Synthesis and Preliminary Pharmacology of 2-Aminophenol Derivatives

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A number of 2-aminophenol derivatives have been prepared which, upon gross pharmacologic screening, were found to stimulate the central nervous system, while several compounds exhibited possible psychosomimetic activity.

In a recent publication, one of the authors (1) has proposed that a single receptor site may be involved in certain actions characteristic of the reserpine and phenothiazine-type depressants. Also, in the same paper, the structural similarities of reserpine and chlorpromazine were pointed out as additional evidence for a unitary concept of receptor site activity. Subsequently, a series of 2-aminobenzenethiol agents, which incorporated these structural requirements, were prepared, subjected to gross pharmacologic screening, and shown to possess analeptic activity within the central nervous system (2).

Because an oxygen atom is often bioisosteric with a sulfur atom in a similar series of compounds, several derivatives of 2-aminophenol were prepared to compare the relative toxicity and efficacy of 2-aminophenol compounds with those of 2-aminobenzenethiol derivatives which had previously been observed. It was presumed that the 2-aminophenol compounds, which possessed the required structural relationships of the latter agents, would follow a similar general pharmacologic pattern but with less toxicity and central nervous system stimulation.

The benzenesulfonamides (II) of *o*-anisidine and *o*-phenetidine and the corresponding *N*-methyl derivatives (III) were prepared by a procedure reported by Nieforth (1) and were hydrolyzed (IV) in hydrochloric or phosphoric acid. Preparation of the *o*-alkoxy-*N*-methyl-*N*-dialkylaminoalkylaniline (V) derivatives was carried out by two modifications of a procedure reported by Huttrer (3). These compounds were screened for gross pharmacologic activity using the Hippocratic screening technique (4).

EXPERIMENTAL

Melting points were taken on a Thomas melting point apparatus and corrected. Microanalyses were carried out by Drs. G. Weiler and F. B. Strauss, Oxford, England, and in this laboratory.

N-Benzenesulfonyl-o-alkoxyanilide (II).—Pyridine (0.29 mole) and the *o*-alkoxyaniline (0.29 mole) were dissolved in 100 ml. of ether. Benzenesulfonyl chloride (0.29 mole) was added dropwise to the solution with stirring at room temperature. After the addition was complete, the mixture was stirred for 30 min. The ether was removed, and the remaining solid dissolved in 10% sodium hydroxide by heating to 70°. The mixture then was acidified with 10% HCl and the resulting solution extracted with benzene. The benzene was removed and the solid recrystallized (5, 6).

N - Methyl - N - benzenesulfonyl - o - alkoxyanilines (III).—*N*-Benzenesulfonyl-*o*-alkoxyaniline (0.24 mole) was dissolved in 2 L. of a 50% ethanol/water solution containing sodium hydroxide (0.24 mole). Methyl iodide (0.24 mole) was added dropwise at room temperature with stirring and the mixture allowed to stand for 24 hr. The solution then was concentrated to a volume of 500 ml. The immiscible oil which separated from the mixture was extracted with several portions of ether. Ligroin (65–110°) was added to the ethereal solution until crystallization occurred (7). *N*-Methyl-*N*-benzenesulfonyl-*o*-phenetidine.

Anal.—Calcd. for N, 4.81%. Found: 4.70%.

Hydrolysis of N-Methyl-N-benzenesulfonyl-o-anisidine (IV).—The sulfonamide (0.2 mole) was added to 500 ml. of a 25% hydrochloric acid solution and heated under reflux for 6 hr. The mixture was cooled, made basic with 10% sodium hydroxide, and extracted with ether. The ether was removed and the oil distilled, b.p. 107°/12 mm. (8).

Hydrolysis of N-Methyl-N-benzenesulfonyl-o-phenetidine (IV).—The sulfonamide (0.3 mole) was placed in twice its weight of 85% phosphoric acid and the mixture heated at 140–160° with stirring for 12 hr. The reaction mixture was made basic with sodium hydroxide and extracted while hot with toluene. The toluene was removed and the oil distilled, b.p. 116–118°/18 mm. (9).

