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Synthesis and Biological Activity of Spiro[isocoumarin-piperidines] and Reltaed Compounds. I

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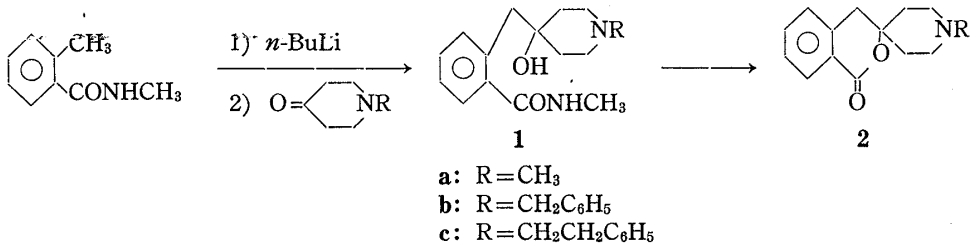
1′-Alkylspiro[isochroman-3,4′-piperidin]-1-ones (**2**) and 1′-alkylspiro[isochroman-4,4′-piperidines] (**4** and **5**) were prepared and examined for analgesic activity. Several of the 4-spiro compounds (**3**, **4**, and **5**) were found to have analgesic activity as potent as that of aminopyrine, while the 3-spiro compounds (**2**) were inactive. Further pharmacological studies revealed that several of these compounds inhibited the histamine release induced by compound 48/80 from isolated rat peritoneal mast cells.

Keywords——isocoumarin; isochroman; piperidine; analgesic activity; histamine-release inhibition; structure–activity relationship: spiro compound

In a search for unique and potent analgesics, we prepared two types of 3,4-dihydroisocoumarins with a piperidine ring in spiro form, namely, 1′-alkylspiro[isochroman-3,4′-piperidin]-1-ones (**2**) and 1′-alkylspiro[isochroman-4,4′-piperidin]-1-ones (**5**). These compounds were designed by reference to the molecular theory for narcotic analgesics proposed by Beckett and Casy.[1] Several compounds showed analgesic activity as potent as that of aminopyrine, but there were no compounds having activity as potent as that of morphine. Further pharmacological studies revealed that several of these compounds inhibited the histamine release induced by compound 48/80 from isolated rat peritoneal mast cells. The results are reported herein.

1′-Alkylspiro[isochroman-3,4′-piperidin]-1-ones (**2**)[2] were prepared as shown in Chart 1. Thus, 1-alkyl-4-hydroxy-4-(2-N-methylcarbamoylbenzyl)piperidines (**1**), prepared according to the method of Vaulx, *et al.*,[3] were cyclized by heating in a mixture of acetic and sulfuric acids to give **2**.



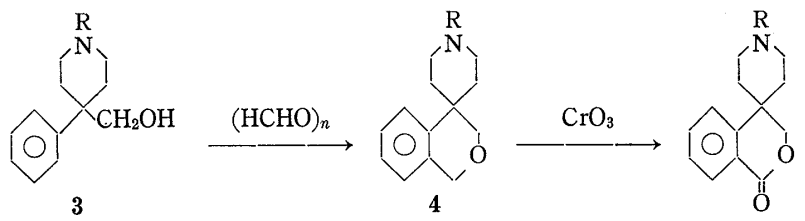


Chart 1

1′-Alkylspiro[isochroman-4,4′-piperidines] (**4**) were prepared by cyclization of 1-alkyl-4-hydroxymethyl-4-phenylpiperidines (**3**) according to the method of Iorio *et al.*[4] Oxidation of **4** with chromic acid gave 1′-alkylspiro[isochroman-4,4′-piperidin]-1-ones (**5**).

The analgesic activities of the compounds listed in Table I were determined by the phenylquinone method.

The compounds of types **3**, **4**, and **5**, 4-spiro compounds, were active while the compounds of type **2**, 3-spiro compounds, were inactive. This result may be explained in terms of the marked structural difference between the two types: The phenyl group of the former, bonds directly with quarternary carbon atom, whereas in the latter, a methylene group lies between the phenyl group and the quarternary carbon atom. The effect of N-substituents on the activity was examined. The N-methyl analogs (**3a**,[4] **4a**,[4] and **5a**) showed activity comparable to that of aminopyrine, while the N-benzyl (**3b**,[5] **4b**, and **5b**) and N-phenethyl (**3c**,[4] **4c**,[4] and **5c**) analogs showed markedly lower activity. The lactone carbonyl group seemed not to be essential for the activity, because no remarkable differences were observed among the activities of the isochroman (**4**) and isocoumarin (**5**) derivatives.

