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Cyanoglycosylation products of 17-*O*-acetyl-testosterone

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Abstract

17-*O*-Acetyl testosterone, which has no susceptible hydroxyl or carboxyl group for glycosylation, was glycosylated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of a mixed catalyst, $Hg(CN)_2$ and $HgBr_2$, in benzene–nitromethane. Reaction occurred on the α, β-unsaturated ketone on the six–membered A-ring to give six 3-*O*-glycosides, each bearing a cyano group at the 3- or 5-position of the aglycon, and a 3-*O*-glycoside bearing a $CONH_2$ group at the 3-position. Structural analyses of these products were carried out by various NMR (^1H, ^{13}C NMR, ^1H–^1H and ^1H–^{13}C COSY, HMBC, and DEPT), FABMS and X-ray analyses. The mechanisms of the formations of the products are discussed. It was determined that mercuric cyanide was essential as a catalyst for the progress of the cyanoglycosylation.

Keywords: Testosterone; α, β-unsaturated ketone; Glycosylation; Mercury(II) cyanide

1. Introduction

During research for the preparation of triterpenoidal saponins, which consisted of glycyrrhetic acid (**1**) as an aglycon and several kinds of sugar components at the *O*-3 position [1–5], we incidentally found [6,7] that an α, β-unsaturated ketone on the six-membered C-ring of methyl 3-*O*-acetyl-glycyrrhetinate (**2**) was easily subject to glycosylation with 2,3,4,6-tetra-*O* acetyl-α-D-glucopyranosyl bromide (**3**) by the use of silver trifluoromethanesulfonate (AgOTf) [8–10] as a catalyst to give an enol α-glycoside (**4**) in good yield. This finding led us to attempt to synthesize an enol glycoside such as **5** from the steroidal hormone, testosterone [androst-4-ene-3-one-17β-ol, (**6**)]. This aglycon was chosen because this steroidal hormone is a readily available compound of a representative group of compounds having an α, β-unsaturated ketone in the six-membered A-ring. Furthermore, the enol glycoside (**5**) may be capable of recognizing target organs and cells and be hydrolysed with a glycosidase to release testosteorone (**6**) when it is used

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as pharmacophore. As we previously reported [11], the reaction of 17-*O*-acetyl-testosterone (**7**) with a large excess of (**3**) in the presence of AgOTf as a catalyst afforded 17-*O*-acetylandrost-4-ene-3,6-dione-17β-ol (**8**) and 17-*O*-acetyl-7α-bromoandrost-4-ene-3,6-dione-17β-ol (**9**).

However, when mercuric cyanide and mercuric bromide were used as a mixed catalyst instead of AgOTf according to the reported procedures [5,12,13], the glycosylation at the α, β-unsaturated ketone on the A-ring of **7** with **3** proceeded to afford several glycosides. In this paper, we report the glycosylation at the α, β-unsaturated ketone of **7** and the structural analyses of the products. Furthermore, a mechanism for the formation of the glycosides has been formulated.

2. Results and discussion

Glycosylation has generally been considered to occur on either a hydroxyl or a carboxyl group [3,5,14–16]. Although 17-*O*-acetyltestosterone [17-*O*-acetylandrost-4-ene-3-one-17β-ol, (**7**)] has no such a susceptible group for glycosylation, the reaction of **7** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**3**) in the presence of $Hg(CN)_2$ and $HgBr_2$ as a mixed catalyst in dry 1:1 benzene–nitromethane at 60 °C in accordance with the modified methods of Paulsen and Bunshe [13] and Saito et al. [5] gave compounds **10–16** in yields of 36.7, 4.6, 1.9, 18.0, 3.6, 7.5 and 3.7%, respectively. Products **10–16** exhibited signals for protons on a glucopyranose ring in their ^{1}H NMR spectra (Table 1), which suggests that **10–16** are monoglycoside derivatives. In the fast-atom-bombardment mass (FABMS) spectra, compounds **10, 11** and **13–16** showed the same quasimolecular ion peak at m/z 710 $[M+Na]^+$, which was a higher mass number by 27 amu than that of the expected product such as **17** (m/z 683 $[M+Na]^+$), which corresponds to acetylated product of **5**. Compound **12** showed a quasimolecular ion peak at m/z 728 $[M+Na]^+$ that was also at a higher mass number by 45 amu than that of **17**. It was evident that all these compounds **10–16** contained a nitrogen atom by their elemental analyses (Table 2). As it was first thought that the nitrogen atom came from nitromethane used as one of the solvents, the reaction was carried out in the absence of nitromethane. However, the reaction of **7** with **3** in the presence of only $Hg(CN)_2$ and $HgBr_2$ in dry benzene gave the same products as the foregoing reaction. Since another source of a nitrogen atom could be from the $Hg(CN)_2$ used as one of the catalysts, the reaction of **7** with **3** was carried out using only $HgBr_2$ as a catalyst in dry 1:1 benzene–nitromethane. In this case, the reaction gave no product. From these results, together with the FABMS spectral data of products **10–16** that showed a higher mass number than that of the expected product **17**, by 27 amu in **10, 11** and **13–16**, and by 45 amu in **12**, it is suggested that compounds **10, 11** and **13–16** are monoglycosides bearing a CN group on their aglycons, and compound **12** is monoglycoside bearing a $CONH_2$ group (Schemes 1 and 2).

Compounds **10** (amorphous powder) and **11** (mp 208–210 °C) revealed similar ^{13}C NMR spectra (Table 3). Each of them showed 36 carbon signals, which is a larger number by one than that for **17**. In their ^{1}H NMR spectra, **10** and **11** exhibited anomeric proton signals with the coupling constants of 3.7 and 4.0 Hz at δ 5.56 and 5.57, respectively, which suggests that **10** and **11** are α-glycosides. Compounds **10** and **11** showed a few easily assignable signals of protons on the aglycons: H-4(s), H-17 (dd, $J_{16,17}$ and $J_{16',17}$ 8.9 and 8.2 Hz), 18-CH_3 (s) and 19-CH_3 (s) at δ 5.39, 4.58, 0.81 and 1.06, respectively, in **10**, and H-4 (s), H-17 (dd, $J_{16,17}$ and $J_{16',17}$ 8.9 and 7.9 Hz), 18-CH_3 (s) and 19-CH_3 (s) at δ 5.56, 4.6, 0.81 and 1.05, respectively, in **11** (Table 1). All carbon signals of **10** and **11** in the ^{13}C NMR spectra were assigned as listed in Table 3 using these assignable proton signals on the basis of the heteronuclear multiple bond connection (HMBC) spectral data with help of the ^{1}H–^{1}H and ^{1}H–^{13}C correlated spectroscopy (COSY) and distortionless enhancement by polarization transfer (DEPT) spectral data [17]. In the ^{13}C NMR spectra (Table 3), carbon signals at the 3-positions of **10** and **11** were sifted to higher fields (δ 73 and 68.2, respectively) than that of the starting material **7** (δ 199.3) [11]. In the HMBC spectra, the anomeric proton signals at δ 5.56 and 5.57 of **10** and **11** correlated to the carbon signals at δ 73.0 and 68.2, respectively, and the H-4 signals (δ 4.68 and 4.90) of **10** and **11** correlated to the newly introduced carbon (CN) signals at δ 119.2 and 120.9 of **10** and **11**, respectively (Fig. 1). From these NMR spectral data, together with FABMS data and the elemental analyses (Table 2), it was determined that both compounds **10** and **11** were 17-*O*-acetyl-3-cyano-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-

Table 1
^{1}H NMR spectral data for compounds **10–16** and **26**[a]

