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THE SYNTHESIS OF 24-NOR-23(ALKYL TELLURO) STEROIDS

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ABSTRACT

A synthetic method has been developed for the introduction of tellurium into the steroid sidechain. A modification of the Hünsdiecker degradation was used for the conversion of 3α-acetoxy-5β-cholanic acid and 3β-acetoxy-5α-cholanic acid to the corresponding 24-norbromides. The 24-norbromides were then coupled with sodium alkyl tellurols to form the 24-nor-23-(alkyl telluro) steroids. In this manner 3α-hydroxy-24-nor-23-(isopropyl telluro)-5β-cholane, 3α-hydroxy-24-nor-23-(isopentyl telluro)-5β-cholane, 3β-hydroxy-24-nor-23-(isopropyl telluro)-5α-cholane and 3β-hydroxy-24-nor-23-(isopentyl telluro)-5α-cholane have been prepared. The physical properties of these interesting compounds have been studied in detail.

INTRODUCTION

Adrenal imaging using I-131 labeled 3β-hydroxy-19-iodo-cholest-5-ene [1,2] has proven to be an important noninvasive procedure for the diagnosis of adrenal disorders. This agent has been used successfully in the diagnosis of Cushing's syndrome [3,4], adrenal adenoma [5,6], and aldosteroma of the adrenal cortex [7,8,9,10]. In addition, adrenal remnants have been identified in patients with persistent Cushing's syndrome following adrenalectomy [5,6,11]. Both metastatic adrenal carcinoma [12] and the gall bladder [13] have also been visualized with this agent. The uniqueness of this noninvasive method for the diagnosis of adrenal disease and related disorders has resulted in the preparation

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of other steroids labeled with a variety of gamma-emitting nuclides. High adrenal/tissue ratios have been demonstrated in laboratory animals after the administration of I-131-labeled 3β-hydroxy-19-nor-6β-(iodomethyl)-cholest-5(10)-ene (NP-59) [14,15,16] and Se-75-labeled 3β-hydroxy-10-(methyl seleno)-cholest-5-ene [17]. The success of these studies has suggested that the preparation of potential adrenal imaging agents labeled with gamma-emitting nuclides with properties more attractive than those exhibited by I-131 and Se-75 should be further explored.

The Te-123m nuclide decays (120 day half-life) via an isomeric transition with emission of a single gamma photon with an energy of 159 keV. We have initiated a broad program directed towards the preparation of a variety of Te-123m-labeled substances which may have diagnostic potential in nuclear medicine. Although the general field of selenium organic chemistry is well documented [18] the chemistry of tellurium organic compounds has evidently not been well established because of a lack of interest in this area and also because of the difficulty in preparing and handling many of these substances [19]. In addition, selenium is an important trace element and in comparison tellurium has no established biological importance.

Considerable effort is therefore now being exerted in this laboratory directed towards developing synthetic techniques for the preparation of tellurium organic compounds that may be of biological interest. We have recently reported the preparation of Te-123m-labeled 17β-hydroxy-2,3-bisnor-A-tellura-5α-androstane [20] and the efficacy of this substance as a prostatic imaging agent is presently being investigated. Our interest has now focused upon the introduction of tellurium into the steroid sidechain rather than the nucleus since we wished to prepare a

3β-hydroxy steroid in which the total *trans* geometry of the steroid nucleus was maintained. These requirements suggested that introduction of the heteroatom into the steroid nucleus would be difficult. Furthermore, the degrees of freedom of the sidechain suggested that the tellurium heteroatom could be accommodated in this region of the molecule. The latter approach was also attractive because of the ready availability of bile acids which were envisioned as starting materials for the proposed route. In the present paper the strategy that has been developed for the introduction of tellurium into the steroid sidechain is discussed and the synthesis and physical properties of several 24-nor-23-(alkyl telluro) steroids are described in detail.

EXPERIMENTAL

General

The 3α-hydroxy-5β-cholanic acid (lithocholic acid), 3β-hydroxy-5α-cholanic acid (allolithocholic acid) and 3β-hydroxy-22,23-bisnor-5α-cholanic acid were purchased from Steraloids, Inc. (Wilton, NH). Isopropyl iodide and isopentyl iodide were obtained from Eastman Organic Chemicals (Rochester, NY) and were used without further purification. Tellurium metal was purchased from Alpha Inorganics (Danvers, MA) and was ground to a fine 45-micron powder prior to use. Analytical thin-layer chromatographic analyses (t.l.c.) were performed using precoated 250-micron thick silica gel GF plates purchased from Analtech, Inc. (Newark, DE). For preparative t.l.c. analyses glass plates were coated with 1-mm thick layers of silica gel PF-254 (Merck, Darmstadt). The following solvent systems were used: S-1, chloroform; S-2, ether-hexane, 5:95; S-3, ethyl acetate-chloroform, 30:70. Analytical plates were sprayed with an ammonium molybdate-sulfuric acid reagent and then heated at 80-100°C to visualize the spot colors [21]. The telluro steroids were also detected as dark areas on both analytical and preparative plates by viewing the plates under a 254-nm ultraviolet light source. In this manner, bands were detected and scraped from the preparative plates. The material was then eluted from the silica gel with chloroform. Gas liquid chromatographic analyses (g.l.c.) were performed using a Varian model 1400 gas chromatograph equipped with a flame ionization detector. A 4% Dexil column (1/4 in. x 10 ft) was programmed from 28 to 310° at 6°/min using helium as a carrier gas at a flowrate of 20 cm^3/min. Column chromatographic analyses were performed as described in the text (*vide infra*). Melting points are uncorrected and were determined in capillary tubes using a Büchi SMP-20 apparatus. The

ultraviolet spectra (u.v.) were determined in ethanol solution using a Beckman DB double beam spectrophotometer. Infra-red spectra (i.r.) were determined using KBr pellets with a Beckman Model 18A spectrophotometer. Low resolution mass spectral analyses (m.s.) were performed using the Oak Ridge National Laboratory low resolution instrument [22] under the following conditions: ionizing energy, 70 eV; probe temperature, 200-220°C; source temperature, 120°C; trap current, 100 μA. Naturally occuring tellurium contains the following stable isotopes: Te-130, 34%; Te-128, 32%; Te-126, 19%; Te-125, 7%; Te-124, 5%: Te-123, 1%; Te-122, 3% [23]. Because of the complex clusters of peaks that are detected for ions that contain tellurium, only those ions that contain the Te-130 isotope are tabulated in the mass spectral data. To conserve space, only those ions of mass greater than m/z 200 have been tabulated. High resolution mass spectral measurements were determined with an MS 50 mass spectrometer equipped with a DS 50 data system. Spectra were recorded under the following conditions: ionizing energy, 70 eV; source temperature, 150°C; probe temperature, ~200°C; trap current, 500 μA; resolution, 10^4; scan rate, 10 sec/decade; accelerating potential, 8000 volts. The nuclear magnetic resonance spectra (n.m.r.) were determined using a Varian XL-100 spectrometer. Samples were dissolved in $CDCl_3$ solution and resonances are reported downfield (δ) from the internal tetramethylsilane standard.

Preparation of Dialkyl Ditellurides

The dialkyl ditellurides were prepared by the reaction of alkyl iodides with sodium ditelluride in liquid ammonia [24]. In a typical preparation tellurium powder (127 mg, 1 mmole) was added to 25 ml of liquid ammonia that had been distilled into the reaction vessel under an argon atmosphere. Freshly cut sodium metal (25 mg, 1.1 mmole) was added and the solution was stirred two hours. The alkyl iodide (1 mmole) was added to the deep red sodium ditelluride solution and the mixture was stirred two hours. The ammonia was evaporated under an argon stream and benzene was added to the residue. Extraction of the residue with ether or methanol resulted in emulsification of the amorphous tellurium residue which necessitated a filtration step. This problem was not encountered during benzene extraction. The orange-colored benzene extract was decanted from the residue which consisted of amorphous tellurium and other insoluble products. Upon evaporation of the solvent *in vacuo* an orange gum was obtained. The small scale of this reaction made even microscale distillation of the oxygen- and light-sensitive ditellurides impractical. Typical yields averaged from 50-70%. The dialkyl ditelluride yields were determined in independent experiments by using the isotopic tracer [25]. The ditellurides consisted of pungent smelling red oils and were used directly for subsequent reactions without further purification.

3β-Acetoxy-20ξ-bromo-5α-pregnane (II)

The modified Hünsdiecker degradation [26] of 3β-acetoxy-22,23-bisnor-5α-cholane (I) gave 3β-acetoxy-20ξ-bromo-5α-pregnane (II) in 20% yield. The product crystallized from methanol-water as microcrystals, m.p. 106-109°C; i.r., ν_{max}^{KBr} 1735 cm^{-1} (acetate carbonyl); m.s., 426 (M,

absent), 366 and 364 (M-CH_3COOH, 2%, 2%), 344 (M-HBr, 29%), 329 (M-CH_3-HBr, 22%), 315 (M-sidechain, 45%), 284 (M-Br-CH_3COOH, 26%), 269 (M-CH_3-HBr-CH_3COOH, 76%), 255 (63%), 241 (4%), 230 (12%), 227 (13%), 215 (30%), and 201 (20%). Although the crystalline product appeared homogeneous by both column (neutral alumina) and t.l.c. analyses (S-1, R_f 0.82), the n.m.r. analysis indicated this material was a mixture apparently containing both (20R)- and (20S)-3β-acetoxy-20-bromo-5α-pregnane. An analogous mixture of (20R)- and (20S)-bromides has been obtained upon classical Hünsdiecker degradation of 3β,12α-diacetoxy-23,24-bisnor-desoxycholic acid [27].

Attempted Preparation of 3β-Hydroxy-20ξ-(isopentyl telluro)-5α-pregnane (IV)

The 3β-acetoxy-20ξ-bromo-5α-pregnane (II, 43 mg, 100 μmoles) was added to a solution of sodium isopentyl tellurol (200 μmoles) in liquid ammonia prepared from diisopentyl ditelluride in the usual manner [24]. Upon evaporation of the solvent only unreacted (II) was recovered. The coupling of 3β-acetoxy-20ξ-bromo-5α-pregnane with sodium isopentyl tellurol was also attempted in refluxing methanol. Diisopentyl ditelluride (81 mg, 200 μmoles) was dissolved in methanol under an argon atmosphere. Sodium borohydride was added in small portions until the orange color discharged and the solution became colorless. A 1 *N* sodium hydroxide solution (0.5 ml, 500 μmoles) was added and then 3β-acetoxy-20ξ-bromo-5α-pregnane (43 mg, 100 μmoles). The mixture was refluxed one hour, poured into water and extracted with ether. Following drying over anhydrous sodium sulfate, the solvent was removed *in vacuo* to yield an orange gum. Analysis of an aliquot by t.l.c. (S-1) on PF-254 plates indicated only one u.v. absorbing component chromatographing with the expected mobility of diisopentyl ditelluride (R_f 0.94). Upon being sprayed with sulfuric acid-ammonium molybdate spray, a second spot appeared (R_f 0.29). This material was purified by t.l.c. and proved to be 3β-hydroxy-20ξ-bromo-5α-pregnane (III), 33 mg, m.p., 125-129°C (softening at 122°C); i.r., ν_{max}^{KBr} 3340 cm^{-1} (-OH); m.s., 366 and 364 (M-H_2O, 3% and 3%), 351 and 349 (M-H_2O-CH_3, 2% and 2%), 312 and 310 (7% and 8%), 302 (29%), 300 (9%), 287 (58%), 285 (48%), 284 (M-HBr-CH_3, 53%), 273 (24%), 269 (M-CH_3-HBr-H_2O, 100%), 255 (45%), 245 (18%), 243 (8%), 230 (32%), 227 (20%), 215 (32%), 214 (17%), 213 (12%), 203 (19%), 202 (22%), and 201 (23%).

3α-Acetoxy-24-nor-23-bromo-5β-cholane (VII)

The preparation of 3α-acetoxy-24-nor-23-bromo-5β-cholane by modified Hünsdiecker degradation of 3α-acetoxy-5β-cholanic acid has been described earlier [28].

3α-Hydroxy-24-nor-23-(isopentyl telluro)-5β-cholane (IX)

The 3α-acetoxy-24-nor-23-bromo-5β-cholane (91 mg, 200 μmoles) was added to a methanolic solution of sodium isopentyl tellurol (400 μmoles) prepared as described earlier. The solution was refluxed one hour, after which time t.l.c. analysis of an aliquot indicated the reaction to be complete. The solution was poured into water and extracted with

chloroform. Analysis by t.l.c. (S-1) using PF-254 plates followed by u.v. irradiation indicated the presence of diisopentyl ditelluride (R_f 0.95) and a second u.v. absorbing material (R_f 0.25). This product was purified by t.l.c. to give a 3α-hydroxy-24-nor-23-(isopentyl telluro)-5β-cholane (IX) as a thick gum, 34 mg (32%). Trituration with ether gave only a semi-solid which could not be crystallized; u.v., λ_{max} 234 (log ε 4.1) and 221 nm (log ε 3.4); i.r., ν_{max}^{KBr} 3330 cm^{-1} (-OH); m.s., 532 (M, 14%), 514 (M-H_2O, 14%), 461 (M-C_5H_{11}, 15%), 443 (M-H_2O-C_5H_{11}, 7%), 415 (1%), 394 (3%), 392 (3%), 353 (2%), 331 (5%), 328 (6%), 313 (12%), 302 (7%), 299 (7%), 297 (10%), 285 (8%), 278 (12%), 273 (9%), 257 (M-sidechain, 44%), 243 (9%), 229 (15%), 215 (52%), and 201 (25%); high resolution m.s., 532.2884 (calculated for $C_{23}H_{50}OTe$: 532.2898); n.m.r., 0.62 (s, 3H, C-18-CH_3), 0.92 (s, 3H, C-19-CH_3), 0.93 (d, J = 6Hz, 3H, C-21-CH_3), 2.60 (complex multiplet, 4H, methylene hydrogens flanking the tellurium) and 3.58 (m, 1H, C-3α-H).

3α-Hydroxy-24-nor-23-(isopropyl telluro)-5β-cholane (XI)

A solution of sodium isopropyl tellurol was prepared by reduction of diisopropyl ditelluride (135 mg, 400 μmoles) with sodium borohydride in basic methanol in the usual manner. The 3α-acetoxy-24-nor-23-bromo-5β-cholane (90 mg, 200 μmoles) was added, and the mixture was refluxed two hours and the product obtained as described earlier. Purification by t.l.c. (solvent, chloroform; R_f 0.19) gave 3α-hydroxy-24-nor-23-(isopropyl telluro)-5β-cholane, 31.6 mg, as a thick gum. Even trituration with ether gave only a gummy semi-solid that could not be adequately crystallized despite numerous attempts from a variety of solvents; i.r., ν_{max}^{KBr} 3360 (-OH); u.v. λ_{max} 235 (log ε 4.1) and 222 nm (log ε 3.4); m.s., 504 (M, 8%), 486 (M-H_2O, 12%), 461 (M-isopropyl, 2%), 443 (M-H_2O-isopropyl, 8%), 349 (1%), 332 (3%), 330 (4%), 313 (16%), 299 (9%), 297 (14%), 285 (8%), 283 (6%), 278 (8%), 273 (23%), 271 (8%), 260, (9%), 257 (43%), 255 (55%), 243 (7%), 241 (8%), 233 (6%), 231 (10%), 230 (15%), 229 (13%), 219 (5%), 217 (12%), 215 (58%), 211 (6%), 203 (22%), and 201 (28%); high resolution m.s., 504.2564 (calculated for $C_{26}H_{46}OTe$: 504.2584); n.m.r., 0.63 (s, 3H, C-18-CH_3), 0.92 (s, 3H, C-19-CH_3), 0.93 (d, J = 6Hz, 3H, C-21-CH_3), 1.60 (d, J = 6Hz, 6H, C-26, and C-27-CH_3's), 2.64 (m, 2H, C-23-H's), 3.38 (m, 1H, C-25-H), and 3.57 (m, 1H, C-3α-H).

3β-Acetoxy-24-nor-23-bromo-5α-cholane (VIII)

The 3β-acetoxy-24-nor-23-bromo-5α-cholane was prepared by the modified Hünsdiecker degradation of 3β-acetoxy-5α-cholanic acid (II) and was purified as described earlier [28]. The product was crystallized from ethanol-water to give fine needles (178 mg, 20%), m.p. 198°C. The product was homogeneous as determined by t.l.c. analysis (S-1, R_f 0.84; S-2, R_f 0.23); i.r., ν_{max}^{KBr} 1730 cm^{-1} (acetate carbonyl); m.s., 454 and 452 (M, 4%, 4%), 439 and 437 (M-CH_3, 3%, 3%), 394 and 392 (M-CH_3COOH, 16%, 17%), 379 and 377 (M-CH_3-CH_3COOH, 15%, 15%), 340 and 338 (8%, 9%), 325 and 323 (2%, 2%), 315 (7%), 312 (6%), 290 (8%), 286 and 284 (6%, 7%), 276 (15%), 275 (22%), 257 (M-sidechain-CH_3COOH, 28%),

255 (11%), 241 (3%), 230 (40%), 215 (100%), and 201 (24%); n.m.r., 0.65 (s, 3H, C-18-CH_3), 0.81 (s, 3H, C-19-CH_3), 0.91 (d, 3H, J = 6Hz, C-21-CH_3), 2.05 (s, 3H, acetate CH_3), 3.37 (m, 2H, C-23 H's), and 4.64 (m, 1H, C-3αH).

3β-Hydroxy-24-nor-23-(isopentyl telluro)-5α-cholane (X)

Sodium isopentyl tellurol (200 μmoles) and 3β-acetoxy-24-nor-23-bromo-5α-cholane (VIII, 45.4 mg, 100 μmoles) were coupled in refluxing methanol as described earlier. The reaction mixture was poured into water and the crude product extracted with chloroform. Purification by t.l.c. (S-1) gave a thick gum (7 mg, 14%). The product was homogeneous by t.l.c. analysis (S-1, R_f 0.14) and trituration with a small volume of ether gave a white solid, m.p. 78-80°; u.v. λ_{max} 234 (log ε 4.1) and 221 nm (log ε 3.4); i.r., ν_{max}^{KBr} 3300 cm^{-1} (-OH); m.s., 532 (M, 48%), 514 (M-H_2O, 57%), 461 (M-C_5H_{11}, 10%), 443 (M-H_2O-C_5H_{11}, 17%), 415 (2%), 349 (2%), 331 (3%), 313 (21%), 303 (2%), 299 (3%), 297 (7%), 285 (12%), 273 (15%), 257 (M-sidechain, 30%), 255 (19%), 243 (8%), 229 (39%), 215 (28%), and 201 (20%); high resolution m.s., 532.2843 (calculated for $C_{26}H_{50}OTe$: 532.2883); n.m.r., see Fig. 3.