N - Methyl - N - dialkylaminoalkyl - o - alkoxyanilines (V).—*Procedure A.*—In a three-necked 250-ml. flask fitted with a stirrer, dropping funnel, and reflux condenser (calcium chloride tube) was placed 100 ml. of toluene and sodium amide (0.4 mole). The flask was placed in an oil bath, heated to 98–102°, and *N*-methyl-*o*-alkoxyaniline (0.1 mole) added dropwise. After 18 hr., the dialkylaminoalkyl chloride hydrochloride (0.1 mole) was added and the mixture heated at 100° for 24 hr. The mixture then was cooled to room temperature; the liquid was decanted from the solid and 10–15 ml. of ethanol added to the solid to destroy the excess sodium

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amide. The ethanolic mixture was combined with the toluene mixture and the solvents removed. The residue was treated with a saturated solution of potassium carbonate. This mixture was extracted with several portions of ether, and the combined ether extracts were dried over anhydrous sodium sulfate for 12 hr. The ether was removed and the oil distilled under reduced pressure.

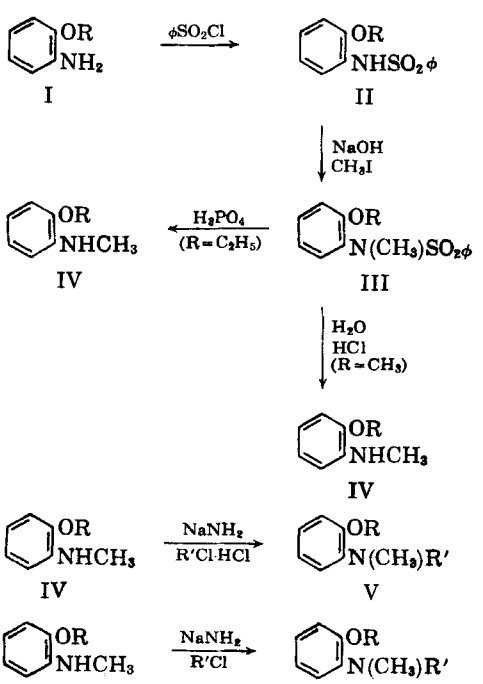
Procedure B.—*N*-Methyl-*o*-alkoxyaniline (0.05 mole) and sodium amide (0.07 mole) were added to 100 ml. of toluene in a three-necked 250-ml. flask fitted with a stopper, stirrer, and reflux condenser. This mixture was heated at reflux for 24 hr. at 115°. The mixture was cooled to room temperature and placed in an ice-salt bath. The dialkylaminoalkylchloride hydrochloride was placed in a mortar and triturated with sodium hydroxide pellets. The free base (0.05 mole) was added to the reaction vessel immediately with stirring. After 24 hr., the temperature was raised to 70°, and the mixture was stirred for 1 hr. Upon cooling to room temperature, the toluene was removed, a saturated solution of potassium carbonate added to the residue, and the mixture extracted with ether. The ether solution was dried over anhydrous sodium sulfate for 12 hr., concentrated, and the oil distilled. (See Scheme I.)

Fumarate Salts

The purified amine was dissolved in anhydrous ether and a saturated ethereal solution of fumaric acid added. Upon standing, the fumarate salt crystallized and was filtered off. Recrystallization solvents are given in Table I.

Pharmacology

A preliminary gross observational screen was performed on compounds *1–12* identified in Table I. The test animals used were both male and female nonfasted intact, unanesthetized, albino rats of the



