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## Nuclear Medicine Technology Progress Report for Quarter Ending December 31, 1977

J. K. Poggenburg

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NUCLEAR MEDICINE TECHNOLOGY PROGRESS REPORT  
FOR QUARTER ENDING DECEMBER 31, 1977

J. K. Poggenburg

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NUCLEAR MEDICINE TECHNOLOGY PROGRESS REPORT  
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SUMMARY

Progress is reported for the applications of  $^{11}\text{C}$ ,  $^{195\text{m}}\text{Pt}$ ,  $^{33}\text{P}$ , and  $^{123\text{m}}\text{Te}$ . Of note in this report is the first human clinical testing of  $^{11}\text{C}$ -1-aminocyclobutanecarboxylic acid and microscale synthesis and tissue distributions for a number of new  $^{195\text{m}}\text{Pt}$ -labeled chloroamine-platinum(II) complexes. A project to investigate the labeling of beta-adrenergic myocardial agents was begun. A two-dimensional Fast Fourier Transform is now operational for the PDP-11 version of the Oak Ridge Imaging System.

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CARBON-11

*T. A. Butler*

The collaboration with Oak Ridge Associated Universities (ORAU) in the clinical testing of  $^{11}\text{C}$ -labeled amino acids for tumor localization studies continued during this quarter with six production runs. Twenty patients were scanned with the positron tomographic instrument (ECAT). Three patients received  $^{11}\text{C}$ -1-aminocyclobutanecarboxylic acid ( $^{11}\text{C}$ -ACBC) which comprised the first human clinical studies with this labeled compound. Previous animal studies at ORAU had indicated that  $^{11}\text{C}$ -ACBC may be superior to the cyclopentane analog ( $^{11}\text{C}$ -ACPC) as a general tumor localization agent. Five patients were administered  $^{11}\text{C}$ -ACPC and the remaining 12 patients received  $^{11}\text{C}$ -DL-tryptophan. Uptake of  $^{11}\text{C}$ -DL-tryptophan in the pancreas is high, and excellent images of the gland continue to be obtained with the ECAT scanner.

The primary yield of  $^{11}\text{C}$  oxides from the  $\text{B}_2\text{O}_3$  targets remains good, averaging about 4 Ci per run. This appears to be the maximum for the current target design using natural  $\text{B}_2\text{O}_3$ . To increase the yield of  $\text{H}^{11}\text{CN}$  (used in the Strecker amino acid synthesis) from the catalytic conversion of the oxides, the nickel catalyst was improved by using smaller particles to increase the surface area and by reducing the catalyst bed length to assure a more uniform temperature within the furnace heat zone. Preliminary tests of the modified system indicated that about 90% conversion efficiency can be obtained vs an average of 60% conversion experienced using the former system.

#### PLATINUM-195m

*J. D. Hoeschele and T. A. Butler*

As part of the continuing cooperative program to study antitumor compounds, three shipments of  $^{195\text{m}}\text{Pt}$ -labeled  $\text{Na}_2\text{PtCl}_6$  and one shipment of  $^{195\text{m}}\text{Pt}$ -labeled cis- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$  (cis-DDP) were made to the University of Southern California. Two shipments of  $^{195\text{m}}\text{Pt}$ -labeled  $\text{Na}_2\text{PtCl}_6$  were made to the University of Kentucky Medical Center. Also,  $^{195\text{m}}\text{Pt}$ -labeled cis- and trans-DDP were prepared for the Biology Division (ORNL) for use in cooperative studies of subcellular distribution and Pt-DNA binding.

#### Synthesis and Tissue Distribution Studies of Chloroamineplatinum(II) Compounds

Microscale syntheses and tissue distribution studies of chloroamineplatinum(II) complexes of the general formula  $[\text{Pt}(\text{NH}_3)_{4-x}\text{Cl}_x]^{2-x}$  have been initiated. These studies are being pursued as part of our interest in platinum antitumor agents and in conjunction with medical cooperative

programs employing some of these agents. The purpose of these studies is to obtain distribution/retention information of a systematic nature which can be correlated with physiochemical, physiological, and structure-activity data available for platinum antitumor agents. Such correlations will be generally useful in understanding and perhaps predicting the fate and utility of these and related agents in biological systems. A further objective is to determine if any of these complexes exhibit unique tissue distribution patterns suggesting their use as scanning/visualization agents.

We report here general details of the microscale syntheses developed and some initial empirical observations derived from tissue distribution studies of the following  $^{195\text{m}}\text{Pt}$ -labeled compounds:  $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$  (I),  $[\text{Pt}(\text{NH}_3)_3\text{Cl}]\text{Cl}$  (II), cis-DDP (III), trans-DDP (IV),  $\text{K}[\text{Pt}(\text{NH}_3)\text{Cl}_3]$  (V), and  $\text{K}_2\text{PtCl}_4$  (IV). Details of synthesis were presented previously (ORNL/TM-6044) for cis- and trans-DDP, which are preferred starting materials for compounds I, II, and V. General details of the microscale syntheses ( $\leq 0.1$  millimole) of the remaining four compounds as well as a schematic outline of  $^{195\text{m}}\text{Pt}$ -labeled chloroamineplatinum(II) syntheses are presented below. The number in parentheses following each synthesis heading is keyed to the schematic outline (\* denotes  $^{195\text{m}}\text{Pt}$ , A represents  $\text{NH}_3$ ).

#### Synthesis of $\text{K}_2\text{Pt}^*\text{Cl}_4$ (2)

Potassium tetrachloroplatinate (II) was prepared from  $\text{Na}_2\text{Pt}^*\text{Cl}_4$ , an intermediate in the synthesis of cis-DDP, by adding solid  $\text{K}_2\text{CO}_3$  to an acidic solution of the latter followed by evaporation to dryness.

Purification was achieved by dissolving the crude residue in a minimum volume of H<sub>2</sub>O followed by addition of 12 M HCl to induce crystallization at 0°C.

#### Synthesis of [Pt<sup>\*</sup>(NH<sub>3</sub>)<sub>4</sub>]Cl<sub>2</sub> (5)

Platinum(II) tetrammine chloride was synthesized from the starting material cis-[Pt<sup>\*</sup>A<sub>2</sub>Cl<sub>2</sub>] (see Fig. 1). Excess 15 M NH<sub>4</sub>OH was added to a slurry of cis-[Pt<sup>\*</sup>A<sub>2</sub>Cl<sub>2</sub>] in saline and the mixture heated to insure complete conversion to the product. The resultant solution was evaporated just to dryness and the white residue (compound + NaCl) dissolved in the requisite volume of H<sub>2</sub>O to restore isotonicity.

#### Synthesis of K[Pt<sup>\*</sup>(NH<sub>3</sub>)Cl<sub>3</sub>] (4)

The synthesis of K[Pt<sup>\*</sup>ACl<sub>3</sub>] was adapted from the procedure of Elleman et al.<sup>1</sup> The key step in this synthesis is the preparation of HPt<sup>\*</sup>ACl<sub>3</sub> (in situ) by refluxing cis-[Pt<sup>\*</sup>A<sub>2</sub>Cl<sub>2</sub>] in 6 M HCl in the presence of Pt metal powder as catalyst. Subsequently, the [Pt<sup>\*</sup>ACl<sub>3</sub>]<sup>-</sup> anion is precipitated as part of the golden complex electrolyte, [PtA<sub>4</sub>][Pt<sup>\*</sup>ACl<sub>3</sub>]<sub>2</sub>, which after recrystallization, is converted to K[Pt<sup>\*</sup>ACl<sub>3</sub>] by selectively removing the PtA<sub>4</sub><sup>2+</sup> cation either by (a) adsorption onto a strong cation resin (in K<sup>+</sup> form) or (b) by adding K<sub>2</sub>PtCl<sub>4</sub> to precipitate the salt, [PtA<sub>4</sub>][PtCl<sub>4</sub>].

#### Synthesis of [Pt<sup>\*</sup>(NH<sub>3</sub>)<sub>3</sub>Cl]Cl (7)

A three step reaction scheme was used in synthesizing [Pt<sup>\*</sup>A<sub>3</sub>Cl]Cl in relatively low yield from trans-[Pt<sup>\*</sup>A<sub>2</sub>Cl<sub>2</sub>], the preferred isomer for this synthesis. The critical first step involves reacting trans-[Pt<sup>\*</sup>A<sub>2</sub>Cl<sub>2</sub>]

