RADIOLABELING OF THE B- AND C-RING OF ESTRADIOL USING NO-CARRIER-ADDED BROMINE-77

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SUMMARY

Two estradiol derivatives, Δ^6- and Δ^9-estradiol 3-acetate (1) and (2) were prepared and allowed to react with $Na^{77}Br$ and N-chlorosuccinimide (NCS) in methanol. The rapid addition of ^{77}Br-OCH_3 to the B-ring ($\Delta^{6,7}$) and C-ring ($\Delta^{9,11}$) double bonds produced brominated adducts 3a and 4b, respectively, in excellent radiochemical yields. Product 3a showed excellent radiochemical stability over a 3-day period; however, attempts to prepare the free phenol 5 by removal of the 3-acetyl group with base, resulted in loss of all activity from the molecule. Contrary to this, the the 3-acetyl group of 4b was readily removed with base without loss of radioactivity and the resultant phenol 6 could be isolated in 73% radiochemical yield. Unfortunately 6 was moderately unstable since 80% of the activity was dissociated from the compound within 48 hours.

Key Words: bromine-77, B- and C-radiobrominated estradiol, Δ^6- and Δ^9-estradiol, radiolabeling.

INTRODUCTION

Radiohalogenated estrogens are of interest in nuclear medicine since they have the potential of being used to detect and/or determine the course of therapy of hormone dependent tumors (1). Subsequently, there has been a large effort in the design and synthesis of these compounds (2-4). Important factors in the design of new radiolabeled compounds include high specific activity, good

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in vivo stability and high target specificity.

The primary emphasis in the area has involved the preparation of A- and D-ring radiohalogenated estrogens. Iodination with stable iodine in the 6- and 7-positions (B-ring) have been reported (5), and 6-[^{125}I]iodo-$\Delta^{6,7}$-estradiol 3-acetate has been prepared (6). To our knowledge, this is the only radiohalogenation involving the B- or C-ring reported to date. The object of this investigation was to prepare B- and C-ring radiobrominated estradiol derivatives and to investigate the radiochemical stability of the obtained compounds.

DISCUSSION

The preparation of the electrophilic brominating agent, BrCl, from NCS and NaBr, followed by addition of this reactive species to a double bond has been previously reported (7). Preliminary reactions of 1 and 2 were performed with NaBr and NCS in methanol, effectively adding "Br-OCH_3" to the nonaromatic double bond. Treatment of 1 with in situ generated Br-OCH_3 in the described manner, yielded the product 3 in 80% yield. Since a bromonium ion would be a conceivable intermediate and attack by -OMe would be favored at the benzylic 6-position, it is likely that the bromine atom is attached to carbon-7 of the product rather than carbon-6. The stereochemistry at those positions has not been confirmed.

Treatment of 1 with no-carrier-added (nca) Na^{77}Br/NCS in methanol generated 3a in 80% radiochemical yield, with an estimated specific activity of 500-1000 Ci/mmol. Millicurie quantities of 3a could be isolated by HPLC and less than 5% radiochemical decomposition had occurred over a 3-day period (8).

All attempts to remove the acetyl group of 3a under basic conditions resulted in immediate loss of radiobromine from the molecule, presumably through loss of H^{77}Br.

When 2 was allowed to react with NaBr/NCS in methanol under similar conditions to those used for 1, the brominated addition product 4 was obtained. We were unable to characterize the product since upon isolation, rapid decomposition was observed. When the acetyl group of 2 was replaced by a 3-benzyloxy

as in 2a, the corresponding brominated adduct 4a was readily obtained and sufficiently stable to allow characterization (9). Based on previous studies, it is evident that the bromine is attached to carbon-11 (10). The stereochemistry has not been unambiguously determined; however, by comparison with analog examples, it is likely that 11β-bromo-9α-methoxy epimer of 4a is the major product.

Reaction of 2 with nca $Na^{77}Br$/NCS generated 4b in over 90% radiochemical yield. Subsequent removal of the acetyl group from the adduct 4b under basic conditions yielded 6 in 60-70% overall radiochemical yield from 2. Following purification by HPLC, from 10 mCi of $Na^{77}Br$, up to 7 mCi of 6 could be readily obtained. Stability tests showed that the compound was not exceedingly stable in acetonitrile-water since 80% of the activity was dissociated from 6 within 48 h. In conclusion, the brominated adducts of 2 (R=Ac) were unstable, thus, chemical characterization was not possible. The proposed structures of 4, 4b, and 6 are based on comparisons made with the synthesized analog 4a (9).

