME 1



10 as a single product. The position of the acetonide ring was assigned by NOESY correlations from H-5 (δ_H 3.58) and H-7 (δ_H 3.94) to α-methyl (δ_H 1.20) of the isopropylidene group. Since α- and *β*-methyl groups of the isopropylidene group resonated at δ_C 19.3 and 30.1, respectively, the 1,3-diol at C-5 and C-7 was assigned as a *syn* relation according to the Rychnovsky rule.[10] The relative stereochemistry of C-7 and C-9 remained undefined, since the 7,9-isopropylidene derivative of **7** was not obtained. However, the carbon chemical shift of C-7 (δ_C 68.9) in the ^{13}C NMR spectrum of **3** in methanol-d_4 corresponded well to that of C-5 (δ_C 68.4) for the 3,7-*anti* form of decane-1,3,5,7-tetraol,[11] indicating a C-5/C-9-*anti* relation for **3**.

Compound **10** was converted into the 9,13-bis-(*S*)- and -(*R*)-MTPA esters **14a** and **14b**, respectively, through MTPA esterification and then deprotection of the acetonide. Δδ values obtained from the ^{1}H NMR spectra of **14a** and **14b** suggested that the absolute configurations at C-9 and C-13 were *S* and *R*, respectively[12] (Figure 3). Therefore, the absolute configurations at C-5, C-7, C-9, and C-13 in **1** were concluded to be *S*, *R*, *S*, and *R*, respectively. Since the stereochemical relationship between C-12 and C-13 was not derived from the NMR data, the relative stereochemistry was elucidated as described later.

Absolute Configurations at C-15, C-16, C-17, C-19, C-21, and C-23 in 1. The 17,27-bis-*O*-pivaloyl ester of the C-17−C-27 segment (**8**) was converted into 19,21- and 21,23-isopropylidene derivatives **11** and **12**, respectively. The NOESY spectrum of **11** showed correlations for H-19 (δ_H 3.89)/α-CH$_3$ (δ_H 1.31) of the acetonide and H-21 (δ_H 4.11)/*β*-CH$_3$ (δ_H 1.36) of the acetonide, while NOESY correlations for H-21 (δ_H 4.14)/*β*-CH$_3$ (δ_H 1.38) of the acetonide and H-23 (δ_H 3.68)/α-CH$_3$ (δ_H 1.32) of the acetonide were observed for **12**. Two methyl carbons of the acetonide in **11** resonated at δ_C 24.7 and 24.8, suggesting the 19,21-*anti* relation.[9] The 21,23-*anti* relation in **12** was deduced from the ^{13}C chemical shifts of two methyls (δ_C 24.8 and 25.0) of the acetonide. The absolute configuration at C-19 in **12** was elucidated to be *S* on the basis of application of the modified Mosher method[11] (Figure 4).

Therefore, the absolute configurations of the C-15−C-23 portion including the tetrahydropyran ring in **1** were assigned as 15*S*, 16*R*, 17*S*, 19*R*, 21*S*, and 23*R*.

Absolute Configurations of C-28−C-37 in 1. Treatment of the C-28−C-37 segment (**9**) with 2,2-dimethoxypropane gave the 35,37-isopropylidene derivative **13**. The relative stereochemistry of the tetrahydropyran portion in **13** was implied to be 29,30-*anti*, 30,31-*syn*, 31,32-*anti*, and 32,33-*anti* relations by NOESY correlations for H-28/H$_3$-39, H-29/H-34, and H-30/H$_3$-40 and *J*(H-29,H-30), *J*(H-30,H-31), *J*(H-31,H-32), and *J*(H-32,H-33) values (Figure 5). The 35,37-*syn* relation was confirmed by ^{13}C chemical shifts of two acetonide methyl groups (α-CH$_3$, δ_C 30.7; *β*-CH$_3$, δ_C 19.8) obtained by the HMQC spectrum of **13**.[11] NOESY correlations for H-35 (δ_H 3.70) and H-37 (δ_H 3.56) to *β*-CH$_3$ (δ_H 1.52) in the acetonide and ^{1}H−^{1}H coupling constants for H-35/H-36 (9.0 Hz) and H-36/H-37 (9.8 Hz) suggested that the relative configurations of H-35/H-36 and H-36/H-37 were both *trans*-diaxial orientations. On the other hand, the relative configurations at C-33/C-34 and C-34/C-35 were implied to be both *erythro* by NOESY correlations for H-34/H$_3$-42, H-36/H$_3$-41, and H-35/H-32 and *J*(H-33/H-34) (9.0 Hz) and *J*(H-34/H-35) (<2 Hz) values.

Δδ values obtained from ^{1}H NMR data of the (*S*)- and (*R*)-MTPA esters **16a** and **16b**, respectively, of **13** indicated the 31*R* configuration in **13** (Figure 6). Therefore, the absolute configurations of the C-28−C-37 portion including the *trans*-epoxide ring in **1** were concluded to be 28*S*, 29*S*, 30*S*, 31*R*, 32*S*, 33*S*, 34*R*, 35*R*, 36*R*, and 37*S*.

