vacuo, and the oily residue was used for HPLC analysis.

HPLC Studies. HPLC studies were performed with use of a Waters apparatus, equipped with two M6000A pumps, a U6K injector, a M660 solvent programmer, and a M-450UV detector (Waters).

For analytical determinations, a 10-μm μ-Bondapak C_{18} column (Waters, 3.9 × 300 mm) was used. The separation of the diastereoisomers was obtained on a semipreparative 10-μm μ-Bondapak C_{18} column (Waters, 7.8 × 300 mm).

The eluted peaks were monitored at 220 or 260 nm when the eluent contained acetic acid.

The eluents used for the separation of the diastereoisomers **10a** and **10b** were 2% acetic acid in water/CH_3CN (65/35), for the mixture **13a** and **13b** 2% acetic acid in water/MeOH (55/45), and for the peptides **14a** and **14b** ammonium acetate buffer (pH 4.2)/CH_3CN (94/6).

Enzymatic Studies. Enkephalinase was purified to homogeneity from rabbit kidney by the method of Almenoff and Orlowski.[38] A single species was observed by polyacrylamide gel electrophoresis in the presence of $NaDodSO_4$.

The enkephalinase activity was checked following the procedure previously described.[26] The enzyme (at a final concentration of 0.9 ± 0.2 pmol/100 μL) was preincubated for 15 min at 25 °C with and without increasing concentrations of inhibitor in 100-μL total volume of 50 mM Tris-HCl buffer. [^{3}H]-D-Ala^2-Leu-enkephalin (k_m = 30 μM) was added to 20 nM final concentration, and the reaction was stopped after 30 min by adding 25 μL of 0.2 N HCl. The tritiated metabolites formed were separated on polystyrene beads.[39] Determination of IC_{50} values were performed as already described in detail.[13]

(38) Almenoff, J.; Orlowski, M. *Biochemistry* **1965**, *22*, 590.
(39) Vogel, Z.; Altstein, M. *FEBS Lett.* **1977**, *80*, 332.

Acknowledgment. We are grateful to Dr. Ann Beaumont for stylistic revision and to Annick Bouju for typing the manuscript. This study was supported by grants from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, and the Université René Descartes.

Registry No. **1a**, 83024-49-1; **1b**, 100484-62-6; **2a**, 95909-00-5; **2b**, 95908-99-9; **3a**, 100484-63-7; **3b**, 100484-64-8; **4a**, 100431-27-4; **4b**, 100431-28-5; **5a**, 100431-29-6; **5b**, 100431-30-9; **6a**, 100431-31-0; **6b**, 100431-32-1; **7a**, 100484-65-9; **7b**, 100484-66-0; (R,R)-**8**, 100431-33-2; (R,S)-**8**, 100431-34-3; (R,R)-**9**, 100431-35-4; (R,S)-**9**, 100431-36-5; (R,R)-**10**, 100431-37-6; (R,S)-**10**, 100431-38-7; (*R*,*R*)-**11**, 100431-39-8; (*R*,*S*)-**11**, 100431-40-1; (*R*,*R*)-**12**, 100431-41-2; (*R*,*S*)-**12**, 100431-42-3; (*R*,*R*)-**13**, 100431-43-4; (*R*,*S*)-**13**, 100431-44-5; **14a**, 100431-45-6; **14b**, 100431-46-7; enkephalinase, 82707-54-8; (*S*)-*N*-[2-(benzoylthio)methyl]-1-oxo-3-phenylpropyl]glycine benzyl ester, 100431-47-8; (*R*)-*N*-[2-(benzoylthio)methyl]-1-oxo-3-phenylpropyl]glycine benzyl ester, 100431-48-9; glycine benzyl ester *p*-tosylate, 1738-76-7; (*S*)-*N*-[2-(benzoylthio)methyl]-1-oxo-3-phenylpropyl]-L-alanine methyl ester, 100431-49-0; (*R*)-*N*-[2-(benzoylthio)methyl]-1-oxo-3-phenylpropyl]L-alanine methyl ester, 100431-50-3; L-alanine methyl ester hydrochloride, 2491-20-5; L-phenylalaninol, 3182-95-4; D-phenylalaninol, 5267-64-1; monoethyl malonate, 1071-46-1; 1-hydroxybenzotriazole, 2592-95-2; potassium thioacetate, 10387-40-3; methylmalonic acid (half ethyl ester), 81110-31-8; 2-(methylpropyl)malonate (half ethyl ester), 78220-81-2; *N*-[(*R*,*S*)-2-(ethoxycarbonyl)-1-oxopropyl]-L-phenylalanine *tert*-butyl ester, 100431-51-4; L-phenylalanine *tert*-butyl ester hydrochloride, 15100-75-1; *N*-[(*R*,*S*)-2-carboxy-1-oxypropyl]-L-phenylalanine *tert*-butyl ester, 100431-52-4; *N*-[(*R*,*S*)-2-(aminocarbonyl)-1-oxopropyl]-L-phenylalanine *tert*-butyl ester, 100431-53-6; *N*-hydroxysuccinimide, 6066-82-6.

Preparation and Evaluation of Radioiodinated (Iodophenyl)cholines and Their Morpholinium and Piperidinium Analogues as Myocardial Perfusion Imaging Agents

Pamela L. Zabel, Yolanda Zea-Ponce, G. William Brown, Gary Morrissey, Duncan H. Hunter,*† Albert A. Driedger, and Michael J. Chamberlain

Radiopharmaceutical Development Group, Departments of Nuclear Medicine and Chemistry, University of Western Ontario, London, Ontario, Canada. *Received July 24, 1985*

A series of nine radioiodinated quaternary ammonium salts related to phenylcholine were synthesized, characterized, and radiolabeled by exchange. These compounds were evaluated as myocardial perfusion imaging agents in mice, pigs, and humans. Mice biodistribution studies showed that five of the nine compounds were taken up in the heart to the same extent as $^{201}Tl^+$ at 5 min. At 60 min myocardial retention was significantly better than $^{201}Tl^+$ for six of the compounds. Several of the compounds showed more favorable heart/blood and heart/liver ratios when compared to $^{201}Tl^+$. Evaluation of three of the more promising compounds in pigs and humans however revealed no selective myocardial uptake.

Although $^{201}Tl^+$ is widely used for myocardial perfusion imaging, its properties are less than ideal.[1] Self-absorption of low-energy photons, redistribution during the imaging period, and interference of interpretation due to nearby hepatic or pulmonary activity are disadvantages of $^{201}Tl^+$ that have encouraged investigators to search for alternative myocardial perfusion imaging agents.

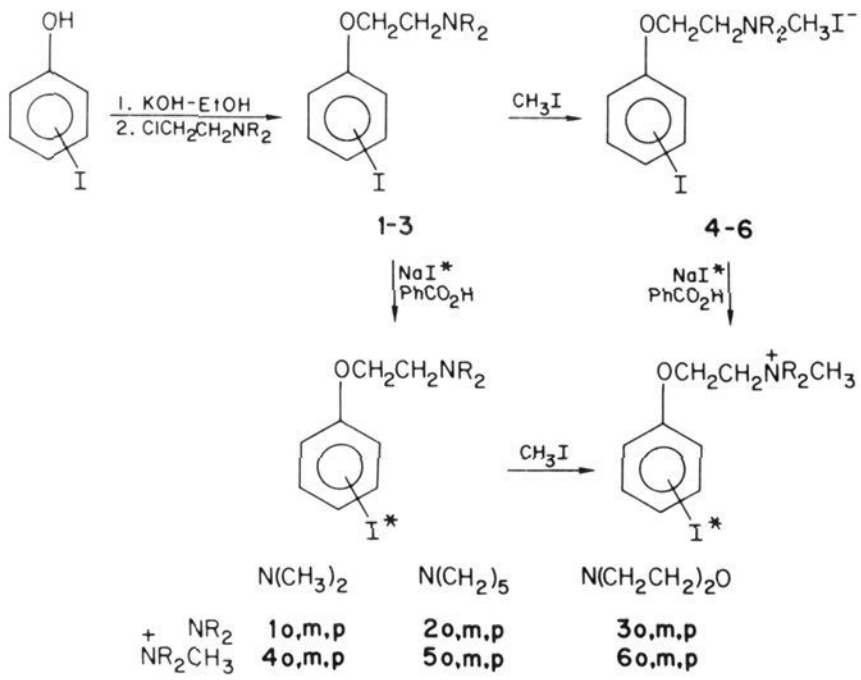
The favorable imaging qualities of ^{123}I and the in vivo stability of most aryliodo compounds[2,3] have stimulated research into radioiodinated aromatic quaternary ammonium compounds. A radioiodinated bretylium analogue (RIBA: (*o*-[^{125}I]iodobenzyl)trimethylammonium),[4] (*p*-[^{125}I]iodophenyl)trimethylammonium,[3] and a radioiodinated benzoylcholamine derivative[5] were all found to have initial high myocardial uptake in rats or mice. Unfortunately rapid myocardial washout or extensive hepatic uptake in higher species has not indicated promise for any of these agents to replace $^{201}Tl^+$ for myocardial perfusion imaging.

