Synthesis and Selective Activity of Cholinergic Agents with Rigid Skeletons. I*

SHOJI TAKEMURA, YASUYOSHI MIKI, FUSAO KOMADA,
KEIKO TAKAHASHI, and Arito S. SUZUKI

Faculty of Pharmaceutical Sciences, Kinki University and Department of Pharmacology, Kinki University School of Medicine

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A common conformation was assumed to be involved in the potent muscarinic activities of 1-(+)-muscine, acetylcholine, and (+)-trans-2S-acetoxyccyclopropyl-1S-trimethylammonium. The conformation of other muscarinic agents was also considered from this point of view; three types of quaternary salts having semi-rigid piperidine ring structures which satisfy the hypothetical requirements were synthesized, and their muscarinic activities were examined. These synthetic compounds showed selective muscarinic activity.

Keywords—cholinergic agents; muscarinic activity; design and synthesis; semi-rigid conformation; quaternary salts of piperidine derivatives; dose-response curve

Many conformational hypotheses have been presented in relation to the interaction of acetylcholine with the muscarinic receptor. These proposals were largely based on the crystal structures of energetically favored conformations in solution of acetylcholine and its agonists. However, the conformation interacting with the receptor may or may not be similar to that in the crystals or in solution. This paper considers the conformation of cholinergic agents binding on the receptor from an alternative point of view, and describes the synthesis of some compounds with semi-rigid structures designed on the basis of our working hypothesis, together with their biological activities.

Acetylcholine (Fig. 1-3) has an open-chain of six atoms from the methyl carbon of the acetyl group (C\(_1\)) to the ammonium nitrogen (N\(^8\)), and most muscarinic agents commonly possess terminal methyls (C\(_1\)) and basic ammonium or tertiary nitrogen atoms (N\(^8\)). Semi-rigid moieties are found in 1-(+)-muscine (Fig. 1-1) and (+)-trans-2S-acetoxyccyclopropyl-1S-trimethylammonium (Fig. 1-2), which are both well-known potent muscarinic agents. Their skeletons can be superposed on each other, as well as on that of acetylcholine, as shown in Fig. 1-4. Thus the six atoms from C\(_1\) to N\(^8\) of each compound are located in nearly the same relative positions. The approximate distances between the main atoms and the torsion angles of the bonds in this conformation of acetylcholine are also given in Fig. 1. The skeletons

1) A part of this work was reported at the 98th Annual Meeting of the pharmaceutical Society of Japan, Okayama, 1978.
2) Location: a) Kowakae, Higashi-Osaka, 577, Japan; b) 380, Nishiyama, Sayama-cho, Minamikawachigun, Osaka, 589, Japan.
of other known potent muscarinic agonists and antagonists are shown for comparison. Fig. 2 shows the three-dimensional projections of such compounds.\textsuperscript{13}

All these compounds (1—3, 5—7, 9 and 11) have two oxygen atoms which probably correspond to the oxygen atoms in the ester moiety of acetylcholine. Fig. 3 shows the positions of four atoms (C\textsuperscript{1}, O\textsubscript{a}, O\textsubscript{b} and N\textsuperscript{6}) of each of these compounds located as close as possible to the corresponding atoms in the acetylcholine conformation 3. The projection A (Fig. 3) shows that O\textsubscript{b} of these compounds is located to the right. This implies a corresponding arrangement of the sites in the muscarinic receptor.

On this basis, some semi-rigid compounds, types A, B, and C, were designed (Fig. 4).

Compound 17 (one of the type A compounds) was obtained by acetylation of 3-hydroxy-1-methylpiperidine (15) followed by quaternization with methyl iodide to yield the desired methiodide (Chart 1). The synthesis of the second type, B, was performed starting from 4-carboethoxy-1-methyl-3-oxopiperidine (13), which was obtained by a modification of McElain's method.\textsuperscript{14} In the Dieckmann cyclization of the diester (12), the best result was obtained using sodium ethoxide in a toluene–benzene mixture. The reduction of the keto-ester (13) with lithium aluminum hydride was unsuccessful. Dreiding\textsuperscript{15} reported that β-keto-

\textsuperscript{13} The matching was done with Dreiding stereomodels. When the activities of both enantiomers were found in the literature, the more active one was chosen.


esters were generally not reduced to diols by lithium aluminum hydride, but gave complex mixtures. Since a large excess of sodium borohydride often successfully reduces carboxy-esters to hydroxymethyl groups,\textsuperscript{16} the reduction was carried out by refluxing the hydrochloride of

