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Molecular Requirements of the Active Site of Cholinergic Receptors XV: Synthesis and Biological Activity of 2,3-Dehydrodeoxamuscarone and 2,3-Dehydrodeoxamuscarines

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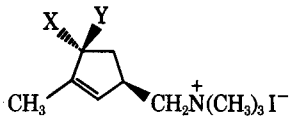
Abstract □ To elucidate the molecular requirements of the active sites of cholinergic receptors, 3-methyl-4-oxo-1-(*N,N*-dimethylaminomethyl)cyclopent-2-ene methiodide (2,3-dehydrodeoxamuscarone) and *cis*- and *trans*-3-methyl-4-hydroxy-1-(*N,N*-dimethylaminomethyl)cyclopent-2-ene methiodides (*cis*- and *trans*-dehydrodeoxamuscarines) were synthesized and tested. The results, compared with those of the corresponding oxygenated compounds, seem to indicate that 2,3-dehydrodeoxamuscarines and muscarine bind at the same site while 2,3-dehydrodeoxamuscarone interacts with the site normally occupied by muscarone. Furthermore, the previously suggested hypothesis that the unpolar site might somehow incorporate that of muscarone was considered.

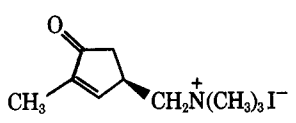
Keyphrases □ Cholinergic receptors—molecular requirements of active sites, synthesis of 2,3-dehydrodeoxamuscarone and *cis*- and *trans*-dehydrodeoxamuscarines □ Structure–activity relationships—2,3-dehydrodeoxamuscarone and *cis*- and *trans*-dehydrodeoxamuscarines, molecular requirements of cholinergic receptors, dualism of receptor active sites □ 2,3-Dehydrodeoxamuscarone and 2,3-dehydrodeoxamuscarine—synthesis and biological activity

To investigate the molecular requirements of cholinergic receptors, many compounds incorporating a cyclopentane nucleus were synthesized in the past few years (1). As a consequence of these studies, the hypothesis of Triggle and Triggle (2) concerning an accessory site of reduced polarity and low steric demand of the cholinergic receptor gained further support (3–5). In fact, most of these compounds, although lacking oxygenated functions, are fairly active on both nicotinic and muscarinic receptors. However, their specificity is generally low when compared with the corresponding oxygenated compounds (*i.e.*, muscarine).

To gain more information on the dualism of the active site of the cholinergic receptors, *cis*- and *trans*-3-methyl-4-hydroxy-1-(*N,N*-dimethylaminomethyl)cyclopent-2-ene methiodides (*cis*- and *trans*-2,3-dehydrodeoxamuscarines, I*a* and I*b*) and 3-methyl-4-oxo-1-(*N,N*-dimethylaminomethyl)cyclo-pent-2-ene methiodide (2,3-dehydrodeoxamuscarone, II) were synthesized and tested. Their pharmacological results were compared with those of 3-methyl-1-(*N,N*-dimethylaminomethyl)cyclopent-2-ene methiodide (III), because it incorporates a



I*a*: X = H, Y = OH
I*b*: X = OH, Y = H
III: X = Y = H



II

double bond at the same position 2 of the cyclopentyl moiety as a basic feature and is one of the most active compounds among those lacking oxygenated functions (5).

EXPERIMENTAL

Melting points[1] were taken in sealed capillaries and are uncorrected. NMR spectra were recorded on a 90-MHz apparatus[2] with tetramethylsilane or 3-(trimethylsilyl)propanesulfonic acid sodium salt as the internal standard. Chromatographic separations were performed on silica gel (Kieselgel[3] 60, 0.063–0.200 mm) columns. Organic solutions were dried over anhydrous sodium sulfate.

3-Methyl-4-oxo-1-carbomethoxycyclopent-2-ene (VI)—Bromine (7.56 ml) in carbon tetrachloride (100 ml) was added to a vigorously stirred solution of V (21.9 g) (6) in carbon tetrachloride (100 ml) at room temperature. After the reaction started, the addition was continued with cooling at 0°. The solvent then was evaporated to give an oil, which was kept at 120° for 15 min under 50 mm pressure and then distilled, bp 130–135°/30 mm (12.7-g yield); IR[4] (liquid film): 1640 (C=C), 1710 (C=O), and 1735 (COO) cm^{-1}; NMR (chloroform-*d*): δ 1.70 (broad s, 3H, 3-CH_3), 2.60 (d, 2H, 5-H_2), 3.70 (s, 3H, OCH_3), 3.50–4.00 (m, 1H, 1-H), and 7.13 (m, 1H, 2-H) ppm.

