Synthesis and Structure-Activity Relationship of Spiro[isochromanpiperidine] Analogues for Inhibition of Histamine Release. 1

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Received December 18, 1979

Two types of 1'-alkylspiro[isochroman-3,4'-piperidines] and 1'-alkylspiro[isochroman-4,4'-piperidines] were prepared and examined for their biological activity. Several of the compounds inhibited the compound 48/80 induced histamine release from isolated rat peritoneal mast cells. The structural requirements for this activity in the present series are discussed.

In a search for unique and potent analgesics, we prepared 3,4-dihydroisocoumarins with a piperidine ring in the spiro form, namely, two types of 1'-alkylspiro[isochroman-3,4'-piperidin]-1-ones (**2**) and 1'-alkylspiro[isochroman-4,4'-piperidin]-1-ones (**7**). Some compounds (**5b**, **6b**, and **7b**) showed weak analgesic activity; however, there were no compounds possessing activity as potont as morphine. Further pharmacological studies revealed that several of these compounds inhibited the compound 48/80 induced release of histamine from isolated rat peritoneal mast cells. Therefore, some spiro[isochromanpiperidine] derivatives and related compounds were synthesized. Their activities were tested and compared with disodium cromoglycate, which has been shown to be effective in the treatment of bronchial asthma by inhibiting the release of mediators of the allergic reaction.[1] We now describe the structure–activity relationships of a new series of spiro[isochromanpiperidines] and rleated compounds for inhibition of histamine release.

Chemistry. 1'-Alkylspiro[isochroman-3,4'-piperidin]-1-ones (**2**), except compounds **2e–i**, were prepared as shown in Scheme I (method A): 1-Alkyl-4-hydroxy-4-[2-(methylcarbamoyl)benzyl]-piperidines (**1**) were prepared according to the method of Vaulx et al.;[2] they were cyclized by heating to give **2**. Compounds **2e–i** were prepared from 1'-benzylspiro[isochroman-3,4'-piperidin]-1-one (**2p**) (Scheme II, method B).[3] Method B is preferable to method A for the synthesis of **2**.

The intermediates (**3**), obtained by the Stobbe condensation of ethyl homophthalate with 1-alkyl-4-piperiodones, were cyclized by heating with a mixture of sodium acetate, acetic acid, and acetic anhydride to give 4-(ethoxycarbonyl)spiro[isochroman-3,4'-piperidin]-4-ones (**4**) (Scheme III, method C).

Spiro[isochroman-4,4'-piperidines] (**6**) were prepared by cyclization of 4-(hydroxymethyl)piperidines (**5**).[4] Oxidation of **6** with chromic acid gave spiro[isochroman-4,4'-piperdin]-1-ones (**7**) (Scheme IV, method D).

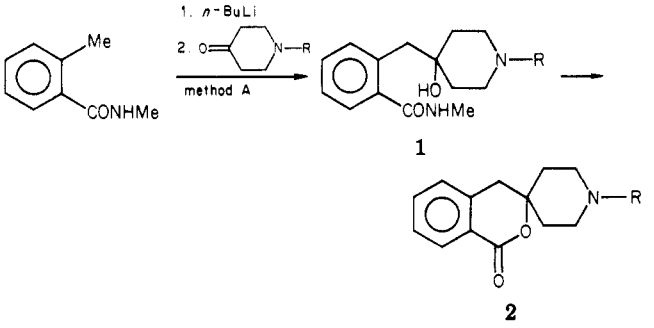
Spiro[cyclohexane-1,3'-isochroman]-1'-one (**9**) was prepared according to method A by using 4-benzylcyclohexanone instead of 1-alkyl-4-piperidones.

4-[(*N*-Benzyl-*N*-methylamino)ethyl]-4-methylisochroman (**12**) was synthesized from α-methyl-α-(β-chloroethyl)phenylacetonitrile, prepared according to the method of Hasting and Cloke[5](Scheme V, method E).

