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Notes

Potential Organ- or Tumor-Imaging Agents. 18. Radioiodinated Diamines and Bisquaternaries

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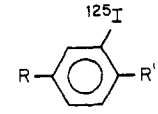
The purpose of this research was to employ diamines and their quaternary derivatives as carrier molecules for γ-emitting radiation. The diamine putrescine is widespread in nature and has been reported to selectively concentrate in the rat ventral prostate and pancreas. This study confirms the selective uptake of radioactivity in the rat ventral prostate, but not in the pancreas, following administration of [^{14}C]putrescine. The radioiodinated analogues of putrescine showed no predilection for either of these organs. On the other hand, radioactivity associated with a radioiodinated quaternary derivative (**3**) was found to accumulate in cartilaginous tissues such as trachea, intervertebral disks, and chondrosarcoma tumor in a manner simular to hexamethonium.

The polyamines spermidine and spermine and their precursor putrescine are ubiquitous in nature. They have been reported to occur in high concentrations in tissues such as the rat ventral prostate and pancreas.[1] The recent report in the literature that [^{3}H]putrescine can be selectively concentrated in the rat ventral prostate and pancreas,[2] as well as the fact that high levels of polyamines have been reported in the serum and urine of cancer patients,[3,4] has raised interest in analogues of polyamines as possible carrier molecules for γ-emitting nuclides useful for imaging organs such as prostate and pancreas or tumors.

In addition, the rapid accumulation of the bisquaternary drug hexamethonium in poorly vascularized tissue such as cartilage[5,6] suggests that a bisquaternary ammonium analogue labeled with a suitable γ-emitting nuclide may be useful for imaging cartilaginous tissues and other tissues containing high concentrations of glycosaminoglycans.

Radioiodine was selected as the γ-emitting nuclide for our preliminary studies. The reactivity of aliphatic iodides with amines necessitated incorporation of the radioiodine into an aromatic system. This feature also afforded the needed stability to minimize in vivo deiodination, a general finding with radioiodinated aliphatic compounds.[7]

On this basis, appropriate derivatives of 2-iodo-*p*-xylylenediamine were synthesized as iodinated analogues of putrescine and hexamethonium. Introduction of iodine-125 was achieved by isotope exchange of the iodinated primary or tertiary diamines with sodium iodide-125 and afforded compounds **1** and **2** The radioiodinated hexamethonium analogue **3** was obtained by quaternization of **2** with CH_3I.



1, R = R′ = CH_2NH_2
2, R = R′ = $CH_2N(CH_3)_2$
3, R = R′ = $CH_2N^+(CH_3)_3$
4, R = H; R′ = $CH_2N^+(CH_3)_3$

The tissue distribution of **1**–**3** in rats was compared with that found for ^{14}C-labeled putrescine and hexamethonium. The distribution of all three diamines was studied at a minimum of three time periods. Because of the excretion of these polar compounds, most of the radioactivity had disappeared from tissues by 24 h and most certainly by 48 h. For this reason, a comparison of the distribution profiles for the compounds was restricted to early time periods, namely, 0.5 and 2 h.

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Table I. Distribution of Radioactivity Following Intravenous Injection of Diamines [^{14}C]Putrescine, 1 and 2 to Rats

