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HIGHLY POTENT ANTIMETABOLITES OF SEROTONIN WITH LITTLE SEROTONIN-LIKE ACTION

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Abstract—Six new analogues of serotonin have been synthesized. All were made by condensation of N-phthalyl-1-benzyl-2-methyl-5-hydroxytryptamine with suitable alkyl halides to give alkyl indole ethers. The phthalyl group was removed by hydrazinolysis. Special condensing conditions were required for the alkylations because of the unreactivity of the phenol. Some of the new analogues were more potent as antimetabolites of serotonin than those previously known, when tested either on isolated rat uterus or *in vivo*. In addition, most of them had greatly reduced serotonin-like action on clam heart; in one this activity was undetectable. The most potent analogue, both as an anti-serotonin and as one without serotonin-like action in the heart-test, was 1-benzyl-2-methyl-5-tryptaminoxyacethydrazide. The importance of the lack of serotonin-like activity is pointed out.

STUDIES of the clinical effects of the antimetabolite of serotonin known as BAS, or 1-benzyl-2-methyl-5-methoxytryptamine, in the treatment of hypertension,[1–4] have raised two pharmaceutical chemical questions which the present work has sought to answer. These are: (a) can antimetabolites of serotonin be made which are even more potent than BAS? and, (b) can the gastric distress which is called forth in a small percentage of the patients by BAS be eliminated by modifying of the structure in such a way as to eliminate the serotonin-like action of BAS? Although BAS showed no serotonin-like effects on such test objects as the isolated rat uterus and in the blood pressure test with anaesthetized dogs,[2,5,6] it did exhibit such serotonin-like action to a slight extent in the blood pressure test with human beings[4] and in the clam heart assay. Because gastric contractions can be caused with serotonin, and because these might be associated with the gastric distress occasionally seen in a few patients taking BAS, it might be that this undesirable side-effect of the drug is the expression of its residual serotonin-like action on some tissues. Therefore, it seemed worth while to attempt to synthesize antimetabolites of serotonin which would lack detectable serotonin-like action on suitable test objects (e.g. clam hearts), and which would still possess the high anti-serotonin action of BAS in mammalian smooth muscles. These would also need to retain the other desirable features of BAS,[1] such as oral effectiveness, and especially the failure to induce psychotic changes.

Antimetabolites of serotonin lacking detectable serotonin-like action also might be useful for psychiatric experiments. As we have previously pointed out,[6–8] the fact that most existing analogues of serotonin which induce mental disturbances can be shown to be serotonin-like in tests such as that using clam hearts or human oligo-

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dendroglia[9] obscures the interpretation whether schizophrenia is to be viewed as a deficiency of cerebral serotonin or as an excess of it.[10] If an antimetabolite could be made which lacked serotonin-like action on any test object, but which still penetrated into the brain freely, some light might be thrown on this important question. In addition, if the disease should prove to be the result of excess cerebral serotonin, such an antimetabolite would have obvious therapeutic possibilities.

Several ideas for increasing the antiserotonin potency of BAS have been explored:

(a) Since it is well known that doubling the acetylcholine molecule, as in succinylcholine, or of its analogues, as in hexamethonium, leads to the formation of potent antagonists of this hormone, the doubling of the BAS molecule might similarly enhance potency. Compound (I) was therefore synthesized, but was found to be slightly less potent than BAS. Congeners of (I), in which the number of $-CH_2-$ groups bridging the indole rings was two and four instead of ten, were also made, but were not successfully purified. However, because the potency of the crude materials indicated that no great additional enhancement of potency was being realized, this approach was not pursued further.