† Department of Chemistry, University of Western Ontario, London, Ontario, Canada N6A 5B7.

(1) Pohost, G. M.; Alpert, N. M.; Ingwall, J. S.; Strauss, H. W. *Semin. Nucl. Med.* **1980**, *10*, 70.
(2) Huang, C. C.; Friedman, A. M.; Rayudu, G. V. S.; Clark, P. *J. Nucl. Med.* **1980**, *21*, 679.
(3) Burns, H. D.; Marzilli, L. G.; Dannals, R. F.; Dannals, T. E.; Trageser, T. C.; Conti, P.; Wagner, H. N. *J. Nucl. Med.* **1980**, *21*, 875.
(4) Counsell, R. E.; Yu, T.; Ranade, V. V.; Buswinke, A. A.; Carr, E. A.; Carroll, M. *J. Nucl. Med.* **1974**, *15*, 991.
(5) Dannals, R. F.; Burns, H. D.; Marzilli, L. G.; Trageser, T. C.; Wagner, H. N. *J. Pharm. Sci.* **1981**, *70*, 439.

0022-2623/86/1829-0757$01.50/0

Scheme I



Replacement of the ester linkage of a radioiodinated benzoylcholine by an amide led to increased in vivo stability and heart accumulation of radioactivity in mice.[5] Encouraged by this observation, we synthesized an analogous ether, (*p*-iodophenyl)choline, and investigated its biodistribution in mice.[6] Selective heart accumulation of 18–20% injected dose/g and heart to blood ratios of 15–18:1 during the first 15-min postinjection prompted us to extend this investigation to a series of related compounds.

Attempts were made to optimize the heart selectivity and retention both by altering the position of the radioiodine and by incorporating the ammonium nitrogen into heterocyclic rings.

This report details the synthesis and a structure–biodistribution relationship in mice of nine radioiodinated (iodophenyl)choline analogues and the further investigation of three of the nine compounds in both pigs and humans.

Results and Discussion

Chemistry and Radiochemistry. As illustrated, nine amines (**1**–**3**) and nine quaternary ammonium salts (**4**–**6**) have been prepared and characterized (Scheme I). These two sets of nine compounds represent the ortho, meta, and para iodo isomers of a dimethylamino (**1** and **4**), a piperidino (**2** and **5**), and a morpholino (**3** and **6**) derivative of β-phenoxyethylamine and their *N*-methylammonium iodide derivatives. All nine amines were prepared from the corresponding iodophenols in good yield by the method previously published[6] and were purified and characterized as either the free amine or as its hydrochloride salt. All gave confirmatory mass and proton NMR spectra.

These nine amines were converted to the corresponding methyl quaternary ammonium iodides by reaction with methyl iodide.[6] These compounds gave satisfactory elemental analyses and proton NMR spectra.

Both the amines and the quaternary ammonium salts were labeled with ^{131}I or ^{123}I by exchange labeling with use of ca. 1 mg of the cold (nonradioactive) iodo compounds and 0.5–2 mCi (18–74 MBq) of radioiodide. The exchange product was purified by TLC. The biological evaluation of the radioiodinated amines will be published elsewhere.[7]

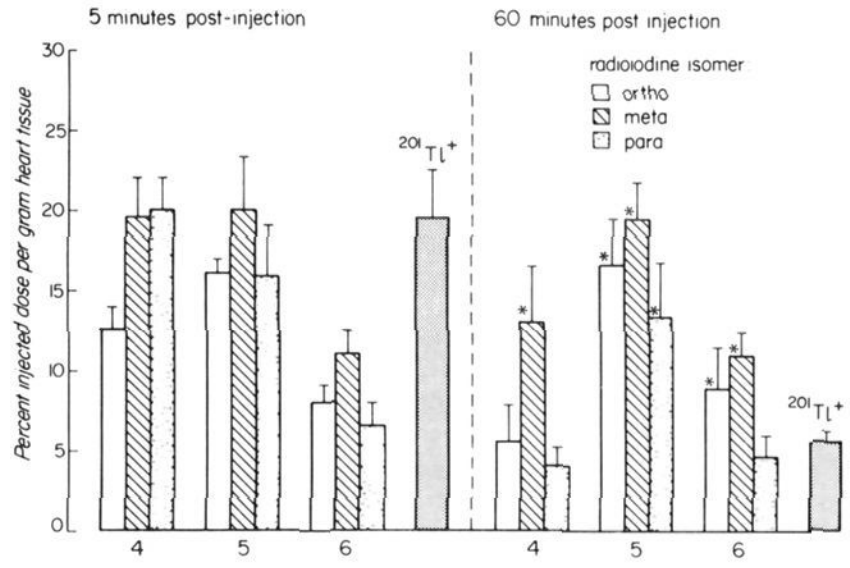


Figure 1. Comparative heart uptake (percent injected dose per gram) of the ortho, meta, and para isomers of compounds **4**–**6** and of $^{201}Tl^+$ at 5- and 60-min postinjection in Swiss white mice, mean + SD, $n = 4$, * = significantly higher than $^{201}Tl^+$ at the same period ($p < 0.05$).

Exchange labeling of the amines with sodium iodide-131 resulted in variable radiochemical yields with no obvious pattern of reactivity emerging, but material of specific activity to the range of 50–400 mCi/mmol (1.8–15 GBq/mmol) was produced. These specific activities were calculated from the observed activity assuming 1 mg of the starting material remained after exchange and purification by TLC. Thus the specific activities are minimum values.

Attempts to improve the specific activity by decreasing the amount of substrate much below 1 mg resulted in reduced radiochemical yields. Exchange reactions run with higher activity radioiodide resulted in similar percentages of exchange labeling and thus higher specific activity products.

The radioiodinated amines were converted to the corresponding quaternary ammonium iodides by reaction with an excess of methyl iodide. After purification by TLC, radiochemical yields in the range of 80–90% were realized, and these products should have the same specific activity as the starting amines (50–400 mCi/mmol or 1.8–15 GBq/mmol).

The alternate synthetic route to the radioiodinated quaternary ammonium salts was to introduce the radioiodine in the final step. The quaternary ammonium iodides were converted to the acetate salts with use of an anion-exchange resin and were thereafter successfully exchange labeled with radiochemical yields in the range of 50–80%. In this manner both the ^{131}I- and ^{123}I-labeled quaternary ammonium acetates were prepared with specific activities in the range of 50–2000 mCi/mmol (1.8–74 GBq/mmol).

In the case of the amine **3p**, the radioiodine (^{125}I) was introduced via a diazonium salt intermediate produced from the corresponding aniline.[8] This reaction in the absence of added carrier iodide proceeded in very low yield (~3%) but presumably provided "no-carrier added" amine **3p**. This amine was converted to a quaternary ammonium iodide **6p**.

