Oxygen-Functionalization of C_{13}-Angular Methyl Group in Pregnane Steroid by Means of Intramolecular Carbonyl-Mediated Anodic Oxidation

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An oxygen-functionalization of the C_{13}-angular methyl group in 3β-acetoxy-5α-pregnan-20-one and its tetra-*O*-acetyl-β-D-glucopyranosyl derivative has been effected by means of an anodic oxidation mediated by the C_{20}-carbonyl residue in the steroid skeleton.

Keywords anodic oxidation; angular methyl oxidation; pregnane steroid; pregnane steroid glucoside; carbonyl-mediated electrolysis

During the course of our study on selective cleavage methods for the glucuronide linkage in oligoglycosides,[1] we developed a new cleavage method by means of anodic decarboxylation.[2] Afterwards, a versatile conversion method for synthesizing various oxygen-functionalized oleanene-triterpenoidal oligoglycosides was developed by using anodic oxidation as a key reaction, and several oleanene-oligoglycosides with interesting biological activity were synthesized.[3] As an extension of these studies, we also reported a skeletal rearrangement of an olean-12-en-11-one (**i**) to its A-nor-B-homo analog (**v**) which was effected *via* an intramolecular carbonyl-mediated indirect electrooxidation.[4] The abstraction of the 1β-hydrogen by an oxygen-cation-radical electrochemically generated at the C_{11}-carbonyl residue (**ii**) was presumed to be the initial step in the skeletal rearrangement (**ii**→**iii**→**iv**→**v**).

It has been anticipated that the stereochemical environment of the 1β-H and the C_{11}-carbonyl residue in the olean-12-en-11-one derivative (**vi**), favorable for the hydrogen abstraction, may be occur or less duplicated by the C_{13}-angular methyl group and the C_{20}-carbonyl residue in various conformations of pregnane steroids (*e.g.*, **vii** for **1**). Thus, we have examined the oxygen-functionalization reaction of the C_{13}-angular methyl group in a pregnane steroid as a possible application of chemical modification of natural products by means of the intramolecular carbonyl-mediated anodic oxidation. This paper deals with the results of our recent investigation.

When 3β-acetoxy-5α-pregnan-20-one (**1**), which was prepared from pregnenolone by acetylation, catalytic hydrogenation and pyridinium chlorochromate (PCC) oxidation, was subjected to constant current electrolysis (Pt electrode, 10 mA/cm^2) in acetonitrile–water (10 : 1) using $NaClO_4$ as a supporting electrolyte, three products (**2**, **3**, **4**) were obtained in 33%, 8%, and 6% yield, respectively, together with recovered **1** (51%).[5]

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the major product **2**, which was obtained as colorless needles, $C_{23}H_{36}O_4$, showed the signals assignable to 18-methylene protons adjacent to an oxygen function, but lacked the C_{13}-methyl proton signal observed in **1**. The infrared (IR) spectrum of **2** exhibited a hydroxyl absorption band (3595 cm^{-1}), but lacked the absorption band due to the 20-ketone group. Furthermore, the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum suggested the presence of a ketal moiety (δ_C 107.5, C-20; δ_C 73.0, C-18) in **2** (Table I). On the basis of these spectral data, the structure of **2** has been formulated as 3β-acetoxy-18,20-epoxy-5α-pregnan-20-ol.[6]

The ^{1}H-NMR spectrum of **3**, colorless needles, $C_{23}H_{34}O_4$, showed the signal ascribable to a formyl group (δ 9.82, 1H, s), but again it lacked the signal of the 13-methyl group. The IR spectrum of **3** exhibited two carbonyl absorption bands due to a formyl moiety (1708 cm^{-1}) and a ketone moiety (1725 cm^{-1}), the latter being indicative of the retention of the 17-methylcarbonyl function of **1**. Thus, the structure of **3** has been assigned as 3β-acetoxy-18-oxo-5α-pregnan-20-one.

