Syntheses of 16,17-Dihydroxy-17-methyl-5α-androstan-3,2-cisoxazoles

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(Received March 8, 1973)

In order to clarify the metabolic fate of 17β-hydroxy-17α-methyl-5α-androstan-3,2-cisoxazole, preparation of the titled compounds as its potential metabolites has been undertaken. Treatment of the appropriate 5α-androstan-3-ones (I, XIa, and XIb) with ethyl formate provided the 2-hydroxymethylene derivatives (II, XIIa, and XIIb), which in turn were condensed with hydroxylamine to yield the desired isoxazole fused steroids (IV, XIIIa, and XIIIb), respectively.

In recent years numerous kinds of the modified steroids have been presented for the chemotherapeutic purpose. One of these modifications involves the fusion of a hetero ring to the inherent steroidal nucleus. A heterocycle fused steroid, 17β-hydroxy-17α-methyl-5α-androstan-3,2-cisoxazole (androisoxazole), has been shown to exhibit the potent anabolic activity. However, the metabolic fate of this drug still remains unsolved. It has already been reported that the principal metabolites of the analogous steroids were the 16-oxygenated derivatives. In addition the occurrence of epimerization at the 17α-methyl-17β-ol structure in man has recently been demonstrated. These findings prompted us to synthesize the titled compounds as potential metabolites.

Chart 1

An initial project was focused on preparation of a compound having the 16β,17β-glycol structure. First, 16β,17β-dihydroxy-17α-methyl-5α-androstan-3-one acetonide (I), obtainable from dehydroepiandrosterone in several steps, was employed as a starting material. Condensation with ethyl formate in the presence of sodium methoxide proceeded with ease yielding the 2-hydroxymethylene derivative (II). Formation of isoxazole attached to ring A was effected by refluxing with hydroxylamine in aqueous pyridine. The mode of cycliza-

2) Location: Aobayama, Sendai.
tion into 5x-androstano[3,2-c]isoxazole under these conditions was unequivocally justified based upon the fact that the product (III) did resist the ring opening when treated with sodium methoxide.\textsuperscript{9)} The protecting group at C-16, 17 was readily eliminated with p-toluenesulfonic acid to afford the desired 16β,17β-dihydroxy-17α-methyl-5α-androstano[3,2-c]isoxazole (IV).

![Chemical Structures]

\begin{align}
\text{VIIIa: } R &= H \\
\text{VIIIb: } R &= \text{Ac}
\end{align}

\begin{align}
\text{IXa: } 17\alpha-\text{CH}_3, R &= H \\
\text{IXb: } 17\alpha-\text{CH}_3, R &= \text{Ac} \\
\text{IXc: } 17\beta-\text{CH}_3, R &= H \\
\text{IXd: } 17\beta-\text{CH}_3, R &= \text{Ac}
\end{align}

![Chart 2]

The preparation of C-17 epimeric 16α-hydroxy compounds was then undertaken. Introduction of an oxygen function to C-16 was accomplished by the method worked out by Gallagher and his co-workers.\textsuperscript{9)} First, isandrosterone 3-methyl ether (V) was transformed into the 16\textsuperscript{A}-enol acetate (VI) by treatment with isopropenyl acetate and a catalytic amount of sulfuric acid in a fairly good yield. Oxidation with m-chloroperbenzoic acid furnished solely the 16α,17α-epoxide (VII), which on brief exposure to sulfuric acid in aqueous methanol was converted into the 16β-hydroxy-17-ketone (VIIIa). Grignard reaction of the 16-acetate (VIIIb) with methylmagnesium iodide afforded two epimeric 17-methyl-17-hydroxy compounds (IXa, IXc) in a ratio of ca. 3 to 1. The cis-glycol structure of IXc was definitely established by leading to the 16,17-acetonide (X) in the usual manner. It is to be noted that formation of IXc reflects the influence of the steric hindrance due to the 16α-substituent.

