Convenient synthesis of 18-hydroxylated cortisol and prednisolone

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18,20-Epoxy-11β,17α,20β,21-tetrahydroxypregn-4-en-3-one was synthesized by the application of hypoiodite reaction to the cortisol acetonide. The intermediary 18-iodo derivative was converted to the 11-oxo steroid by chromic acid prior to silver ion-assisted solvolysis. Removal of the protective group with hydrochloric acid was finally carried out to give the desired 11β,17α,18,21-tetrahydroxypregn-4-ene-3,20-dione as the hemiacetal form. 18,20-Epoxy-11β-17α,20β,21-tetrahydroxypregna-1,4-dien-3-one was also prepared from prednisolone through a similar reaction sequence. (*Steroids* **57**:426–429, 1992)

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Introduction

18,20-Epoxy-11β,17α,20β,21-tetrahydroxypregn-4-en-3-one (18-hydroxycortisol) is a steroid found in the urine of patients with primary aldosteronism caused by adrenal adenoma[1] and lack of glucocorticoid or mineralocorticoid activity.[2,3] This steroid is secreted by the adrenal glands in normal humans, and its urinary levels in patients with glucocorticoid-remediable hyperaldosteronism are significantly greater than those in normal subjects or in patients with idiopathic zona glomerulosa hyperplasia.[4]

Recent studies have shown that this new steroid may be useful for the differential diagnosis of primary aldosteronism.[5] To develop the physiologic and pathophysiologic studies and to assess the utility of this steroid for the diagnosis, reference 18-hydroxylated cortisol and prednisolone are required. One method has been reported for the preparation of 18-hydroxycortisol starting from prednisolone.[6] However, the synthetic method requires poisonous nitrosyl chloride to produce the 11β-nitrite derivative for activation of the 18-methyl group in prednisolone. Several methods for oxygenation of an angular methyl group in steroid by involving thermal or photolytic lead tetraacetate oxidation and hypohalite reaction have appeared in the literature.[7,8] We report a convenient synthesis of 18-hydroxycortisol and 18,20-epoxy-11β,17α,20β,21-tetrahydroxypregna-1,4-dien-3-one (18-hydroxyprednisolone) using the hypoiodite reaction (Scheme 1).

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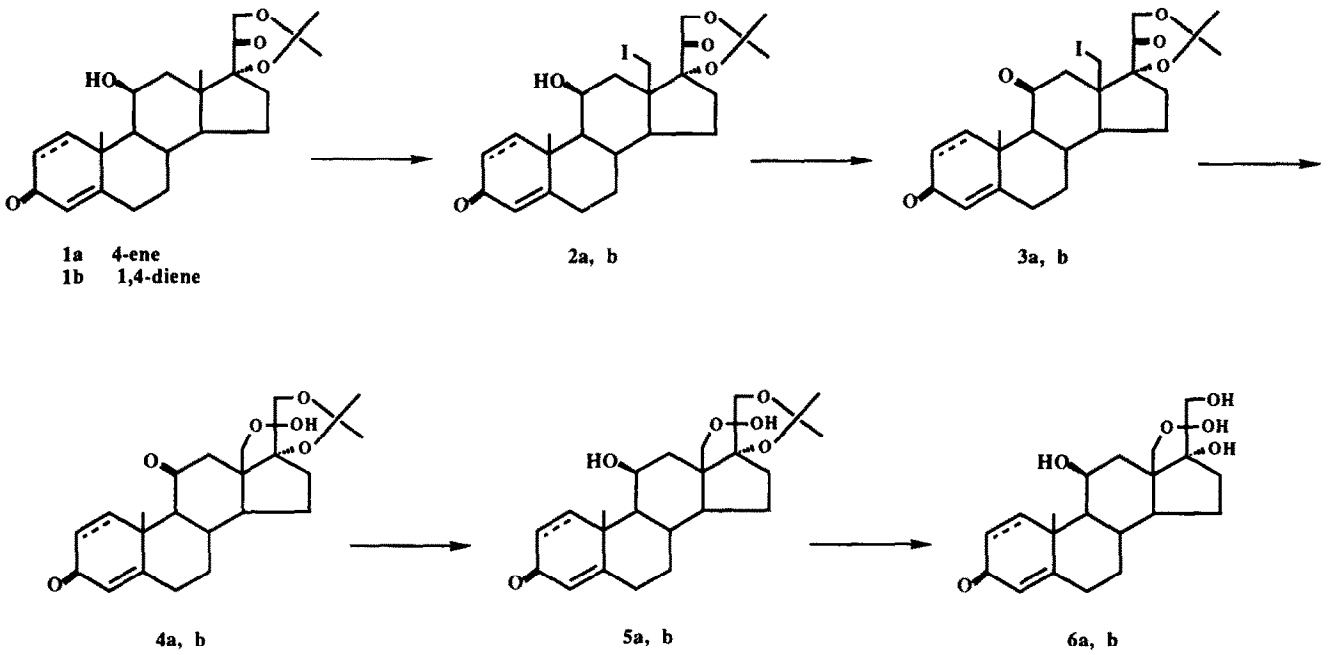
Experimental