	10	**11**	**12**	**13**	**14**	**15**	**16**	**26**
H-1a	1.61*	1.34*	1.65*	1.80*	1.75–1.82*	1.76–1.82*	1.73–1.80*	1.49*
H-1b	1.80*	1.66*	1.92*	1.84*	1.75–1.82*	1.76–1.82*	1.73–1.80*	2.30*
H-2a	1.83*	2.17*	2.05*	2.23*	2.13*	2.02*	0.95*	5.13 (dd, 4.4, 0.5)
H-2b	2.72*	2.27*	2.27*	2.28*	2.26*	2.15*	1.51*	—
H-4a	5.39 (s)	5.56 (s)	4.90 (d, 1.5)	4.82 (s)	4.68 (s)	4.90 (s)	4.68 (s)	2.32 (d, 16.1)
H-4b	—	—	—	—	—	—	—	2.05*
H-6a	2.09*	1.68*	1.98*	1.48*	1.63*	0.90*	1.76*	1.46*
H-6b	2.22*	2.30*	2.08*	2.19*	1.66*	1.52*	2.18*	1.60*
H-7a	0.97*	0.90*	0.95*	1.62*	1.25*	1.80*	1.75*	1.69*
H-7b	1.78*	1.80*	1.74*	1.72*	1.57*	2.15*	2.08*	1.18*
H-8	1.52*	1.52*	1.52*	1.53*	1.55*	1.40*	1.40*	1.44*
H-9	0.88*	0.62*	0.91*	1.37*	1.38*	1.09*	1.20*	1.27*
H-11a	1.33*	1.37*	1.30*	1.28*	1.25*	1.38–1.42*	1.40*	1.34*
H-11b	1.50*	1.45*	1.53*	1.59*	1.68*	1.38–1.42*	1.43*	1.56*
H-12a	1.17*	1.18*	1.14*	1.26*	1.24*	1.34*	1.18*	1.25*
H-12b	1.77*	1.78*	1.74*	1.77*	1.77*	1.78*	1.75*	1.78*
H-14	1.02*	0.99*	1.02*	1.23*	1.22*	0.99*	1.05*	1.15*
H-15a	1.31*	1.33*	1.32*	1.31*	1.55*	1.29*	1.26*	1.35*
H-15b	1.63*	1.66*	1.63*	1.65*	1.78*	1.62*	1.58*	1.66*
H-16a	1.50*	1.52*	1.47*	1.50*	1.50*	1.52*	1.47*	1.50*
H-16b	2.18*	2.15*	2.15*	2.20*	2.18*	2.15*	2.17*	2.22*
H-17	4.58 (dd, 8.9, 8.2)[b]	4.60 (dd, 8.9, 7.9)	4.56 (dd, 8.6, 8.6)	4.60 (dd, 8.9, 8.2)	4.60 (dd, 8.5, 8.2)	4.59 (dd, 9.2, 8.2)	4.56 (dd, 8.9, 7.9)	4.59 (dd, 8.9, 7.9)
18-CH_3	0.81 (s)	0.81 (s)	0.81 (s)	0.80 (s)	0.80 (s)	0.78 (s)	0.78 (s)	0.79 (s)
19-CH_3	1.06 (s)	1.05 (s)	1.06 (s)	0.92 (s)	0.89 (s)	1.20 (s)	1.20 (s)	0.87 (s)
Others	—	—	6.32 and 6.57 (NH×2)	—	—	—	—	—
H-1′	5.56 (d, 3.7)	5.57 (d, 4.0)	5.38 (d, 3.7)	5.54 (d, 3.4)	4.93 (d, 7.9)	5.54 (d, 4.0)	4.97 (d, 8.0)	5.40 (d, 3.6)
H-2′	4.90 (dd, 10.4, 3.7)	4.80 (dd, 10.4, 4.0)	4.90 (dd, 10.1, 3.7)	5.00 (dd, 10.1, 3.4)	5.10 (dd, 9.5, 7.9)	4.92 (dd, 10.1, 4.0)	5.13 (dd, 9.5, 8.0)	4.94 (dd, 10.2, 3.6)
H-3′	5.47 (dd, 10.4, 9.7)	5.52 (dd, 10.4, 9.5)	5.47 (dd, 10.1, 9.8)	5.08 (dd, 10.1, 9.5)	5.08 (dd, 9.5, 9.5)	5.53 (dd, 10.1, 9.5)	5.25 (dd, 9.5, 9.5)	5.52 (dd, 10.2, 9.2)
H-4′	5.09 (dd, 9.7, 9.7)	5.10 (dd, 10.1, 9.5)	5.05 (dd, 9.8, 9.8)	5.08 (dd, 9.8, 9.5)	5.07 (dd, 9.5 8.9)	5.08 (dd, 9.8, 9.5)	5.08 (dd, 9.8, 9.5)	5.07 (dd, 9.8, 9.2)
H-5′	4.16 (ddd, 9.7, 4.3, 2.1)	4.35 (ddd, 10.1, 4.0, 2.1)	4.20 (ddd, 9.8, 4.9, 2.0)	3.90 (ddd, 9.8, 4.9, 2.1)	3.82 (ddd, 8.9, 6.1, 2.1)	4.02 (ddd, 9.8, 4.6, 2.1)	3.82 (ddd, 9.8, 4.9, 2.8)	3.97 (ddd, 9.8, 4.6, 2.0)
H-6′a	4.28 (dd, 12.2, 4.3)	4.38 (dd, 12.8, 4.0)	4.23 (dd, 11.9, 4.9)	4.21 (dd, 12.5, 4.9)	4.26 (dd, 12.5, 4.6)	4.30 (dd, 12.2, 4.6)	4.19 (dd, 12.2, 4.9)	4.26 (dd, 12.5, 4.6)
H-6′b	4.06 (dd, 12.2, 2.1)	4.17 (dd, 12.8, 2.1)	4.04 (dd, 11.9, 2.0)	4.02 (dd, 12.5, 2.1)	4.12 (dd, 12.5, 2.1)	4.09 (dd, 12.2, 2.1)	4.16 (dd, 12.2, 2.8)	4.07 (dd, 12.2, 2.0)
$COCH_3$	2.08, 2.08, 2.04, 2.03, 2.02	2.09, 2.04, 2.03, 2.03, 2.01	2.09, 2.07, 2.04, 2.04, 2.03	2.10, 2.08, 2.04, 2.03, 2.02	2.11, 2.05, 2.04, 2.04, 2.02	2.12, 2.09, 2.04, 2.04, 2.03	2.08, 2.07, 2.05, 2.03, 2.02	2.08, 2.06, 2.04, 2.04, 2.02

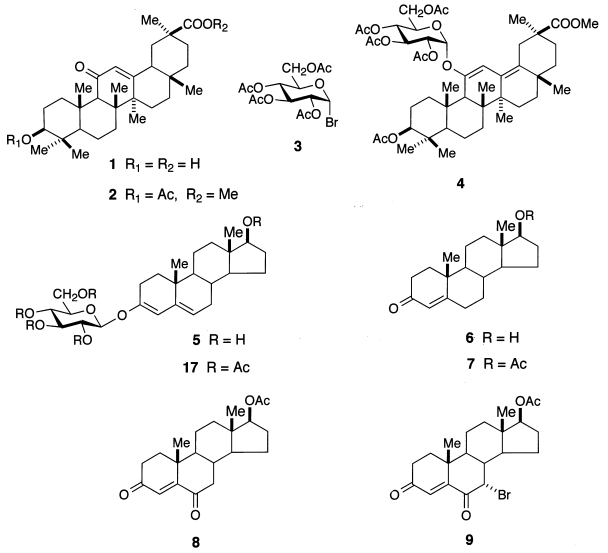
[a] Spectra were obtained in $CDCl_3$. Chemical shifts are in ppm from internal $(CH_3)_4Si$. Signal assignments were based on ^{1}H–^{1}H, ^{1}H–^{13}C COSY and ^{1}H–^{13}C long-range COSY spectral data.
[b] Coupling constants (J in Hz) are given in parentheses.
* These proton signals were overlapped with one or more signals, and the chemical shifts were obtained on the bases of ^{1}H–^{13}C COSY.

Table 2
Elemental analysis data of compounds **10–16**

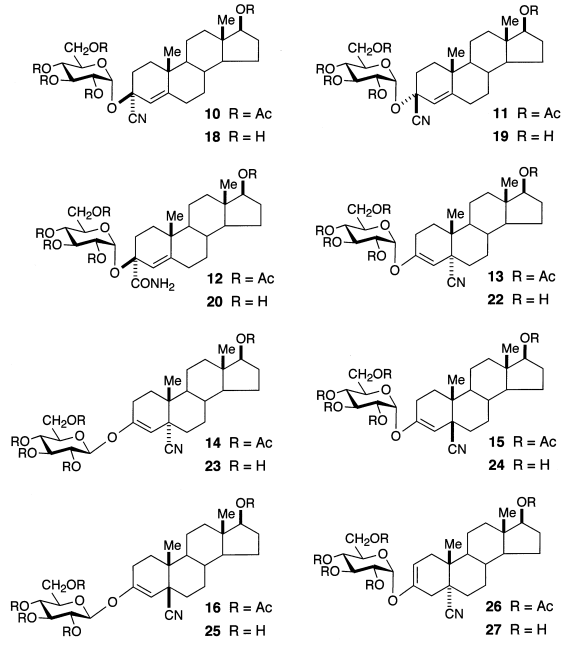
Compound	Formula	Calcd			Found		
		H	C	N	H	C	N
10	$C_{36}H_{49}NO_{12}$	7.18	62.87	2.04	7.19	62.54	1.96
11	$C_{36}H_{49}NO_{12}$	7.18	62.87	2.04	7.09	62.78	2.00
12	$C_{36}H_{52}NO_{13}$	7.28	61.26	1.98	7.20	60.91	1.91
13	$C_{36}H_{49}NO_{12}$	7.18	62.87	2.04	7.19	62.49	1.96
14	$C_{36}H_{49}NO_{12}$	7.18	62.87	2.04	7.09	62.61	2.07
15	$C_{36}H_{49}NO_{12}$	7.18	62.87	2.04	7.00	62.92	2.02
16	$C_{36}H_{49}NO_{12}$	7.18	62.87	2.04	7.19	62.77	2.02

α-D-glucopyranosyl)androst-4-ene-17β-ol, and were diastereoisomers with respect to the 3-position with each other.

The absolute structure of **11** was unequivocally confirmed by X-ray diffraction study of single crystals (Fig. 2) [18,19]. In the crystal the cyano group and the pyranosyl group substituted at the 3-position of **11** arranged in β and α, respectively. Consequently, the cyano group and pyanosyl group on the 3-position of **10** could be assigned to have α- and β-configuration, respectively. The crystal data for compound **11** are listed in Table 4. Alkaline hydrolyses of **10** and **11** gave corresponding deacetylated glycosides **18** and **19** in 82.3 and 75.7% yields, respectively, which showed the same quasimolecular ion peak at m/z 500 $[M+Na]^+$ in the FABMS spectra. Acid hydrolysis of both **18** and **19** gave testosterone 6, which was identified with an authentic sample by HPLC and ^{13}C NMR spectroscopy.



Scheme 1.



Scheme 2.

