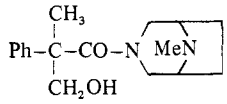
Table I. Affinity for Postganglionic Acetylcholine Receptors of the Isolated Guinea-Pig Ileum

Compound		Log K^a	SSI[b]
8-Methyl-3α-methyltropoyl-3,8-diazabicyclo[3.2.1]octane			
Base	(−)	8.165 + 0.029 (7)	1140:1
	(+)	5.107 + 0.039 (6)	
Methiodide	(−)	7.527 + 0.042 (6)	46:1
	(+)	5.867 + 0.019 (6)	
Hyoscyamine			
Base	*S*	9.380	330:1
	R	6.861	
Methiodide	*S*	9.666	87:1
	R	7.725	

[a] Estimates of the mean value of log *K* are shown with the standard error and number of results. Values for hyoscyamine and hyoscyamine methiodide, included for comparison, were obtained by exactly the same method by Barlow, Franks, and Pearson.[2] [b] The ratio of the affinity constants of the enantiomers is referred to as the stereospecific index (SSI).

ity of the (+) and (−) forms of 8-methyl-3α-methyltropoyl-3,8-diazabicyclo[3.2.1]octane (I). The enantiomers were described by Scarselli, Cignarella, and Maffii[1] and samples were obtained from Professor G. Nathansohn (Gruppo Lepetit SPA). The (+) enantiomer was in the form of the base (monohydrate) and the (−) enantiomer was the hydro-



I

chloride and there was sufficient material for the preparation of small quantities of the methiodides. These had identical ir spectra (KCl disks) and mp 214–216° dec. The molar rotations ($c = 5 \times 10^{-2} M$, water) at 300 nm were estimated to be −635 and +669°

The affinity constants for the postganglionic acetylcholine receptors of the guinea-pig ileum at 37° were measured as described previously[2] in conditions in which the antagonists were allowed time to come into equilibrium with the tissue, with carbachol as the agonist and in the presence of hexamethonium. The results are shown in Table I, which includes values for (*R*)- and (*S*)-hyoscyamine to which the compounds bear some resemblance.

The enantiomeric forms of the base differ over 1000-fold in affinity, indicating their high stereochemical purity.[3] The stereospecific index is reduced 25-fold by methylation, however, which decreases the affinity of the more active enantiomer of the base whereas it increases the affinity of the less active enantiomer. As the compounds are amides of α-methyltropic acid, it is unlikely that quaternization has been accompanied by racemization or hydrolysis. The difference between the molar rotations suggests that the more active enantiomer may be slightly less stereochemically pure but this would make little difference to the stereospecific index and is almost certainly due to errors in the measurement of the rotations.

The values for the enantiomeric forms of hyoscyamine which have been included in Table I show that in spite of their superficial resemblance to the diazabicyclooctane compounds they have about ten times the affinity for the receptors. This may be because the amide link is less flexible than an ester group but, whatever the cause of the difference in affinity, it is remarkable that the bases with lower affinity are nevertheless highly stereospecific and that this stereospecificity is drastically reduced by methylation. Although the bases may not be completely ionized at pH 7.6, so the estimates of log affinity constant may be less than the true value for the ion, the stereospecific index should not be affected as both enantiomers will have the same pK_a It appears, then, that increasing the size of the onium group of the more active (−) enantiomer appreciably disturbs the binding to the receptor and possibly this disturbance is greater in the rather inflexible amides than in the esters, such as hyoscyamine methiodide where it may be offset to some extent by changes in conformation.

Acknowledgments. I am most grateful to Professor G Nathansohn for the samples and to Mrs. F. Franks and Miss. M. Harrison for the biological measurements.

