# Synthesis of Various Geometric and Enantiomeric Oxime *O*-($\alpha$- and $\beta$-Methylcholinyl) Ethers as Potential Anticholinergic Agents

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**Abstract** □ Various enantiomeric and geometric oxime *O*-($\alpha$- and $\beta$-methylcholinyl) ethers were synthesized as potential anticholinergic agents. The synthesis, separation, resolution, and structural characterization of these compounds are reported. The first step of the synthetic pathway involved an oxime formation, with subsequent *O*-alkylation of the respective oxime with 2-chloro-*N*,*N*-dimethylpropylamine hydrochloride. The separation of the $\alpha$- and $\beta$-structural isomers utilized vacuum fractional distillation and/or column chromatography, and the resolution of the enantiomers was accomplished *via* the formation of tartrate diastereoisomers. A preliminary pharmacological evaluation for anticholinergic activity was conducted using a rat ileum assay. Structure–activity relationships, including some stereochemical properties and antimuscarinic activity, are discussed.

**Keyphrases** □ Oxime *O*-($\alpha$- and $\beta$-methylcholinyl) ethers—synthesized, enantiomeric and geometric isomers separated, anticholinergic activity evaluated □ *O*-($\alpha$- and $\beta$-Methylcholinyl) ether derivatives of oximes—synthesized, enantiomeric and geometric isomers separated, anticholinergic activity evaluated □ Anticholinergic activity—evaluated in series of oxime *O*-($\alpha$- and $\beta$-methylcholinyl) ethers □ Structure–activity relationships—various oxime *O*-($\alpha$- and $\beta$-methylcholinyl) ethers, anticholinergic activity evaluated

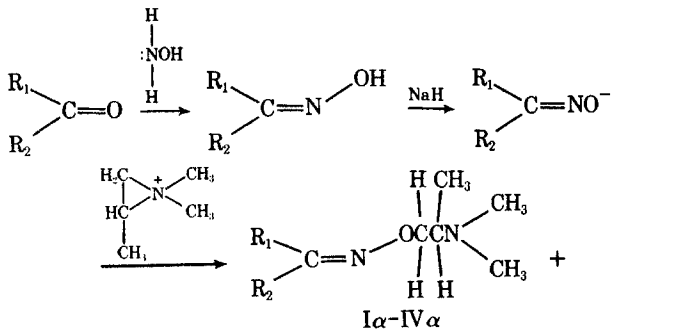
The premise that a compound must possess optimal stereochemical properties for significant antimuscarinic activity has been well established by structure–activity relationship studies of atropine (1, 2), hyoscine (2–4), $\beta$-methylcholine esters of $\alpha$-methyltropic acid and cyclohexylphenylglycolic acid (5, 6), and 2-substituted 4-dimethylaminomethyl-1,3-dioxolanes (7, 8).

Furthermore, the work dealing with $\beta$-methylcholine esters of $\alpha$-methyltropic acid and cyclohexylphenylglycolic acid (5, 6) concluded that the stereochemistry of the amino alcohol moiety was not important for antimuscarinic activity, since the enantiomeric potency ratios were not very large when the absolute configuration of the $\beta$-methylcholine moiety was varied. However, in the same reports (5, 6) and in earlier work (9), the data indicated that $\beta$-methyl substitution reduced anticholinergic activity but that the converse was true for $\alpha$-methyl substitution. Therefore, $\alpha$-methylcholine derivatives should be studied as potential anticholinergic agents.

In view of this earlier work and of the fact that oxime *O*-ethers exhibited high antimuscarinic activity (10–12) and served as good models for studying geometric isomerism (12), a series of geometric and enantiomeric oxime *O*-($\alpha$- and $\beta$-methylcholinyl) ethers was synthesized. A preliminary pharmacological evaluation was also conducted, since these data could lead to postulated receptor interactions if certain limitations are considered, such as comparing data of analogous compounds (13) and ensuring relative stereochemical purity (14).

## DISCUSSION

**Synthesis**—The major steps in the synthesis of the racemic *O*-($\alpha$- and $\beta$-methylcholinyl) ethers of 1-methyl-4-piperidone oxime (I$\alpha$ and I$\beta$), benzophenone oxime (II$\alpha$ and II$\beta$), (*E*)-phenyl 2-pyridyl oxime (III$\alpha$ and III$\beta$), and (*Z*)-phenyl 2-pyridyl oxime (IV$\alpha$ and IV$\beta$) (Table I) are depicted in Scheme I.