Compound **12** (mp 207–208 °C) exhibited ^1H and ^{13}C NMR spectra similar to the corresponding spectra of **10**. The anomeric proton of **12** was observed as a doublet with coupling constants of 3.7 Hz at δ 5.38, which suggests that **12** is α-glycoside. The ^{13}C NMR spectrum of **12** showed 36 signals as the same as those of **10** and **11**. Since the FABMS spectrum of **12** showed a quasimolecular ion peak at m/z 728 that had a higher mass number by 18 than those of either **10** or **11**, the newly introduced substituent was thought to be an amide ($CONH_2$) group instead of a cyano group in the cases of **10** and **11**. The α-glycosyl and amide groups of **12** were also determined to substitute at the 3-position on the basis of the HMBC spectral data. Alkaline hydrolysis of **12** gave deacetylated compound **20** that showed a quasimolecular ion peak at m/z 518 $[M+Na]^+$ in the FABMS spectrum. Compound **20** was also obtained by the reaction of **18** with Na in methanol in 9.6% yield, though most starting material was recovered. Therefore, the glycosyl and amide group on the 3-position of **12** were confirmed to have β and α configurations, respectively. The acid hydrolysis of **20** gave quantitatively compound **21** (Scheme 3), which showed a quasimolecular ion peak at m/z 338 $[M+Na]^+$. Compound **21** exhibited two vinyl protons at the 4- and 6-positions as singlet and double-doublet ($J_{6,7}$ and $J_{6,7'}$ 5.1 and 2.2 Hz) at δ 7.19 and 5.67, respectively, in the ^1H NMR spectrum and

Table 3
^{13}C NMR Spectral data of compounds **10–16** and **26**[a]

	10	**11**	**12**	**13**	**14**	**15**	**16**	**26**
C-1	33.4[b]	31.6	34.3	30.7	30.6	30.7	30.8	35.4
C-2	29.9	32.4	32.4	24.4	24.6	23.9	25.9	100.1
C-3	73.0	68.2	81.6	154.4	156.1	156.0	156.5	146.9
C-4	116.4	112.6	116.8	101.3	101.8	102.0	101.3	35.5
C-5	153.3	157.6	155.9	44.5	45.0	43.2	42.9	43.4
C-6	32.0	31.1	28.0	27.3	27.5	26.0	24.0	27.7
C-7	31.9	32.3	31.9	30.6	30.9	32.6	32.8	31.0
C-8	35.4	35.4	35.5	34.4	34.6	34.4	34.4	34.6
C-9	53.3	54.3	52.8	48.1	48.2	40.5	40.1	49.1
C-10	37.5	37.7	37.0	37.9	38.0	36.2	36.3	36.7
C-11	20.4	20.7	20.4	20.9	21.0	21.3	21.1	20.8
C-12	36.4	36.8	36.5	36.3	36.5	37.0	36.5	36.4
C-13	42.4	42.6	42.3	42.5	42.8	42.5	42.4	42.5
C-14	50.0	50.3	50.0	49.8	50.0	50.2	49.7	49.9
C-15	23.3	23.4	23.3	23.1	23.3	23.3	23.3	23.4
C-16	27.4	27.4	27.4	27.3	27.5	27.4	27.4	27.5
C-17	82.4	82.4	82.6	82.3	82.5	82.4	82.3	82.5
C-18	11.9	12.0	11.9	12.0	12.2	12.0	11.9	12.0
C-19	18.7	17.8	18.8	12.4	12.5	19.1	19.0	12.4
Other	119.2 (CN)	120.9 (CN)	175.6 ($CONH_2$)	123.0 (CN)	122.8 (CN)	123.3 (CN)	123.4 (CN)	122.9 (CN)
C-1′	92.2	91.6	90.9	92.4	97.0	93.5	97.1	92.7
C-2′	70.1	71.0	70.6	69.5	71.1	70.4	70.6	70.3
C-3′	69.6	69.8	70.0	69.8	72.6	70.0	72.5	70.1
C-4′	68.2	68.6	68.4	68.1	68.3	68.1	68.1	68.2
C-5′	68.1	68.2	67.5	67.8	72.0	68.1	72.1	67.9
C-6′	61.5	61.2	61.8	61.7	62.1	61.6	62.1	61.6
$COCH_3$	171.0, 170.5, 170.0, 170.0, 169.4	171.1, 170.6, 170.1, 170.0, 169.6	171.0, 170.4, 170.1, 169.5, 169.5	170.8, 170.3, 169.9, 169.8, 169.3	171.1, 170.6, 170.2, 169.4, 169.1	171.2, 170.7, 170.1, 170.1, 169.6	171.0, 170.3, 170.0, 169.3, 169.2	171.0, 170.6, 170.0, 170.0, 169.6
$COCH_3$	21.0, 20.6, 20.6, 20.5, 20.5	21.1, 20.8, 20.7, 20.7, 20.6	21.0, 20.6, 20.6, 20.6, 20.5	20.9, 20.5, 20.5, 20.5, 20.4	21.2, 20.7, 20.6, 20.6, 20.6	21.1, 20.8, 20.8, 20.7, 20.6	21.1, 20.6, 20.5, 20.5, 20.5	21.1, 20.7, 20.7, 20.7, 20.6

[a] Spectra were obtained in $CDCl_3$.
[b] Chemical shifts are in ppm from internal $(CH_3)_4Si$. Signal assignments were based on DEPT, ^1H–^1H, ^1H–^{13}C COSY and ^1H–^{13}C-long-range COSY spectral data.

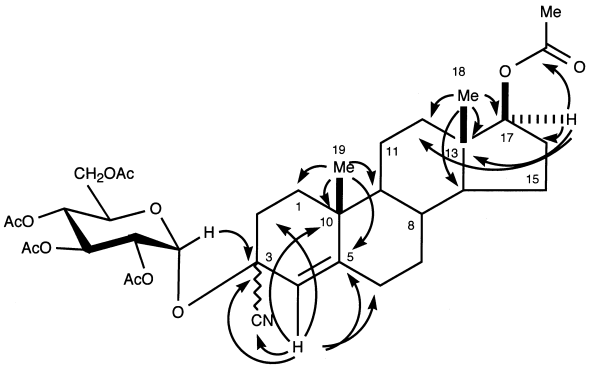


Fig. 1. ^{1}H–^{13}C Long-range correlations observed for compounds **10** and **11**.

the amide carbon at the 3-position at δ 170.2 in the ^{13}C NMR spectrum (see Experimental).

Compounds **13** (104–106 °C), **14** (mp 232–233 °C), **15** (mp 199–201 °C) and **16** (mp 260–261 °C) showed the same quasimolecular ion peak at m/z 710 $[M+Na]^+$ as those of **10** and **11**. Compounds **13–16** exhibited the proton signals due to a 2′,3′,4′,6′-tetra-*O*-acetyl-D-glucopyranosyl residue in the ^{1}H NMR spectra, and 36 carbon signals in the ^{13}C NMR spectra. These spectral data suggest that **13–16** are also monoglycoside derivatives in which a cyano group is substituted at the other position than the 3-position on their aglycons. The proton and carbon signals observed in the ^{1}H and ^{13}C NMR spectra of **13–16** were assigned on the basis of ^{1}H–^{1}H and ^{1}H–^{13}C COSY, DEPT and

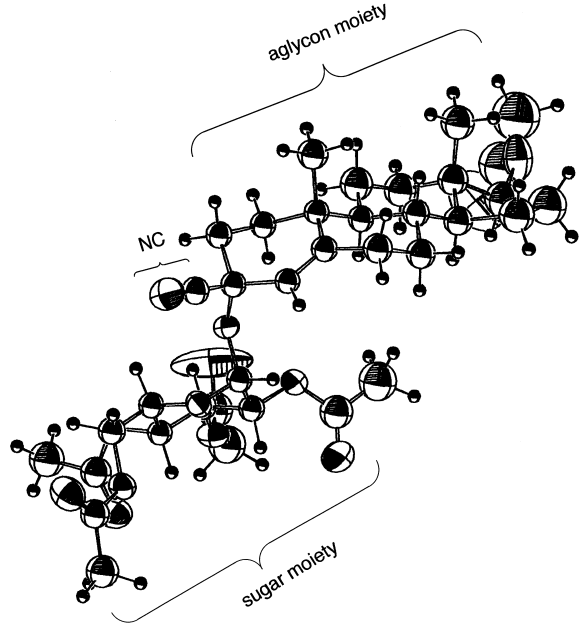


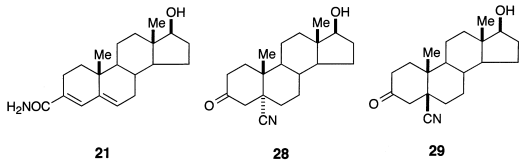
Fig. 2. Crystal structure of compound **11**.

Table 4
X-Ray crystal data for compounds **11** and **28**

	11	**28**
Molecular formula	$C_{36}H_{49}NO_{12}$	$C_{20}H_{29}NO_2 \cdot 0.33H_2O$
Molecular weight	687.795	321.464
Crystal color	colorless	colorless
External form	prismatic crystal	prismatic crystal
Crystal dimensions (mm)	0.5×0.2×0.2	0.5×0.4×0.3
Crystal system	orthorhombic	hexagonal
Space group	$P2_12_12_1$	$P6_5$
Cell dimensions		
a(Å)	10.583(1)	21.302(5)
b(Å)	46.222(5)	—
c(Å)	7.614(1)	7.110(3)
α(°)	90.0	90.0
β(°)	90.0	90.0
γ(°)	90.0	120.0
V(Å^3)	3725(6)	2794(2)
Z value	4	6
Dx(g/cm^3)	1.226	1.125
Final R	0.080	0.105
R_w	0.046	0.086
Goodness of fit (GOF)	5.479	17.142

HMBC spectral data, which are listed in Tables 1 and 3, respectively. In the HMBC spectra of **13–16** (Fig. 3), the correlations between anomeric protons and carbons at the 3-positions were observed, and those between the protons at the 4- and 6-positions and the carbons of cyano groups at the 5-positions were also observed. Furthermore, in the ^{13}C NMR spectra of **13–16**, the carbons at the 5-positions shifted to higher fields at δ 42.9–45 than that of the starting material **7** (δ 170.9) [11].