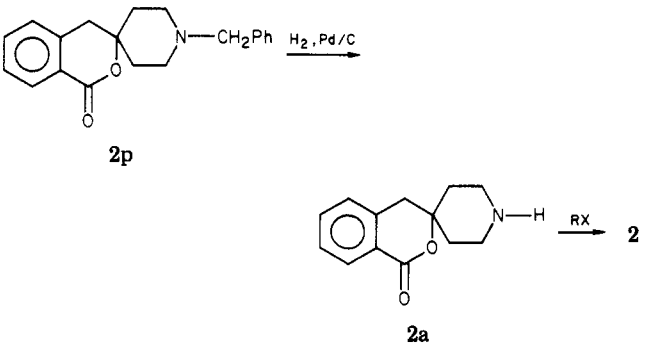
Results and Discussion

All compounds listed in Tables I–III were tested for their inhibitory action on the compound 48/80 induced release of histamine from isolated rat peritoneal mast cells.

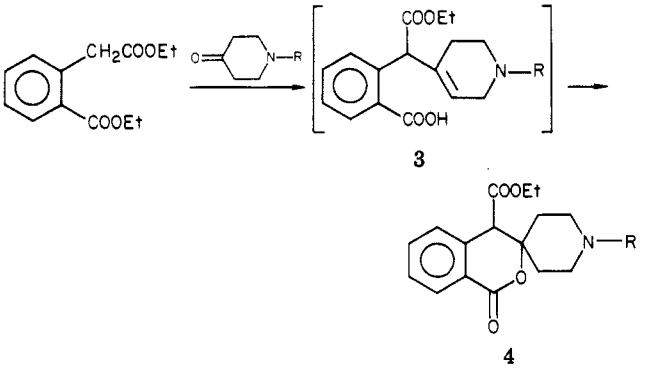
Scheme I. Method A



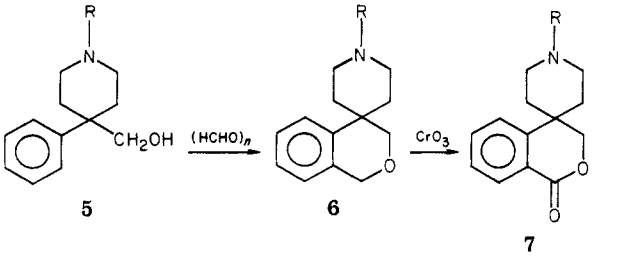
Scheme II. Method B



Scheme III. Method C



Scheme IV. Method D



(1) T. S. C. Orr, *Acta Allergol.*, **30** (Suppl 12), 13 (1975).
(2) R. L. Vaulx, W. H. Puterbaugh, and C. R. Hauser, *J. Org. Chem.*, **29**, 3514 (1964).
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(4) M. A. Iorio, F. Gatta, and E. Menichini, *Farmaco, Ed. Sci.*, **32**, 212 (1977).
(5) R. Hasting and L. B. Cloke, *J. Am. Chem. Soc.*, **56**, 2136 (1934).

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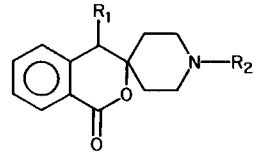
Table I. Inhibition of Histamine Release from Isolated Rat Peritoneal Mast Cells by Spiro[isochroman-3,4′-piperidin]-1-ones



