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STEROIDAL ALKALOIDS FROM THE BULBS OF *FRITILLARIA PERSICA*

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Key Word Index—*Fritillaria persica*; Liliaceae; bulbs; steroidal alkaloids; cyclic AMP phosphodiesterase; inhibitory activity.

Abstract—The methanolic extract of the fresh bulbs of *Fritillaria persica* has yielded five new steroidal alkaloids. Their structures have been shown by spectral analysis and chemical evidence to be (25*R*)-22,26-epimino-3β-hydroxy-5α-cholest-22(*N*)-en-6-one 3-*O*-β-D-glucopyranoside; (25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-ene-6,22-dione and its 3-*O*-glucoside, and their C-20 epimers. The inhibitory activity of the alkaloids on cyclic AMP phosphodiesterase was examined.

INTRODUCTION

Fritillaria species have been extensively investigated and a large number of steroidal alkaloids have been isolated [1, 2]. Bulbs of some *Fritillaria* plants are used in traditional Chinese medicine [3]. *Fritillaria persica* L. native to Cyprus, southern Turkey and Iran is one of the tallest species with large bulbs [4]; nothing has been published so far on its constituents. The chemical compounds in the bulbs have been analysed as a part of a systematic study of the Liliaceae plants, resulting in the isolation of five new steroidal alkaloids. We report the structural elucidation of the alkaloids and their inhibitory activity on cyclic AMP phosphodiesterase.

RESULTS AND DISCUSSION

The fresh bulbs of *F. persica* were extracted with hot methanol. The crude extract was partitioned between 1-butanol and water. A series of chromatographic separations of the 1-butanol-soluble phase gave compounds **1**–**5**, which presented a positive Dragendorff reaction on TLC, indicative of alkaloids.

The molecular formula of **1**, $C_{33}H_{53}NO_7$, was confirmed from an accurate $[M]^+$ peak at *m/z* 575.3827 (calcd: 575.3824) in the EI mass spectrum. The presence of a β-D-glucopyranosyl unit in the molecule was demonstrated by the appearance of an anomeric proton signal at δ 5.02 (*d*, $J=7.6$ Hz) in the ^{1}H NMR spectrum and also by characteristic ^{13}C NMR signals at δ 102.2, 75.3, 78.6, 71.9, 78.5 and 63.0 (Table 1). Acid hydrolysis of **1** with 1 M hydrochloric acid yielded D-glucose and a steroidal alkaloid (**1a**), $C_{27}H_{43}NO_2$. The ^{1}H NMR spectrum of **1a** showed two three-proton singlet signals at δ 0.78 and 0.75, indicating the presence of two angular methyl groups, two three-proton doublet signals at δ 1.00 ($J=6.9$ Hz) and 0.85 ($J=6.6$ Hz) attributable to two secondary methyl groups, and a multiplet signal centred at δ 3.84 ($W_{1/2}=21.2$ Hz), which may be assigned to the α-hydrogen adjacent to the 3β-hydroxy group. Evidence for the presence of a carbonyl group and a six-membered cyclic azomethine group in **1a** were obtained from the IR [1710 cm^{-1} (C=O); 1658 cm^{-1} (C=N)], UV[λ_{max} 295 nm, log ε 2.18 (C=O); λ_{max} 246 nm, log ε 2.64 (C=N)] and ^{13}C NMR [δ 210.2 (C=O); δ 173.5 (C=N)] [5] spectra. The ^{1}H NMR chemical shift of the 10-methyl group and the ^{13}C NMR signals arising from the A/B rings of **1a** closely corresponded to those of 3β-hydroxy-6-oxo steroidal compounds with the ring junction being A/B *trans* (5α) [6, 7]. The EI mass spectrum of **1a** showed two prominent fragment ion peaks at *m/z* 125 and 111, which are typical of a $\Delta^{22(N)}$-unsaturated side chain moiety [8, 9]. The ion at *m/z* 125 was formed as the result of the cleavage of the C-17/C-20 bond with the migration of a hydrogen from the D-ring. The above data indicated the structure of **1a** to be 22,26-epimino-3β-hydroxy-5α-cholest-22(*N*)-en-6-one. The CD spectrum of **1a**, measured in dioxane, showed a negative Cotton effect at 295 nm due to the C-6 carbonyl group, indicating that **1a** has the normal absolute steroidal stereostructure, and a positive Cotton effect at 255 nm due to the cyclic azomethine group, which was consistent with data for other cyclic azomethines of this type with the 25*R* configuration [10]. Thus, the structure of **1** was elucidated as (25*R*)-22,26-epimino-3β-hydroxy-5α-cholest-22(*N*)-en-6-one 3-*O*-β-D-glucopyranoside.