	% administered dose/g[a]					
	[^{14}C]putrescine		compd 1		compd 2	
tissue	0.5 h	2 h	0.5 h[b]	2 h	0.5 h	2 h
adrenal	0.580 ± 0.125	0.426 ± 0.058	1.732 ± 0.216	1.577 ± 0.114	2.287 ± 0.167	1.840 ± 0.194
bone marrow	0.657 ± 0.089	0.629 ± 0.059	0.440 ± 0.061	0.682 ± 0.040	0.483 ± 0.042	0.569 ± 0.062
brain	0.041 ± 0.002	0.064 ± 0.016	0.063 ± 0.006	0.088 ± 0.005	1.791 ± 0.139	1.255 ± 0.202
diaphragm	0.093 ± 0.005	0.081 ± 0.007	0.380 ± 0.063	0.293 ± 0.024	0.241 ± 0.037	0.114 ± 0.015
duodenum	1.198 ± 0.106	0.747 ± 0.106	0.485 ± 0.071	0.343 ± 0.014	1.053 ± 0.074	0.349 ± 0.022
heart	0.212 ± 0.008	0.170 ± 0.025	0.553 ± 0.062	0.440 ± 0.026	0.367 ± 0.059	0.174 ± 0.010
kidney	0.965 ± 0.080	0.384 ± 0.066	1.926 ± 0.222	1.670 ± 0.096	3.598 ± 0.381	2.225 ± 0.301
liver	0.401 ± 0.050	0.274 ± 0.036	1.008 ± 0.150	0.949 ± 0.072	1.069 ± 0.081	1.042 ± 0.087
lung	0.706 ± 0.079	0.468 ± 0.052	2.384 ± 0.220	2.261 ± 0.079	1.993 ± 0.264	1.065 ± 0.085
pancreas	0.360 ± 0.065	0.364 ± 0.017	0.657 ± 0.060	0.617 ± 0.024	0.737 ± 0.072	0.349 ± 0.032
pituitary	0.439 ± 0.031	0.342 ± 0.051	0.533 ± 0.048	0.681 ± 0.036	0.899 ± 0.052	0.684 ± 0.144
spleen	0.440 ± 0.046	0.354 ± 0.028	0.844 ± 0.081	0.988 ± 0.056	1.402 ± 0.154	1.511 ± 0.057
thyroid	0.297 ± 0.043	0.294 ± 0.043	2.457 ± 0.335	3.476 ± 0.134	5.001 ± 0.865	3.114 ± 0.399
testes	0.051 ± 0.004	0.056 ± 0.010	0.036 ± 0.011	0.048 ± 0.004	0.154 ± 0.011	0.163 ± 0.018
prostate						
dorsolateral	0.497 ± 0.082	0.274 ± 0.034	0.320 ± 0.056	0.436 ± 0.139	0.657 ± 0.130	0.614 ± 0.128
ventral	0.941 ± 0.201	0.639 ± 0.070	0.296 ± 0.047	0.293 ± 0.029	0.673 ± 0.091	0.588 ± 0.120
blood	0.086 ± 0.004	0.066 ± 0.007	0.073 ± 0.005	0.041 ± 0.002	0.050 ± 0.003	0.038 ± 0.002

[a] Values represent mean ± SEM for five rats, unless otherwise noted. [b] Three rats.

Table II. Distribution of Radioactivity Following Intravenous Injection of Bisquaternary Compounds [^{14}C]Hexamethonium and 3 to Rats

	% administered dose/g[a]			
	[^{14}C]hexamethonium		compd 3	
tissue	0.5 h	2 h	0.5 h	2 h
adrenal	0.104 ± 0.017	0.043 ± 0.004	0.094 ± 0.012	0.043 ± 0.005
bone marrow			0.132 ± 0.021	0.078 ± 0.010
brain	0.018 ± 0.004	0.018 ± 0.003	0.044 ± 0.008	0.038 ± 0.005
diaphragm	0.094 ± 0.021	0.014 ± 0.002	0.080 ± 0.014	0.022 ± 0.002
duodenum			0.085 ± 0.007	0.033 ± 0.007
heart	0.145 ± 0.021	0.018 ± 0.004		
auricle			0.203 ± 0.024	0.108 ± 0.014
ventricle			0.121 ± 0.012	0.018 ± 0.001
intervertebral disk	1.094 ± 0.531	0.144 ± 0.021	0.607 ± 0.068	0.087 ± 0.022
kidney	2.616 ± 0.302	1.384 ± 0.236	3.305 ± 0.651	1.834 ± 0.269
liver	0.112 ± 0.012[b]	0.049 ± 0.006	0.129 ± 0.016	0.085 ± 0.011
lung	0.377 ± 0.056	0.137 ± 0.018	0.257 ± 0.029	0.087 ± 0.011
meninges	0.301 ± 0.063	0.413 ± 0.020	0.467 ± 0.066	0.658 ± 0.138
pituitary			0.118 ± 0.012	0.082 ± 0.011
pancreas	0.079 ± 0.013	0.016 ± 0.002	0.075 ± 0.019	0.030 ± 0.006
spleen	0.123 ± 0.017	0.051 ± 0.005	0.137 ± 0.013	0.094 ± 0.010
testes	0.097 ± 0.012	0.022 ± 0.009	0.076 ± 0.010	0.034 ± 0.014
thyroid	0.206 ± 0.046	0.047 ± 0.015	0.095 ± 0.010	0.060 ± 0.008
trachea	1.350 ± 0.476	0.127 ± 0.035	0.798 ± 0.085	0.089 ± 0.018
blood	0.310 ± 0.028[b]	0.020 ± 0.006	0.277 ± 0.029	0.015 ± 0.002

