Studies on Cardiac Ingredients of Plants. VII[1]: Chemical Transformation of Proscillaridin by Means of the Diels–Alder Reaction and Biological Activities of Its Derivatives

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The Diels–Alder reactions of a cardiac glycoside, proscillaridin (1), with some dienophiles were investigated. The reaction of 1 with alkenes such as methyl vinyl ketone and methyl acrylate afforded 3-oxo-2-oxabicyclo[2.2.2]oct-7-enes (2—5) and *para*-substituted benzene derivatives (6 and 7), while 1 reacted with alkynes (3-butyn-2-one, methyl propiolate) to yield *para*- or *meta*-substituted benzene derivatives (6—9). The biological activities of the resulting derivatives were evaluated by the use of isolated guinea-pig papillary muscle preparations and Na^+,K^+-adenosine triphosphatase (ATPase) preparation from dog kidney. Among the proscillaridin derivatives, compounds 4 and 7 moderately inhibited Na^+,K^+-ATPase activity. Furthermore, the concentration range of 7 over which its positive inotropic effect on guinea-pig papillary muscle preparations, increased from 5% to 95% of maximum was broader than that of 1, *i.e.*, concentration dependency was maintained over a greater range of concentration.

Keywords proscillaridin; cardiac glycoside; chemical transformaion; Diels–Alder reaction; guinea-pig papillary muscle; Na^+,K^+-ATPase; positive inotropic effect

The cardiac glycoside, proscillaridin (**1**) has been widely used in the treatment of congestive heart failure.[3] However, this drug develops its positive inotropic effect (PIE) over a very narrow concentration range and sometimes causes arrhythmia.[4] The narrow therapeutic range of this drug makes it desirable to develop improved proscillaridin analogues with a lower risk of toxicity. We have recently reported extensive chemical modifications of **1** and have found that C_{22}–C_{23} hydrogenated proscillaridin, 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxybufa-4,20-dienolide has a greatly expanded concentration range of PIE development on guinea-pig papillary muscle preparation and shows a reduced occurrence of arrhythmia.[5] As part of our continuing program aimed at the development of therapeutically advantageous drugs with a high margin of safety, we modified the δ-lactone group of **1** mainly by means of the Diels–Alder reactions with alkenes and alkynes. In this paper, we describe the preparation, the structure determination and the biological evaluation of the Diels–Alder cycloadducts.

Chemistry In a previous paper, we reported that the pyridinone molecule derived from the α-pyrone of **1** underwent the Diels–Alder reaction with dimethyl acetylenedicarboxylate to give the 1,4-cycloadduct in moderate yield.[2] Thus, we initiated the Diels–Alder reaction of **1** with several dienophiles such as methyl vinyl ketone, methyl acrylate, 3-butyn-2-one, and methyl propiolate as shown in Chart 1.

Compound **1** was allowed to react with methyl vinyl ketone in boiling dioxane for 10 h to give a product showing a single spot on thin-layer chromatography (TLC). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum, however, revealed that the product is a mixture of two compounds, which were easily separable by high-performance liquid chromatography (HPLC) using an ODS column without endcapping of residual silanol groups to furnish **2** (54%) and **3** (38%). The infrared (IR) spectrum of **2** (a crystalline powder, mp 155—156 °C, $C_{34}H_{48}O_9$) showed lactone and ketone carbonyl absorptions at 1740 and 1720 cm^{-1}. The ^{1}H-NMR spectrum exhibited signals due to the C-8 olefinic proton at δ 5.99 (1H, dd, J=1.8, 6.3 Hz), the C-4 bridgehead proton at δ 3.76 (1H, dd, J=2.5, 6.3 Hz), the C-5 methine proton at δ3.02 (1H, ddd, J=2.5, 3.8, 9.8 Hz) and the C-1 bridgehead proton at δ 5.38 (1H, br s). The stereochemistry at C-1 and C-4 of **2** was established by circular dichroism (CD) spectroscopy, which showed a positive Cotton effect at 232 nm ([θ] $+1.2\times10^4$). Based on the octant rule, we concluded that compound **2** possesses the 1*R*,4*R*-configuration as illustrated in Fig. 1-i. The configuration at C-5 was then deduced as follows. The signal of an *exo* proton of the 3-oxo-2-oxabicyclo[2.2.2]oct-7-ene ring system is known to be observed at lower field than that of an *endo*-proton in the ^{1}H-NMR spectrum,[6] because of shielding by the double bond between

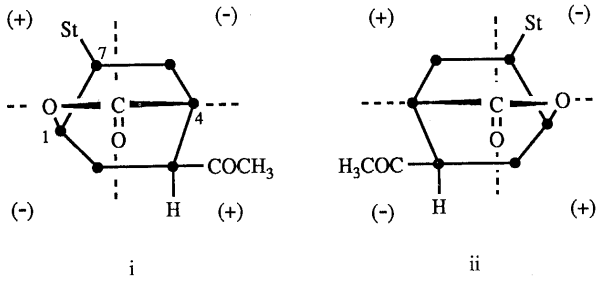

Fig. 1. Perspective Representation of 3-Oxo-2-oxabicyclo[2.2.2]oct-7-ene Derivatives (**2** and **3**)

St: steroid nucleus.

