Synthesis and Bronchodilator Activity of *endo*-2-(2-Cyclopentyl-2-hydroxy-2-phenyl)acetoxy-7-methyl-7-azabicyclo-[2.2.1]heptane Methobromide, a Potent and Long-Acting Anticholinergic Agent

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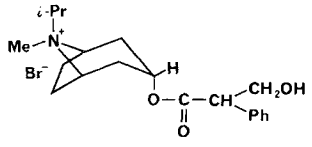
Abstract □ The synthesis of the α-cyclopentylmandelate ester of quaternized *endo*-7-methyl-7-azabicyclo[2.2.1]heptan-2-ol (**4**, RS-11635) is described. The key step of this synthesis consists of the intramolecular *trans*-diaxial epoxide opening of 4-(*N*-methylamino)-1,2-epoxycyclohexane (**8**) to form the *endo*-azabicyclic structure **9**. Evaluation of anticholinergic bronchodilator activity by intravenous administration in methacholine-challenged guinea pigs indicated **4** to be approximately twice as potent as ipratropium bromide (ED_{50} of 1.1 versus 2 μg/kg) and to have a duration of action nearly five times as long (230 versus 50 min). Evaluation of anticholinergic bronchodilator activity by aerosol administration in methacholine-challenged dogs also indicated **4** to be approximately twice as potent as ipratropium bromide and to have a duration of action nearly three times as long.

Anticholinergic agents have been used in the treatment of asthma and bronchitis since the 19th century.[1,2] Although in disfavor for such utility in this country, largely as a consequence of the potential occurrence of well-known side effects, evidence has been presented which shows that aerosolized anticholinergic agents, particularly when quaternized so as to avoid penetration of the blood–brain barrier, provide a highly efficacious and selective means by which to treat chronic obstructive lung disease.[3,4] In particular, a large number of reports on ipratropium bromide (**1**) (Atrovent, Boehringer Ingelheim) have supported this type of therapeutic regimen.[5] We felt that a more potent, longer-acting agent would be desirable, especially from the point of view of increased patient compliance and, possibly, drug stability.

The structure–activity relationships of anticholinergic agents have been reviewed in detail.[6] From the literature, it appears that the overwhelming majority of the therapeutically most active anticholinergics are aminoalkyl esters of 2,2-disubstituted glycolic acids, as exemplified by glycopyrrolate (**2**). In our hands, this agent was about twice as potent as ipratropium bromide (**1**), but had a similar duration of action. In vivo, ipratropium bromide (and presumably most, if not all other aminoalkyl ester anticholinergics) is deactivated mainly by hydrolytic or enzymatic cleavage of the ester function.[7] For the hydrolytic cleavage of quaternized aminoalkyl esters (**3**), an electrostatic interaction between the positively charged nitrogen atom and the ester carbonyl group has been proposed.[8] This interaction increases the polarization of the carbonyl group and, consequently, its susceptibility to nucleophilic attack. In a bicyclic, rigid system such as the tropine moiety of **1**, the nitrogen atom cannot, for purely steric reasons, approach the ester carbonyl group, a feature that should contribute to improved hydrolytic stability. On the other hand, in 2,2-disubstituted glycolates such as **2**, the steric hindrance brought about by the two α-substituents is known to effectively stabilize the ester linkage.[9] We thought that the 7-azabicyclo[2.2.1]heptan-2-ol derivative **4** (RS-11635) would constitute an attractive synthetic target, since it combines the salient structural features of both ipratropium bromide (**1**) and glycopyrrolate (**2**).



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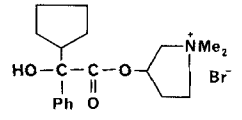

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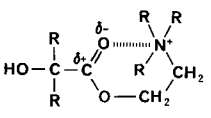

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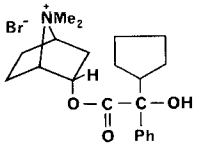

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Results and Discussion

Chemistry—Alkylation of 4-(*N*-trifluoroacetylamino) cyclohexene (**5**)[10] with methyl iodide and sodium hydride in dimethylformamide gave the *N*-methyl derivative **6**, which was transformed into the epoxide **7** (*syn*/*anti* ratio ~1:3) with *m*-chloroperoxybenzoic acid. Although the spontaneous cyclization of epoxy amines to form relatively unstrained azapolycyclic systems under mild deacylation conditions (potassium carbonate in aqueous methanol) has been described,[11,12] only the methylamino epoxide **8** could be isolated under these conditions. Heating **8** in a variety of solvents led to either resinification of the substrate or, with protic solvents, formation of polar products assumed to arise via intermolecular opening of the epoxide by the solvent. Partial success in cyclizing **8** to **9**[13] was achieved using refluxing dimethylformamide, but polar products, presumably formed by reaction of **8** with dimethylamine from the slowly decomposing solvent, were still being obtained. This side-reaction could be suppressed by utilizing the thermally more-stable *N*-methylpyrrolidone instead. Azeotropic distillation of the desired *endo*-bicylic amino alcohol **9** with the solvent allowed for facile isolation of practically pure **9**. (This compound is a mixture of the two possible enantiomers. Only the (*S*)-isomer is shown.) Transesterification of methyl α-cyclopentylmandelate[14] with **9**, followed by quaternization with methyl bromide, completed the synthesis of **4** (Scheme I).