3β-Hydroxy-24-nor-23-(isopropyl telluro)-5α-cholane (XII)

Sodium isopropyl tellurol (400 μmoles) and 3β-acetoxy-24-nor-23-bromo-5α-cholane (VIII, 45 mg, 100 μmoles) were coupled in refluxing methanol and the product obtained and purified in the usual manner to give a thick gum (15.6 mg, 31%). Trituration with ether gave a white solid which was homogeneous by t.l.c. analysis (S-1, R_f 0.14), m.p. 118-119°C; u.v., λ_{max} 235 (log ε 4.1) and 222 nm (log ε 3.4); i.r., ν_{max}^{KBr} 3310 cm^{-1} (-OH); m.s., see Fig. 2 for complete spectrum; high resolution m.s., 504.2610 (calculated for $C_{26}H_{46}OTe$: 504.2629); n.m.r., see Fig. 4.

RESULTS AND DISCUSSION

General

The goal of the present study was to develop a method for the introduction of tellurium into the steroid sidechain. There are a variety of methods known for the incorporation of tellurium into organic molecules [19]. In the present study a synthetic method that used elemental tellurium was attractive since the eventual preparation of Te-123m-labeled 24-nor-23-(alkyl telluro) steroids from the elemental form of the nuclide was anticipated. The reaction scheme that was envisioned resembled the coupling of an alkyl halide (R'X) with a sodium alkyl

tellurol (NaTeR). A number of dialkyl tellurides (R-Te-R') have been prepared by this general method [19]. The sodium alkyl tellurols can be generated from symmetrical dialkyl ditellurides (R_2Te_2) and a convenient system for the preparation of the latter intermediates involves the alkylation of sodium ditelluride (Na_2Te_2) with an alkyl halide. The sodium ditelluride is unstable but may be formed *in situ* by the reaction of elemental tellurium with a stoichiometric amount of sodium metal in liquid ammonia [24]. The dialkyl ditelluride thus formed may be cleaved with sodium in liquid ammonia [24] or by sodium borohydride reduction in a basic medium [19]. The resulting sodium alkyl tellurols can then be reacted with the appropriate alkyl halide to form the desired unsymmetrical dialkyl telluride. These transformations are summarized below.

$$Te + Na \xrightarrow{NH_3} Na_2Te_2 \xrightarrow{RX}$$

$$R_2Te_2 \xrightarrow{\text{reduction}} NaTeR \xrightarrow{R'X} R\text{-}Te\text{-}R'$$

Preparation of 24-Nor-23-(Alkyl Telluro) Steroids

In the present study bile acids were readily converted to the corresponding norbromides via a modification of the Hünsdiecker reaction [26,28]. Fabrication of the alkyl-telluro sidechain was then accomplished by reaction of the norbromide with the appropriate sodium alkyl tellurol. The sodium alkyl tellurols were prepared by sodium borohydride reduction of the precursor dialkyl ditellurides in basic-methanol solution. By choice of the appropriate dialkyl ditelluride a variety of alkyl-telluro steroids can be prepared by this method. The present study was limited to the attempted synthesis of 20-(alkyl telluro) and 23-(alkyl telluro) steroids. The general scheme that was

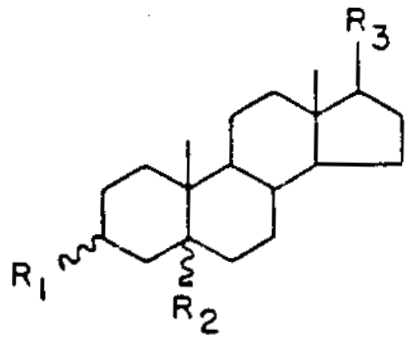
developed is outlined below.

3-Acetoxy steroid-R-COOH $\xrightarrow{HgO\text{-}Br_2}$

3-Acetoxy steroid-R-Br $\xrightarrow{Na\text{-}Te\text{-}R'}$

3-Hydroxy steroid-R-Te-R'

In an initial experiment 3β-acetoxy-22,23-bisnor-5α-cholanic acid (I) was converted to 3β-acetoxy-20ξ-bromo-5α-pregnane (II). Although apparently homogeneous upon t.l.c. analysis, this product was found by n.m.r. studies to be a mixture of two species, presumably the (20R)- and (20S)-bromides. An analogous mixture of C-20 isomers was also obtained upon phosphorus pentabromide treatment of the sodium borohydride reduction product of 3β-acetoxy-preg-5-en-20-one [29]. The bromide mixture (II) did not react with sodium isopentyl tellurol in either liquid ammonia or refluxing methanol. In the latter system 3β-hydroxy-20ξ-bromo-5α-pregnane (III) was isolated as the hydrolysis product. The failure to form 3β-hydroxy-20ξ-(isopentyl telluro)-5α-pregnane (IV) was attributed to steric hindrance in the vicinity of the secondary C-20 bromide. In an attempt to determine the reactivity of a steroid containing a primary halide in the sidechain, 3α-acetoxy-24-nor-23-bromo-5β-cholane (VII) was prepared as a model substrate via Hünsdiecker degradation of the readily available lithocholic acid acetate (V). Reaction of (VII) with sodium isopentyl tellurol in refluxing methanol gave 3α-hydroxy-24-nor-23-(isopentyl telluro)-5β-cholane (IX) in moderate yield (32%). The isomeric 3β-hydroxy-24-nor-23-(isopentyl telluro)-5α-cholane (X) was prepared in an analogous manner from 3β-acetoxy-24-nor-23-bromo-5α-cholane (VIII). The latter intermediate was prepared by Hünsdiecker degradation of allolithocholic acid acetate (VI). These studies indicated that 24-nor-23-(alkyl telluro) steroids could be



Compound	R_1	R_2	R_3
I	β-OAc	α-H	$-CH(CH_3)COOH$
II	β-OAc	α-H	$-CH(CH_3)Br$ (20R)-
III	β-OAc	α-H	$-CH(CH_3)Br$ (20S)-
IV	β-OH	α-H	$-CH(CH_3)Te(CH_2)_2CH(CH_3)_2$
V	α-OAc	β-H	$-CH(CH_3)CH_2CH_2COOH$
VI	β-OAc	α-H	$-CH(CH_3)CH_2CH_2COOH$
VII	α-OAc	β-H	$-CH(CH_2)CH_2CH_2Br$
VIII	β-OAc	α-H	$-CH(CH_2)CH_2CH_2Br$
IX	α-OH	β-H	$-CH(CH_3)(CH_2)_2Te(CH_2)_2CH(CH_3)_2$
X	β-OH	α-H	$-CH(CH_3)(CH_2)_2Te(CH_2)_2CH(CH_3)_2$
XI	α-OH	β-H	$-CH(CH_3)(CH_2)_2TeCH(CH_3)_2$
XII	α-OH	β-H	$-CH(CH_3)(CH_2)_2TeCH(CH_3)_2$

Fig. 1. Steroid Structures.

readily prepared by this methodology.

The 3-hydroxy-24-nor-23-(alkyl telluro) steroids are relatively insoluble in ether. The telluro steroids could not be efficiently extracted from reaction mixtures with ether but were readily extracted with chloroform or ethyl acetate. Following thin layer chromatographic purification and extraction of the telluro steroids from the absorbant with chloroform-methanol, evaporation of the solvent gave gummy solids. Trituration of the 3β-hydroxy-24-nor-23-(alkyl telluro) steroids with ethyl ether gave white solids. Melting points were obtained on samples prepared in this manner since the small amounts of relatively unstable products could not be adequately crystallized. Similar trituration of the 3α-hydroxy-24-nor-23-(alkyl telluro) steroids gave only thick glasses which did not exhibit well-defined melting points. The telluro steroids are more stable upon exposure to light and oxygen than a number of simple dialkyl tellurides that have been prepared in this laboratory [29]. The 3α-hydroxy-24-nor-23-(alkyl telluro) steroids appear less stable than the isomeric 3β-hydroxy-24-nor-23-(alkyl telluro) analogs and the former compounds slowly decomposed when stored in the dark under argon at 4°C. The 3β-hydroxy-24-nor-23-(alkyl telluro) steroids were stable when stored as dry solids under these conditions for periods of up to several months.

As described below, an analysis of the physical properties of these unusual 24-nor-23-(alkyl telluro) steroids demonstrated that the structures assigned to the synthetic products are consistent with the proposed structures. The ultraviolet spectral properties of the isomeric 24-nor-23-(isopentyl telluro) steroids (IX) and (X) are very similar. The isomeric 24-nor-23-(isopropyl telluro) steroids (XI) and

(XII) also exhibit similar spectral properties. Tellurides absorb strongly in the ultraviolet region as a result of an $n \rightarrow \sigma^*$ transition [19]. The 24-nor-23-(alkyl telluro) steroids prepared in the present study all exhibit essentially identical ultraviolet spectra. The ultraviolet spectrum of 3β-hydroxy-24-nor-23-(isopropyl telluro)-5α-cholane (XII), for example, contains a maximal absorption at 231 nm and the high extinction coefficient (log ε 4.1) can be used to determine accurately the concentration of solutions of the telluro steroids. Such measurements should facilitate the determination of the specific activity of Te-123m-labeled 24-nor-23-(alkyl telluro) steroids.

Although the mass spectra of these 24-nor-23-(alkyl telluro) steroids have not been studied in detail, several general comments can be made concerning the origin of a number of ions found in the spectra of these compounds. The mass spectra of the four telluro steroids (IX), (X), (XI), and (XII) are qualitatively very similar and the mass spectrum of 3β-hydroxy-24-nor-23-(isopropyl telluro)-5α-cholane (XII) will serve as an illustration to discuss the general characteristics of the electron-impact-induced fragmentation of such compounds. The high mass region of the mass spectrum of 3β-hydroxy-24-nor-23-(isopropyl telluro)-5α-cholane (XII) is illustrated in Fig. 2. Ions containing the tellurium heteroatom are generally easily recognized by the presence of a cluster of peaks representing the various tellurium isotopes (*vide ante*). Ions containing the Te-130 isotope are found at m/z 504 (M), 486 ($M-H_2O$), 461 ($M-C_3H_7$), 443 ($M-H_2O-C_3H_7$) and 257 (M-sidechain). The moderately abundant m/z 461 ion is formed by loss of the isopropyl group indicating preferential cleavage of the smaller alkyl group. One unique feature of the mass spectra of (XII) and other 24-nor-23-(alkyl telluro)

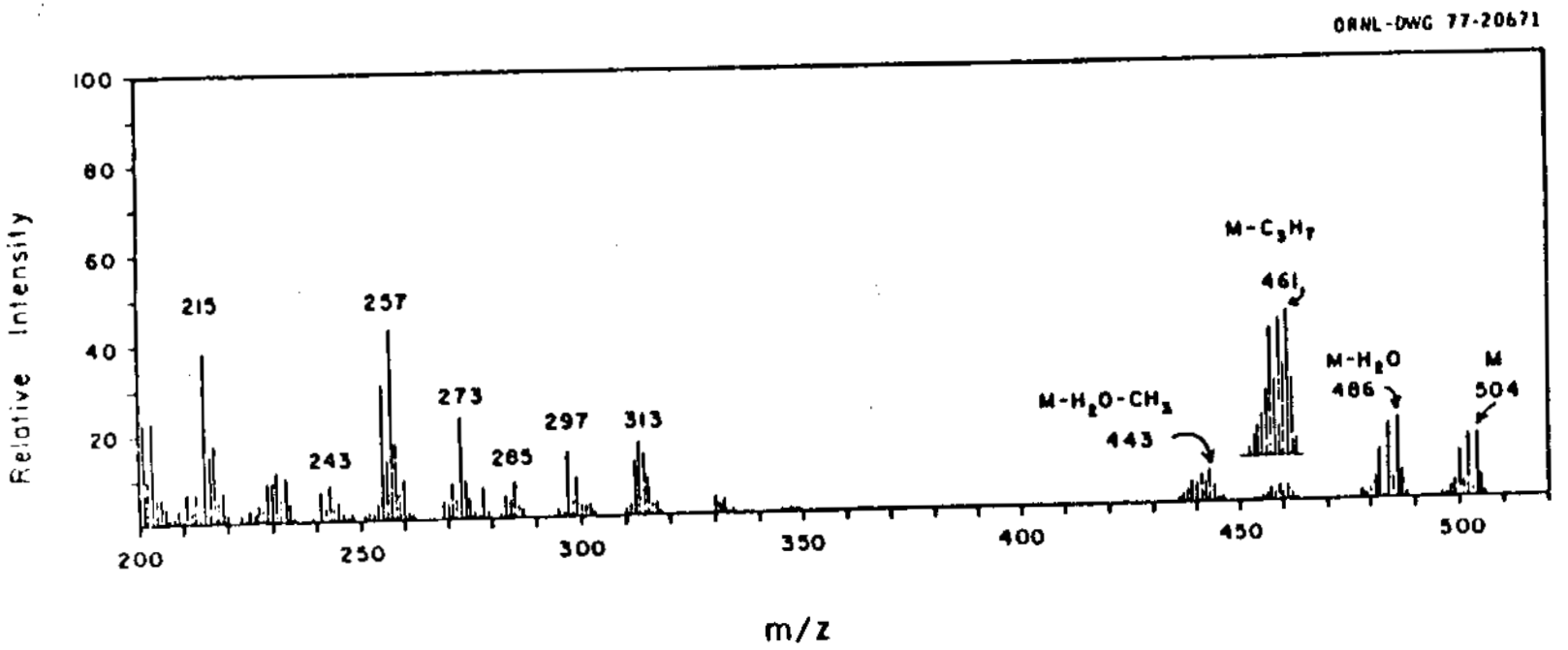


Fig. 2. The 70-eV Mass Spectrum of 3β-Hydroxy-24-nor-23-(isopropyl telluro)-5α-cholane (XII).

steroids appears to be the absence of ions corresponding to methyl loss.

The nuclear magnetic resonance spectra of 24-nor-23-(alkyl telluro) steroids contain resonances that are easily assigned, and the spectrum of 3β-hydroxy-24-nor-23-(isopentyl telluro)-5α-cholane (X) is illustrated in Fig. 3. The methyl group resonances are easily assigned (Table I) and the four proton multiplet centered at 2.65 ppm undoubtedly represents the protons of the two deshielded methylene groups that flank the tellurium heteroatom. These protons are further coupled to the adjacent methylene protons, and we have not yet attempted to further delineate this complex coupling pattern. The nuclear magnetic resonance spectrum of 3β-hydroxy-24-nor-23-(isopropyl telluro)-5α-cholane is very instructive (Fig. 4). The methyl resonances are clearly recognized (Table I) and the coupling patterns of the methylene and methine protons flanking the tellurium can be easily extracted. The two-proton multiplet centered at 2.66 ppm represents the C-23 methylene protons and appears to be a ABA'B' four-spin system as might be expected if the system is subjected to restrained rotation. The septet centered at 3.41 ppm represents the methine proton of the terminal isopropyl group and is partially obscured by the one-proton multiplet at 3.58 ppm. The latter resonance represents the axial C-3α proton. These nuclear magnetic resonance data are summarized in Table I and are compared to data for the isomeric 5β-steroids.

The methodology that has been developed in the present study should be applicable for the preparation of a wide variety of 24-nor-23-(alkyl telluro) steroids. The preparation of such compounds could be of considerable practical importance since we have recently demonstrated the concentration in rat adrenals of Te-123m-labeled 3β-hydroxy-24-nor-23-

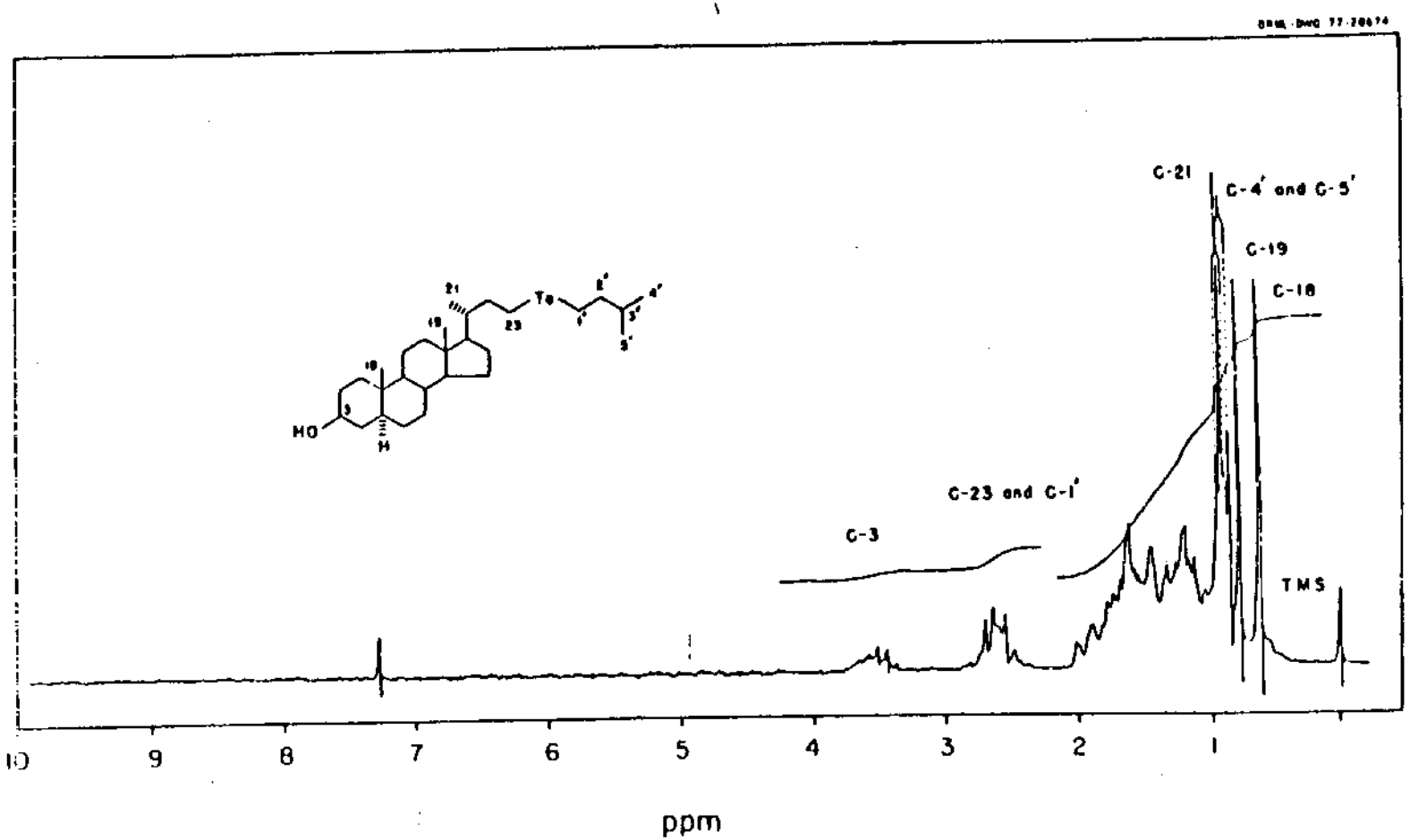


Fig. 3. The 100 MHz Nuclear Magnetic Resonance Spectrum of 3β-Hydroxy-24-nor-23-(isopentyl telluro)-5α-cholane (X).

