Electrochemical A-Ring Bromination of Estrogens

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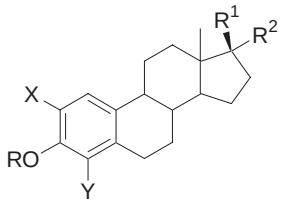
A-ring bromination of estrogens has been achieved by constant current electrolysis of the solutions of these substrates and Et_4NBr in appropriate solvents. Thus, electrolysis consuming $2\,F\,mol^{-1}$ charge gave mixtures of 2- and 4-estrogens (1:1.1–2.5; up to 97%), whereas $4\,F\,mol^{-1}$ charge experiments yielded 2,4-dibromoestrogens as the sole products.

One of the major metabolism pathways of estrogens, which are important mammalian hormones, is A-ring functionalization[1] to give products, which are themselves active compounds, characterized by different biological properties.[2–5] A-ring halogenated estrogens, which are not naturally occurring compounds, have been used in a variety of medical applications, such as diagnostic radio-imaging agents,[6] inhibitors of steroid metabolizing enzymes,[7] and metabolic probes of estrogen carcinogenesis.[8,9] From a synthetic point of view, these compounds are interesting because they are good starting materials for the synthesis of the other A ring mono- and disubstituted derivatives. Thus, deuterium-labeled[10–12] and tritiated estrogens[13] have been prepared starting from the corresponding brominated compounds. Naturally occurring A-ring-oxygenated compounds, which are metabolites of estrogens, have been synthesized by halogen–oxygen exchange reactions,[14–18] while the bromo–nitrogen exchange reaction had been used to synthesize 2-aminoestrogens.[19] Therefore, there is strong interest in the synthesis of A-ring-brominated estrogens.

The first well documented direct bromination of an estrogen has been reported by Woodward,[20] who obtained 2,4-dibromo-α-estradiol in a 68% yield by treating α-estradiol with *N*-bromoacetamide (NBA) in ethanol (EtOH). A better yield has been achieved in a similar dibromination of estriol by using *N*-bromosuccinimide (NBS).[12] Selective bromination of estrogens at the 2- or 4-position and dibromination with elemental bromine in acetic acid (AcOH) has been reported in 1962.[21] However, it has not been confirmed in other laboratories.[22] Wilbur and O'Brien[23] have tested several systems for bromination of estradiol, such as NCS/NaBr/EtOH, NCS/LiBr/THF, NBA/EtOH, NBS/EtOH, pyridinium bromide perbromide (PBPB)/EtOH, PBPB/THF, PBPB/AcOH, and Br_2/AcOH. Their investigations have shown that brominations with one equivalent of NCS and NBS independent of the solvent used give 2- and 4-bromo derivatives in a similar ratio (1:2.38–2.80), along with 2,4-dibromide. However, this ratio dramatically changes if Br_2 or PBPB in AcOH are used for bromination (1:1.27 and 1:0.85, respectively). All later reports concerning this reaction mainly confirm these results.[15,17,18,24,25]



1a (R = R^2 = X = Y = H; R^1 = OH)
1b (R = X = Y = H; R^1, R^2 = O)
2a (R = R^2 = Y = H; R^1 = OH; X = Br)
2b (R = Y = H; R^1, R^2 = O; X = Br)
3a (R = R^2 = X = H; R^1 = OH; Y = Br)
3b (R = X = H; R^1, R^2 = O; Y = Br)
4a (R = Ac; R^1 = OAc; R^2 = Y = H; X = Br)
5a (R = Ac; R^1 = OAc; R^2 = X = H; Y = Br)
6a (R = R^2 = H; R^1 = OH; X = Y = Br)
6b (R = H; R^1, R^2 = O; X = Y = Br)

Scheme 1.

In continuation of our investigations of electrochemically generated agents and electrochemical halogenation of steroids,[26,27] we decided to subject β-estradiol (**1a**) and estrone (**1b**) to reaction conditions, in which bromine is generated at the anode. This technique has already been used in the bromination of organic compounds,[28] among which phenols have been used as substrates.[29] Although oxidation potentials of phenols strongly depend on the number and types of substituents,[30] the oxidation of bromides is possible using potential uncontrolled electrolysis.[29,30] Our focus has been to examine whether this methodology is applicable to the synthesis of brominated estrogens.