Anal.—Calc. for $C_8H_{10}O_3$: C, 62.32; H, 6.54. Found: C, 62.20; H, 6.60.

***cis*- and *trans*-3-Methyl-4-hydroxy-1-carbomethoxycyclopent-2-enes (VII*a* and VII*b*)**—The overall procedure recommended by Brown and Hess (7) was followed. Thus, a solution of 0.55 *M* 9-borabicyclo[3.3.1]nonane in tetrahydrofuran[5] (36.5 ml) was added dropwise over 2 hr to a stirred and cooled (0°) solution of VI (3 g, 19.5 mmoles) in dry tetrahydrofuran (5 ml) under a dry nitrogen stream. After 4 hr at 0°,

[1] Büchi SMP-20 apparatus.
[2] Model EM-390, Varian.
[3] Merck.
[4] Perkin-Elmer 297 spectrophotometer.
[5] Aldrich.

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the solution was stirred at 25° for 2 hr. Then methanol (0.5 ml) was added, and the solvent was removed under reduced pressure. Ethanolamine (1.23 g) and *n*-pentane (100 ml) were added, and the sticky precipitate was extracted several times with *n*-pentane. Evaporation of the solvent gave an oil (1.64 g), which was purified by column chromatography using ethyl acetate–cyclohexane (1:1) as the eluting system. The first fraction was the starting material.

The second fraction was VII*a* (0.42 g); IR (chloroform): 1720 (C=O), 3460 (bonded OH), and 3580 (free OH) cm^{-1}; NMR (chloroform-*d*): δ 1.80 (broad s, 3H, 3-CH_3), 1.90–2.78 (m, 2H, 5-H_2), 2.78 (s, 1H, OH), 3.32 (m, 1H, 1-H), 3.65 (s, 3H, OCH_3), 4.38 (dd, 1H, 4-H), and 5.38 (m, 1H, 2-H) ppm.

Anal.—Calc. for $C_8H_{12}O_3$: C, 61.52; H, 7.75. Found: C, 61.65; H, 7.84.

The third fraction was VII*b* (0.26 g); IR (chloroform): 1730 (C=O), 3480 (bonded OH), and 3590 (free OH) cm^{-1}; NMR (chloroform-*d*): δ 1.78 (broad s, 3H, 3-CH_3), 1.80–2.70 (m, 2H, 5-H_2), 2.65 (s, 1H, OH), 3.30–3.80 (m, 1H, 1-H), 3.60 (s, 3H, OCH_3), 4.62 (t, 1H, 4-H), and 5.40 (m, 1H, 2-H) ppm.

Anal.—Calc. for $C_8H_{12}O_3$: C, 61.52; H, 7.75. Found: C, 61.74; H, 7.84.

***cis*-3-Methyl-4-hydroxy-1-(*N,N*-dimethylcarboxamido)cyclopent-2-ene (VIII*a*)**—*Method A*—Dimethylamine (10 ml) was added to VII*a* (or VII*b*) (0.5 g) and heated at 95–100° in a sealed tube for 48 hr. Workup of the reaction mixture gave 0.25 g of VIII*a*, which was used without further purification; IR (chloroform): 1620 (C=O), 3360 (bonded OH), and 3590 (free OH) cm^{-1}; NMR (chloroform-*d*): δ 1.82 (broad s, 3H, 3-CH_3), 1.95–2.50 (m, 2H, 5-H_2), 2.92 (s, 3H, NCH_3), 3.11 (s, 3H, NCH_3), 3.50–3.90 (m, 1H, 1-H), 4.25 (dd, 1H, 4-H), 4.58 (s, 1H, OH), and 5.31 (m, 1H, 2-H) ppm.