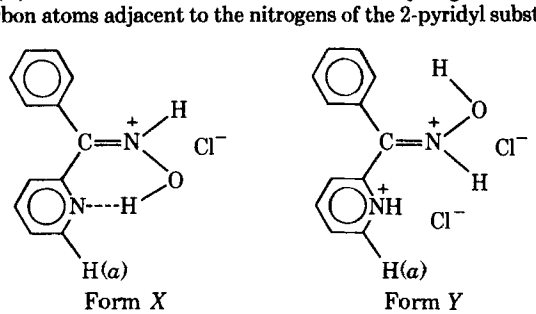



*Scheme I*

Oxime formation was the first step (Scheme I). The oxime from 1-methyl-4-piperidone was produced with a method adapted from Kochhar *et al.* (10). Benzophenone, (*E*)-phenyl 2-pyridyl, and (*Z*)-phenyl 2-pyridyl oximes were obtained by utilizing the Wylie *et al.* (15) base-catalyzed procedure. However, the synthetic procedure employing phenyl 2-pyridyl ketone formed a mixture of the (*E*)- and (*Z*)-geometric isomers.

Separation of the geometric isomers was accomplished by selective crystallization of the oximes when the pH of the acidic solution containing the oxime hydrochloride salts was adjusted to 2.5 with an aqueous 10% sodium hydroxide solution. Each of two consecutive adjustments to a pH value less than 2.5 with the sodium hydroxide solution yielded the (*Z*)-isomer, while a third adjustment to a pH of 2.5 produced a mixture of both isomers. Subsequent adjustments to a pH value greater than 2.5 caused the precipitation of the (*E*)-isomer. The (*Z*)-isomer was precipitated first, since it existed primarily in the monoprotonated form (*X*) and was the stronger conjugate acid. The (*E*)-isomer remained in solution until a higher pH value was reached, because the protonated pyridyl nitrogen (*Y*) was the weaker conjugate acid.

The difference in the degree of availability of the electrons of the pyridyl nitrogen in the formation of the hydrochloride salts was accounted for by variations in intramolecular hydrogen bonding. The IR spectrum for the (*E*)-isomer in chloroform demonstrated a free hydroxyl group at 3550 $cm^{-1}$, but a similar one was absent in the spectrum of the (*Z*)-isomer. The NMR data also substantiated the intramolecular hydrogen bonding in the (*Z*)-isomer when the chemical shifts of the hydrogen atoms (a) on the carbon atoms adjacent to the nitrogens of the 2-pyridyl substituents



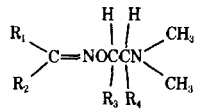


**Table I—Oxime *O*-(α- and β-Methylcholinyl) Ethers**

| Compound | $R_1$ | $R_2$ | $R_3$, NMR Chemical Shift (τ) | $R_4$, NMR Chemical Shift (τ) | Boiling Point | Yield, g, % | Oxime, g, mole | Sodium Hydride, g, mole | Alkyl Halide, g, mole |
|---|---|---|---|---|---|---|---|---|---|
| (±)-Iα | $CH_3NC_5H_8$ | | H (6.11) | $CH_3$ (9.06) | 92–94° (1.15 mm Hg) | 32, 79 | 25.6, 0.2 | 18.8, 0.4 | 31.6, 0.2 |
| (±)-Iβ | $CH_3NC_5H_8$ | | $CH_3$ (8.82) | H (7.60) | 88–90° (2 mm Hg) | 30, 71 | 29.55, 0.15 | 14.4, 0.309 | 23.7, 0.15 |
| (±)-IIα | $C_6H_5$ | $C_6H_5$ | H (5.88) | $CH_3$ (9.06) | 133–137° (0.15 mm Hg) | 28, 89 | 22, 0.112 | 10.4, 0.224 | 17.6, 0.112 |
| (±)-IIβ | $C_6H_5$ | $C_6H_5$ | $CH_3$ (8.73) | H (7.62) | 106–109° (0.1 mm Hg) | 15, 95 | 11, 0.056 | 5.2, 0.112 | 8.8, 0.056 |
| (±)-IIIα | $C_5H_4N$ | $C_6H_5$ | H (5.88) | $CH_3$ (9.06) | — | — | — | — | — |
| (±)-IIIβ | $C_5H_4N$ | $C_6H_5$ | $CH_3$ (8.73) | H (7.60) | — | — | — | — | — |
| (±)-IVα | $C_5H_4N$ | $C_6H_5$ | H (5.88) | $CH_3$ (9.06) | — | — | — | — | — |
| (±)-IVβ | $C_5H_4N$ | $C_6H_5$ | $CH_3$ (8.73) | H (7.62) | — | — | — | — | — |

were studied. The NMR spectrum of Form *X* did not demonstrate a significant variation in the chemical shifts of the analogous (*a*) protons when compared to the NMR spectrum of the free oxime, because protonation did not take place on the pyridyl nitrogen since its electrons were already involved in intramolecular hydrogen bonding. However, the analogous (*a*) proton in Form *Y* was shifted downfield (0.80 τ) relative to the (*a*) proton (1.40 τ) of the (*E*)-isomer, since it was deshielded when the pyridyl nitrogen was protonated.

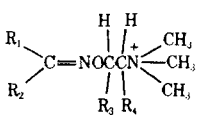
The next step of the synthetic pathway (Scheme I) consisted of the generation of the anionic oxime derivatives when sodium hydride was added. The etherification mechanism generally encompasses a nucleophilic attack by the anionic oxime derivative on the alkyl halide, resulting in the displacement of the halide ion. However, in this investigation the interaction between the nucleophiles and 2-chloro-*N*,*N*-dimethylpropylamine did not proceed exactly as in the other *O*-alkylation reactions, since α- and β-methyl structural isomers were obtained from the same reaction mixtures.

