isopropylcarbodiimide (0.1 mol) in 30 ml of benzene was added to a stirred solution of the aromatic amine hydrochloride (0.1 mol) in 100 ml of ethyl alcohol. The mixture was heated at reflux for 1 hr. Products were isolated and purified as described in method 1.

Method 9. 2-Anilino-2-imidazolines. A mixture of the aromatic thiourea (0.1 mol) and 20 ml of ethylenediamine was stirred and heated at 135° for 2.5 hr. The reaction mixture was cooled and mixed with 100 ml of water. The mixture was acidified to pH 2 by the addition of concentrated hydrochloric acid and was cooled in an ice bath. The mixture was filtered and the filtrate was adjusted to pH 8.0 by addition of aqueous 25% sodium hydroxide solution. The mixture was extracted twice with 50-ml portions of ether. The aqueous layer was made basic (pH 11–12) with aqueous 25% sodium hydroxide solution. The mixture was cooled to 0° and the precipitated solid was collected on a filter. The products were purified by recrystallization from aqueous methanol.

2-(1-Indolino)-2-imidazoline (62). Compound **83** (0.1 mol) was heated to 65° with 20 ml of ethylenediamine for 4 hr. The product was isolated as described in method 9.

Method 10. Aromatic Biguanides. The aromatic amine hydrochloride, dicyandiamide, and isopropyl alcohol in a molar ratio of 1.0:1.5:15 were heated to reflux for 20 hr. The products were isolated and purified using the procedures described in method 1.

Method 11. 3,4-Dihydroxyphenylguanidines and Biguanides. A mixture of 0.1 mol of the 3,4-dibenzyloxyphenylguanidine or biguanide hydrochloride, 250 ml of ethyl alcohol, and 0.5 g of 5% palladium on charcoal was shaken under an initial hydrogen pressure of 50 psi. After 4 hr of agitation the reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was recrystallized from the solvents described in Tables I and II. Compound **63** was obtained from the reaction mixture by filtration to remove the catalyst and evaporation of the filtrate to a viscous residue. The residue was dissolved in 100 ml of water and a solution of 17.0 g of silver nitrate in 100 ml of water was added. After stirring for 10 min in the absence of light, the reaction mixture was filtered to remove the precipitated silver chloride. The filtrate was evaporated under reduced pressure. The residue was recrystallized from isopropyl alcohol.

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Synthesis of a Bifunctional Coordination Complex of Osmium with Curariform Activity

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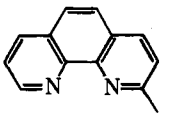
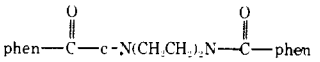
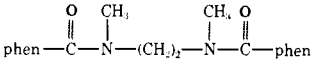
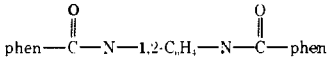
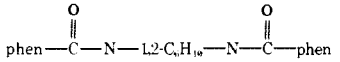
Based on the known curariform action of tris(bipyridyl)iron(II) sulfate and other complex ions, two series of bifunctional ligands designed to hold transition metal ions at approximately the same distance apart as the interquaternary ammonium distance in the potent neuromuscular blocking agents were synthesized. In the first series two 1,10-phenanthrolines (R_1) were joined at the 2 position to form four compounds: $R_1CO\text{-}c\text{-}N(CH_2CH_2)_2N\text{-}COR_1$, $R_1CONH\text{-}1,2\text{-}C_6H_{10}\text{-}NHCOR_1$, $R_1CONH\text{-}1,2\text{-}C_6H_4\text{-}NHCOR_1$, and $R_1CON(CH_3)(CH_2)_2N(CH_3)COR_1$. In the second series two terpyridines (R_2) were joined by different chains to give $R_2(CH_2)_2CH{=}CH(CH_2)_2R_2$, $R_2CH_2C(CH_3)(OH)(CH_2)_2C(CH_3)(OH)CH_2R_2$, $R_2CH_2C(CH_3)(OH)C(CH_3)(OH)CH_2R_2$, and $R_2CH_2(OH)\text{-}1,4\text{-}C_6H_{10}\text{-}(OH)CH_2R_2$. Three other ligands in which the terpyridines were joined by 5-, 6-, and 7-methylene groups were also made. The ligands were converted to nickel(II) complexes and the coordination of each nickel ion was completed by adding terpyridine. These were assayed by the intravenous mouse LD_{50} method. The most potent ligand, the dihydroxy compound $R_2CH_2(OH)\text{-}1,4\text{-}C_6H_{10}\text{-}(OH)CH_2R_2$, was then converted to the bis(pyridinebipyridine)diosmium-(II) coordinated complex and assayed by the iv mouse LD_{50} method and by the ED_{50} isolated guinea-pig diaphragm method. By the iv mouse LD_{50} method, it was about twice as potent as *d*-tubocurarine and by the isolated diaphragm method, it was 16 times more potent. The compound has been called dihydroxyosmarine tetrachloride or DHO for short. The term "transarine" ions is proposed for transition metal coordination complexes having curariform action. The position of the transarine ions is discussed in the classification of cholinergic ligands, in structure–action relationships, and in relation to some current ideas on receptor mechanisms.

