A RAPID SYNTHESIS OF A-RING BROMINE-77-LABELLED ESTROGENS WITH HIGH SPECIFIC ACTIVITY

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SUMMARY

A rapid method of no-carrier-added radiobrominations of A-ring estrogens has been demonstrated. The method employs N-chlorosuccinimide and high specific activity sodium bromide-77 to generate an electrophilic brominating agent, which when reacted with phenolic steroids, yields three primary products. Two of the radiobrominated products have been shown to be the phenolic ortho brominated regioisomers, and the third product is believed to be a reaction intermediate. The use of HPLC afforded a method of separation and isolation of radiobrominated products. The 2-bromo-^{77}Br- and 4-bromo-^{77}Br- isomers were found to be quite stable *in vitro*, whereas the suspected intermediates were not. A specific activity range of 600-1200 Ci/mmole and radiochemical yields of 25-30% were obtained for the 4-bromo-^{77}Br regioisomers.

Key Words: Bromination, Bromine-77, Estrogens, Estradiol, 17α-Ethynylestradiol

INTRODUCTION

Radiolabelling steroids to study their potential applications as diagnostic agents for hormone-associated tumors has received a renewed research interest in the last couple of years (1-9). This resurgence of interest has been brought about, in part, by the findings that good target tissue localization of the radiolabelled steroids is dependent upon having a very high specific activity product (1).

Most early investigations involving radiolabelled steroids were conducted with A-ring radioiodinated estrogens (10-14). Unforunately, the results from many of these studies are fraught with ambiguities because of the low specific activities used, and because of the questionable isomeric and radiochemical purity of the radioiodine labelled estrogens (15).

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This report describes an investigation of radiolabelling in the A-ring of estrogens, specifically estradiol 1 and 17α-ethynylestradiol 2, where the main emphasis of the investigation was directed towards obtaining high specific activity products, pure radiolabelled compounds, and reasonable radiochemical yields. Bromine-77 was used as the radiolabel, instead of iodine-131 or -123, because the aromatic bromine-carbon bond is stronger (16), which should result in the bromine being less labile than iodine, and because the bromine atom, being a less sterically bulky substituent than iodine, should be less likely to affect the receptor binding affinities of the labelled estrogens.

RESULTS AND DISCUSSION

The radiobrominations of estrogens, 1 and 2, were accomplished by the reaction of these compounds with N-chlorosuccinimide (NCS) and no-carrier-added sodium bromide-^{77}Br in absolute ethanol. Under these reaction conditions the bromide ions are oxidized to an electrophilic brominating agent in-situ. This method of radiobromination was chosen because the radioactive bromine-77 is received as bromide, and there are presently no methods of introducing an electrophilic brominating agent in a no-carrier-added state (17) that does not either leave a great deal of activity in the generating flask or use copious quantities of chlorine to carry the radiobromine into the reaction vessel (18). A similar method of oxidizing bromide-77 has been described by Coenen, Machulla, and Stöcklin (19). In their study they used N-chlorotetrafluorosuccinimide (NCTFS) as the oxidizing agent; however, the fact that NCTFS is not commercially available and that its use is restricted to completely anhydrous conditions (dry box, etc.), made this compound unattractive for our investigation.

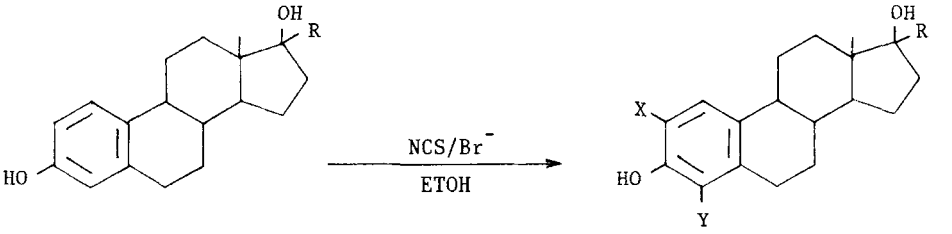
The bromine-77 used in this study was produced at this facility by proton induced spallation reaction on a molybdenum target (20). Inital experiments utilized the bromide-77 as obtained from the processing of the molybdenum target, however, other ions introduced during processing appear to promote

side reactions in the radiobrominations. A more satisfactory bromide-77 sample was obtained by the separation of the bromide ions from other ions with ion chromatography (21).