Melting points (mp) were measured on a Mitamura melting point apparatus (Mitamura Riken Co., Tokyo, Japan) and are uncorrected. Infrared (IR) spectra were obtained using an IRA-102 spectrometer (JASCO Co., Tokyo, Japan) in Nujol and are expressed in cm^{-1}. ^1H Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz with a JEOL JNM-EX 400 spectrometer (JEOL Co., Tokyo, Japan). Chemical shifts are given as the δ value with tetramethylsilane as the internal standard. The abbreviations used are s (singlet), d (doublet), and dd (doublet of doublets). Column chromatography was done using silica gel CQ-2 (74-147 μm, Fuji Gel Hanbai Co., Tokyo, Japan).

General methods

Hypoiodite reaction of the acetonides 1a and 1b. To a stirred mixture of 10 g of 11β-hydroxy-17α,21-isopropylidenedioxypregn-4-ene-3,20-dione (**1a**) or 11β-hydroxy-17α,21-isopropylidenedioxypregna-1,4-diene-3,20-dione (**1b**) (obtained by the reported method[9]), I_2 (6 g) in dry benzene (250 ml), and cyclohexane (250 ml) was added lead tetraacetate (7.5 g) and calcium carbon-



Scheme 1

ate (10 g). The reaction was carried out by irradiation with a 500-W tungsten lamp under ice cooling for 2 hours. The mixture was filtered through a bed of Celite and the residue was washed with EtOAc. The filtrate was washed with 5% $Na_2S_2O_3$ and H_2O, and was dried (Na_2SO_4) in the presence of a trace amount of pyridine. Evaporation of the solvent gave the crude product, which was purified by column chromatography with $CHCl_3$/MeOH (100 : 1 v/v) and recrystallized to afford the 18-iodinated steroid (**2a** or **2b**).

*11β-Hydroxy-18-iodo-17α,21-isopropylidenedioxypregn-4-ene-3,20-dione (**2a**).* Compound **1a** yielded 75% (9.8 g) of the title steroid **2a**: mp 76 C (decomp) (colorless prisms from MeOH). IR: 3,420 (OH), 1,740 (C=O), 1,660 (C=O). ^{1}H NMR ($CDCl_3$): 1.39 and 1.46 (each 3H, s, isopropylidenedioxy), 1.43 (3H, s, 19-H), 3.26 and 3.96 (each 1H, d, J = 10 Hz, 18-H), 4.29 and 4.52 (each 1H, d, J = 18.6 Hz, 21-H), 4.50 (1H, broad s, 11α-H), 5.69 (1H, s, 4-H). Analysis calculated for $C_{24}H_{33}O_5I$: C, 54.55; H, 6.29. Found: C, 54.62; H, 6.31.