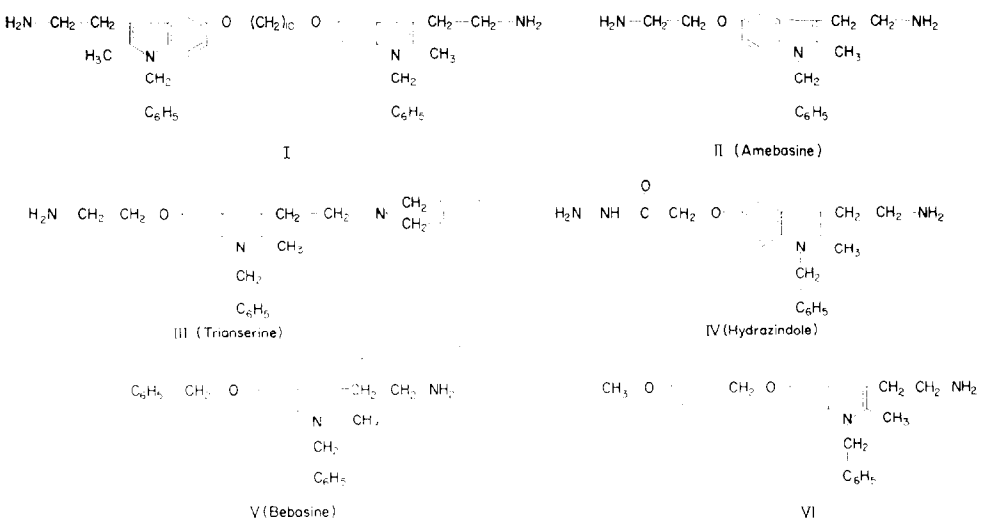

FIG. 1. Structures of new antimetabolites of serotonin.

(b) Because Woolley[11] had shown that suitably substituted phenoxyethylamines were antimetabolites of serotonin, it was thought that if the phenoxy part were changed to oxytryptamine, anti-serotonin potency might be enhanced. Such an analogue would be twice an antiserotonin, once for the phenoxyethylamine moiety, and once for the alkyltryptamine moiety. Compound (II) was therefore prepared, and found to be very potent. Compound (III) was next prepared because it would be thrice an antiserotonin, once for the phenoxyethyl part, once for the alkyltryptamine part and once for the alkyl*iso*indolinyl part. Davis had found N-methylbenz*iso*indoline to be antagonistic to serotonin.[12] Unfortunately, this substance (III) was less potent than

(II). However, it had the advantage of being able to penetrate into the central nervous system, as is shown by its effects on animals.

(c) When (II) and (III) were tested for serotonin-like action on clam hearts they were found to have much less of this sort of activity than had BAS. Therefore, it seemed possible that mere increase in size of the alkyl group which formed the ether part of BAS might be the way to decrease serotonin-like action. To test this idea, (IV), (V) and (VI) were synthesized and tested. The results indicated that more than size alone was involved. Nevertheless, one of these compounds, (IV), proved to have the properties desired (high anti-serotonin-activity and no serotonin-like activity).

The synthesis of these compounds was difficult. The amino group of BAS-phenol (1-benzyl-2-methyl-5-hydroxytryptamine) was protected by phthalylation,* and the phenolic hydroxyl was then reacted with a suitable alkyl halide, followed by removal of the phthalyl group by hydrazinolysis. The difficulty was that the phenolic group was so weakly acidic that it would scarcely react under the usual conditions with alkyl halides. The weakness of the phenol was readily shown by the fact that it (*viz.* 1-benzyl-2-methyl-3-phthalimidoethyl-5-hydroxyindole) could not be extracted from organic solvents by 0·1 N NaOH, and remained as the free phenol, not the sodium salt. With active halides, such as benzyl chloride or chloroacetamide, the desired products were obtained by long heating with the phenol in ethanolic sodium ethoxide, but with less reactive halides it was necessary to prepare the dry sodium salt of the phenol with sodium ethoxide, and to heat this dry salt for long periods with the halide.

Because of their high potency as anti-serotonins, and other desirable properties, some of these new compounds may possibly become useful both as laboratory tools and in the treatment of disease. The chemical names are too unwieldy for general use, and numbers lack distinguishability. Therefore, the trivial names shown in the figure were given to the most promising ones.

