Stereochemical Preferences for Curarimimetic Neuromuscular Junction Blockade III: Enantiomeric Bisquaternary Amines Related to Benzoquinonium as Probes

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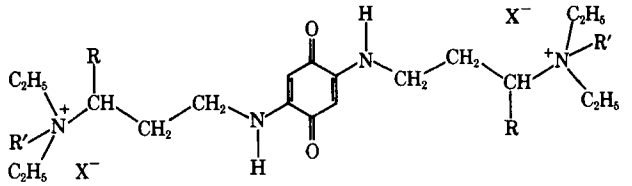
Abstract □ Enantiomeric neuromuscular junction blocking agents, which are of the benzoquinonium type but which have a methyl group introduced adjacent to the quaternary moieties to provide an asymmetric center, were synthesized and tested to determine whether the neuromuscular junction exhibits the relatively modest *(R) > (S)* superiority shown toward previously tested bisquaternaries. Testing included a mouse inclined screen assay and an *in vivo* cat hypoglossal nerve–tongue preparation, as well as standard estimations of anticholinesterase activity since the candidate compounds are known to have such a component in their activity spectrum. The observed 2:1 difference in blocking activity favoring the compound with an *(R)*-configuration is the same as that for previously tested bisquaternaries, both in direction and magnitude. Furthermore, it cannot be accounted for by preferential inhibition of acetylcholinesterase by the *(S)*-enantiomer. Absolute configurations of the enantiomers were assigned on the basis of comparisons with compounds of known configuration.

Keyphrases □ Curarimimetic activity—stereochemical preferences for neuromuscular junction blockade, enantiomeric bisquaternary amines related to benzoquinonium as probes □ Neuromuscular junction blockade (curarimimetic)—stereochemical preferences, enantiomeric bisquaternary amines related to benzoquinonium as probes □ Benzoquinonium—related enantiomeric bisquaternary amines as probes for stereochemical preferences for curarimimetic neuromuscular junction blockade □ Amines, enantiomeric bisquaternary, related to benzoquinonium—probes for determining stereochemical preferences for curarimimetic neuromuscular junction blockade

Previous studies (1, 2) were concerned with stereochemical preferences exhibited by the neuromuscular junction toward nondepolarizing blocking agents. It is, of course, recognized that asymmetry is not requisite to activity, but it is possible that differences in blocking activity by enantiomeric species could be of use in defining the characteristics of the anionic site of the hypothetical neuromuscular junction receptor.

The previously examined bisquaternaries (2) could be assumed to have answered the question of stereochemical preference, since both enantiomeric pairs showed a statistically significant *(R) > (S)* superiority in blocking potency. However, both of the previously reported probes were formally related to tetrahydroisoquinoline and, even though they were somewhat related to (+)-tubocurarine in this respect, it seemed desirable to test the generality of the *(R) > (S)* superiority in a nontetrahydroisoquinoline system.

An obvious candidate was benzoquinonium (I), since it has been recognized as a nondepolarizing blocker, presumably of the curare type, although it has no asymmetric centers. Nevertheless, synthetic introduction of a methyl group adjacent to the quaternary moiety was considered possible and would lead to a desired asymmetric probe without, hopefully, eliminating neuromuscular junction blocking activity. Although the introduction of the methyl group adjacent to the quaternary moiety was accomplished without difficulty, it proved to be impossible to quaternize the tertiary amine with the required benzyl halide, presumably because of the steric hindrance introduced by the adjacent methyl group.