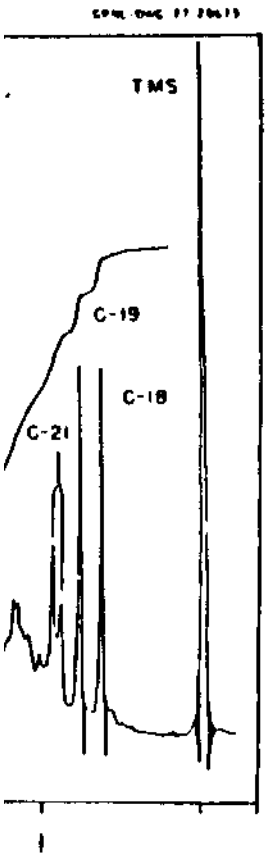
Table I. The Chemical Shifts and Coupling Constants for Selected Protons Detected In the 100-MHz Nuclear Magnetic Resonance Spectra of 3-hydroxy-24-nor-23-(alkyl telluro) Steroids

Proton	24-Nor-23-(isopentyl telluro)		24-Nor-23-(isopropyl telluro)	
	5β-Cholan-3α-ol (IX)	5α-Cholan-3β-ol (X)	5β-Cholan-3α-ol (XI)	5α-Cholan-3β-ol (XII)
C-3	∿3.58[a] (m)	∿3.62 (m)	∿3.57 (m)	∿3.58 (m)
C-18	0.62 (s)	0.69 (s)	0.63 (s)	0.66 (s)
C-19	0.92 (s)	0.84 (s)	0.92 (s)	0.79 (s)
C-21	∿0.93[b]	0.97 (d)	0.93 (d)	0.92 (d)
		J ≃6Hz	J ≃6Hz	J ≃6Hz
C-23 C-25	2.60[c] (m)	2.60[c] (m)	∿2.64 (m) 3.38 (h)	∿2.66 (m) 3.41 (h)
C-26, 27	0.95 (d)	0.94 (d)	1.60 (d)	1.61 (d)
	J ≃6.5Hz	J ≃6.5Hz	J ≃6.5Hz	J ≃6.5Hz

[a] Only approximate (∿) chemical shift values are given for broad or overlapping resonances.

[b] The downfield wing of this doublet could not be determined due to overlapping resonances.

[c] Only approximate centers could be determined for these broad, overlapping resonances. In these two instances, however, the total area under the multiplets integrated for four protons.



y–

(isopropyl telluro)-5α-cholane [30,31]. These studies have shown that Te-123m-labeled 24-nor-23-(alkyl telluro) steroids represent a new class of potential adrenal imaging agents.

ACKNOWLEDGEMENTS

The author would like to thank B. M. Benjamin and L. L. Brown for performing the nuclear magnetic resonance analyses and D. C. Canada, C. A. Pritchard, and W. T. Rainey, Jr., for helpful discussions and determination of the mass spectral measurements.

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Exhibit A

OAK RIDGE NATIONAL LABORATORY
OPERATED BY
UNION CARBIDE CORPORATION
NUCLEAR DIVISION

POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37830

Dr. Albert Segaloff
Alton Ochsner Medical Foundation
1520 Jefferson Highway
New Orleans, LA 70121

Dear Dr. Segaloff:

Please find enclosed three copies of a paper entitled "The Synthesis of 24-Nor-23-(Alkyl Telluro) Steroids" which we are submitting for consideration for publication in Steroids.

Sincerely yours,

Furn F. Knapp, Jr., Ph.D.

FFK:nyw

Enclosures

DISCLOSURE

FROM

UNION CARBIDE CORPORATION, NUCLEAR DIVISION

DA CASE NO.: S-49,068 CNID NO. 3649

NVENTOR: Furn F. Knapp, Jr.

BJECT: IMPROVED TISSUE-SPECIFIC SCINTIGRAPHIC IMAGING AGENTS

STRACT: The present development is a new composition of matter comprising steroids, amino acids, and the like labeled with the radioactive nuclide tellurium-123m. These ^{123m}Te-labeled compounds are useful as tissue-specific, scintigraphic imaging agents in diagnostic nuclear medicine procedures. This particular nuclide has multiple advantages as a radioactive label over some prior art nuclides.

SCRIPTION: Background

Adrenal disease is a commonly observed clinical disorder and the excessive or subnormal production of adrenal steroids (corticosteroids) results in severe physiological consequences. The hypercorticoid condition (Cushing's Disease) results from the overproduction of corticosteroids and can have a variety of primary causes. Large amounts of corticosteroids are produced by carcinomas and ademas of the adrenal glands. The involvement of corticosteroid-producing tumors is often unilateral, involving only one adrenal gland. Since there are two adrenal glands it is essential for the physician to locate the specific gland which is involved in the overproduction of corticosteroids. Clinical procedures which are presently used to assess adrenal function include the determination of steroid levels by adrenal venography and

the use of contrast venography to visualize the adrenal gland by normal x-ray transmission analysis. It is generally recognized that adrenal vein catheterization is a difficult and dangerous procedure which few physicians are adequately trained to perform. The use of an agent labeled with a gamma-emitting radionuclide which would concentrate in the adrenal glands would greatly aid the physician in the diagnosis of adrenal disorders. In addition, this diagnostic method would also serve as an adjunct in cases where the use of an invasive procedure is also required. Such a noninvasive procedure would guide the surgeon to the specific adrenal gland that must be removed, or from which a tumor must be excised.

Presently both ^{131}I- and ^{75}Se-labeled steroids are being evaluated by other groups as potential adrenal imaging agents. Results in laboratory animals with these agents have indicated acceptable adrenal accumulation of these agents and good quality images of dog adrenal glands have been obtained. One of these compounds, ^{131}I-19-iodocholest-5-en-3β-ol, has undergone extensive human tests and has been used to successfully diagnose a number of adrenal disorders. The radioiodinated steroids are very unstable in vivo. As a result of this in vivo instability, a high thyroid accumulation of radioactive iodine is encountered which cannot be entirely overcome by predisposition of the patient with thyroid blocking agents. The use of the ^{131}I-labeled agents also results in a high patient beta-adsorbed dose due to the high beta yield from this nuclide (major β^- at 610 keV). In addition, the short physical half-life of the ^{131}I nuclide results in a short

shelf life. The relatively time-consuming multi-step synthesis of the ^{131}I-labeled steroids coupled with their short shelf life indicates that patient costs will be relatively high.

The ^{75}Se-labeled steroid overcomes problems associated with the short shelf life, high beta dose and in vivo instability encountered with the ^{131}I-labeled agents. The ^{75}Se nuclide, however, decays with the emission of several high energy photons which results in inefficient collimation and poor quality images.

The early diagnosis of pancreatitis and pancreatic carcinoma is a common clinical problem. The use of ^{75}Se-labeled selenomethionine (Fig. 1, V.b.) was originally developed because of the biological importance of the amino acid, methionine, and the availability of the gamma-emitting ^{75}Se nuclide. Furthermore, ^{75}Se was readily incorporated into selenomethionine by both microbiological and chemical methods. Selenomethionine (V.b.) behaves similarly to methionine (V.a.) in vivo and is concentrated in a number of animal species by the pancreas and other tissues that are involved in active protein synthesis. The multiple high-energy gamma emissions of ^{75}Se, however, result in poor images with an unnecessary radiation dose to the patient.

Summary of the Invention

In structurally-related organic compounds which are tissue-specific to absorption in special organs, said organic compounds being derivatized with a radionuclide for the purpose of scintigraphic examination of said special organs in vivo, the present invention is an improvement comprising organic compounds derivatized with the radionuclide ^{123m}Te.

Detailed Description

Because of the importance and need for radionuclide-labeled steroids, as pointed out in the Background Section, extensive application of the invention is embodied in these compounds. The general scheme for the synthesis of steroids labeled in a sidechain with ^{123m}Te is by coupling steroidal halides or other functionalized forms of a steroid substrate with ^{123m}Te-labeled reagents. This general scheme is illustrated below.

$$\text{Steroid-X} + \text{R-}(^{123m}\text{Te})\text{-Y}$$

$$\text{Steroid-}(^{123m}\text{Te})\text{-R} + \text{Y}$$

The steroid substrate contains a group (X) that can be a halide, p-toluene sulfonyl, methyl sulfonyl, or similar functionality. The ^{123m}Te-labeled reagent contains the group (Y) which would generally be an alkali metal. The general strategy is that the driving force for the coupling of substrate and reagent is the formation of the (X-Y) species. The (R) group can represent a variety of organic moieties.

A more specific route involves the coupling of steroidal sidechain halides with alkali metal salts of alkyl tellurols. The steroidal sidechain halides can be prepared from bile acids or related steroidal acids via a Hunsdiecker-type degradation or by the action of reagents such as acyl halides or phorphorus halides on steroidal sidechain alcohols. An alternate route involves the reaction of the steroidal sidechain alcohols with the R_3P_2 species formed by reaction of triphenyl phosphine and a carbon tetrahalide. The reaction of steroidal sidechain halides with alkali metal salts of alkyl tellurols is an attractive route and has been studied in detail. Since these unusual

steroids have not been previously prepared, a detailed description of the preparation and physical and chemical properties of a representative 24-nor-23-(alkyl telluro) steroid is presented (Example I). A monohydroxy bile acid such as lithocholic acid or allolithocholic acid can be conveniently converted to the corresponding 24-norbromide via a modification of the Hunsdiecker degradation as illustrated in Fig. II. This reaction proceeds in reasonable yield and the synthesis and purification of the norbromides can be completed in a single day.

The steroidal norbromides can be coupled with sodium alkyl tellurols (NaTeR) which are formed by reduction of dialkyl ditellurides (Te_2R_2) in basic solution. The ditellurides are formed by alkylation of sodium ditelluride (Na_2Te_2). One convenient method for the generation of the sodium ditelluride that can be conveniently adapted to the microscale consists of reacting the metallic tellurium with a stoichiometric amount of metallic sodium in liquid ammonia. The sodium ditelluride is then alkylated by the addition of the desired alkyl halide, alkyl sulfate, etc. The dialkyl ditelluride can be reduced by one of a variety of methods. One method which is simple and uses reagents, whose products do not interfere with subsequent transformations, is reduction of the ditelluride with sodium borohydride in an organic solvent in the presence of base. The sodium alkyl tellurol is generated in this manner <u>in situ</u> and is readily available for subsequent reaction with the steroid substrate. The final product from this series of reactions is the 24-nor-23-(alkyl telluro) steroid and it can be easily purified either by thin-layer chromatography or by absorption column chromatog-

Using this method essentially any 24-nor-23-(alkyl telluro) steroid can be prepared. To alleviate disadvantages of the use of ^{75}Se as described earlier, ^{123m}Te-labeled telluromethionine (V.e.) has been previously suggested as a superior alternative for pancreatic imaging. Attempts to prepare ^{123m}Te-labeled telluromethionine by microbiological techniques have been unsuccessful. Our first attempts to prepare telluro amino acids by methods involving the introduction of the benzyl telluro moiety failed primarily because of the extreme instability of dibenzyl ditelluride. In addition, the benzyl methylene carbon-tellurium bond is unstable even in simple benzyl alkyl tellurides. These properties preclude the preparation of the requisite benzyl telluro intermediates. Our early studies of factors affecting the formation and stability of ditellurides and tellurides proved that phenyl alkyl tellurides are much more stable than simple dialkyl tellurides because of the stabilizing effect of the aromatic ring. Thus a scheme was devised for the preparation of a representative α-amino acid containing the phenyl telluro moiety (Fig. I). The method developed should be of general applicability for the synthesis of a variety of telluro amino acids. Its success results from strictly avoiding any attempts to isolate the tellurol intermediates. Such intermediates (I) are quite useful synthetically when generated in situ, however, and are formed by reduction of the precursor ditellurides under an inert atmosphere. The method is illustrated in Examples III and IV.

Embodiments of the Invention

Compounds that have been prepared in this laboratory using the

method described above include:

24-nor-23-(isopropyl telluro)-5α-cholan-3β-ol

24-nor-23-(isopropyl telluro)-5β-cholan-3α-ol

24-nor-23-(isopentyl telluro)-5α-cholan-3β-ol

24-nor-23-(isopentyl telluro)-5β-cholan-3α-ol

24-nor-23-(phenyl telluro)-5β-cholan-3α-ol

24-nor-23-(octyl telluro)-5α-cholan-3β-ol

24-(isopropyl telluro)-chol-5-en-3β-ol

24-(isopropyl telluro)-chol-5-en-3β-OMe

17 -(isopropyl telluro methyl)-androst-5-en-3β-ol

Example I

The synthesis of 24-nor-23-(isopropyl telluro)-5α-cholan-3β-ol is described. Diisopropyl ditelluride (70 mg, 200 μmoles) was dissolved in 10 ml of methanol in an argon atmosphere with gentle warming. Sodium borohydride was added in small portions to the orange solution until a clear, colorless solution was obtained. Following the addition of 1 N sodium hydroxide solution (1 ml, 1 mmole) the 24-nor-23-bromo-5α-cholan-3β-yl acetate (45 mg, 100 μmoles, prepared by Hunsdiecker degradation of allolithocholic acid) was added. The solution was refluxed one hour after which time thin layer chromatographic analysis indicated the reaction to be complete. The solution was poured into water and extracted thoroughly with chloroform. Chloroform or ethyl acetate (but not ether) efficiently extract the product from the crude mixture. The combined chloroform extracts were washed thoroughly with water, dried over anhydrous sodium sulfate, and the solvent

removed in vacuo to give a thick gum. This material was purified by preparative thin-layer chromatography (solvent system, chloroform, R_f 0.14). The purified product consisted of 15.6 mg of a thick gum which solidified to a white solid upon trituration with ether, m.p. 118-119°C. The ultraviolet spectrum (EtOH) contained maxima at 225, 237, and 268 nm. The infrared spectrum (KBr) contained a maximum absorbence at 3420 cm^{-1} (-OH). High resolution mass spectral measurements indicated a molecular formula of $C_{26}H_{46}OTe$ (found: 502.2509; calculated for $C_{26}H_{46}OTe$:502.2589).

The tissue distribution, metabolic fate, and excretion of radioactivity following intravenous administration of ^{123m}Te-labeled 24-nor-23-(isopropyl telluro)-5α-cholan-3β-ol have been studied in detail in both male and female Fischer strain rats. The results of these studies with male rats are summarized in Figs. III through V. Male rats were injected via the tail vein with the ^{123m}Te-labeled steroid (6-15 μCi) in a saline-ethanol-Tween 80 emulsion and were sacrificed at various time intervals varying from one hour to three weeks later. The organs were removed, weighed and counted, and in this manner the distribution of radioactivity was determined (% dose/g). These data are shown in Figs. III and IV. The urine and feces of these animals were also monitored for radioactivity and these results (Fig. V) indicated that approximately 50% of the administered radioactivity was excreted in

in five days. In addition, several animals were injected with a large dose of the ^{123m}Te-labeled steroid (100 μCi) and examined with a rectilinear scanner at various time periods from one hour to three weeks after injection. The adrenal glands of these animals were clearly visible even one day after injection, and very clear images were obtained one week later. Representative scintigraphic images obtained with both a rectilinear scanner equipped with a 60-hole gold collimator and an RC type proportional counter with a xenon gas detector are illustrated in Figs. VI and VII. Some animals were sacrificed after such studies and the adrenals, liver and lungs removed, homogenized in Folch medium, and the resulting organic soluble material chromatographed on silicic acid columns. The resulting chromatographic profiles indicated that the administered ^{123m}Te-labeled steroid was converted to a number of metabolites in these rat tissues. Since the tissues were treated in the same manner, the differences observed upon chromatographic analysis of the extracted lipid soluble material must indicate that the detected radioactivity did not represent artifacts that accumulated during the tissue manipulation. These combined results have demonstrated that radioactivity from ^{123m}Te-labeled steroid accumulates in the rat adrenal glands, that the steroid is evidently metabolized by rat tissues, and that excellent images of rat adrenal glands can be obtained.