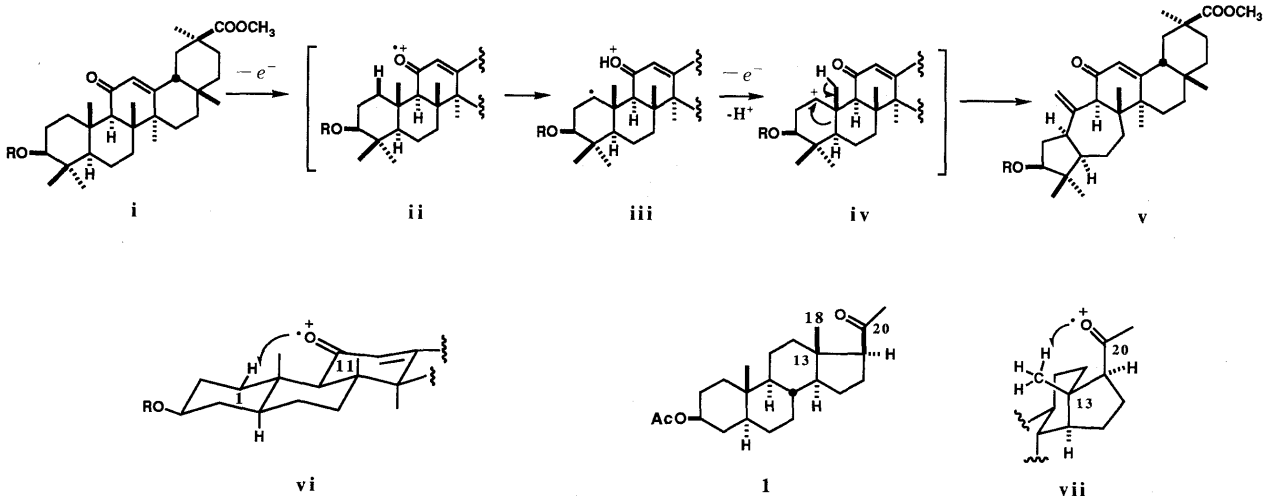
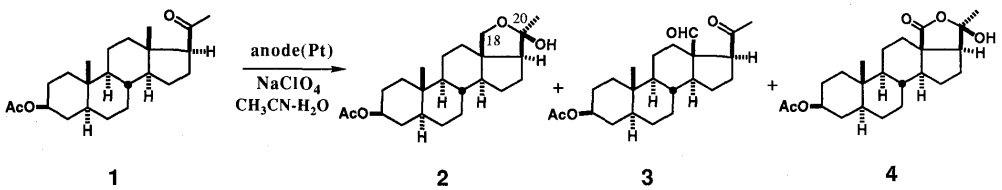
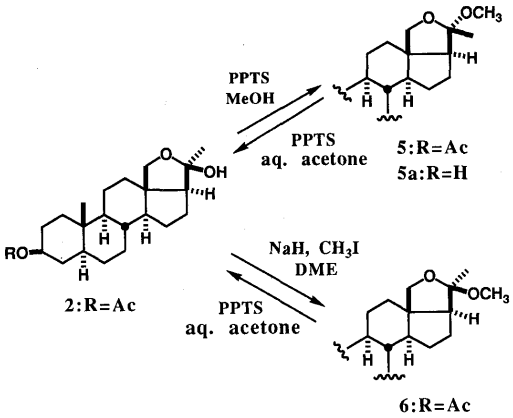


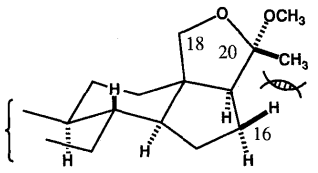
Chart 1



1 2 3 4

Chart 2





viii

Chart 3

The IR spectrum of **4**, colorless needles, $C_{23}H_{34}O_5$, showed hydroxyl absorption bands (3570, 3360 cm^{-1}) and an ester carbonyl absorption band (1750 cm^{-1}), but lacked the ketone absorption band. In the ^{1}H-NMR spectrum of **4**, the signal due to the 20-methyl group (δ 1.64, 3H, s) appeared at lower field than that observed in the case of **2** (δ 1.47). Thus, the structure of **4** has been formulated as 3β-acetoxy-20-hydroxy-5α-pregnan-20,18-olide.