I: R = H, R′ = $CH_2C_6H_5$, X = Cl
II: R = R′ = CH_3, X = I

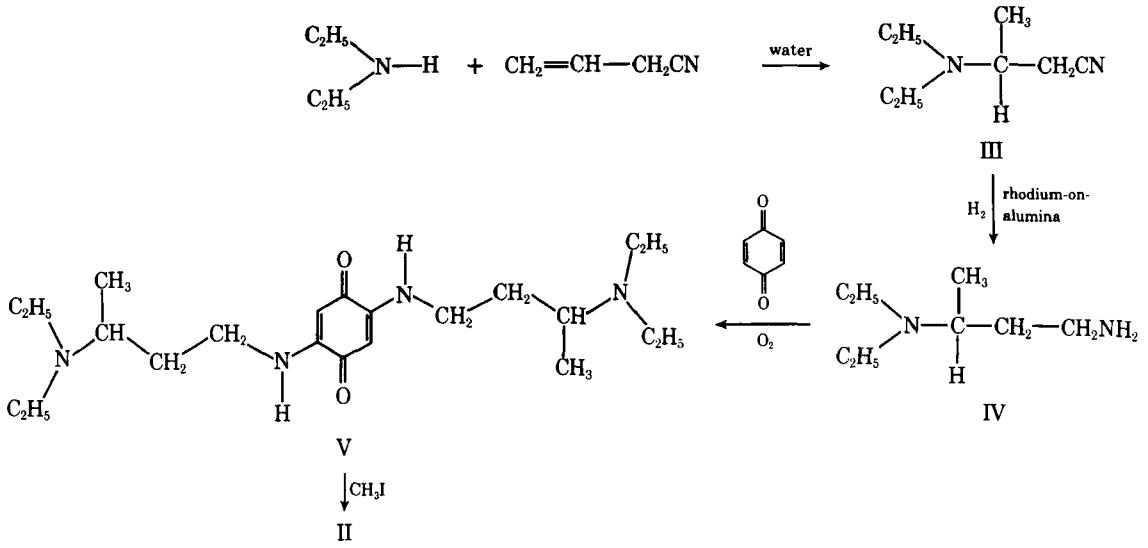
Therefore, methyl iodide as a quaternizing agent was employed, primarily because the original paper (3) describing the synthesis of benzoquinonium indicated that methyl quaternization provided blocking agents with activity comparable to compounds having benzyl as the quaternizing moiety. The present study bears out the earlier findings, since the substitution of a methyl for benzyl still provides compounds with activity comparable to the parent compound.

DISCUSSION

The synthetic route to 2,5-bis[(3-diethylamino)butylamino]-1,4-benzoquinone dimethiodide (II)[1] is shown in Scheme I. Attempted preparation of 3-diethylaminobutyronitrile (III) utilizing crotononitrile was unsuccessful. Apparently, a combination of steric and electronic factors exerted by the methyl group make the double bond resistant to nucleophilic addition, since diethylamine has been reported to add readily to acrylonitrile (4). However, the amine adds readily to allyl cyanide to give III, according to the method of Dahlbom (5), in good yield. In this case, the reaction mechanism is probably electrophilic in nature.

Compound IV was prepared in good yield by hydrogenation of III over a rhodium-on-alumina catalyst at moderate pressures. Purification of IV by the usual methods, *e.g.*, fractional distillation, could not be effected because of excessive foaming which was not alleviated by the usual methods. However, the structural identity of the compound was substantiated by IR (loss of CN stretching frequency and the appearance of NH stretching frequencies) and

[1] Because of the several nomenclature changes reflected in *Chemical Abstracts* for I, the present authors sought the advice of Dr. Kurt L. Loening, Director of Nomenclature at the Chemical Abstracts Service, who kindly furnished the most recent systematic nomenclature for the new compound: 4,4′-[(3,6-dioxo-1,4-cyclohexadiene-1,4-diyl)diimino]bis[*N*,*N*-diethyl-*N*-methyl-2-butanaminium] diiodide. Since this nomenclature is not suitable for the primary literature, the use of the alternative nomenclature as given in the text is suggested.



Scheme I—Synthetic route for preparation of II

NMR (appearance of new protons in the methylene region and complication of the methylene splitting patterns).

The reduction product (IV) was used without further purification to prepare V by reaction with either hydroquinone or benzoquinone in dioxane in the presence of oxygen. The product, obtained in rather poor yield, was in the form of bright-orange crystals, with the major part of the reaction being in the form of intractable tars. The original intention was to convert V to the benzyl quaternary salt since this would have mimicked most closely the parent compound, benzoquinonium (I). However, all attempts to prepare the benzyl quaternary derivative were unsuccessful, probably due to the steric influence of the methyl group adjacent to the tertiary nitrogens.