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Potential Organ- or Tumor-Imaging Agents. 14.† Myocardial Scanning Agents

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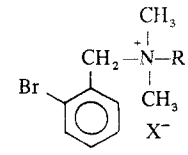
Received February 15, 1973

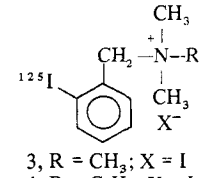
A γ-emitting radiopharmaceutical capable of selectively concentrating in cardiac muscle would be of potential clinical value for the diagnosis of a variety of myocardial disorders. Although several agents have undergone clinical evaluation, no myocardial scanning agent is currently available for routine clinical use.

Carr and associates[1-3] have made the most concerted effort to find a myocardial scanning agent. A survey of radionuclides among elements of group IA in the periodic table showed cesium-131 to be the most promising. Suitable myocardial scans in humans have been obtained within 3 hr after the administration of 1.25 mCi of carrier-free cesium-131.[2] Unfortunately, this radionuclide is also retained in skeletal muscle for a long period and prevents rescanning the patient for at least 5 weeks.[3]

The rather high uptake of Toluidine Blue by cardiac tissue following intravenous administration to rats[4] and dogs[5] prompted evaluation of radioiodinated Toluidine Blue as a myocardial imaging agent. Although preliminary results were encouraging, the use of this labeled compound required a priming dose of stable Toluidine Blue, a serious practical disadvantage.[3]



1, R = CH_3; X = I
2, R = C_2H_5; X = $CH_3C_6H_4SO_3$



3, R = CH_3; X = I
4, R = C_2H_5; X = I
5, R = H; X = Cl

As illustrated by previous papers in this series, our approach to specific organ- or tumor-localizing agents has been based to a considerable degree on well-established biochem-

† This work was supported by CA-08349 from the National Cancer Institute, National Institutes of Health.

Table I. Tissue Distribution Profiles for 3 and 4 in Male Rats at Various Time Periods

Tissue	Methyl quaternary 3				Ethyl quaternary 4	
	0.5 hr	2 hr	6 hr	18 hr	2 hr	6 hr
Adrenal	[a]	478 ± 36[b]	427 ± 60	71 ± 9	396 ± 71	289 ± 187
Blood	207 ± 53	78 ± 27	69 ± 33	0	1,116 ± 151	591 ± 167
Brain	7 ± 2	7 ± 2	7 ± 2	0		
Fat	131 ± 104	73 ± 27	144 ± 67	0		
Heart, auricle	767 ± 124	1180 ± 193	971 ± 120	20 ± 18	364 ± 44	220 ± 140
Heart, ventricle	996 ± 196	1447 ± 213	1140 ± 273	22 ± 13	309 ± 31	180 ± 62
Intestine			276 ± 133		778 ± 93	451 ± 207
Kidney	1160 ± 424	391 ± 60	213 ± 69	9 ± 4	655 ± 33	329 ± 49
Liver	822 ± 222	373 ± 98	207 ± 38	16 ± 2	358 ± 27	200 ± 64
Lung	384 ± 182	353 ± 40	124 ± 24	9 ± 4	724 ± 111	351 ± 93
Muscle	71 ± 7	149 ± 67	284 ± 44	27 ± 16		
Spleen	231 ± 184	144 ± 33	153 ± 51	4 ± 2	431 ± 58	213 ± 60
Thyroid	280 ± 20	313 ± 56	536 ± 89	376 ± 178	16,449 ± 1,280	14,731 ± 2,633

[a] Samples displayed excessive standard deviation. [b] Values represent mean dpm/mg for three rats ± standard error.

ical and/or pharmacological information. Accordingly, the report by Boura, *et al.*,[6] citing the predilection of bretylium (**2**) for the cat heart prompted us to explore similar molecules labeled with a γ-emitting nuclide as potential myocardial imaging agents. Interestingly, tissue distribution studies with a methyl quaternary analog of bretylium (**1**) showed a similar distribution profile in the cat, but this quaternary was much less active as an adrenergic-neurone blocker.[7]

In keeping with the earlier bretylium studies, compounds **3** and **4** were synthesized for tissue distribution studies. The use of an appropriate radionuclide of iodine was necessitated by the lack of a suitable γ emitter among C, H, and N. Moreover, the radiation from bromine-84 (444 keV, $t_{1/2}$ = 36 hr) is of sufficiently high energy as to limit its clinical utility. Consequently, iodine-125 (35 keV, $t_{1/2}$ = 60 days) was selected for our initial studies because its low energy and reasonably long half-life minimized handling and storage problems. In addition, a subsequent switch to iodine-131 (364 keV, $t_{1/2}$ = 8 days) or iodine-123 (159 keV, $t_{1/2}$ = 13.3 hr) would represent no major change in methodology.

