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Iodo-Bis(quaternary ammonium) Salts. Potential Cartilage-Selective X-Ray Contrast Agents

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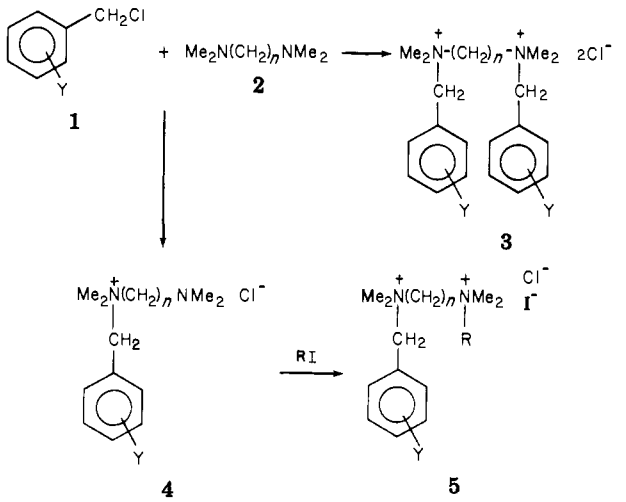
Bis(quaternary ammonium) compounds in which one or both quaternary nitrogens bear iodinated benzyl moieties and the charged centers are separated by 2, 4, 6, or 10 methylene units have been synthesized and evaluated for (a) binding to cartilaginous material, (b) radiocontrast characteristics, and (c) in vitro pharmacological effects. Also prepared was 1,5-diiodo-2,4-bis(β-trimethylammonioethyl)benzene diiodide, an analogue in which the iodinated aryl group lies between the charged nitrogen centers. Biological studies show that these compounds bind to cartilage but at relatively slow rates and with low persistence, resulting in low and transient levels of radiopacity. In common with simpler bisquaternary compounds, these compounds block synaptic transmission.

The density and anatomical location of cartilage is such that, under ordinary conditions of clinical radiography, it is indistinguishable from adjacent soft tissues and body fluids. Thus, to date, the visualization of cartilage-lined joint spaces has required the intraarticular injection of artificial contrast agents (radiolucent gases or radioopaque fluids). It has recently been demonstrated by Asghar and Roth,[1] by Wassermann,[2] and by Shindo et al.[3] that radioactive (^{14}C, ^{3}H) bis(quaternary ammonium) salts [e.g., hexamethonium, $Me_3N^+(CH_2)_6N^+Me_3$] administered intravenously or intraperitoneally to rats or mice are bound selectively to cartilaginous structures.[4] These reports suggested to us that iodinated bis(quaternary ammonium) compounds might be useful for the selective radiographic visualization of cartilage. To test this hypothesis a number of bis(quaternary ammonium) compounds bearing one or two iodinated aryl moieties have been synthesized and evaluated for (a) binding to cartilaginous material, (b) radiocontrast characteristics, and (c) in vitro pharmacological effects.

Synthesis. A series of bis(quaternary ammonium) compounds bearing iodinated benzyl groups was prepared as shown in Scheme I. Quaternization of an α,Ω-bis(dimethylamino)alkane (**2**) with 2 equiv of 3-iodobenzyl chloride[5] (**1**, Y = 3-I) in acetone or dimethylformamide occurred readily to yield the corresponding bis(quaternary ammonium) compounds **3a–c** (see Table I). However, when more highly substituted benzyl chlorides [e.g., 2,4,5-triiodobenzyl chloride[6] (**1**), Y = 2,4,5-I] were used, these reaction conditions produced, in low yield, a mixture of mono- and bis(benzyl quaternary ammonium) salts (**4** and **3**, respectively). Use of 4 equiv (i.e., 100% excess) of the substituted benzyl chloride **1** and prolonged reaction time (~60 h) allowed preparation of bis(iodobenzyl) quaternary compounds **3e–j** although, in most instances, it was necessary to separate some monoquaternary compound **4** which was also present in the crude reaction product. This separation of **3** from **4** was accomplished readily since the monoquaternary salts **4** are soluble in

Scheme I



ethanol at room temperature whereas the poly(iodobenzyl)-bisquaternary compounds **3** are not. Changing the stoichiometry of the reactions such that the bis(tertiary amine) **2** was present in excess allowed the monoquaternary compounds **4** to be prepared as intermediates for synthesis of the unsymmetrical bis(quaternary ammonium) compounds **5**. It was necessary to exercise care in the purification of compounds in the mono series (**4**) since heating solutions of **4** sometimes led to disproportionation, producing mixtures of **2** and **3**.