Since the original studies (3) had indicated that methyl quaternization, as previously mentioned, was not inimical to activity, it was attempted by treating V with a 10-fold excess of methyl iodide in dioxane–ethanol (2:1) with heating at moderate temperatures for several hours. The desired product (II) was obtained as an orange solid, which appeared to decompose on attempted crystallization. Therefore, it was best treated by careful washing of the obtained precipitate using dioxane–ethanol (2:1).

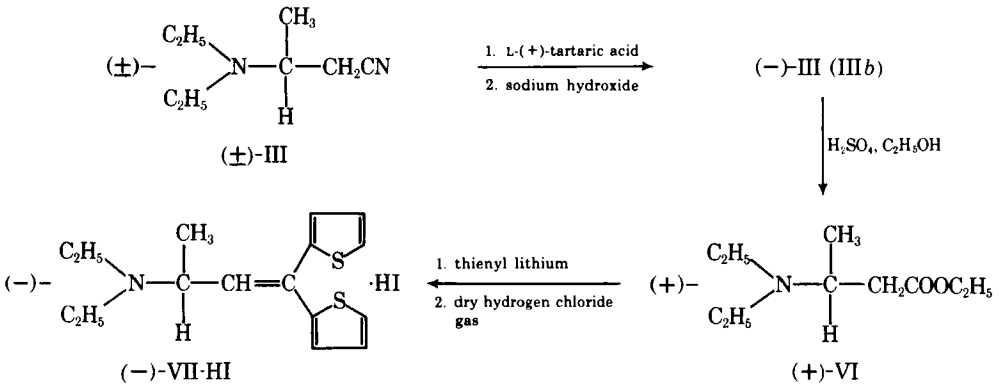
Since there are three possible isomers (a racemic pair and a *meso*-form) when II is prepared from racemic materials, it was necessary to deracemize at a stage prior to the final condensation. The deracemization was most conveniently carried out through the diastereomeric bitartrates of III; the rotatory data are given in Table I. The enantiomeric forms, *IIIa and IIIb, allowed the preparation* of the enantiomeric forms of IV, V, and II according to the methods developed on the racemic materials. The corresponding rotatory data are recorded in Table I together with the absolute configurations as determined here.

Because the objective of this study was to determine stereochemical relevancy to neuromuscular junction blocking ability, it was necessary to determine the absolute configuration of the blocking agents which was most readily attainable by determining this parameter for the enantiomeric forms of III. Scheme II depicts the transformations used to relate III*b* of unknown configuration to (+)-VII-HI of known configuration.

Table I—Physical Data on Compounds Prepared

Compound	Prepared from	Yield, %	Boiling Point (mm) or Melting Point	n_D^{23}	$[\alpha]^{20}$ 589	578	546	436	365	Solvent, mg/ml	Absolute Configuration
II	(±)-V	61	234–235°	—	—	—	—	—	—	—	—
II*a*	(+)-V	87	233.5–234.5°	—	—	34[a]	−74[a]	−446[a]	—	Water (0.6)	(*R*)
II*b*	(−)-V	87	233–234°	—	—	−33[a]	73[a]	452[a]	—	Water (0.6)	(*S*)
III	—	86	72–73° (10)	1.4362	—	—	—	—	—	—	—
III*a*	(±)-III	41[b]	—	1.4353	17.8	18.4	—	37.2	61.0	Absolute ethanol (20)	(*R*)
III*b*	(±)-III	40[b]	—	1.4350	−18.7	−19.5	—	−38.9	−63.8	Absolute ethanol (20)	(*S*)
IV	(±)-III	86	—	1.4420	—	—	—	—	—	—	—
IV*a*	(+)-III	80	—	1.4419	44.7	45.7	—	91.1	146.0	Absolute ethanol (20)	(*R*)
IV*b*	(−)-III	81	—	1.4418	−45.2	−47.2	—	−94.4	−147.5	Absolute ethanol (20)	(*S*)
V	(±)-IV	35	131–132°	—	—	—	—	—	—	—	—
V*a*	(+)-IV	38	124–125°	—	—	131[a]	—	—	—	Absolute ethanol (0.6)	(*R*)
V*b*	(−)-IV	44	124–125°	—	—	−135[a]	—	—	—	Absolute ethanol (0.6)	(*S*)
VI	(−)-III	72	96–97° (15)	1.4298	15.5	—	—	—	—	Absolute ethanol (1.0)	(*S*)
VII	(+)-VI	44	142–144°	—	−101.0	—	—	—	—	Absolute ethanol (0.9)	(*S*)