The initial observations of the curariform action of a transition metal complex were made by Beccari in 1938[1–3] who noted that the doubly charged coordination complex of ferrous iron and three 2,2′-bipyridyls caused paralysis and death by failure of ventilation in rabbits. He estimated the minimum lethal subcutaneous dose to be about 35 mg/kg. The compound was not found anywhere in the brain. He also noted that the complex cations showed high chemical stability and were excreted in the urine.

Following Beccari's reports the biological activity of a series of related transition metal complexes was further investigated by Dwyer et al.[4–7] The substances they studied consisted of chelates formed by uniting a variety of ligands to a central ion of one of the transition elements. The most active ligands found were 2,2′-bipyridyl, 1,10-phenanthroline, and 2,2′,2″-terpyridyl. Ions of iron, nickel, cobalt, ruthenium, and osmium functioned adequately as the central element of the complexes. All the complexes of the above ions and ligands they tested caused characteristic reversible curariform paralysis of isolated rat diaphragm and of mice upon intraperitoneal injection. Only positively charged complexes were active and on the isolated dia-

Table I. Bifunctional Ligands from 1,10-Phenanthroline

phen = 

Compd no.	Structure	Mp, °C	Formula	Analyses[a]	Mouse LD_{50} of complex,[b] μmol/kg ± SD
1		Charred	$C_{30}H_{22}N_6O_2$	C, H, N	Not done
2		290–291	$C_{30}H_{24}N_6O_2$	C, H, N	0.62 ± 0.08
3		230 dec	$C_{32}H_{20}N_6O_2 \cdot H_2O$	C, H, N	0.45 ± 0.02
4		248	$C_{32}H_{26}N_6O_2 \cdot 2H_2O$	C, H, N	0.31 ± 0.02
	d-Tubocurarine				0.18 ± 0.01

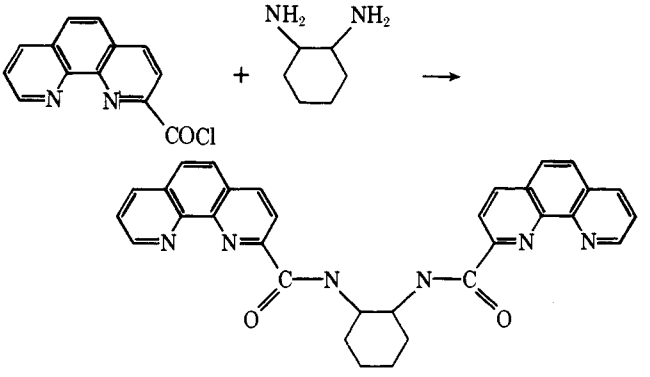
[a]Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values. [b]These are complexes with nickel(II) and terpyridine. They have not been isolated nor has their stereochemistry been studied.

phragm all were reversible and were antagonized by K^+ ions, eserine, and neostigmine. The bis(terpyridine) complex of ruthenium(II) had a potency one-tenth that of *d*-tubocurarine on the isolated rat diaphragm. The action of acetylcholine on the toad rectus was also reversibly blocked. Paralyzed nerve muscle preparations responded to direct electrical stimulation. Junctional transmission was blocked in the isolated superior cervical ganglion of the rabbit.

All the metal complexes referred to above are coordinatively saturated. They have no specific active groups or centers and are highly stable chemically. Their redox potentials are out of the biological range. The ruthenium and osmium complexes are not attacked by boiling concentrated acids or alkalis, and dissociation of these complexes can be regarded as vanishingly small. Although chelating agents themselves react with biological systems in various ways, these stable, fully saturated complexes in which metal and chelate are firmly united retain their saturated structures in the body. Beccari[2] demonstrated that tris(bipyridyl)iron(II) is excreted unchanged by frogs and rabbits, and Brandt et al.[8] state that the highly stable ruthenium(II) and osmium(II) complexes are best from the standpoint of stability for biological investigation. Tris(1,10-phenanthroline)ruthenium(II)[9] was not metabolized by rats or mice and was excreted mainly in the urine.

The main objective of this work was to see if more potent curariform agents could be made by joining two such complex cations together so that the distance between the metals was about the same as the interionic distance in the diquaternary neuromuscular blocking drugs. To achieve our objective, two series of bifunctional ligands were prepared. In the first set two 1,10-phenanthrolines were joined together and in the second two terpyridyls were joined (Tables I and II). The choice of 1,10-phenanthroline and 2,2′,2″-terpyridine as a basis for the two series of ligands was made because these gave the most potent monofunctional complexes and the metal coordination shell in each case could be closed by terpyridine or a closely related ligand.

Synthesis of Bifunctional Ligands from 1,10-Phenanthroline. The synthetic method used to join the phenanthrolines was facilitated by the availability of a reactive group at the 2 position. The 2-carboxyl derivative was made by the methods of Corey et al.[10] and converted to the acid chloride by the method of Sigman et al.[11] The acid chloride (2 equiv) was then condensed with 1 equiv of 1,2-diaminocyclohexane in chloroform solution containing purified triethylamine as a proton sink to give ligand I [2,2′-bis(1,10-phenanthroline)-1,2-cyclohexanediamide] in good yield.



