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Synthesis and Structure–Activity Relationships of Selected Tricyclic Oxime *O*-Ethers as Potential Anticholinergic Agents

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Abstract □ Selected isomeric and nonisomeric oxime *O*-ether derivatives of thioxanthone oxime were synthesized and evaluated for anticholinergic activity. The oxime *O*-ethers were prepared *via O*-alkylation of the oximate anion with appropriate aminoaklyl halides. Separation and isolation of the structural isomers were accomplished through dry-column chromatography. The racemic α-methyl isomer was resolved *via* formation of tartrate diastereomers, which were subsequently isolated. All synthesized compounds exhibited significant antimuscarinic activity. A comparison of the antimuscarinic activities of these compounds revealed that the racemic α-methyl isomer was the most potent and that the racemic β-methyl isomer was the least potent. Structure–activity relationships among the oxime *O*-ether derivatives synthesized are discussed.

Keyphrases □ Anticholinergics, potential—selected tricyclic oxime *O*-ethers, synthesis, structure–activity relationships □ Structure–activity relationships—selected tricyclic oxime *O*-ethers as potential anticholinergics, synthesis □ Antimuscarinic activity—comparison of synthesized tricyclic oxime *O*-ethers □ Oxime derivatives—synthesis, comparison as potential anticholinergics

Various structurally and stereochemically different esters exhibiting anticholinergic activity were studied previously (1–4) to obtain information concerning stereospecificity of the parasympathetic postganglionic acetylcholine receptor. One report (3), dealing with α- and β-methylcholine esters of 2-cyclohexyl-2-hydroxy-2-phenylacetic acid, concluded that α-methyl substitution significantly increased antimuscarinic activity and that such an increase was independent of the configuration of the α-substituted carbon atom. Based on these findings, the report indicated that the stereochemistry of the aminoalcohol moiety was not significant in determining antimuscarinic activity.

Previous work (5, 6) demonstrated that oxime derivatives are useful model compounds for the study of structure–activity relationships. More recent studies related geometric (7, 8) and enantiomeric (8) isomerism in oxime *O*-ethers to their anticholinergic activity. These findings (8), however, were not in complete agreement with earlier reports (1–3) concerning the stereochemical significance of the aminoalcohol moiety in determining antimuscarinic potency.

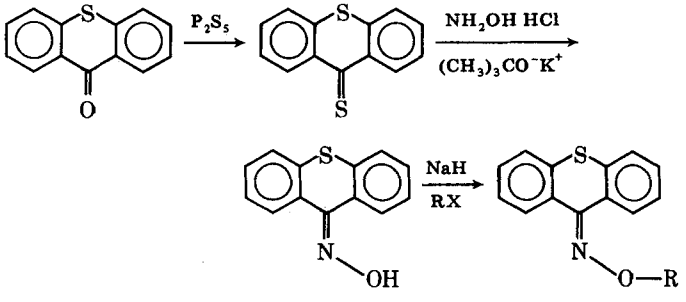
The present study was a further investigation of the possible relationships between structural and stereochemical properties and antimuscarinic activity among oxime *O*-ethers. Selected isomeric and nonisomeric oxime *O*-ethers of thioxanthone oxime (Table I) were synthesized, and a preliminary pharmacological evaluation was conducted to determined their antimuscarinic activity.

DISCUSSION

Preparation of the oxime directly from thioxanthone was attempted *via* methods described by Gomer *et al.* (6), for the synthesis of xanthoxime, and Wylie *et al.* (9), for the preparation of benzophenone oxime, but was unsuccessful. This failure can be explained by the fact that, in thioxanthone, the carbonyl carbon is considerably less electrophilic and, therefore, less susceptible to nucleophilic attack by the hydroxylamine nitrogen. Subsequently, thioxanthone oxime was prepared by a modified method of Nagrajan *et al.* (10). Accordingly, thioxanthone was converted first to the thione by refluxing it with excess phosphorus pentasulfide in xylene. The thione then was refluxed with hydroxylamine hydrochloride and potassium *t*-butoxide in anhydrous ethanol to yield the oxime in fair yields. Based on the reported mechanism (8, 11) involved in the synthesis of oximes from highly aromatic ketones, a general base-catalyzed mechanism was proposed for the synthesis of thioxanthone oxime. The oxime was quite unstable when exposed to direct sunlight, decomposing to the ketone. The major steps involved in the synthesis of the oxime and the oxime *O*-ethers are depicted in Scheme I.

