Chem. Pharm. Bull. **42**(6) 1286—1290 (1994) Vol. 42, No. 6

Synthesis and Muscarinic Properties of (1*S**,3*R**,5*R**)-Trimethyl(1-methyl-6-oxabicyclo[3.1.0]hex-3-yl)methyl Ammonium Iodide[1)]

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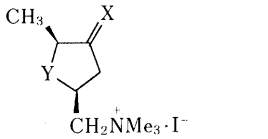
Received October 25, 1993; accepted January 5, 1994

To acquire more information about the so-called "muscarinic subsite", compound 4 was synthesized and tested. The results show that in comparison with deoxamuscarine (23) the muscarinic potency of 4 on M_2 and M_3 subtypes is not significantly altered by the presence of an epoxidic function, which confirms the donor–acceptor hydrogen bonding character of this receptive site. Conversely, there is a negative influence on the transduction processes. In addition, a second hydroxylic function bound on the carbon carrying the terminal methyl of the fourth substituent on the nitrogen dramatically affects the muscarinic behavior; the resulting compounds (11—14) lack any agonist or antagonist activity.

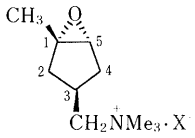
Keywords muscarinic potency; muscarinic affinity, efficacy; cyclopentane carrier; epoxide derivative

Functionally, there seem to be at least three different muscarinic receptor aggregations, M_1 (neuronal type), M_2 (cardiac type), and M_3 (smooth muscle-glandular type). These forms correspond to the first m_1-m_5 types, whose presence was predicted using molecular cloning techniques.[2)] In all cases, the interaction of the agonists with every species of muscarinic populations seems to be governed by recognition at the level of oxygenated moieties of the so-called "muscarinic subsite", which acts as a donor or acceptor for hydrogen bonding.[3)] It is for this reason, for example, that muscarine (**1**) and muscarone (**2**) are 10- to 100-fold more potent than the respective methylene analog **3**, which is unable to supply the drug–receptor complexation process with the energy of the corresponding charge transfer interaction.[4)] However, the electronic cloud has to be supplied by the lone-pairs of the heteroatom rather than by an *endo-* or *exo*-cyclic electron-rich double bond.[5)] Moreover, this receptive area, unlike the site interacting with the ether oxygen of AcCh and muscarine, appears to be relatively large since it can accommodate, without excessively compromising the activity, functions such as propoxy or benzyloxy.[6)]

While carrying forward the study of muscarinic agonists with cyclopentane nuclei,[7)] it seemed useful to employ an epoxidic moiety as the oxygenated function of the ligand interacting with the corresponding muscarinic subsite; for this reason, compound **4** was synthesized and tested. Since its oxygenated function can act as an acceptor for hydrogen bonding in the same way as a carbonyl but has a spatial arrangement analogous to that of a hydroxyl, this compound should be a useful probe of the agonist–receptor interaction.



1 : X=H, OH*trans*, Y=O
2 : X=Y=O
3 : X=H_2, Y=O
23 : X=H, OH *trans*, Y=CH_2



4a : X=Br
4b : X=I

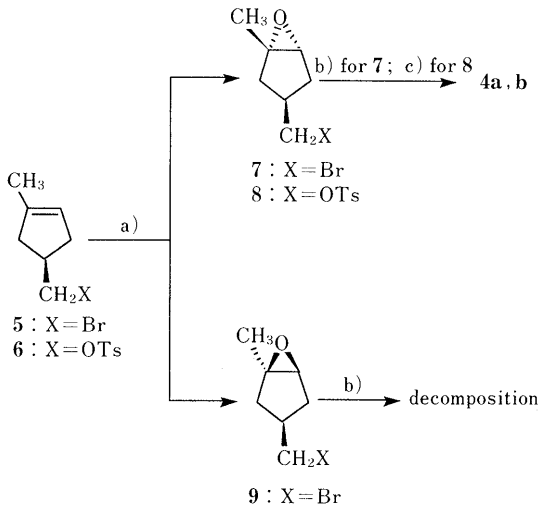
Fig. 1

Chemistry

Compound **4** (bromide **4a** or iodide **4b**) was prepared according to the reaction sequence reported in Chart 1. The configurational isomer of **4** could not be prepared, either because its precursor (the geometric isomer of **8**) is not formed or because subsequent treatment with Me_3N of **9** only induced its decomposition.

