# A New Class of 5-Hydroxytryptamine Antagonists

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A series of benzyl and substituted benzyl quaternary salts of N,N-dimethyl- and -diethyltryptamine and of N,N-dimethyl-5-hydroxytryptamine (bufotenine) were prepared and tested against the stimulant actions of 5-hydroxytryptamine (5-HT) on peripheral nervous receptors of the cat and dog (autononic ganglia, chemoreceptors) and on smooth muscles. *meta*-Substituted benzyl quaternary derivatives of N,N-dimethyltryptamine and bufotenine exhibited marked blocking potency against 5-HT on the nervous receptors. The most potent member of the series was N-(*m*-chlorobenzyl)-N,N-dimethyl-5-hydroxy-3-indolylethylammonium bromide. The blocking actions were predominant against the ganglionic and cardiorespiratory reflex actions of 5-HT. Cholinergic stimulation of autonomic ganglia was unaffected by the more potent members of the series in the dose range used. Compounds exhibiting marked antagonistic actions against neural effects of 5-HT were fairly inactive on smooth muscle preparations. In this respect members of this series were entirely different from other, well known "musculotropic" antagonists of 5-HT (*i.e.*, LSD, chlorpromazine, BAS, and cyporheptadine). As side effect a cholinergic type of ganglion stimulation was observed with a few compounds of the series.

Several antagonists of 5-hydroxytryptamine (5-HT) are pharmacologically potent on smooth muscle preparations, but are ineffective or very weak on nervous preparations.[2] The aim of the present investigation was to find new antagonists which may act primarily on nervous receptors sensitive to 5-HT. Such receptors can be found in different autonomic ganglia[3-5] and at the endings of visceral afferent nerves.[6,7] These types of agents besides their theoretical importance, may also have therapeutic value in disease and syndromes where 5-HT might be of pathological significance, *e.g.*, chromaffin tumors, abnormal cardiovascular and cardiorespiratory reflexes, certain types of visceral pain, vascular headaches, such as migraine, and allergic diseases. Only a few of these pathological manifestations, many of which were expected in the past to be amenable to 5-HT antagonist therapy, responded to known agents which block 5-HT, *e.g.*, methysergid, cyproheptadine, BAS, and chlorpromazine. The failures with these agents might have been due to their incomplete spectrum of action against 5-HT. Therefore, it was felt that by devising a new class of 5-HT antagonists two criteria must be satisfied: (1) The molecules preferably should have a close structural resemblance to 5-HT (such structural similarity is desirable in the present stage of limited knowledge concerning pharmacological antagonisms and structure–activity relationships). (2) The new compounds should have a markedly different spectrum of action as compared to known antagonists of 5-HT covering those pharmacological actions of 5-HT which are not influenced by commonly known 5-HT antagonists.

Utilizing the previous observations of Gyermek and Nador[8,9] that substituted aralkyl quaternary ammonium compounds have high affinity for ganglionic cholinergic nervous receptors, this pharmacologically potent chemical group was built into indolealkylamines, *e.g.*, N,N-dimethyl- and -diethyltryptamine and N,N-dimethyl-5-hydroxytryptamine (bufotenine), which themselves exhibit 5-HT like and also mild 5-HT blocking properties.[5,10]

## Experimental[11]

The compounds were prepared by quaternization of N,N-dialkylaminoethyl-3-indoles with the corresponding aralkyl halides in absolute acetone or ethanol. Quaternization took place usually at room temperature and was complete in most cases within a few hours. The quaternary salts were washed with the solvents and recrystallized.

**Example. N-(Benzyl)-N,N-dimethyl-β-(3-indolylethyl)ammonium Bromide.**—N,N-Dimethyltryptamine (200 mg.) was dissolved in 4 ml. of absolute acetone and 210 mg. (20% excess) of benzyl bromide was added dropwise. A white precipitate formed immediately. By adding an additional 2 ml. of absolute ether, a gummy quaternary salt precipitated. After discarding the supernatant and taking up the residue in a few ml. of absolute ether, a white solid formed. The quaternary salt was recrystallized from methanol–ether. The rest of the compounds were prepared essentially by a similar procedure. Data of the chemical structure, preparation, and physical constants of the compounds are presented in Table I.

