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Synthesis of 3β,6α-Dihydroxycholesta-9(11),17(20),24-trien-23-one, the Aglycone of Acrosome Reaction-Inducing Steroidal Saponin Co-ARIS II

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3β,6α-Dihydroxycholesta-9(11),17(20),24-trien-23-one (**2**), the aglycone of acrosome reaction-inducing steroidal saponin Co-ARIS II (**1**), was synthesized from asterone diacetate (**3**) in 8 steps. The side chain was constructed by the coupling reaction of the allyl chloride (**8**) with the dithiane anion (**9**). Evidence for 17(20)-*E* stereochemistry of **2** was obtained by nuclear Overhauser effect (NOE) experiments in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **1** confirmed the structure assignment of the latter compound.

Keywords——Co-ARIS; steroid; steroid saponin; acrosome reaction; dithiane; allyl chloride

Introduction

The acrosome reaction in the starfish, *Asterias amurensis*, is induced by a combination of sulfated glycoprotein, ARIS, and steroidal saponin, Co-ARIS.[2] We have purified three different, but closely related, steroidal saponins (Co-ARIS, I, II and III) from the egg jelly of *A. amurensis*[3] and elucidated their structures on the basis of chemical and spectroscopic evidence.[4] Co-ARIS II (**1**) possesses a unique structural feature, a 17(20),24-dien-23-one moiety, on the steroid side chain. To confirm the structure of Co-ARIS II and to supply a source material for the synthetic study of Co-ARIS II itself, we have synthesized 3β,6α-dihydroxycholesta-9(11),17(20),24-trien-23-one, the steroid part (**2**) of Co-ARIS II.

Results and Discussion

Chart 1 shows our synthetic route, in which the side chain was constructed by the coupling reaction of the unstable allyl chloride **8** with the dithiane anion **9**. The starting steroid, 3β,6α-diacetoxypregn-11-en-20-one (asterone diacetate) (**3**) was prepared in six steps from commercial 11-oxoprogesterone in 27% overall yield according to the literature procedure.[5,6]

Treatment of **3** with KCN in AcOH/EtOH at 0 °C afforded the cyanohydrin **4** in 75% yield (after one recycle). Dehydration of **4** with $POCl_3$ in pyridine afforded the nitrile **5** quantitatively. Reduction of **5** with diisobutylaluminum hydride and subsequent acetylation afforded the conjugated aldehyde **6** in 50% yield. Since this reaction sequence (from 20-ketone to conjugated aldehyde) is known to furnish the 17(20)*E*-compound,[7] the structure of **6** was assumed to be the 17(20)*E*-aldehyde. Reduction of **6** with sodium borohydride in the presence of cerium chloride[8] afforded the 17(20)*E*-allyl alcohol **7** in 80% yield.

In our preliminary studies using a model compound, cholesta-5,17(20)-diene-3β,22-diol

