ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2015, Vol. 41, No. 2, pp. 178–185. © Pleiades Publishing, Ltd., 2015.
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Curare-Like Camphor Derivatives and Their Biological Activity

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Received June 25, 2014; in final form, August 27, 2014

Abstract—The synthesis of symmetric dimeric camphor derivatives containing two quaternary nitrogen atoms was performed and their myorelaxant activity in mice was evaluated. For the comparison, some salts derived from *meta*- and *para*-xylylene dibromides were synthesized and their activity was tested.

Keywords: camphor, muscle relaxant activity, rota-rod test, inclined plane test

DOI: 10.1134/S1068162015020156

INTRODUCTION

One of the most effective current approaches to the synthesis of biologically active compounds is functionalization of the known biologically active molecules with various pharmacophoric groups. Natural resources serve as the major source of primary biomolecules. Now researchers specializing in the field of medicinal chemistry pay special attention to the synthesis and studies of symmetrical molecules bearing two or more natural fragments in the backbone. Structures of these natural fragments are very different. They can be of the alkaloid [1, 2], steroid [3], mono- or diterpene [4, 5], etc. nature. Symmetrical nitrogen-containing compounds with natural fragments joined with linkers and bearing in the backbone two quaternary nitrogen atoms display a wide spectrum of biological activity. However, these compounds are most known for their neuroblocking properties and are also called curare-like compounds.

The biological activity of curare, concentrated extracts of South American plants of the *Strychnos* and *Chondodendron* species, has been studied for several centuries. Curare and curare-like compounds are used in medicine as skeletal muscle relaxants, first of all, in surgery. Compounds of different chemical classes belong to such drugs, particularly, alkaloids containing one or several tertiary amino groups, mono- and di quaternary nitrogen fragments within various backbones, as well as the structures with positively charged heteroatoms other than nitrogen [6].

Since 1953, when the structure of the active compounds of the pipe curare, alkaloid d-tubocurarin (**I**), was found, many of its analogues causing muscular relaxation have been approved for application in anaesthesiology [7] (formula). Atracurium (**II**), which is structurally similar with tubocurarin, is a synthetic compound used in medical practice. Dithylin (succinylcholine) (**III**) is a major representative of depolarizing neuromuscular relaxants, which blocks neuromuscular activation and causes relaxation of skeletal muscles. This drug demonstrates a rapid and short term effect and in spite of a number of side reactions [8] is widely used in medicine. Neuromuscular relaxants with a steroidal backbone, being nondepolarizing blocking agents with the myorelaxant activity close to that of tubocurarin, do not display the hormonal activity. For example, arduan (**IV**) and pancuronium (**V**) are approved for medical use [9] (formula). Until now, steroidal myorelaxant drugs have displaced dithylin in many hospitals due to a higher safety when used for the relaxation of skeletal muscles.

However, none of the presently available muscle relaxants meets the criteria for an ideal neuromuscular blocking agent [10]. These criteria defined in the 1970s include the rapid onset and the predetermined duration of action, antidepolarizing mechanism of action, rapid development of the effect, the lack of the cumulative action, the lack of cardiovascular side effects, rapid and complete reversal with anticholinesterases, and fast elimination from the body without regard to renal/liver function or biotransformation into inactive metabolites [11].

In the 1970s, D.A. Kharkevich et al. systemically searched for the most active derivatives of truxillic acid [12]. Anatruxonium [13] and cyclobutonium [14], the most effective compounds of this series, were implemented in medical practice in the USSR. Currently, almost no studies on the search of new synthetic myorelaxants are in progress. An exception is the studies of

