Table I—Standard Solution

Trial	Theophylline		Hydroxyethyltheophylline		Diphenylpyraline Hydrochloride	
	Sample, mg	Recovery, %	Sample, mg	Recovery, %	Sample, mg	Recovery, %
1	100.7	97.9	9.3	98.9	8.6	104.7
2	99.3	99.1	10.7	98.1	7.9	101.3
3	101.5	100.8	11.1	97.3	8.1	97.5
4	98.9	101.2	9.8	98.0	8.3	97.6
5	99.2	102.1	10.5	100.0	7.9	102.5
6	99.1	99.4	10.5	98.1	8.4	97.2
Mean relative error, %	1.26		1.43		2.63	
$\bar{X}$	100.1		98.4		100.1	
SD, %	±1.55		±0.93		±3.4	
RSD, %	1.55		0.95		3.4	

Table II—Production Lot of Syrup

	Theophylline	Hydroxyethyltheophylline	Diphenylpyraline Hydrochloride
Theoretical concentration, mg/30 ml	100.0	10.0	8.0
$\bar{X}$ of 15 samples	99.3	10.5	8.3
RSD, %	1.87	1.20	3.50

hydroxyethyltheophylline. Diphenylpyraline hydrochloride appeared not to undergo any chemical reaction in methanol and reaction mixtures as shown by the retention time. A preliminary 1-μl injection of reagent alone showed a small precursor peak just before the diphenylpyraline hydrochloride peak. This peak may insignificantly affect measurement of diphenylpyraline hydrochloride.

Linearity was also determined. Linear curves passing through the origin were obtained for solutions containing 40–100% of the quantities of drug ingredients being sought. These curves related the peak area and drug concentration.

The initial pH 3.8 of the sample and standard solutions was necessary to ensure the extraction of theophylline and hydroxyethyltheophylline.

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Stereochemical Preferences for Curarimimetic Neuromuscular Junction Blockade IV: Monoquaternary Ammonium Probes Possessing Carbon and Nitrogen Asymmetry

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Abstract □ Two enantiomeric pairs of neuromuscular junction blocking agents were prepared in which an asymmetric carbon atom is adjacent to an asymmetric quaternized nitrogen moiety. The blocking agents were obtained from the enantiomers of laudanosine by stereoselective quaternization with benzyl and ethyl iodides. Curarimimetic potencies were measured with an *in vivo* cat hypoglossal nerve–tongue muscle preparation. The studies suggest that the asymmetry present in these structures does not lead to significant differences in blocking potency between enantiomers.

Keyphrases □ Enantiomers—benzyl- and ethyllaudanosinium iodides synthesized, curarimimetic potencies compared □ Neuromuscular junction blocking agents—enantiomers of benzyl- and ethyllaudanosinium iodides synthesized, curarimimetic potencies compared □ Curarimimetic potency—compared in enantiomers of benzyl- and ethyllaudanosinium iodides □ Quaternary ammonium probes—enantiomers of benzyl- and ethyllaudanosinium iodides synthesized, curarimimetic potencies compared □ Structure–activity relationships—enantiomers of benzyl- and ethyllaudanosinium iodides, curarimimetic potencies compared

Previous reports (1–4) described stereochemical preferences exhibited by the neuromuscular junction toward nondepolarizing blocking agents having carbon asymmetry alpha to quaternary ammonium functionality. Initial studies (1) employed monoquaternary structures (hemicurares) as probes and indicated a modest blocking preference for the (*S*)-enantiomers; subsequent investigations (2, 3) employed bisquaternary probes and indicated that the order of blocking preference is (*R*) > (*S*) for these structures. Since the quaternary ammonium function is the most distinguishing pharmacophore of this pharmacological class, structures having nitrogen asymmetry could possess larger enantiomeric potency differences that might also be independent of other, more remote structural modification.