Example II

Preparation of ^{123m}Te-labeled 24-nor-23-(isopropyl telluro)-5α-cholan-3β-ol

The ^{123m}Te nuclide can be prepared via neutron irradiation of isotopically enriched ^{122}Te. The microscale synthesis of the ^{123m}Te-labeled steroid was accomplished by the general method described earlier for the synthesis of 24-nor-23-(isopropyl tellura)-5α-cholan-3β-ol (Example I). The system was easily adaptable to the 200-500 mole scale. In a typical preparation reactor-produced ^{123m}Te (22 mg, 25.78 mCi) was combined with carrier tellurium (45 micron powder) to yield a final specific activity of 25.78 mCi/mmole. Under an argon atmosphere approximately 25 ml of liqiid ammonia was distilled into the reaction vessel which contained the tellurium powder. The vessel was cooled to -60 to -70°C in an acetone-CO_2 bath. Freshly cut metallic sodium (25 mg, 1 mmole) was added to the rapidly stirred slurry. The solution was stirred two hours and progressed through the typical color change: yellow green blue red. Isopropyl iodide (174 mg, 1 mmole) was added by means of a Hamilton syringe inserted through a rubber septum. The initial deep red color of the solution slowly turned to a yellow-amber hue concomitant with the appearance of colloidal tellurium. After being stirred for one hour the ammonia was allowed to evaporate under a stream of argon yielding a residue consisting of an orange gum containing metallic tellurium. The residue was extracted with several small portions of benzene (15 ml

total volume) and the orange-colored combined extract washed well with water. The benzene solution was diluted with methanol to 25 ml in a volumetric flask and aliquots taken for counting. The benzene-extracted material consisted of 10.75 mCi of activity indicating a 42% yield of ^{123m}Te-labeled diisopropyl ditelluride. The ditelluride solution was combined with an additional 25 ml of methanol and the mixture stirred vigorously under an argon atmosphere. Small portions of sodium borohydride were added until a colorless solution was obtained, indicating complete reduction to ^{123m}Te-labeled isopropyl tellurol. Gentle warming of the ditelluride solution was generally required for the vigorous reductive process to be initiated. Approximately 80 mg (2 mmole) of sodium hydroxide was added to the colorless solution and the mixture then brought to a gentle reflux. Should the solution turn light yellow it indicates that the ditelluride has re-formed as a result of oxygen being introduced. Sodium borohydride can therefore be added in small aliquots until an amber solution is obtained. The 24-nor-23-bromo-5α-cholan-3β-ol acetate (Fig. II) (112 mg, 250 μmole) was added as a slurry in a small volume of benzene and the mixture refluxed for one hour. At this time, thin layer chromatographic analysis of an aliquot indicated the reaction to be complete. The solution was poured into water and the organic layer washed several times with water. The yellow-colored

benzene solution was applied to a silicic acid column slurried in benzene. Fractions 25 ml in volume were collected by elution with increasing concentrations of ethyl ether in benzene. Aliquots (100 µl) of each fraction were taken for counting.

The ^{123m}Te-labeled steroids listed below have been prepared by this procedure.

24-nor-23-(isopropyl telluro)-5β-cholan-3α-ol

24-nor-23-(octyl telluro)-5α-cholan-3β-ol

24-(isopropyl telluro)-chol-5-en-3β-ol

24-(isopropyl telluro)-chol-5-en-3β-OMe

17β-(isopropyl telluro methyl)-androst-5-en-3β-ol

Example III

For the preparation of a model telluro amino acid, diphenyl ditelluride was reduced with sodium borohydride in methanol under an argon atmosphere. The resulting phenyl tellurol was then coupled with 5-(bromoethyl) hydrantoin, Fig. I (III), which was conveniently prepared by known methods from DL-homoserine, Fig. I (II). The reaction resulted in a high yield of 5-(phenyl telluro ethyl)-hydrantoin (IV.a.). This unusually substituted hydrantoin is stable when stored in the dark as a solid at 4°C. It was fully characterized and exhibited the expected physical and chemical properties. Treatment of this hydrantoin with 1 N NaOH in a teflon-lined bomb at 160°C resulted in hydrolysis to DL-α-amino-γ-(phenyl telluro)

butyric acid (V.d.). Although some decomposition was detected, the product was isolated in reasonable yield and was fully characterized by the usual methods. The ^{123m}Te-labeled amino acid (V.e.) was prepared from reactor-produced ^{123m}Te. The microscale synthesis of diphenyl ditelluride was accomplished by reaction of the ^{123m}Te with phenyl magnesium chloride. The ^{123m}Te-labeled diphenyl ditelluride was then reduced, coupled with the hydantoin, and the labeled butyric acid (V.e.) then obtained by the methods described above. The physical properties of the labeled amino acid were identical to those determined for the unlabeled product, and radiochemical homogeneity was established chromatographically.

Example IV

Using this same method we have synthesized telluromethionine (V.c.). Special precautions must be taken in this case because of the high volatility of dimethyl ditelluride and methyl tellurol (I.b.). In addition, the 5-methyl telluro ethyl) hydantoin (IV.b.) must be isolated by a different method from that used to isolate 5-(phenyl telluro ethyl) hydantoin (IV.a.). Dimethyl ditelluride has been prepared by reaction of methyl iodide with sodium ditelluride. Reduction of the ditelluride to methyl tellurol with subsequent coupling with 5-(bromo ethyl) hydantoin (III) then gave (IV.b.). The 5-(methyl telluro ethyl) hydantoin has been purified and characterized.

The invention should not be considered as being limited to the tissue-specific compositions herein described. In addition to the introduction of the ^{123}Te nuclide into the C-24 position of the steroid sidechain this method can be adapted for the introduction of this nuclide into any other desired position of the steroid sidechain. Examples would include the following fabrications: C-17-(alkyl telluro)-, 23-nor-22-(alkyl telluro)-, C-20-(alkyl telluro)-, C-25-(alkyl telluro)-, etc. The limiting factor for such syntheses would be the availability of the appropriate steroid starting materials. In addition, essentially any desired alkyl group could be introduced into the steroid sidechain using this method. These could include functionalized alkyl groups or groups that would be amenable to functionalization after their incorporation into the sidechain. In the latter case the introduction of such substituents would be a function of (a) the compatibility of the presence of the alkyl substituents to the coupling procedures and/or (b) the availability of the desired alkyl group. With regard to scintigraphic analyses using these agents, other tissues besides the adrenal glands could conceivably be imaged with this agent.

The general method was developed also for the preparation of telluro amino acids. The first such compound, DL-α-amino-γ-(phenyl telluro ethyl) butyric acid and tellurmethionine have been prepared. The synthesis is easily adapted to the microscale and, providing the requisite halo-alkyl hydantoin intermediates are available, the preparation of a variety of telluro amino acids is possible.

.ATED ART: The related art of this invention is contained in the Background Section of this disclosure.

)BABLE
.UE: The use of a ^{123m}Te-labeled agent would overcome a number of disadvantages. The ^{123m}Te nuclide decays with the emission of a single gamma photon in 84% abundance with an energy of 159 keV which is ideally suited for the scintigraphic instruments used clinically in nuclear medicine. Secondly, the ^{123m}Te-labeled steroid can be prepared in a simple three-step sequence from readily available starting materials. The long physical half-life and the attractive radionuclidic properties would suggest a relatively low patient cost. Furthermore, the reasonably short biological half-life of this agent in rats would suggest that the long physical half-life of ^{123m}Te (e.g., 119 days) would not be as severe a problem as might be anticipated. Finally the per cent adrenal dose is much higher for the ^{123m}Te steroid than that reported for the ^{131}I- and ^{75}Se-labeled steroids. This would indicate that a lower overall dose would be required for adequate adrenal imaging.

It is anticipated that ^{123m}Te-labeled steroids will be useful adrenal imaging agents for the clinical diagnosis of adrenal disorders and that the likewise-labeled amino acids will be useful in the imaging of the pancreas. The greatest interest and subsequent utilization of such ^{123m}Te-labeled agents will probably be generated in the general medical community. Favorable response would indicate that the preparation and distribution of ^{123m}Te-labeled tissue imaging agents would be developed also in the private industrial sector.

MENDATION: The filing of a patent application may be warranted.

PERTINENT FACTS:

Information obtained from: Report of Possible Invention or Discovery form

Verbal reference number: 4818 (ORNL)

Contract involved: W-7405-eng-26

Inventors' home addresses: [redacted]

[redacted] Tennessee [redacted]

Date of origin of idea: ------ Recorded in: A-7619 (RK-1-131)

Date of first sketch or drawing: Recorded in: A-7705 (RK-2-117)

Date of first written description: Recorded in: "

Date of first model or test unit: Recorded in: "

Date of first test of invention: Recorded in: A-7789 (RK-3-107)

Date of First Disclosure to Others:

(a) Name: J.K.Poggenburg Date [redacted] Recorded in: A-7860 (RK-4-75)

(b) Name: D.V.Woo Date: " Recorded in: "

Date of First Written or Oral Disclosure to Public (Abstract)

Where Disclosed: J1. Nuc. Med., V. 18, No. 6, p. 600

EPARED BY: [signature] DATE:

PROVED BY: [signature] DATE:

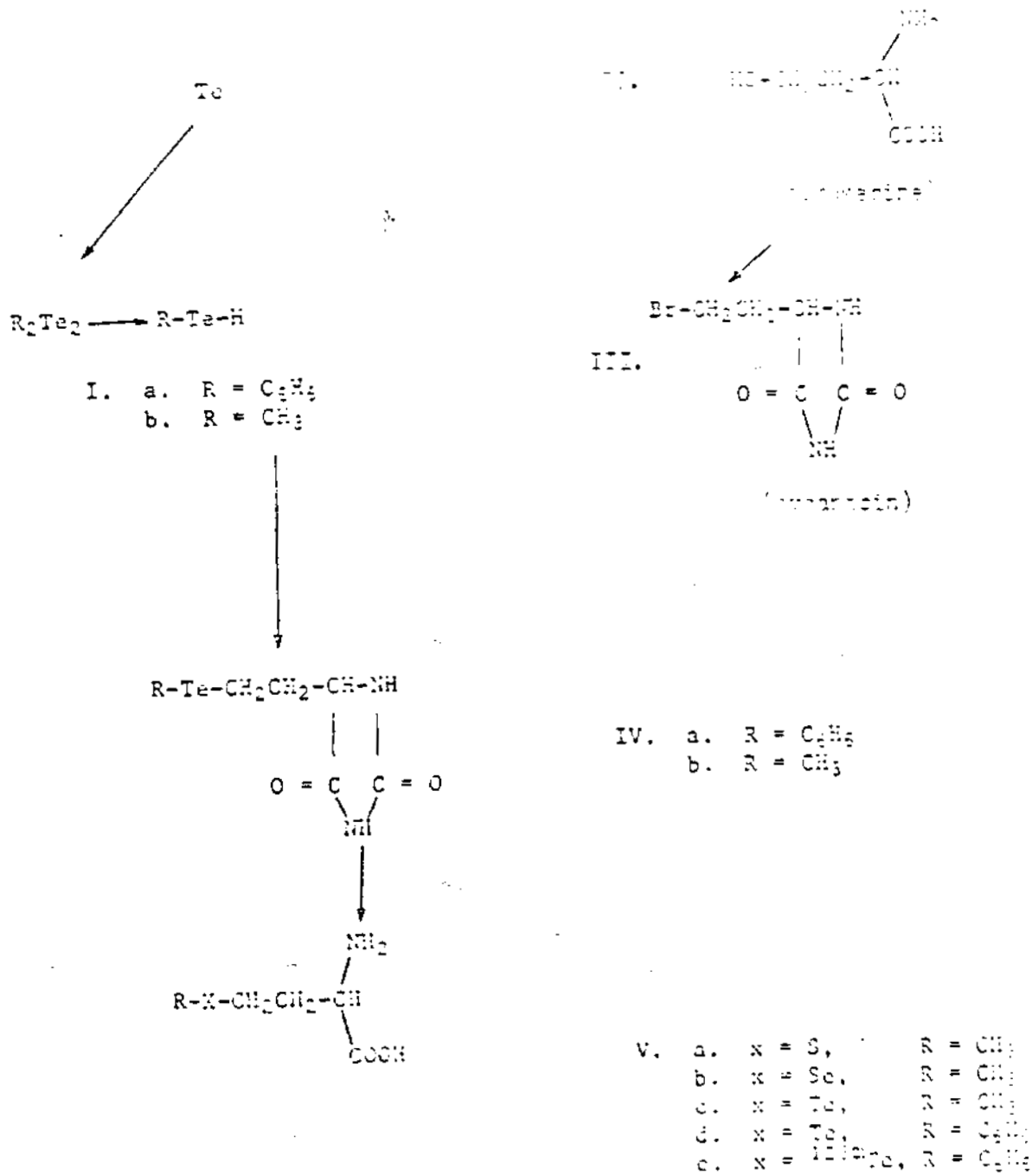

Fig. 1. The Synthesis of Tellurium Amino Acids.

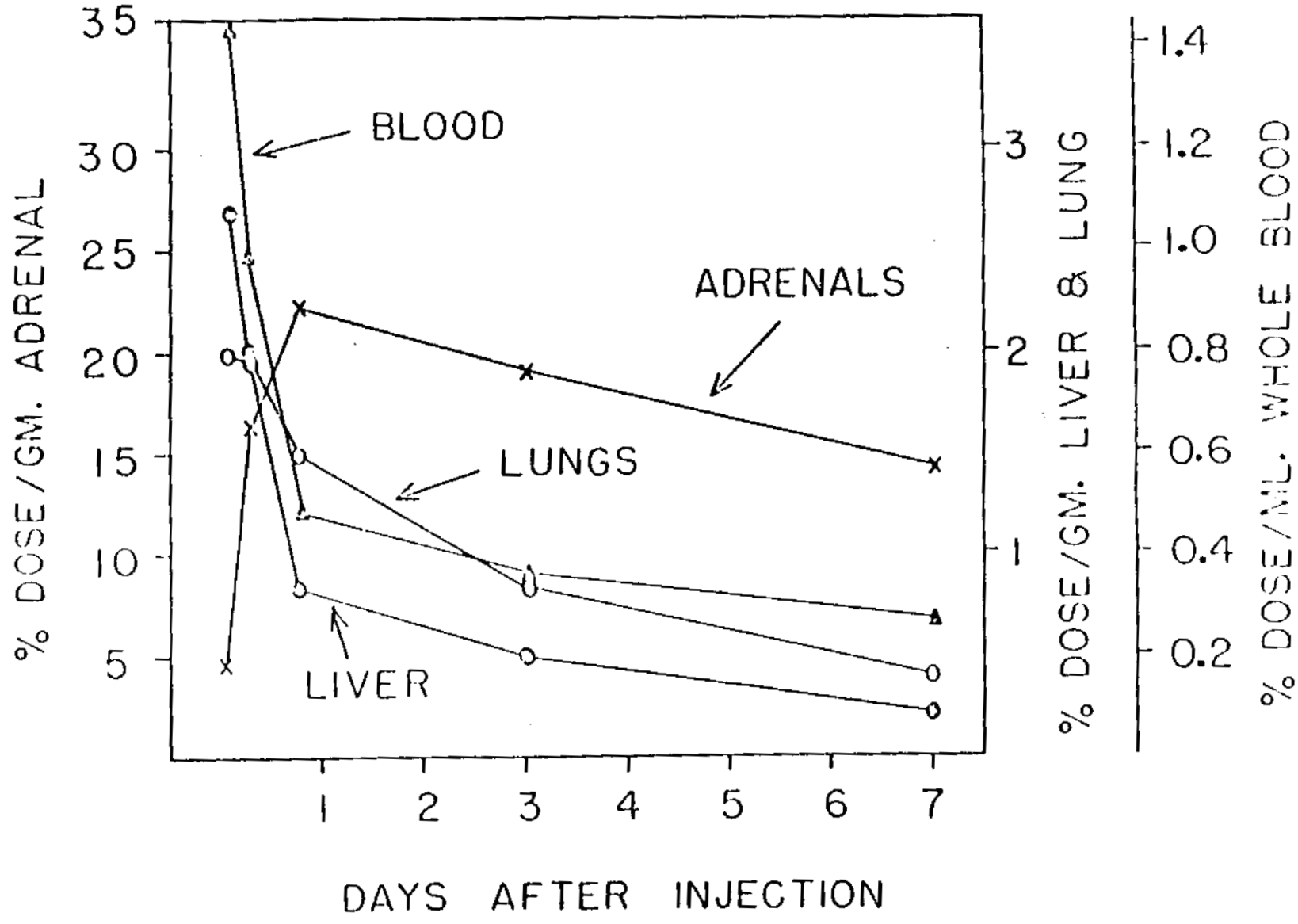

Figure III. The per cent dose of administered radioactivity to selected organs reflecting [illegible] of [illegible] labeled 3-(isopropyl telluro)-3α-cholane to male [illegible].