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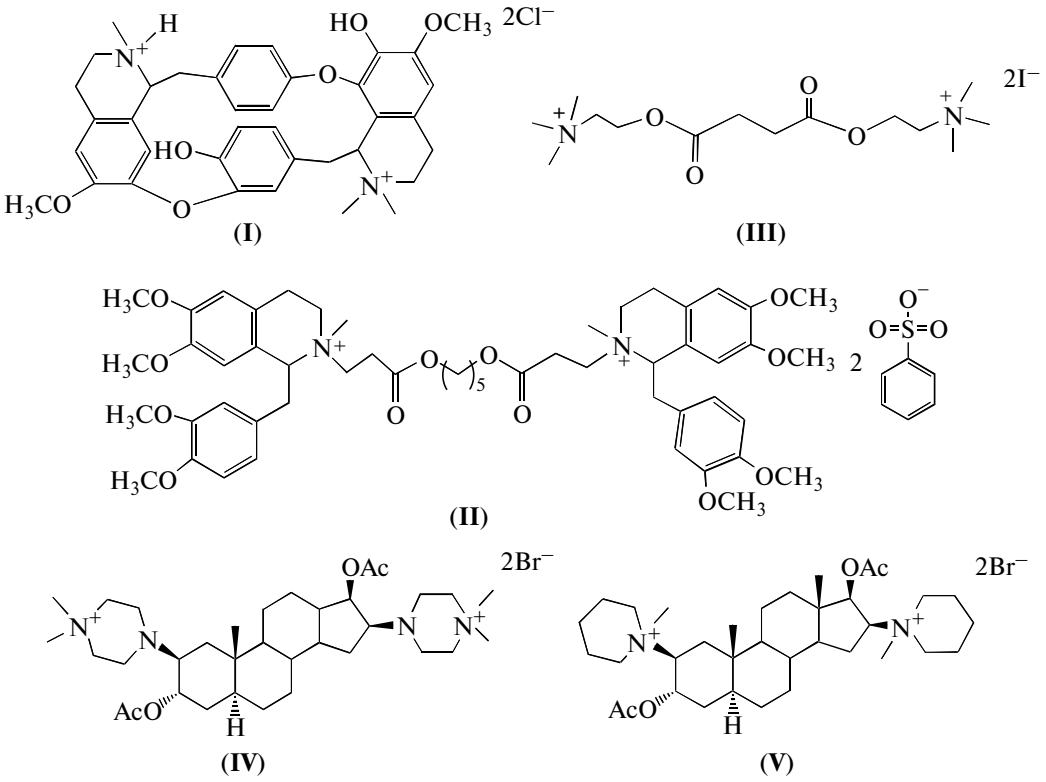
Kazan researchers on the potential myorelaxant properties of some uracil derivatives [15]. Experiments with animals are complicated by a significant drawback of neuroblocking agents: an extremely small difference exists between the doses used for treatment and those paralyzing respiratory muscles and causing apnoea.

The goal of this work was the synthesis of symmetrical curare-like compounds containing in the backbone two fragments of natural camphor molecule and two quaternary nitrogen atoms joined with linkers of different length and rigidity. We studied myorelaxant activities of the compounds synthesized, compared the activities with those of some structurally similar compounds, and evaluated the structure–activity relationship within the series synthesized.

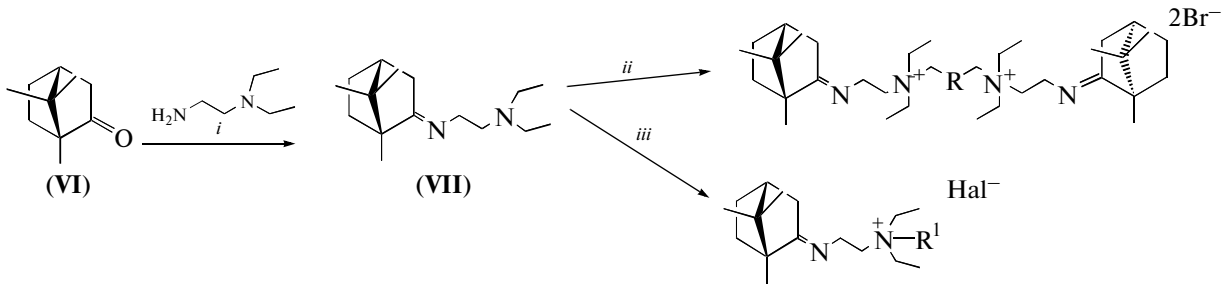
RESULTS AND DISCUSSION

As a basis for the synthesis of symmetrical derivatives we took a common natural molecule of the monoterpene series, (+)-camphor (**VI**). Previously, it was shown that symmetrical camphor imino derivatives displayed a marked antiviral activity [16]; amidoalcohols based on the camphor backbone were demonstrated to be highly effective antituberculosis agents [17]. A characteristic feature of the camphor structure is a rigid carbon backbone to some extent similar to the adamantane backbone. It is known that adamantane derivatives with quaternary ammonium atoms joined with aliphatic chains manifest myorelaxant properties [6].