We have also prepared a bis(quaternary ammonium) compound, 1,5-diiodo-2,4-bis(β-trimethylammonioethyl)benzene diiodide (**13**), in which the iodine-bearing aryl group is inserted in the carbon chain separating the charged nitrogens (Scheme II). 1,4-Bis(β-acetamidoethyl)benzene (**9**), prepared in four steps from 1,4-benzenedimethanol (**6**) as described previously,[7] was iodinated using iodine and periodic acid[8] to yield the 1,5-bisiodo derivative **10**. Hydrolysis of the amide groups

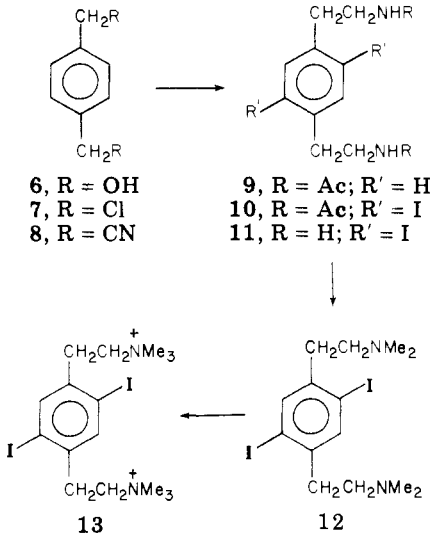
Table I. Iodobenzyl-Bis(quaternary ammonium) Compounds Prepared

$Me_2N^+(CH_2)_nN^+Me_2\ 2X^-$ (with R_1 and R_2)

No.[a]	R_1	R_2	X	n	Method of prepn	% yield	Mp, °C
3a	3-Iodobenzyl		Cl	2	A	73	218-220
3b	3-Iodobenzyl		Cl	4	A	80	219-222
3c	3-Iodobenzyl		Cl	6	A	77	223-226
3d	3-Iodobenzyl		Cl	10	A	89	236-238
3e	2,4,5-Triiodobenzyl		Cl	4	B	50	166-167
3f	2,4,5-Triiodobenzyl		Cl	6	B	29	180-183
3g	2,4,5-Triiodobenzyl		Cl	10	B	57	209-212
3h	5-Amino-2,4-diiodobenzyl		Cl	4	B	33	158-161
3i	5-Amino-2,4-diiodobenzyl		Cl	6	B	40	152-157
3j	5-Amino-2,4-diiodobenzyl		Cl	10	B	33	165-167
3k	5-Acetamido-2,4-diiodobenzyl		Cl	10	B	80	188-194
4a	5-Amino-2,4-diiodobenzyl	H		10	C[b]		
4b	3-Amino-2,4,6-triiodobenzyl	H		6	C[b]		
5a	5-Amino-2,4-diiodobenzyl	Me	Cl, I	10	C	65	187-190
5b	3-Amino-2,4,6-triiodobenzyl	Ethylacetyl	Cl, I	6	C	92	158-163

[a] All compounds (except 4a-c) were analyzed for % C, H, and N and yielded experimental values within the limits; theoretical value ±0.3%. [b] Intermediate in the synthesis of asymmetric bisquaternary compounds 5.

Scheme II



6, R = OH; 7, R = Cl; 8, R = CN

9, R = Ac; R' = H; 10, R = Ac; R' = I; 11, R = H; R' = I

13 12

of **10** under basic conditions and methylation using formic acid–formamide yielded the bis(tertiary amine) **12** which, upon treatment with methyl iodide, yielded the desired iodinated bis(quaternary ammonium) compound **13**.

Biological Studies. (a) Radiocontrast Properties. White rats, anesthetized with pentobarbital, were given injections of compounds **3k** and **3d** intravenously and subcutaneously, respectively. Three rats given **3k** intravenously (4, 6, and 7 mg/kg) showed no x-ray enhancement of cartilaginous structures, compared to preinjection x-rays, during the 1–2.5 h postinjection examination period.