The formation of Iα–IVα and Iβ–IVβ may be rationalized *via* the etherification procedures involving a cyclic aziridinium-ion intermediate, which was formed when the neighboring amino group anchimerically assisted the displacement of the halide ion and became internally bonded to two different positions in the molecule (16, 17). The reactions proceeded *via* a nucleophilic attack on the cyclic immonium ion, producing Iα–IVα when the attack took place on the secondary carbon atom but Iβ–IVβ when the reactive site was the tertiary carbon atom. The α/β isomeric ratios demonstrated that the nucleophilic attack was more predominant at the least substituted carbon atom.

The α- and β-structural isomers were separated and purified with two techniques. Compounds Iα, Iβ, IIα, and IIβ were isolated *via* vacuum fractional distillation. Compounds IIα and IIβ were also separated with adsorption column chromatography, which proved successful in the separation of IIIα, IIIβ, IVα, and IVβ. The distillations and chromatographic separations were monitored using GLC, comparison of refractive indexes, TLC, and NMR and IR spectra.

**Resolution**—The two most commonly used methods for the resolution of racemic modifications (18, 19) are stereospecific synthesis and diastereoisomer formation. The stereospecific synthesis of the optically pure

**Table II—Oxime *O*-(α- and β-Methyltrimethylaminoethyl) Ether Bromides**



| Compound | Melting Point | Empirical Formula | Analysis, % Calc. | Found |
|---|---|---|---|---|
| (±)-Iα $CH_3Br$ | 218–222° | $C_{13}H_{29}Br_2N_3O$ | C 38.72 | 38.51 |
| | | | H 7.19 | 7.16 |
| | | | N 10.42 | 10.65 |
| (±)-Iβ $CH_3Br$ | 209–211° | $C_{13}H_{29}Br_2N_3O$ | C 38.72 | 38.06 |
| | | | H 7.19 | 7.37 |
| | | | N 10.42 | 9.70 |
| (±)-IIα $CH_3Br$ | 189–190° | $C_{19}H_{25}BrN_2O$ | C 60.49 | 60.76 |
| | | | H 6.63 | 6.87 |
| | | | N 7.43 | 7.38 |
| (±)-IIβ $CH_3Br$ | 165–166° | $C_{19}H_{25}BrN_2O$ | C 60.49 | 60.46 |
| | | | H 6.63 | 6.75 |
| | | | N 7.43 | 7.89 |

enantiomers was not considered because: (*a*) the synthetic pathway (Scheme I) was designed to take advantage of the formation of both structural isomers since the research proposal involved α- and β-isomeric derivatives, and (*b*) it has not been established that *O*-alkylation of oximes with 2-chloro-*N*,*N*-dimethylpropylamine proceeds exclusively *via* an $S_N2$ mechanism and any products formed by an $S_N1$ mechanism would lead to racemization.

The decision was made to resolve these racemates by the formation of diastereoisomeric derivatives and subsequent separation of the two different components *via* fractional crystallization. The first resolving agent, *d*-10-camphorsulfonic acid, did not prove satisfactory. The diastereoisomeric salts did not precipitate from the reaction mixture, and the mixture resisted separation with column chromatography when silica gel was used as the adsorbent material. However, (+)-tartaric acid formed stable diastereoisomeric derivatives. Subsequent treatment of the tartrates with triethylamine regenerated the free enantiomers, which were converted to the methylbromide derivatives (Tables II and III).

The relative purity of the diastereoisomers and corresponding methylbromide derivatives of the enantiomers from IIα and IIIα was determined by repeated recrystallizations until constant melting points and optical rotations were achieved (Table III). The methylbromide derivatives exhibited opposite optical rotations for the *dextro*- and *levo*-enantiomers (Table III), which also verified their relative purity. These physical properties have been employed previously (18) to substantiate the relative purity of diastereoisomers and crystalline enantiomeric derivatives.

Both types of derivatives, diastereoisomers and methylbromides, were subjected to elemental analyses (Table III) to substantiate their characterizations. An NMR spectral analysis of the diastereoisomers did not supply unambiguous data, even though some diastereoisomers exhibit significant differences when the appropriate solvent system is utilized (20).