To establish the structure of **4**, diols **11**—**14** were prepared according to the methods reported in Chart 2. Treating the amide **15**[8)] with $KMnO_4$ leads almost solely to the *cis* diol **18**, from which the isomer **19** can be obtained by inversion at C_1 by treatment with MeONa, according to the procedure reported for a similar epimerization reaction.[9)] The *trans* diols **20** and **21** were instead prepared from the corresponding epoxides **16** and **17** by treatment with strong acids ($HClO_4$ in the present case) that favor S_N1 opening reaction at C_1.[10)]

By subjecting the precursor of **4a** (compound **7**) to the



a) MCPBA; b) Me_3N ; c) NaI/Me_3N

Chart 1

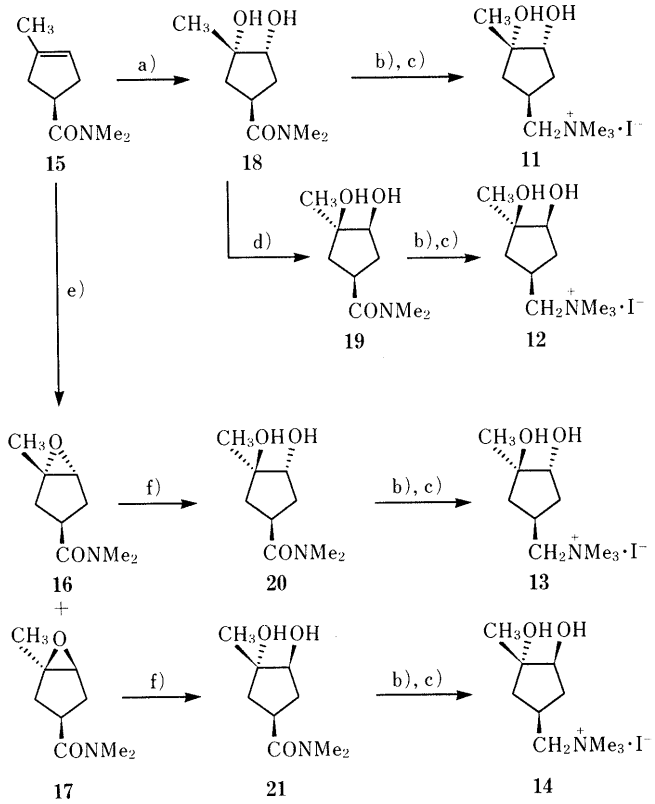


Chart 2

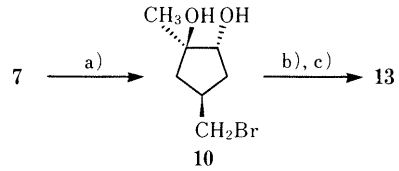


Chart 3

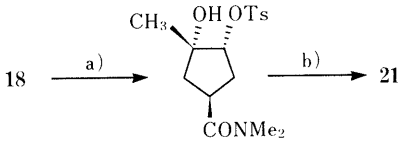


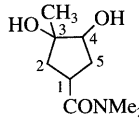
Chart 4

same reaction, one can also generate the methiodide **13** (Chart 3); the structure of **4a** is determined unequivocally by this correlation.

Further stereochemical information was obtained by the inversion of the amide **18** to its isomer **21** *via* the tosylate **22** according to the reported procedure,[11] as illustrated in Chart 4.

Stereochemical Assignments The structures of the amides **16**—**21** were determined on the basis of 1D and 2D ^{1}H-NMR spectra and, in the cases of **18**—**21**, of ^{13}C-NMR spectra (Table I) to confirm, by means of

TABLE I. ^{13}C-NMR Spectral Data for Amides **18**—**21** (ppm, in $CDCl_3$)



C	Compd.			
	18	**19**	**20**	**21**
1	36.14	35.64	37.32	37.81
2	40.73	40.72	40.16	41.72
3	78.66	77.88	80.96	82.96
4	77.79	78.62	81.20	79.99
5	35.44	36.17	37.48	36.17
CH_3	25.11	23.72	20.50	22.22
$N(CH_3)_2$	35.76	36.09	36.05	35.99
	37.11	37.81	37.77	37.77
CO	175.46	178.77	178.91	178.72