Bearing in mind the reaction conditions of classical brominations of estrogens with bromine described in the literature,[20,22] we started with constant current electrolysis (20 mA) of the substrate **1a** in a $0.05\,mol\,L^{-1}$ glacial acetic acid solution of tetraethylammonium bromide. When this electrolysis was performed in an undivided electrolytic cell using a platinum anode and a graphite cathode, consuming $2\,F\,mol^{-1}$ charge, which generates one equivalent of bromine, a complex mixture of products was obtained. On the basis of the position of aromatic protons signals in ^1H NMR spectra, this mixture contained unconsumed **1a**, both 2- (**2a**) and 4-bromoestradiol (**3a**) (Scheme 1), and some unknown products. Approximate calculations from the ^1H NMR spectrum showed that more than 50% of **1a** remained. That prompted us to perform an experiment with $4\,F\,mol^{-1}$ charge consumption. However, even then considerable amounts of **1a** remained.

In the next experiment substrate **1a** was electrolyzed under the same conditions, but in a divided electrolytic cell with a ceramic membrane with $2\,F\,mol^{-1}$ charge consumption. In the ^1H NMR spectrum of the obtained crude reaction mixture, compounds **2a** and **3a** and only negligible amounts of **1a** were observed. Since monobromo derivatives have very similar R_f values in many solvents and in mixtures, the mixture was treated with acetyl chloride and pyridine, and the obtained diacetates (**4a** and **5a**) were separated by column chromatography (SiO_2/ethyl acetate–petrol ether 99:1). After hydrolysis, **2a** and **3a** were obtained as pure compounds in the ratio of

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Table 1. Bromination of Estrogens

Substrate	Solvent	Method[a)]	Products	Yield /%	Ratio (**2**:**3**)
1a	AcOH	A	**2a** + **3a**	94	1:1.16[b)]
		B	**6a**	95	
	DMSO	A	**2a** + **3a**	97	1:2.15[b)]
		B	**6a**	92	
1b	AcOH	A	**2b** + **3b**	93	1:1.09[c)]
		B	**6b**	90	
		C	**2b** + **3b**	87	1:1.20[c)]
		D	**2b** + **3b**	91	1:1.18[c)]
	DMSO	A	**2b** + **3b**	96	1:2.50[c)]
		B	**6b**	91	
		C	**2b** + **3b**	90	1:2.30[c)]
		D	**2b** + **3b**	96	1:2.60[c)]

a) See experimental. b) Based on the isolated bromides after acetylation/hydrolysis procedure. c) Estimated from ^{1}H NMR spectra of the mixture.

1:1.16 (94%), respectively.

On the other hand, when electrolysis was carried out under the same experimental conditions, but with 4 F mol^{-1} charge consumption, 2,4-dibromoestradiol (**6a**, Scheme 1) was obtained as the sole product (95%), which could be identified by ^{1}H and ^{13}C NMR spectroscopies.

Similar results were obtained with estrone (**1b**), as shown in Table 1 and Scheme 1. However, in this case, regioisomers **2b** and **3b** could not been separated, even after acetylation.

Since in classical bromination of estrogens with elemental bromine,[20,22] the solvent affects the distribution of 2- and 4-bromo products, several additional experiments were carried out using different solvents. The addition 5–10% of water or 20–30% of acetic anhydride to acetic acid did not affect either the overall yield or the ratio of **2** and **3** in the 2 F mol^{-1} charge consumption experiments. Electrolysis in dichloromethane also gave similar results. However, the use of dimethyl sulfoxide as the solvent significantly changed the ratio of the 2- and 4-bromo isomers, although the overall yield remained almost the same (see Table 1). This solvent effect is not specific for the electrochemical reaction, and it has been found in chemical bromination of estrone with elemental bromine using the same solvents with or without Et_4NBr (see Table 1).

In conclusion, we have shown that β-estradiol and estrone can be smoothly brominated by anodically generated bromine from tetraethylammonium bromide dissolved in acetic acid or DMSO, in a divided electrolytic cell. Controlling the charge consumption, this method allows for the preparation of the corresponding 2- and 4-monobromo- and 2,4-dibromoestrogens in high yields. The regiochemistry of the monobrominated product depended on the solvent used. The process compares favorably in yield and ease of operation with classic bromination reactions but avoids the use of hazardous brominating reagents. Since the necessary equipment is very simple, inexpensive, and readily available, this methodology should replace classical ones.