Surprisingly, a search of the literature revealed that the chemical or biological properties of **3** and **4** had not been previously reported. Although some technical difficulties were encountered, the synthesis of these compounds was straightforward and requires little comment. Treatment of *o*-iodobenzyl bromide with excess dimethylamine in benzene gave essentially a quantitative yield of *o*-iodobenzyldimethylamine isolated as the hydrochloride salt (**5**). This amine served as the substrate for introduction of iodine-125 by isotope exchange in refluxing NH_4OH. The radioiodinated tertiary amine was not isolated but directly quaternized with CH_3I or C_2H_5I in MeOH. As expected, quaternization with C_2H_5I was much slower than with CH_3I. The radioiodinated quaternary derivatives displayed physical properties in agreement with the stable compounds.

Preliminary tissue distribution data for the radioiodinated bretylium analogs are recorded in Table I. In contrast to the methyl quaternary **3**, the ethyl analog **4** underwent considerable *in vivo* deiodination as reflected by the high concentration of radioactivity in the thyroid. It is interesting to speculate that the increased steric strain associated with **4** may contribute to this marked difference in metabolic behavior. Undoubtedly, it is this propensity of **4** to deiodinate that largely accounts for the poor uptake of radioactivity by heart tissue.

On the other hand, the distribution profile of radioactivity from **3** shows many similarities to the previous data for *o*-bromobenzyltrimethylammonium iodide-^{14}C (**1**) in cats. Such organs as heart, adrenal, liver, and kidney accumulate concentrations of radioactivity far in excess of blood at early time periods. The negligible amount of radioactivity in the brain agrees with the well-known inability of ionized drugs to cross the blood-brain barrier. As noted in previous studies with bretylium,[6,8] most of the tissue radioactivity had dissipated by 18 hr. In man, 72–81% of intramuscularly administered bretylium is excreted in the urine during the first 24 hr.[8]

From an organ-imaging standpoint, a comparison of the target-to-nontarget ratios is an important practical consideration. Since the liver is generally the most troublesome interfering organ in myocardial scanning, a compound must show a good heart to liver ratio before it can be considered for follow-up studies. In our study, **3** displayed ventricle/liver ratios of 4.57 ± 1.35 and 5.51 ± 0.71 at 2 and 6 hr, respectively. This compares favorably with the value of 1.24 reported for the corresponding bromo analog **1** at 3 hr in the cat.[7] Moreover, the heart ventricle/blood ratios at 2 and 6 hr were more than double the value of 9.0 found in the cat at 3 hr. Changes in the auricle- and ventricle/liver ratios with time are discernable from Table I. The apparent preferential uptake of radioactivity from **3** in the myocardium of the rat shortly after administration has prompted more detailed studies in rats and dogs and these will be reported at a later date.

Experimental Section‡

***O*-Iodobenzyldimethylamine Hydrochloride (5).** *O*-Iodobenzyl bromide[9] (9 g, 30 mmol) was prepared according to the method of Sloviter[10] and dissolved in C_6H_6 (25 ml). A solution of 20% dimethylamine in C_6H_6 (25 ml) was added dropwise with cooling and the mixture allowed to stand at room temperature overnight. After heating the mixture under reflux for 1 hr, the benzene and excess dimethylamine were removed by distillation under reduced pressure. Ether (50 ml) was added to the residue and the insoluble dimethylamine hydrobromide removed by filtration. The filtrate was washed with H_2O and dried (Na_2SO_4), and the solvent was removed *in vacuo*. Treatment of the residue with MeOH–HCl and recrystallization of