Absolute Configurations at C-12 and C-27 in 1. From the experiments described above, absolute configurations at 24 of 26 chiral centers in **1** have been

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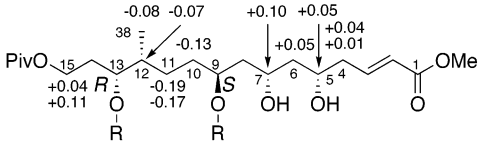
TABLE 1. ^{1}H and ^{13}C NMR Data of 1 and Its Aglycon 3 in CD_3OD

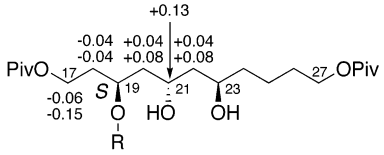
	1		**3**	
position	δ_C	δ_H (m, *J* (Hz))	δ_C	δ_H (m, *J* (Hz))
1	169.26		168.27	
2	125.34	5.99 (d, 15.6)	125.55	5.97 (d, 15.7)
3	148.98	7.07 (dt, 15.6, 7.7)	140.90	7.07 (dt, 15.7, 7.6)
4	41.69	2.50 (dt, 14.6, 7.4)	41.44	2.55 (m)
		2.40 (m)		2.47 (m)
5	70.40	3.97 (m)	70.51	4.00 (m)
6	45.66	1.67 (m)	45.31	1.62 (m)
7	68.81	4.04 (m)	68.91	4.06 (m)
8	46.24	1.55 (m)	45.99	1.59 (m)
9	70.15	3.79 (m)	70.23	3.84 (m)
10	36.94	1.52 (m)	37.96	1.53 (m)
11	31.64	1.62 (m)	31.06	1.67 (m)
		1.19 (m)		1.22 (m)
12	41.15	1.52 (m)	41.44	1.50 (m)
13	71.91	3.80 (m)	72.07	4.03 (m)
14	45.52	1.99 (m)	44.49	1.89 (br d, 5.1)
		1.83 (m)		
15	100.53		100.87	
16	77.83	3.39 (m)	78.72	3.31 (m)
17	70.40	3.89 (m)	70.36	3.91 (m)
18	41.93	1.93 (m)	41.89	1.97 (br d, 10.8)
		1.34 (m)		1.38 (m)
19	66.75	4.17 (br t, 10.4)	67.34	4.20 (br t, 9.2)
20	45.86	1.58 (m)	45.00	1.66 (m)
21	69.83	3.79 (m)	70.03	3.88 (m)
22	46.66	1.49 (m)	45.92	1.62 (m)
23	66.85	4.06 (m)	67.57	4.01 (m)
24	39.69	1.47 (m)	39.18	1.56 (m)
		1.41 (m)		
25	23.40	1.62 (m)	23.40	1.72 (m)
		1.19 (m)		1.47 (m)
26	34.63	1.45 (m)	34.02	1.82 (m)
		1.34 (m)		1.46 (m)
27	71.70	3.45 (br d, 8.6)	74.13	3.61 (m)
28	64.60	2.79 (dd, 3.8, 1.9)	78.78	3.74 (dd, 4.5, 8.7)
29	61.17	2.74 (dd, 8.4, 1.9)	78.64	3.88 (m)
30	41.04	1.52 (m)	32.85	2.42 (m)
31	75.95	5.33 (br d, 10.5)	78.72	5.14 (dd, 3.9, 6.7)
32	39.23	1.95 (m)	34.07	2.17 (m)
33	78.67	3.36 (br d, 9.3)	83.34	3.83 (m)
34	36.67	1.79 (m)	36.87	2.15 (m)
35	80.34	3.45 (br d, 8.6)	79.74	3.55 (br d, 9.4)
36	41.15	1.97 (m)	44.19	1.80 (m)
37	74.95	4.04 (m)	70.99	4.03 (m)
38	15.44	0.92 (d, 6.6)	15.04	0.93 (d, 6.7)
39	16.38	1.04 (d, 7.0)	15.44	1.09 (d, 6.9)
40	10.17	0.86 (d, 6.8)	16.20	1.02 (d, 6.9)
41	5.74	0.89 (d, 6.8)	7.79	0.99 (d, 6.9)
42	10.89	0.79 (d, 6.8)	12.09	0.80 (d, 6.9)
43	15.23	1.08 (d, 6.5)	16.20	1.13 (d, 6.7)
1′	98.49	4.95 (d, 3.2)		
2′	34.63	1.89 (m)		
		1.68 (m)		
3′	67.73	3.95 (m)		
4′	73.09	3.56 (br s)		
5′	68.37	3.95 (m)		
6′	17.93	1.19 (d, 6.5)		

determined, while those of C-12 and C-27 remain unsolved. To obtain the C-1−C-15 segment possessing an acetal ring at C-9−C-13 and the C-17−C-37 segment with a 1,2-diol at C-27−C-28, another oxidative degradation of **1** was carried out as shown in Scheme 2. Compound **1** was treated with sodium borohydride, sodium methoxide, sodium periodate, and sodium borohydride in turn to yield a mixture of C-1−C-15 and C-17−C-37 segments, the latter of which possessed a sugar unit at C-37. The mixture was treated with HCl/methanol to remove the



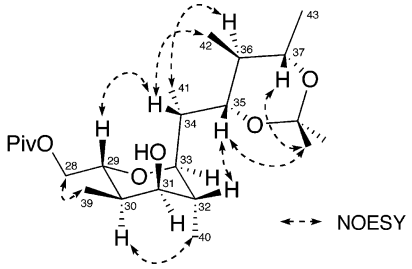
14a : R=(*S*)-MTPA
14b : R=(*R*)-MTPA

FIGURE 3. $\Delta\delta$ values [$\Delta\delta_H$ (ppm) = $\delta_S - \delta_R$] obtained for the 9,13-bis-(*S*)- and (*R*)-MTPA esters **14a** and **14b**, respectively, of the C-1−C-15 segment (**7**) in **1**.