An early example of variation in curarimimetic potency due to nitrogen asymmetry is the report (5) that the diastereomers derived from asymmetric quaternization of coniine show small potency differences. Larger potency differences were reported for the stereoisomers resulting from quaternization of canadine (6, 7). Similarly, certain optically active inorganic onium ions also showed small

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Table I—Neuromuscular Junction Blocking Potency Data for the Pharmacological Probes

Probe	Stereochemistry	ED_{50}, mg/kg	ED_{50} Potency Ratio to (+)-Tubocurarine (1/N)	Slope Comparison[a], F_{slope} (dF', dF)	Potency Ratio of Enantiomers	Elevation Comparison[b], $F_{elevation}$ (dF', dF)
(+)-Tubocurarine		0.09	1			
I	C-1 (*S*); N (*R*)	1.81	24	0.38 (2, 20)	2:1 (I:II)	5.79 (1, 19)
II	C-1 (*R*); N (*S*)	3.64	48	+		+
III	(*S,S*)	5.33	79	0.65 (2, 15)	1:1 (III:IV)	0.06 (1, 15)
IV	(*R,R*)	5.23	78	+		−

[a] Statistical comparisons were made between the regression line slopes of the probes and (+)-tubocurarine (+ indicates that the slopes are not statistically dissimilar). [b] Statistical comparisons were made between the line elevations of enantiomeric pairs to evaluate the significance of the difference in ED_{50} values (+ indicates a statistically significant difference, and − indicates that the difference is not statistically significant).

enantiomeric differences in curariform activity (8). More recent reports considered nitrogen asymmetry present in bisquaternary isomers related to tubocurarine (9) and in monoquaternary structures derived from norcoralydine (10).

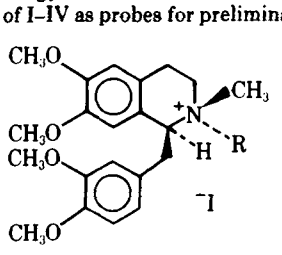
DISCUSSION

Chemistry—Simple hemicurare structures (1) having a single asymmetric nitrogen were sought as probes. Stereoselective quaternization (11–14), exploiting carbon asymmetry alpha to a tertiary amine to direct an incoming alkyl halide, provided a ready method for the synthesis of structures containing the requisite features of the desired probes. The enantiomeric laudanosines were chosen as candidates for stereoselective quaternization with a large (benzyl iodide) and small (ethyl iodide) alkylating agent. Preparation of the laudanosine racemate and enantiomers was as previously described (1). Stereoselective quaternization of these materials followed the method reported by Kobor *et al.* (12), who synthesized the racemic derivatives of these compounds.

When (±)-laudanosine was quaternized with benzyl iodide, the *trans*-material represented 95% of the initial reaction product mixture and crystallization of the pure *trans*-material could be effected from either ethanolic or aqueous solutions. When (±)-2-benzyl-1,2,3,4-tetrahydropapaverine (VI) [prepared *via* benzylation of papaverine to give V followed by sodium borohydride reduction (1)] was quaternized with methyl iodide, the reaction product mixture showed a substantial increase in the *cis*-material. Repetition of this latter process with methyl iodide-d_3 supported the NMR chemical shift assignments of Kobor *et al.* (12) for the *trans*-(δ 3.43) and *cis*-(δ 3.09) N^+CH_3 protons since the resonances at these locations were decreased. Finally, when chloroformic solutions of either the pure *trans*-product or the increased *cis*-product mixture were subjected to heated bomb conditions, the same equilibrium relationship between *cis*- and *trans*-products was obtained, as predicted previously (12).

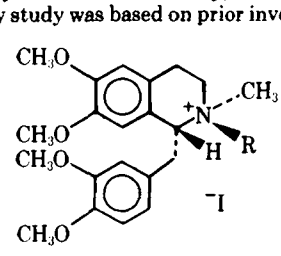
When each of the enantiomeric laudanosines was quaternized with benzyl iodide, the initial product mixtures again showed 95% *trans*-material. Precipitates obtained from aqueous ethanol were taken up in acetone and, after evaporation of this solvent, the resulting amorphous powders gave acceptable elemental analyses, showed only the *trans*-spot on thin-layer chromatograms, and yielded identical NMR spectra corresponding to the *trans*-diastereomer. These compounds represent pharmacological probes (1*S*,2*R*)-*N*-benzyllaudanosinium iodide (I) and (1*R*,2*S*)-*N*-benzyllaudanosinium iodide (II). Probes (1*S*,2*S*)-*N*-ethyllaudanosinium iodide (III) and (1*R*,2*R*)-*N*-ethyllaudanosinium iodide (IV) were prepared similarly by quaternization with ethyl iodide and yielded crystalline materials from 95% ethanol.