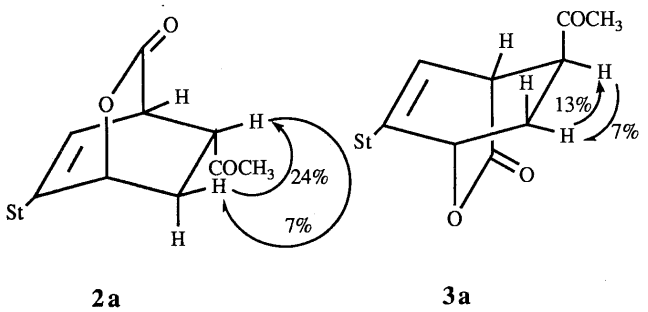

Fig. 2. NOE Data for **2a** and **3a**

C-7 and C-8. Therefore, the signal observed at 2.30 ppm (1H, ddd, $J=3.7$, 9.8, 13.5 Hz) in the ^{1}H-NMR spectrum of **2** was assigned to *exo* 6-H and the signal at 2.23 ppm (1H, dd, $J=3.8$, 13.5 Hz) was assigned to *endo* 6-H. From the coupling constants between the C-5 and C-6 protons, the stereochemistry of the C-5 acetyl group was determined to be *endo*. The assigned configuration is consistent with Alder's rule. The stereochemical assignment was further corroborated by difference nuclear Overhauser effect (NOE) studies of the tri-*O*-acetate of **2** (**2a**); irradiation of *exo* 6-H led to 24% enhancement of the proton resonance at *exo* 5-H, while irradiation at *exo* 5-H resulted in 7% enhancement of *exo* 6-H (Fig. 2). From the observed NOEs, the chemical structure of **2** was confirmed to be (1*R*,4*R*,5*R*)-5-acetyl-7-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]-3-oxo-2-oxabicyclo[2.2.2]oct-7-ene. In contrast, the minor isomeric product (**3**) showed a negative Cotton effect in the CD spectrum, (Fig. 1-ii). In addition, NOE enhancements between *exo* 6-H (δ 2.33, 1H, ddd, $J=3.2$, 9.4, 13.9 Hz) and *exo* 5-H (δ 3.02, 1H, ddd, $J=2.1$, 4.0, 9.4 Hz) were observed in the ^{1}H-NMR spectrum of the tri-*O*-acetate of **3** (**3a**). Consequently, the structure of **3** was determined to be (1*S*,4*S*,5*S*)-5-acetyl-7-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]-3-oxo-2-oxabicyclo[2.2.2]oct-7-ene.

Similarly, the Diels–Alder reaction of **1** with methyl acrylate gave a mixture of 3-oxo-2-oxabicyclo[2.2.2]oct-7-ene derivatives, which were separated by HPLC to give **4** (43%) and **5** (32%). The spectral features of **4** and **5** were very similar to those of **2** and **3** except for those due to the carbomethoxy moiety. The CD spectra of **4** and **5** showed a positive Cotton effect at 228 nm ([θ] 2.3×10^4) and a negative Cotton effect at 221 mn ([θ] -1.4×10^4), respectively. Accordingly, the structures of **4** and **5** were established to be methyl (1*R*,4*R*,5*R*)-7-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]-3-oxo-2-oxabicyclo[2.2.2]oct-7-ene-5-carboxylate and methyl (1*S*,4*S*,5*S*)-7-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-en-17β-yl]-3-oxo-2-oxabicyclo[2.2.2]-oct-7-ene-5-carboxylate.

We then conducted the same Diels–Alder reactions at elevated temperature. When compound **1** and methyl vinyl ketone were heated at 150 °C in a sealed tube, compound **6** was obtained as a sole product in 68% yield. The IR spectrum of **6** (a crystalline powder, mp 143—145 °C, $C_{33}H_{46}O_7$) exhibited an absorption band ascribable to an α,β-unsaturated carbonyl group at 1670 cm^{-1}, and the ^{1}H-NMR spectrum showed A_2B_2 type proton signals at 7.41 (2H, d, $J=8.4$ Hz) and 7.91 ppm (2H, d, $J=8.4$ Hz). Based on the above spectral data, compound **6** was confirmed to be 4-[3β-[(6-deoxy-α-L-mannopyranosyl)-oxy]-14β-hydroxyandrost-4-en-17β-yl]phenylethanone, which is obviously formed from the 1,4-cycloadducts (**2** and **3**) by elimination of carbon dioxide *via* a cycloreversion reaction followed by aromatization.[7] In a similar manner, the reaction of **1** with methyl acrylate gave compound **7** in 78% yield. The structure of **7** was established to be methyl 4-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]benzoate by analysis of the spectral data described in Experimental.

On the other hand, the Diels–Alder reactions of **1** with some alkynes such as 3-butyn-2-one[8] and methyl propiolate were expected to afford the isomeric benzenes possessing the substituents at the *meta* and *para* positions. Thus, we undertook the reaction of **1** with 3-butyn-2-one to obtain a mixture of two regioisomers, which were readily separated by HPLC to yield **6** and 3-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]phenylethanone (**8**) in 52% and 43% yields, respectively, as shown in Chart 1. In the ^{1}H-NMR spectrum of **8**, the characteristic signals due to *meta*-substituted benzene [δ 7.35 (1H, dd, $J=7.7$, 7.7 Hz), 7.62 (1H, d, $J=7.7$ Hz), 7.75 (1H, d, $J=7.7$ Hz), 7.93 (1H,s)] were observed. These data were consistent with the assigned structure **8**. The reaction of **1** with methyl propiolate also provided **7** and methyl 4-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-