Five rats given **3d** (140–320 mg/kg) subcutaneously, and examined by x-ray at intervals from 1 to 24 h after injection, exhibited no change in radiocontrast of cartilage when compared to their preinjection films.

Injections of 0.1–0.3-ml volumes of aqueous solutions of compounds **3b**, **3c**, and **3d** were made into the tail and knee joints of five dogs anesthetized with sodium pentobarbital. The solutions were made to provide the following molar concentration ranges of iodine: **3b**, 2.3–4.4 $\times 10^{-1}$ M; **3c**, 1.5–4.4 $\times 10^{-1}$ M; **3d**, 1.7–8.3 $\times 10^{-1}$ M. In several of the dogs the radiopacity of solutions, equimolar with respect to iodine, of the test compounds and Renografin was compared.

These experiments confirmed the radiopacity of concentrated solutions of the test compounds, as well as demonstrating that the test compounds and Renografin were equally radiopaque when iodine-equivalent solutions were compared. However, both the test compounds and Renografin were nearly completely absorbed from the joint spaces within 1 h, and there was no augmented or persistent radiopacity of cartilage at any time during or after the experiment.

(b) Binding to Cartilage and to Chondroitin Sulfate. Experiments with compounds **3c** and **3i**, performed by the technique of equilibrium dialysis, have been published[9] and are referred to in the Discussion.

(c) Synaptic Transmission Block. The transmission of nerve impulses from pre- to postganglionic neurons of the isolated bullfrog sympathetic ganglion was blocked by all the iodinated bisquaternary compounds, as would be expected because of their similarity to transmission-blocking drugs such as *d*-tubocurarine, hexamethonium, and decamethonium. Generally, 10^{-6} M solutions of the iodinated bisquaternary compounds produced complete block of synaptic transmission within 15 min after introduction into the recording chamber.

Discussion

In the biological studies the synaptic transmission block reveals that iodination does not interfere with the fundamental blocking activity common to bisquaternary compounds. Furthermore, the blocking potency of the iodinated compounds matches very closely the potency of *d*-tubocurarine.[10]

The absence of persistent radiocontrast properties in the in vivo experiments may be attributable to several factors. For example, in a previously published study[10] we have shown, by in vitro equilibrium dialysis techniques, that two of the test compounds have a high degree of binding to chondroitin sulfate, but the binding proceeds slowly. In that report[9] solutions of compounds **3c** (10^{-5} M) and **3i** (1.3×10^{-5} M), equilibrated for 16 h with 10 mg/ml of pure chondroitin sulfate, were bound to the extents of 6.7×10^{-7} and 9.4×10^{-7} mol/g of chondroitin sulfate, respectively. Since equilibrium was not achieved until 6–8 h in the in vitro study it is likely that, in vivo, absorption of the iodinated compound from the joint space, coupled with urinary excretion, may be rapid enough to prevent accumulation of radiopaque quantities in cartilage. The hydrophobic character of these iodinated compounds,

relative to aliphatic or noniodinated aryl bis(quaternary amines), could be a factor in the negative in vivo radiocontrast results, but it is important to note that the binding of *d*-tubocurarine dimethyl ether iodide (dTC) and decamethonium bromide (C 10) to chondroitin sulfate is also high and quantitatively greater than their binding to plasma proteins.[9] Nevertheless, if the binding of the iodinated bis(quaternary amines) to plasma proteins is similar to that of dTC and C 10, the large mass of well perfused protein binding sites in vivo could preclude attainment of high levels of the iodinated compounds at cartilaginous sites.

Conclusions

Although the iodo-bis(quaternary ammonium) salts do bind to chondroitin sulfate and cartilage, the rate of binding is too slow, relative to absorption and redistribution from joint spaces, to attain clinically useful radiopacity. Also these compounds display the synaptic blocking potency characteristic of bisquaternary compounds.