The oxime *O*-ethers included in this study (Table I) were prepared *via O*-alkylation of the oximate anion with the appropriate aminoalkyl halides. The mechanism of *O*-alkylation involves a nucleophilic attack by the base-generated anionic oximate species on the aminoalkyl halide, resulting in the displacement of the halide ion (12).

The procedure of Huerta *et al.* (8) was used to prepare the α- and β-methylcholine *O*-ethers of the oxime. Accordingly, 2-chloro-*N*,*N*-



RX = aminoalkyl halide

Scheme I

0022-3549/81/0500-0558$01.00/0

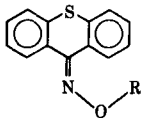
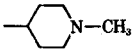


Table I—Oxime *O*-Aminoalkyl Ethers

Compound[a]	Yield, %	R	NMR Data[b], ppm				IR Data[c], cm^{-1}		
			OCH_2	NCH_2	$N(CH_3)_2$	Aromatic H	C=N	C–O	N–O
I	40	$-CH_2CH_2N(CH_3)_2$	4.37	2.73	2.20	7.13–8.60	1640	1030	980
II	40		4.42–3.92[d]	2.75–1.58[e]	2.15[f]	7.00–8.43	1620	1030	980
III	70	$-CH_2CH_2CH_2N(CH_3)_2$	4.16	2.50–1.50[g]	2.13	6.90–8.17	1640	1030	980
(±)-IV	31	$-CH_2CHN(CH_3)_2$ CH_3	4.67–4.00	3.25–2.83[h]	2.33	7.20–8.57	1620	1040, 1030	980
(±)-V	15	$-CHCH_2N(CH_3)_2$ CH_3	4.83–4.42[d]	2.83–2.40	2.38	7.17–8.30	1640	1065, 1030	980

[a] Chromatographed on silica gel dry column. [b] Chemical shifts are expressed as δ; deuterochloroform was used as the solvent in all determinations except with II where carbon tetrachloride was used. [c] Frequency of absorptions for neat samples. [d] The OCH proton. [e] The $N(CH_2)_2$ and $C(CH_2)_2$ protons. [f] The NCH_3 protons. [g] The NCH_2–CH_2 protons. [h] The NCH proton.

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

Compound	Recrystallization Solvent	Melting Point	$[\alpha]_D^{25}$	Empirical Formula	Analysis, %	
					Calc.	Found
IV-Tartrate	Ethanol	118–120°	+8.13°	$C_{22}H_{26}N_2O_7S$	C 57.13	56.98
					H 5.67	5.71
					N 6.06	5.98
(+)-IV · CH_3Br	Isopropyl alcohol–isopropyl ether	219–220°	+16.33	$C_{19}H_{23}BrN_2OS$	C 56.02	56.20
					H 5.69	5.80
					N 6.88	6.81
IV-Tartrate	Isopropyl alcohol–isopropyl ether	128–130°	+18.60°	$C_{22}H_{26}N_2O_7S$	C 57.13	57.17
					H 5.67	5.80
					N 6.06	5.79
(−)-IV · CH_3Br	Isopropyl alcohol	216–218°	−16.43°	$C_{19}H_{23}BrN_2OS$	C 56.02	55.73
					H 5.69	5.85
					N 6.88	6.58

dimethylpropylamine was used as the alkylating agent, resulting in the synthesis of both structural isomers (IV and V) in the same reaction mixture. The proposed mechanism (13) implicates the generation of a cyclic aziridinium-ion intermediate, followed by a nucleophilic attack by the oximate anion on the two possible nucleophilic sites of the cyclic aziridinium ion. An attack on the secondary carbon results in the formation of the α-methyl isomer (IV), and a corresponding attack on the tertiary carbon results in the β-methyl isomer (V). It was apparent from the α–β isomeric ratio that a nucleophilic attack on the secondary carbon of the aziridinium ion was favored.

An attempt to separate the structural isomers by high-vacuum distillation failed because of the high thermal decomposition of the oily mixture. Dry-column chromatography was effective for large-scale separation. This technique allowed direct application of the optimal conditions, previously determined by using TLC, to a column adsorbent of the same composition. The separation and purification of the structural isomers were monitored by TLC, and they were identified conclusively based on their NMR and IR spectra.