Reverse-phase high performance liquid chromatography (HPLC) was used to follow the reaction progress of the radiobrominations. Bromide-77 ion was observed to elute with the solvent front which made it possible to follow the reaction progress as a function of percent of radioactivity in the radiochromatographic peaks eluting after the solvent front. Identification of the radioactive peaks that corresponded to the A-ring brominated products was achieved by correlation of the HPLC retention times to the stable brominated compounds.

Prior to the radiobrominations of 1 and 2, these compounds were brominated with stable bromide and NCS. The A-ring brominated isomers of estradiol, 3 and 4, were isolated and fully characterized (22), then compared to those described in the literature (23, 24). The previously unreported A-ring brominated isomers of 17α-ethynylestradiol, 5 and 6, were also fully characterized (22).

The radiobrominations with NCS/$^{77}Br^-$ were found to be very rapid with most (>95%) of the radioactivity being retained in radiochromatographic peaks that



1: R = H
2: R = -C≡CH

3: R = H; X = Br, Y = H
4: R = H; X = H, Y = Br
5: R = -C≡CH; X = Br; Y = H
6: R = -C≡CH; X = H; Y = Br

elute after the solvent front after only 15 minutes reaction time. The radiobrominations yielded nearly a 1:2 ratio of 2-bromo-^{77}Br- to 4-bromo-^{77}Br- isomers, which is the same as observed for the stable brominations (25). However, the activity associated with the A-ring brominated peaks accounted for only about half of the activity in the radiochromatograms (e.g., Figures 1 and 2). In the radiobromination of both 1 and 2, a major peak of activity eluted just prior to the 2-bromo-^{77}Br isomer. Interestingly, the retention time of this radiobrominated species is identical to that observed (by U.V. detection) for a transient species in the corresponding stable brominations. In fact, the percentage of total activity associated with this species is found to decrease if the reaction is allowed to proceed for longer periods of time. For instance, the activity associated with the unidentified peak after 15 minutes reaction time is found to be approximately 50% of total activity, but after 5 hours reaction time has decreased to approximately 25%.

In choosing the reaction time, a compromise must be made between the radiochemical yields and the specific activity. The use of NCS to oxidize the bromide introduces A-ring chlorinated estrogens into the reaction mixture. Fortunately, the A-ring chlorinations are much slower than brominations with less than 1% reaction occuring after 7 hours reaction time. However, even after only 30 minutes reacton time some of the chlorinated side reaction products could be observed by HPLC (U.V. detection). Although the chlorinated products can be separated from the brominated products, minimizing the chlorinated products by minimizing the reaction time makes the separation much easier. The better the separation, the less chance of the radiobrominated products being contaminated by chlorinated species, which ultimately would yield lower then theoretical specific activity.

The *in vitro* stabilities of the high specific activity bromine-77 labelled estrogens, 3-6, were of interest and were, therefore, measured by HPLC as a

function of time. Both the 2-bromo-^{77}Br- isomers, 3 & 5, and the 4-bromo-^{77}Br- isomers, 4 & 6, were found to be quite stable *in vitro* as shown in Table I. Not surprisingly, the unknown radiobrominated species were found to be unstable *in vitro*, decomposing to yield a new radiochromatographic peak near the solvent front as well as considerable activity in the solvent front (e.g., Figure 3).

The 4-bromo-^{77}Br- isomers, 4 & 6, were the compounds of most interest for *in vivo* studies because the receptor binding affinity of the 4-bromo- isomer of 1 had been found to be much higher than the 2-bromo- isomer (26). Therefore, the specific activities and radiochemical yields were only calculated for 4 and 6. The specific activity range calculated for both compounds was 600-1200 Ci/mmole

Table I: In Vitro Stabilities of the Isolated Radiobrominated Reaction Compounds

Radioactive Component	Time from Isolation	% Radioactivity in Parent Peak
Estradiol Radiobrominations		
2-Bromo-^{77}Br (3)	21 hours	96%
	5 days	96%
4-Bromo-^{77}Br (4)	45 minutes	98%
	3 days	97%
	6 days	97%
Unknown Species	30 minutes	75%
	21 hours	<1%
17α-Ethynylestradiol Radiobrominations		
2-Bromo-^{77}Br (5)	3 hours	93%
	30 hours	89%
4-Bromo-^{77}Br (6)	20 hours	97%
	5 days	90%
Unknown Species	1 hour	59%
	30 hours	<0.5%

(see experimental). The difference between the observed specific activity range and the maximum theoretical specific activity (approx. 54,000 Ci/mmole) is believed to be due, in part, to the introduction of trace quantities of stable bromine during the processing of the bromide-77 (20). Isolated radiochemical yields for 4 and 6 were generally about 30% of the activity injected into the HPLC, but on an average only about 80% of the activity in the reaction flask went into the HPLC. Longer reaction times and optimization of experimental technique in the transfer of activity to the HPLC could possibly increase the radiochemical yields of 4 and 6 to 40-50%.