Biology—In addition to their ready chemical accessibility, the selection of I–IV as probes for preliminary study was based on prior investigations (1, 15) where closely related structures were shown to possess modest curarimimetic activity. Also, biological comparison between probes alkylated with both large and small groups could provide additional information about the importance of steric parameters associated with any stereochemical features related to the anionic site of the neuromuscular junction receptor.



I: R = CH_2—⬡

III: R = CH_2CH_3



II: R = CH_2—⬡

IV: R = CH_2CH_3

The cat hypoglossal nerve–tongue muscle procedure was employed to assess the blocking potencies of I–IV. Probes were tested in three animals at a minimum of three concentrations. Probit values of the percent decrease from control response levels due to inhibition by the probes were plotted against log dose *via* a linear regression program (1).

As expected, I–IV possessed only modest blocking activity. That the nature of this blockade was curarimimetic was established by the observation that a characteristic relief of the blockade could be effected by subsequent administration of edrophonium and by a statistically nonsignificant difference of linear regression slopes between I–IV and (+)-tubocurarine (performed by analysis of covariance). The enantiomeric potency relationships for I and II and III and IV are presented in Table I. Based on previous studies (1), it seems reasonable to assume that the tabulated potencies reflect differences in interaction with neuromuscular junction receptors rather than differences in distribution patterns or in interactions with acetylcholinesterase.

Since III and IV did not show a significant potency difference and I and II showed only a small difference with the same trend [C-1 (*S*) > C-1 (*R*)] obtained for related probes having carbon asymmetry only (1), these preliminary results suggest that the effect of nitrogen asymmetry on neuromuscular junction receptor interaction with these structural types is not significant. However, relatively few compounds were studied; a more generalized conclusion would require a wider range of candidate compounds, which are currently being prepared.

EXPERIMENTAL[1]

Laudanosine Bases—The preparation and resolution of racemic laudanosine followed published methods (1).

***N*-Benzylpapaverinium Iodide (V)**—Papaverine (24 g, 0.0695 mole) was added in portions, with stirring, to a solution of benzyl iodide in acetone[2] (0.278 mole in 400 ml). The mixture was refluxed for 4 hr, and then the volume was reduced to 200 ml. After standing at room temperature, yellow crystals separated; they were collected and washed with ether to give 25 g (67%), mp 202–204°.

(±)-2-Benzyl-1,2,3,4-tetrahydropapaverine (VI)—Compound V (10 g, 0.018 mole) was suspended in methanol (250 ml); 2 ml of water was added, and the mixture was cooled in an ice bath. Sodium borohydride (10 g, 0.263 mole) was added in small portions, with vigorous stirring, after which the mixture was gently refluxed for 2 hr. Water (40 ml) was then added, the mixture was cooled, and the resulting precipitate was collected and recrystallized in a similar fashion to give 7.0 g (90%), mp 84–86° [lit. (13) mp 91–92° (from ether)].

Quaternization with Benzyl Iodide—Racemic, (*S*)-, or (*R*)-laudanosine (1.87 g, 0.0052 mole) was dissolved in acetone (50 ml). Benzyl iodide (0.0052 mole) in acetone (25 ml) was added, and the combined solutions were refluxed gently for 3 days under nitrogen while protected

[1] Melting points were determined on a Thomas-Hoover apparatus. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Analyses were performed by M-H-W Laboratories, Garden City, Mich. TLC was conducted on Eastman chromatogram sheet 6060 silica gel developed in butanol–water–acetic acid (7:2:1), and visualization was by a UV lamp and iodine vapor. NMR spectra were measured with a Varian Associates model A-60 D NMR spectrometer with tetramethylsilane as an internal standard.

[2] Acetone solutions of benzyl iodide (0.7 *M*) were prepared by quantitative Finkelstein conversion of benzyl bromide plus sodium iodide.