Pharmacology—The anticholinergic bronchodilator activity of **4** was compared with ipratropium bromide (**1**) in anesthetized guinea pigs and dogs according to the methodology described in the *Experimental Section*. In both the guinea pig

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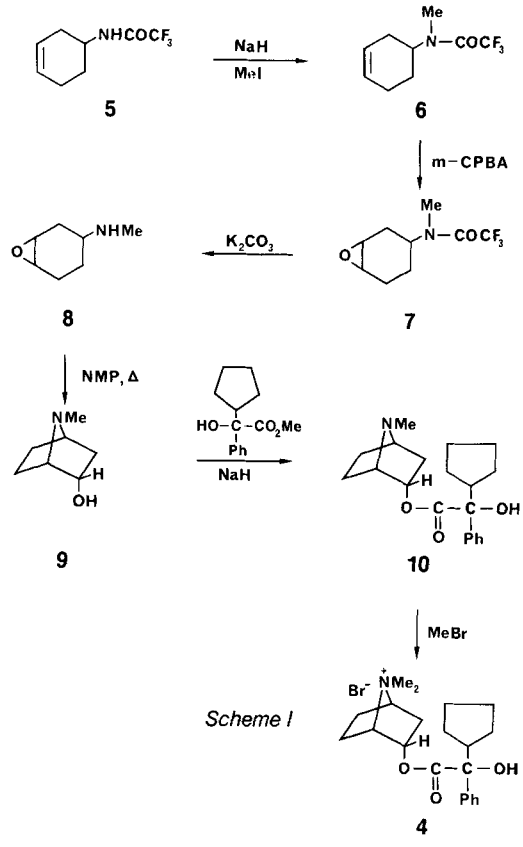

Scheme I

and the dog, **4** was approximately twice as potent as ipratropium bromide at inhibiting increases in tracheal pressure induced by methacholine. It should be emphasized that in both species, the slopes of the dose–response curves differed and that relative potencies were calculated at only the ED_{50} level, which was 1.1 μg/kg for **4**, and 2 μg/kg for ipratropium bromide. Of greater interest, the duration of action as expressed by $t_{1/2}$ (i.e., the time required for peak inhibition of metacholine-induced tracheal pressure increases to be reduced by 50%) was 230 ± 15 min for **4** versus 48 ± 5 min for ipratropium bromide in guinea pigs and 660 ± 90 min for **4** versus 216 ± 8 for ipratropium bromide in dogs. Thus, **4** was observed to be not only more potent, but longer acting than ipratropium bromide.

Experimental Section

Melting points (uncorrected) were obtained on a Mel-Temp apparatus, IR spectra with a Perkin-Elmer 237 grating instrument, NMR spectra using a Varian A-60 or HA-100 spectrometer, and mass spectra with either an Atlaswerke CH-4 or CH-7 instrument. Combustion analyses were performed by Syntex Analytical Research and Atlantic Microlab, Atlanta.

4-(*N*-Methyl-*N*-trifluoroacetylamino)cyclohexene (6)—A stirred solution of **5** (105.9 g, 0.548 mol) and methyl iodide (106 mL, 1.7 mol) in dry dimethylformamide (675 mL) was cooled in an ice bath. Sodium hydride (50% oil dispersion, 29.6 g, 0.617 mol) was added in portions over 1 h. The ice bath was removed, and stirring was continued at ambient temperature for 1 h. The mixture was poured into water (4 L) containing acetic acid (50 mL) and extracted with ether. The organic phase was washed four times with water, dried over magnesium sulfate, filtered, and concentrated by distillation at normal pressure on a steam bath. The residue was distilled at the aspirator through a short Vigreux column to afford 101.7 g (90%) of **6**, bp 107–109°C (12 mm); IR (neat): 1670 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.55–2.0 (br m, 2), 2.0–2.45 (br m, 4), 2.95 (s, 3), 3.7–4.8 (br m, 1), and 5.65 ppm (s, 2).

Anal.—Calc. for $C_9H_{12}F_3NO$: C, 52.17; H, 5.84; N, 6.76. Found: C, 52.41; H, 5.82, N, 6.62.

