Histamine Releasers. II. Synthesis of a Trimer in the Formaldehyde–*p*-Methoxyphenethylamine Series of Histamine Releasers

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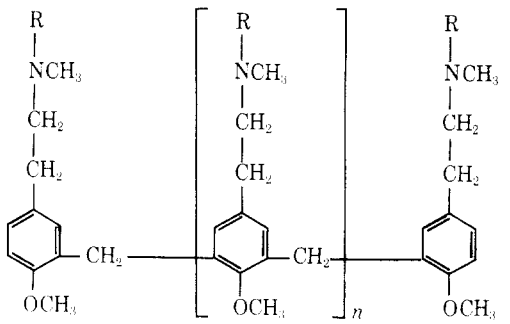
An unequivocal synthesis is described for the N,N-dimethyl "trimer" (Ib) of the formaldehyde–*p*-methoxyphenethylamine series of histamine releasers first reported by Baltzly, *et al.*[1] The substance was found to be devoid of histamine-releasing properties, contrary to the literature. An unsuccessful attempt to prepare the more important N-methyl "trimer" is also reported.

In 1949, Baltzly, *et al.*,[1] reported the preparation of a family of potent histamine releasers derived from the condensation of formaldehyde and *p*-methoxyphenethylamines. These closely related, but ill-defined, substances were regarded to be "dimers," "trimers," etc., of the basic structure I (n = 0, 1, 2). The most potent of these was tentatively identified as the "trimer" (Ia); the N,N-dimethyl derivative (Ib) was also found to possess strong histamine-releasing properties. The "dimers" (Ic and Id) were reported to have only weak activity.

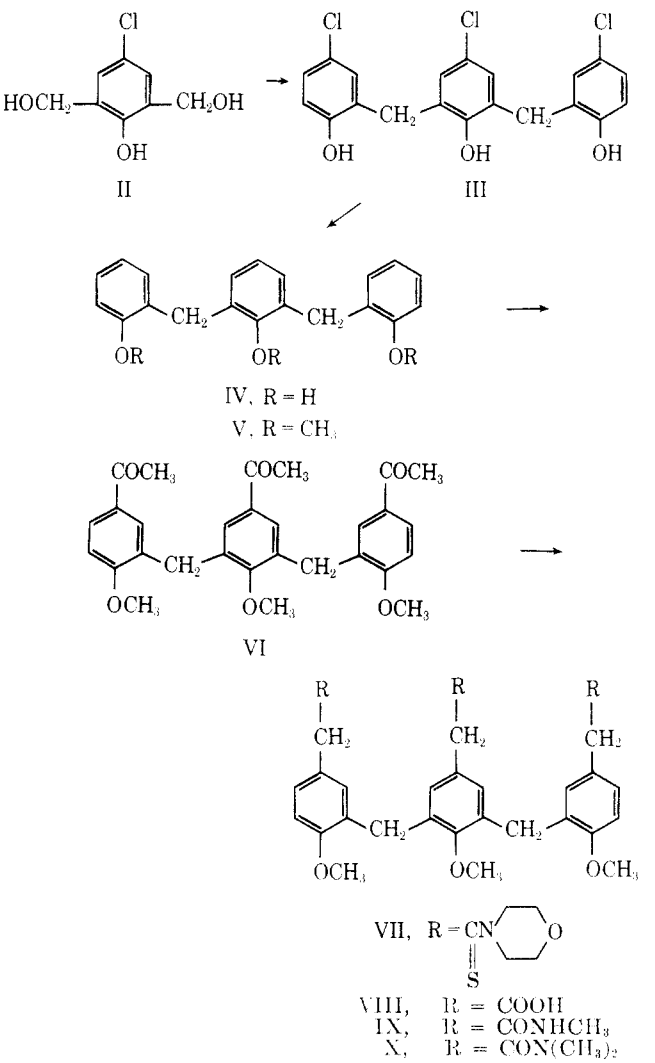
In a previous publication[2] we described an unequivocal synthesis of the "dimer" (Id) and a proof of structure for Ic by its subsequent conversion to Id. We were unable to demonstrate any histamine-releasing ability for compounds Ic and Id. We then turned our attention to the preparation of the "trimers" (Ia and Ib), utilizing some of the chemistry developed in the synthesis of the "dimers." Our goals were only partially achieved; we were able to synthesize Ib but were unsuccessful in the preparation of the more important compound, Ia.