These spectral data suggest that **13–16** are 17-*O*-acetyl-5-cyano-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-D-glucopyranosyl)androst-3-ene-17β-ol derivatives. In the ^{1}H NMR spectra, **13** and **15** exhibited anomeric protons with coupling constants of 3.4 and 4.0 Hz, respectively; on the other hand, **14** and **16** showed signals for anomeric protons with coupling constants of 7.9 and 8.0 Hz, respectively. Therefore, it was determined that **13** and **15** were α-glycosides and **14** and **16** were β-glycosides. Alkaline hydrolyses of **11–16** gave deacetylated glycosides



Scheme 3.

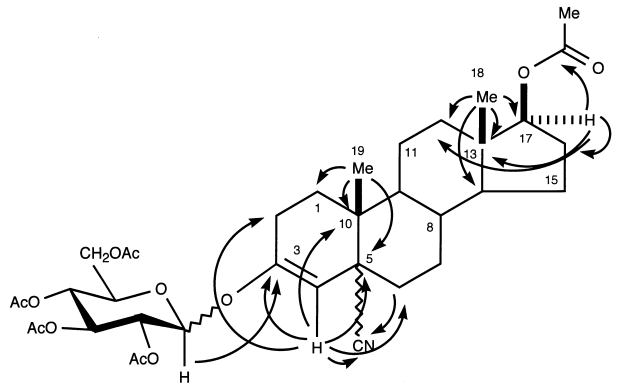


Fig. 3. ^{1}H–^{13}C Long-range correlations observed for compounds **13–16**.

22–25, respectively, which showed the same quasimolecular ion peak at m/z 500 $[M+Na]^+$ in the FABMS spectra. The ^{1}H and ^{13}C NMR spectral data of **22–25** are listed in Tables 5 and 6, respectively. Acid hydrolyses of **22** and **23** gave compound **28** in the yields of 71.9 and 56.6%, respectively, and those of **24** and **25** compound **29** in the yields of 60.5 and 66%, respectively. Compounds **28** and **29** showed the same quasimolecular ion peak at m/z 338 $[M+Na]^+$. In the ^{1}H NMR spectrum (see Experimental), protons at the 4-position of **28** were observed as an AB quartet (J 15.6 Hz) at δ 2.44 and 2.55, and also those of **29** as an AB quartet (J 15.9 Hz) at δ 2.35 and 3.02. The carbons at the 3-positions of **28** and **29** appeared at δ 205.6 and 207.1, respectively, which suggests that those carbons are carbonyl carbons. These spectral data indicated that **28** and **29** were diastereoisomers with respect to the 5-position, and seemed to be the same compounds already obtained by the hydrocyanation of testosterone **6** [20]. The configurations at the 5-positions of **28** and **29** were estimated by the optical rotatory dispersion experiments compared with the analogs from cholest-4-ene-3-one [20,21]. However, as the melting point of **28** was different from the reported one [20], the absolute structure of **28** was confirmed

Table 5
^{1}H NMR Spectral data for compounds **18–20, 22–25** and **27**[a]

	18	**19**	**20**	**22**
Assignable protons on aglycon				
Vinyl proton	5.84 (s, H-4)	5.78 (s, H-4)	5.85 (s, H-4)	5.24 (s)
H-17	3.87 (dd, 8.2, 7.0)[b]	3.76 (dd, 8.6, 8.5)	3.82 (dd, 8.6, 8.6)	3.82 (dd, 8.6, 8.6)
CH_3-18	0.87 (s)	0.91 (s)	0.94 (s)	0.93 (s)
CH_3-19	0.94 (s)	0.93 (s)	1.10 (s)	0.93 (s)
Protons on pyranose				
H-1′	5.91 (d, 3.7)	5.79 (d, 4.0)	5.76 (d, 4.0)	5.92 (d, 3.0)
H-2′	4.19 (dd, 9.8, 3.7)	4.15 (dd, 9.8, 4.0)	4.17 (dd, 9.5, 4.0)	4.22 (dd, 9.2, 3.0)
H-3′	4.61 (dd, 9.8, 9.1)	4.58 (dd, 9.8, 9.1)	4.64 (dd, 9.5, 9.2)	4.64 (dd, 9.5, 9.1)
H-4′	4.33 (dd, 9.1, 9.1)	4.36 (dd, 9.5, 9.1)	4.30 (dd, 9.2, 9.2)	4.24 (dd, 9.5, 9.1)
H-5′	4.54 (m)	4.60–5.46 (m)	4.37–4.45 (m)	4.16 (m)
H-6′a	4.54 (dd, 12.5, 2.1)	4.60–5.46 (m)	4.37–4.45 (m)	4.43 (dd, 11.6, 1.8)
H-6′b	4.46 (dd, 12.5, 5.2)	4.60–5.46 (m)	4.37–4.45 (m)	4.35 (dd, 11.6, 4.9)
	23	**24**	**25**	**27**
Assignable protons on aglycon				
Vinyl	5.32 (s, H-4)	5.24 (s, H-4)	5.27 (s, H-4)	5.49 (dd, 4.8, 1.0, H-2)
H-17	3.82 (dd, 8.5, 8.5)	3.77 (dd, 8.6, 8.5)	3.62 (dd, 8.5, 8.5)	3.82 (dd, 8.5, 8.5)
CH_3-18	0.88 (s)	0.90 (s)	0.86 (s)	0.80 (s)
CH_3-19	0.92 (s)	1.20 (s)	1.20 (s)	0.91 (s)
Protons on pyranose				
H-1′	5.42 (d, 7.6)	5.91 (d, 3.4)	5.46 (d, 7.9)	5.80 (d, 3.1)
H-2′	4.37 (dd, 9.0, 7.6)	4.20 (dd, 9.8, 3.4)	4.14 (dd, 9.2, 7.9)	4.21 (dd, 9.5, 3.1)
H-3′	4.03 (dd, 9.0, 9.0)	4.62 (dd, 9.8, 9.8)	4.26 (dd, 9.2, 8.9)	4.66 (dd, 9.5, 9.5)
H-4′	4.35 (dd, 9.5, 9.0)	4.31 (dd, 9.8, 9.8)	4.13 (dd, 9.5, 8.9)	4.36 (dd, 9.5, 9.5)
H-5′	4.00 (m)	4.26 (m)	4.02 (ddd, 9.5, 5.8, 2.4)	4.30–4.45 (m)
H-6′a	4.49 (dd, 11.9, 2.0)	4.32 (dd, 11.9, 4.0)	4.50 (dd, 11.9, 2.4)	4.30–4.45 (m)
H-6′b	4.39 (dd, 11.9, 4.9)	4.32 (dd, 11.9, 2.7)	4.23 (dd, 11.9, 5.8)	4.30–4.45 (m)

[a] Spectra were obtained in d_5-pyridine. Chemical shifts are in ppm from Internal $(CH_3)_4Si$. Signal assignments were based on ^{1}H–^{1}H, ^{1}H–^{13}C COSY and ^{1}H–^{13}C long-range COSY spectral data.
[b] Coupling constants (J in Hz) are given in parentheses.
* These proton signals were overlapped with one or more signals, and the chemical shifts were obtained on the bases of ^{1}H–^{13}C COSY.

Table 6
^{13}C NMR Spectral data for compounds **18–20, 22–25** and **27**[a]

	18	**19**	**20**	**22**	**23**	**24**	**25**	**27**
Aglycon								
C-1	34.5[b]	31.8	33.1	31.3	31.0	31.4	31.0	35.9
C-2	30.7	31.8	32.9	31.2	25.3	24.0	25.3	100.4
C-3	71.8	69.4	80.7	155.3	157.0	156.6	157.0	148.9
C-4	119.0	114.9	119.1	101.3	100.9	102.4	100.9	36.1
C-5	152.5	156.0	152.7	45.2	45.5	43.8	45.6	43.7
C-6	32.1	32.5	30.8	28.0	28.0	26.2	28.0	28.1
C-7	32.3	32.5	32.8	31.2	31.2	33.1	32.2	31.2
C-8	35.9	35.7	36.2	34.9	35.0	34.9	35.0	35.0
C-9	54.4	54.1	52.3	49.1	49.1	40.7	49.1	49.8
C-10	37.7	37.8	37.6	38.3	38.3	36.4	38.3	36.9
C-11	20.9	21.2	21.3	21.5	21.6	21.7	21.6	21.2
C-12	37.0	37.0	37.1	37.1	37.2	37.1	37.1	36.9
C-13	43.2	43.3	43.7	43.5	43.6	43.3	43.6	43.5
C-14	50.6	50.4	50.8	50.6	50.7	50.4	50.7	50.5
C-15	23.6	23.6	23.6	23.5	23.5	23.6	23.5	23.5
C-16	30.8	30.8	30.8	30.7	30.8	30.8	30.8	30.7
C-17	81.2	81.0	81.2	80.9	81.0	80.9	81.0	80.9
C-18	11.7	11.7	11.8	11.8	11.9	11.7	11.9	11.7
C-19	18.5	17.9	19.8	12.6	12.5	19.3	12.4	12.3
Other	121.1 (CN)	122.3 (CN)	177.8 ($CONH_2$)	124.1 (CN)	123.9 (CN)	124.2 (CN)	123.9 (CN)	133.8 (CN)
Pyranose								
C-1′	97.7	97.3	96.0	97.2	101.1	98.0	101.1	98.2
C-2′	73.3	73.4	73.4	73.1	74.7	73.2	74.7	73.3
C-3′	75.0	74.9	75.2	75.2	74.8	75.2	78.4	75.2
C-4′	71.8	71.6	71.6	71.5	71.0	71.4	71.0	71.5
C-5′	75.1	75.2	74.1	75.0	78.6	74.9	78.6	74.7
C-6′	62.3	61.8	62.2	62.3	62.1	62.1	62.1	62.1