4-(*N*-Methyl-*N*-trifluoroacetylamino)-1,2-epoxycyclohexane (7)—To a solution of **6** (101.6 g, 0.49 mol) in dichloromethane (2 L), stirred in an ice bath, was added *m*-chloroperoxybenzoic acid (85%, 117 g, 0.576 mol) in portions. After stirring for 4 h at room temperature, the excess peracid was destroyed by addition of aqueous potassium iodide followed by sodium sulfite. The organic phase was separated, washed three times with sodium bicarbonate, and dried over magnesium sulfate. Filtration and evaporation left 107.3 g (98%) of **7** as an oil consisting of 64.9% *anti*- and 25.2% *syn*-isomer (GC). An aliquot was further purified by chromatography (silica gel, hexane:acetone, 4:1) and kugelrohr distillation at 75–90°C (0.3 mm); IR (neat): 3350 and 1670 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.2–2.4 (br m, 6), 2.88 (s, 3), 3.14 (m, 2), and 3.6–4.5 ppm (br m, 1).

Anal.—Calc. for $C_9H_{12}F_3NO_2$: C, 48.43; H, 5.42; N, 6.28. Found: C, 48.82; H, 5.42; N, 6.04.

4-(*N*-Methylamino)-1,2-epoxycyclohexane (8)—To a solution of **7** (107.3 g, 0.48 mol) in methanol (300 mL) was added a solution of potassium carbonate (84.5 g, 0.61 mol) in water (300 mL). After stirring at room temperature for 5 h, most of the methanol was removed on a rotary evaporator. The residue was extracted with ether in a continuous extractor for 48 h. The extract was dried over potassium carbonate, filtered, and evaporated to afford 40.9 g (67%) of **8** as a brown oil; IR (neat): 3270 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.3–1.95 (br m, 4), 2.0–2.4 (br m, 3), 2.35 (s, 3), and 3.10 ppm (d, J = 2 Hz, 2).

***endo*-7-Methyl-7-azabicyclo[2.2.1]heptan-2-ol (9)**—A solution of **8** (40.9 g, 0.322 mol) in dry *N*-methylpyrrolidone (400 mL) was heated under a nitrogen blanket at 155–160°C in an oil bath for 48 h. The dark mixture was subjected to distillation under reduced pressure, and the distillate [bp 86–90°C (12 mm)] was made slightly acidic with concentrated hydrochloric acid. Distillation of the solvent left 23.4 g (44%) of the hydrochloride of **9**, mp 256–259°C. An analytical sample (from methanol:ethyl acetate) had mp 261–262°C. The free base [obtained by continuous extraction of a basified, aqueous solution of the hydrochloride followed by kugelrohr distillation at 70°C (0.3 mm)] formed colorless, easily sublimable crystals, mp 45°C; IR (neat): 3150 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.12 (m, 2), 1.68 (m, 4), 2.21 (s, 3), 3.10 (m, 2), 3.60 (m, 1), and 4.13 ppm (s, 1); ^{13}C NMR ($CDCl_3$): δ 1.59, 24.71, 34.56, 43.72, 60.17, 68.43, and 74.48 ppm; MS: *m/z* 127 (M^+), 98, and 83.

Anal.—Calc. for $C_7H_{14}ClNO$: C, 51.38; H, 8.62; N, 8.56. Found: C, 51.55; H, 8.90; N, 8.82.

***endo*-2-(2-Cyclopentyl-2-hydroxy-2-phenyl)acetoxy-7-methyl-7-azabicyclo[2.2.1]heptane (10)**—To a solution of **9** (500 mg, 3.94 mmol) and methyl α-cyclopentylmandelate (1.05 g, 4.49 mmol) in *n*-heptane (30 mL) was added sodium hydride (50% oil dispersion, 10 mg, 0.21 mmol), and the resulting mixture was refluxed for 18 h through molecular sieves (type 3A) contained in an addition funnel. The cooled mixture was diluted with ether (40 mL) and washed with water, sodium bicarbonate, and again with water. The organic phase was extracted three times with 5% citric acid solution (30 mL each). The acidic aqueous phase was made basic with potassium carbonate and extracted with ether. After drying over magnesium sulfate, the extract was filtered, and the filtrate was evaporated to dryness to give 1.1 g (85%) of **10** as a viscous oil, homogeneous on TLC (silica gel, ethyl acetate: methanol:*tert*-butylamine, 90:10:1).

***endo*-2-(2-Cyclopentyl-2-hydroxy-2-phenyl)acetoxy-7-methyl-7-azabicyclo[2.2.1]heptane Methobromide (4)**—A solution of methyl bromide (4.0 g, 42.1 mmol) in 2-butanone (20 mL) was added to a solution of **10** (1.1 g, 3.33 mmol) in the same solvent (20 mL). After the mixture had stood at room temperature for 48 h, the precipitate was removed by filtration and recrystallized from methanol:ethyl acetate to

furnish 1.15 g (82%) of **4**, mp 205–206°C; IR (nujol mull): 3200 and 1720 cm^{-1}; ^1H NMR (Me_2SO): δ 1.1–2.4 (br m, 15), 3.07 (s, 6), 4.22 (m, 2), 5.05 (m, 1), 5.82 (s, 1), and 7.43 ppm (m, 5).