The NMR spectral data were particularly conclusive. In the α-methyl isomer, the α-methyl group is influenced only by the methine proton adjacent to the amino nitrogen; thus, it was represented by a doublet peak at δ 1.10. The corresponding doublet for the β-methyl group in the β-methyl isomer appeared slightly downfield at δ 1.40, indicating that the methine group influencing the β-methyl group must be adjacent to the ether oxygen. Other differences in the NMR spectra also were useful in assigning the correct structures (Table I).

Resolution of the racemic α-methyl structural isomer into its respective enantiomers was accomplished by preparing diastereomeric salts with tartaric acid, followed by repeated recrystallizations until each diastereomer exhibited a constant melting point and specific rotation. The enantiomeric free bases were liberated from the diastereomers separately by treatment with triethylamine and derivatized to their respective methyl bromide salts. These methyl bromide salts, after several recrystallizations, exhibited essentially equal but opposite specific rotations. All of the established criteria (14) for confirming the relative optical purity of enantiomers were met during resolution. Elemental analyses and optical rotation data of the diastereomers and enantiomers are summarized in Table II.

The β-methyl isomer was not resolved because preliminary pharmacological screening indicated that the α-methyl isomer was several times more potent as an antimuscarinic agent. The chemical purity and structural characterizations of the oxime *O*-ether methyl halide derivatives prepared were confirmed by elemental analyses (Table III).

EXPERIMENTAL[1]

Oxime—*Thioxanthone Oxime*—A previous method (10) was adopted with modifications. Thioxanthone (212 g, 1 mole) was combined with distilled phosphorus pentasulfide (444 g, 2 moles) and refluxed in xylene (2000 ml) for 24 hr. The reaction was monitored with TLC using benzene as the developing solvent. The reaction mixture was filtered while hot, and the filtrate was washed quickly with five 100-ml portions of 2 *N* NaOH followed by five 100-ml portions of distilled water. The filtrate was evaporated under reduced pressure, and the resulting residue was recrystallized from hot absolute ethanol. The crystalline material, thioxanthione (160 g, 70%), melted at 167–168° [lit. (10) mp 168°].

Potassium *t*-butoxide (224 g, 2 moles) was added in small portions to a stirred solution of thioxanthione (160 g, 0.7 mole) in absolute ethanol (1000 ml). Hydroxylamine hydrochloride (140 g, 2 moles) was added to this stirred mixture, which then was refluxed for 8 hr. The reaction mixture was filtered while hot, and the filtrate was evaporated under reduced pressure. The resulting residue was washed several times with xylene and then recrystallized from hot benzene. The analytically pure oxime (63 g, 40%) melted at 193–194° [lit. (10) mp 192–193°]; IR (KBr

[1] Melting points were determined on a Thomas–Hoover Uni-Melt apparatus and are uncorrected. A Beckman IR-8 spectrophotometer was used to obtain IR spectra. Proton NMR spectra were obtained on JEOL C-60HL and Perkin-Elmer R-12A spectrometers with tetramethylsilane and dimethyl sulfoxide as internal standards. Optical rotation data were obtained on a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, N.Y. Pharmacological determinations were made on a Marco physiograph desk model DMP-4A.

Table III—Quaternary Oxime *O*-Aminoalkyl Ether Methyl Halides

Compound	Melting Point	Empirical Formula		Analysis, % Calc.	Found
I · CH_3Br	233–234.5°	$C_{18}H_{21}BrN_2OS$	C	54.96	55.25
			H	5.38	5.42
			N	7.12	7.18
I · CH_3I	223–225°	$C_{18}H_{21}IN_2OS$	C	49.09	49.29
			H	4.81	4.90
			N	6.36	6.34
II · CH_3Br	277–278°	$C_{20}H_{23}BrN_2OS$	C	57.28	56.99
			H	5.53	5.61
			N	6.68	6.72
III · CH_3Br	211–212.5°	$C_{19}H_{23}BrN_2OS$	C	56.02	55.76
			H	5.69	5.90
			N	6.88	6.77
(±)-IV · CH_3Br	218–219°	$C_{19}H_{23}BrN_2OS$	C	56.02	55.89
			H	5.69	5.79
			N	6.88	6.72
(±)-IV · CH_3I	177–179°	$C_{19}H_{23}IN_2OS$	C	50.22	50.46
			H	5.10	5.23
			N	6.17	6.20
(±)-V · CH_3Br	209–211°	$C_{19}H_{23}BrN_2OS$	C	56.02	56.07
			H	5.69	5.85
			N	6.88	6.93
(±)-V · CH_3I	176–178°	$C_{19}H_{23}IN_2OS$	C	50.22	49.84
			H	5.10	4.90
			N	6.17	5.90

disk): 1620 (C=N) and 980 (N–O) cm^{-1}; NMR (dimethylsulfoxide-d_6): δ 7.13–8.50 (m, 8H, aromatic H) and 13.07 (s, 1H, NOH).

