NO-CARRIER-ADDED BROMINATION OF ESTROGENS WITH CHLORAMINE-T AND $Na^{77}Br$

J.K. Mazaitis, B.E. Francis, W.C. Eckelman, R.E. Gibson, R.C. Reba,
J.W. Barnes*, G.E. Bentley*, P.M. Grant* and H.A. O'Brien, Jr.*
Radiopharmaceutical Chemistry, George Washington University Medical Center,
Washington, D.C. and
*Los Alamos Scientific Laboratory, Los Alamos, NM

SUMMARY

The syntheses of monobromohexestrol and 17α-bromoethynylestradiol are described as examples of bromination of phenolic and ethynyl moieties using Chloramine-T. This technique represents a unique method of producing no-carrier-added radiobromo-ethynylderivatives. However, more efficient chromatography is needed to separate the chloroderivative, which is a side product of the reaction, from the radio-bromoderivative.

INTRODUCTION

Radiobrominated estrogens are potential receptor binding radiotracers for use in the diagnosis and staging of breast cancer (1,2,3). Of the readily available substrates we chose hexestrol and 17α-ethynylestradiol because of their high affinity for the estrogen receptor (1,4) and promising in vivo studies with halogenated 3-methoxyethynylestradiol (5). Recently N-Cl succinimides and N-Cl phthalmides were suggested as reagents for the introduction of no-carrier-added ^{77}Br in aromatic, benzylic and allylic position (6) but no one has used Chloramine-T, a similar but readily available reagent, to radiobrominate phenolic and ethynyl moieties.

Monobromohexestrol (Scheme 1) was prepared using Chloramine-T and NaBr, with a large excess of hexestrol to ensure the formation of a monobrominated hexestrol only. The reaction and isolation procedures are essentially the same as the ones used to prepare no-carrier-added monoiodohexestrol (7).

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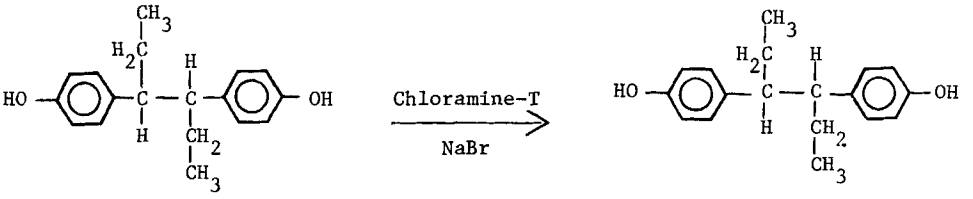
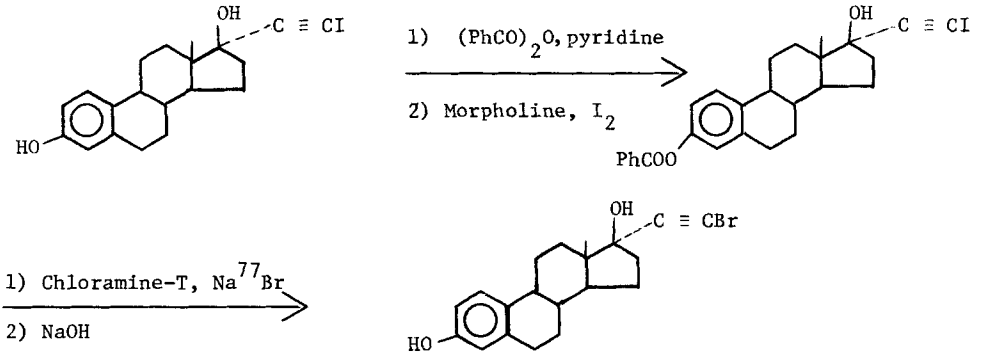


Scheme 1

Preparation of [^{77}Br] 17α-bromoethynylestradiol:



Scheme 2

Although the ethynyl group can be brominated by a variety of methods such as NaOBr in base (8) or F_3CBr in liquid NH_3 (9), neither is suitable for a simple no-carrier-added procedure as is our method using Chloramine-T and $Na^{77}Br$ in tetrahydrofuran (THF) at low pH.