Compound **2** ($C_{27}H_{41}NO_3$) gave an IR spectrum consistent with the presence of a hydroxy group (3474 cm^{-1}), a carbonyl group (1707 cm^{-1}) and an α,β-unsaturated carbonyl group (1690 cm^{-1}). The ^{1}H NMR chemical shifts of the two angular methyl protons (H-18 and H-19) at δ 0.75 and 0.76 agreed with those of **1a**. Furthermore, the ^{13}C NMR signals arising from the A–D rings were almost superimposable on those of **1a** (Table 1). The prominent fragment ion peak at *m/z* 140 in the EI mass spectrum of **2** was characteristic of the piperidine or pyrrolidine side chain steroidal alkaloids with a carbonyl group [8, 11]. In the ^{1}H NMR spectrum of **2**, a three-proton doublet signal at δ 1.04 ($J=6.9$ Hz) was assigned to the H-27 methyl protons. By tracing out the

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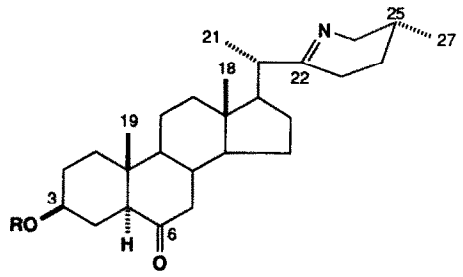
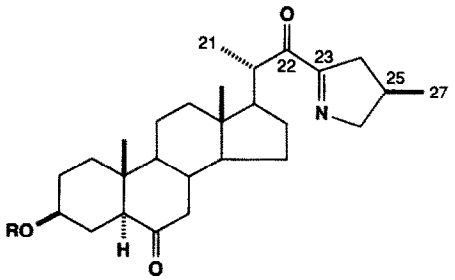
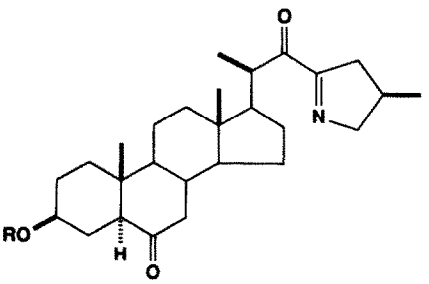


R
1 β-D-Glcp
1a H



R
2 H
3 β-D-Glcp



R
4 H
5 β-D-Glcp

Table 1. ^{13}C NMR spectral data of compounds **1**, **1a** and **2**–**5**

C	1	1a	2	3	4	5
1	36.9	37.2	36.7	36.9	36.7	36.8
2	29.5	31.9	30.7	28.9	30.7	28.8
3	76.8	70.0	70.6	77.9	70.6	77.9
4	27.1	31.3	30.1	26.4	30.0	26.5
5	56.5	57.0	56.8	56.8	56.8	56.8
6	209.7	210.2	210.7	212.1	210.6	212.1
7	46.7	46.8	46.7	46.8	46.6	46.7
8	37.9	38.0	37.9	38.2	37.9	38.2
9	53.9	54.0	53.9	54.1	53.9	54.0
10	41.0	41.0	40.9	41.4	40.8	41.4
11	21.6	21.8	21.5	21.7	21.4	21.6
12	38.1	38.2	39.4	39.5	38.6	38.8
13	43.2	43.2	43.3	43.6	42.6	42.8
14	56.3	56.4	56.0	56.1	55.9	56.0
15	23.9	23.9	24.2	24.4	23.6	23.7
16	27.4	27.4	27.2	27.4	26.3	26.5
17	53.5	53.6	52.3	52.6	53.1	53.4
18	12.1	12.1	12.4	12.4	12.8	13.0
19	13.1	13.3	13.2	13.2	13.1	13.1
20	46.2	46.6	42.6	43.0	40.8	41.1
21	18.1	18.2	16.8	16.9	17.3	17.3
22	173.2	173.5	203.7	204.1	204.4	204.9
23	26.9	26.8	173.9	174.4	173.7	174.1
24	28.0	28.3	41.6	41.9	41.7	41.9
25	27.8	28.0	31.1	31.2	31.1	31.2
26	57.4	57.2	69.9	69.9	69.9	69.9
27	19.4	19.6	20.1	20.1	20.2	20.3
1′	102.2			101.3		101.3
2′	75.3			73.8		73.8
3′	78.6[a]			76.7[a]		76.7[a]
4′	71.9			70.5		70.5
5′	78.5[a]			76.1[a]		76.1[a]
6′	63.0			62.2		62.2