Oxime O-Ethers—Elemental analyses data and appropriate physical constants for all final compounds are summarized in Tables I–III.

Thioxanthone Oxime *O*-(*N*-Dimethylaminoethyl) Ether (I) and Methyl Halides—The oxime (7.95 g, 0.035 mole) was dissolved in warm absolute ethanol (100 ml) and placed in a three-necked round-bottom flask. Sodium hydride (3.28 g of a 51.6% mineral oil dispersion, 0.07 mole) was added in small portions to the stirred solution. 2-Chloro-*N*,*N*-dimethylethylamine hydrochloride (1.89 g, 0.035 mole) was dissolved in hot absolute ethanol (100 ml) and added dropwise to the reaction mixture over 30 min. The stirred mixture was refluxed for 72 hr and filtered while hot. The filtrate was evaporated under reduced pressure, and the resulting oily residue was dissolved in petroleum ether. The solution was filtered, and the solvent was evaporated under reduced pressure.

The oily product (6.27 g, 60%) was purified further *via* dry-column chromatography after proper conditions (with respect to solvent and column material) were established with silica gel TLC. A 50.8-cm strip of nylon column was packed with silica gel powder (300 g, 60–200 mesh), and the crude oily product was eluted with ethyl acetate–methanol (10:1). The elution was stopped when the solvent front reached the bottom of the column, which then was sliced into 2.54-cm sections. The contents of each section were extruded into separate beakers and extracted with absolute ethanol (25 ml). The extract in each beaker was analyzed by TLC, and the extracts containing the pure oxime *O*-ether were combined. The combined extracts were filtered, and the filtrate was evaporated under reduced pressure. The oily residue was dissolved in petroleum ether, and the mixture was filtered. Then the solvent was evaporated under reduced pressure to yield the pure oxime *O*-ether (4.37 g, 40%).

The methy bromide derivative (I · CH_3Br) was prepared by treating a solution of the free base in absolute ethanol with excess methyl bromide. The mixture was placed in a freezer for 24 hr, and the resulting precipitate was collected on a filter. Recrystallization from absolute ethanol yielded an analytically pure sample, mp 233–234.5°. The methyl iodide derivative was prepared similarly by treating a solution of the free base in absolute ethanol with excess methyl iodide, mp 223–225°.

Thioxanthone Oxime *O*-[4-(1-Methylpiperidinyl)] Ether (II) and Methyl Bromide Derivative—Sodium hydride (1.6 g of a 51.6% mineral oil dispersion, 0.034 mole) was added in small portions to a stirred solution of the oxime (3.85 g, 0.017 mole) in dimethyl sulfoxide (100 ml). 4-Chloro-1-methylpiperidine hydrochloride (2.08 g, 0.017 mole) was added to the stirred mixture in small portions. Then the mixture was refluxed for 4 hr, treated with distilled water (200 ml), and extracted with five 100-ml portions of chloroform. The combined chloroform extracts were washed twice with distilled water (100 ml) and dried over anhydrous calcium carbonate. The mixture was filtered, and the solvent was evaporated under reduced pressure. The crude oily material, on purification *via* dry-column chromatography using ethyl acetate–petroleum ether–ethanol (1:1:0.1), yielded the pure oxime *O*-ether (2.2 g, 40%). The methyl bromide derivative was prepared and recrystallized from absolute ethanol, mp 277–278°.

Thioxanthone Oxime *O*-(*N*-Dimethylaminopropyl) Ether (III) and Methyl Bromide Derivative—The procedure described for the synthesis of II was adopted for the synthesis of III. Sodium hydride (1.9 g of a 51.6% mineral oil dispersion, 0.04 mole) and 3-chloro-*N*,*N*-dimethylpropylamine hydrochloride (3.2 g, 0.02 mole) were added to a stirred solution of the oxime (4.54 g, 0.02 mole) in dimethyl sulfoxide, and the mixture was refluxed for 4 hr. The reaction mixture was combined with distilled water (200 ml) and extracted with five 100-ml portions of chloroform. The combined chloroform extracts were washed twice with distilled water (100 ml) and dried over anhydrous calcium carbonate. The mixture was filtered, and the solvent was evaporated under reduced pressure. The resulting crude product, on purification *via* dry-column chromatography using ethyl acetate–petroleum ether–ethanol (1:1:0.1), yielded the pure oxime *O*-ether (4.4 g, 70%). The methyl bromide derivative was prepared and recrystallized from absolute ethanol, mp 211–212.5°.