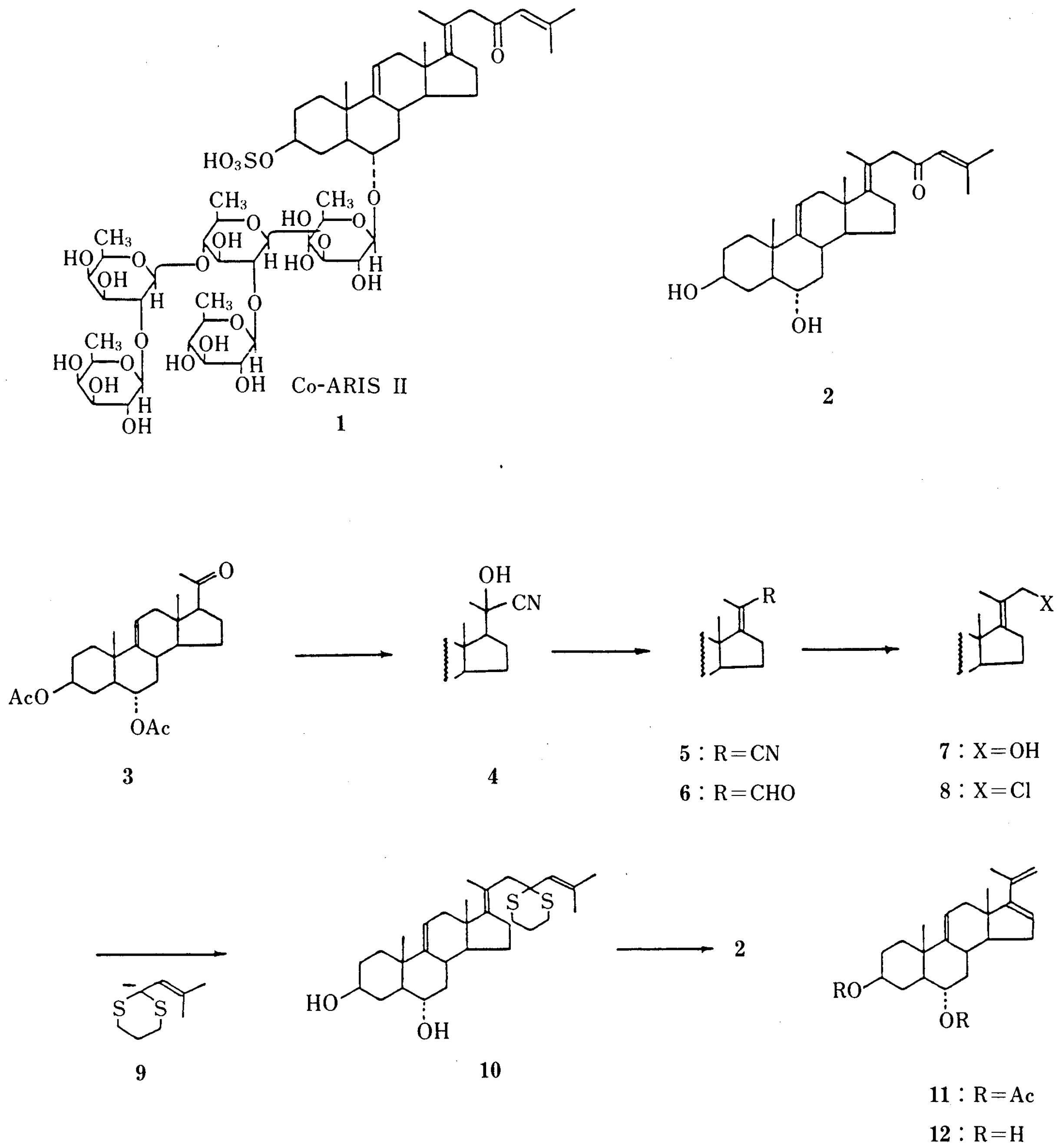

Chart 1

3-acetate,[9] attempted sulfonate formation under various conditions[10] afforded exclusively the 16,20(22)-diene, whereas introduction of a leaving group at the 22-position was effected in a moderate yield by treatment with thionyl chloride and pyridine.[11] Thus, brief treatment of **7** with thionyl chloride and pyridine in tetrahydrofuran at $-10\,^{\circ}\mathrm{C}$ afforded a mixture of the chloride **8** and the diene **11** in *ca.* 1 : 1 ratio. Since the chloride **8** was unstable, the mixture was immediately used for the next reaction. Coupling reaction of the mixture with excess 2-(2-methylpropen-1-yl)-1,3-dithiane anion **9**[12] afforded the dithioacetal **10** in 26% yield (from **7**) after reversed-phase (ODS) chromatographic separation from the diene **12**. Treatment of **10** with silver nitrate or silver nitrate/*N*-bromosuccinimide gave a complex mixture, while use of thallium (III) trinitrate (TTN)[13] afforded the crystalline enone **2** in 60% yield.

