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ACYLATED CHOLESTANE GLYCOSIDES FROM THE BULBS OF *ORNITHOGALUM SAUNDERSIAE*

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Key Word Index—*Ornithogalum saundersiae*; Schilloideae; Liliaceae; bulbs; acylated cholestane glycosides; cyclic AMP phosphodiesterase; inhibitory activity.

Abstract—Phytochemical examination of the bulbs of *Ornithogalum saundersiae* led to the isolation of three new acylated cholestane glycosides. Their structures were elucidated, on the basis of the spectroscopic data and chemical evidences, and by comparing them with those of known compounds, as 3β,16β,17α-trihydroxycholest-5-en-22-one 16-*O*-β-D-xylopyranosyl-(1→3)-(2-*O*-acetyl-α-L-arabinopyranoside), 3β,16β,17α-trihydroxycholest-5-en-22-one 16-*O*-(2-*O*-4-methoxybenzoyl-β-D-xylopyranosyl)-(1→3)-(2-*O*-acetyl-α-L-arabinopyranoside) and 3β,16β,17α-trihydroxycholest-5-en-22-one 16-*O*-(2-*O*-3,4-dimethoxybenzoyl-β-D-xylopyranosyl)-(1→3)-(2-*O*-acetyl-α-L-arabinopyranoside). Inhibitory activity on cyclic AMP phosphodiesterase of the cholestane glycosides was evaluated.

INTRODUCTION

The genus *Ornithogalum* with some 150 species belongs to the subfamily Schilloideae in Liliaceae [1]. Several cardenolide glycosides have been found in some species of *Ornithogalum* [2, 3]. We have shown the bulbs of *O. thyrsoides* to be devoid of cardenolide glycosides and to contain cholestane bisdesmosides as the characteristic constituents [4]. We have now carried out a phytochemical screening of the bulbs of *O. saundersiae*, on which no previous chemical study has been done. This resulted in the isolation of three new acylated cholestane glycosides (**1–3**). We describe the structural elucidation and inhibitory activity on cyclic AMP phosphodiesterase of the cholestane glycosides.

RESULTS AND DISCUSSION

Compounds **1–3** were obtained as amorphous powders and their molecular formulae shown by negative-ion FAB mass spectrometry and ^{13}C NMR spectroscopy to be $C_{39}H_{62}O_{13}$, $C_{47}H_{68}O_{15}$ and $C_{48}H_{70}O_{16}$, respectively.