Ia, R = H; n = 1
b, R = CH_3; n = 1
c, R = H; n = 0
d, R = CH_3; n = 0

Our synthetic work in the trimer series was initiated from 2,6-bishydroxymethyl-4-chlorophenol (II).[3] This compound, when condensed with an excess of *p*-chlorophenol under acid catalysis, gave 2,6-bis(2-hydroxy-5-chlorobenzyl)-4-chlorophenol (III) in 82% yield (Scheme I). The chloro groups were readily removed by reduction with sodium in liquid ammonia and the resulting triphenol (IV) was exhaustively methylated with methyl iodide and potassium carbonate in boiling acetone to afford 2,6-bis(2-methoxybenzyl)-anisole (V). Treatment of the ether (V) with acetic anhydride in polyphosphoric acid at 60° afforded the triketone (VI) in 72% yield. The ultraviolet spectrum of VI in ethanol showed maxima at 229 and 270 mμ which strongly supports the *p*-methoxy ketone structure.[2,4] This method of acetylation was adopted because of its effectiveness when applied to the acetylation of bis-2-anisylmethane in the "dimer" series.[2]

Scheme I



IV, R = H
V, R = CH_3
VI
VII, R =
VIII, R = COOH
IX, R = $CONHCH_3$
X, R = $CON(CH_3)_2$

(1) R. Baltzly, J. S. Buck, E. J. DeBeer, and F. J. Webb, *J. Am. Chem. Soc.*, **71**, 1301 (1949).

(2) J. I. DeGraw, V. H. Brown, S. A. Ferguson, N. E. Kontaxis, and W. A. Skinner, *J. Med. Chem.*, **9**, 292 (1966).

(3) M. Weiler and K. Berres, German Patent 510,447 (May 18, 1929); *Chem. Abstr.*, **25**, 974 (1931).

(4) N. A. Valyashko and Y. S. Rozum, *J. Gen. Chem. USSR*, **17**, 755 (1947).

Reaction of the triketone (VI) with sulfur and morpholine under the usual Kindler–Pesche conditions[5] proved to be unsatisfactory for preparation of the tris(acetic acid) compound (VIII) *via* the thiomorpholineamide intermediate (VII). However, when we used the modified procedure of Mayer and Wehl,[6] which involved prior formation of the morpholine enamine of VI, a 97% yield of the crude thiomorpholineamide (VII) was obtained. The amide could be recrystallized from methanol to give material melting at 95–100° whose analysis was barely acceptable. Alkaline hydrolysis of VII gave a poor yield of the tris acid (VIII) after extensive purification.

The N-methyl- (IX) and N,N-dimethylamides (X) were prepared from VIII *via* the acid chloride. Reduction of IX with either lithium aluminum hydride or diborane[7] gave low yields of an unidentifiable syrup. However, reduction of the dimethylamide (X) afforded the N,N-dimethyl "trimer" Ib, characterized as its tripicrate.

Biological Evaluation.—The tripicrate of Ib (57.6 mg) was converted to the syrupy trihydrochloride (29.0 mg, 93%) by stirring with 1.0 g of Dowex 2 (chloride) resin in 20 ml of 95% methanol for 16 hr. The nearly colorless hydrochloride possessed an infrared spectrum similar to that of the dimer hydrochloride (Id).[2] Aqueous solutions (see Table I) of the hydrochloride salt were administered intravenously to anesthetized dogs,[8] and a commercial preparation of the trimer Ia was run as a control. Blood samples (2–3 ml) were obtained prior to, and at 2- and 30-min intervals following injection. The blood pressures were continuously recorded while histamine levels were measured by the procedure of Shore, *et al.*[9]

TABLE I

BLOOD PRESSURE AND HISTAMINE LEVELS

Compd	Dose, μg/kg	Time after injection, min	Blood pressure, mm[a]	Plasma histamine level, μg/100 ml[b]
Ia[c]	...	0	128	2.0
	100	2	60	41.4
	100	30	90	3.5
Ib[c]	...	0	180	1.2
	100	2	178	1.2
	100	30	182	1.4
	250	2	182	1.9
	250	30	178	1.5
Ib[d]	...	0	180	1.8
	200	2	185	1.5
	200	30	175	1.7
	500	2	150	4.5
	500	30	165	2.4

[a] Blood pressure measurements were ±5% of average. [b] Histamine measurements were ±10% of average. [c] Average of two animals. [d] Single animal.

