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SOINE MEMORIAL SECTION

The articles in this Section came from the former students, colleagues, and friends of the late Professor T. O. Soine in honor and in memory of his outstanding contributions to the pharmaceutical sciences.

Synthesis, Stereochemical Analysis, and Neuromuscular Blocking Activity of Selected *E,E*- and *Z,Z*-Isomeric *O*-Ethers of 4-Androstene-3,17-dione Dioximes

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Abstract □ A number of selected geometric oxime ethers were synthesized as potential neuromuscular blocking agents. The study consisted of the synthesis, separation, and stereochemical analysis of selected compounds followed by a preliminary pharmacological evaluation for neuromuscular blockade. Synthesis of the compounds began with the double oximination of 4-androstene-3,17-dione followed by TLC, column chromatography, and fractional crystallization as the purification methods. These procedures yielded two of the possible four isomers. Configurational assignments on the isolated pair were based on experiments using IR, UV, and NMR spectroscopy. Isomerically pure diethers were prepared by two methods: (*a*) *O*-alkylation of the stereochemically pure dioximes with the appropriate aminoalkyl halide, and (*b*) *O*-alkylation of the dioxime mixture followed by quaternization and subsequent isolation of configurationally pure salts *via* fractional crystallization. A preliminary pharmacological evaluation was conducted by using mice on a treadmill apparatus. Some structure–activity relationships are discussed.

Keyphrases □ 4-Androstene-3,17-dione dioximes—*E,E*- and *Z,Z*-isomeric *O*-ethers, synthesis, stereochemical analysis, and evaluation for neuromuscular blocking activity □ Neuromuscular blocking agents, potential—*E,E*- and *Z,Z*-isomeric *O*-ethers of 4-androstene-3,17-dione dioximes, synthesis, stereochemical analysis, and pharmacological testing

Recent reports from these laboratories discussed oxime derivatives possessing significant anticholinergic activity (1–4). Furthermore, some of the studies (3, 4) supported the idea that the isomeric character of the oxime function results in potency differences among isomeric pairs that could characterize further the cholinergic receptor. On the basis of these findings, this investigation was initiated to study the neuromuscular blockage and receptor activity of oxime ethers.

The oximino ethers chosen were derived from 4-androstene-3,17-dione dioxime and are bisquaternary ammonium compounds (I). These compounds differ from the more classical neuromuscular blocking agents in that their quaternary nitrogens are bridged by 17 or 19 atoms instead of the usual nine to 11 atoms. This feature places the series in the second peak of potency reported by Barlow and Zoller (5).

DISCUSSION

Synthesis and Resolution—The dioximes of 4-androstene-3,17-dione were prepared in good yield using a reported method (6). This method was applied on the assumption that four geometrical isomers would result (II–V). However, numerous attempts by several methods failed to produce four compounds. TLC, column chromatography, and fractional crystallization proved to be suitable and yielded apparently two isomerically pure compounds (fast and slow[1]). NMR spectroscopy (PMR and ^{13}C-NMR) was utilized to verify their isolation.

[1] Fast and slow designate the relative migration rates of the two compounds on the TLC plates.

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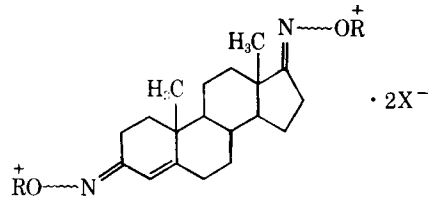
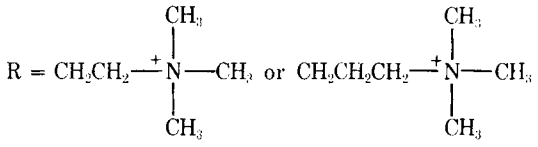


I

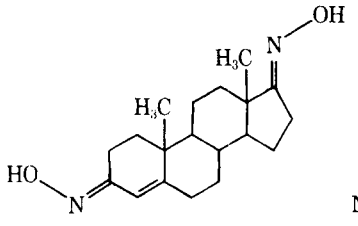


The isolation (and the apparent existence of only two of the possible four isomers) is perplexing since the dioximes that were isolated and stereochemically characterized possess all possible configurations at each oxime site. Furthermore, earlier studies (7–10) involving steroid monooximes showed that the isomeric pairs are separable and distinguishable using available methods. At present, the possibility of specific stereochemical conversion of the *Z,E*- and *E,Z*-isomers to the *E,E*- and *Z,Z*-isomers, respectively, is under consideration. This conversion may be a consequence of the isolation or purification procedures. Indirect evidence for this hypothesis comes from PMR data, which clearly show that the two zones obtained from a dry silica gel column represent configurationally pure compounds. The dioxime mixture is readily distinguishable, with respect to all configurations at both oxime sites, when subjected to PMR analysis.

