Compound 48/80. Structure–Activity Relations and Poly-THIQ, a New, More Potent Analog

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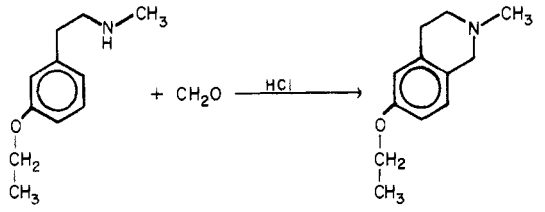
Derivatives of *p*-methoxyphenethylmethylamine were synthesized from which formaldehyde copolymers analogous to compound 48/80 were prepared. Measurement of the hypotensive activity of these analogs revealed that potency was not enhanced by changing the group in the para position, by varying the length of the alkyl group, or by altering the degree of methylation of the amine. However, when the ethylamine side chain was cyclized to form 7-methoxytetrahydroisoquinoline, the copolymer prepared from this derivative (poly-THIQ) was seven times more potent than compound 48/80. The hypotensive action of poly-THIQ was found to result from the liberation of histamine, as with compound 48/80.

In 1937, Ide and Buck[1] described the synthesis of a number of tetrahydroisoquinolines (THIQ's) which were prepared by cyclization of the appropriate phenethylamines (Scheme I). Closure of the heterocyclic ring was achieved by using formaldehyde in acid as a source of methylene groups to link the amine of the side chain to the aromatic ring.

Scheme I

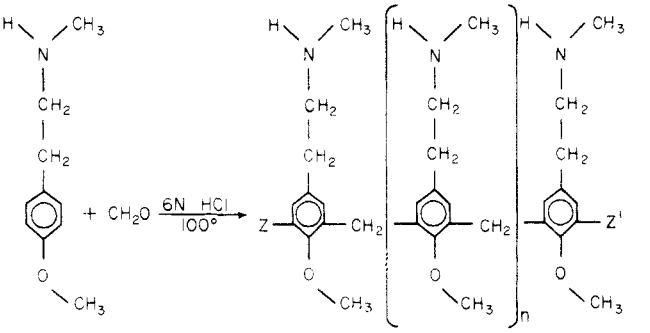


In 1938, Fasset and Hjort[2] described the hypotensive effects of some of these THIQ's. One of them, 6-ethoxy-*N*-methyl-THIQ, was especially potent. In a study of the purity of this particular product, Hjort, *et al.*,[3] found that in addition to the THIQ, it contained an unidentified by-product which was responsible for the strong hypotensive effect.

In 1949, Baltzly, *et al.*,[4] described their studies of the by-product and reported that the yield of hypotensive activity could be greatly increased if the structure of the phenethylamine starting material were altered slightly. Ide and Buck had been inducing closure of the heterocyclic ring by positioning an activating alkoxy group para to the cyclization point on the aromatic ring. Baltzly, *et al.*, found that about a tenfold increase in activity could be obtained if the alkoxy group instead were placed para to the origin of the side chain. They found that optimum activity was obtained when equimolar concentrations of formaldehyde and *p*-methoxyphenethylmethylamine were allowed to react together in 6 *N* HCl for 4 hr at 100°. They proposed that the product (compound 48/80) was a family of copolymers of the two reactants as shown in Scheme II (Z and Z′ may be H, CH_2OH, or CH_2Cl). If the hypotensive activity discovered by Baltzly, *et al.*, is related to the hypotensive activity synthesized by Ide and Buck, it would seem likely that the Ide and Buck by-product contained formaldehyde copolymers with either 6-ethoxy-*N*-methyl-THIQ or *m*-ethoxyphenethylmethylamine (the reactant for the production of the 6-ethoxy-*N*-methyl-THIQ) or both.

By distillation, Baltzly's group separated compound 48/80 into fractions which indicated that the hypotensive activity was associated with the trimeric and tetrameric members of the family. They also studied the structure–

Scheme II



activity relations of other reactants and found that the *p*-methoxyphenethyldimethylamine family was quite active.

In 1966, DeGraw, *et al.*,[5,6] reported that the *p*-methoxyphenethyldimethylamine dimer and trimer synthesized by an alternate route were inactive. They concluded that the active constituents of the family must be higher molecular weight oligomers.