compd	R_1	R_2	synth method	mp, °C	recrystn solvent	yield, %	formula[a]	% inhibn at various dcses[e]		
								2×10^{-4} mol	5×10^{-4} mol	10^{-3} mol
2a	H	H		294–304[b]	Me_2CO–EtOH	75	$C_{13}H_{15}NO_2 \cdot HCl$		inactive	
2b	H	CH_3	A	290–294 dec	EtOH	14	$C_{14}H_{17}NO_2 \cdot HCl$		inactive	
2c	H	$(CH_2)_2CH_3$	A	253–257	Me_2CO–EtOH	25	$C_{16}H_{21}NO_2 \cdot HCl$		inactive	
2d	H	$(CH_2)_3CH_3$	A	258–262	Me_2CO–EtOH	9	$C_{17}H_{23}NO_2 \cdot HCl$	18 ± 0.6	43 ± 1.5	85 ± 8.8
2e	H	$(CH_2)_4CH_3$	B	230–239	EtOH	57	$C_{18}H_{25}NO_2 \cdot HCl$	23 ± 0.8	35 ± 2.6	79 ± 6.1
2f	H	$(CH_2)_5CH_3$	B	235–241	EtOH	58	$C_{19}H_{27}NO_2 \cdot HCl$	32 ± 2.1	67 ± 3.4	
2g	H	$(CH_2)_6CH_3$	B	224–231 dec	EtOH	43	$C_{20}H_{29}NO_2 \cdot HCl$	64 ± 2.7	100	100
2h	H	$(CH_2)_7CH_3$	B	220–221	EtOH	26	$C_{21}H_{31}NO_2 \cdot HCl$		inactive	
2i	H	$(CH_2)_9CH_3$	B	215–219	EtOH	67	$C_{23}H_{35}NO_2 \cdot HCl$		inactive	
2j	H	$CH_2CH{=}CH_2$	A	226–230	EtOH	21	$C_{16}H_{19}NO_2 \cdot HCl$		inactive	
2k	H	$CH_2CH(CH_3)_2$	A	262–271 dec	Me_2CO–EtOH	44	$C_{17}H_{23}NO_2 \cdot HCl$		inactive	
2m	H	cyclohexyl	A	270–275 dec	Me_2CO	2	$C_{19}H_{25}NO_2 \cdot HCl$		inactive	
2n	H	CH_2-c-C_6H_{11}	A	272–280	Me_2CO–EtOH	26	$C_{20}H_{27}NO_2 \cdot HCl$		47 ± 1.7	83 ± 8.3
2o	H	C_6H_5	A	159–159.5	MeOH	9	$C_{19}H_{19}NO_2$		*d*	
2p	H	$CH_2C_6H_5$	A	281–285 dec[c]	Me_2CO–EtOH	37	$C_{20}H_{21}NO_2 \cdot HCl$	20 ± 0.5	40 ± 1.6	76 ± 1.2
2q	H	$(CH_2)_2C_6H_5$	A	260–266 dec	Me_2CO	5	$C_{21}H_{23}NO_2 \cdot HCl$		6 ± 0.2	86 ± 1.8
2r	H	$(CH_2)_3C_6H_5$	A	249–253	Me_2CO–EtOH	34	$C_{22}H_{25}NO_2 \cdot HCl$	5 ± 0.3	18 ± 0.6	85 ± 2.6
4b	$COOC_2H_5$	CH_3	C	119–121	cyclohexane	21	$C_{17}H_{21}NO_4$		inactive	
4j	$COOC_2H_5$	$CH_2CH{=}CH_2$	C	208–210 dec	Me_2CO–EtOH	25	$C_{19}H_{23}NO_4 \cdot HCl$		inactive	
4p	$COOC_2H_5$	$CH_2C_6H_5$	C	195–197 dec	Me_2CO–EtOH	24	$C_{23}H_{25}NO_4 \cdot HCl$		12 ± 1.1	84 ± 2.9
disodium cromoglycate								31 ± 1.3	58 ± 1.9	80 ± 3.1

[a] All compounds were analyzed for C, H, and N, and the results were within ±0.4% of calculated values. [b] For free base, see ref 3 (mp 130–131.5 °C). [c] For free base, see ref 3 (mp 68.5–70.5 °C). [d] See Table IV. [e] $p < 0.05$ using Student's *t* test.

Scheme V. Method E

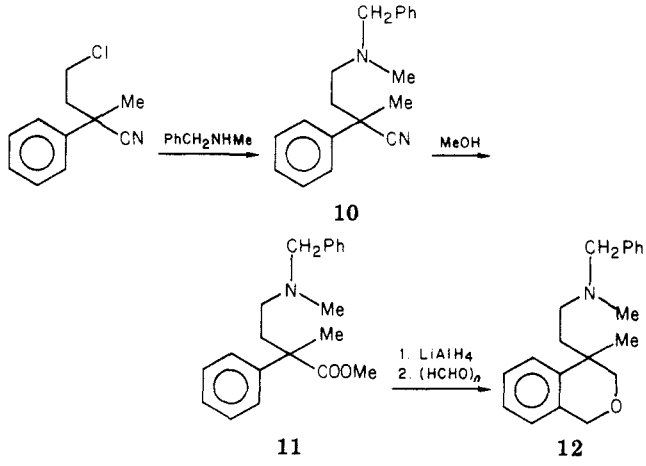
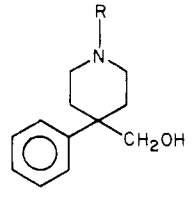