Thioxanthone Oxime *O*-(α- and β-Methylcholinyl) Ethers (IV and V) and Methyl Halide Derivatives—A reported method (8) was adopted. Sodium hydride (9.3 g of a 51.6% mineral oil dispersion, 0.2 mole) was added in small portions to a stirred solution of the oxime (20.30 g, 0.1 mole) in absolute ethanol. A solution of 2-chloro-*N*,*N*-dimethylpropylamine hydrochloride (15.8 g, 0.1 mole) in absolute ethanol was added dropwise to the stirred, refluxing, reaction mixture over 1 hr. The mixture was refluxed further for 12 hr, during which the reaction was monitored with TLC using ethyl acetate–petroleum ether–ethanol (1:1:0.1). The hot reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The resulting oily residue was extracted with isopropyl ether, and the combined extracts were filtered. Evaporation of the solvent under reduced pressure yielded a crude oily mixture of the α- and β-methyl structural isomers (21.88 g, 70%).

Separation and Isolation of Structural Isomers—The initial separation of the structural isomers was accomplished on a small scale, with silica gel TLC using ethyl acetate–petroleum ether–ethanol (1:1:0.1). One isomer had an R_f value of 0.14, and the other isomer exhibited an R_f value of 0.28. These TLC results were applied directly to silica gel dry-column chromatography for the large-scale separation. A 91.4-cm nylon column was packed with silica gel (1200 g, 60–200 mesh) powder, previously activated at 115° for 1 hr. The isomeric mixture (20 g) was adsorbed on a separate portion of adsorbent and added to the top of the column. The column then was eluted with a solvent mixture identical to the one used during TLC separation. Development of the column was stopped when the solvent front reached the bottom of the column. The column then was cut into 2.54-cm sections, and the adsorbent in each section was extracted with absolute ethanol into separate beakers.

The contents of each beaker were analyzed by TLC, and extracts containing a single compound with identical R_f values were combined and filtered. Evaporation of the filtrates under reduced pressure yielded the pure α-methyl (9.5 g) and β-methyl (4.5 g) isomers. Based on their IR and NMR spectral data, the isomer with an R_f value of 0.14 was identified as the α-methyl isomer; the isomer with an R_f value of 0.28 was identified as the β-methyl isomer. The IR spectrum of the α-isomer exhibited a charcteristic C=O stretching band at 1040 cm^{-1}, while the β-isomer had a corresponding band at 1065 cm^{-1}. The NMR spectrum of the α-isomer exhibited a characteristic doublet at δ 1.10 for the α-methyl protons, while the corresponding doublet for the β-methyl protons appeared further downfield at δ 1.40 due to the greater influence of the oxygen atom. Other characteristic differences also were observed in the NMR spectra (Table I) of these structural isomers.

Methyl bromide and methyl iodide derivatives were prepared. The analytically pure methyl bromide derivative of the racemic α-methyl isomer melted at 218–219°, and the methyl iodide derivative melted at 177–179°. The analytically pure methyl bromide derivative of the racemic β-methyl isomer melted at 209–211°, and the methyl iodide derivative melted at 176–178°.

Resolution—The racemic α-methyl isomer (6.72 g, 0.018 mole) was dissolved in absolute ethanol (50 ml), and the solution was combined with an equimolar solution of tartaric acid (5.02 g, 0.018 mole) in absolute ethanol (50 ml). The mixture was stirred for 4 hr and allowed to stand for 24 hr. Since no precipitate formed, anhydrous ether was added to the solution, and the mixture was allowed to stand for an additional 12 hr. The resulting crystalline precipitate was collected on a filter and washed several times with anhydrous ether. The crystalline material (9.6 g, mp 110–127°) was redissolved in hot absolute ethanol (30 ml), and the solution was allowed to stand for 8 hr.