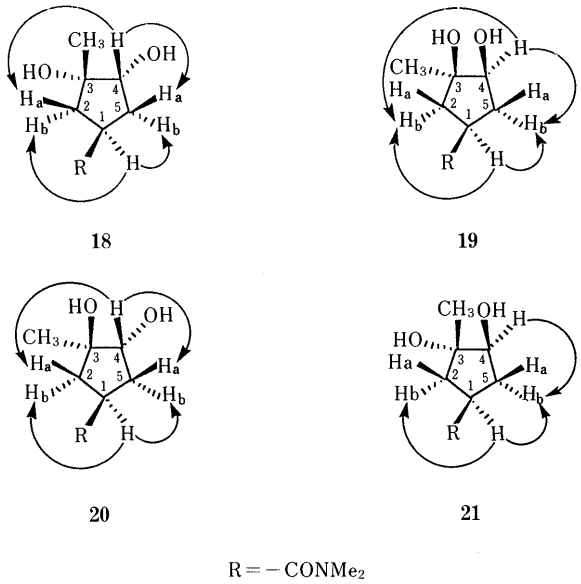


Fig. 2. Principal Correlations Observed in the NOE Spectra

heteronuclear correlation (HETCOR),[12] the attribution of the proton signals. The deshielding effect of the amidic carbonyl function appeared distinctively in the *cis* groups, thereby providing a useful clue for the determination of the structure: CH_3 and C_5-H of **16** that appear, respectively, at δ 1.48 and 3.32, must be in a *cis* relationship with $CONMe_2$, unlike in **17** where the same groups appear at δ 1.44 and 3.25, respectively. Similarly, the chemical shift of C_4-H is δ 3.95 in **18**, and δ 3.70 in **19**; in the same way, C_4-H of **20** is shifted to lower field (δ 3.88) than the corresponding proton of **21** (δ 3.60).

The structures of the amides **18** and **19** were confirmed by 2D-nuclear Overhauser effect (2D-NOE) (NOESY) measurements.[13] Compound **18** showed cross peaks between C_4-H and C_5-H_a and C_2-H_a and between C_1-H and C_5-H_b and C_2-H_b, thus indicating a *trans* relationship between C_1-H and C_4-H. Conversely, in compound **19**, both C_4-H and C_1-H show cross peaks with C_5-H_b and C_2-H_b, confirming a mutual *cis* relationship (Fig. 2). The structures of the amides **20** and **21** were satisfactorily

TABLE II. Comparison of Potency (ED_{50}) and Affinity (K_A) of **4b**, (±)-Deoxamuscarine (**23**), and (±)-Muscarine (**1**) for Muscarinic Receptors of Guinea Pig Left Atrium, Ileum and Bladder[a]

Compd.	Left atrium (M_2)				Ileum (M_3)				Bladder (M_3)			
	$ED_{50}\pm$S.E. $[pD_2]$[d]	$K_A\pm$S.E. $[pK_A]$[d]	^rp[b]	e_r[c]	$ED_{50}\pm$S.E. $[pD_2]$[d]	$K_A\pm$S.E. $[pK_A]$[d]	^rp[b]	e_r[c]	$ED_{50}\pm$S.E. $[pD_2]$[d]	$K_A\pm$S.E. $[pK_A]$[d]	^rp[b]	e_r[c]
4b	$8.5\pm1.8\times10^{-7}$ [6.09±0.096]	$1.35\pm0.085\times10^{-6}$ [5.87±0.028]	1.45	0.086	$6.3\pm1.07\times10^{-7}$ [6.21±0.076]	$8.62\pm0.12\times10^{-6}$ [5.07±0.007]	1.2	0.26	$2.00\pm0.15\times10^{-5}$ [4.72±0.033]	$2.39\pm1.39\times10^{-5}$ [4.73±0.28]	0.85	0.54
23	$1.17\pm1.02\times10^{-6}$ [5.93±0.05]	$3.39\pm0.04\times10^{-5}$ [4.47±0.16]	1	1	$7.41\pm1.3\times10^{-7}$ [6.13±0.07]	$4.17\pm1.03\times10^{-5}$ [4.38±0.12]	1	1	$1.62\pm0.2\times10^{-5}$ [4.79±0.05]	$5.01\pm1.6\times10^{-5}$ [4.30±0.12]	1	1
1	$2.00\pm0.48\times10^{-7}$ [6.69±0.09]	$2.00\pm0.44\times10^{-5}$ [4.70±0.09]	5.75	3.37	$7.90\pm0.66\times10^{-8}$ [7.10±0.04]	$1.10\pm0.32\times10^{-6}$ [5.96±0.12]	9.33	0.26	$2.00\pm0.51\times10^{-6}$ [5.69±0.09]	$2.40\pm0.45\times10^{-5}$ [4.62±0.08]	7.94	3.18

a) All compounds studied behaved as full agonists (compound **23** as reference), except for compound **4b** at the bladder ($E_{max}=0.86$). The results are the means (±S.E.) of four to six independent experiments. *b*) Relative potency calculated with reference to compound **23**.[6] *c*) Relative efficacy determined according to the method previously described.[14-16] *d*) $-\log ED_{50}$ and $-\log K_A$, respectively.

determined by 1D-NOE measurements. The C_1-H and C_4-H *trans* relationship in compound **20** is demonstrated by the observation that irradiation of C_4-H causes NOE at C_5-H_a (2%), C_5-H_b (1%) and C_2-H_a (0.33%), while irradiation of C_1-H gives the same effect at C_2-H_b (1.5%), C_5-H_b (1.6%) and C_2-H_b (0.3%). On the contrary, in compound **21** irradiation of C_4-H causes NOE at C_5-H_b (2.5%) and C_5-H_a (1.2%), while irradiation of C_1-H gives NOE at C_5-H_b (2.5%), C_2-H_b (2.5%), C_5-H_a (0.8%) and C_2-H_a (0.5%) (Fig. 2).