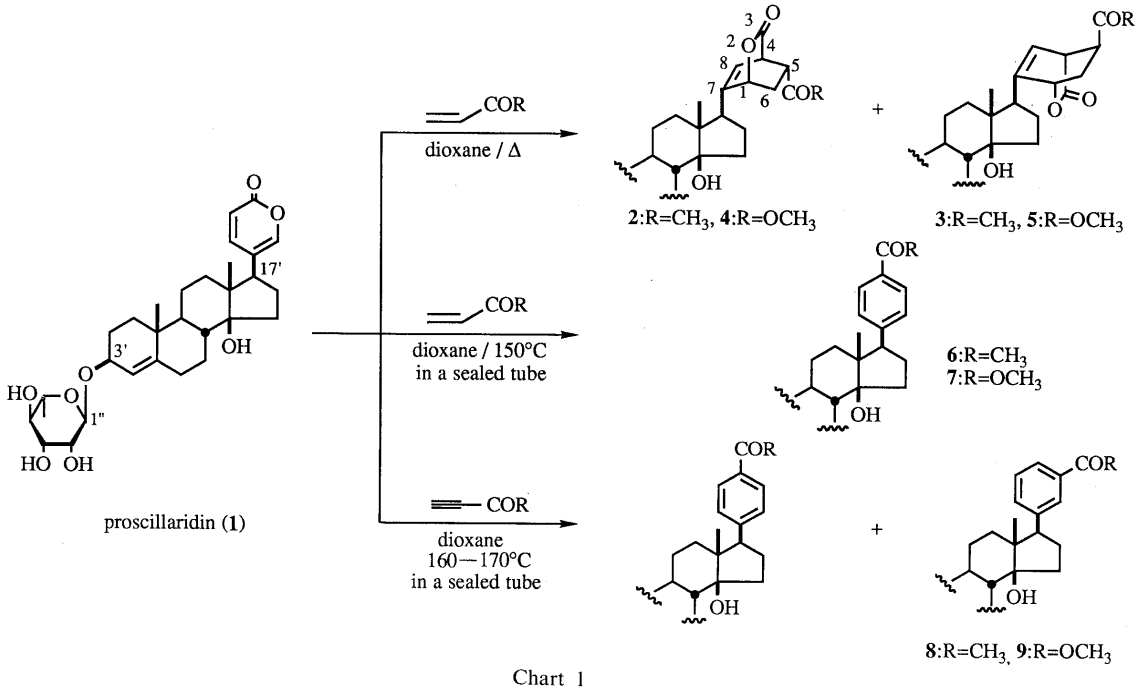

Chart 1

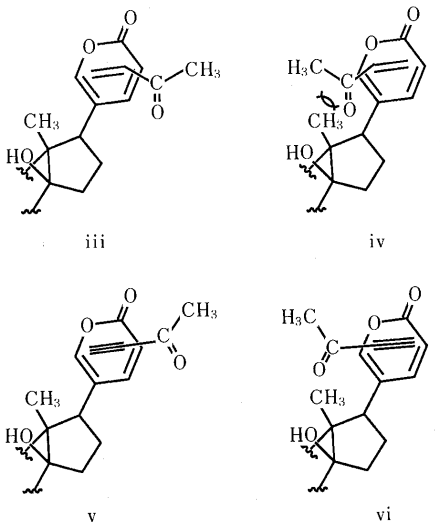

Fig. 3. Possible Transition State in the Diels–Alder Reaction of **1**

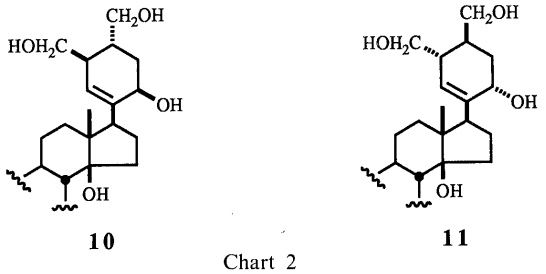

Chart 2

4-en-17β-yl]benzoate (**9**) in 36% and 50% yields. The structures of **7** and **9** were confirmed by the spectral data (see Experimental).

The orientation in the Diels–Alder reactions of **1** with the alkenes is considered to be determined by the steric interaction between the 13′-methyl group and the carbonyl residue in the alkene (Fig. 3). Namely, the cycloaddition may proceed through the transition state (iii) to give the 3-oxo-2-oxabicyclo[2.2.2]oct-7-enes (**2**—**5**) exclusively. In contrast, such selectivity never occurred in the reaction of **1** with the alkynes, since the interactions between the 13′-methyl group and the dienophiles seemed to be negligible (Fig. 3, transition states v and vi).

We finally treated compounds **4** and **5** with lithium aluminum hydride at room temperature. The reductive cleavage of the lactone ring was accomplished within 5 min to give the corresponding alcohols, (1*R*,4*S*,5*S*)-2-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]]-4,5-dihydroxymethyl-2-cyclohexen-1-ol (**10**) and (1*S*,4*R*,5*R*)-2-[3β-[(6-deoxy-α-L-mannopyranosyl)-oxy]-14β-hydroxyandrost-4-en-17β-yl]-4,5-dihydroxy-methyl-2-cyclohexen-1-ol (**11**) quantitatively. The ^{1}H-NMR spectrum of **10** showed one olefinic proton signal (δ 6.04, 1H, d, J=1.8 Hz) and the oxymethine proton signal (δ 5.53, 1H, br s) as well as the signals due to the steroidal and the sugar moieties, while the IR spectrum indicated the absence of a carbonyl group. The spectral data of **11** were almost the same as those of **10**. Based on these data, the structures of **10** and **11** were proved to be as depicted in Chart 2.