The proton nuclear magnetic resonance (^{1}H-NMR) data for **2**, along with those for **1**, are listed in Table I. They are in good agreement with each other, confirming the correctness of

TABLE I. ^{1}H-NMR Data of Compound (**2**) and Co-ARIS II (**1**) Aglycone Part (CD_3OD–D_2O, 1 : 1)

	Compound (**2**)	Co-ARIS II (**1**)
18-H_3	0.83 (s)	0.84
19-H_3	0.98 (s)	1.00
21-H_3	1.71 (br t, $J=2.1$ Hz)	1.70
26-$H_3{}^{a)}$	1.92 (br d, $J=1.3$ Hz)	1.90
27-$H_3{}^{a)}$	2.11 (br d, $J=1.3$ Hz)	2.11
22-H_2	3.02 (s)	3.01
11-H	5.40 (br d, $J=5.2$ Hz)	5.40
3α-H	3.45 (m)	4.22
6β-H	3.45 (m)	$^{b)}$
24-H	6.16 (br t, $J=1.3$ Hz)	6.16

a) The methyl group *trans* to C-23 was numbered as C-26. *b*) Obscure due to the overlap of sugar oxymethine signals.

TABLE II. ^{13}C-NMR Data for Compound (**2**) and Co-ARIS II (**1**) Aglycone Part

	Compound (**2**)$^{a)}$ (CD_3OD)	Co-ARIS II (**1**) (CD_3OD–D_2O)
C-1	37.0 (t)	36.4
C-2	32.0 (t)	29.0
C-3	71.9 (d)	81.0
C-4	33.4 (t)	30.6
C-5	51.1 (d)	$^{b)}$
C-6	69.8 (d)	80.2
C-7	43.4 (t)	41.1
C-8	36.2 (d)	36.1
C-9	147.5 (d)	146.5
C-10	39.4 (s)	39.1
C-11	117.8 (d)	117.8
C-12	41.1 (t)	40.7
C-13	44.0 (s)	43.7
C-14	55.3 (d)	54.8
C-15	26.4 (t)	26.1
C-16	31.6 (t)	31.5$^{c)}$
C-17	148.5 (s)	148.4
C-18	16.9 (q)	16.8
C-19	19.7 (q)	19.6
C-20	121.2 (s)	120.7
C-21	19.0 (q)	19.0
C-22	53.1 (t)	52.8
C-23	202.1 (s)	203.9
C-24	124.1 (d)	123.5
C-25	157.3 (s)	158.8
C-26	27.7 (q)	28.0
C-27	20.6 (q)	21.1

a) Multiplicity was obtained by INEPT experiments. *b*) The signal is buried under the CD_3OD signal. *c*) The value δ: 22.9 was incorrectly reported in ref. 4 because of the weak intensity of the signal.

the structure of **1**, in particular, of the 17(20),24-dien-23-one moiety. The stereochemistry of the 17(20) double bond of **2** was firmly established by ^{1}H-NMR nuclear Overhauser effect (NOE) experiments. Thus, irradiation of the 18-H_3 (δ 0.83) caused apparent NOE at 21-H_3 (δ 1.71), but not at 22-H_2 (δ 3.02), indicating *E* configuration for the 17(20) double bond. In the previous paper$^{4)}$ we described the chemical shifts of carbon signals of the steroid part of Co-ARIS II. Although most of the ^{13}C signals of **1** could be assigned in comparison with the data$^{14)}$ reported for related compounds such as asterosaponins, versicoside A and 3β,6α-dihydroxy-23-oxocholesta-9(11),24-diene, there remained some ambiguity with respect to the assignment of several signals, *i.e.* C-15 *vs.* C-16, C-7 *vs.* C-12, and C-18 *vs.* C-21. Detailed NMR studies, including ^{13}C INEPT and ^{1}H–^{13}C COSY and long-range COSY experiments, on compound **2**, asterone and 23-oxocholesta-5,17(20)*E*,24-trien-3β-ol$^{9)}$ allowed us to assign all the carbon signals of **2**, as shown in Table II. With the established assignment of ^{13}C signals of **2**, we were able to assign the ^{13}C signals of the steroid part of **1** (Table II). As expected from the structure of **1**, C-3 and C-6 carbon signals of **1** appeared downfield from the respective signals of **2**, whereas the carbon resonances of C-2, C-4, C-5 and C-7 were shifted upfield slightly.