Results and Discussion

The biological profile of the epoxide **4b** at peripheral muscarinic receptors, assessed on isolated guinea pig atrium, ileum and bladder, is compared with those of (±)-deoxamuscarine (**23**) and (±)-muscarine (**1**) (Table II). On all three preparations, it behaves as a full agonist with potency similar to that of (±)-deoxamuscarine and 4- to 10-fold less than that of (±)-muscarine. The affinity is significantly higher than that of the reference agonist **23** in atrium and ileum, and higher than that of (±)-muscarine in atrium. With respect to ligand **23**, compound **4b** displays an affinity about 25-fold higher in the left atrium (M_2 receptors), 5-fold higher in the ileum (M_3 receptors), and 2-fold higher in the bladder (M_3 receptors). As a consequence, while agonist **23** does not discriminate the tissues studied, compound **4b** shows a distinct selectivity (6- to 18-fold) for M_2 receptors. However, the greater lipophilicity of the epoxidic function compared with the hydroxylic one, of which it preserves the same spatial arrangement, seems clearly to favor the receptor recognition process; the contribution is such as to compensate for the absence of the dipole–dipole bond that the ether oxygen of **1** (substituted by a CH_2 in **4b**) establishes with the corresponding receptor site.

Conversely, the relative efficacy of the epoxide **4b** is generally less than that of the reference compound, with a more than ten-fold maximum in atrium. Therefore, the transduction processes of the effect are negatively affected, as suggested previously,[15] by the absence at the muscarinic site of a strong dipole or a polarizable function. This finding confirms that it is not possible to establish *a priori* and unequivocally a correlation between potency and affinity and efficacy.[17] Furthermore, it is interesting to note that in ileum, **4b** displays the same efficacy as (±)-muscarine, about four times lower than that of the reference compound **23**.

As with other compounds structurally correlated to the epoxide **4b**, differences in potency and efficacy between various muscarinic preparations may be attributable to the differences in tissue sensitivity derived from a smaller effective receptor reserve in bladder[18]; this may be the result of a low receptor density or of a less efficient coupling mechanism.

Finally, the methiodides **11**—**14** display neither agonist nor antagonist activity, since they do not show any effect at concentrations below 1×10^{-5}. The introduction of a second hydroxylic group, despite the correct spatial arrangement of the other active functions (compound **11**), causes such steric hindrance that the receptor is unable to recognize these ligands.

Experimental

Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Varian Gemini-200 (200 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) and spin multiplicities are given as s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), dt (double triplet), ddd (3-fold doublet), br (broad), or m (multiplet). ^{13}C-NMR spectra were recorded on a Varian VXR-300 operating at 75.429 MHz, using a 5-mm broad band and probe. They were measured in $CDCl_3$ at room temperature (22 °C), and chemical shifts are given in ppm with reference to $CDCl_3$ (77.00 ppm). The assignments were carried out by running standard Varian distortionless enhancement by polarization transfer (DEPT) and HETCOR sequences. The microanalyses were performed by the Microanalytical Laboratory of our department, and the elemental compositions of the compounds agreed to within ±0.4% with the calculated values. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040—0.063, Merck) by flash chromatography.

4-Bromomethyl-1-methyl-cyclopentene (5) LiBr (4.72 g, 54.34 mmol) was added to a stirred solution of tosylate **6** (5 g, 18.77 mmol)[8] in dry acetone (100 ml) over a period of 30 min at room temperature. After being stirred at 60 °C for 10 h, the mixture was evaporated *in vacuo* and the residue was dissolved in ether. This solution was washed with cold water, dried over Na_2SO_4, and concentrated *in vacuo* to give an oil, which was distilled under reduced pressure: bp 98 °C (45 mmHg) (2.68 g, 82%). ^{1}H-NMR ($CDCl_3$) δ: 1.70 (3H, s, CH_3), 2.01—2.20 (2H, m, cyclo), 2.39—2.58 (2H, m, cyclo), 2.75 (1H, m, cyclo), 3.44 (2H, d, $J=6.8$, CH_2Br), 5.28 (1H, m, CH=C).