Formulas. Chemical structures of myorelaxant agents used in medical practice.

For the synthesis of the target compounds we synthesized nitrogen-containing camphor derivatives bearing imine and tertiary amine groups in the backbone (**VII**–**XIII**) using the interaction of halogenides with tertiary amines (Scheme 1). Previously, we described the synthesis of compounds (**VIII**–**XIII**) and demonstrated their effective inhibitory properties against the influenza virus. Iminoamine (**VII**) used as a starting compound in the synthesis of the derivatives was obtained in a yield of 85–90% by the reaction of camphor (**VI**) with *N*,*N*-diethylethan-1,2-diamine with azeotropic distillation of water in toluene in the presence of $BF_3 \cdot Et_2O$ (Scheme 1). For the preparation of symmetrical dimeric molecules, aliphatic dihalogenides with the varied length of the chain were used as linkers (R, Scheme 1). For the evaluation of the impact of the linker rigidity and electron structure we synthesized compound (**XI**) bearing an aromatic ring (Scheme 1). The reaction of methyl iodide or ethyl bromide and the starting iminoamine (**VII**) resulted in the corresponding quaternary ammonium bases (**XII**) and (**XIII**) (Scheme 1).

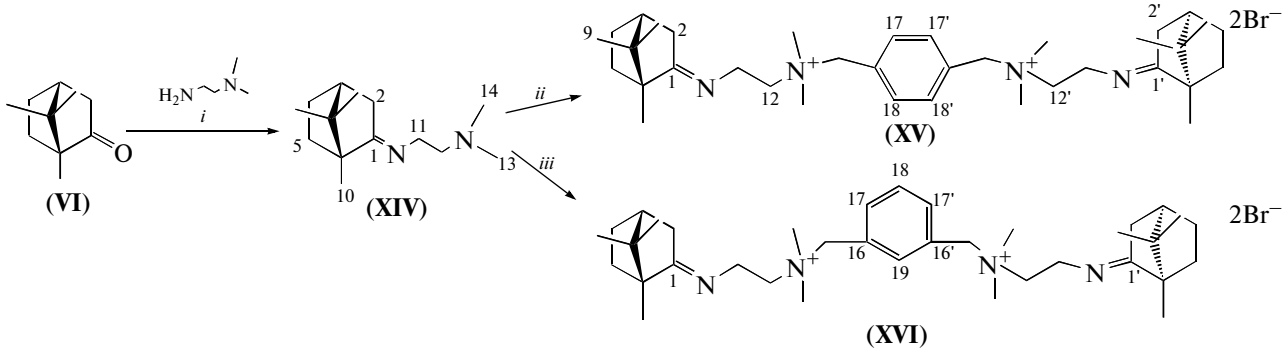


Scheme 1. The synthesis of compounds **(VII)**–**(XIII)**.
i) PhMe, $BF_3 \cdot Et_2O$ (1–5 mol %); *ii*) Br-CH_2-R-CH_2-Br (0.5 mol), CH_3CN; *iii*) R^1Hal, CH_3CN.
–R– **(VIII)** $-(CH_2)_3-$; **(IX)** $-(CH_2)_4-$; **(X)** $-(CH_2)_6-$; **(XI)** *p*-Ph–R^1; **(XII)** $-CH_3$, Hal = I; **(XIII)** $-C_2H_5$, Hal = Br.

The study of the neuroblocking activity of compounds **(VIII)**–**(XIII)** bearing a camphor backbone and quaternary nitrogen atoms showed that only compound **(XI)** containing a *para*-substituted aromatic ring in the linker chain could cause muscle relaxation in mice at doses of 10 mg/kg and 15 mg/kg (table). In the rota-rod test the capacity of the animals to remain on the rod after the injection of compound **(XI)** reduced and the time of the first fall from the rod essentially decreased. The control animals did not fall during the whole experimental time, whereas the animals with dithylin fell immediately after they were placed on the rod. Some animals (14 to 33%, depending on the dose) that were administered compound **(XI)** could not keep the position on the inclined plane, which served another evidence of the neuroblocking activity of the compound under study. In this test the control animals went up the inclined plane, whereas the animals with the induced muscle relaxation went down the plane and fell down as it was observed for the dithylin group (table).