In 1972, Read and Lenney[7] described studies on the rate of dialysis and the gel filtration characteristics of the hypotensive activity in compound 48/80 and reported that the size of the active constituents approximated that of a hexamer.

Thus, the active members of the family seem to be built up out of the smallest members, possibly as simple linear copolymers. However, there is some evidence which indicates that intramolecular reactions may also be important. For example, the conditions Ide and Buck used to synthesize their THIQ's favored cyclization rather than copolymerization. Therefore, it would be likely that if polymers were produced, many of them would have contained THIQ's. In addition, we have examined the mass spectra of several purified fractions of compound 48/80 and found that many of the ethylamine side chains do not fragment in the same way that free ethylamine side chains do (unpublished data), suggesting that cyclization might have occurred.

In order to explore the nature of the active constituents in compound 48/80, we performed a series of structure activity relations studies. Although Baltzly's group had already done this to some extent, there were additional reactants we wished to consider. In addition, the earlier copolymerizations were run for 4 hr, the optimal time for the preparation of compound 48/80. However, it seemed possible that some of the reactants might combine at different rates, so we also studied the relationship between the duration of the copolymerization and the activity of the product.

Methods

Materials. Four of the ten phenylalkylamines were obtained commerically (phenethylamine, *p*-methoxyaniline, and *p*-methoxybenzylamine from Aldrich Chemical Co., and tyramine hydrochloride from City Chemical Corp.); the other six were synthesized as described below.

***p*-Methoxyphenethylamine** was prepared from *p*-anisyl alcohol *via* *p*-methoxybenzyl chloride and *p*-methoxyphenylacetonitrile, by the procedure of Kiefer,[8] in 75% overall yield. It was identified as the hydrochloride, mp 212-214°, by mass spectroscopy [*m/e* 151 (M^+), 122, 121, 30, 28]. The two intermediates were characterized by ir and nmr spectroscopy.

***N*-Methyl-*p*-methoxyphenethylamine** was prepared in 77% overall yield by dimethyl sulfate methylation of the *N*-benzylidene derivative of *p*-methoxyphenethylamine as described by Kiefer:[8] hydrochloride mp 185.5-186.5°; mass spectral ions at *m/e* 165 (M^+), 121, and 44; nmr (D_2O-DSS) δ 2.80 (s, 3 H), 3.00 and 3.26 (AA'BB', 4 H), 3.82 (s, 3 H), 7.01 and 7.33 (aromatic AA'BB', 4 H).

***N,N*-Dimethyl-*p*-methoxyphenethylamine** was prepared in 31% overall yield by reduction of the corresponding dimethylamide with lithium aluminum hydride in ether, using an adaptation of a standard procedure.[9] It was identified as the hydrochloride: mp 163-165°; mass spectrum *m/e* 179 (M^+, 1%), 58 ($CH_2{=}NMe_2^+$, 100%); nmr (D_2O-DSS) δ 2.99 (s, 6 H), 3.05 and 3.36 (AA'BB', 4 H), 3.84 (s, 3 H), 7.03 and 7.35 (AA'BB', 4 H). The oily amide, prepared in the usual way in 92% yield from *p*-methoxyphenylacetic acid *via* the acid chloride and aqueous dimethylamine, was identified by nmr spectroscopy (nonequivalent *N*-methyl singlets in addition to characteristic *p*-methoxybenzyl signals).

***N,N,N*-Trimethyl-*p*-methoxyphenethylammonium chloride** was prepared by methylation of the above tertiary amine with excess methyl iodide in ether; the crystalline methiodide which precipitated was converted to the chloride by stirring its aqueous solution with silver chloride for 1 hr at room temperature. Filtration and evaporation of the filtrate gave quantitative yields of silver iodide and the quaternary ammonium chloride, respectively: nmr (D_2O-DSS) δ 3.20 (s, 9 H), 3.49 (AA'BB', 4 H), 3.82 (s, 3 H), 6.95 and 7.30 (AA'BB', 4 H).

