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Isolation of the Trimer of Compound 48/80 and Determination of Its Histamine-Releasing Activity

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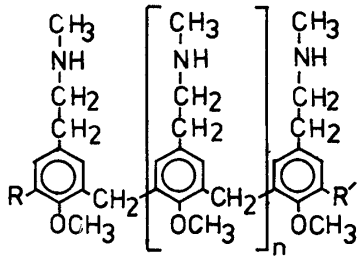
The trimer of compound 48/80 was isolated and its potency for histamine release from rat peritoneal mast cells was examined. ED_{50} (the concentration giving 50% histamine release) of the trimer was 2×10^{-5} M. The result indicated that the trimer is not as inactive as was previously considered.

Keywords——compound 48/80; *p*-methoxy-*N*-methylphenethylamine; polycation; trimer; structure–activity relationship; histamine release; mast cell

In 1949, a mixture of polymers, showing potent hypotensive action, was prepared by condensation of *p*-methoxy-*N*-methylphenethylamine and formaldehyde.[1] This compound was named "compound 48/80" from its code number "BW 48/80." Paton and others showed that the hypotensive action of 48/80 was attributable to histamine release from tissues.[2] Despite the remarkable pharmacological activity of 48/80, the active component of this compound was poorly defined. It was proposed that the most potent polymer was probably the trimer (Fig. 1, $n=1$).[1] In 1966, DeGraw *et al.*[3] succeeded in preparing the trimer of the "*N*, *N*-dimethyl" analog of phenethylamine; however, they were unable to prepare the more important "N-monomethyl" trimer of 48/80. The trimer that they prepared was inactive as regards hypotensive action.[3]

Structure assignment of the active component of 48/80 has been attempted by several workers. Dialysis and gel filtration[4] and ^{13}C- nuclear magnetic resonance (NMR) studies[5] showed that the most active polymer was probably the hexamer (Fig. 1, $n=4$), though it was difficult to identify the structures of individual components of 48/80. On the other hand, many studies have shown that various polyamines (including diamine and triamine) induce histamine secretion from tissues and mast cells.[6-10] However, the structure–activity relationship required for histamine secretion has not yet been clarified in detail; there is a tendency for an increase in the number of cationic groups to enhance the activity for histamine release.[10] Therefore, it is expected that the dimer and trimer of 48/80 should also induce histamine release depending on the number of cationic groups.



R, R'= H or CH_2OH

Fig. 1. Proposed Structure of Compound 48/80

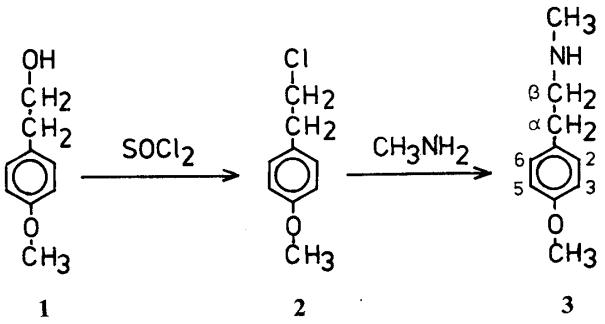
The above considerations prompted us to investigate the dose–response relation of the dimer and trimer of 48/80 for histamine release from mast cells. This approach should also help to clarify the nature of the binding site of 48/80 on mast cells. The present paper describes the preparation of the monomer, dimer and trimer of 48/80, and reports the histamine-releasing activities of these compounds on rat peritoneal mast cells.

Synthesis

The monomer of 48/80, *p*-methoxy-*N*-methylphenethylamine (**3**) was prepared from the corresponding phenethyl alcohol (**1**). Compound **1** is commercially available (Aldrich Chemical Co.). Treatment of **1** with thionyl chloride afforded phenethyl chloride (**2**) in almost quantitative yield. The reaction of **2** with methylamine in methanol solution gave **3** in 73% yield. This procedure was rather convenient compared to the previous preparation,[11] because the present method permits easy separation of the reaction product and avoids the use of poisonous NaCN. The reaction could also be applied to the preparation of analogous amines, and we obtained *p*-methoxy-*N*, *N*-dimethylphenethylamine from the reaction of **2** with dimethylamine (overnight reaction at room temperature gave the product in 41% yield). The dimer (**4**) and trimer (**5**) of 48/80 were prepared by the reaction of the monomer (**3**) with formaldehyde in aqueous solution containing perchloric acid, followed by chromatographic separation. Originally, this reaction was utilized for preparation of the dimer;[1] however, we found that the trimer was also present in the reaction mixture. We separated the dimer (**4**) and trimer (**5**), and their structures were unambiguously identified by 500 MHz ^{1}H-NMR spectrometry. This is the first attempt to isolate the trimer of 48/80.