EXPERIMENTAL

N-*Phthalyl*-1-*benzyl*-2-*methyl*-5-*hydroxytryptamine*. Phthalic anhydride (4·5 g) and 1-benzyl.-2-methyl-5-hydroxytryptamine (7·7 g) (free gase)[5]† in 100 ml of toluene (dried over Na) were heated at 160–170°C in a open flask for 1 hr. The remaining toluene was removed under reduced pressure, and the residue was dissolved in 35 ml of ethanol plus 300 ml of ethyl acetate. This solution was washed once with 50 ml of cold aqueous 5 per cent sodium bicarbonate, then with water, dried with $MgSO_4$, and freed of solvent under reduced pressure. Whenever this or other phthalyl compounds were treated with aqueous alkali it was done rapidly in the cold. Even warm aqueous sodium bicarbonate (65° for 15 min) caused considerable opening of the phthalimide ring to give an acid (m.p. 90 °C) which was the *o*-carboxybenzoyl derivative of the tryptamine. The tarry residue from evaporation of the ethyl acetate was triturated very thoroughly for a long time with 600 ml of boiling benzene, and the soluble portion was concentrated under reduced pressure to 50 ml and stored overnight at 4 °C to give 8·0 g of crystals, m.p. 186–188 °C. Analyses are in Table 1.

*In preliminary work the amino group was protected by acetylation. However, when attempts were made to remove this group by acid hydrolysis of the final products extensive decomposition of the indole occurred even though the acid-labile positions (1) and (2) of the indole ring were protected.

†A quantity of this compound was generously supplied by Dr. K. Pfister of Merck, Sharp and Dohme.

TABLE 1. N-PHTHAPLYL-1-BENZYL-2-METHYL-TRYPTAMINES

Additional substituent	Recrystallizing solvent	M.P. (°C)	Empirical formula	Calculated C H N	Found C H N
5-Hydroxy	C_6H_6	186–188	$C_{26}H_{22}N_2O_3$	76·1 5·4 6·8	76·4 5·7 6·8
5-Oxyacetamide	$CHCl_3$-C_2H_5OH	218–220	$C_{28}H_{25}N_3O_4$	71·9 5·4 9·0	71·8 5·5 9·0
1′:10′-Decamethylenebis-(5-oxy)	C_2H_5OH	86–90	$C_{62}H_{62}N_4O_6$	77·7 6·5 5·8	77·0 6·8 6·0
5-Benzyloxy	C_2H_5OH	164–168	$C_{33}H_{28}N_2O_3$	79·2 5·6 5·6	79·0 5·8 5·8
5-(*p*-Methoxy-benzyloxy)	C_6H_6-hexane*	83-91†	$C_{34}H_{30}N_2O_4$	77·0 5·7 5·3	76·7 6·3 5·0
5-Phthalimidoethoxy	C_2H_50H	—	$C_{36}H_{29}N_3O_5$	74·1 5·0 7·2	73·4 5·2 7·0

*Oily from ethanol; crystals could only be obtained by rapid addition of 5 vols. of hexane to a 10% solution in benzene.
†M.P. not sharpened by further purification.

N-Phthalyl-1-benzyl-2-methyl-5-tryptaminoxy acetamide; general method for alkylation of the above phenol with active halides. N-phthalyl-1-benzyl-2-methyl-5-hydroxytryptamine (10·5 g) was added to 100 ml of absolute ethanol in which had previously been dissolved 620 mg of sodium. Solution was effected by heating, and then 2·55 g of chloroacetamide was added. The mixture was refluxed anhydrously for 72 hr. Ethanol was removed under reduced pressure, the residue was washed with 100 ml of water and then heated with 500 ml of refluxing ethanol. The mixture was not filtered, but was cooled thoroughly and the crystals (6·4 g), m.p. 215–218 °C., collected. An additional 150 mg was recovered from the mother liquor. Recrystallization from chloroform by addition of ethanol raised the m.p. to 218–220 °C. Analyses are given in Table 1.