[a] Values represent mean ± SEM for five rats. [b] Ten rats.

In contrast to a previous report,[2] we saw no marked accumulation of radioactivity in the pancreas following administration of [^{14}C]putrescine (Table I). During the first 2 h, the tissues with the highest uptake of radioactivity were bone marrow, duodenum, kidney, and ventral prostate. The distribution of radioactivity following the administration of **1** (Table I) showed very little similarity to that of [^{14}C]putrescine. In this case, tissues with high concentration of radioactivity were adrenal, kidney, lung, and thyroid. Like putrescine, however, low levels of radioactivity were noted in brain.

The distribution profile of **2** also showed little similarity to that for putrescine (Table I). Moreover, in this instance high levels of radioactivity were observed in brain, in addition to adrenal, kidney, lung, spleen, and thyroid. Similar to the radiohalogenated amphetamines recently described by Sargent et al.,[8,9] compound **2** when labeled with iodine-131 could have potential use for imaging certain brain lesions.

The most interesting finding was with the radioiodinated bisquaternary derivative **3**, an analogue of hexamethonium. Previous autoradiographic studies by Wasserman[5] and Asghar and Roth[6] had shown that ^{14}C- or ^{3}H-labeled hexamethonium concentrated in cartilaginous tissues of mice and rats. We obtained similar results in rats with [^{14}C]hexamethonium. Moreover, the distribution profile of the radioiodinated analogue **3** was remarkably similar to that observed for hexamethonium. Cartilaginous tissues such as trachea, intervertebral disks, and meninges all had initially high levels of activity. Since the affinity of bisquaternaries for these tissues has been ascribed to their high content of polyanionic glycosaminoglycans such as chondroitin sulfate, we recently administered **3** to rats bearing a transplantable chondrosarcoma tumor. The high uptake of radioactivity achieved in the tumor (0.639 ± 0.062% dose/g at 0.5 h postinjection) was similar to that found in trachea and intervertebral disks. The presence of the bisquaternary moieties appears to be important for

selective localization, since studies with the monoquaternary compound 4[10,11] resulted in no appreciable concentration of radioactivity in cartilaginous tissues or chondrosarcoma tumor (0.044 ± 0.002% dose/g at 0.5 h postinjection). Preparation of the ^{131}I-labeled compounds for use in imaging studies is now in progress.

Experimental Section

Except for the radioiodinated compounds, all products and intermediates were subjected to the standard spectral analyses for proper characterization. IR spectra of KBr pellets were recorded on either a Perkin-Elmer Model 281 or 337 spectrophotometer. NMR spectra were obtained with a Varian A-60A or EM-360 spectrophotometer. $CDCl_3$, $CDCl_3$–Me_2SO-d_6, or Me_2SO-d_6 were used as solvents and trimethylsilane served as the internal standard. Mass spectra (MS) were recorded on a Dupont 21-490 mass spectrometer. Results of these analyses were all consistent with the proposed structures. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich., or by Midwest Microlab, Ltd., Indianapolis, Ind. All results were within ±0.4% of the theoretical values for these structures. Thin-layer chromatography (TLC) with at least two solvent systems was performed on all radiolabeled compounds to ascertain purity. In all cases, the radioactivity was coincident with the R_f value of the authentic unlabeled compound run side by side. Plates were scanned for radioactivity using an Atomic Associates RCS-363 radiochromatogram scanner with a Baird Atomic ratemeter Model 432A or a Berthold radiochromatogram scanner Model 6000. Total radioactivities were determined with a Picker nuclear isotope calibrator.