Next effort was directed to the cleavage of the methyl ether at C-3. Concerning with this transformation chromium trioxide oxidation of 3-methoxyxysteroid into the formyloxy derivative has already been demonstrated.\textsuperscript{10)} Hence, the attempt was made on the utilization of this method for the subsequent elaboration. Being treated with chromium trioxide in glacial acetic acid and dry methylene chloride, 3β-methoxy-17α-methyl-5α-androstane-16α,17β-diol 16-acetate (IXb) was oxidized into the corresponding 3-oxo compound (XIIa) in a satisfactory yield. It seemed very likely that the reaction might proceed in such a way that the resulting formate underwent the spontaneous hydrolysis followed by further oxidation. Reaction with ethyl formate gave rise to the 2-hydroxymethylene derivative (XIIa), which in turn was condensed with hydroxylamine to yield the desired heterocycle fused compound (XIIIa).

The synthesis of its C-17 epimer was then carried out in the same reaction sequence as described above. Oxidation of 3β-methoxy-17β-methyl-5α-androstane-16α,17α-diol 16-

acetate (IXd) with chromium trioxide furnished the 3-ketone (XIIb), which in turn was led to the 2-hydroxymethylene derivative (XIIIb) by treatment with ethyl formate and sodium methoxide. The facile cyclization with hydroxylamine was attained to provide the isoxazole fused steroid (XIIIb) in a reasonable yield.

The 16-oxygenated compounds thus prepared will serve as useful reference for the studies on the metabolism of Androisoxazole.

**Experimental**

16β,17β-Dihydroxy-17α-methyl-5α-androstano[3,2-c]isoxazole 16,17-Acetonide (III)—To a ice-cooled solution of 16β,17β-dihydroxy-17α-methyl-5α-androst-3-one 16,17-acetonide (I) (50 mg) and MeONa (143 mg) in benzene (7 ml) was added dropwise ethyl formate (2 ml) over a period of 2 hr under a N₂ gas stream and stirred at room temperature for 17 hr. The resulting solution was acidified with 15% AcOH and extracted with benzene. The organic layer was separated, washed with 5% NaHCO₃ and H₂O, and dried over anhydrous Na₂SO₄. Evaporation of solvent gave 2-hydroxymethylene-16β,17β-dihydroxy-17α-methyl-5α-androst-3-one 16,17-acetonide (II) as a yellow oil. UV λ_{max} nm: 285. To a solution of II (50 mg) in pyridine (3 ml) was added an aq. solution of NH₂OH·HCl (23 mg in 0.5 ml) and refluxed for 4 hr. The resulting solution was diluted with ether, washed with 5% HCl and H₂O, dried over anhydrous Na₂SO₄, and evaporated. An oily residue was submitted to preparative TLC using benzene-AcOEt (4:1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.73) with AcOEt and recrystallization of the eluate from acetone–hexane gave III (25 mg) as colorless plates. mp 244.5—245.5° (c=0.10). Anal. Calcd. for C₉₉H₅₂O₇N: C, 74.76; H, 9.15; N, 3.63. Found: C, 74.71; H, 9.12; N, 3.49. NMR (5% solution in CDCl₃) δ: 0.75 (3H, s, 18-CH₃), 0.92 (3H, s, 18-CH₃), 1.31 (3H, s, 17α-CH₃), 1.36 (3H, s, acetone-CH₃), 1.46 (3H, s, acetone-CH₃), 4.20 (1H, q, J=7, 5 Hz, 16β-H), 8.06 (1H, s, isoxazole-5-H).