Fig. 4. Three-dimensional Projections of the Designed Compounds having Semi-rigid Skeletons

Chart 1
13 with a large excess of sodium borohydride and sodium hydroxide in methanol, yielding a mixture of cis and trans diols, 18a and 18b, in a ratio of approximately 4:1. A milder reduction of 13 with sodium borohydride gave a mixture of hydroxy-esters, 19a and 19b. The isomer 19a was converted to 18a by reduction with a large amount of sodium borohydride. The diols, 18a and 18b, gave the ketals, 20a and 20b, on reaction with acetone, in the presence of a molecular sieve and p-toluenesulfonic acid, respectively. Each ketal was then quaternized with methyl iodide to yield methiodide (21a and 21b).

In compounds 18 to 21, the “a” series was concluded to have cis-configuration, and the “b” to be trans on the basis of the nuclear magnetic resonance spectra (NMR) of 19 and the hydrogenation of 13 with rhodium-aluminum oxide. In the NMR of the hydroxy-ester, 19a, the proton signal of C-3 appeared at 4.3 ppm (m, $W_{1/2}=7.5$ Hz), and its coupling constant with the C-4 proton was estimated to be not larger than 5 Hz, while in compound 19b, the C-4 proton with a somewhat larger coupling constant, 11 Hz. These observations suggest that compounds of the series “a” have cis configuration and those of “b” are trans. It is known that the hydrogenation of $\beta$-keto-esters in the presence of a rhodium-aluminum oxide catalyst gives cis adducts selectively. Therefore, the keto-ester, 13, was hydrogenated with 5% rhodium on alumina at 50—60° under pressure. The product was identified as 19a by thin-layer chromatography. The series “a” is thus confirmed to have cis configuration.

The synthesis of the compounds of type C, 24, 25, and 27, was started from 1-methyl-3-piperidone. The reactions of 14 with d-l,1,2-propanediol and 2,3-butanediol in the presence of sulfuric acid gave the corresponding ketals, 22, and 23, respectively. Each spiroketal was converted to the quaternary salt (24 and 25) with methyl iodide. The reaction of 14 with d-l-lactic acid in the presence of sulfuric acid gave a lactone, 26, which was quaternized with methyl iodide to give 27. The ketal-amine, 22, was found to be a mixture of two isomers (22a and 22b) on the basis of its NMR spectrum. The proton signals of C-CH$_3$ appeared as two doublets at 1.13 ppm (6.0 Hz) and 1.28 ppm (5.5 Hz). The ratio of the components was estimated from the integral values of the signals to be 3:2. One of the isomers (22a) was obtained by stirring the mixture with aluminum oxide in ether. This product showed only one doublet of C-CH$_3$ signal at 1.13 ppm. The other isomer (22b) could not be isolated in a pure state. The conversion of 22 to 24 was carried out using the isomer “a”. Similarly, the ketal, 23, was also a mixture of two isomers (23a and 23b) which exhibited two doublets of C-CH$_3$ signals at 1.24 ppm (6H, 5.5 Hz) and 1.14 ppm (6H, 6.5 Hz). The ratio of the components was estimated to be 4:1 by comparison of the integral values. Treatment of the mixture with aluminum oxide gave one component (23a) which exhibited a C-CH$_3$ signal at 1.24 ppm. The other isomer (23b) could not be isolated. The stereochemistries of 22a and 23a were not established. Compound 25 was obtained from 23a.

The lactone, 26, gave one spot on thin-layer chromatography, exhibited one doublet corresponding to C-CH$_3$ in NMR, and appeared to be a single component on distillation under reduced pressure, but no definitive evidence was obtained on its stereochemistry.

**Pharmacology and Discussion**

The cholinomimetic activities of the compounds 17, 21a, 21b, 24, 25 and 27 were examined. The compounds tested, except for compound 25, produced contraction; compound 25 showed relaxation. The contractions produced by these compounds were inhibited by the selective ganglion blocking agent atropine, but were not inhibited by the selective ganglion blocking.

20) The details of the pharmacological studies will be presented shortly.
agent hexamethonium. These results suggest that the compounds 17, 21a, 21b, 24 and 27 act on muscarinic receptors, but not on nicotinic receptors. The contractions produced by these compounds were not potentiated by the selective cholinesterase inhibitor, eserine, unlike those by ACh. This suggests that the compounds tested may be not destroyed by cholinesterase.