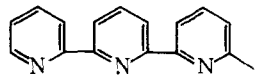
I

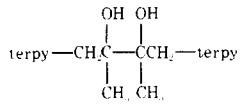
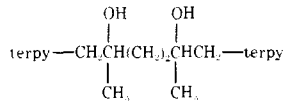
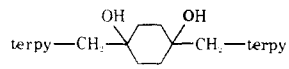
Similar compounds in which the binding bases were piperazine, *o*-phenylenediamine, and *N,N′*-dimethylethylenediamine were also made (Table I). The compounds were recrystallized and their formulations confirmed by mass spectroscopy. The ligand formed by joining two phenanthrolines with piperazine was too insoluble for further use.

Synthesis of Bifunctional Ligands from 2,2′,2″-Terpyridine. The problem of attempting to join two terpyridines is that of providing a suitably placed reactive site on the terpyridine ring system. This was achieved by making terpyridine with a methyl group at position 2, as such methyl groups attached to pyridine have acidic hydrogens and can be used to generate carbanions. In general terpyridine syntheses are slow, difficult, low-yield processes and few methods are available.

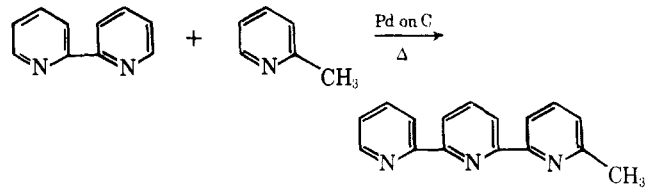
The method used was that of Rosvear and Sasse,[12] who made a series of 4-alkyl-substituted terpyridines by refluxing the corresponding 4-alkylpyridines with 5% palladium on carbon. Using the same method, an equimolar mixture of 2,2′-bipyridine and purified 2-picoline was refluxed for 3 days with the catalyst. Although the yield of 2-methylter-

Table II. Bifunctional Ligands from Terpyridine

terpy = 

Compd no.	Structure	Mp, °C	Formula	Analyses[a]	Mouse LD_{50} of complex,[b] μmol/kg ± SD
5	terpy—CH_3	112	$C_{16}H_{13}N_3$	C, H, N	Not done
6	terpy—$(CH_2)_5$—terpy	141	$C_{35}H_{30}N_6 \cdot 0.5H_2O$	C, H, N	0.23 ± 0.01
7	terpy—$(CH_2)_6$—terpy	151	$C_{36}H_{32}N_6$	C, H, N	0.26 ± 0.03
8	terpy—$(CH_2)_2CH{=}CH(CH_2)_2$—terpy	193	$C_{36}H_{30}N_6$	C, H, N	0.24 ± 0.04
9	terpy—$(CH_2)_7$—terpy	133	$C_{37}H_{34}N_6$	C, H, N	0.27 ± 0.01
10		225–238	$C_{36}H_{32}N_6O_2 \cdot 0.5H_2O$	C, H, N	0.30 ± 0.03
11		195	$C_{38}H_{36}N_6O_2$	C, H, N	0.19 ± 0.01
12		192	$C_{38}H_{34}N_6O_2 \cdot 0.5H_2O$	C, H, N	0.11 ± 0.02
	d-Tubocurarine				0.18 ± 0.01

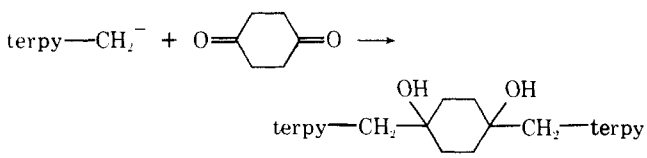
[a]Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values. [b]These are complexes with nickel(II) and terpyridine. They have not been isolated nor has their stereochemistry been studied.



pyridine was low (about 1.5%) and some quaterpyridine was formed, the bulk of the unreacted starting materials could be recovered and used again.

The attack then involved the replacement of one of the acidic hydrogens with a lithium atom. The resultant 2-methylterpyridine carbanion was allowed to react with electrophilic bridging compounds. When *n*-butyllithium was used to generate the carbanion low yields were encountered, perhaps because of a reaction similar to the action of *n*-butyllithium on pyridine,[13] where the 2 position is butylated and the lithium is on the nitrogen atom. To discourage this tendency, the lithiation was carried out by employing lithium diisopropylamide[14,15] with a stoichiometric amount of lithium chloride added at the beginning of the synthesis.

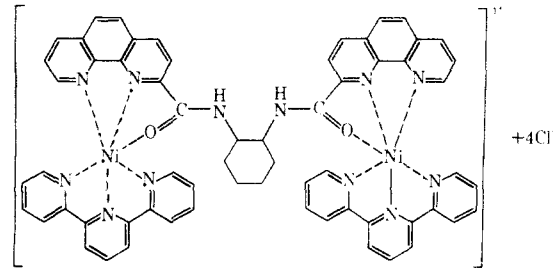
The 2-methylterpyridine carbanion was then allowed to react with 1,4-cyclohexanedione or another suitable elec-



trophile. In adding the lithium chloride at the beginning, use was made of the fact that lithium chloride dehydrates just below 200° to ensure anhydrous conditions. When alkyl halides were used as bridging compounds, the diiodides gave the highest yields.

As the ligands were synthesized it was necessary to have an efficient way to complete their conversion to a suitable coordination complex in order to obtain an estimate of the relative biological activity of the members of the series.