Table II. Inhibition of Histamine Release from Isolated Rat Peritoneal Mast Cells by 4-Phenyl-4-(hydroxymethyl)piperidines



compd	R	formula	mp, °C	% inhibn at 10^{-3} mol dose[c]
5b	CH_3	$C_{13}H_{19}NO$	134-135 (136-137)[a]	inactive
5p	$CH_2C_6H_5$	$C_{19}H_{23}NO$	119-121 (120-122)[b]	inactive
5q	$(CH_2)_2C_6H_5$	$C_{20}H_{23}NO$	136-138 (138)[a]	49 ± 1.8

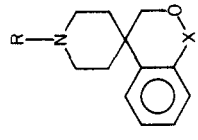
[a] See ref 4. [b] See ref 6. [c] $p < 0.05$ using Student's t test.

Compounds **2o**, **9**, and **12** were insoluble in physiological buffer solution. They were suspended in carboxymethylcellulose (CMC) buffer solution and tested for their ability to inhibit the compound 48/80 induced degranulation of rat mast cells.

The structure–activity relationships were as follows: (1) Compound **2** was synthesized to study the substituents in the piperidine ring. Compounds without a substituent (**2a**) or with a lower alkyl group, e.g., methyl (**2b**), propyl (**2c**), and allyl (**2j**), were inactive (Table I). Analogues from butyl (**2d**) to heptyl (**2g**) showed potent activity; however, increasing the R_2 to octyl (**2h**) or decyl (**2i**) resulted in the loss of the activity. These results suggest that the optimum number of carbon atoms in the R_2 group is 4 to 7. However, branching, as in the isobutyl compound **2k**, resulted in the loss of the activity. Compounds in which R_2 was a cyclic group were synthesized. Compounds with cyclohexyl (**2m**) and phenyl (**2o**) attached directly to the nitrogen atom of the piperidine ring were inactive, while compounds with cyclohexylmethyl (**2n**), benzyl (**2p**), phenethyl (**2q**), and 3-phenylpropyl (**2r**), in which the alkyl chain lies between the nitrogen atom and the cyclic group, were active.

(2) Compounds of type **7** with a piperidino group in spiro form at position 4 of the isocoumarin nucleus were synthesized. As typical substituents of the piperidino group, methyl, benzyl, and phenethyl groups were selected (Table

Table III. Inhibition of Histamine Release from Isolated Rat Peritoneal Mast Cells by Spiro[isochroman-4,4′-piperidines]



compd	X	R	synth method	mp, °C	recrystn solvent	yield, %	formula[a]	% inhibn at various doses[c] 2×10^{-4} mol	5×10^{-4} mol	10^{-3} mol
6b	CH_2	CH_3	D	302-307 (307-308)[b]	Me_2CO-EtOH	41	$C_{14}H_{19}NO \cdot HCl$		inactive	
6p	CH_2	$CH_2C_6H_5$	D	285-299 dec	Me_2CO-EtOH	45	$C_{20}H_{23}NO \cdot HCl$		28 ± 1.4	72 ± 5.4
6q	CH_2	$(CH_2)_2C_6H_5$	D	290-295 (293 dec)[b]	Me_2CO-EtOH	56	$C_{21}H_{25}NO \cdot HCl$	28 ± 1.5	96 ± 4.0	100
7b	CO	CH_3	E	298-300	Me_2CO-EtOH	35	$C_{14}H_{17}NO_2 \cdot HCl$		inactive	
7p	CO	$CH_2C_6H_5$	E	281-288 dec	Me_2CO-EtOH	32	$C_{20}H_{21}NO_2 \cdot HCl$	5 ± 0.7	31 ± 2.2	80 ± 3.6
7q	CO	$(CH_2)_2C_6H_5$	E	104-104.5	cyclohexane	37	$C_{21}H_{23}NO_2$	15 ± 0.5	94 ± 2.8	100

[a] See footnote *a* in Table I. [b] See ref 4. [c] $p < 0.05$ using Student's t test.