Biological Evaluation in Mice. The biodistribution of the nine radioiodinated aromatic quaternary ammonium compounds was compared to $^{201}Tl^+$ in mice. Figure 1 illustrates the comparative heart uptake (percent injected dose per gram of heart tissue) at 5- and 60-min postinjection. At 5-min postinjection the heart uptake of radioactivity following iv administration of compounds **4m**,

(6) Hunter, D. H.; Zea-Ponce, Y.; Brown, G. W.; Chamberlain, M. J.; Driedger, A. A.; Morrissey, G. *Can. J. Chem.* **1984**, *62*, 2015.
(7) Zabel, P. L.; Zea-Ponce, Y.; Hunter, D. H.; Chamberlain, M. J.; Driedger, A. A.; Morrissey, G., unpublished results.
(8) Hunter, D. H.; Payne, N. C.; Rahman, A.; Richardson, J. F.; Zea-Ponce, Y. *Can. J. Chem.* **1983**, *62*, 421.

Table 1. Biodistribution[a] of 5m in Swiss White Mice (Mean ± SD, $n = 4$)

tissue	time, min					
	5	15	30	60	90	120
heart	20.0 ± 3.3	21.6 ± 3.3	23.1 ± 1.0	19.5 ± 2.3	18.1 ± 1.0	17.7 ± 1.8
blood	1.9 ± 0.2	2.4 ± 0.4	1.9 ± 0.1	1.5 ± 0.4	1.2 ± 0.1	1.4 ± 0.3
lung	7.3 ± 3.4	5.5 ± 0.5	4.6 ± 0.6	3.4 ± 0.7	2.7 ± 0.4	2.6 ± 0.4
liver	12.5 ± 3.7	4.9 ± 0.7	4.1 ± 0.9	2.4 ± 0.6	2.3 ± 0.4	2.0 ± 0.4
spleen	5.0 ± 0.4	3.6 ± 0.8	3.4 ± 0.9	2.6 ± 0.4	2.1 ± 0.2	2.1 ± 0.4
kidneys	40.1 ± 3.2	24.0 ± 8.0	14.1 ± 4.3	7.4 ± 1.8	5.3 ± 0.9	6.0 ± 1.3
muscle	0.9 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.4
bone	1.6 ± 0.5	2.0 ± 0.4	2.0 ± 0.1	1.5 ± 0.3	1.3 ± 0.1	1.3 ± 0.1

[a] Percent injected dose/gram of tissue.

4p, 5o, 5m, and **5p** was comparable to $^{201}Tl^+$ (no significant difference at $p < 0.05$, unpaired t test). This very high initial heart uptake in animal studies has also been observed for a few other aromatic[3,9] and aliphatic quaternary ammonium compounds[2] as well as some organic phosphonium cations.[10,11] Rapid hydrolysis and/or elimination has prevented significant heart uptake of quaternary ammonium compounds such as radiolabeled benzoylcholine derivatives[5] or a radioiodinated analogue of hexamethonium.[12] However the unreactive ether linkage in our series of compounds seems to allow heart accumulation to occur in competition with either metabolism or elimination.

Selective heart retention of radioactivity in the mice occurred for our series of compounds so that at 60 min six of the nine compounds (**4m, 5o, 5m, 5p, 6o, 6m**) exhibited a heart uptake that was significantly ($p < 0.05$, unpaired t test) better than that observed for $^{201}Tl^+$ (see Figure 1). The other three compounds (**4o, 4p, 6p**) were not significantly different from $^{201}Tl^+$.

The trimethylammonium compounds **4** showed an exponential clearance curve in mouse hearts very similar to that seen for $^{201}Tl^+$. In contrast, both the *N*-methylpiperidinium **5** and *N*-methylmorpholinium **6** compounds showed prolonged retention and very little clearance from the heart with levels being quantitatively greater for the *N*-methylpiperidinium series **5**.

It is desirable that the myocardial retention of a heart imaging agent is sufficiently prolonged that no significant redistribution occurs within the time needed for imaging. This is particularly important when single photon emission computerized tomography (SPECT) techniques with imaging times up to 30 min are used. More prolonged heart retention may also allow imaging to be delayed until more favorable heart to background ratios are achieved but defeats the use of the agent to obtain exercise and redistribution images in one and the same study as is commonly done with $^{201}Tl^+$.

Although a few quaternary ammonium compounds[2,9] and phosphonium compounds[10,11] have also been found to show sustained retention in animal heart tissue, many quaternary ammonium compounds[3-5,12,13] or cationic compounds[14-16] have exhibited rapid heart clearance.

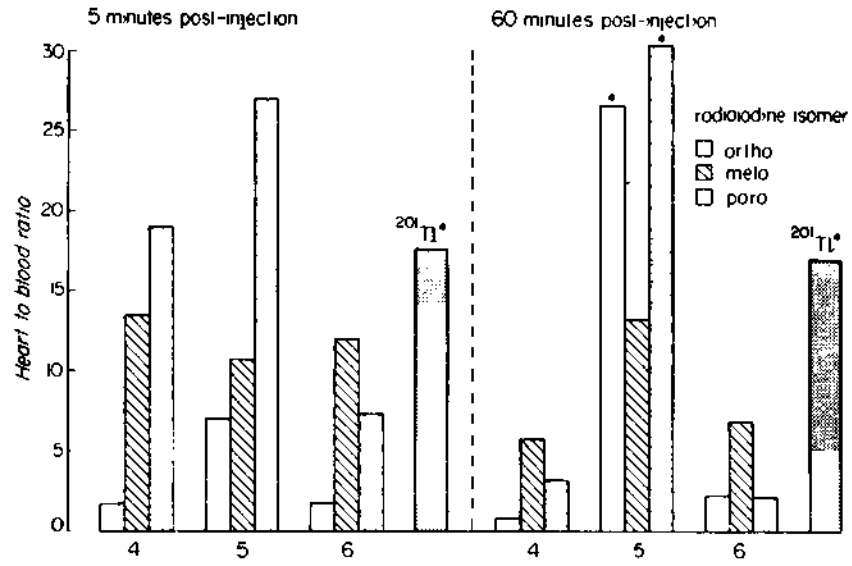


Figure 2. Comparative heart to blood ratios of the ortho, meta, and para isomers of compounds **4-6** and of $^{201}Tl^+$ at 5- and 60-min postinjection in Swiss white mice, $n = 4$, * = significantly higher than $^{201}Tl^+$ at the same period ($p < 0.05$).

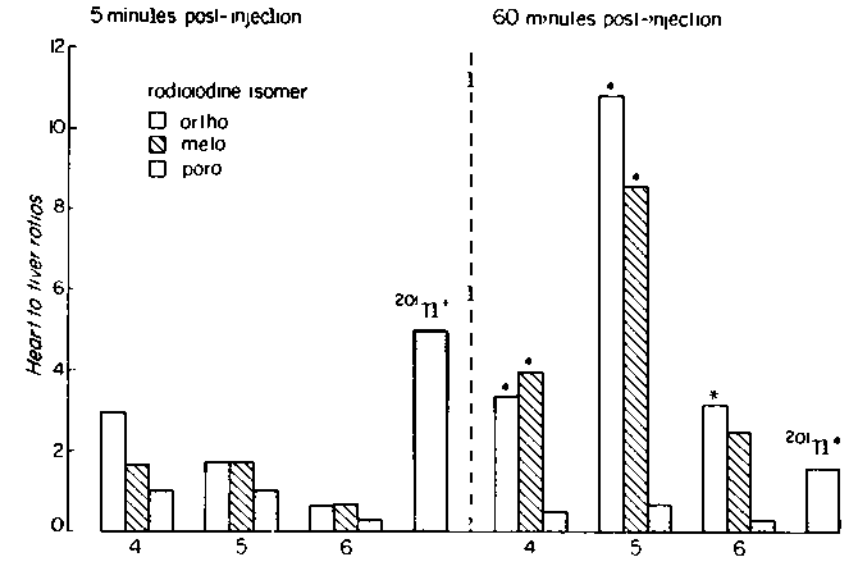


Figure 3. Comparative heart to liver ratios of the ortho, meta, and para isomers of compounds **4-6** and of $^{201}Tl^+$ at 5- and 60-min postinjection in Swiss white mice, $n = 4$, * = significantly higher than $^{201}Tl^+$ at the same period ($p < 0.05$).