The resulting precipitate was recrystallized five times from hot absolute

ethanol to yield a crystalline product {2.0 g, mp 128–130°, $[\alpha]_D^{25}$ +18.60° (*c*, 1.5%, absolute methanol)}. The combined filtrate remaining after isolation of the pure diastereomer (mp 128–130°) was evaporated under reduced pressure. The residue (6.6 g) was dissolved in hot isopropyl alcohol (30 ml) and allowed to stand at room temperature for 12 hr. The precipitate (0.6 g, mp 128–130°) was filtered off, and anhydrous isopropyl ether was added to the filtrate. The precipitate formed; after the solution was allowed to stand for 12 hr, it was collected and recrystallized three times from isopropyl alcohol–isopropyl ether {2.5 g, mp 118–120°, $[\alpha]_D^{25}$ +8.13° (*c*, 2.5%, absolute methanol)}.

The methyl bromide derivative of (+)-IV was prepared from the lower melting diastereomer after regeneration of the free base. The diastereomer (2.5 g) was dissolved in distilled water (20 ml), and the solution was treated with excess triethylamine. The mixture was stirred for 1 hr, and anhydrous isopropyl ether (20 ml) was added. The mixture then was stirred for 30 min. The isopropyl ether layer was isolated, and the aqueous layer was extracted twice with isopropyl ether (10 ml). The isopropyl ether extracts were combined, dried over calcium carbonate, and filtered, and the solvent was evaporated under reduced pressure. The resulting oily residue was redissolved in anhydrous isopropyl ether (20 ml), and the solution was treated with excess methyl bromide. The mixture was placed in a freezer for 24 hr, and the resulting precipitate (1.2 g, mp 217–220°) was recrystallized four times from isopropyl alcohol–isopropyl ether {0.4 g, mp 219–220°, $[\alpha]_D^{25}$ +16.33° (*c*, 5%, absolute methanol)}.

The methyl bromide derivative of (−)-IV was prepared from the higher melting diastereomer after regeneration of the free base. Excess triethylamine was added to the diastereomer (2.6 g) in distilled water (20 ml). The mixture then was stirred for 1 hr, isopropyl ether was added, and the mixture was stirred for an additional 30 min. The isopropyl ether layer was isolated, and the aqueous layer was extracted twice with isopropyl ether (10 ml). The combined extracts were dried over anhydrous calcium carbonate and filtered, and the solvent was evaporated under reduced pressure. The oily residue was dissolved in anhydrous isopropyl ether, and the solution was treated with excess methyl bromide. The mixture was placed in a freezer for 24 hr, and the resulting precipitate (1.6 g, mp 206–212°) was collected and recrystallized three times from isopropyl alcohol {0.5 g, mp 216–218°, $[\alpha]_D^{25}$ −16.43° (*c*, 5%, absolute methanol)}.

Melting points, optical rotation data, and elemental analyses of the diastereomers (tartrates) and enantiomers (methyl bromide) of the α-methyl isomer (IV) are presented in Table II.

PHARMACOLOGY

Methyl bromide derivatives of the synthesized isomeric and nonisomeric oxime *O*-ethers were evaluated for antimuscarinic (spasmolytic) activity. Various test compound concentrations were used to obtain recordable dose–response curves. The spasmogen (urecholine) concentration was 2.55×10^{-4} mmole/90 ml for each dose increment. The atropine sulfate concentration used in all determinations was 2.00×10^{-7} mmole/90 ml. All test solutions were freshly prepared aqueous solutions.

Method—The method involved measurement of the inhibition (by a spasmolytic agent) of a spasmogen-induced contraction in a smooth muscle tissue (rat ileum) (15). The apparatus consisted of a glass cylinder (100-ml capacity) with an inlet for introducing the Tyrode bathing solution (90 ml) and an outlet for drainage. The bath solution was aerated continuously. The smooth muscle tissues were uniform strips of ileum taken from an unanesthetized, recently sacrificed, albino rat. The normal contractions of the ileum (0% response) were recorded on a physiograph through a myograph transducer. The spasmogen-induced contractions were produced by successive additions of the spasmogen dose increments at regular intervals until maximum response (100%) was achieved. There were no washes between the cumulative additions of the spasmogen.

Determination of antimuscarinic activity of the test compounds was initiated with a standard spasmogen curve, as already described, followed by washings of the muscle tissue. After the muscle strip had resumed its natural, spontaneous contractions, an appropriate dose of the test compound or atropine sulfate was introduced into the bath solution. After a brief interval (1.5 min), spasmogen dose increments were added to the bath until maximum response was achieved. The muscle strip then was washed and the whole process was repeated after 5–10 min for the next determination.