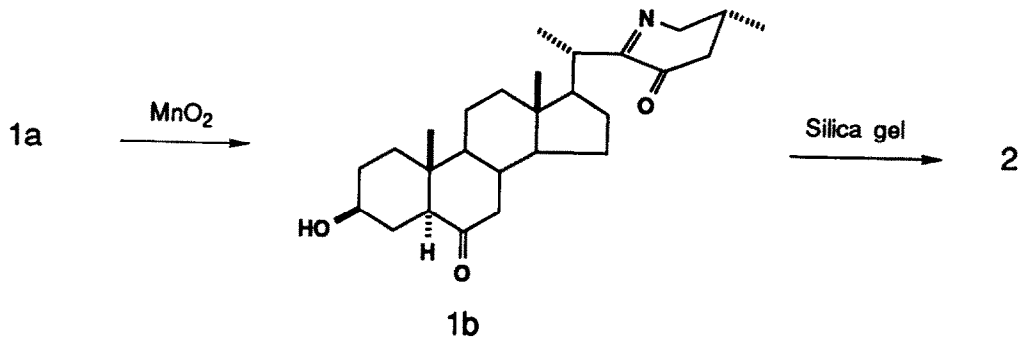
Spectra of **1** and **1a** were measured in pyridine-d_5, and those of **2**–**5** in $CDCl_3$.

[a] Assignments may be interchangeable within each column.

proton–proton coupling systems from the methyl group through the ^1H–^1H COSY spectrum, a partial structure CH_2–CH(Me)–CH_2 was revealed. Another three-proton doublet signal at δ 1.13 (J = 6.9 Hz) was assigned to the H-21 methyl protons, which was shifted to lower field by 0.23 ppm as compared with that of cholesterol. The H-20 methine proton at δ 3.70 was shown to couple only with the H-21 methyl protons and the H-17 methine proton at δ 1.73 in the ^1H–^1H COSY spectrum. The ^{13}C NMR signal at δ 173.9 was due to the carbon of an azomethine group and this was shown to be conjugated with the carbonyl group by the UV spectrum (λ_{max} 344 nm, log ε 1.73; λ_{max} 270 nm, log ε 2.28, overlapping with the absorption of the C-6 carbonyl group) [11]. Thus, the structure of **2** was presumed to be 23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-ene-6,22-dione. Further evidence for the structure of **2** including the C-25 configuration was obtained from the following chemical correlation. Compound **1a** was oxidized with active manganese dioxide in chloroform, in which the 23-oxo compound (**1b**) of **1a** was expected, and the compound was deposited on a plate coated with silica gel for 3 days to yield a product, all the spectral data of which were identical to those of **2**. Thus, the structure of **2** was determined to be (25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-ene-6,22-dione.

Compound **3** ($C_{33}H_{51}NO_8$) was the 3-*O*-β-D-glucoside of **2**, which was shown by the ^1H and ^{13}C NMR spectra and by enzymatic hydrolysis. Therefore, the structure of **3** was determined to be (25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-ene-6,22-dione 3-*O*-β-D-glucopyranoside.

The ^1H NMR spectrum of **4** ($C_{27}H_{41}NO_3$) was identical with that of **2** with the exceptions of the H-17, H-18,



Scheme 1.