In order to gain a better perspective of the striking selectivity that some of the nine radiolabeled compounds exhibited for the heart in the mouse studies, the whole body distribution of radioactivity following injection of **5m** is given in Table I. Aside from initially high kidney values at 5 and 15 min, the localization of radioactivity was most selective for the heart from all the tissues evaluated.

In Figure 2, the heart to blood ratios of the nine compounds are graphically compared to $^{201}Tl^+$ at 5- and 60-min postinjection. The initial high heart to blood ratios observed for the *N*-methylpiperidinium series **5** at 5 min increased with time so that compounds **5o** and **5p** had ratios significantly better ($p < 0.05$) than $^{201}Tl^+$ at 60 min.

(9) Hanson, R. N.; Hasson, M. A. *J. Nucl. Med.* 1985, *26*, P122.
(10) Srivastava, P. C.; Knapp, F. F. *J. Med. Chem.* 1984, *27*, 978.
(11) Woo, D. V. *Circulation, Suppl.* 1982, *66* (Suppl. II), II-124.
(12) Huang, C. C.; Korn, N.; Counsell, R. E. *J. Med. Chem.* 1979, *22*, 449.
(13) Friedland, R. P.; Mathis, C. A.; Budinger, T. F.; Moyer, B. R.; Rosen, M. *J. Nucl. Med.* 1983, *24*, 812.
(14) Gerson, M. C.; Deutsch, E. A.; Nishiyama, H.; Libson, K. F.; Adolph, R. J.; Grossman, L. W.; Sodd, V. J.; Fortman, D. L.; Vanderheyden, J. L.; Williams, C. C.; Saenger, E. L. *Eur. J. Nucl. Med.* 1983, 8, 371.
(15) Sullivan, P. J.; Werre, J.; Elmaleh, D. R.; Okada, R. D.; Kopiwoda, S. Y.; Castronovo, F. P.; McKusick, K. A.; Strauss, H. W. *Int. J. Nucl. Med. Biol.* 1984, *11*, 3.
(16) Gerson, M. C.; Deutsch, E. A.; Libson, K. F.; Adolph, R. J.; Ketring, A. R.; Vanderheyden, J. L.; Williams, C. C.; Saenger, E. L. *Eur. J. Nucl. Med.* 1984, *9*, 403.

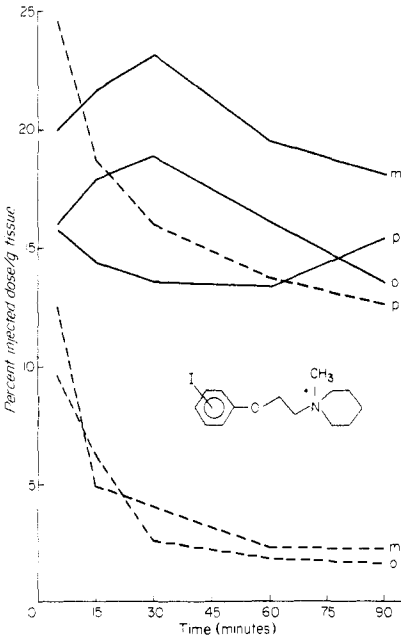


Figure 4. Heart (–) and liver (- -) levels of radioactivity (percent injected dose per gram of tissue) for the ortho, meta, and para isomers of compound **5** between 5- and 90-min postinjection in Swiss white mice.

Similar heart values but a higher blood level at 60 min produced a slightly lower heart to blood ratio for compound **5m** more comparable to that observed for $^{201}Tl^+$.

Although the exponential clearance of heart radioactivity was similar for the trimethylammonium compounds **4** and $^{201}Tl^+$, the simultaneous blood clearance seen for $^{201}Tl^+$ did not occur for this series of compounds. Therefore the heart to blood ratios were not as favorable at 60 min as observed for $^{201}Tl^+$. The *N*-methylmorpholinium series **6** exhibited heart to blood ratios that were also not as favorable as seen for $^{201}Tl^+$.

Due to the large mass and proximity of the liver to the heart, high liver uptake of a myocardial imaging agent may prevent it from being diagnostically useful.[14] Therefore the comparative heart to liver ratios in mice are illustrated graphically in Figure 3. While $^{201}Tl^+$ is initially selectively concentrated in the heart, a rapid heart clearance and a steady liver concentration leads to a decreasing heart to liver ratio with time. In contrast, the ortho and meta isomers of **4–6** show initially high liver levels followed by rapid liver elimination. In combination with selective heart retention this leads to improvement of heart to liver ratios with time. Therefore by 60 min compounds **4o**, **4m**, **5o**, **5m**, and **6o** were all found to have heart to liver ratios that were significantly better ($p < 0.05$) than that of $^{201}Tl^+$. In particular, compounds **5o** and **5m** exhibited heart to liver ratios at 60 min that were 5 and 7 times, respectively, larger than that of $^{201}Tl^+$.

The para-radioiodinated isomers had a tendency to show very high liver uptake and retention that resulted in low heart to liver ratios for **4p**, **5p**, and **6p** (Figure 3). To illustrate the substantial difference between the liver uptake of the ortho, meta, and para isomers, the heart and liver time–activity curves of **5o**, **5m**, and **5p** are shown in Figure 4. While heart levels tended to be substantially higher than liver values for the ortho and meta isomers, the para isomer exhibited selective liver uptake and retention as great or greater than that seen in the heart.

Biodistribution studies with radioiodinated aromatic ortho-, meta-, and para-radioiodinated isomers of a bretylium analogue revealed that the para isomer showed the greatest liver uptake and slowest liver elimination following iv injection in rats.[4] A metabolic pathway followed by many aromatic compounds results in para hydroxylation.[17,18] The presence of iodine in the para position may block this metabolic pathway in mice and thereby lead to the liver retention. Presumably studies of the metabolic products found in the plasma, bile, urine, and feces following injection of these compounds would indicate if this is indeed the case.

Quaternary ammonium compounds of molecular weight greater than 300 with an intermediate length chain separating a positively charged ammonium group and one or more nonpolar ring structures tend to undergo relatively extensive biliary excretion.[19–22] Although we have not attempted studies of biliary metabolism and excretion, we anticipate that the rapid liver clearance observed for the ortho and meta series may have been due to the rapid biliary elimination of the compounds themselves, their hydroxylated metabolites, or conjugates of the hydroxylated metabolites.[20]

Most of the compounds investigated showed a very high initial kidney uptake followed by a rapid curvilinear clearance. The presence of a positive charge in quaternary ammonium compounds allows them to be eliminated by active secretion in the proximal tubules of the kidneys.[22,23] A number of proposed cationic myocardial imaging agents such as a radioiodinated bretylium analgue,[4] (*p*-iodophenyl)trimethylammonium,[3] and (*o*-iodobenzoyl)cholamine[5] were all found to be rapidly excreted unchanged.

Two of the nine compounds investigated, **4o** and **6o**, demonstrated relatively high spleen uptake which gradually increased over the first 30–60-min postinjection. The 30-min spleen uptake of 3.6%/intact spleen or 32%/g of tissue for compound **4o** was 6–7 times that observed for its meta and para isomers. Likewise a spleen uptake of 0.7% (9%/g of tissue) for compound 6o was 3–4 times that seen for **6m** or **6p**. This spleen uptake is unlikely to be due to colloidal uptake in the reticuloendothelial system since liver values were low for these compounds (e.g., 1.9%/g and 3.6%/g for **4o** and **6o**, respectively, at 30 min). The blood levels were also slightly elevated for these two compounds. Binding of these compounds of their metabolites to red blood cells could possibly lead to localization in the spleen due to its ability to remove old or damaged red blood cells. The para-radioiodinated analogue of bretylium also showed relatively high spleen uptake and retention in both the rat and dog following intravenous injection.[4,24] The smooth muscle of the spleen is proposed to have a high-capacity, low-affinity, extraneuronal uptake mechanism for certain compounds.[24] In some species, this same uptake mechanism exists in the heart and may be responsible for part of the heart localization of a wide range of substrates such as radiolabeled catecholamines and bretylium.[24]

(17) Fuller, R. W.; Hines, C. W. *J. Pharm. Sci.* **1967**, *56*, 302.
(18) Goodman, L. S.; Gilman, A. "The Pharmacological Basis of Therapeutics"; 5th ed.; MacMillan: New York, 1975; Chapter 1.
(19) Schanker, L. S. In "The Biliary System"; Taylor, W., Ed; Oxford Blackwell: London, 1965; p 469.
(20) Ryfeldt, A. *Acta Pharmacol. Toxicol.* **1973**, *32*(suppl III), 7.
(21) Meijer, D. F. K. In "Intestinal Permeation"; Excerpta Medica: Amsterdam, Netherlands, 1977; p 178.
(22) Neef, C.; Oosting, R.; Meijer, D. K. F. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *328*, 103.
(23) Nielson, P.; Rusmussen, F. *Life Sci.* **1975**, *17*, 1495.
(24) Carr, E. A. In "Animal Models in Radiotracer Design"; Lambrecht, R. M., Eckelman, W. C.; Eds.; Springer-Verlag: New York, 1983; Chapter 2.