A = NH<sub>3</sub>  
 \* = <sup>195m</sup>Pt

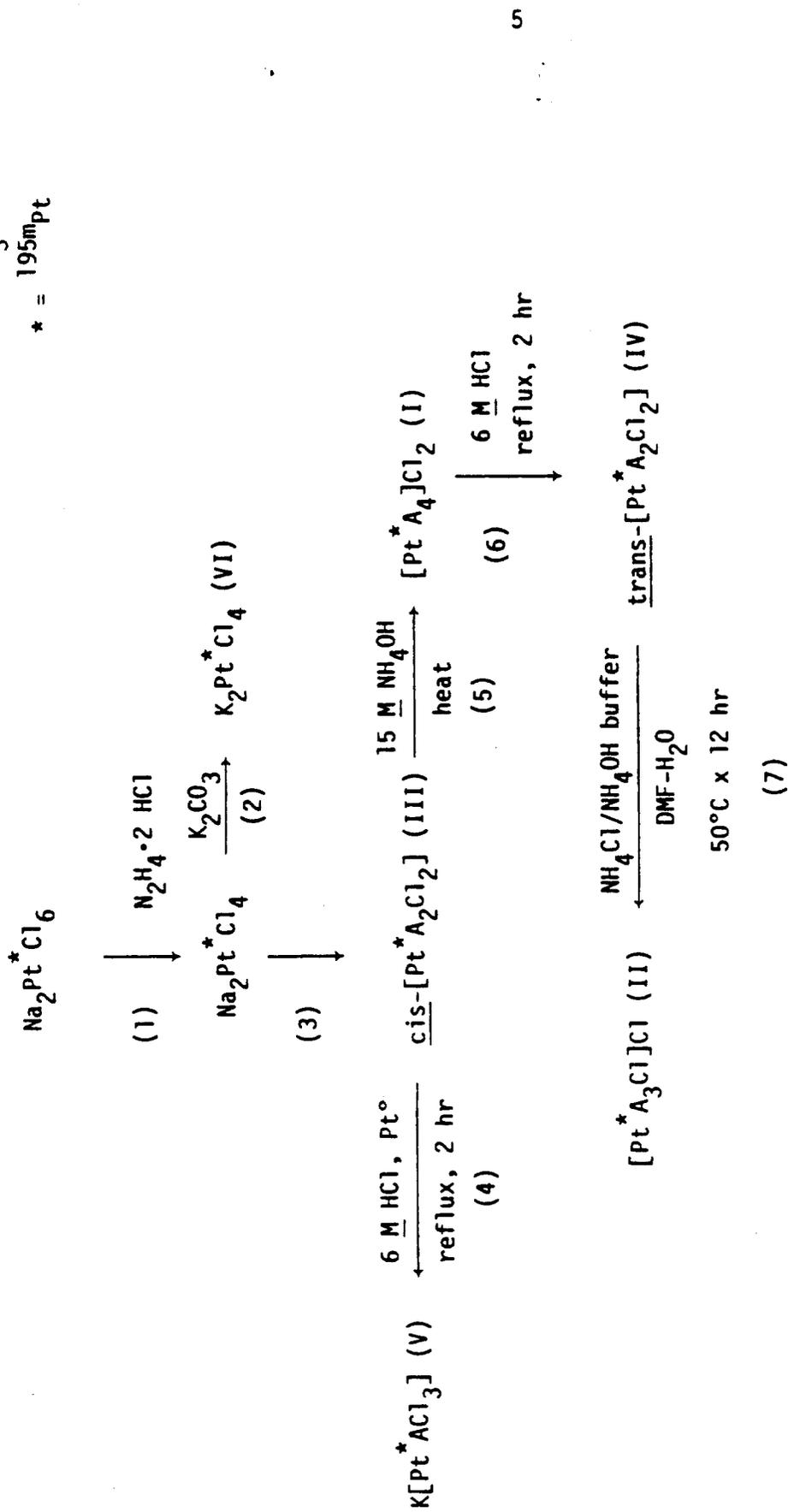


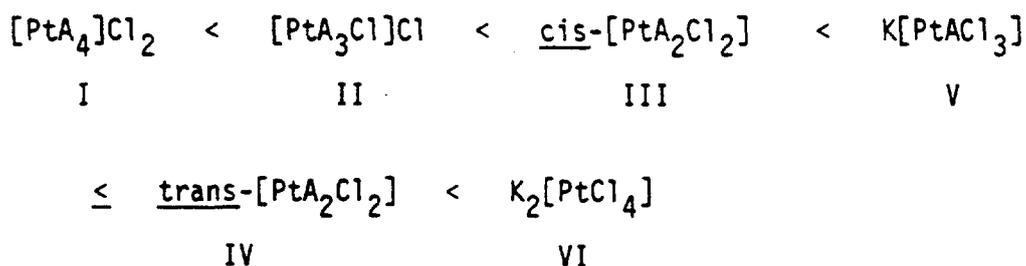
Fig. 1. Schematic outline of the syntheses of <sup>195m</sup>Pt-radiolabeled chloroamineplatinum (II) compounds.

with  $\text{NH}_4\text{OH}$  in a  $\text{DMF-H}_2\text{O}$  medium at  $55^\circ\text{C}$  for 12-hr (pH buffered using  $\text{NH}_4\text{Cl}$ ;  $\text{NH}_4\text{OH}/\text{Pt(II)}$  ratio of 1.10). Potassium tetrachloroplatinate was added to the acidified reaction mixture to precipitate a mixture of the complex electrolytes,  $[\text{Pt}^*\text{A}_4][\text{PtCl}_4]$  (green) and  $[\text{Pt}^*\text{A}_3\text{Cl}]_2[\text{PtCl}_4]$  (red). The desired red component was dissolved away from the insoluble green component, recrystallized, and then converted to  $[\text{Pt}^*\text{A}_3\text{Cl}]\text{Cl}$  by selectively removing the  $\text{PtCl}_4^{2-}$  anion either by (a) adsorption onto a strong anion resin ( $\text{Cl}^-$  form) or (b) adding a stoichiometric amount of  $[\text{PtA}_4]\text{Cl}_2$  to precipitate the salt,  $[\text{PtA}_4][\text{PtCl}_4]$ .

#### Tissue distribution of chloroamineplatinum(II) complexes

Comparisons of the preliminary tissue distribution data for these complexes are shown in Tables 1 to 3. Thus far, the following empirical observations have been gleaned from these data (% dose/g-tissue, organ/blood ratios, and relative tissue distributions) at 24 hr postinjection in the female Fischer 344 rat:

(1) Organ levels generally increase in the order



which appears to parallel trends in reactivity, the net negative charge on the complex, and/or the number of chloride ligands attached to the central metal atom, Pt(II). The cationic complexes, I and II, exhibit the lowest retention in all tissues, except that compound I exhibits the

Table 1. Tissue distribution of chloroamineplatinum (II) complexes of the general formula  $[\text{Pt}(\text{NH}_3)_{4-x}\text{Cl}_x]^{2-x}$  in the Fischer (female) 344 rat at 24 hr postinjection (i.v., tail vein) (A =  $\text{NH}_3$ )

Complex:	$[\text{PtA}_4]\text{Cl}_2$	$[\text{PtA}_3\text{Cl}]\text{Cl}$	<u>cis</u> - $[\text{PtA}_2\text{Cl}_2]$	<u>trans</u> - $[\text{PtA}_2\text{Cl}_2]$	$\text{K}[\text{PtACl}_3]^a$	$\text{K}_2[\text{PtCl}_4]$
Dose (mg/Kg)	11	3.0	4.0	2.15	3.5	11.1
Tissue	% dose/g tissue					
Blood	0.0066	0.050	0.24	1.14	1.31	0.66
Liver	0.14	0.28	0.40	0.41	0.38	0.67
Spleen	0.063	0.063	0.26	0.75	0.92	0.71
Pancreas			0.17		0.20	0.48
Stomach	0.042	0.014		0.084	0.11	0.31
S. intestine	0.076	0.057		0.17	0.15	0.35
Adrenals			0.33		0.28	0.27
Kidneys	0.25	1.20	2.02	3.59	2.78	0.37
Heart	0.033	0.042	0.084	0.33	0.23	0.41
Lungs			0.23	0.58	0.54	0.72
Brain	0.048	0.0075	0.013	0.019	0.018	0.011
Ovaries			0.20			0.65
Fallopian tubes/ uterus	0.069	0.055	0.32		0.51	0.11
Carcass		0.041	0.19		0.19	0.34
Total		7.6			25.0	

<sup>a</sup>One rat/data point; duplicate for others.

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Table 2. Tissue/blood ratios<sup>a</sup> for platinum (II) chloroamine complexes in the Fischer 344 rat at 24 hr postinjection (i.v., tail vein) (A = NH<sub>3</sub>)

Complex:	[PtA <sub>4</sub> ]Cl <sub>2</sub>	[PtA <sub>3</sub> Cl]Cl	cis-[PtA <sub>2</sub> Cl <sub>2</sub> ]	trans-[PtA <sub>2</sub> Cl <sub>2</sub> ]	K[PtACl <sub>3</sub> ] <sup>a</sup>	K <sub>2</sub> [PtCl <sub>4</sub> ]
Tissue	% dose/g tissue					
Liver	20.9	5.54	1.69	0.36	0.29	1.0
Spleen	9.6	1.26	1.11	0.67	0.70	1.07
Pancreas			0.71	0.073	0.16	0.72
Stomach	6.5	0.29				0.47
S. intestine	11.6	1.15		0.15	0.086	0.35
Adrenals			1.40		0.21	0.41
Kidneys	37.6	24.2	8.5	3.15	2.13	0.55
Heart	5.1	0.86	0.35	0.29	0.17	0.62
Lungs			0.98	0.58	0.41	1.09
Brain	7.3	0.15	0.054	0.017	0.014	0.017
Ovaries			0.84			0.98
Fallopian tubes/ uterus	10.6	1.12	1.34		0.39	1.67
Carcass		0.82			0.14	0.52

<sup>a</sup>One rat/data point; duplicate for others.

Table 3. Relative tissue distribution of chloroamine platinum (II) complexes of the general formula,  $[\text{Pt}(\text{NH}_3)_4-x\text{Cl}]_2^{2-x}$  in the Fischer rat (female)<sup>a</sup> at 24 hr postinjection (i.v., tail vein) (A =  $\text{NH}_3$ )

Complex:	$[\text{PtA}_4]\text{Cl}_2$	$[\text{PtA}_3\text{Cl}]\text{Cl}$	<u>cis</u> - $[\text{PtA}_2\text{Cl}_2]$	<u>trans</u> - $[\text{PtA}_2\text{Cl}_2]$	$\text{K}[\text{PtACl}_3]$ <sup>a</sup>	$\text{K}_2[\text{PtCl}_4]$
Dose (mg/Ke)	11.1	3.0	4.0	2.15	3.5	11.1
Tissue	Ratios of % dose/g tissue relative to the compound with the lowest level in the same tissue type					
Blood	$*6.6 \times 10^{-3}$	7.6	36.5	174	+200	101
Liver	$*1.4 \times 10^{-1}$	2.0	2.9	3.0	2.7	+4.9
Spleen	$*6.3 \times 10^{-2}$	1.0	4.2	11.9	+14.6	11.3
Pancreas			1.0		1.2	+2.8
Stomach	2.97	*0.014		5.8	7.9	+21.8
S. intestine	1.33	*0.057		3.1	2.6	+6.1
Adrenals		+1.2			1.0	*0.27
Kidneys	$*2.5 \times 10^{-1}$	4.9		+14.6	11.3	1.5
Heart	$*3.3 \times 10^{-2}$	1.3		9.8	6.8	+12.4
Lungs			1.0	2.5	2.3	+3.1
Brain	+6.4	*0.0075	1.8	2.5	2.4	1.5
Ovaries <sup>c</sup>			3.6			
Fallopian tubes/ uterus	1.2	*0.055	5.8		9.2	+11.7
Carcass		*0.041	4.6		4.7	8.5
Total		7.6			25.0	

\*Compound with lowest level per given tissue; in % dose/g tissue.

