Bifunctional Chelates of Rh-105 and Au-199
as Potential Radiotherapeutic Agents

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INTRODUCTION

Since last year we have

- 1) Investigated the production of Rh-105 by the Szilard-Chalmers process using $\text{Ru}(\text{acac})_3$ targets,
- 2) Synthesized several new ligands and their rhodium complexes,
- 3) Done preliminary studies of the radiochemical properties of some of these complexes of Rh-105 at 10^{-4} - 10^{-3} M concentration, and
- 4) Done preliminary animal studies on one of the complexes.

1. Production of Rh-105 by the Szilard-Chalmers Process

Last year we described a method for separating and purifying Rh-105 from neutron-irradiated ruthenium metal targets. The separation is based on the oxidation of ruthenium metal to RuO_4 in basic NaClO solution followed by distillation of RuO_4 after acidifying. The RuO_4 is collected in CCl_4 or HCl traps and the Rh-105 activity remains in the distillation flask. The method works well for targets up to ~ 100 mg and is now being used routinely by University of Missouri Research Reactor (MURR) personnel to produce ~ 50 mCi of high purity Rh-105 each week from 10-mg 99%-enriched Ru-104 targets.

Large scale production (50-100 Ci/wk, for example) will require the use of natural ruthenium (to reduce cost and insure adequate supply of target) in quantities of 50-100 g. We are interested in finding a production method which will not require dissolving of such large amounts of the metal or distillation of such large amounts of RuO_4 .

One such possibility is the use of the Szilard-Chalmers method (1)

to produce high-specific-activity Ru-104 which would then decay to Rh-105 which might then be separated from the bulk target. We chose ruthenium acetylacetonate, $\text{Ru}(\text{acac})_3$, because earlier work (2) with $\text{Co}(\text{acac})_3$ indicated the feasibility of its use, its ready solubility in a number of organic solvents, and its commercial availability.

In a typical experiment, 10 mg of $\text{Ru}(\text{acac})_3$ (97% pure) was sealed in a quartz vial and placed inside a high density polyethylene vial. This target was irradiated for 30 min at 8×10^{13} n/cm²/sec or 60 min at 4×10^{13} n/cm²/sec to give an integrated flux of 1.4×10^{17} n/cm². The target was allowed to decay for 24 hr and then opened and dissolved in benzene. Portions of this benzene solution were extracted with twice their volume of 3M NaOH, 0.001 M HCl, or 1 M HCl. The basic solution was chosen to give one from which Rh-105 could be purified as before and the acids to give solutions from which Rh^{3+} complexes might be prepared directly.

Since the target was natural ruthenium, Ru-97 and Ru-103 were also produced. The various fractions were counted on a Ge(Li) detector which allowed easy resolution of the gamma ray peaks of each radionuclide. Each sample was extracted twice. The experiment was repeated for some benzene solutions which were allowed to stand for 24 hrs before separating. The results are shown in Table 1. Results for Ru-97 and Ru-103 were very similar and only those for Ru-103 are shown. Approximately 10% less total activity could be extracted the second day.

Some of the aqueous extracts were analyzed for ruthenium by a spectrophotometric method and we estimate that less than 5% of the total ruthenium is in the first HCl extracts and less than 7% in the NaOH. This leads to an enrichment factor of ~ 10 . We found, however, that $\sim 3\%$

of the ruthenium could be extracted from unirradiated $\text{Ru}(\text{acac})_3$ so that the actual enrichment factor may be greater.

Table 1. Extraction of benzene solutions of neutron-irradiated $\text{Ru}(\text{acac})_3$.

Extracting Solution	3M NaOH	0.001M HCl	1M HCl
Percent activity in first extract			
Rh-105	74±6	53±3	58±7
Ru-103	75±1	47±3	50±6
Percent activity in second extract			
Rh-105	8±1	9±2	9±3
Ru-103	7±1	9±2	10±2
Percent activity left in benzene			
Rh-105	18	38	33
Ru-103	18	44	40

Two runs using 10 mg and 25 mg were made for 24 hr in a flux of $8 \times 10^3 \text{ n/cm}^2/\text{sec}$ for an integrated flux of $7 \times 10^{18} \text{ n/cm}^2$. Results were somewhat poorer with 40-50% of the activity in the first extracts.