16β,17β-Dihydroxy-17α-methyl-5α-androstano[3,2-c]isoxazole (IV)—A solution of III (38 mg) and p-TsOH·H₂O (6.9 mg) in 99% EtOH (3 ml) was heated at 60—70° for 45 hr. After evaporation of solvent the residue was diluted with ether–CH₂Cl₂, washed with 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄, and evaporated. The crude product was submitted to preparative TLC using benzene–AcOEt (4:1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.16) with AcOEt and recrystallization of the eluate from acetone–hexane gave IV (15 mg) as colorless needles. mp 232—234.5° (c=0.14). Anal. Calcd. for C₂₉H₂₄O₇N: C, 73.00; H, 9.05; N, 4.05. Found: C, 72.98; H, 9.03; N, 3.85. UV λ_{max} nm: 222. NMR (4% solution in CDCl₃) δ: 0.76 (3H, s, 19-CH₃), 0.87 (3H, s, 18-CH₃), 1.12 (3H, s, 17α-CH₃), 3.70 (1H, q, J=6, 4 Hz, 16α-H), 8.06 (1H, s, isoxazole-5-H). IR v_{max} cm⁻¹: 1616 (C=O).

3β-Methoxy-5α-androstan-16-en-17-ol Acetate (V)—To a solution of 3β-methoxy-5α-androst-17-one (V) (1.1 g) in isopropyl acetate (10 ml) was added the catalyst solution (2 ml) (isopropenyl acetate (5 ml) and conc. H₂SO₄ (0.1 ml)) and refluxed for 1.5 hr. The reaction mixture was concentrated to one-half of its volume by slow distillation over a period of 1 hr. The resulting solution was diluted with ether and washed with cold 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄, and evaporated. An oily residue was submitted to column chromatography on silica gel. Elution with hexane–hexane (4:1 to 2:1) and recrystallization of the eluate from MeOH gave VI (940 mg) as colorless needles. mp 102° (c=0.13). Anal. Calcd. for C₂₉H₃₄O₇: C, 76.26; H, 9.89. Found: C, 76.29; H, 10.00. NMR (4% solution in CDCl₃) δ: 0.82 (3H, s, 19-CH₃), 0.86 (3H, s, 18-CH₃), 2.11 (3H, s, 17-OCH₃), 3.30 (3H, s, 3β-OCH₃), 5.45 (1H, m, 16-H).

3β-Methoxy-16α-hydroxy-5α-androstan-17-one (VIIa)—To a solution of VI (460 mg) in CHCl₃ (20 ml) was added m-chloroperbenzoic acid (260 mg) and allowed to stand at room temperature overnight. The resulting solution was diluted with ether, washed with 5% Na₂S₂O₅, 5% NaHCO₃ and H₂O, and dried over anhydrous Na₂SO₄. Evaporation of solvent gave 3β-methoxy-16α,17α-epoxy-5α-androst-17β-ol acetate (VII) (460 mg) as colorless needles. To a solution of VII in MeOH (40 ml) was added 1N H₂SO₄ (3 ml) under ice-cooling and allowed to stand at room temperature for 15 min. The resulting solution was diluted with ether, washed with 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄, and evaporated. Recrystallization from acetone–hexane gave VIIa (450 mg) as colorless prisms. mp 132—133° (c=0.12). Anal. Calcd. for C₂₉H₂₄O₇: C, 74.96; H, 10.06. Found: C, 75.31; H, 10.31. NMR (5% solution in CDCl₃) δ: 0.80 (3H, s, 19-CH₃), 0.95 (3H, s, 18-CH₃), 3.34 (3H, s, 3β-OCH₃), 4.35 (1H, t, J=4.5 Hz, 16β-H).

11) All melting points were taken on a micro-hot stage apparatus and are uncorrected. Optical rotations were measured in CHCl₃ unless otherwise specified. Ultraviolet (UV) and infrared (IR) spectra were run on Hitachi Model 124 and JASCO Model IR-S spectrophotometers, respectively. Nuclear magnetic resonance (NMR) spectra were recorded on Hitachi Model R-20 spectrometer at 60 MHz employing tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, t=triplet, q=quartet, and m=multiplet. For preparative thin-layer chromatography (TLC) silica gel H (E. Merck AG, Darmstadt) was used as an adsorbent.
3β-Methoxy-16α-hydroxy-5α-androstan-17-one Acetate (VIIIb)—Treatment of VIIa (450 mg) with Ac₂O (10 ml) and pyridine (10 ml) in the usual manner followed by recrystallization from MeOH gave VIIb (439 mg) as colorless prisms. mp 154—155°. [α]D^2 +182.6° (c=0.08). Anal. Calcd. for C₂₃H₃₆O₄: C, 72.81; H, 9.45. Found: C, 72.81; H, 9.53. NMR (6% solution in CDCl₃) δ: 0.82 (3H, s, 19-CH₃), 0.96 (3H, s, 18-CH₃), 2.09 (3H, s, 16α-OOCCH₃), 3.12 (3H, s, 3β-CH₃), 5.40 (1H, q, J=6.8, 3 Hz, 16β-H).