**Pharmacological. (1) Cat Inferior Mesenteric Ganglion Preparation.**—Postganglionic action potentials of the hypogastric nerves were recorded as described earlier.[4] 5-HT and its blocking agents were administered intraaortically through the cannulated inferior mesenteric artery. Minimal effective doses of the blocking agents inhibiting the stimulant action of 2–20 γ/kg. of 5-HT on the interior mesenteric ganglion were determined.

**(2) Dog Pelvic Nerve–Bladder Preparation.**—Action of 5-HT and its antagonists on the pelvic ganglia was studied in dogs under pentobarbital anesthesia with pretreatment of methysergid as described earlier.[12] $ED_{50}$ values (doses which reduced to 50% the bladder contractions elicited by repeated (every 2 to 3 min.) constant intraaortic doses of 0.5 to 2.0 γ/kg. of 5-HT) were determined on the basis of 2 to 3 dose levels of the blocking agents.

**(3) Pelvic Afferent Nerves.**—Cats were prepared as in 1, but instead of the hypogastric nerve, some branches of the pelvic plexus were isolated, cut centrally, and placed on a bipolar platinum electrode under light mineral oil at 37°. Injections of 5–20 γ/kg. of 5-HT and its blocking agents were given through the cannulated inferior mesenteric artery. Different doses of one blocking agent (XII) were tested against the stimulant action of intraarterially given 5-HT. 5-HT was given before the blocking

(1) Syntex Laboratories, Palo Alto, Calif.
(2) L. Gyermek, *Pharmacol. Rev.*, **13**, 399 (1961).
(3) U. Trendelenburg, *Brit. J. Pharmacol.*, **11**, 74 (1956).
(4) E. Hertzler, *ibid.*, **17**, 407 (1961).
(5) L. Gyermek and E. Bindler, *J. Pharmacol. Exptl. Therap.*, **135**, 344 (1962).
(6) G. S. Dawes and J. H. Comroe, *Physiol. Rev.*, **34**, 167 (1954).
(7) J. C. Mott and A. S. Paintal, *Brit. J. Pharmacol.*, **8**, 238 (1954).
(8) L. Gyermek and K. Nador, *Acta Physiol. Acad. Sci. Hung.*, **4**, 341 (1953).
(9) L. Gyermek and K. Nador, *J. Pharm. Pharmacol.*, **9**, 209 (1957).
(10) R. B. Bawlow and I. Kahn, *Brit. J. Pharmacol.*, **14**, 99, 553 (1959).
(11) Melting points were determined with a Thomas melting point apparatus and are corrected.
(12) L. Gyermek, *Arch. Intern. Pharmacodyn.*, **137**, 137 (1962).

agent and 30 sec. and 4 min. after the blocking agent. Minimal effective doses were determined in three experiments.

(4) **Cardiovascular and Respiratory Reflexes.**—Cats under chloralose and urethane and dogs under pentobarbital sodium were used. Blood pressure (femoral artery) and respiration were recorded on a polygraph. Compounds were given either i.v. into the femoral vein or through one of the lingual arteries into the common carotid artery. After the reflex actions to selected doses of 5-HT (the doses of i.v. administration ranged between 10–160 γ/kg., for intracarotid administration between 5–40 γ/kg.), *i.e.*, drop in blood pressure, bradycardia, reflex apnea (cat and dog), and respiratory stimulation (dog) had been established, the blocking agents were given and the challenging dose of 5-HT was repeated, usually 2, 5, and 10 min. later. Degrees of the inhibitions produced by the blocking agents to 5-HT were determined.