[a] Spectra were obtained in pyridine-d_5.
[b] Chemical shifts are in ppm from $(CH_3)_4Si$.
Signal assignments were based on DEPT, ^{1} H–^{1}H and ^{1}H–^{13}C COSY and HMBC spectral data.

by X-ray diffraction study (Fig. 4) [18,19]. The crystal data for compound **28** are listed in Table 4. As shown in Fig. 4, it determined that the cyano group at the 5-position of **28** was α. This consequently revealed that the cyano group at the 5-position of **29** had the β-configuration. Therefore, it became apparent that the cyano groups at the 5-positions of **13** and **14** were arranged α, while those of **15** and **16** were arranged β. Compounds **13–16** gave glycosides **22–25**, respectively, by alkaline hydrolysis. These resulting glycosides showed the same quasimolecular ion peak at m/z 500 $[M+Na]^+$ in the FABMS spectra.

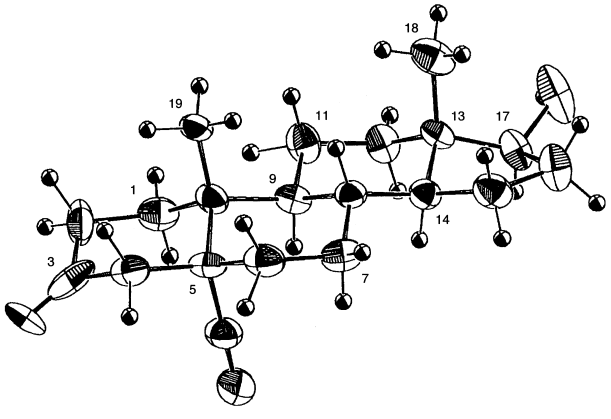


Fig. 4. Crystal structure of compound **28**.

Interestingly, acetylation of glycoside **22** gave two acetates **13** and **26** in 39.5 and 42.2% yields, respectively, although glycosides **23–25** gave only the corresponding acetates **14, 15** and **16**, respectively. Compound **26** showed the same quasimolecular ion peak at m/z 710 $[M+Na]^+$ as **13** in the FABMS spectrum. Alkaline hydrolysis of **26** yielded deacetyl glycoside **27** that showed the same quasimolecular ion peak at m/z 500 $[M+Na]^+$ as **22**. Compound **27** quantitatively gave **28** by acid hydrolysis. These results suggest that **13** and **26**, resultingly **22** and **27**, are isomers with each other. In the ^{1}H NMR spectrum of **26**, the anomeric proton was observed as a doublet having the coupling constant of 3.6 Hz, which indicates that **26** is an α-glycoside as well as **13**. A vinyl proton of **26**

was observed as doublet of doublets with the coupling constants of 4.4 and 0.5 Hz different from the vinyl proton at the 4-position of **13**, which was observed as a singlet. All this chemical and spectral evidence suggests that **26** is 17-*O*-acetyl-5α-cyano-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-glucopyranosyl)-androst-2-ene-17β-ol. The suggestion was confirmed by the HMBC spectrum of **26**; the vinyl proton at the 2-position correlated to C-1, C-3 and C-4 observed at δ 35.4, 146.9 and 35.5, respectively, and the anomeric proton of the pyranose correlated to the C-3.

The two more unstable conformers of **22** (the α-pyranoside of the 5α-cyano derivative) [Fig. 5(a) and (b)] were compared with those of **23** (the β-pyranoside of the 5α-cyano derivative) [Fig. 6(a) and (b)] in stereomodels that were obtained by molecular dynamics calculations (MM2) (Chem 3D plus, Chembridge Scientific Company, Inc.). When the pyranose rings were rotated along the O-1′–C-3 bonds, the pyranose ring in **22** came closer to the 19-CH_3 group (Fig. 5a) or to the α-cyano group (Fig. 5b) on the aglycon than that in **23**, which seemed to indicate that the α-pyranose ring of **22** was subject to a greater steric hindrance from the bulky aglycon than the β-pyranose ring of **23**. To relax the steric hindrances in **22**, the rearrangement of the double bond from C-3–C-4 to C-2–C-3 during the acetylation of **22** took place, resulting in **26**. The two more unstable conformers

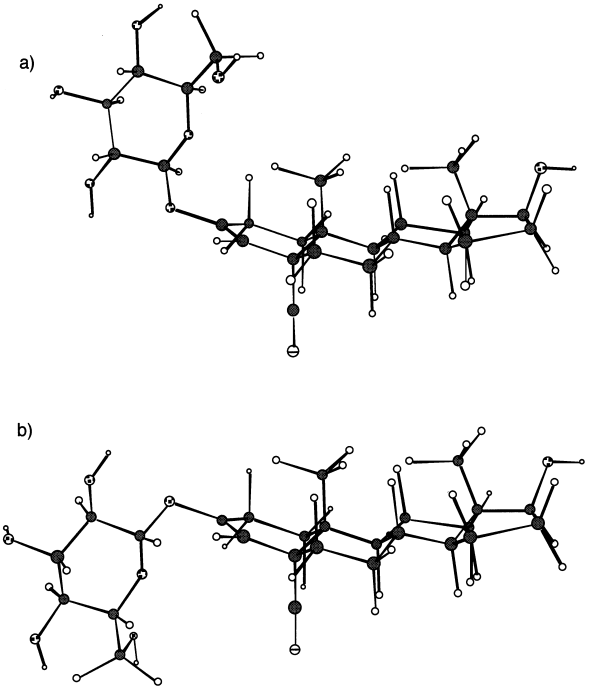


Fig. 6. Two of the less stable conformers of compound **23** (MM2-based, Chem, 3D plus). θ, N; ⊙, O; ●, C; ○, H.

[Fig. 7(a) and (b)] of **27**, which is the deacetylated product of **26**, showed that the α-pyanose ring in **27** was further removed from the 19-CH_3 and 5α-cyano groups on the aglycon than in **22**. This rearrangement was also observed when compound **22** was heated in 1:1 pyridine–H_2O at 200 °C for

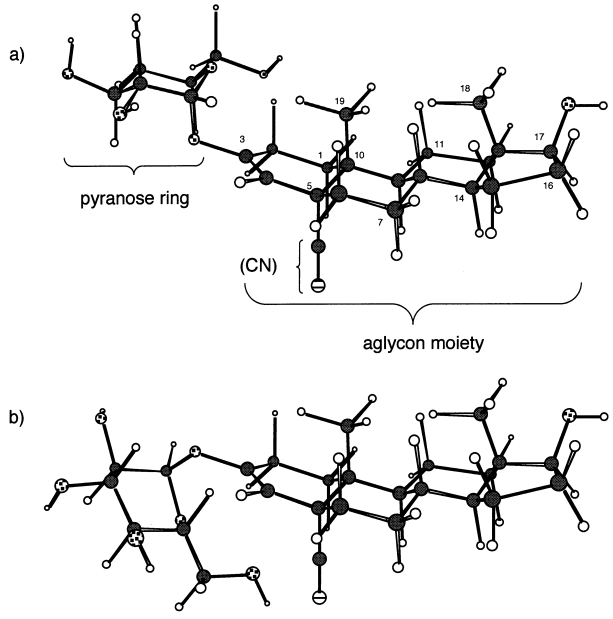


Fig. 5. Two more unstable conformers of compound **22** (MM2-based, Chem 3D plus). θ, N; ⊙, O; ●, C; ○, H.

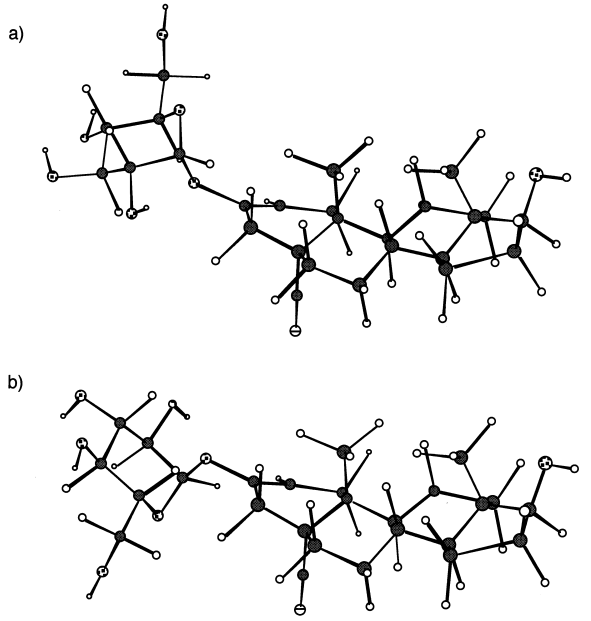
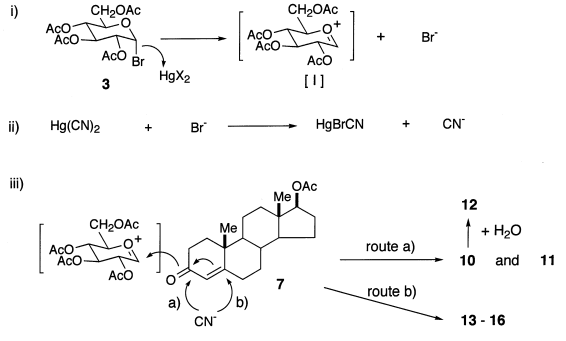


Fig. 7. Two of the less stable conformers of compound **27** (MM2-based, Chem 3D plus). θ, N; ⊙, O; ●, C; ○, H.