Anal.—Calc. for $C_{21}H_{30}BrNO_3$: C, 59.43; H, 7.12, N, 3.30. Found: C, 59.33; H, 7.30; N, 3.24.

Pharmacological Methods—The anticholinergic bronchodilator activity of **4** was evaluated in guinea pigs and dogs and compared with ipratropium bromide. In the guinea pig studies, animals were anesthetized with sodium pentobarbital, 30–40 mg/kg ip, and ventilated mechanically with room air via a tracheal cannula attached to a Harvard respirator set at a volume of 10 mL/kg of body weight and frequency of 60 strokes per minute. The right jugular vein was cannulated for intravenous drug administration, and the left carotid artery was cannulated for blood pressure measurement. Inflation pressure was measured with a differential pressure transducer with the high side of the gauge connected to the tracheal cannula, while the low side was exposed to atmospheric pressure.

In the dog studies, animals were anesthetized with sodium pentobarbital (35 mg/kg iv). A midsternal thoracotomy was performed, and the dogs were ventilated mechanically with room air via a Harvard respirator. Tracheal pressure was recorded by a Statham venous pressure transducer attached to a cannula inserted into rubber tubing connecting the endotracheal tube to the respirator air hoses. An ultrasonic nebulizer was placed between the endotracheal tube and respirator air hoses to aerosolize **4** or ipratropium bromide. A femoral vein was cannulated for intravenous administration of methacholine challenges, and a femoral artery was cannulated for measurement of blood pressure. Heart rate was recorded by a cardiotachometer triggered by the R wave of a limb lead II electrocardiogram.

Bronchoconstriction was elicited by intravenous injection of methacholine (2–10 μg/kg in guinea pigs or 1–1.5 μg/kg in dogs). In each animal, a dose of methacholine was selected which produced a moderate increase in tracheal inflation pressure with a return to baseline within 5 min. This dose was repeated at least twice in the control period until approximately equal bronchoconstrictive responses were obtained. Compound **4** or ipratropium bromide was administered to guinea pigs at doses of 0.01–10 μg/kg iv and by aerosol to dogs at concentrations of 0.05 and 0.1 mg/mL, respectively. Guinea pigs were challenged with methacholine at 30 s, at 5, 10, 20, and 30 min, and at every 30 min thereafter as long as significant inhibition was observed. Dogs were challenged with methacholine at 5 min and every 15 min for up to 8 h following administration of **4** or ipratropium bromide.

References and Notes

1. Sigmund, C. G. *Lancet* **1836**, *ii*, 392.
2. Abbott, A. O.; Van Vleet, W. E.; Roberts, A. E.; Saloraone, D. P. *J. Thorac. Surg.* **1955**, *30*, 564.
3. Vastag, E.; Nagy, L.; Miskovits, G. *Eur. J. Clin. Pharmacol.* **1976**, *10*, 201.
4. Ulmer, W. T.; Dorsch, J.; Iravani, J.; Schuler, K. G.; Stempel, G.; Vastag, E. *Arzneim.-Forsch.* **1973**, *23*, 468.
5. Pakes, G. E.; Brogden, R. N.; Heel, R. C.; Spright, T. M.; Avery, G. S. *Drugs*, **1980**, *20*, 237.
6. Rama Sastry, B. V. "Medicinal Chemistry," 3rd ed.; Wiley-Interscience: New York, 1970; p 1544.
7. Adlung, J.; Höhle, K. D.; Zeren, S.; Wahl, D. *Arzneim.-Forsch.* **1976**, *26*, 1005.
8. Hansen, B. *Acta Chem. Scand.* **1962**, *16*, 1927.
9. Nogami, H.; Kigasawa, K.; Ikari, N.; Nakamura, K.; Ohtani, H.; Takeuchi, K.; Haga, S. *Yakugaku Zasshi* **1970**, *90*, 979.
10. Pfister, J. R.; Wymann, W. E. *Synthesis* **1983**, 38.
11. Kozikowski, A. P.; Schmiesing, R. *J. Chem. Soc., Chem. Commun.* **1979**, 106.
12. Glass, R. S.; Deardorff, D. R.; Gains, L. H. *Tetrahedron Lett.* **1978**, 2965.
13. Since the epoxide opening occurs by back-side attack of the methylamino group, only the *anti*-isomer of **8** can react in this fashion. The resulting *endo* stereochemistry is mandated by the *trans*-diaxial nature of the epoxycyclohexane ring opening.
14. Franko, B. V.; Lunsford, C. D. *J. Med. Chem.* **1960**, *2*, 523.

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