Biological Results and Discussion

The biological activities (pIC_{50} and pD_2 values) of proscillaridin derivatives (**2**—**11**) were examined by means of measurements of the enzyme activity of an Na^+,K^+-adenosinetriphosphatase (ATPase) preparation from dog kidney[9] and of PIE in isolated guinea-pig papillary muscle. The results are summarized in Table I.

Table I. Biological Activities of Proscillaridin Derivatives

Compound	pIC_{50}[a]	pD_2[b]
1	7.44±0.02	7.41±0.14
2	5.42±0.03	—
3	5.43±0.08	—
4	<5.0	—
5	<5.0	—
6	6.79±0.01	6.27±0.04
7	6.38±0.10	5.92±0.04
8	6.07±0.02	—
9	5.95±0.09	—
10	<5.0	—
11	<5.0	—

a) pIC_{50} is the concentration of the test compounds required for 50% of the maximum inhibition of Na^+, K^+-ATPase from dog kidney (means±S.E.). *b*) pD_2 is the concentration of the test compounds required for 50% of the maximum PIE in guinea-pig papillary muscles (means±S.E.).

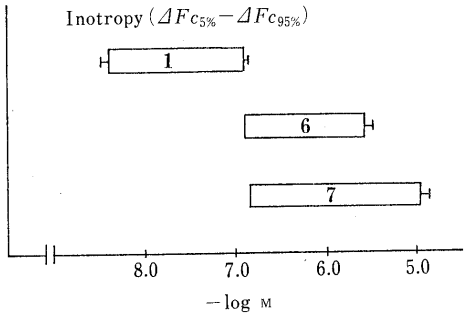

Fig. 4. Concentration-Dependent Range of **1**, **6**, and **7**

The concentration dependency was determined by measuring the range from **5** to 95% of full development of PIE.

Although the biological activities (pIC_{50}) of **2**—**11** were less potent than those of the parent compound (**1**), compounds **6** and **7** bearing a benzene ring at the C-17 position showed moderately potent enzyme-inhibitory activity compared with the other derivatives. Moreover, it is noticeable that the pIC_{50} values of *para*-substituted benzene derivatives (**6** and **7**) were greater than those of *meta*-substituted analogues (**8** and **9**). Compounds **6** and **7** also showed appreciable pD_2 values, and in particular the development of PIE by compound **7** occurred over a rather wider concentration range as compared with **1** (Fig. 4). These biological results suggest that there may be some correlation between the position of the carbonyl oxygen on the C-17 benzene ring and biological activity.

In summary, the δ-lactone of proscillaridin (**1**) was readily transformed to 3-oxo-2-oxabicyclo[2.2.2]oct-7-ene (**2**—**5**) and isomeric benzenes (**6**—**9**) in sufficient yields without prior protection of all the hydroxyl groups in the sugar moiety. Thus, the present modification procedures may be applicable to other cardiac glycosides containing on α-pyrone moiety in the molecule. Additional studies on the correlation between the biological activity and the chemical structure of substituents at C-17 are in progress.

Experimental

All melting points were determined on a Yanagimoto micro melting

point apparatus and are uncorrected. The ultraviolet (UV) spectra were recorded with a Shimadzu UV-2100 spectrometer, the IR spectra with a JASCO IRA-2 spectrometer, and the CD spectra with a JASCO J-600 spectrometer. The ^{1}H-NMR spectra were measured with JEOL JNM-FX-100 and JEOL GSX-400 spectrometers using tetramethylsilane as an internal standard. The following abbreviations are used; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Optical rotations were measured on a JASCO DIP-4 digital polarimeter. HPLC was performed using a JASCO 880-PU pump, and a Shodex RI, SE-11 differential refractometer. Medium pressure chromatography (MPLC) was performed on a C.I.G. column system (Kusano Scientific Co., Ltd., Tokyo; pump KPW-20, UV detector KU-331) with a prepacked column, 20 mm i.d. × 100 mm (octadecyl silica, 20 μm). TLC was carried out on Merck precoated Kieselgel 60F_{254} silanized plates, and spots were detected by illumination with an ultraviolet lamp, or 5% vanillin–70% $HClO_4$, 1% $Ce(SO_4)_2$–10% H_2SO_4 followed by heating. Column chromatography was performed on Silica gel BW-200 or BW-300 (Fuji Davison Chemicals Co., Ltd.).