Compound Ib was found to be without significant effect on the animals' blood pressure or histamine level, while the control was quite active at 100 μg/kg. However, Baltzly, *et al.*,[1] reported that a preparation rich in this "trimer" (Ib) exerted a strong blood pressure lowering effect. Unfortunately, we were unable to prepare Ia for direct comparison, but the data for Ib led us to suspect that the pure N-methyl "trimer" (Ia) may also be inactive. Thus, the possibility exists that the true histamine releasers may be very active, higher molecular weight species which contaminate the different preparations described in the literature.[1]

Experimental Section

2,6-Bis(2-hydroxy-5-chlorobenzyl)-4-chlorophenol (III).—To a melt (40°) of 50 g (0.39 mole) of *p*-chlorophenol were added 10.0 g (53 mmoles) of 2,6-bis(hydroxymethyl)-4-chlorophenol[3] and 1.0 ml of concentrated HCl. The mixture was stirred at 40° for 4.5 hr, and excess *p*-chlorophenol was removed by distillation at reduced pressure. The white, crystalline residue was washed with benzene and the crystals were collected to give 19.3 g (82%) of product, mp 234–238°. An analytical sample, recrystallized from benzene–methanol, had a melting point of 233–235°.

Anal. Calcd for $C_{20}H_{15}Cl_3O_3$: C, 58.6; H, 3.68; Cl, 26.0. Found: C, 58.6; H, 3.68; Cl, 25.7.

2,6-Bis(2-hydroxybenzyl)phenol (IV).—To 600 ml of liquid NH_3 in a Dry Ice–methanol bath (−75°) was added dropwise, 18.5 g (0.8 g-atom) of sodium and 19.3 g (47 mmoles) of III in 300 ml of anhydrous tetrahydrofuran. The mixture was stirred at −75° for 6 hr, then allowed to warm up to room temperature over a period of 14 hr. Excess sodium was destroyed with NH_4Cl, followed by the cautious addition of a little water. The mixture was acidified (pH 2) with 6 *N* HCl and extracted with CH_2Cl_2. The CH_2Cl_2 layer was separated, dried over $MgSO_4$, and evaporated *in vacuo* to leave a gummy residue. The gum was taken up in hot toluene. After cooling, 11.9 g (82%) of tan crystalline material was collected; mp 158–160°; Bender, *et al.*,[10] reported mp 161–162°.

2,6-Bis(2-methoxybenzyl)anisole (V).—To a suspension of 43.0 g of pulverized K_2CO_3 and 162 ml of acetone were added 9.5 g (31 mmoles) of 2,6-bis(2-hydroxybenzyl)phenol and 81 ml (1.3 moles) of methyl iodide. The mixture was refluxed 42 hr, the potassium salts were filtered off, and the filtrate was evaporated *in vacuo* to yield an orange syrup. The syrup was taken up in $CHCl_3$ and washed with water, and the $CHCl_3$ layer was dried over anhydrous $MgSO_4$. Evaporation *in vacuo* gave an orange syrup which crystallized at room temperature (8.85 g). Recrystallization from cyclohexane yielded 8.1 g (75%) of white crystals, mp 100–103°. An analytical sample from cyclohexane had a melting point of 101–103°.

Anal. Calcd for $C_{23}H_{24}O_3$: C, 79.3; H, 6.94. Found: C, 78.9; H, 6.91.

2,6-Bis(2-methoxy-5-acetylbenzyl)-4-acetylanisole (VI).—A mixture of 23.4 g (67.3 mmoles) of V, 66.5 ml (0.71 mole) of acetic anhydride, and 580 ml of polyphosphoric acid was stirred at 60° for 16 hr. The resulting, dark solution was cooled to −5° and 2 l. of water was added over 4 hr with stirring (temperature below 17°). Failure to adhere to these conditions for destruction of the phosphate complex resulted in a dark, tarry product. The yellow precipitate was collected by filtration and resuspended in 700 ml of ice water. The pH was adjusted to 8 by the addition of 20 ml of concentrated NH_4OH at 0–5°. The solid was collected and washed with water and 200 ml of cyclohexane. The damp material was dissolved in $CHCl_3$, dried over $MgSO_4$, and twice treated with decolorizing carbon. The $CHCl_3$ was evaporated *in vacuo* and the residue was recrystallized from 300 ml of EtOAc to yield 21.4 g (67%) of white crystals, mp 150–153°; a second crop of 1.6 g (5%) was obtained by concentration of the mother liquors. An analytical sample, mp 156.5–158.5°, was obtained from EtOAc; λ_{max}^{EtOH} 229 mμ (ε 40,700), 270 mμ (ε 36,700).