The glycosidic nature of **1** was indicated by the strong absorption bands at 3450 and 1045 cm^{-1} in the IR spectrum. The presence of a carbonyl group in the molecule was shown by the IR (1695 cm^{-1}) and ^{13}C NMR (δ218.9) spectra, and the presence of an acetyl group by the IR (1740 cm^{-1}), ^{1}H NMR [δ2.34 (3H, s)] and ^{13}C NMR (δ170.0 and 21.5) spectra. Acid hydrolysis of **1** with 1 M hydrochloric acid (dioxane–H_2O, 1:1) gave D-xylose and L-arabinose as the carbohydrate compounds, the absolute configurations of which were confirmed by HPLC analysis of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the sugars [5–8]. The aglycone decomposed under acid conditions. Alkaline treatment of **1** with 3% sodium methoxide in methanol yielded a deacetyl derivative, $C_{37}H_{60}O_{12}$ (**1a**), of **1**. The ^{1}H NMR spectrum of **1a** contained signals for two tertiary methyl protons at δ1.08 and 0.94 (each *s*), three secondary methyl protons at δ1.32 (*d*, *J* = 7.4 Hz), 0.92 (*d*, *J* = 6.4 Hz) and 0.87 (*d*, *J* = 6.3 Hz), two anomeric protons at δ5.16 (*d*, *J* = 7.5 Hz) and 4.49 (*d*, *J* = 5.8 Hz), and an olefinic proton at δ5.38 (*br d*, *J* = 4.2 Hz). The ^{13}C NMR spectrum of **1a** gave 27 carbons for the aglycone residue and 10 carbons for the disaccharide composed of D-xylose and L-arabinose. Comparison of the ^{13}C signals due to the sugars with those of reference methyl glycosides [9] allowed us to assign the structure of the disaccharide as β-D-xylopyranosyl-(1→3)-α-L-arabinopyranose through the glycosylation-induced shift. The 27 carbons due to the aglycone were readily separated in five methyl, nine methylene, eight methine and five quaternary carbon groups with the help of the DEPT spectra. The signals at δ219.5 (C), 141.9 (C) and 121.2 (CH), 88.9 (CH), 86.2 (C) and 71.3 (CH) were assigned to a carbonyl carbon, a pair of olefinic carbons, and carbons bearing oxygen functions, respectively. The above data were indicative of the fundamental structure of **1a** being a cholestene derivative with a carbonyl group and three hydroxyl groups, one of which bears the disaccharide moiety. The substituted positions of the functional groups were clarified by the following spectral data. The presence of a 3β-hydroxy-5-ene group in **1a** was readily recognized by the agreement of the ^{1}H signal due to the H-19 methyl proton and ^{13}C signals due to the A and B ring carbons between **1a** and cholesterol. The downfield-shifted ^{13}C signal at δ88.9, which it seemed pertinent to assign to the glycosyloxymethine carbon of the five-membered ring (D-ring) of the cholestane skeleton, showed a correlation with the methine proton signal at δ4.22 (*dd*, *J* = 7.9, 5.1 Hz) in the ^{1}H–^{13}C COSY spectrum. A NOE correlation was observed between the anomeric proton signal of the arabinose at δ4.49 (*d*, *J* = 5.8 Hz) and the methine proton signal

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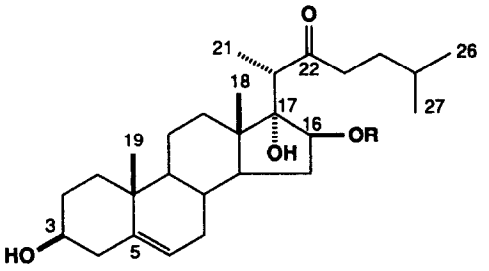

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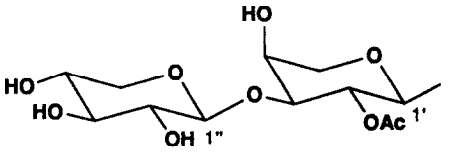
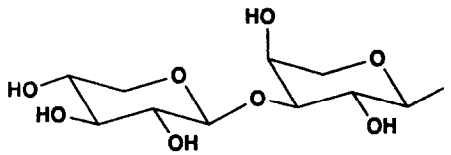


1a



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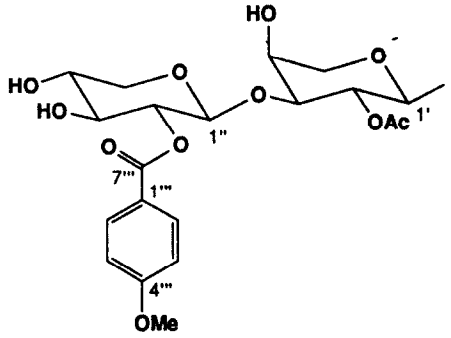

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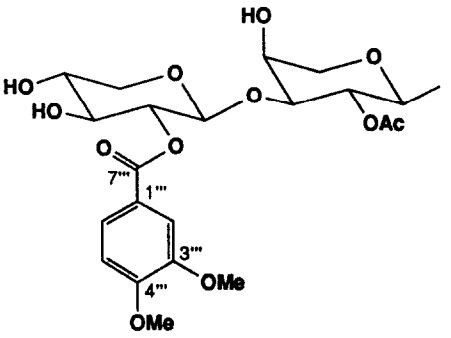