The addition of 2 equiv of nickel(II) to the insoluble ligand I caused it to dissolve. The further addition of 2 equiv of insoluble terpyridine also dissolved and presumably formed compound II [μ-[1,2-cyclohexylbis(1,10-phenanthralino-2-amido)]bis(2,2′,2″-terpyridine)dinickel(II) chloride] in which the 1,10-phenanthroline carbonamide grouping is shown as a tridentate ligand in accordance with the structure proposed by Goodwin and Smith[16] who showed that the >C=O group can be a donor in such compounds.



II

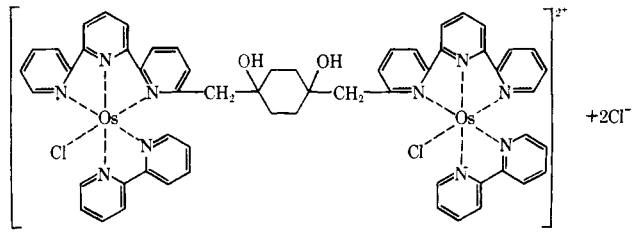
The other bifunctional ligands in Tables I and II were treated similarly. The ligands were all insoluble in water but the addition of 2 mol of nickel(II) caused their solution. Nickel was used as the transition ion because its valence state is stable. The nickel complexes have not been isolated nor has their stereochemistry been studied. The complexes of the ligands were given to mice intravenously and assayed by the LD_{50} up and down method.[17]

Insertion of the Osmium Ions. Although coordination complexes between transition ions such as Fe, Ni, Co, etc., and pyridine ring ligands can be formed readily by mixing the ligands and the metal ions in solution, this method cannot be used for the insertion of osmium mainly because the affinity of osmium for oxygen makes simple salts difficult to prepare and isolate. Indirect methods and more forceful conditions are necessary, using osmium-containing reagents having insulating ligands to protect the metal from polymerization and oxidation. The methods used were essentially the same as those developed by Buckingham et al.[18-20]

The ligand selected for osmium insertion was the dihydroxycyclohexane compound (no. 12, Table II) because of

its high potential biological potency as a curariform ion and because of its two hydroxyl groups where membrane probes or other groups might be added. Our attempts to achieve a one-step insertion of osmium(II) terpyridine complexes using (trichloroterpyridine)osmium(III) as the starting material and forcing conditions did not lead to the desired complex or a suitable intermediate species.

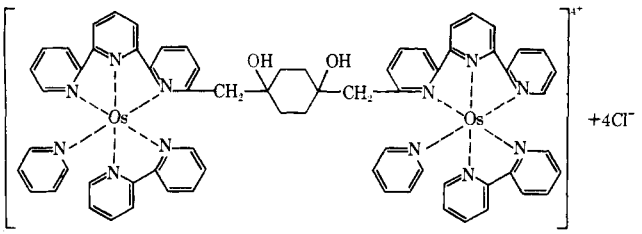
An indirect method developed by Buckingham et al.[20] was then used because they had already prepared the monofunctional osmium complexes (chlorobipyridineterpyridine)osmium(II) and (pyridinebipyridineterpyridine)osmium(II) corresponding to compounds III and IV below. By treating the ligand with (tetrachlorobipyridine)osmium(IV)[20] at 190° in ethylene glycol the (chlorobipyridine)osmium(II) dichloride derivative III [dichloro-μ-[1,4-cyclo-



III

hexylbis(2,2′,2″-terpyridyl-6″-methyl)-1,4-diol]bis(2,2′-bipyridine)diosmium(II) chloride] was prepared in high yield as a dark brown or black product.

The (chlorobipyridine)osmium(II) dichloride derivative III was refluxed in aqueous pyridine for 48 hr[20] to give compound IV [μ-[1,4-cyclohexylbis(2,2′,2″-terpyridyl-6″-



IV

methyl)-1,4-diol]bis(2,2′-bipyridine)bis(pyridine)diosmium(II) chloride].

This compound has been named dihydroxyosmarine tetrachloride or DHO for short. It has potential uses as an electron dense probe in electron microscopy and as a heavy metal carrier for isomorphous replacement studies in X-ray diffraction analysis. The two hydroxyl groups are possible sites for attachment of fluorescent and spin probes. The stereochemistry of compounds III and IV has not been studied.

Pharmacological Results. In addition to the nickel(II) terpyridine complexes of the bifunctional ligands in Tables I and II, several monofunctional transition element complexes were also assayed (Table III) as well as a commercial sample of bis(2,2′-bipyridine)europium(III) nitrate. In the bipyridine complexes (Table III), the ruthenium derivative was the most potent and cobalt the least, with nickel and iron being intermediate. Several complexes had either low potencies or were essentially inactive. These were bis(bipyridyl)europium(III) nitrate $[Eu^{3+}(bpy)_2](NO_3)_3$, hexaamminecobalt(III) pentachlorocuprate $[Co^{3+}(NH_3)_6]CuCl_5$, hexaamminecobalt(III) chloride $[Co^{3+}(NH_3)_6]Cl_3$, bis(ethylenediamine)carbonatocobalt chloride $[Co^{+}(en)_2CO_3]Cl$, and *trans*-bis(ethylenediamine)cobalt(III) chloride, *trans*-$[Co^{+}(en)_2Cl_2]Cl$. These results essentially confirm those of Dwyer et al.