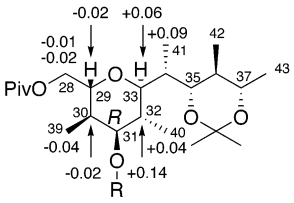
15a : R=(*S*)-MTPA
15b : R=(*R*)-MTPA

FIGURE 4. $\Delta\delta$ values [$\Delta\delta_H$ (ppm) = $\delta_S - \delta_R$] obtained for the 19-(*S*)- and (*R*)-MTPA esters **15a** and **15b**, respectively, of the C-17−C-27 segment (**8**) in **1**.



13

FIGURE 5. NOESY correlations and relative stereochemistry for the 35,37-*O*-isopropyridene C-28−C-37 segment (**13**) in **2**. *J* (Hz) (H/H): 29/30, 7.8; 30/31, <2; 31/32, <2; 32/33, <2; 33/34, 9.0; 34/35, <2; 35/36, 9.0; 36/37, 9.8.



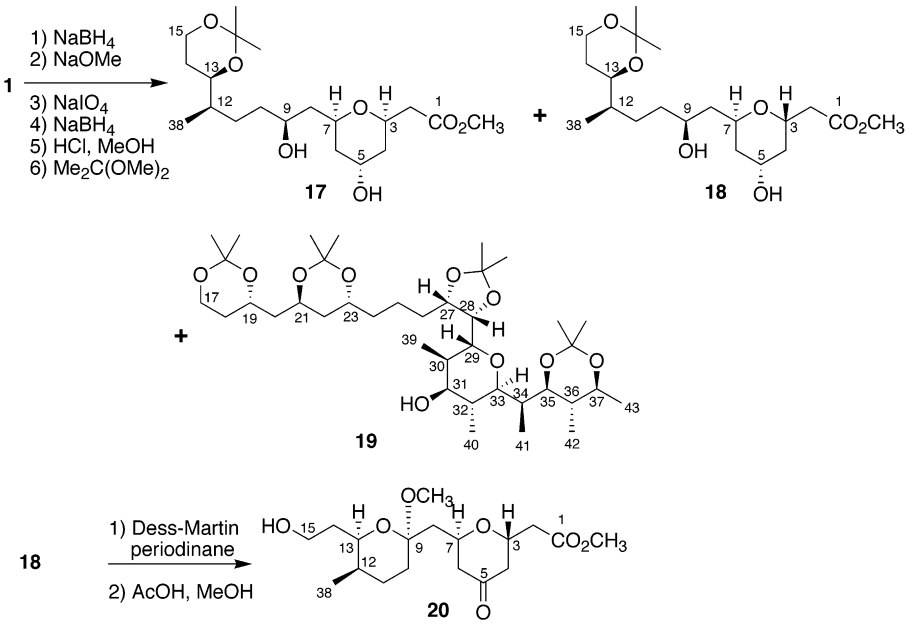
16a : R=(*S*)-MTPA
16b : R=(*R*)-MTPA

FIGURE 6. $\Delta\delta$ values [$\Delta\delta_H$ (ppm) = $\delta_S - \delta_R$] obtained for the 31-(*S*)- and (*R*)-MTPA esters **16a** and **16b**, respectively, of the 35,37-*O*-isopropylidene C-28−C-37 segment (**13**) in **1**.

sugar unit, and then subjected to acetonidation with 2,2-dimethoxypropane to afford three degradation products, **17**−**19**.

The molecular formula of compound **19** was elucidated to be $C_{38}H_{68}O_{10}$ by HRESIMS data [*m*/*z* 707.4714 (M + Na)$^+$, Δ −0.4 mmu]. The ^{1}H NMR spectrum of **19** disclosed eight singlet methyl signals due to four isopropyridene acetals and five doublet methyls. Analysis of the ^{1}H−^{1}H COSY and TOCSY spectra revealed that the carbon chain of **19** corresponded to the C-17−C-37 part of **1**. The NOESY spectrum showed the correlation for H-28 (δ_H 4.45)/H-33 (δ_H 3.42), indicating the presence of a tetrahydropyran ring at C-29−C-33. Three six-mem-

SCHEME 2



bered acetonide rings were revealed to be formed at C-17−C-19, C-21−C-23, and C-35−C-37 by NOESY correlations for H-17 (δ_H 3.69) and H-19 (δ_H 4.08) to a methyl signal at δ_H 1.39, H-21 (δ_H 4.28) to a methyl at δ_H 1.47, H-23 (δ_H 3.91) to a methyl at δ_H 1.49, and H-35 (δ_H 3.70) and H-37 (δ_H 3.53) to a methyl at δ_H 1.43. The residual acetonide was implied to be a five-membered ring formed at C-27−C-28 by NOESY correlations for H-27 (δ_H 4.32) and H-28 (δ_H 4.45) to one (δ_H 1.38) of the acetonide methyls, suggesting an H-27/H-28-*syn* relation (27,28-*erthro*). Thus, the absolute configuration at C-27 in **19** was elucidated to be *S*.