Table IV. Inhibitory Effect of Various Compounds on Compound 48/80 Induced Rat Mast Cell Degranulation (0.25 μg/mL)

compd	% inhibn of degranulation[a]
2o	29.1 ± 2.5
free base of 2p	94.5 ± 3.9
9	52.0 ± 3.3
12	−8.0 ± 0.3

[a] Compounds were suspended in 0.5% CMC solution (final concentration 10^{-3} mol/L) and incubated for 15 min at 37 °C prior to the addition of compound 48/80.

III). The effect of the substituents on the level of activity were similar to type 2. Although no remarkable difference was observed in the activity of the two types of 3- and 4-spiro compounds, the latter tended to be more active.

(3) Comparable levels of activity were observed for both isochroman (6) and isocoumarin (2 and 7) analogues, suggesting that the carbonyl group of the isocoumarin ring is not essential for the appearance of activity (Table III). Compounds of type 5, the structure of which corresponds to that of 6 with the isochroman ring opened, were inactive (Table II).

(4) Compound 12, the structure of which corresponds to that of 6p with the piperidine ring opened, was inactive. Compound 9, in which the nitrogen atom of the piperidine ring in 2p is replaced by a methylene group, was active. These results suggest that the nitrogen atom is not critically involved in binding to the active site and plays a role in determining the conformation of the ring system (Table IV).

(5) Conversion of the nitrogen atom in the piperidine ring of 2p into tetraalkylammonium iodide (13) resulted in remarkable reduction of activity; inhibition of histamine release was 28% at 2×10^{-3} mol.

Several compounds of this series were nearly equal in potency to disodium cromoglycate, especially 2f, 2g, 6q, and 7q which were more effective than disodium cromoglycate at 5×10^{-4} mol. Our results suggest that these spiro[isochromanpiperidine] derivatives may possess antiallergic activity similar to that of disodium cromoglycate.

Systematic investigations are under way to determine the structural requirements for the inhibition of compound 48/80 induced histamine release and to study the antiallergic activity of this series.

Experimental Section

Melting points, determined on a Yanagimoto micro melting point apparatus, are uncorrected. Where indicated by the symbols of the elements, the analytical values were within 0.4% of the theoretical values. NMR and/or mass spectra were obtained for all compounds; they were consistent with the structures. NMR spectra were recorded on a Hitachi R-22 spectrometer, and mass spectra were recorded on a Shimadzu LKB-9000 spectrometer.

Method A. 1′-Methylspiro[isochroman-3,4′-piperidin]-1-one (2b). To a cold solution of *N*-methyl-*o*-toluamide (6.4 g, 42 mmol) in dry THF (10 mL) was added dropwise 15% *n*-butyllithium–hexane solution (63 mL, 148 mmol) under a N_2 atmosphere, and the mixture was stirred at room temperature for 40 min. Then, a solution of 1-methyl-4-piperidone (4.8 g, 42 mmol) in dry Et_2O (60 mL) was added over a 30-min period, and the mixture was stirred at room temperature for 2 h and diluted with H_2O. This solution was made basic with 10% aqueous NaOH and extracted with AcOEt. After the solvent was removed, the resulting oily residue was used without further purification. A mixture of the oil, concentrated H_2SO_4 (10 mL), and 50% AcOH (30 mL) was allowed to reflux for 2 h. After cooling, the solution was made basic with 20% aqueous NaOH, and extracted with Et_2O. After the solvent was removed, the salt was formed in benzene with dry HCl. The crude hydrochloride was recrystallized from EtOH to give 1.5 g (14%) of **2b**·HCl, mp 290–294 °C dec. Anal. ($C_{14}H_{18}ClNO_2$) C, H, N.

The other new compounds, prepared in a similar manner, are listed in Table I. Compound 2o was purified by column chromatography (charcoal, CH_2Cl_2) and recrystallized from MeOH to give needles, mp 159–159.5 °C.