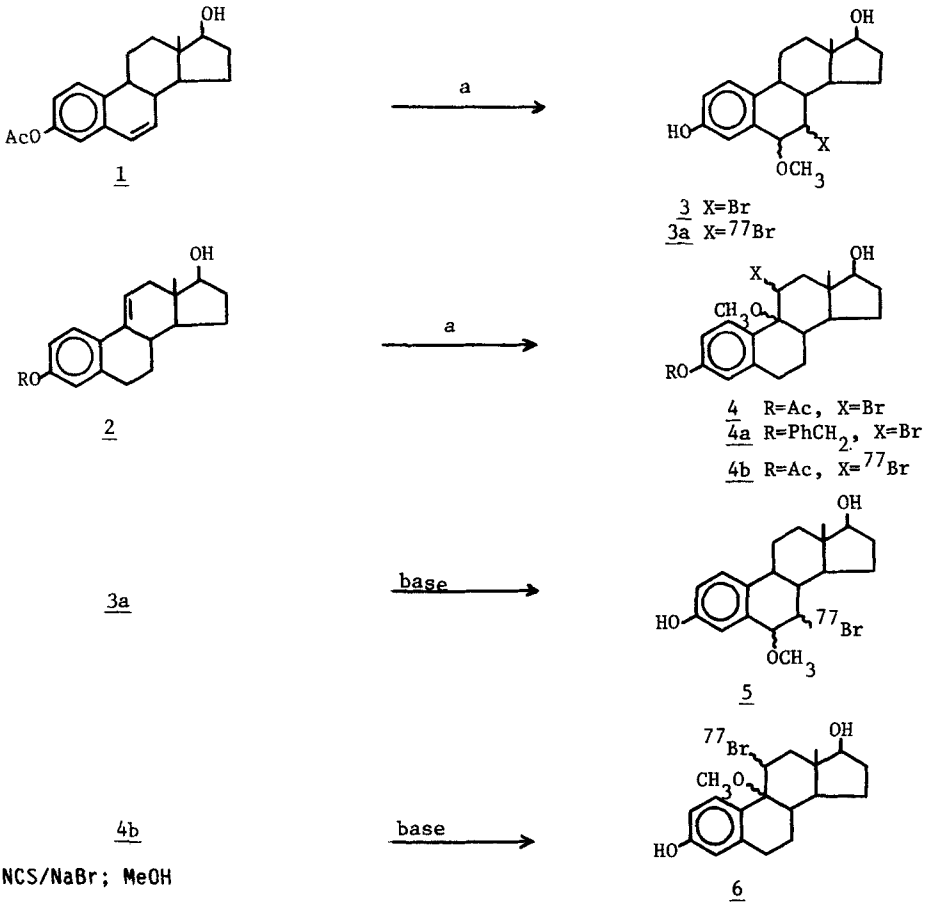
EXPERIMENTAL

Materials and Methods.

Melting points were obtained on an Electrothermal melting point apparatus and are uncorrected. Proton NMR were recorded at 60 MHz on an Hitachi Perkin-Elmer R-24B instrument and are referenced to TMS as an internal standard. Carbon NMR were obtained on a Varian FT-80 spectrometer and chemical shifts obtained are referenced to the center peak of the deuterated solvent used. Mass spectra were obtained on a Finnigan Model 4510 using a solid probe insert.

Product purity and reaction progress were detected by analytical thin-layer chromatography using Baker plates coated with silica gel Gf or by high performance liquid chromatography (HPLC). The Spectra Physics 8700 HPLC used is equipped with a UV detector at 254 nm, Waters Z-module equipped with a C_{18} reversed phase radial compression column, and a Canberra NIM BIN module with counter and NaI crystal detector in sequence with the UV detector. Acetonitrile-water mixtures were generally used as the mobile phase.

SCHEME I



a) NCS/NaBr; MeOH

Medium pressure liquid chromatography (MPLC) was performed at 80 psi using a Fluid Metering pump, 9 mm x 1000 mm Altex glass column an Woelm 32-64 micro-silica gel as the stationary phase. Ethyl acetate-toluene mixtures were used as the eluting solvents. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride under N_2 prior to use. NaBr and NCS were obtained from Aldrich and finely pulverized prior to use. Methanol was used directly as obtained from Burdick and Jackson. Steroidal precursors were used as obtained from Sigma.

7-[^{77}Br]bromo-6-Methoxyestradiol 3-Acetate (3a). To a vial containing 2.44 mCi of dry Na^{77}Br in 30 µL of methanol, was added 15 µL of a methanolic solution of NCS (2 mg/mL). After 5 min, 30 µL of a methanolic solution of 1 (1 mg/mL) was added. The reaction mixture was allowed to stir at 25°C for

15 min at which time 25 µL (0.83 mCi) of the reaction mixture was injected on HPLC (45/55 H_2O/CH_3CN, flow rate = 1.5 mL/min). The labeled material was collected at a retention time of 8.0 min which was identical to that of unlabeled material. A radiochemical efficiency (11) of 90% (0.75 mCi) was obtained.