**Table III—Oxime *O*-(α-Methylcholinyl) Ether Tartrates and Methylbromides**

| Compound | Melting Point | $[\alpha]_D^{26}$ | Empirical Formula | Analysis, % Calc. | Found |
|---|---|---|---|---|---|
| IIα Tartrate | 128–130° | +8.38° | $C_{22}H_{28}N_2O_7$ | C 61.11 | 60.91 |
| | | | | H 6.48 | 6.58 |
| | | | | N 6.48 | 6.52 |
| (+)-IIα $CH_3Br$ | 177–179.5° | +17.28° | $C_{19}H_{25}BrN_2O$ | C 60.49 | 60.36 |
| | | | | H 6.63 | 6.61 |
| | | | | N 7.43 | 7.33 |
| IIα Tartrate | 155–156° | +19.33° | $C_{22}H_{28}N_2O_7$ | C 61.11 | 60.65 |
| | | | | H 6.48 | 6.58 |
| | | | | N 6.48 | 6.36 |
| (−)-IIα $CH_3Br$ | 188–190° | −17.26° | $C_{19}H_{25}BrN_2O$ | C 60.49 | 60.76 |
| | | | | H 6.63 | 6.88 |
| | | | | N 7.43 | 7.40 |
| IIIα Tartrate | 136–139° | +8.42° | $C_{21}H_{27}N_3O_7$ | C 58.19 | 58.31 |
| | | | | H 6.23 | 6.15 |
| | | | | N 9.70 | 9.87 |
| (+)-IIIα $CH_3Br$ | 176–178° | +20.4° | $C_{18}H_{24}BrN_3O$ | C 57.16 | 57.07 |
| | | | | H 6.35 | 6.51 |
| | | | | N 11.11 | 11.31 |
| IIIα Tartrate | 156–158° | +19.4° | $C_{21}H_{27}N_3O_7$ | C 58.19 | 58.13 |
| | | | | H 6.23 | 6.54 |
| | | | | N 9.70 | 9.80 |
| (−)-IIIα $CH_3Br$ | 185–187° | −23.46° | $C_{18}H_{24}BrN_3O$ | C 57.16 | 57.03 |
| | | | | H 6.35 | 6.28 |
| | | | | N 11.11 | 11.16 |

Table IV—Summary of Pharmacological Data of Oxime *O*-(α-Methyltrimethylaminoethyl) Ether Bromides

| Compound (as Methylbromide Salt) | Dose[a], m*M*/70 ml | $ED_{50}$[b], m*M* | Potency[c] Ratio | Determinations[d] |
|---|---|---|---|---|
| (±)-IIα | $1.33 \times 10^{-5}$ | $4.14 \times 10^{-3}$ [e] | 2.11 | 4 |
| (±)-IIβ | $1.33 \times 10^{-5}$ | $1.96 \times 10^{-3}$ [e] | | |
| (+)-IIα | $7.96 \times 10^{-6}$ | $1.89 \times 10^{-3}$ [e] | 1.18 | 4 |
| (−)-IIα | $7.96 \times 10^{-6}$ | $1.60 \times 10^{-3}$ [e] | | |
| (+)-IIα | $7.96 \times 10^{-6}$ | $10.3 \times 10^{-5}$ [f] | 1.05 | 4 |
| (−)-IIα | $7.96 \times 10^{-6}$ | $9.82 \times 10^{-5}$ [f] | | |
| (+)-IIIα | $1.19 \times 10^{-5}$ | $1.37 \times 10^{-3}$ [e] | 1.61 | 4 |
| (−)-IIIα | $1.19 \times 10^{-5}$ | $2.21 \times 10^{-3}$ [e] | | |
| (+)-IIIα | $1.19 \times 10^{-5}$ | $3.70 \times 10^{-5}$ [f] | 1.69 | 4 |
| (−)-IIIα | $1.19 \times 10^{-5}$ | $6.24 \times 10^{-5}$ [f] | | |
| (+)-IIIα | $9.92 \times 10^{-6}$ | $4.43 \times 10^{-5}$ [f] | 1.79 | 6 |
| (−)-IIIα | $9.92 \times 10^{-6}$ | $7.93 \times 10^{-5}$ [f] | | |

[a] Dose of spasmolytic agent introduced prior to the addition of the spasmogen dose increments. [b] Spasmogen doses necessary to elicit a 50% response after pretreatment with the spasmolytic agent. [c] Comparison of the spasmolytic activities based on the respective $ED_{50}$. [d] Number of consecutive determinations using the same muscle strip. [e] Urecholine as spasmogen. [f] Carbachol as spasmogen.

Compounds IIβ and IVα resisted resolution, and only one diastereoisomer was obtained in each case. The isolation of the enantiomers of IIβ was not pursued, since preliminary pharmacological data substantiated that (±)-IIα possessed greater anticholinergic activity than (±)-IIβ (Table IV). Resolution of IIIβ and IVβ was not attempted for the same reason.

Since it was confirmed previously (12) that dimethylaminoethyl *O*-ether derivatives of (*E*)- and (*Z*)-phenyl 2-pyridyl oximes were equipotent, the recovery of the corresponding diastereoisomer of IVα was discontinued. Elemental analyses of Iα and Iβ indicated that diquaternization occurred, which complicated the resolution procedure; therefore, the synthesis of these compounds as antimuscarinic agents was not pursued.