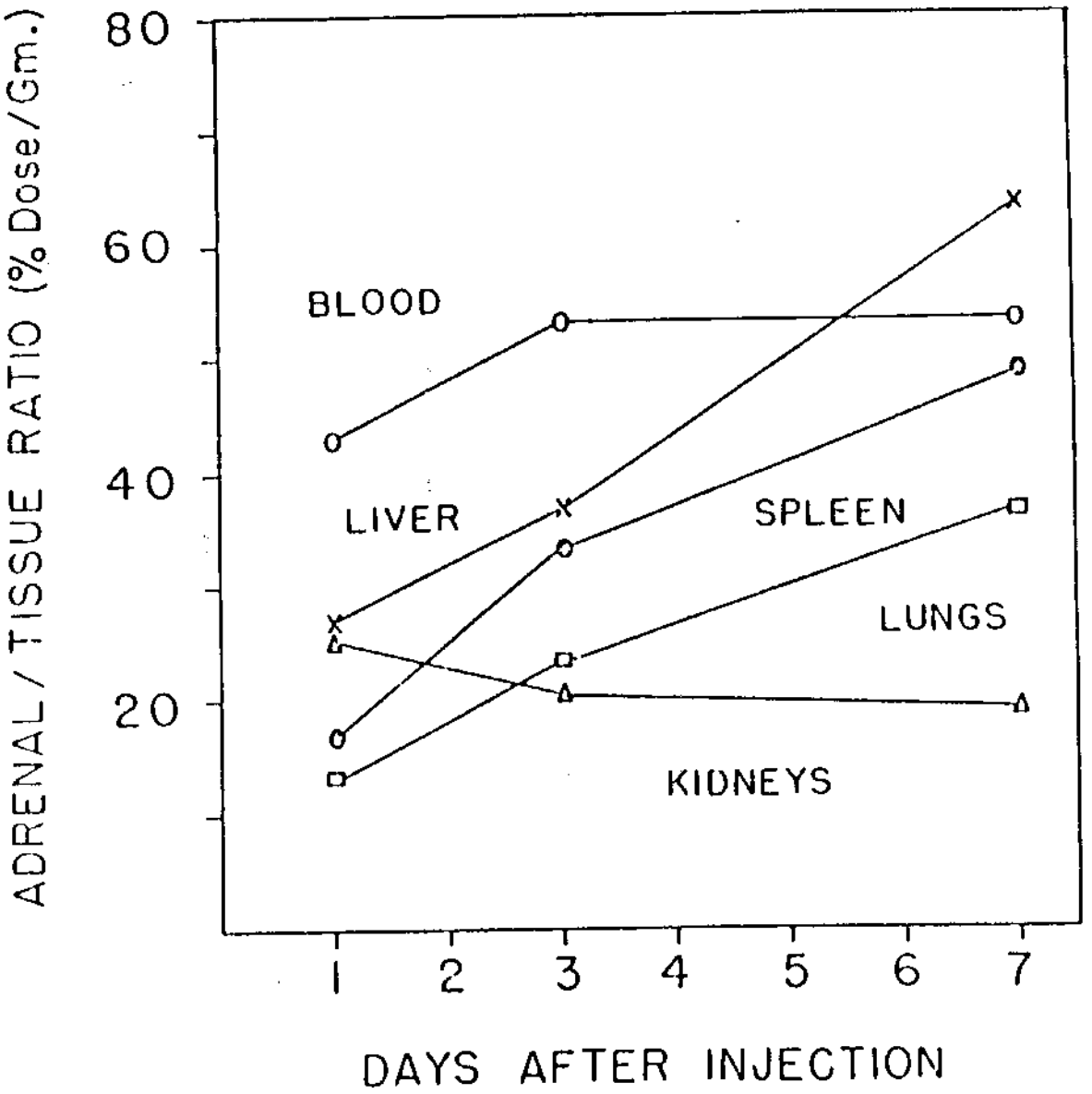

Figure IV. The adrenal/tissue ratios in male rats following the administration of [illegible]

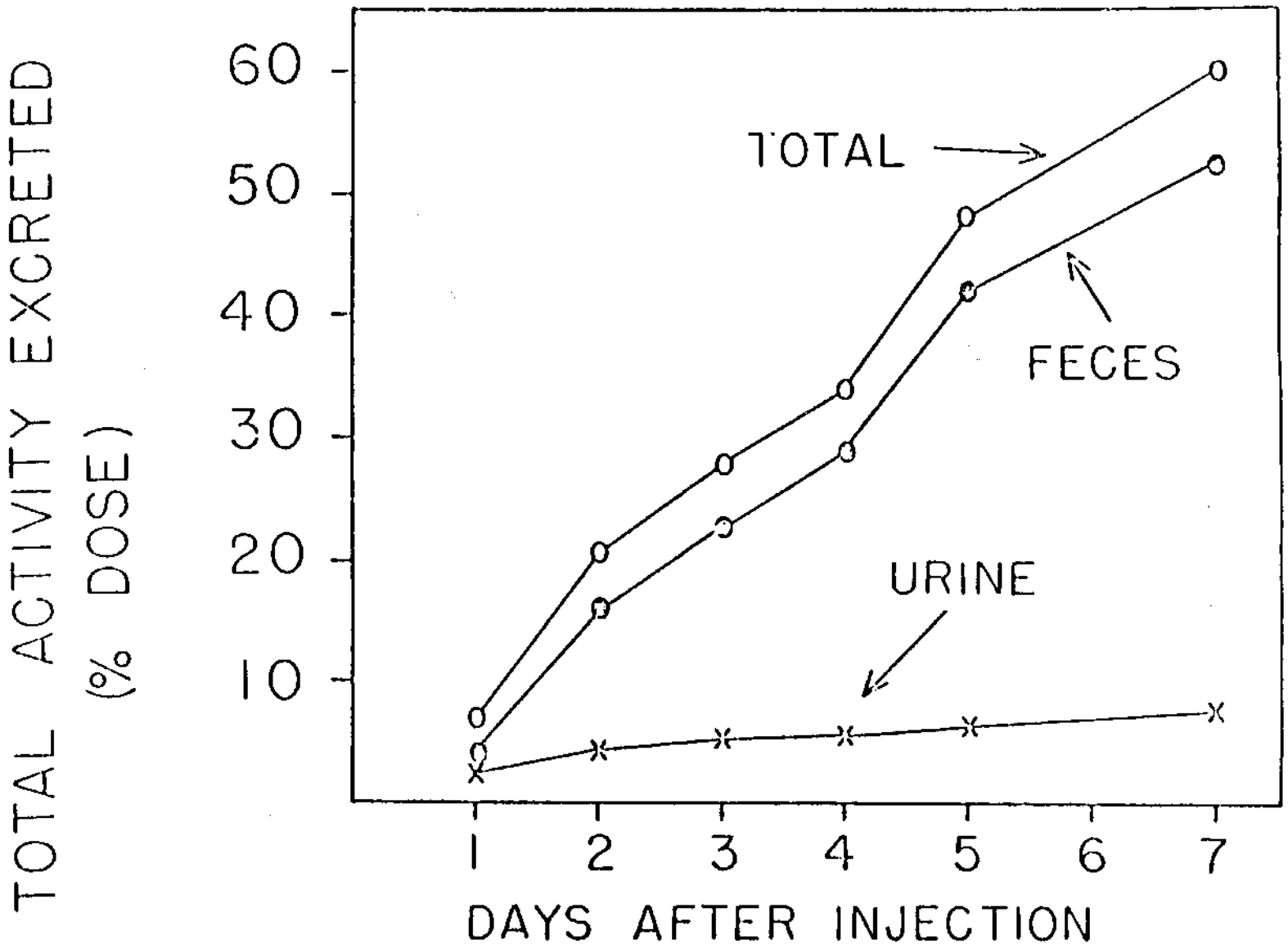

Figure V. Excretion of radioactivity in the urine and feces following the administration of ^{123m}Te-labeled 3β-hydroxy-25-nor-27-(isopropyl telluro)-5α-cholane to male rats.

Figure VI a

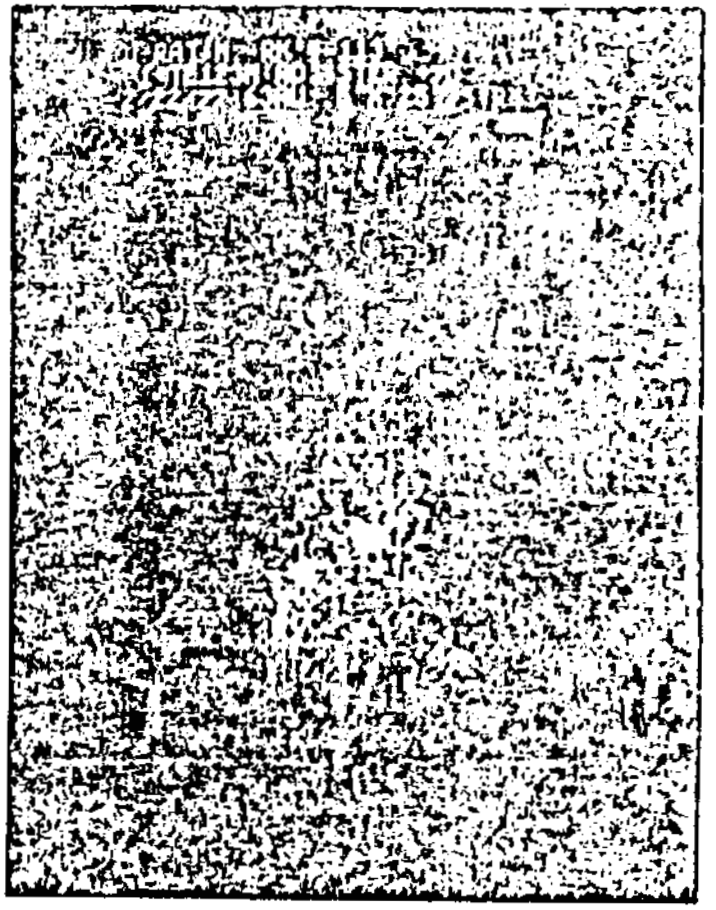

Figure VI b

Figure VI. Posterior views of a male rat seven days after the administration of 185 μCi of ^{123m}Te-labeled β-hydroxy-3h-nor-2β-(isopropyl tellure)-17α-ethane. The image shown in Figure Va was obtained with an RC-type proportional counter camera with a xenon gas detector. The same rat was imaged (Figure Vb) with a rectilinear scanner equipped with a high resolution gold collimator. In both images the centrally located hot (bright spots are the adrenal glands.

ORNL-PHOTO 0273-77

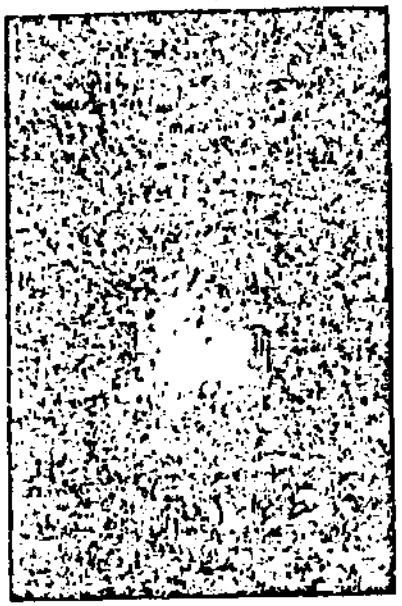

Figure VII a

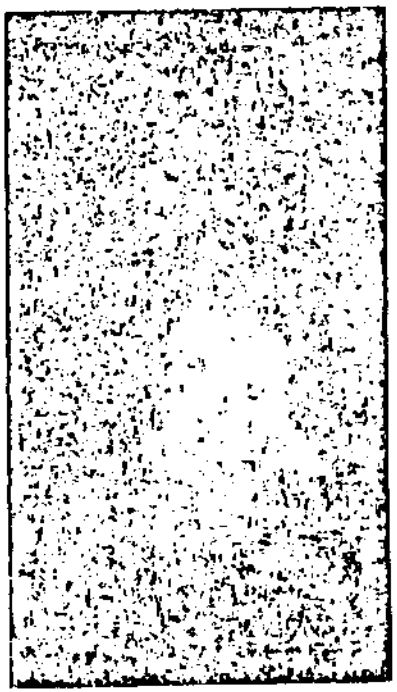

Figure VII b

Figure VII. Posterior views of a female rat two days after the administration of [^{125}I]-labeled 17β-hydroxy-4-nor-3-(isopropyl tolimo)-17α-ethynyl-. The image shown in (a) was obtained with an E2-type proportional counter camera with a xenon gas detector. The rat was imaged (Figure VIb) with a rectilinear scanner equipped with a high resolution collimator. In both images the two centrally located hot (bright) spots are the uterine horns and the two smaller peripherally located hot spots are the ovaries.

Exhibit C

Synthesis of Te-123m Labeled D,L-α-Amino-β-(Phenyl Telluro) Butyric Acid

General Scheme similar to that described on RK-7-71, etc. :

$$\text{Te-123m} + \phi MgCl \longrightarrow \longrightarrow {}^{123m}Te_2\phi_2 -$$

$$\phi\,{}^{123m}Te\text{-}H + Br\text{-}CH_2CH_2CH(\text{succinimide})$$

$$\downarrow$$

$$\phi\text{-}{}^{123m}Te\text{-}CH_2CH_2CH(\text{succinimide})$$

$$\xrightarrow{NaOH}$$

$$D,L\text{-}\ \phi\text{-}{}^{123m}Te\text{-}CH_2CH_2\text{-}CH(NH_2)(COOH)$$

Te-123m (see 7-58; Batch #2; 30.84 mCi; prepared by AFC) + Te carrier (45 μg) = total 25[illegible]

TF-123X DIPHENYL DITELLURIDE

100 MICROLITERS/25 ML

00 0 022222

20 CM/200 SEC.

SAMPLE #1

310288 310288 310288 310288 000000
318874 318874 318874 318874
$\frac{30702}{200} \times \frac{1}{.84} \times 1.34\times10^{-3} \times \frac{25}{.100} \times \frac{1}{3.7\times10^{7}} = 9.06$ mC.

SSAMPLE #2
313558 313558 313558 000000
000000 000001C
322146 322146 322146 000000

9.11 mC.

$\frac{30496}{200} \times \frac{1}{.84} \times \frac{1}{1.34\times10^{-3}} \times \frac{25}{.100} \times \frac{1}{3.7\times10^{7}} = 9.15$ mC

13.72

1077230

Te stirred in r.b. flask in 5 ml. A.R. THF + small crystal of benzoyl peroxide. 1 ml. of $\phi MgCl$ (1.92 M ; ~ 2 mmoles, in THF) added & solution stirred — no red color until brought to reflux momentarily & cooled in an ice bath — allowed to equilibrate to r.t. c̄ stirring for 30 min. — some Te↓ and dark red solution.

Filtered into 25 ml. volumetric flask, etc. washed c̄ benzene which was used to dilute the THF solution of $^{123m}Te-\phi_2Te_2$ to 25 ml. — Aliquots counted

Benzene solution added to reaction flask flushed c̄ Argon — 25 ml. MeOH added & $NaBH_4$ until a colorless solution was obtained

The diphenyl ditelluride was assumed based upon activity to consist of 0.9 mmoles - To insure an excess of Φ-Te-N, 0.7 mmoles 149 mg., of 5-(bromo ethyl)-hydantoin (Lg. RK #7-) was used. The hydantoin was dissolved in ca 3 ml. MeOH and added to the Φ-Te-H solution & refluxed 30 min. - T.L.C. indicated complete reaction.

The mixture was diluted c̄ H_2O (20 ml.) & ext. thoroughly c̄ C_6H_6 (2X) and Et_2O (2X) to remove the $Φ_2Te_2$, $Φ_2Te$, etc. (Lg. the lack of much color indicated no or very little $Φ_2Te_2$ to be present suggesting the presence of unreacted Bromo hydantoin which was indeed substantiated by t.l.c.). The aqueous layer ⟶ pH 1-2 c̄ 6N HCl & extracted thoroughly c̄ Et_2O - large amount of activity - See continuation #5-74.

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save
moles

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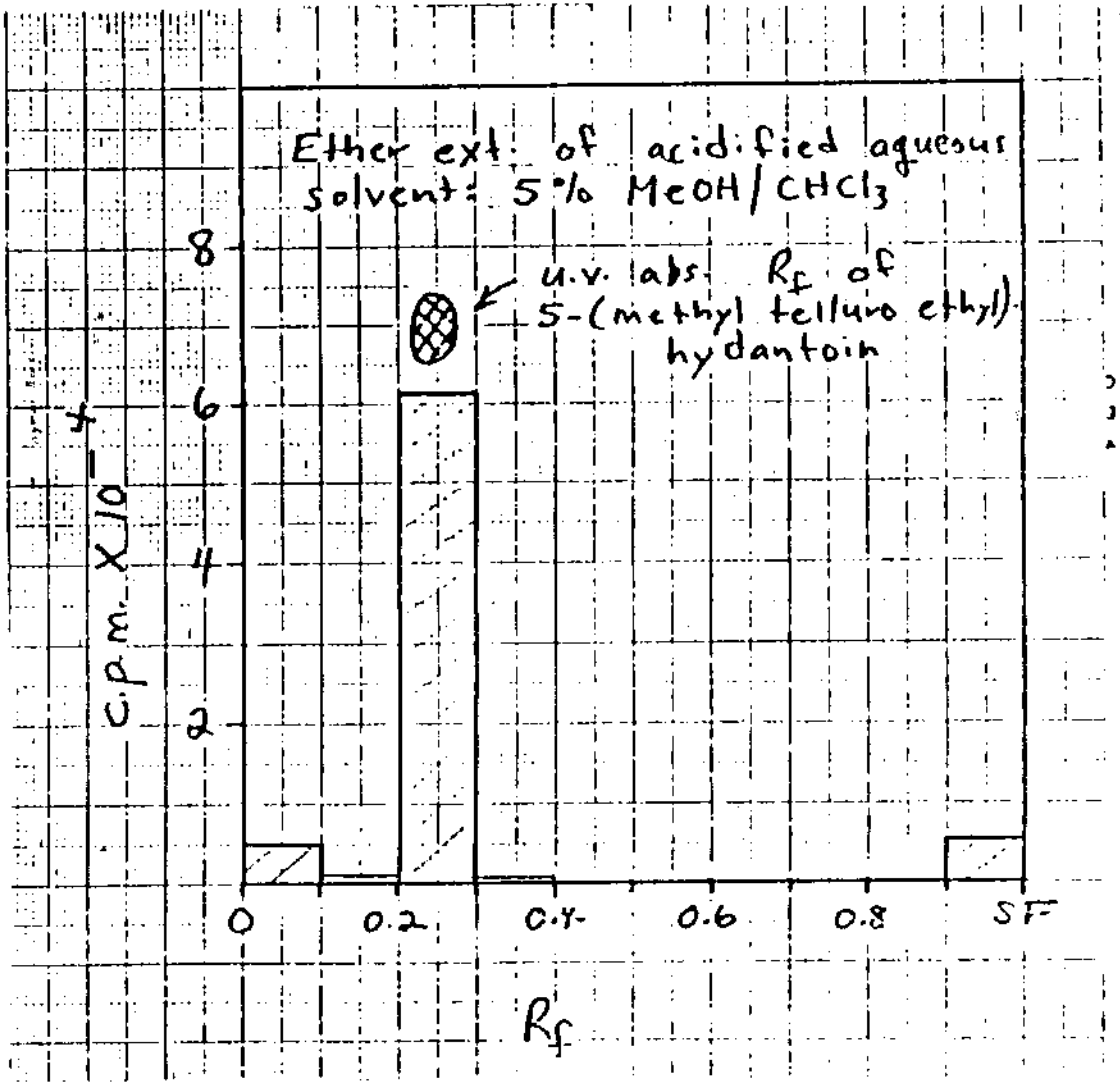
ons

0 —

200SEC 2CM TE-123M ETHER EXT. 100LAMDA OF 100ML 1430HRS

040552 040552 000000
040552 040552 000000
041533 041533 041533 000000
[illegible]
040076 040076 040076 000000
041085 041085 041085 000000

[illegible]


Ether ext. of acidified aqueous
solvent: 5% MeOH/CHCl3
u.v. abs. Rf of
5-(methyl telluro ethyl)
hydantoin
c.p.m. X10⁻⁴
8
6
4
2
0
0.2
0.4
0.6
0.8
S.F.
Rf

The ethereal solution of Te-123m labeled 5-(phenyl telluro ethyl hydantoin) was taken to dryness in vacuo. small sample saved for (Eg. white crystalline solid):

m.p.
i.r.
u.v.
m.s.