10 h to give a mixture of **22** and **27** in the ratio of 1:2.

Thus, the reaction of 17-*O*-acetylandrost-4-ene-3-one-17β-ol (**7**) with acetylated glucopyranosyl bromide (**3**) in the presence of $Hg(CN)_2$ and $HgBr_2$ as catalysts in 1:1 benzene–nitromethane gave glycoside derivatives **10–16**. However, when the same reaction was carried out without $Hg(CN)_2$, no glycosylated product was obtained. Furthermore, the reaction without acetylated pyranosyl bromide **3** gave no product. From this chemical evidence, the reaction mechanisms for the formations of **10–16** by the reaction of **7** with **3** in the presence of $Hg(CN)_2$ and $HgBr_2$ were presumed as follows (Scheme 4): (*i*) Acetylated pyranosyl bromide **3** reacts with $Hg(CN)_2$ or $HgBr_2$ to produce a pyranose oxonium cation intermediate such as [I], and bromine anion (Br^-) is simultaneously released. (*ii*) The bromine anion reacts with $Hg(CN)_2$ to generate a cyanide anion (CN^-). (*iii*) When the attacking of the cyanide anion on the carbonyl carbon at the 3-position of **7** and the electron-transfer from the carbonyl oxygen to the anomeric carbon of the carbonium cation intermediate [I] take place at the same time, compounds **10** and **11** are obtained (route a). Addition of H_2O, which might come from water of crystallization of bromide **3** ($C_{14}H_{19}BrO{\cdot}0.5H_2O$), to the cyano group of **10** gives minor compound **12**. On the other hand, synchronous change by the attacking of the cyanide anion to the carbon at the 5-position of **7** and the electron-transfer from the carbonyl oxygen at the 3-position to the anomeric carbon of the intermediate [I], accompanied by the rearrangement of the double bond from C-4–C-5 to C-3–C-4, produces compounds **13–16** (route b). Compound **26**, which is an isomer of **13** with the respect to the position of the double bond, is produced during the acetylation of **22**.



Scheme 4. Proposed mechanisms for the formation of **10–16**.

3. Conclusions

It was not known that ketone and α,β-unsaturated ketone groups were susceptible to glycosylation before we found that the α,β-unsaturated ketone on the C-ring of methyl 3-*O*-acetylglycyrrhetinate (**2**) underwent glycosylation with acetylated sugar bromide **3** in the presence of AgOTf as a catalyst to give enol α-glycoside **4** [6,7]. However, the reaction of 17-*O*-acetyltestosterone (**7**), which has an α,β-unsaturated ketone on the A-ring, with **3** in the presence of AgOTf gave no glycoside, but 6-oxo- and 7α-bromo-6-oxo-derivatives **8** and **9** [11]. In the reaction of **7** with **3**, when a mixed catalyst, $Hg(CN)_2$ and $HgBr_2$, was employed instead of AgOTf, the glycosylation occurred on the α,β-unsaturated ketone to afford several glycosides **10–16**. The structures of these glycosides were determined to be 3-*O*-glycosides bearing CN groups at the 3α-position in **10**, at the 3β-position in **11**, at the 5α-positions in **13** and **14** and at the 5β-positions in **15** and **6** on the bases of elemental analyses, FABMS and various NMR spectral data and X-ray analyses. Compound **12** was thought to be H_2O-adduct on the cyano group of **10**.

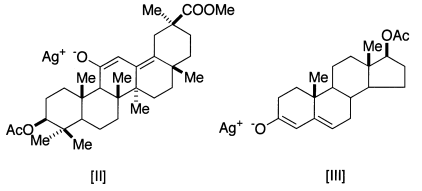
The reaction mechanisms for the formation of the glycosides **10–16** in the reaction of **7** with **3** in the presence of $Hg(CN)_2$ and $HgBr_2$ were deduced as follows: Attack of the cyanide anion (CN^-), generated from $Hg(CN)_2$ at the 3-position, and electron-transfer from the carbonyl oxygen at the 3-position of **7** to the carbonium cation at the anomeric carbon of the intermediate [I], which is produced from **3** during the reaction occurred at the same time, gives products **10** and **11**. On the other hand, when the attack of CN^- at the 5-position of **7** and the same electron-transfer occurred at the same time, accompanied by rearrangement of the double bond from C-4–C-5 to C-3–C-4, products **13–16** were obtained.

Thus, the glycosylation of 17-*O*-acetyltestosterone **7** with **3** occurred when $Hg(CN)_2$ was used as catalyst to give various glycosides bearing CN groups at either the 3- or 5-position on the aglycon. These results are different from the glycosylation of methyl 3-*O*-acetylglycyrrhetinate (**2**) with **3** in the presence of AgOTf to give only an enol α-glycoside **4** [6,7]. Although the reason for this difference has not yet been determined, it is known that glycyrrhizin (**1**) is easily epimerized at the 18-position [22]. Therefore, it might be thought that the α,β-unsaturated ketone on the C-ring of **2** is easily

enolized to produce an intermediate such as [II] that reacts with **3** to give enol glycoside **4**. From the result wherein the reaction of **7** with **3** in the presence of AgOTf gave no glycoside, it was presumed that no isomerization of α,β-unsaturated ketone on the A-ring of **7** occurred to produce an enolate intermediate such as [III]. When the reaction of **7** with **3** was carried out in the presence of only $Hg(CN)_2$ as a catalyst, the same products **10–16** were obtained in almost the same yields as the case of the reaction in the presence of both $Hg(CN)_2$ and $HgBr_2$ in the step (*i*) in Scheme 4, though longer reaction times were necessary. This may only indicate that $HgBr_2$ acts to release a bromine anion from **3** much faster than $Hg(CN)_2$ releases a CN^- ion (Scheme 5).

4. Experimental

General procedures.—Dry benzene was obtained by refluxing with Na, followed by distillation. 17-*O*-Acetyltestosterone (**7**) [23] was obtained according to the conventional method. Other chemicals and solvents were of reagent grade and were obtained from commercial sources. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The thin-layer chromatography (TLC) utilized Kieselgel 60-F_{254} (E. Merck), and spots were detected by spraying the plates with 1:9 $Ce(SO_4)_2$/10% H_2SO_4 reagent, followed by heating at 100 °C for 10 min. Column chromatography was carried out on a Wakogel C-200, and the eluates were monitored by TLC. An SSC-6300 HPLC instrument (Senshu Scientific Co. Ltd) was employed for analytical HPLC using a DOCOSIL (4.6×250 mm; flow rate, 0.2 mL/min, column temp, 40 °C) column, and was further equipped with an SSC autoinjector 6310 and an SSC traction collector 6320 for preparative HPLC using a DOCOSIL (10×250 mm; flow rate, 1 mL/min, column temp, 40 °C) preparative column. ^1H and ^{13}C NMR at 500 and 125 MHz, respectively, as well as ^1H–^1H and ^1H–^{13}C COSY, DEPT and HMBC spectra, were obtained with a JEOL JNM-A500 FT NMR spectrometer. Tetramethylsilane was used as the internal standard, unless otherwise stated, and chemical shifts are given in ppm. Multiplicities of ^1H NMR signals are indicated as s (singlet), d (doublet), dd (doublet of doublets) and m (multiples). Fast-atom-bombardment mass spectra (FABMS) were recorded on a JEOL JMS-DX 300 mass spectrometer. The optical rotations of 1% MeOH solution of samples were recorded on a JASCO J 20A automatic recording spectropolarimeter.



Scheme 5. Proposed structures of silver enolate intermediates [II] and [III].

*Reaction of 7 with **3** in the presence of $Hg(CN)_2$ and $HgBr_2$ in benzene–nitromethane*.—To a solution of **7** (5 g, 15.1 mmol) in dry benzene (10 mL) and nitomethane (10 mL) was added **3** (9.3 g, 22.7 mmol), $Hg(CN)_2$ (3.8 g, 15.1 mmol) and $HgBr_2$ (5.5 g, 15.2 mmol), then the mixture was stirred at room temperature for 16 h. Dichloromethane (300 mL) was added, and the resulting mixture was filtered. The filtrate was successively washed with satd aq $NaHCO_3$ and water, dried over anhydrous $MgSO_4$, then filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–10% EtOAc in benzene), followed by application of preparative HPLC (35:65 H_2O–acetone), to give compounds **10** (white foam, 3.8 g, 36.7%, FABMS *m/z*: 710 $[M+Na]^+$), **11** (mp 208–210 °C after recrystallization from ether, 480 mg, 4.6%, FABMS *m/z*, 710 $[M+Na]^+$), **12** (mp 207–208 °C after recrystallization from ether–petroleum ether, 170 mg, 1.9%, FABMS *m/z*: 718 $[M+Na]^+$), **13** (mp 104–106 °C after recrystallization from ether–petroleum ether, 1.87 g, 18%, FABMS *m/z*. 710 $[M+Na]^+$), **14** (mp 232–233 °C after recrystallization from ether, 375 mg, 3.6%, FABMS *m/z*: 710 $[M+Na]^+$), **15** (mp 199–201 °C after recrystallization from ether, 760 mg, 7.5%, FABMS *m/z*: 710 $[M+Na]^+$) and **16** (mp 260–261 °C after recrystallization from ether, 384 mg, 3.7%, FABMS *m/z*: 710 $[M+Na]^+$). ^1H and ^{13}C NMR spectra are listed in Tables 1 and 3, respectively. Elemental analyses for compounds **10–16** are listed in Table 2.