Reaction of 1 with Methyl Vinyl Ketone A solution of **1** (200 mg, 0.38 mmol) and methyl vinyl ketone (1.6 ml) in dry dioxane (6.4 ml) was heated under reflux for 10 h. The solvent was removed, and the residue was chromatographed on silica gel with $CHCl_3$–$CHCl_3$: MeOH = 8 : 1 to give a mixture of **2** and **3** (213 mg). The mixture was separated by HPLC (Develosil ODS A-5, MeOH : H_2O = 65 : 35, 2.0 ml/min) to yield **2** (123 mg, 54%) and **3** (87 mg, 38%). **2**: a crystalline powder. mp 155—156 °C (MeOH–isopropyl ether). $[\alpha]_D^{26}$ −40.1° (c = 0.5, MeOH). CD (c = 0.02, MeOH) $[\theta]^{25}$ (nm): +1.2 × 10^4 (232) (positive maximum). IR (KBr) cm^{-1}: 3440 (OH), 1740 (C=O), 1720 ($COCH_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.80 (3H, s, 13′-CH_3), 1.04 (3H, s, 10′-CH_3), 1.30 (3H, d, J = 6.0 Hz, 5″-CH_3), 2.18 (3H, s, $COCH_3$), 2.23 (1H, dd, J = 3.8, 13.5 Hz, *endo* 6-H), 2.30 (1H, ddd, J = 3.7, 9.8, 13.5 Hz, *exo* 6-H), 3.02 (1H, ddd, J = 2.5, 3.8, 9.8 Hz, 5-H), 3.45 (1H, dd, J = 9.4, 9.4 Hz, 4″-H), 3.76 (1H, dd, J = 2.5, 6.3 Hz, 4-H), 3.75—3.80 (2H, m, 3″-H, 5″-H), 3.92 (1H, br s, 2″-H), 4.12 (1H, dd, J = 7.5, 8.1 Hz, 3′-H), 4.96 (1H, s, 1″-H), 5.31 (1H, s, 4′-H), 5.38 (1H, br s, 1-H), 5.99 (1H, dd, J = 1.8, 6.3 Hz, 8-H). *Anal.* Calcd for $C_{34}H_{48}O_9 \cdot 1/2\,H_2O$: C, 67.00; H, 8.05. Found: C, 66.86; H, 8.03. **3**: a crystalline powder. mp 174—176 °C (MeOH–isopropyl ether). $[\alpha]_D^{26}$ −43.5° (c = 0.5, MeOH). CD (c = 0.02, MeOH). $[\theta]^{25}$ (nm): −7.0 × 10^3 (222) (negative maximum). IR (KBr) cm^{-1}: 3450 (OH), 1740 (C=O), 1710 ($COCH_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.79 (3H, s, 13′-CH_3), 1.03 (3H, s, 10′-CH_3), 1.30 (3H, d, J = 6.0 Hz, 5″-CH_3), 1.97 (1H, dd, J = 4.0, 13.9 Hz, *endo* 6-H), 2.18 (3H, s, $COCH_3$), 2.33 (1H, ddd, J = 3.2, 9.4, 13.9 Hz, *exo* 6-H), 3.02 (1H, ddd, J = 2.1, 4.0, 9.4 Hz, 5-H), 3.45 (1H, dd, J = 9.4, 9.4 Hz, 4″-H), 3.74 (1H, dd, J = 2.1, 6.2 Hz, 4-H), 3.72—3.80 (2H, m, 3″-H, 5″-H), 3.92 (1H, br s, 2″-H), 4.12 (1H, dd, J = 7.5, 9.3 Hz), 3′-H), 4.95 (1H, s, 1″-H), 5.31 (1H, s, 4-H), 5.38 (1H, dd, J = 2.1, 3.2 Hz, 1-H), 5.88 (1H, dd, J = 2.2, 6.2 Hz, 8-H). *Anal.* Calcd for $C_{34}H_{48}O_9 \cdot 1/2\,H_2O$: C, 67.00; H, 8.05. Found: C, 66.86; H, 8.03.

Acetylation of 2 and 3 Ac_2O (0.5 ml) was added dropwise to an ice-cooled solution of **2** (10 ml, 0.017 mmol) in pyridine (1.0 ml). The mixture was allowed to stand at room temperature for 10 h, then poured into ice-water and extracted with EtOAc. The EtOAc extract was successively washed with 5% HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a product which was purified by column chromatography (SiO_2) to furnish **2a** (12 mg, quant.). Acetylation of **3** (10 mg) in the same manner afforded the corresponding acetate (**3a**, 12 mg, quant.). **2a**: a crystalline powder. mp 129—131 °C (EtOH). $[\alpha]_D^{25}$ −38.6° (c = 0.6, $CHCl_3$). IR (KBr) cm^{-1}: 3500 (OH), 1750 (C=O). ^{1}H-NMR ($CDCl_3$, 400 Hz) δ: 0.79 (3H, s, 13′-CH_3), 1.04 (3H, s, 10′-CH_3), 1.20 (3H, d, J = 6.3 Hz, 5″-CH_3), 1.98, 2.04, 2.15, 2.18 (3H each, all s, OAc), 2.23 (1H, dd, J = 4.2, 13.3 Hz, *endo* 6-H), 2.30 (1H, ddd, J = 3.7, 9.6, 13.3 Hz, *exo* 6-H), 3.02 (1H, ddd, J = 2.6, 4.2, 9.6 Hz, 5-H), 3.76 (1H, dd, J = 2.6, 6.2 Hz, 4-H), 3.97 (1H, dq, J = 6.3, 9.9 Hz, 5″-H), 4.11 (1H, m, 3′-H), 4.89 (1H, d, J = 1.6 Hz, 1″-H), 5.05 (1H, dd, J = 9.9, 9.9 Hz, 4″-H), 5.21 (1H, dd, J = 1.6, 3.4 Hz, 2″-H), 5.31 (1H, s, 4′-H), 5.32 (1H, dd, J = 3.4, 9.9 Hz, 3″-H), 5.38 (1H, d-like, 1-H), 5.99 (1H, dd, J = 1.9, 6.2 Hz, 8-H). *Anal.* Calcd for $C_{40}H_{54}O_{12}$: C, 66.17; H, 7.50. Found: C, 65.92; H, 7.61. **3a**: a crystalline powder. mp 115—117 °C (EtOH). $[\alpha]_D^{26}$ −41.6° (c = 0.7, $CHCl_3$). IR (KBr) cm^{-1}: 3440 (OH), 1755 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.79 (3H, s, 13′-CH_3), 1.04 (3H, s, 10′-CH_3), 1.20 (3H, d, J = 6.3 Hz, 5″-CH_3), 1.96 (1H, dd, J = 4.2, 13.7 Hz, *endo* 6-H), 1.98, 2.04, 2.15, 2.18 (3H each, all s, OAc), 2.33 (1H, ddd, J = 4.1, 9.7, 13.7 Hz, *exo* 6-H), 3.02 (1H, ddd, J = 4.2, 6.0, 9.7 Hz, 5-H), 3.73 (1H, dd, J = 2.4, 6.0 Hz, 4-H), 3.97 (1H, dq, J = 6.3, 9.9 Hz, 5″-H), 4.10 (1H, m, 3′-H), 4.89 (1H, d, J = 1.6 Hz, 1″-H), 5.05 (1H, dd, J = 9.9, 9.9 Hz, 4″-H), 5.20 (1H, dd, J = 1.6, 3.5 Hz, 2″-H), 5.30 (1H, s, 4′-H), 5.32 (1H, dd, J = 3.5, 9.9 Hz, 3″-H), 5.83 (1H, d-like, 1-H), 5.87 (1H, dd, J = 2.0, 6.0 Hz, 8-H). *Anal.* Calcd for $C_{40}H_{54}O_{10}$: C, 66.17; H, 7.50. Found: C, 66.24; H, 7.40.