‡ Melting points were taken on a Fisher-Johns melting point apparatus and are corrected. Elemental analyses were performed by Midwest Microlab Ltd., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Ir spectra were taken on a Perkin-Elmer 337 spectrophotometer. The nmr spectra were obtained with a Varian A-60 spectrometer in $CDCl_3$ and TMS as an internal standard. Tlc were run with Eastman chromagrams cut in 1-in. wide strips and spots detected under uv light. Chromatograms of radioiodinated compounds were scanned with an Atomic Associate RCS-363 radiochromatogram scanner. Specific activities were ascertained using a Beckman LS-200 liquid scintillation counter.

the product from MeOH–Et_2O gave **5** (8.7 g, 96%), mp 156–157°. *Anal.* ($C_9H_{13}ClIN$) C, H.

O-**Iodobenzyltrimethylammonium Iodide** (3). This above HCl salt (3 g, 10 mmol) was converted to the free base in the usual manner. The product without further purification was dissolved in a mixture of absolute MeOH (5 ml) and CH_3I (2 g, 14 mmol). The mixture was allowed to stir at room temperature for 2 hr whereupon most of the solvent was removed under reduced pressure. Trituration of the residue with Me_2CO (10 ml) afforded a crystalline product which was collected by filtration, washed with Et_2O, and allowed to air dry. Recrystallization from MeOH–Et_2O afforded pure 3 (3.9 g, 97%), mp 175–176° dec. *Anal.* ($C_{10}H_{15}I_2N$) C, H.

O-**Iodobenzyldimethylethylammonium Iodide (4).** In a similar manner treatment of the amine with C_2H_5I for 18 hr afforded the ethiodide as an oil (quantitative yield). Trituration of this oil with EtOH afforded a solid which was recrystallized from the same solvent to give **4** as a white solid, mp 187–188°.§ *Anal.* ($C_{11}H_{17}I_2N$) C, H.

Isotope Exchange and Quaternization. A solution of *O*-iodobenzyldimethylamine hydrochloride (**5**, 75 mg) and $Na^{125}I$ (5 mCi) in reagent grade NH_4OH (4 ml) was refluxed with stirring under an atmosphere of N for 24 hr. The solution was allowed to cool and poured into excess 10% NaOH (20 ml). A $CHCl_3$ extract was washed with H_2O and dried (Na_2SO_4), and the solvent was removed under a slow stream of air. Radioanalysis of the product (62 mg) indicated a specific activity of 76 μCi/mg (94% exchange). Tlc using absolute Et_2O showed a single spot coincident with the radioactive peak displayed on a radiochromatogram (R_f 0.45). The product was dissolved in absolute MeOH (1 ml) and CH_3I (40 mg) added. The solution was stirred at room temperature for 2 hr and the solvent removed under a slow stream of air Precipitation and recrystallization as above afforded radioiodinated **3** (98 mg): mp 177–178° dec; specific activity, 42 μCi/mg. Tlc using MeOH–$CHCl_3$ (1:2) gave a single spot coincident with the radioactive peak displayed on the radiochromatogram. Similarly, quaternization with C_2H_5I afforded **4**, specific activity, 10 μCi/mg.