Table II—Physical and Analytical Data for Probe Molecules and New Compounds [a]

Compound	Melting Point	Optical Rotation [b], $[\alpha]_D^{25}$	Formula		Analysis, % Calc.	Found
I	112–114° [c]	+29°	$C_{28}H_{34}INO_4$	C	58.4	58.16
				H	5.9	6.12
				I	22.1	21.90
				N	2.4	2.11
II	116–118° [c]	−30°	$C_{28}H_{34}INO_4$	C	58.4	58.20
				H	5.9	5.77
				I	22.1	22.46
				N	2.4	2.34
III	166–168°	+74°	$C_{23}H_{32}INO_4 \cdot H_2O$	C	52.0	51.78
				H	6.4	6.24
				I	23.9	24.19
				N	2.6	2.47
IV	164–168° [c]	−72°	$C_{23}H_{32}INO_4 \cdot H_2O$	C	52.0	51.83
				H	6.4	6.57
				I	23.9	23.73
				N	2.6	2.49
V	202–204°	0°	$C_{27}H_{28}INO_4$	C	58.2	58.21
				H	5.0	4.99
				I	22.8	22.66
				N	2.5	2.25
VI	84–86°	0°	$C_{27}H_{31}NO_4$	C	74.8	74.57
				H	7.2	7.33
				N	3.2	3.03

[a] Reported only for new compounds and for VI because of a discrepancy in melting-point data for this compound. [b] All rotations determined as (c 0.5, ethanol). [c] Obtained as amorphous solids.

from light. The solvent was then removed under reduced pressure, and the racemic derivative was crystallized from either ethanol or water while enantiomeric derivatives yielded soft, tar-like materials. The enantiomeric tars were taken up in acetone, which was then evaporated to produce fine amorphous powders. Final yields were: racemic material, 75%; I, 67%; and II, 17%. Physical data for probe compounds are recorded in Table II.

Quaternization with Ethyl Iodide—Either (*S*)- or (*R*)-laudanosine (3.57 g, 0.01 mole) was dissolved in ethanol (100 ml), ethyl iodide (1.6 ml, 0.02 mole) was added, and the mixture was refluxed gently for 12 hr. The solution was evaporated, and the residue was recrystallized (six times) from ethanol until its NMR spectrum and TLC showed only the *trans*-product. Final yields were: III, 14%; and IV, 10%.

Quaternization of VI—To a solution of VI (2.17 g, 0.005 mole) in methanol (50 ml) was added methyl iodide or methyl iodide-d_3 (0.01 mole). The mixtures were refluxed gently for 6 hr under nitrogen while protected from light. The solvent was removed by evaporation, and the initial reaction product mixture residues were used for NMR studies.

***cis–trans*-Equilibration Reactions**—A chloroformic solution of *trans*-(±)-*N*-benzyllaudanosinium iodide or of the residue obtained from quaternization of VI was subjected to the conditions specified previously (12, 16), except that reactions were carried out in a stainless steel bomb apparatus. Residues obtained after solvent evaporation were utilized for NMR studies.

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Phenazines with Two Cationic Side Chains as Potential Antimalarials

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Received July 12, 1978, from the *Department of Chemistry, Georgia State University, Atlanta, GA 30303.* Accepted for publication September 13, 1978.

Abstract □ 1,9-Phenazine-bis(dialkylaminocarboxamides) were prepared for screening as potential antimalarials. No significant activity against *Plasmodium berghei* was observed. The phenazine targets were prepared from 1,9-phenazinedicarboxylic acid by standard methods. The reaction between 1,9-phenazinedicarboxylic acid and thionyl chloride in the presence of dimethylformamide unexpectedly gave 4-chloro-1,9-phenazinedicarbonyl chloride.

Keyphrases □ Phenazines—with two cationic side chains, synthesized and screened as potential antimalarials □ Antimalarials, potential—synthesis and screening of phenazines with two cationic side chains

As part of a research program to develop new antimalarials (1), some selected phenazine derivatives were synthesized because of indications of antimalarial activity for several phenazine types. Clofazimine, which has proven safe for human use against leprosy, has been reported to suppress parasitemia completely in mice infected with *Plasmodium berghei* (2). Modest activity against *P. berghei* in mice has been observed for several simple phenazines, including 7-chloro-2-phenazinenitrile, methylene violet/Bernthsen, and diazine black[1].

While the mode of antimalarial action of these compounds is unknown, the fact that phenazine antibiotics such as myxin and iodimin (3), as well as other phenazines (4, 5), related phenoxazines (6) (actinomycins), and related

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