H-20 and H-21 resonances; the signal of H-17 was shifted to lower field by 0.16 ppm and the signals of H-18, H-20 and H-21 to upper field by 0.10, 0.06, and 0.09 ppm, respectively. Furthermore, the ^{13}C NMR spectra of **2** and **4** were almost identical, with the only remarkable difference being the C-20 signal which was 1.8 ppm upfield in the latter compound (Table 1). The above data suggested that **4** was the C-20 epimer of **2**, which was confirmed by comparison of the NOESY spectra between **2** and **4**. In both compounds, the NOE correlation between the H-20 signal and H-18 methyl proton signal was observed, but no NOE between the H-20 and the H-17 signals, indicating that H-20 conformationally prefers to lie toward the 13-methyl group. The H-21 signal showed the NOE correlations with the H-12β, H-17, and H-18 signals in **2**, and the H-21 with the H-16α, H-16β, and H-17 in **4** (Fig. 1). Thus, the absolute configuration of **4** at C-20 was unequivocally determined to be *R*. The full structure of **4** is formulated as (20*R*, 25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-ene-6,22-dione.

Compound **5** ($C_{33}H_{51}NO_8$) was the 3-*O*-β-D-glucoside of **4**, which was shown by the ^1H and ^{13}C NMR spectra and by enzymatic hydrolysis. Thus, the structure of **5** was determined to be (20*R*,25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-ene-6,22-dione 3-*O*-β-D-glucopyranoside.

Compounds **1**–**5** are new steroidal alkaloids. The piperidine and pyrrolidine side chain alkaloids occur widely in plants of the genera *Solanum* and *Veratrum*, but are rare in plants of the genus *Fritillaria* [1, 2]. The concentrations of the alkaloids required to give 50% inhibition (IC_{50} values) of cyclic AMP phosphodiesterase were determined as shown in Table 2 [12, 13].

Table 2. Inhibitory activity on cyclic AMP phosphodiesterase of compounds **1**–**5**

	IC_{50} ($\times 10^{-5}$ M)
1	>500
2	10.6
3	67.9
4	21.4
5	17.1

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 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) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo Kasei Kogyo, 1 cm i.d. × 25 cm, ODS, 5 μm) or an HPLC system (pump, Tosoh CCPM-M; controller, Toşoh CCP controller PX-8010; detector, ERMA ERC-7530) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo Kasei Kogyo, 2 cm i.d. × 25 cm, ODS, 5 μm).

Extraction and isolation. Fresh bulbs of *F. persica* L. (7.2 kg) purchased from Heiwaen, Japan, were cut into pieces and extracted with hot MeOH. The *n*-BuOH-soluble phase was fractionated on a silica gel column with a $CHCl_3$–MeOH gradient system to give five frs. Fr. 1 was purified by a silica gel column with EtOAc–$CHCl_3$–hexane (2:1:1) and an ODS col-

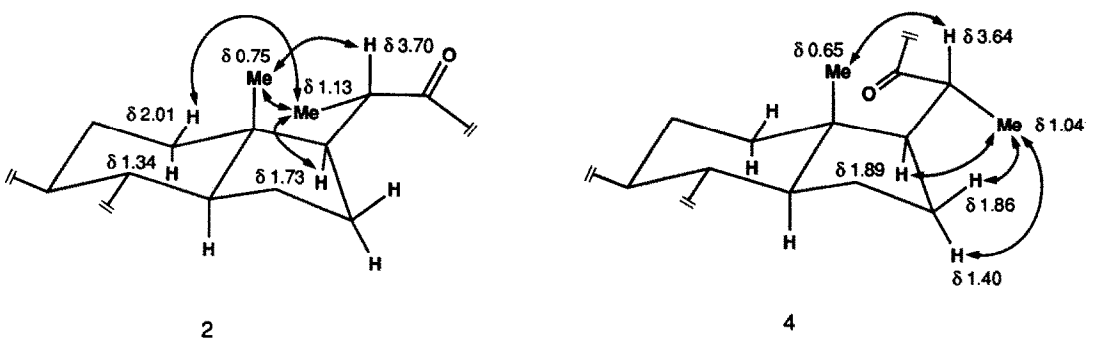


Fig. 1. NOEs of **2** and **4** in $CDCl_3$.