Other N-phthalyl-1-benzyl-2-methyltryptamines. All other phthalyltryptamines, except the one described in the following section, were made by the general procedure described above. Because these other compounds were more soluble in ethanol, more concentrated solutions in this solvent were needed for crystallization. Gums which crystallized only with difficulty were frequently encountered. Details and analyses are summarized in Table 1. The alkyl halides used were: for the third compound, 1:10-decamethylene bromide; for the fourth, benzyl chloride; and for the fifth, *p*-methoxybenzyl chloride.

N-Phthalyl-1-benzyl-2-methyl-5-(β-phthalimidoethoxy)-tryptamine. N-Phthalyl-1-benzyl-2-methyl-5-hydroxytryptamine (4·1 g) was dissolved in 100 ml of hot absolute ethanol in which had been previously dissolved 242 mg of sodium. Dry toluene (25 ml) was added and ethanol was removed by several evaporations under reduced pressure followed by additions of dry toluene, each time without complete removal of solvent. β-Bromoethylphthalimide (2·6 g) dissolved in 25 ml of dry toluene was added, and the resulting suspension was concentrated under reduced pressure to 10 ml. The flask was then heated under anhydrous conditions at 160–170 °C for 72 hr. The product was purified by several fractional crystallizations from ethanol. At first it came out of solution as a liquid, but it was finally obtained crystalline by cautious cooling of an ethanolic solution. Yield 3·44 g, m.p. indistinct, with extensive sintering beginning at 116 °C, and final melting at 126 °C. All attempts to prepare this substance by use of ethanol in the reaction mixture during the condensation (as in the preceding examples) failed. Analyses are given in Table 1.

1-*Benzyl-2-methyl*-3-iso*indolinylethyl*-5-(*β-aminoethoxy*)-*indole* (*compound* (III)). A solution of 500 mg of lithium aluminium hydride in 100 ml of anhydrous ether was treated with 467 mg of N-phthalyl-1-benzyl-2-methyl-5-tryptaminoxy acetamide, and the mixture was stirred and refluxed under anhydrous conditions for 4 days. Excess hydride was decomposed by cautious addition of 6 ml of 50 % aqueous sodium potassium tartrate. The filtered ether phase was extracted with 55 ml of 0·1 N hydrochloric acid. The acid extract was immediately made alkaline with sodium hydroxide solution and the gummy precipitate which formed was extracted into ethyl acetate. The solvent was removed from the extract under reduced pressure and the residue was dissolved in ethanol and converted to the hydrochloride with two equivalents of ethanolic hydrogen chloride. The hydrochloride was crystallized by addition of ether; m.p. 158–162 °C. (Found: C, 66·8; H, 6·5; N, 8·2. Calc. for $C_{28}H_{33}Cl_2N_3O$; C, 67·4; H, 6·6; N, 8·4.)

1′:10′-*Decamethylenebis*-(1-*benzyl*-2-*methyl*-5-*oxytryptamine*) *dihydrochloride* (*compound* (I)). 1′:10′-Decamethylenebis-(*n*-phthalyl-1-benzyl-2-methyl-5-oxytryptamine) (86 mg) was dissolved in 30 ml ethanol plus 0·3 ml hydrazine hydrate and the solution was refluxed 7 hr. Most of the hydrazine was removed by repeated evaporation under reduced pressure, with additions of ethanol. The residue was suspended in 3 ml of 3 N ethanolic hydrogen chloride, heated to 50 °C for 5 min, cooled, and made alkaline with aqueous potassium hydroxide. The alkaline solution was heated for 15 min to open the phthalimide ring, thus rendering unextractable any unchanged starting material, and concentrated under reduced pressure to dryness. The residue was suspended in water, and the suspension was extracted twice with benzene. The benzene extract was evaporated and the residue was converted to the dihydrochloride by solution in ethanol, acidification, and evaporation. The dihydrochloride was then crystallized from hot water, in which it was only slightly soluble. Analyses and m.p. are in Table 2. The slight solubility of this compound in water severely limited its use in biological experiments.