Spiro[isochroman-3,4′-piperidin]-1-one (2a). A solution of **2p**·HCl (1.8 g, 5 mmol) in EtOH (160 mL) and H_2O (30 mL) was hydrogenated over 10% Pd/C (0.35 g). After the absorption of H_2 was completed, the solvent was evaporated. The residue was recrystallized from Me_2CO–EtOH to give 1.0 g (75%) of **2a**·HCl, mp 294–304 °C. Anal. ($C_{13}H_{16}ClNO_2$) C, H, N.

Method B. 1′-Hexylspiro[isochroman-3,4′-piperidin]-1-one (2f). To a solution of **2a**·HCl (0.3 g, 1.2 mmol) in dry THF (5 mL) were added K_2CO_3 (0.34 g, 2.5 mmol) and hexyl bromide (0.2 g, 1.2 mmol). The mixture was heated at 80 °C for 2 h, diluted with H_2O, and extracted with Et_2O. After the solvent was removed, the salt was formed in benzene with dry HCl. The crude hydrochloride was recrystallized from EtOH to give 0.23 g (58%) of **2f**·HCl, mp 235–241 °C. Anal. ($C_{19}H_{26}ClNO_2$) C, H, N.

Method C. 4-(Ethoxycarbonyl)-1′-methylspiro[isochroman-3,4′-piperidin]-1-one (4b). To a solution of diethyl homophthalate (2.8 g, 12 mmol) and 1-methyl-4-piperidone (1.4 g, 12 mmol) in dry benzene was added portionwise a 60% dispersion of NaH in mineral oil (0.5 g, 12 mmol) during cooling in ice–water. The mixture was stirred for 3 h, diluted with H_2O, adjusted to pH 6 with 10% HCl, and concentrated. The residue was chromatographed on a column of anion-exchange resin (Dowex 1-X8) and eluted with a mixture of H_2O, MeOH, and AcOH (5:5:1). The eluate was concentrated, and the residue was desalted by column chromatography over a cation-exchange resin (Amberlite IR120) and eluted with a mixture of H_2O, MeOH, and aqueous NH_3 (10:10:1). Removal of the solvent gave 4.4 g of an oil; this was used without further purification. A mixture of the oil (1.8 g), Ac_2O (20 ml), AcONa (1.0 g), and AcOH (10 mL) was allowed to reflux for 10 h. After cooling, the mixture was made basic with 10% K_2CO_3 and extracted with AcOEt. After the solvent was removed, the residue was recrystallized from cyclohexane to give 0.39 g (21%) of **4b**, mp 119–121 °C. Anal. ($C_{17}H_{21}NO_4$) C, H, N.

Compounds 4j and 4p were prepared in a similar manner, and their salts were formed by the usual method.

Method D. 1′-Benzylspiro[isochroman-4,4′-piperidine] (6p). A mixture of 1-benzyl-4-(hydroxymethyl)-4-phenylpiperidine (**5p**)[6] (8.0 g, 28 mmol), paraformaldehyde (6.0 g, 199 mmol), concentrated HCl (70 ml), and dioxane was allowed to reflux for 10 h with bubbling in dry HCl. After cooling, the mixture was adjusted to pH 9–10 with 20% aqueous NaOH and extracted with Et_2O. After the solvent was removed, the salt was formed in benzene with dry HCl. The crude hydrochloride was recrystallized from Me_2CO–EtOH to give 4.2 g (45%) of **6p**·HCl, mp 285–299 °C dec. Anal. ($C_{20}H_{24}ClNO$) C, H, N.

Method E. 1′-Benzylspiro[isochroman-4,4′-piperidin]-1-one (7p). To a mixture of CrO_3 (4.4 g, 44 mmol), AcOH (50 ml), and H_2O (10 mL), was added dropwise at 30–35 °C **6p** (3.2 g, 11 mmol) in AcOH (30 mL). After the mixture stirred for 2.5 h, isopropyl alcohol was added, and the solution was concentrated in vacuo and extracted with AcOEt. After the solvent was removed, the salt was formed in Et_2O with dry HCl. The crude hydrochloride was recrystallized from Me_2CO–EtOH to give 1.3 g (32%) of **7p**·HCl, mp 281–288 °C. Anal. ($C_{20}H_{22}ClNO_2$) C, H, N.

Compound 7q was purified by column chromatography (charcoal, CH_2Cl_2) and recrystallized from cyclohexane to give needles, mp 104–104.5 °C.