## EXPERIMENTAL[1]

**Oximes**—*1-Methyl-4-piperidone*—A modified method of Kochhar *et al.* (10) was used. Sodium bicarbonate (79.2 g, 0.84 mole) was added, with constant stirring, to a 600-ml aqueous solution containing hydroxylamine hydrochloride (66 g, 0.84 mole). The reagent, 1-methyl-4-piperidone (67.2 g, 0.6 mole), was added next, and the reaction mixture was stirred at room temperature for 5 hr with a magnetic stirrer. The mixture was then saturated with sodium carbonate and extracted with five 50-ml portions of chloroform. The combined chloroform extracts were evaporated under reduced pressure. The crude product, after recrystallization from anhydrous benzene–petroleum ether (7:1), yielded a crystalline material (56 g, 75%) that melted at 128–129°. The IR spectrum demonstrated characteristic bands for C=N (1630 $cm^{-1}$) and NOH (920–970 $cm^{-1}$).

*Benzophenone*—The method described by Wylie *et al.* (15) was followed by combining benzophenone (100 g, 0.53 mole), previously dissolved in 200 ml of warm ethanol (95%), with hydroxylamine hydrochloride (50 g, 0.89 mole), which was previously dissolved in 40 ml of distilled water. Sodium hydroxide pellets (110 g, 2.75 moles) were added to the mixture with constant stirring and cooling when necessary. The reaction mixture was allowed to cool to room temperature prior to its combination with 2 liters of distilled water containing 300 ml of 36% hydrochloric acid. The precipitate was recrystallized from 95% ethanol, and the pure oxime (88 g, 81%) melted at 143–144°. Its IR spectrum indicated the presence of the characteristic bands for the C=N (1580 $cm^{-1}$) and aromatic (2920 and 1900 $cm^{-1}$) moieties.

*Phenyl 2-Pyridyl Ketone*—A modified method of Wylie *et al.* (15) was employed by adding a 50-ml aqueous hydroxylamine hydrochloride (50 g, 0.89 mole) solution to a solution of phenyl 2-pyridyl ketone (100 g, 0.53 mole) in 350 ml of warm 95% ethanol. Sodium hydroxide (110 g, 2.75 moles) was added in small portions to the mixture with stirring and cooling when the heat of reaction became excessive. After allowing the reaction mixture to cool to room temperature, an aqueous hydrochloric acid solution (300 ml of 37% HCl in 2 liters of distilled water) was added to the mixture. A mixture of the (*E*)- and (*Z*)-geometric isomers precipitated but dissolved upon continued addition of the acidic solution and subsequent heating. A precipitate (2.8 g) formed after the solution cooled to room temperature. This precipitate was recrystallized from 95% ethanol, and its melting point (166–168°) coincided with the one reported by Haney (12) for the (*Z*)-isomer.

The pH (1.8) of the acidic solution containing the soluble hydrochloride salts was adjusted to 2.1 with an aqueous sodium hydroxide solution (10%). Precipitation occurred, and the solution was heated to allow the solid material to dissolve. The precipitate that formed upon subsequent cooling to room temperature was recrystallized from ethanol (95%) and melted at 165–168° (9 g). A second adjustment was made to pH 2.3, and the solution was heated to dissolve the precipitate that had formed. The solid material that formed when this last solution cooled to room temperature was recrystallized from ethanol (95%), mp 164–166° (7 g).

The melting point and NMR data demonstrated that each product obtained from the first and second pH adjustments was the (*Z*)-geometric isomer. However, a third pH adjustment to 2.5 afforded a mixture (13.4 g) of the (*Z*)- and (*E*)-geometric isomers. A fourth adjustment to pH 6 produced the (*E*)-isomer (32.3 g), mp 151–153°, after recrystallization from ethanol (95%). The NMR spectra demonstrated characteristic chemical shifts for the protons adjacent to the pyridyl nitrogen in the (*Z*)-(1.25 τ) and (*E*)- (1.40 τ) isomers.

**Oxime *O*-Ethers**—Pertinent preparative data and physical constants for each synthesized compound are given in Tables I–III.

*Synthesis*—The respective oxime was dissolved in warm absolute ethanol and placed in a three-necked round-bottom flask equipped with an electric stirrer, a water condenser, and a dropping funnel. Sodium hydride as a 51.6% mineral oil dispersion was added to this solution in small portions with constant stirring and cooling. The alkyl halide, 2-chloro-*N,N*-dimethylpropylamine hydrochloride, was dissolved in hot absolute ethanol and placed in a dropping funnel. The solution in the funnel was heated with a heating tape when precipitation of the alkyl halide occurred while it was being added dropwise to the refluxing reaction mixture. The hot reaction mixture was filtered, and the solvent was evaporated under reduced pressure. The resultant oily material containing the α- and β-structural isomers was extracted with anhydrous petroleum ether for I and II, but isopropyl ether was used for III and IV. The mixture was filtered, and the solvent was evaporated under reduced pressure.

*Separation*—The α- and β-structural isomers of I were separated *via* vacuum fractional distillation utilizing a microdistillation apparatus with a heated 14-cm fractionating column. The isomers of II were separated by both vacuum fractional distillation, using an 18-cm vacuum-jacketed fractionating column, and column chromatography. In addition, the structural isomers of III and IV were separated by column chromatography.