at δ4.22 in the phase-sensitive NOESY (PHNOESY) spectrum of **1a**. The methine proton was shown to couple with the adjacent methylene protons at δ2.42 (*ddd*, J = 11.7, 7.9, 7.5 Hz) and 1.92 (*br dd*, J = 11.7, 5.1 Hz) in the ^{1}H–^{1}H COSY spectrum. The signal at δ2.42 showed a NOE correlation in the PHNOESY spectrum with the signal at δ 4.22, and the signal at δ 1.92 with the signal at δ0.94 due to the H-18 methyl proton. The ^{1}H signal at δ1.32 was assigned to the H-21 secondary methyl proton and the signal at δ3.40 (*q*, J = 7.4 Hz) to the H-20 methine proton. The H-20 methine proton was shown to couple only with the H-21 methyl proton in the ^{1}H–^{1}H COSY spectrum, indicating that the C-17 and C-22 carbons possess no hydrogen. Furthermore, the H-20 methine proton signal showed a NOE correlation with the H-18 methyl proton signal. Thus, the existence of a 16β-glycosyloxy group, a 17α-hydroxyl group and a 22-carbonyl group was evident (Fig. 1). The ^{1}H NMR chemical shift of the H-18 methyl group (δ0.94) (that of cholesterol appeared at δ0.70 in pyridine-d_5) was consistent with the orientation of the C-16 substituted group being β [10, 11]. The presence of the 17α-hydroxyl group was also supported by comparison of the ^{13}C NMR spectrum of **1a** with that of (22*S*)-cholest-5-en-3β,16β,22-triol 16-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-galactopyranoside (schubertoside B) [12]. The signal arising from the C-12 and C-14 of **1a** were displaced upfield by 4.0 and 6.6 ppm, respectively, while the C-13 was moved downfield by 4.0 ppm as compared with those of schubertoside B. The ^{13}C NMR chemical shifts due to the side-chain carbons were assigned as shown in Table 1; the signal due to C-23 was shifted downfield by 8.6 ppm and the signal due to C-24 upfield by 7.2 ppm as compared with those of cholesterol, which was consistent with the presence of a 22-carbonyl group. Accordingly, the structure of **1a** was determined to be 3β,16β,17α-trihydroxycholest-5-en-22-one 16-*O*-β-D-xylopyranosyl-(1→3)-α-L-arabinopyranoside.

The position of attachment of the acetyl group in **1** was proved to be C-2 of the α-L-rhamnopyranosyl moiety by the following data. In the ^{1}H NMR spectrum of **1**, the signal due to arabinose H-2 appeared at δ5.80 (*dd*, J = 8.2, 6.5 Hz), and was shifted downfield by 1.33 ppm as compared with that of **1a**. Furthermore, the ^{13}C signals due to the arabinose C-1 and C-3 of **1** was shifted upfield by 4.0 ppm and 3.7 ppm, respectively, as compared with those of **1a**. From the data presented above, the structure of **1** was elucidated as 3β,16β,17α-trihydroxycholest-5-en-22-one 16-*O*-β-D-xylopyranosyl-(1→3)-(2-*O*-acetyl-α-L-arabinopyranoside).

The spectral data of **2** were essentially analogous with those of **1** and suggestive of a cholestane glycoside of the same type. The existence of a 4-methoxybenzoyl group in addition to an acetyl group in the molecule was indicated by the IR [1690 cm^{-1} (C=O); 1600 and 1510 cm^{-1} (aromatic ring)], UV (λ_{max} 259 nm, log ε 3.90), ^{1}H NMR [δ8.32 and 7.08 (each 2H, *d*, J = 8.9 Hz); δ3.75 (3H, *s*)] and ^{13}C NMR [δ123.0 (C), 132.4 (CH) × 2, 114.2 (CH) × 2, 163.9 (C), 165.5 (C) and 55.5 (Me)] spectra. Alkaline hydrolysis of **2** with 4% potassium hydroxide in ethanol yielded **1** and 4-methoxybenzoic acid. In the ^{1}H NMR spectrum of **2**, the signals due to the arabinose H-2 and xylose H-2 were shifted downfield by 1.07 and 1.68 ppm, respectively, as compared with those of **1a** to appear at δ5.54 (*dd*, J = 7.9, 6.0 Hz) and 5.67 (*dd*, J = 8.9, 7.6 Hz). Furthermore, the ^{13}C signal due to the arabinose C-1 and C-3, and xylose C-1 and C-3 were shifted upfield by 4.6 and 2.8, and 3.2 and 3.0 ppm, respectively, as compared with those of **1a**. Mild treatment of **2** with 10% ammonia solution in methanol–water (2:1) furnished **1** as a partial hydrolysis product. The above data clearly accounted for linkage of the acetyl moiety to the C-2 hydroxyl of arabinose, and the 4-methoxybenzoyl moiety to the C-2