Direct bromination (analogous to iodination) of the ethynyl group using Chloramine-T and NaBr did not occur. However with the iodoethynyl group as starting material the reaction yielded the bromoethynyl product. The first two steps in the preparation of 17α-bromoethynylestradiol have been described before (7). The 3-benzoate ester of 17α-ethynylestradiol protects the aromatic ring from iodination and bromination and can be easily hydrolyzed afterwards. The same scheme applies to cold and no-carrier-added bromination. The reaction time of no-carrier-added bromination was 2 hrs. All purifications were done using reverse phase HPLC.

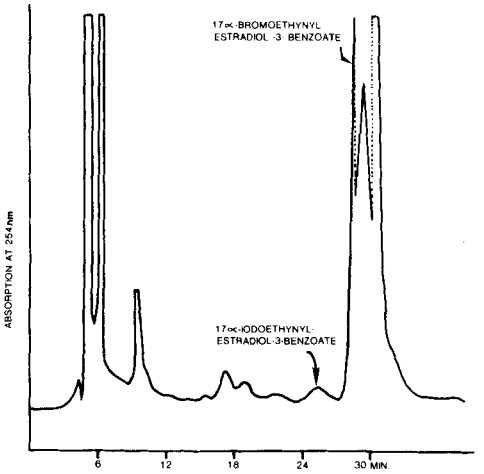


Fig 1: HPLC reverse phase separation of 17α-iodoethynyl estradiol-3-benzoate and 17α-bromoethynyl-3-benzoate.

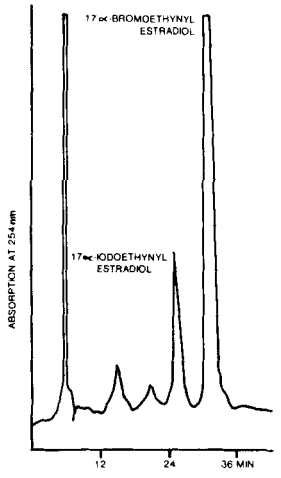


Fig 2: HPLC reverse phase separation of 17α-iodoethynylestradiol and 17α-bromoethynylestradiol.

In Fig. 1 we show a reverse phase HPLC separation of a reaction mixture containing equimolar amounts of 17α-iodoethynylestradiol-3-benzoate ester, Chloramine-T and NaBr. The UV absorption at 25.5 min coincides with that of authentic 17α-iodoethynylestradiol-3-benzoate ester and the absorption at 29 min with the product 17α-bromoethynylestradiol-3-benzoate ester. Fig. 2 shows a reverse phase HPLC separation of the reaction mixture after hydrolysis. The UV absorption at 25 min coincides with 17α-iodoethynylestradiol, and at 31 min with 17α-bromoethynylestradiol.

EXPERIMENTAL

17α-Bromoethynylestradiol:

80 mg of 17α-iodoethynylestradiol-3-benzoate (.15 mmoles), 17 mg NaBr (.17 mmoles) and 47 mg Chloramine-T (.17 mmoles) were dissolved in 6.4 ml THF and 1.6 ml of 1N HCl and stirred overnight at room temperature. After evaporation of THF the iodo- and bromoethynylestradiol-3-benzoates were extracted in ether and washed with water. The ether was evaporated and the residue dissolved in 4 ml methanol and hydrolyzed by 3 drops of 1N NaOH, stirring for 2 hrs at room temperature. The bromoethynylestradiol was separated from the iodoethynylestradiol using HPLC. (Altex reverse-phase LiChrosorb RP 18, 10 μ column, 25 cm long, 1 cm internal diameter, solvent system methanol-water (70-30) at a flow rate of 2 ml/min). 17α-Iodoethynylestradiol has a retention time of 25 min and 17α-bromoethynylestradiol 31 min. This procedure yielded 36 mg pure 17α-bromoethynylestradiol. The UV spectrum (Cary 15) in methanol is similar to that of 17α-ethynylestradiol, indicating an unchanged A ring. The IR spectrum (Perkin Elmer 700) in KBr disc showed no bands at 3300 cm^{-1} (≡C-H) or at 2130 cm^{-1} RC≡CH), but showed a RC≡CBr band at 2210 cm^{-1}. Elemental analysis for $C_{20}H_{23}O_2Br \cdot 1.5\ H_2O$ was in good agreement with the calculated value (C 59.70, H 6.52; found C 59.62, H 6.41).