(1*S,3*R**,5*R**)-3-Bromomethyl-1-methyl-6-oxabicyclo[3.1.0]hexane (7) and (1*R**,3*R**,5*S**)-3-Bromomethyl-1-methyl-6-oxabicyclo[3.1.0]-hexane (9)** 3-Chloroperbenzoic acid (purity 60%, 7.0 g, 24.34 mmol) was added to an ice-cooled solution of **5** (3.8 g, 21.7 mmol) in CH_2Cl_2 (30 ml) under stirring over a period of 1 h. The mixture was stirred for 1 h at 0 °C and left one night in a refrigerator. The precipitate that appeared was filtered off and the filtrate was successively washed with

aqueous $NaHSO_3$, $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was column-chromatographed using cyclohexane–AcOEt (19 : 1) as the eluent. The first fraction afforded the epoxide **9** (0.45 g, 11%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (3H, s, CH_3), 1.89—1.99 (4H, m, cyclo), 2.48 (1H, m, cyclo), 3.30 (1H, s, C_5-H), 3.38 (2H, d, J=7.6, CH_2Br). The second fraction afforded the isomer **7** (0.72 g, 17%). ^{1}H-NMR ($CDCl_3$) δ: 1.45 (3H, s, CH_3), 1.46—1.60 (2H, m, cyclo), 2.05—2.32 (3H, m, cyclo), 3.29 (1H, s, C_5-H), 3.41 (2H, d, J=5.4, CH_2Br).

(1*S,3*R**,5*R**)-(1-Methyl-6-oxabicyclo[3.1.0]hex-3-yl)methyl *p*-Toluenesulfonate (8)** In the same way, compound **6** was converted to **8** in 21% yield after column chromatography using petroleum ether–Et_2O (7 : 3) as the eluent. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, s, CH_3), 1.40—1.49 (2H, m, cyclo), 1.89—2.15 (3H, m, cyclo), 2.42 (3H, s, $ArCH_3$), 3.20 (1H, s, C_5-H), 3.92 (2H, d, J=5.9, CH_2O), 7.30 (2H, d, ArH), 7.73 (2H, d, ArH).

(1*S,3*R**,5*R**)-Trimethyl(1-methyl-6-oxabicyclo[3.1.0]hex-3-yl)-methyl Ammonium Iodide (4b)** A mixture of **8** (0.6 g, 2.12 mmol) and NaI (1 g, 6.67 mmol) in dry acetone (50 ml) was stirred at 60 °C for 1 h. After cooling it was filtered and concentrated *in vacuo*; the residue was dissolved in $CHCl_3$, washed with water and 2 M $NaHSO_3$, dried over Na_2SO_4 and evaporated *in vacuo*. The resulting iodo intermediate in dry Et_2O (15 ml) was reacted with an excess of anhydrous trimethylamine (7 ml) in a sealed tube at room temperature for 3 d. The white solid was filtered off and crystallized from a dry EtOH–Et_2O mixture to give **4b** (0.4 g, 64%); mp 149—151 °C. ^{1}H-NMR (DMSO) δ: 1.40 (3H, s, CH_3), 1.49 (2H, m, cyclo), 2.10—2.28 (3H, m, cyclo), 3.05 (9H, s, NMe_3), 3.34 (3H, s, CH_2N, C_5-H). *Anal.* Calcd for $C_{10}H_{20}INO$: C, 40.42; H, 6.78; N, 4.71. Found: C, 40.68; H, 6.81; N, 4.66.

The methylbromide **4a** was obtained by allowing **7** to react with an excess of anhydrous trimethylamine in a sealed tube at room temperature for 5 d. The resulting white solid was crystallized from dry iso-PrOH–Et_2O to give **4a** (0.31 g, 33%); mp 139—140 °C. *Anal.* Calcd for $C_{10}H_{20}BrNO$: C, 48.01; H, 8.06; N, 5.60. Found: C, 48.22; H, 8.21; N, 5.84.

(1*S,3*R**,5*R**)-*N*,*N*-Dimethyl-1-methyl-6-oxabicyclo[3.1.0]-hexane-3-carboxamide (16) and (1*R**,3*R**,5*S**)-*N*,*N*-Dimethyl-1-methyl-6-oxabicyclo[3.1.0]-hexane-3-carboxamide (17)** The epoxidation of the amide **15**[8] was carried out by a method similar to that described above. The resulting oil was column-chromatographed using Et_2O–AcOEt (4 : 1) as the eluent. The first fraction afforded the amide **16** in 33% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (3H, s, CH_3), 1.90—2.21 (4H, m, cyclo), 2.89 (1H, m, C_3-H), 2.90—3.01 (6H, ds, NMe_2), 3.32 (1H, s, C_5-H).