+Compound with highest level per given tissue; relative to \*.

<sup>a</sup>12-14 weeks old.

<sup>b</sup>1.0 mg/ml except for trans-DDP.

<sup>c</sup>Ovaries and fallopian tubes combined except for trans-DDP; value for  $[\text{PtA}_3\text{Cl}]\text{Cl}$  used as reference.

highest retention in the brain. The doubly-charged anionic complex VI shows the highest tissue retention in 8/13 comparisons. The singly-charged anionic complex V exhibits the highest blood (1.31% dose/g) and spleen levels.

(2) Organ selectivity, as judged by organ/blood ratios, appears optimal for  $[\text{PtA}_4]\text{Cl}_2$ . Values for the liver, kidney, and brain are 21, 38, and 7, respectively (ref. Table 2). An intriguing result is the relatively high uptake of I into the brain. The % dose/g-tissue and brain/ blood ratios for I are the highest of any of the complexes, i.e., 3.7 and 135 times the values for cis-DDP, respectively. Interestingly, the level for I (a dipositive cation) is 6.4 times that for II (a monopositive cation). This apparent selectivity for a dipositive complex cation, which would not be expected to pass the "blood-brain barrier," might come about by transport as an ion pair and/or by a mechanism similar to the transport of quaternary amines. It also suggests potential utility of I (or congeners) as brain scanning and/or a radiation sensitization agent, in conjunction with radiation therapy of brain tumors.

(3) Kidney levels increase markedly in the series, I to IV and then fall abruptly at  $\text{K}_2\text{PtCl}_4$  (10X less than for trans-DDP). The data for the adrenals suggests a similar but less pronounced trend; however, the full set of data is not available as yet. It is not known at this point whether the relatively low kidney levels for  $\text{K}_2\text{PtCl}_4$  reflect a generalization that doubly-charged anionic platinum (metal) complexes lead to lesser kidney retention (and potential damage). What does appear clear however is that doubly-charged chloroamineplatinum (II)

species (cation and anion) are taken up in the kidney far less than their monopositively charged analogs. Intrinsic reactivity as well as species mobility (transport) may contribute to the apparent selectivity.

Additional correlations will be forthcoming in the next report.

### PHOSPHORUS-33

*D. V. Woo and K. R. Ambrose*

Experiments conducted during this quarter have been concerned with developing techniques to be used in the in vitro evaluation of cyclophosphamide and its  $^{33}\text{P}$ -labeled analog. Previously the hepatic 9000Xg homogenate (S-9) from the Fischer rat which was used for the activation of cyclophosphamide had shown significant cytotoxicity at low concentrations. At sub-toxic concentrations there was an insufficient amount of microsomal enzymes for activation of the cyclophosphamide. The liver homogenate (S-9) obtained from a different rat strain (Sprague-Dawley) was tolerated at higher concentrations, indicating a lesser degree of cytotoxicity to the KB tumor cell line which has been used in published studies of cyclophosphamide cytotoxicity. The optimization of the microsomal activating system and necessary techniques to conduct an in vitro assay of cyclophosphamide will continue in the next quarter.

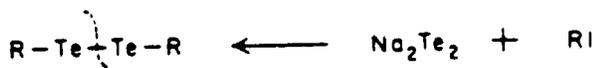
### TELLURIUM-123m

*F. F. Knapp and K. R. Ambrose*

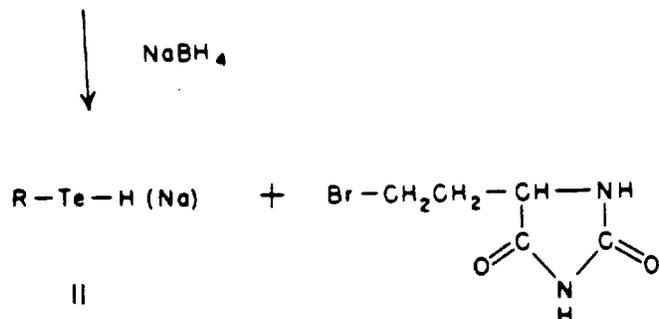
#### Telluro Amino Acids

A large scale preparation of 5-[ $\beta$ (methyl telluro) ethyl] hydantoin (IVb, Fig. 2) has recently been completed. We have found that the yield of (IVb) formed upon reaction of methyl telluro (IIb) with 5-( $\beta$ -bromo ethyl) hydantoin is critically dependent upon the reaction conditions

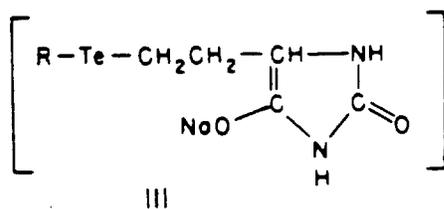
Step 1



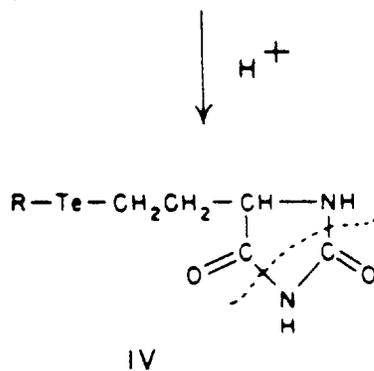
Step 2



Step 3



Step 4



Step 5

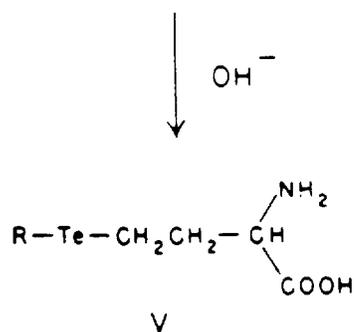


Fig. 2. The synthesis of telluro amino acids.  
(a = C<sub>6</sub>H<sub>5</sub>, b = CH<sub>3</sub>)

(Fig. 2). When (IVb) was prepared by the same methods that had been used successfully to prepare (IVa) in high yield, the 5-[ $\beta$ -(methyl telluro) ethyl] hydantoin (IVb) was obtained in only a low yield (20%). Attempts to increase the yield of (IVb) involved a detailed analysis of the various reaction parameters: the solvent used in Steps 2 and 3, the acid used in Step 4, the solvent used for extraction in Step 4, and the solvent used for crystallization of the final product (IVb). The initial product formed in Step 3 is presumably the sodium salt of an enolic isomer of (IIIb) since the reduction conditions are strongly basic (e.g.,  $\text{NaBH}_4$ -ROH; pH 8-9) and the product is water soluble. Conversion of (IIIb) or an equivalent structure to the neutral product (IVb) is critically dependent upon the nature of the acid used in the acidification step. While (IVa) can be obtained in high yield by acidification of (IIIa) with 1 N HCl, careful acidification of a solution of (IIIb) with this acid resulted in the formation of a black tellurium precipitate and only modest amounts of (IVb) were isolated under these conditions. The majority of the product isolated consisted of a polar decomposition product as determined by chromatography. Experiments are now in progress to identify this material. The use of  $\text{H}_2\text{SO}_4$  in Step 4 dramatically increased the yield of (IVb). Optimal extraction of (IVb) after Step 4 was accomplished with ethyl acetate. Finally, crystallization of crude (IVb) was best accomplished with acetone-petroleum ether. These careful studies have resulted in the optimization of the reaction conditions and 5-[ $\beta$ -(methyl telluro) ethyl] hydantoin (IVb) has now been prepared in 55% yield.

The potential use of  $^{123m}\text{Te}$ -labeled telluromethionine as a pancreatic imaging agent is one of our interests, and our immediate goal is to determine the tissue distribution of telluromethionine in rats. Our present method of preparation of telluromethionine originates from the generation of dimethyl ditelluride ( $\text{CH}_3\text{-Te-Te-CH}_3$ , Ib) which is a highly volatile intermediate. Our present method also involves numerous manipulations, and the generation and use of  $^{123m}\text{Te}$ -labeled dimethyl ditelluride by these methods would require complex containment procedures. These problems are a result of the apparent necessity of introducing the tellurium at the initial step of the synthetic scheme. For these reasons we are presently developing the methodology for preparation of DL-2-tritio-telluromethionine (2-tritio-Vb) for the tissue distribution studies. The tritium label can be introduced in a high-yield one-step reaction by hydrolysis of the 5-[ $\beta$ -(methyl telluro) ethyl] hydantoin (IVb) in basic solution of tritiated water (Fig. 2). The results of the preliminary tissue distribution experiments with the  $^3\text{H}$ -labeled telluromethionine will determine whether the development of a method for the preparation of the  $^{123m}\text{Te}$ -labeled amino acid is warranted.

#### Telluro Steroids

A large-scale preparation of 3 $\beta$ -hydroxy-24-(isopropyl telluro)-chol-5-ene has recently been completed. Attempts are now in progress to obtain crystals of this material that will be suitable for x-ray analysis. Such studies would unequivocally confirm the structure of this unusual and potentially useful steroid. In addition, the x-ray analysis of 3 $\beta$ -hydroxy-24-(isopropyl telluro)-chol-5-ene would also represent an interesting structural problem.