Method B—Ethyl chloroformate (0.62 g, 5.7 mmoles) in chloroform (10 ml) was added to a stirred and cooled solution of XV*a* (0.81 g, 5.7 mmoles) and triethylamine (0.58 g, 5.7 mmoles), followed after 5 min by 33% dimethylamine in benzene (5.6 ml). The mixture was allowed to stand at room temperature for 30 min and then was washed with 2 *N* HCl and saturated sodium bicarbonate solution and dried. Evaporation of the solvent gave 0.74 g of VIII*a*.

***cis*-3-Methyl-4-hydroxy-1-(*N,N*-dimethylaminomethyl)cyclopent-2-ene (IX*a*)**—A solution of VIII*a* (0.34 g, 2.01 mmoles) in dry tetrahydrofuran (100 ml) was added to a suspension of aluminum lithium hydride (0.15 g, 4.02 mmoles) in dry tetrahydrofuran (100 ml) with stirring and cooling. The suspension then was heated to reflux for 5 hr. After cooling, the excess aluminum lithium hydride was decomposed with ethyl acetate (50 ml) and water (5 ml). The organic layer was decanted, and the solid was washed with ethyl acetate (2 × 50 ml). Evaporation of the dried solvent gave a nearly pure oil (0.26 g), which was used without further purification; IR (chloroform): 3350 (bonded OH) and 3500 (free OH) cm^{-1}; NMR (chloroform-*d*): δ 1.17–1.60 (m, 1H, 5-H), 1.79 (broad s, 3H, 3-CH_3), 2.00–2.90 (m, 4H, 1-CH_2, 1-H, and 5-H), 2.26 [s, 6H, $N(CH_3)_2$], 4.19 (s, 1H, OH), 4.26 (dd, 1H, 4-H), and 5.37 (broad s, 1H, 2-H) ppm.

***cis*-2,3-Dehydrodeoxamuscarine (I*a*)**—An excess of methyl iodide (2 ml) was added to a solution of IX*a* (0.2 g) in anhydrous ether (20 ml), and the solution was left to stand overnight at room temperature. The white solid was filtered off and recrystallized (0.23 g) from anhydrous ethanol–ether, mp 153–154°; IR (mineral oil): 3350 (OH) cm^{-1}; NMR (water-*d*): δ 1.30–1.80 (m, 1H, 5-H), 1.76 (s, 3H, 3-CH_3), 2.66–3.66 (m, 4H, 1-H, 4-H, and 1-CH_2), 3.17 [s, 9H, $^+N(CH_3)_3$], 4.70 (4H, obscured by water), and 5.50 (broad s, 1H, 2-H) ppm.

Anal.—Calc. for $C_{10}H_{20}INO$: C, 40.42; H, 6.78; N, 4.71. Found: C, 40.38; H, 6.92; N, 4.64.

3-Methyl-4-oxo-1-(*N,N*-dimethylaminomethyl)cyclopent-2-ene (X)—A solution of chromium trioxide (1.13 g, 11.0 mmoles) in 2.5 *M* H_2SO_4 (10 ml) was added slowly to a stirred and cooled (0°) solution of IX*a* (1.76 g, 11.0 mmoles) in 2.5 *M* H_2SO_4 (5 ml). After standing at room temperature for 2 hr, the solution was made basic with 5 *N* NaOH and extracted with methylene chloride. Evaporation of the dried solvent gave X (1.0 g), which was purified by column chromatography with chloroform–petroleum ether–methanol–concentrated ammonium hydroxide (250:150:50:5) as the eluting system; IR (liquid film): 1635 (C=C) and 1705 (C=O) cm^{-1}; NMR (chloroform-*d*): δ 1.72 (d, 3H, 3-CH_3), 2.20 [s, 6H, $N(CH_3)_2$], 2.10–3.50 (m, 5H, 5-H_2, 1-H, and 1-CH_2), and 7.28 (m, 1H, 2-H) ppm.

Anal.—Calc. for $C_9H_{15}NO$: C, 70.55; H, 9.87; N, 9.14. Found: C, 70.41; H, 9.79; N, 9.21.