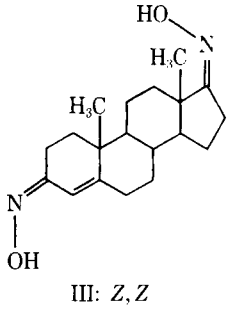
Two general methods were used in the preparation of isomerically pure oxime ethers. Method A utilized isomerically pure dioximes followed by *O*-alkylation with the appropriate aminoalkyl halide. Method B generated a mixture of ethers from *O*-alkylation of isomerically mixed dioximes. Stereochemically pure material in Method B was obtained by exhaustive quaternization of the free base followed by fractional crystallization.

Stereochemical Analysis—Because of the importance of the stereochemical features of the oxime ethers to the research objectives, several methods were used to identify the configurations at each oxime site. Configurational characterization was restricted mainly to the dioximes and was based on NMR, IR, and UV spectroscopy.

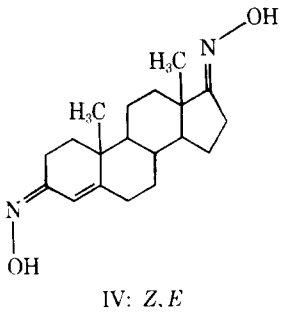
Preliminary NMR Data—There is evidence that, in some cases, proximity of an electronegative atom such as oxygen causes deshielding of protons on adjacent groups (11, 12). Karabotsos *et al.* (13) noted that the α-methylene protons and the imino proton experience greater deshielding as a consequence of the oxime hydroxyl group being in closer proximity; the β-methylene protons are reported to experience a shielding effect under the same conditions (Fragment A). These effects on the α- and β-protons have been verified for this system in carbon tetrachloride,



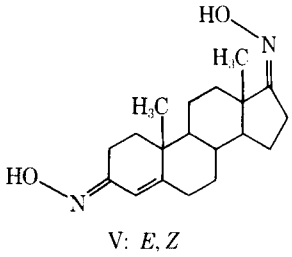
II: *E,E*



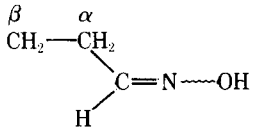
III: *Z,Z*



IV: *Z,E*



V: *E,Z*



Fragment A

benzene, methylene bromide, and nitrobenzene. The effect on the imino proton appears to be independent of the solvent and the structure. Whereas the imino proton can be used confidently to determine *E*- and *Z*-configurations, assignments based on α- and β-protons are complicated because the structure as well as the solvent reverses the resonance of *E*- and *Z*-hydrogens.

In the present study, preliminary NMR data on the dioximes were generated in dimethyl sulfoxide. A previous study on the oximes of isophorone (compounds resembling the A-ring of the dioximes) supports the suggestion that closer proximity of the oxime hydroxyl group causes deshielding of protons on adjacent groups of the dioximes (11).

For the fast isomer, the chemical shifts were δ 5.63 and 0.94 for the olefinic and 18-methyl protons, respectively. For the slow isomer, the chemical shifts were δ 6.26 and 0.98, respectively.

With the assumption that closer proximity of the oxygen to a group causes deshielding of protons on that group, tentative configurational assignments of *E,E* and *Z,Z* were made for the fast and slow isomers, respectively.

Aromatic Solvent-Induced Shifts—To support the tentative configurational assignments, the two compounds were analyzed *via* the technique of aromatic solvent-induced shifts. Wolkowski *et al.* (14) reported data derived from this technique that allowed identification of the oximes of isophorone.