All the compounds listed in Table I were tested for inhibitory action on the compound 48/80-induced release of histamine from isolated rat peritoneal mast cells. Their activities were compared with that of disodium cromolycate (DSCG), which has been shown to be effective in the treatment of bronchial asthma.[6]

Both types of spiro compounds, 3- (**2**) and 4- (**4** and **5**) spiro compounds, were active. However, the compounds of type **3**, having a structure which corresponds to that of **4** with the isochroman ring opened, were inactive. The effects of substituents of the piperidine ring on the level of activity of these compounds were similar. Namely, the N-methyl derivatives (**2a**, **4a**, and **5a**) were inactive, while the N-benzyl (**2b**, **4b**, and **5b**) and N-phenethyl (**2c**, **4c**, and **5c**) derivatives were nearly equal in potency to DSCG. In particular, compounds **4c** and **5c** were more effective than DSCG at 5×10^{-4} mol.

These results suggest that these spiro[isochroman-piperidines] may possess antiallergic activity similar to that of DSCG. This finding led us to study the structure-activity relationship of this series. The results will be reported in the near future.

Table I. Biological Activities

Compd.[a] No.	Analgesic activity			% inhibition of histamine release at various doses		
	Dose mg/kg *p.o.*	Effect %	ED_{50}	2×10^{-4} mol	5×10^{-4} mol	10^{-3} mol
2a	25	1.9	—		Inactive	
	100	—	—			
2b	50	2.3	—			
	100	—	—	20±0.5	40±1.6	76±1.2
3a	25	47.7	27		Inactive	
	100	84.2				
3b	100	12.6	—		Inactive	
3c	100	31.5	100	—	—	49±1.8
4a	5	20.8	12		Inactive	
4b	100	45.6	100	—	28±1.4	72±5.4
4c	100	92.6	54	28±1.5	96±4.0	100
5a	25	60.6	20		Inactive	
	100	88.7				
5b	100	38.9	100	5±0.7	31±2.2	80±3.6
5c	100	83.3	60	15±0.5	94±2.8	100
Aminopyrine	100	54.6	91	—	—	—
DSCG	—	—	—	31±1.3	58±1.9	80±3.1

a) All compounds, except **3a—c** and **5c**, were tested as their hydrochlorides.

Experimental

Melting points, determined on a Yanagimoto micromelting point apparatus, are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained on a Hitachi 22-FTS spectrometer at 90 MHz, with tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Shimadzu LKB-9000 spectrometer, and infrared (IR) spectra on a Nipponbunko A-102 spectrometer.

4-Hydroxyl-1-methyl-4-(2-N-methylcarbamoylbenzyl)piperidine (1a)——To a cold solution of N-methyl-*o*-toluamide (6.4 g, 42 mmol) in dry THF (10 ml), 15% *n*-butyl lithium-hexane solution (63 ml, 148 mmol) was added dropwise under an N_2 atmosphere. 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. The whole was stirred at room temperature for 2 hr and diluted with H_2O, then made acidic with 10% HCl. This solution was made basic with 10% NaOH solution and extracted with AcOEt. The solvent was evaporated off, and the residue was recrystallized from AcOEt to give 5.2 g (46%) of **1a**, mp 148—152°. *Anal.* Calcd for $C_{15}H_{22}N_2O_2$: C, 68.67; H, 8.45; N, 10.68. Found: C, 68.90; H, 8.30; N, 10.51. NMR (in $CDCl_3$) δ: 1.40—1.65 (4H, m, C_3–H and C_5–H), 2.15 (3H, s, NCH_3), 2.05—2.26 (4H, m, C_2–H and C_6–H), 2.72 (2H, s, CH_2), 2.85 (3H, d, $J=6$ Hz, $NHC\underline{H}_3$), 3.10 (1H, b, $N\underline{H}CH_3$), 5.60 (1H, s, OH), 7.05—7.46 (4H, m, Ar–H).

1′-Methylspiro[isochroman-3,4′-piperidin]-1-one (2a)——A mixture of **1a** (1.8 g, 69 mmol), conc. H_2SO_4 (7 ml), and 50% AcOH (28 ml) was allowed to reflux for 2 hr, made basic with 20% NaOH solution, and extracted with Et_2O. The extract was evaporated to dryness, and the salt was formed in dry Et_2O with dry HCl. The crude hydrochloride was recrystallized from EtOH to give 1.2 g (76%) of **2a**·HCl, mp 290—294° (dec.). *Anal.* Calcd for $C_{14}H_{18}ClNO_2$: C, 62.80; H, 6.73; N, 5.23. Found: C, 62.35; H, 6.76; N, 5.31. IR ν_{max}^{KBr} cm^{-1}: 1715. NMR (in D_2O) δ: 2.98 (3H, s, NCH_3), 3.16 (2H, s, C_4–H), 7.95 (1H, dd, $J=1$, 6 Hz, C_8–H).