The preliminary results reported earlier (ORNL/TM-6044) described the synthesis and tissue distribution studies of two  $^{123m}\text{Te}$ -labeled neutral steroids which effectively concentrate in rat adrenals: 3 $\beta$ -hydroxy-24-nor-23-(isopropyl telluro)-5 $\alpha$ -cholane (VI) and 3 $\beta$ -hydroxy-24-(isopropyl telluro)-chol-5-ene (VII). A comparative study of the tissue distribution of  $^{123m}\text{Te}$ -labeled (VI) and (VII) in male and female rats indicated a higher adrenal uptake of both compounds in females when compared to the results of similar studies conducted with male rats. In addition, a somewhat higher adrenal concentration of  $^{123m}\text{Te}$ -labeled (VII) as compared to  $^{123m}\text{Te}$ -labeled (VI) was detected in female rats.

To determine the relative concentration of the labeled steroids within the adrenal cortex and medulla, rats were injected intravenously with  $^{123m}\text{Te}$ -labeled (VI) or (VII). Two days following the injection, the adrenals were removed, dissected to separate the medulla and cortex and the radioactive contents of each section then determined. In one experiment (Table 4) female rats showed a higher medullary concentration of  $^{123m}\text{Te}$ -labeled (VII), whereas the concentration of radioactivity within the cortex and medulla of male rat adrenals was approximately equal. In another experiment female rats injected with  $^{123m}\text{Te}$ -labeled (VI) again showed the higher medullary concentration of radioactivity. Other workers<sup>2</sup> have reported a high uptake of  $^{75}\text{Se}$ -19-(seleno methyl)-cholesterol in the adrenal medulla of female dogs, although the relative concentration of radioactivity varied between the cortex and medulla at different time periods.

We are unable at this time to explain these interesting results using the  $^{123m}\text{Te}$ -labeled steroids. Although we are unaware of the

possible significance of the high medullary concentration of radioactivity following administration of the  $^{123m}\text{Te}$ -labeled steroids we feel this interesting observation should be studied further. During the next quarter the possible sex differences in the relative concentration of radioactivity in the medullary and cortical tissues following injection of  $^{123m}\text{Te}$ -labeled (VI) will be determined. We also plan to initiate studies that will compare the concentration and retention of radioactivity in rat adrenals following injection of the two  $^{123m}\text{Te}$ -labeled steroids.

Table 4. Adrenal (medulla/cortex) ratios<sup>a</sup> two days following intravenous administration of  $^{123m}\text{Te}$ -labeled steroids

$^{123m}\text{Te}$ -labeled steroid	Female rats	Male rats
3 $\beta$ -Hydroxy-24-nor-23-(isopropyl telluro)-5 $\alpha$ -cholane (VI)	2.34 $\pm$ 0.78	b
3 $\beta$ -Hydroxy-24-(isopropyl telluro)-chol-5-ene (VII)	2.07 $\pm$ 0.24	1.04 $\pm$ 0.14

<sup>a</sup>The ratios are calculated from % dose/gm of tissue data.

<sup>b</sup>These data are not yet available.

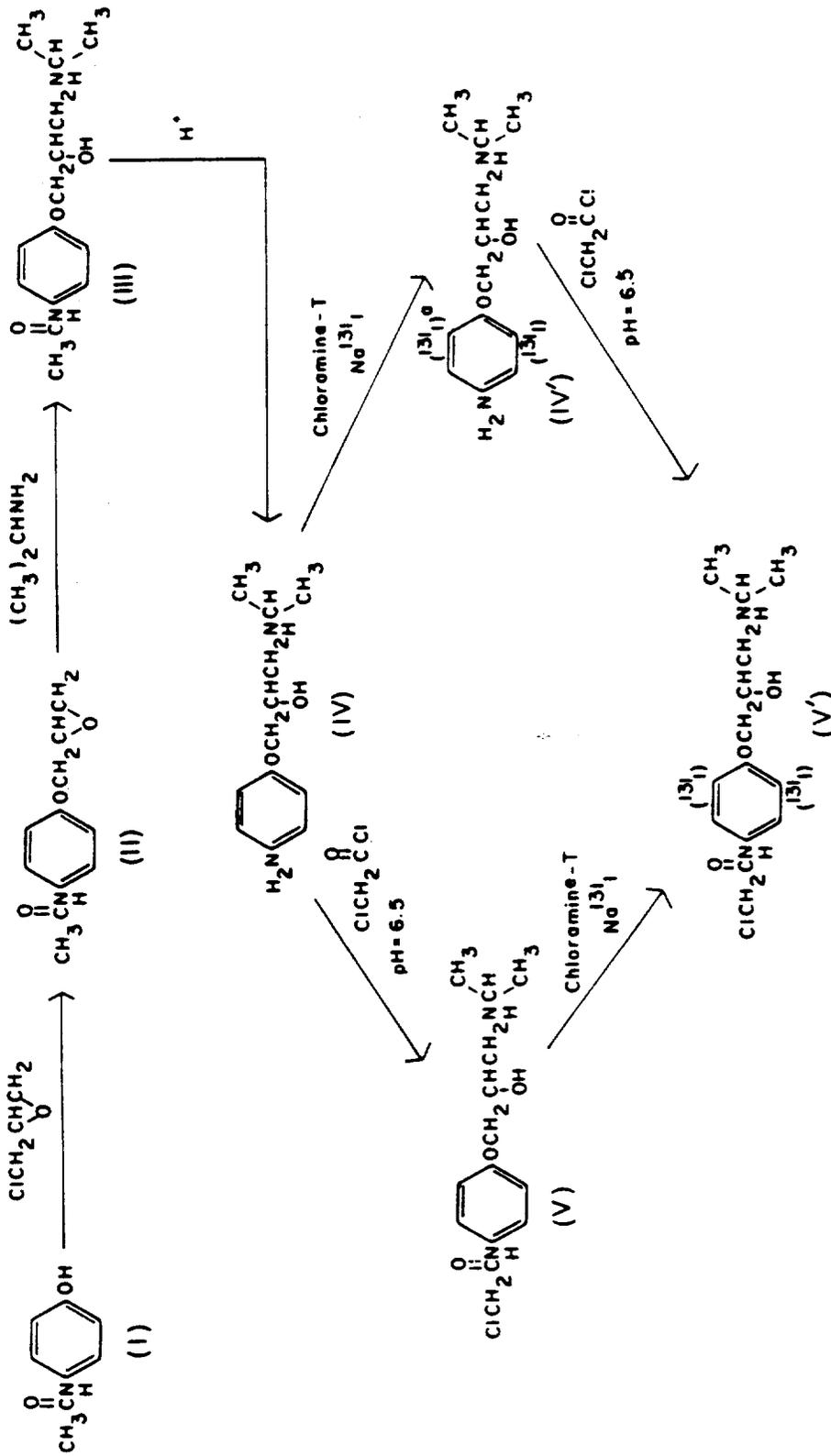
#### BETA-ADRENERGIC MYOCARDIAL AGENTS

*D. V. Woo*

Because of our longstanding involvement in Medical Cooperatives with 43K and myocardial imaging, a synthesis program has been initiated to develop specific radio-labeled beta-adrenergic antagonists for possible myocardial imaging. In published reports similar compounds have been

studied in vitro using high specific activity radio-labeled analogs. These compounds bind to beta-adrenergic receptors in various model systems (turkey erythrocyte membrane, cardiac tissue) in the presence of other competing uptake mechanisms. Chloropractolol, which has been reported by Erez et al.<sup>3</sup> to bind specifically and irreversibly to the cardiac beta-adrenergic receptor, appears to be a potential candidate for labeling with a gamma-emitting radionuclide. If labeled in sufficiently high specific activity, chloropractolol could conceivably be used to image the heart with current nuclear medicine instrumentation and thereby delineate the dynamic physiological function of these receptors in intact animals during normal and pathophysiologic conditions.

The synthesis of chloropractolol (V), 1-(4-chloro-acetamidophenoxy)-3-isopropylamino-2-propanol, involves the following reaction scheme shown in Fig. 3. Compound I, p-acetamido phenol, is reacted with an excess of 1-chloro-2,3-epoxypropane in a methanolic-sodium hydroxide solution at room temperature. After stirring approximately 16-18 hr the solvents are evaporated off in vacuo. The residue is dissolved in ethyl acetate, washed with distilled water, dried with  $MgSO_4$ , and the organic solvent is evaporated in vacuo. The intermediate II, 1-(4-acetamido-phenoxy)-2,3-epoxy propane, is recrystallized from isopropanol and further reacted with an excess of isopropyl amine in isopropanol at room temperature. The reaction is stirred for approximately 16 hr; the isopropanol and unreacted isopropylamine are evaporated in vacuo. The resulting compound III, 1-(4-acetamido-phenoxy)-3-isopropylamine-2-propanol, is recrystallized from an ethanol-ethyl



<sup>a</sup> Probable site of iodination

Fig. 3. The synthesis of  $^{131}\text{I}$ -labeled chloropractolol.

acetate solution. Deacetylation of the acetyl group on III via acid hydrolysis (concentrated HCl in ethanol) resulted in IV, 1-(4-aminophenoxy)-3-isopropylamine-2-propanol, dihydrochloride (after in vacuo evaporation of solvents). Selective chloroacetylation of the aniline function of IV in a 0.1 M phosphate buffer at pH 6.5 resulted in chloropractolol (V). The pH of the reaction was maintained at 6.5 with a 5 N NaOH during the addition of an excess of chloroacetyl chloride. After 2 hr of stirring (1 hr at 5-10°C and 1 hr at room temperature), the solution was filtered, cooled (5-10°C), and made alkaline to pH 10-11. The chloroacetylated product (V) is observed as crystalline plates forming in the solution. Presently, all of the compounds (I-V) have been synthesized. Characterization of these compounds by nuclear magnetic resonance ( $^{13}\text{C}$  and H), infrared and mass spectral analysis have provided spectra consistent with the chemical structures of the compounds. Alternatively, the high temperature of the inlet system used during mass spectral analysis could have resulted in thermal decomposition of (V) prior to ionization.

Optimization of the reaction conditions for selective acylation of the aniline function will be reported during the next quarter. The synthesis of radio-labeled chloropractolol (V') using Iodine-131 will follow similar iodination procedures reported in the literature. Radioiodination of chloropractolol (V) or the deacetylated intermediate (IV) using chloramine-T and carrier-free  $\text{Na}^{131}\text{I}$  in a buffered media will be tested to determine which substrate is the most stable under the reaction conditions required for labeling.