The second fraction gave the isomer **17** in 32% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (3H, s, CH_3), 1.95 (1H, dd, J=10.1, 14.0, C_2-H), 2.05 (1H, ddd, J=1.5, 10.1, 14.3, C_4-H), 2.40 (2H, m, C_2-H, C_4-H), 2.91 (6H, s, NMe_2), 3.07 (1H, s, C_3-H), 3.25 (1H, d, J=1.5, C_5-H).

(1*R,3*S**,4*R**)-*N*,*N*-Dimethyl-3,4-dihydroxy-3-methyl-1-cyclopentane Carboxamide (18)** A solution of 1 M $KMnO_4$ (15.2 ml) was added dropwise to a stirred solution of amide **15** (2 g, 13 mmol) in acetone (50 ml) over 1 h at room temperature. The solution was stirred for 3 h, filtered over Celite and evaporated to dryness *in vacuo*. The residue was chromatographed using $CHCl_3$–MeOH (19 : 1) as the eluent to yield **18** (1.3 g, 53%). ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, s, CH_3), 1.88 (1H, dd, J=8.4, 13.6, C_2-H_b), 1.90 (1H, ddd, J=6.4, 6.9, 13.3, C_5-H_b), 2.05 (1H, dd, J=9.0, 13.7, C_2-H_a), 2.23 (1H, ddd, J=5.8, 7.5, 13.3, C_5-H_a), 2.30 (1H, s, OH), 2.45 (1H, d, J=5.2, OH), 2.90 and 3.03 (6H, ds, NMe_2), 3.34 (1H, m, C_1-H), 3.95 (1H, dt, J=7.0, 5.2, C_4-H).

(1*R,3*R**,4*S**)-*N*,*N*-Dimethyl-3,4-dihydroxy-3-methyl-1-cyclopentane Carboxamide (19)** A stirred solution of **18** (0.5 g, 2.67 mmol) in dry iso-PrOH (15 ml) was refluxed for 24 h with CH_3ONa (0.14 g, 2.67 mmol). The solvent was evaporated *in vacuo* and the residue, after treatment with diluted HCl, was extracted with $CHCl_3$. The solution was dried over Na_2SO_4 and evaporated to dryness, affording an oil, which was chromatographed using $CHCl_3$–MeOH (97 : 3) as the eluent: (0.1 g, 20%). ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, s, CH_3), 1.70 (1H, ddd, J=4.6, 6.4, 14.0, C_5-H_a), 1.85 (1H, dd, J=9.5, 14.3, C_2-H_b), 2.0 (1H, dd, J=3.0, 14.3, C_2-H_a), 2.35 (1H, ddd, J=7.0, 9.8, 14.0, C_5-H_b), 2.97 and 3.08 (6H, ds, NMe_2), 3.19 (1H, m, C_1-H), 3.58 (1H, br s, OH), 3.70 (1H, br t, C_4-H), 4.90 (1H, br s, OH).

(1*R,3*R**,4*R**)-*N*,*N*-Dimethyl-3,4-dihydroxy-3-methyl-1-cyclopentane Carboxamide (20)** A solution of 3 M $HClO_4$ (1.5 ml) was added dropwise to a stirred solution of **16** (0.3 g, 1.77 mmol) in dry tetrahydrofuran (THF) (10 ml) at 0 °C over a period of 20 min. The solution was kept at room temperature for 1 h, then neutralized with cold 30% NH_4OH. The solvent was evaporated *in vacuo* and the residue was chromatographed using $CHCl_3$–MeOH–concentrated NH_4OH (9 : 1 : 0.01) to give **20** (0.15 g, 45%). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, s, CH_3), 1.83 (1H, dt, J=1.5, 9.7, 13.7, C_2-H_a), 1.97 (1H, ddd, J=1.2, 9.8, 14.3, C_5-H_b), 2.07 (1H, dd, J=9.8, 13.7, C_2-H_b), 2.18 (1H, ddd, J=4.9, 6.4, 14.3, C_5-H_a), 2.63 (1H, d, J=2.1, OH), 2.91 and 3.06 (6H, ds, NMe_2), 3.40 (1H, m, C_1-H), 3.88 (1H, m, C_4-H), 5.36 (1H, s, OH).

(1*R,3*S**,4*S**)-*N*,*N*-Dimethyl-3,4-dihydroxy-3-methyl-1-cyclopentane Carboxamide (21)** A) In the same way, compound **17** was converted to **21** in 37% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, s, CH_3), 1.78 (1H, dd, J=6.7, 14.1, C_2-H_a), 1.90 (1H, br d, J=14.1, C_5-H_a), 2.00 (1H, dd, J=9.5, 14.0, C_2-H_b), 2.38 (1H, ddd, J=4.6, 9.8, 14.0, C_5-H_b), 2.90 and 3.08 (6H, ds, NMe_2), 2.90 (1H, s, OH), 3.38 (1H, m, C_1-H), 3.60 (1H, d, J=4.6, C_4-H), 4.9 (1H, s, OH).