The intrinsic activities of those compounds on ACh receptors were examined. The maximum contraction produced by compound 17 was similar to that produced by ACh. That is, the intrinsic activity of compound 17 is the same as that of 1. However, the dose-response curve was shifted to the right compared with that of ACh. This suggests that the affinity of this compound for ACh receptors may be lower than that of ACh. The cis-ketal, 21a, showed the same type of dose-response curve as ACh but at a much higher concentration region, while the intrinsic activity of the trans-isomer, 21b was smaller than that of 21a (about 0.6). This suggests that some structural requirement for binding is not satisfied in 21b. The affinities of the compounds 24 and 27 were almost the same as that of 21b, whereas their intrinsic activities were smaller. It is interesting that the addition of one methyl group in 24 (compound 25) gave an exceptional compound, causing relaxation. This suggests the presence of strict steric requirements near the methyl group in the ACh receptor.

![Chemical structures](image)

**Fig. 5. Dose-response Curves (guinea-pig ileum)**

**Experimental**

3-Acetoxy-1-methylpiperidine (16) — A mixture of 3-hydroxy-1-methylpiperidine$^{21}$ (15, 780 mg), pyridine (1 ml) and Ac$_2$O (1 ml) was refluxed for 5 min. The solvent was removed by distillation in vacuo, and H$_2$O was added to the residue. The mixture was neutralized with K$_2$CO$_3$ and extracted with CHCl$_3$. The CHCl$_3$ layer was dried over Na$_2$SO$_4$ and the solvent was evaporated off. The residue was distilled to give

an oil, 110—115° (bath temperature, 980 mg, 77%). This was directly converted to the methiodide (17).
IR νmax cm⁻¹ → 1720 (CH₃COO).

3-Acetoxy-1-methylpiperidine Methiodide (17)—Compound 16 (500 mg) in acetonitrile with an excess of MeI (500 mg) was allowed to stand for 2 hr with occasional stirring. The separated crystals were collected by filtration and recrystallized from iso-PrOH, mp 148—149.5° (1.29 g, 82%). Anal. Calcd. for C₃H₅NO₂: C, 36.14; H, 6.07; N, 4.68. Found: C, 36.04; H, 6.11; N, 4.65. IR νmax cm⁻¹ → 1725 (CH₃COO). NMR (DMSO-d₆): δ → 1.5—2.0 (m, 4H, 4-CH₂), 2.07 (s, 3H, CH₃COO), 3.22 (s, 6H, N⁺(CH₃)₂), 3.3—3.7 (m, 4H, 2-CH₂, 6-CH₂), 5.1 (m, 1H, 3-CH).

4-Ethenylocarbonyl-1-methyl-3-oxopiperidine (13)—Benzene (135 ml) was added to a solution of toluene (24 ml), EtOH (88 ml), and Na (4.73 g) and was azetropically distilled with excess EtOH under stirring. Ethyl 1-ethoxyethenylmethyl-1-methyl-γ-aminobutyrate (12, 45.2 g) was added to the stirred residue and the EtOH formed was distilled with C₂H₅. After heating for 1 hr at 110—120°, H₂O was added and the mixture was washed with ether, made alkaline by saturating it with K₂CO₃ and extracted with ether. The ether extract was dried (K₂CO₃) and treated with dry HCl, giving the hydrochloride of 13, mp 171—173° dec. (33.8 g, 78%)

 cis- and trans-Ethenylocarbonyl-3-hydroxy-1-methylpiperidine (18a and 18b)—i) Direct Reduction of 13 with Excess NaBH₄; NaBH₄ (8.54 g) was added in portions to a mixture of NaOH (0.99 g) in MeOH (30 ml) and the hydrochloride, 13 (5.0 g) at room temperature. After reflux for 3 hr, H₂O (10 ml) was added to the mixture and the MeOH was distilled off in vacuo. The residue in water was continuously extracted with CHCl₃, dried (K₂CO₃) and separated by preparative TLC to give 18a and 18b in a ratio estimated of 4: 1. The overall yield (18a + 18b) was 58%. 18a was obtained as crystals, mp 116—117°. Anal. Calcd. for C₃H₅NO₂: C, 57.90; H, 10.41; N, 9.65. Found: C, 57.63; H, 10.41; N, 9.55. The trans isomer (18b) was obtained as an oil. As it seemed to be unstable, the crude substance was used for the next step.

ii) Reduction of 19a to 18a: NaBH₄ (170 mg) was added in small portions to the solution of the cis-hydroxy-ester 19a (109 mg) in MeOH (5 ml). After decomposing excess NaBH₄ with H₂O, the reaction mixture was concentrated in vacuo. The residue was dissolved in H₂O, saturated with K₂CO₃, and continuously extracted with CHCl₃. Evaporation of the dried (K₂CO₃) CHCl₃ extract yielded crystals, mp 110° (274 mg, 28.3%). This product was identical with the sample obtained by method “a” as regards spectral data and by mixed melting point determination.