3β-Methoxy-17α-methyl-5α-androstan-16α,17β-diol (IXa), 3β-Methoxy-17β-methyl-5α-androstan-16α,17α-diol (IXc)—To an ethereal solution of MeMgBr freshly prepared from Mg ribbon (7 g) and MeI (43 g) in ether (150 ml) was added a solution of VIIb (2 g) in benzene (200 ml) under ice-cooling and stirred at room temperature for 8 hr. To the resulting solution were added moist ether, ice-water and 5% HCl, successively and extracted with AcOEt. The organic layer was separated, washed with H₂O, dried over anhydrous Na₂SO₄ and evaporated. An oily residue was submitted to column chromatography on silica gel. Elution with benzene–AcOEt (2:1) and recrystallization of the eluate from acetone–hexane gave IXa (400 mg) as colorless needles. mp 199—200°. [α]D^2 +0.2° (c=0.25). Anal. Calcd. for C₂₅H₃₈O₃: C, 74.95; H, 10.78. Found: C, 75.08; H, 10.83. NMR (4.5% solution in CDCl₃) δ: 0.69 (3H, s, 18-CH₃), 0.80 (3H, s, 19-CH₃), 1.16 (3H, s, 17β-CH₃), 3.32 (3H, s, 3β-CH₃), 3.40 (1H, q, J=6.8, 3 Hz), 4.05 (1H, t, J=7.8, 3 Hz), 5.05 (1H, m, 16β-H).

3β-Methoxy-17α-methyl-5α-androstan-16β-diol (IXb)—Treatment of IXa with Ac₂O and pyridine in the usual manner followed by recrystallization from MeOH gave IXb as colorless needles. mp 153—154°. [α]D^2 +3.7° (c=0.14). Anal. Calcd. for C₂₅H₃₈O₃: C, 72.97; H, 10.12. Found: C, 73.15; H, 9.94. NMR (4.5% solution in CDCl₃) δ: 0.81 (3H, s, 19-CH₃), 0.92 (3H, s, 18-CH₃), 1.05 (3H, s, 17α-CH₃), 2.08 (3H, s, 16α-OOCCH₃), 3.31 (3H, s, 3β-CH₃), 5.05 (1H, m, 16β-H).

3β-Methoxy-17β-methyl-5α-androstan-16α,17α-diol (IXd)—Treatment of IXc with Ac₂O and pyridine in the usual manner followed by recrystallization from MeOH gave IXd as colorless needles. mp 196—197.5°. [α]D^2 +18.3° (c=0.08). Anal. Calcd. for C₂₅H₃₈O₃: C, 72.97; H, 10.12. Found: C, 72.86; H, 10.20. NMR (3.4% solution in CDCl₃) δ: 0.72 (3H, s, 18-CH₃), 0.81 (3H, s, 19-CH₃), 1.14 (3H, s, 17β-CH₃), 2.11 (3H, s, 16α-OOCCH₃), 3.33 (3H, s, 3β-CH₃), 5.05 (1H, m, 16β-H).