There was no evidence however of pressure build-up in the vials or of significant radiation damage to the samples. The vials which we use are too small to permit use of larger samples, but we believe these results are encouraging and we intend to pursue these studies. (See section on proposed work).

Under the conditions above we made 0.12 mCi/mg $\text{Ru}(\text{acac})_3$. Assuming a 50% extraction of Rh-105 and a 5% extraction of ruthenium this gives a product with a specific activity of 480 mCi Rh-105/mmol ruthenium. Further purification of this extract with a separation factor of only 10,000 would give 4800 Ci Rh-105/mmol ruthenium, a specific activity high enough for antibody labeling.

If we can work with targets as large as 10g with similar results, we should be able to obtain ~600 mCi of Rh-105 and only 125 mg. of ruthenium, a quantity which we can easily handle with our present system. Use of enriched Ru-105 would require only 2g of $\text{Ru}(\text{acac})_3$ to make the same amount of activity but the ruthenium in the solution would be only 25 mg. Cost of the Ru-104 for 2 g of $\text{Ru-104}(\text{acac})_3$ would be about \$1,500 but a large part of it, perhaps as high as 90% could be recovered for reuse. Therefore, we believe that continued investigation of this production method is warranted.

(This work was abstracted from a University of Missouri M.S. thesis by Yau-Kai Wong, August 1987. The work was in collaboration with Dr. Alan Ketring, MURR).

2. SYNTHESIS OF LIGANDS AND RHODIUM COMPLEXES

We have concentrated thus far on development of appropriate stable, inert rhodium (III) complexes which are or can be modified to be bifunctional chelating ligands for Rh-105. Our approach has been to modify known ligands (3) to give stable rhodium (III) complexes; these are amine-oxime ligands. By loss of one oxime proton and coordination of two chloride ligands, two amine nitrogens, and two oxime nitrogens, a stable, neutral, octahedral rhodium (III) complex is formed.

The following reaction schemes are being used to produce new amine oxime ligands:

where R and/or R' in all four final products (2), (3), (6) and (11) would ideally contain the functionality to bind the antibody.

For (2) we have succeeded in preparing ligands with $R=\phi$, ϕCH_2 , $\phi\text{CH}_2\text{CH}_2$, $\text{NO}_2\phi\text{CH}_2$, and $\text{NH}_2\phi\text{CH}_2$. For the first four we have isolated and characterized the rhodium(III) complexes. Proton NMR spectra of these indicate the expected octahedral complexes. X-ray structures of the first three show the usual Rh coordination (see Figures 1 thru 3). The phenyl groups project out from the metal center which should allow attachment to antibodies after inclusion of appropriate functionalities on the phenyl groups. We have also isolated a rhodium product from the reaction of the $\text{NH}_2\phi\text{CH}_2$ ligand, but characterization is incomplete. We are presently studying solution compositions in the syntheses of the metal complexes by examining the UV-vis spectra of the products and the reaction mixtures.

In an attempt to react a tetraamine with the chlorooxime (1) in methanol, we isolated

and have obtained a very stable rhodium complex of that ligand (Figure 4). We then succeeded in making the same ligand with triethylamine replacing the tetraamine. This ether oxime has been reported (4) by another route.

For (3) we have succeeded in obtaining the ligand with $R=\phi\text{CH}_2$ and $R'=\text{H}$ and have isolated and characterized by x-ray diffraction the Ni(II) complex. In addition we obtained the tridentate ligand (HL)

Fig. 1

Fig. 2

Fig. 4

as a second product and have isolated and structurally characterized its Rh(III) complex, $[\text{RhLCl}_3]$ (Figure 5) which with appropriate substituents on the phenyl group might be useful for attachment to antibodies.

Presently work is in progress to attach benzyl groups to both amine nitrogens. The first step of this involving production of $\phi\text{SO}_2\text{NHCH}_2\text{CH}_2\text{CH}_2\text{NHSO}_2\phi$ has been completed.

In the case of (6) we are in final stages of synthesis of the $\text{R}=\text{CH}_2\phi$ product and are beginning work on the $\text{R}=\text{CH}_2\phi\text{NO}_2$ product while for reaction scheme (4) we have reached (9) with $\text{R}=\text{CH}_2\phi$ as a model for future substitution on ϕ .