Ethyl cis- and trans-3-Hydroxy-1-methyl-piperidine-4-carboxylate (19a and 19b)—A solution of the hydrochloride of 13 (1 g) in MeOH (6 ml) was stirred with solid K₂CO₃ (0.63 g) for 1 hr, adding NaBH₄ (90 mg) portionwise with stirring. After removal of the solvent in vacuo, the solution was diluted with H₂O and extracted with CHCl₃. The CHCl₃ layer was dried over K₂CO₃ and condensed to give a mixture of 19a and 19b (0.43 g, 58%), which was separated by preparative TLC. 19a: Anal. Calcd. for C₃H₅NO₂: C, 57.73; H, 9.15; N, 7.48. Found: C, 57.77; H, 9.15; N, 7.55. NMR (CDCl₃): δ → 1.29 (3H, t, CH₂CH₃), 1.6—3.2 (7H, m, 2, 5, 6-CH₂, and 4-CH₂), 2.88 (3H, s, N-CH₃), 4.17 (2H, q, CH₂CH₃), 4.30 (1H, m, 3-CH). 19b: Anal. Calcd. for C₃H₅NO₂: C, 57.73; H, 9.15; N, 7.48. Found: C, 57.93; H, 9.32; N, 7.67. NMR (CDCl₃): δ → 1.26 (3H, t, CH₂CH₃), 1.6—3.20 (7H, m, 2, 5, 6-CH₂, 4-CH₂), 2.27 (3H, s, N-CH₃), 3.7—4.1 (1H, d-t, J = 11 Hz, 3-CH), 4.17 (2H, q, CH₂CH₃).

 cis-3-Hydroxy-4-hydroxymethyl-1-methylpiperidine Acetonide (20a)—A mixture of the diol 18a (101.8 mg), acetone (5 ml), TSOH (147 mg), and molecular sieve (type 4Å) (500 mg) was refluxed for 9 hr, then filtered, and the filtrate was made alkali with saturated aqueous K₂CO₃. The solution was distilled in vacuo to remove acetone extracted with CHCl₃, dried (K₂CO₃), and passed through a short column of Al₂O₃. The eluate with CHCl₃ was distilled to give an oil, bp 127° (bath temperature) (95 mg, 72%). The acetonide was methiodized to the (21a) without further purification.

trans-3-Hydroxy-4-hydroxymethyl-1-methylpiperidine Acetonide (20b)—Compound 18b (56 mg) was treated with acetone as described for 20a to give the desired ketal, 20b (46 mg, 71%), bp 105—110° (bath temperature). This was used to prepare methiodide, 21b, without further purification.

 cis-3-Hydroxy-4-hydroxymethyl-1-methylpiperidine Acetonide Methiodide (21a)—A mixture of the ketal 20a (750 mg) in acetonitrile (10 ml) and MeI (5 ml) was allowed to stand overnight. The resulting crystals were filtered off and washed with acetone (935 mg, 71%). The crude 21a was recrystallized from iso-PrOH, mp 191—192°. Anal. Calcd. for C₄H₁₄NO₂: C, 40.38; H, 6.78; N, 4.28. Found: C, 40.50; H, 6.80; N, 4.20.

trans-3-Hydroxy-4-hydroxymethyl-1-methylpiperidine Acetonide Methiodide (21b)—The ketal 20b (40 mg) was treated as described for the preparation of 21a to give crystals of 21b (64 mg, 94%) which were recrystallized from iso-PrOH, mp 252—253°. Anal. Calcd. for C₄H₁₄NO₂: C, 40.38; H, 6.78; N, 4.28. Found: C, 40.17; H, 6.52; N, 4.25.

