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CINNAMAMIDES AS STRUCTURAL ANALOGS AND ANTAGONISTS OF SEROTONIN

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Abstract—Cinnamamides with aminoalkyl groups on the amide nitrogen were conceived as structural analogs of serotonin which would act as antimetabolites of it. Several such amides were synthesized and tested for antiserotonin activity on the isolated rat uterus and in living animal. All such amides that were tested acted as antagonists of the hormone. When the structural analogy to serotonin was increased by introduction of a *meta*-methoxyl group in the benzene ring, antiserotonin potency was increased considerably. A further increase in potency was achieved by introduction of a benzyl group on the amide nitrogen. The most active cinnamamide proved to be more active as a serotonin antagonist in the rat uterus assay than the previously known and highly potent 1-benzyl-2-methyl-5-methoxytryptamine (BAS), and was much easier to synthesize. It was, however, less active than BAS in the *in-vivo* assay that was used. The compounds were not antagonistic toward acetylcholine or bradykinin on rat uterus. It was suggested that some previously known cinnamamides may owe some of their pharmacological activity (e.g. local anesthetic) to their ability to act as antimetabolites of serotonin.

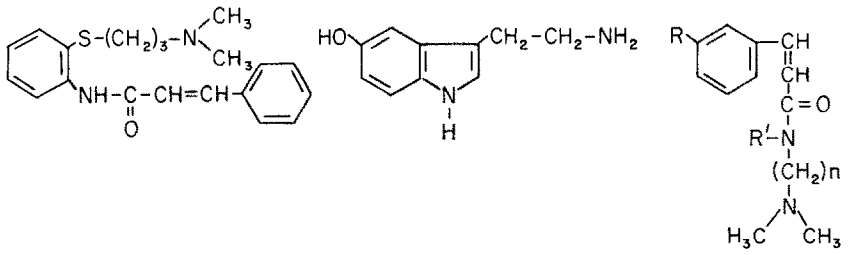
RECENTLY, Krapcho *et al.*[1] described a new class of highly potent serotonin antagonists namely 2′-(3-dimethylaminopropylthio)cinnamanilide (I) and related compounds. They seemed to imply by their manner of representation of the structural formula of (I) that these compounds were analogous in structure to serotonin (II), and that the analogy resided in the anilide moiety of the antagonist molecule. Various structural modifications of (I) were studied, and the effects of these on antiserotonin activity were determined.

An alternative idea that occurred to us was that the structural analogy to serotonin might reside in the cinnamamide portion of the molecule rather than in other parts. A dimethylaminoalkyl cinnamamide can be structurally represented in such a way (cf. formula III) as to appear somewhat analagous to an opened indole ring of a serotonin-like molecule in which the aminoalkyl side chain is located on the nitrogen atom rather than in its normal position. The —NH— of an opened indole ring is thus replaced by a —CONH— in the cinnamamide molecule (Fig. 1).

To test this idea, various simple cinnamamides were synthesized and their antiserotonin activities were measured. The results showed that the dimethylaminoalkyl cinnamamides (III) themselves, without an aromatic group interspersed between the cinnamamide portion and the dimethylaminoalkyl group as in compound (I), were active antiserotonin compounds. The potency could be enhanced by introduction of a

* With the technical assistance of B. W. Gommi. All elemental analyses were carried out by Mr. T. Bella.

methoxyl group into the *meta* position. This position of the cinnamamide corresponds to that occupied by the hydroxyl group in serotonin. Introduction of a benzyl group on the amide nitrogen of these substituted cinnamamides further increased the anti-serotonin potency. It is therefore evident that suitable structural alterations on the dimethylaminoalkyl cinnamamide molecule (III) can lead to very active antagonists of serotonin.



I II III

FIG. 1. Structures of serotonin and cinnamamides.