3β-Methoxy-17α-methyl-5α-androstan-16α,17β-diol 16,17-Acetonide (X)—To a solution of IXc (140 mg) in acetone (8 ml) was added 2 drops of 70% HClO₄ under ice-cooling and stirred at room temperature for 1 hr. The resulting solution was poured into ice-water and extracted with AcOEt. The organic layer was separated, washed with 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄ and evaporated. An oily residue was submitted to preparative TLC using benzene–AcOEt (15:1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.41) with AcOEt and recrystallization of the eluate from MeOH gave X (71 mg) as colorless needles. mp 120—120.5°. [α]D^2 -15.9° (c=0.13). Anal. Calcd. for C₂₅H₃₈O₅: C, 76.55; H, 10.71. Found: C, 76.64; H, 10.81. NMR (4% solution in CDCl₃) δ: 0.66 (3H, s, 18-CH₃), 0.80 (3H, s, 19-CH₃), 1.31 (3H, s, 17β-CH₃), 2.42 (6H, s, -C(CH₃)₃), 3.33 (3H, s, 3β-CH₃), 4.34 (1H, broad d, J=4.5 Hz, 16β-H).

16α,17β-Dihydroxy-17α-methyl-5α-androstan-3-one 16-Acetate (XIA)—To a suspended solution of CrO₃ (120 mg) in AcOH (6 ml)—anhydrous CH₂Cl₂ (1 ml) was added dropwise a solution of IXb (100 mg) in anhydrous CH₂Cl₂ (5 ml) under ice-cooling and stirred over a period of 30 min and then stirred at room temperature for 1.5 hr. After careful addition of 5% Na₂SO₄ to decompose the excess reagent the resulting solution was extracted with ether. The organic layer was separated, washed with 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄ and evaporated. An oily residue was submitted to preparative TLC using benzene–AcOEt (4:1) as developing solvent. Elution of the adsorbent corresponding to the spot (Rf 0.26) and recrystallization of the eluate from MeOH gave XIA (30 mg) as colorless needles. mp 202—204°. [α]D^2 +47.6° (c=0.10). Anal. Calcd. for C₂₃H₃₆O₄: C, 72.89; H, 9.45. Found: C, 72.93; H, 9.37. NMR (4% solution in CDCl₃) δ: 0.96 (3H, t, J=17.8, 3 Hz), 1.03 (3H, s, 19-CH₃), 1.05 (3H, s, 17α-CH₃), 2.09 (3H, s, 16α-OOCCH₃), 5.01 (1H, q, J=6, 3 Hz, 16β-H).

16α,17β-Dihydroxy-17α-methyl-5α-androstan-3-one 3-Acetate (XIB)—To a suspended solution of CrO₃ (630 mg) in AcOH (6 ml)—anhydrous CH₂Cl₂ (3 ml) was added a solution of IXd (140 mg) in anhydrous CH₂Cl₂ (2 ml) under ice-cooling and stirred over a period of 30 min and then stirred at room temperature for 1.5 hr. After careful addition of 5% Na₂SO₄ to decompose the excess reagent the resulting solution was extracted with ether. The organic layer was separated, washed with 5% NaHCO₃ and H₂O, dried over anhydrous Na₂SO₄ and evaporated. Recrystallization from MeOH gave XIB (80 mg) as colorless needles. mp 206.5—208°. [α]D^2 -71.7° (c=0.02). Anal. Calcd. for C₂₃H₃₆O₄: C, 72.89; H, 9.45. Found: C, 72.82; H, 9.46. NMR (4.5% solution in CDCl₃) δ: 0.75 (3H, s, 19-CH₃), 1.02 (3H, s, 19-CH₃), 1.14 (3H, s, 17β-CH₃), 2.09 (3H, s, 16α-OOCCH₃), 5.00 (1H, q, J=9.3, 2.7 Hz, 16β-H).

