A SHORT EFFICIENT SYNTHESIS OF 16-OXYGENATED ESTRATRIENE 3-SULFATES

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ABSTRACT

A novel synthesis of sodium 17-oxo-16α-hydroxy-1,3,5(10)-estratrien-3-yl sulfate (4), sodium 16α,17β-dihydroxy-1,3,5(10)-estratrien-3-yl sulfate (5) and sodium 16-oxo-17β-hydroxy-1,3,5(10)-estratrien-3-yl sulfate (6) is described. 16α-Bromo-3-hydroxy-1,3,5(10)-estratrien-17-one (1) was efficiently synthesized in one step with 70-97% yield by bromination of 3-hydroxy-1,3,5(10)-estratrien-17-one with cupric bromide. 3,16α-Dihydroxy-1,3,5(10)-estratrien-17-one (3) was quantitatively obtained by controlled stereospecific hydrolysis of the bromoketone 1 with sodium hydroxide in aqueous pyridine. The bromoketone 1 was converted to the 16α-hydroxy-17-ketone 3-sulfate 4 by sulfation with chlorosulfonic acid in pyridine and a subsequent controlled hydrolysis in a high yield without formation of the other ketols. Treatment of the sulfate 4 with sodium borohydride gave the triol sulfate 5. The sulfate 4 was also rearranged to the 17β-hydroxy-16-ketone 6 with sodium hydroxide in water in a quantitative yield.

INTRODUCTION

16α-Hydroxylation of estrogens, which produces 16α-hydroxyestrone and estriol, is one of the main pathways for estrogen metabolism in the human [1]. These estrogen metabolites are also major estrogens in pregnancy [2] and are mainly found as sulfates in the plasma [3-5]. Estriol is now recognized as a potent uterotropic agent equivalent to estradiol [6]. On the other hand, the quantitative importance of 16α-hydroxyestrone in pregnancy [7] and non-pregnancy [8] bile and its new biological activities in man [9] have been reported. Such estrogen sulfates, however, are not readily available, primarily because of the difficulties of their synthesis. Fex *et al.* [10] reported the chemical synthesis of estriol 3-sulfate (5) which involves four steps from

estriol with sulfation of its 16,17-diacetate as a key reaction. This method has an overall yield of approximately 31%. On the other hand, chemical syntheses of 16α-hydroxyestrone 3-sulfate (4) and 16-oxo-estradiol 3-sulfate (6) have not been achieved, although formation of the sulfates 4 and 6 is observed in biological systems [2].

We recently discovered the controlled stereospecific alkaline hydrolysis of 16-bromo-17-ketoandrostanes [11] and also developed the hydrolytic method for the synthesis of several 16α-hydroxy-17-keto-androstanes [12]. We report here an efficient synthesis of 16α-bromo-estrone (1) and the novel syntheses of estriol 3-sulfate (5), 16α-hydroxyestrone 3-sulfate (4) and 16-oxoestradiol 3-sulfate (6) by using the controlled alkaline hydrolysis of the bromoketone 1 as a key reaction with high overall yields.

RESULTS AND DISCUSSION

The bromination of estrone with cupric bromide [13, 14] in dry methanol was initially explored in order to obtain 16α-bromoestrone (1) directly from estrone in a high yield. As shown in Table I, when

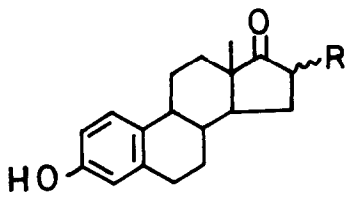
Table I. Bromination of estrone with cupric bromide in methanol.

Conditions		Relative amount of product (%)*		
$CuBr_2$ (mol. equiv.)	Time (hr)	Estrone	Bromoketone 1	By-products
1.2	24	75	15	10
2	20	38	56	6
2	36	32	48	20
3	24 (expt. 1)	< 1	97	< 2
3	24 (expt. 2)	10	70	20

* Relative amount of product was obtained by the weight of products isolated by TLC together with by peak areas corresponding to both the C-16 proton and C-18 angular methyl of NMR spectra of a mixture of 1 and estrone. TLC: estrone, Rf= 0.30; bromoketone 1, Rf= 0.33; by-products, Rf= 0.41; *n*-hexane/ ethyl acetate= 2/ 1,v/v. NMR ($CDCl_3$): estrone δ 0.90 (18-CH_3).