p-Methoxyphenylpropylamine was prepared in low yield from β-(*p*-methoxyphenyl)propionic acid by $LiAlH_4$ reduction of the primary amide in a manner analogous to that described for *N,N*-dimethyl-*p*-methoxyphenethylamine above, except that the acid chloride was added to concentrated ammonia instead of dimethylamine. The nmr spectrum ($CDCl_3$-TMS) of the crude amide, mp 130-132°, showed signals at δ 2.49 (t, 2 H), 2.88 (t, 2 H), 3.77 (s, 3 H), 5.88 (broad s, 1 H), 6.09 (broad s, 1 H), and 6.80 and 7.16 (aromatic AA'BB', 4 H). The amine was identified by mass spectroscopy: *m/e* 165 (M^+, 12%), 148 ($M - NH_3$, 100%), 121 ($MeOC_6H_4CH_2^+$, 31%), 30 ($CH_2{=}NH_2^+$, 36%).

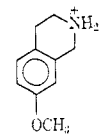
7-Methoxytetrahydroisoquinoline cannot be prepared by the conventional (Bischler-Napieralski) route from formaldehyde and *p*-methoxyphenethylamine; it was synthesized by the modified Pomeranz-Fritsch procedure described by Bobbitt, *et al.*,[10] starting with *m*-methoxybenzaldehyde and β-aminoacetaldehyde diethyl acetal. The product was characterized as the hydrochloride: mp 235-236° (reported[10] 233-234°); mass spectrum *m/e* 163 (M^+, 98%), 134 ($M - CH_2{=}NH$, 100%); nmr in agreement with the assigned structure.

Copolymerization. Each phenylalkylamine was allowed to react with equimolar formaldehyde according to the method of Baltzly, *et al.*,[4] but for periods of time ranging from 1 min to 128 hr. Immediately following the reaction period, the product was dried *in vacuo* at 100° and then stored in the dry state until chromatographed and assayed.

Gel filtration chromatography was used to determine the extent of the copolymerization reaction. A Sephadex G-25 (medium) column 111 × 1.5 cm was perfused at a flow rate of 12 ml/hr with a solution of 0.03 *N* AcOH adjusted to pH 2.5 with HCl. Samples (10 mg) of the phenylalkylamines and their reaction products from each different time period of copolymerization were run separately. The absorption of the effluent at 280 nm was recorded.

Blood Pressure Assay. Male Wistar rats weighing 300-500 g were anesthetized with pentobarbital sodium (50 mg/kg, Abbott), and their arterial blood pressure was recorded on a Grass Model 7 polygraph. Pressure was detected with a Statham P23Dc transducer connected by a cannula to the right femoral artery. Heparin sodium (10 MG/kg, Nutritional Biochemicals), antagonists, and the products to be assayed were given through a cannula inserted into the right femoral vein.

Table I. Hypotensive Activities of Some Phenylalkylamines Copolymerized to Form Analogs of Compound 48/80

Phenylalkylamine		Copolymerization time for optimum activity, min	Dose to lower blood pressure 50%, μg/kg
$(CH_3)_n$ $^+NH_{(3-n)}$ $(CH_2)_2$ Ph OCH_3	$n = 0$	240	91 (83–106)
	$n = 1$[a]	240	75 (74.6–76.2)
	$n = 2$	240	132 (126–139)
	$n = 3$	240	1250 (1170–1360)
$^+NH_3$ $(CH_2)_n$ Ph OCH_3	$n = 0$	*b*	Inactive
	$n = 1$	480	419 (409–428)
	$n = 2$	240	91 (83–106)
	$n = 3$	60	329 (296–365)
$^+NH_3$ $(CH_2)_2$ Ph R	R = H	*b*	Hypertensive[c]
	R = OH	4	195 (191–200)
	R = OCH_3	240	91 (83–106)
		960	11 (10.5–11.5)

[a] Phenylalkylamine of compound 48/80. [b] Did not copolymerize. [c] Activity from unreacted phenylalkylamine.

The optimum duration for copolymerization of each phenylalkylamine was determined by comparing the average blood pressure depressions obtained in two to seven rats 5 min after administration of each product. (Five minutes was selected because at that time the blood pressure was stable at the new level and representative of the potency of the product). The most potent product from each phenylalkylamine was then tested in 10-22 rats to obtain the relationship between dose and blood pressure depression 5 min after administration. The dose required to produce a 50% reduction in blood pressure was then used to compare the products of the different phenylalkylamines.

