THE SYNTHESIS OF ^{123m}Te-Labeled 17β-HYDROXY-2-TELLURA-A-NOR-5α-ANDROSTANE

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SUMMARY

A microscale synthesis has been developed for the preparation of 17β-hydroxy-2-tellura-A-nor-5α-androstane. Reactor-produced ^{123m}Te has been used to prepare the ^{123m}Te labeled steroid. The radiochemical synthesis and purification of this potential prostate imaging agent are described in detail.

Key Words: ^{123m}Te Steroid, Androgen, Prostate Imaging

INTRODUCTION

The concentration of a substance labeled with a gamma-emitting nuclide in the prostate gland would be an attractive method for prostate visualization. The use of such a non-invasive technique to determine the size of the prostate could prove to be a useful adjunct in the diagnosis and management of prostate disease. It is well known that both the prostate and seminal fluid have a high concentration of zinc (1) and that the human prostate accumulates radioactive zinc (2). There have been attempts to use the ^{65}Zn (3) and ^{69m}Zn (4) nuclides as imaging agents. The results of these studies have illustrated the practical limitations of using such agents. Since the prostate is known to bind estrogens (5) attempts have also been reported to utilize ^{131}I-diethyl stilbestrol (6) and ^{131}I-estradiol (7) as prostate imaging agents. As a consequence of either *in vivo* instability or minimal prostate uptake, the use of these agents has not been successful. Recent reports (8,9) have documented the potent androgenic activity of the androgen analog 17β-hydroxy-2-tellura-A-nor-5α-androstane (III, Fig. I). The androgenic properties of this substance would suggest that it binds to the high affinity receptor sites present in prostate tissue (1). The goal of the present

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investigation was to prepare ^{123m}Te-labeled 17β-hydroxy-2-tellura-A-nor-5α-androstane (IV). The ^{123m}Te nuclide is readily available via neutron irradiation of isotopically enriched ^{122}Te(n,γ) and decays by isomeric transition with the emission of a single gamma photon with an energy of 159 keV. The superior radionuclidic properties of ^{123m}Te compared to those exhibited by ^{75}Se make the ^{123m}Te nuclide an attractive candidate for use in nuclear medicine (11). The potential of ^{123m}Te-labeled substances as organ-imaging agents has not yet, however, been demonstrated. The problems associated with the production of the ^{123m}Te nuclide and the details of the radiochemical synthesis and purification of ^{123m}Te-labeled 17β-hydroxy-2-tellura-A-nor-5α-androstane are described in the present paper.

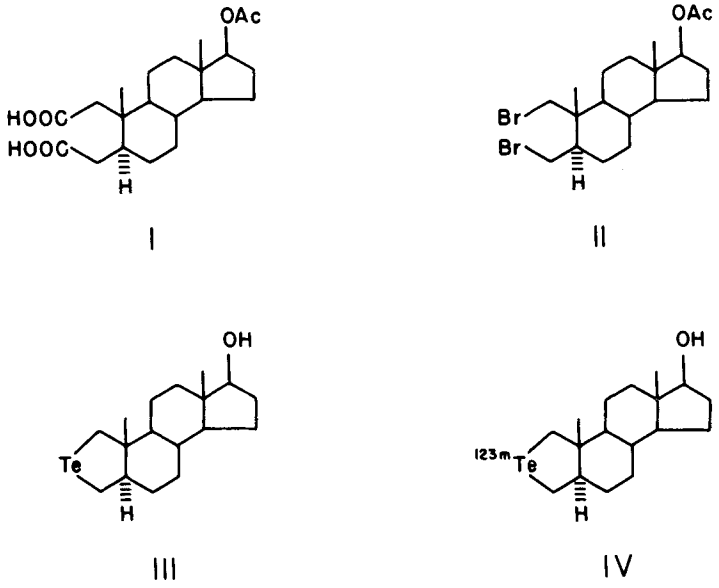

Fig. 1. Structures of 17β-acetoxy-2,3-seco-5α-androstan-2,3-dioic acid (I), 17β-acetoxy-1,4-dibromo-1,4-seco-2,3-bisnor-5α-androstane (II), 17β-hydroxy-2-tellura-A-nor-5α-androstane (III) and ^{123m}Te-labeled 17β-hydroxy-2-tellura-A-nor-5α-androstane (IV).