HRESIMS data of compounds **17** [*m*/*z* 411.2363 (M + Na)$^+$, Δ +0.5 mmu] and **18** [*m*/*z* 411.2360 (M + Na)$^+$, Δ +0.2 mmu] suggested the common molecular formula $C_{20}H_{36}O_7$. In the ^{1}H NMR spectrum of **17** in pyridine-d_5, oxymethine (δ_H 4.76, H-3) and methylene (δ_H 2.54 and 2.68, H_2-2) proton signals were observed. The NOESY correlations for H-13 (δ_H 3.67) and H-15α (δ_H 3.80) to α-CH_3 (δ_H 4.76) of the acetonide suggested that the isopropylidene acetal was attached to C-13 and C-15. Since the lower field shifts of H-5 and H-9 were observed by esterification of **17** with (MTPA)Cl, C-5 and C-9 were elucidated to be adjacent to a hydroxyl group. The presence of a tetrahydropyran ring with a 3,7-*syn* relation at C-3−C-7 in **17** was indicated by the NOESY correlation for H-3/H-7. The structure of **17** was assigned as the 3,7-*syn*-3,7-oxa-13,15-isopropylidene derivative of the C-1−C-15 segment. Analysis of the ^{1}H−^{1}H COSY and TOCSY spectra revealed that compound **18** had the same gross structure as **17**. The NOESY correlation for H-2/H-7 was suggestive of a 3,7-*trans* relation for **18**. The tetrahydropyran ring might be generated from Michael addition of the 7-hydroxyl group to the C-2−C-3 double bond.

To determine the relative stereochemistry at C-12−C-13, formation of a ketal ring at C-9−C-13 was performed as follows. Oxidation of two hydroxyl groups at C-5 and C-9 in compound **18** using Dess−Martin periodinane and then deprotection of the acetonide at C-13−C-15 with acetic acid in methanol yielded compound **20** having the ketal ring at C-9−C-13, which was indicated by the NOESY correlation for H-13/9-OCH_3 (δ_H 3.14) (Figure 7). The 12,13-*erythro* relation was deduced from NOESY correlations for H-10β (δ_H 1.62)/H_3-38 (δ_H 0.85) and H-11α (δ_H 2.10)/H-13 (δ_H 4.36) and *J*(H-11α/H-12) (<3 Hz), *J*(H-11β/H-12) (5.0 Hz), and *J*(H-12/H-13) (2.7 Hz) values. Thus, the absolute configuration at C-12 in **1** was concluded to be *R*.

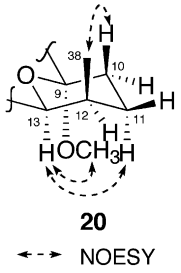


FIGURE 7. NOESY correlations and relative stereochemistry for the C-9−C-13 portion of the 9,13-acetal derivative **20** of the C-1−C-15 segment (**18**) in **1**. *J* (Hz) (H/H): 10a/11a, 4.7; 10a/11b, 2.4; 10b/11a, 13.8; 10b/11b, 4.6; 12/13, 2.7.

From the results described above, the absolute configurations at 26 chiral centers in **1** were determined to be 5*S*, 7*R*, 9*S*, 12*R*, 13*R*, 15*S*, 16*R*, 17*S*, 19*R*, 21*S*, 23*R*, 27*S*, 28*S*, 29*S*, 30*S*, 31*R*, 32*S*, 33*S*, 34*R*, 35*R*, 36*R*, 37*S*, 1′*R*, 3′*S*, 4′*S*, and 5*S*.

Absolute Stereochemistry of 2. 2 was subjected to the hydrolysis in water to give an aglycon (**3**) and **4**. ^{1}H and ^{13}C NMR data as well as the $[\alpha]_D$ value of the aglycon **3** obtained from **2** were completely identical with those of **3** derived from **1**. Therefore, the absolute configurations of **2** were concluded as 5*S*, 7*R*, 9*S*, 12*R*, 13*R*, 15*S*, 16*R*, 17*S*, 19*R*, 21*R*, 23*R*, 27*S*, 28*S*, 29*S*, 30*S*, 31*R*, 32*S*, 33*S*, 34*R*, 35*R*, 36*R*, 37*S*, 1′*R*, 3′*S*, 4′*R*, and 5*S*.

Biological Activity of 2 and C 1. 2 exhibited suppressive activity on mouse mixed lymphocyte reaction (MLR) with an IC_{50} value of 0.625 μg/mL. Although the immunosuppressive activity of **2** was less effective than

those of cyclosporin A (IC_{50} 0.016 μg/mL) and ascomycin (IC_{50} 0.040 μg/mL), **2** showed no toxicity in a dose of 500 mg/kg in mice. Therefore, **2** may be a favorable immunosuppressive reagent. On the other hand, **1** showed no suppressive activity on mouse MLR at 20 μg/mL. These results suggested that 23-*O*-malonyl and 3′-*O*-pentanoyl groups were important for the immunosuppressive activity of **2**. Furthermore, **1** showed cytotoxicity against murine lymphoma P388 cells (IC_{50} 8.3 μg/mL), while **2** was not cytotoxic at 100 μg/mL.