(5) **Isolated Rat Uterus (Musculotropic Action).**—Uterine horns of estrogen-sensitized rats were used. The blocking agents were given into the oxygenated organ bath at 30° for 10 min. preceding the challenging dose of 5-HT (0.05–0.5 γ/ml.). Dose ratios of 5-HT were determined as described by Gaddum and Picarelli[13] in the presence of two concentrations (1 and 10 γ/ml.) of the blocking agents. Dose ratios to ACh were determined at one (10 γ/ml.) concentration.

## Results

**Blocking Actions Against 5-HT. (a) Inferior Mesenteric Ganglion Preparation.**—Most of the compounds showed marked blocking action against ganglionic stimulation induced by 5-HT (Table II). Of the quaternary derivatives of N,N-dimethyltryptamine the *meta*-substituted benzyl compounds (IV, VI, and especially V) surpassed the nonsubstituted benzyl quaternary derivative (I), and also those which carried substitutions in the *ortho* (II, III) and *para* positions (VII, VIII). N-(*m*-Chlorbenzyl)-N,N-diethyl-β-(3-indolylethyl)ammonium bromide (IX) was markedly less potent than any of the N,N-dimethyl derivatives.

The three derivatives of bufotenin, X, XI, and XII, were all markedly more potent than the corresponding members of the N,N-dimethyltryptamine series. An outstanding potency was observed with the *m*-chlorobenzyl quaternary derivative (XII). This was the most potent member of the group.

Selectivity of the ganglionic actions to 5-HT of some compounds is illustrated in Table II. It is shown that several compounds were 50–100 times as potent as LSD in blocking the ganglionic action of 5-HT. These agents, V, XI, and XII, did not block the effect of DMPP in the same doses (LSD at the high dose which blocks 5-HT also blocks DMPP; a cholinergic type of ganglionic blocking agent, hexamethonium, inhibits the action of DMPP more readily than that of 5-HT). Less potent members of the series were not tested against DMPP in doses beyond those employed against 5-HT. These doses were usually not effective against DMPP.

(b) **Pelvic Ganglia (Table III).**—Potencies of the compounds against the 5-HT induced ganglionic stimulation on the dog bladder ran quite parallel with those obtained on the mesenteric ganglia of the cat; XI was the only exception. It was considerably more active in the dog than in the cat. The $ED_{50}$ values obtained on the pelvic ganglia were usually lower than on the cat ganglion preparation. Selectivity of two potent compounds (V, XII) was investigated and found to be high for 5-HT. Doses 10–25 times higher than those effective against 5-HT were found ineffective against DMPP. Five of the 12 compounds were at least 80 times more potent in blocking the ganglionic action of 5-HT than either bromo LSD or chlorpromazine.

(c) **Pelvic Afferent Nerves.**—In 3 experiments, XII, in intraarterial doses of 0.5, 1, and 2 γ/kg., respectively, blocked the stimulant action of 5-HT on the afferent nerves. Selectivity to the 5-HT induced stimulation was indicated by the finding that the same doses of XII were ineffective against the afferent nerve stimulation elicited by DMPP.

(d) **Isolated Rat Uterus Preparation.**—The musculotropic 5-HT blocking action of the compounds expressed in dose ratios are shown in Table IV. None of the compounds, except XI, produced a high dose ratio to 5-HT at 1 and 10 γ/ml. LSD under similar conditions at 0.06 γ/ml. gives a dose ratio as high as 32. The musculotropic 5-HT blocking action of these agents with the exception of XI is, therefore, negligible.

**Reflex Actions. (a) Cat.**—XII in 25 γ/kg., i.v., inhibited the reflex blood pressure drop, bradycardia, and apneic response of 50 γ/kg. of 5-HT, i.v. Two γ/kg. given into the common carotid artery inhibited the same reflex actions produced by 10 γ/kg. intraarterially administered 5-HT. It is, therefore, possible that the site of interaction between 5-HT and the antagonist is within the carotid sinus area. Doses of XII which inhibited these reflex actions were ineffective against the musculotropic stimulant action of 5-HT on the nictitating membrane. A dose of 320 γ/kg., i.v., of V also inhibited the reflex BP fall and respiratory arrest produced by 5-HT given i.v. Lower (20–80 γ/kg.) doses of V were ineffective.

