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Synthesis of sodium androst-5-ene-17-one-3β-methylene sulfonate

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The synthesis of sodium androst-5-ene-17-one-3β-methylene sulfonate **2**, *a stable analog of memory-enhancing neurosteroid dehydroepiandrosterone sulfate, is described. The synthesis of compound* **2** *is carried out in six steps from dehydroepiandrosterone.* (*Steroids* **62**:543–545, 1997) © *1997 by Elsevier Science Inc.*

Keywords: steroids; steroid sulfatase; memory enhancement; dehydroepiandrosterone sulfate

Introduction

Neurosteroid dehydroepiandrosterone sulfate (DHEAS) **1** (Figure 1) was reported to enhance memory.[1,2] The mechanism of this enhancement is unknown, but DHEAS is known to inhibit the actions mediated by γ-aminobutyric acid type A receptor and facilitate those mediated by *N*-methyl-D-aspartate receptors.[3,4] The unsulfated analog of DHEAS, dehydroepiandrosterone (DHEA), can also enhance memory.[1] Because the interconversion of DHEA and DHEAS occurs within the central nervous system and periphery, it is uncertain whether the sulfated or unsulfated forms produce memory-enhancing effects independently or via metabolism from one analog to the other. Recently, we reported that estrone-3-*O*-sulfamate, a steroid sulfatase inhibitor, could significantly enhance the potency of DHEAS in reversing scopolamine-induced amnesia.[5] These data indirectly indicated that DHEAS is responsible for the memory-enhancing properties of this neurosteroid. It occurred to us that a sulfonate analog of DHEAS, such as sodium androst-5-ene-17-one-3β-methylene sulfonate **2**, would be stable toward the hydrolytic activity of steroid sulfatase and might mimic the memory enhancing-effect of DHEAS. Compound **2** might also be useful in investigating other unknown biological properties of DHEAS, in addition to acting as a neurosteroid and a precursor of DHEA.

Experimental

General methods

Chemicals and silica gel were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin, USA). The chemicals were checked for purity by thin-layer chromatography and NMR. Melting points were determined on a Thomas Hoover capillary melting point apparatus and were uncorrected. IR spectral data were obtained with a Perkin–Elmer Corp. (Norwalk, Connecticut, USA) 1430 ratio recording spectrophotometer. Proton NMR spectra were obtained with a Bruker (Billerica, Massachusetts, USA). Negative ion cesium Liquid-SIMS was performed by the Analytical Instrument Group (Raleigh, North Carolina, USA) on an AMD Intectra GMBH mass spectrometer, with thioglycerol as the matrix.

3β-Chloro-androst-5-ene-17-one (4)

Dehydroisoandrosterone (**3**) (2.88 g, 10 mmol) was dissolved in 20 mL of a 2 M solution of thionyl chloride in CH_2Cl_2. The reaction mixture was stirred at room temperature (r.t.) for 1.5 h and then concentrated in vacuo. The residue was purified by chromatography on silica gel using petroleum ether/ethyl acetate (3:1) to give **4** (2.88 g, 91.5%): m.p. 156–157°C (Lit.[6] 156–157°C); ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 3.76 (m, 1H, CHCl), 5.38 (m, 1H, vinyl proton). Analysis calculated for $C_{19}H_{27}ClO$: C, 74.36; H, 8.87. Found: C, 74.22; H, 8.91.

3β-Chloro-17,17-ethylenedioxyandrost-5-ene (5)

p-Toluenesulfonic acid (133 mg, 0.7 mmol) and ethylene glycol (1.0 mL) were added to a solution of compound **4** (2.7 g, 8.82 mmol) in benzene (70 mL). The reaction mixture was refluxed for 18 h using a Dean–Stark apparatus to separate the water. The reaction mixture was cooled, washed with water (60 mL) and brine (60 mL), and dried with Na_2SO_4. After evaporation of the solvent at reduced pressure, the residue was purified by silica gel chromatography using petroleum ether/ethyl acetate (6:1) to yield pure **5** (2.8 g, 90.7%): m.p. 144–145.5°C; ^{1}H NMR ($CDCl_3$) δ 0.86 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 3.75 (m, 1H, CHCl), 3.87 (m, 4H, $-OCH_2CH_2O-$), 5.35 (m, 1H, vinyl proton). Analysis calculated for $C_{21}H_{31}ClO_2$: C, 71.88; H, 8.90. Found: C, 72.05; H, 8.82.