Table 1. ^{13}C NMR spectral data for compounds **1**, **1a**, cholesterol, schubertoside B, **2** and **3** (in pyridine-d_5)

C	1	1a	Cholesterol	Schubertoside B*	2	3
1	37.8	37.8	37.9	37.8	37.8	37.8
2	32.3	32.3	32.3	32.1	32.3	32.3
3	71.3	71.3	71.3	71.3	71.3	71.3
4	43.5	43.5	43.5	43.5	43.5	43.5
5	142.0	141.9	142.0	142.0	142.0	142.0
6	121.1	121.2	121.2	121.3	121.1	121.1
7	32.6	32.5	32.7	32.7	32.7	32.7
8	32.1	32.2	32.2	31.9	32.1	32.1
9	50.2	50.2	50.6	50.6	50.2	50.2
10	36.9	36.9	37.0	36.9	36.9	36.9
11	21.0	21.0	21.4	21.2	21.0	21.0
12	35.0	36.1	40.1	40.1	34.7	34.7
13	46.6	46.5	42.6	42.5	46.6	46.6
14	48.6	48.7	57.0	55.3	48.6	48.6
15	39.5	39.5	24.6	37.1	39.3	39.3
16	88.4	88.9	28.5	82.5	88.4	88.4
17	85.8	86.2	56.5	58.0	85.7	85.8
18	13.6	13.7	12.1	12.8	13.6	13.7
19	19.6	19.6	19.7	19.7	19.6	19.6
20	46.4	46.2	36.1	36.0	46.3	46.4
21	11.9	12.2	19.0	13.5	11.9	11.9
22	218.9	219.5	36.6	72.1	218.9	218.9
23	32.9	32.8	24.2	33.4	32.7	32.7
24	32.8	32.6	39.8	36.8	32.7	32.7
25	27.9	27.9	28.3	28.8	27.8	27.7
26	22.8	23.0	23.0	23.1	22.8	22.8
27	22.6	22.6	22.7	23.1	22.5	22.4
1′	101.4	105.4			100.8	100.8
2′	72.2	71.7			72.1	72.1
3′	80.0	83.7			80.9	80.9
4′	68.6	68.8			67.8	67.7
5′	66.4	67.0			65.5	65.4
1″	106.6	106.8			103.6	103.6
2″	74.2	75.1			76.3	76.3
3″	78.2	78.2			75.2	75.3
4″	70.9	71.0			70.7	70.7
5″	67.2	67.3			67.0	67.0
Ac	170.0				169.3	169.3
	21.5				20.9	20.9
1‴					123.0	†
2‴					132.4	113.7
3‴					114.2	†
4‴					163.9	154.1
5‴					114.2	111.3
6‴					132.4	124.4
7‴					165.5	165.6
OMe					55.5	55.9 × 2

*Data for the sugar moiety are omitted.

†Signals are unclear due to overlapping with solvent signals.

of xylose. Thus, the structure of **2** was elucidated as 3β,16β,17α-trihydroxycholest-5-en-22-one 16-*O*-(2-*O*-4-methoxybenzoyl-β-D-xylopyranosyl)-(1→3)-(2-*O*-acetyl-α-L-arabinopyranoside).