Experimental Section

Nuclear magnetic resonance spectra were determined with a Varian HA-100 spectrometer. Melting points were determined on a microscope hot stage and are uncorrected. Elemental analyses were by Heterocyclic Chemical Corp., Harrisonville, Mo. 3-Iodobenzyl chloride,[5] 2,4,5-triiodobenzyl chloride,[6] 5-amino-2,4-diiodobenzyl chloride,[6] 3-amino-2,4,6-triiodobenzyl chloride,[6] 5-acetamido-2,4-diiodobenzyl chloride,[6] and *N,N,N′,N′*-tetramethyl-1,10-decanediamine[11] were prepared as previously described; other reagents were obtained commercially.

Iodobenzyl-Bis(quaternary ammonium) Compounds. Procedure A. To a solution of 0.01 mol of the appropriate *N,N,N′,N′*-tetramethyl-α,Ω-alkanediamine in 20 ml of acetone was added 5.04 g (0.02 mol) of 3-iodobenzyl chloride.[5] The reaction mixture was stirred for 24 h; the product which had appeared as a precipitate was then removed by filtration and dissolved in a minimum volume of boiling ethanol. After the solution had cooled ether was added until the solution became cloudy. The solution was then heated gently causing crystals to form and after overnight refrigeration the product was collected and dried.

Procedure B. To a solution of 1–3 mmol of 2,4,5-triiodobenzyl chloride,[6] 5-amino-2,4-diiodobenzyl chloride,[6] or 5-acetamido-2,4-diiodobenzyl chloride[6] in 5–15 ml of dimethylformamide was added 0.25 equiv of the appropriate *N,N,N′,N′*-tetramethyl-α,Ω-alkanediamine. The reaction mixture was stirred for 60 h and then poured into 50–75 ml of ether. The resulting precipitate was removed by filtration, digested in 50 ml of boiling chloroform, again filtered, suspended in 50 ml of ethanol, and stirred for 20 min at room temperature. The crude product was collected by filtration and treated with a second 50-ml portion of ethanol as described. Finally, the product was recrystallized twice using a mixture of ethanol and ether.

Procedure C. To a solution of 1 mmol of an iodobenzyl chloride 1 in 10 ml of dimethylformamide was added 4 equiv of the appropriate *N,N,N′,N′*-tetramethyl-α,Ω-alkanediamine (**2**). The resulting solution was stirred for 48–60 h at room temperature and then poured into 75 ml of ether. The iodobenzyl monoquaternary compound which precipitated was filtered, washed with ether, and used directly. This material was dissolved in 100 ml of ethanol maintained at 50–60 °C under reflux conditions and 5 ml of methyl iodide (or 1 ml of ethyl iodoacetate) was added dropwise. After alkyl iodide addition was complete, the reaction mixture was cooled to room temperature (becoming turbid in the process) and an additional 0.5 portion of alkyl iodide was added. After an additional 3–4 h, the reaction mixture was poured into 100 ml of ether and the resulting precipitated product was removed by filtration and recrystallized from ethanol.

1,4-Bis(β-acetamidoethyl)-2,5-diiodobenzene (10). A mixture consisting of 1.18 g (4.8 mmol) of 1,4-bis(β-acetamidoethyl)benzene[7] (**9**), 1.01 g (4.4 mmol) of periodic acid dihydrate, 2.27 g (8.9 mmol) of iodine, 17 ml of acetic acid, 0.6 ml of concentrated sulfuric acid, and 3 ml of water was heated at 70–80 °C for 4 h. The reaction mixture was then poured into a dilute aqueous solution of sodium bisulfite. The resulting precipitated crude product was removed by filtration and recrystallized from ethanol to yield 1.09 g (46%) of **10**: mp 253–256 °C; NMR (F_3CCOOH) δ 8.64 (NH), 7.73 (Ar), 3.82 (CH_2N), 3.10 ($ArCH_2$), 2.45 (Ac). Anal. ($C_{14}H_{18}N_2O_4I_2$) C, H, N.