estrone was heated in methanol under reflux, for 24 hr, with 3 mol. equivalents of cupric bromide, the bromoketone 1 was obtained in 70-90% yield, together with a small amount of by-products (2-20%) and the starting material (1-10%). The NMR spectra of the by-products showed that they would be approximately 1:1 mixture of 2,16α-dibromoestrone [δ 0.95 (18-CH_3), 4.57 (16β-H), 6.67 (4-H) and 7.33 (1-H)] and 4,16α-dibromoestrone [δ 0.95 (18-CH_3), 4.57 (16β-H), 6.83 (4-H) and 7.14 (1-H)]. Fishman *et al.*[15] previously reported a three-step synthesis of the bromide 1 from estrone involving the bromination of the 17-enol acetate with bromine. To our knowledge, regio- and stereospecific direct (one-step) bromination of estrone has not been previously reported.

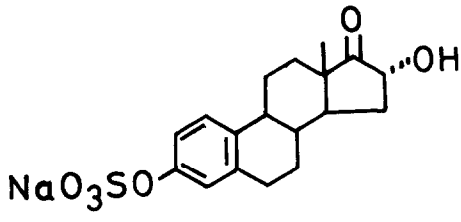
Alkaline hydrolysis of the 16α-bromo ketone 1 with sodium hydroxide in aqueous pyridine was examined in order to clarify whether 16α-hydroxyestrone 3 could be isolated in a high yield without formation of the other ketols as previously reported [11, 12]. The dynamic aspects of equilibrium between the 16α-bromo ketone 1 and its 16β-isomer 2, and of production of the 16α-hydroxy-17-ketone 3 are shown in Table II. Treatment of the bromoketone 1 with 0.12 equivalent of sodium hydroxide caused an appoximate 1:1.3 equilibrium between the bromoketones 1 and 2, in favor of the 16β-isomer 2 without formation of the ketol 3. An increasing production of the ketol 3 in proportion to the amount of the base and reaction time was observed, maintaining bromoketones 1 and 2 in the same equilibrium. The ketol 3 was stereoselectively obtained in 90% yield from the bromoketone 1 by using 6 or 3.3 hr reaction time and 1.2 or 1.8 equivalents of alkali, respectively. The ketol 3 was identical in every respect with an authentic sample. These results indicate



1 R=···Br

2 R=—Br

3 R=···OH



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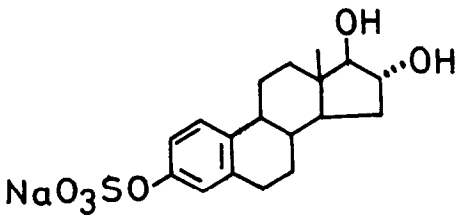

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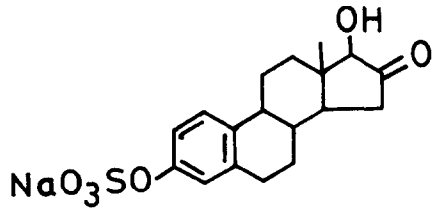

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Table II. Epimerization of the 16-bromo ketone 1 and formation of the 16α-hydroxy-17-ketone 3 in sodium hydroxide-aqueous pyridine system.

	Conditions		Relative amount of product (%)*		
	NaOH (equiv)	Time (min)	1	2	3
A	0.012	10	92	8	0
B	0.06	10	48	52	0
C	0.12	10	43	57	0
D	0.6	10	37	52	11
E	1.2	10	27	36	37
F	1.2	360	0	0	>99
G	1.8	200	0	0	>99

* Relative amount of product was obtained by integrating the peak areas corresponding to both the C-16 proton and C-18 angular methyl of NMR spectra of the reaction mixtures without isolation.

a nucleophilic displacement of the 16β-bromine substituent of bromide 2 by hydroxide ion, in analogy with that reported for the C_{19} steroids [11, 12].

The short efficient synthesis of sodium 17-oxo-16α-hydroxy-5-androsten-3-yl sulfate by controlled alkaline hydrolysis of the corresponding 16-bromoketone was previously reported by us [11]. In order to develop this synthetic method and also to obtain hitherto not available 16α-hydroxyestrone 3-sulfate (4), we determined the optimal conditions for the hydrolysis of 16α-bromoestrone 3-sulfate in the presence of excess amounts of chlorosulfonic acid. Treatment of the bromoketone 1 with 6 mol. equivalents of chlorosulfonic acid in pyridine quantitatively gave the 3-sulfate of compound 1. The reaction mixture was poured into a chilled sodium hydroxide solution. The 16α-hydroxy-17-ketone 3-sulfate 4 was isolated from the solution by means of an Amberlite XAD-2 resin. The structure of the sulfate 4 was determined by the IR and NMR spectra. Elemental analysis and solvolysis of the sulfate 4 also supported the structure of the ketol sulfate 4. We could not detect the formation of the other ketols in alkaline hydrolysis.