Experimental Section

Cultivation. The voucher specimen of *N. brasiliensis* (strain IFM 0406) was deposited at the Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University (deposition no. FERM BP-5498).[5] This actinomycete was grown in broth [glycerol (2.0%), polypepton (1.0%), and meat extract (0.5%) in H_2O, pH 7.0]. Cultures were incubated in a 150 L jar fermentor at 32 °C for 4 days with stirring at 250 rpm and a 150 L/min aeration rate and were centrifuged.

Extraction and Separation. The supernatant of the fermentation broth (80 L) was passed through a Diaion HP-20 column, washed with 2 M NaCl(aq) (20 L) and H_2O (20 L), and then eluted batchwise with MeOH/H_2O (1:1, 20 L) and MeOH (20 L). The fraction eluted with MeOH was chromatographed on a silica gel column eluted with a stepwise gradient of $CHCl_3$/MeOH to yield a fraction (13.7 g) which was separated by a C_{18} column with a stepwise gradient of MeOH/H_2O. The fraction eluted with 50−70% MeOH/H_2O was further separated by C_{18} HPLC (YMC-Pack ODS R&D, YMC Co., Ltd., 2 × 25 cm; MeOH/H_2O, 70:30; flow rate 10 mL/min; UV detection at 205 nm) and then C_{18} HPLC again [YMC-Pack ODS R&D; MeOH/H_2O, 65:35 → 67:33, containing CF_3CO_2H (25 ppm); flow rate 10 mL/min; UV detection at 205 nm] to afford **1** (285 mg) and **2** (50 mg). Data for **1**: colorless solid; $[\alpha]_D^{22}$ −21° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3402, 1703, and 1651 cm^{-1}; UV (MeOH) λ_{max} 210 nm (ϵ 15400); ^1H and ^{13}C NMR (Table 1); FABMS *m*/*z* 1019 $(M + Na)^+$; HRFABMS *m*/*z* 1019.5760 $(M + Na)^+$, calcd for $C_{49}H_{88}O_{20}Na$ 1019.5767.

Hydrolysis of 1. **1** (50 mg) was dissolved in H_2O (500 μL) and heated at 110 °C for 9 h. The mixture was purified by C_{18} HPLC to afford an aglycon (**3**; 9.6 mg) and **4** (1.0 mg). Data for **3**: colorless solid; $[\alpha]_D^{21}$ +22° (*c* 1.0, MeOH); ^1H and ^{13}C NMR (Table 1); FABMS *m*/*z* 867 $(M + H)^+$ and 889 $(M + Na)^+$; HRFABMS *m*/*z* 867.5356 $(M + H)^+$, calcd for $C_{43}H_{79}O_{17}$ 867.5374.

A solution of **4** (1.0 mg) in MeOH (200 μL) was treated with 6 M HCl(aq) (5 μL) at 100 °C for 30 min. After dilution with H_2O and then evaporation of organic solvent, the residue was subjected to an HP-20 column (H_2O and then MeOH). The fraction eluted with MeOH was purified on a silica gel column (acetone/hexane) to afford **5** (0.5 mg): $[\alpha]_D$ −140° (*c* 0.1, H_2O); ^1H NMR ($CDCl_3$) δ 1.28 (3H, d, J = 6.7 Hz, H_3-6′), 1.77 (1H, dt, 3.9 and 13.1 Hz, H-2), 1.91 (1H, dd, 5.4 and 13.1 Hz, H-2), 3.32 (3H, s, OMe), 3.63 (1H, m, H-4′), 3.90 (1H, q, J = 6.7 Hz, H-5′), 3.99 (1H, m, H-3′), and 4.78 (1H, d, J = 3.6 Hz, H-1′); ESIMS *m*/*z* 185 $(M + Na)^+$; HRESIMS *m*/*z* 185.0775 $(M + Na)^+$, calcd for $C_7H_{14}O_4Na$ 185.0790.