The promising heart uptake and selectivity observed for many of our compounds in the mouse biodistribution studies encouraged extension of the studies to other species.

Acute Toxicity. Before evaluating the utility of any of these compounds in other animal species, the LD_{50} values were determined in mice as an indication of acute toxicity.

The acute toxic symptoms were characteristic of ganglionic blockade and included labored breathing, gasping, muscular weakness, paralysis, exopthalmos, and twitching. The mice that expired did so within 2 min of injection. Surviving mice appeared to be without adverse effects by 5-min postinjection and had a lifespan similar to control mice not injected with the compounds. The intravenous LD_{50} values in Swiss white mice were determined to be 14.5 mg/kg for **4p**, 6.7 mg/kg for **5m**, and 6.2 mg/kg for **6m**.

Another halogenated aromatic quaternary ammonium compound, bretylium, has been reported to have an intravenous mouse LD_{50} of 16 mg/kg.[25] A group of halogenated nonaromatic choline ethers have been found to be about 1000-fold more toxic in mice with LD_{50} in the 9–13 μg/kg range.[26]

Pig and Human Studies. After intravenous injection of each of the ^{131}I-labeled compounds, **4p**, **5m**, and **6m**, into three anesthetized pigs, radioactivity in the region of the heart cleared at the same rate as that of the blood pool generally. In marked contrast to mice, in no case was there evidence of myocardial uptake. Concentration and retention of activity were evident in the liver, lungs, and kidney. There was rapid renal clearance and also evidence of biliary excretion.

In three human volunteers ^{123}I-labeled compounds **4p**, **5m**, and **6m** also failed to show significant uptake and retention in heart tissue. Initially the kidneys had the most prominant localization for all three compounds with activity peaking between 5 and 9 min followed by rapid clearance. Rapid initial uptake in the liver and/or lungs was followed by a more gradual increase over the next 30 min. By 1 h all three compounds showed predominately liver localization with differing degrees of lung uptake. As an example the 1-h computer-smoothed image of the distribution with **4p** is shown in Figure 5a.

Varying rates of liver and lung clearance occurred. As seen in Figure 5b, by 24 h **4p** was predominately in the lung and gut due probably to prior biliary excretion. In contrast, for **5m** the gall bladder had the greatest density of radioactivity followed by the lungs and liver.

The observed liver uptake and biliary excretion of these three compounds is consistent with observations of other quaternary ammonium compounds of a molecular weight above 300.[19-22,27] The prolonged liver retention of compound **4p** that occurred in mice was not observed in the human study. The lung accumulation of these compounds may be due partially to the porosity of pulmonary epithelium, which has been shown to allow diffusion of various quaternary ammonium compounds.[26]

After the very promising biodistribution results in mice, the lack of heart uptake of compounds **4p**, **5m**, and **6m** in both pigs and humans was very disappointing. Interspecies differences have plagued various proposed myocardial imaging agents.[28] A β-adrenergic antagonist derivative was found to have heart to blood and heart to lung ratios that were high in the rat but low in both guinea pigs and rabbits.[29] A radioiodinated organic phosphonium cation had a promising heart uptake and retention in rats. However, rapid myocardial washout, high liver uptake, rapid hepatobiliary, and urinary excretion occurred in dogs.[10] A number of recently synthesized ^{99m}Tc cations have also shown a distinct species dependance.[14,15,30] The cation $^{99m}Tc[dmpe]_2Cl_2^+$ showed high myocardial uptake in rat, rabbit, and dog but little in the heart of pigs or humans.[14,15] Also $^{99m}Tc[dmpe]_3^+$ was promising in laboratory animals,[16] but a prolonged level of blood radioactivity and intense hepatic uptake in man produced heart images inferior to $^{201}Tl^+$. The radioiodinated (*o*-iodobenzyl)trimethylammonium cation, RIBA, is structurally similar to **4o**. The heart to liver ratios of RIBA in rats was found[4,24] to be 7:1 at 2-h postinjection. In contrast, although some heart localization of RIBA did occur in dogs, a predominant liver uptake was observed so that heart to liver ratios remained below 0.5:1 at all time points up to 6 h.[4] Insignificant heart

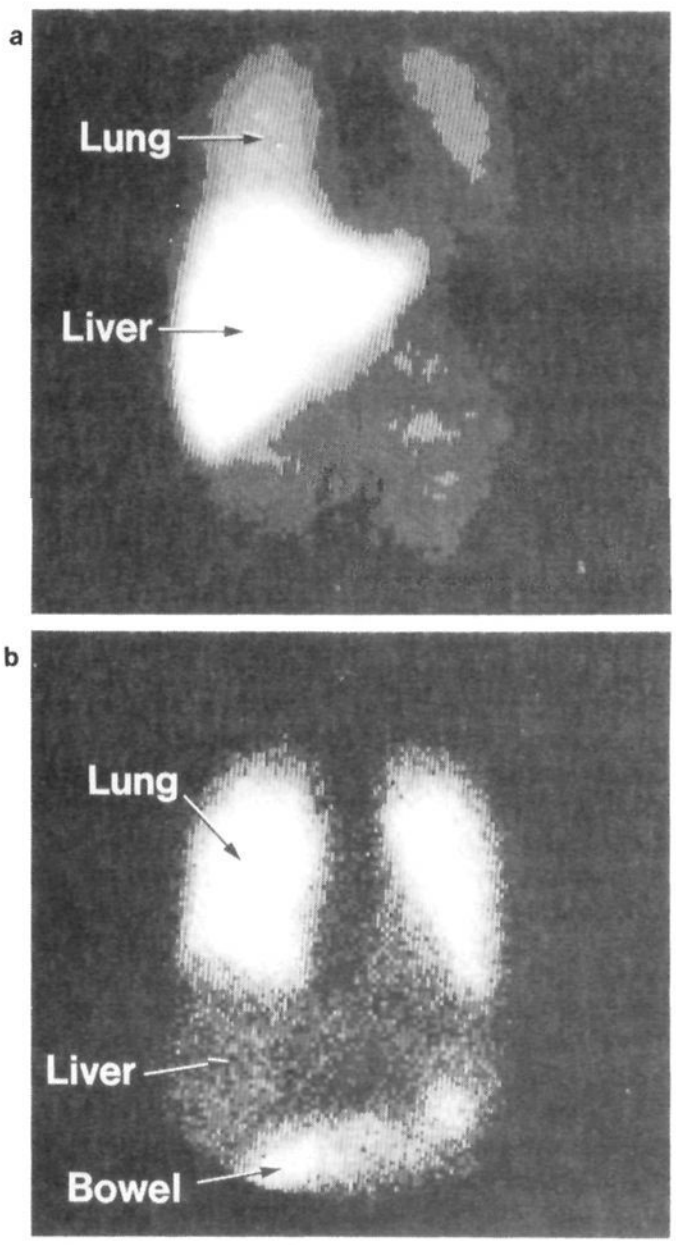


Figure 5. Anterior image of human trunk following intravenous injection of ^{123}I-labeled **4p** at (a) 1 h and (b) 24 h. The 1-h image is computer smoothed.