(b) **Dog.**—The respiratory stimulation produced by 5-HT in the dog was not inhibited by doses of 10–160 γ/kg., i.v., of XII and V. The increased pulse amplitude and apnea after 5-HT, however, was blocked by 50 γ/kg. of XII.

**Other Pharmacological Effects. Acute Toxicity.**—The approximate $LD_{50}$ of XII in mice is 55 mg./kg., i.p. (20 animals). Toxic symptoms developed within 5 to 10 min. and consisted of sedation, dyspnea, anoxic convulsions, and finally, respiratory failure. The minimal lethal doses of II, V, VII, and VIII in mice were all above 50 mg./kg. The minimal lethal doses of XII in rats were 40 mg./kg., i.p. Toxic symptoms were similar to those observed in mice.

**Autonomic Side Effects.**—The most characteristic effect of this class of agents was a strong inhibitory action at certain 5-HT sensitive neural receptor sites. In addition, most of the compounds exhibited varying degrees of stimulant actions on the autonomic nervous system. A rise in blood pressure was sometimes preceded by a short drop, respiratory stimulation, contraction of the cat nictitating membrane, and rapid, short contraction of the bladder of the dog. These stimulant actions were quite marked in the case of XII and were found to be due to cholinergic type of ganglionic stimulation. The effects could be inhibited by hexamethonium to the same extent as those of DMPP, indicating a similarity between the actions of the two agents. No stimulation of the cholinergic postganglionic receptor sites have been observed on the dog bladder and in isolated rat uterus experiments.

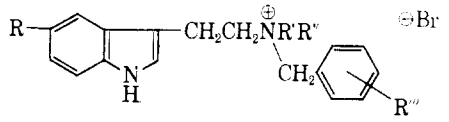
## Discussion

It is shown that compounds of the general formula

(13) J. H. Gaddum and Z. Picarelli, *Brit. J. Pharmacol.*, **12**, 323 (1957).

TABLE I

ARALKYL QUARTERNARY DERIVATIVES OF INDOLE ALKYLAMINES



| Compound | R | R' | R'' | R''' | M.p., °C.[c] | Yield, % | Formula | Quarternization[a] Time,[b] hr. | Medium | Cryst. Solvent |
|---|---|---|---|---|---|---|---|---|---|---|
| I | H | $CH_3$ | $CH_3$ | H | 202–204 | 90 | $C_{19}H_{23}BrN_2$ | 1 | Acetone | Methanol–ether |
| II | H | $CH_3$ | $CH_3$ | *o*-$CH_3$ | 204–205 | 70 | $C_{20}H_{25}BrN_2$ | 24 | Acetone | Ethanol |
| III | H | $CH_3$ | $CH_3$ | *o*-Cl | 181–183.5 | 79 | $C_{19}H_{22}BrClN_2$ | 4 (45°) 24 | Ethanol | Ethanol |
| IV | H | $CH_3$ | $CH_3$ | *m*-$CH_3$ | 181.5–185 | 87 | $C_{20}H_{25}BrN_2$ | 24 | Acetone[d] | [e] |
| V | H | $CH_3$ | $CH_3$ | *m*-Cl | 204–207 | 79 | $C_{19}H_{22}BrClN_2$ | 6 (45°) 24 | Ethanol | Methanol–ether |
| VI | H | $CH_3$ | $CH_3$ | *m*-$NO_2$ | 214–217 | 76 | $C_{19}H_{22}BrN_3O_2$ | 1 | Acetone | Acetone–ether |
| VII | H | $CH_3$ | $CH_3$ | *p*-$CH_3$ | 189.5–192 | 87 | $C_{20}H_{25}BrN_2$ | 6 (45°) 24 | Ethanol | Ethanol–ether |
| VIII | H | $CH_3$ | $CH_3$ | *p*-Br | 204–205 | 70 | $C_{19}H_{22}Br_2N_2$ | 2 | Acetone[d] | Ethanol–benzene |
| IX | H | $C_2H_5$ | $C_2H_5$ | *m*-Cl | 188.5–191 | 75 | $C_{21}H_{26}BrClN_2$ | 1 | Acetone[d] | Ethanol–ether |
| X | OH | $CH_3$ | $CH_3$ | H | 170–175 | 47 | $C_{19}H_{23}BrN_2$ | 24 | Acetone[d] | Methanol–ether |
| XI | OH | $CH_3$ | $CH_3$ | *m*-$CH_3$ | 169–172 | 75 | $C_{20}H_{25}BrNO_2$ | 24 | Acetone[d] | Methanol–ether[d] |
| XII | OH | $CH_3$ | $CH_3$ | *m*-Cl | 205–206 | 85 | $C_{19}H_{22}BrClNO_2$ | 5 | Acetone[d] | [e] |