The spectral data of **3** were almost identical with those of **2**. In the ^{1}H NMR spectra of **2** and **3**, the aromatic proton signals observed as an AA′BB′ system in **2** were replaced by the signals of an ABC system [δ8.05 (1H, *dd*, J=8.4, 1.7 Hz), 7.93 (1H, *d*, J=1.7 Hz) and 7.06 (1H, *d*, J=8.4 Hz)]. The ^{13}C NMR spectrum of **3** was completely superimposable on that of **2**, except for the aromatic region signals due to the substituted benzoyl moiety. Alkaline hydrolysis of **3** yielded **1** and 3,4-dimethoxybenzoic acid. Thus, the structure of **3** was determined to be 3β,16β,17α-trihydroxycholest-5-en-22-one 16-*O*-(2-*O*-3,4-dimethoxybenzoyl-β-D-xylopyranosyl-(1→3)-(2-*O*-acetyl-α-L-arabinopyranoside). Compounds **1**–**3** and the deacyl derivative (**1a**) prepared by alkaline treatment are

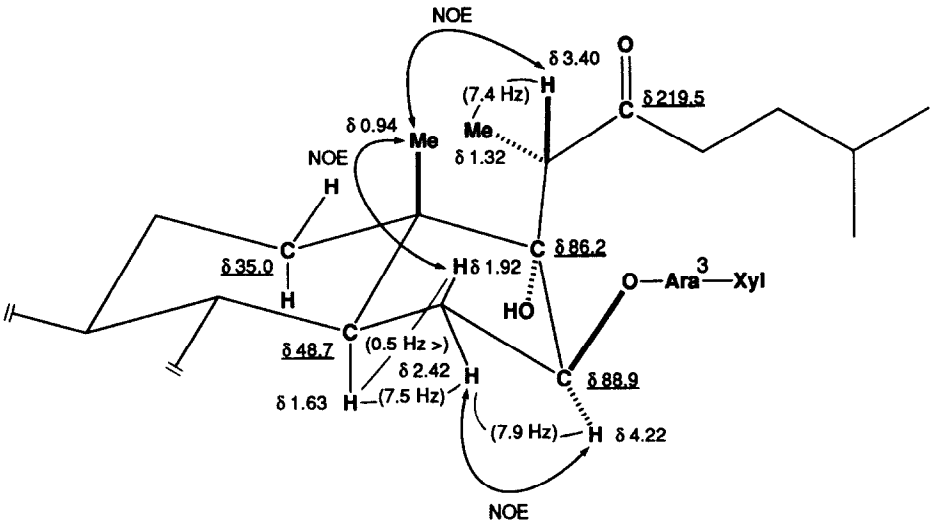

Fig. 1. ^1H and ^{13}C NMR (underlined figures) chemical shifts, ^1H–^1H spin-coupling constants, and NOE correlations of **1a** in pyridine-d_5.

Table 2. Inhibitory activity on cyclic AMP phosphodiesterase of compounds **1**, **1a**, **2**, **3** and papaverine

Compound	IC_{50} ($\times 10^{-5}$ M)
1	—
1a	23.0
2	5.5
3	0.5
Papaverine	3.0

new cholestane glycosides. The inhibitory activity of the glycosides on cyclic AMP phosphodiesterase was examined (Table 2) [13, 14]. Compounds **2** and **3** showed considerable inhibitory activity. Benzoyl derivative attached to the sugar moiety seems to enhance the activity.

EXPERIMENTAL

General. NMR: 1D (Bruker AM-400) and 2D (Bruker AM-500); CC: silica gel (Fuji Davison), ODS (Nacalai Tesque) and Sephadex LH-20 (Pharmacia); TLC: precoated Kieselgel 60 F_{254} (0.25 mm or 0.5 mm thick, Merck) and RP-18 $F_{254}S$ (0.25 mm thick, Merck); HPLC: a Tosoh HPLC system (pump, CCPM; controller, CCP controller PX-8010; detector, RI-8010 or UV-8000) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo Kasei Kogyo, 10 mm i.d. × 250 mm, ODS, 5 μm or 4.6 mm i.d. × 250 mm, ODS, 5 μm).