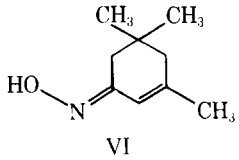
Using the formula $\Delta\delta = \nu CCl_4 - \nu C_6D_6$, Wolkowski *et al.* (14) obtained $\Delta\delta$ values of −21 and −14 Hz for the olefinic proton of the *Z*-(VII) and *E*-(VI) isomers, respectively. By utilization of the same formula, with the substitution of deuterochloroform for the aliphatic solvent and pyridine (*d*-5) for the aromatic solvent[2], similar calculations were conducted on data derived from the isolated oximes. The fast isomer demonstrated $\Delta\delta$ values of −24 and −3.4 Hz for the olefinic and 18-methyl protons, respectively. The slow isomer showed $\Delta\delta$ values of −32 and −4.4 Hz for the same parameters. Since Wolkowski *et al.* obtained greater negative values for the *Z*-isomer of isophorone oxime, the data indicate that the fast isomer has the *E,E*-configuration and the slow isomer must possess the *Z,Z*-configuration.

NMR Shift Studies—Earlier investigations (15) involving the oximes of isophorone and the shift reagent tris(dipivalomethanato)europium revealed that a greater shift of the olefinic proton is found in the *Z*-isomer. Wolkowski *et al.* (14) extrapolated to an equimolar concentration of the shift reagent and obtained shifts of 27.0 and 22.0 ppm for the *Z*- and *E*-isomers, respectively. However, in the present study, in which the shift reagent tris(1,1,1,1,1,2,2-heptafluoro-7,7-dimethyloctanedione)-europium and the oximes of isophorone were used, the opposite effect was observed (*i.e.*, a greater shift of the olefinic proton in the *E*-isomer). No attempt was made to investigate this apparent anomaly.

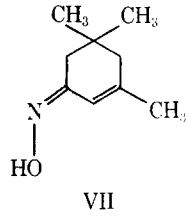
Subjecting the dioximes of 4-androstene-3,17-dione to a similar study using the shift reagent tris(1,1,1,1,1,2,2-heptafluoro-7,7-dimethyloctanedione)europium resulted in a greater shift of the olefinic and 18-methyl protons in the fast isomer compared to the shifts in the slow isomer. Since the olefinic proton of the *E*-isomer in isophorone oxime also exhibited greater movement using the same shift reagent, it was concluded that the fast isomer also must possess the *E*-configuration on the A-ring.

The assignments of *E*- and *Z*-configurations at the D-ring to the fast and slow isomers, respectively, unfortunately were not substantiated with a model shift study; consequently, the evidence is not as strong.

IR Data—Several factors have been cited as affecting the stretching



VI



VII

[2] The equation $\Delta\delta = \nu_{aliphatic} - \nu_{aromatic}$ also applies to the substitution of chloroform for carbon tetrachloride and of pyridine for benzene.

frequency of the carbonyl group (16). The position of this particular band in the IR spectrum can be affected by the physical state, electrical and mass effect, conjugation, hydrogen bonding, and ring strain. Since the same factors also are expected to affect the oximino function (C=N) in the same qualitative manner, generalities that apply to a carbonyl system were extrapolated to the oximino systems of the dioximes.

Since ring strain tends to raise the frequency of the C=N group, the higher frequencies in the region of the C=N frequency were assigned to the C=N group of the D-ring. Configurational assignments on the D-ring were made on the basis of data reported by Göndös *et al.* (9). After establishing the configuration of some 17-oximino steroids *via* the Beckman rearrangement reaction, these investigators assigned the higher frequency to the *E*-isomer. Thus, the slow isomer in the present work, which exhibited a frequency of 1681 cm^{-1}, was assigned the *Z*-configuration; the fast isomer, which displayed a frequency of 1704 cm^{-1}, was assigned the *E*-configuration.

By inspection of the oxime function on the A-ring using Dreiding stereomodels, it was noted that more steric hindrance would be realized if the oxime hydroxyl group were in the *Z*-configuration. Since steric hindrance tends to interfere with the coplanarity of the conjugated system, a higher frequency was expected for the isomer with the *Z*-configuration at this site. For the fast isomer, a singlet was recorded at 1634 cm^{-1}; for the slow isomer, a doublet was found at 1642 and 1618 cm^{-1}.

Of significance in these recordings is the fact that the slow isomer did show one elevated frequency (1642 cm^{-1}), but perhaps more informative is the observation that a distinct doublet was detected. This observation possibly could indicate a hybrid situation where perfect overlap between the C=N and C=C groups is not allowed because of the *peri-peri* interactions encounted by the oxime hydroxyl group and the olefinic hydrogen. Thus, on this basis, the slow isomer must be assigned the *Z*-configuration and the fast isomer must have the *E*-configuration.