An isomeric mixture (19.7 g) of II was chromatographed on silica gel (200 g). The eluting solvents, benzene, benzene–ether (95:5), benzene–ether (4:1), and ether, successfully separated IIα and IIβ. Another chromatographic separation also proved successful when 45.5 g of the reaction mixture (II) was introduced on a column packed with 355 g of silica gel and eluted with cyclohexane, cyclohexane–benzene (3:1), cyclohexane–benzene (1:1), cyclohexane–benzene (1:3), benzene, benzene–ether (4:1), and benzene–ether (1:1).

A mixture (24.4 g) of α- and β-isomers from III was incorporated on a chromatography column packed with 200 g of silica gel. Separation was accomplished when the column was eluted with successive portions of benzene, benzene–ether (9:1, 85:15, 4:1, 3:1, and 1:1), ether, ether–ethanol (1:1), and ethanol. An isomeric mixture (14.2 g) of IV was also successfully separated using 200 g of silica gel and a similar eluting system.

The IR spectra of the α-isomers displayed a characteristic carbonyl stretching band at 1040 $cm^{-1}$, while the β-isomers' corresponding bands were at 1060 or 1075 $cm^{-1}$. The separations also were monitored with TLC, GLC, and NMR spectroscopy. Correct elemental analyses of the methylbromide derivatives also were used to characterize the structural isomers of I and II. These derivatives were obtained by recrystallization of the precipitates that developed when the respective isomers, while in isopropyl ether, were treated with excess bromomethane and placed in the freezer for 24 hr.

*Resolution*—The diastereoisomers from IIα were prepared with (+)-tartaric acid. The oxime *O*-ether (12.51 g, 0.044 mole) was dissolved

[1] The melting points are uncorrected; a Thomas-Hoover Uni-Melt apparatus was used. Elemental analyses were conducted by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. IR spectra of all final products were measured on a Beckman IR-8 spectrophotometer. NMR spectra were obtained with a Varian A-60 spectrometer (tetramethylsilane and dimethyl sulfoxide as the internal standards). Optical rotations were obtained with a Perkin-Elmer 141 polarimeter. Pharmacological determinations were made on a Marco physiograph desk model DMP-4A.

in absolute ethanol (200 ml), and this mixture was combined with 200 ml of absolute ethanol containing an isomolar amount of (+)-tartaric acid (6.66 g, 0.044 mole). The reaction mixture was refluxed for 1 hr, but heavy precipitation necessitated the addition of 80 ml more of absolute ethanol. The precipitate resulting after the mixture stood at room temperature for 1 hr was purified by recrystallizing it twice from absolute ethanol [6.9 g, mp 155–156°, $[\alpha]_D^{26}$ +19.33° (c 1.5%, absolute methanol)]. Anhydrous isopropyl ether (150 ml) was added to the original filtrate to promote precipitation of the other diastereoisomer. The precipitate (6 g, mp 125–130°) was collected and recrystallized four times from isopropyl alcohol [2 g, mp 128°–130°, $[\alpha]_D^{26}$ +8.38° (c 5%, absolute methanol)].

The methylbromide derivative of (−)-IIα from the higher melting diastereoisomer was prepared following the regeneration of the free base. The diastereoisomeric salt (3 g) was dissolved in 16 ml of distilled water, and this mixture was combined with triethylamine (10 ml) and stirred for 30 min at room temperature. Anhydrous isopropyl ether (30 ml) was added, and the reaction mixture was stirred for an additional 15 min. The isopropyl ether layer was isolated, and the solvent was evaporated under reduced pressure. The residue was dissolved in anhydrous isopropyl ether, and this mixture was treated with excess bromomethane. Then the mixture was placed in the freezer for 12 hr, and the resulting precipitate was recrystallized three times from isopropyl alcohol. The analytically pure sample melted at 188–190° (0.4 g) and possessed an optical rotation of $[\alpha]_D^{26}$ −17.26° (c 5%, chloroform).

The methylbromide derivative of (+)-IIα from the lower melting diastereoisomer was prepared following the regeneration of the free base. The diastereoisomeric salt (2 g) was dissolved in 12 ml of distilled water, and this mixture was combined with 7 ml of triethylamine. The reaction mixture was stirred for 30 min at room temperature. Anhydrous isopropyl ether (20 ml) was then added, and the reaction mixture was stirred for an additional 15 min. The isopropyl ether layer was separated from the aqueous layer, and the solvent was evaporated under reduced pressure. The residue was dissolved in anhydrous isopropyl ether, and this mixture was treated with excess bromomethane.