The nickel(II) terpyridine complexes of the ligands in Table I are all less active than *d*-tubocurarine when tested by intravenous injection in mice. Activity increases as the bridging compound changes from dimethylethylenediamine to phenylenediamine to diaminocyclohexane. The piperazine derivative was too insoluble to work with.

Table III. Monofunctional Coordination Complexes of Bipyridine

	Mouse LD_{50}, μmol/kg ± SD	Diaphragm ED_{50}, μmol/l. ± SD
Tris(bipyridine)ruthenium(II) chloride hexahydrate, $[Ru^{2+}(bpy)_3]Cl_2 \cdot 6H_2O$	2.67 ± 0.20	3.52 ± 0.79
Tris(bipyridine)nickel(II) chloride, $[Ni^{2+}(bpy)_3]Cl_2$	4.88 ± 0.25	45.8 ± 1.32
Tris(bipyridine)iron(II) chloride, $[Fe^{2+}(bpy)_3]Cl_2$	6.06 ± 0.69	44.1 ± 1.7
Tris(bipyridine)cobalt(III) perchlorate, $[Co^{3+}(bpy)_3](ClO_4)_3$	20.13 ± 3.66	Slightly active
d-Tubocurarine	0.18 ± 0.01	0.63 ± 0.2

The least active of the nickel(II) terpyridine complexes of the bifunctional ligands in Table II was as potent as the most potent derivative from Table I. The derivatives of ligands **5**–**9** differ mainly in the intermetallic distance, being 9 atoms in no. 5 and 11 in no. **9**. They show little difference in potency as the chain length is altered. The next three ligands are dihydroxy compounds of increasing activity and the last ligand gave a nickel(II) bis(terpyridine) complex somewhat more active than *d*-tubocurarine itself and was chosen for the introduction of osmium atoms. In general the effects of the nickel(II) terpyridine complexes of the ligands in Table II took some 5–10 sec to appear, whereas the paralyzing effects of *d*-tubocurarine appeared in less than 5 sec.

The introduction of the osmium ions (Table IV) not only influenced the potency of the complexes but prolonged the time taken for the action to appear compared with the action of *d*-tubocurarine. More time was thereby provided for distribution, excretion, and binding by acceptors or inactive sites of loss. Presumably such delayed action would tend to diminish the activity of the osmium compound when administered in this way. In spite of this, no. IV (Table IV) is over twice as potent as *d*-tubocurarine. Although compound no. III resembles IV closely in structure, the coordination of two chloride ions instead of two pyridine molecules causes each osmium(II) atom to be associated with a single positive charge, whereas the Os atoms in no. IV provide two charges each. Associated with this is the lower pharmacological action of III.

Because of the longer period of onset of action by the intravenous route, dihydroxyosmarine (IV) was assayed on indirectly stimulated slips of diaphragm from young guinea pigs where its ED_{50} was about 1/16th that of *d*-tubocurarine.

In the isolated diaphragm method there is about 50 mg of muscle in a bath of 30 ml of fluid and an amount of drug is added which is just sufficient to cause a complete paralysis. The concentration of drug is then reduced until a detectable response appears and becomes steady. This gives an estimate of the percent response, at a drug concentration which is in equilibrium with the receptors.[21] Several such points obtained at decreasing drug concentrations can be used to construct an equilibrium dose–response curve and from which the ED_{50} can be calculated. In this way a more basic comparison of curariform activity is obtained.

Table IV. Assay Values of Osmium Complexes of Ligand No. 12

Compd no.	Osmium coord complex of ligand no. 12	Formula	Analyses[a]	Mouse LD_{50}, μmol/kg ± SD	Diaphragm ED_{50}, μmol/l. ± SD
III	Bis(chlorobipyridine)	$C_{58}H_{50}N_{10}O_2Os_2Cl_4 \cdot 3H_2O$	C, H, N; Cl[b]	1.2 ± 0.097	
IV	Bis(pyridinebipyridine)-dihydroxyosmarine (DHO)	$C_{68}H_{60}N_{12}O_2Os_2Cl_4 \cdot 4H_2O$	C, H, N	0.08 ± 0.008	0.04 ± 0.008
	d-Tubocurarine			0.18 ± 0.01	0.63 ± 0.2

[a] Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of the theoretical values. Their stereochemistry has not been studied. [b] Cl: calcd, 9.48; found, 10.18.

The pharmacology of the monofunctional transarine ions studied by Dwyer et al. has been summarized in the introduction and they are all typical nondepolarizing curariform agents.

Dihydroxyosmarine like *d*-tubocurarine and unlike the depolarizers can produce quite steady states of partial paralysis.[21,22] The drug can be antagonized by reducing the temperature to 25° and by neostigmine. It did not cause the frog rectus to contract. Dihydroxyosmarine behaves as a typical antidepolarizer and no depolarizing activity was detected at any time. It had no effect on directly stimulated muscle.

Discussion

Terminology. Since active complex ions can be formed by coordinating at least five transition elements with a greater number of ligands, the possible number of combinations is large and it seems justifiable to have a name for this class of compounds. Based on representative nomenclature we would like to propose the term "transarine" ions for transition metal coordination complexes having curariform actions. The term transarine lends itself to logical development in that for any given transition element the "trans" part of the term can be replaced by an abbreviation of the elements, and complexes of osmium for example could be referred to as osmarine ions; thus the osmium complex IV (Table IV) from ligand no. 12 in Table II would be dihydroxyosmarine tetrachloride.