2-[^{125}I]Iodo-*p*-xylylenediamine (1). A mixture of 2-iodo-*p*-xylene (2.9 g, 12.5 mmol) and NBS (5.0 g, 28.1 mmol) in CCl_4 (20 mL) was refluxed under N_2 in the presence of benzoyl peroxide (0.3 g) for 18 h. The mixture was cooled and filtered. Removal of the solvent afforded a solid, which was recrystallized from hexane to give α,α'-dibromo-2-iodo-*p*-xylene (34%), mp 109–110.5 °C. Anal. ($C_8H_7Br_2I$) C, H. Treatment of this product (1.15 g, 2.95 mmol) with liquid NH_3 (25 mL) in C_6H_6 (15 mL) was carried out at 55 °C for 12 h in a pressure vessel. The solution was cooled and 2 N HCl (100 mL) was added. The mixture was washed with CCl_4 (80 mL). Neutralization of the aqueous solution with 5% NaOH, followed by extraction with CH_2Cl_2 (2 × 150 mL) gave an oil which was chromatographed on alumina. Elution with CH_2Cl_2 afforded 2-iodo-*p*-xylylenediamine, which was converted to the HCl salt by bubbling HCl into an ether solution of the amine (0.80 g, 81%), mp 289–290 °C. Anal. ($C_8H_{11}IN_2$·2HCl) C, H. This compound was radiolabeled with ^{125}I by means of an exchange reaction using 60 mg of the HCl salt and 3 mCi of Na^{125}I in H_2O (1 mL) at 135 °C for 24 h. The mixture was cooled, and one pellet of NaOH (~100 mg) was added to ensure a basic solution. The solution was extracted with $CHCl_3$ (3 × 1 mL) and the extract passed through a short column of anhydrous Na_2SO_4. Removal of the solvent under a stream of N_2 gave the radioiodinated diamine. Incorporation of 17% of the radioiodine was achieved under these conditions. TLC on silica gel plates developed with methylcellosolve–propionic acid–water (7:1.5:1.5) saturated with NaCl gave an R_f 0.56. Cellulose plates developed in 0.1 N HCl–EtOH (1:1) gave an R_f 0.67.

2-[^{125}I]Iodo-*N,N,N′,N′*-tetramethyl-*p*-xylylenediamine (2). A mixture of α,α'-dibromo-2-iodo-*p*-xylene (1.2 g, 3.1 mmol), prepared as described above, in C_6H_6 (15 mL) and dimethylamine (5 mL) in C_6H_6 (5 mL) was stirred at room temperature for 4 h. The precipitate was removed by filtration. Concentration of the filtrate gave an oil which was chromatographed on alumina. Elution with C_6H_6 gave 2-iodo-*N,N,N′,N′*-tetramethyl-*p*-xylylenediamine (0.929 g, 93%). The HCl salt was prepared by bubbling HCl into an ether solution of the amine: mp 256.5–257 °C. Anal. ($C_{12}H_{19}IN_2$·2HCl) C, H. The exchange reaction for radiolabeling was performed on the free amine which was obtained by neutralization of the salt with K_2CO_3, followed by extraction with ether. The free amine (84 mg) and 3 mCi of Na^{125}I were placed in NH_4OH (2.1 mL) and stirred at 135 °C for 40 h. The radioiodinated diamine was isolated as described above for 1. Incorporation of 83% of the radioiodine was achieved. TLC on alumina plates developed in $CHCl_3$ gave an R_f 0.53. On the other hand, compound and radioactivity remained at the origin when the silica gel plate was developed with concentrated HCl–EtOH (1:1).

2-[^{125}I]Iodo-*N,N,N,N′,N′,N′*-hexamethyl-*p*-xylylenediammonium Diiodide (3). The tertiary diamine 2 was used directly for the preparation of 3. Thus, the radioiodinated diamine resulting from the exchange reaction (~80 mg) was assayed for purity and immediately dissolved in a mixture of MeOH (1 mL) and CH_3I (0.5 mL). The solution was warmed at 80 °C for 5 min and then allowed to stand at room temperature with occasional swirling. Addition of ether afforded the bisquaternary derivative in essentially quantitative yield. Purification was achieved by digestion in boiling EtOH and filtration of the cooled mixture. A standard of the stable compound was prepared in the same manner from stable diamine, mp 243 °C dec. Anal. ($C_{14}H_{25}I_3N_2$) C, H. TLC on silica gel plates developed in methylcellosolve–propionic acid–water (7:1.5:1.5) saturated with NaCl gave an R_f 0.09. The compound and radioactivity remained at the origin when the plate was developed with MeOH.