*11β-Hydroxy-18-iodo-17α,21-isopropylidenedioxypregna-1,4-diene-3,20-dione (**2b**).* Compound **1b** yielded 84% (11.1 g) of the title steroid **2b**: mp 82 C (decomp) (colorless prisms from MeOH). IR: 3,400 (OH), 1,710 (C=O), 1,660 (C=O). ^{1}H NMR ($CDCl_3$): 1.37 and 1.43 (each 3H, s, isopropylidenedioxy), 1.44 (3H, s, 19-H), 3.24 and 3.97 (each 1H, d, J = 10.2 Hz,18-H), 4.29 and 4.51 (each 1H, d, J = 18.5 Hz, 21-H), 4.54 (1H, broad s, 11α-H), 6.03 (1H, broad s, 4-H), 6.29 (1H, d, J = 9.5 Hz, 2-H), 7.25 (1H, d, J = 9.5 Hz, 1-H). Analysis calculated for $C_{24}H_{31}O_5I$: C, 54.76; H, 5.94. Found: C, 54.60; H, 6.02.

Oxidation of the iodides 2a and 2b with Jones reagent. Compound **2a** (6.5 g) or **2b** (8.7 g) was dissolved in acetone (60 ml) and CH_2Cl_2 (40 ml). To this solution was added dropwise 10 ml of Jones reagent with stirring below 5 C, and the mixture was allowed to stir for 20 minutes. After this time, 10 ml of MeOH was added to destroy the excess reagent; the mixture was diluted with H_2O and then extracted with CH_2Cl_2. The extract was washed with H_2O and dried (Na_2SO_4). Evaporation of the solvent gave a crude product, which was recrystallized to afford the 11-oxo steroid (**3a** or **3b**).

*18-Iodo-17α,21-isopropylidenedioxypregn-4-ene-3,11,20-trione (**3a**).* Compound **2a** yielded 82% (5.3 g) of the title steroid **3a**: mp 116 C (decomp) (colorless prisms from MeOH). IR: 1,720 (C=O), 1,700 (C=O), 1,660 (C=O). ^{1}H NMR ($CDCl_3$): 1.407 and 1.45 (each 3H, s, isopropylidenedioxy), 1.414 (3H, s, 19-H), 2.87 (1H, d, J = 13.2 Hz, 12-H), 3.05 (1H, d, J = 11.7 Hz, 18-H), 3.08 (1H, dd, J = 13.2 and 1.9 Hz, 12-H), 3.18 (1H, dd, J = 11.7 and 1.9 Hz, 18-H), 4.31 and 4.32 (each 1H, d, J = 18.6 Hz, 21-H), 5.74 (1H, s, 4-H). Analysis calculated for $C_{24}H_{31}O_5I$: C, 54.76; H, 5.94. Found: C, 54.55; H, 6.14.

*18-Iodo-17α,21-isopropylidenedioxypregna-1,4-diene-3,11,20-trione (**3b**).* Compound **2b** yielded 93% (8.1 g) of the title steroid **3b**: mp 125 C (decomp) (colorless prisms from MeOH). IR: 1,720 (C=O), 1,700 (C=O), 1,660 (C=O). ^{1}H NMR ($CDCl_3$): 1.41 and 1.46 (each 3H, s, isopropylidenedioxy), 1.45 (3H, s, 19-H), 2.91 (1H, d, J = 13.2 Hz, 12-H), 3.07 (1H, dd, J = 13.2 and 1.5 Hz, 12-H), 3.09 (1H, d, J = 11.7 Hz, 18-H), 3.20 (1H, dd, J = 11.7 and 1.5 Hz, 18-H), 4.31 and 4.51 (each 1H, d, J = 19 Hz, 21-H), 6.09 (1H, s, 4-H), 6.23 (1H, d, J = 10 Hz, 2-H), 7.66 (1H, d, J = 10 Hz, 1-H). Analysis calculated for $C_{24}H_{29}O_5I$: C, 54.97; H, 5.57. Found: C, 54.72; H, 5.48.