[^{77}Br]-17α-Bromoethynylestradiol:

80 μl THF, 20 μl 0.5N HCl, 0.7 mg of 17α-iodoethynylestradiol-3-benzoate, and 0.35 mg Chloramine-T were added to a vial containing 5.3 mCi high specific activity Na^{77}Br, prepared at Los Alamos Scientific Laboratory, Los Alamos, NM. Acid stronger than 0.5N might be needed depending on the amount of base present in the ^{77}Br. The reaction was monitored for the production of 17α-bromoethynylestradiol-3-benzoate by TLC (Silicagel F 254 glass plates EM Laboratories) in solvent system toluene-ethylacetate 8-2, followed by a radio TLC scan (Vanguard Model 930 autoscan). (Rf values: ^{77}Br associated with Chloramine-T .15, [^{77}Br]-17α-bromoethynylestradiol-3-benzoate .55.) To isolate the radiobrominated 17α-ethynylestradiol-3-benzoate the

reaction mixture was chromatographed over an Altex reverse-phase LiChrosorb RP 18,10 μ column, 25 cm long, 1 cm internal diameter, solvent system methanol-water 85-15, at a flow rate of 3 ml/min. The retention time of 17α-iodoethynylestradiol-3-benzoate is 25.5 min, of 17α-bromoethynylestradiol-3-benzoate 29 min.

0.745 mCi of 17α-bromoethynylestradiol-3-benzoate was hydrolyzed with 35 μl 1N NaOH while most of the solvent was evaporated. The hydrolysis was monitored by rad TLC scans (toluene-ethylacetate 8-2; Rf values: [^{77}Br]-17α-bromoethynylestradiol .36, [^{77}Br]-17α-bromoethynylestradiol-3-benzoate .55). 17α-Bromoethynylestradiol was isolated and purified by HPLC using methanol-water 70-30 as solvent, at a flow rate of 2 ml/min. The retention time of 17α-bromoethynylestradiol is 31 min. After synthesis and purification (2 days) the yield is 90 μCi [^{77}Br]-17α-bromoethynylestradiol of >99% radiochemical purity.

DISCUSSION

One of the major side products of the bromination reaction of hexestrol with Chloramine-T is monochlorohexestrol. However, it could easily be separated from monobromohexestrol by HPLC. We did not prepare radiobromohexestrol because in in vitro studies using immature rat uterine cytosol the behavior of the cold compound is like that of monoiodohexestrol (7) in that it has too much nonspecific binding for it to be a useful compound.

Theoretically no-carrier-added bromination leads to a product of high specific activity (with Br-77 56,000 Ci/mmole). Our results with 17α-ethynylestradiol were different. In a competitive binding study as described in (7) using immature rat uterine cytosol, an excess of estradiol did not displace [^{77}Br]-17α-bromoethynylestradiol from the estrogen receptor. Yet, in in vivo studies in immature rats, a preinjection of estradiol blocked the accumulation of [^{77}Br]-17α-bromoethynylestradiol in the uterus. These results can be explained by assuming a low specific activity (<<100 Ci/mmole) of the [^{77}Br]-17α-bromoethynylestradiol.

A possible source of carrier Br^- could be Chloramine-T. However, its amount of Br^-, 14 ppm, is not enough to cause such a decrease in specific activity. A more likely explanation is a competing chlorination reaction (sources of Cl^- are Chloramine-T, HCl and the $^{77}Br^-$ preparation). This reaction is not noticeable when equimolar Br^- is present, but interferes at the no-carrier-added level. The product of this reaction, 17α-chloroethynylestradiol, cannot easily be separated from the 17α-bromoethynylestradiol. This in contrast to the bromination of hexestrol, where it is comparatively easy to separate the monochlorohexestrol from the monobromohexestrol. A similar observation of reduced specific activity has been reported using HOCl as the oxidizing agent for electrophilic bromination (10).

Currently we are working on a method of separation of 17α-chloro- and bromoethynylestradiol. At the the same time we are investigating a no-carrier-added method of bromination of 17α-ethynylestradiol that does not involve Cl^-.

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