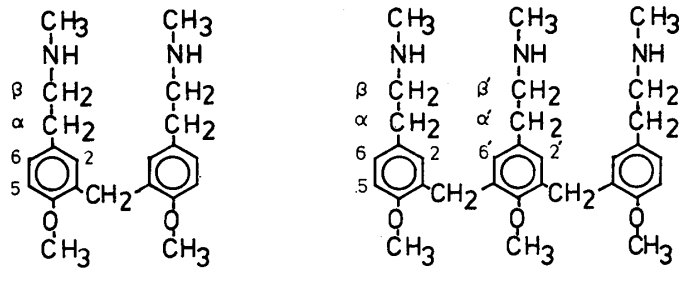
Histamine-Releasing Activity

Figure 2 shows the histamine-releasing activities of **3**, **4**, and **5** from rat peritoneal mast cells, along with that of compound 48/80 (Sigma Chemical Co., Lot No. 21F-0153). The dose–response curves clearly depend on the number of cationic groups of the compounds. In accordance with the results of the preceding studies,[4,5] compound 48/80 had a higher degree



1 2 3

Chart 1



4 5

Chart 2

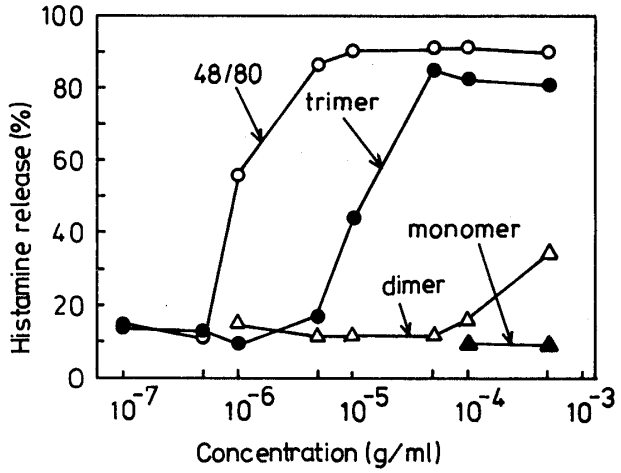


Fig. 2. Histamine-Releasing Activities of the Monomer (**3**), Dimer (**4**), and Trimer (**5**) of Compound 48/80

The corresponding hydrochlorides were used. The figure also shows the dose–response curve of commercial compound 48/80 (Sigma Chemical Co., Lot No. 21F-0153). Each point represents the mean value from 3 experiments. Standard error at each point was less than 3%.

of polymerization than the trimer. It is noteworthy that the potency of the trimer was comparable to those of various other polycationic releasers reported previously.[10] ED_{50} (the concentration giving 50% histamine release) of the trimer was 2×10^{-5} M. This indicates that the trimer of 48/80 is not as inactive as was previously considered.[3,10]

Experimental

^{1}H-NMR spectra were obtained with a JEOL JNM-GX500 FT NMR spectrometer (500 MHz) with tetramethylsilane as an internal standard. Melting points are uncorrected.

***p*-Methoxy-*N*-methylphenethylamine (3)**——A large excess of methylamine dissolved in methanol solution (40 w/w%, 135 g) was added to *p*-methoxyphenethyl chloride (**2**) (5.7 g), which was prepared by refluxing thionyl chloride (5.8 g) and *p*-methoxyphenethyl alcohol (**1**) (5 g) for 30 min, followed by removal of excess thionyl chloride by evaporation. The mixture was stirred for a week at room temperature, since an overnight reaction of **2** with methylamine gave **3** in low yield (28%). The mixture was evaporated, and the residue was acidified with 300 ml of 1 N HCl, then washed with ether (200 ml × 3) to remove unreacted **2**. The acidic layer was basified with 300 ml of 2 N KOH, and the liberated amine was extracted with ether (200 ml × 3). The combined ether extracts were washed with 100 ml of H_2O, dried over $MgSO_4$, and evaporated to dryness under a vacuum to give 4.0 g (73%) of **3** as a colorless oil. The oily **3** was treated with 20% HCl–methanol solution to yield the corresponding white crystalline hydrohloride, which was recrystallized from ethanol–ether; mp 185—186 °C (lit.,[11] 185.5—186.5 °C). *Anal.* Calcd for $C_{10}H_{16}ClNO$: C, 59.55; H, 8.00; N, 6.94. Found: C, 59.38; H, 8.07; N, 6.91. ^{1}H-NMR (in CD_3OD) δ: 2.70 (3H, s, N–CH_3), 2.90 (2H, t, J=8 Hz, α-CH_2–), 3.18 (2H, t, J=8 Hz, β-CH_2–), 3.76 (3H, s, O–CH_3), 6.88 (2H, d, J=9 Hz, aromatic 3-H and 5-H), 7.19 (2H, d, J=9 Hz, aromatic 2-H and 6-H).