[a] All compounds were colorless crystals except VI which was a yellow crystalline product and X which yielded light tan crystals. [b] Time of standing or heating and temperature. [c] Melting points were determined by a Thomas type melting point apparatus and are corrected. [d] Precipitated with ether, the oily precipitate was refrigerated for 24 hr. and formed a gummy solid. [e] At room tempera-

TABLE II

BLOCKING POTENCIES ON THE INFERIOR MESENTERIC GANGLION OF THE CAT

| Compound | Minimal effective dose, γ/kg. against 5-HT[a] | N[b] | Minimal effective dose, γ/kg. against DMPP[a] | N[b] |
|---|---|---|---|---|
| I | 30 (5–80) | 4 | | |
| II | ~80 (>20, 80) | 2 | ~80 (>20, 80) | 2 |
| III | 50 (20–80) | 3 | >20 | 1 |
| IV | ~20 (20, 20) | 2 | | |
| V | 7 (1.2–20) | 4 | >5 | 1 |
| VI | ~20 (20, 20) | 2 | | |
| VII | ~80 (>20, 80) | 3 | >20 | 1 |
| VIII | 20 (20, 20, 20) | 3 | >20 | 2 |
| IX | ~80 | 2 | | |
| X | 4.2 (1–8) | 4 | | |
| XI | 8.3 (1–16) | 3 | >8 | 1 |
| XII | 0.66 (0.08–1.2) | 11 | >2.0 | 3 |
| LSD[c] | 400 | | 400 | |
| Methysergid[d] | >200 | | >200 | |
| Hexamethonium[c] | >200 | | <25 | |

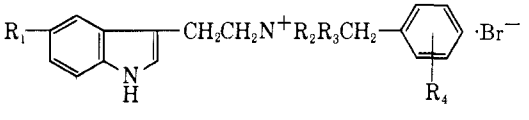
[a] Mean values and range. [b] Number of experiments. [c] See ref. 5. [d] Gyermek, unpublished data.

TABLE III

BLOCKING POTENCIES ON THE PELVIC GANGLIA OF THE DOG

| Compound | $ED_{50}$, γ/kg. against 5-HT[a] | N[b] | $ED_{50}$, γ/kg. against DMPP[a] | N[b] |
|---|---|---|---|---|
| I | 11 (8–16) | 3 | | |
| II | 20 (20, 20) | 2 | | |
| III | >20 | 1 | | |
| IV | 8 (8, 8) | 2 | | |
| V | <1 | 3 | >10 | 2 |
| VI | 2.5 (1, 4) | 2 | | |
| VII | ~20 | 1 | | |
| VIII | 20 (20, 20) | 2 | | |
| IX | ~20 | 1 | | |
| X | 2 (0.5–3.0) | 3 | | |
| XI | 0.3 (0.12–0.5) | 2 | | |
| XII | 0.4 (0.025–0.05) | 5 | >12 | 3 |
| Bromo-LSD[c] | >200 | | | |
| Chlorpromazine[d] | >200 | | | |
| Hexamethonium[d] | >500 | | <100 | |