Projected studies using high specific activity radioiodinated chloropractolol will determine basic pharmacokinetic parameters in test animals in collaboration with N. Revis of the Biology Division. Also, in vitro studies using isolated cardiac tissue preparations will be used to determine the specificity, affinity, and kinetics of binding of chloropractolol and its radio-labeled analog, along with other known competitive agonists and antagonists. After characterization and evaluation of the labeled product, further studies with experimentally induced infarcted animals will determine relative myocardial distribution of the labeled product between normal and damaged tissue. If there are apparent differences in distribution, then external imaging may allow one to visualize such differences.

#### IMAGING AND INSTRUMENTATION

*P. R. Bell and J. M. Dougherty*

Translation of ORIS from PDP-8 to PDP-11

##### The resident program

The resident program of the Oak Ridge Imaging System (ORIS) for the PDP-11 has been expanded to include comment text provisions so that processed images include the text describing the subject, dose, and other parameters entered by the operator in free format. The text provision also includes the automatic appending of processing tags so that photographs show the image processing performed on the image photographed.

A command was added to type out the current image comments since we have no display with the borrowed PDP-11. The quantitation marks and typeouts, as well as a full set of "display level" and subtract

(thresholding) commands similar to those available in the -8 version, have been added to the resident program. Although the PDP-11/05 has no display, these provisions became essential while we were debugging the -11 version of the Fast Fourier Transform program, TWOD. Areas of the processed image could thus be typed out for examination in array format without having to go through the cumbersome and time-consuming process of writing the tentative image to the diskette and transferring the floppy disk unit to the PDP-8 for examination after each small program test. Commands were also provided to scale the values of the image elements before writing on the diskette image file since the PDP-11 word is larger than the PDP-8 word. The TWOD program has automatic integer arithmetic scaling which takes full advantage of the accuracy afforded by the available word size in the computer. This automatic scaling leaves the image too large to be transferred to the PDP-8 without scaling. This problem is rarely or never encountered with unprocessed clinical images.

#### The Fast Fourier Transform Module TWOD

The image processing program for the PDP-11 most desired by the Vanderbilt group was the Fast Fourier Transform (FFT). The TWOD module of ORIS containing this procedure was translated and is now operating properly. The TWOD procedure gave us considerable difficulty as it is a very complicated mathematical process. Besides the main program, it has 27 subroutines and 3 function tables. Added to the ordinary program errors generated in the translation, we had to find four of the concealed and value-dependent kind of errors. The performance of the TWOD is now excellent. Its difference residuals are about one-third that of the PDP-8

program for the forward and reverse transformations of a relatively intense 64 x 64 clinical image, because of improved numerical significance of the larger word size of the PDP-11.

The TWOD module performs the following operations:

1. Reads the image to be transformed.
2. Zeros the edge elements to reduce dissymmetry effects.
3. Determines the average value of all the elements in the image and subtracts this from all elements to remove the zero-frequency element in the frequency domain. This element is usually by far the largest and its removal allows the program to achieve higher precision via the automatic scaling.
4. Performs a phase transformation on the element values so that zero frequency lies in the center of the frequency domain, making radially symmetric filters easier to use.
5. Transforms all image lines by the FFT process in one dimension.
6. Transposes both the real and imaginary images.
7. Again applies the FFT to the real and imaginary lines as in 5, producing the real and imaginary Fourier images.
8. Tests a flag in the radial filter function table and, if it is set, carries out the adaptive filter process on the real and imaginary images.
9. Converts the radial filter function into a full field filter and multiplies both the real and imaginary images by the filter point-by-point.
10. Performs the inverse FFT on all lines of the image.
11. Transposes the real and imaginary images.

12. Again applies the inverse FFT as in 10.
13. Applies the phase transform to the real image to return it to normal.
14. Corrects the image average value for any net scale increase or decrease during the transformations and adds it to the image restoring the average value.
15. Rewrites the transformed image to the working area on the disk and appends the FFT tag (with or without the adaptive filter tag) to the image comments.

#### FILE program

A program to access raw data images from the Digital Equipment Corporation (DEC) GAMMA-11 image files and make them available for processing and for storing the results of processing back into GAMMA-11 files must be written. This task is made somewhat difficult due to the complex way in which the image condition is given in the GAMMA-11 administrative blocks and the changes that are being made by DEC in these blocks. The program itself is not too difficult, although a considerable variety of image formats are used. A temporary program performing some of the functions is now being used to access patient files from copies of a disk file from the VA Hospital at Lexington.

#### Development of an adaptive filter

A nonlinear imaging processing method involving operations in the spatial frequency domain was introduced by D. L. Kirch and D. W. Brown in 1972.<sup>4</sup> This method has great potential and complements the nonlinear methods in the spatial domain since it can attack image faults inaccessible

in signal space. The method had a considerable fault as originally proposed, due to the large intensity and wide-spread presence of frequency components arising from the truncation of intense parts of the image at the edge of the camera field -of- view. The strong components of this artifact obscured many of the unwanted components and shielded them from treatment by the frequency domain process they called an "adaptive filter."

In order to provide a practical version of this process, we found it necessary to develop an automatic method to reduce the effects of image truncation. A program was developed to find the edge of a camera image (which is not always in the same place in the computer image array) and apply a cosine rolloff before performing the direct FFT.

The adaptive filter process consists of testing each real and imaginary component of the frequency domain and if neither component is larger than a comparison value previously established for the image, then the sum of their squares is compared with the square of the comparison value. If the sum of the squares is less than the square of the test value, both real and imaginary components are set to zero. In this way much of the image noise and, equally important, many weak nonrandom interfering components are removed. After this adaptive process, the normal smoothing or a smoothing-and-resolution-sharpening filter, such as a Weiner filter, is applied to the frequency domain before inverse FFT transformation back to the signal domain. Preliminary tests of this adaptive filter are encouraging and the translated version is included in ORIS-11 as an option.

ORIS report

Volume II of the ORIS report (ORNL/TM-5875/V2), which serves as a program description and an operator's manual, has been completed and is available from the ORNL Biomedical Computing Technology Information Center as a part of the MED/8-ORIS code package. The program listings and comments are in late stages of completion and will appear as ORNL/TM-5875/V3. Plans for writing the algorithm descriptions and system performance are being completed. This part will be issued as Volume 1 of the report. A separate, considerably smaller report will be written for the ORIS-11 system when it is in operation in conjunction with GAMMA-11 at Vanderbilt or at Lexington.

## MISCELLANEOUS

Six shipments of  $^{43}\text{K}$  were made this quarter. Three shipments were to the University of Mississippi for their coronary disease studies in comparison with similar images obtained using  $^{201}\text{Tl}$ . Two shipments went to the V.A. Center, Wood, Wisconsin, and one shipment was sent to the National Institute for Environmental Health.

Three batches of  $^{64}\text{Cu}$  were supplied to ORAU for their continuing study of the tumor localization of  $^{64}\text{Cu}$  citrate and  $^{64}\text{Cu}$  bleomycin in tumored rats.

A cooperative program was initiated this quarter with the ORNL Biology Division to examine the usefulness of neutron activated chrysotile "A" asbestos fibers in their study of the distribution of asbestos in rodents following the deposition of fibers in transplanted tracheas. The principal radionuclides produced by neutron irradiation are  $^{51}\text{Cr}$ ,

$^{59}\text{Fe}$ ,  $^{60}\text{Co}$ , and  $^{46}\text{Sc}$ . The proposed method of determining translocations of the fibers in rodents is separation of the fibers from various tissue samples and measurement of the quantity by determining the radioactive content. However, previous investigations have shown that the radioactive species will leach from the asbestos in vivo and thus alter the radioactive concentrations. The purpose of this cooperative study is to determine the leach rate of the principal radionuclides in appropriate media and to determine whether corrections can be made to allow using the radioactivity present in a sample as a measure of the amount of asbestos.

Visitors this period include: a tour of Nuclear Medicine Technology students from Hillsborough Community College, Florida; Dr. Robert West, University of Minnesota, to discuss synthesis of organotin compounds of potential biological interest; and Dr. Michael Zalutsky, of Franklin McLean Memorial Research Institute to discuss preparation of short-lived radiopharmaceuticals.

F. F. Knapp presented a paper at the 18th Annual Meeting of the Southeastern Chapter of the Society of Nuclear Medicine, Winston-Salem, North Carolina, October 12-15, 1977 and also presented a seminar at ORAU entitled "Tellurium-123 Labeled Steroids: A New Class of Potential Adrenal Imaging Agents." R. R. Bell attended the Nuclear Science Symposium of the IEEE in San Francisco, California, October 17-20, 1977 where he presented a teaching session in the IEEE Short Course on Nuclear Medicine. He also presented an ORAU Traveling Lecture entitled "Origin of the Solar System," at Huntington College, Montgomery, Alabama on December 6, 1977.

## PAPERS AND PUBLICATIONS

## Papers

- F. F. Knapp, Jr., "Potential Pancreatic Imaging Agents-The Synthesis of Te-123m Labeled Telluro Amino Acids," Proceedings of the 18th Annual Meeting of the Southeastern Chapter of the Society of Nuclear Medicine, Winston-Salem, N. C., Oct. 13, 1977.
- P. R. Bell and J. M. Dougherty, "Nonlinear Image Processing Methods," IEEE Symposium on Nuclear Science, Short Course on Nuclear Medicine, San Francisco, Calif., October 18-21, 1977.

## Journals

- Karl F. Hubner, Gould A. Andrews, Raymond L. Hayes, J. Kenneth Poggenburg, Jr., and Alan Solomon, "The Use of Rare-Earth Radionuclides and Other Bone-Seekers in the Evaluation of Bone Lesions in Patients with Multiple Myeloma or Solitary Plasmacytoma," Radiology 125:171-176, October, 1977.
- Karl F. Hubner, Gould A. Andrews, Lee Washburn, Bruce W. Wieland, William D. Gibbs, Raymond L. Hayes, Thomas A. Butler, and John D. Winebrener, "Tumor Location with 1-Aminocyclopentane[<sup>11</sup>C]Carboxylic Acid: Preliminary Clinical Trials with Single-Photon Detection," J. Nucl. Med. 18(12):1215-1221, December, 1977.

