# Preparation of Tritiated Substituted Estratrienes

A.D. Fraser*, S.J. Clark and H.H. Wotiz

Department of Chemistry, Boston University School of Medecine,

Boston, Massachusetts 02118.

Received on June 6, 1975
Revised on October 10, 1975

## SUMMARY

2,4-Dibromo-1,3,5(10)-estratriene-3,6β,17β-triol, 2,4-dibromo-1,3,5(10)-estratriene-3,7α,17β-triol and 2,4-dibromo-1,3,5(10)-estratriene-3,16β,17β-triol were prepared by bromination with N-bromosuccinimide. 2,4-Diiodo-1,3,5(10)-estratriene-3,7α,17β-triol, 2,4-diiodo-1,3,5(10)-estratriene-3,11β,17β-triol and 2,4-diiodo-1,3,5(10)-estratriene-3,16α-diol were formed by direct iodination with $I_2$. Hydrogenolysis of the dihalo estrogen derivatives by tritium gas over 5% palladium/$Al_2O_3$ gave the 2,4-tritiated parent compounds in good yield with high specific activity.

## INTRODUCTION

Studies on the kinetics of steroid binding to the uterine estrogen specific receptor necessitated preparation of a series of estratrienes labelled by tritium of high specific activity at biochemically stable positions. (The usual method involving saturation at C6-C7 double bonds by tritium could not be used for estratrienes having hydroxyl groups at C6 or C7.) An alternative procedure, halogenation of the aromatic A ring followed by reductive displacement of halogen with tritium, was therefore used.

## EXPERIMENTAL

The steroids used in this work were either purchased from commercial sources or supplied by National Institutes of Health from the National

Steroid Collection. They were checked for purity by gas chromatography as the trimethylsilyl ethers. All were better than 99% pure, the major impurities being non-steroid in nature. Particular attention was paid to the detection of estradiol-17β, which, if present, would seriously affect the results of binding studies. Sensitivity of detection was such that 0.01% would have been detected, but in no case was this amount of estradiol-17β found.

1. Bromination[1]-The steroid (25 mg) was dissolved in ethanol (10 ml) and N-bromosuccinimide (40 mg) added. The reaction mixture was allowed to stand for 18 hours at room temperature and the solvent was then removed in vacuo at 20°. The residue was dissolved in chloroform (20 ml) and washed 3 times with water (10 ml each). The solution was dried over $Na_2SO_4$, the solvent evaporated and the residue crystallized from methanol.

2. Iodination[2],[3]-The steroid (20 mg) was dissolved in ammonia:methanol (1:1, 20 ml). The solution was cooled to 4° and a solution of iodine in methanol added dropwise until a slight excess of iodine was present, as judged by a persistent yellow color in the solution. The solution was stirred magnetically for one hour at 4°, neutralized (pH7) with dilute acetic acid, diluted to approximately 300 ml with distilled water and extracted 3 times with 100 ml portions of diethyl ether. The ether solution was dried over $Na_2SO_4$ and the solvent removed by evaporation. The residue was crystallized from ethanol.

3. Tritiation[4],[5]-Approximately 20 mg of compound was dissolved in ethyl acetate (7 ml). Catalyst (100 mg 5% $Pd/Al_2O_3$) was added and the reaction mixture stirred overnight in the presence of 10 Ci of tritium gas. Labile tritium was removed in vacuo using ethanol as solvent and the solution filtered to remove catalyst. The compound of interest was isolated from the mixture received from New England Nuclear by thin layer chromatography using pre-coated TLC plates (Silica Gel F-254 on glass, E. Merck A. G., Darmstadt, Germany) in chloroform:ethanol (1:1) solvent system.

4. Analytical Procedures[6],[7]-The halogenated compounds were checked

for purity by gas chromatography as the trimethylsilyl ethers (3%, SE-30, 240-280° programmed at 3°/min.) on a Perkin Elmer Model 990 gas chromatograph and by mass spectrometry (Hitachi-Perkin Elmer Model RMU-6). The chromatographic effluent was split, approximately 50% going to the chromatograph's flame ionization detector and 50% to the mass spectrometer via a modified Watson-Biemann separator. Infra-red spectra of the compounds were determined in KBr pellets on a Perkin Elmer Model 237 spectrometer.

## DISCUSSION

The iodination procedure produced acceptable results only for 1,3,5(10)-estratriene-3,11β,17β-triol and 1,3,5(10)-estratriene-3,16α-diol (Table 1). However, it was found necessary to use a large excess of iodine to force the reaction to completion. Iodination of 1,3,5(10)-estratriene-3,16β-diol and 1,3,5(10)-estratriene-3,6α,17β-triol was not successful despite several attempts using a variety of reaction conditions and pure diiodo derivatives were not isolated. 2,4-Dibromo-1,3,5(10)-estratriene-3,7α,17β-triol was successfully prepared as judged by gc-ms evidence, but the product formed during tritiation corresponded to 1,3,5(10)-6-estratetraene-3,17β-diol. Apparently the 1,3,5(10)-estratriene-3,7α,17β-triol is very susceptible to dehydration catalyzed by hydriodic acid generated by the tritiation procedure.

Bromination of 1,3,5(10)-estratriene-3,6β,17β-triol, 1,3,5(10)-estratriene-3,16β-diol, and 1,3,5(10)-estratriene-3,7α,17β-triol proceeded smoothly in good yield (Table 1). The dibromo compounds were converted to tritiated derivatives of acceptable specific activities (Table 2).