Experimental

General. All chemicals were commercially available and used as received, except for the solvents which were purified by distillation. A Uniwatt Beha Labor-Netzgerät (NG 394) was used as a direct current source for the electrolysis. A cylindrical glass vessel equipped with a magnetic stirrer, a cylindrical platinum foil as the anode ($\phi = 2.5$ cm), a ceramic tube as the membrane ($\phi = 1.5$ cm), and a graphite stick as the cathode ($\phi = 0.5$ cm) was assembled as the divided electrochemical cell. NMR spectra were recorded on a Varian Gemini (200 MHz) spectrometer, using $(CD_3)_2SO$ as the solvent. Chemical shifts are expressed in δ (ppm). IR measurements were carried out with a Perkin-Elmer 457 grating FT instrument in KBr tablets. For TLC, silica gel 60 on Al plates, layer thickness 0.2 mm (Merck), was used.

General Procedure for Electrochemical Bromination, Methods A and B. Substrate **1a** or **1b** (100 mg, ≈0.37 mmol) and a 0.05 M solution of Et_4NBr in the corresponding solvent (20 mL) were placed in the anodic compartment of the cell (outside the ceramic tube). The same solution of Et_4NBr (2.5 mL) was used as the catholyte. Constant current electrolysis (20 mA) was stopped after 60 (method A) or 120 min (method B) in order to provide 2 or 4 F mol^{-1} charge. If acetic acid was used the solvent was removed by evaporation, H_2O (20 mL) was added to the residue, and the mixture was extracted with three portions of ether (3 × 30 mL). The organic layers were first collected and washed with a solution of $NaHSO_3$ (10%, 40 mL) to remove residual amounts of bromine, then with saturated $NaHCO_3$ (40 mL), brine (40 mL), and H_2O (40 mL). After drying over anhydrous Na_2SO_4 overnight, the solvent was evaporated, and the crude reaction mixture was subjected to column chromatography on SiO_2 (5–10 g). The elution (dichloromethane) was monitored by TLC. Monobromides **2** and **3** were eluted as one fraction, whereas dibromides **6a** and **6b** were isolated as the pure compounds. In the case of substrate **1a** the two isomeric monobromides were separated through an acetylation/hydrolysis procedure (see below).

If DMSO was used as the solvent, the mixture was diluted after electrolysis with water and extracted with Et_2O, then worked up as it is described above.

General Procedure for Chemical Bromination, Methods C and D. To a solution of Br_2 (60 mg, 0.37 mmol) in 20 mL of the corresponding solvent **1b** (100 mg, 0.37 mmol) was added, and the mixture was stirred 2 h. Work up was the same as previous experiments (method C). For method D, Et_4NBr (210 mg) was added.

Acetylation/Hydrolysis Procedure for Separation of Monobromoestradiols. To a dichloromethane solution of crude reaction mixture obtained after the 2 F mol^{-1} charge consumption electrolysis of β-estradiol (≈130 mg/10 mL), acetyl chloride (0.1 mL) and pyridine (0.1 mL) were added, and the resulting mixture was stirred vigorously overnights with a magnetic stirrer. The mixture was diluted with dichloromethane (20 mL), washed with water (30 mL), 1 mol L^{-1} HCl, saturated $NaHCO_3$, brine and water subsequently and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the mixture was separated by column chromatography (SiO_2/petrol ether–ethyl acetate 99:1) to give pure **4a** and **5a**. The separated diacetates were dissolved in 10 mL of 0.5 mol L^{-1} KOH in methanol and the obtained solution was refluxed 2 h. After evaporation of the methanol, to the resulting residue was added 20 mL of water, and the mixture extracted with ether (3 × 20 mL). Evaporation of the solvent gave pure compounds **2a** and **3a**.

2-Bromo-β-estradiol (2a): White solid; mp 189–192 °C (lit.: 197–198,[22] 191–193,[23] 200–207 °C[31]); IR (cm^{-1}): 3248, 2955, 1604, 1500, 1414, 1342, 1255, 1053, 1026, 1008, 884, 730; ^{1}H NMR: δ 0.65 (s, 3H, 18-Me), 4.50 (d, $J = 4.7$ Hz, 1H, C17H),

6.62 and 7.26 (two s, 2H, C1 and C4), 9.81 (s, 3-OH); ^{13}C NMR: δ 11.4, 23.0, 26.2, 26.9, 28.8, 30.1, 36.7, 39.5, 43.0, 43.4, 49.6, 80.2, 106.6, 116.3, 129.5, 132.9, 137.0, 151.7.

2- and 4-Bromoestrone (2b and 3b): ^{1}H NMR: δ 0.80 (s, 3H, 18-Me of both isomers), 6.64 and 7.27 (two s, 2H, C1H and C4H of **2a**), 6.75 and 7.10 (two d, $J = 8.5$ Hz, 2H, C1H and C2H of **3a**), 9.55 (s, 1H, OH of **2a**), 9.97 (s, 1H, OH of **3a**).

