Table III—Comparison of Direct Deproteinization Method to Extraction Method for the HPLC Determination of Procainamide and *N*-Acetylprocainamide in Plasma

Clinical Study[a]			Metabolic Study of Procainamide in Rats[b]					
Steady-State Plasma Concentrations of *N*-Acetylprocainamide, μg/ml			Procainamide Concentration, μg/ml			*N*-Acetylprocainamide Concentration, μg/ml		
Deproteinization Method (I)	Extraction Method (II)	I/II	Deproteinization Method (I)	Extraction Method (II)	I/II	Deproteinization Method (I)	Extraction Method (II)	I/II
3.27	3.33	0.982	0.720	0.762	0.945	0.672	0.776	0.866
4.10	4.20	0.976	0.833	0.897	0.929	0.610	0.617	0.987
4.08	4.09	0.998	2.23	2.06	1.08	0.904	0.970	0.932
4.39	4.33	1.01	1.67	1.68	0.994	0.877	1.06	0.827
5.65	5.82	0.971	0.698	0.706	0.948	1.09	1.15	0.948
4.32	4.52	0.956	0.281	0.351	0.801	2.27	2.22	1.02
4.49	4.58	0.980	0.338	0.310	1.09	0.397	0.411	0.966
6.95	6.89	1.01	0.737	0.744	0.991	0.820	0.823	0.996
5.54	5.50	1.01	1.34	1.33	1.01	1.17	1.13	1.04
4.34	4.40	0.986	1.02	1.02	1.00	0.726	0.767	0.947
Mean		0.988			0.979			0.953
SD		0.0186			0.082			0.066

[a] A normal subject received 500 mg of *N*-acetylprocainamide hydrochloride every 6 hr for 54 hr. [b] Rats received procainamide hydrochloride mixed in the diet of 1280 mg/kg/day for 6 months.

exogenous compounds. Plasma samples often are heparinized, and traces of heparin can be a source of interference with the drug peaks, particularly in the deproteinization techniques. This problem was observed with at least two HPLC columns differing in their polarity and under various elution systems. Unless a suitable chromatographic system is selected for a given compound, heparin probably will be a source of error in the assay of drugs. In this method, the retention time of heparin was 2.2 min and that of components of coffee or a carbonated beverage was <2 min. Previous investigators (8, 9) noticed that chemicals contained in vacutainer stoppers may interfere with extraction procedures for lidocaine and procainamide for a GLC measurement.

The application of both methods to human and rat plasma samples produced results with no statistically significant differences (paired *t* test) between the method described in this study and the specific extraction method (7) with respect to *N*-acetylprocainamide in humans and procainamide and *N*-acetylprocainamide in rats (Table III). The concentration ratio of the deproteinization method to the extraction method averaged 95.3–97.9%.

The proposed assay offers the same simplicity of the deproteinization procedure published previously (5, 7). However, the use of the alkylphenyl column with an appropriate mobile phase produces more consistent and reliable results. This procedure was tested with numerous samples obtained from subjects or animals given *N*-acetylprocainamide, procainamide, or both without any difficulties or specificity problems.

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Synthesis and Biological Evaluation of *p*-Bromospiperone as Potential Neuroleptic Drug

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Received February 19, 1980, from the **Chemistry Division, Argonne National Laboratory, Argonne, IL 60439,* and the ‡*Department of Radiology and the* §*Department of Psychiatry, University of Chicago, Chicago, IL 60637.* Accepted for publication March 18, 1980.

Abstract □ *p*-Bromospiperone was prepared from the reaction of spiperone with bromine. It was tested for dopamine receptor binding affinity *in vitro* and its ability to stimulate prolactin secretion *in vivo*. The results indicate no significant change of biological activities due to the bromination of spiperone.