Another approach has been to look at an even simpler ligand, dien, $\text{NH}_2\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{NH}_2$, and its derivatives. It has been shown that such ligands form complexes of the type RhLCl_3 with rhodium (5).

Furthermore, a relatively simple scheme for protecting the primary amines while alkylating the secondary is known (6). We have utilized that scheme as shown to prepare the benzyl compound and its derivatives.

Y = phthalic anhydride
 X = -H, -NO₂, -CO₂H, -NH₂

A complex of the ligand (16) for which X=H with rhodium has been prepared and is shown in Fig. 6. It was crystallized from DMSO and the

Fig. 5

Fig. 6

site trans to the tertiary amine is coordinated by DMSO rather than Cl^- giving a positive complex.

This ligand may be even more useful because it is now possible to add to the primary amines to form pentadentate ligands. We have, for example, made an amine oxime.

This complexes Rh^{+3} readily but the complex is not yet characterized. Similar reactions with the triamines described above will lead to bifunctional pentadentate ligands. A similar compound has been prepared by reacting (1) with (16).

We can also make bifunctional unsymmetrical tetradentate ligands by alkylating the secondary amine of the protected dimethyl dien

deprotecting and adding to the lone primary amine. This series of reactions can therefore lead to a group of bifunctional tri, tetra, and pentadentate ligands.

An important feature of all the structures shown is that the phenyl group is directed away from the rhodium core and functional groups such as $-\text{NH}_2$ or $-\text{CO}_2\text{H}$ at the para position should not be involved in coordination and should therefore be available for protein linking reactions. If necessary a "spacer" group can be added to them to make the separation even greater.

3. Radiochemistry of Some Rhodium Complexes.

We have used the Ru-105 produced by the Szilard-Chalmers process

described earlier to make complexes of rhodium propylene amine oxime and benzylamine oxime.

They were chosen because RhCl_2PnAO is a well-known complex which we use as a model compound and because the benzylamine oxime complex afforded an opportunity to investigate the properties of one of the benzyl complexes described earlier.

In a typical experiment, $\sim 8 \times 10^{-4}$ or 8×10^{-3} mmol of rhodium carrier as $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ was added to 10 ml of 0.001M HCl containing the no-carrier-added Rh-105 as well as ~ 5 – 10×10^{-4} mmol ruthenium with Ru-97 and Ru-103. (Note that in actual use the Rh-105 would be further purified. The ruthenium was left to serve as an indicator of the extent of complexing of the Ru-97 and Ru-103.) This quantity of rhodium carrier was chosen to approximate conditions which would be encountered in actual use. As an example, assume that the Rh-105 product is 100 mCi/ml and that the labeled antibody is to be 10 mCi/mg (i.e., $\sim 1.5 \times 10^6$ mCi/mmol). If an average of one rhodium per antibody is added the specific activity of the Rh-105 must be the same. Therefore the amount of carrier to be added to 10 ml of 100 mCi/ml Rh-105 is $\sim 7 \times 10^{-4}$ mCi/ml. On the other hand, if 1000 mCi/ml Rh-105 is available and only 1 mCi/mg antibody is needed, as much as 7×10^{-2} mmol carrier could be used.

The solutions were refluxed to promote exchange between the Rh-105

and the rhodium carrier and then 10 ml of an alcoholic solution of a two-fold excess of the ligand was added and the solution refluxed again. Most of the alcohol was removed by evaporation and the complex extracted with two 10-ml portions of CHCl_3 or CH_2Cl_2 . The amount of Rh-105 and Ru-103 in each of the organic fractions was determined.

With 8×10^{-3} mmol carrier 50-60% of the complexed activity could be extracted into CH_2Cl_2 or CHCl_3 . With 8×10^{-4} mmol carrier only 20-30% was extracted. However, when excess ligand was added (~ 10 -fold excess), again 50-60% of the complex could be extracted. (Note that a complex with the benzyl groups trans to each other would probably be charged and not as readily extracted. The total complex yield may be higher than reported here.) When the organic fractions were back extracted into saline, ~ 25 -30% could be extracted, indicating a CHCl_3 /saline distribution of $\sim (2-3)/1$. Results were similar for propylene amine oxime and benzyl amine oxime.