Tissue Distribution Studies. Radioiodinated compounds were given by subcutaneous injection to immature male Sprague-Dawley albino rats weighing 175–200 g. The dose administered was approximately 50 μCi per rat and the vehicle used as isotonic saline. Groups of three animals were killed by exsanguination through ventricle 2, 6 and 18 hr postinjection. The major organs such as liver, kidney, lung, spleen, auricle, and ventricle were excised, weighed, and homogenized. These organs were washed thoroughly with isotonic saline to remove blood, dried and minced with scissors, and placed in a homogenizer tube containing 20 ml of H_2O in the case of liver and 2 ml of H_2O in the case of other major organs. Homogenates were not prepared for small organs such as adrenal and thyroid. Several samples of homogenates, heparinized blood and plasma specimens, and entire adrenal, thyroid, and other tissue samples such as fat and muscle were placed in scintillation counting vials. To each vial, 0.3 ml of 2.5 *M* NaOH solution was added and left overnight and then heated for at least 10 min at 60° in a water bath to complete the digestion. The vials were allowed to cool and 0.7 ml of 1.1 *M* HOAc, 0.05 ml of 30% H_2O_2, and 10 ml of Aquasol# cocktail were added successively to each vial and the contents shaken using a vortex mixer. The vials were kept in a cool dark place for at least 4 hr before counting. Radioactivity was assayed in a Beckman LS-200 liquid scintillation spectrometer. Sufficient counts were accumulated to reduce the probable error of counting to less than 5%. All counts were corrected for quenching by using ^{125}I-quench standards curves.

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§ The bromide salt was reported in U. S. Patent 3,037,910 (1962), mp 145–146°.

Xylene-based liquid scintillation counting solution was obtained from New England Nuclear, Boston, Mass.

Antimalarials. 5. 2-Aryl-6-trifluoromethyl-4-pyridinemethanols

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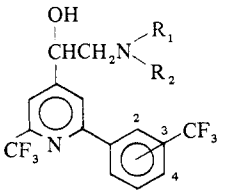
Received April 6, 1973

We have previously reported[1] the synthesis of a series of 2,6-bis(aryl)-4-pyridinemethanols containing Cl, Br, F, OCH_3, and CF_3 substituents on the phenyl rings. These compounds were shown to possess a high degree of activity against *Plasmodium berghei* in mice.† Later,[3] a series of styryl- and benzoyl-containing 4-pyridinemethanols were reported which also showed significant antimalarial activity.

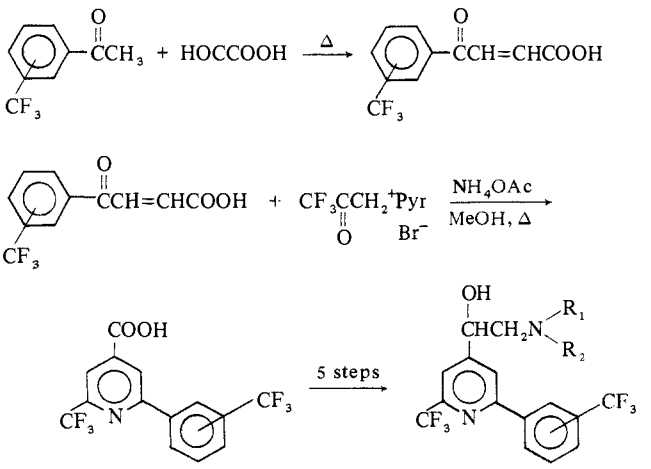
In a continuing effort to maximize the activity of the 4-pyridinemethanols, a series of 2-aryl-6-trifluoromethyl-4-pyridinemethanols (represented by structure **I**) was synthesized.



I

Chemistry. The requisite 2,6-disubstituted isonicotinic acids were prepared *via* the modified Zecher-Krohnke ring-closure method previously described.[1,4] The intermediate trifluoromethyl-substituted benzoylacrylic acids were prepared by reacting the appropriate acetophenone with glyoxylic acid.[1b] Conversion to the 4-pyridylethylene oxide was by the procedure developed by Lutz and coworkers.[5] Ring opening with the appropriate mono- or dialkylamine afforded the eight α-*N*-alkylaminomethyl-2-aryl-6-trifluoromethyl-4-pyridinemethanols shown in Table I. The sequence is shown in Scheme I.

Scheme I



† The antimalarial tests were performed by Dr. Leo Rane of the University of Miami.[2] See footnote *a*, Table II. Testing results were supplied through the courtesy of Drs. Thomas R. Sweeney and Bing T. Poon of the Walter Reed Army Institute of Research.