TABLE 2. 1-BENZYL-2-METHYL TRYPTAMINES

Compound no.	Additional substituents	Recrystallizing solvent	M.P. (°C)	Empirical formula	Calculated C H N	Found C H N
(I)	1′:10′-Decamethylene bis-(5oxy) dihydrochloride	H_2O	146	$C_{46}H_{60}Cl_2N_4O_2$	– – 7·3	– – 7·5
(II)	5-(β-Aminoethoxy) dihydrochloride	C_2H_5OH	249*	$C_{20}H_{27}Cl_2N_3O$	60·6 6·8 10·6	61·7† 7·1 10·4
(IV)	5-Oxyacethydrazide	C_6H_6	131–134	$C_{20}H_{24}N_4O_2$	68·2 6·9 15·9	68·2 7·0 15·6
	5-Oxyacethydrazide monohydrochloride	C_2H_5OH‡	233–236	$C_{20}H_{25}ClN_4O_2$	61·7 6·4 14·4	61·9 6·8 14·3
(V)	5-Benzyloxyhydrochloride	H_2O	147–150	$C_{25}H_{27}ClN_2O$	– – 6·9	– – 6·9§
(VI)	5-(*p*-Methoxybenzyloxy)	C_6H_6-hex.	96–100	$C_{26}H_{28}N_2O_2$	78·0 7·1 7·0	77·6 7·1 6·8

*Began to soften at 238 °C.
†Compound was difficult to dry. Analyses after drying at 78 °C indicated 0·5 mole ethanol of crystallization. Analyses shown were of a sample dried 3 hr at 100 °C.
‡Can also be recrystallized from water.
§Dried at 60 °C; long drying at 100 °C caused some decomposition.

1-*Benzyl*-2-*methyl*-5-(β-*aminoethoxy*)-*tryptamine dihydrochloride* (*compound* (II)). Hydrazinolysis of N-phthalyl-1-benzyl-2-methyl-5-phthalimidoethoxytryptamine was carried out as in the preceding example, except that a greater excess of hydrazine (10 ml hydrazine hydrate plus 8 ml ethanol for 1·4 g) was required. The free base was a solid (m.p. 193 °C) which was extracted from the aqueous phase with chloroform more completely than with benzene. The dihydrochloride was soluble in water but was precipitated by excess chloride ions. This kind of behaviour has been found with several other tryptamines in this and preceding investigations.

1-*Benzyl*-2-*methyl*-5-*tryptaminoxyacethydrazide* (*compound* (IV)). When attempts were made to prepare the aminoethoxy analogue (compound (II)) by hydrazinolysis of N-phthalyl-1-benzyl-2-methyl-5-tryptaminoxyacetamide, followed by hydride reduction of the amide group, there was no success. The difficulty was that when the phthalyl compound was heated with 1 equivalent of hydrazine a mixture of starting material and the corresponding hydrazide was always obtained. It therefore proved impossible to prepare the unprotected tryptamine with the acetamide grouping on the ether oxygen by this route. For preparation of the hydrazide (compound (IV)) in acceptable yield, the following conditions were found suitable. N-Phthalyl-1-benzyl-2-methyl-5-tryptaminoxyacetamide (6·13 g) was dissolved by warming in 30 ml of hydrazine hydrate and 50 ml of absolute ethanol. The solution was heated on a steam bath for 1 hr and concentrated to dryness under reduced pressure. Ethanol was added and the evaporation repeated three times, in order to remove the excess hydrazine as completely as possible. Hydrolysis with hydrochloric acid and extraction of the free amine were carried out as described in the preceding examples, except that ethyl acetate rather than benzene was required for the extraction, and five extractions were necessary. Because of the basic nature of the hydrazide group it was necessary to adjust the pH to above 10 before making the ethyl acetate extractions. When this was not done, and only a slight excess of alkali was used as in the preceding methods, the yield was low, and the monohydrochloride of the desired product crystallized from the aqueous phase after long standing in the cold. The free base in the ethyl acetate extracts was freed of solvent and recrystallized by solution in ethanol, addition of benzene, and gradual replacement of the ethanol with benzene by frequent partial evaporations under reduced pressure. The yield was 2·7 g.