[a] Mean values (range or individual values). [b] Number of experiments. [c] See ref. 11. [d] Gyermek, unpublished observations.



where $R_1$ is H or OH, $R_2$ and $R_3$ is lower alkyl, and $R_4$ is H, $CH_3$, $NO_2$, Cl, or Br, possess very marked antagonistic properties against certain stimulant actions of 5-HT. Pharmacological tests used in this study demonstrated that the blocking action of these agents is primarily directed to nervous receptors of 5-HT in autonomic ganglia and in chemoreceptors. While receptors at the inferior mesenteric, pelvic ganglia, and carotid chemoreceptors are very sensitive to the above agents, other receptor types of 5-HT present in smooth muscles (*i.e.*, rat uterus and dog bladder) are resistent to them.

Although the number of compounds in this series and the number of observations, especially with the less potent members of the series, was relatively small, some conclusions regarding structure–activity relationships could be drawn as follows:

(1) **Substituents of the Benzyl Groups.**—Nonsubstituted benzyl and *ortho*- and *para*-substituted benzyl quaternary salts of N,N-dimethyltryptamine showed approximately the same degree of potency against 5-HT at autonomic ganglia. The minimal effective doses of these compounds ranged between 20–80 γ, on the two ganglion preparations used. The *meta*-substituted benzyl quaternary derivatives usually showed the highest potency. Their minimal effective doses were

| % Calcd. | | | | | % Found | | | | |
|---|---|---|---|---|---|---|---|---|---|
| C | H | Br | Cl | N | C | H | Br | Cl | N |
| 63.5 | 6.41 | 22.3 | | 7.80 | 63.43 | 6.49 | 21.91 | | 7.84 |
| 64.6 | 6.70 | 21.42 | | 7.53 | 65.4 | 6.81 | 22.01 | | 7.70 |
| 57.86 | 5.59 | 20.30 | 9.01 | 7.12 | 58.13 | 5.80 | 20.36 | 9.22 | 7.82 |
| 64.5 | 6.71 | 21.40 | | 7.51 | 64.47 | 7.12 | 21.45 | | 7.70 |
| 57.86 | 5.59 | 20.30 | 9.01 | 7.12 | 58.18 | 5.79 | 20.13 | 9.32 | 6.89 |
| 56.50 | 5.45 | 19.80 | | 10.40 | 56.22 | 5.44 | 19.99 | | 10.31 |
| 64.61 | 6.70 | 21.42 | | 7.53 | 64.00 | 7.06 | 21.42 | | 7.50 |
| 52.07 | 5.06 | 36.5 | | 6.39 | 52.29 | 5.74 | 36.86 | | 6.08 |
| 59.8 | 6.18 | 19.0 | | 6.75 | 59.97 | 6.52 | 19.11 | | 6.90 |
| 60.90 | 6.18 | 21.4 | | 7.46 | 60.77 | 6.08 | 21.2 | | 7.35 |
| 61.70 | 6.42 | 20.60 | | 7.21 | 61.57 | 6.62 | 20.76 | | 7.30 |
| 55.74 | 5.38 | 19.55 | | 6.84 | 55.45 | 5.71 | 19.26 | | 7.00 |

ture. [f] Precipitated with ether. [g] The precipitate was taken up in absolute benzene before recrystallization. [h] Washed with acetone. [i] Washed twice with ether.