The hydantoin was dissolved in 2 ml. 1N NaOH and heated in a bomb 1 hr. at 160°C, cooled & extracted ē C_6H_6 & Et_2O - some yellow color - majority of radioactivity in the aqueous phase → pH 7-7.2 ē bN & 1N HCl, light brown solution - diluted to 5 ml. in volumetric ē H_2O and aliquots counted (See 7-75) - 258.1 μCi - filtered through millipore into sterile vial → clear solution + 5 ml normal saline - see t.l.c. 7-76

10712235

TE-123M AMILO ACID 50 LAMBDA OD 5ML OAM 000 2CM 0022 200SEC
1310HRS

SAMPLE #1

284669 284669 284669 284669
291577 291577 291577 000000

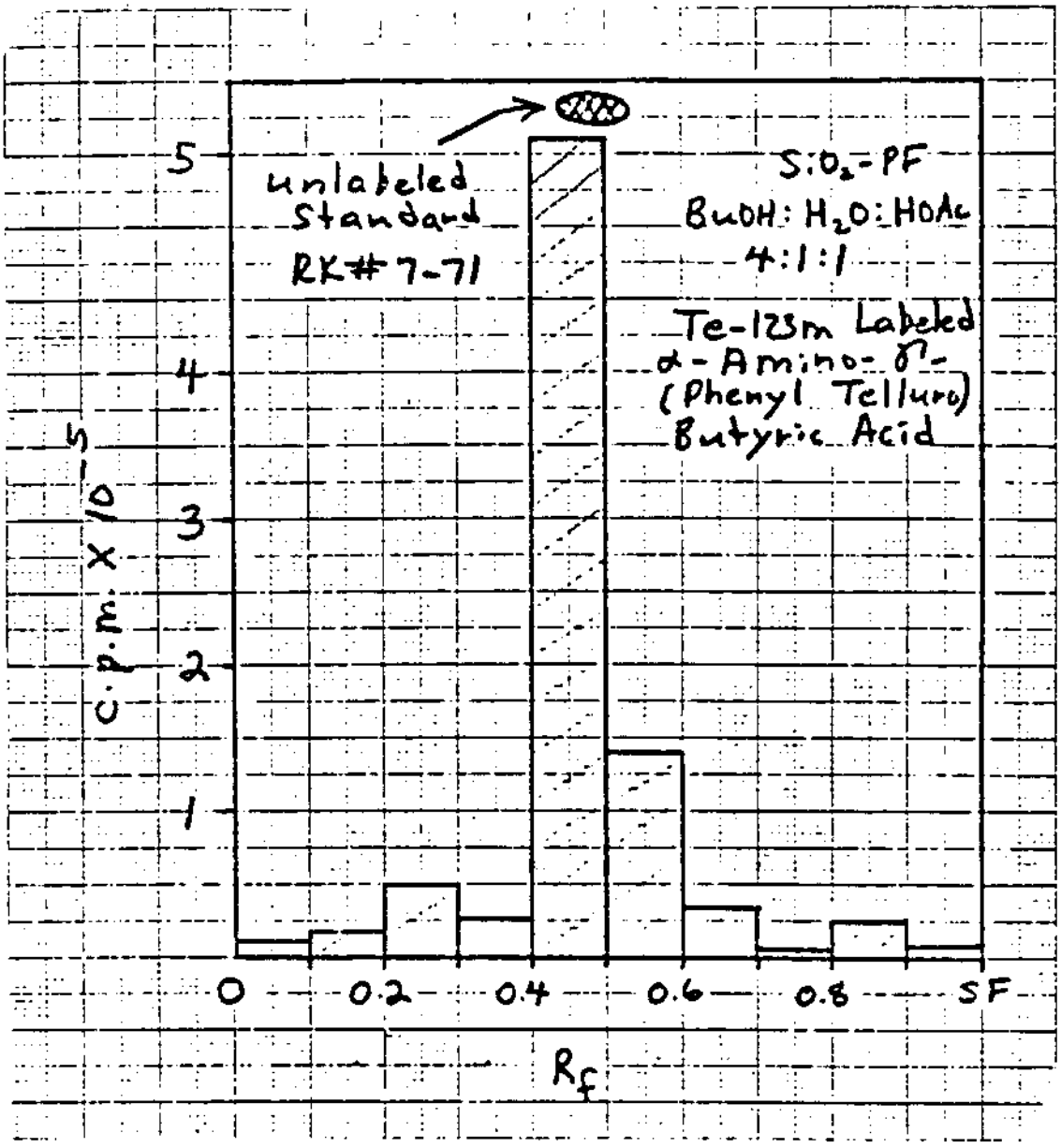
$\frac{277K}{300} \times \frac{1}{.84} \times \frac{1}{1.71 \times 10^{-16}} \times \frac{5}{6.24} \times \frac{1}{3.7 \times 10^{4}} = 261.3$

SAMPLE #2

277654 277654 000000
284432 284432 284432 000000

$= 254.8$

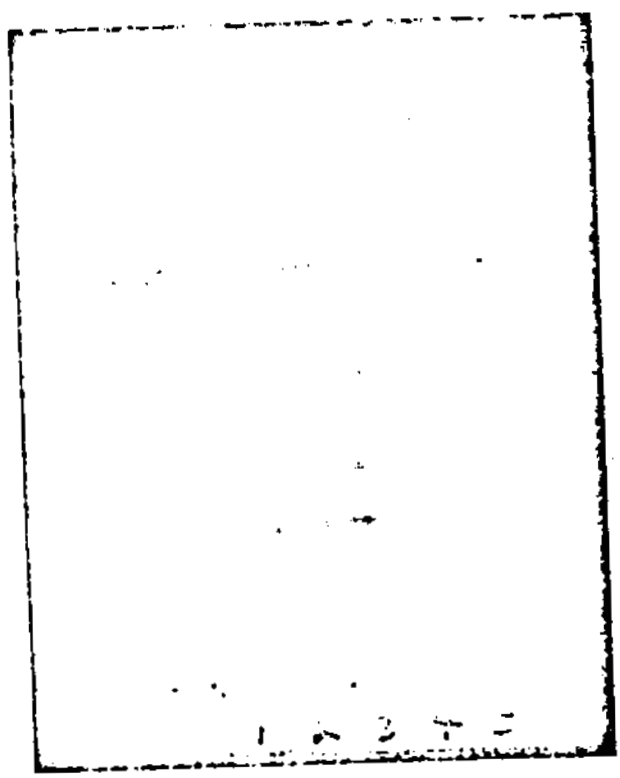
$= 258.1 \pm 6.2$



#5

105
100
466959
507358

Butanol–Acetic Acid – Water, 4:1:1
& Ninhydrin Spray (A)



SF ————————

O —
1 2 3 4 5

1. L–Alanine
2. L–Methionine
3. L–Homoserine
4. D,L–α–Amino–γ–(phenyl tellur... butyric acid (CK–7–...)
5. Crude 5–7X

1077238

Ex. 6.HD

Preparation of 5-(Methyl Telluro Ethyl)-Hydantoin

Continuation of RK-7-101, 7-97 & 7-91

Te, 762 mg, slurried in ~50 ml. of liq. NH_3 under Argon at −70°C − Na, 150 mg, added and solution stirred two hours.

CH_3I, 375 µl., added and solution stirred 1 hr. & NH_3 evap. off → dark brown gummy solid, some Te. ppt.

Me_2Te_2 extracted from the residue ε 15 ml. C_6H_6, ~ 3 portions → deep orange solution added to 15 ml. MeOH; $NaBH_4$ added in small portions → colorless

under Argon

solution.

5-(bromo ethyl) hydantoin (440 mg.) added in 5 ml. MeOH - orange colored appeared., additional $NaBH_4$ added → colorless, stirred at r.t. 20 min. - t.l.c. showed complete conversion - no

starting hydantoin detected ∴ losses must occur during the acidification - isolation step.

Taken to low volume at 61°C in vacuo → some Te, light orange solution (Eg. most Me_2Te_2 distilled over) poured into 100 ml. Et_2O-MeOH, 1 N HCl added → pH ~7

ext. c Et_2O → #1

6 N HCl added to H_2O layer

until H_2O ; large Te ppt; #2
ext. c Et_2O →

Et_2O exts. evap. in vacuo → light yellow solids dissolved in small volume acetone & hexane added → light yellow ppts. ; filtered under Argon, dried in vacuo

#1 – 45.5 mg., m.p. 123-124°C, d

#2 – 76 mg., m.p. ~110-111°C, d

light

$$100 \times \frac{115}{\frac{272}{208} \times 440} = \underline{20\% \text{ yield}}$$

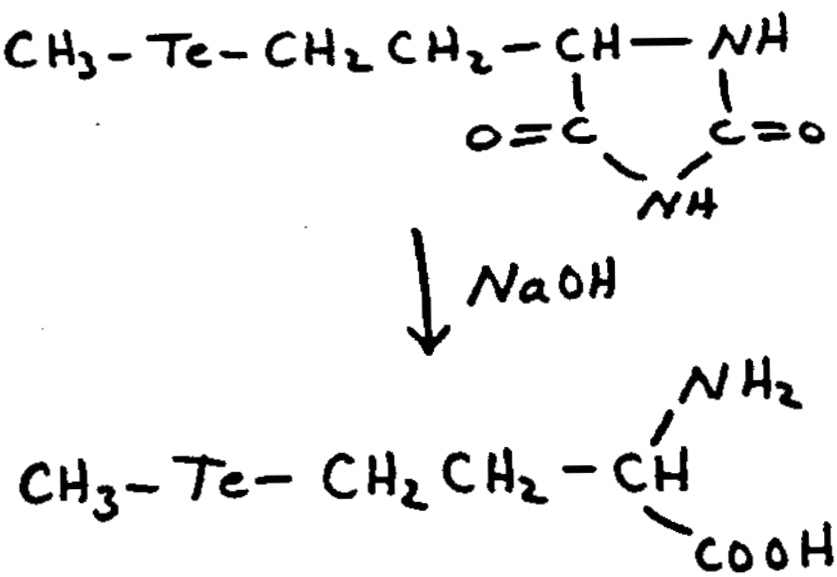
OH,

By t.l.c. (5% MeOH – $CHCl_3$) homogeneous – same R_f as #1

The Synthesis of Telluromethionine

Basic Hydrolysis of 5-(Methyl Telluro Ethyl)-Hydantoin

(RK # crup used thro stud



D,L-α-Amino-γ-(Methyl Tellur
Butyric Acid (Eg. Telluromethi

#1 10 mg. of the Hydantoin (Eg. 0.037 mmoles) dissolved in 500 µl. of 1N NaOH (Eg. 0.500 mmoles) in the teflon-liner of the reactio bomb — 160°C for 45 min Cooled & opened ⇒ Lu...

analyzed by t.l.c. (See 7-133) ninhydrin-positive product with expected R_f of telluromethionine! Since the teflon liner may have been contaminated from previous synthesis (Eg. 7-119)- the basic hydrolysis repeated below in glass ampoule

#2 Performed as in #1 but in a glass tube (Eg. n.m.r. tube, cut-off) placed in the teflon liner- t.l.c. indicated expected product (See 7-133)

#3 Performed as in #2 (Eg. 10 mg. hydantoin + 500 µl. 1N NaOH in glass tube- 160° - 45 min.)- t.l.c. again indicated presence of expected product!

Eg

Note: If the chromatograms were air dried at ~90-100°C to evaporate the solvents prior to u.v. analyses gray spots (Eg. Tet) were detected & R_f of product.

O.
m

When these samples were left in the dark overnight at 4°C, however, t.l.c. indica the product had decomposed Evidently, the same decompos was detected when the reac mixture was neutralized with hydrochloric acid (Eg. See RK #7-99)!

To study the effects of
a. the 160°C treatment and
b. the presence of only catalytic amounts of NaO the following experiments were performed.

#4
5 mg. of the hydantoin was heated with 200 µl. of H_2O at 160°C for 45 min.

#5 and
5 mg. of the hydantoin was heated with 0.001 mm (Eg. 0.010 ml. of 0.1 N NaOH,

Eg. 5 mg. = 0.018 mmoles

1077244 160°C for 45 min - see

The t.l.c. of #4 & #5 showed no significant decomposition and the unhydrolyzed starting material was isolated unchanged

The two samples below were therefore treated with increased concentrations of base:

#6 5 mg. hydantoin (Eq. 0.037 mmoles) + 0.05 ml. of 1 N NaOH + .15 ml. H_2O (Eq. .05 mmole) 160°C, 45 min.

#7 5 mg. hydantoin + 0.20 ml. 1 N NaOH (Eq. 0.20 mmoles), 160°, 45 min.

These combined results indicate:

1) We have prepared telluromethionine
2) Telluromethionine is very unstable to oxygen, acid, etc., as was expected
3) The substituted (CH_3 Te ethyl) hydantoin is stable at 160°C in H_2O (Eq. neutral conditions)
4) A stoichiometric amount of base is required for hydrolysis of the hydanto-

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H_2O
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H_2O
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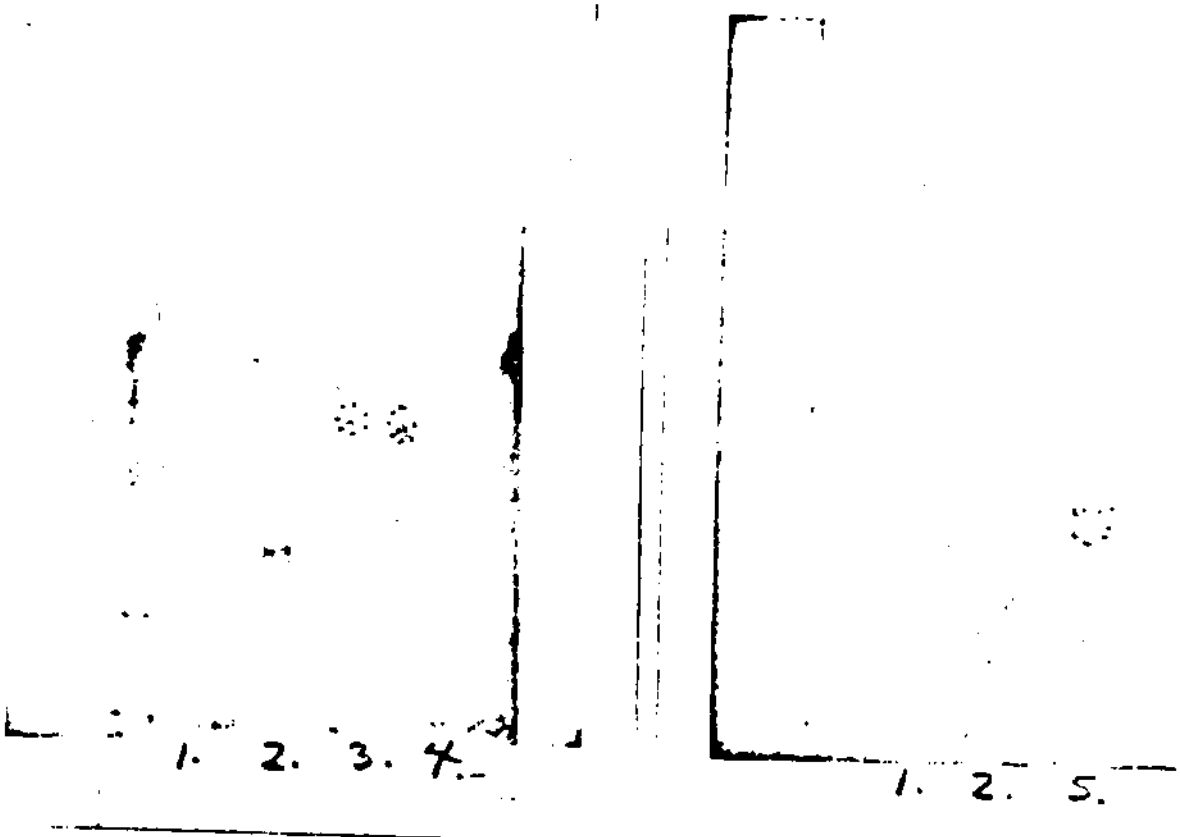


Solvent System:
$BuOH:Ac_2O:H_2O$ 4:1
(+ Ninhydrin Spray)

1. D,L- Methionine
2. D,L- Selenomethi
3. Sample # 4
4. Sample # 5
5. Sample # 2
6. Sample # 6
7. Sample # 7

7.7. Ku

DRAFT
Uzzell:dr
5/25/78

Exhibit E

S-49,068

"IMPROVED TISSUE-SPECIFIC SCINTIGRAPHIC IMAGING AGENTS"

Inventor: Furn F. Knapp, Jr.
[redacted] Tenn. [redacted]

Background of the Invention

This invention was made in the course of, or under, a contract with the United States Department of Energy. It relates to the preparation of ^{123m}Te-labeled organic compounds useful as tracers for the study of metabolic pathways and physiological research. Additionally, compounds of this invention have ~~potential~~ utility as radioactive imaging agents for the detection of systemic or organal disorders.

The use of radioactively labeled organic compounds in the study of biochemical reactions is well known. Tritium, ^{14}C and ^{32}P have been used extensively since their corresponding stable isotopes are present in practically all important cellular components. Biochemical agents labeled with ^{99}Tc and ^{75}Se have also found application as scintigraphic imaging agents for the detection of ~~cancinomas, (What else?)~~ [illegible]

Steroids labeled with ^{131}I and ^{75}Se have been proposed as adrenal imaging agents. Results in laboratory animals, i.e. mice, dogs, [illegible] have shown acceptable adrenal accumulation of the agents good quality images of dog adrenals have been obtained. The use of ^{75}Se-labeled 3 Beta-hydroxy-10-(methyl seleno)-cholest-5-ene is described in S. D. Sarkar et al. in Journal of Nuclear Medicine, Vol. 16, p.1038 (1975).

One disadvantage ^{131}I labeled adrenal agents is that the radioiodinated steroids were very unstable in vivo resulting in a high thyroid accumulations of radioactive iodine. Additionally, ^{131}I has [illegible]

a limited shelf life and results in a high Beta absorbed dose. The ^{75}Se nuclide decays with the emission of two high energy photons which result in inefficient collimation and poor quality images.

Certain of the prior art difficulties could be avoided by the use of ^{123m}Te-labeled agents as suggested in Radioactive Pharmaceuticals, Andrews et al. CONG-651111, Springfield, Virginia, National Bureau of Standards 1966 p.118. German Patent 2,553,408 also suggests the use of ^{123m}Te-labeled compounds and describes the synthesis of a steroid having nonradioactive tellurium present at position 19.