The configurations at C-20 in **2** and **4** have been substantiated by the following evidence. Treatment of **2** with pyridinium *p*-toluenesulfonate (PPTS) in methanol gave a methyl ether **5** in 95% yield with inversion of the C_{20}-configuration of **2**. On the other hand, methylation of **2** with sodium hydride and methyl iodide in 1,2-dimethoxyethane (DME) afforded quantitatively another methyl ether **6** which retained the C_{20}-configuration of **2**. These stereochemical correlations are well explained by a stereochemical consideration of both reactions. Treatment of **5** and **6** with PPTS in aqueous acetone regenerated **2** both in excellent yields. In a comparison of the ^{13}C-NMR spectra of **2**, **5** and **6**, the signal due to the C_{20}-methyl carbon (C-21) of **5** was observed at a higher field (δ_C 17.6) than those of **2** (δ_C 24.8) and **6** (δ_C 23.8) (Table I). These findings indicate that the C_{20}-methyl group of **5** is in a more sterically congested configuration (especially involving the 16-methylene group, as shown in **viii**), than those of **2** and **6**, thus resulting in the appearance of the carbon signal at higher field. Consequently, the C_{20}-configuration of **2** has been assigned as *R*. As for the C_{20}-configuration of **4**, it has also been assigned as *R* on the presumption that ring formation between C-18 and C-20 occurs analogously to that in the case of **2** (*vide infra*). The signal of the C_{20}-methyl carbon was deshielded (δ_C 25.2) as compared with that of **2**.

TABLE I. ^{13}C-NMR Data for **1**, **2**, **3**, **4**, **5**, **6** and **10** (δ_C)[a]

	1	2	3	4	5	6	10
1	36.8	36.9	36.7	36.9	36.8	36.8	37.3
2	27.5	27.5[b]	27.3	27.5	27.4	27.5	28.9
3	73.5	73.6	73.3	73.8	73.5	73.6	78.6[c]
4	31.9	32.3	31.8	32.3	32.2	32.5	32.5
5	44.7	44.7	44.4	44.7	44.5	44.7	44.5
6	28.5	28.5	28.0	28.4	28.4	28.4	30.0
7	34.0	34.0	33.8	34.0	34.0	34.0	34.8
8	35.5	37.8	36.8	33.8	37.7	37.2	37.8
9	54.2	55.6	53.9	54.1	55.4	56.2	55.6
10	35.5	35.5	35.4	35.5	35.4	35.7	35.7
11	21.2	23.2	21.1	21.0	23.2	22.2	23.5
12	39.1	38.2	44.4	34.3	38.1	35.8	38.6
13	44.2	54.5	55.5	55.8	54.4	54.6	54.7
14	56.6	56.6	57.5	56.4	57.0	58.8	57.4
15	24.4	27.4[b]	25.5	27.0	26.8	26.8	27.2
16	22.9	26.2	24.6	26.4	26.2	24.8	26.6
17	63.8	53.4	63.9	53.5	53.4	53.8	53.7
18	13.4	73.0	206.5	179.0	73.2	69.5	73.4
19	12.2	12.2	12.0	12.4	12.1	12.2	12.2
20	209.1	107.5	208.1	105.8	109.9	107.4	110.3
21	31.4	24.8	31.8	25.2	17.6	23.8	17.9
OAc	170.4	170.6	170.3	170.9	170.3	170.5	
	21.3	21.4	21.1	21.5	21.2	21.4	
OCH_3					47.3	48.8	47.4
1′							102.2
2′							75.4
3′							78.7[c]
4′							71.8
5′							77.2
6′							63.0

a) The spectra of **1**—**6** were measured in $CDCl_3$ and the spectrum of **10** was measured in pyridine-d_5. *b*, *c*) Assignments may be interchanged.

Various attempts to improve the chemical yield in the present anodic oxidation have been made, but without success. It was found that the constant current electrolysis of **3** and **4** under the same reaction conditions as used for **1** gave complex degradation products, while the electrolysis of **2** provided **4** in 63% yield. Therefore, it has been assumed that the unsatisfactory yield may be due to the degradation of the initially formed products (*e.g.*, **3**, **4**) under the anodic oxidation conditions.[5]

Since we knew that the glycoside linkage is usually unaffected under the anodic oxidation conditions,[2,3] we next applied this electrooxidation to the tetra-*O*-acetyl-β-D-glucoside of **1** (**9**). The Koenigs-Knorr type glycosidation of 3β-hydroxy-5α-pregnan-20-one (**7**) with 2,3,4,6-tetra-*O*-