TABLE IV

BLOCKING POTENCIES ON THE ISOLATED RAT UTERUS PREPARATION

| Compound | Concentration, γ/ml. | Dose ratios[a] against 5-HT | N[b] | Dose ratios[a] against ACh | N |
|---|---|---|---|---|---|
| I | 1 | 1.7 | 2 | | |
| | 10 | 2 | 3 | 2.5 | 2 |
| II | 1 | 1 | 5 | | |
| | 10 | 1.4 | 5 | 1.5 | 2 |
| III | 1 | 1 | | | |
| | 10 | <4 | 2 | <4 | 3 |
| IV | 1 | 2.5 | 2 | | |
| | 10 | 16 | 2 | 2.5 | 2 |
| V | 1 | 1 | 2 | | |
| | 10 | 9 | 5 | <4 | 2 |
| VI | 1 | 1 | 2 | | 2 |
| | 10 | 2 | 2 | 1 | |
| VII | 1 | 1 | 2 | | |
| | 10 | <4 | 2 | 1 | 2 |
| VIII | 1 | 2 | 2 | | |
| | 10 | 4 | 2 | 1 | 2 |
| IX | 1 | 1 | 4 | | |
| | 10 | 1 | 3 | 1 | 2 |
| X | 1 | 1 | 3 | | |
| | 10 | 13 | 6 | 1 | 2 |
| XI | 1 | 3 | 3 | | |
| | 10 | 64 | 3 | 1 | 2 |
| XII | 1 | 1.3 | 3 | | |
| | 10 | 13 | 6 | 2.5 | 3 |
| LSD | 0.06 | 32 | | | |

[a] For dose ratios see Methods. [b] Number of experiments.

found to be between 7–20 γ in the cat prepartion, and 1–8 γ in the dog. Of the different types of substituents the chlorine substituent proved to be superior to the others (*i.e.*, $CH_3$, Br, and $NO_2$).

(2) **N-Alkyl Groups.**—Exchange of the terminal two methyl groups for ethyl groups markedly decreased the blocking potency (V *vs.* IX).

(3) **5-OH Substitution of the Indole Ring.**—This substitution favorably influenced the 5-HT blockade on autonomic ganglia. Derivatives of bufotenine were found to be 2.4–25 times more potent than the corresponding N,N-dimethyltryptamine derivatives.

Regarding their spectrum of 5-HT blocking actions the indolylalkylbenzylammoniun salts resemble certain indole acetamidines. 5-Hydroxy-3-indoleacetamidine, for example, exhibited a similar type of blocking action to 5-HT on peripheral nervous receptors.[14] The quaternary indole compounds and the indoleacetamidines, so far, occupy a unique position among blocking agents of 5-HT. Most of the well known antagonists of 5-HT do not have appreciable action at peripheral nervous receptors sensitive to 5-HT. The list of those 5-HT antagonists which are practically inactive at the above mentioned receptors includes agents like LSD, chlorpromazine, BAS, and cyproheptadine; although morphine and cocaine are fairly potent in blocking the actions of 5-HT on nervous receptors,[13,15] their other pharmacological actions overshadow this effect.

Members of the present series show considerable selectivity in autonomic ganglion-preparations antagonizing the action of 5-HT to a greater extent than that of cholinergic stimulants (*i.e.*, DMPP); however, they are not without an affinity to cholinergic ganglionic receptors. Some of the agents, especially XII, exhibited fairly marked stimulant actions at the ganglia which were of the "nicotinic" type. It is not known whether this affinity to cholinergic receptors of the ganglia plays any role in the blocking action to 5-HT. Nevertheless, it was assumed that the two phenomena might somehow be related to each other. In the presence of hexamethonium blockade, however, which eliminates the ganglionic stimulant action of XII,

(14) L. Gyermek, *Nature*, **192**, 465 (1961).