[^{14}C]Hexamethonium Diiodide. A mixture of *N,N,N′,N′*-tetramethyl-1,6-hexanediamine (5.4 mg, 0.03 mmol) and $^{14}CH_3I$ (7.9 mg, 0.06 mmol, 1.0 mCi) in MeOH (3 mL) in a stoppered flask was allowed to stand at room temperature for 12 h. CH_3I (10 mg) was added, and the mixture was kept at room temperature for an additional 40 h. The solution was filtered through glass wool, and the filtrate was allowed to evaporate to dryness under a stream of N_2. The resulting crystals were washed with anhydrous ether to give a pure product with a specific activity of 21 μCi/mg. The compound and radioactivity remained at the origin upon TLC on silica gel plates and development with $CHCl_3$–MeOH (1:2) or concentrated HCl–EtOH (1:1).

Tissue Distribution Studies. Adult male Sprague-Dawley rats (Spartan Research Animals, Inc., Hasslett, Mich.) weighing 200–420 g were used as experimental animals. The rats were housed in temperature and light-controlled quarters and had access to food (Teklad 4% Rat and Mouse Diet) and water ad libitum. Compounds were administered intravenously via the tail vein while rats were under ether anesthesia. [1,4-^{14}C]Putrescine dihydrochloride, obtained from New England Nuclear, Boston, Mass., was received in 2.5 mL of 0.01 N HCl solution, which was diluted with 2.5 mL of saline (0.78 mg/5.0 mL). [^{14}C]Hexamethonium diiodide was dissolved in saline (15 mg/5.0 mL), compound 1 was dissolved in 0.1 N HCl (47 mg/3.0 mL), 2 was dissolved in 0.5 N HCl (53.25 mg/7.5 mL), and 3 was dissolved in saline (26 mg/2.0 mL). Aliquots of each solution were assayed to quantitate radioactivity. TLC analysis of each solution was performed just prior to administration to confirm radiochemical purity.

Each of 48 rats received either 10.6 μCi of [^{14}C]putrescine (51.8 mCi/mmol) in 0.2 mL of vehicle (ten rats), 6.3 μCi of [^{14}C]-hexamethonium (20.9 μCi/mg) in 0.1 mL of vehicle (ten rats), 6.2–10.4 μCi of 1 (6.5–7.0 μCi/mg) in 0.10–0.15 mL of vehicle (eight rats), 11.9–12.1 μCi of 2 (8.4–8.6 μCi/mg) in 0.2 mL of vehicle (ten rats), or 11.7–12.3 μCi of 3 (9.0–9.5 μCi/mg) in 0.1 mL of vehicle (ten rats). Five rats per compound were killed at 0.5 and 2 h postinjection, except for 1 where only three rats were included in the 0.5-h interval. Rats were killed by exsanguination under ether anesthesia. The major organs were excised, rinsed to remove blood, blotted dry, and weighed. Large organs were minced with scissors. Duplicate samples were taken. Samples of tissues from rats that received ^{125}I-labeled compounds were placed in tared cellulose acetate capsules and weighed. The cellulose acetate capsules were then placed in counting tubes and assayed for radioactivity in a well-scintillation counter (Searle 1185 Automatic Gamma System). Counting efficiency for ^{125}I was 87%. Samples of tissues from rats that received ^{14}C-labeled compounds were prepared for counting as previously described[11] and assayed for radioactivity in a liquid scintillation counter (Beckman LS 150 liquid scintillation spectrometer). The observed counts per minutes were corrected for quench by use of the external standard–channels ratio method.