UV Data—Since oximes show no absorption in the UV spectrum for the C=N group unless it is involved in conjugation (17), the UV analysis was restricted to the oxime function on the A-ring. In this connection, certain α,β-unsaturated carbonyl compounds have been known to possess lower absorptivities when the coplanarity is affected unfavorably (18). Therefore, a lower absorptivity for the *Z*-isomer is expected since, in this isomer, the oxime hydroxyl group would interact significantly with the olefinic hydrogen. Experimentally, the faster isomer demonstrated an absorptivity of 26,898; the slow isomer registered an absorptivity of 22,943. Therefore, the fast isomer was assigned the *E*-configuration and the slow isomer must have the *Z*-configuration at the C-3 oxime site. These assignments are in complete agreement with NMR and IR data.

Conclusion—Based on the evidence, it is concluded that the fast and slow isomers must possess *E,E*- and *Z,Z*-configurations, respectively. These assignments are substantiated by the rule predicting the isomeric ratio (19). The rule predicts that the more nearly equal in bulk the substituent groups attached to the oxime function are, the more nearly equal will be the isomeric ratio.

Oxime Ethers—Studies in these laboratories (3, 4) showed that oximes in general are relatively configurationally stable to the etherification reaction. Thus, in the systems investigated, starting with a configurationally pure oxime will yield a configurationally enriched oxime ether. In the present study, etherification of configurationally enriched dioximes did not produce detectable levels of alternate configurations, as evidenced by PMR spectroscopy. For example, the *Z,Z*- and *E,E*-isomers of the *N,N*-dimethylaminopropyl ethers (free bases) are readily distinguishable by comparison of the chemical shifts for the olefinic protons and the 18-methyl protons. In the *Z,Z*-isomer, the δ values were 5.56 and 1.03, respectively; for the *E,E*-isomer, the same groups had δ values of 6.22 and 1.01.

NMR analysis also was conducted on the quaternary ammonium salts to ensure the configurational purity of the final compounds.

EXPERIMENTAL[3]

Synthesis—4-Androstene-3,17-dione dioximes were prepared according to the method of Crabbé *et al.* (6).

Oxime Ethers—Two general methods were employed for the preparation of isomerically pure oxime ethers. The first method consisted of reacting isomerically pure dioximes (as determined by TLC and NMR spectroscopy) with the appropriate aminoalkyl halide. No problems with configurational conversion were encountered. The second method involved etherification of the mixture of the two dioximes with the appropriate aminoalkyl halide followed by quaternization and fractional crystallization of the mixture produced. Both methods were reliable.

All ethers were prepared by first dissolving the appropriate dioxime in absolute ethanol followed by the addition of the base, potassium *tert*-butoxide. The proper aminoalkyl halide hydrochloride then was added in one portion. All solutions were refluxed and stirred for the prescribed time in a three-necked flask. Subsequent to the reaction interval, the solutions were filtered by suction and evaporated on a rotary evaporator *in vacuo*. This general procedure afforded an oily residue. Variations in reactant quantities and specific procedures, as applicable to the individual ethers, will be given; physical data also are included.

Quaternization of the free bases was effected by dissolving the oily material in absolute ethanol in the presence of excess methyl bromide. The salts were purified by crystallization from a mixture of absolute ethanol and isopropyl ether.

E,E-*4-Androstene-3,17-di*(N,N-*dimethylaminoethyl oximino ether*) *Dimethobromide* (*VIII*)—*E,E*-4-Androstene-3,17-dione dioxime (1.65 g, 0.0052 mole) was dissolved in 90 ml of absolute ethanol. Potassium *tert*-butoxide (2.33 g, 0.021 mole) and 2-dimethylaminoethyl chloride (1.5 g, 0.0104 mole) then were added with constant stirring. After 4.5 hr, the solvent was evaporated, giving an oily residue that was treated with two 25-ml portions of isopropyl ether. Separation of the isopropyl ether layers and evaporation gave a material that subsequently was treated with 20 ml of petroleum ether. The petroleum ether extract was cooled in an acetone–dry ice bath and then removed and allowed to warm to room temperature. Decanting and subsequent evaporation of the warmed petroleum ether extract yielded the first crop of the dioximino ether.