Reaction of 1 with Methyl Acrylate A solution of **1** (200 mg, 0.38 mmol) and methyl acrylate (2.0 ml) in dry xylene (3.0 ml)–dioxane (3.0 ml) was heated under reflux for 24 h. The solvent was removed, and the residue was chromatographed on silica gel using $CHCl_3$–$CHCl_3$: MeOH = 7 : 1 as an eluent to give a mixture of **4** and **5** (190 mg). The mixture was separated by HPLC (Develosil ODS A-5, MeOH : H_2O = 70 : 30, 3.0 ml/min) to yield **4** (100 mg, 43%) and **5** (75 mg, 32%). **4**: a crystalline powder. mp 153—154 °C (MeOH–isopropyl ether). $[\alpha]_D^{26}$ −30.1° (c = 0.6, MeOH). CD (c = 0.01, MeOH) $[\theta]^{25}$ (nm): +2.3 × 10^4 (228) (positive maximum). IR (KBr) cm^{-1}: 3440 (OH), 1735 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.79 (3H, s, 13′-CH_3), 1.04 (3H, s, 10′-CH_3), 1.30 (3H, d, J = 6.2 Hz, 5″-CH_3), 2.26 (1H, dd, J = 3.3, 13.5 Hz, *endo* 6-H), 2.40 (1H, ddd, J = 3.4, 9.9, 13.5 Hz, *exo* 6-H), 3.02 (1H, ddd, J = 2.7, 3.3, 9.9 Hz, 5-H), 3.45 (1H, dd, J = 9.2, 9.2 Hz, 4″-H), 3.68 (3H, s, CO_2Me), 3.79 (1H, dd, J = 2.7, 6.1 Hz, 4-H), 3.73—3.81 (2H, m, 3″-H, 5″-H), 3.92 (1H, d, J = 1.8 Hz, 2″-H), 4.12 (1H, dd, J = 6.8, 8.2 Hz, 3′-H), 4.95 (1H, s, 1″-H), 5.31 (1H, s, 4′-H), 5.38 (1H, br s, 1-H), 6.04 (1H, dd, J = 2.0, 6.1 Hz, 8-H). *Anal.* Calcd for $C_{34}H_{48}O_{10} \cdot 1/2\,H_2O$: C, 65.28; H, 7.84. Found: C, 64.98; H, 7.54. **5**: a crystalline powder. mp 175—176 °C (MeOH–isopropyl ether). $[\alpha]_D^{26}$ −43.8° (c = 0.5, MeOH). CD (c = 0.02, MeOH) $[\theta]^{25}$ (nm): −1.4 × 10^4 (221) (negative maximum). IR (KBr) cm^{-1}: 3440 (OH), 1735 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.79 (3H, s, 13′-CH_3), 1.04 (3H, s, 10′-CH_3), 1.30 (3H, d, J = 6.4 Hz, 5″-CH_3), 1.98 (1H, dd, J = 3.3, 13.4 Hz, *endo* 6-H), 2.43 (1H, ddd, J = 3.3, 9.6, 13.4 Hz, *exo* 6-H), 3.01 (1H, ddd, J = 2.6, 3.3, 9.6 Hz, 5-H), 3.45 (1H, dd, J = 9.4, 9.4 Hz, 4″-H), 3.78 (1H, dd, J = 2.6, 6.1 Hz, 4-H), 3.75—3.80 (2H, m, 3″-H, 5″-H), 3.92 (1H, br s, 2″-H), 4.12 (1H, dd, J = 7.5, 7.9 Hz, 3′-H), 4.95 (1H, br s, 1″-H), 5.31 (1H, s, 4′-H), 5.82 (1H, br s, 1-H), 5.93 (1H, dd, J = 2.0, 6.1 Hz, 8-H). *Anal.* Calcd for $C_{34}H_{48}O_{10} \cdot 1/2\,H_2O$: C, 65.28; H, 7.84. Found: C, 65.53; H, 8.00.