4-Benzylspiro[cyclohexane-1,3′-isochroman]-1′-one (9). 4-Benzyl-1-[(*N*-methylcarbamoyl)benzyl]cyclohexanol (8) was prepared from *N*-methyl-*o*-toluamide and 4-benzylcyclohexanone, as described for 2b, and recrystallized from EtOH: mp 181–181.5 °C; yield 21%. Anal. ($C_{22}H_{27}NO_2$) C, H, N. Compound 8 (2.5 g, 7 mmol) was heated for 6 h in tetralin (30 mL) at 180–200 °C. After the tetralin was evaporated in vacuo, the residue was purified

(6) M. Protiva, M. Rajser, V. Trcka, M. Vanecek, J. Nemec, and Z. Sedivy, *Collect. Czech. Chem. Commun.*, **40**, 3904 (1975).

by column chromatography (silica gel; benzene) to give 1.52 g (67%) of **9**, mp 91.5–92 °C (from EtOH). Anal. ($C_{21}H_{22}O_2$) C, H.

***N*-Benzyl-3-cyano-*N*-methyl-3-phenylbutylamine (10).** A mixture of α-methyl-α-(β-chloroethyl)phenylacetonitrile (13 g, 67 mmol) and *N*-methylbenzylamine (2 g, 165 mmol) was heated for 2 h at 140 °C, allowed to cool, and then diluted with H_2O. The solution was washed with Et_2O, made basic with 20% aqueous NaOH, and extracted with Et_2O. After the solvent was removed, the residue was distilled in vacuo to give 13.4 g (72%) of **10**, bp 190–195 °C (5 mmHg). Anal. ($C_{19}H_{22}N_2$) C, H, N.

***N*-Benzyl-3-(methoxycarbonyl)-*N*-methyl-3-phenylbutylamine (11).** A mixture of **10** (12 g, 43 mmol), absolute MeOH (20 mL), and concentrated H_2SO_4 (13 g) was heated in a sealed tube for 25 h at 140 °C. After cooling, the mixture was diluted with H_2O, made basic with 20% aqueous NaOH, and extracted with Et_2O. Evaporation of the solvent gave 5.1 g (38%) of **11** as a colorless oil. Anal. ($C_{20}H_{25}NO_2$) C, H, N.

4-[(*N*-Benzyl-*N*-methylamino)ethyl]-4-methylisochroman (12). Compound **11** (3.2 g, 10 mmol) was reduced with $LiAlH_4$ in Et_2O by the usual method to give a colorless paste, which was dissolved in dry dioxane (20 mL). To this solution were added paraformaldehyde (4 g) and concentrated HCl (30 mL). The mixture was allowed to reflux for 10 h with bubbling in dry HCl, allowed to cool, made basic with 20% aqueous NaOH, and extracted with Et_2O. After the solvent was removed, the residue was purified by column chromatography (Al_2O_3; benzene) to give 0.6 g (25%) of **12** as an oil. Anal. ($C_{20}H_{25}NO$) C, H, N.

1'-Benzyl-1'-methyl-1-oxospiro[isochroman-3,4'-piperidinium] Iodide (13). A mixture of **2p** (0.5 g, 1.6 mmol), methyl iodide (2 g), and dry Et_2O (20 mL) was stirred for 24 h at room temperature. The precipitate of **13** which separated out was filtered, washed with Et_2O, and dried in vacuo: mp 168–171 °C; yield 0.7 g (95%). Anal. ($C_{21}H_{24}INO_2$) C, H, N.