3β-Hydroxymethyl-17,17-ethylenedioxyandrost-5-ene (6)

Methyl magnesium iodide [3 M solution in tetrahydrofuran (THF); 16.1 mL, 50.1 mmol] was added at r.t. to a solution of compound

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Received October 16, 1996; accepted March 31, 1997.

Steroids 62:543–545, 1997

655 Avenue of the Americas, New York, NY 10010

0039-128X/97/$17.00
PII S0039-128X(97)00039-1

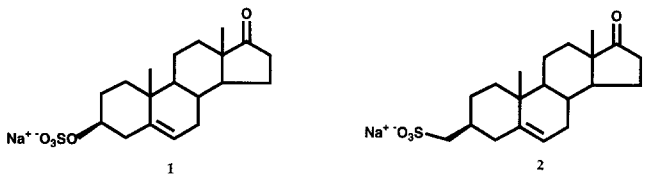


Figure 1 Structure of DHEAS **1** and sodium androst-5-ene-17-one-3β-methylene sulfonate **2.**

5 (8 g, 22.86 mmol) in THF (150 mL). The reaction mixture was refluxed for 72 h; solid paraformaldehyde (5 g) was added to the refluxing solution, and the heating was continued for another 26 h. The reaction mixture was cooled in an ice bath, and a cold aqueous solution of ammonium chloride (NH_4Cl; 10 g in 50 mL of water) was slowly added. The mixture was extracted with ethyl acetate (3 × 100 mL), and the organic layer was separated, washed with water (2 × 150 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography using petroleum ether/ethyl acetate (2:1) to afford **6** (3 g, 38%): m.p. 136–137°C (Lit.[7] 135.5–137.5°C); ^{1}H NMR($CDCl_3$) δ 0.84 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 3.46 (d, 2H, J = 6.0 Hz, CH_2OH), 3.85 (m, 4H, -OCH_2CH_2O-), 5.30 (m, 1H, vinyl proton). Analysis calculated for $C_{22}H_{34}O_3$: C, 76.26; H, 9.89. Found: C, 76.10; H. 9.95.

3β-Iodomethyl-17,17-ethylenedioxy-androst-5-ene (7)

To the mixture of triphenylphosphine (7.93 g, 30.3 mmol), imidazole (2.06 g, 30.3 mmol), iodine (7.72 g, 30.3 mmol), and potassium acetate (3.97 g, 60.5 mmol) in CH_2Cl_2 (250 mL), a solution of compound **6** (2.8 g, 8.1 mmol) in CH_2Cl_2 (30 mL) was added in one portion. After the reaction mixture was stirred at r.t. for 2 h, a saturated aqueous solution of sodium bicarbonate ($NaHCO_3$, 150 mL) was added, and the stirring continued for another 30 min. The organic layer was then separated and washed with saturated aqueous solution of $Na_2S_2O_3$ (2 × 60 mL) and water (60 mL), dried (Na_2S0_4), and evaporated under reduced pressure. The residue was purified by silica gel chromatography using petroleum ether/ethyl acetate (4:1) to yield **7** (3.18 g, 86.2%): m.p. 143.5–144.5°C; ^{1}H NMR ($CDCl_3$) δ 0.84 (s, 3H, CH_3), 0.95 (s,3H, CH_3), 3.10 (d, 2H, J = 6.0 Hz, CH_2I), 3.86 (m, 4H, -OCH_2CH_2O-), 5.31 (m, 1H, vinyl proton). Analysis calculated for $C_{22}H_{33}O_2I$: C, 57.90; H, 7.29. Found: C, 57.79; H, 7.31. MS *(m/z)* 456 (M^+).

3-[2'-(Trimethylsilyl)ethane sulfonyl methyl]-17,17-ethylenedioxy-androst-5-ene (8)

Compound **7** (1.3 g, 2.85 mmol), sodium 2-(trimethylsilyl)ethanesulfinate (5.8 g, 30.85 mmol), and tetraethyl ammonium chloride (4.2 mg, 0.086 mmol) were added to a mixture of methanol/isopropanol/dioxane (2:2:1, 30 mL). The heterogeneous mixture was stirred at 70–80°C for 120 h. The solvent was evaporated under reduced pressure, followed by addition of CH_2Cl_2 (100 mL) and water (50 mL). The organic layer was separated and washed with water (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica gel chromatography using petroleum ether/ethyl acetate (4:1) to yield **8** (0.99 g, 70.3%): m.p. 178.5–180°C; ^{1}H NMR ($CDCl_3$) δ 0.06 (s, 9H, TMS), 0.87 (S, 3H, CH_3), 1.00 (s, 3H, CH_3), 2.85 (m, 4H, -$CH_2SO_2CH_2$-), 3.85 (m, 4H, -OCH_2CH_2O-), 5.34 (m, 1H, vinyl proton). MS *(m/z)* 494 (M^+).