(25) Christensen, H. E.; Lunginbyhl, T. T. "Toxic Substances List"; U.S. Department of Health, Education, and Welfare: Rocksville, MD, 1974; p 51.
(26) Chaturved, A. K.; Rao, N. G. S.; Rama Sastry, B. V. *Toxicol. Appl. Pharmacol.* **1980**, *54*, 265.
(27) Hemberger, J. A.; Schanker, L. S. *Drug Metab. Dispos.* **1983**, *11*, 73.
(28) Lambrecht, R. M.; Eckelman, W. C.; Rescigno, A. In "Animal Models in Radiotracer Design"; Lambrecht, R. M., Eckelman, W. C., Eds.; Springer-Verlag: New York, 1983; Chapter 1.
(29) Eckelman, W. C.; Gibson, R. E.; Rzeszptarski, W. J.; Jiang, V.; Mazaiti, J. K.; Paik, C.; Komai, T.; Reba, C. In "Receptor Binding Radiopharmaceuticals"; CRC Press: Boca Raton, FL, 1982; Vol. 1, p 251.
(30) Neirinckx, R. D.; Glavan, K. A.; Eakins, M. N.; Kronauge, J. F. "Proceedings of the Third World Congress of Nuclear Medicine and Biology"; Raynaud, C., Ed.; Permagon Press: Paris, 1982; Vol. 1, p 698.

localization was seen in monkeys and man.[24] The 2-h image of RIBA in man[24] is very similar to those of compounds **4p** at 1 h (Figure 5a).

Potential Uptake Mechanisms. RIBA and bretylium are thought to be concentrated in the heart primarily by an extraneuronal, low-affinity, high-capacity mechanism termed "uptake$_2$".[24] The high-capacity uptake of bretylium in rat hearts is therefore not easily saturated with carrier but uptake$_2$ inhibitors such as corticosterone and estradiol inhibit its heart localization.[24] The uptake$_2$ mechanism is species dependent. It is very poorly developed in the heart tissues of guinea pig, rabbit, monkey, and man but well developed in rat, cat, and dog hearts.[24] If the mechanism of localization of our series of compounds is by uptake$_2$, the poorly developed system in man may partially account for the lack of myocardial uptake and retention. Studies to see if isolated rat heart preparations show diminished uptake of our series of quaternary ammonium compounds in the presence of corticosteroids or estradiol would indicate if their uptake is indeed via uptake$_2$.

Bretylium is an antiarrhythmic drug that like our compounds contains a quarternary ammonium nitrogen and an aromatic ring. Other antiarrhythmic compounds with the same structural features are clofilium,[31] *N,N*-dimethylpropanolol,[32] and *N*-methyllidocaine,[33] and they all have delayed onset and prolonged action compared to antiarrhythmic drugs lacking a quaternary ammonium nitrogen. Clofilium and *N,N*-dimethylpropanolol have both been shown to have very prolonged retention in the heart tissues of dogs and rats[31,32,34] and are thought to be trapped in the myocardial cells.[33,34]

The anionic centers of cholinergic receptors or acetylcholinesterase represent another potential site for localization of a large number of organic cations such as quaternary ammonium compounds.[35] Isolated tissue experiments using frog rectus abdominus muscle and (*p*-iodophenyl)choline (**4p**) have revealed that it is a partial agonist at nicotinic receptors.[36] The bromo and chloro analogues of **4m** and the chloro analogue of **4p** have been found to have ganglionic stimulant activity that were 160, 100, and 5 times, respectively, as potent as acetylcholine.[37,38] A limited-size hydrophobic shell surrounding the anionic center of cholinergic receptors generally allows best interaction with a trimethylammonium group[39] while bulkier structures, such as morpholinium and piperidinium salts, are generally less active.[37,39]

It is difficult to extrapolate from these observations and to use in vitro binding assays and pharmacological assays to predict the usefulness of a compound as a diagnostically useful imaging agent. Receptor binding in vivo is complicated by numerous competing and complicating factors including nonspecific binding, protein binding, blood flow, elimination, metabolism, receptor concentration, and affinity constants.[29] Structural changes that may increase binding to a receptor may also limit its availability to the receptor due to a simultaneous increasing contribution of one or more competing factors.

More studies are required to understand the exact mechanism of the mice heart uptake. If receptor binding or uptake$_2$ is involved, high specific activity compounds should be prepared for use in comparative biodistribution studies in the presence and absence of compounds known to specifically inhibit the receptor binding or uptake mechanisms involved. Depending on the results, additional studies may need to be done in higher animal species such as pigs or humans also utilizing the compounds prepared to high specific activities.

In summary, a series of nine radioiodinated quaternary ammonium salts related to phenylcholine were synthesized and characterized. Mouse biodistribution studies revealed that five of the nine compounds were initially taken up in heart tissue to the same extent as $^{201}Tl^+$ at 5 min. Prolonged retention in myocardial tissue of mice produced 60-min values that were significantly better than $^{201}Tl^+$ for six compounds. Several of the compounds were found at various time points to have better heart to blood and/or heart to liver ratios than that observed for $^{201}Tl^+$. The para-radioiodinated isomers tended to display high liver uptake and retention.

Extension of the evaluation of three of the compounds to pig and human imaging revealed no evidence of selective heart uptake or retention. Rapid liver or kidney accumulation occurred with subsequent renal and hepatobiliary/gut excretion. Prolonged retention tended to occur in the liver and/or lung. Thus this attempt to find a myocardial perfusion imaging agent for human use has proven unsuccessful after initial promise in an experimental animal, illustrating the unfortunate problem of interspecies variation.

Experimental Section

Proton NMR spectra were recorded on either a Varian T60 or a Varian XL200 spectrometer. Mass spectra were performed in a MAT 311A spectrometer. Melting points were determined with a Gallenkamp apparatus and are uncorrected. Radioactivity was assayed with a Radioisotope Calibrator (Capintec Model CRC-10) and in a LKB 1280 Ultra γ counter. Combustion elemental analyses were obtained from Guelph Chemical Laboratories Ltd.

Materials. 3-Iodophenol, 4-iodophenol, 2-(dimethylamino)ethyl chloride hydrochloride, and *N*-(2-chloroethyl)piperidine hydrochloride were purchased from Aldrich Chemical Co. and used without further purification. *N*-(2-Chloroethyl)morpholine hydrochloride was purchased from Pfaltz and Bauer Inc.

2-Iodophenol. A solution of 2-iodoaniline (10 g, 45.7 mmol) in 70 mL of 15% sulfuric acid was reacted at ice-bath temperature with sodium nitrite (4 g, 58 mmol) in 5 mL of water. After 5 min 30 mL of an urea solution (0.5 g, 8.3 mmol) was added to destroy any excess nitrite. This solution was then added dropwise to a heated (~90 °C) solution of 18 g of sodium sulfate and 15 mL of sulfuric acid in 300 mL of water.

The cooled reaction mixture was extracted with ether. After washing with water and bicarbonate, the ether layer was extracted with 10% sodium hydroxide. The alkaline extract was then acidified with hydrochloric acid and the 2-iodophenol was extracted with ether. After washing with water and drying, the ether was removed to yield 7.75 g of material. Purification by sublimation yielded 5 g (50% yield) of a material of mp 40–41 °C[40]

(31) Lindstrom, T. D.; Murphy, P. J.; Smallwood, J. K.; Wiest, S. A.; Steinberg, M. I. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 584.
(32) Gibson, J. K.; Korn, N. J.; Counsell, R. E.; Lucchesi, B. R. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1979**, *38*, 697.
(33) Kniffen, F. J.; Lomas, T. E.; Nobel-Allen, N. L.; Lucchesi, B. R. *Circulation* **1974**, *49*, 264.
(34) Patterson, E.; Stetson, P.; Lucchesi, B. R. *J. Pharmacol. Exp. Ther.* **1980**, *214*, 449.
(35) Burns, H. D.; Dannals, R. F.; Waud, J.; Dannals, T. E.; Marzilli, L. G.; Wagner, H. N. In "Radiopharmaceuticals. Structure Activity Relationships"; Spencer, R. P., Ed.; Grune and Stratton: New York, 1981; Chapter 28.
(36) Brown, G. W.; Philp, R. B. "Proceedings of the 4th International Symposium of Radiopharmacology"; Permagon Press: Toronto, in press.
(37) Hey, P. *Br. J. Pharmacol.* **1952**, *7*, 117.
(38) Ambache, N.; Robertson, P. A. *Br. J. Pharmacol.* **1953**, *8*, 147.
(39) Michelson, M. J.; Zeimal, E. V. "Acetylcholine. An Approach to the Molecular Mechanism of Action"; Permagon Press: New York, 1973; Chapter 4.
(40) "Handbook of Chemistry and Physics"; The Chemical Rubber Publishing Co.; Cleveland, OH, 1960.