When the 3-sulfate of bromoketone 1 obtained as described above, was hydrolyzed with a large excess of base at room temperature, 16-oxoestradiol 3-sulfate (6) was obtained as the sole product in 62% yield. The structure of the sulfate 6 was determined by elemental analysis and the IR and NMR spectra. Solvolysis of the sulfate 6 gave 16-oxoestradiol as the sole product. The sulfate 6 was also obtained by the ketol rearrangement of the 16α-hydroxy-17-ketone 3-sulfate 4 with sodium hydroxide in aqueous methanol at room temperature.

Estriol 3-sulfate (5) was obtained by sodium borohydride reduction of 16α-hydroxyestrone 3-sulfate (4) in methanol at 0°. The crude product was purified by preparative TLC. The structure of the triol sulfate 5 was determined by the IR and NMR spectra and solvolysis.

Recently, the controlled hydrolysis has been shown to represent a potent method for synthesis of estriol 16-glucuronide [16]. The present results, together with the previous ones, suggest that a controlled hydrolysis of α-halo ketones might represent a useful method for the stereoselective synthesis of other steroidal ketols.

EXPERIMENTAL

General methods. Melting points were measured on Yanagimoto melting point apparatus and were uncorrected. IR spectra were recorded on a Shimadzu 400 spectrophotometer in KBr pellets. NMR spectra were obtained with a JEOL PMX 60 spectrometer at 60 MHz using tetramethylsilane as an internal standard. Thin-layer chromatography (TLC) was carried out on a plate coated with a layer (0.25 mm thick) of silica gel G (E. Merck AG).

Bromination of 3-hydroxy-1,3,5(10)-estratrien-17-one with $CuBr_2$ in MeOH. A solution of estrone (3.7 mmol.) and $CuBr_2$ (1.2, 2 and 3 mol. equiv.) in dry MeOH (250 ml) was heated under reflux for an appropriate time, respectively. The reaction mixture was poured into ice-cold water and then extracted with AcOEt (100 ml x 2). The organic layer was dried (Na_2SO_4) and evaporated to give the crude brominated products (1.28-1.40 g).

16α-Bromo-3-hydroxy-1,3,5(10)-estratrien-17-one (1). The crude product obtained by using 3 mol. equivalents of $CuBr_2$ was recrystallized from MeOH to give 1 (1.23 g, 95%) as colorless plates, mp 223-227° (lit. [15] mp 225-228°). IR (KBr): ν_{max} 3400 (OH), 1741 (C=O). NMR ($CDCl_3$): δ 0.93 (3H, s, 18-CH_3), 4.60 (1H, m, 16β-H), 6.53-7.23 (3H, m, aromatic protons).

Epimerization of the bromoketone 1 and formation of the 16α-hydroxy-17-ketone 3 in sodium hydroxide-aqueous pyridine system. To a solution of 1 (0.41 mmol.) in 75% aqueous pyridine (8 ml) was added 0.48 ml of NaOH solution and the mixture was allowed to stand at room temperature for an appropriate time. The mixture was poured into 1% HCl solution and then extracted with AcOEt (20 ml x 2). The organic layer was washed with 5% $NaHCO_3$ and H_2O and dried (Na_2SO_4). After evaporation of the solvent the residue obtained (110-130 mg) was submitted to NMR analysis.

16β-Bromo-3-hydroxy-1,3,5(10)-estratrien-17-one (2). Upon a fractional

crystallization of the epimerized residue of 1 obtained by condition D (Table II), 2 (25 mg, 17%) was obtained as colorless needles, mp 225-227° (lit. [15] mp 225-228°). IR (KBr): ν_{max} 3400 (OH), 1742 (C=O). NMR ($CDCl_3$): δ 1.13 (3H, s, 18-CH_3), 4.16 (1H, t, J= 8 Hz, 16α-H), 6.51-7.23 (3H, m, aromatic protons).

3,16α-Dihydroxy-1,3,5(10)-estratrien-17-one (3). The hydrolyzed residues of 1 obtained by conditions F and G were recrystallized from MeOH to give 3 (110mg, 90%) as colorless needles, mp 203-206° (lit. [17] 205-207°). IR (KBr): ν_{max} 3400 (OH), 1735 (C=O). NMR ($CDCl_3$): δ 0.97 (3H, s, 18-CH_3), 4.40 (1H, m, 16β-H), 6.51-7.20 (3H, m, aromatic protons).