11-[^{77}Br]bromo-9-methoxyestradiol 3-Acetate (4b) and 11-[^{77}Br]bromo-9-methoxyestradiol (6). To a vial containing 10.08 mCi of dry $Na^{77}Br$ in 10 µL of methanol was added 30 µL of a methanolic solution of NCS (2 mg/mL). After 5 min 30 µL of a methanolic solution of 2 (1 mg/mL) was added. Reaction progress was checked after 20 min by HPLC (55/45 H_2O/CH_3CN, flow = 2.0 mL/min) and showed 90% radiochemical conversion to 4b.

Twenty minutes after the addition of 5 µL of 1 N NaOH in methanol to the initial reaction mixture, 8.43 mCi of the mixture was injected on HPLC (60/40 H_2O/CH_3CN, flow = 2.0 mL/min. The desired product 6 (6.15 mCi) was isolated at a retention time of 15.0 min in an overall radiochemical yield of 73%.

$\Delta^{6,7}$-Estradiol 3-Acetate (1). To a solution of 87 mg (0.32 mmol) of 6-dehydroestradiol (12) in 10 mL of dry THF, was added 77 mg (3.2 mmol) of NaH. The resulting suspension was allowed to stir at room temperature and under a N_2 atmosphere for 4 h, after which, 120 µL (1.28 mmol) of Ac_2O was added. The reaction mixture was then allowed to stir at room temperature for an additional 12 h. Hydrolysis was carried out by pouring the reaction mixture into 20 mL of 2% HCl solution. The resulting cloudy mixture was then extracted with ether (2x15 mL), the organic layer was washed with water (2x15 mL), dried over anhydrous $MgSO_4$, filtered and evaporated at reduced pressure to give a yellow oil. This oil was purified by MPLC, using 15% ethyl acetate in toluene as the elutant. After collection and evaporation of the solvents, 73 mg (73% yield) of colorless crystals, mp 125-125.5°C (reported (13) 126-127°C), was obtained.

TLC: R_f = 0.27 (25% ethyl acetate in toluene) ^{1}H NMR ($CDCl_3$): δ 7.20-6.80 (m, 3H); 6.62-6.25 (d, 1H, J=11 Hz); 5.99-5.82 (d, 1H, J=11 Hz); 3.80-1.33 (m, 16H); 0.79 (s, 3H).

^{13}C NMR ($CDCl_3$): δ 169.31, 149.23, 136.51, 135.55, 133.05, 127.34, 124.17,

119.42, 118.66, 81.50, 48.42, 43.77, 42.28, 38.39, 36.17, 30.46, 24.02, 22.97, 20.87, 10.68.

7-Bromo-6-methoxyestradiol 3-Acetate (3). To a suspension of 33 mg (0.25 mmol) of N-chlorosuccinimide (NCS) in 4.5 mL of dry methanol, under a N_2 was added 26 mg (0.25 mmol) of NaBr. After 15 min a yellow homogeneous atmosphere, solution was obtained to which was added a solution of 70 mg (0.22 mmol) of 1 in 3 mL of methanol. The resultant reaction mixture was allowed to stir at room temperature for 45 min. The methanol was evaporated under reduced pressure and the obtained semi-solid was dissolved in 20 mL of ethyl acetate. This solution was washed with water (2x15 mL), dried over anhydrous $MgSO_4$, filtered and the solvent removed under reduced pressure to afford 80 mg of colorless oil. Purification was performed by means of a MPLC system using 10% ethyl acetate in toluene as the elutant. After collection of the proper fractions and evaporation of the solvents, 63 mg (68% yield) of colorless crystals was obtained, mp 105-107°C. An analytical sample was obtained by recrystallization from water-methanol.

TLC: R = 0.26 (25% ethyl acetate in toluene).

^{1}H NMR ($CDCl_3$): δ 78.50-7.03 (m, 3H); 4.50-1.51 (m, 15H); 3.52 (s, 3H); 0.81 (s, 3H) MS (m/e, relative intensity): 424 (M^++1, 0.65); 422 (M^+-1, 0.56).

Anal. Calcd for $C_{21}H_{27}BrO_4$: C, 59.57; H, 6.38. Found: C, 59.68; H, 6.38.