umn with MeCN–H_2O (8:1), and finally by HPLC with MeOH–H_2O–2-methoxyethanol (12:2:1) to yield compounds **2** (76.7 mg) and **4** (114 mg). Fr. 2 was subjected to silica gel CC with $CHCl_3$–MeOH–28% NH_3 soln (90:10:1), Sephadex LH-20 CC with $CHCl_3$–MeOH (2:3) and to ODS CC with MeOH–H_2O (7:3) to yield a mixt. of **3** and **5**, which was separated by HPLC with MeOH–H_2O–2-methoxyethanol (14:6:1) to give **3** (32.8 mg) and **5** (83.0 mg) as the pure compounds. Fr. 4 was chromatographed on silica gel with $CHCl_3$–MeOH–28% NH_3 soln (150:10:1) and Sephadex LH-20 with MeOH to yield **1** with a few impurities, which was purified by prep. TLC developed twice with $CHCl_3$–MeOH (4:1) to give **1** (78.4 mg).

Compound **1**. Amorphous powder, $[\alpha]_D^{29}$ −4.4° (MeOH; *c* 0.50). EIMS *m/z* (rel. int.): 575.3827 $[M]^+$ (17), calcd for $C_{33}H_{53}NO_7$: 575.3824, 396 (16), 164 (14), 125 (100), 111 (70); UV $\lambda_{max}^{dioxane}$ nm (log ε): 247 (2.42), 290 (2.14); CD (dioxane; *c* 6.96 × 10^{-3}) nm (θ): 253 (+1149), 295 (−2586); IR ν_{max}^{film} cm^{-1}: 3423 (OH), 2946 and 2869 (CH), 1708 (C=O), 1655 (C=N), 1459, 1421, 1380, 1261, 1164, 1079, 1040, 958; ^1H NMR (pyridine-d_5): δ 5.02 (1H, *d*, *J* = 7.6 Hz, H-1′), 4.57 (1H, *br d*, *J* = 11.6 Hz, H-6′a), 4.38 (1H, *dd*, *J* = 11.6, 5.4 Hz, H-6′b), 4.30–4.18 (2H, overlapping, H-3′ and H-4′), 4.05–3.92 (3H, overlapping, H-3, H-2′ and H-5′), 1.01 (3H, *d*, *J* = 6.8 Hz, H-21), 0.84 (3H, *d*, *J* = 6.5 Hz, H-27), 0.74 (3H, *s*, H-18), 0.65 (3H, *s*, H-19); for ^{13}C NMR, see Table 1.

Acid hydrolysis of compound **1**. Compound **1** (60.0 mg) was dissolved in 1 M HCl in dioxane–H_2O (1:1) and the mixt. refluxed for 2 hr under N_2. After being cooled, the reaction mixt. was neutralized by passing through an Amberlite IRA-93ZU (OH^- form) and purified by silica gel CC with $CHCl_3$–MeOH (15:1,4:1) to furnish D-glucose (7.2 mg) and an aglycone (**1a**) (16.0 mg). D-Glucose: $[\alpha]_D^{30}$ +55.5° (H_2O; *c* 0.72); TLC, R_f 0.38 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). Compound **1a**: amorphous powder, $[\alpha]_D^{28}$ +35.5° ($CHCl_3$; *c* 0.22). EIMS *m/z* (rel. int.): 413 $[M]^+$ (19), 396 (18), 164 (15), 150 (20), 125 (100), 111 (66); UV $\lambda_{max}^{dioxane}$ nm (log ε): 246 (2.64), 295 (2.18); CD (dioxane; *c* 3.87 × 10^{-3}) nm (θ): 255 (+1292), 295 (−3359); IR ν_{max}^{film} cm^{-1}: 3418 (OH), 2938 and 2869 (CH), 1710 (C=O), 1658 (C=N), 1453, 1380, 1360, 1239, 1066, 966, 753; ^1H NMR (pyridine-d_5): δ 3.84 (1H, *br m*, $W_{1/2}$ = 21.2 Hz, H-3), 1.00 (3H, *d*, *J* = 6.9 Hz, H-21), 0.85 (3H, *d*, *J* = 6.6 Hz, H-27), 0.78 (3H, *s*, H-19), 0.75 (3H, *s*, H-18); for ^{13}C NMR, see Table 1.