2,3-Dehydrodeoxamuscarone (II)—Compound II was obtained from X as a white solid following the procedure described for I*a* (from anhydrous ethanol–ether) in an 80% yield, mp 230–231°; IR (mineral oil): 1710 (CO) cm^{-1}; NMR (water-*d*): δ 1.79 (broad s, 3H, 3-CH_3), 2.58 (m, 1H, 1-H), 2.82 (m, 2H, 5-H_2), 3.20 [s, 9H, $^+N(CH_3)_3$], 3.52 (m, 2H, 1-CH_2), and 7.40 (m, 1H, 2-H) ppm.

Anal.—Calc. for $C_{10}H_{18}INO$: C, 40.49; H, 6.15; N, 4.74. Found: C, 40.77; H, 6.27; N, 4.88.

***trans*-3-Methyl-4-hydroxy-1-(*N,N*-dimethylaminomethyl)cyclopent-2-ene (IX*b*)**—*Method A*—Compound IX*b* was obtained starting from X (1.1 g) following the procedure described for VII and was purified by column chromatography with chloroform–petroleum ether–methanol–concentrated ammonium hydroxide (250:150:50:5) as the eluting system. The first fraction was the starting material (0.2 g). The second fraction was IX*a* (0.12 g). The third fraction was IX*b* (0.35 g); IR (chloroform): 3380 (bonded OH) and 3600 (free OH) cm^{-1}; NMR (chloroform-*d*): δ 1.79 (broad s, 3H, 3-CH_3), 1.84 (t, 2H, 5-H_2), 2.06–2.45 (m, 1H, 1-H), 2.21 [s, 6H, $N(CH_3)_2$], 2.86 (s, 1H, 4-OH), 2.96 (m, 2H, 1-CH_2), 4.59 (t, 1H, 4-H), and 5.42 (m, 1H, 2-H) ppm.

Anal.—Calc. for $C_9H_{17}NO$: C, 69.63; H, 11.04; N, 9.02. Found: C, 69.57; H, 10.96; N, 9.13.

Method B—Compound IX*b* was also synthesized starting from VIII*b* (0.09 g) by the procedure described for IX*a*; 0.059 g of nearly pure oil was obtained and was used without further purification.

***trans*-2,3-Dehydrodeoxamuscarine (I*b*)**—Compound I*b* was obtained starting from IX*b* by the procedure described for I*a* and was recrystallized from anhydrous ethanol–ether (85% yield), mp 151–152°; IR (mineral oil): 3410 (OH) cm^{-1}; NMR (water-*d*): δ 1.76 (s, 3H, 3-CH_3), 2.13 (t, 2H, 4-H_2), 3.13 [s, 9H, $^+N(CH_3)_3$], 2.66–3.66 (m, 3H, 1-H and 1-CH_2), 4.69 (t, 1H, 4-H), and 5.56 (m, 1H, 2-H) ppm.

Anal.—Calc. for $C_{10}H_{20}INO$: C, 40.42; H, 6.78; N, 4.71. Found: C, 40.39; H, 6.95; N, 4.75.

***cis*-3-Methyl-4-hydroxy-1-hydroxymethylcyclopent-2-ene (XI*a*)**—A solution of VII*a* (1.14 g, 7.3 mmoles) in anhydrous tetrahydrofuran (20 ml) was added to a stirred and cooled (0°) suspension of aluminum lithium hydride (0.34 g, 8.9 mmoles) in anhydrous tetrahydrofuran (20 ml). The suspension then was heated to reflux for 48 hr and worked up as described for IX*a* to give an oil, which was used without further purification (0.93 g); IR (liquid film): 3320 (OH) cm^{-1}; NMR (chloroform-*d*): δ 1.0–2.6 (m, 3H, 5-H_2 and 1-H), 1.79 (s, 3H, 3-CH_3), 3.3–4.5 (m, 3H, 1-CH_2 and 4-H), 3.26 (s, 2H, OH), and 5.50 (broad s, 1H, 2-H) ppm.

***trans*-3-Methyl-4-hydroxy-1-hydroxymethylcyclopent-2-ene (XI*b*)**—Compound XI*b* was obtained in an 85% yield starting from VII*b* by the procedure described for XI*a*; IR (liquid film): 3300 (OH) cm^{-1}; NMR (chloroform-*d*): δ 1.0–2.3 (m, 3H, 5-H_2 and 1-H), 1.86 (s, 3H, 3-CH_3), 3.3–4.3 (m, 3H, 1-CH_2 and 4-H), 3.59 (s, 2H, OH), and 5.56 (m, 1H, 2-H) ppm.