Diphenhydramine hydrochloride (Parke, Davis & Co.) and tripelennamine hydrochloride (Purity Organics) were used to antagonize the hypotensive effects of compound 48/80 (Burroughs Wellcome Co.) and the product from the copolymerized 7-methoxy-THIQ (poly-THIQ). Compound 48/80 and poly-THIQ were also used to deplete rats of their mass cell histamine.

Histamine Release Assay. Rat peritoneal mast cells were used to measure histamine release *in vitro*. The cells were obtained by injecting 10 ml of a solution of 0.1% glucose in 0.9% saline into the peritoneal cavity, agitating for 90 sec, and then decanting the suspension of cells through an abdominal incision. Histamine released from the mast cells by compound 48/80 or poly-THIQ was measured by the technique of Shore.[11] The data are presented as percentages of the amount of histamine releasable by lysis with distilled water.

Statistics. Where appropriate, standard errors were calculated and appear in the tables as ranges or ± values and in the figure as vertical lines.

Results and Discussion

All of the phenylalkylamines yielded hypotensive products except phenethylamine and *p*-methoxyaniline (Table I). In contrast to the others, each of these preparations gave only one peak in gel filtration chromatography, and each of these peaks had the same elution volume as its unreacted phenylalkylamine. Thus, it is concluded that little or no copolymerization occurred during the periods

Table II. Effect of Antihistamines against the Hypotension Produced by Compound 48/80 and Poly-THIQ

Pretreatment			Challenge		Mean blood pressure		
Drug	Dose, mg/kg	No. of animals	Drug	Dose, μg/kg	Initial, mm	5 min after challenge, mm	Treated as % of initial
None (control)		7	48/80	100	147	55	38 ± 6
None (control)		5	Poly-THIQ	15	138	38	28 ± 3
Diphenhydramine	30	4	48/80	100	156	92	59 ± 3
Diphenhydramine	32	5	Poly-THIQ	15	153	61	40 ± 1
Tripelennamine	13	4	48/80	100	150	79	53 ± 4
Tripelennamine	10	4	Poly-THIQ	15	151	68	45 ± 2

Table III. Development of Tolerance to Compound 48/80 or Poly-THIQ after Pretreatment with Compound 48/80 or Poly-THIQ

		Challenge		Mean blood pressure		
Pretreatment	No. of animals	Drug	Dose, μg/kg	Initial, mm	5 min after challenge, mm	Treated as % of initial
None (control)	7	48/80	100	147	55	38 ± 6
None (control)	5	Poly-THIQ	15	138	38	28 ± 3
Compound 48/80[a]	4	48/80	100	158	150	96 ± 3
Compound 48/80[a]	5	Poly-THIQ	15	156	138	88 ± 6
Poly-THIQ[b]	4	48/80	100	151	142	94 ± 3
Poly-THIQ[b]	3	Poly-THIQ	15	165	164	99 ± 2

[a] Compound 48/80 given subcutaneously for 10 days, beginning with 0.1 mg/kg and increasing by 0.1 mg/kg per day, such that on day 10 the animals were receiving 1 mg/kg. Challenge occurred on day 11. [b] Poly-THIQ given subcutaneously for 10 days, beginning with 0.015 mg/kg and increasing by 0.015 mg/kg per day, such that on day 10 the animals were receiving 0.15 mg/kg. Challenge occurred on day 11.

that phenethylamine (1, 2, 4, and 8 hr) and *p*-methoxyaniline (1, 2, 4, 8, and 12 hr) were exposed to the conditions for copolymerization. The rest of the phenylalkylamines produced products in which the number of constituents and their molecular weights increased as the copolymerization reaction progressed. The maximal depressions obtained with the active products were all about the same and only varied in the doses necessary to produce them.

Replacing the *p*-methoxy group with a hydroxy group had only a minor effect on the activity of the product relative to compound 48/80 but substantially accelerated the rate of the reaction.

When the length of the alkyl group between the amine and the ring was varied, hypotensive activity was greatest with an ethylene bridge and decreased as the chain length was made longer or shorter. The fact that phenylethylamine would cyclize to a six-membered ring more readily than the other phenylalkylamines would to form five- or seven-membered rings suggests that ring formation might be essential for activity.