Another useful class of tracer compounds are radioactively labeled amino acids. Labeled amino acids have been used in the study of protein metabolism and synthesis, For example, that taking place in the pancreas. Labeled amino acids are also useful in the study of the effects of various pharmaceuticals on protein metabolism. [Can we provide some references which describe the use of radioactively-labeled amino acids in the study of metabolism etc.?]

^{123m}Te-labeled amino acids are likely to be isoteric with the sulfur analogs and behave similarly in vivo. Additionally, the high quality scintigraphic images produced by the ^{123m}Te nuclide is a substantial improvement over labeled amino acids. Prior art attempts to prepare telluro-amino acids by microbiological methods have been unsuccessful, see Kolar Z., Int. J. Appl. Radiat. Isot. 25 330 (1974).

Summary of the Invention

It is an object of this invention to provide ^{123m}Te-labeled biochemicals.

It is a further object to provide a method of synthesis for ^{123m}Te-labeled biochemicals.

These and other objects are achieved in a method for the preparation of ^{123m}Te-labeled organic compounds comprising the steps of (a) reacting a di-alkali metal ditellurol M_2 ^{123m}Te with a halogen-

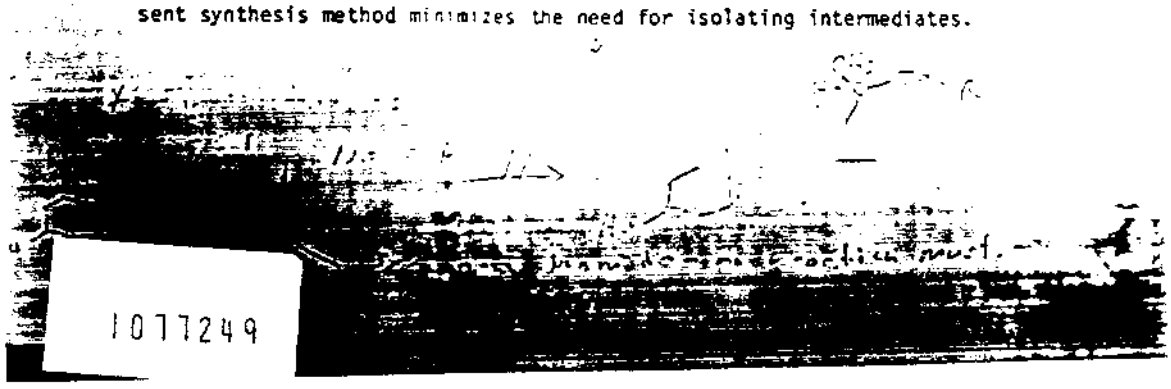
substituted organic compound, R-X, R being an alkyl or aryl group, to form a symmetric diorgano-ditelluride $^{123m}Te_2$, (b) reacting said diorgano-ditelluride with a reducing agent to form an alkali metal organo telluride of the formula R-^{123m}Te-M, (c) reacting said alkali metal organo telluride with a halogenated organic compound with a formula R'-X, R being an amino acid group, a group hydrolyzable to an amino acid, or a steroid side chain to form an organo telluro of the formula R'-^{123m}Te-R.

Detailed Description

One aspect of this invention involves a synthesis of ^{123m}Te-labeled steroids by methods which introduce the Te into the side chain rather than the steroid nucleus. As used herein, the steroidal side chain group is defined as the well known cyclopentanophenanthrene nucleus ~~with~~ an alkyl group from the No. 17 carbon atom as shown in drawing. Without departing from the spirit of this invention, the steroid nucleus can be substituted with _____ in positions. _____. The side chain attached to the number 17 carbon atoms can be any alkyl group of ~~up to~~ _____ carbon atoms and can also contain _____ groups. [Are there any steric limitations on what can be substituted on the carbon atom adjacent to the Te? Please explain.]

According to the methods described herein the introduction of the tellurium into the side chain rather than the steroid nucleus preserves the trans geeometry of the steroid nucleus [Why is this important?]

Due to the general instability of telluro organic compounds, the present synthesis method minimizes the need for isolating intermediates.



The reaction sequence leading to a steroid having a tellurium labeled side chain involves the reaction of a steroid-nor-halide with an alkali metal alkyl tellurol to provide a steroidal alkyl tellurol.

A. General Procedures for Preparation of ^{123m}Te

^{123m}Te is conveniently prepared from isotopically-enriched ^{122}Te, obtainable from the isotope sales office of the Oak Ridge National Laboratory, Oak Ridge, Tennessee, 37830. The radiation of this isotope in a neutron flux provides a ^{123m}Te isotope. Generally, irradiation will result in melting of target metal resulting in a hard mass upon cooling. The target is taken into solution in an acid solution such as aqua regia, _____, etc. which is evaporated to dryness to provide a tellurium salt. The salt can be redissolved in acid such as _____ to assure complete dissolution of metallic Te and again evaporated to dryness. The resulting solid is dissolved in water. A salt ~~such as~~ ~~NaBr~~ is added (Why?) _____

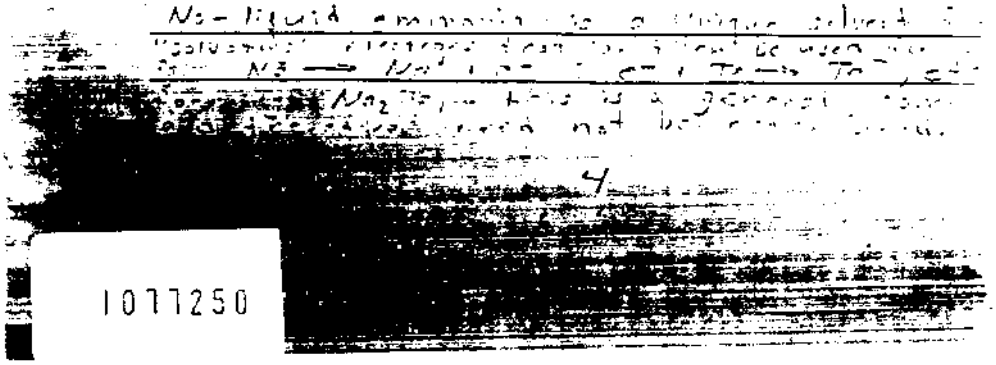
and the solution is boiled for one-half hour (Why? _____

and cooled. ~~SO_2~~ is bubbled through the solution to cause the precipitation of tellurium metal which can be recovered by filtration, etc. [Is this a known method of tellurium precipitation?]

^{123m}Te can be combined with carrier (non-radioactive) Te in the initial Te dissolution steps to reduce the specific activity to the desired level while providing isotopic homogeniety. The remainder of the synthesis will be described with reference to Te, with the understanding that some or all of the Te is in the ^{123m}Te form.

B. Preparation of An Alkali Metal Alkyl Tellurol

The preparation of a di-alkali metal ditelluride is achieved by the direct reaction of tellurium powder with an alkali metal. This can be conveniently achieved by reaction in liquid ammonia to form M_2Te_2, M being any alkali metal. [Would any other non-aqueous solvent be suitable?



M_2Te_2 is then converted to the desired di-alkyl-di-telluride. Since M_2Te_2 is unstable, it is reacted in situ by the direct addition of the appropriate alkyl halide to form a dialkyl-ditelluride (Is it essential that the R-X be added only after the Na_2Te_2 is formed? Please explain) _____

The reaction product is extracted with a suitable organic solvent such as benzene, _____ capable of extracting dialkyl di-telluride. (3.) The extracted dialkyl di-telluride is reduced to form an alkali metal alkyl telluride by the addition of a reducing agent such as alkali metal borohydride, _____

[Does the reducing agent have to be an alkali metal compound?] _____

NaOH is added to the mixture and refluxed [Why?] _____

The reduced product is an alkali metal alkyl tellurol. According to the method of this invention, the alkali metal alkyl tellurol M-Te-R is reacted with any halogenated organic compound R'-X to form R-Te-R'. [Are there any limitations on the composition of R'? Please explain.] _____

C. Preparation of Steroidal nor-Halides

~~The~~ preferred preparation procedure is a modified Hunsdiecker degradation of bile acids and other steroids containing carboxylic groups in the side chain. [Is a carboxylic acid group necessary? Why?] _____

This degredation comprises reaction of an appropriate steroid with mercuric

oxide bromide, Hg_2O-Br_2 in refluxing carbon tetrachloride, and is described in detail in Cristol, S. J. et al. J. Org. Chem. 26, 280 (1971) (Meerstein,) herein incorporated by reference. The result of the modified Hunsdicker degradation is a steroidal nor-halide. [Do you know of other methods of preparing steroidal nor-halides? What does -nor- signify?] _____

_____

_____

D. Preparation of 24-nor-23 Alkyl Telluro Steroids

The steroidal-nor-halide is reacted directly with the alkyli metal tellurol product of step B. This can be performed by direct addition of the nor-halide as a slurry in a suitable organic solvent, for example benzene. The resulting telluro steroid can be recovered by ~~ion exchange.~~ [Any other recovery methods?] _____

_____

E. Preparation of ^{123m}Te-labeled Amino Acids

The preparation of telluro amino acids involves the reaction of a halogenated organic compound with an alkali metal telluride. Unlike the seleno compounds used in the prior art, alkyl telluro compoounds are too unstable for use even at the site of formation. Attempts to introduce tellurium from benzyl tellurides into amino acids have been unsuccessful. [How were they unsuccessful? Did no product appear, or was it very low yields, etc? Were other alkyl tellurides attempted?] _____

_____

_____

_____

[Why can alkyl tellurides be used in steroid labeling and not amino acid labeling?] _____

_____

_____

It has been found that the instability of alkyl tellurides can be overcome by the use of phenyl tellurides. Following is described the general procedure for labeling amino acids with ^{123m}Te.

A. Preparation of ^{123m}Te-labeled Diphenyl Ditellurides

Phenyl magnesium chloride is reacted with ^{123m}Te to form a diphenyl telluride. [Please describe this step in detail.]

[Is there any reason why the diphenyl telluride cannot be prepared by the same method used in the preparation of dialkyl tellurides for steroid labeling?]

B. Preparation of Phenyl Tellurol

The diphenyl di-tellurol from step A. is reacted with a reducing agent such as an alkali metal borohydride, or _____

_____

[Please describe this reaction in detail. What is the resultant product? Sodium alkyl tellurol?]

C. Preparation of Telluro Amino Acid

This is readily accomplished by reaction with a hallogenated compound, ~~hydrolyzable~~ to form an amino acid, for example, hydantoin. This method is similar to prior art methods of preparing seleno amino acids, See Klosterman, H. J. et al., J. Am. Chem. Soc. 69 2009 (1947). (Please describe this step in detail. Also please list other materials than hydantoins, which can be used.)

The following examples illustrate the preparation of several representative ^{123m}Te-labeled compounds.

EXAMPLE I

Preparation of ^{123m}Te

Isotopically enriched ^{122}Te (94.71%), obtained from the isotope sales office of the Oak Ridge National Laboratory, was irradiated for [illegible] days in the Oak Ridge High Flux Isotope Reactor at 2×10^{15} neutrons/cm^2·sec. The reactor irradiation of the metallic tellurium resulted in the formation of a molten target mass which had solidified during cooling. The target was dissolved in an aqua regia and the solution is taken to insipient dryness. The solid residue was dissolved in concentrated hydrochloric acid and taken to dryness again. The acid treatment was repeated again and the resulting solid was dissolved in 200 ml of water. (About how much solid was there?) [illegible] After the addition of NaBr (5 grams) the solution was boiled for one-half hour, cooled, and SO_2 was passed through the solution at the rate of two bubbles per second for two hours. The tellurium metal precipitated as very fine particles and was recovered by centifugation. The recovered particles were washed three times with water and dried in an oven at 140°C. The recovery of ^{123m}Te is consistently better than 90% by this method.

EXAMPLE II

Preparation of di-sodium di-telluride.

22 milligrams, 25.8 mCi was combined with carrier tellurium, (45 micron powder) to yield a sample with a specific activity of 25.8 mCi/mmole. Approximately, 25 ml liquid ammonia was condensed into the

reaction vessel containing the tellurium powder. The ammonia is maintained in the liquid state by inserting the flask in a bath of _____

The reaction vessel had been previously flushed with argon connected to a small trap to maintain a slight argon pressure during the reaction. Freshly cut pieces of metallic sodium (23 mg, 1 mole) were added to the rapidly stirred slurry. The solution was stirred 2 hours and progressed through the color changes yellow, green, blue, red. The red color indicated the completion of Na_2Te_2 formation.

EXAMPLE III

Preparation of dialkyl di-telluride.

Isopropyl iodide (174 mg, 1 mmole) was added to the reaction vessel of Example II by means of a syringe inserted through a rubber septum. The initial deep red color of the solution slowly turns to yellow amber concomitant with the appearance of colloidal tellurium. After one hour of stirring the ammonia was allowed to evaporate under a stream of argon yielding a residue consisting of an orange gum containing metallic tellurium. The residue was extracted with several small portions of benzene (15 ml.) and the combined extracts were washed with water several times. The benzene solution was diluted with methanol to 25 ml. and aliquots were taken for counting. The benzene extracted material indicated a 42% yield of ^{123m}Te di-isopropyl di-telluride.

EXAMPLE IV

Preparation of sodium alkyl telluride.

The ^{123m}Te di-isopropyl di-telluride solution from Example III was combined with 25 ml. of methanol (Why?) _____ and the mixture stirred vigorously under an argon atmosphere. Small portions of sodium borohydride were then added until a colorless solution was obtained which indicated complete reduction of the di-telluride to the sodium isopropyl tellurol. In some cases gentle warming of the di-telluride solution is needed to initiate the reduction.

EXAMPLE V

Preparation of steroidal-nor-bromides.

[Please describe the Hundiecker synthesis of 3 beta-acetoxy-24-nor-bromo-5-alpha-cholane in detail, emphasing any features you regard as new.]

EXAMPLE VI

Preparation of 3-beta-hydroxy-24-nor-23(isobutyl telluro)-5-beta-cholane.

To the solution of Example IV was added about 80 mg. (2 mmole) of sodium hydroxide and the mixture was then refluxed (What is the purpose of this step?)

112 milligrams of 3 Beta-24-nor-23-bromo-5 alpha-cholane from Example V was added to the colorless solution as a slurry in a small volume of benzene and the mixture was refluxed for 1 hour. After this time period the reaction was completed as indicated by thin layer chromatographic analysis. The resulting solution was poured into water and the organic layer washed several times with water. The yellow-colored benzene solution was applied to a silicic acid column, slurried in benzene. (Does this silicic acid material have a trade name or other identifying characteristics?)

25 ml fractions were collected by dilution with increasing concentrations of ethyl ether in benzene. Aliquots (100 microliters) of each fraction were taken for counting. The specific activity of the ^{123m}Te-labeled steroid product was 26 mci/mmole, indicating more than ___% yield. [Why did the acetoxy group convert to hydroxy? Is this necessary or desirable?]

EXAMPLE VII

Preparation of 3-alpha-hydroxy-24-nor-23-(isopentyl telluro)-5-beta-colane.

3-alpha-acetoxy-24-nor-23-bromo-5-~~alpha~~-cholan, prepared by a modified Hunsdiecker synthesis from _____ was added as described in Example V. [Any difference in preparation?]

to a methanolic solution of sodium isopentyl tellurol prepared as in Example IV from di-isopentyl di-tellurides prepared as in Example ____. The solution was refluxed for one hour after which time thin layer chromotography indicated the reation to be complete. The solution was poured into water and extracted with chloroform. The yield was 34 milligram (32%). The material was purified by a thin layer chromotography. [Is there anything special about the TLC? What instrument, solvent and absorbant were used?]

EXAMPLE VIII

Preparation of3-alpha-hydroxy-24-nor-23-(isopropyl telluro)-5-beta-cholane.

A solution of sodium isopropyl tellurol was prepared by reduction of diisopropyl di-telluride (135 mg, 400 micro moles) with sodium boronydride in basic methanol in the manner of Examples 1-12. 3-alpha-acetoxy-24nor-23-bromo-5-beta-cholane (90 mg, 200 micro moles) was added and the mixture was refluxed two hours. Purification was performed by thin layer chromotography using chloroform solvent.) (This material and the material of Example VI could not be crystallized, how would you propose purifying the product?) _____

EXAMPLE IX

Preparation of 3-Beta-hydroxy-24-nor-23-(isopentyl telluro)-5-alpha-cholane.

3-beta-acetoxy-24-nor-23-bromo-5-alpha-cholane was prepared by the modified Hunsdiecker degradation of 3-beta-acetoxy-5-alpha-cholanic acid and purified, (How?) _____

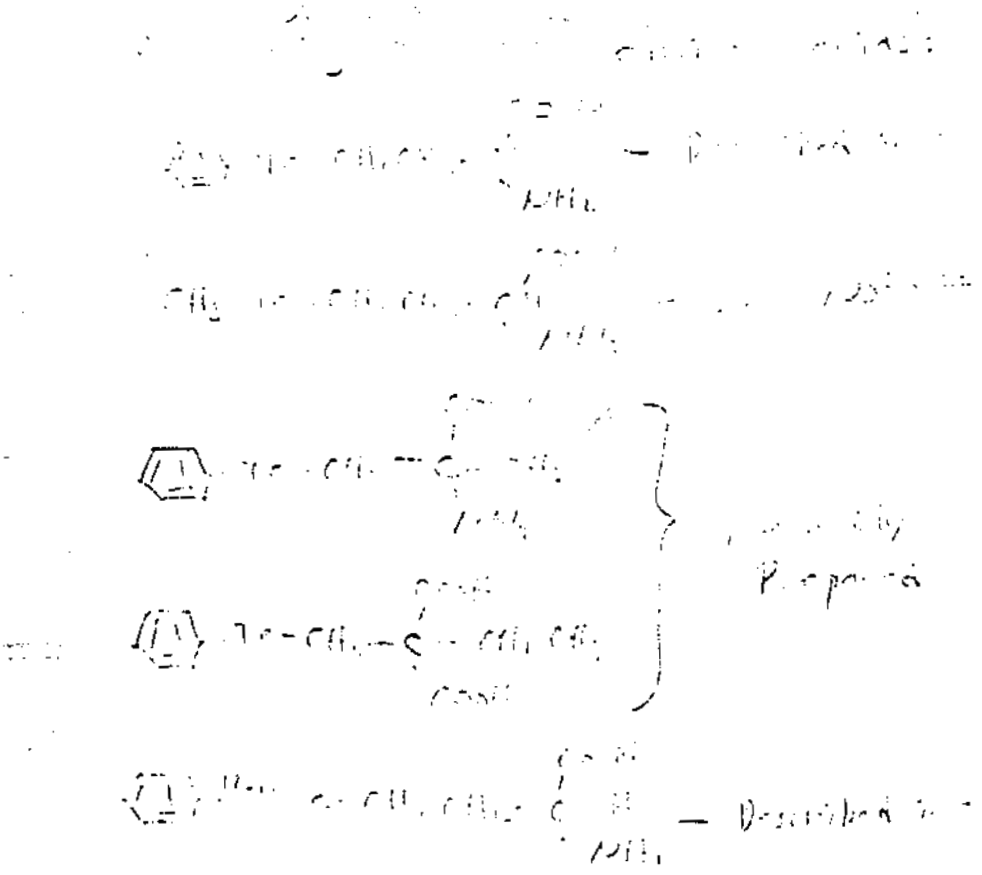
The product was crystallized from methanol and water to give fine needles. To this material was added 200 mu moles of sodium isopentyl tellurol. In refluxing methanol the reaction mixture was poured into water and the crude product extracted with chloroform. Purification by thin layer chromotography gave a thick gum the product was homogeneous by thin layer chromotography analysis and trituration with a small volume of ether gave a solid having a melting point of 78 to 80°C (How has this material purified?) _____

The 3-hydroxy-24-nor-23-(alkyl telluro) steroids are relatively insoluble in ether but are readily extracted from reaction mixtures

with chloroform or ethyl acetate. [What is the expected difference between alpha and beta hydroxy steroids? Which would be preferred for imaging?]