Dialkylaminoethyl cinnamamides have previously been known to possess pharmacological activity as local anesthetics.[2] The present finding of specific antiserotonin activity for this kind of compound suggests that the local anesthetic property may be related to the involvement with serotonin. There is now considerable evidence, principally of an indirect kind but also some of a direct nature, which suggests that serotonin plays a role in the functioning of certain nerves. However, there is as yet no direct evidence that serotonin functions in the conduction of pain impulses in those nerves (especially the C-fibers) that are involved in local anesthesia, and for this reason the idea just mentioned is only a suggestion that awaits further study

MATERIALS AND METHODS

Starting materials. N,N-Dimethylaminoethylamine[3] and N,N-dimethylaminopropylamine[4] were purchased from Aldrich Chemical Co., Milwaukee, Wis. They were dried first over potassium hydroxide, then over barium oxide, and distilled at atmospheric pressure prior to use. The fractions of N,N-dimethylaminoethylamine and N,N-dimethylaminopropylamine boiling at 107–108° and 130–138°, respectively, were used.

N,N-Dimethyl-N′-benzylethylenediamine was prepared as described by Villani *et al.*[5] by reaction of benzaldehyde with N,N-dimethylaminoethylamine followed by hydrogenation of the Schiff base with platinum oxide as a catalyst.

Cinnamoyl chloride was obtained from Aldrich Chemical Co. The acid chlorides of the *ortho-*, *para-*, and *meta*-methoxycinnamic acids were prepared by refluxing the corresponding acids in thionyl chloride followed by evaporation of excess thionyl chloride. The acid chlorides were all purified by distillation *in vacuo*. *m*-Methoxycinnamoyl chloride is a crystalline solid, m.p. 40–42°, b.p. 163–164°/10 mm; *p*-methoxycinnamoyl chloride[6] is a crystalline solid, m.p. 51–53°, b.p. 188–191°/13 mm; *o*-methoxycinnamoyl chloride is a liquid, b.p. 182–184°/17 mm.

TABLE 1. PHYSICAL AND ANALYTICAL PROPERTIES OF DIMETHYLAMINOALKYL CINNAMAMIDE SALTS

Compound number	R*	R′	n	Recrystallization solvent†	Salt	m.p. °C	Calculated C	Calculated H	Calculated N	Found C	Found H	Found N
(IV)	H	H	2	A	Hydrochloride	165–166	61·29	7·52	11·00	61·64	7·33	10·76
(V)	*m*-OCH_3	H	2	B	Bioxalate	155–156	56·79	6·55	8·28	56·87	6·47	8·16
				C	Picrate	158–159	50·31	4·86	14·67	50·30	4·76	14·62
(VI)	*m*-OCH_3	H	3	D	Bioxalate	150–151	57·94	6·87	7·95	57·76	6·94	7·95
(VII)	*m*-OCH_3	$CH_2C_6H_5$	2	E	Hydrochloride	173–174	67·27	7·26	7·47	67·11	7·35	7·47
				D	Bioxalate	168–169	64·47	6·59	6·54	64·70	6·70	6·43
(VIII)	*o*-OCH_3	$CH_2C_6H_5$	2	E	Hydrochloride	167–168	67·27	7·26	7·47	67·26	7·23	7·27
(IX)	*p*-OCH_3	$CH_2C_6H_5$	2	A	Hydrochloride	134–136	67·27	7·26	7·47	66·83	7·06	7·41

* R, R′ and n refer to the positions as shown in structure III of Fig. 1.
† A, isopropyl alcohol–ether; B, acetone; C, 95% ethanol; D, water–acetone; E, isopropyl alcohol.

Synthesis of the compounds. All the dimethylaminoalkyl cinnamamides were prepared by the reaction of the acid chloride of the cinnamic acid with the dimethylaminoalkylamine in benzene solution. The free bases were all viscous oils, and each was isolated and characterized as the hydrochloride or, if the hydrochloride was oily, as the bioxalate salt. The recrystallization solvents, melting points, and elemental analyses for these salts are given in Table 1. A specific illustration of the general synthetic method is described below.

N-(β-Dimethylaminoethyl)-N-benzyl-*m*-methoxycinnamamide (VII). To a stirred benzene solution containing 2·30 g (12·9 mmole) N,N-dimethyl-N′-benzylethylenediamine[5] was added dropwise a benzene solution of 2·55 g (12·9 mmole) *m*-methoxycinnamoyl chloride. After all the acid chloride was added, the reaction mixture was refluxed for 60 min. The cooled reaction mixture was extracted with 25 ml 1 N sodium hydroxide. The benzene layer was washed with water, dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. The viscous oily residue was dissolved in 40 ml anhydrous ether, and to this solution was added dropwise a saturated solution of ethereal hydrogen chloride until precipitation was complete. The hydrochloride, which was oily, crystallized when cooled and scratched. It was filtered and washed with ether to give 3·6 g (74 per cent) of hydrochloride salt, m.p. 165–170°. It was recrystallized from isopropyl alcohol to give white crystals, m.p. 173–174°.