Keyphrases □ Spiperone—*p*-bromo analog, synthesis and biological evaluation as a potential neuroleptic □ Neuroleptics, potential—*p*-bromospiperone, synthesis and biological evaluation □ Prolactin secretion—stimulation by *p*-bromospiperone

Spiperone (I) is one of the most potent neuroleptic drugs in clinical use. This drugs binds extensively to the dopamine receptors in the brains of rats and mice (1, 2). Attempts have been made to study the receptor–drug inter-

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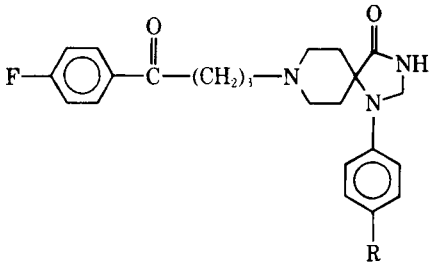

I: R = H
II: R = Br

action by using suitable gamma or positron radionuclides. Since the usual techniques for incorporation of fluorine 18 into an aromatic ring result in compounds of low specific activity, the use of radiobromines (bromine 77, 76, and 75) was investigated. Substitution of bromine for hydrogen at one aromatic ring may represent a minimum structural alteration without much of a change in pharmacological activity. As an essential step in this procedure, the nonradioactive brominated analog of spiperone was prepared to determine the effect of this substitution on its biological utility as a neuroleptic compound.

This report describes the synthesis and biological testing of the brominated spiperone (II).

EXPERIMENTAL[1]

Synthesis—To a solution of spiperone (80 mg, 0.2 mmole) in methylene chloride (5 ml) was added dropwise bromine (35 mg, 0.22 mmole) in carbon tetrachloride (5 ml). The solution was stirred at 0° for 30 min. At the end of the reaction, an equal volume of an aqueous solution of 5% NH_4OH and 1% $Na_2S_2O_3$ was added. The organic phase was separated and dried over anhydrous sodium sulfate. Removal of the solvent gave a white powder. Recrystallization from a mixture of methylene chloride and carbon tetrachloride gave pure II (70 mg, 74%), mp 204–205°.

Anal.—Calc. for $C_{23}H_{25}BrFN_3O_2$: C, 58.23; H, 5.31; Br, 16.85. Found: C, 58.01; H, 5.36; Br, 16.52.

The mass spectrum of the compound showed molecular ions at *m/e* 474 (M) and 476 (M + 2) of equal intensity and other fragmentation peaks at 123, 165, 322, 324, 336, and 338; NMR ($CDCl_3$): δ 1.5–2.2 (m, 4H), 2.3–3.2 (m, 10H), 4.66 (s, 2H), 6.68 (d, 2H *ortho* to N, J = 9 Hz), 6.9–7.5 (m, 4H), 7.97 (q, 2H *ortho* to carbonyl group), and 8.89 (s, 1H); IR (KBr): ν_{max} 814, 839, 1230, 1387, 1507, 1606, and 1717 cm^{-1}; TLC [reversed-phase silica gel[2] with methanol–water (80:20)]: R_f 0.18 for II and 0.23 for I.

Receptor Binding Assay—The ability of bromospiperone to displace [^{3}H]spiperone from its specific binding sites in calf caudate and anterior pituitary homogenates was tested by methods described previously (3). The tissues were homogenized in cold 50 m*M* tris(hydroxymethyl)aminomethane hydrochloride buffer (pH 7.7) and centrifuged twice at 50,000×*g* for 10 min, with rehomogenization of the intermediate pellet in the same buffer. The final pellet was resuspended in a cold incubation medium containing 50 m*M* tris(hydroxymethyl)aminomethane hydrochloride, 10 m*M* pargyline hydrochloride, 0.1% ascorbic acid, 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, and 1 m*M* $MgCl_2$ (pH 7.1) at 25°.

The final concentration of [^{3}H]spiperone was 0.25 n*M* for pituitary and 0.13 n*M* for caudate. Each tube contained ~0.4 mg of protein and was incubated at 37° for 10 (pituitary) or 15 (caudate) min. The assay was terminated by rapid filtration through glass fiber filters[3]. The filters were washed with three 3-ml portions of cold 50 m*M* tris(hydroxymethyl)-aminomethane hydrochloride buffer (pH 7.7), and the radioactivity trapped by the filters was counted with an efficiency of 40–45%. Nonspecific binding was obtained by displacement using 1 μ*M* *d*-butaclamol (3). Specific binding was defined as the difference between the total and the nonspecific binding and ranged from 40 to 50% of the total binding.

Plasma Prolactin Levels—Male Sprague–Dawley rats (175–200 g) were housed six per cage in a temperature- (26°) and humidity- (60%) regulated room with a 12-hr light–dark cycle. The animals had free access to rat chow[4] and water at all times. Rats were decapitated 30 min following the intraperitoneal injection of a test compound or saline. Trunk blood was collected. Serum then was separated and stored at −20° until it was assayed for prolactin by the double-antibody radioimmunoassay as described previously (4).