The reaction mixture was stored in the freezer for 12 hr. The precipitate (0.9 g, mp 182–185°) was recrystallized three times from a mixture of anhydrous isopropyl ether (2%) and isopropyl alcohol, but a significant optical rotation was not achieved. The filtrates from these recrystallizations were combined and placed in the freezer. The solid material (mp 178–183°) was recrystallized four times from a solvent system composed of anhydrous isopropyl ether (5%) and isopropyl alcohol. An analytically pure sample (0.2 g) was obtained [mp 177–179.5°, $[\alpha]_D^{26}$ +17.28° (c 5%, chloroform)].

The diastereoisomers from IIIα were formed with (+)-tartaric acid. The oxime *O*-ether (3.74 g, 0.0132 mole) was dissolved in 200 ml of absolute ethanol, and this mixture was combined with 200 ml of absolute ethanol containing an isomolar quantity of (+)-tartaric acid (1.98 g, 0.0132 mole). The reaction mixture was refluxed for 1 hr and stirred for 12 hr at room temperature. Then the mixture was heated to dissolve the solid material and allowed to stand at room temperature for 12 hr. The precipitate [2.1 g, mp 143–150°, $[\alpha]_D^{26}$ +13.88° (c 2.5%, absolute methanol)] was recrystallized three times from absolute ethanol.

The analytically pure sample (1 g) melted at 156–158° with an optical rotation of $[\alpha]_D^{26}$ +19.4° (c 2.5%, absolute methanol). Anhydrous isopropyl ether (450 ml) was added to the original filtrate while heating and stirring, and the mixture was allowed to stand at room temperature until precipitation occurred. The precipitate [1.7 g, mp 132–136°, $[\alpha]_D^{26}$ +9.52° (c 5%, absolute methanol)] was recrystallized three times from a mixture of anhydrous isopropyl ether and absolute methanol. An analytically pure sample (0.8 g) was obtained [mp 136–139°, $[\alpha]_D^{26}$ +8.42° (c 5%, absolute methanol)].

The methylbromide derivative of (−)-IIIα from the higher melting diastereoisomer was prepared after the regeneration of the free base. Excess triethylamine was added to the diastereoisomer (1 g), and the mixture was stirred for 15 min. Distilled water (20 ml) was then added, and the reaction mixture was stirred until all gummy material dissolved. Anhydrous isopropyl ether (20 ml) was combined with the mixture, and the resultant mixture was stirred for an additional 15 min. The isopropyl ether layer was segregated and evaporated under reduced pressure.

The residual oil (0.7 g) was dissolved in anhydrous isopropyl ether, and this mixture was treated with excess bromomethane. The reaction mixture was stored in the freezer for 12 hr. The precipitate was recrystallized three times from a mixture of anhydrous isopropyl ether and isopropyl alcohol. The analytically pure derivative (0.3 g) melted at 185–187°, $[\alpha]_D^{26}$ −23.46° (c 5%, chloroform).

The methylbromide derivative of (+)-IIIα from the lower melting diastereoisomer was prepared after the regeneration of the free base. Excess triethylamine was added to the diastereoisomer (0.8 g), and the mixture was stirred for 15 min. Distilled water (20 ml) was added, and the reaction mixture was stirred until all gummy material dissolved. Anhydrous isopropyl ether (20 ml) was combined with the mixture, and this final mixture was stirred for an additional 15 min. The isopropyl ether layer was separated and evaporated under reduced pressure.

The residual oil was dissolved in anhydrous isopropyl ether, and this mixture was treated with excess bromomethane. The reaction mixture was stored in the freezer for 12 hr. The precipitate was recrystallized six times from a mixture of anhydrous isopropyl ether and isopropyl alcohol. The analytically pure derivative (0.1 g) melted at 176–178°, $[\alpha]_D^{26}$ +20.4° (c 5%, chloroform).

## PHARMACOLOGY

A preliminary pharmacological evaluation was conducted to determine the antimuscarinic activity of the methylbromide derivatives of (±)-IIα, (±)-IIβ, (+)-IIα, (−)-IIα, (+)-IIIα, and (−)-IIIα. Antimuscarinic activity was determined relative to atropine sulfate ($3.60 \times 10^{-7}$ mmole/ml) when urecholine and/or carbachol were utilized as spasmogens. The concentrations of the spasmogen solutions were determined so that $5.1 \times 10^{-4}$ mmole of urecholine and $1.23 \times 10^{-5}$ or $1.64 \times 10^{-5}$ mmole of carbachol were added per increment.

**Method**—The general method (6) consisted of the inhibition of spasmogen-induced contractions by a spasmolytic agent on excised strips of rat ileum. The ileum strip was kept at constant temperature (37 ± 0.5°) in an aerated 80-ml glass chamber that was filled to 70 ml with a Tyrode bathing solution and was attached to a myograph transducer connected to a physiograph.

**Results**—A summary of the experimental data is found in Table IV. The synthesized compounds were tested as the methylbromide salts. The pharmacological data demonstrated that the racemic IIα structural isomer was more active than the corresponding (±)-IIβ isomer, which was in accord with previous results (9).