Extraction and isolation. Fresh bulbs of *O. saundersiae* (16.2 kg) purchased from Heiwaen, Japan, were cut into pieces and extracted with MeOH under reflux. The extract was concd to almost dryness under red. pres., and the crude residue, after diln with H_2O, was extracted with *n*-BuOH. The *n*-BuOH-sol. phase was fractionated on a silica gel column with a gradient mixt. of $CHCl_3$–MeOH. Frs with the same TLC profile were combined. Four frs (I–IV) were recovered. Fr. I was further separated by silica gel CC with $CHCl_3$–MeOH (9:1, 6:1, 2:1) into 2 frs (Ia and b). Fr. Ia was submitted to ODS CC with MeOH–H_2O (4:1) and to prep. HPLC with MeOH–H_2O (12:1, 9:1) and MeCN–H_2O (4:1) to yield **1** (25 mg), **2** (439 mg) and **3** (23.5 mg). Further analysis of other frs is now under way.

Compound **1**. $[\alpha]_D^{25}$ −35.7° (MeOH; *c* 0.50). Negative-ion FAB-MS *m/z* 737 $[M-H]^-$, 695 $[M-Ac]^-$; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2975, 2950, 2930 and 2895 (CH), 1740 and 1695 (C=O), 1470, 1380, 1260, 1240, 1170, 1135, 1045, 970, 895, 805, 785, 700; ^1H NMR (pyridine-d_5): δ 5.80 (1H, *dd*, *J* = 8.2, 6.5 Hz, H-2′), 5.39 (1H, *br d*, *J* = 4.2 Hz, H-6), 4.90 (1H, *d*, *J* = 7.4 Hz, H-1″), 4.65 (1H, *d*, *J* = 6.5 Hz, H-1′), 4.44 (1H, *br s*, H-4′), 4.31 (1H, *dd*, *J* = 11.1, 4.8 Hz, H-5″a), 4.26 (1H, *dd*, *J* = 12.3, 3.8 Hz, H-5′a), 4.23 (1H, *dd*, *J* = 8.4, 5.4 Hz, H-16), 4.22 (1H, *dd*, *J* = 8.2, 3.6 Hz, H-3′), 4.13 (1H, *ddd*, *J* = 9.9, 8.6, 4.8 Hz, H-4″), 4.07 (1H, *dd*, *J* = 8.6, 8.6 Hz, H-3″), 3.85 (1H, *dd*, *J* = 8.6, 7.4 Hz, H-2″), 3.81 (1H, *m*, H-3), 3.78 (1H, *br d*, *J* = 12.3 Hz, H-5′b), 3.68 (1H, *dd*, *J* = 11.1, 9.9 Hz, H-5″b), 3.34 (1H, *q*, *J* = 7.4 Hz, H-20), 2.83 (2H, *t*-like, *J* = 7.1 Hz, H_2-23), 2.34 (3H, *s*, Ac), 1.32, (3H, *d*, *J* = 7.4 Hz, H-21), 1.09 (3H, *s*, H-19), 0.98 (3H, *s*, H-18), 0.96 (3H, *d*, *J* = 6.1 Hz, H-26 or H-27), 0.92 (3H, *d*, *J* = 6.0 Hz, H-26 or H-27).

Acid hydrolysis of compound **1** *and determination of the absolute configurations of sugars.* A soln of **1** (15.0 mg) in 1 M HCl (dioxane–H_2O, 1:1) was heated at 100° for 1 hr under a N_2 atmos. The reaction mixt. was neutralized by passage through an Amberlite IRA-93ZU (Organo) column and subjected to silica gel CC with $CHCl_3$–MeOH (19:1) and $CHCl_3$–MeOH–H_2O (20:10:1) to yield uncharacterized compounds and a mixt. of xylose and arabinose (4.0 mg). The sugar mixt. was treated with (−)-α-methylbenzylamine (0.4 ml) and $Na[BH_3CN]$ (15 mg) at 40° for 4 hr, followed by acetylation with Ac_2O in pyridine containing a catalytic amount of 4-(dimethylamino)pyridine (5 mg). The reaction mixt. was concd. under red. pres. and passed through a Sep-Pak C_{18} cartridge (Waters) with H_2O–EtOH (4:1) and then with EtOH. The EtOH eluate was purified by prep. TLC with hexane–EtOH (19:1) to give a mixt. of 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetates of xylose and arabinose, which was analysed by HPLC under the following conditions: column, Kaseisorb LC ODS-120-5 (4.6 mm, i.d. × 250 μm, ODS, 5 mm); solvent, MeCN–H_2O (2:3); flow rate, 0.8 ml min^{-1}; detection, UV (230 nm). R_t (min): L-arabinose derivative, 21.6; D-xylose derivative, 23.0.