To a solution of 2-deoxyfucopyranoside **5** (0.5 mg) in pyridine (100 μL) were added 4-(dimethylamino)pyridine (1 mg), triethylamine (1 μL), and benzoyl chloride (2 μL), and the mixture was stirred at 37 °C for 8 h and then at 80 °C for 2 h. After addition of saturated aqueous NH_4Cl, the reaction mixture was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, H_2O, and brine. The organic phase was evaporated, and the residue was separated on a silica gel column (hexane/EtOAc, 95:5 and 90:10) to afford methyl 3,4-bis-*O*-benzoyl-2-deoxy-α-L-fucopyranose (**6**; 0.055 mg): λ_{ext} 237 ($\Delta\epsilon$ −20.5) and 222 (+7.6) nm; ^1H NMR (CD_3OD) δ 1.26 (3H, d, J = 6.6 Hz, H_3-6′), 2.14 (1H, ddd, J = 12.4, 5.1, and 1.2 Hz, H-2′), 2.33 (1H, dt, J = 12.4 and 3.3 Hz, H-2′), 3.47 (3H, s, OMe), 4.32 (1H, q, J = 6.6 Hz, H-5′), 5.06 (1H, d, J = 3.3 Hz, H-1′), 5.58 (1H, br, H-4′), 5.63 (1H, ddd, J = 3.1, 5.1, and 12.4 Hz, H-3′), 7.37 (2H, d, J = 7.9 Hz, Ph), 7.57 (3H, t, J = 7.6 Hz, Ph), 7.70 (1H, tt, J = 7.5 and 1.3 Hz, Ph), 7.83 (2H, d, J = 8.2 and 1.2 Hz, Ph), and 8.11 (2H, dd, J = 8.2 and 1.2 Hz, Ph); ESIMS *m*/*z* 393 $(M + Na)^+$; HRESIMS *m*/*z* 393.1308 $(M + Na)^+$, calcd for $C_{21}H_{22}O_6Na$ 393.1314.

Oxidative Degradation of the Aglycon 3 of 1. The aglycon **3** (9.6 mg) of **1** was treated with $NaBH_4$ (1.2 mg) in MeOH (500 μL) at room temperature for 40 min. After addition of 5% aqueous AcOH (200 μL) and then evaporation of the organic solvent, the residual aqueous solution was subjected to an HP-20 column (H_2O and then MeOH). The fraction eluted with MeOH was evaporated in vacuo to give a residue (9.9 mg). To a solution of the residue in MeOH (300 μL) was added a 28% solution of NaOMe in MeOH (18.5 μL), and the mixture was stirred at room temperature for 6 h. After addition of saturated aqueous NH_4Cl, evaporation of the organic solvent, and chromatography on an HP-20 column (H_2O and then MeOH), the MeOH-eluted portion (9.3 mg) was treated with $NaIO_4$ (4.9 mg) in MeOH/H_2O (1:1; 600 μL) at room temperature for 30 min. To this reaction mixture was added $NaBH_4$ (2.2 mg), and stirring was continued at room temperature for 30 min. After evaporation of the organic solvent, and chromatography on an HP-20 column (H_2O and then MeOH), the MeOH-eluted fraction (7.7 mg) was treated with pivaloyl chloride (16 mg) and pyridine (400 μL) at room temperature for 30 min. The reaction mixture was partitioned between EtOAc and saturated aqueous $NaHCO_3$, and the organic phase was washed with saturated aqueous NH_4Cl, H_2O, and then brine and evaporated in vacuo. The residue was subjected to C_{18} HPLC [Develosil ODS-UG-5, Nomura Chemical Co., Ltd., 10 × 250 mm; eluent CH_3CN/H_2O, 45:55 (∼5 min) to 50:50 (∼15 min); flow rate 2.5 mL/min; UV detection at 210 nm] to afford the 15-pivaloyl ester of the C-1−C-15 segment (**7**; 0.4 mg, t_R 11 min), the 17,27-bis-pivaloyl ester of the C-17−C-27 segment (**8**; 1.3 mg, t_R 24 min), and the 28-pivaloyl ester of the C-28−C-37 segment (**9**; 1.6 mg, t_R 15 min).

5,7-Isopropyridene Acetal of 7 (10). To a solution of the 15-pivaloyl ester of the C-1−C-15 segment (**7**, 0.4 mg) in MeOH (10 μL) were added 2,2-dimethoxypropane (40 μL) and TsOH·H_2O (0.02 mg), and the mixture was stirred at room temperature for 40 min. After addition of saturated aqueous $NaHCO_3$, the mixture was extracted with EtOAc, and the organic layer was washed with H_2O and brine and evaporated in vacuo. The residue was separated by preparative TLC (hexane/EtOAc, 1:1; four times) to afford compound **10** (0.4 mg): colorless oil; ^1H NMR (C_6D_6) δ 0.90 (3H, d, J = 6.8 Hz, H_3-38), 0.92 (1H, m, H-6β), 1.14 (1H, m, H-6α), 1.18 (1H, m, H-11), 1.18 (1H, m, H-10), 1.20 (9H, s, Piv), 1.25 (3H, s, β-CH_3 of acetonide), 1.38 (1H, m, H-12), 1.42 (3H, s, α-CH_3 of acetonide), 1.43 (1H, m, H-8), 1.44 (1H, m, H-11), 1.52 (1H, m, H-8), 1.60 (2H, m, H_2-14), 1.70 (1H, m, H-10), 1.99 (1H, J = 7.0 and 14.3 Hz, H-4β), 2.21 (1H, dd, J = 7.4 and 14.3 Hz, H-4α), 3.45 (3H, s,1-OCH_3), 3.51 (1H, m, H-13), 3.58 (1H, m, H-5), 4.88 (1H, m, H-9), 3.94 (1H, m, H-7), 4.15 (1H, dt, J = 10.9 and 5.6 Hz, H-15), 4.32 (1H, dt, J = 10.9 and 7.3 Hz, H-15), 5.96 (1H, d, J = 15.4 Hz, H-2), and 7.12 (1H, dt, J = 15.4 and 7.6 Hz, H-3); ESIMS *m*/*z* 495 $(M + Na)^+$; HRESIMS *m*/*z* 495.2927 $(M + Na)^+$, calcd for $C_{25}H_{44}O_8Na$ 495.2934.