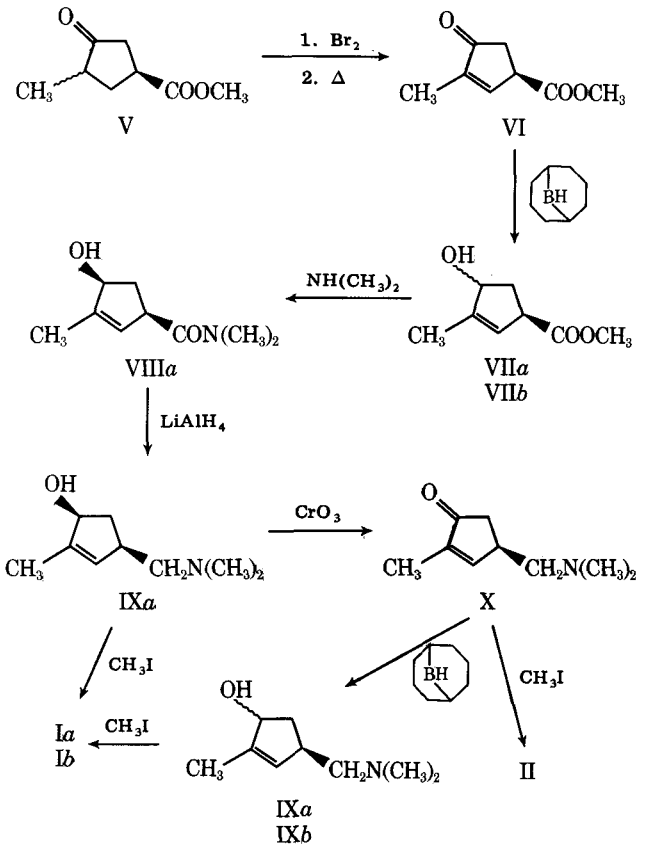
***cis*-3-Methyl-4-hydroxy-1-methyl-*p*-toluenesulfonatecyclopent-2-ene (XII*a*)**—*p*-Toluenesulfonyl chloride (1.42 g) was added portionwise to a stirred and cooled (0°) solution of XI*a* (0.97 g) in anhydrous pyridine (10 ml). The mixture was kept 4 days at room temperature and then acidified with 2 *N* HCl and extracted with methylene chloride. The organic layer was washed with a saturated sodium bicarbonate solution and then evaporated at room temperature to give a nearly pure oil (0.47 g), which was used without further purification; NMR (chloroform-*d*): δ 1.65 (broad s, 3H, 3-CH_3), 1.40–2.30 (m, 2H, 5-H_2), 2.30–3.00 (m, 1H, 1-H), 2.38 (s, 3H, aromatic CH_3), 3.78 (d, 2H, 1-CH_2), 4.12 (m, 2H, OH and 4-H), 5.12 (m, 1H, 2-H), and 7.38 (q, 4H, aromatic H) ppm. The compound has to be stored at low temperature because of its tendency to polymerize.

***trans*-3-Methyl-4-hydroxy-1-methyl-*p*-toluenesulfonatecyclopent-2-ene (XII*b*)**—Compound XII*b* was obtained starting from XI*b* (0.31 g) as described for XII*a* (0.17 g); NMR (chloroform-*d*): δ 1.65 (broad s, 3H, 3-CH_3), 1.40–2.10 (m, 2H, 5-H_2), 2.38 (s, 3H, aromatic CH_3), 2.92 (m, 1H, 1-H), 3.62 (s, 1H, 4-OH), 3.68 (d, 2H, 1-CH_2), 4.35 (t, 1H, 4-H), and 5.10 (s, 1H, 2-H) ppm. It has to be stored at low temperature.