Alkaline hydrolysis of compound **1**. Compound **1** (7.5 mg) in 3% NaOMe in MeOH was kept at ambient temp. for 1 hr. The reaction mixt. was neutralized by passage through an Amberlite IR-120B column and purified by Sephadex LH-20 CC with MeOH to produce **1a** (5.0 mg). Compound **1a**: $[\alpha]_D^{25}$ −29.6° (MeOH; *c*0.25). Negative-ion FAB-MS *m/z* 695 $[M-H]^-$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2960, 2940 and 2880 (CH), 1685 (C=O), 1465, 1385, 1340, 1260, 1165, 1135, 1075, 1045, 970, 795, 700; ^1H NMR (pyridine-d_5): δ5.38 (1H, *br d*, *J* = 4.2 Hz, H-6), 5.16 (1H, *d*, *J* = 7.5 Hz, H-1″), 4.49 (1H, *d*, *J* = 5.8 Hz, H-1′), 4.47 (1H, *dd*, *J* = 7.4, 5.8 Hz, H-2′), 4.42 (1H, *br s*, H-4′), 4.35 (1H, *dd*, *J* = 11.3, 4.9 Hz, H-5″a), 4.25 (1H, *dd*, *J* = 11.1, 2.8 Hz, H-5′a), 4.22 (1H, *dd*, *J* = 7.9, 5.1 Hz, H-16), 4.18 (1H, *ddd*, *J* = 9.8, 8.5, 4.9 Hz, H-4″), 4.12 (1H, *dd*, *J* = 8.5, 8.5 Hz, H-3″), 4.09 (1H, *dd*, *J* = 7.4, 4.3 Hz, H-3′), 3.99 (1H, *dd*, *J* = 8.5, 7.5 Hz, H-2″), 3.82 (1H, *m*, H-3), 3.73 (1H, *br d*, *J* = 11.1 Hz, H-5′b), 3.71 (1H, *dd*, *J* = 11.3, 9.8 Hz, H-5″b), 3.40 (1H, *q*, *J* = 7.4 Hz, H-20), 3.33 (1H, *ddd*, *J* = 18.3, 9.5, 5.2 Hz, H-23a), 2.76 (1H, *ddd*, *J* = 18.3, 9.5, 5.5 Hz, H-23b), 2.42 (1H, *ddd*, *J* = 11.7, 7.9, 7.5 Hz, H-15a), 1.92 (1H, *br dd*, *J* = 11.7, 5.1 Hz, H-15b), 1.32 (3H, *d*, *J* = 7.4 Hz, H-21), 1.08 (3H, *s*, H-19), 0.94 (3H, *s*, H-18), 0.92 (3H, *d*, *J* = 6.4 Hz, H-26 or H-27), 0.87 (3H, *d*, *J* = 6.3 Hz, H-26 or H-27).