The Dimer (4) and Trimer (5) of 3——One ml of aq. 20% perchloric acid and 42 μl of formalin were added to 91 mg of **3** at room temperature. The mixture was heated at around 90 °C for 4 h on a water bath and then placed in a refrigerator. The supernatant was decanted and the residual gummy product was dissolved in 2 ml of 20% HCl–methanol solution. This solution was basified with 100 ml of 1 N KOH, and the liberated amines were extracted with ether (100 ml × 4). The combined ether extracts were washed with H_2O (50 ml × 2), dried over $MgSO_4$, and evaporated to dryness under a vacuum. A methanolic solution of the residue was sujbected to preparative thin layer chromatography (20 cm × 20 cm × 0.7 mm; 4 plates; Kieselgel 60 PF_{254}; solvent system, ethanol : 28% ammonia water = 25 : 7.5, v/v%). Three main bands (*Rf*'s of *ca.* 0.9, 0.7, and 0.5) corresponding to the monomer (**3**), dimer (**4**), and trimer (**5**) were visualized under ultraviolet (UV) light. The dimer (**4**) and trimer (**5**) were eluted with ethanol–28% ammonia water (2 : 1, v/v%). The eluates were each concentrated, followed by extraction with ether. The extracts were dried and the solvent was evaporated off. The separated amines were converted to the corresponding hydrochlorides by treatment with 20% HCl–methanol solution. Each precipitate was dissolved in distilled water and purified by treatment with activated charcoal powder, followed by evaporation of the water. The dimer (**4**) and trimer (**5**) were identified as the corresponding hydrochlorides. The hydrochlorides of **4** and **5** were hygroscopic: yield; 29 mg (26%) for **4**, and 7 mg (6%) for **5**; mp > 300 °C for both **4** and **5**. *Anal.* of **4** Calcd for $C_{21}H_{32}Cl_2N_2O_2 \cdot H_2O$: C, 58.20; H, 7.91; N, 6.46. Found: C, 58.65; H, 7.62; N, 6.53. *Anal.* of **5** Calcd for $C_{32}H_{48}Cl_3N_3O_3 \cdot 3H_2O$: C, 56.26; H, 7.97; N, 6.15. Found: C, 56.62; H, 7.49; N, 5.98. ^{1}H-NMR of **4** (in CD_3OD) δ: 2.66 (6H, s, N–CH_3), 2.84 (4H, t, J=8 Hz, α-CH_2–), 3.13 (4H, t, J=8 Hz, β-CH_2–), 3.80 (6H, s, O–CH_3), 3.88 (2H, s, –CH_2– bridge), 6.91 (2H, d, J=8 Hz, aromatic 5-H), 6.92 (2H, s, aromatic 2-H), 7.08 (2H, d, J=8 Hz, aromatic 6-H). ^{1}H-NMR of **5** (in CD_3OD) δ: 2.63

(3H, s, N′–CH_3), 2.67 (6H, s, N–CH_3), 2.78 (2H, t, $J=8$ Hz, α′-CH_2–), 2.87 (4H, t, $J=8$ Hz, α-CH_2–), 3.07 (2H, t, $J=8$ Hz, β′-CH_2–), 3.15 (4H, t, $J=8$ Hz, β-CH_2–), 3.67 (3H, s, O′–CH_3), 3.78 (6H, s, O–CH_3), 3.96 (4H, s, –CH_2– bridge), 6.80 (2H, s, aromatic 2′-H and 6′-H), 6.93 (2H, d, $J=8$ Hz, aromatic 5-H), 6.98 (2H, s, aromatic 2-H), 7.11 (2H, d, $J=8$ Hz, aromatic 6-H).

Histamine Release from Mast Cells——Male Wistar rats weighing 300—350 g were stunned and exsanguinated by cutting the carotid arteries. Physiological saline (10 ml) containing 154 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 5.6 mM glucose and 5 mM Hepes-NaOH (pH 7.4) was injected into the abdominal cavity, and the abdominal wall was gently massaged for 90 s. The fluid in the abdominal cavity was collected and centrifuged at $100\times g$ for 5 min at 4 °C. The pellet was resuspended in fresh physiological saline. The cell suspension (*ca.* 5×10^4 mast cells/1.9 ml in each tube) was pre-warmed at 37 °C for 5 min. Thereafter, a test compound, dissolved in 0.1 ml of the same saline, was added, and incubation was continued for another 12 min. The histamine-releasing process was stopped by chilling the tube in ice-cold water. Cells were centrifuged at $100\times g$ for 5 min at 4 °C. Amounts of histamine in the supernatant and the pellet were determined fluorometrically after conversion of histamine to the fluorescent product by reaction with *o*-phthalaldehyde.[12] The fluorescence measurements were carried out on a Hitachi 650-10S fluorospectrophotometer by using excitation and emission wavelengths of 356 and 440 nm, respectively. Histamine release was expressed as the ratio of the amount of histamine in the supernatant to the total amount of histamine in the supernatant and the pellet. Each point shown in Fig. 2 represents the mean value from 3 experiments. Standard error at each point was less than 3%.

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