EXPERIMENTAL

General

Tellurium metal was purchased from Alfa Inorganics (Danvers, MA) and was ground to a 45-micron powder before use. The 17β-acetoxy-5α-androstan-3-one

was obtained from Steraloids, Inc. (Wilton, NH). Column chromatography was performed using silicic acid (60-100 mesh) obtained from Sigma Chemical Co. (St. Louis, MO). Thin-layer chromatography (t.l.c.) was performed on 250-micron-thick layers of PF-254 impregnated silica gel G(Analtech, Inc., Newark, DE). The following solvent systems were used: S-1, chloroform; S-2, ether-hexane, 10:90; S-3, ethyl acetate-chloroform, 30:70. The plates were heated at 80-100°C after being sprayed with molybdic acid-sulfuric acid spray (12). The steroids detected as blue spots. The telluro steroids were also detected as dark spots on the t.l.c. plates by irradiation with a 254-nm ultraviolet source. Melting points were determined in capillary tubes with a Buchi model SM-200 apparatus and are uncorrected. The ultraviolet spectra were determined with a Beckman DB instrument in ethanol solution and infrared spectra were obtained on KBr pellets using a Beckman Model 18A spectrophotometer. Low resolution mass spectral analyses were obtained using the Oak Ridge National Laboratory low resolution instrument (13) under the following conditions: source, 120°C; ionizing energy, 70 eV; trap current, 100 μA; probe, 200-200°C. The nuclear magnetic resonance spectra were determined with a Varian XL-100 instrument. Spectra were recorded in $CDCl_3$ solution and chemical shifts (δ) are reported downfield from the internal tetramethylsilane standard. A Nuclear Data Model 2200 multichannel analyzer equipped with a Ge(Li) detector was used for radio-activity determinations. Thin-layer radiochromatographic analyses were performed using Baker silica gel G-coated plastic sheets. The sheets were cut into sections which were placed into vials for counting. Samples of the telluro steroid were handled in subdued lighting to minimize the possibility of photochemical decomposition.

17β-Acetoxy-2,3-seco-5α-androstan-2,3,-dioic Acid (I)

The dioic acid (I) was prepared by the method described by Wolff et al (14). The 17β-acetoxy-5α-androstan-3-one (4 g) was dissolved in acetic acid (80 ml) at 50°C. After the addition of chromium trioxide (4 g) dissolved in water-acetic acid (1:1, 24 ml) the solution was stirred six hours at 67°C. The resulting dark green solution was poured into ice water and the suspension left to stand over-

night. The precipitate was collected and dissolved in 10% sodium bicarbonate solution and washed thoroughly with ether. Following acidification of the aqueous layer with hydrochloric acid the product was extracted with ether. The organic extract was washed well with water, dried over anhydrous sodium sulfate and the solvent distilled *in vacuo* to give a white solid. Three crystallizations from methanol-water gave micro crystals, 1.6 g, m.p. 227-228°C. The product was homogeneous upon thin layer chromatographic analysis (S-1, R_f 0.00; S-3, R_f 0.11). The infrared spectrum contained absorbances at 2940 (-OH), 1730 (acetate carbonyl) and 1660 cm^{-1} (carboxylate carbonyls). The molecular ion (M^+; m/z 380) was not detected upon low resolution mass spectral analysis. Ions were detected at m/z 362 (M^+-H_2O; 6%), 334 (4%), 329 (9%), 320 (M^+-CH_3COOH; 30%), 302 ($M^+-H_2O-CH_3COOH$; 70%), 287 ($M^+-H_2O-CH_3COOH-CH_3$; 24%), 284 (12%), 278 (17%), 277 (9%), 276 (20%), 274 (72%), 269 (12%), 261 (57%), 260 (65%), 259 (50%), 258 (82%), 243 (94%), 235 (19%), 234 (17%), 233 (22%), 232 (24%), 231 (18%), 230 (29%), 221 (33%), 219 (24%), 217 (59%), 215 (100%), 214 (36%), 213 (35%), 203 (30%), 202 (28%), and 201 (70%). The nuclear magnetic resonance spectrum exhibited resonances at 0.79 (s, 3H, C-18-CH_3), 0.81 (s, 3H, C-19-CH_3), 2.02 (s, 3H, acetate methyl), 4.59 (m, 1H, C-17α-H), and 9.96 (m, ∿2H, acid OH's).