The monoquaternized salts of camphor iminoamine **(XII)** and **(XIII)** containing a charged nitrogen atom were inactive in any of the tests at the doses of 10 to 20 mg/kg (table). Among compounds **(VIII)**–**(X)**, no agent was found which could induce noticeable neuroblockage in animals. It is noteworthy that the elongation of the linker chain in this series did not affect the capacity of the compounds to induce muscle relaxation but affected the compound toxicity: compound **(X)** with the longest aliphatic chain was the most toxic (LD_{50} 4.1 mg/kg), whereas LD_{50} values for the other members of this group were within the range of 9.2 to 12.5 mg/kg.

With the goal of studying the effects of shielding the charged nitrogen atom in camphor imino derivatives on the biological activity of the compounds tested, we synthesized another starting compound, iminoamine **(XIV)**, by the interaction of camphor with *N*,*N*-dimethyl-1,2-diamine. Compound **(XV)** was obtained under similar conditions, by the interaction of iminoamine **(XIV)** with *para*-xylylene dibromide. For the studies of the positional relationship of the linker aromatic ring substituents and the distance between the quaternary nitrogen atoms of the "dimeric" molecule, we prepared compound **(XIV)** using a similar reaction of *meta*-xylylene dibromide with compound **(XVI)** (Scheme 2).



Scheme 2. The synthesis of compounds **(XIV)**–**(XVI)**.
i) PhMe, $BF_3 \cdot Et_2O$ (1–5 mol %); *ii*) *p*-$BrCH_2$–Ph–CH_2Br (0.5 mol), CH_3CN;
iii) *m*-$BrCH_2$–Ph–CH_2Br (0.5 mol), CH_3CN.

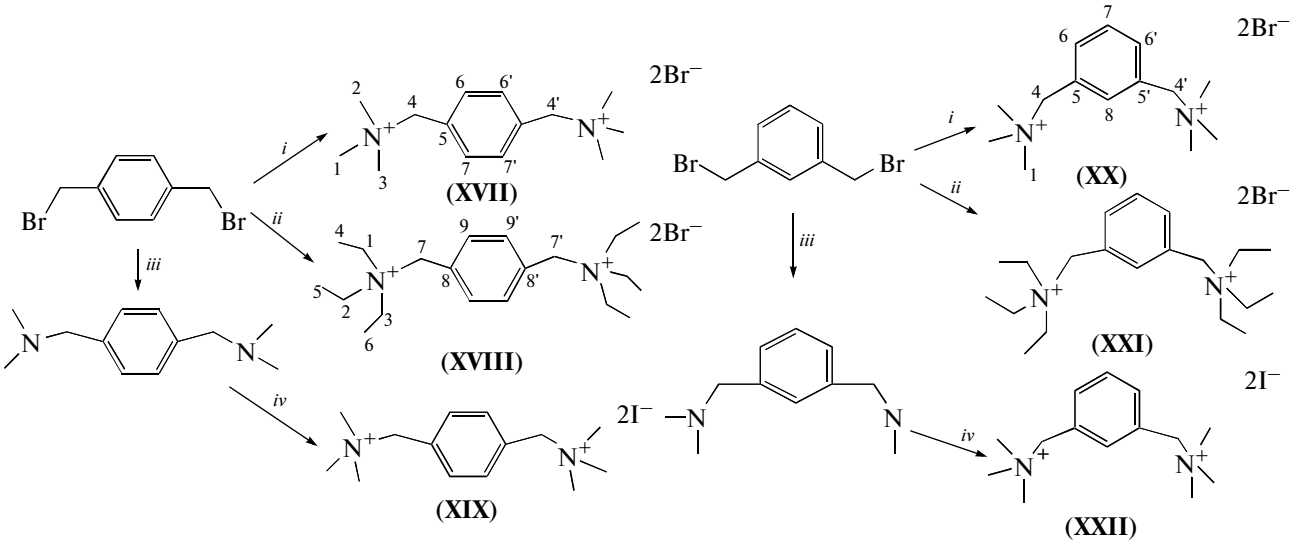
Biological activity of compounds (**VIII**–**XIII**) in mice