Scheme I

Table I.—Physical Properties

Compd. No.	R	R′	B.p./mm.	% [a] Yield	Refract. Index	PR.	M.p., [b] (salt)	N [c] Calcd. %	N [c] Found	Recryst. [c] Solvents
1	CH_3	$CH(CH_3)CH_2N(CH_3)_2$	146–149/16	47	n_D^{28} 1.5223	A	173–174	9.49	9.75	iPro-EA
2	CH_3	$CH_2CH_2N(CH_3)_2$	169–170/15	46	n_D^{25} 1.5273	A	103–104	8.64	8.58	iPro-PE(20–40)
3	CH_3	$CH_2CH_2CH_2N(CH_3)_2$	149–152/13	63	n_D^{24} 1.5223	A	117–118	8.28	8.33	iPro-PE(20–40)
4	CH_3	$CH_2CH(CH_3)CH_2N(CH_3)_2$	152/15	43	n_D^{25} 1.5154	A	125–126	7.95	7.98	iPro-PE(20–40)
5	CH_3	$CH_2CH_2CH_2N$⟨⟩	196–198/17	42	n_D^{28} 1.5212	A	99–100	7.40	7.37	iPro-PE(20–40)
6	CH_3	$CH_2CH_2CH_2N$⟨⟩NCH_3	206–208/17	45	n_D^{28} 1.5286	A	193–194	10.68	10.35	iPro-PE(60–90)
7	C_2H_5	$CH(CH_3)CH_2N(CH_3)_2$	137–139/15	76	n_D^{32} 1.5190	B	160–161	22.93	22.46 [d]	iPro-EA
8	C_2H_5	$CH_2CH_2N(CH_3)_2$	136–137/15	45	n_D^{28} 1.5212	A	91–92	8.28	7.96	iPro-*n*-hexane
9	C_2H_5	$CH_2CH_2CH_2N(CH_3)_2$	158–162/18	51	n_D^{27} 1.5158	A	111–112	7.95	7.87	iPro-*n*-hexane
10	C_2H_5	$CH_2CH(CH_3)CH_2N(CH_3)_2$	142–144/14	76	n_D^{25} 1.5109	B	83–84	7.64	7.55	*n*-Bu-PE(65–110)
11	C_2H_5	$CH_2CH_2CH_2N$⟨⟩	190/14	55	n_D^{24} 1.5298	B	116–117	7.14	6.89	*n*-Bu
12	C_2H_5	$CH_2CH_2CH_2N$⟨⟩NCH_3	192–194/15	68	n_D^{23} 1.5305	B	200–201	10.31	10.30	*n*-Bu

[a] Per cent yields of only one synthesis. [b] Salts are fumarates, except *1* and *7*, which are dihydrochlorides. [c] Analyses are of the salts. [d] Analyzed for per cent chloride. [e] iPro = isopropanol; PE = petroleum ether; EA = ethyl acetate; *n*-Bu = *n*-butanol.

Wistar strain. Qualitative and semiquantitative symptomatology was carefully observed using a standardized work sheet (4). Injections were made intraperitoneally over a wide range of logarithmically spaced doses, ranging from inactive to lethal concentrations. Promazine hydrochloride, promazine fumarate (m.p. 120–122°) (10), and fumaric acid were used as chemical and pharmacological prototypes for control purposes. All dosages were calculated as milligrams per kilogram of free base using a constant injection volume (5 ml./Kg.) and aqueous 0.25% agar solution as the vehicle.

RESULTS AND DISCUSSION

A characteristic phenothiazine dose–response pattern was observed using the hydrochloride and fumarate salts of the promazine base. Essentially, the qualitative pharmacologic effects of the two promazine salts were the same, although the fumarate may add to the general symptomatology and toxicology, inasmuch as fumaric acid is not a completely innocuous substance. A dose of 10 mg./Kg. of fumaric acid showed signs of hepatotoxicity, tremors, and hypothermia. Larger doses of fumaric acid produced extreme hypothermia, decrease in motor activity, diuresis, and gross evidence of severe hepatotoxicity. Promazine fumarate, at 100 mg./Kg., showed a lethality more acute than the hydrochloride salt. The observed progressive symptomatology of promazine hydrochloride, at 100 mg./Kg., was: a prompt decrease in motor activity, mydriasis refractory to light, ataxia, loss of righting reflex, decrease in muscular strength, splaying out of the hind feet, decrease in respiratory rate with some compensatory increase in depth, a slight burst of clonic convulsions, enophthalmos, pseudoblepharoptosis, skin plasticity, and extreme hypothermia. Lower doses of both salts showed a decrease in motor activity, mydriasis, enophthalmos, skin plasticity, passivity, and a marked degree of hypothermia. In all the animals receiving the lethal dosage of 316 mg./Kg. of promazine, death occurred within 6 min. after intraperitoneal injection. In each case, there was only the slightest indication of a convulsive state, and this was noted after the onset of severe dyspnea. Oxygen lack resulted in cyanosis, anoxic convulsions, and death.