All of the material that was not soluble in the petroleum ether or isopropyl ether was solubilized in ~20 ml of dimethyl sulfoxide and extracted with five 20-ml portions of petroleum ether. Small amounts of water were added between each extraction to reduce the solubility of the ether in the dimethyl sulfoxide. The petroleum ether extracts were combined and treated as before using the acetone–dry ice bath. Evaporation of the petroleum ether yielded a second crop. This procedure yielded a total of 0.7 g of the dioximino ether (29.3% yield); IR: λ_{max} (neat) 1619 (C=N, conjugated), 1667 (C=N, unconjugated), and 1035 (O—C) cm^{-1}; NMR[4]: δ 1.04 (s, 3H, 18-methyl), 2.19 (s, 12H, *N*-methyl protons), ~2.50 (β-methylene protons from both side chains; two almost overlapping A_2X_2 systems), and 5.58 (s, 1H, HC=).

The methobromide salt melted at 221° dec. and was analyzed for carbon, hydrogen, and nitrogen.

Anal.—Calc. for $C_{29}H_{52}Br_2N_4O_2$: C, 53.70; H, 8.08; N, 8.64. Found: C, 53.79; H, 8.13; N, 8.38.

Z,Z-*4-Androstene-3,17-di*(N,N-*dimethylaminoethyl oximino ether*) *Dimethobromide* (*IX*)—A mixture of *Z,Z*- and *E,E*-4-androstene-3,17-dione dioximes (3.0 g, 0.0095 mole) was dissolved in 80 ml of absolute ethanol. While stirring, potassium *tert*-butoxide (4.32 g, 0.039 mole) and 2-dimethylaminoethyl chloride (2.77 g, 0.019 mole) were added. The reaction was allowed to proceed for 8 hr, giving an oily residue upon evaporation of the solvent. The crude product was extracted with several portions of petroleum ether, and the extracts were combined and chilled in an acetone–dry ice bath to precipitate impurities. After the petroleum ether extracts warmed to room temperature, the solvent was decanted and evaporated. This procedure yielded 2.3 g of the ether (40.0% yield).

The oil obtained was dissolved in ~80 ml of absolute ethanol. Excess methyl bromide was added to the alcoholic solution followed by standing for several hours in a stoppered flask. Isopropyl ether was added to the solution of the methobromide salt until cloudiness persisted, and the milky solution was placed on a hot plate until the cloudiness dissipated. Allowing the solution to cool slowly over ~10 hr gave the first crop (0.65 g) of crystals, characterized (*via* NMR) as a mixture of *Z,Z*- and *E,E*-isomers in a 2:1 ratio.

A second crystallization in the same manner afforded a material in which no *E,E*-isomer was detectable. This material was recrystallized four times to ensure the isomeric purity of the product, mp 251° dec.; IR: λ_{max}[3] 1619 (C=N, conjugated), 1646 (C=N, unconjugated), and 1084 (C—O) cm^{-1}; NMR[5]: δ 1.09 (s, 3H, 18-methyl), 3.10 (s, 18H, *N*-methyl protons), and 6.26 (s, 1H, HC=).

[3] Melting points are uncorrected; a Thomas-Hoover Uni-Melt apparatus was used. Elemental analysis were conducted by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. IR spectra were obtained with a Beckman IR-8 spectrophotometer; spectra of oils were obtained using sodium chloride windows; spectra of salts utilized potassium bromide dispersion pellets. PMR spectra were produced on Varian A-60, Varian 100-MHz, and Jeol C-60hl spectrometers. ^{13}C-NMR spectra were taken on a Bruker WH 90 spectrometer. Optical rotations were obtained with a Perkin-Elmer 141 polarimeter.

[4] Chloroform-*d*; tetramethylsilane was the reference.

[5] Dimethyl sulfoxide-*d* was the solvent; the pentet for dimethyl sulfoxide-*d* at δ 2.5 was used as the reference.

Anal.—Calc. for $C_{29}H_{52}Br_2N_4O_2$: C, 53.70; H, 8.08; N, 8.64. Found: C, 53.63; H, 8.20; N, 8.47.