Sulfation of the bromoketone 1 with chlorosulfonic acid in pyridine. A SO_3-pyridine complex was prepared by adding dropwise 1 g of $ClSO_3H$ to 22.5 ml of dry pyridine cooled at -10°. The bromoketone 1 (500 mg) in pyridine (7.5 ml) was added to this complex and the reaction mixture was stirred at 30° for 2.5 hr. The starting material was completely sulfated under this condition according to TLC analysis (Rf= 0.50, $CHCl_3$/ MeOH/ NH_4OH= 15/ 5/ 1, v/v).

Controlled hydrolysis of the 3-sulfate of compound 1 with NaOH in aqueous pyridine. The reaction mixture containing the 3-sulfate of compound 1 obtained above was poured into an ice-cold 0.05N NaOH solution (800 ml) and then stirred at 0° for 2.5 hr. After this time, the reaction mixture was diluted with 1000 ml of H_2O and then passed through Amberlite XAD-2 column (4 x 60 cm). After washing with H_2O (1000 ml), the adsorbed steroids were eluted with MeOH (800 ml). The MeOH fraction was evaporated to dryness under reduced pressure at 50° to give crude sulfate 4 (500 mg, 95%) as a colorless solid.

Solvolysis of the crude product 4. The crude product 4 (25 mg) obtained above was dissolved in H_2O (50 ml). To this solution, 50% H_2SO_4 was added until pH 1 was reached and, subsequently, NaCl to saturation. The solution was extracted with AcOEt (50 ml x 2) and then allowed to stand at 37° for 8 hr. The organic layer was washed with 5% $NaHCO_3$ and H_2O, and dried (Na_2SO_4) and evaporated to dryness to yield a solid (13 mg, 73%), mp 204-206°. The ketol 3 was identified by the IR and NMR spectra of the solid and the formation of the other ketones were not observed.

Sodium 17-oxo-16α-hydroxy-1,3,5(10)-estratrien-3-yl sulfate (4). The ketol sulfate obtained above was recrystallized from MeOH to give 4 as colorless plates, mp 222-225°. Yield: 65% form 1. TLC: Rf= 0.50, $CHCl_3$/ MeOH/ NH_4OH= 15/ 5/ 1, v/v. IR (KBr): ν_{max} 3300-3500 (OH), 1735 (C=O), 1230 (SO_4). NMR (CD_3OD): δ 0.96 (3H, s, 18-CH_3), 4.36 (1H, m, 16β-H), 6.97-7.33 (3H, m, aromatic protons). $[\alpha]_D^{22}$ + 4.8 (c= 0.2, MeOH).

Anal. Calcd. for $C_{18}H_{21}O_6SNa \cdot H_2O$: C, 53.20; H, 5.70; S, 7.88.
Found C, 53.40; H, 5.70; S, 7.74.

Sodium 16α,17β-dihydroxy-1,3,5(10)-estratrien-3-yl sulfate (5). A

solution of 4 (95 mg) and $NaBH_4$ (20 mg) in MeOH (12 ml) was allowed to stand at 0° for 30 min. The reaction mixture was poured into ice-cold H_2O (100 ml) and the passed through Amberlite XAD-2 column (2.5 x 30 cm). After washing with H_2O (200 ml), the adsorbed steroids were eluted with MeOH (300 ml). The steroid fraction was condensed and then purified by preparative TLC ($CHCl_3$/ MeOH/ NH_4OH= 15/ 5/ 1, v/v). The area corresponding to 5 (Rf= 0.31) was scrapped off and then eluted with MeOH and recrystallized from the same solvent to give 5 (45 mg, 47%) as colorless needles, mp 255-260° (lit. [18] 212-218°. IR (KBr): ν_{max} 3100-3400 (OH), 1230 (SO_4). NMR (CD_3OD): δ 0.83 (3H, s, 18-CH_3), 3.56 (1H, d, J= 5 Hz, 17α-H), 4.50 (1H, m, 16β-H), 6.87-7.36 (3H, m, aromatic protons). $[\alpha]_D^{22}$ + 26.6 (c= 0.15, 80% MeOH)

Anal. Calcd. for $C_{18}H_{23}O_6SNa \cdot H_2O$: C, 52.93; H, 5.68; S, 7.85.
Found C, 53.15; H, 5.55; S, 7.80.