The 12 compounds studied were qualitatively quite similar to one another pharmacologically, although there were some exceptions that will be discussed. None of the agents could be classified as promazine-like. While the lethal dose ranges for the promazine hydrochloride, the fumarate, and this series were quite similar, the modes of action for causing death were significantly different. All of the *o*-alkoxyaniline series caused severe intermittent tonic and clonic convulsions, which eventually terminated in asphyxiant state and death. In contrast, the promazine salts caused ataxia, general central nervous system depression, dyspnea, cyanosis terminating with transient anoxia-induced convulsions, and death. Briefly, the *o*-alkoxyanilines were prompt and direct convulsants, whereas the promazine compounds were progressive central nervous system depressants.

The characteristic marked hypothermia produced by promazine was not observed in this series of compounds, except as secondary effects after doses causing convulsions. In these cases, the hypothermia was attributed to the toxic manifestations and fatigue associated with the convulsive state. The typical passivity during provocation caused by the promazine salts was not seen with the experimental agents, since the observed passivity with these agents was uniformly associated with post-convulsive states where fatigue closely mimicked passivity.

An example of the effects by a member of this series can be shown with compound *1*. At 100 mg./Kg., death by respiratory arrest occurred within 15 min. after injection; prior to death, there were intermittent tonic and clonic convulsions, ataxia, and cyanosis. Autopsy showed the heart beating normally, although abnormally engorged with blood. At 31.6 mg./Kg., there was observed an initial premonitory indication of convulsion; after the convulsions, the animal was hyper-reactive and quite aggressive. Some equivocal decrease in motor activity was also noted as well as body tremors, enophthalmos, abdominal griping, and hypothermia reaching a maximum fall of 2.1° 4 hr. after injection. The gross autopsy revealed possible hepatotoxicity. At 10 mg./Kg., there was no significant drop in rectal temperature. Fine body tremors were noted associated with equivocal hyperemia. On the second day, repetitive chewing motions were seen. The gross autopsy revealed numerous adhesions and possible nephro- and hepatotoxicity. Compounds qualitatively like *1* were *3*, *4*, *6*, *8*, *9*, and *12*. Animals receiving these agents routinely showed both tonic and clonic convulsion and 7 days after injection displayed either little or no loss in body weight, an indication of a relative lack of delayed toxicity.

Compound *6* showed a different and interesting dose–response pattern. At 316 mg./Kg., there was abdominal griping, intermittent clonic convulsions with frothing at the mouth, tremors, mydriasis, skin plasticity, ataxia, loss of righting reflex, and hypothermia. Eight hours after injection, the animal showed a noticeable increase in motor activity; after 4 days, the animal was still very fearful, would not remain on the inverted screen, and showed indications of disorientation. Two days after injection, the animal became increasingly passive and after 7 days had lost 32 Gm. of body weight. At 100 mg./Kg., initial griping was noted, and the dyspneic animals assumed odd positions and had a tendency to remain in one of the corners of the observational rink. After 4 hr., the animals became very active and would not remain in the observation rink, indicative of biphasic activity. The gross autopsy revealed nephro- and hepatotoxicity. At 31.6 mg./Kg., there were little or no acute effects other than reduced motor activity, equivocal hypothermia, and some micturition. There were some indications of a change in personality from a more aggressive to a more passive state; this passivity and reduced motor activity was sustained until autopsy, at which time the animal had lost 25–32 Gm. of body weight. The gross autopsy showed adhesions and nephro- and hepatotoxicity. At 10 mg./Kg., within 1 hr. there was a slight decrease in motor activity, which was maintained for 4 days concurrent with body tremors and piloerection. After 6 hr., there were indications of increasing passivity. The autopsy showed possible nephrotoxicity, the animal had lost 32 Gm. of body weight. Compound *6* displayed only clonic convulsions and some delayed disorientation and

aggressive reaction patterns that in time gave way to increased passivity and malaise; by the time of gross autopsy, the animals had lost a considerable amount of body weight and showed definite signs of nephro- and hepatotoxicity. Compounds *5* and *9* were similar to *6*.