The data pertinent to (+)-IIα, (−)-IIα, (+)-IIIα, and (−)-IIIα indicated that, relative to atropine sulfate, the first two compounds possessed higher antimuscarinic potency than the latter two and that (−)-IIα and (+)-IIα were equipotent. On the other hand, (−)-IIIα was more active than (+)-IIIα, demonstrating enantiomeric potency ratios of 1.61 and 1.79 when urecholine and carbachol were used, respectively, as spasmogens. However, these data were not consistent with Pfeiffer's rule (21), which states that the more active compounds should possess a higher degree of stereospecificity.

Recent reports (13, 14, 22) elaborated on the fact that minor structural changes in a compound produce unusually large differences in affinity which cannot be ascribed simply to the binding or failure to bind of one group. These studies illustrated the need to consider the effect of a new group on the binding of the rest of the molecule with the receptor since it may force a realignment of other important functional groups. This concept can account for the difference in activity between (±)-IIα and (±)-IIβ. The α-methyl group in (±)-IIα demonstrated an overall positive contribution regardless of whether it produced the preferred conformation or supplied an additional site for a greater interaction with the receptor.

The activities of the enantiomers, (+)-IIα, (−)-IIα, (+)-IIIα, and (−)-IIIα, may be discussed similarly, since it has been reported (14) that differences in stereospecificity arise from greater disturbances in the binding of one enantiomer than in the other corresponding one.

Compounds (−)-IIIα and (+)-IIIα possess an unsymmetrical flat umbrella-like moiety at one end of the molecule while (−)-IIα and (+)-IIα possess a symmetrical one. If the theory that the receptor demonstrates selective affinity for the phenyl and 2-pyridyl functions is invoked as in the study where the effect of a cyclohexyl group was compared with a phenyl group (22), a change in the overall conformation of (+)-IIα and (−)-IIα would not affect the binding with the receptor, but this would not be the case with (+)-IIIα and (−)-IIIα.

Therefore, the absence and presence, respectively, of enantiomeric potency ratios in (−)-IIα, (+)-IIα, (−)-IIIα, and (+)-IIIα may be rationalized on the basis that the α-methyl group alters the overall binding of the molecule with the receptor. In (+)-IIα and (−)-IIα, the alteration did not demonstrate a significant enantiomeric potency ratio, since the opposite end of the molecule consisted of a symmetrical umbrella-like moiety. On the other hand, a significant one was observed in (−)-IIIα and (+)-IIIα, since they possess an unsymmetrical umbrella-like moiety. This observation, however, is not in complete agreement with the study concluding that an α-methyl group contributed considerably to antiacetylcholine potency but that the contribution was not dependent on stereochemistry (23).

In view of existing evidence, it appears that a methyl group alpha to the alkylamino function influences anticholinergic activity. Furthermore, the postulated ability of this group to alter the orientation between the molecule and the receptor should further substantiate the premise that the binding of an important moiety of an antagonist may be affected considerably by modifying other parts of the same molecule.

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# Chelates of Dicumarol I: Preparation and Structure Identification of Magnesium Chelate


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**Abstract** □ A magnesium chelate of dicumarol was prepared by reacting a suspension of dicumarol and magnesium oxide in 50% water–methanol. GLC, thermogravimetric, and elemental analyses showed that this compound has a 2:1 ligand–metal stoichiometry with 2 moles of water associated with the complex. Although the chelate does not melt, two endothermic peaks at 205 and 274° were observed in the thermogram, in contrast to a single endothermic peak corresponding to a melting point of 288° for dicumarol. IR spectroscopy indicated that the magnesium is bonded between the carbonyl at C-2 and the oxygen at C-4′ (or vice versa).



**Keyphrases** □ Dicumarol—chelate with magnesium prepared, structure elucidated □ Chelates—dicumarol–magnesium, prepared, structure elucidated □ Magnesium—chelate with dicumarol prepared, structure elucidated □ Metals—magnesium, chelate with dicumarol prepared, structure elucidated □ Anticoagulants—dicumarol, chelate with magnesium prepared, structure elucidated


Dicumarol [3,3′-methylenebis(4-hydroxycoumarin)] (I) is an oral anticoagulant utilized for the prevention and therapy of thromboembolic vascular disease. However, many problems are associated with its use: concomitant administration with certain drugs can inhibit or potentiate its anticoagulant effect (1), it is slowly and erratically absorbed from tablet dosage forms (2), and its bioavailability from tablets depends on the type and amount of excipients in the formulation (3).

Reported bioavailability differences from various tablet formulations provided the stimulus for a study of potential solid–solid interactions between I and various metal-containing excipients (4). Chemisorption of I occurred with several excipients, but the interaction with the magnesium-containing excipients was postulated to be chelation.

In subsequent studies (5, 6) in dogs and humans, concomitant administration of I with magnesium-containing adjuvants resulted in faster and more complete drug absorption. These investigators postulated that the enhanced bioavailability may be due to chelate formation within the GI tract.

This investigation was undertaken to prepare, isolate, and study the physicochemical properties and bioavailability of the magnesium chelate of dicumarol. This report details the preparation, stoichiometry, and structure