17β-Acetoxy-1,4-dibromo-1,4-seco-2,3-bisnor-5α-androstane (II)

The 17β-acetoxy-2,3-seco-5α-androstan-2,3-dioic acid (1.5 g) and red mecuric oxide (1.1 g) were vigorously stirred under reflux in the dark. Bromine (1.1 g) was added dropwise and the mixture was stirred for 1.5 hr. The suspension was filtered while hot and the dark precipitate washed well with carbon tetrachloride. The combined filtrates were evaporated to dryness *in vacuo* to yield a gummy yellow mass which was triturated thoroughly with hexane. Following concentration, the hexane solution was applied to a short silicic acid column which was eluted with ether. Evaporation of the ether solution yielded a white solid. Crystallization from ethanol-water gave 738 mg of micro crystals, m.p. 159-161°C [Lit. m.p. 155-158°C, (15)]. The product was homogeneous upon t.l.c. analysis (S-1, R_f 0.88; S-2, R_f 0.39). The infrared spectrum contained an absorbance at

1715 cm^{-1} (acetate carbonyl). The low resolution mass spectrum contained ions at m/z 452, 450 and 448 (M^+; 0.2%, 0.5%, and 0.2%, respectively), 437, 435, and 433 (M^+-CH_3; 0.1%, 0.2%, and 0.1%, respectively), 420, 418, and 416 (M^+; 0.2%, 0.2%, and 0.1%, respectively), a series of peaks centered at 407 (0.6%), 392, 390, and 388 (M-CH_3COOH; 7%, 15%, and 8%, respectively), 377, 375, and 373 (M^+-CH_3-CH_3-COOH; 2%, 6%, and 3%, respectively), 371 and 369 (M^+-HBr; 6% and 6%), 364, 362, and 360 (4%, 6%, and 2%, respectively), 356 and 354 (M-HBr-CH_3; 10% and 11%, respectively), 351, 349, and 347 (2%, 6%, and 3%, respectively), 311 and 309 (M^+-CH_3COOH-HBr; 95% and 100%, respectively), 297 and 295 (58% and 60%, respectively), 289 (21%), 285, 283, and 281 (8%, 13%, and 6%, respectively), 275 (6%), 271, 269, and 267 (20%, 28%, and 10%, respectively), 257, 255, and 253 (10%, 17% and 8%, respectively), 247 (3%), 243, 241, and 239 (6%, 11%, and 6%, respectively), 229 (50%), 227 (17%), 215 and 213 (22% and 18%, respectively), 203 (8%) and 201 (18%). The nuclear magnetic resonance spectrum contained resonances at 0.77 (s, 3H, C-18-CH_3), 0.88 (s, 3H, C-19-CH_3), 2.02 (s, 3H, acetate CH_3), 3.02 (t, J = 5Hz, 1H, one of the C-4-H's), 3.44 (s, 2H, C-1-H's), 3.54 (m, 1H, one of the C-4-H's), and 4.62 (m, 1H, C-17α-H). The resonances observed at 3.02 and 3.54 δ must represent the two nonequivalent hydrogens at C-4 which are each coupled to the C-5α-hydrogen.

17β-Hydroxy-2-tellura-A-nor-5α-androstane (III)

Our initial attempts to couple sodium telluride and 17β-acetoxy-1,4-dibromo-1,4-seco-2,3-bisnor-5α-androstane in liquid ammonia were unsuccessful. The microscale preparation of the telluro steroid in an aqueous system was performed as described below. Finely divided tellurium powder (45 microns, 20mg, 154 μmoles) and sodium formaldehyde sulfoxylate (e.g., Rongalite C, 48.5 mg, 308 μmoles) were stirred under argon in a mixture of ethanol and water (7:8, 15 ml). Sodium hydroxide solution (1 *N*, 1 ml = 40 mg, 1 mmole) was added and the solution refluxed. After 30 minutes 17β-acetoxy-1,4-dibromo-1,4-seco-2,3-bisnor-5α-androstane (23 mg, 154 μmoles) was added to the purple solution. After 30 minutes t.l.c. analysis (S-1) of an aliquot indicated the reaction to be complete. The mixture was poured into water and extracted with ether. The organic layer