Experimental

Melting points were determined on a Yazawa BY-1 micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a JEOL FX-200 spectrometer in $CDCl_3$ solution with tetramethylsilane as an internal reference. Infrared (IR) spectra were determined on a JASCO IR-810 spectrometer. Ultraviolet (UV) spectra were obtained on a Shimadzu UV 200 spectrometer. Elemental analysis was done on a Perkin-Elmer 240 analyzer.

3β,6α-Diacetoxy-20-hydroxypregn-9(11)-ene-20-carbonitrile (4)——Potassium cyanide (27 g) was added to a cooled (0 °C) solution of **2** (4.5 g) in 95% ethanol (186 ml) and acetic acid (39 ml). Stirring was continued at 0 °C for 1 h and then at room temperature for 4 h. Next, 2 N HCl (2 ml) and water (4 ml) were added and the mixture was concentrated under reduced pressure. The residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography with hexane–ethyl acetate (6 : 1) as an eluting solvent to give unreacted starting material (2.1 g) and the cyanohydrin (2.2 g). The recovered starting material was subjected to the same sequence. The cyanohydrin **4** (3.4 g, 75% yield after one recycle) was obtained as a solid, mp 171—173 °C (from methanol). ^{1}H-NMR δ: 0.83 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.64 (3H, s, 21-H_3), 2.05 (3H, s, CH_3CO), 2.05 (3H, s, CH_3CO), 4.65 (1H, m, 3-H), 4.85 (1H, dt, J=3.8, 11.4 Hz, 6-H), 5.35 (1H, br s, 11-H). *Anal.* Calcd for $C_{26}H_{37}NO_5$: C, 70.45; H, 8.41; N, 3.16. Found: C, 70.20; H, 8.41; N, 3.38.

3β,6α-Diacetoxypregna-9(11),17(20)-diene-20-carbonitrile (5)——Cyanohydrin **4** (4.1 g) was added to a mixture of dry pyridine (80 ml) and phosphorus chloride (7.74 ml) and the solution was heated at 90 °C for 1 h. After cooling, the mixture was poured into ice and extracted with ethyl acetate. The organic layer was washed with 2 N HCl, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give **5** (4.1 g, 100% recovery yield) as a solid, mp 79—80 °C (from hexane–methylene chloride). ^{1}H-NMR δ: 0.88 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.83 (3H, s, 21-H_3), 2.04 (3H, s, CH_3CO), 2.04 (3H, s, CH_3CO), 4.66 (1H, m, 3-H), 4.86 (1H, dt, J=3.8, 11.4 Hz, 6-H), 5.45 (1H, br s, 11-H). *Anal.* Calcd for $C_{26}H_{35}NO_4$: C, 73.38; H, 8.29; N, 3.29. Found: C, 73.10; H, 8.35; N, 3.34.

3β,6α-Diacetoxy-20-formylpregna-9(11),17(20)-diene (6)——Diisobutylaluminum hydride (25% solution in hexane, 7.0 ml) was added to a solution of the nitrile **5** (2.55 g) in dry toluene (57 ml) with stirring at −78 °C under argon. The mixture was stirred for 10 min at the same temperature and poured into ccoled saturated aqueous NH_4Cl. The whole mixture was further acidified to pH 3 by addition of 2 N H_2SO_4 and extracted with methylene chloride. The organic layer was washed with water and saturated $NaHCO_3$, dried over Na_2SO_4, and concentrated. The product was acetylated in a standard manner (acetic anhydride and pyridine). The crude acetate was purified by silica gel column chromatography with hexane–ethyl acetate (6 : 1) as an eluting solvent to give the aldehyde **6** (1.28 g, 50%), mp 89 °C (from ethyl acetate). ^{1}H-NMR δ: 0.89 (3H, s, 18-H_3), 1.05 (3H, s, 19-H_3), 1.80 (3H, s, 21-H_3), 2.05 (3H, s, CH_3CO), 2.06 (3H, s, CH_3CO), 4.66 (1H, m, 3-H), 4.88 (1H, dt, J=3.8, 11.4 Hz, 6-H), 5.41 (1H, br d, J=2.9 Hz, 11-H), 9.98 (1H, s, CHO). *Anal.* Calcd for $C_{26}H_{35}O_5$: C, 72.86; H, 8.47. Found: C, 73.59; H, 8.46.