In essentially all transarines the coordination bonds joining the ligands to the transition metal ion involve electron donation by the nitrogen atoms, causing the charge on the positive central atom to be partially transferred to, and spread out over, the six coordinating pyridine rings (Pauling[23]). This marked charge delocalization is where the transarines differ markedly from all the other cholinergic agents.

The monofunctional transarines such as those in Table III, and others, have six ligand atoms coordinated to the central transition metal and possess no residual bonding capacity. They behave rather as large positively charged pseudospheres and are held to negatively charged receptor groups by a combination of electrostatic, hydrophobic, and van der Waals forces.[7] All the transarines so far examined are antidepolarizers and have electrovalencies of two or four. The question is, "where do the mono- and bifunctional transarine complexes fit into the general structure–action relationships of curariform compounds?"

In general, charged nitrogens of the diquaternaries are thought of as binding electrostatically to two receptors about 10–12 Å apart, or perhaps to a receptor and an anionic acceptor (inactive binding or anchoring site) separated by a similar distance,[24] while the rest of the organic structure is nonspecifically bound by hydrophobic bonds and van der Waals forces.

Until the bifunctional transarine compounds had been synthesized there was no evidence that they would be more potent than the corresponding monofunctional ions. The question as to whether a monofunctional transarine with two positive charges was analogous to a monoquaternary or to a hypothetical diquaternary with zero interchange distance was equally unclear. It now appears that the monofunctional and bifunctional transarines are more closely related to mono- and diquaternaries than to anything else but this leaves the question of the significance of the double charge on the monotransarine ions or on the ends of bis(transarine) ions unresolved, especially since there is evidence that stoichiometric ion exchange is occurring at the receptors.[25,26] Stoichiometric ion exchange requires DHO to exchange for ions carrying four positive charges, while it would take two diquaternary ions to displace the same number of charges.

The evidence that the joining of two transarines can increase potency is clear. The fact that each end of DHO carries two positive charges and these charges are well spread out over the six pyridines rather than being sharply localized to a quaternary nitrogen group reinforces the doubt that DHO and the diquaternaries combine with the receptor in the same way. This difference may be resolved by a receptor group arrangement previously suggested. Evidence was presented[26] that the cholinergic receptor site had a pair of negatively charged groups separated by less than 4 Å. This was based on the marked selectivity of the site for the bivalent Mg^{2+} and Ca^{2+} over the monovalent Na^+ and K^+ ions, a condition which requires the negative charges to be less than 4 Å apart.[27] These paired groups are normally occupied by Mg^{2+} and Ca^{2+} and two acetylcholine ions are thought to react by displacing one bivalent inorganic ion by stoichiometric ion exchange. This system has been referred to as the ion-exchange receptor pair model, and the initiation of depolarization may be due to the displacement of the inorganic ions rather than the combination with the acetylcholine ions.

Evidence was also obtained that in the case of decamethonium an anchoring site[26] is involved as well as a receptor site and that the anchoring site is a pair of negatively charged groups normally occupied by a calcium ion, the receptor and anchoring sites being about 10 Å apart. Two C-10 ions would then bridge the two sites and displace one bivalent inorganic ion from each. Since DHO carries two positive charges at each end, only one DHO ion would be required to occupy both the receptor and the anchoring sites.

Experimental Section

Acid Chloride of 1,10-Phenanthroline-2-carboxylic Acid.[11] 2-Carboxy-1,10-phenanthroline (1 g) and 50 ml of thionyl chloride were refluxed until complete dissolution was effected (2 hr). The solution was evaporated on a rotary evaporator to about 10 ml and the acid chloride allowed to crystallize for about 10 min. Dry benzene (20 ml) was added and the solution was allowed to stand for 0.5 hr to complete the crystallization. The solvents were evapo-

rated off and the evaporation was repeated twice with 20-ml portions of benzene.

Bis(1,10-phenanthroline) Ligands. The general method of preparation of the four bis(1,10-phenanthroline) ligands was the same except for minor details of the separation and crystallization of the end products. Chloroform (5 ml) was added to the phenanthroline acid chloride above followed by 0.5 mol equiv of linking base in 5 ml of chloroform and 2 ml of triethylamine (dried and distilled from 2% phenyl isocyanate). The mixture was refluxed for 0.5 hr and cooled and the solvent evaporated on the rotary evaporator. The product was washed with water and collected. Ligand no. 1 was recrystallized from boiling dimethyl sulfoxide. Ligand no. 4 was converted to its 3,5-dinitrobenzoate, recrystallized from methanol, suspended in water, basified, and extracted with chloroform. The chloroform was evaporated and the product crystallized from ether. Ligands 3 and 4 were recrystallized from chloroform–methanol.