Reaction of the iodides 3a and 3b with silver acetate. To a solution of compound **3a** (6.2 g) or **3b** (4.2 g) in dioxane (70 ml) and H_2O (12 ml) was added silver acetate (8.1 g for **3a**; 5.24 g for **3b**). The mixture was heated under reflux with stirring for 4 hours. Removal of the solvent afforded a solid, which was dissolved in EtOAc and filtered through a bed of Celite. The filtrate was washed with H_2O and dried (Na_2SO_4). Evaporation of the solvent gave a crude product, which was purified by column chromatography with $CHCl_3$/MeOH (50 : 1 v/v) and was recrystallized to afford the 18-hydroxylated derivatives as hemiacetal form (**4a** or **4b**).

*18,20-Epoxy-20β-hydroxy-17α,21-isopropylidenedioxypregn-4-ene-3,11-dione (**4a**).* Compound **3a** yielded 80% (3.9 g) of the

title steroid **4a**: mp 235 to 238 C (colorless prisms from MeOH containing a few drops of triethylamine). IR: 3,500 (OH), 1,700 (C=O), 1,665 (C=O), 1,615 (C=C). ^{1}H NMR ($CDCl_3$): 1.27 and 1.42 (each 3H, s, isopropylidenedioxy), 1.37 (3H, s, 19-H), 3.37 and 3.678 (each 1H, d, J = 9.8 Hz, 18-H), 3.61 and 3.675 (each 1H, d, J = 11.7 Hz, 21-H), 5.73 (1H, s, 4-H). Analysis calculated for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 69.00; H, 7.63.

*18,20-Epoxy-20β-hydroxy-17α,21-isopropylidenedioxypregna-1,4-diene-3,11-dione (**4b**).* Compound **3b** yielded 84.8% (2.8 g) of the title steroid **4b**: mp 224 to 226 C (colorless needles from MeOH containing a few drops of triethylamine). IR: 3,400 (OH), 1,700 (C=O), 1,665 (C=O), 1,620 (C=C). ^{1}H NMR ($CDCl_3$): 1.26 and 1.42 (each 3H, s, isopropylidenedioxy), 1.40 (3H, s, 19-H), 3.40 (1H, d, J = 9.3 Hz, 18-H), 3.61 and 3.68 (each 1H, d, J = 11.7 Hz, 21-H), 3.70 (1H, dd, J = 9.3 and 2 Hz coupled with 12-H, 18-H), 6.09 (1H, d, J = 1.5 Hz, 4-H), 6.21 (1H, d, J = 10.13 and 1.5 Hz, 2-H), 7.70 (1H, d, J = 10.3 Hz, 1-H). Analysis calculated for $C_{24}H_{30}O_6$: C, 69.54; H, 7.30. Found: C, 69.35; H, 7.12.

18,20-Epoxy-11β,20-dihydroxy-17α,21-isopropylidenedioxy-pregn-4-en-3-one (5a). Compound **4a** (3.2 g) in MeOH (50 ml) and CH_2Cl_2 (25 ml) at 0 C was stirred during careful addition of sodium borohydride (1.5 g) and allowed to stand for 1 hour. The excess of the reagent was destroyed by adding acetic acid. EtOAc was added and the mixture was washed with H_2O and dried (Na_2SO_4). Thin-layer chromatographic analysis showed the complete reaction to contain approximately 100% of the reduced product. The crude product was dissolved in dry dioxane (25 ml). 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ, 2 g) was added to this solution and the mixture was stirred at room temperature for 18 hours. The reaction mixture was diluted with benzene and filtrated on a bed of Celite. Evaporation of the solvent gave an oily product, which was purified by column chromatography on silica gel with $CHCl_3$/MeOH (100 : 2 v/v) and recrystallized from n-hexane/acetone containing a few drops of triethylamine to give **5a** (2.3 g, 72%) as colorless needles: mp 247 to 251 C. IR: 3,400 (OH), 1,655 (C=O), 1,610 (C=C). ^{1}H NMR ($CDCl_3$): 1.28 and 1.42 (each 3H, s, isopropylidenedioxy), 1.41 (3H, s, 19-H), 3.67 and 3.676 (each 1H, d, J = 11.7 Hz, 21-H), 3.65 and 4.75 (each 1H, d, J = 9.3 Hz, 18-H), 4.53 (1H, broad s, 11α-H), 5.68 (1H, s, 4-H). Analysis calculated for $C_{24}H_{34}O_6$: C, 68.87; H, 8.19. Found: C, 68.70; H, 8.31.