Compound	Dose, mg/kg	Latency of the fall from the rod, s (120 s max)			Neuromuscular block on an inclined plane, % of animals	LD_{50}, mg/kg
		attempt 1	attempt 2	attempt 3		
Control		120	120	120	0	
(VIII)	5	120	120	120	0	9.2
	10	115 ± 5	110 ± 10	120	33.3	
(IX)	10	120	120	120	0	10.3
	15	109 ± 8	87 ± 16	98 ± 13	25	
(X)	5	120	120	120	0	4.1
	7	114 ± 6	91 ± 19	93 ± 14	25	
(XI)	5	120	120	120	0	12.3
	10	117 ± 2	65 ± 15*	106 ± 6	14.3	
	15	103 ± 8	63 ± 21*	90 ± 30	33.3	
(XII)	10	120	120	120	0	not determined
	20	120	120	120	0	
(XIII)	5	120	120	120	0	not determined
	10	120	120	120	0	
	20	120	115 ± 5	120	0	
(XV)	5	120	120	120	0	14.9
	10	109 ± 8	53 ± 13**	108 ± 5	16.7	
	15	104 ± 9	43 ± 14***	48 ± 16**	33.3	
(XVI)	10	120	60 ± 35	115 ± 5	0	12.5
(XVII)	10	120	75 ± 14*	110 ± 5	0	27.2
	15	98 ± 9*	56 ± 15**	49 ± 9***	0	
	20	120	25 ± 6**	68 ± 13***	0	
(XVIII)	5	120	120	120	0	13.9
	10	85 ± 9**	32 ± 5***	84 ± 11*	20	
(XIX)	10	118 ± 3	30 ± 30**	68 ± 21*	0	22.7
	15	100 ± 14	20 ± 20***	100 ± 20	0	
	20	90 ± 30	–	–	0	
(XX)	10	95 ± 15	98 ± 13	118 ± 3	0	20.6
	15	94 ± 13	60 ± 19*	90 ± 19	0	
(XXI)	5	10	–	–	0	4.3
(XXII)	10	120	83 ± 13**	108 ± 6*	0	30.4
	15	120	35 ± 16***	105 ± 6*	0	
	20	110 ± 10	40 ± 20*	55 ± 5*	0	
Dithylin	2.5	0***	0***	107 ± 8	100	1.8

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to control.

The activity studies of salts (**XV**) and (**XVI**) demonstrated that compound (**XV**) displayed myorelaxant properties similar to those of compound (**XI**): at a dose of 10 mg/kg the latency to fall off the rod was reduced and 16% of the animals could not keep themselves on the inclined plane. Salt (**XVI**) did not show the neuroblocking activity: the reduction of the time to fall from the rod was not reliable and no positive results were observed in the inclined plane test. The toxicity of these salts did not differ from the toxicity of compound (**XI**). Thus, we could assume that shielding of the charged nitrogen atom (the replacement of ethyl by methyl radicals) in the "dimeric" molecules under study did not have any influence on the neuroblocking activity, whereas a decrease in the distance between quaternary nitrogen atoms resulted in a reduced capacity to relax muscles in animals.

For the evaluation of the role of the terpene backbone in the salts tested for biological activity we synthesized a series of bis-quaternized compounds (**XVII**)–(**XXII**) derived from the corresponding *para*- and *meta*-xylylene dibromides (Scheme 3). Salts (**XVII**), (**XVIII**), (**XX**), and (**XXI**) were obtained by a direct interaction of the aromatic dibromo derivative with the corresponding tertiary amine, and salts (**XIX**) and (**XXII**), via the formation of tertiary amines followed by quaternization with methyl iodide.



Scheme 3. The synthesis of compounds (**XVII**)–(**XXII**). *i*) NMe_3, CH_3CN; *ii*) NEt_3, CH_3CN; *iii*) $NHMe_2$, CH_3CN; *iv*) CH_3I, CH_3CN.

The study of the myorelaxant activity of these compounds showed that nearly all of them at doses of 10 to 20 mg/kg following intra-abdominal administration considerably reduced the time taken for the animal to remain on the rod. However, only compound (**XVIII**) affected the capacity to remain on the inclined plane (table). Hence, in this case we can observe the violation of coordination of movements in animals caused by neurotoxic effects rather than by the myorelaxant activity of the compounds tested. The fact that nearly all of the compounds of this group (except (**XVIII**)) caused tremor, and even convulsions in some cases, after physical exercises (the attempts of the animals to remain on the rod) is in favor of this hypothesis.