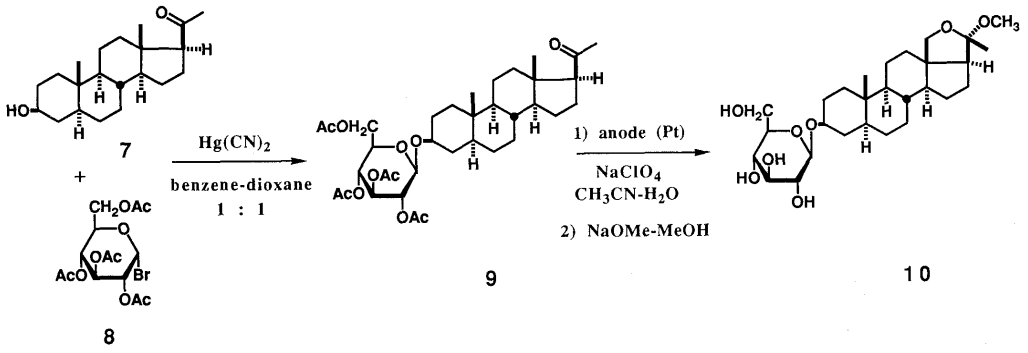


Chart 4

acetyl-α-D-glucopyranosyl bromide (**8**) gave the desired β-glucoside **9** in 76% yield. The structure of **9** was corroborated by its physicochemical properties [*e.g.*, ^{1}H-NMR: δ 0.58, 0.78, 1.99, 2.00, 2.02, 2.06, 2.09, (3H each, all s); IR: 1746, 1705 cm^{-1}; MS: 648 (M^+)]. Constant-current electrolysis of **9** under the same reaction conditions as mentioned above gave a labile product which could not be isolated.

In order to facilitate the isolation of the reaction product, the crude product was subjected to alkaline hydrolysis with sodium methoxide in methanol to furnish a glucoside **10**, although the yield was still unsatisfactory: 52% conversion, 10% yield from **9**.

The ^{1}H-NMR spectrum of **10** showed characteristic signals [δ 3.44, 3.70 (2H, ABq, J=9.0 Hz)] of the 18-methylene protons in the 18,20-epoxy moiety, while the IR spectrum of **10** lacked the carbonyl absorption band which was observed in the case of **9**. As for the configuration at C-20 of **10**, it has been presumed that the initial anodic oxidation product of **9** had a 20*R* configuration as in **2** and the introduction of the methoxyl group at C-20 must occur from the less-hindered side (*i.e.* the α-side) upon treatment with sodium methoxide. The chemical shifts of the signals due to C-18 (δ_C 73.4), C-20 (δ_C 110.3) and C-21 (δ_C 17.9) in the ^{13}C-NMR spectrum of **10**, indicated not only the presence of an 18,20-epoxy moiety but also the 20*S* configuration of **10**. Finally, hydrolysis of **10** with β-glucosidase in MeOH–acetate buffer (pH 5.0) afforded in a moderate yield an aglycone (**5a**), which was converted to **5** by acetylation. Thus, the structure of **10** with a 20*S* configuration has been determined.

In regard to the reaction pathway from **1** to **2**, **3** and **4**, a possible scheme is shown in Chart 5. Namely, an initial one-electron oxidation at 20-CO of **1** may give rise to a cation-radical (**ix**), which would abstract 18-H to yield another cation-radical (**x**). Deprotonation followed by one-electron oxidation at C-18 would give a cation (**xi**). Hydration of the cation may give rise to 3β-*O*-acetyl-18-hydroxy-5α-pregnan-20-one (**xii**), which would cyclize to yield **2** having a less-hindered 20*R* configuration in the oxabicyclo[3.3.0]octane system. The successive oxidation of the keto-alcohol (**xii**) may provide the 13-formyl derivative (**3**) and the hydroxy lactone (**4**) is presumably formed through a keto carboxylic acid (**xiii**) which may be generated by further oxidation of **3**. On the other hand, the exclusive formation of the 20(*R*)-hemiketal (**2**) in the electrooxidation of **1** may be rationalized as indicating that the hydroxymethyl group at C-13 of **xii** would preferentially

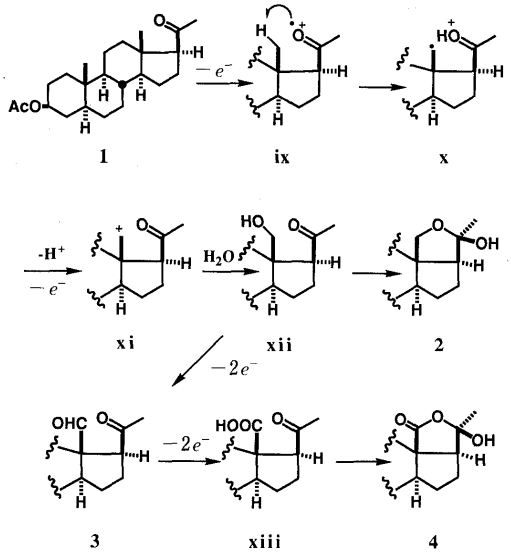


Chart 5

attack the 20-CO group so as to orient the C_{20}-methyl group to the less hindered *exo* side during the formation of the 2-oxabicyclo[3.3.0]octane moiety. In addition, the fact that treatment of **5** and **6** with PPTS in aqueous acetone selectively regenerated **2** may also substantiate this presumption.