These results suggest that rhodium complexes of the Rh-105 specific activities required for use can be made. However, another separation scheme may be needed since the complexes containing $-\text{NH}_2$ or $-\text{CO}_2\text{H}$ functional groups may not extract as well.

(This portion of the work was also abstracted from the M.S. thesis of Yau-Kai Wong).

We have also prepared Tc-99m complexes of benzyl amine oxime and find there properties very similar to those of the Rh-105 complex. We suspect that the technetium structure includes the $\text{Tc}(v)\text{O}_2^+$ core.

4. Biodistributions.

Biodistributions studies have just been started. It is our intention to do biodistribution for each promising bifunctional chelate

as the uncoupled chelate as well as after coupling to a protein.

RhCl_2PnAO was chosen as our first model compound because of its similarity to the previously studied TcOPnAO and $\text{TcO}_2\text{PentAO}$.

RhCl_2PnAO was prepared as described above using Rh-105 which had been prepared from irradiation of enriched Ru-104 metal. $\text{RhCl}_3 \cdot 3\text{H}_2\text{O}$ carrier (0.04 mmol) was added to 5 ml stock Rh-105 solution and refluxed. One 1-ml portion was set aside. A second 1-ml portion was diluted to 5ml with 10^{-3}M HCl and an alcoholic solution containing 0.015 mmol PnAO added. The complex was prepared and extracted as before. The CHCl_3 was evaporated and the residue taken up in 1 ml physiological saline and this solution filtered.

Sprague-Dawley rats ($\sim 250\text{g}$) were given 200- μl injections of the complex solution as well as the uncomplexed Rh-105. Three rats were used for each test. The animals were sacrificed at 30-min and the major organs removed for counting. Results are shown in Table 2 along with other results from our laboratory for two similar complexes of Tc-99m.

Table 2. Biodistributions. Percent dose per organ.

	Uncomplexed ^a			
	<u>Rh-105</u>	<u>RhCl_2PnAO</u>	<u>TcOPnAO</u>	<u>$\text{TcO}_2\text{PentAO}$</u>
Liver	28 $\pm$ 3	8 $\pm$ 2	16	8
Kidneys + Urine	17 $\pm$ 2	16 $\pm$ 3	17	30
Intestines	3 $\pm$ 1	18 $\pm$ 5	47	30
Blood	9 $\pm$ 1	3 $\pm$ 1	6	5

^a $\sim 40\%$ of injected activity localized in tail vein.
 Organ distributions are normalized to remainder of dose.

These results do not of course, serve to predict the biodistribution of labeled proteins. They do, however, show a clear difference between the uncomplexed Rh-105 and the complex as well as a similarity to related Tc-99m complexes. Less than 1% was found in the heart or lungs and less than 0.1% in the brain. An apparent anomaly is that only ~40-50% of the total injected dose was found for the Rh-105 samples compared to 70-90% for the Tc-99m. This may indicate a high bone or muscle uptake and will be investigated more carefully.

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5. Publications and Presentations.

- a. D.E. Troutner, "Chemical and Physical Properties of Radionuclides", Nucl. Med. Biol. 14, 171 (1987). (reprint enclosed)
This paper was also presented as an oral presentation at the meeting of the Missouri Valley Chapter of the Society of Nuclear Medicine, Sept. 1986, Kansas City.
- b. B. Grazman and D.E. Troutner, "Rh-105 as a Potential Radiopharmaceutical" submitted to Int. J. App. Radiation and Isotopes. (preprint enclosed)
- c. Yau-Kai Wong "Production of Rh-105 by the Szilard-Chalmers Process", M.S. Thesis, Univ. of Missouri, 1987.
- d. Tonja Lynde-Kernell and E.O. Schlemper, "Preparation, Characterization, and Crystal Structure of Two Amine-Oxime Rhodium Complexes", Coordination Chemistry, in press. (page proofs enclosed)
- e. Tonja Lynde-Kernell and E.O. Schlemper, "Trans-Dichlorobis(ethylenediamine) rhodium(III) Complexes as Potential Radiopharmaceuticals", oral presentation, American Chemical Society, New Orleans, Sept. 1987.