Table I—IC_{50} Values[a] for Binding of [^{3}H]Spiperone to Calf Anterior Pituitary and Caudate

Drug	IC_{50}, n*M*	
	Caudate	Anterior Pituitary
Spiperone	2.6	2.2
p-Bromospiperone	2.8	3.2

[a] The IC_{50} values are concentrations for 50% reduction in binding of [^{3}H]spiperone.

Table II—Effect of a Low Dose of Spiperone or Its Brominated Derivative on Serum Prolactin in Male Rats

Group	Dose, mg/kg	Prolactin Concentration[a], ng/ml	Comparison	*p*
1 Saline	—	2.0 ± 1.0	—	—
2 Spiperone	0.01	22.0 ± 5.9	1–2	<0.01
3 Bromospiperone	0.01	30.2 ± 8.4	1–3	<0.05
			2–3	NS

[a] Mean serum prolactin level ± *SEM* for a group of five rats. The significance of the difference was assessed using the Student *t* test.

RESULTS AND DISCUSSION

Chemistry—*p*-Bromospiperone (II) was synthesized by direct bromination of spiperone with bromine. Structural identification of II was obtained from various spectral evidence. The mass spectrum of II showed the molecular ions at *m/e* 474 (M) and 476 (M + 2) of approximately equal intensity, characteristic of the presence of a bromine atom. The brominated product was assigned a *para*-position based on the fact that the aniline-type structure favors *ortho*- or *para*-substitution in the electrophilic reaction because of the electron-donating character of nitrogen. In this case, the steric interference would render *ortho*-substitution less favorable. In addition, the NMR spectrum showed a characteristic doublet (δ 6.68) for two protons *ortho* to nitrogen.

The IR spectrum of II did not show much difference from that of I. However, careful examination revealed some information as to the structural identity of the compound. For example, the disappearance of the peak at 755 cm^{-1} is characteristic of monosubstitution due to a C–H bending vibration. This disappearance was accompanied by the appearance of the peak at 814 cm^{-1}, which is indicative of 1,4-disubstitution due to the bromination (5). The peak at 839 cm^{-1}, present in both I and II, probably is due to the CH bending from the other fluorine-substituted aromatic ring.

The similarities in the chemical behavior of spiperone and *p*-bromospiperone can be demonstrated by TLC. TLC on silica gel in several solvent systems (acetone, methanol, methylene chloride, and carbon tetrachloride–methanol) did not separate I and II. However, the separation could be accomplished by reversed-phase TLC[2] with methanol–water (80:20) as the solvent.

Biological Testing—The IC_{50} values determined by the binding assay are shown in Table I. Spiperone and *p*-bromospiperone were essentially equipotent in displacing [^{3}H]spiperone from specific binding sites in both calf caudate and pituitary. This finding indicates that bromination does not interfere with the binding of the spiperone nucleus to dopamine receptors *in vitro*.

Dopamine receptor blockers stimulate prolactin secretion *in vivo* be-

[1] Melting points were determined with an Electrothermal melting-point apparatus and are uncorrected. NMR spectra were obtained with a Varian A-60 spectrometer using tetramethylsilane as the internal standard and deuterochloroform as the solvent. IR spectra were recorded on a Beckman IR 12 spectrophotometer. TLC was performed on silica gel with a fluorescence indicator (Eastman) on plastic sheets or on reversed-phase silica gel with a fluorescence indicator (Whatman KC_{18}); visualization was with either UV light or iodine vapor. The mass spectrum was obtained with a Bendix time-of-flight mass spectrometer. Elemental analyses were performed by Galbraith Laboratory, Knoxville, Tenn. [^{3}H]Spiperone was obtained from New England Nuclear with a specific activity of 25.64 Ci/mmole. Spiperone was a gift of Janssen Pharmaceuticals, Belgium, and Dr. P. Seeman, Toronto, Canada, and *d*-butaclamol was a gift of Ayerst Laboratories.

[2] Whatman KC_{18} silica gel.

[3] Whatman GF/B.