## Reports

- P. R. Bell and R. S. Dillon, ORIS - The Oak Ridge Imaging System Operation's Manual, ORNL/TM-5875/V2, December, 1977.

## LIST OF REFERENCES

1. T. S. Elleman, J. W. Reishus, and D. S. Martin, Jr., J. Am. Chem. Soc. 80, 536 (1958).
2. Sarkar, et al., J. Nucl. Med. 17, 212 (1976).
3. Erez, et al., Nature 255, 635 (1975).
4. D. L. Kirch and D. W. Brown, "Recent Advances in Digital Processing Static and Dynamic Scintigraphic Data," pp. 27-54 in Proceedings of Second Symposium on Sharing of Computer Programs and Technology in Nuclear Medicine, CONF 720430, Oak Ridge, Tennessee (1972).

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Exhibit G

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S-49,068

"IMPROVED TISSUE-SPECIFIC SCINTIGRAPHIC IMAGING AGENTS"

Inventor: Furn F. Knapp, Jr.  
[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 <sup>123m</sup>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, <sup>14</sup>C and <sup>32</sup>P have been used extensively since their corresponding stable isotopes are present in practically all important cellular components. Biochemical agents labeled with <sup>99m</sup>Tc and <sup>75</sup>Se and many other radioisotopes have also found application as scintigraphic imaging agents for the detection of ~~various~~ cinomas, (What else?) pathophysiological conditions, which includes the detection of various diseases such as cancer, and as a means to detect abnormal functioning tissues.

\* Steroids labeled with <sup>131</sup>I and <sup>75</sup>Se have been proposed as adrenal imaging agents. Results in laboratory animals, i.e. mice, dogs,                      have shown acceptable adrenal accumulation of the agents good quality images of dog adrenals have been obtained. The use of <sup>75</sup>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 <sup>131</sup>I labeled adrenal agents is that the radioiodinated steroids were very unstable in vivo resulting in a high thyroid accumulations of radioactive iodine. Additionally, <sup>131</sup>I has

\* Note - These <sup>131</sup>I- and <sup>75</sup>Se-labeled agents have also been used in humans on a limited basis, and good images can be

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a limited shelf life and results in a high  $\beta$  absorbed dose. The  $^{75}\text{Se}$  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  $^{123m}\text{Te}$ -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  $^{123m}\text{Te}$ -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.?] We will have to discuss this a very general and well documented area.

$^{123m}\text{Te}$ -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  $^{123m}\text{Te}$  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  $^{123m}\text{Te}$ -labeled biochemicals.

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

These and other objects are achieved in a method for the preparation of  $^{123m}\text{Te}$ -labeled organic compounds comprising the steps of (a) reacting a di-alkali metal ditelluro  $\text{M}_2^{123m}\text{Te}$  with a halogen-

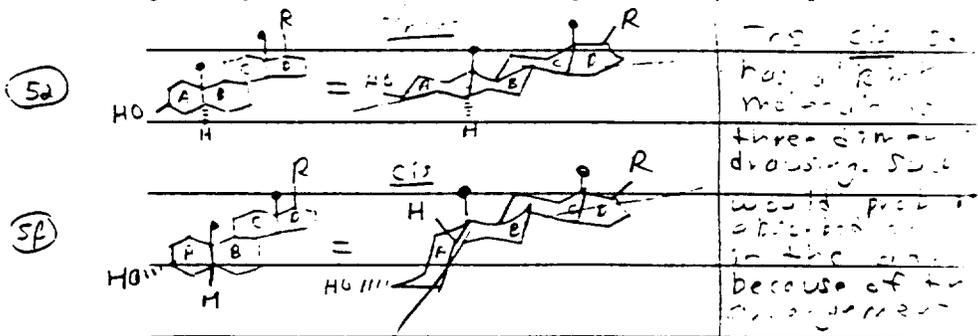
substituted organic compound, R-X, R being an alkyl or aryl group, to form a symmetric diorgano-ditelluride  $^{123m}\text{Te}_2$ , (b) reacting said diorgano-ditelluride with a reducing agent to form an alkali metal organo telluride of the formula  $\text{R}-^{123m}\text{Te-M}$ , (c) reacting said alkali metal organo telluride with a halogenated organic compound with a formula  $\text{R}'\text{-X}$ , 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  $\text{R}'\text{-}^{123m}\text{Te-R}$ .

Detailed Description

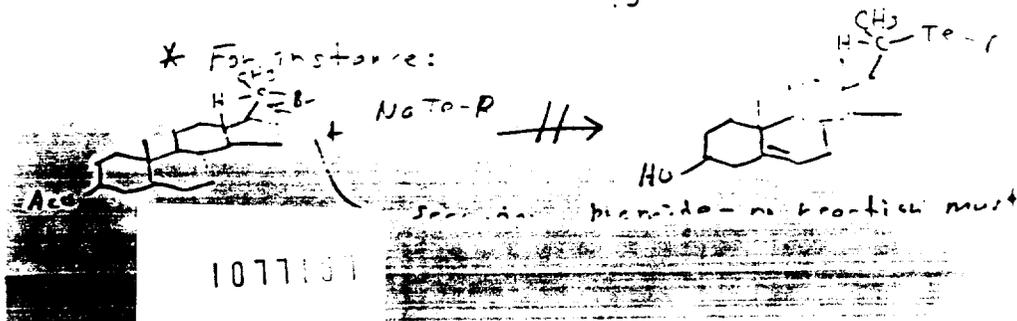
One aspect of this invention involves a synthesis of  $^{123m}\text{Te}$ -labeled steroids by methods which introduce the Te into the side chain rather than the steroid nucleus. As used herein, the steroidal side chain group is defined as the well known cyclopentanophenanthrene nucleus with an alkyl 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.]  
 we have found secondary positions are with certain alkyl tellurides - see page 2

According to the methods described herein the introduction of the tellurium into the side chain rather than the steroid nucleus preserves the trans geometry 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 $^{123m}\text{Te}$

$^{123m}\text{Te}$  is conveniently prepared from isotopically-enriched  $^{122}\text{Te}$ , 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  $^{123m}\text{Te}$  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.~~, etc. which is evaporated

to dryness to provide a tellurium salt. The salt can be redissolved in acid such as ~~HCl~~ HCl to assure complete dissolution of metallic Te and again evaporated to dryness. The resulting solid is dissolved in water. A ~~salt~~ reducing agent such as ~~NABr~~ (NaBr) is added (Why?) The NaBr reduces Te(IV)  $\rightarrow$  Te(II) and the ~~SO<sub>2</sub>~~ SO<sub>2</sub> then reduces the Te(IV) down to metallic tellurium [Eg. Te(0)].

and the solution is boiled for one-half hour (Why?) The reaction is more efficient at higher temperatures - by energy needed -

and cooled. ~~SO<sub>2</sub>~~ SO<sub>2</sub> 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?] Yes - It is a modification of a general method of H. P. Hupf, J. S. Elvinge and J. E. Beaver, Internat. J. Appl. Radiat. & Isotopes, 1<sup>o</sup>, 345-351 (1960).

$^{123m}\text{Te}$  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 homogeneity.~~ 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  $^{123m}\text{Te}$  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  $\text{M}_2\text{Te}_2$ , M being any alkali metal. [Would any other non-aqueous solvent be suitable?]

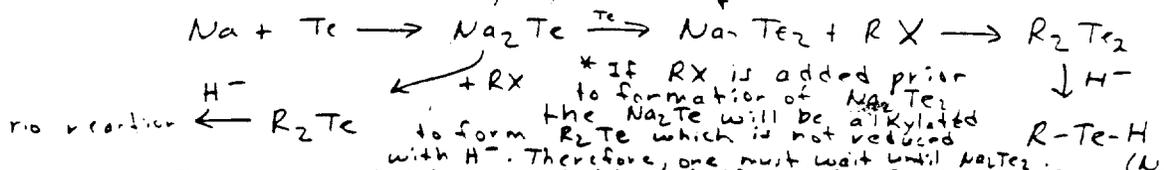
Na - liquid ammonia is a unique solvent for "solvating" electrons that can then be used for reduction -  
Eg.  $\text{Na} \rightarrow \text{Na}^+ + e^-$ ;  $e^- + \text{Te} \rightarrow \text{Te}^-$ , etc.  
forming  $\text{Na}_2\text{Te}_2$  - this is a general, common reaction and therefore need not be referenced.

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M<sub>2</sub>Te<sub>2</sub> is then converted to the desired di-alkyl-di-telluride. Since M<sub>2</sub>Te<sub>2</sub> 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<sub>2</sub>Te<sub>2</sub> is

formed? Please explain) Yes, the sequence of reactions is:



The reaction product is extracted with a suitable organic solvent such as benzene.

Note: almost any organic solvent can be used, diethyl ether, ethyl acetate, methanol, ethanol, hexane, etc.... Benzene is best because the inorganic by-products (Te, etc.) are not emulsified with this solvent and benzene can be used in the reductive step.

(3.) The extracted dialkyl ditelluride is reduced to form an alkali metal alkyl telluride by the addition of a reducing agent such as alkali metal borohydride,

Any hydride reducing agent will probably work but sodium borohydride is best because it readily reduces the -Te-Te- bond, but will not reduce many other functional groups.

[Does the reducing agent have to be an alkali metal compound?] No, sodium metal in the appropriate solvent (NH<sub>3</sub>), sodium borohydride, sodium borohydride and many others will work.

NaOH is added to the mixture and refluxed [Why?] To make sure the sodium salt of R-Te-H is formed (eg. R-Te-Na) and also to hydrolyze the steroid acetate ester to the free alcohol. In this manner an additional step is not needed since the free alcohol is the form we want.