(15) L. Gyermek, *Proc. XXII. Intern. Physiol. Congr.* (Leiden), **1**, 28 (1962).

this compound was still potent against 5-HT. Since quantitative aspects of this interaction were not studied, suggestions regarding the relation of cholinergic stimulation to 5-HT blockade produced by the same agent at the ganglia cannot be made. When cholinergic stimulation and blockade of the ganglia is produced by different specific agents (*i.e.*, DMPP or hexamethonium), and stimulation and blockade of the 5-HT receptors by other distinct agents (*i.e.* 5-HT and 5 hydroxy-3-indoleacetamidine), a clear separation of effects regarding stimulation as well as blockade can be obtained between the two different types of ganglionic receptors. Thus, their independence from each other can be established. The situation with molecules which combine cholinergic and 5-HT-like ganglionic stimulant properties (*i.e.*, bufotenine)[16] or cholinergic stimulant and 5-HT blocking properties (members of the present series) is not so clear. It is obvious that further studies are needed in order to clarify certain points in the ganglionic action of this novel type of agents.

Some members of the above series proved to be the most potent agents of their class, and, therefore, in spite of their incompletely known mode of action, will merit further interest as pharmacological tools.

**Acknowledgment.**—The author wishes to express his thanks to the Microanalytical Department (Dr. Schneller) for the analytical data, to Miss Y. Yosh, Mr. E. Bindler, and Mr. L. Soffer for their technical help.

(16) L. Gyermek, L. Bindler, and L. Soffer, *Federation Proc.*, **21**, 335 (1962).

# Syntheses of Some 4-Hydroxycoumarins and Their Condensation Products with Aldehydes and Carboxylic Acids. The Anticoagulant Activity of Some 4-Hydroxycoumarin Derivatives

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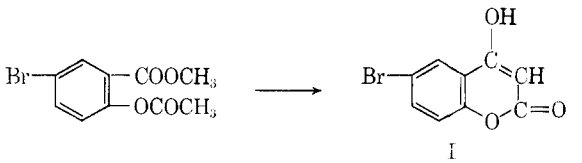
The syntheses of 6-bromo-4-hydroxycoumarin and 4-hydroxy-6,7-benzocoumarin and their condensation products with various aldehydes and carboxylic acids are described. Some of these compounds show anticoagulant activity in experimental animals.

Many compounds of the dicoumarin type have been prepared[1-3] by condensing 4-hydroxycoumarin with aldehydes. A great number of them show an intensive anticoagulant activity and therefore they are used in the therapy of thromboembolisms. This article describes analogous condensations with 6-bromo-4-hydroxycoumarin and 4-hydroxy-6,7-benzocoumarin, prepared in the hope that they may yield new substances with improved anticoagulant activity.

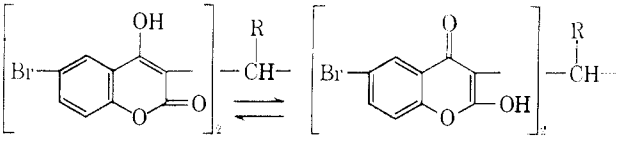
The starting material for the synthesis of 6-bromo-4-hydroxycoumarin was methyl 5-bromosalicylate[4] which on acetylation furnished methyl 2-acetoxy-5-bromobenzoate.[5] From this ester 4-hydroxy-6-bromocoumarin (I) was prepared according to a modified Pauly–Lockemann method.[6]



I

On introducing bromine into position 6 of the aromatic nucleus of 4-hydroxycoumarin its reaction with aldehydes was not essentially changed, so that we were able to prepare 3,3′-alkylidenebis(6-bromo-4-hydroxycoumarin) and 3,3′-arylidenebis(6-bromo-4-hydroxycoumarin) in the usual way. All the compounds obtained are shown in Table I. One of the compounds (3,3′-methylenebis-6-bromo-4-hydroxycoumarin) had been prepared by Huebner and Link.[7]

Because of the coumarin–chromone tautomerism[8] these compounds can be represented in two tautomeric structural formulas (IIa and IIb).



IIa IIb

4-Hydroxy-6,7-benzocoumarin (III) was synthesized starting from methyl 3-hydroxy-2-naphthoate, which by acetylation gave the acetyl derivative.[9] The reaction of methyl 3-acetoxy-2-naphthoate with sodium in hot liquid paraffin caused the lactone ring to close, giving III.

III

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