Bioassay methods. All analogs were assayed and their concentrations expressed as the hydrochloride salts. A bioxalate salt was converted to a hydrochloride by adding one molar equivalent of both calcium chloride and sodium hydroxide to an aqueous solution of the bioxalate salt of the cinnamamide. The precipitated calcium oxalate was separated by centrifugation, and the supernatant was an aqueous neutral solution of hydrochloride salt. This solution was diluted to the desired concentration and used for assay.

All compounds were assayed for their antiserotonin activity on the isolated rat uterus, according to the method previously described.[7, 8] As explained in these papers and others referred to therein, the amount of analog was determined that was necessary to inhibit half maximally the contraction caused by a specified amount of serotonin. The amount of the challenging dose of serotonin was determined for each uterine horn by measurement of the responses to graded doses of the hormone. A dose–response curve for the hormone with each tissue was plotted, and the minimal quantity of serotonin that elicited a contraction near to the maximal one possible was read from this curve and used in the remainder of each experiment. Because, as is well known from the shape of such dose–response curves, there is some error involved in the determination of the precise amount giving the maximal response possible, this maximum was deduced from the asymptote of the curve. The challenging dose was thus an amount of serotonin that elicited nearly maximal response. For most tissues this challenging dose was 0·1 μg serotonin creatinine sulfate/10 ml bath fluid.

When the uterine horn had been standardized in its response in this way, graded doses of an analog were applied to it. The first dose was always less than the amount required to produce an effect, and subsequent doses were twofold or threefold greater than each preceding one. Each amount of analog was allowed to remain in contact with the tissue for 10 min; the challenging dose of serotonin was then applied and the response was measured quantitatively. The reasons for careful control of the time of

contact of the analog with the tissue, and for the choice of a 10-min time, have been explained in previous papers (e.g. Ref. 8). It is well known that potencies of analogs of serotonin may vary with time of contact with the tissue before the challenge of serotonin is applied. The doses of analog were increased until the amount that caused complete inhibition was found. The amount of analog required to inhibit half maximally the response of the uterine horn to the challenging dose of serotonin was determined graphically by construction of a dose–response curve which related the dose of analog to the size of the contraction produced by the challenge of serotonin. From this curve the quantity of analog that caused 50 per cent inhibition was ascertained.

Each analog was tested in this way on a number of uterine horns from different rats. The average value required for half maximal antagonism was then determined.

In order to verify the accuracy of the comparison of potencies of two analogs, the following method (the reason for which was previously described[8]) was used. One horn of the uterus from an individual rat was used for the assay of one analog and the other horn from the same rat was used for the assay of the other analog. Comparison of the two values then allowed a conclusion about the relative potencies of the two analogs on strictly comparable tissues. The two analogs were then assayed again on another pair of tissues from an individual rat in order to obtain a second value for relative potency. This was done in order to make sure that the average potencies (the average value from tests with several rats) did not merely reflect individual variations among animals.

These methods of assay were accurate enough to permit valid rating of the relative potencies of the various analogs tested. However, the precision was not great enough to allow discrimination between compounds which differed only slightly in potency. For the purposes of the present study, however, the precision was good enough to establish answers to the questions raised.

Some of the analogs were also tested in this same system and in the same way as antagonists to acetylcholine and to bradykinin.

Serotonin-like activity of each analog was also determined on the isolated rat uterus. This was done by noting whether the compound itself, without serotonin, caused contraction.

The ability to act *in vivo* as an antiserotonin was measured according to the method of Woolley.[9] Graded amounts of the analog were injected into mice which were subsequently challenged with 5-hydroxytryptophan. The amount of analog hydrochloride required to protect half of a group of mice from diarrhea was determined as previously described,[9] and compared to the activity of 1-benzyl-2-methyl-5-methoxytryptamine (BAS), which was used as a reference compound.