B) A mixture of **22** (1 g, 2.93 mmol) and CH_3COOK (1.47 g, 14.98 mmol) in *N*,*N*-dimethylformamide (DMF) (37.5 ml) and H_2O (1.25 ml) was refluxed for 24 h. After evaporation of the solvent *in vacuo*, the residue was chromatographed to give **21** (0.2 g, 36%).

Methiodides 11—14 A solution of an amide **18—21** (0.35 g, 1.86 mmol) in THF (20 ml) was added dropwise to a stirred mixture of $LiAlH_4$ (0.4 g, 10.52 mmol) in dry THF (20 ml) at 0 °C over a period of 20 min. The mixture was kept at room temperature for 5 h, then decomposed with H_2O (0.4 ml), a diluted solution of NaOH (0.4 ml) and H_2O (2 ml). After stirring for 1 h, the solid was filtered off and the filtrate was dried over Na_2SO_4. The solvent was evaporated *in vacuo* and the residue was chromatographed using $CHCl_3$–MeOH–concentrated NH_4OH (9 : 3 : 0.01) as the eluent. The resulting amine (75—80%), dissolved in dry Et_2O, was treated with an excess of MeI; after 2 d, the solid was collected by filtration and recrystallized from iso-PrOH to afford the corresponding product, **11—14** (85—90%) (Table III). *Anal.* Calcd for $C_{10}H_{22}INO_2$: C, 38.11; H, 7.03; N, 4.44. For **11** Found: C, 38.32; H, 7.08; N, 4.48. For **12** Found: C, 38.38; H, 6.87; N, 4.40. For **13** Found: C, 37.89; H, 7.19; N, 4.53. For **14** Found: C, 38.25; H, 6.91; N, 4.67.

The methiodide **13** was also obtained by treating compound **10** (0.22 g, 1.05 mmol) in dry ether (20 ml) with an excess of Me_2NH (3 ml) in a sealed tube at 80 °C for 3 d. The resulting white solid was filtered off and the solution was evaporated to dryness. The residue was treated in the same way as above with an excess of MeI to afford **13** (0.24 g, 73%).

(1*R,2*R**,4*R**)-4-Bromomethyl-1-methyl-cyclopentane-1,2-diol (10)** A THF solution of **7** (0.35 g, 1.83 mmol) was treated with 3 M $HClO_4$ as described above. Chromatography of the residue using $CHCl_3$–MeOH–concentrated NH_4OH (9 : 1 : 0.02) as the eluent gave **10**: (0.2 g, 52%), mp

TABLE III. Physicochemical Properties of Methiodides **11—14**

Compd. No.	mp (°C)	^{1}H-NMR (DMSO)									
		OH	OH	C_4-H	CH_2N	$N(CH_3)_3$	C_1-H	C_2-H	C_5-H	C_5-H	C_2-H	CH_3
11	148—150	4.62 (d) J=5.5	4.06 (s)	3.60 (m)	3.38 (m)	3.00 (s)	2.60 (m)	1.90 (dd) J=8.2, 13.1	1.85 (ddd) J=6.7, 9.8, 14.1	1.65 (ddd) J=6.7, 7.9, 14.1	1.25 (dd) J=9.7, 13.1	1.12 (s)
12	169—170	4.62 (d) J=5.2	4.04 (s)	3.45 (m)	3.32 (m)	3.00 (s)	2.30 (m)	1.82 (dd) J=9.0, 12.7	2.10 (m)	1.41 (m)	1.43 (m)	1.10 (s)
13	131—134	4.62 (br)	4.45 (br)	3.62 (m)	3.38 (m)	3.00 (s)	2.60 (m)	2.00 (dd) J=10.0, 13.2	1.80 (m)	1.80 (m)	1.35 (dd) J=5.1, 13.1	1.12 (s)
14	164—165	4.78 (d) J=4.3	4.38 (s)	3.55 (m)	3.30 (m)	3.00 (s)	2.55 (m)	1.78 (dd) J=7.6, 12.5	2.40 (ddd) J=5.5, 9.5, 13.0	1.20 (ddd) J=2.4, 5.5, 13.1	1.40 (dd) J=9.5, 12.9	1.12 (s)

85—86 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, s, CH_3), 1.59 (1H, m, cyclo), 1.60 (1H, s, OH), 1.66 (1H, s, OH), 1.80—2.10 (3H, m, cyclo), 2.65 (1H, m, cyclo), 3.46 (2H, d, J=11.5, CH_2Br), 3.92 (1H, m, C_2-H). *Anal.* Calcd for $C_7H_{13}BrO_2$: C, 40.21; H, 6.27. Found: C, 40.33; H, 6.41.