Compound **2**. Amorphous powder, $[\alpha]_D^{29}$ +18.4° ($CHCl_3$; *c* 0.50). EIMS *m/z* (rel. int.): 427.3080 $[M]^+$ (17), calcd for $C_{27}H_{41}NO_3$: 427.3088, 399 (5), 164 (13), 140 (100), 111 (36); UV λ_{max}^{MeOH} nm (log ε): 270 (2.28), 344 (1.73); IR ν_{max}^{film} cm^{-1}: 3474 (OH), 2941, 2870 and 2851 (CH), 1707 and 1690 (C=O), 1616, 1457, 1447, 1430, 1376, 1277, 1258, 1237, 1063, 989, 964, 755; ^1H NMR ($CDCl_3$): δ 4.23 (1H, *dddd*, *J* = 17.7, 8.8, 2.3, 2.3 Hz, H-26a), 3.70 (1H, *m*, H-20), 3.69 (1H, *m*, H-26b), 3.57 (1H, *m*, H-3), 2.88 (1H, *dddd*, *J* = 17.7, 8.8, 2.3, 2.3 Hz, H-24a), 2.45 (1H, *m*, H-25), 2.36 (1H, *m*, H-24b), 1.73 (1H, *m*, H-17), 1.13 (3H, *d*, *J* = 6.9 Hz, H-21), 1.04 (3H, *d*, *J* = 6.9 Hz, H-27), 0.76 (3H, *s*, H-19), 0.75 (3H, *s*, H-18); for ^{13}C NMR, see Table 1.

Compound **3**. Amorphous powder, $[\alpha]_D^{29}$ −19.6° (MeOH; *c* 0.50). (Found: C, 65.34; H, 8.54; N, 2.39. Calc. for $C_{33}H_{51}NO_8 \cdot H_2O$: C, 65.21; H, 8.46; N, 2.41%.) EIMS *m/z* (rel. int.): 589 $[M]^+$ (6), 456 (6), 426 (11), 410 (100), 382 (7), 301 (12), 177 (9), 140 (93), 108 (37); UV λ_{max}^{MeOH} nm (log ε): 270 (2.22), 344 (1.69); IR ν_{max}^{film} cm^{-1}: 3385 (OH), 2941 and 2871 (CH), 1696 (C=O), 1620, 1457, 1429, 1374, 1277, 1259, 1164, 1105, 1077, 1041, 1025, 991, 960; ^1H NMR ($CDCl_3$): δ 4.41 (1H, *d*, *J* = 7.7 Hz, H-1′), 4.23 (1H, *dddd*, *J* = 17.7, 8.8, 2.3, 2.3 Hz, H-26a), 3.86 (1H, *dd*, *J* = 12.0, 3.1 Hz, H-6′a), 3.75 (1H, *dd*, *J* = 12.0, 4.9 Hz, H-6′b), 3.72–3.62 (3H, overlapping, H-3, H-20 and H-26b), 3.45–3.39 (2H, overlapping, H-3′ and H-4′), 3.28 (1H, *m*, H-5′), 3.24 (1H, *dd*, *J* = 9.4, 7.7 Hz, H-2′), 2.90 (1H, *dddd*, *J* = 17.7, 8.8, 2.3, 2.3 Hz, H-24a), 2.48 (1H, *m*, H-25), 2.37 (1H, *m*, H-24b), 1.14 (3H, *d*, *J* = 6.9 Hz, H-21), 1.05 (3H, *d*, *J* = 6.9 Hz, H-27), 0.76 (3H × 2, *s*, H-18 and H-19); for ^{13}C NMR, see Table 1.

Enzymatic hydrolysis of compound **3**. A mixt. of **3** (20.0 mg) and β-glucosidase (10 mg) in HOAc–NaOAc buffer (pH 5) was incubated at 37° for 72 hr. The reaction mixt. was chromatographed on silica gel with EtOAc–hexane–$CHCl_3$ (2:1:1) and $CHCl_3$–MeOH (4:1) to give pure D-glucose (1.2 mg) and an aglycone with a few impurities, which was further purified by HPLC with MeOH–H_2O–2-methoxyethanol (14:2:1) to yield **2** (3.0 mg) as the pure compound.