4-Bromo-β-estradiol (3a): White solid; mp 205–207 °C (lit.: 213–215,[22] 207–208.5,[23] 209–211,[31] 209–212 °C[32]); IR (cm^{-1}): 3399, 3153, 2924, 2854, 1467, 1378, 1292, 976, 790; ^{1}H NMR: δ 0.64 (s, 3H, 18-Me), 4.50 (d, $J = 4.7$ Hz, 1H, C17H), 6.74 and 7.08 (two d, $J = 8.4$ Hz, 2H, C1H and C2H), 9.83 (s, 1H, 3-OH); ^{13}C NMR: δ 11.4, 22.9, 26.4, 27.2, 30.1, 31.0, 36.7, 37.8, 42.9, 43.9, 49.6, 80.2, 112.7, 113.3, 125.1, 132.9, 136.5, 152.0.

2-Bromo-3,17β-estradiol Diacetate (4a): White solid; mp 165–168 °C (lit.: 166–168 °C[22]); IR (cm^{-1}): 3067, 2964, 2927, 1770, 1728, 1486, 1375, 1255, 1197, 1042, 910; ^{1}H NMR: δ 0.78 (s, 3H, 18-Me), 2.00 (s, 3H, COMe), 2.28 (s, 3H, COMe), 4.60 (t, $J = 8.1$ Hz, 1H, C17H), 6.96 and 7.49 (two s, 2H, C1H and C4H); ^{13}C NMR: δ 12.1, 20.8, 21.1, 23.0, 25.8, 26.4, 27.4, 28.6, 36.6, 37.7, 42.7, 43.4, 49.2, 82.0, 112.5, 124.1, 129.8, 138.0, 140.2, 145.6, 168.8, 170.7.

4-Bromo-3,17β-estradiol Diacetate (5a): White solid; mp 168–171 °C (lit.: 175.5–177.5,[22] 171–173 °C[32]); IR (cm^{-1}): 2968, 2930, 2874, 2852, 1769, 1721, 1470, 1370, 1261, 1197, 1043, 1019; ^{1}H NMR: δ 0.77 (s, 3H, 18-Me), 2.01 (s, 3H, COMe), 2.29 (s, 3H, COMe), 4.62 (t, $J = 8.0$ Hz, 1H, C17H), 7.04 and 7.37 (two d, $J = 8.4$ Hz, 2H, C1H and C2H); ^{13}C NMR: δ 12.1, 20.1, 21.1, 23.0, 26.1, 26.8, 27.4, 29.2, 30.9, 36.6, 37.1, 37.7, 42.6, 43.8, 49.2, 82.1, 118.8, 120.9, 125.8, 137.4, 140.3, 146.1, 168.8, 170.7.

2,4-Dibromo-β-estradiol (6a): White solid; mp 219–222 °C (lit.: 223–226,[22] 225–226,[23] 220–222 °C[33]); IR (cm^{-1}): 3585, 3293, 1542, 1464, 1269, 1180, 1014, 761; ^{1}H NMR: δ 0.62 (s, 3H, 18-Me), 4.52 (d, $J = 4.7$ Hz, 1H, C17H), 7.37 (s, 1H, C1H), 9.50 (s, 1H, 3-OH); ^{13}C NMR: δ 11.3, 22.9, 26.3, 27.0, 30.1, 31.2, 36.6, 37.6, 42.8, 43.5, 49.5, 80.1, 108.8, 116.6, 128.5, 135.5, 136.5, 148.5.

2,4-Dibromoestrone (6b): White solid; mp 224–226 °C (lit.: 220–226,[33] 235–237 °C[34]); IR (cm^{-1}): 3270, 2937, 2869, 1723, 1545, 1464, 1275, 1171, 762; ^{1}H NMR: δ 0.79 (s, 1H, 18-Me), 7.40 (s, 1H, C1H), 9.53 (s, 1H, OH); ^{13}C NMR: δ 13.6, 21.2, 25.6, 26.1, 31.0, 31.3, 35.5, 36.8, 43.4, 47.3, 49.5, 108.9, 115.6, 128.6, 134.9, 135.4, 148.6, 219.6.