RESULTS

Antiserotonin activity on isolated rat uterus

The relative potencies of the various cinnamamides are summarized in Table 2. N-(β-Dimethylaminoethyl)cinnamamide (IV) possessed appreciable activity. The *m*-methoxy derivative (V) was considerably more active than (IV). Lengthening the dimethylaminoalkyl chain of (V) by one carbon atom to give N-(γ-dimethylaminopropyl)-*m*-methoxycinnamamide (VI) resulted in a compound having nearly the same antiserotonin activity *in vitro* as the lower homolog. Replacement of the hydrogen

atom of the amide nitrogen in (V) with a benzyl group gave N-(β-dimethylaminoethyl)-N-benzyl-*m*-methoxycinnamamide (VII) which was more active than the unbenzylated compound. The *ortho*- and *para*-methoxy isomers (VIII) and (IX) of (VII), however, were considerably less active than the *m*-methoxy compound. Compound (VII) was the most potent antiserotonin found in this series of analogs. It was more active than BAS, which is a well-known, highly active antagonist of serotonin.[7, 8]

TABLE 2. RELATIVE ANTISEROTONIN POTENCIES OF SUBSTITUTED CINNAMAMIDES

Compound number	Cinnamamide	Antiserotonin activity Rat uterus* Mean, μg/10 ml	Rat uterus* Range, μg/10 ml	Mice,† mg/kg
(IV)	N-(β-Dimethylaminoethyl)	40 ± 8	30–50	75
(V)	N-(β-Dimethylaminoethyl)-*m*-methoxy	3·5 ± 1·5	2–5	60
(VI)	N-(γ-Dimethylaminopropyl)-*m*-methoxy	2·5 ± 0·5	2–3	75
(VII)	N-(β-Dimethylaminoethyl)-N-benzyl-*m*-methoxy	0·6 ± 0·3	0·3–1	45
(VIII)	N-(β-Dimethylaminoethyl)-N-benzyl-*o*-methoxy	7 ± 0	7–7	45
(IX)	N-(β-Dimethylaminoethyl)-N-benzyl-*p*-methoxy	10 ± 0	10–10	60
	1-Benzyl-2-methyl-5-methoxytryptamine (BAS)	2·5 ± 0·5	2–3	12

* Values shown are means of amounts of analogs required to cause half maximal inhibition of the contraction of the isolated rat uterus stimulated by a fully effective dose (0·1 μg/10 ml) of serotonin creatinine sulfate. The numbers following the ± signs are standard deviations calculated in the usual way; the range represents the lowest amount found for any single uterine horn and the highest amount for any horn.

† Values shown are amounts of analogs per kilogram body weight required to protect half the mice from diarrhea caused by 1 mg DL-5-hydroxytryptophan per mouse.

The actions of most of the cinnamamides shown in Table 2 were difficult to reverse with serotonin. In order to restore the sensitivity of the tissues, it was necessary to wash them for a long time, and even then the reactivity to the original amount of serotonin was not always regained. Increases in the challenging dose of serotonin in such tissues led to normal contractions which again could be prevented by increases in the dose of the analogs. Such irreversible effects of other antimetabolites of serotonin are well known[8] and often reflect high potency.

Specificity

Experiments on isolated rat uteri showed that, although the cinnamamides antagonized the action of serotonin, they did not overcome the effects of acetylcholine or of

TABLE 3. RESPONSE OF AN ISOLATED RAT UTERUS TO N-(β-DIMETHYLAMINOETHYL)-*m*-METHYOXYCINNAMAMIDE TO SHOW SPECIFICITY TOWARD SEROTONIN

Serotonin, μg/10 ml	Acetylcholine, μg/10 ml	Analog, μg/10 ml	Contraction, cm
0·1	0	0	7·3
0	1·0	0	7·7
0·1	0	1·0	7·4
0·1	0	10·0	0·2
0	1·0	10·0	7·7

bradykinin. The data in Table 3 illustrate this phenomenon with respect to acetylcholine and compound (V). These observations on specificity were repeatedly confirmed in several experiments.