The infra-red spectra of the iodinated and brominated estrogens are nearly identical with the spectrum of the parent compound in those regions representing vibration of groups in the same molecular environment such as the methyl rocking vibration (1130 $cm^{-1}$), and the region of aliphatic C-O stretching and O-H bending (near 1050 $cm^{-1}$). The major changes in the spectra due to halogen introduction were: (1) When compared to the parent

Table 1. Halogenated Compounds

| Compound | Melting Point (°C) | Gas Chromatography Retention Time * (min) | Mass Spectra* M+(m/e) |
|---|---|---|---|
| 2,4-dibromo-1,3,5(10)-estratriene-3,6β,17β-triol | 213-215 | 12.7 | 662,664,666 |
| 2,4-dibromo-1,3,5(10)-estratriene-3,7α,17β-triol | 239 | 22.5 | 662,664,666 |
| 2,4-dibromo-1,3,5(10)-estratriene-3,16β,17β-triol | 233-235 | 21.0 | 662,664,666 |
| 2,4-diiodo-1,3,5(10)-estratriene-3,7α,17β-triol | 209-211 | 21.5 | 756 |
| 2,4-diiodo-1,3,5(10)-estratriene-3,11β,17β-triol | 213-214 | 18.5 | 756 |
| 2,4-diiodo-1,3,5(10)-estratriene-3,16α-diol | 228-230 | 17.0 | 668 |

Table 2. Tritiated Compounds

| Compound | Specific Activity (Ci/mmol) | Gas Chromatography Retention Time (min) | Mass Spectra M+(m/e) |
|---|---|---|---|
| 1,3,5(10)-estratriene-3,6β,17β-triol-2,4-$^3H_2$ | 26.5 | 11.0 | M+2(506),M+4(508) |
| 1,3,5(10)-estratriene-3,7α,17β-triol-2,4-$^3H_2$ | 15.5 | 10.5 | M+2(506),M+4(508) |
| 1,3,5(10)-estratriene-3,11β,17β-triol-2,4-$^3H_2$ | 27.6 | 10.5 | M+2(506),M+4(508) |
| 1,3,5(10)-estratriene-3,16α-diol-2,4-$^3H_2$ | 23.0 | 9.5 | M+2(418),M+4(420) |
| 1,3,5(10)-estratriene-3,16β,17β-triol-2,4-$^3H_2$ | 16.6 | 14.5 | M+2(506),M+4(508) |

* Trimethylsilyl ether derivative

compounds the dihalogenated compounds show both a reduction of the relative intensities and decrease in frequencies of the doublet at 1610-1615 $cm^{-1}$ and 1585 $cm^{-1}$. The 1610-1615 $cm^{-1}$ band was less intense and broad in the dihalogenated compounds and the 1585 $cm^{-1}$ band was shifted approximately 50 $cm^{-1}$ to 1535 $cm^{-1}$ (2). The decrease ($\Delta\nu$) of the 1500 $cm^{-1}$ ring vibration in the dihalo compounds was 40-50 $cm^{-1}$. The intensity of this band is much greater for the dihalo compounds and is at least partially due to band overlapping with methyl and methylene bending vibrations. [8](3) Absorption of the lone aromatic C-H out-of-plane bending vibration in the dihalo steroids cannot be recognized and (4) all the dihalogenated compounds show a strong new absorption band at 745-755 $cm^{-1}$, evidently associated with the presence of the two C-Br and C-I groups.[8]

The mass spectra of the halogenated derivatives are generally similar to those of the parent compounds with the appropriate increase in m/e for those ions containing halogen. The expected triplet for ions containing two bromine atoms was always observed.

All attempts to produce the tritiated derivative of 1,3,5(10)-estratriene-3,6α,17β failed and it seems likely that this compound is even more susceptible to dehydration than 1,3,5(10)-estratriene-3,7α,17β-triol.

The reason(s) why the specific activities of the tritiated compounds failed to reach the theoretical maximum for the introduction of 2 tritium atoms per molecule (about 58 Ci/mmol) was not determined. One possibility may have been acid catalyzed exchange of tritium with the ethyl acetate solvent. However, since the tritiation was performed commercially by New England Nuclear, the reduction procedure was not investigated in an attempt to explain the low specific activities obtained.

ACKNOWLEDGEMENT

This research was supported by Contract #NO1-HD-3-2787 of the National Institute of Child Health and Human Development, National Institute of Health, Bethesda, Maryland.

REFERENCES

(1) Slaunwhite, W. R. and Neely, L. - J. Org. Chem. 27:1749 (1962).

(2) Barnes, J. H., Borrows, E. T., Elks, J., Hems, A., and Long, A. G.- J. Chem. Soc.: 2824 (1950).

(3) Nunez, J., Jacquemin, A., and Roche, J., - Internat. J. Appl. Radiation and Isotopes 13:573 (1962).

(4) The catalytic reduction was performed commercially by the New England Nuclear Corporation, Boston, Massachusetts.

(5) Coombs, M. M. and Roderick, H. R. - Steroids 11:925 (1968).

(6) Rao, P. N. - Steroids 18:219 (1971).

(7) Faulstich, H. and Trischman, H. - Anal. Biochem. 62:615 (1974).

(8) Winckelmann, H. J., Smakula, E. and Wotiz, H. H. - Spectrochimica Acta 16:1446 (1960).