The reduced product is an alkali metal alkyl telluroal. According to the method of this invention, the alkali metal alkyl telluroal 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.] The composition of R' is essentially unlimited and would depend upon R' being soluble and stable under the reaction conditions generally used.

C. Preparation of Steroidal nor-Halides

One 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?] No - the acids have been used essentially because they are readily available and easily converted to the halides by this reaction.

This degradation 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) herein incorporated by reference. <sup>(Hunsdicker copy)</sup> 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?] <sup>for means a</sup> ~~carbon has been removed - and in this case, with the concomitant introduction of a halide. There are many other methods of preparing steroids with a halogen in the side chain. This reaction was chosen because of its simplicity.~~

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

The steroidal-nor-halide is reacted directly with the alkyl 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 <sup>absorption chromatography.</sup> ion-exchange. [Any other recovery methods?] <sup>Any other absorption chromatographic reaction</sup> ~~could be used which includes: thin-layer chromatography, high-pressure liquid chromatography, etc.~~

#### 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, <sup>some</sup> alkyl telluro compounds 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?] ~~No other alkyl tellurides attempted.~~

*N.I.H.*  
~~benzyl telluride in the side chain. Benzyl telluride was used in the synthesis of telluro amino acids. Methyl telluride was also used to make telluroaminoacetic acid. See the pre-print for a description of this synthesis.~~

[Why can alkyl tellurides be used in steroid labeling and not amino acid labeling?] <sup>This is an interesting question and must</sup> ~~be a function of the inherent stability of the molecules.~~

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  $^{123m}\text{Te}$ -labeled Diphenyl Ditellurides

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

See Note # 1

[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?] Yes, aryl halides will not react with  $\text{MgTe}_2$  in liquid ammonia unless an electron withdrawing or electron donating group is present. Bromobenzene contains no such activating group and diphenyl ditelluride must therefore be prepared by an alternate route

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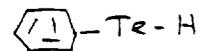
### B. Preparation of Phenyl Telluro

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

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[Please describe this reaction in detail. What is the resultant

product? Sodium alkyl telluro?] → aryl telluro



See Note # 2

### C. Preparation of Telluro Amino Acid

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

See Note # 3

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

EXAMPLE I

Preparation of  $^{123m}\text{Te}$

Isotopically enriched  $^{122}\text{Te}$  (94.71%), obtained from the isotope sales office of the Oak Ridge National Laboratory, was irradiated for 14 days in the Oak Ridge High Flux Isotope Reactor at  $2 \times 10^{15}$  neutrons/cm<sup>2</sup>·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?) not important  
After the addition of NaBr (5 grams) the solution was boiled for one-half hour, cooled, and SO<sub>2</sub> 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 centrifugation. The recovered particles were washed three times with water and dried in an oven at 140°C. The recovery of  $^{123m}\text{Te}$  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/mole. Approximately, 25 ml liquid ammonia was condensed into the

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reaction vessel containing the tellurium powder. The ammonia is maintained in the liquid state by inserting the flask in a bath of acetone and dry ice (-70-70°C)

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  $\text{Na}_2\text{Te}_2$  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  $^{123}\text{mTe}$  di-isopropyl di-telluride.

#### EXAMPLE IV

##### Preparation of sodium alkyl telluride.

The  $^{123}\text{mTe}$  di-isopropyl di-telluride solution from Example III was combined with 25 ml. of methanol (Why?) also, the substrates are more soluble and react more readily in the benzene-methanol mixture reduce the substrate in neat benzene. Sodium borohydride will not. 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, emphasizing any features you regard as new.]

See Note # 4

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?) See previous comment - the extra base induces formation of the Na-Te-R salt and also hydrolyzes the steroid acetate to the free alcohol.

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?)

The silicic acid column was prepared by slurring the silicic acid in benzene and packing it into a column. -//-

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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  $^{123m}\text{Te}$ -labeled steroid product was 26 mci/mole, indicating more than \_\_\_% yield.

[Why did the acetoxy group convert to hydroxy? Is this necessary or desirable?] See previous note - both necessary and desirable

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EXAMPLE VII

Preparation of 3-alpha-hydroxy-24-nor-23-(isopentyl telluro)-5-beta<sup>h</sup>-cholane.

3-alpha-acetoxy-24-nor-23-bromo-5-alpha<sup>e</sup>-cholane prepared by a modified Hunsdiecker synthesis from lithocholic acid acetate was added as described in Example V. [Any difference in preparation?]

no

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to a methanolic solution of sodium isopentyl telluro 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 chromatography indicated the reaction 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 chromatography. [Is there anything special about the TLC? What instrument, solvent and absorbant were used?]

yes is a standard TLC technique in this case. the absorbant was used as a standard. the solvent was chloroform.

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EXAMPLE VIII

Preparation of 3-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 borohydride in basic methanol in the manner of Examples 1-12.

3-alpha-acetoxy-24-nor-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 chromatography using chloroform solvent. (This material and the material of Example VI could not be crystallized, how would you propose purifying the product?)

*See TLC prep. of purification - there were earlier experiments actually giving good results. It is preferable to use a different solvent system.*

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?)

*Sample II - See Note #4*

The product was crystallized from methanol and water to give fine needles. To this material was added 200  $\mu$  moles of sodium isopentyl telluro. In refluxing methanol the reaction mixture was poured into water and the crude product extracted with chloroform. Purification by thin layer chromatography gave a thick gum the product was homogeneous by thin layer chromatography 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?)

*by TLC*

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



Th...  
adrenal imaging

Adrenal imaging

Time = D...

①

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  $^{123m}\text{Te}$ -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 anesthetized with ether. The rats were sacrificed at select times after being anesthetized with ether. Blood was drained from the carcass into a beaker containing a small amount of sodium citrate solution. (Is this to prohibit clotting?)

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

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

4) Male and female rats were injected with the  $^{123m}\text{Te}$ -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.

6) The animals were anesthetized after intraperitoneal injection of a sodium pentobarbital solution (30-50 mg/kg) and scans were obtained using a rectilinear scanner equipped 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?] \_\_\_\_\_

7) 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 intravenous administration of the  $^{123m}\text{Te}$ -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, <sup>e</sup>adrenals and lungs all contained significant levels of

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The mean percent dose/gram of the liver was 2.71 after one hour decreasing to 0.84 after eighteen 18 hours. The % dose for the spleen decreased from 2.48 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 for the adrenals increased from 4.51 after one hour to 22.17 after 18 hours.

15B

After one day the % dose <sup>in</sup> for the liver was 1.01 decreasing to 0.23 after 7 days. The % dose <sup>in</sup> for the spleen was 1.59 <sup>after one day</sup> decreasing to 0.29 after 7 days. The % dose <sup>in</sup> for the lungs decreased from 2.12 after 1 day to 0.40 after seven 7 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 <sup>substantially</sup> constant <sup>at 7 days</sup>.

17c

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 7 days to 0.05 after 21 days. The % dose in the thyroid decreased <sup>remained</sup> constant at 0.12-0.15 throughout the 7-21 day period. The % dose of adrenals decreased from 5.56% at 7 days to 1.81% after 21 days.

radioactivity. The concentration of radio activity increased rapidly in the adrenals, however, while the levels of radioactivity decreased or remained constant, in the other organs described above. <sup>← A</sup> 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.~~

B → These results are further substantiated by similar data obtained from animals in the third group <sup>which</sup> and were sacrificed 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, II,~~

~~and summary,~~ 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.

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

Tissue	Mean Percent Dose/gram, $\pm$ s.d.		
	1 hour after dose	6 hours after dose	18 hours after dose
Blood	1.48 $\pm$ 0.35	0.95 $\pm$ 0.04	0.49 $\pm$ 0.02
Liver	2.71 $\pm$ 0.30	2.03 $\pm$ 0.07	0.84 $\pm$ 0.09
Spleen	2.98 $\pm$ 0.15	2.75 $\pm$ 0.27	1.29 $\pm$ 0.09
Pancreas	0.16 $\pm$ 0.02	0.24 $\pm$ 0.06	0.19 $\pm$ 0.01
Stomach	0.11 $\pm$ 0.04	0.07 $\pm$ 0.02	0.05 $\pm$ 0.01
Small Intestine	0.71 $\pm$ 0.19	0.71 $\pm$ 0.09	0.39 $\pm$ 0.02
Large Intestine	0.06 $\pm$ 0.03	0.72 $\pm$ 0.23	0.86 $\pm$ 0.10
Adrenals	4.51 $\pm$ 1.16	16.51 $\pm$ 1.31	22.17 $\pm$ 3.05
Kidneys	0.63 $\pm$ 0.04	0.76 $\pm$ 0.05	0.77 $\pm$ 0.08
Prostate	0.07 $\pm$ 0.01	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01
Testes	0.04 $\pm$ 0.003	0.08 $\pm$ 0.01	0.09 $\pm$ 0.01
Heart	0.48 $\pm$ 0.09	0.53 $\pm$ 0.09	0.43 $\pm$ 0.05
Lungs	2.01 $\pm$ 0.09	1.99 $\pm$ 0.08	1.52 $\pm$ 0.18
Thyroid	0.42 $\pm$ 0.05	2.07 $\pm$ 0.78	0.53 $\pm$ 0.22
Brain	0.04 $\pm$ 0.01	0.05 $\pm$ 0.001	0.04 $\pm$ 0.004

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

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

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

	Mean Percent Dose/gram, $\pm$ s.d.		
	7 days after dose	14 days after dose	21 days after dose
Blood	0.19 $\pm$ 0.05	0.16 $\pm$ 0.04	0.09 $\pm$ 0.02
Liver	0.14 $\pm$ 0.03	0.06 $\pm$ 0.01	0.03 $\pm$ 0.01
Spleen	0.32 $\pm$ 0.12	0.20 $\pm$ 0.03	0.11 $\pm$ 0.03
Pancreas	0.12 $\pm$ 0.01	0.07 $\pm$ 0.01	0.04 $\pm$ 0.01
Stomach	0.05 $\pm$ 0.02	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01
Small Intestine	0.38 $\pm$ 0.49	0.03 $\pm$ 0.003	0.02 $\pm$ 0.01
Large Intestine	0.09 $\pm$ 0.01	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01
Adrenals	5.56 $\pm$ 1.38	4.59 $\pm$ 0.45	1.81 $\pm$ 0.57
Kidneys	0.49 $\pm$ 0.12	0.29 $\pm$ 0.05	0.16 $\pm$ 0.01
Prostate	0.06 $\pm$ 0.01	0.04 $\pm$ 0.01	0.02 $\pm$ 0.004
Testes	0.07 $\pm$ 0.01	0.05 $\pm$ 0.01	0.03 $\pm$ 0.004
Heart	0.09 $\pm$ 0.03	0.03 $\pm$ 0.02	0.02 $\pm$ 0.001
Lungs	0.22 $\pm$ 0.04	0.11 $\pm$ 0.01	0.05 $\pm$ 0.004
Thyroid	0.13 $\pm$ 0.07	0.15 $\pm$ 0.08	0.12 $\pm$ 0.06
Brain	0.04 $\pm$ 0.01	0.06 $\pm$ 0.03	0.03 $\pm$ 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 elution with solvents of increasing polarity. The columns were initially eluted 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 extract 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.