9,13-Bis-(*S*)-MTPA Ester of the C-1−C-15 Segment (14a). To a solution of **10** (0.1 mg) in pyridine (50 μL) were added DMAP (0.1 mg) and (*R*)-(−)-(MTPA)Cl (0.2 μL), and stirring was continued at 37 °C for 9 h. *N*,*N*-Dimethyl-1,3-propanediamine (1 μL) and saturated aqueous $NaHCO_3$ were added, and the reaction mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine and dried by a N_2 stream. The residue was purified by preparative TLC (hexane/EtOAc, 7:3) to afford a 9,13-bis-(*S*)-MTPA ester of **10** (0.1 mg) as a colorless oil. This MTPA ester was treated with AcOH/MeOH/H_2O (1:8:1; 50 μL) at 50 °C for 1 h. The solvent

was removed by a N_2 stream to afford **14a** (0.1 mg) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, d, J = 6.9 Hz, H_3-38), 0.91 (1H, m, H-11), 1.20 (9H, s, Piv), 1.20 (1H, m, H-11), 1.49 (2H, m, H_2-6), 1.53 (2H, m, H_2-10), 1.59 (2H, m, H_2-8), 1.65 (1H, m, H-12), 1.83 (2H, m, H_2-14), 2.32 (1H, m, H-4), 2.39 (1H, m, H-4), 3.49 (3H, s, OCH_3 of MTPA), 3.51 (3H, s, OCH_3 of MTPA), 3.65 (1H, m, H-7), 3.79 (3H, s, 1-OCH_3), 3.89 (1H, m, H-5), 3.90 (1H, m, H-15), 4.11 (1H, m, H-15), 5.09 (1H, m, H-13), 5.15 (1H, m, H-9), 5.90 (1H, d, J = 15.1 Hz, H-2), 6.96 (1H, dd, J = 7.7 and 15.1 Hz, H-3), 7.43−7.39 (6H, m, Ph), 7.50 (2H, m, Ph), and 7.53 (2H, m, Ph); ESIMS m/z 887 $(M + Na)^+$; HRESIMS m/z 887.3436 $(M + Na)^+$, calcd for $C_{42}H_{54}F_6O_{12}Na$ 887.3417.

31-(*S*)-MTPA Ester of 13 (16a). To a solution of **13** (0.3 mg) in pyridine (100 μL) were added DMAP (0.06 mg) and (*R*)-(−)-MTPACl (0.4 μL), and stirring was continued at 37 °C for 9 h. *N*,*N*-Dimethyl-1,3-propanediamine (1 μL) and saturated aqueous $NaHCO_3$ were added, and the reaction mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine and dried by a N_2 stream. The residue was purified on a silica gel column (hexane/EtOAc, 92:8) to afford the 31-(*S*)-MTPA ester of **13** (**16a**; 0.1 mg) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.63 (3H, d, J = 6.9 Hz, H_3-42), 0.86 (3H, d, J = 6.9 Hz, H_3-39), 0.96 (3H, d, J = 6.9 Hz, H_3-41), 1.13 (3H, d, J = 7.2 Hz, H_3-40), 1.15 (3H, d, J = 6.1 Hz, H_3-43), 1.22 (9H, s, Piv), 1.31 (3H, s, CH_3 of acetonide), 1.36 (3H, s, CH_3 of acetonide), 1.39 (1H, m, H-36), 2.08 (1H, m, H-32), 2.12 (1H, m, H-30), 2.15 (1H, m, H-34), 3.46 (3H, s, OCH_3 of MTPA), 3.47 (1H, m, H-35), 3.52 (1H, dd, J = 6.3 and 9.9 Hz, H-37), 3.56 (1H, dd, J = 3.9 and 8.5 Hz, H-33), 3.64 (1H, m, H-29), 4.13 (1H, dd, J = 5.8 and 11.8 Hz, H-28), 4.18 (1H, dd, J = 3.0 and 11.8 Hz, H-28), 5.14 (1H, t, J = 3.0 Hz, H-31), 7.43 (3H, m, Ph), and 7.54 (2H, m, Ph); ESIMS m/z 653 $(M + Na)^+$; HRESIMS m/z 653.3279 $(M + Na)^+$, calcd for $C_{33}H_{49}F_3O_8Na$ 653.3277.