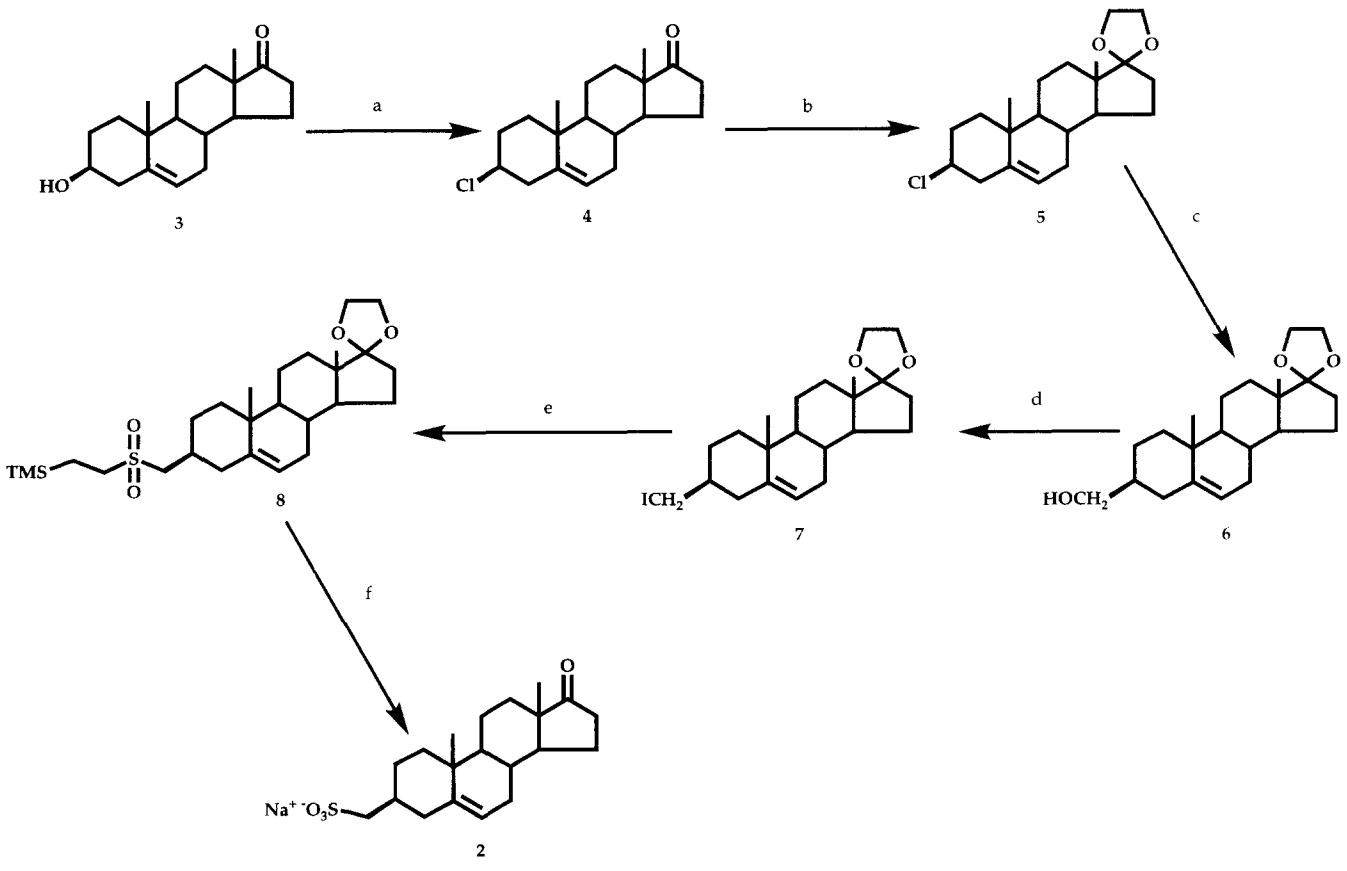
Sodium androst-5-ene-17-one-3β-methylene sulfonate (2)