3α-Cyano-3-O-(α-D-glucopyranosyl)androst-4-ene-17β-ol (**18**).—A solution of compound **10** (3.5 g, 5.1 mmol) in 5 N KOH in 1:1 EtOH–H_2O (10 mL) was stirred for 16 h at room temperature. Acetic acid was added at 0 °C to neutralize. The neutralized solution was passed through a Diaion HP-20 column chromatography and eluted first

with distilled water, then with MeOH. The MeOH eluent was evaporated to give a residue that was subjected to preparative HPLC (45:55 H_2O–MeOH) to obtain compound **18** as a white solid (mp 231–233 °C after recrystallization from 2-propanol, 2.1 g, 82.3%). FABMS *m/z*: 500 $[M+Na]^+$. $[\alpha]_D^{22}$ +158.6. ^1H and ^{13}C NMR spectra are listed in Tables 5 and 6, respectively. Anal. Calcd for $C_{26}H_{39}NO_7 \cdot 0.5H_2O$: C, 64.18; H, 8.29; N, 2.88. Found; C, 64.41; H, 8.10; N, 2.62.

3β-Cyano-3-O-(α-D-glucopyranosyl)androst-4-ene-17β-ol (**19**).—The general procedure was employed with **11** (400 mg, 580 μmol) dissolved in 5 N KOH in 1:1 EtOH-H_2O (3 mL) to give a residue that was subjected to preparative HPLC (40% H_2O in MeOH) to afford compound **19** as a white solid (mp 189–192 °C after recrystallization from MeOH–H_2O, 210 mg, 75.7%). FABMS *m/z*: 500 $[M+Na]^+$. $[\alpha]_D^{22}$ +149.1. ^1H and ^{13}C NMR spectra are listed in Tables 5 and 6, respectively. Anal. Calcd for $C_{26}H_{39}NO_7 \cdot 0.5H_2O$: C, 64.18; H, 8.29; N, 2.88. Found; C, 64.09; H, 8.38; N, 2.69.

3α-Amido-3-O-(α-D-glucopyranosyl)androst-4-ene-17β-ol (**20**).—The general procedure was employed with **12** (150 mg, 210 mmol) dissolved in 5 N KOH in 1:1 EtOH–H_2O (1 mL) to give a residue that was subjected to preparative HPLC (1:1 H_2O–MeOH) to afford compound **20** as a white solid (mp 244–246 °C after recrystallization from MeOH–H_2O, 76 mg, 72.2%). FABMS *m/z*: 518 $[M+Na]^+$. $[\alpha]_D^{22}$ +130.2. ^1H and ^{13}C NMR spectra are listed in Tables 5 and 6, respectively. Anal. Calcd for $C_{26}H_{41}NO_8 \cdot 0.5H_2O$: C, 60.49; H, 7.33; N, 1,96. Found; C, 60.71; H, 7.20; N, 1.91.

5α-Cyano-3-O-(α-D-glucopyranosyl)androst-4-ene-17β-ol (**22**).—The general procedure was employed with **13** (1.7 g, 2.47 mmol) dissolved in 5 N KOH in 1:1 EtOH–H_2O (5 mL) to give a residue that was subjected to preparative HPLC (35:65 H_2O–MeOH) to afford compound **22** as a white solid (mp 284–286 °C after recrystallization from MeOH–H_2O, 910 mg, 77.1%). FABMS *m/z*: 500 $[M+Na]^+$. $[\alpha]_D^{22}$ +60.9. ^1H and ^{13}C NMR spectra are listed in Tables 5 and 6, respectively. Anal. Calcd for $C_{26}H_{39}NO_7 \cdot 0.5H_2O$: C, 64.18; H, 8.29; N, 2.88. Found., C, 64.01; H, 8.35; N, 2.90.

5α-Cyano-3-O-(β-D-glucopyranosyl)androst-4-ene-17β-ol (**23**).—The general procedure was employed with **14** (350 mg, 523 mmol) dissolved in 5 N KOH in 1:1 EtOH–H_2O (2 mL) to give a residue that was subjected to preparative HPLC (35:65 H_2O–MeOH) to afford compound **23** as a white solid (mp 278–279 °C after recrystallization from EtOH, 170 mg, 68.0%). FABMS *m/z*: 500 $[M+Na]^+$. $[\alpha]_D^{22}$ +71.0. ^1H and ^{13}C NMR spectra are listed in Tables 5 and 6, respectively. Anal. Calcd for $C_{26}H_{39}NO_7 \cdot 0.5H_2O$: C, 64.18; H, 8.29; N, 2.88. Found; C, 64.51; H, 8.18; N, 2.82.

5β-Cyano-3-O-(α-D-glucopyranosyl)androst-4-ene-17β-ol (**24**).—The general procedure was employed with **15** (320 mg, 465 mmol) dissolved in 5 N KOH in 1:1 EtOH–H_2O (2 mL) to give a residue that was subjected to preparative HPLC (35:65 H_2O–MeOH) to afford compound **24** as a white foam (380 mg, 78.2%). FABMS *m/z*: 500 $[M+Na]^+$. $[\alpha]_D^{22}$ +169.7. ^1H and ^{13}C NMR spectra are listed in Tables 5 and 6, respectively. Anal. Calcd for $C_{26}H_{39}NO_7 \cdot 0.5H_2O$: C, 64.18; H, 8.29; N, 2.88. Found; C, 64.50; H, 8.32; N, 2.62.

5β-Cyano-3-O-(β-D-glucopyranosyl)androst-4-ene-17β-ol (**25**).—The general procedure was employed with **16** (350 mg, 520 μmol) dissolved in 5 N KOH in 1:1 EtOH–H_2O (2 mL) to give a residue that was subjected to preparative HPLC (35:65 H_2O–MeOH) to afford compound **25** as a white foam (150 mg, 67.5%). FABMS *m/z*: 500 $[M+Na]^+$. $[\alpha]_D^{22}$ +79.6. ^1H and ^{13}C NMR spectra are listed in Tables 5 and 6, respectively. Anal. Calcd for $C_{26}H_{39}NO_7 \cdot H_2O$: C, 63.01; H, 8.34; N, 2.83. Found; C, 62.96; H, 8.30; N, 2.71.

Acid hydrolysis of glycoside **18**.—A solution of **18** (200 mg, 419 μmol) in 1 N H_2SO_4 (2 mL) was refluxed for 18 h. After cooling to room temperature, satd aq $BaCO_3$ was added to the reaction mixture to the point of neutrality, and the mixture then centrifuged to give a solution. The solution was then extracted with AcOEt (20 mL×3), and the combined EtOAc extracts were evaporated to give a residue that was subjected to column chromatography to obtain testosterone **6** (97 mg, 73.4%) that was identified with an authentic sample by HPLC and ^{13}C NMR spectroscopy [24].

Acid hydrolysis of glycoside **19**.—The general procedure was employed with **19** (50 mg, 105 μmol), which was dissolved in 1 N H_2SO_4 (1 mL) to give testosterone **6** (20 mg, 60.6%).

Acid hydrolysis of glycoside **20**.—The general procedure was employed with **20** (70 mg, 140 μmol), which was dissolved in 1 N H_2SO_4 (1 mL) to give 3-amidoandrost-3,5-diene-17β-ol (**21**) as a white solid (mp 203–205 °C after recrystallization from acetone–H_2O, 32 mg, 81.8%). FABMS *m/z*: 338 $[M+Na]^+$; ^1H NMR ($CDCl_3$) (only assignable signals were listed) δ 7.96

and 7.60 (each br s, 1 H, NH), 7.19 (s, 1 H, 4-H), 5.67 (dd, 1 H, $J_{6,7}$ and $J_{6,7'}$, 5.1, 2.2 Hz, 6-H), 5.00 (br s, 1 H, OH), 3.83 (dd, 1 H, $J_{16,17}$ and $J_{16',17}$ 8.9, 8.5 Hz, 17-H), 0.95 (s, 3 H, 18-CH_3), 0.93 (s, 3 H, 19-CH_3); ^{13}C NMR ($CDCl_3$) δ 170.2 ($CONH_2$), 140.8 (C-5), 133.2 (C4), 128.9 (C-3), 128.7 (C-6), 80.4 (C-17), 50.9 (C-14), 47.8 (C-9), 42.6 (C-13), 36.4 (C-12), 34.3 (C-10), 33.2 (C-1), 31.7 (C-7), 31.3 (C-8), 29.9 (C-16), 22.9 (C-15), 21.8 (C-2), 20.3 (C-11), 18.4 (C-19), 10.9 (C-18); Anal. Calcd for $C_{20}H_{29}NO_2$: C, 76.15; H, 9.27; N, 4.44. Found: C, 75.98; H, 9.33; N, 4.16.