Compound **2**. $[\alpha]_D^{25}$ −43.2° (MeOH; *c*0.25). Negative-ion FAB-MS *m/z* 871 $[M-H]^-$, 735 $[M-4\text{-methoxybenzoyl}-2H]^-$; UV λ_{max}^{MeOH} nm (log ε): 259 (3.90); IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 2950, 2905 and 2875 (CH), 1740, 1715 and 1690 (C=O), 1600 and 1510 (aromatic ring), 1460, 1365, 1315, 1255, 1170, 1130, 1070, 1040, 985, 970, 840, 760, 695; ^1H NMR (pyridine-d_5): δ8.32 (2H, *d*, *J* = 8.9 Hz, H-2‴ and H-6‴), 7.08 (2H, *d*, *J* = 8.9 Hz, H-3‴ and H-5‴), 5.67 (1H, *dd*, *J* = 8.9, 7.6 Hz, H-2″), 5.54 (1H, *dd*, *J* = 7.9, 6.0 Hz, H-2′), 5.38 (1H, *br d*, *J* = 3.9 Hz, H-6), 5.12 (1H, *d*, *J* = 7.6 Hz, H-1″), 4.58 (1H, *d*, *J* = 6.0 Hz, H-1′), 3.75 (3H, *s*, OMe), 3.20 (1H, *q*, *J* = 7.4 Hz, H-20), 1.97 (3H, *s*, Ac), 1.29 (3H, *d*, *J* = 7.4 Hz, H-21), 1.09 (3H, *s*, H-19), 1.03 (3H, *s*, H-18), 0.88 (3H, *d*, *J* = 6.3 Hz, H-26 or H-27), 0.86 (3H, *d*, *J* = 6.3 Hz, H-26 or H-27).

Alkaline hydrolysis of compound **2**. Compound **2** (70.0 mg) was treated with 4% KOH in EtOH at ambient temp. for 1 hr. The reaction mixt. was neutralized by passage through an Amberlite IR-120B column and purified by Sephadex LH-20 CC with MeOH to yield **1a** (35.0 mg) and 4-methoxybenzoic acid (4.4 mg), which were identified by the ^1H NMR spectra.

Mild hydrolysis of compound **2**. Compound **2** (30.0 mg) was treated with 10% aq. NH_3 in MeOH–H_2O (2:1) at ambient temp. for 3 hr. The reaction soln was evapd under red. pres. and the crude product was chromatographed on silica gel with $CHCl_3$–MeOH (9:1, 6:1) to yield **1** (13.7 mg), which was identified by the ^1H NMR spectrum.

Compound **3**. $[\alpha]_D^{25}$ −37.7° (MeOH; *c* 0.50). Negative-ion FAB-MS *m/z* 901 $[M-H]^-$, 734 $[M-3,4\text{-dimethoxybenzoyl}-2H]^-$; UV λ_{max}^{MeOH} nm (log ε): 293 (3.67), 262 (3.95); IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 2950, 2925 and 2885 (CH), 1720 and 1695 (C=O), 1605 and 1515 (aromatic ring), 1465, 1420, 1370, 1345, 1270, 1225, 1180, 1135, 1045, 970, 880, 800, 760; ^1H NMR (pyridine-d_5): δ8.05 (1H, *dd*, *J* = 8.4, 1.7 Hz, H-6‴), 7.93 (1H, *d*, *J* = 1.7 Hz, H-2‴), 7.06 (1H, *d*, *J* = 8.4 Hz, H-5‴), 5.70 (1H, *dd*, *J* = 8.9, 7.6 Hz, H-2″), 5.54 (1H, *dd*, *J* = 7.8, 5.8 Hz, H-2′), 5.39 (1H, *br d*, *J* = 4.2 Hz, H-6), 5.14 (1H, *d*, *J* = 7.6 Hz, H-1″), 4.58 (1H, *d*, *J* = 5.8 Hz, H-1′), 3.82 and 3.81 (each 3H, *s*, OMe), 3.21 (1H, *q*, *J* = 7.4 Hz, H-20), 2.00 (3H, *s*, Ac), 1.31 (3H, *d*, *J* = 7.4 Hz, H-21), 1.08 (3H, *s*, H-19), 1.02 (3H, *s*, H-18), 0.88 (3H, *d*, *J* = 6.0 Hz, H-26 or H-27), 0.86 (3H, *d*, *J* = 6.0 Hz, H-26 or H-27).

Alkaline hydrolysis of compound **3**. Compound **3** (15.0 mg) was treated with 4% KOH in EtOH as for **2** to yield **1a** (5.0 mg) and 3,4-dimethoxybenzoic acid (1.0 mg), which were identified by the ^1H NMR spectra.

Assay of cyclic AMP phosphodiesterase activity. The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described in the previous paper [13].

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