Sulfone **8** (350 mg, 0.71 mmol) and cesium fluoride (CsF, 1.08 g, 7.1 mmol) were added to anhydrous dimethylformamide (DME) (5 mL). The reaction mixture was stirred at 80°C for 120 h and then cooled to r.t. HCl (2 N; 3 mL) and dioxane (5 mL) were added, and the clear solution was stirred at r.t. for 80 min. Brine (50 mL) was added to the above solution, and the product was precipitated out immediately. The solution was kept at room temperature for 3–4 h, the mixture was filtered, and the solid was dried in vacuo overnight, yielding the desired compound **2** (185 mg, 67.3%): m.p. >250°C (dec.); ^{1}H NMR (DMSO-d_6) δ 0.79 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 2.34 (d, 2H, J = 5.9 Hz, CH_2SO_3Na), 5.25 (m, 1H, vinyl proton); positive fast atom bombardment mass spectrometry 411 ($RSO_3^-Na^+$+Na); High-resolution negative ion cesium Liquid-SIMS: calculated for $[C_{20}H_{29}O_4S]^-$: 365.17889. Found: 365.17869.

Results and discussion

Compound **2** is an analog of DHEAS **1,** with the sulfate group isosterically replaced by a methylene sulfonate group. The isosteric replacement of other naturally occurring sulfates, such as nucleoside sulfates,[8] glucose-6-sulfate,[9] cholesterol sulfate,[10] and estrone sulfate,[11] with sulfonate esters has been reported. Cholesterol sulfonate was demonstrated to be as effective as cholesterol sulfate in inhibiting cholesterol side chain cleavage enzyme.[10] Thus, it is possible that compound **2** would be stable toward the hydrolytic activity of steroid sulfatase and might mimic the memory-enhancing effect of DHEAS.

The synthesis of **2** (Scheme 1) started with the commercially available DHEA **3.** Treatment of **3** with excess thionyl chloride at r.t. yielded the chloride **4** (91.5%). The 17-keto group of **4** was then protected as ketal to yield **5** (90.7%). Chloride **5** was transformed to the Grignard reagent by treatment with methyl magnesium iodide in THF at reflux for 72 h, followed by reaction with excess paraformaldehyde, yielding the alcohol **6** (38%).[12] Iodination of alcohol **6** with tripenylphosphine/iodine/imidazole in the presence of potassium acetate afforded the iodide **7** (86.2%). We first attempted to displace the iodide in **7** with excess K_2SO_3 using 18-crown-6 as the phase transfer catalyst to obtain the final sulfonate. However, it proved to be unsatisfactory. Iodide **7** was then reacted with large excess of sodium 2-(trimethylsilyl)ethanesulfinate[10] in the presence of a catalytic amount of tetraethylammounium chloride to give the sulfone **8** (70.3%). Compound **8** was then reacted with CsF in DMF followed by treatment with 2 N HCl to cleave the ketal. To the above reaction mixture, saturated NaCl solution was added, and the product precipitated out immediately. According to the literature procedure,[10] treatment of the similar sulfone with fluoride ion (CsF) elicited the fragmentation to give the sulfinic acid, and it took another two steps to transform the sulfinic acid into the corresponding sulfonate. However, the solid obtained was unambiguously confirmed by fast atom bombardment MASS Spectra to be the desired final compound sulfonate **2.** Because the fragmentation of compound **8** was not under nitrogen atmosphere, the sulfinic acid that was formed could be converted to sulfonic acid through air oxidation.



Scheme 1 (a) $SOCl_2$, r.t., 1.5 h, 91.5%; (b) ethylene glycol, benzene, *p-toluenesulfonic acid, reflux 18 h, 90.7%; (c) 1. CH_3MgI, THF, reflux 72 h; 2. paraformaldehyde, reflux 24 h; (d) triphenylphosphine/iodine/imidazole, potassium acetate, CH_2Cl_2, r.t., 2 h, 86.2%; (e) $TMSCH_2CH_2SO_2^-Na^+$, Et_4NCl, methanol/isopropanol/dioxane (2:2:1), reflux 120 h, 70.3%; (f) 1. CsF, DMF, 80°C, 120 h; 2: dioxane, 2 N HCl, r.t., 80 min; 3. saturated NaCl, 67.3%.*

In conclusion, sodium androst-5-ene-17-one-3β-methylene sulfonate **2**, a stable analog of memory-enhancing neurosteroid DHEAS **1** was synthesized in six steps from DHEA. Biological studies of its memory-enhancing effects are now in progress. We will report the results in due course.

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