In conclusion, the C_{13}-angular methyl groups in 3β-acetoxy-5α-pregnan-20-one (**1**) and its glucoside (**9**) were oxygen-functionalized by means of anodic oxidation mediated by the intramolecular C-20 carbonyl residue. Although the conversions were not satisfactory as regards the chemical yield, this seems to be the first example of the oxidation of the angular methyl group in a steroid by carbonyl-mediated electrochemical reaction. The 18,20-hemiketal and the 20,18-olide moieties introduced by the present anodic oxidation are important partial structures in various biologically active natural products: *e.g.*, antitumor condurangoglycosides from *Mersmedia condurango*[7] and antifungal holotoxins from the sea cucumber *Sticopus japonicus*.[8]

Experimental

The following instruments were used to obtain physical data: a Jasco DIP-181 digital polarimeter for specific rotations; a Hitachi 260-30 infrared spectrometer for IR spectra; a JEOL JNM FX-90Q (90 MHz), a JEOL JNM EX-270, or a JEOL JNM GX-500 (500 MHz) NMR spectrometer for ^{1}H- and ^{13}C-NMR spectra [in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard unless otherwise specified]; a JEOL

JMS-D300 mass spectrometer or a JEOL JMS-01SG-2 mass spectrometer for mass spectrum (MS) and high-resolution MS. Silica gel (Merck, Kieselgel 60, 70—230 mesh) and pre-coated thin-layer chromatography (TLC) plates (Merck, Kieselgel $60F_{254}$) were used for column chromatography and TLC. Spots on TLC plates were detected by spraying 1% $Ce(SO_4)_2$–10% H_2SO_4 or 5% vanillin–concentrated H_2SO_4 with subsequent heating. For preparative anodic oxidations, a potentiostat/galvanostat apparatus (Hokuto Denko Co., Model HA 105) was used.

Preparation of 3β-Acetoxy-5α-pregnan-20-one (1) Acetic anhydride (2.5 ml) was added dropwise to a solution of pregnenolone (2.0 g) in pyridine (5.0 ml) under cooling in an ice-water bath, then the reaction mixture was allowed to stand at room temperature for 5 h. The reaction mixture was poured into ice-water and the whole was extracted with EtOAc. Work up of the EtOAc extract in a usual manner gave 3-*O*-acetylpregnenolone (2.27 g). A solution of this acetate (2.27 g) in 95% EtOH (50 ml) containing PtO_2 (200 mg) was hydrogenated under 6 kg/cm² pressure at room temperature for 6 h. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to afford 3β-acetoxy-5α-pregnan-20-ol quantitatively. A solution of this alcohol (2.25 g) in CH_2Cl_2 (20 ml) was treated with PCC (2.41 g) at room temperature for 1 h. After dilution with dry ether (50 ml), the reaction mixture was passed through a Florisil (5 g) column. Removal of the solvent from the eluate under reduced pressure gave a product, which was purified by column chromatography (SiO_2, *n*-hexane : EtOAc = 10 : 1) to furnish 3β-acetoxy-5α-pregnan-20-one (**1**, 2.16 g, 95% from pregnenolone).

1: Colorless fine crystals, mp 141—142 °C (EtOAc). $[\alpha]_D^{20}$ +79.0° (c = 1.6, $CHCl_3$). IR (CCl_4): 1728, 1703, 1243 cm^{-1}. ^{1}H-NMR (90 MHz, δ): 0.61 (3H, s, 13-CH_3), 0.83 (3H, s, 10-CH_3), 2.02 (3H, s, 21-H_3), 2.11 (3H, s, OAc), 4.69 (1H, m, 3α-H). ^{13}C-NMR: Table I. MS *m/z* (%): 360 (M^+, 18), 300 ($M^+ - AcOH$, 100), 285 ($M^+ - AcOH - CH_3$, 33). High-resolution MS *m/z*: Calcd for $C_{23}H_{36}O_3$: 360.266. Found: 360.266 (M^+).