was washed well with water, dried, and the solvent distilled *in vacuo* to yield a white solid. Crystallization from ether-hexane gave needles, 7 mg, m.p. 149-151°C [Lit. m.p. for (III), 146-147°C (9)]. The product was homogeneous upon t.l.c. analysis (S-1, R_f 0.31; S-3, R_f 0.77). The ultraviolet spectrum contained a maximum absorbance at 237 nm (log ε = 3.68). The infrared spectrum exhibited a maxima at 3425 cm^{-1} (hydroxyl). Low resolution mass spectral analysis indicated the presence of ions at m/z 378 (M^+, ^{130}Te; 78%), 360 (M^+-H_2O, ^{130}Te; 9%), 278 (8%), 258 (3%), 247 (3%), 229 (47%) ,and 187 (62%). The nuclear magnetic resonance spectrum contained resonances at 0.73 (s, 3H, C-18-CH_3), 0.82 (s, 3H, C-19-CH_3), 2.63 (m, 1H, one of the C-4-H's), 3.01 (d, 2H, J = 2Hz, C-1-H's), 3.22 (m, 1H, one of the C-4-H's), and 3.65 (m, 1H, C-17α-H).

^{123m}Te-labeled 17β-Hydroxy-2-tellura-A-nor-5α-androstane (IV)

The reactor-produced ^{123m}Te (20 mg, 154 μmoles, ∿1 mCi/mg) was placed in a 25-ml round bottom flask. Following the addition of water (8 ml), ethanol (7 ml), sodium formaldehyde sulfoxylate (48.5 mg), and a 1 *N* sodium hydroxide solution (1 ml), the system was flushed with argon and the reaction mixture refluxed for 30 minutes. The solution turned red quickly and then became a deep purple color. To the resulting ^{123m}Te sodium telluride solution was added 17β- acetoxy-1,4-dibromo-1,4-seco-2,3-bisnor-5α-androstane (23 mg, 154 μmoles) in 1 ml of ethanol. The mixture was refluxed one hour under argon and cooled. The solution was poured into water at which time a fine, collodial suspension of tellurium was formed. This material was recovered by filtration for future use. The aqueous solution was extracted thoroughly with ether, and the combined organic extracts were washed with water and dried over anhydrous sodium sulfate. Aliquots of the solution were taken for counting and the remaining solvent was removed under a slow stream of argon. The resulting white solid was dissolved in a small volume of benzene and applied to a silicic acid column (2 x 20 cm) which was slurried in benzene. Fractions 20 ml in volume were eluted with increasing volumes of ether in benzene and the resulting radiochromatographic profile is shown in Fig. II.

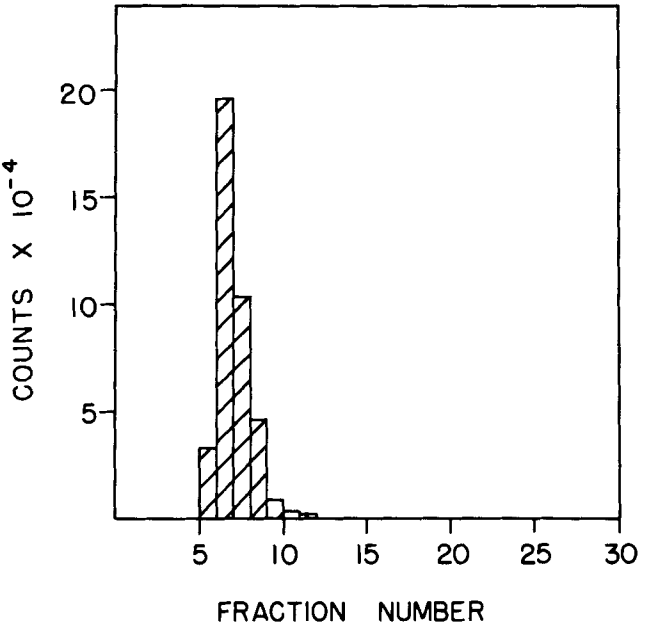

Fig. II. Silicic acid column chromatographic purification of ^{123m}Te-labeled 17β-hydroxy-2-tellura-A-nor-5α-androstane (IV).