1-*Benzyl*-2-*methyl*-5-*benzyloxytryptamine hydrochloride* (*compound* (V)). This compound was made by hydrazinolysis of N-phthalyl-1-benzyl-2-methyl-5-benzyloxytryptamine according to the method described for compound (I) except that the refluxing was for 3 hr, and 1·2 equivalents of hydrazine were used. The free base could be crystallized from benzene, m.p. 116 °C after softening from about 110 °C.

1-*Benzyl*-2-*methyl*-5-(p-*methoxybenzyloxy*)-*tryptamine* (*compound* (VI)). Hydrazinolysis of the corresponding N-phthalyltryptamine with an excess of hydrazine as described for compounds (II) and (IV), yielded compound (VI). Chloroform rather than benzene was required to extract the free base from the alkaline aqueous solution. The hydrochloride was an oil which could not be crystallized, and which was extractable by water from chloroform only with difficulty. The free base was precipitated by addition of sodium hydroxide to an aqueous solution of the hydrochloride, and was crystallized from benzene by addition of hexane.

Antiserotonin activity in vitro *in rat uterus.* All compounds were compared in potency to BAS for their antiserotonin activity on the isolated rat uterus. In this assay the

amount of analogue was determined which would prevent the contraction induced by an effective dose of serotonin as described earlier.[2,11] To increase the accuracy of comparison with BAS, one horn of the uterus of each rat was used to determine potency of BAS, and the other horn was used for assay of the new analogue. The same horn could not be used for both because all these new analogues, just like BAS,[2] were irreversible anti-serotonins. In all tests each dilution of the analogue was held in contact with the tissue for 10 min prior to addition of the challenging dose of serotonin. It is known that potency increases with increasing time of exposure to such analogues.[2,11] The specificity of most of the new anti-serotonins was demonstrated by showing that they did not antagonize the contractile action of acetylcholine. The results of the assays are summarized in Table 3, which contains the averages of at least three trials for each compound.

TABLE 3. SEROTONIN-LIKE AND ANTISEROTONIN ACTIVITIES OF SOME 1-BENZYL-2-METHYL TRYPTAMINES

Compound no.	Additional substituents	Antiserotonin activity		Serotonin-like‡ activity
		Rat uterus* (μg/ml.)	Mouse assay† (μg/mouse)	(%)
	5-Methoxy (or BAS)	0·2	250	3·0
	5-Hydroxy (or BAS-phenol)	—	50	0·3§
(I)	1′:10′-Decamethylenebis-(5-oxy)	0·5	—	
(II)	5-(β-Aminoethoxy)	0·1	100	0·1§
(III)	N-*Iso*Indoline-5-(β-aminoethoxy)	0·5	75	0·05**
(IV)	5-Oxyacethydrazide	0·05	10	less than 0·04**††
(V)	5-Benzyloxy	0·4	—	0·2-1·0‡‡
(VI)	5-(p-Methoxybenzyloxy)	0·02	5	1·0

*Expressed as the amount required to cause half-maximal inhibition of a contraction elicited by an amount of serotonin just sufficient to cause maximal contraction. All values were in direct comparison with the standard analogue (viz. BAS) as explained in the text.

†Amount required to protect half of the mice against diarrhoea induced by 1 mg 5-hydroxytryptophane per mouse.

‡Expressed as percentage of the activity of serotonin creatinine sulphate in causing increased amplitude of beat of clam hearts. Thus, 1 per cent means that 100 μg of analogue caused the same response as 1 μg of serotonin creatinine suphate.

§Dose–response curve less steep than for serotonin.

**The analogue decreased the base beat in two of five hearts (anti-activity?). In one of five no detectable serotonin-like or antiserotonin action.

††Detectable serotonin-like activity in only one of five hearts.

‡‡Considerable variation from heart to heart; almost inactive for some.

The potency of compounds (II), (IV) and (VI) was noteworthy. These are the most potent anti-serotonins known, as shown by the fact that the contraction of sensitive uteri (which will respond maximally to 0·01 μg of serotonin creatinine sulphate per ml) was reduced 50 per cent by 0·05 μg of compound (IV) or 0·02 μg of compound (VI) per ml. None of the new analogues showed any serotonin-like action on the rat uterus.