Varying the degree of methylation of the amine from primary to tertiary made little difference in the activity. However, the product of the quarternary ammonium derivative was only one-sixteenth as active as compound 48/80. It is not known whether this relative inactivity was due to the increased size of the amine, the presence of a permanent charge, or the inability of the phenylalkylamine to cyclize.

When the side chain of the phenylalkylamine was cyclized to form the THIQ, the potency of the product (poly-THIQ) was increased sevenfold over that of compound 48/80. Because the structure of the THIQ was quite different from that of the other phenylalkylamines, it was considered necessary to determine if the hypotensive action of poly-THIQ resulted from histamine release as is the case with compound 48/80.[12] In Table II the antagonism by diphenhydramine and tripelennamine of the depressor responses to compound 48/80 and poly-THIQ is shown. It is apparent that both compound 48/80 and poly-THIQ were inhibited by these antihistamines. (The absence of a total blockade is typical of this type of experiment and is possibly due to the appearance of other hypotensive agents beside histamine.) The ineffectiveness of poly-THIQ to lower the blood pressure of rats depleted of their mast cell histamine by compound 48/80 is shown in Table III. In addition, the tolerance that develops to treatment with poly-THIQ is apparent, as is the cross tolerance between compound 48/80 and poly-THIQ. Finally, Figure 1 shows the dose-response curves for the *in vitro* release of mast cell histamine by both compound 48/80 and poly-THIQ. Comparison of the doses necessary to release 50% of the histamine reveals that poly-THIQ is several times more potent than compound 48/80 in this assay. Thus, it appears that compound 48/80 and poly-THIQ have the same mechanism of action, which is to cause the release of histamine.

During the pretreatment of rats with compound 48/80 and poly-THIQ to deplete their stored histamine, some comparison of the general toxicity of the two agents was obtained. We find that pretreatment with compound 48/80 invariably kills some of the rats about half way through the regimen. In the past this has amounted to 37% (15/41). In contrast, none of the seven rats receiving poly-THIQ died during the pretreatment regimen.

The possibility that the active constituents of compound 48/80 might contain THIQ's should be considered because several factors suggest that cyclization could be important for histamine liberating activity.

(1) The hypotensive activity which led to the discovery of compound 48/80 was produced during the synthesis of a THIQ. Because the synthesis favored cyclization more than polymerization, it is probable that if any copolymers were formed, they would have contained THIQ's.

(2) Some of the ethylamine side chains in compound

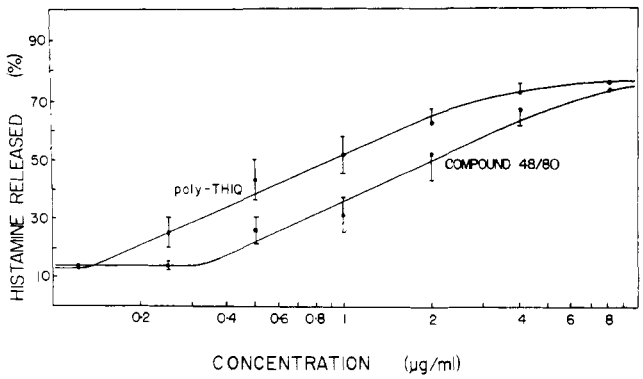


Figure 1. Dose-response curves for the release of histamine from rat peritoneal mast cells by compound 48/80 and poly-THIQ. Histamine released is expressed as a per cent of the total histamine releasable by distilled water.

48/80 do not fragment in the usual manner when examined mass spectrographically. Cyclization could account for this behavior.

(3) When the nitrogen of the phenethylamine is quaternary so that cyclization cannot occur, the product after copolymerization is nearly inactive.

(4) When the alkyl group in the phenylalkylamine is longer or shorter than the ethyl group in compound 48/80, the products after copolymerization are less active. A phenethylamine would cyclize more readily than the other phenylalkylamines.

(5) Cyclization of the phenethylamine prior to copolymerization increases the activity of the product.

Proof of the presence or absence of cyclized phenethylamines in the active constituents of compound 48/80 will have to await the isolation of pure members of the family.

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