The data (percent response) were plotted on a probability scale (ordinate) *versus* the log of the spasmogen dose, in micromoles per 90 ml (abscissa). The spasmogen dose necessary to elicit a 50% response (ED_{50}) after pretreatment with the respective test compound or atropine sulfate was determined graphically. The relative potencies of the test compounds were calculated by comparing their respective ED_{50} values with those of atropine sulfate. The pharmacological data are summarized in Table IV.

Table IV—Summary of Pharmacological Data [a]

Compound	Dose[b], m*M*/90 ml	ED_{50}[c], m*M*	Relative[d] Potency	Isomeric[e] Potency Ratio
I · CH_3Br	5.00×10^{-6}	3.23×10^{-3}	0.074	—
II · CH_3Br	5.00×10^{-6}	2.26×10^{-3}	0.052	—
III · CH_3Br	5.00×10^{-6}	1.46×10^{-3}	0.033	—
(±)-IV · CH_3Br	1.00×10^{-6}	2.44×10^{-3}	0.279	—
(±)-V · CH_3Br	5.00×10^{-6}	1.25×10^{-3}	0.029	9.62
(+)-IV · CH_3Br	1.00×10^{-6}	2.33×10^{-3}	—	1.09
(−)-IV · CH_3Br	1.00×10^{-6}	2.12×10^{-3}	—	—

[a] Four consecutive determinations using the same muscle strip were made for each compound. [b] Dose of spasmolytic agent. [c] Spasmogen dose necessary to elicit a 50% response after pretreatment with the spasmolytic agent. [d] Spasmolytic activity of the test compound when compared with the spasmolytic activity of atropine sulfate. [e] Comparison of spasmolytic activities of the isomers based on their respective ED_{50}.

Results—All test compounds exhibited significant antimuscarinic activity (Table IV). Among the compounds tested, (±)-IV was the most potent (1/3 as potent as atropine sulfate) and (±)-V was the least potent (1/35 as potent as atropine sulfate).

The fact that (±)-IV was at least five times more potent than the corresponding *O*-(α-methylcholinyl) ether derivative of benzophenone oxime, which was reported (16) to be 1/22 as potent as atropine sulfate, indicated that the introduction of a sulfur atom in the umbrella-like portion of the oxime *O*-ether increased its antimuscarinic activity. This observation may indicate that the flat, nonpolar area of the muscarinic receptor, with which the umbrella-like moiety is believed to interact, is fairly extensive.

Compounds I, II, and III were 1/14, 1/19, and 1/30 as potent as atropine sulfate, respectively. The data indicate that antimuscarinic activity is optimal when a two-atom *O*-alkyl chain separates the ether oxygen from the amino nitrogen.

The fact that the racemic α-methyl isomer, (±)-IV, was considerably more potent than the racemic β-methyl isomer, (±)-V, was in agreement with previous reports (2, 3, 8). Furthermore, (±)-IV was more potent than the unsubstituted *O*-cholinyl ether derivative, I. These observations, coupled with the fact (17) that, at the receptor site involved, the activity of an antagonist is determined by its ability to bind with the receptor, may explain the α-methyl group's overall positive effect on the binding of (±)-IV with the muscarinic receptor. Such a contribution could be due to the production of a preferred conformation or to binding at an additional site on the receptor. The fact that the β-methyl isomer was less active than I would lead to an opposite conclusion: that the β-methyl group makes a negative overall contribution to the binding of (±)-V with the receptor.

It may be that the relatively large potency difference between the structural isomers cannot be due merely to binding or failure to bind of the methyl group but rather to the effect of the methyl group on binding of the rest of the molecule with the receptor. Such a conclusion would be entirely consistent with earlier reports (17–19) which observed that apparently minor structural changes in a compound can produce unusually large differences in affinities for the muscarinic receptor; such differences cannot be attributed to binding or failure to bind of any one group but rather are due to marked changes in the binding ability of the rest of the molecule.

Finally, no significant enantiomeric potency ratio was observed between (+)-IV and (−)-IV, which is consistent with earlier reports (3, 8). It may be concluded that the α-methyl group contributed positively to the binding of the rest of the molecule; however, configurational changes in the α-carbon atom did not alter significantly the binding of the rest of the molecule with the muscarinic receptor. Hence, no significant potency ratio was observed between the enantiomers.