Constant-Current Electrolysis of 1 A solution of **1** (200 mg) in CH_3CN (100 ml)–H_2O (10 ml) containing $NaClO_4 \cdot H_2O$ (1.21 g) was subjected to constant current electrolysis (Pt electrode, 10 mA/ cm^2) in an ice-cooling bath for 3 h. The reaction mixture was poured into aqueous saturated and the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated 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 10 g, *n*-hexane : EtOAc = 5 : 1→2 : 1) to furnish **2** (35 mg, 33%), **3** (8.5 mg, 8%), and **4** (7.0 mg, 6%) together with recovered **1** (102 mg, 51% recovery).

2: Colorless needles, mp 156—157 °C (from EtOAc). $[\alpha]_D^{25}$ +34.9° (c = 0.84, $CHCl_3$). IR (CCl_4): 3595, 1731, 1245 cm^{-1}. ^{1}H-NMR (90 MHz, δ): 0.76 (3H, s, 10-CH_3), 1.47 (3H, s, 20-CH_3), 2.02 (3H, s, OAc), 3.69 (2H, s, 18-H_2), 4.68 (1H, m, 3α-H). ^{13}C-NMR: Table I. MS *m/z* (%): 358 ($M^+ - H_2O$, 100), 316 ($M^+ - AcOH$, 60), 301 ($M^+ - AcOH - CH_3$, 28). *Anal.* Calcd for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.58; H, 9.51. **3**: Colorless needles, mp 124—126 °C (from EtOAc). $[\alpha]_D^{25}$ +46.4° (c = 0.44, $CHCl_3$). IR (CCl_4): 1725, 1708, 1235 cm^{-1}. ^{1}H-NMR (90 MHz, δ): 0.75 (3H, s, 10-CH_3), 2.01 (3H, s, OAc), 2.11 (3H, s, 20-CH_3), 4.68 (1H, m, 3α-H), 9.82 (1H, s, CHO). ^{13}C-NMR: Table I. MS *m/z* (%): 374 (M^+, 100), 359 ($M^+ - CH_3$, 25). High-resolution MS *m/z*: Calcd for $C_{23}H_{34}O_4$: 374.246. Found: 374.244 (M^+). **4**: Colorless needles, mp 198—200 °C (EtOAc). $[\alpha]_D^{20}$ +3.0° (c = 0.62, $CHCl_3$). IR ($CHCl_3$): 3570, 3360, 1750, 1708 cm^{-1}. ^{1}H-NMR (90 MHz, δ): 0.91 (3H, s, 10-CH_3), 1.64 (3H, s, 20-CH_3), 2.03 (3H, s, OAc), 4.64 (1H, m, 3α-H). ^{13}C-NMR: Table I. MS *m/z* (%): 390 (M^+, 14), 372 ($M^+ - H_2O$, 48), 330 ($M^+ - AcOH$, 50), 301 (100). High-resolution MS *m/z*: Calcd for $C_{23}H_{34}O_5$: 390.241. Found: 390.241 (M^+).

Constant-Current Electrolysis of 2 A solution of **2** (20 mg) in CH_3CN (10 ml)–H_2O (1 ml) containing $NaClO_4 \cdot H_2O$ (121 mg) was subjected to constant current electrolysis (Pt electrode, 5 mA/cm^2) in an ice-cooling bath for 2 h. Work up of the reaction mixture as described above gave a product, which was purified by column chromatography (SiO_2 5 g, *n*-hexane : EtOAc = 3 : 1) to furnish **4** (13 mg, 63%). The product was shown to be identical with the above-described **4** by IR, ^{1}H-NMR, and MS comparisons.

Methylation of 2 with MeOH and PPTS Giving 5 A solution of **2** (15 mg) in MeOH (2.0 ml) was treated with PPTS (2 mg) at room temperature for 5 min. The reaction mixture was poured into aqueous saturated $NaHCO_3$, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and 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 3 g, *n*-hexane : EtOAc = 3 : 1) to furnish **5** (15 mg, 95%).

5: Colorless needles, mp 158—159 °C (from MeOH). $[\alpha]_D^{20}$ +21.6° (c = 0.61, $CHCl_3$). IR (CCl_4): 1731, 1244, 1066 cm^{-1}. ^{1}H-NMR (90 MHz, δ): 0.76 (3H, s, 10-CH_3), 1.35 (3H, s, 20-CH_3), 2.03 (3H, s, OAc), 3.18 (3H, s, OCH_3), 3.40, 3.66 (2H, ABq, J = 9.0 Hz, 18-H_2), 4.69 (1H, m, 3α-H). ^{13}C-NMR: Table I. MS *m/z* (%): 375 ($M^+ - CH_3$, 86), 359 ($M^+ - OCH_3$, 100). *Anal.* Calcd for $C_{24}H_{38}O_4$: C, 73.79; H, 9.81. Found: C, 73.91; H, 9.58.