Sodium 16-oxo-17β-hydroxy-1,3,5(10)-estratrien-3-yl sulfate (6).
(A) The reaction mixture of the sulfation of the bromoketone 1 (56 mg) was poured into 0.05 NaOH solution (50 ml) and allowed to stand at room temperature for 12 hr. The ketol sulfate 6 was isolated by means of Amberlite XAD-2 as above. Recrystallization from MeOH gave 6 (34 mg, 62%) as colorless needles, mp >290°. IR (KBr): ν_{max} 3400 (OH), 1735 (C=O), 1220 (SO_4). NMR (CD_3OD): δ 0.78 (3H, s, 18-CH_3), 3,97 (1H, s, 17α-H), 6,97-7,32 (3H, m, aromatic protons). $[\alpha]_D^{22}$ -38.9 (c=0.08, 80% MeOH).

Anal. Calcd. for $C_{18}H_{21}O_6SNa \cdot H_2O$: C, 53.20; H, 5.70; S, 7.88.
Found C, 52.96; H, 5.44; S, 7.95.

(B) The sulfate 4 (50 mg) was dissolved in 50% aqueous MeOH (30 ml). One ml of 0,5N NaOH solution was added to this solution and the mixture was allowed to stand at room temperature for 12 hr. The rearranged product was isolated by means of Amberlite XAD-2 and recrystallized from MeOH to give 6 (38 mg, 76%) as colorless needles, mp >290°. The sulfate 6 obtained by the method B was identified by the IR and NMR spectra.

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REFERENCES

The following trivial names have been used in this paper:

Estrone= 3-hydroxy-1,3,5(10)-estratrien-17-one
16α-Hydroxyestrone= 3,16α-dihydroxy-1,3,5(10)-estratrien-17-one
16α-Bromoestrone= 16α-bromo-3-hydroxy-1,3,5(10)-estratrien-17-one
2,16α-Dibromoestrone= 2,16α-dibromo-3-hydroxy-1,3,5(10)-estratrien-17-one
4,16α-Dibromoestrone= 4,16α-dibromo-3-hydroxy-1,3,5(10)-estratrien-17-one
16-Oxoestradiol= 3,17β-dihydroxy-1,3,5(10)-estratrien-16-one

Estriol= 1,3,5(10)-estratriene-3,16α,17β-triol

1. Fishman, J., Guzik, H. and Dixon, D., BIOCHEMISTRY, 8, 4304 (1969).
2. Musey, P. I., Wright, K., Preedy, J. R. K. and Collins, D.C., In "STEROID BIOCHEMISTRY", Vol. II, 83; ed. by Hobkirk, R., CRC Press, Boca Raton (1979).
3. Smith, O. W. and Hagerman, D. D., J. CLIN, ENDOCRINOL. METAB., 25, 732 (1965).
4. Brown, J. B. and Smyth, B. J., REPROD. FERTIL., 24, 142 (1971).
5. Levitz, M., Kander, S. and Young, B. K., STEROIDS, 27, 287 (1976).
6. Clark, J. H., Paszko, Z. and Peck, E. J., ENDOCRINOLOGY, 100, 91, (1977).
7. Adlercreutz, H., Ervast, H.-S., Tenhunen, A. and Tikkanen, M. J., ACTA ENDOCRINOL., 73, 543 (1973).
8. Adlercreutz, H., Tikkanen, M. J. and Hunneman, J., J. STEROID BIOCHEM, 5, 211 (1974).
9. Fishman, J. and Martucci, C., J. CLIN. ENDOCRINOL. METAB., 51, 611 (1980).
10. Fex, H., Lundvall, K. E. and Olsson, A., ACTA CHEM. SCAND., 22, 254 (1968).
11. Numazawa, M. and Osawa, Y., J. AM. CHEM. SOC., 102, 5402 (1980).
12. Numazawa, M. and Osawa, Y., STEROIDS, accepted for publication.
13. Glazier, E. R., J. ORG. CHEM., 27, 4397 (1962).
14. Numazawa, M. and Osawa, Y., STEROIDS, 32, 516 (1978).
15. Fishman, J. and Biggerstaff, W. R., J. ORG. CHEM., 23, 1190 (1958).
16. Numazawa, M., Nagaoka, M., Tsuji, M. and Osawa, Y., J. CHEM. SOC. CHEM. COMM., 1981, 383.
17. Biggerstaff, W. R. and Gallagher, T. F., J. ORG. CHEM., 22, 1220 (1957).
18. Hadd, H. R. and Blickenstaff, R. T., In "CONJUGATES OF STEROID HORMONES", 63, Academic Press, New York (1969).