DISCUSSION

A modification of the method reported by Wolff et al (9) was used to prepare 17β-hydroxy-2,3-bisnor-A-tellura-5α-androstane (III). The requisite 17β-acetoxy-1,4-dibromo-1,4-seco-2,3-bisnor-5α-androstane (II) was readily available via Hunsdiecker degradation of 17β-acetoxy-2,3-seco-5α-androstan-2,3-dioic acid (I). Attempts to couple (II) with sodium telluride generated in liquid ammonia were unsuccessful. Alternatively, sodium telluride was formed from elemental tellurium and sodium formaldehyde sulfoxylate in a basic solution of aqueous ethanol. Reaction of (II) with sodium telluride in this system gave 17β-hydroxy-2-tellura-A-nor-5α-androstane in moderate yield. The strong basic conditions also hydrolyzed the 17β-acetate. Hydrolysis concomitant with the coupling reaction simplified the radiochemical synthesis since an additional hydrolysis step would not be required. Isotopically enriched ^{122}Te (94%) was irradiated in the Oak Ridge High Flux Isotope Reactor (HFIR). The gamma spectrum of the ^{123m}Te product nuclide (Fig. III) indicated that the product was free of any significant long-

lived radionuclide contaminants. Assuming negligible burnup of the ^{123m}Te product one calculates an approximate theoretical specific activity of 37 mCi/mg (4.8 Ci/mmole) upon irradiation of enriched ^{122}Te in the HFIR for one full cycle (23 days at ∿2 x 10^{15} n/cm^2·sec). The experimentally determined specific activity obtained under these conditions was 1-2 mCi/mg. This low value probably indicates a high burn-up cross section for the ^{123m}Te product. The feasibility of altering the neutron energy spectrum with suitable shielding or of using carrier-free cyclotron-produced ^{123m}Te of high specific activity are problems that are presently being explored (16).

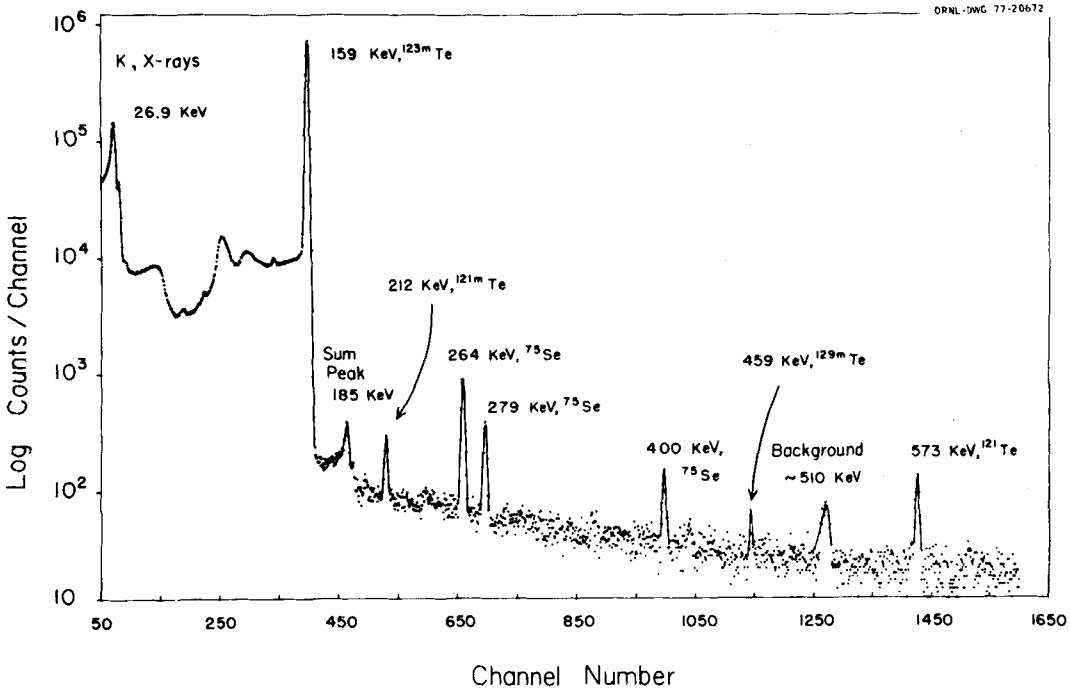

Fig. III. The gamma spectrum of ^{123m}Te prepared by neutron irradiation of isotopically enriched ^{122}Te.