Reaction of **18** *with Na*.—Sodium (100 mg, 4.4 mmol) was added to a solution of **18** (200 mg, 420 μmol) in dry MeOH (3 mL), and the solution was then refluxed for 2 h. After cooling to room temperature, the reaction mixture was neutralized with AcOH. The solution was evaporated to give a residue that was dissolved in MeOH (5 mL), and insoluble suspended particles were filtered off. The filtrate was evaporated to afford a residue that was subjected to preparative HPLC (35:65 H_2O–MeOH) to give **20** (20 mg, 9.6%) and **18** (135 mg, 67.5% recovery).

Acid hydrolysis of glycoside **22**.—The general procedure was employed with **22** (200 mg, 420 μmol) dissolved in 1 N H_2SO_4 (2 mL) to give 3α-cyanoandrost-3-one-17β-ol (**28**) as a white solid (mp 242–243 °C after recrystallization from MeOH, 95 mg, 71.9%) (lit., [20] mp 234–236 °C). X-Ray data were obtained with this crystal (see Fig. 6 and Table 4). FABMS *m/z*: 338 $[M+Na]^+$, ^{1}H NMR ($CDCl_3$) (only assignable signals were listed) δ 5.50 (br s, 1 H, OH), 3.71 (dd, 1 H, $J_{16,17}$ and $J_{16',17}$ 8.6, 8.5 Hz, 17-H), 2.55 and 2.44 (AB quartet, 2 H, *J* 15.6 Hz, 4-Ha and 4-Hb), 2.36–2.49 (2 H, m, 2-Ha and 2-Hb), 1.12 (s, 3 H, 19-CH_3), 0.82 (s, 3 H, 18-CH_3); ^{13}C NMR ($CDCl_3$) δ 205.6 (C-3), 121.6 (CN), 80.2 (C-17), 49.7 (C-14), 49.0 (C-9), 46.8 (C-4), 42.8 (C-5), 42.5 (C-13), 37.4 (C-10), 36.6 (C-2), 35.9 (C-12), 34.3 (C-8), 33.7 (C-1), 30.8 (C-7), 29.7 (C-16), 27.1 (C-6), 22.7 (C-15), 20.6 (C-11), 11.7 (C-19), 10.8 (C-18); Anal. Calcd for $C_{20}H_{29}NO_2 \cdot 0.33H_2O$: C, 74.73; H, 9.09; N, 4.36. Found: C, 74.36; H, 9.3 5; N, 4.11.

Acid hydrolysis of glycoside **23**.—The general procedure was employed with **23** (100 mg, 0.2 mmol), which was dissolved in 1 N H_2SO_4 (1 mL) to give **28** (36 mg, 56.6%).

Acid hydrolysis of glycoside **24**.—The general procedure was employed with **24** (200 mg, 420 μmol), which was dissolved in 1 N H_2SO_4 (2 mL) to give 3β-cyanoandrost-3-one-17β-ol (**29**) as a white solid (mp 207–209 °C after recrystallization from acetone–H_2O, 80 mg, 60.5%) (lit., [20] mp 216–216.5 °C after recrystallization from MeOH). FABMS *m/z*: 338 $[M+Na]^+$ ^{1}H NMR ($CDCl_3$) (only assignable signals were listed) δ 5.48 (br s, 1 H, OH), 3.64 (dd, 1 H, $J_{16,17}$ and $J_{16',17}$ 8.9, 8.6 Hz, 17-H), 3.02 and 2.35 (AB quartet, 2 H, *J* 15.9 Hz, 4-Ha and 4-Hb), 2.32 (m, 1 H, 2-Ha), 1.26 (s, 3 H, 19-CH_3), 0.76 (s, 3 H, 18-CH_3); ^{13}C NMR ($CDCl_3$) δ 207.1 (C-3), 122.5 (CN), 81.1 (C-17), 50.4 (C-14), 45.7 (C-5), 44.0 (C-4), 42.7 (C-13), 40.1 (C-9), 37.0 (C-10), 36.4 (C-2), 36.3 (C-12), 34.2 (C-8), 33.3 (C-1), 30.8 (C-7), 30.1 (C-16), 24.9 (C-6), 23.0 (C-15), 20.7 (C-11), 19.3 (C-19), 10.9 (C-18); Anal. Calcd for $C_{20}H_{29}NO_2$: C, 76.15; H, 9.27; N, 4.44. Found: C, 76.29; H, 9.28; N, 4.47.

Acid hydrolysis of glycoside **25**.—The general procedure was employed with **21** (100 mg, 210 μmol), which was dissolved in 1 N H_2SO_4 (1 mL) to give **29** (42 mg, 66.0%).

Reaction of **7** *with* **3** *in the presence of* $Hg(CN)_2$ *and* $HgBr_2$ *in benzene*.—The general procedure was performed with **7** (2.0 g, 6.1 mmol), **3** (5.0 g, 12.2 mmol), $Hg(CN)_2$ (1.6 g, 6.3 mmol) and $HgBr_2$ (1.6 g, 4.4 mmol) in dry benzene (10 mL) for 14 h. The analytical HPLC (3:7 H_2O–acetone) exhibited signals due to compounds **10–16** showing peak height ratios similar to that of the reaction of **7** with **3** in the presence of $Hg(CN)_2$ and $HgBr_2$ in benzene–nitromethane. After purification by successive column chromatography and preparative HPLC, compounds **10** (1.3 g, 31.2%), **11** (210 mg, 5.0%), **12** (30 mg, 0.7%), **13** (650 mg, 15.6%), **14** (115 mg, 2.8%), **15** (230 mg, 5.5%) and **16** (170 mg, 4.1%) were obtained.

Reaction of **7** *with* **3** *in the presence of* $Hg(CN)_2$ *in benzene–nitromethane*.—The general procedure was performed with **7** (1.0 g, 3.1 mmol), **3** (2.5 g, 6.1 mmol) and $Hg(CN)_2$ (0.8 g, 3.2 mmol) in dry benzene (3 mL) and nitromethane (3 mL) for 38 h. The analytical HPLC (35:65 H_2O–acetone) exhibited signals due to compounds **10–16** showing peak height ratios similar to that of the foregoing reaction in the presence of $Hg(CN)_2$ and $HgBr_2$ in benzene–nitromethane.

Acetylation of glycoside **22**.—A solution of **22** (510 mg, 1.1 mmol) in Ac_2O (2 mL) and dry pyridine (2 mL) was allowed to stand overnight at room temperature. The reaction mixture was coevaporated with toluene (50 mL×3) to give a residue. The residue was subjected to preparative

HPLC (1:1 H_2O–acetone) to obtain compounds **13** (290 mg, 39.5%) and **26** as a white foam (420 mg, 42.2%). FABMS of **26** *m/z*, 710 $[M+Na]^+$. ^1H and ^{13}C NMR spectra are listed in Tables 1 and 3, respectively. Anal. Calcd for $C_{36}H_{49}NO_{12}$: C, 62.87; H, 7.18; N, 2.04. Found; C, 62.44; H, 7.19; N, 1.96.

5α-Cyano-3-O-(α-D-glucopyranosyl) androst-2-ene-17β-ol (**27**).—The general procedure was employed with **26** (200 mg, 291 μmol) dissolved in 5 N KOH in 1:1 EtOH–H_2O (1.5 mL) to give a residue that was subjected to preparative HPLC (4:6 H_2O–MeOH) to afford compound **27** as a white solid (mp 291–292 °C after recrystallization from EtOH, 98 mg, 70.1%). FABMS *m/z*: 500 $[M+Na]^+$. ^1H and ^{13}C NMR spectra are listed in Tables 5 and 6, respectively. Anal. Calcd for $C_{26}H_{39}NO_7 \cdot 0.5H_2O$: C, 64.18; H, 8.29; N, 2.88. Found; C, 64.61; H, 8.21; N, 2.81.

Acid hydrolysis of **27**.—The general procedure was employed with **27** (100 mg, 210 μmol) dissolved in 1 N H_2SO_4 (1 mL) to give **28** (48 mg, 72.7%), which was identified by HPLC and ^{13}C NMR spectroscopy as identical with **28** that was obtained by the acid hydrolysis of **22**.

Isomerization of **22**.—A solution of **22** (10 mg, 21 μmol) in pyridine (0.5 mL) and H_2O (0.5 mL) was heated at 200 °C for 10 h. The solution showed two peaks due to **22** and **27** in the ratios of ca. 1:2 by analytical HPLC (4:6 H_2O-MeOH).

X-Ray structure determinations.—The intensity data were measured on a Mac Science MXC 3KHF diffractometer using graphite-monochromated Cu-K_α radiation ($\lambda = 1.5418$ Å) with a scan rate $12° \, min^{-1}$ in range 3–140° and scan width of $\delta(2\theta) = (1.82 + 0.5 \tan\theta)$. Background intensities were measured 3 s at each end of a scan. Crystal data are given in Table 4. Total of 3509 and 2077 reflections for **11** and **28**, respectively, were collected using the $\omega - 2\theta$ scan method. The structures were solved by direct method using CRYSTAN. The structures were refined by full-matrix least-squares procedure with 2687 and 1971 reflections ($F_0 > 3.0 \, \sigma|F_0|$) for **11** and **28**, respectively. Calculations were carried out with the Xtal 3.2 [18].

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