2,5-Diiodo-1,4-bis(β-dimethylaminoethyl)benzene (12). A solution of 2.95 g (5.9 mmol) of **10** in 37 ml of 40% aqueous potassium hydroxide and 95 ml of ethanol was heated under reflux for 12 h. The reaction mixture was then concentrated to remove the ethanol, resulting in formation of a yellow oil. Addition of water and cooling in an ice bath caused the oil to solidify. The solvent was removed and 20 g of formic acid (0.4 mol) and 4.5 g (55 mmol) of a 37% formaldehyde solution were added to the solid. The reaction mixture was then placed on a steam bath. After several minutes carbon dioxide evolution began and periodic removal of the reaction mixture from the steam bath was necessary to moderate the reaction. After vigorous reaction ceased, the mixture was heated on a steam bath under reflux for 8 h. Then 18 ml of 4 N hydrochloric acid was added and the reaction mixture was concentrated to near dryness under reduced pressure. To the resulting dark liquid was added 50 ml of 9 N sodium hydroxide solution causing the product to precipitate. This crude product was removed by filtration, the filtrate was extracted with benzene, and the combined crude product in benzene solution was dried over magnesium sulfate and treated with charcoal. Removal of solvent produced a yellow-white solid which was recrystallized from hexane to yield 1.35 g (67%) of **12**: mp 138–143 °C; NMR ($CDCl_3$) δ 7.63 (Ar), 2.81, 2.49, 2.31. Anal. ($C_{14}H_{22}N_2I_2$) C, H, N.

2,5-Diiodo-1,4-bis(β-trimethylammonioethyl)benzene Diiodide (13). To 0.6 g (1.2 mmol) of **12** in 50 ml of ethanol was added 7 ml (large excess) of methyl iodide. After stirring for 4 h the reaction mixture was poured into 100 ml of ether and the resulting precipitated crude product was collected by filtration. This material recrystallized from water following treatment with charcoal to yield 0.46 g (48%) of **13**, mp 300–305 °C dec. Anal. ($C_{16}H_{28}I_4N_2$) C, H, N.

Biological Studies. Radiocontrast Properties. White rats, of either sex, weighing 200–300 g were allowed water and a standard laboratory chow ad libitum. Prior to x-ray studies they were anesthetized with pentobarbital sodium (60 mg/kg ip). After control x-rays were taken in the prone position, test compounds were administered by tail vein or ip and serial x-rays taken for periods from 2 to 24 h after injection. Experiments with pentobarbital-anesthetized dogs and intraarticular injection of test compounds differed only in that the dogs were fasted overnight prior to the experiment.

Binding to Chondroitin Sulfate. Binding studies were performed by the equilibrium dialysis technique, as described in detail by Olsen et al.[9] Pure chondroitin sulfate (ICN Nutritional Biochemicals Corp.) was placed inside the dialysis sac (20 mg in 2 ml of 0.01 M phosphate-buffered 0.15 M NaCl) and known concentrations of the test compound were added to the buffered NaCl solution (10 ml) outside the sac. Dialysis with constant agitation was continued for 16 h. Samples of the solution outside the sac were then analyzed by uv spectrometry in a Cary 15 recording spectrophotometer. Samples were scanned from 360 to 200 nm and peak absorbance was at 234 nm. Binding of test compounds to the dialysis sac was also measured to permit accurate calculation of binding to chondroitin sulfate.[9]

Synaptic Transmission Block. Studies of synaptic transmission were conducted in the isolated bullfrog sympathetic ganglion according to methods previously described.[10] The preganglionic nerve trunk was stimulated supramaximally (0.1 Hz) and the postganglionic compound action potential response was recorded by extracellular electrodes on the postganglionic ramus. After control records were obtained in drug-free Ringer's solution the ganglion was transferred to Ringer's solution containing a known concentration of one of the iodinated bisquaternary test compounds. Stimulation (0.1 Hz) was continued, and measurements of the compound action potential amplitude were plotted against time and expressed as a percentage of the control amplitude.