3β,6α-Diacetoxy-20-(hydroxymethyl)pregna-9(11),17(20)-diene (7)——A mixture of the aldehyde **6** (2.5 g) in ethyl acetate (2.5 ml), methanol (25 ml) and tetrahydrofuran (THF) (25 ml) containing a catalytic amount of cerium chloride heptahydrate was stirred for 10 min at 0 °C. The solvent was removed and the residue was partitioned between ethyl acetate and water. The organic layer was washed with 2 N HCl, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by silica gel chromatography with hexane–ethyl acetate (4 : 1) as an eluting solvent to give the allyl alcohol **7** (2.0 g, 80%) as colorless crystals, mp 92—95 °C (from methanol). ^{1}H-NMR δ: 0.79 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.79 (3H, s, 21-H_3), 2.04 (3H, s, CH_3CO), 2.04 (3H, s, CH_3CO), 4.04 (2H, s, 22-H_2), 4.66 (1H, m, 3-H), 4.88 (1H, dt, J=3.8, 11.4 Hz, 6-H), 5.38 (1H, br d, J=2.9 Hz, 11-H). *Anal.* Calcd for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.31; H, 8.70.

3β,6α-Diacetoxy-20-(chloromethyl)pregna-9(11),17(20)-diene (8)——Thionyl chloride (244 μl) and dry pyridine (244 μl) were added to a solution of the allyl alcohol **7** (940 mg) in THF (14 ml) at −10 °C under argon. The mixture was stirred at the same temperature for 10 min. The reaction mixture was filtered through a pad of Celite 545 and the filtrate was concentrated under reduced pressure while the bath temperature was maintained below 25 °C. The residual yellow oil (1.14 g) was dried *in vacuo* in a dessicator containing P_2O_5. Thin layer chromatography (TLC) and ^{1}H-NMR indicated that this sample is a *ca.* 1 : 1 mixture of the allyl chloride **8** and the diene **11**. This sample was immediately used for the next reaction. ^{1}H-NMR δ: 0.79 (3H, s, 18-H_3), 1.03 (3H, s, 19-H_3), 1.81 (3H, s, 21-H_3), 2.03 (3H, s, CH_3CO), 2.04 (3H, s, CH_3CO), 4.01 (2H, s, 22-H_2), 4.65 (1H, m, 3-H), 4.85 (1H, dt, J=3.8, 11.4 Hz, 6-H), 5.38 (1H, br d, J=2.9 Hz, 11-H).

^{1}H-NMR of the Diene **11**, δ: 0.85 (3H, s, 18-H_3), 1.06 (3H, s, 19-H_3), 1.89 (3H, s, 21-H_3), 2.04 (3H, s, CH_3CO), 2.04 (3H, s, CH_3CO), 4.63 (1H, m, 3-H), 4.86 (1H, dt, J=3.8, 11.4 Hz, 6-H), 4.87 (1H, s, 22-Ha), 5.04 (1H, s, 22-Hb), 5.40 (1H, br d, J=3.8 Hz, 11-H), 5.74 (1H, s, 16-H).