Antiserotonin activity in vivo

All the cinnamamides were active *in vivo* in the mouse diarrhea assay (Table 2). They all had rather similar potencies, which were less than that of BAS. At the dose that protected the animals in this very stringent test for antiserotonin activity, most of the compounds caused signs of toxicity, which included paralysis of the hind legs and marked drowsiness. Higher doses caused convulsions and death.

DISCUSSION

The results of the present investigation show that simple dimethylaminoalkyl cinnamamides exhibit rather specific activity as antagonists of serotonin. The activity *in vitro* can be increased by introduction into the benzene ring of a methoxyl group in such a position (the *meta* position) as to make the compounds even more analogous in structure to serotonin. It can be increased further by introduction of a planar group such as a benzyl on the amide nitrogen. Introduction of a methoxyl group in either the *ortho* or *para* position of the cinnamamide molecule (instead of in the *meta* position), thus decreasing the analogy to serotonin, reduced the antiserotonin potency. These facts are compatible with the idea that certain cinnamamides have a structural analogy to serotonin which is great enough to allow them to act as antimetabolites of the hormone. With respect to the enhancement in activity brought about by introduction of the benzyl group on the amide nitrogen, it should be remarked that earlier studies of indolic antimetabolites of serotonin had shown clearly that such a planar group both increased antiserotonin activity and conferred an irreversible character on the antagonism to the hormone.[7, 10–12] The present findings would fit in with this conclusion. It must be noted, however, that the various structural alterations on the cinnamamide molecule had only negligible effects on the activities of these compounds *in vivo*, as shown by the results obtained in the assay with living mice (Table 2).

The most potent compound of the present series, compound (VII), ranks high among the most powerful antagonists of serotonin known, but it is not the most active. Thus BAS, which has been much used, was about ten times less potent as an antagonist in the rat uterus assay.[7, 8, 12] Hydrazindole, however, a derivative of BAS, was considerably more potent (50 times BAS).[8] The compound of Krapcho *et al.*,[1] shown as structure (I) of Fig. 1, was approximately 150 times as potent as BAS in the same kind of assay. Nevertheless, compounds of the present series exhibited rather high potency.

High potency in any one assay system is not the only criterion of usefulness of an antagonist of serotonin. The ability to act *in vivo*, or to be orally active, or long acting, or to be able to affect certain tissues and not others may all be of great importance.[10, 12, 13] For example, the present compounds are all tertiary amines and for this reason would be expected (cf. Refs. 12 and 14) to exert effects on nerves. If one wished to produce compounds that would not do this but, rather, would be restricted to action on smooth muscles, a primary amine would be indicated. Consequently, it would not be surprising to find that compound (VII) would be active in assays involving primarily

the nervous system, whereas the corresponding analog with an amino group instead of the dimethylamino group would be relatively inactive in such an assay. This relationship has been clearly shown for BAS and its N,N-dimethyl analog.[14] The reason why the compound of Krapcho *et al.*[1] was active in the head-twitch assay, whereas BAS was not, probably revolves around this point.

The great advantage of the cinnamamides as antagonists of serotonin lies in the fact that they are easy to make. Most previously known antimetabolites of serotonin are complicated indoles. Because of the great difficulties in the syntheses of such indoles, the really potent antagonists of serotonin have been scarce and, consequently, little used. The ready availability of the simple aminoalkyl cinnamamides makes possible their widespread use in experimentation. It will quite probably be possible to make such compounds, which are even more potent than (VII) and which have other desirable features in their spectrum of activity.

Some comment about the higher potency of the compound of Krapcho *et al.*,[1] (compound I of Fig. 1) in comparison with compound (VII) of the present series may be permissible. To judge by the graphic representation of Krapcho *et al.*, this compound was regarded by its discoverers as an analog of serotonin in which the structural analogy resided in the noncinnamic portion of the molecule. The present study has shown that the aminoethyl cinnamamide moiety itself is analogous to the hormone, and sufficient to confer on the molecule an antiserotonin potency. However, it is entirely possible that compound (I) is twice an analog of serotonin, once because of its cinnamamide portion, and once because of its noncinnamic part. Such 'double analogs' have sometimes proved to be of unusually high potency.[8]

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