CONCLUSIONS

The reaction of NCS with high specific activity bromide-77 in absolute ethanol yields an electrophilic brominating species which can be utilized to radiobrominate estrogens (27). The optimum reaction time is only fifteen minutes, if high specific activity products are desired, or five plus hours if higher radiochemical yields for the A-ring regioisomers are desired. HPLC can be utilized to purify and isolate the desired radiobrominated products in a minimum amount of time. The *in vitro* stability of isolated products can be determined by HPLC analysis.

EXPERIMENTAL

General

Estradiol and 17α-ethylestradiol were purchased from Sigma Chemical Company and were used without further purification. HPLC analyses were performed with Waters Associates 6000A pumps, UK6 injector, Model 450 U.V. Detector (at 254 nm), System Controller, and Data Module. A flow-past radioactivity detector was utilized to detect radioactive species. This radiochromatographic detection system was made up of a 2-inch NaI crystal coupled to an Ortec power bin, high voltage supply, ratemeter, amplifier, counter & timer, and lineprinter.

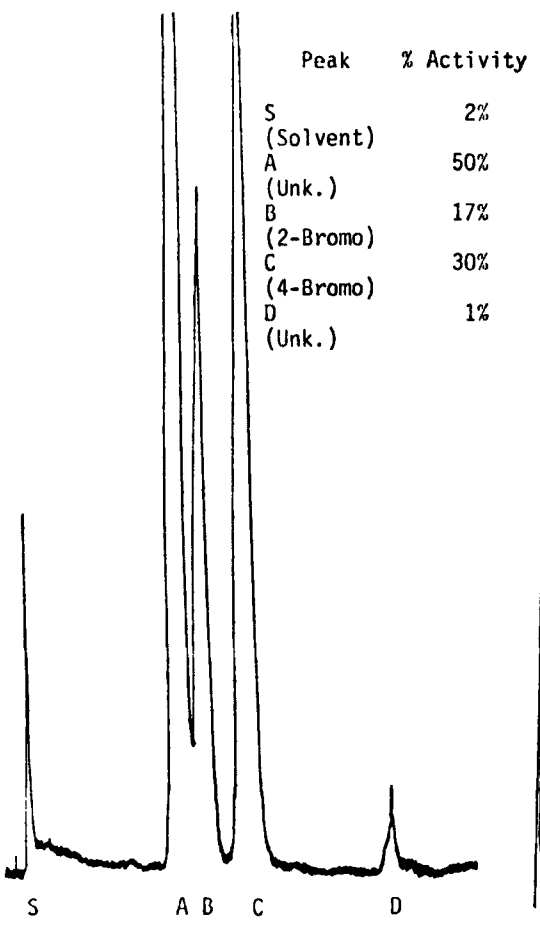


Figure 1: Radiochromatogram of Reaction of Estradiol.

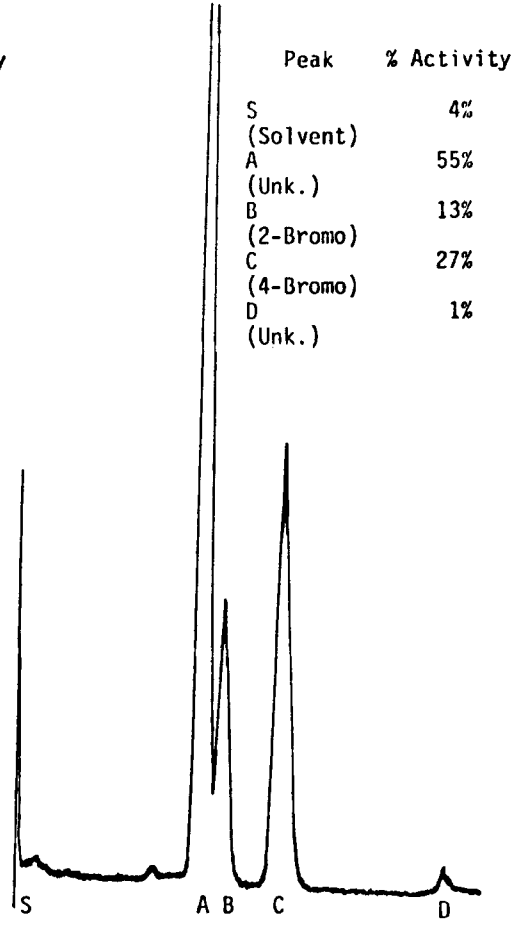


Figure 2: Radiochromatogram of Reaction of 17 α-Ethynylestradiol.