3β,6α-Dihydroxy-5α-cholesta-9(11),17(20),24-trien-23-one Dithioacetal (10)——*n*-Butyl lithium (1.59 M hexane solution, 5.75 ml, 8.95 mmol) was added to a stirred solution of 2-(2-methylpropen-1-yl)-1,3-dithiane (1.62 g, 9.21 mmol) in THF (17 ml) over a period of 5 min at −78 °C under argon. The solution was stirred at −20 °C for 20 min and cooled to −78 °C. The crude allyl chloride **8** (1.14 g) in dry THF (17 ml) was added to the dithiane anion solution over 5 min. The mixture was reacted for 10 min at −78 °C and then saturated aqueous NH_4Cl and ether were added. The ether extract was washed with 0.2 N HCl, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to leave a yellow oil (3.8 g). This was chromatographed on a silica gel (packed with benzene–ethyl acetate (1 : 1)) column with ethyl acetate as an eluting solvent to give a mixture of the dithioacetal **10** and the diene **12**, formed from **11** by deacetylation. The mixture was purified by reversed-phase (SP-C-ODS, Chemco) column chromatography with methanol as an eluting solvent to give the diene **12** (386 mg, 27% from **7**) and the dithioacetal **10** (297 mg, 26% from **7**), mp 92—95 °C (from methanol). ^{1}H-NMR δ: 0.80 (3H, s, 18-H_3), 0.96 (3H, s, 19-H_3), 1.78 (3H, s, 21-H_3), 1.77 (3H, br d, J=1.5 Hz, 26-H_3), 1.94 (3H, br d, J=1.4 Hz, 27-H_3), 2.60—3.05 (4H, m, 2×CH_2S), 3.60 (2H, m, 3-H, 6-H), 5.38 (1H, br d, J=5.2 Hz, 11-H), 5.60 (1H, br d, J=1.5 Hz, 24-H). *Anal.* Calcd for $C_{30}H_{47}O_2S_2$: C, 71.52; H, 9.40. Found: C, 71.36; H, 9.24.

^{1}H-NMR of the Diene **12**, δ: 0.86 (3H, s, 18-H_3), 0.99 (3H, s, 19-H_3), 1.89 (3H, s, 21-H_3), 3.6 (2H, m, 3-H, 6-H), 4.85 (1H, s, 22-Ha), 5.06 (1-H, s, 22-Hb), 5.38 (br d, J=1.9 Hz, 11-H), 5.75 (1H, br t, J=2.2 Hz, 16-H).

3β,6α-Dihydroxycholesta-9(11),17(20),24-trien-23-one (2)——A solution of thallium (III) trinitrate (65 mg) in methanol (1.67 ml) was added dropwise to a solution of the dithioacetal **10** (30 mg) in methanol (1.67 ml) and ether (1.25 ml) with stirring at −60 °C over 5 min. The mixture was stirred for 5 min at the same temperature and filtered through Hyflo Super Cell (Wako). The filtrate was concentrated and the residue was partitioned between chloroform and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by preparative TLC (Merck silica gel 60 F_{254} pre-coated plate 20×20 cm^2, 0.5 mm thickness) with chloroform–methanol (5 : 1) and then hexane–ethyl acetate (1 : 1) as developing solvents (*Rf* 0.35) to give the ketone **2** (17 mg, 60%) as crystals, mp 114 °C (from methanol–acetone). IR $\nu_{max}^{CHCl_3}$: 3625, 1680 cm^{-1}. UV $\lambda_{max}^{methanol}$: 240 nm (ε 10000). ^{1}H-NMR (CD_3OD) δ: 0.83 (3H, s, 18-H_3), 0.97 (3H, s, 19-H_3), 1.72 (3H, s, 21-H_3), 1.87 (3H, s, 26-H_3), 2.14 (3H, s, 27-H_3), 3.00 (2H, s, 22-H_2), 3.60 (2H, m, 3-H, 6-H), 5.40 (1H, br d, J=5.2 Hz, 11-H), 6.07 (1H, br t, J=1.3 Hz, 24-H). The ^{1}H-NMR data recorded in CD_3OD–H_2O (1 : 1) are listed in Table I. *Anal.* Calcd for $C_{31}H_{44}O_5$: C, 74.96; H, 8.93. Found: C, 75.23; H, 8.80.

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