Oxidative Degradation of 1. To a solution of **1** (20 mg) in MeOH (1 mL) was added $NaBH_4$ (4.0 mg), and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with H_2O, concentrated in vacuo, and subjected to an HP-20 column (H_2O and then MeOH). The MeOH-soluble materials (19.2 mg) were treated with NaOMe (25 mg) in MeOH (750 μL) at room temperature for 2 h. After addition of saturated aqueous NH_4Cl and then concentration of the solvent, the reaction mixture was subjected to an HP-20 column (H_2O and then MeOH) to give a residue (17.3 mg). To a solution of the residue in MeOH/H_2O (2:1; 1 mL) was added $NaIO_4$ (4.4 mg), and the mixture was stirred for 20 min at room temperature. After addtion of $NaBH_4$ (2.5 mg), stirring was continued at room temperature for 30 min. After evaporation of the solvent, the reaction mixture was subjected to a HP-20 column (H_2O and then MeOH) and then a silica gel column ($CHCl_3$/MeOH, 90:10) to give two fractions, **a** (1.6 mg) and **b** (1.6 mg). To a solution of fraction **a** in MeOH (50 μL) were added 2,2-dimethoxypropane (50 μL) and TsOH·H_2O (0.1 mg), and the mixture was stirred at room temperature for 10 min. After addition of saturated aqueous $NaHCO_3$, the reaction mixture was extracted with EtOAc. The organic layer was washed with H_2O and brine and evaporated in vacuo. The residue was purified by preparative TLC (hexane/acetone, 6:4) to afford compounds **17** (0.49 mg) and **18** (0.32 mg). Fraction **b** was treated with 6 M HCl (0.16 μL) in MeOH (200 μL) at 100 °C for 4 h. After concentration, the residue was treated with 2,2-dimethoxypropane (50 μL) and TsOH·H_2O (0.1 mg) in MeOH (50 μL) at room temperature for 1 h. After addition of saturated aqueous $NaHCO_3$, the reaction mixture was extracted with EtOAc. The organic layer was washed with H_2O and brine and evaporated in vacuo. The residue was subjected to a silica gel column (hexane/EtOAc, 3:1) to afford compound **19** (0.1 mg).

9,13-Acetal Derivative of 18 (20). To a solution of **18** (0.77 mg) in CH_2Cl_2 (50 μL) were added Dess−Martin periodinane (4.0 mg) and $NaHCO_3$ (1.2 mg) at room temperature, and the mixture was stirred in a shield tube at 37 °C for 16 h. After addition of saturated aqueous $NaHCO_3$, the mixture was extracted with EtOAc. The organic phase was washed with H_2O and brine and evaporated. The residue was treated with AcOH (10 μL) in MeOH (80 μL) at 40 °C for 210 min. After the solvent was removed under a N_2 stream, the residue was separated by preparative TLC (hexane/acetone, 6:4) and afforded **20** (0.15 mg) as a colorless oil: ^{1}H NMR (C_6D_6) δ 0.85 (3H, d, J = 7.0 Hz, H_3-38), 1.19 (1H, m, H-14), 1.23 (1H, dt, J = 13.1 and 5.0 Hz, H-11β), 1.31 (1H, m, H-12), 1.40 (1H, ddt, J = 13.8, 4.7, and 2.4 Hz, H-10α), 1.57 (1H, dd, J = 5.0 and 14.8 Hz, H-8), 1.62 (1H, dt, J = 4.6 and 13.8 Hz, H-10β), 1.69 (1H, m, H-14), 1.92 (1H, dd, J = 6.0 and 14.7 Hz, H-8), 1.96 (1H, dd, J = 7.2 and 14.4 Hz, H-4α), 2.05 (1H, m, H-2), 2.05 (1H, m, H-6α), 2.10 (1H, m, H-11α), 2.17 (1H, bd, J = 4.9 and 14.4 Hz, H-4β), 2.24 (1H, dd, J = 5.0 and 14.2 Hz, H-6β), 2.43 (1H, dd, J = 8.2 and 15.3 Hz, H-2), 3.14 (3H, s, 9-OCH_3), 3.35 (3H, s, 1-OCH_3), 3.60 (1H, m, H-15), 3.63 (1H, m, H-15), 3.83 (1H, dt, J = 10.0 and 2.7 Hz, H-13), 4.20 (1H, m, H-7), 4.36 (1H, m, H-3); ESIMS m/z 381 $(M + Na)^+$; HRESIMS m/z 381.1892 $(M + Na)^+$, calcd for $C_{18}H_{30}O_7Na$ 381.1889.

Hydrolysis of 2. **2** (30 mg) was dissolved in H_2O (500 μL), and the mixture was heated at 110 °C for 9 h. The mixture was purified by C_{18} HPLC to afford an aglycon (**3**; 3.2 mg) and **4** (1.0 mg). Data for **3**: colorless solid; $[\alpha]_D^{23}$ +18° (*c* 0.56, MeOH); ^{1}H and ^{13}C NMR (Table 1); FABMS m/z 867 $(M + H)^+$ and 889 $(M + Na)^+$; HRFABMS m/z 867.5356 $(M + H)^+$, calcd for $C_{43}H_{79}O_{17}$ 867.5374.

Acknowledgment. We thank Dr. E. Fukushi and Prof. J. Kawabata, Graduate School of Agriculture, Hokkaido University, for measurements of HETLOC spectra and Ms. S. Oka and Miss M. Kiuchi, Center for Instrumental Analysis, Hokkaido University, for ESIMS and FABMS measurements. This work was partly supported by a Grant-in-Aid from the Takeda Science Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Supporting Information Available: Data of **7**, **8**, **9**, **11**, **12**, **13**, **14b**, **15a**, **15b**, **16b**, **17**, **18**, **19**, and the MTPA esters of **17** and NMR spectra of **1**, **3**, **10**−**13**, **19**, and **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO035773V