***cis*-3-Methyl-4-hydroxycyclopent-2-ene-1-carboxylic Acid (XV*a*)**—Compound VII*a* (1.64 g) was dissolved in 2 *N* NaOH (5 ml) and heated at 70° for 10 min. After cooling, the solution was washed with chloroform, acidified with 2 *N* HCl and then extracted with chloroform to give a white solid. This solid was recrystallized (0.97 g) from benzene, mp 69–71°; IR (mineral oil): 1685 (C=O), 2460, and 3340 (OH) cm^{-1}; NMR (chloroform-*d*): δ 1.82 (d, 3H, 3-CH_3), 1.70–2.70 (m, 2H, 5-H_2), 3.38 (m, 1H, 1-H), 4.43 (dd, 1H, 4-H), 5.48 (broad s, 1H, 2-H), and 7.20 (s, 2H, OH) ppm.

Anal.—Calc. for $C_7H_{10}O_3$: C, 59.56; H, 6.43. Found: C, 59.44; H, 6.51.

The same reaction on VII*b* gave a sticky oil, which could not be re-



Scheme I

crystallized and which was possibly a mixture of the two isomeric acids.

3-Methyl-4-oxo-1-(*N,N*-dimethylcarboxamido)cyclopent-2-ene (XIV)—Bromine (4 ml, 78.0 mmoles) in carbon tetrachloride (50 ml) was added to a solution of XIII (6) (13 g, 77.0 mmoles) in carbon tetrachloride (100 ml) at room temperature under vigorous stirring. After the reaction started, the addition was continued with cooling at 0°. The solvent then was evaporated to give a solid, which was treated with a saturated sodium bicarbonate solution (100 ml) and extracted with chloroform. Removal of solvent gave an oil, which was distilled to give 5.23 g of XIV, bp 137–140°/0.25 mm. Much of XIV decomposed to a gummy product during the distillation; IR (liquid film): 1640 and 1700 (CO) cm^{-1}; NMR (chloroform-*d*): δ 1.78 (broad s, 3H, 3-CH_3), 2.60 (m, 2H, 5-H_2), 2.98 (s, 3H, NCH_3), 3.18 (s, 3H, NCH_3), 2.80–3.50 (m, 1H, 1-H), 4.00 (m, 1H, 1-H), and 7.20 (m, 1H, 2-H) ppm.

Anal.—Calc. for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.81; H, 7.70; N, 8.24.

***trans*-3-Methyl-4-hydroxy-1-(*N,N*-dimethylcarboxamido)cyclopent-2-ene (VIII*b*)**—Compound VIII*b* was obtained starting from XIV (0.83 g) by the procedure described for VII and was purified by column chromatography with chloroform–ethyl acetate–ethanol (90:5:5) as the eluting system. The first fraction was VIII*a* (0.35 g). The second fraction was VIII*b* (0.09 g); IR (chloroform): 1620 (CO), 3370 (bonded OH), and 3600 (free OH) cm^{-1}; NMR (chloroform-*d*): δ 1.80 (d, 3H, 3-CH_3), 1.60–2.80 (m, 2H, 5-H_2), 2.88 (s, 3H, NCH_3), 3.08 (s, 3H, NCH_3), 3.30–4.10 (m, 2H, 1-H and OH), 4.70 (t, 1H, 4-H), and 5.38 (s, 1H, 2-H) ppm.

Anal.—Calc. for $C_9H_{15}NO_2$: C, 63.88; H, 8.94; N, 8.28. Found: C, 64.03; H, 8.70; N, 8.41.

Pharmacological Testing—Testing was accomplished using guinea pig ileum and frog rectus abdominis preparations according to the protocol described previously (8).