Biological Tests. Method I. Male Wister rats weighing 300–350 g were stunned and exsanguinated by cutting the corotid arteries. Ten milliliters of a buffered physiological salt solution [NaCl, 154 mmol; KCl, 2.7 mmol; $CaCl_2$, 0.9 mmol; glucose, 5.6 mmol; and Tris-HCl buffer, 10 mmol (pH 7.4)] were injected into the abdominal cavity, and the abdominal wall was gently massaged for 90 s. The fluid of the abdominal cavity was collected and centrifuged at 100 g for 5 min at 4 °C. The pellet was resuspended in fresh buffered physiological salt solution. The peritoneal cell suspension contained approximately 10% mast cells. The cell suspension (1.8 mL in each test tube) was prewarmed at 37 °C for 5 min, the test compound, dissolved in 0.1 mL of the same buffer solution, was added, and incubation was continued for 15 min. Thereafter, compound 48/80, dissolved in 0.1 mL of buffer solution, was added to obtain a final concentration of 0.25 μg/mL, and incubation was continued for another 15 min. The histamine-releasing process was stopped by chilling the test tube in ice–water; this was followed by centrifugation at 400 g for 5 min at 0 °C. A few drops of 1 N HCl were added to the supernatants and to suspensions of the sediments; they were resuspened in 2 mL of a fresh buffer solution and placed in boiling water for 5 min. Histamine in those samples was extracted and determined by the *o*-phthalaldehyde spectrofluorometric procedure of Shore et al.[7] All measurements were carried out in duplicate. In many cases, the fluorescence of the histamine-OPT fluorophore was not affected by the test compound after repeated extractions. However, in some cases, fluorophore formation was appreciably hampered even after several extractions. In those cases, the recovery of histamine from media containing various amounts of histamine and test compounds was measured beforehand, and the histamine content in the samples was estimated, based on this corrected standard curve. Histamine release was expressed in percent of total histamine content of the cells. The spontaneous release, ranging from 1 to 5%, was subtracted. The percent inhibition of histamine release was calculated according to the formula $[(A - B)/A]100$, where A represents the mean percentage of histamine release induced by compound 48/80 alone and B represents the mean percentage of histamine release elicited by compound 48/80 following pretreatment of the test compound. All data were expressed as mean values ± SEM and each value was calculated from data obtained for at least four rats. The statistical significance of the results was determined by Student's t test ($p < 0.05$). If inhibition of histamine release was less than 10%, the test compound was considered as inactive.

Method II. Mast cells, harvested as described above, were incubated for 15 min at 37 °C in media containing the test compounds in CMC suspension. A few drops of mast cell solution were placed in a 37 °C microbath and exposed for 15 min to compound 48/80 solution (final concentration 0.25 μg/mL). Morphological changes of the mast cells were observed under an inverted phase-contrast microscope (Nikon MD, ×600) and in each case more than 100 mast cells were counted. The percent inhibition of degranulation was calculated, using the formula $100[1 - [(X - Z)/(Y - Z)]]$, where X represents the mean percentage of degranulated cells pretreated with the test compound and then treated with compound 48/80, Y represents the mean percentage of degranulated mast cells exposed to compound 48/80 alone, and Z represents the mean percentage of cells undergoing degranulation in the control medium.

(7) For a method of the fluorometric assay of histamine in tissues, see P. A. Shore, A. Burkhalter, and V. J. Cohn, Jr., *J. Pharmacol. Exp. Ther.*, **28**, 127, 182 (1959).

Quantitative Structure–Activity Relationships by Distance Geometry: Thyroxine Binding Site[1]

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The binding data for 27 analogues of thyroxine interacting with human prealbumin have been fitted to a simple distance geometry model of the binding site. The novel aspect of the calculation is that a general definition of chirality has been implemented, so that the model site exhibits the observed stereospecificity. The calculated free energies of binding of the ligands fit the experimental data with a root mean square deviation of 0.5 kcal/mol. Three additional analogues not included in the original data set fit equally well. The geometry of the proposed binding site matches the X-ray crystal structure of the tetraiodothyronine–prealbumin complex with a root mean square deviation of 1.0 to 1.6 Å.

This is the third paper in a series on a novel method for deducing the geometry and chemical nature of a macromolecular binding site, given only the chemical structures and observed free energies of binding for a series of ligands. The first paper[2] discussed the basic simplifying assumptions employed and the format for framing up one's hypothesis about the modes of binding of the ligands. The second article[3] elaborated on additional algorithms de-

(1) This work was supported by National Science Foundation Grant PCM8002602.

(2) Crippen, G. M. *J. Med. Chem.* **1979**, *22*, 988.
(3) Crippen, G. M. *J. Med. Chem.* **1980**, *23*, 599.

0022-2623/81/1824-0198$01.00/0