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References

1 J. Fishman, *Endocr. Metab.* **1963**, *23*, 207.

2 E. L. Cavalieri, D. E. Stack, P. D. Devanesan, R. Todorovic, I. Dwivedy, S. Higginbotham, S. L. Johansson, K. D. Patil, M. L. Gross, J. K. Gooden, R. Ramanathan, R. L. Cerny, E. G. Rogan, *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 10937.

3 J. G. Liehr, *Endocr. Rev.* **2000**, *21*, 40.

4 P. Ball, R. Knuppen, *Acta Endocrinol., Suppl.* **1980**, *232*, 1.

5 N. J. Macluskey, F. Naftolin, L. C. Krey, S. Franks, *J. Steroid Biochem.* **1981**, *15*, 111.

6 J. A. Spicer, D. F. Preston, R. J. Baranczuk, E. Harvey, M. M. Guffey, D. L. Bradshaw, R. G. Robinson, *J. Nucl. Med.* **1979**, *20*, 761.

7 M. Numazawa, S. Satoh, *J. Steroid Biochem.* **1989**, *33*, 111.

8 J. G. Liehr, *Mol. Phamacol.* **1983**, *23*, 278.

9 J. J. Li, R. H. Purdy, E. H. Appelman, J. K. Klicka, S. A. Li, *Mol. Pharmacol.* **1985**, *27*, 559.

10 J. Pinkus, D. Charles, S. C. Chattoraj, *J. Biol. Chem.* **1971**, *246*, 633.

11 J. Fishman, H. Guzik, L. Hellman, *Biochemistry* **1970**, *9*, 1593.

12 B. H. Albrecht, D. D. Hagerman, *Steroids* **1972**, *19*, 177.

13 M. M. Coombs, H. R. Roderick, *Steroids* **1968**, *11*, 925.

14 P. N. Rao, J. E. Burdett, Jr., *Synthesis* **1977**, 168.

15 X. Zheng, W. Wang, Z. Zhong, Z. Xu, H. Zhao, *Steroids* **1982**, *40*, 121.

16 M. Numazawa, Y. Ogura, K. Kimura, M. Nagaoka, *J. Chem. Res., Synop.* **1985**, 348.

17 D. J. Pert, D. D. Ridley, *Aust. J. Chem.* **1989**, *42*, 421.

18 A. J. Lee, S. J. Walter, W. E. Cotham, T. B. Zhu, *Steroids* **2004**, *69*, 61.

19 M. Numazawa, K. Kimura, *Steroids* **1983**, *41*, 675.

20 R. B. Woodward, *J. Am. Chem. Soc.* **1940**, *62*, 1625.

21 W. R. Slaunwhite, Jr., L. Neely, *J. Org. Chem.* **1962**, *27*, 1749.

22 T. Utne, R. B. Jobson, F. W. Landraf, *J. Org. Chem.* **1968**, *33*, 1654.

23 D. S. Wilbur, H. A. O'Brien, *J. Org. Chem.* **1982**, *47*, 359.

24 Z. Szendi, G. Dombi, I. Vincze, *Steroids* **1991**, *56*, 392.

25 P. C. Bulman Page, F. Hussain, N. M. Bonham, P. Morgan, J. L. Maggs, B. K. Park, *Tetrahedron* **1991**, *47*, 2871.

26 S. Milisavljević, R. D. Vukićević, *J. Serb. Chem. Soc.* **2004**, *69*, 941.

27 S. S. Milisavljević, K. Wurst, G. Laus, M. D. Vukićević, R. D. Vukićević, *Steroids* **2005**, *70*, 867.

28 *Organic Electrochemistry an Introduction and Guide*, 3rd ed., ed. by H. Lund, M. M. Baizer, Marcel Dekker, New Yurk, **1991**.

29 T. Bejerano, E. Gileadi, *Electrochim. Acta* **1976**, *21*, 231.

30 M. M. Ngundi, O. A. Sadik, T. Yamaguchi, S. Suye, *Electrochem. Commun.* **2003**, *5*, 61, and literature cited there.

31 A. J. Lee, J. W. Sowell, W. E. Cotham, B. T. Zhu, *Steroids* **2004**, *69*, 61.

32 A. D. Cross, E. Denot, R. Acevedo, R. Urquiza, A. Bowers, *J. Org. Chem.* **1964**, *29*, 2195.

33 M. Cushman, H.-M. He, J. A. Katzenellenbogen, R. K. Varma, E. Hamel, C. M. Lin, S. Ram, Y. P. Sachdeva, *J. Med. Chem.* **1997**, *40*, 2323.

34 E. Schwenk, C. G. Castle, E. Joachim, *J. Org. Chem.* **1963**, *28*, 136.