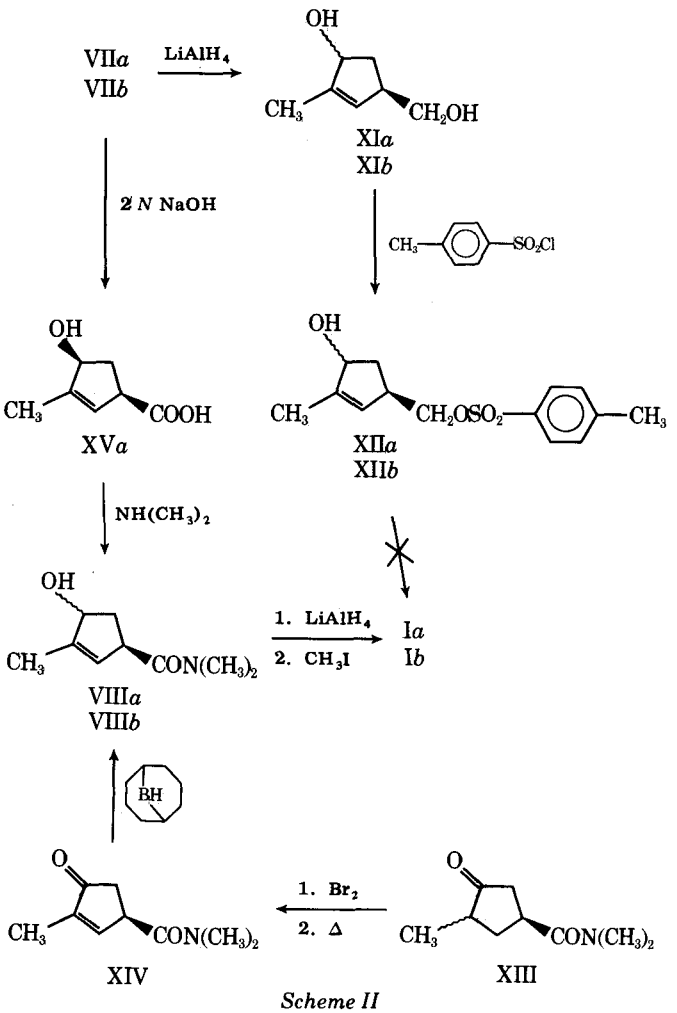
RESULTS AND DISCUSSION

Chemistry—Compounds I*a*, I*b*, and II were synthesized according to Scheme I. The introduction of the double bond in V (6) went smoothly and only at position 2, as revealed by NMR spectra. The reduction of the carbonyl group was eventually achieved with 9-borabicyclo[3.3.1]nonane (9) since other common reducing agents gave saturation of the double bond through a 1,4-addition to the enone system (7, 10). The reaction of either VII*a* or VII*b*, separated by column chromatography, with dimethylamine in a sealed tube at 80° invariably gave the *cis*-isomer (VIII*a*) through isomerization, possibly at C-1 (11, 12). The reduction of VIII*a* with aluminum lithium hydride followed by the reaction of IX*a* with methyl iodide gave I*a*. The *trans*-isomer (I*b*) was obtained through the oxidation of IX*a* to X followed by reduction with 9-borabicyclo-[3.3.1]nonane to yield IX as a *cis–trans* mixture, which was separated by column chromatography and the final reaction of IX*b* with methyl iodide. Compound II was easily obtained from X through the reaction with methyl iodide.

Scheme II shows some pathways that were explored to obtain the two isomers of I in a simpler way. In a first attempt, VII*a* and VII*b* were reduced to the corresponding alcohols (XI*a* and XI*b*) followed by reaction with *p*-toluenesulfonyl chloride to give a selective tosylation of primary hydroxyl groups. Unfortunately, both XII*a* and XII*b* are difficult to handle since they decompose quickly into dark solids showing no double bond. The hydrolysis of VII*a* and VII*b* was unsatisfactory with acids. In one case (VII*a*), 2 *N* NaOH afforded the *cis* acid (XV*a*); in the other case (VII*b*), a *cis–trans* mixture of XV was obtained. Finally, the reduction of amide XIV, obtained in poor yield from XIII (6), afforded VIII*a* and only traces of the *trans*-isomer (VIII*b*), which were separated by column chromatography.

Identification of Compounds—The structures of VI and XIV were established by NMR spectra, which showed a broad singlet for the methyl group at position 3 (δ 1.70 and 1.78, respectively), thus proving the double bond at position 2.