18,20-Epoxy-11β,20-dihydroxy-17α,21-isopropylidene-dioxypregna-1,4-dien-3-one (5b). Reduction of compound **4b** (2.2 g) in MeOH (30 ml) and CH_2Cl_2 (15 ml) with sodium borohydride (0.5 g) was carried out according to the method described for **5a** (0.5 g) and the crude product was recrystallized from n-hexane/acetone containing a few drops of triethylamine to afford **5b** (1.78 g, 81%) as colorless prisms: mp 240 to 242.5 C. IR: 3,400 (OH), 3,350 (OH) (C=O), 1,655 (C=O), 1,610 (C=C). ^{1}H NMR ($CDCl_3$): 1.37 and 1.43 (each 3H, s, isopropylidenedioxy), 1.44 (3H, s, 19-H), 3.24 (1H, dd, J = 10.2 and 1.5 Hz coupled with 12-H, 18-H), 3.93 (1H, d, J = 10.2 Hz, 18-H), 4.29 and 4.51 (each 1H, d, J = 18.5 Hz, 21-H), 4.54 (1H, broad s, 11α-H), 6.04 (1H, d, J = 1.4 Hz, 4-H), 6.29 (1H, dd, J = 10.2 and 1.4 Hz, 2-H), 7.24 (1H, d, J = 10.2 Hz, 1-H). Analysis calculated for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 68.95; H, 7.68.

18,20-Epoxy-11β,17α,20β,21-tetrahydroxypregn-4-en-3-one (6a). Acid hydrolysis of compound **5a** (2.1 g) with 2 M hydrochloric acid (5 ml) in tetrahydrofuran was allowed to stand for 1.5 hours. After usual work-up, the crude product was recrystallized from MeOH containing a few drops of triethylamine to give **6a** (1.7 g, 89.5%) as colorless prisms: mp 176 to 179 C. IR: 3,450 (OH), 1,655 (C=O), 1,615 (C=C). ^{1}H NMR (pyridine-d_5): 1.57 (3H, s, 19-H), 4.21 and 4.29 (each 1H, d, J = 11.2 Hz, 21-H), 4.20 and 5.02 (each 1H, d, J = 9.8 Hz, 18-H), 4.56 (1H, broad s, 11α-H), 5.84 (1H, s, 4-H). Analysis calculated for $C_{21}H_{30}O_6$: C, 66.64; H, 7.99. Found: C, 66.50; H, 7.78.

18,20-Epoxy-11β,17α,20β,21-tetrahydroxypregna-1,4-dien-3-one (6b). Compound **5b** (1.8 g) was treated with 2 M hydrochloric acid (4 ml) in tetrahydrofuran (40 ml) at room temperature for 1.5 hours. After usual work-up, the crude product obtained was recrystallized from EtOAc/MeOH containing a few drops of triethylamine to give **6b** (1.32 g, 81%) as colorless prisms: mp 227 to 230 C. IR: 3,540 (OH), 3,450 (OH), 3,350 (OH), 1,655 (C=O), 1,615 (C=C). ^{1}H NMR (pyridine-d_5): 1.61 (3H, s, 19-H), 4.12 and 4.28 (each 1H, d, J = 12.7, 21-H), 4.15 and 4.62 (each 1H, d, J = 10.2 Hz, 18-H), 4.64 (1H, broad s, 11α-H), 6.05 (1H, d, J = 1.4 Hz, 4-H), 6.42 (1H, dd, J = 9.8 and 1.4 Hz, 2-H), 7.36 (1H, d, J = 9.8 Hz, 1-H). Analysis calculated for $C_{21}H_{28}O_6$: C, 67.00; H, 7.50. Found: C, 66.87; H, 7.36.