[a] Because of their orange color, the optical rotations were difficult to obtain. Concentrations were very dilute; consequently, the error in the smaller rotations (less than 100°) is probably larger than normal due to instrument noise and variability. [b] A 100% yield is equivalent to 50% of the starting racemic form.



Scheme II—Reactions involved in absolute configuration studies

The absolute configuration of (+)-VII-HI rests on the work of Beckett and Casy (6), who showed that D-(−)-alanine and (+)-VII-HI possess identical configurations through an independent synthetic conversion. Since VII-HI derived from III*b* (see Scheme II) had a negative optical rotation, it follows that III*b* has a configuration opposite to *(R)*- or D-(−)-alanine and, therefore, must have a configuration identical with *(S)*- or L-(+)-alanine. It may be added, parenthetically, that the present paper is the first to establish the absolute configuration of (+)-VI to be of the *(S)* variety, although the fact that an optically active form of it was used in the preparation (6) of (+)-VII-HI is obvious.

As a result of the failure to find a solid configurational statement for VI, the anticipated simple conversion of III to VI to determine configuration ended up in the necessity of converting III to VII. The present study provides a firm absolute configurational assignment for the enantiomers of III and VI and obviates the necessity of preparing (+)- or (−)-VII-HI to reach a decision. Finally, because III*b* has been shown to be of the *(S)*-configuration, it follows that II*b* derived from it has the same configuration and that II*a*, of necessity, is *(R)*.

The pharmacological testing employed the mouse inclined screen assay and the cat tongue–hypoglossal nerve assay, both of which were described previously (1, 2). In the mouse assay (Table II), the *(S)*-isomer was found to be about one-half as toxic as its enantiomer and about three-fourths as effective as a blocking agent. The cat assay (Table III) was more informative for reasons discussed previously (1) and showed an approximate 2:1 ratio of superiority for the *(R)*-configuration over the *(S)* in a preparation that is probably more meaningful than the mouse assay. This finding is in agreement with previous findings (2) with bisquaternaries obtained from tetrahydroisoquinoline compounds.

The examination of preferential inhibition of acetylcholinesterase (Table IV) was of interest. Although the compounds under study, as well as the parent compound (II), are strong inhibitors of acetylcholinesterase, it has been shown rather convincingly that the differences in enzyme-blocking ability cannot account for potency differences.

EXPERIMENTAL[2]

3-Diethylaminobutyronitrile (III)—This compound was prepared, according to the method of Dahlbom (5), from allyl cyanide treated with dimethylamine to give a product agreeing with the literature values, bp 83–85°/11 mm, n_D^{20} 1.4362.

3-Diethylaminobutylamine (IV)—This compound was prepared, according to the method of Freifelder (7), utilizing a rhodium-on-alumina catalyst. Evaporation of the solvent (CH_3OH) afforded 86% of a light-yellow liquid, which could not be distilled because of its foaming properties. The spectral characteristics of the yellow oil were consistent with those expected for IV. Therefore, the product was used as such without further purification, since all attempts to purify it seemed to cause further degradation.

2,5-Bis[(3-diethylamino)butylamino]-1,4-benzoquinone (V)—The preparation of this compound was carried out essentially according to the procedure of Cavallito *et al.* (3). The amine (IV) (5.0 g, 37 mmoles) in 10 ml of dry dioxane was added (10 min) to a cooled and stirred solution of benzoquinone (1.9 g, 18 mmoles) in 30 ml of dioxane. After addition was complete, the reaction mixture was allowed to come to room temperature and a slow stream of oxygen was passed through the solution with stirring for about 15 hr.