The attribution of structures to VII*a*, VII*b*, VIII*a*, and VIII*b* depended mainly on IR spectra at different dilutions, which showed a nearly constant ratio between the intensity of free and bonded hydroxy absorption for the *cis*-isomers (VII*a* and VIII*a*). The *trans*-isomers (VII*b* and VIII*b*) presented a strong variation of the same ratio upon dilution. NMR spectra confirmed this attribution since the proton at position 4 of VII*b* [δ 4.62 (t)] and VIII*b* [δ 4.70 (t)] was more deshielded than the corre-



Scheme II

Table I—Comparative Biological Activity on Guinea Pig Terminal Ileum and Frog Rectus Abdominis [a]

Compound	EPMR[b] Guinea Pig Ileum[c]	EPMR[b] Frog Rectus Abdominis[d]	$EPMR_N/EPMR_M$[e]
Ia	1000	94	0.1
Ib	50	>500	>10
II	5.85	0.66	0.1
III	26.6[f]	3.1[f]	0.1

[a] The choice, performance, and evaluation of the bioassay were described in detail in Ref. 8. [b] EPMR = equipotent molar ratios between ED_{50} of compound and ED_{50} of acetylcholine calculated through the regression of the angular transformate of the fractional effects *versus* the log concentrations of the agonists. The statistical significance of the EPMR averages was estimated by the t test at the $p \leq 0.05$ level. Experiments were repeated at least four times. The standard error of the mean ED_{50} values for the compounds was <10%. [c] The ED_{50} value of acetylcholine = 3.2×10^{-7} M. [d] The ED_{50} value of acetylcholine = 2.58×10^{-5} M. [e] Ratio between frog rectus abdominis (N) and guinea pig ileum (M) EPMR values. [f] Data from Ref. 5.

sponding proton of VII*a* [δ 4.38 (dd)] and VIII*a* [δ 4.25 (dd)], owing to the anisotropic effect of the groups at position 1. In the *cis* series, the proton at position 4 appeared as a double doublet; in the *trans* series, it was a triplet. This difference allowed a safe identification of the derivatives of VII and VIII. Accordingly, XV shows a *cis* structure.

Pharmacology—The cholinergic activities of I*a*, I*b*, and II on guinea pig ileum and frog rectus abdominis preparations are listed in Table I together with those of III (5). Introduction of the carbonyl group in III to give II improved both muscarinic and nicotinic activities. The specificity of II, as far as muscarinic activity is concerned as revealed by the $EPMR_N/EPMR_M$ ratio, remained low; its activity was 10 times lower than that of the corresponding muscarones but nearly equivalent to that of deoxamuscarone (11). The influence of the double bond, which imposed a planar configuration to the methyl group at position 2, did not seem to be relevant in this case. The difference of activity between *cis*- and *trans*-muscarone is practically insignificant (1).

The introduction of a hydroxyl group had a totally different effect. With I*a*, there was a drop of two orders of magnitude in both muscarinic and nicotinic activities. In fact, muscarinic activity was nearly lost while nicotinic activity fell dramatically and the specificity remained low. With I*b*, there was a small drop in muscarinic activity, but the specificity was greatly increased since nicotinic activity was practically absent. Compound I*b* had a pattern of activity fairly similar to that of muscarine. Moreover, since I*b* was also less active than deoxamuscarine as a muscarinic agent, the planar configuration of the methyl group seems to be of some relevance in this case, unlike the corresponding carbonyl compound (II). Introduction of the hydrophilic hydroxyl group dramatically changed the biological profile of the molecule, giving further support to the hypothesis of Triggle and Triggle (2). The same conclusions were reached by Barlow (13) who evaluated the consequences of the introduction of a hydroxyl group in a series of alkyltrimethylammonium compounds. In any case, the hydroxyl group that drives away the molecule from the unpolar binding site has to be oriented properly to allow interaction with the polar binding site.

The similarity of the pattern of activity of II, III, muscarones, and deoxamuscarone suggests that such compounds act at the same binding site, which seems to be quite different from where I*b*, muscarine, and deoxamuscarine interact. This difference is further stressed by different stereochemical requirements of the site interacting with the methyl group. It is known that the enantiomers of muscarine show high stereospecificity toward the muscarinic receptor. Should I*b* interact with the same muscarine binding site (as was previously suggested), the prediction that the chiral effects of the two enantiomers of I*b* would manifest themselves could be made.

It also is well known that the stereospecificity of muscarone is fairly different from that of muscarine qualitatively and quantitatively. Various rationalizations have been advanced; one of these implies a different site of action for muscarone (14). The results reported herein, while adding further evidence to the hypothesis of Belleau and Puranen (14), suggest the intriguing possibility that the accessory unpolar area (2) might incorporate the site of action of muscarone (14). Work is in progress to test this hypothesis.

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