1-Methylpiperidine-3-spiro-2-(4'-methylhydroxymethylene) (22)—1 A mixture of 1-methyl-3-piperidone hydrobromide (14) (1.0 g) in propylene glycol (1 ml) and H₂SO₄ (0.1 ml) was heated at 80° for 2 hr. A cold solution of K₂CO₃ was added, and the mixture was extracted with ether. The extract was dried (K₂CO₃) and the solvent removed in vacuo to leave an oil (0.42 g, 28%) which appeared to be a mixture of 22a and 22b, as discussed previously.

ii) The hydrobromide (14) (1.0 g) in CHCl₃ (20 ml) was bubbled through with dry HCl gas (309 mg) under cooling. Propylene glycol (2 ml) and a molecular sieves (type 4Å, 1 g) were added and the mixture
was stirred at room temperature for 4.5 hr. Ice and a saturated solution of K₂CO₃ were added to the mixture, which was then extracted with ether and dried (K₂CO₃). The solvent was removed to give an oily residue (0.71 g, 40.5%) which was shown to be a mixture of 22a and 22b.

ii) Isolation of One Isomer (22a): The mixture (0.25 g) in ether (2 ml) was stirred with Al₂O₃ (Merck neutral Art 1077, 3 g) for 3 hr at room temperature, then filtered, and the filtrate was condensed to leave an oily residue (0.09 g, 36%). This was confirmed to be a single component of 22, as described previously, and was converted to the methiodide (24) without further purification.

1-Methylpiperidine-3-spiro-2'-(4'-methylidioxolane) Methiodide (24)—One isomer of 22 (22a) (100 mg) in acetone (2 ml) and MeI (1 ml) was allowed to stand at room temperature, yielding crystals. These were dissolved in CHCl₃, and ether was added to precipitate pure crystals, mp 179—180°. Anal. Calcd. for C₁₈H₂₃INO₄: C, 38.35; H, 6.44; N, 4.47. Found: C, 38.19; H, 6.44; N, 4.51.

1-Methylpiperidine-3-spiro-2'-(4',5'-dimethylidioxolane) (23)—i) A mixture of the hydrobromide (14) (1.0 g) in 2,3-butanediol (1 ml) and H₂SO₄ (0.1 ml) was heated at 80° for 3 hr. A cold saturated solution of K₂CO₃ was added, and the mixture was extracted with ether. The extract was dried (K₂CO₃), and the solvent removed to leave a mixture of 23a and 23b, as described previously.

ii) Isolation of One Isomer (23a): The mixture of 23a and 23b (275 mg) in ether (2 ml) was stirred with Al₂O₃ (Merck neutral Art 1077, 3 g) for 5 hr at room temperature and filtered. The filtrate was condensed to leave an oil which gave a single spot on TLC; it was confirmed to be a single component of 23 by NMR as described previously, and was used to prepare the methiodide (25).

1-Methylpiperidine-3-spiro-2'-(4',5'-dimethylidioxolane) Methiodide (25)—A mixture of the ketal (23a) in acetone (2 ml) and MeI was allowed to stand overnight. The resulting crystals were collected by filtration and recrystallized from EtOH; mp 239—240° (80 mg, 63%). Anal. Calcd. for C₁₃H₂₅INO₄: C, 40.38; H, 6.78; N, 4.28.

1-Methylpiperidine-3-spiro-2'-(4'-methyl-5'-oxidoxolane) (26)—H₂SO₄ (4 g) was dropped into a mixture of 1-methyl-3-piperidone (14) hydrobromide (1 g) and dl-lactic acid (1 ml) under ice-cooling with stirring. The mixture was made alkaline with K₂CO₃ under cooling after adding a small amount of ice, then extracted with ether. The organic layer was dried (K₂CO₃), and the solvent removed to leave an oil (1 g, 61.7%). The product was converted to the methiodide (27).

1-Methylpiperidine-3-spiro-2'-(4'-methyl-5'-oxidoxolane) Methiodide (27)—A solution of the dioxolane (26) (1 g) in acetone (2 ml) and MeI (1 ml) was allowed to stand overnight in a refrigerator. The resulting crystals of crude methiodide were collected by filtration and recrystallized from MeOH—EtOH (1.2 g, 69.6%), mp 219—220°. Anal. Calcd. for C₁₃H₂₅INO₄: C, 36.71; H, 5.55; N, 4.28. Found: C, 36.65; H, 5.36; N, 4.51. IR νmax cm⁻¹: 1815 (COO).

Hydrogenation of 13 to 19a with Rhodium on Alumina—A solution of the keto-ester (13) (1 g) in AcOH (20 ml) was shaken with 5% Rh—Al₂O₃ (200 mg) at 50—60° under H₂ (70 atom) for 9 hr. After cooling to room temperature, the catalyst was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in H₂O (10 ml), made alkaline with K₂CO₃ and extracted with CHCl₃ (15 ml x 3). The extract was dried (K₂CO₃) and the solvent removed by distillation to leave an oily residue which was distilled, bₚ4,7 110—130° (bath temp.) (588 mg, 70%). The product was identical with 19a (TLC and NMR spectra).

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