EXAMPLES X

(Please describe the successful synthesis of several ^{123}Te-amino acids in detail. Where is it most desirable to label the amino acids?]

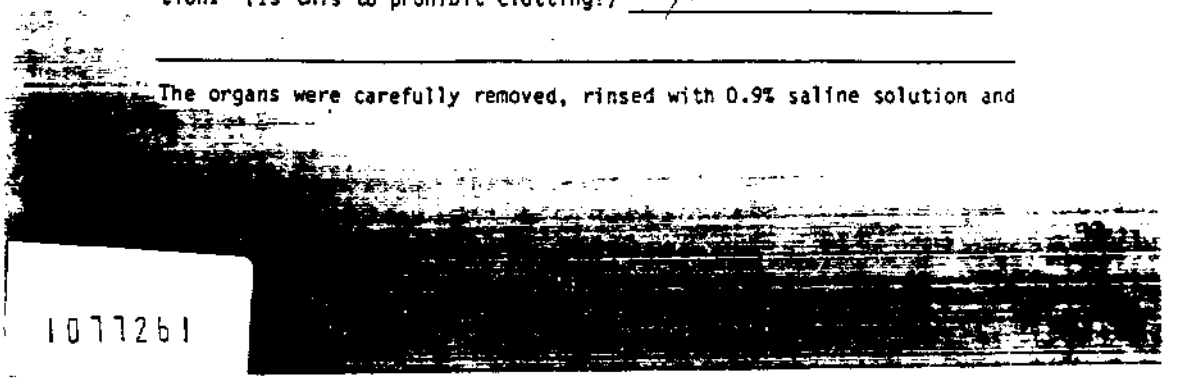


Adrenal Imaging

Fisher strain white albino rats were used for the following investigation. The animals were six to ten weeks old, male rats weighed 225-300 grams and the female rats weighed 160-180 grams. Food and water were allowed ad libitum prior to injection throughout the duration of the experiment. Benzene solution of the ^{123m}Te-labeled steroid of Example IX was taken to dryness under argon and the solid dissolved in ethanol. The solution was filtered through a millipore filter directly into a sterile vial containing a physiological saline solution containing 10% Polysorbate 80. (What was the Polysorbate for?) _____

The final ethanol concentration of this solution was 10%. The steroid solution (1 ml, 6-15 micro ci) was injected via the tail vein of rats that were anesthesized with ether. The rats were sacrificed at select times after being anesthesized with ether. Blood was drained from the carcass into a beaker containing a small amount of sodium citrate solution. (Is this to prohibit clotting?) Yes

The organs were carefully removed, rinsed with 0.9% saline solution and



blotted dry prior to weigning. The tissue distribution data were analyzed through a multifactorial analysis of variance computer program.

Male and female rats were injected with the ^{123m}Te-labeled steroid of Example IV (100-300 microcuries) as described above. After three days the animals were sacrificed and the adrenals, livers, lungs and ovaries were removed. Tissues were homogenized in 45 ml of a chloroform methanol mixture (2-1, Folch medium) at 5000 rpm for 30 seconds using a Sorvall Omni-Mix device. The homogenates were filtered through cheese cloth and after addition of an equal amount of water the phases were allowed to separate. Aliquots of the lower organic phase and upper aqueous phase were counted. The aqueous phase contained very little radioactivity. The organic layers were separated and evaporated to dryness in vacuo and the resulting residues dissolved in a small volume of chloroform and applied to silicic acid columns (600-200 mesh, 2 x 30 cm). Fractions 25 ml in volume were collected by elution with increasing volumes of ether in benzene. Aliquots of each fraction were counted.

The animals were anesthesized after intraperitonial injection of a sodium pentabarbitol solution (30-50 mg/kg) and scans were obtained using a rectilinear scanner equiped with a 63 hole gold collimator at a focal distance of 3 cm. The animals were scanned at .25 inches per minute. The camera images were obtained with an RC-type proportional counter camera utilizing a xenon gas field detector [Do you have further description of the model number, etc. of the scanner which was used?] _____

The distribution of radioactivity in tissues of male rats was determined at a variety of time intervals of 1 hour to 21 days following the intervenous administration of the ^{123m}Te-labeled steroid. The major organs were removed, weighed and counted directly in a multichannel analyzer. The first group distribution of radioactivity was determined at 1,6 in 18 hours after administration. ~~The results are presented in Table I~~. At the early time intervals the liver, spleen, adrenals and lungs all contained significant levels of

15B

After one day the % dose in the liver was 1.07 decreasing to 0.23 after 7 days. The % dose in the spleen was 1.59, after one day decreasing to 0.24 after 7 days. The % dose in the lungs decreased from 2.12 after 1 day to 0.40 after seven days. The adrenals contained 26.37% of the dose after 1 day, decreasing to 14.48% after seven days. The % dose in the thyroid was 1.23 after 1 day, decreasing to 0.78 after 7 days, remaining constant for 7 days.

15C

The % dose in the liver decreased from 0.14 after 7 days to 0.03 after 21 days. The % dose in the spleen decreased from 0.32 after 7 days to 0.11 after 21 days. The % dose in the lungs decreased from 0.22 after seven days to 0.05 after 21 days. The % dose in the thyroid decreased somewhat but remained at 0.12-0.15 throughout the 7-21 day period. The % dose of adrenals decreased from 5.56% in 7 days to 1.81% after 21 days.

radioactivity. The concentration of radio activity increased rapidly in the adrenals, however, while the lows of radioactivity decreased or remained constant, in the other organs described above. In the second group of animals the distribution of radioactivity was determined at one, three and seven days after injection of the labeled steroid. ~~These~~ results are ~~presented in Table II. The results indicate that the~~ percent of the administered radioactivity in the ~~adrenal~~ began to decrease after one day. These results are further substantiated by similar data obtained from animals in the third group ~~and~~ were sacrified at seven, fourteen and twenty-one days after injection with the labeled steroid. ~~The results of the third group are presented in Table III. As is seen from the data of tables I-III~~ the radioactive contents of the blood, liver and lungs are very high at early time intervals decreasing rapidly with a concomitant increase in the radioactive contents of the adrenal glands. The adrenal glands reached a maximum concentration at one to two days after injection. Female rats showed generally parallel concentrations except that the concentration of radioactivity in the ovaries was also high.

19 4

The mean percent dose / gram of the liver was 2.71 after one hour decreasing to 0.84 after 18 hours. The % dose of the spleen decreased from 2.15 after one hour to 1.29 after 18 hours. The % dose of the lungs decreased from 2.01 after one hour to 1.52 after 18 hours. The % dose of the adrenals increased from 4.51 after one hour to 22.17 after 18 hours.

TABLE 1. EXPERIMENT 1. DISTRIBUTION OF RADIOACTIVITY IN MALE RAT TISSUES 1, 6 AND 18 HOURS AFTER INTRAVENOUS INJECTION OF ^{123m}Te-3β-HYDROXY-24-NOR-23-(ISOPROPYL TELLURO)-5α-CHOLANE

Tissue	Mean Percent Dose/gram, ± s.d.		
	1 hour after dose	6 hours after dose	18 hours after dose
Blood	1.48 ± 0.35	0.95 ± 0.04	0.49 ± 0.02
Liver	2.71 ± 0.30	2.03 ± 0.07	0.84 ± 0.09
Spleen	2.98 ± 0.15	2.75 ± 0.27	1.29 ± [illegible]
Pancreas	0.16 ± 0.02	0.24 ± 0.06	0.19 ± [illegible]
Stomach	0.11 ± 0.04	0.07 ± 0.02	0.05 ± [illegible]
Small Intestine	0.71 ± 0.19	0.71 ± 0.09	0.39 ± [illegible]
Large Intestine	0.06 ± 0.03	0.72 ± 0.23	0.86 ± [illegible]
Adrenals	4.51 ± 1.16	16.51 ± 1.31	22.17 ± [illegible]
Kidneys	0.63 ± 0.04	0.76 ± 0.05	0.77 ± [illegible]
Prostate	0.07 ± 0.01	0.09 ± 0.01	0.09 ± [illegible]
Testes	0.04 ± 0.003	0.08 ± 0.01	0.09 ± [illegible]
Heart	0.48 ± 0.09	0.53 ± 0.09	0.43 ± [illegible]
Lungs	2.01 ± 0.09	1.99 ± 0.08	1.52 ± [illegible]
Thyroid	0.42 ± 0.05	2.07 ± 0.78	0.53 ± [illegible]
Brain	0.04 ± 0.01	0.05 ± 0.001	0.04 ± [illegible]

TABLE 2. ~~EXPERIMENT 2:~~ DISTRIBUTION OF RADIOACTIVITY IN MALE RAT TISSUES 1, 3 AND 7 DAYS AFTER INTRAVENOUS INJECTION OF ^{123m}Te-3β-HYDROXY-24-NOR-23-(ISOPROPYL TELLURO)-5α-CHOLANE

Tissue	Mean Percent Dose/gram, ± s.d.		
	1 day after dose	3 days after dose	7 days after dose
Blood	0.61 ± 0.04	0.37 ± 0.03	0.28 ± 0.04
Liver	1.01 ± 0.23	0.53 ± 0.04	0.23 ± 0.02
Spleen	1.59 ± 0.28	0.58 ± 0.07	0.29 ± 0.05
Pancreas	0.33 ± 0.07	0.30 ± 0.03	0.21 ± 0.008
Stomach	0.25 ± 0.02	0.32 ± 0.13	0.14 ± 0.01
Small Intestine	0.77 ± 0.24	0.49 ± 0.20	0.23 ± 0.02
Large Intestine	1.82 ± 0.93	1.89 ± 1.99	0.59 ± 0.14
Adrenals	26.39 ± 1.13	19.27 ± 2.40	14.48 ± 1.76
Kidneys	1.08 ± 0.14	0.94 ± 0.06	0.75 ± 0.07
Prostate	0.18 ± 0.06	0.17 ± 0.02	0.09 ± 0.04
Testes	0.14 ± 0.02	0.12 ± 0.01	0.09 ± 0.01
Heart	0.59 ± 0.09	0.26 ± 0.04	0.12 ± 0.03
Lungs	2.12 ± 0.35	0.85 ± 0.14	0.40 ± 0.06
Thyroid	1.23 ± 0.09	0.73 ± 0.07	0.74 ± 0.27
Brain	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.001

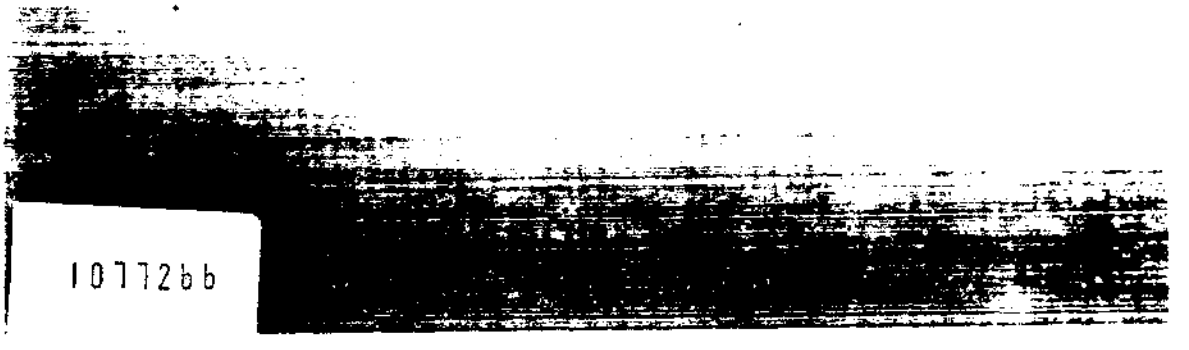


TABLE 3. EXPERIMENT-3. DISTRIBUTION OF RADIOACTIVITY IN MALE RAT TISSUES 7, 14 AND 21 DAYS AFTER INTRAVENOUS INJECTION OF ^{123m}Te-3β-HYDROXY-24-NOR-23-(ISOPROPYL TELLURO)-5α-CHOLANE

	Mean Percent Dose/gram, ± s.d.		
	7 days after dose	14 days after dose	21 days after dose
Blood	0.19 ± 0.05	0.16 ± 0.04	0.09 ± 0.02
Liver	0.14 ± 0.03	0.06 ± 0.01	0.03 ± 0.01
Spleen	0.32 ± 0.12	0.20 ± 0.03	0.11 ± 0.03
Pancreas	0.12 ± 0.01	0.07 ± 0.01	0.04 ± 0.01
Stomach	0.05 ± 0.02	0.01 ± 0.01	0.01 ± 0.01
Small Intestine	0.38 ± 0.49	0.03 ± 0.003	0.02 ± 0.01
Large Intestine	0.09 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Adrenals	5.56 ± 1.38	4.59 ± 0.45	1.81 ± 0.57
Kidneys	0.49 ± 0.12	0.29 ± 0.05	0.16 ± 0.01
Prostate	0.06 ± 0.01	0.04 ± 0.01	0.02 ± 0.004
Testes	0.07 ± 0.01	0.05 ± 0.01	0.03 ± 0.004
Heart	0.09 ± 0.03	0.03 ± 0.02	0.02 ± 0.001
Lungs	0.22 ± 0.04	0.11 ± 0.01	0.05 ± 0.004
Thyroid	0.13 ± 0.07	0.15 ± 0.08	0.12 ± 0.06
Brain	0.04 ± 0.01	0.06 ± 0.03	0.03 ± 0.02

Experiments were also conducted to determine if the labeled steroid was metabolized by the adrenals and other tissues of rats. Three days following intravenous administration of the labeled steroid male and female rats were sacrificed and selected tissues removed, weighed, counted and homogenized in Folch medium. The organic phases from the Folch extracts were chromatographed on silicic acid columns by ellution with solvents of increasing polarity. The columns were initially eluded with benzene followed by solvent mixtures containing increasing proportions of ether and benzene and were finally washed with methanol. The profiles from male rats suggested that the labeled steroid was metabolized to several products by the male adrenals. The adrenal exract from a female rat contained a nonpolar radioactive component and also significant radioactivity in a region resembling the original steroid which appears to indicate a significant portion of the agent was not metabolized. The presence of non-polar radioactive components would indicate at least partial metabolism. Among the tissues which were examined the components that were observed upon chromatographic analysis of extracted lipids were consistently different and would indicate that the radioactive components represent true metabolites. The metabolism of such adrinal imaging agents is important because

[redacted]

The adrenal glands of the male rats were clearly imaged one day after administration of the ^{123m}Te-labeled steroid. Both the adrinals and ovaries of female rats were also imaged following the injection of the agent both a rectalinear scanner and an RC-type proportional counter camera.

Tests with other steroids indicate a complex relationship between steroid structure, relative rates of entry and exit from the various body components. Two steroids prepared according to the above-described procedure, 3-beta hydroxy-24-nor-23-octyl-telluro-chol-5-ene and 3-beta methoxy-24-isopropyl-telluro-5-ene accumulate slowly in the

[redacted]

adrinals. The steroid 3-beta-hydroxy-24-isopropyl telluro-chol-5-ene showed a slightly greater adrinal uptake than the steroid in the above test. Two other steroids (3-alpha hydroxy-24-nor-23-(isopropyl telluro)-5-beta-cholan and 3-beta hydroxy-[(isopropyl telluro) methyl]-androsed-5-ene did not concentrate in the adrinals.

It is seen that the general synthesis method of this invention can be adapted to the preparation of any alkyl telluro steroid merely by providing a suitable allogenated reaction site and such steroids are contemplated at equivalents of the specific steroids described herein.

Exhibit F

Department of Energy
Oak Ridge Operations
P.O. Box E
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June 23, 1978

Dean E. Carlson, Chief, Prosecution Branch, Patents, HQ
Germantown, CGXI, A2-3018

DOE CASE S-49,068

Enclosed are an application in the above case and the following additional papers:

( ) Bristol Board Drawing(s) ( ) Record of Invention

( ) Prior Art Letter (in dup.) ( )

( ) Assignment (in dup.) ( )

Fees payable are:

Basic Fee $65

Additional Fees:
Total claims in excess of ten, times $2. 16

Number of independent claims minus one, times $10. . 30

Total Filing Fee. . . . . $ 111

Filing prior to as soon as possible is necessary.

Publication status: A brief abstract was published June 13, 1977. We believe that this is not a statutory bar and will argue it in the patent office. Other publications have been released since then at numerous times.

Foreign filing is recommended. The following countries should be considered. Canada

Stephen D. Hamel
Assistant Patent Counsel
for Patent Prosecution
Oak Ridge

DCP:AHU/br

Enclosures:
As stated above

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