E,E-*4-Androstene-3,17-di*(N,N-*dimethylaminopropyl oximino ether*) *Dimethobromide* (*X*)—*E,E*-4-Androstene-3,17-dione dioxime (3.0 g, 0.0095 mole) was reacted with potassium *tert*-butoxide (4.3 g, 0.0383 mole) and 3-dimethylaminopropyl chloride (3.0 g, 0.019 mole). A reaction time of ~18 hr was allowed. After filtration and evaporation of the solvent, the resulting material was extracted with 150 ml of petroleum ether. The extract was cooled in an acetone–dry ice bath, allowed to come to room temperature, and decanted; the solvent was evaporated to yield a viscous yellow oil. Further purification was effected by redissolving the material in 40 ml of petroleum ether followed by a second cooling in the acetone–dry ice bath, decantation, and evaporation as already described.

A second crop of the desired material was obtained by dissolving all of the material that was not soluble in the petroleum ether in 17 ml of dimethyl sulfoxide. Several extractions of the dimethyl sulfoxide with petroleum ether afforded additional material, which was purified by employing the acetone–dry ice bath as described. Small amounts of water were added between extractions. The total yield was 2.1 g (35.0% yield); IR: λ_{max} (neat) 1634 (C=N, conjugated), 1730 (C=N, unconjugated), and 1044 (C—O) cm^{-1}; NMR[6]: δ 1.03 (s, 3H, 18-methyl), 2.11 (s, 12H, *N*-methyl), 3.88 (γ-methylene protons from both side chains; two overlapping A_2X_2 systems), and 5.56 (s, 1H, HC=).

Quaternization of the free base followed by crystallization from a mixture of absolute ethanol and isopropyl ether gave a salt that melted at 244° dec.

Anal.—Calc. for $C_{31}H_{56}Br_2N_4O_2$: C, 55.02; H, 8.34; N, 8.29. Found: C, 55.39; H, 8.36; N, 8.28.

Z,Z-*4-Androstene-3,17-di*(N,N-*dimethylaminopropyl oximino ether*) *Dimethobromide* (*XI*)—The *Z,Z*-dioxime of 4-androstene-3,17-dione (2.0 g, 0.0063 mole) was dissolved in 80 ml of absolute ethanol. Potassium *tert*-butoxide (2.85 g, 0.0254 mole) and 3-dimethylaminopropyl chloride (2.0 g, 0.0127 mole) were added, and the mixture was allowed to reflux for 23 hr. The oily residue that resulted after evaporation of the alcohol was extracted several times with petroleum ether. The petroleum ether extracts were combined, and the solvent was evaporated, yielding an oily residue.

The residue was purified further by retreating it with petroleum ether followed by separation of the ether layer and evaporation. This procedure was continued until only petroleum ether-soluble material remained. The procedure yielded 1.4 g of a viscous yellow oil (35% yield), mp 236° dec.; IR: λ_{max} (neat) 1634 (C=N, conjugated), 1734 (C=N, unconjugated), and 1044 (C—O) cm^{-1}; NMR[6]: δ 1.01 (s, 3H, 18-methyl), 2.11 (s, 12H, *N*-methyl protons), 4.08 (γ-methylene protons from both side chains; two overlapping A_2X_2 systems), and 6.22 (s, 1H, HC=).

Crystallization of the methobromide salt from absolute alcohol and isopropyl ether yielded an analytically pure sample.

Anal.—Calc. for $C_{31}H_{56}Br_2N_4O_2$: C, 55.03; H, 8.34; N, 8.28. Found: C, 54.82; H, 8.63; N, 7.98.

Separation Procedures (Dioximes)—*Method I* (*TLC*)—Separation by TLC was accomplished using a cylindrical developing chamber (25 × 5.5 cm) lined with filter paper to enhance saturation. The developing solvent consisted of benzene–ethyl acetate (10:3). Silica gel strips[7] (20 cm) were activated for 30 min at 110° and allowed to cool in a desiccator. After the system reached equilibrium (1 hr), the plates were developed to a distance of 15 cm, resulting in two distinct zones. The fast isomer was at R_f 0.17, and the slow isomer was at R_f 0.11.

Method II (*Dry Column Chromatography*)—With the same solvent system described for TLC and silica gel[8] as the support, column chromatography afforded semipreparative amounts of isomerically pure dioximes.

Method III (*Fractional Crystallization*)—4-Androstene-3,17-dione dioxime (mixture) (10.7 g) was dissolved in 425 ml of hot acetone and filtered. The solution was placed in a 500-ml beaker and covered with aluminum foil. Several small holes were provided near the center of the foil to allow slow and controlled evaporation of the acetone. When the solvent had reached the 350-ml level, the acetone was decanted and the crystals that had formed were washed with 5 ml of fresh acetone.