$\Delta^{9,11}$-Estradiol 3-Acetate (2): In like manner to that described for the synthesis of 1, a solution of 250 mg (0.93 mmol) of $\Delta^{9,11}$-estradiol in 10 mL of THF, was allowed to react with 220 mg (9.3 mmol) of NaH followed by addition of 260 μL (2.8 mmol) of acetic anhydride. After workup and purification, 220 mg (76% yield) of colorless crystals, mp 129-131°C (reported (10) 123-126°C), was obtained.

TLC: R_f = 0.32 (25% ethyl acetate in toluene).

^{1}H NMR ($CDCl_3$): δ 7.65-6.76 (m, 3H); 6.20 (s, 1H); 3.82-1.10 (m, 14H); 2.25 (s, 3H); 0.73 (s, 3H).

$\Delta^{9,11}$-Estradiol 3-Benzyloxy Ether (2a): A mixture of 0.30 g (0.11 mmol) of $\Delta^{9,11}$-estradiol and 0.80 g (6.39 mmol) of potassium carbonate in 11 mL of DMF under a N_2 atmosphere and at room temperature was allowed to stir for 20

min. After that time, 0.28 mL (1.61 mmol) of benzyl bromide was added and the resultant reaction mixture was allowed to stir overnight. The cloudy suspension obtained was filtered under reduced pressure and the residue was washed with 20 mL of ethyl acetate. The filtrate was placed in a separatory funnel and the organic layer was washed with water (2x30 mL), saturated NaCl solution (1x50 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to afford 380 mg of a light yellow oil. This material was purified by means of a MPLC system using 15% ethyl acetate in toluene as the elutant. After collecting the fractions, evaporating the solvents and drying under reduced pressure, 340 mg (94% yield) of a light beige solid, mp 132-134°C, was obtained. An analytical sample, mp 133.5-135°C was obtained by recrystallization from methanol-H_2O.

TLC: R_f = 0.35 (25% ethyl acetate in toluene). ^1H NMR ($CDCl_3$): 7.62-6.70 (m, 8H); 6.13 (s, 1H); 5.02 (s, 2H); 3.79 (s, 1H), 2.90-1.25 (m, 13H); 0.78 (s, 3H).

^{13}C NMR: 157.32, 137.17, 136.93, 134.77, 128.23, 127.53, 127.11, 124.91, 117.36, 114.23, 113.12, 81.55, 69.64, 47.17, 41.28, 38.82, 38.61, 30.43, 29.83, 27.97, 23.70, 10.78.

MS (m/e, relative intensities): 360 (M^+, 20.03).

Anal. Calcd. for $C_{25}H_{28}O_2$: C, 83.29; H, 7.83. Found: C, 83.13; H, 7.63.

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REFERENCES AND NOTES

1. Krohn, K. A., J. Nucl. Med., 21, 593 (1980).

2. Longcope, C., Arunachalam, T. Rafkind, I., and Caspi, E., Steroid Biochem., 14: 261.(1981).

3. Katzenellenlogen, J. A., Herman, D. F., Carlson, K. E., and Lloyd, J. E., In "Receptor-Banding Radiotracers," Eckelman, W. C., Ed., Vol. 1, CRC Press, Boca Raton, Fl, 1982, p.93.

4. Njar, V.C.O., Arunachalam, J., and Caspi, E., J. Org. Chem., 48: 1007 (1983).

5. Arunchalamn, T. Longcope, C., and Caspi, R., J. Biol Chem., 254: 5900 (1979).

6. Longcope, C., Arunachalam, T., Rafkind, I, and Caspi, E., J. Steroid Biochem., 14: 261 (1982).

7. Wilbur, D. C. and Anderson, J. W., J. Org. Chem., 47: 358 (1982).

8. The radiolabeled compound was purified by HPLC (see Experimental). The radiochemical stability was determined by reinjecting a portion of the solution containing the compound into the HPLC at various intervals.

9. In an unoptimized run, a product with a larger R_f value than 2+ was isolated by MPLC. MS showed a M^+=471 which corresponds to the addition product. A peak corresponding to M^+-31 (loss of $-OCH_3$) was also observed. ^1H NMR showed a peak at δ 3.85 which can be assigned as a $-OCH_3$ signal.

10. Tsuda, K., Nopzoe, S., and Okada, Y., Chem. Pharm. Bull., 11: 1022 (1963)

11. Radiochemical efficiency is defined as: $\frac{\text{mCi injected in HPLC}}{\text{mCi isolated from HPLC}} \times 100.$

12. Obtained by reduction ($LiAl(OtBu)_3H$) of commercially available 6-dehydroestrone.

13. Ferrara, G., Boffi, G., Torri, G., and Corbellini, A., Steroids, 8, 111 (1966).