(S) The metabolism of such adrenal imaging agents is important because

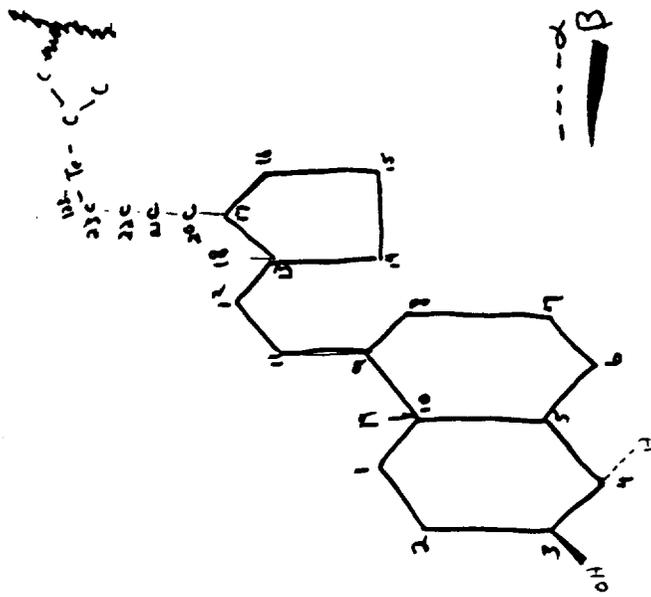
(T) The adrenal glands of the male rats were clearly imaged one day after administration of the  $^{123m}\text{Te}$ -labeled steroid. Both the adrenals and ovaries of female rats were also imaged following the injection of the agent both a rectilinear scanner and an RC-type proportional counter camera.

(C) 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-cholesterol-5-ene and 3-beta methoxy-24-isopropyl-telluro-5-ene accumulate slowly in the

adrenals. The steroid 3-beta-hydroxy-24-isopropyl telluro-cho1-5-ene showed a slightly greater adrenal 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 adrenals.

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.

1077177





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June 23, 1978

Dean E. Carlson, Chief, Prosecution Branch, Patents, HQ  
Germantown, CXXI, 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. . . . . 15

    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

MCP:AHU/br  
Enclosures:  
As stated above

11/21/78

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT : Fern F. Knapp, Jr. :  
SERIAL NO.: 920,411(70) : GROUP 124  
FILED : June 29, 1978 : EXAMINER  
FOR :  $^{123}\text{mTe}$ -LABELED BIOCHEMICALS AND  
METHOD OF PREPARATION

DECLARATION UNDER 37 CFR 1.131

I, Fern F. Knapp do hereby declare and affirm:

THAT, I am the applicant of U. S. Patent Application S.N. 920,411, entitled " $^{123}\text{mTe}$ -Labeled Biochemicals and Method of Preparation" filed June 29, 1978, and the inventor of the subject matter described and claimed therein;

THAT, prior to June 1, 1978, I had completed my invention as described and claimed in said application in the United States of America as evidenced by the following:

a) Prior to June 1, 1978 I, or others under my supervision and control, had prepared the following  $^{123}\text{mTe}$ -labeled organic compounds of the general formula  $\text{R-}^{123}\text{mTe-R}'$ , R being either alkyl, substituted alkyl, and or substituted aryl and R' being a steroid side chain, alkyl amino acid, or amino acid group

- a)  $^{123}\text{mTe}$ -24-nor-23-(isopropyl telluro)-5 $\beta$ -cholan-3 $\alpha$ -01
- b)  $^{123}\text{mTe}$ -24-nor-23-(isopropyl telluro)-5 $\alpha$ -cholan-3 $\beta$ -01
- c)  $^{123}\text{mTe}$ -24-(isopropyl telluro)-chol-5-en-3 $\beta$ -01
- d)  $^{123}\text{mTe}$ -24-(isopropyl telluro)-chol-5-en-3 $\beta$ -0Me
- e) 24-nor-23-(isopropyl telluro)-5 $\alpha$ -cholan-3 $\beta$ -01
- f) 24-nor-23-(isopentyl telluro)-5 $\alpha$ -cholan-3 $\beta$ -01
- g) 24-nor-23-(isopentyl telluro)-5 $\beta$ -cholan-3 $\alpha$ -01
- h) 24-nor-23-(phenyl telluro)-5 $\beta$ -cholan-3 $\alpha$ -01

(Please list other  $^{123}\text{mTe}$  steroids you had prepared prior to the date of June 1, 1978. Indicate which of the above were prepared with  $^{123}\text{mTe}$  and which were prepared with stable Te.

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DL- $\alpha$ -amino- $\alpha$ -(phenyl telluro ethyl)butyric acid.

(Please list other amino acids or alkyl amino acids prepared prior to June 1, 1978.)

as evidenced by attached exhibit <sup>A</sup> #;

THAT each of the above-listed  $^{123m}\text{Te}$ -labelled or stable Te-organic chemicals were prepared by a method which comprised the steps of

a) reacting a  $^{123m}\text{Te}$ -symmetric diorgano ditelluride  $\text{R}_2^{123m}\text{Te}_2$ , with  $\text{NaBH}_4$  which is a hydride reducing agent and a source of alkali metal ions to form  $\text{Na-}^{123m}\text{Te-R}$ , an alkali metal organo telluride, in which R was either an isopropyl, octyl, phenyl, pentyl, (others<sup>?</sup>)

and;

b) reacting the  $\text{Na-}^{123m}\text{Te-R}$  with a primary halogenated organic compound  $\text{Ra'-X}$  in which X was either Br, \_\_\_\_\_ and Ra' was either (steroidal side chain groups)

or (groups hydrolyzeable to an alkyl amino acid group)

THAT in the preparation of the above-listed  $^{123m}\text{Te}$ -labelled biochemicals, the symmetric diorgano ditellurides,  $\text{R}_2^{123m}\text{Te}_2$  were prepared by reacting  $\text{Na}_2^{123m}\text{Te}_2$  with a halogenated organic compound  $\text{R-X}$  where X was \_\_\_\_\_;

THAT in the preparation of said  $^{123}\text{mTe}$ -3-beta-hydroxy-24-nor-23-(isopropyl telluro)-5-alpha-cholane, the primary halogenated organic compound  $\text{Ra}'\text{-X}$  was 3-beta-acetoxy-24-nor-23-bromo-5-alpha-cholane and was reacted with  $^{123}\text{mTe}$ -sodium isopropyl telluro under basic conditions provided by conducting the reaction in refluxing methanol as evidenced in the attached exhibit <sup>B</sup>; exhibits I and II

THAT in the preparations of the following  $^{123}\text{mTe}$ -labeled amino acids,  $\text{R-}^{123}\text{mTe-CH}_2\text{-R}'$  [please list the amino telluro  $\rightarrow$   $^{123}\text{mTe}$  telluro amino acids prepared]

(10 lines)

were carried out by reacting the following  $\text{Na-}^{123}\text{mTe-R}$  compounds

(10 lines)

with a primary halogenated organic compound  $\text{Re}'\text{-X}$ ,  $\text{Re}'$  being a hydantoin group or a 5-alkyl hydantoin group to form the products \_\_\_\_\_

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which were hydrolyzed under basic conditions by (describe briefly what was done.)

to form the following  $^{123}\text{mTe}$ -labelled amino acids

THAT the preparation of the above-described compounds is evidenced by the following documents which were prepared by me or at my direction prior to June 1, 1978. (I believe I have sufficient documents attached to show how all the compounds were prepared. If possible, could you see if you have any records such as letters to journals, etc. which substantiate the date the articles were prepared. If you have any such documents, please describe them briefly.)

Exhibit A was prepared prior to June 1, 1978 as evidenced by

its date which is ~~dated~~ prior to June 1, 1978

etc.

Exhibit B was prepared prior to June 1, 1978 as evidenced by its date on page 17 which is deleted.

~~Exhibit~~

C-E:  
Please describe the remaining exhibits A what were they? when were they written?, etc.

THAT, on May 25, 1978, a draft of the above-identified patent application, <sup>Exhibit F</sup> was typed and thereafter forwarded to me for review;

THAT within about ~~2-5-78~~ days during which I also carried on my normal duties associated with my position of \_\_\_\_\_ at the Nuclear Medicine Technology Group at the Oak Ridge National Laboratory, I reviewed the draft application and made extensive corrections as indicated by the attached exhibit E;

THAT, after my review, my attorney, Mr. Allen Uzzell, incorporated my corrections and revisions into the application, had it retyped, and I read and executed the application as filed on June 23, 1978;

THAT the executed application was forwarded to the Department of Energy Headquarters on June 23, 1978 as indicated by the attached Exhibit <sup>H</sup> F;