(1*R,2*S**,4*R**)-4-Dimethylcarbamoyl-2-hydroxy-2-methyl-1-cyclopentyl *p*-Toluenesulfonate (22)** *p*-Toluenesulfonyl chloride (1.5 g, 7.86 mmol) was added to a stirred solution of **18** (1.3 g, 6.94 mmol) in pyridine (13 ml) at 0 °C. The mixture was stirred for 3 h at 0 °C, allowed to stand for one night in the refrigerator, poured over ice and concentrated HCl (20 ml) and extracted with $CHCl_3$. The organic phase was washed with 2 M HCl, $NaHCO_3$ solution and water (3×25 ml), and dried over Na_2SO_4. After evaporation of the solvent *in vacuo*, the residue was recrystallized from AcOEt to give **22** (1.21 g, 51%), mp 104—106 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, s, CH_3), 1.75 (1H, br s, OH), 1.78 (1H, m, C_3-H), 2.00 (1H, m, C_3-H), 2.02 (1H, m, C_5-H), 2.18 (1H, m, C_5-H), 2.43 (3H, s, $ArCH_3$), 2.90—2.99 (6H, ds, NMe_2), 3.38 (1H, m, C_4-H), 4.55 (1H, t, C_1-H), 7.39 (2H, d, ArH), 7.80 (2H, d, ArH). *Anal.* Calcd for $C_{16}H_{23}NO_5S$: C, 45.61; H, 5.50; N, 3.32. Found: C, 45.53; H, 5.61; N, 3.29.

Biological Evaluation Procedures Male guinea pigs (200—300 g) were killed by cervical dislocation, and the organs required were set up rapidly under 1 g of tension in 20-ml organ baths containing a physiological salt solution of the following composition (mmol): NaCl (118), $NaHCO_3$ (23.8), KCl (4.7), $MgSO_4 \cdot 7H_2O$ (1.18), KH_2PO_4 (1.18), $CaCl_2$ (2.52), glucose (11.7). The solution was kept at 37 °C (ileum and bladder) or 30 °C (heart) and aerated with 5% CO_2–95% O_2. Heart left atrium was stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 5—10 V). Tissues were equilibrated for 30 min (2 h in the case of heart) and dose–response curves were obtained at 30-min intervals by cumulative addition of carbachol, the first one being discarded and the second taken as control. A third dose–response curve was constructed with the agonist under study. For the antagonist activity, when studied, the preparation was incubated with a test compound for 30 min before obtaining the third dose–response curve to carbachol. Constractions were recorded isotonically (ileum) or isometrically (heart and bladder) by means of a force transducer connected to a two-channel Gemini polygraph.

Potency was expressed as ED_{50} plus or minus S.E. derived from dose–response curves and represents the concentration of agonist required to produce 50% of the maximum contraction.

The agonist dissociation constant (K_A) and relative efficacy (e_r) were determined according to the method of Furchgott and Bursztyn,[14] as previously described.[15,16] After determining the dose–response curve, the preparation was treated with an adequate amount of dibenamine (1—40 μmol for 20 min) to occlude a fraction of the receptors. The tissue was then washed for 20 min and a new dose–response curve constructed for the dibenamine-treated tissue. Several equipotent doses of the agonist before (A) and after (A′) dibenamine treatment were determined graphically. 1/A was plotted *vs.* 1/A′, and the points were fitted to a straight line by linear regression analysis. The dissociation constant (K_A) was calculated from the slope of the regression line and the intercept on the ordinate scale. In some experiments, K_A values were shown to be independent of the percentage of receptor inactivation, since further incubation with the irreversible antagonist gave the same results. The efficacy of the agonist under study (e_r) relative to that of (±)-deoxamuscarine (**23**) used as standard compound, was evaluated by the ratio RA **23**/RA X, where RA **23** and RA X are the percentages of the receptor to be occupied by **23** and the compound under study, respectively, to elicit 50% of the maximal response.

Statistical Analysis: Data are presented as means ± S.E. of four to six independent experiments. Differences between mean values were tested for significance by Student's t test.

Acknowledgments This work was supported by a grant from the Italian Research Council (CNR), "Progetto finalizzato, Chimica fine II". We are grateful to Prof. P. Angeli for helpful discussions and suggestions, and to Dr. F. Paparelli for technical assistance.

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