[4] Purina.

cause they interfere with tonic dopaminergic inhibition of prolactin release (6). Previous work showed that spiperone, one of the most potent dopamine receptor antagonists, elevates plasma prolactin levels in rats (3). In the present study, prolactin levels were elevated significantly 30 min after the administration of a low dose of this neuroleptic to male rats (Table II). As shown in Table II, the same low dose of *p*-bromospiperone resulted in a comparable rise in serum prolactin levels. These results indicate that the bromination of the spiperone molecule does not significantly affect its ability to act as a dopamine receptor blocker in pituitary receptors *in vivo*, a finding consistent with results from the *in vitro* binding study. This brominated analog thus has potential as a neuroleptic drug. In addition, the radiobrominated compound may prove to be an extremely useful pharmacological tool for dopamine receptor studies in humans using positron emission tomography.

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New Compounds: N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine and Analogs as Potential Iron-Chelating Drugs

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Abstract □ N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine was synthesized and evaluated as an iron-chelating drug. Homologs were prepared and evaluated together with a series of *N,N'*-bis(2,3-dihydroxybenzoyl)-α,ω-diaminoalkanes. Analogous 2-hydroxybenzoyl compounds also were synthesized and evaluated.

Keyphrases □ N^1,N^8-Bis(2,3-dihydroxybenzoyl)spermidine—synthesis and evaluation as iron-chelating drug □ Chelating agents—N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine and analogs, synthesis and evaluation as iron-sequestering agents

An orally effective, iron-chelating drug is needed to treat patients with Cooley's anemia. Patients suffering from this genetic disease must be transfused every 2–4 weeks. Since the body lacks a physiological means of excreting the iron present in transfused erythrocytes, these patients develop a secondary iron overload, which leads to damage of vital organs and eventually to death. It was suggested that 2,3-dihydroxybenzoic acid might be an orally effective, iron-chelating drug (1). Although subsequent clinical evaluation confirmed this report (2–4), it was not possible to maintain patients in iron balance.

A chelating agent with a greater affinity for iron presumably would be more effective. Several iron-sequestering agents produced by microbes are conjugates of 2,3-dihydroxybenzoic acid (5–9). These compounds all possess affinities for iron that are many orders of magnitude greater than the affinity of the parent compound. One of these compounds, N^1,N^8-bis(2,3-dihydroxybenzoyl)spermidine (8), removes iron from the ferritin of cultured Chang cells and from transferrin, the principal iron-binding protein of serum (10).

The synthesis of N^1,N^8-bis(2,3-dihydroxybenzoyl)-spermidine hydrochloride (XVII) together with a series of simpler analogs is presented in this report. Preliminary evidence suggests that this type of compound may be useful in iron-chelation therapy whereas the corresponding 2-hydroxybenzoyl derivatives are inactive. The *in vitro* and *in vivo* evaluation of these compounds will be presented later.

DISCUSSION

Prior to the synthesis of XVII, a series of simpler analogs, *i.e.*, *N,N'*-bis(2,3-dihydroxybenzoyl)-α,ω-diaminoalkanes, was prepared. Like the spermidine derivative, these compounds would be expected to bind iron strongly, their affinities for iron and perhaps their biological activities being related to the separation of the 2,3-dihydroxybenzoyl moieties. The compounds were obtained readily in a 40–50% yield *via* the reaction of 2,3-dioxosulfinylbenzoyl chloride (11) with the appropriate α,ω-diaminoalkane in tetrahydrofuran (Procedure A). Since the goal of this program was to obtain enough pure material for biological testing, no attempt was made to optimize the yield of this or subsequent reactions.

All compounds were obtained as solids which were purified by recrystallization. The ethane (I) and propane (II) derivatives were somewhat unique in that they were crystallized from aqueous methanol as their hydrates. Compound I was obtained as a monohydrate, with the water of crystallization being lost upon drying under vacuum at 78° for 4 hr. Compound II was obtained from aqueous methanol as the dihydrate. Drying caused the loss of both moles of water, as evidenced by elemental analysis.

The identity of I, II, and V was confirmed *via* independent synthesis. Two moles of 2,3-diacetoxybenzoic acid were condensed with ethylenediamine, 1,3-diaminopropane, or 1,6-diaminohexane *via* activation with *N,N'*-dicyclohexylcarbodiimide (Procedure B). Subsequent hydrolysis of the protecting groups yielded products (40%) identical to those obtained by Procedure A. Compound II also was prepared by fusing methyl 2,3-dihydroxybenzoate and 1,3-diaminopropane at 130° under nitrogen (Procedure C). The latter procedure was used to prepare an analogous series of *N,N'*-bis(2-hydroxybenzoyl)-α,ω-diaminoalkanes (X–XIV).

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