4-[3β-[(6-Deoxy-α-L-mannopyranosyl)oxy-14β-hydroxyandrost-4-en-17β-yl]phenylethanone (6) A solution of **1** (100 mg, 0.19 mmol) and methyl vinyl ketone (0.8 ml) in dioxane (3.2 ml) was heated at 150 °C for 4 h in a sealed tube. The solvent was removed, and the residue was chromatographed on silica gel with $CHCl_3$–$CHCl_3$: MeOH (10 : 1) to give a crude product, which was purified by MPLC (MeOH : H_2O = 70 : 30, 5.0 ml/min) to yield **6** (71 mg, 68%). **6**: a crystalline powder. mp 143—145 °C (MeOH–isopropyl ether). $[\alpha]_D^{26}$ −49.6° (c = 0.5, MeOH). IR (KBr) cm^{-1}: 3440 (OH), 1670 (C=O). UV (λ_{max}, MeOH) nm (ε): 259.9 (1.4 × 10^4). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.56 (3H, s, 13′-CH_3), 1.03 (3H, s, 10′-CH_3), 1.30 (3H, d, J = 6.2 Hz, 5″-CH_3), 2.58 (3H, s, $COCH_3$), 2.92 (1H, dd, J = 7.1, 9.3 Hz, 17′-H), 3.46 (1H, dd, J = 9.4, 9.4 Hz, 4″-H), 3.75—3.79 (2H, m, 3″-H, 5″-H), 3.93 (1H, s, 2″-H), 4.12 (1H, dd, J = 7.5, 7.9 Hz, 3′-H), 4.96 (1H, s, 1″-H), 5.32 (1H, s, 4′-H), 7.41 (2H, d, J = 8.4 Hz, 3-H, 5-H), 7.91 (2H, d, J = 8.4 Hz, 2-H, 6-H). *Anal.* Calcd for $C_{33}H_{46}O_7 \cdot 1/2\,H_2O$: C, 69.21; H, 8.44. Found: C, 69.11; H, 8.23.

Methyl 4-[3β-(6-Deoxy-α-L-mannopyranosyl)oxy-14β-hydroxyandrost-4-en-17β-yl]benzoate (7) A solution of **1** (100 mg, 0.19 mmol) and methyl acrylate (1.0 ml) was heated at 150 °C for 9.5 h in a sealed tube. The solvent was removed, and the resulting residue was chromatographed on silica gel using $CHCl_3$–$CHCl_3$: MeOH = 7 : 1 as an eluent to give a crude product, which was purified by HPLC (Develosil ODS A-5, MeOH : H_2O = 65 : 35, 3.0 ml/min) to yield **7** (84 mg) in 78% yield. **7**: a crystalline powder. mp 128—130 °C (MeOH–isopropyl ether). $[\alpha]_D^{26}$ −44.8 °C (c = 0.6, MeOH). IR (KBr) cm^{-1}: 3450 (OH), 1720 (C=O). UV (λ_{max}, MeOH) nm (ε): 246.0 (2.0 × 10^4). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.56 (3H, s, 13′-CH_3), 1.03 (3H, s, 10′-CH_3), 1.30 (3H, d, J = 6.2 Hz, 5″-CH_3), 2.92 (1H, dd, J = 8.0, 8.0 Hz, 17′-H), 3.46 (1H, dd, J = 9.4, 9.4 Hz, 4″-H), 3.75—3.79 (2H, m, 3″-H, 5″-H), 3.89 (3H, s, CO_2Me), 3.93 (1H, s, 2″-H), 4.12 (1H, dd, J = 7.5, 7.9 Hz, 3′-H), 4.96 (1H, s, 1″-H), 5.32 (1H, s, 4′-H), 7.41 (2H, d, J = 8.4 Hz, 3-H, 5-H), 7.91 (2H, d, J = 8.4 Hz, 2-H, 6-H). *Anal.* Calcd for $C_{33}H_{46}O_8 \cdot 1/2\,H_2O$: C, 68.37; H, 8.17. Found: C, 68.25; H, 8.31.

Reaction of 1 with 3-Butyn-2-one A solution of **1** (200 mg, 0.38 mmol) and 3-butyn-2-one (0.4 ml) in dry dioxane (4.0 ml) was heated at 170 °C for 15 h in a sealed tube. The reaction mixture was concentrated under reduced pressure, then the residue was chromatographed on silica gel with $CHCl_3$: MeOH = 9 : 1 to give a mixture of **6** and **8**. The mixture was separated by HPLC (Develosil ODS-5, MeOH : H_2O = 80 : 20, 2.0 ml/min) to yield **6** (110 mg, 52%) and **8** (91 mg, 43%). **8**: a crystalline powder. mp 135—137 °C (MeOH–isopropyl ether). $[\alpha]_D^{26}$ −51.9° (c = 0.7, MeOH).

IR (KBr) cm^{-1}: 3430 (OH), 1670 (C=O). UV (λ_{max}, MeOH) nm (ε): 250.2 (9.3×10^3). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.57 (3H, s, 13′-CH_3), 1.04 (3H, s, 10′-CH_3), 1.31 (3H, d, J=6.2 Hz, 5″-CH_3), 2.59 (3H, s, $COCH_3$), 2.94 (1H, dd, J=7.0, 9.2 Hz, 17′-H), 3.45 (1H, dd, J=9.3, 9.3 Hz, 4″-H), 3.75—3.81 (2H, m, 3″-H, 5″-H), 3.94 (1H, s, 2″-H), 4.14 (1H, dd, J=7.5, 8.1 Hz, 3″-H), 4.97 (1H, d, J=1.3 Hz, 1″-H), 5.32 (1H, s, 4′-H), 7.35 (1H, dd, J=7.7, 7.7 Hz, 5-H), 7.62 (1H, d, J=7.7 Hz, 4-H), 7.75 (1H, d, J=7.7 Hz, 6-H), 7.93 (1H, s, 2-H). *Anal.* Calcd for $C_{33}H_{46}O_7 \cdot H_2O$: C, 69.21; H, 8.44. Found: C, 69.51; H, 8.44.