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Synthesis and Antihypertensive Activity of Some Imidazoindole Derivatives

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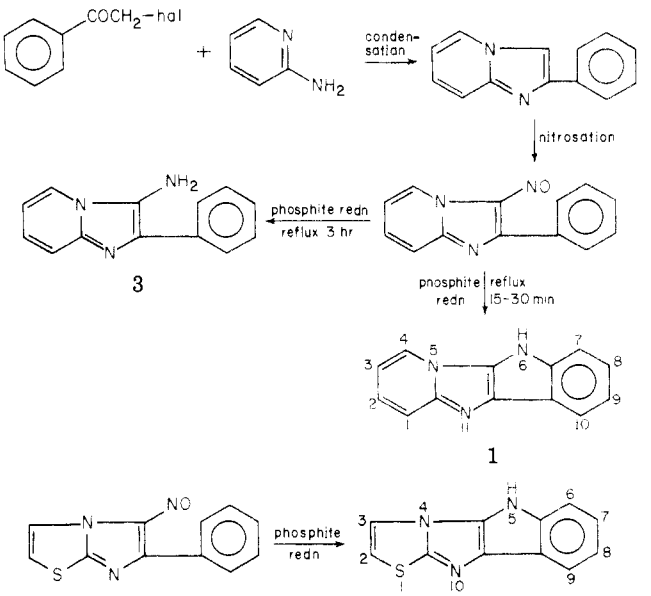
The synthesis of pyridino[1,2-*a*]imidazo[5,4-*b*]indole (1) and thiazolo[3,2-*a*]imidazo[5,4-*b*]indole (**2**) has been achieved by phosphite reduction of 3-nitroso-6-phenylimidazo[1,2-*a*]pyridine and 5-nitroso-6-phenylimidazo[2,1-*b*]thiazole. Compound 1 has shown strong antihypertensive activity in spontaneously hypertensive rats while compound **2** showed similar bioactivity both in spontaneously hypertensive rats and in normotensive dogs. A tricyclic amino derivative, 3-amino-2-phenylimidazo[1,2-*a*]pyridine, which has structural resemblance to compound 1, showed no hypotensive activity.

Some known antihypertensive agents like hydralazine, catapres, and the amidines[1-3] have the –N=CNH– group common to their structures which we believe is the basis for the bioactivity of these compounds. We wanted to know if other compounds, in which –N= of the above-mentioned bioactive group was replaced by a bridgehead nitrogen, would show some kind of bioactivity. We were also interested to ascertain the role of higher cyclic order in the bioactivity of such compounds. The objective of the following research was to synthesize some tetracyclic imidazoindole derivatives and to compare the bioactivity of these tetracyclic compounds with a tricyclic compound of similar structure.

Chemistry. The reduction of a nitroso compound by triethyl phosphite, as described by Cadogan,[4] was adopted by us for the reduction of 3-nitroso-2-phenylimidazo-[1,2-*a*]pyridine and 5-nitroso-6-phenylimidazo[2,1-*b*]-thiazole. The nitroso intermediates of pyridine and thiazole derivatives were synthesized by condensation of ω-bromoacetophenone respectively with 2-aminopyridine and with 2-aminothiazole as described by Almirante et al.[5,6] and then nitrosation of the resulting bases with sodium nitrite and acetic acid as described by LaRocca et al.[7] In the case of the pyridine derivative, the phosphite reduction of the nitroso intermediate to the tetracyclic indole derivative, pyridino[1,2-*a*]imidazo[5,4-*b*]indole (**1**), was complete with 15–30 min of refuxing. Further heating yielded gradual decomposition of compound 1 and simultaneous formation of the tricyclic amine, 3-amino-2-phenylimidazo[1,2-*a*]pyridine (**3**), which was the single product obtained after 3 h of refluxing. It could not be ascertained at this stage whether **3** was formed due to the cleavage of the indolic bond of 1 or by the triethyl phosphite reduction of the nitroso intermediate. Compound **3** is the zinc–acetic acid reduction product of 3-nitroso-2-phenylimidazo[1,2-*a*]pyridine[5] and its formation by phosphite reduction is rather unexpected. (The hypothesis that the phosphite reduction occurs via formation of a nitrene intermediate does not favor such a reduction product. We are in the process of conducting additional experiments to resolve this problem.) The phosphite reduction of the nitroso intermediate of thiazole yielded the expected tetracyclic indole derivative, thiazolo[3,2-*a*]imidazo[5,4-*b*]indole (**2**), and no further change of product or yield occurred by prolonging the reaction period (Scheme I).

Scheme I. Synthesis of Indole Derivatives



The assumed structures of **1** and **2** are consistent with their elemental analysis and ir spectra shown in the Experimental Section. In addition, the compounds show