Results and discussion

The starting materials for the projected synthesis were suitably protected isopropylidenedioxy derivatives (**1a** and **1b**), which were prepared from commercially available cortisol and prednisolone according to the known method.[9] With 11β-hydroxy steroids, functionalization of the angular methyl group by hypoiodite reaction is frequently complicated by bifunctional attack at the C-18 or C-19 position to form 11,18- and/or 11,19-ether linkages.[10] Hydrogen abstraction by hypohalite reaction in saturated steroids with the 11β-hydroxyl group occurs almost exclusively at position C-19, whereas that in ring A unsaturated steroid favorably takes place at position C-18.[11,12] In some instances, it has been possible to isolate the intermediate.[13] An initial attempt was therefore undertaken to isolate the 18-iodo derivatives.

When the acetonides (**1a** and **1b**) were reacted with lead tetraacetate and iodine at 0 C in the presence of calcium carbonate to scavenge generated acid, preferential iodination occurred at position C-18 to give the respective 18-monoiodo derivatives (**2a** and **2b**) in satisfactory yield. During this sequence, the cleavage of side chain and the formation of 19-iodomethyl derivatives were not observed. The only minor product was the 11,18-epoxy compound.* The structural assignments of these iodomethyl derivatives were unequivocally determined by their NMR spectra, in which the 18-methylene protons were observed at 3.2 to 4.0 ppm as a pair of doublets (J = 10 Hz).

In general, direct hydrolysis of the 18-iodo intermediates with silver ion essentially leads to the predominant formation of 11,18-ether linkage. However, oxidation of the 11β-hydroxyl group in the iodides (**2a, 2b**) yielding the 11-ketones (**3a, 3b**) avoided formation of

* 11,18-Epoxycortisol acetonide: mp 194 to 196 C. IR: 1,725, 1,665 cm^{-1}. ^{1}H NMR ($CDCl_3$): 1.26 (3H, s, acetonide), 1.44 (3H, s, 19-H), 1.47 (3H, s, acetonide), 3.77 and 3.64 (each 1H, d, J = 9 Hz, 18-H), 4.15 and 4.30 (each 1H, d, J = 19 Hz, 21-H), 4.41 (1H, d, J = 6.3 Hz, 11-H), 5.72 (1H, s, 4-H).

the ether linkage between the 11-hydroxyl and 18-iodomethyl groups. Indeed, hydrolysis of the 18-iodomethyl group in **3a** and **3b** by silver ion provided the expected 18-hydroxy derivatives (**4a, 4b**) in the hemiacetal form. The spectral and physical data of these compounds were identical with those of the reported data.[6]

Reduction of the 11-oxo group in prednisolone derivative (**4b**) was readily attained with sodium borohydride to give 18-hydroxyprednisolone 17α,21-acetonide (**5b**). In contrast, an analogous reduction of the cortisol derivative (**4a**) gave the 3,11-dihydroxy product, in which the allylic alcohol at position C-3 was selectively oxidized with DDQ to afford 18-hydroxycortisol 17α,21-acetonide (**5a**).

Finally, hydrolysis of acetonides **5a** and **5b** with dilute hydrochloric acid gave 18-hydroxycortisol (**6a**) and 18-hydroxyprednisolone (**6b**), respectively. The spectral and physical data of these synthetic compounds were identical with those reported values.[6]

In this study, photolytic hypoiodite reaction was investigated to synthesize 18-hydroxy derivatives of cortisol and prednisolone. The advantages of this method are easy handling of the reagents, compared with the nitrite method, and preferential attack at the 18-methyl group without concomitance of functionalization at the 19-methyl group. Clinical investigations with these synthesized compounds are now in progress in our laboratory and the details will be reported in the near future.

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