Table II. Yields[a] and Melting Points of (Iodophenoxy)ethylamines and Their Methylammonium Iodides[b]

	ortho		meta		para	
no.	% yield	mp, °C	% yield	mp, °C	% yield	mp, °C
1	50	136–137[c]	73	157–158[d]	79	186–187[d]
2	73	161–162[d,e]	85	166–167[c]	87	212[d,f]
3	95	169–170[d,e]	83	222[d,e]	77	36–37
4	68	151–152	74	179–180	95	246[f]
5	46	123–124	63	153–154	87	191–192
6	50	148–149	59	131–132	83	216[f]

[a] Percent yield of recrystallized product. [b] Recrystallized from water. [c] Recrystallized from chloroform–hexanes. [d] Hydrochloride salt. [e] Recrystallized from alcohol–ether. [f] With decomposition.

Table III. Radiochemical Yields of the Amines and Ammonium Salts Labeled with Iodine-131 Exchange Labeling

no.[a]	yield[b]	no.	yield[c]	sp act.[d]
1o	69	**4o**	85	100
1m	13	**4m**	90	50
1p	65	**4p**	82	380
2o	78	**5o**	81	410
2m	60	**5m**	93	260
2p	31	**5p**	81	100
3o	64	**6o**	78	110
3m	62	**6m**	79	110

[a] Compounds **3p** and **6p** were prepared[6] from the diazonium salt but without added carrier. [b] Percent radiochemical yield of exchange. [c] Percent radiochemical yield of methylation step. [d] Millicuries/millimole. These are only approximate minimum values.

that had an NMR, IR, and UV spectrum consistent with 2-iodophenol.

Ortho, Meta, and Para Isomers of *N*,*N*-Dimethyl-2-(iodophenoxy)ethanamine (1o, 1m, 1p), Ortho, Meta, and Para Isomers of 1-[2-(Iodophenoxy)ethyl]piperidine (2o, 2m, 2p), and Ortho, Meta, and Para Isomers of 4-[2-(Iodophenoxy)ethyl]morpholine (3o, 3m, 3p) and the Corresponding Quaternary Methyl Ammonium Iodides (*N*,*N*,*N*-Trimethyl-2-(iodophenoxy)ethanaminium (4o, 4m, 4p), 1-Methyl-1-[2-(iodophenoxy)ethyl]piperidinium (5o, 5m, 5p), 4-Methyl-4-[2-(iodophenoxy)ethyl]morpholinium (6o, 6m, 6p)). The synthetic procedure for the preparation of the nine amines has been described elsewhere.[6] The yields and melting points for these compounds are contained in Table II. The proton NMR spectra of the amines were consistent with their structures, and they all gave exact masses in agreement with their formulae.

Similarly the synthetic procedure followed for the nine quaternary ammonium iodides has been published.[6] The yields and melting points for these compounds are presented in Table II. Proton NMR spectra were in accord with their structure and combustion analyses (C, H, N, I) were satisfactory.

Quaternary Ammonium Acetates from 4p, 5m, and 6m. Typically 12 g of Rexyn-202 anion-exchange resin was converted to its acetate form by treatment with 200 mL of saturated sodium acetate, and then a column was prepared with use of methanol as the eluent. About 1 g of the quaternary ammonium iodide in methanol was passed through the column and eluted with a few more milliliters of methanol. Solvent removal yielded the acetate salt quantitatively.

Radiochemistry. The sodium iodide-131 was obtained from New England Nuclear, and the sodium iodide-123 was obtained from Atomic Energy of Canada, Ltd. Separations by TLC of radiolabeled products was on plastic-backed alumina sheets from which the band of interest was cut and eluted. The amines **1–3** were isotopically exchanged by procedures already described,[6] and the yields are presented in Table III. These were then converted to the quaternary ammonium salts, and these results are also included in Table III.

Reanalysis of the isolated exchanged amines by TLC indicated that >95% of the radioactivity was at the level of the standard. In one case, **1p**, the exchanged material was mixed with unexchanged **1p** and then recrystallized. The recovered **1p** retained the radioactivity.

Procedure for Exchange Labeling of the Acetate Salts. A solution of 1 mg of the acetate salt, prepared as described above, and 6 mg of benzoic acid in 200 μL of ethanol and 50 μL of sodium iodide-131 (1.5 mCi) in a 1-mL multidose vial was evaporated to dryness by using a rotary evaporator. If the solution was first cooled to dry ice temperatures before evaporation, bumping could be avoided. After sealing with a Teflon-sided septum, the mixture was heated for 1 h at 170 °C. The reaction product was washed with three 1-mL portions of sterile water and passed successively through a Rexyn-202 resin column in the acetate form. The radiolabeled quaternary ammonium acetate in the water eluant was made isotonic by the addition of 25 mg of sodium chloride. Millipore filtration provided solutions ready for injection. The radiochemical yields obtained for the ^{123}I and ^{131}I exchange reactions of the ammonium acetate salts of **4p**, **5m**, and **6p** are presented in Table IV.

Biological Evaluation. Swiss white mice (20–25 g) were initially anesthetized with an intraperitoneal injection of sodium pentobarbital (80 mg/kg of body weight) and then received a 0.1–0.2-mL (74 kBq) intravenous tail injection of the radioiodinated compound or $^{201}Tl^+$. Four mice were sacrificed by cardiac puncture/exsanguination at each postinjection period of 5, 15, 30, and 60 min. In addition, compounds **5o**, **5m**, and **5p** were also evaluated at 90 and 120 min. Selected organs or tissues (heart, lung, liver, spleen, kidneys, muscle, and bone) were excised, washed, patted dry, and weighed along with blood samples. Tissues and injection standards were subsequently assayed for radioactivity. Results were expressed as both percent uptake per organ and percent uptake per gram of wet tissue.

In addition, compounds **4p**, **5m**, and **6m** were evaluated in pigs (Yorks, Landrace, Hampshire, Duroc cross) and following informed consent in human volunteers. The ^{131}I-radiolabeled compounds were used in pigs while the ^{123}I-labeled material was used in the human studies (see Table IV for doses).

The pig studies were acquired on a G.E. Maxi II γ camera with the pig in a right decubitus position. Sixty degree left anterior oblique images with the heart in the middle of the field of view were collected on a computer using a format of one frame every 10 s for the first 30 min and one frame every 300 s for the duration of the study.

The human studies were acquired on the same system with the subjects laying supine. Anterior images of the heart, lungs, and liver were also collected on computer with the heart in the approximate center of the field of view. In the dynamic studies, one frame every second for the first minute and one frame every minute for the duration of the study were obtained for 30–35 min.

Table IV. Radiochemical Yields of Ammonium Acetates from Exchange Labeling with Iodine-131 and Iodine-123

	iodine-131			iodine-123		
no.	yield[c]	injected dose[a]	sp act.[b]	yield[c]	injected dose[a]	sp act.[b]
4p	73	0.14	50	86	2.24	830
5m	75	1.1	450	81	5.13	2080
6m	48	0.88	360	58	1.38	560

[a] Millicurie. [b] Millucuries/millimole. These are only approximate minimum values. [c] Percent radiochemical yield.