The reaction mixture was then concentrated to leave a tarry residue, which resisted recrystallization attempts after providing the product (V) as a dark-red semicrystalline solid. When recrystallized from ethanol–water, this product gave bright-orange-colored crystals, mp 131–132°. IR and NMR spectral characteristics were in accord with the expected structure.

Anal.—Calc. for $C_{22}H_{40}N_4O_2$: C, 67.32; H, 10.27; N, 14.27. Found: C, 67.48; H, 10.25; N, 14.22.

2,5-Bis[(3-diethylamino)butylamino]-1,4-benzoquinone Dimethiodide (II)—The preparation of this compound was carried out as described previously (3). This procedure involved the quaternization of V with methyl iodide (1.1 g, 8.0 mmoles) in absolute ethanol–dioxane (1:2), with 3–5 hr of heating at 40–50°. The precipitate that formed was separated by filtration, washed thoroughly with ethanol–dioxane (1:2), and dried to leave II as a brick-red solid, mp 234–235°. IR and NMR data were in accord with the expected structure.

Resolution of 3-Diethylaminobutyronitrile (III)—Both of the tartaric acids available [(+) and (−)] for use as deracemizing agents were used in the separation of the enantiomeric forms. Thus, L-(+)-tartaric acid (17.3 g, 115 mmoles) dissolved in the minimum volume of hot absolute ethanol was reacted with III (15 g, 115 mmoles) in about 20 ml of absolute ethanol. The solution was allowed to cool to room temperature and then was cooled overnight at 0°. The solvent was decanted and the gummy precipitate was triturated with acetone to give the product as a white solid.

Three recrystallizations from absolute ethanol provided 9.3 g (58%) of the bitartrate salt having a constant rotation of $[\alpha]_D^{20}$ +

Table II—ED_{50}, LD_{50}, and Potency Ratios in the Mouse Assay for Neuromuscular Junction Blocking Potency

Compound	ED_{50}, mg/kg	LD_{50}, mg/kg	Potency Ratios Based on ED_{50} [(+)-Tubocurarine = 100]
(+)-Tubocurarine	0.34	—	100
II*a* (+)	0.35	0.45	97
II*b* (−)	0.50	1.00	68
II*c* (±)	0.45	0.60	75

[2] Melting points were determined on a Thomas-Hoover melting-point apparatus and are uncorrected. Boiling points also are uncorrected. Elemental analyses were performed by M-H-W Microanalytical Laboratories, Garden City, Mich. Moist extracts were dried with anhydrous calcium or sodium sulfate or as otherwise specified. Solvents were routinely removed at about 20 mm pressure, using a rotating-flask evaporator. TLC was routinely carried out on Eastman Chromagram (No. 6060) sheets, and visualization was done with both UV lamps and iodine vapor. Optical rotations were obtained in either absolute ethanol or water, using a Perkin-Elmer 141 polarimeter. NMR spectra were obtained as 10–20% solutions in $CDCl_3$, using tetramethylsilane as an internal standard, on a Varian A-60D or T-60 spectrometer. Physical data on prepared compounds are in Table I.

Table III—ED_{50} and Potency Ratios in the Cat Assay for Neuromuscular Junction Blocking Potency

Compound	ED_{50}, mg/kg	ED_{50} Potency Ratio and Elevation Comparison	ED_{50} Potency Ratio of Enantiomers and Elevation Comparison (Most Potent = 1)
(+)-Tubocurarine	0.09	100	—
II*a* (+)	0.155	90 $F_{el}(dF', dF)^a$ =	1 $F_{el}(dF', dF)^a$ =
II*b* (−)	0.330	45 0.199 (2,24)	2 0.569 (1,12)

[a] Statistical comparisons were made between the line elevations of the isomers to evaluate the significance of the difference in ED_{50} values. Both values indicate that the slopes are not statistically different and that the potency differences are significant.

Table IV—Inhibition of Acetylcholinesterase by II*a* and II*b*

Compound	Wilkinson K_i[a]	Wilkinson V_{max}, moles/unit/min
Acetylcholine	$2.56 \pm 0.02 \times 10^{-4}$ (K_m)	1.008 ± 0.05
(+)-Tubocurarine	$4.29 \pm 0.4 \times 10^{-4}$	1.55 ± 0.7
II*a* (+)	$4.17 \pm 0.16 \times 10^{-6}$	0.385 ± 0.16
II*b* (−)	$4.01 \pm 0.06 \times 10^{-6}$	0.373 ± 0.09

[a] Determined by calculation from computer program (Wilkinson) generated, altered K_m values.