Anti-serotonin potency in vivo *in mice.* Some of the new analogues were compared in potency with BAS in the quantitative assay of Woolley,[13] in which the ability to prevent the contracting action of serotonin on intestines of mice is measured in normal animals. Results are summarized in Table 3. Again, the high potency of compounds

(IV) and (VI) is evident. Half of the animals were protected against the effects of 1 mg of DL-5-hydroxytryptophane by 10 μg. of compound (IV) per mouse or by 5 μg. of compound (VI).

Serotonin-like action in clam hearts. Most of the new compounds, as well as some previously known antimetabolites of serotonin, were assayed with isolated hearts of *Venus mercenaria*, for serotonin-like action.[14,15] Each was compared to serotonin for ability to increase the amplitude of beat as described earlier. Each dilution of analogue was held in contact with the tissue for 15 min in order to detect effects slow to appear.[11] The results are summarized in Table 3.

Although no detectable serotonin-like action of compound (IV) was observed, the results showed that in several hearts treated with this analogue there was some augmentation of the action of serotonin, when analogue and hormone were applied together. This may have indicated a trace of serotonin-like potency of this analogue.

The analogues were also tested on clam hearts for anti-serotonin activity. For this, graded doses of analogue were applied and the response to an effective challenging dose of serotonin was measured, as in the rat uterus assay. None of the compounds showed anti-serotonin activity. However, two of them (III and IV) reduced the amplitude of the beat of some of the hearts. This may have indicated some interference with the action of endogenous serotonin, because the decrease in amplitude could be overcome with serotonin.

DISCUSSION

The results of the present study show that it is possible to synthesize antimetabolites of serotonin with very high anti-serotonin potency for mammalian tissues, and ones which exhibit little, if any, serotonin-like potency on such invertebrate tissues as the clam heart. Because all previously tested analogues of serotonin which are antimetabolites in the usual tests with isolated smooth muscles have eventually been found to possess serotonin-like action on some sort of test object, the present results seem noteworthy. The usual test objects for serotonin-like activity have been mainly invertebrate muscles such as those of clam hearts or liver flukes, and might therefore seem of only academic interest, but when it was shown, as it has been for lysergic acid diethylamide, that such serotonin-like action also can be readily demonstrated on tissues of mammals, e.g. isolated brain cells of human beings,[9] or on those structures which control blood pressure in dogs,[6] one must pay closer attention to this phenomenon, especially since on this point depend some of the current arguments about the causation of schizophrenia,[10] and of hypertension.[1,4] It may be that in clam hearts and in the oligodendroglia of the brain the receptors for serotonin differ in structure from those in smooth muscles such as the rat uterus. This would allow many analogues of serotonin to combine with these heart or brain receptors and function in place of serotonin. In the receptors of the rat uterus, on the contrary, the analogues combine but are unable to carry on the functions of serotonin when they are so combined. Therefore they act as antagonists on these receptors. If this be true, an analogue such as compound (IV) of the present work must be pictured as being quite unable to combine with the receptors in clam hearts, even though it does unite with great avidity with those in rat uterus. The work now in progress,[16] the objective of which is to isolate and determine the chemical structures of the serotonin receptors, may eventually allow one to test these ideas directly. They are now only working hypotheses.

The determination of serotonin-like action on clam hearts may be one useful way to assay an important property of antimetabolites of serotonin. Whether this is an adequate way, or whether an even more suitable test object can be found remains to be seen.*

The present work indicates that a very effective way to reduce, and even to eliminate, this serotonin-like property in 1-benzyl-2-methyl-5-hydroxytryptamines, is to attach certain alkyl groups as ethers to the oxygen at the 5-position. The indication was that to use a basic group such as an aminoethyl or acethydrazide radical was the most effective of those tried.

* The idea that the clam heart test may not be entirely adequate is shown by the following. BAS-phenol showed strong serotonin-like action on dog's blood pressure[5] but on clam heart was rather weak (Table 3).

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