Methylation of 2 with NaH and CH_3I Giving 6 A solution of **2** (15 mg) in dry DME (0.5 ml) was treated for 30 min with NaH (4 mg), which was prepared from 50% NaH (8 mg) by washing with dry pentane. Methyl iodide (0.5 ml) was added to the solution, then the reaction mixture was stirred at room temperature for 20 min. The reaction mixture was poured into ice-water and the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and 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 3 g, *n*-hexane : EtOAc = 3 : 1) to furnish **6** (16 mg, 98%).

6: Colorless needles, mp 121—123 °C (from MeOH). $[\alpha]_D^{25}$ −12.2° (c = 0.90, $CHCl_3$). IR (CCl_4): 1727, 1237, 1022 cm^{-1}. ^{1}H-NMR (90 MHz, δ): 0.78 (3H, s, 10-CH_3), 1.37 (3H, s, 20-CH_3), 2.02 (3H, s, OAc), 3.24 (3H, s, OCH_3), 3.39, 3.55 (2H, ABq, J = 9.0 Hz, 18-H_2), 4.68 (1H, m, 3α-H). ^{13}C-NMR: Table I. MS *m/z* (%): 375 ($M^+ - CH_3$, 86), 359 ($M^+ - OCH_3$, 100). *Anal.* Calcd for $C_{24}H_{38}O_4$: C, 73.79; H, 9.81. Found: C, 73.63; H, 9.71.

Acidic Treatment of 5 and 6 A solution of **5** (10 mg) in 90% aqueous acetone (2 ml) was treated with a catalytic amount of PPTS and was heated under reflux for 30 min. The reaction mixture was poured into aqueous saturated $NaHCO_3$, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and 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 3 g, *n*-hexane : EtOAc = 2 : 1) to furnish **2** (9 mg). In the same manner, treatment of **6** (10 mg) with a catalytic amount of PPTS and subsequent work-up of the reaction mixture in a usual manner gave **2** (9 mg). The identification of the products was conducted by comparisons of their physical data [IR, ^{1}H-NMR, MS] with those of **2** which was obtained above by anodic oxidation of **1**.

Alkaline Hydrolysis of 1 Giving 7 A solution of **1** (200 mg) in MeOH (5 ml) was treated with 10% KOH–MeOH (2 ml) at room temperature for 15 min. The reaction mixture was poured into ice-water and the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and 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 10 g, *n*-hexane : EtOAc = 3 : 1) to furnish **7** (176 mg, quant.).

7: Colorless fine crystals, mp 194—196 °C (from $CHCl_3$–MeOH). $[\alpha]_D^{23}$ +88.3° (c = 1.0, $CHCl_3$). IR ($CHCl_3$): 3595, 3435 (br), 1691 cm^{-1}. ^{1}H-NMR (90 MHz, δ): 0.61 (3H, s, 13-CH_3), 0.81 (3H, s, 10-CH_3), 2.11 (3H, s, 21-H_3), 3.55 (1H, m, 3α-H). MS *m/z* (%): 318 (M^+, 100), 300 ($M^+ - H_2O$, 86), 285 ($M^+ - H_2O - CH_3$, 33). High-resolution MS *m/z*: Calcd for $C_{21}H_{34}O_2$: 318.256. Found: 318.256 (M^+).

Preparation of 3β-[(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-oxy]-5α-pregnan-20-one (9) A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**8**, 770 mg) in dry benzene (1.5 ml)–dry dioxane (1.5 ml) was added dropwise to a solution of **7** (150 mg) and $Hg(CN)_2$ (357 mg) in dry benzene (3.0 mg)–dry dioxane (3.0 ml), then the reaction mixture was heated under reflux for 5 h. The reaction mixture was poured into ice-water and the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and 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 30 g, *n*-hexane : EtOAc = 2 : 1) to furnish **9** (232 mg, 76%).