2-Methyl-2,2′:6″,2″-terpyridine. A mixture of equimolar amounts of bipyridyl (500 g) and 2-picoline (330 ml) was refluxed with 33 g of 5% palladium on carbon for 72 hr with spin bar stirring. The reaction mixture was filtered hot on sintered glass to remove the catalyst and the catalyst washed four times with a little fresh 2-picoline. The filtrate was distilled through a Vigreux column at reduced pressure (water pump) to give 2-picoline and unreacted bipyridine, leaving a brown oil in the flask. On cooling the oil set to a paste and this was mixed with four volumes of *n*-heptane, boiled, and filtered. The extraction of the paste was repeated twice, the filtrates were combined and concentrated, and residual bipyridine was distilled off at 25 mmHg until the distillation temperature reached 220°. Paper chromatography indicated that this removed all of the bipyridine. The residue in the distillation flask was boiled with 4 vol of heptane and immediately cooled by swirling in an ice water bath. Some oil appeared as a milky suspension which later deposited as a dark oil or tar. The heptane solution was poured off and the extraction of the tar with boiling heptane and ice cooling was repeated three times. Evaporation of the pooled extracts gave crude 2-methylterpyridine which was air-dried and recrystallized from heptane: mp 112.5°; yield ~5 g. Anal. ($C_{16}H_{13}N_3$) C, H, N.

Bis(terpyridine) Ligand from 1,4-Cyclohexanedione. The general methods of preparation of the bis(terpyridine) ligands were the same except for details of the purification of the end product. The preparation of the derivative from 1,4-cyclohexanedione will be used as an example of the method for all seven listed compounds.

All glassware was oven dried and desiccator cooled. Preparations were initially carried out at the level of 1 mmol. Lithium chloride (1 mmol) was placed in a two-necked 25-ml flask fitted with a serum cap and an exit tube with a stream of dry N_2 flowing. The bottom of the flask was placed in silicone oil at 250° for 3 min to drive off the water from the salt. The flask was wiped and cooled, a spin bar was added and repeatedly partially evacuated and flushed with N_2, and 3 ml of redistilled (from $LiAlH_4$) tetrahydrofuran was injected. Stirring caused solution of most of the lithium chloride. The flask was placed in acetone–Dry Ice, cooled to −30° for 5 min, and 0.15 ml of dry redistilled diisopropylamine was added followed by 0.47 ml of 2.2 *M* *n*-butyllithium in hexane. After 5 min at −30° Dry Ice was added to the acetone bath and the temperature lowered to about −75°. After 5 min 1 mmol of 2-methylterpyridine in 1 ml of tetrahydrofuran was added and allowed to react for 10 min. Then 0.5 mmol of coupling agent (1,4-cyclohexanedione) in 1 ml of redistilled tetrahydrofuran was added dropwise and the temperature raised to −50° for 5 min. The Dry Ice–acetone bath was replaced by water at 20° and the reaction mixture warmed to room temperature. The reaction was quenched with 1 ml of water.

Excess tetrahydrofuran was evaporated on a rotary evaporator and the small amount of residual water pipetted off. The material was washed with a little water and 2 ml of ether was added to facilitate crystallization. The ether was evaporated and the crystals were suspended in a little acetone and filtered. The crystals were washed with a little acetone, collected, and dried. The yield was ~45%. The crystals were dissolved in a little chloroform and the solvent was evaporated to a small volume; methanol was added and the product was left to crystallize.

The bridging compounds used for ligands 6–12 were 1,3-diiodopropane, 1,4-diiodobutane, 1,4-dibromo-2-butene, 1,5-diiodopentane, 2,3-butanedione, 2,5-hexanedione, and 1,4-cyclohexanedione.

Insertion of Osmium into Ligand No. 12. $OsCl_4bpy$[18] (92 mg) and 50 mg of ligand no. 12 in 0.5 ml ethylene glycol were stirred with a thermometer at reflux in a small test tube in a silicone oil bath for 30 min. After the mixture had cooled, 1 ml of absolute ethanol and 1 ml of diethyl ether were mixed in and the solution was put at 0° overnight. The dark-red brown crystals were filtered off on a sintered disk and washed with two 0.25-ml portions of cold absolute ethanol and 1 ml of cold ether and air-dried overnight. The above chloro compound (50 mg) was boiled with 2 ml of absolute ethanol and the solution decanted onto a filter from some black insoluble material which was further boiled with four more lots of 2 ml of ethanol. The extracts were decanted and filtered as before and the filtrates combined and evaporated to small volume. The almost black crystals were washed with the minimal amount of cold ethanol and ether and air-dried (overall yield ~60%). Anal. ($C_{58}H_{50}N_{10}O_2Os_2Cl_4 \cdot 3H_2O$) C, H, N; Cl: calcd, 9.483; found, 10.18.

(Pyridinebipyridine)osmium(II) Derivative of Ligand No. 12. A solution of 100 mg of the above chloro compound in 4 ml of water and 0.8 ml of redistilled pyridine was refluxed for 48 hr,[20] cooled, and evaporated to dryness. The product was suspended in a mixture of 0.15 ml of water and 0.15 ml of concentrated HCl and left to crystallize. The crystals were collected and washed with anhydrous ether to give very dark brown crystals (yield ~40%). Anal. ($C_{68}H_{60}N_{12}O_2Os_2Cl_4 \cdot 4H_2O$) C, H, N.

Estimation of Biological Activity. The nickel(II) complexes of the ligands in Tables I and II were made by placing 20 mg of the ligand in 2 ml of water and adding 2 mol equiv of nickel sulfate. The solution was left overnight for the ligand to dissolve and 2 mol equiv of terpyridine was added and left overnight again. The complex was diluted and the LD_{50} determined by injection into the warmed tail vein of white mice by the up and down method[17] using *d*-tubocurarine as a standard.