Reaction of 1 with Methyl Propiolate A solution of **1** (500 mg, 0.94 mmol) and methyl propiolate (1.0 ml) in dry dioxane (10.0 ml) was heated at 160 °C for 44 h in a sealed tube. The reaction mixture was concentrated under reduced pressure, then the residue was chromatographed on silica gel with $CHCl_3$: MeOH = 9 : 1 to give a mixture of **7** and **9** (490 mg). The mixture was separated by HPLC (Develosil ODS A-5, MeOH : H_2O = 80 : 20, 3.5 ml/min) to yield **7** (197 mg, 36%) and **9** (272 mg, 50%). **9**: a crystalline powder. mp 144—145 °C (MeOH–isopropyl ether). $[\alpha]_D^{26}$ −51.7° (c=0.7, MeOH). IR (KBr) cm^{-1}: 3440 (OH), 1720 (C=O). UV (λ_{max}, MeOH) nm (ε): 236.0 (8.4×10^3), ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.56 (3H, s, 13′-CH_3), 1.03 (3H, s, 10′-CH_3), 1.31 (3H, d, J=6.3 Hz, 5″-CH_3), 2.92 (1H, dd, J=7.1, 9.3 Hz, 17′-H), 3.47 (1H, dd, J=9.6, 9.6 Hz, 4″-H), 3.76—3.80 (2H, m, 3′-H, 5′-H), 3.90 (3H, s, CO_2Me), 3.94 (1H, s, 2″-H), 4.14 (1H, dd, J=7.3, 8.3 Hz, 3′-H), 4.96 (1H, s, 1″-H), 5.32 (1H, s, 4′-H), 7.32 (1H, dd, J=7.8, 7.8 Hz, 5-H), 7.60 (1H, d, J=7.8 Hz, 4-H), 7.83 (1H, d, J=7.8 Hz, 6-H), 7.98 (1H, s, 2-H). *Anal.* Calcd for $C_{33}H_{46}O_8 \cdot 1/2\,H_2O$: C, 68.37; H, 8.17. Found: C, 68.37; H, 8.50.

$LiAlH_4$ Reduction of 4 $LiAlH_4$ (36 mg, 0.95 mmol) was added portionwise to a solution of **4** (50 mg, 0.073 mmol) in dry THF (10 ml) and the resulting mixture was stirred at room temperature for 10 min under N_2. The reaction mixture was treated with H_2O-saturated ether, H_2O, and 5% aqueous NaOH. The precipitate was removed by filtration, and removal of the solvent from the filtrate gave a product, which was purified by column chromatography (SiO_2, 5 g, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1, lower phase) to furnish **10** (48 mg, quant.). **10**: a crystalline powder. mp 265—267 °C (MeOH–isopropyl ether). $[\alpha]_D^{26}$ −43.3° (c=0.4, MeOH). IR (KBr) cm^{-1}: 3420 (OH). ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.95 (3H, s, 13′-CH_3), 1.23 (3H, s, 10′-CH_3), 1.70 (3H, d, J=5.9 Hz, 5″-CH_3), 3.96—4.10 (4H, m, –$CH_2OH \times 2$), 4.13 (1H, dd, J=5.0, 10.1 Hz, 1-H), 4.30—4.38 (2H, m, 4″-H, 5″-H), 4.42 (1H, dd, J=7.5, 7.9 Hz, 3′-H), 4.54 (1H, dd, J=1.3, 8.6 Hz, 3″-H), 4.58 (1H, br s, 2″-H), 5.53 (1H, s, 1″-H), 5.57 (1H, s, 4′-H), 6.04 (1H, d, J=1.8 Hz, 3-H). *Anal.* Calcd for $C_{33}H_{52}O_9 \cdot H_2O$: C, 64.89; H, 8.93. Found: C, 64.95; H, 9.00.

$LiAlH_4$ Reduction of 5 $LiAlH_4$ (36 mg, 0.95 mmol) was added portionwise to a solution of **5** (50 mg, 0.073 mmol) in dry THF (10 ml) and the resulting mixture was stirred at room temperature for 10 min under N_2. Work-up of the reaction mixture as described above gave a product, which was purified by column chromatography (SiO_2, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1, lower phase) to furnish **11** (48 mg, quant.). **11**: a crystalline powder. mp 266—267 °C. $[\alpha]_D^{24}$ −44.4° (c=0.7, MeOH). IR (KBr) cm^{-1}: 3430 (OH). ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.94 (3H, s, 13′-CH_3), 1.28 (3H, s, 10′-CH_3), 1.71 (3H, d, J=5.7 Hz, 5″-CH_3), 3.96—4.04 (2H, m, 1H in $CH_2OH \times 2$), 4.08 (1H, dd, J=5.6, 10.3 Hz, 1H, in CH_2OH), 4.16 (1H, dd, J=6.0, 10.3 Hz, 1H in CH_2OH), 4.30—4.39 (2H, m, 4″-H, 5″-H), 4.42 (1H, dd, J=7.7, 9.3 Hz, 3′-H), 4.54 (1H, dd, J=1.8, 8.6 Hz, 3″-H), 4.59 (1H, d, J=1.8 Hz, 2″-H), 5.52 (1H, s, 1″-H), 5.57 (1H, s, 4′-H), 6.17 (1H, s, 3-H). *Anal.* Calcd for $C_{33}H_{52}O_9 \cdot H_2O$: C, 64.89; H, 8.93. Found: C, 64.95; H, 9.23.

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