This first crop amounted to 2.2 g of the fast isomer with traces of the slow isomer. A second crystallization from the same solvent afforded 1.7 g of the fast isomer. The mother liquor was allowed to evaporate further in the manner described until the solvent level reached 250 ml. A second crop was obtained and identified as the slow isomer (0.8 g). Further evaporation yielded crystals that were a mixture of both isomers. This procedure gave two analytically pure samples.

The fast isomer had a melting point of 223° dec.; $[\alpha]_D^{25}$ +231° (methanol, 0.32%); UV: λ_{max} (methanol) 241 (ϵ 26,898) nm; IR[3]: λ_{max} 1634 (C=N, conjugated) and 1704 (C=N, unconjugated) cm^{-1}; NMR[5]: δ 0.780 (s, 3H, 19-methyl), 0.940 (s, 3H, 18-methyl), 5.626 (s, 1H, CH=), 9.930 [s, 1H, HO—N=C (D-ring)], and 10.326 [s, 1H, HO—N=C (A-ring)].

Anal.—Calc. for $C_{19}H_{28}N_2O_2$: C, 72.11; H, 8.92; N, 8.86. Found: C, 72.02; H, 8.89; N, 8.59.

The slow isomer melted at 223–226° dec.; $[\alpha]_D^{25}$ +132° (methanol, 0.23%); UV: λ_{max} (methanol) 243 (ϵ 22,943) nm; IR[3]: λ_{max} 1642 and 1618 (C=N, conjugated) and 1681 (C=N, unconjugated) cm^{-1}; NMR[5]: δ 0.780 (s, 3H, 19-methyl), 0.976 (s, 3H, 18-methyl), 6.259 (s, 1H, CH=), 9.930 [s, 1H, HO—N=C (D-ring)], and 10.026 [s, 1H, HO—N=C (A-ring)].

Anal.—Calc. for $C_{19}H_{28}N_2O_2$: C, 72.11; H, 8.92; N, 8.86. Found: C, 72.29; H, 9.09; N, 8.79.

NMR Shift Studies (Dioximes)—Five milligrams of each dioxime was weighed accurately and dissolved separately in 0.5 ml of deuterochloroform. To each sample was added accurately 0.007 ml of a 10% methanol solution in carbon tetrachloride using a microsyringe[9]. Minimal tetramethylsilane was added to avoid precipitation of the sample. Seven spectra were obtained on each sample, with increasing amounts of tris(1,1,1,1,1,2,2-heptafluoro-7,7-dimethyloctanedione)europium added to effect shifts on the signals[10] of the olefinic proton and C-18 methyl protons.

PHARMACOLOGY

Method—The rolling (rotarod) apparatus was used to evaluate neuromuscular blockade. This device was first described by Dunham and Miya (20), who mentioned the possibility of using such an apparatus to detect muscular relaxation. Subsequent reports (21–24) discussed the use of this method to quantify motor incoordination in laboratory animals under the influence of various drugs. Although this method is not specific for the evaluation of neuromuscular blocking agents, it does provide a reliable and simple screening avenue for such agents.

Female albino mice [Sprague–Dawley SH:ARA (ICR) f], 17–29 g, were used to evaluate the neuromuscular blocking activity of the synthesized compounds. The mice were injected intraperitoneally with solutions of the methyl bromide salts of the compounds in double-distilled water. Approximately 0.1 ml of the solution was used to deliver each dose. Immediately after injection, the mice were placed on a rotating motorized drum (7.150 rpm) and the time was recorded.

To eliminate a prolonged training period for each animal, the drum design included a screen wire walking surface with a diameter of 11.4 cm and partitions (17.8 cm in diameter) isolating each animal. A maximum of 30 min was allowed to record observations. If the animal was unable to remain on the rotating drum for the allotted time, a positive response was recorded; no response was noted if the animal remained the full time. No positive responses were observed with the control group that had been injected previously with double-distilled water.

Because the stock solutions lost significant potency over 24 hr, all stock solutions were used within 8 hr; dilutions from the stock solution were used immediately. All stock solutions were made by weighing at least 30 mg of each compound followed by dissolution with an appropriate volume of double-distilled water. Four doses (10 animals per dose) were used for all compounds tested.