22.9° (c 1.0, H_2O). This material was combined with final fractions from two other resolutions (total 26.0 g) and dissolved in 50 ml of water. The aqueous solution was alkalinized with sodium hydroxide and extracted with ether, and the ethereal extract was dried over anhydrous calcium sulfate. Evaporation of the ether left 11.8 g of III*b*.

The same procedure using D-(−)-tartaric acid and III, recovered from the mother liquors of the L-(+)-tartaric acid treatment, afforded 10.1 g of III*a*.

(+)-Ethyl-3-diethylaminobutyrate (VI)—Compound III*b* (4.9 g, 70 mmoles) was dissolved in 15 ml of absolute ethanol and treated with 5.5 ml of concentrated sulfuric acid (8), and the resultant mixture was maintained at 130° for 16 hr in a stainless steel bomb. After cooling, the reaction mixture was poured into 70 g of cracked ice and the alcohol was removed under vacuum. The reaction product was washed with aqueous sodium bicarbonate solution and then distilled under reduced pressure to give 3.4 g of an oily VI. Since the IR and NMR spectral characteristics of this compound were in accord with the expected structure, it was used without further purification for the preparation of VII.

(−)-3-Diethylamino-1,1-di(2′-thienyl)but-1-ene [(−)-VII]—Adamson's (9) procedure was used, in which the initial step was the reaction of VI with thienyl lithium[3] to provide 3-diethylamino-1,1-di(2′-thienyl)butan-1-ol, mp 73–74° [lit. (9) mp 75–76°]. The aminoalcohol (0.23 g) in chloroform (3 ml) was converted to the aminobutene (VII) by passing dry hydrogen chloride into the solution for 10 min. The solvent was removed under reduced pressure, and the residue in water was stirred with charcoal for a few minutes at 60° and then filtered.

Alkalinization of the filtrate with dilute aqueous ammonia, ether extraction of the liberated base, and removal of solvent from the dried (anhydrous sodium sulfate) extract provided 120 mg of (−)-aminobutene [(−)-VII] as an oil. It gave a hydriodide, buff plates (from ether–ethanol), with a melting point and optical rotation substantially in agreement with the literature values (6), mp 139–140° and $[\alpha]_D^{20}$ +109 ± 2°, for its enantiomer.

Neuromuscular Junction Blocking Bioassay—Two different assays were used; the first was an all-or-none-type response and the second was a graded response.

Mouse Assay—Tests for the activity of II, II*a*, and II*b* were carried out on white male Swiss-Webster mice following essentially the procedure of Hoppe (10), which is much the same as that of Cavallito *et al.* (3) except for the time of observation. Groups of six mice were injected intraperitoneally with doses given in a volume of 0.01 ml/g of body weight. The mice were then placed on an inclined screen at a 45° angle. Mice falling off the screen within 15 min were considered to give a positive reaction. The results are summarized in Table II.

Cat Assay—The hypoglossal nerve–cat tongue muscle preparation employed was described previously (1). Table III summarizes the neuromuscular junction blocking potencies obtained by this assay in comparison with (+)-tubocurarine.

Acetylcholinesterase Inhibition—Inhibitory potency against acetylcholinesterase[4] was determined for the enantiomeric II*a* and II*b* as well as for (+)-tubocurarine in essentially the same way as described previously (1, 2). The method employs the pH-stat method of Cocolas *et al.* (11) and provided enzymatic velocity, which was computer analyzed using the Wilkinson method (12) to generate K_m and V_{max} data from which K_i values could be calculated by employing simple Michaelis relationships. The results are recorded in Table IV.

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 3, 1974, from the *Medicinal Chemistry Department, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455*

Accepted for publication July 11, 1974.

Supported by Grant NS08427 from the National Institutes of Health, U. S. Public Health Service, Bethesda, MD 20014

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