The most toxic compound within the monoquaternized (**XVII**)–(**XXII**) was compound (**XXI**): its LD_{50} was 4.3 mg/kg, whereas this value for the other compounds from this group was within 13.9 to 30.4 mg/kg. Also, no basic differences in the biological activity were found between the pairs of the corresponding bromides and iodides (**XVII**)–(**XIX**) and (**XX**)–(**XXII**), which implied the lack of the counterion effect on the activity of the compounds tested (table).

To summarize, we systematically studied a new class of curare-like compounds, diquaternized derivatives of natural bicyclic diterpenoid camphor. On the basis of the research some conclusions can be made. An increase in the length of the aliphatic chain among (**VIII**)–(**X**) led to an increase in the toxicity; shielding of the charged atom, i.e., an ethyl or methyl radical at the quaternary nitrogen atom in compounds (**XI**) and (**XV**) did not essentially affect the neuroblocking activity of the compounds under study; the compounds with *para*-substituted aromatic rings in the linkers of "dimeric" molecules manifested more noticeable myorelaxing properties than their *meta*-substituted counterparts; the counterion nature is not important for biological properties of the compounds tested. A comparison of biological activity of dimeric quaternary bases (**XV**)–(**XVI**) demonstrated that the lack of

a natural camphor fragment provided the lack of the myorelaxant activity and the appearance of the neurotoxic effect.

EXPERIMENTAL

Chemistry

We used in this work (1*R*)-(+)-camphor (Alfa Aesar, 98%, $[\alpha]_D^{25}$ −45.5 ($CHCl_3$, s 0.8)), *p*-xylylene bromide (abcr, 97%), *m*-xylylene bromide (abcr, 97%), N^1,N^1-diethylethane-1,2-diamine (Sigma, 99%), N^1,N^1-dimethylethane-1,2-diamine (Acros, 99%).

^{1}H and ^{13}C NMR spectra (δ, *J*, Hz) of compound (**XIV**) were recorded on a Bruker AV-600 spectrometer (^{1}H: 600.30 MHz, ^{13}C: 150.95 MHz); compounds (**XV**)–(**XVI**), Bruker DRX-500 (^{1}H: 500.13 MHz, ^{13}C: 125.76 MHz); compounds (**XVII**)–(**XXII**), Bruker AV-400 (^{1}H: 400.13 MHz, ^{13}C: 100.78 MHz). As internal standards, chloroform (δ_H 7.24, δ_C 76.90 ppm), DMSO (δ_H 2.50, δ_C 39.50 ppm) and MeOH (δ_H 3.31, δ_C 49.00 ppm) were used. ^{1}H- and ^{13}C NMR spectra of compound (**XV**) were recorded in a 10 : 1 mixture of CD_3OD and NEt_3. The structures of the compounds synthesized were confirmed using ^{1}H and ^{13}C NMR spectra as well as ^{1}H–^{1}H double resonance spectra, two-dimensional homonuclear ^{1}H–^{1}H correlation spectra (^{1}H–^{1}H COSY) and two-dimensional heteronuclear ^{13}C–^{1}H correlation ((C–H–COSY, $^1J_{C,H}$ 160 Hz) spectra, and distant coupling constants (COLOC, $^{2,3}J_{C,H}$ 10 Hz). High resolution mass spectra were recorded on a DFSThermoScientific spectrometer using the exhaustive scanning within *m/z* 0–500; electron impact ionization (70 eV) with a direct injection of samples. Final column chromatography was performed on silica gel (60–200 μm, Masherey-Nagel).

The HPLC–MS system outfitted with an Agilent 1200 chromatograph and a hybrid quadrupole time-of-flight micrOTOF-Q mass-spectrometer (Bruker) (the API-ES mode) was used for the assignment of the structures of diquaternized compounds. The system operated in positive polarity with a scanning mass range of 80 to 3000 *m/z*. The drying gas (nitrogen) flow rate was 4 L/min; the gas temperature was 190°C, nebulizing pressure, 1.0 bar. A solution of the compound in methanol (5 μL) was injected to the nebulizer of the mass spectrometer. The observed relative intensities of the isotopic ions and *m/z* values correlated well with the calculated values for the expected ions.

The atom numeration serves for the assignment of resonances in the NMR spectra and does not correlate with the numeration according to the chemical nomenclature. The solvents were purified using standard protocols. Chloroform for column chromatography was washed with aqueous ammonia and dried with calcined molecular sieves. The synthesis and spectral characteristics of compounds (**VII**)–(**XI**) were described in [18].