Compound **4**. Amorphous powder, $[\alpha]_D^{29}$ +1.6° ($CHCl_3$; *c* 0.50). EIMS *m/z* (rel. int.): 427.3062 $[M]^+$ (12), calcd for $C_{27}H_{41}NO_3$: 427.3088, 399 (4), 164 (5), 140 (100), 111 (19); UV λ_{max}^{MeOH} nm (log ε): 270 (2.23), 344 (1.63); IR ν_{max}^{film} cm^{-1}: 3474 (OH), 2939, 2870 and 2851 (CH), 1708 and 1689 (C=O), 1614, 1456, 1429, 1381, 1251, 1063, 987, 966, 754; ^1H NMR ($CDCl_3$): δ 4.21 (1H, *dddd*, *J* = 17.7, 8.8, 2.3, 2.3 Hz, H-26a), 3.75 (1H, *m*, H-26b), 3.64 (1H, *m*, H-20), 3.54 (1H, *m*, H-3), 2.88 (1H, *dddd*, *J* = 17.7, 8.8, 2.3, 2.3 Hz, H-24a), 2.48 (1H, *m*, H-25), 2.36 (1H, *m*, H-24b), 1.89 (1H, *m*, H-17), 1.04 (3H, *d*, *J* = 6.9 Hz, H-21), 1.03 (3H, *d*, *J* = 6.9 Hz, H-27), 0.72 (3H, *s*, H-19), 0.65 (3H, *s*, H-18); for ^{13}C NMR, see Table 1.

Compound **5**. Needles (MeOH), mp 214–215°, $[\alpha]_D^{29}$ −13.2° (MeOH; *c* 0.50). (Found: C, 63.20; H, 8.52; N, 2.53. Calc. for $C_{33}H_{51}NO_8 \cdot 2H_2O$: C, 63.33; H, 8.21; N, 2.24%.) EIMS *m/z* (rel. int.): 589 $[M]^+$ (7), 456 (6), 426 (6), 410 (100), 301 (8), 177 (8), 140 (85), 111 (21); UV λ_{max}^{MeOH} nm (log ε): 270 (2.17), 344 (1.59); IR ν_{max}^{film} cm^{-1}: 3396 (OH), 2942, 2873 and 2850 (CH), 1702 and 1684 (C=O), 1620, 1458, 1429, 1377, 1252, 1167, 1100, 1078, 1042, 1025, 992, 958; ^1H NMR ($CDCl_3$): δ 4.40 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.22 (1H, *dddd*, *J* = 17.7, 8.8, 2.3, 2.3 Hz, H-26a), 3.86–3.71 (4H, overlapping, H-20, H-26b, H-6′a and H-6′b), 3.64 (1H, *m*, H-3), 3.44–3.39 (2H, overlapping, H-3′ and H-4′), 3.28 (1H, *m*, H-5′), 3.23 (1H, *dd*, *J* = 9.4, 7.8 Hz, H-2′), 2.92 (1H, *dddd*, *J* = 17.7, 8.8, 2.3, 2.3 Hz, H-24a), 2.50 (1H, *m*, H-25), 2.37 (1H, *m*, H-24b), 1.04 (3H, *d*, *J* = 6.9 Hz, H-21), 1.03 (3H, *d*, *J* = 6.9 Hz, H-27), 0.73 (3H, *s*, H-19), 0.66 (3H, *s*, H-18); for ^{13}C NMR, see Table 1.

Enzymatic hydrolysis of compound **5**. Enzymatic hydrolysis of **5** (20.0 mg) was carried out as for **3** to furnish D-glucose (1.0 mg) and **4** (2.0 mg).

Conversion of **1a** *to* **2**. A soln of **1a** (16.0 mg) in $CHCl_3$ (2.5 ml) was stirred with active MnO_2 (160 mg) at room temp. for 4.5 hr under N_2. The inorganic material was filtered off and washed with $CHCl_3$. The combined $CHCl_3$ soln was evapd under red. pres., and the residue absorbed on a plate coated with silica gel, which was left standing at room temp. in the dark for 3 days. Silica gel was scraped from the plate and extracted with EtOAc. The EtOAc eluate was purified by silica gel CC with EtOAc–hexane–$CHCl_3$ (2:1:1), and by HPLC with MeOH–H_2O–2-methoxyethanol (10:2:1) to yield **2** (0.8 mg).

Assay of cyclic AMP phosphodiesterase activity. The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described previously [12].

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