For assays using the isolated guinea-pig diaphragm a slip of muscle with its phrenic nerve from the left side of guinea pigs weighing between 100 and 150 g was used. It was suspended in Krebs fluid gassed with 95% O_2–5% CO_2 at 37° and single maximal shocks were applied to the nerve and muscle alternately[21] so that as the preparation stimulated through the nerve became paralyzed the direct muscle response provided a measurable running control.

Initially a dose of compound sufficient to just cause complete paralysis was added and the drug concentration was then slowly reduced until a response just began to appear and the preparation responded with a steady almost complete paralysis. Such steady paralysis at decreasing drug concentrations represents equilibrium dose–response relationships and gives essentially the same results whether the equilibria are approached from higher or lower drug concentrations.[21] If there is any doubt that the preparation is at equilibrium, a small additional amount of drug is added and if this causes the paralysis to increase then the preparation had essentially reached equilibrium. This test was used routinely with DHO as it showed incipient irreversibility.

Least-squares regression lines were then calculated for the probit percent responses vs. log dose and the ED_{50} values calculated from the regression line constants. Dose–response lines for both *d*-tubocurarine and DHO were obtained from each of five muscles from five different animals. The order of addition of the drugs was reversed in each successive experiment.

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Synthesis and Biological Properties of Some Novel Heterocyclic Homoprostanoids

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In the search for prostaglandin-like structures capable of exerting specific and desirable biological properties, a variety of simple heterocyclic homoprostanoidal derivatives was synthesized from readily available stearic acid derivatives. Compounds **5b** and **5e** were found to be more than 100 times as potent as PGE_1 and PGE_2 in a tracheal chain bioassay and, like **6**, **9**, and **12**, inhibited PGE_2-induced diarrhea. Derivatives **6** and **7a** showed significant PG-synthetase inhibitor activity.

The lack of tissue specificity and the rapid metabolic destruction of the natural prostaglandins have resulted in the search for analogs possessing more desirable biological profiles. Limitations in ready access to these relatively complex structures have imposed additional burdens on their development as potentially useful therapeutic agents.[1]

Encouraged by recent publications indicating the extent to which the chemistry of the natural prostaglandins (especially that of the cyclopentane ring) may be modified without sacrificing biological activity,[2-9] we elected to exploit the ready availability of oleic acid and its 9,10-bifunctionalized (stearic acid) derivatives and their facile conversion to the corresponding heterocyclic homoprostanoidal derivatives.[10] We were additionally attracted to these relatively simple heterocyclic ring systems because of their conformational similarity to the cyclopentane ring of the natural prostaglandins and by our developing appreciation of the state of the art of drug design;[11] calculated estimates of the partition coefficients of the compounds described compared favorably with those of the corresponding PGE and PGF derivatives. The recent disclosure of the modest PGE_2-like activity of certain bis(oxa)prostaglandins[9] prompts us to present our finding at this time.

Chemistry. Oleic acid (1) yields two isomeric 9,10-dihydroxyoctadecanoic acids depending on the mode of synthesis.[12] The higher melting erythro isomer **2** results from treatment of oleic acid with basic permanganate. The lower melting threo isomer **3** is readily obtained when oleic acid is oxidized using hydrogen peroxide under acidic conditions. Esterification yields the corresponding methyl esters **8** and **4** also having characteristic melting points.[12]

The threo ester **4** was chosen for conversion to heterocycles **5a–e** because the resulting compounds would bear a trans configuration of the ring hydrogen atoms and thus correspond to the natural prostaglandins. Accordingly, reaction of **4** with paraformaldehyde and orthophosphoric acid gave the dioxolane **5a**. Treatment of **4** with phosgene, thiophosgene, phosphorus oxychloride–methanol, and thionyl chloride afforded the corresponding homoprostanoids **5b–e**.

Oleic acid (1) was transformed to the cis epoxide[13] **6** using *m*-chloroperbenzoic acid. Reaction with potassium methylxanthate gave a mixture of the trans acid **7a** and the trans ester **7b**. The trans geometry follows from the work of Overberger and Drucker who obtained *trans*-4,5-dimethyl-1,3-dithiolone-2-thione from *cis*-2,3-epoxybutane and potassium methylxanthate.[14]

The NMR spectra of **5b–d** were quite similar; a broad peak near δ 4.5 was assigned to the ring protons. The cyclic sulfite **5e**, however, exhibited two multiplets in this region, one at δ 4.55 and the other at δ 4.01. In their NMR studies of simple cyclic sulfites Pritchard and Lauterbur have shown that the double-bonded oxygen lies out of the plane of the ring.[15] Protons that are cis relative to this oxygen are shifted downfield compared to those that bear a trans relationship to the oxygen. Thus, the NMR spectrum of **5e** is consistent with a cyclic sulfite having a trans arrangement of the protons. A coupling constant of 8.20 Hz for the ring protons (i.e., a dihedral angle of about 160°) indicates the noncoplanarity of the carbon and ring oxygen atoms.[16] It was not determined which of the two possible trans compounds, **5e** or **5d**, was or whether or not it was a 1:1 mixture of the two possible trans diastereoisomers.

An isomeric cyclic sulfite was similarly prepared from *erythro*-9,10-dihydroxyoctadecanoic acid. The NMR spectrum of this compound (**9**) showed only a single ring proton absorption at δ 4.52 corresponding closely to the downfield absorption seen in **5e**. Hence, the probable structure of **9** is