Static images were obtained at 1-, 2-, and 24-h postinjection.

Acute Toxicity. Unanesthetized Swiss white mice (20–25 g) were placed in a restraining tube and injected via a tail vein with different doses of compound. Four mice were injected with each dose, and the number of survivors were determined 5 min after injection. In addition, survivors were examined 24-h postinjection.

Overt symptoms at the time of injection were noted and the LD_{50} value for **4p**, **5m**, and **6m** were determined by using the probit-log dose method.[41]

Acknowledgment. We thank Merck-Frosst Canada Ltd. and the Natural Sciences and Engineering Research Council of Canada for their financial support.

Registry No. **1o**, 99540-52-0; **1o**-^{131}I, 100839-47-2; **1m**, 100839-40-5; **1m**·HCl, 100839-66-5; **1m**-^{131}I, 100839-48-3; **1p**, 100839-41-6; **1p**·HCl, 100839-68-7; **1p**-^{131}I, 100839-49-4; **2o**, 76465-07-1; **2o**·HCl, 100839-64-3; **2o**-^{131}I, 100839-50-7; **2m**, 100839-42-7; **2m**-^{131}I, 100839-51-8; **2p**, 100839-43-8; **2p**·HCl, 100839-69-8; **2p**-^{131}I, 100839-52-9; **3o**, 100839-44-9; **3o**·HCl, 100839-65-4; **3o**-^{131}I, 100839-53-0; **3m**, 100839-45-0; **3m**·HCl, 100839-67-6; **3m**-^{131}I, 100839-54-1; **3p**, 100839-46-1; **3p**-^{125}I, 100857-94-1; **4o**·I^-, 100839-55-2; **4o**-^{131}I·I^-, 100839-70-1; **4m**·I^-, 100839-56-3; **4m**-^{131}I·I^-, 100839-71-2; **4p**·I^-, 100839-57-4; **4p**-^{131}I·I^-, 100839-72-3; **4p**·$CH_3CO_2^-$, 100839-78-9; **4p**-^{131}I·$CH_3CO_2^-$, 100839-84-7; **4p**-^{123}I·$CH_3CO_2^-$, 100839-88-1; **5o**·I^-, 100839-58-5; **5o**-^{131}I·I^-, 100839-73-4; **5m**·I^-, 100839-59-6; **5m**-^{131}I·I^-, 100839-74-5; **5m**·$CH_3CO_2^-$, 100839-80-3; **5m**-^{131}I·$CH_3CO_2^-$, 100839-86-9; **5m**-^{123}I·$CH_3CO_2^-$, 100839-90-5; **5p**, 100839-60-9; **5p**-^{131}I·I^-, 100839-75-6; **6o**·I^-, 100839-61-0; **6o**-^{131}I·I^-, 100839-76-7; **6m**·I^-, 100839-62-1; **6m**-^{131}I·I^-, 100857-93-0; **6m** $CH_3CO_2^-$, 100839-82-5; **6m**-^{131}I·$CH_3CO_2^-$, 100839-92-7; **6m**-^{123}I·$CH_3CO_2^-$, 100839-94-9; **6p**·I^-, 100839-63-2; **6p**-^{125}I·I^-, 100839-96-1; *o*-$IC_6H_4NH_2$, 615-43-0; *o*-IC_6H_4OH, 533-58-4; *m*-IC_6H_4OH, 626-02-8; *p*-IC_6H_4OH, 540-38-5; $Cl(CH_2)_2NMe_2$, 107-99-3; $Cl(CH_2)_2NEt_2$, 100-35-6; *p*-$NH_2C_6H_4OH$, 123-30-8; *p*-$^{125}IC_6H_4OH$, 100839-95-0; *N*-(2-chloroethyl)morpholine, 3240-94-6.

(41) Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.

4-Amino-2-(substituted methyl)-2-butenoic Acids: Substrates and Potent Inhibitors of γ-Aminobutyric Acid Aminotransferase

Richard B. Silverman,*† Susan C. Durkee,‡ and Benedict J. Invergo

Department of Chemistry and Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60201. *Received June 7, 1985*

4-Amino-2-(substituted methyl)-2-butenoic acids, where X (the substituted group) = F, Cl, OH, are synthesized from Cbz-protected *tert*-butyl 4-aminobutanoate. Successive substitutions at the α-carbon by phenylseleno and hydroxymethyl groups, followed by elimination of the selenoxide and halide substitution at the hydroxymethyl group, afford the compounds in good yields. An unexpected degree of stereoselectivity is observed in the selenoxide elimination step, which yields the desired *E* isomer as the sole product. These compounds complement two previously reported series of compounds (Silverman, R. B.; Levy, M. A. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 250–255; *J. Biol. Chem.* **1981**, *256*, 11565–11568) and are used in an approach to map a section of the active site of γ-aminobutyric acid aminotransferase (GABA-T). None of these compounds is a time-dependent inactivator of GABA-T, but all are potent competitive reversible inhibitors; the hydroxy compound has a K_i value of 5 μM. That these compounds are not inactivators suggests that either elimination of X does not occur or that there is no active site nucleophile in the appropriate position for reaction following elimination. With use of the fluoro analogue, enzyme-catalyzed fluoride ion release is demonstrated, indicating that elimination does occur. Unlike the previous two series of compounds (op. cit.) in which exclusive elimination occurs when the substituent is a halogen but exclusive transamination prevails for the hydroxyl-substituted analogues, in the series described here, the fluoro analogue gives a 4:1 ratio of elimination to transamination. This suggests that the 2,3-double bond stabilizes the product of azallylic isomerization of the Schiff base between the fluoro compound and pyridoxal phosphate. The results described here indicate that the design of a mechanism-based inactivator for GABA-T should not be based on electrophile generation near the 2-position of enzyme-bound GABA. Furthermore, substitution of an inhibitor with a 2-hydroxymethyl group (or other hydrogen-bonding substituent) and a 2,3-double bond may lend auspicious binding properties to the molecule for GABA-T.

It has been shown that convulsions can occur when the level of γ-aminobutyric acid (GABA) in the brain diminishes below a critical amount and that direct administration of GABA into the brain terminates the seizures.[1-3] However, GABA does not cross the blood–brain barrier, a protective membrane that prevents xenobiotics from entering the brain. Consequently, GABA is not an effective anticonvulsant agent. Recently, efforts have been directed toward the discovery of compounds that inhibit γ-aminobutyric acid aminotransferase (EC 2.6.1.19; GABA-T),[4-9] a brain enzyme responsible for the catabolism of GABA. This would result in an increase in the concentration of GABA in the brain and could be an effective approach to the design of anticonvulsant agents.

†Recipient of an Alfred P. Sloan Research Fellowship (1981–1985) and a NIH Research Career Development Award (1982–1987).

‡Present address: Hercules, Inc., Research Center, Wilmington, DE 19899.

(1) Krnjević, K.; Schwartz, S. *Exp. Brain Res.* **1967**, *3*, 320–336.
(2) Mandel, P.; DeFeudis, F. V. "Advances in Experimental Medicine and Biology: GABA Biochemistry and CNS Function"; Plenum Press, New York, 1979; Vol. 123.
(3) Roberts, E.; Chase, T. M.; Tower, D. B., Eds. "GABA in Nervous System Function"; Raven Press: New York, 1976.
(4) Silverman, R. B.; Levy, M. A. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 250–255.
(5) Jung, M. J.; Metcalf, B. W. *Biochem. Biophys. Res. Commun.* **1975**, *67*, 301–306.
(6) Lippert, B.; Metcalf, B. W.; Jung, M. J.; Casara, P. *Eur. J. Biochem.* **1977**, *74*, 441–445.
(7) Rando, R. R.; Bangerter, F. W. *Biochem. Biophys. Res. Commun.* **1977**, *76*, 1276–1281.
(8) Metcalf, B. W.; Jung, M. J. *Mol. Pharmacol.* **1979**, *16*, 539–545.
(9) Fletcher, A.; Fowler, L. J. *Biochem. Pharmacol* **1980**, *29*, 1451–1454.

0022-2623/86/1829-0764$01.50/0