The Litchfield–Wilcoxon method (24) was employed for the statistical evaluation of the data and the fitting of curves.

Results—The pharmacological data provided the following information.

1. The slopes of all curves, including the one for decamethonium, were not significantly different (95% confidence limits).
2. The *Z,Z*-isomers were significantly more potent than the *E,E*-isomers.
3. With respect to potency, the molecular modification from the ethylamino side chains to the propylamino side chains was more significant in *Z,Z*-isomerism (the propylamino homolog was more potent).
4. All of the compounds were considerably more potent than decamethonium.
5. The oxime ethers had a slower onset of action compared to decamethonium.

[6] Carbon tetrachloride; tetramethylsilane was the reference.
[7] Eastman 13181 No. 60-60.
[8] J. T. Baker, 60–200 mesh.

[9] The shift of the proton signal on the hydroxyl group of methanol was used as a relative indication of the concentration of tris(1,1,1,1,1,2,2-heptafluoro-7,7-dimethyloctanedione)europium; *i.e.*, the greater the concentration of the shift reagent, the farther downfield the proton signal would appear.
[10] Varian 100-MHz spectrometer.

Table I—Summary of Pharmacological Data

Compound[a]	ED_{50}, μM/kg	95% Confidence Limit, μM/kg	Average Response Time, min
Decamethonium[b]	1.38	1.21–1.57	5.63
VIII: *E,E*-isomer	10.0×10^{-2}	$8.2–12.2 \times 10^{-2}$	11.5
IX: *Z,Z*-isomer	7.05×10^{-2}	$6.47–7.69 \times 10^{-2}$	9.59
X: *E,E*-isomer	9.80×10^{-2}	$9.30–10.3 \times 10^{-2}$	11.1
XI: *Z,Z*-isomer	5.10×10^{-2}	$4.81–5.41 \times 10^{-2}$	15.7

[a] As the methyl bromide salts. [b] Syncurine, Burroughs Wellcome.

Additionally, from gross observations, it appears that the compounds of this study act primarily *via* a depolarizing type of mechanism. Table I summarizes the pharmacological data.

Discussion—Since the slopes of the dose–effect curves of the synthesized compounds and of decamethonium were not significantly different, the mechanism of action must be mainly depolarizing. This conclusion was supported by gross observation of the similarity in behavioral patterns when the animals were under the influence of decamethonium or the synthesized compounds. Both decamethonium and the test compounds resulted in twitching, some piloerection, pronounced heavy deep breathing, bulging eyes, and apparent convulsions subsequent to injection with an overdose. Death always followed the convulsive episode. The behavioral pattern was readily distinguishable from that produced by the antidepolarizing agent (+)-tubocurarine.

Two observations can be made with respect to possible structure–activity relationships implying drug–receptor interactions. First, in both the ethylamino and propylamino series, the *Z,Z*-isomers were significantly more potent than the *E,E*-isomers. However, the distance between the two quaternary nitrogens in the extended conformation was not significantly altered by the isomerism in question. Given the ethylamino side chain, the intercationic distance was not significantly different between the *Z,Z*- and *E,E*-isomers. Furthermore, conformational analysis using Dreiding molecular models revealed significant conformational bulky differences among the side chains of the two isomeric pairs. In the extended conformation, the side chains in the *Z,Z*-isomers could assume an almost parallel alignment (with themselves); however, *E,E*-isomerism directed the side chains at an angle of ~90°.

Second, the molecular modification from ethylamino to propylamino side chains was more significant in *Z,Z*-isomerism. In this respect, the *Z,Z*-dipropylamino congener was significantly more potent than the *Z,Z*-diethylamino compound; the *E,E*-dipropylamino compound was equipotent with the *E,E*-diethylamino congener.

At least two factors may account for the fact that the synthesized compounds were significantly more potent than decamethonium (one compound was ~27 times more potent). Because of the greater hydrophobic to hydrophilic ratio, the steroidal compounds may interact more favorably with the proposed hydrophobic area within or in close proximity to the receptor. Moreover, the steroidal oxime ethers also have the advantage of interacting with the proposed esterophilic portions of the receptor *via* the polarized oximino function; decamethonium obviously is not able to interact by a similar mechanism.

With respect to the onset of action, these compounds were significantly slower than decamethonium, probably because their greater lipid character leads to poorer transfer in the aqueous medium of the biological system.

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