Using the method described earlier, ^{123m}Te-labeled 17β-hydroxy-2-tellura-A-nor-5α-androstane (IV) was prepared from HFIR-produced ^{123m}Te. The crude product was applied in benzene to a silicic acid column previously slurried in the same solvent. The column was eluted with increasing concentrations of ether in benzene and aliquots of each fraction were taken for counting. The results

indicated that a single radioactive component was eluted from the column which corresponded in mobility to 17β-hydroxy-2-tellura-A-nor-5α-androstane (Fig. II) Fractions 5 through 10 contained the radioactivity and were combined. The specific activity of the purified product was ∿127 mCi/mmole. An aliquot was analyzed by thin-layer radiochromatography using chloroform as the developing solvent. When the radioactive sample was allowed to dry on the thin-layer plate prior to development, extensive decomposition became evident. Development of the plate followed by determination of radioactivity on the resulting chromatogram showed activity evenly distributed from the origin to the position where 17β-hydroxy-2-tellura-A-nor-5α-androstane migrated (Fig. IVa). When ∿100 μg of the unlabeled steroid was combined with the radioactive material prior to thin-layer radiochromatographic analysis, however, only one major radioactive component was detected which co-chromatographed with the unlabeled carrier (Fig. IVb). Similar results were obtained using the solvent systems ether-hexane (10:90) and ethyl

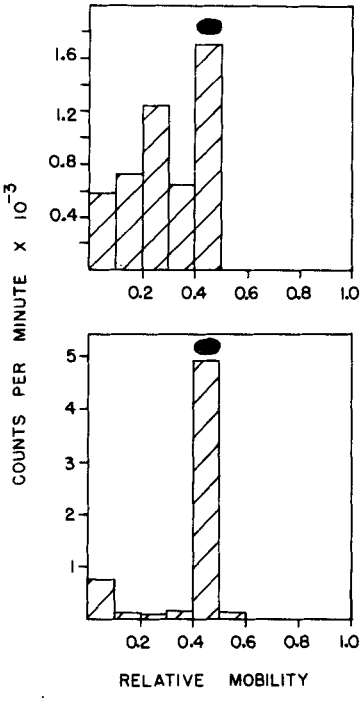

Fig. IV. Thin-layer radiochromatographic analysis of ^{123m}Te-labeled 17β-hydroxy-2-tellura-A-nor-5α-androstane (IV) determined (a) before and (b) after the addition of the unlabeled carrier steroid (III).

acetate-chloroform (30:70). Minimal decomposition was detected after storage for several weeks in dilute benzene solution at 10°C. Organic tellurium compounds absorb strongly in the ultraviolet region as a result of the tellurium $n \rightarrow \sigma^*$ transition (17) and the ultraviolet spectrum of 17β-hydroxy-2-tellura-A-nor-5α-androstane contains a maxima of 237 nm. The concentration of solutions of the telluro steroid can thus easily be determined by measurement of the intense 237-nm absorbance which is linear in the 4-100 μg/ml range. The extinction coefficient of the 237-nm absorbance was found to be 4.77×10^3 (log ε = 3.68).

Steroids of high specific activity are required for the detection of androgen uptake by the high affinity binding sites present in prostate tissue (10, 18). The low specific activity of the ^{123m}Te-labeled 17β-hydroxy-2-tellura-A-nor-5α-androstane described in the present work may limit its usefulness as a potential prostatic imaging agent. Studies are now being conducted to determine the *in vitro* and *in vivo* uptake of this agent. The moderate expense of reactor production, the attractive decay properties and the long shelf life of this nuclide would suggest that the incorporation of ^{123m}Te into other molecules potentially useful in nuclear medicine should be explored.

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