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Transport of Prostaglandins through Silicone Rubber

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Abstract □ The *in vitro* release profiles of the F-series of prostaglandins were determined from a silicone rubber matrix of constant surface area. Silicone rubber was selective toward prostaglandin transport and offers potential as a controlled-release delivery system. Drug release patterns were dependent on the lipophilicity of the prostaglandin molecule. For dinoprost (prostaglandin $F_{2\alpha}$), the following sequence was observed: methyl ester > free acid > tromethamine salt. The biologically potent carboprost methyl [(15*S*)-15-methylprostaglandin $F_{2\alpha}$ methyl ester] was released considerably faster than the methyl ester of the parent dinoprost molecule, while release of the tromethamine salts of the two prostaglandins was similar. Permeability rates of the salts were depressed substantially when compared to their respective C-1 methyl esters. Results from independent membrane transport studies supported the observed dependence of steady-state flux on the chemical structure of the prostaglandin molecule. Plots of the amount released per unit area *versus* the square root of time were linear except for the initial drug release phase, and the total amount of prostaglandin released increased as the initial loading dose was raised. The data were analyzed according to a physical model describing drug release from inert matrix systems. The observed concentration dependence was consistent with the predictions of the model.

Keyphrases □ Prostaglandins—transport through silicone rubber, effect of lipophilicity, *in vitro* release profiles □ Dinoprost—transport through silicone rubber □ Carboprost—transport through silicone rubber □ Controlled-release delivery—transport of prostaglandins through silicone rubber □ Dosage forms—transport of prostaglandins through silicone rubber

Prostaglandins are a class of C_{20} lipid-like substances that produce a wide spectrum of biological responses (1). Their clinical application in fertility control is well recognized since they possess luteolytic and abortifacient properties, alter ovum transport, and induce menses (2). Naturally occurring dinoprost[1] (prostaglandin $F_{2\alpha}$) and dinoprostone[2] (prostaglandin E_2) have been tested clinically by numerous routes of administration in solution, tablet, and suppository dosage forms. The (15*S*)-15-methyl analogs also are being explored as fertility-regulating agents and are considerably more potent (3, 4).

Intrauterine administration reduces undesirable side effects associated with the systemic intravenous route since drug is delivered closer to the uterus. The vaginal route, however, allows for self-administration, and it has been used successfully to terminate pregnancy with dinoprostone suppositories given by a multiple-dosing regimen (5) and with a single carboprost methyl[3] [(15*S*)-15-methylprostaglandin $F_{2\alpha}$ methyl ester] vaginal suppository (6).

BACKGROUND

Studies in these laboratories have been directed toward the development of a prostaglandin delivery module that can be self-administered, is reversible, and provides continuous drug release for the treatment period following a single administration. Structural modifications of polymeric materials have been made to attain satisfactory prostaglandin release rates.

Nuwayser and Williams (7) showed that the permeability rate of dinoprost in deacetylated cellulose acetate was much greater than in other cellulose derivatives; in the rabbit, Akkapeddi *et al.* (8) demonstrated the abortifacient effectiveness of dinoprost and dinoprostone when incorporated into a series of hydrophilic polymeric materials. Polyacrylamide and polyvinylpyrrolidone gels containing dinoprostone or dinoprost also were effective in fertility control in several animal systems (9–12). Silicone rubber, however, was impervious to dinoprost transport and was discounted as a possible delivery system (9). Yet Spilman and Roseman (13) showed that silicone vaginal rings impregnated with carboprost methyl were effective in producing increased uterine muscle activity, luteolysis, and abortion in the Rhesus monkey.

The delivery system design, therefore, not only depends on the delivery module but also on the chemical form of the drug molecule. The present article provides a physicochemical analysis of the transport mechanism of prostaglandins through silicone rubber. The influence of lipophilicity on release is a dominant factor in assessing the utility of silicone rubber as a delivery system. In this regard, these findings parallel an earlier study with steroids (14).

EXPERIMENTAL

The prostaglandins[4], dinoprost, dinoprost tromethamine, dinoprost

[1] Prostin F_2 alpha, The Upjohn Co., Kalamazoo, MI 49001.
[2] Prostin E_2, The Upjohn Co., Kalamazoo, MI 49001.
[3] Prostin M/15, The Upjohn Co., Kalamazoo, MI 49001.
[4] Supplied by the Pharmaceutical Research and Development Division, The Upjohn Co., Kalamazoo, MI 49001.

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