16β,17β-Dihydroxy-17α-methyl-5α-androstan-3,2-cisoxazole (XIIIa)—To an ice-cooled solution of XIA (58 mg) and MeONa (115 mg) in benzene (5 ml) was added ethyl formate (2 ml) and stirred at room temperature for 2 hr. The resulting solution was acidified with 5% HCl and extracted with AcOEt. The organic layer was separated, washed with H₂O, and dried over anhydrous Na₂SO₄. Evaporation of solvent
gave 2-hydroxymethylene-16α,17β-dihydroxy-17α-methyl-5α-androstan-3-one (XIIa) (52 mg) as a yellow oil. UV \( \lambda _{\text{max}}^{\text{nm}} \): 285. To a solution of XIIa (52 mg) in pyridine (10 ml) was added an aq. solution of NH\(_2\)-OH·HCl (40 mg in 0.2 ml) and refluxed for 4 hr. The resulting solution was diluted with AcOEt, washed with 5% HCl and H\(_2\)O, dried over anhydrous Na\(_2\)SO\(_4\), and evaporated. An oily residue was submitted to preparative TLC using benzene–AcOEt (1:2) as developing solvent. Elution of the adsorbent corresponding to the spot (RF 0.21) with AcOEt yielded the eluate of the eluate from acetone–hexane gave XIIa (17 mg) as colorless leaflets, mp 216—218\(^\circ\), \([\alpha]_D^{20} = -0.03^\circ\) (c = 0.13). Anal. Calcd. for C\(_{29}\)H\(_{37}\)O\(_4\)N: C, 73.00; H, 9.05; N, 4.05. Found: C, 72.98; H, 9.16; N, 4.35. UV \( \lambda _{\text{max}}^{\text{nm}} \): 222. NMR (3.5% solution in CDCl\(_3\)) \( \delta \): 0.76 (3H, s, 19-CH\(_3\)), 0.90 (3H, s, 18-CH\(_3\)), 1.20 (3H, s, 17α-CH\(_3\)), 4.30 (1H, q, \( J = 5 \), 9 Hz, 16β-H), 8.11 (1H, s, isoxazole-5-H). IR v\(_{\text{max}}^{\text{cm}^{-1}}\): 1616 (C=N).

16α,17α-Dihydroxy-17β-methyl-5α-androstan-3β,2-α-isoxazole (XIIIb)—To an ice-cooled solution of XIIb (55 mg) and MeONa (116 mg) in benzene (7 ml) was added ethyl formate (2 ml) and stirred at room temperature for 17 hr. The resulting solution was acidified with 5% HCl and extracted with benzene. The organic layer was separated, washed with H\(_2\)O and dried over anhydrous Na\(_2\)SO\(_4\). Evaporation of solvent gave 2-hydroxymethylene-16α,17α-dihydroxy-17β-methyl-5α-androstan-3-one (XIIIb) (50 mg) as a yellow oil. UV \( \lambda _{\text{max}}^{\text{nm}} \): 285. To a solution of XIIIb (50 mg) in pyridine (5 ml) was added an aq. solution of NH\(_2\)-OH·HCl (25 mg in 0.5 ml) and refluxed for 4 hr. The resulting solution was diluted with ether, washed with 5% HCl and H\(_2\)O, dried over anhydrous Na\(_2\)SO\(_4\), and evaporated. An oily residue was submitted to preparative TLC using benzene–AcOEt (4:1) as developing solvent. Elution of the adsorbent corresponding to the spot (RF 0.21) with AcOEt and recrystallization of the eluate from acetone–hexane gave XIIIb (24 mg) as colorless needles. mp 202—203\(^\circ\). \([\alpha]_D^{20} = -23.7^\circ\) (c = 0.08). Anal. Calcd. for C\(_{29}\)H\(_{37}\)O\(_4\)N: C, 73.00; H, 9.05; N, 4.05. Found: C, 73.21; H, 8.98; N, 4.05. UV \( \lambda _{\text{max}}^{\text{nm}} \): 222. NMR (4% solution in CDCl\(_3\)) \( \delta \): 0.72 (3H, s, 18-CH\(_3\)), 0.75 (3H, s, 19-CH\(_3\)), 1.18 (3H, s, 17β-CH\(_3\)), 4.05 (1H, q, \( J = 7 \), 2.7 Hz, 16β-H), 8.05 (1H, s, isoxazole-5-H). IR v\(_{\text{max}}^{\text{cm}^{-1}}\): 1616 (C=N).

**Acknowledgement** The authors are indebted to all the staffs of central analytical laboratory of this Institute for elemental analyses and spectral measurements.