1′-Benzylspiro[isochroman-4,4′-piperidine] (4b)——A mixture of 1-benzyl-4-hydroxymethyl-4-phenylpiperidine (**3b**)[5] (8.0 g, 28 mmol), paraformaldehyde (6.0 g, 149 mmol), conc. HCl (70 ml), and dioxane (50 ml) was allowed to reflux for 10 hr while being bubbled through with dry HCl. After cooling, the mixture was made basic with 20% NaOH solution and extracted with Et_2O. The extract was evaporated to dryness, and the salt was formed in dry benzene with dry HCl. The crude hydrochloride was recrystallized from Me_2CO–EtOH to give 4.2 g (45%) of **4b**·HCl, mp 285—299° (dec.). *Anal.* Calcd for $C_{20}H_{24}ClNO$: C, 72.82; H, 7.33; N, 4.25. Found: C, 72.55; H, 7.35; N, 4.22. NMR (in $CDCl_3$) δ: 3.80 (2H, s, C_3–H), 4.77 (2H, s, C_1–H). MS *m/e*: 293 (M^+).

1′-Benzylspiro[isochroman-4,4′-piperidin]-1-one (5b)——A solution of **4b** (3.2 g, 11 mmol) in AcOH (30 ml) was added dropwise at 30—35° to a mixture of CrO_3 (4.4 g, 44 mmol), AcOH (50 ml), and H_2O (10 ml). The mixture was stirred for 2.5 hr, then isopropylalcohol was added, and the solution was concentrated *in vacuo*, made basic with 20% NaOH solution, and extracted with AcOEt. The extract was evaporated to dryness, and the salt was formed in dry Et_2O with dry HCl. The crude hydrochloride was recrystallized from Et_2O–Me_2CO to give 1.3 g (32%) of **5b**·HCl, mp 281—288° (dec.). *Anal.* Calcd for $C_{20}H_{22}ClNO_2$: C, 69.87; H, 6.45; N, 4.07. Found: C, 69.59; H, 6.30; N, 3.98. IR ν_{max}^{KBr} cm^{-1}: 1715. NMR (in D_2O) δ: 3.53 (2H, s, NCH_2), 4.35 (2H, s, C_3–H), 8.00 (1H, dd, $J=2$, 7 Hz, C_8–H). MS *m/e*: 307 (M^+).

Compounds **5a** and **5c** were prepared in a similar manner. **5a**: Yield 45%. mp 85—88° (from benzene–cyclohexane). *Anal.* Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 73.08; H, 7.53; N, 5.99. IR ν_{max}^{Nujol} cm^{-1}: 1715. NMR (in $CDCl_3$) δ: 2.32 (3H, s, NCH_3), 4.91 (2H, s, C_3–H), 8.06 (1H, dd, $J=2$, 8 Hz, C_8–H). MS *m/e*: 231 (M^+). **5c**: Yield 37%. mp 104—104.5° (from cyclohexane). *Anal.* Calcd for $C_{21}H_{23}NO_2$: C, 78.47; H, 7.21; N, 4.36. Found: C, 78.65; H, 7.28; N, 4.30. IR ν_{max}^{Nujol} cm^{-1}: 1720. NMR (in $CDCl_3$) δ: 4.38 (2H, s, C_3–H), 8.02 (1H, dd, $J=2$, 7 Hz, C_8–H). MS *m/e*: 231 (M^+).

Analgesic Assay——The procedure employed was a modification of the method of Hendershot and Forsaich.[7] The test compounds and aminopyrine were suspended in 0.5% carboxymethylcellulose solution. Drugs were administrated (*p.o.*) to six male dd strain mice weighing 17—25 g 30 min before challenge with phenylquinone. Writhing was induced by an *i.p.* injection of 0.1 ml/10 g of 0.02% phenylquinone in 5% aqueous EtOH in mice. The number of writhings was measured for 20 min after phenylquinone administration.

Inhibition of Histamine Release——Male Wistar rats weighing 300—350 g were stunned and exsanguinated by cutting the carotids. Ten ml 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 sec. The fluid in the abdominal cavity was collected and centrifuged at 100 g for 5 min at 4°. 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° for 5 min, then 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 give 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, and the tube was centrifuged at 400 ***g*** for 5 min at 0°. A few drops of 1 N HCl

were added to the supernatants and to suspensions of the sediments, then they were each resuspended in 2 ml of fresh buffer solution, and placed in boiling water for 5 min. Histamine in the samples was extracted and determined by the *o*-phthalaldehyde spectrofluorometric procedure of Shore *et al.*[8] 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 after several extractions. In these cases, the recovery rate of histamine from media containing various amounts of histamine and test compounds was measured beforehand, and the histamine content in the samples was estimated on the basis of this corrected standard curve. Histamine release was expressed as a percentage of total histamine content of the cells. The spontaneous release, ranging from 1—5%, was subtracted. The % inhibition of histamine release was calculated according to the formula $[(A-B)/A]\times 100$, 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 after pretreatment with the test compound. All data were expressed as mean values $\pm$SEM and each value was calculated from data obtained for at least 4 rats. The statistical significance of the results was determined by means of Student's *t* test ($p<0.05$). If inhibition of histamine release was less than 10%, the test compound was considered as inactive.

References and Notes

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