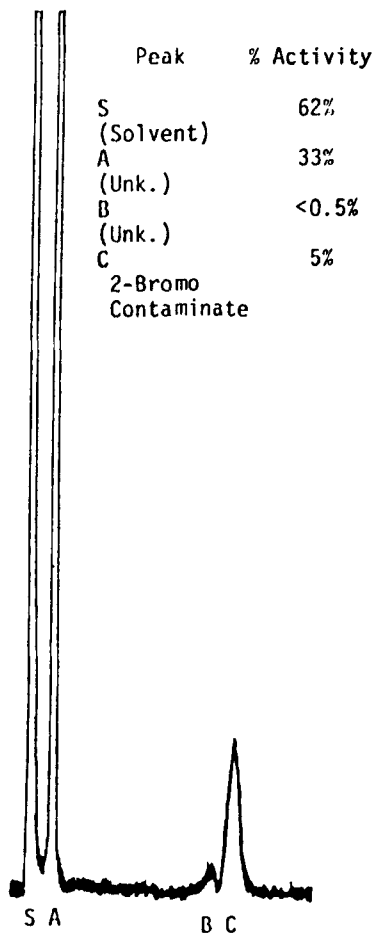


Figure 3: Radiochromatogram of isolated unknown radioactive peak after setting 30 hours.

HPLC separations were performed on a Waters Radial Compression Module with a Radial Pak C_{18} cartridge; eluting with a 45:55 CH_3CN/H_2O mixture at 3 mL/min. for analysis and 1 mL/min. for preparative separations. During a preparative run, individual radioactive fractions were isolated by passing through a Rheodyne valving system into vials. The radioactivity in reaction vessels, syringes, and vials was determined in a Capintec Radioisotope Calibrator.

Estrogen Radiobrominations

An aqueous bromide-^{77}Br solution was placed in a 5 mL septum-stoppered, conical-bottom vial containing a microstirring bar and was evaporated to dryness under reduced pressure at 75-80°C. A 50 μL aliquot of a 1.0 mg/mL absolute ethanol solution of either estradiol 1 or 17α-ethynylestradiol 2 (0.18 μmole and 0.17 μmole, respectively) was added by syringe to the reaction vessel in such a manner as to rinse any activity from the sides of the reaction vessel. This addition was followed by the syringe addition of 50 μL of a 1.0 mg/mL absolute ethanol solution of N-chlorosuccinimide (0.37 μmole) in the same manner. The reaction solution was stirred at room temperature for approx. 30 minutes and the reaction mixture was removed by syringe. The reaction solution in the syringe was injected onto the HPLC and the radiobrominated components were separated and isolated.

Specific Activity and Radiochemical Yield

A typical reaction of estradiol 1 and NCS/Na^{77}Br started with 18.9 mCi of activity. At the end of the reaction period (30 minutes), 15.6 mCi of activity was withdrawn from the reaction vessel (83%). Injection into the HPLC left 1.0 mCi in the syringe (14.6 mCi or 77% of activity). Collection of the 4-bromoestradiol peak yielded ~4 mL of solution containing 4.7 mCi of activity (25% of total or 32% of injected activity). The specific activity of this product could be estimated by observing the U.V. response at the appropriate

retention time. By calibration of the U.V. response at the same flow rate, solvent composition, and injection volumes, a value of 2 μg ± 0.5 μg of stable 4-bromoestradiol was obtained. From this value a specific activity range of 600-1200 Ci/mmole was estimated.

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22. The stable NCS/Br^- chemistry will be presented elsewhere. The isolated compounds were pure by HPLC analysis and were characterized by IR, U.V., M.S., carbon NMR and proton NMR. Differentiation of the 2-bromo- isomers from the 4-bromo- isomers was readily accomplished by proton NMR (60 MHz). The 2-bromo- isomers, 3 and 5, displayed two singlets in the aromatic region; (DMSO-d_6) 7.15δ & 6.52δ and (Acetone-d_6) 7.35δ & 6.70δ, respectively; whereas the 4-bromo- isomers displayed two doublets (DMSO-d_6) 6.99δ & 6.67δ and (Acetone-d_6) 7.17δ & 6.81δ

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