9: Colorless fine crystals, mp 181—183 °C (from $CHCl_3$–MeOH). $[\alpha]_D^{25}$ +26.4° (c = 1.9, $CHCl_3$). IR (CCl_4): 1746, 1705, 1220 cm^{-1}. ^{1}H-NMR (90 MHz, δ): 0.58 (3H, s, 13-CH_3), 0.77 (3H, s, 10-CH_3), 1.99, 2.00, 2.02, 2.06, 2.09 (3H each, all s, OAc × 4 and 21-H_3). MS *m/z* (%): 648 (M^+, 0.1), 588 ($M^+ - AcOH$, 1.0), 301 (100). High-resolution MS *m/z*: Calcd for $C_{35}H_{52}O_{11}$: 648.351. Found: 648.348 (M^+).

Constant-Current Electrolysis Followed by Alkaline Treatment of 9 Giving 10 A solution of **9** (232 mg) in CH_3CN (150 ml)–H_2O (15 ml) containing $NaClO_4 \cdot H_2O$ (1.82 g) was subjected to constant current electrolysis (Pt electrode, 10 mA/cm^2) in an ice-cooling bath for 1.5 h. Work up of the reaction mixture as described above for the electrolysis of **1** gave a product, which was purified by column chromatography (SiO_2 10 g, *n*-hexane : EtOAc = 2 : 1) to afford a mixture of unchanged **9** and the 18, 20

epoxy derivative. The mixture was dissolved in MeOH (2 ml) and the solution was treated with 10% NaOMe–MeOH (1 ml) at room temperature for 15 min. The reaction mixture was then poured into ice-water and the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and 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 4 g, $CHCl_3$: MeOH : H_2O = 10 : 3 : 1, lower phase) to furnish **10** (9.5 mg, 10%) with recovered 3-*O*-*β*-D-glucopyranosyl-5α-pregnan-20-one (84 mg, 49% recovery).

10: Colorless needles, mp 194—196 °C (from MeOH). $[\alpha]_D^{26}$ +17.5° (c = 0.38, MeOH). IR (KBr): 3420 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5, δ): 0.57 (3H, s, 10-CH_3), 1.35 (3H, s, 20-CH_3), 3.19 (3H, s, OCH_3), 3.44, 3.70 (1H each, ABq, J = 9.0 Hz, 18-H_2), 3.90—4.04 (3H, m, 3-H, 2′-H, 5′-H), 4.19 (1H, dd, J = 8.9, 9.2 Hz, 4′-H), 4.28 (1H, dd, J = 8.9, 8.9 Hz, 3′-H), 4.34 (1H, dd, J = 5.5, 11.9 Hz, 6′-H_a), 4.55 (1H, dd, J = 1.5, 11.9 Hz, 6′-H_b), 5.00 (1H, d, J = 8.0 Hz, 1′-H). ^{13}C-NMR: Table I. *Anal.* Calcd for $C_{28}H_{42}O_8$: C, 66.37; H, 8.36. Found: C, 66.51; H, 8.21.

Enzymatic Hydrolysis of 10 with β-Glucosidase Giving 5a A solution of **10** (4 mg) in an acetate buffer (pH 5.0, 1.0 ml)–MeOH (1 ml) mixture was treated with almond *β*-glucosidase (Sigma, 8 mg) at 37 °C for 8 h. The reaction mixture was poured into water and the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and 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 5 g, *n*-hexane : EtOAc = 2 : 1) to furnish **5a** (2.0 mg, 72%).

5a: A white powder. $[\alpha]_D^{26}$ +19.6° (c = 0.18, MeOH). IR (KBr): 3448 cm^{-1}. ^{1}H-NMR (270 MHz, CD_3OD, δ): 0.76 (3H, s, 10-CH_3), 1.29 (3H, s, 13-CH_3), 3.15 (3H, s, OCH_3), 3.48—3.54 (1H, m, 3-H), 3.45, 3.68 (2H, ABq, J = 9.0 Hz, 18-H_2). MS m/z (%): 316 (M^+ − MeOH, 100), 298 (M^+ − MeOH-H_2O, 19). High-resolution MS m/z: Calcd for $C_{21}H_{32}O_2$: 316.238. Found: 316.240 (M^+ − MeOH).

Acetylation of 5a Giving 5 A solution of **5a** (2.0 mg) in pyridine (0.2 ml) was treated with acetic anhydride (0.1 ml) at 0 °C for 1 h. The reaction mixture was poured into ice-water and extracted with EtOAc. Work-up of the EtOAc extract in a usual manner gave a product. Purification of the product by column chromatography (SiO_2 1 g, *n*-hexane : EtOAc = 3 : 1) afforded **5** (1.8 mg, 86%). This product was identical with **5** obtained above by IR, ^{1}H-NMR, and MS comparisons.

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