Anal. Calcd for $C_{29}H_{30}O_6$: C, 73.4; H, 6.37. Found: C, 73.1; H, 6.33.

Trismorpholinethioamide of 2,6-Bis(2-methoxy-5-carboxymethylbenzyl)-4-carboxymethylanisole (VII).—To 3.2 g (6.8 mmoles) of VI was added 0.72 g (22.4 mmoles) of sulfur, 0.1 g

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(6) R. Mayer and J. Wehl, *Angew. Chem. Intern. Ed. Engl.*, **3**, 705 (1964).

(7) H. C. Brown and P. Heim, *J. Am. Chem. Soc.*, **86**, 3566 (1964).

(8) In conducting the research reported herein, the investigator(s) adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

(9) P. A. Shore, A. Burkhalter, and V. H. Cohn, Jr., *J. Pharmacol. Exptl. Therap.*, **127**, 182 (1959).

(10) H. L. Bender, A. G. Farnham, I. W. Guyer, F. N. Apel, and T. B. Gibb, Jr., *Ind. Eng. Chem.*, **44**, 1619 (1952).

(0.5 mmole) of *p*-toluenesulfonic acid, and 6.4 ml (73 mmoles) of morpholine. The mixture was stirred at 130° for 5 hr and cooled to room temperature, and 50 ml of cold 50% MeOH was added. The mixture was stirred in the cold 1 hr, and the yellow solid was collected by filtration and washed with water to afford 5.1 g (97%). This material was used in the next step. An analytical sample, mp 95–100°, was obtained by recrystallization from methanol.

Anal. Calcd for $C_{41}H_{51}N_3O_6S_3$: C, 63.3; H, 6.61; S, 12.4. Found: C, 63.3; H, 6.37; S, 11.8.

2,6-Bis(2-methoxy-5-carboxymethyl)-4-carboxymethylanisole (VIII).—To 5.1 g of the thioamide VII was added 60 ml of 2-methoxyethanol and 60 ml of 20% KOH. The mixture was refluxed for 24 hr and filtered, and the filtrate was evaporated *in vacuo* to near dryness. The brown syrup was taken up in water, chilled, and acidified (pH 1–2) with HCl. The mixture was warmed on a steam bath while stirring for a few minutes, and the supernatant was decanted from the gummy material. The brown gum was crystallized from water, collected by filtration, and taken up in EtOAc. The solution, left in a refrigerator for 2 days, gave a tan crystalline material (1.22 g) which was triturated in fresh EtOAc to give 0.66 g, mp 175–181°. An analytical sample, mp 183–185°, was obtained by recrystallization from methyl ethyl ketone.

Anal. Calcd for $C_{29}H_{30}O_9$: C, 66.6; H, 5.79. Found: C, 66.5; H, 5.79.

Tris-N-methylamide of 2,6-Bis(2-methoxy-5-carboxymethylbenzyl)-4-carboxymethylanisole (IX).—A mixture of 0.70 g of VIII and 15 ml of α,α-dichloromethyl methyl ether was refluxed 15 min and evaporated *in vacuo*. Benzene (20 ml) was added and evaporated *in vacuo*. The residual gum was dissolved in 15 ml of dry CH_2Cl_2 and treated with a slow stream of gaseous methylamine for 10 min. The flask was stoppered and kept at room temperature for 20 hr. The solvent was removed *in vacuo* and the residue was stirred with water for 15 min. The tan solid was collected by filtration, washed with 1.5 *N* NH_4OH and water, and dried to leave 0.69 g (92%) of tan crystals, mp 215–220°. A small portion was recrystallized from ethanol for analysis: mp 221–223°.

Anal. Calcd for $C_{32}H_{39}N_3O_6$: C, 68.4; H, 7.00; N, 7.48. Found: C, 67.9; H, 6.91; N, 7.37.

Reduction of this amide with lithium aluminum hydride in hot tetrahydrofuran gave a negligible amount of amine, while treatment with diborane in tetrahydrofuran gave a syrupy amine whose picrate or hydrochloride could not be made to solidify.