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A New Chemical Series Active against African Trypanosomes: Benzyltriphenylphosphonium Salts

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Antitrypanosomal activity for benzyltriphenylphosphonium salts is reported for the first time. Testing was conducted using *Trypanosoma rhodesiense* infected mice. Of 70 phosphorus-containing compounds tested, 21 were active. Sixteen of these active chemical species were benzyltriphenylphosphonium salts. Four were nonbenzyl triphenyl compounds. The remaining active drug was a benzyldiphenylphosphonium salt.

The effective drug control of human sleeping sickness in the last 35 years has rested principally upon suramin, tryparsamide, pentamidine, and the melaminyl arsenicals, especially melarsoprol (Mel B).[2] The toxicity and other undesirable side effects of these drugs are well documented.[3] Newer chemical agents are urgently needed. In this report, data on the antitrypanosomal activity of a new series of chemical compounds, benzyltriphenylphosphonium salts, are given. Activity of this series against the African trypanosomes has not been previously reported.

Materials and Methods

The test system used to make drug evaluations has been described in detail.[4] Briefly, ICR/HA Swiss mice of either sex, 6 weeks of age weighing 28 to 30 g, are inoculated intraperitoneally with 0.5 mL of a 1:50000 dilution of heparinized heart blood drawn from donor mice infected 3 days earlier with the Wellcome CT strain of *Trypanosoma rhodesiense*. Test compounds, after they have been ground to a fine powder with mortar and pestle, are administered within 2 h of parasite inoculation as a single dose subcutaneously or orally in peanut oil (USP peanut oil, Durkee Foods, Coral Gables, Fla.).

The test system is based on a comparison of the mean survival time of untreated control mice and animals treated with the test compound. An increase of 100% in mean survival time is taken as the minimum response for a candidate compound to be considered as active. Mice alive at the end of 30 days are scored as cured. Deaths prior to the 4th day, before deaths occur in untreated controls, are regarded as from nonparasitic causes and become the basis for an evaluation of chemical toxicity. In calculating mean survival time, toxic deaths and 30-day survivors are not included. Twenty infected, untreated (negative) controls and 20 infected, positive controls are routinely used per test. Positive controls are mice infected and treated at 26.5 mg/kg with either stilbamidine isethionate or hydroxystilbamidine isethionate. Both positive control compounds are essentially 100% curative when given subcutaneously. Stilbamidine and hydroxystilbamidine are 98 and 71% curative, respectively, when given orally.

The phosphonium salts have attracted much attention because of their use in the Wittig reaction.[5-7] A number of such compounds are available commercially and were the source of compounds **7**, **10**, **11**, and **18–21** (Tables I and II). The other compounds were generously supplied by individuals as noted. Compound **8** was reported by Novotny et al.[8] Phosphonium salt **9** was prepared after the method of Coombs and Houghton[9] and Hirao et al.[10] Those numbered **12** through **16** were disclosed by McErven et al.[11,12] Compound **17** was made by Aguiar.[13] In the general mode for preparation of such salts (cf. ref 5 and 6) the tris-substituted phosphine and requisite halide are reacted in a suitable solvent, usually one of low polarity such as benzene, xylene, or diethyl ether. The phosphonium salt is then collected and crystallized to afford a good yield of the product.

Biological Results and Discussion

Use of the triphenylmethane dyes marked a point of departure from arsenicals in early work devoted to finding effective trypanocidal agents.[14] That departure ultimately led to the synthesis of suramin sodium, the present drug of choice in the treatment of the early stages of African trypanosomiasis. Several of these dyes have activity against the African trypanosomes. The structures of five such agents are shown in Figure 1. These compounds also have activity against other parasites. The salt of the triphenylmethane dye *p*-rosaniline and pamoic acid, TAC pamoate, is active against schistosomiasis,[15] schizotrypanosomiasis,[16] as well as African trypanosomiasis.[4]

In view of evidence such as this, it follows that substitution of phosphorus, which has important and multifaceted functions in the biochemistry of host and parasite metabolism, would likely result in compounds with trypanocidal properties. A total of 70 phosphorus-containing compounds were tested. Forty-nine of these were drugs having three phenyl moieties connected directly to the phosphorus atom of the chemical species. Of the 70 tested, 21 were active (Tables I and II). Twenty of these