Compound *7* showed some symptomatology of disorientation at 31.6 and 10 mg./Kg. The animals were very jittery with increased motor activity and mydriasis. This compound may have some psychosomimetic activity. At high doses, it produced clonic convulsions preceded by Straub tail erection. It has a pattern of activity similar in many respects to pentylenetetrazol. Compound *10* showed considerable resemblance to *7* with regard to hyperreflexia, but also could be considered to be somewhat like *1*.

This gross pharmacologic screen has established the general lack of promazine-like activity in the *1–12* series of compounds.

REFERENCES

(1) Nieforth, K. A., This Journal, **52**, 1136(1963).
(2) Nieforth, K. A., and Robichaud, R. C., *ibid.*, **53**, 529(1964).
(3) Huttrer, C. P., *et al.*, *J. Am. Chem. Soc.*, **68**, 1999 (1946).
(4) Malone, M. H., and Robichaud, R. C., *Lloydia*, **25**, 320(1962).
(5) Diepolder, E., *Ber. Deut. Chem. Ges.*, **32**, 3517(1899).
(6) Shriner, R. L., Fuson, R. C., and Curtin, D. Y., "The Systematic Identification of Organic Compounds," 4th ed., John Wiley & Sons, Inc., New York, N. Y., 1956, p. 290.
(7) Diepolder, E., *Ber. Deut. Chem. Ges.*, **32**, 3518 (1899).
(8) *Ibid.*, **32**, 3515(1899).
(9) Hjort, A. M., *et al.*, *J. Pharmacol. Exptl. Therap.*, **55**, 152(1935).
(10) French pat. 1,186,821 (September 2, 1959) Societe des Usines Chimiques Rhone-Poulenc; through *Chem. Abstr.*, **56**, 3494(1962).

Complex Interaction of Starches with Certain Drug Pharmaceuticals

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A study has been made of the complexing behavior of potato and arrowroot starch with a number of pharmaceutical compounds. By means of the solubility method of analysis, it has been found that the starches form complexes in solution with benzoic acid, salicylic acid, *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, ethyl *p*-aminobenzoate, and propyl *p*-hydroxybenzoate. No evidence of complex formation was detected between the starches and caffeine. It is postulated that attractive forces and inclusion formation are responsible for the interaction observed.

Starch, one of the most widely distributed naturally occurring organic compounds, is mainly composed of polysaccharides of the glucan type. The view now is accepted generally that the majority of starches contain molecules which can be classified according to one of two quite different structural patterns (1). One type is a linear polymer, and the polymeric bonds are substantially 1-4 α-glucosidic linkages. This linear fraction is called amylose. The other type has a branched structure due to connection of another chain of glucose units to the primary chain by a 1-6 glucosidic linkage. This nonlinear fraction is called amylopectin. When the spatial distribution of the chain of glucose units in amylose is considered, it is found to assume a spiral form. Support for this helical concept comes from X-ray, ultracentrifuge, viscometric, and other studies of the amylose–iodine complex. Results indicate that the period of each spiral is six glucose units, and in the amylose–iodine complex, it has been shown that the iodine atoms are situated in the core of helically oriented amylose molecules (2). Amylopectin, most likely, has a large and ramified structure with short linear branches. This concept of structure is supported by the results of studies employing enzymatic degradation (3).

Separation of amylose and amylopectin can be made by adding to a starch dispersion certain agents such as butanol, nitropropane, nitrobenzene, and thymol, which form complexes with amylose and cause it to precipitate in semicrystalline form. The amylose complex is collected by centrifugation, and the amylose is regenerated by adding hot water or ethanol. Amylopectin then is isolated from the mother liquor by precipitation with alcohol or by freeze-drying (4–6).

Starch is used in many pharmaceutical preparations. It is used extensively as an absorbent in "weeping" types of dermatosis. It is applied

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