Tris-N,N-dimethylamide of 2,6-Bis(2-methoxy-5-carboxymethylbenzyl)-4-carboxymethylanisole (X).—The acid chloride from 0.60 g (1.1 mmoles) of VIII was taken up in 10 ml of anhydrous CH_2Cl_2, chilled, and treated with a mixture of 3 ml (45 mmole) of anhydrous dimethylamine in 5 ml of anhydrous CH_2Cl_2. The mixture was stirred briefly, then the flask was stoppered and allowed to stand at room temperature for 21 hr. The mixture was evaporated to dryness *in vacuo*, and the brown material was extracted with EtOAc. The EtOAc extract was washed with water, dried over $MgSO_4$, and evaporated in vacuo giving 0.64 g (93%) of brown gum, λ_{max}^{film} 6.05 μ (C=O of amide). This material did not crystallize and was considered to be of sufficient purity for the next step.

2,6-Bis(2-methoxy-5,β-dimethylaminoethylbenzyl)-4,β-dimethylaminoethylanisole (Ib) Tripicrate.—To a chilled slurry of 0.43 g (11 mmoles) of lithium aluminum hydride in 25 ml of anhydrous tetrahydrofuran was added dropwise a mixture of 0.61 g (1 mmole) of the dimethyl amide (X) in 20 ml of anhydrous tetrahydrofuran. The mixture was refluxed 5.5 hr, and excess hydride was decomposed by the careful addition of absolute EtOH. The reaction mixture was then treated with water, stirred briefly, and evaporated *in vacuo* to near dryness. The pasty material was extracted with ether, and the ethereal extract was dried over $MgSO_4$ and evaporated *in vacuo* to give 176 mg of clear syrup. A chilled solution of 126 mg (0.2 mmole) of the free amine in 10 ml of dry ether was treated with dry HCl, giving white, hygroscopic material. The ether was removed by decantation, and the hydrochloride salt was taken up in absolute EtOH and added to a saturated solution of 0.17 g (0.7 mmole) of picric acid in water. The mixture was left at room temperature for 15 hr. The alcohol–water mixture was decanted, and the gummy material was crystallized from water to yield 215 mg. The yellow solid was taken up in a warm ethanol 2-methoxyethanol mixture and the solution was allowed to stand at room temperature for 15 hr. The alcohol mixture was decanted from the gummy material, and 2-propanol was added, giving yellow crystals, mp 90–100°. A 140-mg portion was twice recrystallized from 80% 2-methoxyethanol to give 70 mg of yellow crystals, mp 171–174°.

Anal. Calcd for $C_{53}H_{61}N_{12}O_{21}$: C, 51.0; H, 4.84; N, 13.4. Found: C, 51.1; H, 5.05; N, 13.3.

Antiinflammatory Compounds Exhibiting Fibrinolytic Activity

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A group of diphenyldioxopyrazolidine derivatives possessing antiinflammatory activity was subjected to the von Kaulla test for fibrinolytic activity. The compounds which had exhibited antithrombotic effectiveness in the clinic showed also a marked fibrinolytic activity. These findings allow the assumption of a parallelity between the antithrombotic properties of some antiinflammatory compounds and their capability of an active participation in the fibrinolytic process.

The clinical application of antiphlogistic drugs is based on a broad pharmacodynamical spectrum of antiphlogistic, analgetic, antipyretic, and uricosuric activities; a more general classification differentiates their antirheumatic and antithrombotic effectiveness. As to the chemical structure, the principal representatives of antiphlogistic drugs are salicylates, corticoids, derivatives of diphenyldioxopyrazolidine, antimalarial drugs, and indole derivatives. In phlebitis and thrombosis, the application of diphenyldioxopyrazolidine derivatives has been constantly spreading in recent time. Neither the principle nor the mechanism of the action of this group, however, have been fully elucidated yet.

Recently von Kaulla[1] elaborated a test for screening the fibrinolytic activity of synthetic organic compounds. This *in vitro* test consists essentially of the formation of clots from recalcified citrated human plasma and the incubation of the clots in media containing the compound to be tested in a number of graded concentrations. The fibrinolysis-inducing capacity is measured and expressed by the specific optimal concentration of the compound studied at which a complete lysis occurs. In a series of papers,[1-3] von Kaulla studied the de-

(1) K. N. von Kaulla, *J. Med. Chem.*, **8**, 164 (1965).
(2) K. N. von Kaulla, *Experientia*, **21**, 439 (1965).
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