The purity of the active substance in the samples used in biological tests was at least 98%.

Analytical and spectral tests were performed in the Chemical Service Center for Joint Use, Siberian Branch of Russian Academy of Sciences.

(*E*)-N^1,N^1-Dimethyl-N^2-((1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yliden)ethan-1,2-diamine (XIV). N^1,N^1-Diethylethan-1,2-diamine (5.8 g, 66 mmol) and $BF_3 \cdot Et_2O$ (3.9 mmol) in 3 mL toluene was added to a solution of (1*R*)-(+)- camphor (10 g, 66 mmol) in toluene (80 mL) and the reaction mixture was refluxed for 15 h with a Dean–Stark adaptor. Brine (10 mL) was added, extracted with methylene chloride, dried with Na_2SO_4, and the solvent was evaporated. The residue was distilled in vacuum to give compound (**XIV**) (9.8 g, 67%); bp 98°C (5 mm Hg). ^{1}H NMR: 0.57 (3H, s, Me-9), 0.74 (3H, s, Me-10), 0.77 (3H, s, Me-8), 1.02 (1H, ddd, ^2J = 12.3, $J_{4endo,\ 5endo}$ = 9.3, $J_{4endo,5exo}$ = 4.2, $H4_{endo}$), 1.16 (1H, ddd, ^2J = 12.3, $J_{5endo,\ 4endo}$ = 9.3, $J_{5endo,\ 4\ exo}$ = 4.5, $H5_{endo}$), 1.47 (1H, ddd, $^2J = J_{5exo,\ 4exo}$ = 12.3, $J_{5exo,\ 4endo}$ = 4.2, $H5_{exo}$), 1.67 (1H, d, ^2J = 16.9, $H2_{exo}$), 1.67 (1H, dddd, $^2J = J_{4exo,\ 5exo}$ = 12.3, $J_{4exo,\ 5endo} = J_{4exo,\ 3}$ = 4.5, $J_{4exo,\ 2exo}$ = 3.2, $H4_{exo}$), 1.78 (1H, dd, $J_{3,\ 2exo} = J_{3,\ 4exo}$ = 4.5, H3), 2.10 (6H, s, Me-13 and Me-14), 2.18 (1H, ddd, ^2J = 16.9, $J_{2exo,\ 3}$ = 4.5, $J_{2exo,\ 4exo}$ = 3.2, $H2_{exo}$), 2.35 (2H, t, $J_{12,\ 11}$ = 7.6, H12), 3.14 and 3.19 (two 1H, dt, ^2J = 12.1, $J_{11,\ 12}$ = 7.6, H11). ^{13}C NMR: 182.18 s (C1), 59.56 t (C12), 53.11 s (C6), 50.60 t (C11), 46.55 s (C7), 45.51 q (Me-13 and Me-14), 43.40 d (C3), 35.10 t (C2), 31.80 t (C5), 27.07 t (C4), 19.14 q (Me-9), 18.58 q (Me-10), 11.01 q (Me-8). $[\alpha]_D^{22}$ −19.8 ($CHCl_3$, *c* 1.1). Found: *m/z* 222.2092 $[M]^+$ $C_{14}H_{26}N_2$. Calc.: *M* 222.2091.

(*R*,*R*,*E*)-*N*,*N*'-(1,4-Phenylenbis(methylene))bis-(*N*,*N*-dimethyl-2-((*E*)-((1*R*,4*R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yliden)amino)ethanaminium)dibromide (XV). *p*-Xylylene dibromide (0.26 g, 1 mmol) was added to a solution of compound (**XIV**) (0.5 g, 2 mmol) in dry acetonitrile (10 mL), and the mixture was refluxed for 20 h. The precipitate was filtered off and loaded onto silica gel column (5 g) eluting with chloroform–ethyl acetate + 1% methanol (100 : 0 → 0 : 100) to give compound (**XV**) (0.5 g, 35%); mp 207–210°C. In the spectra recorded, the substitution with deuterium in position 2-exo achieved 95%. The resonances of the deuterium-substituted compound differed from the unsubstituted are shown with asterix. ^{1}H NMR: 0.80 (6H, s, Me-9), 0.96 (6H, s, Me-8), 0.98 (6H, s, Me-10), 1.28–1.40 (4H, m, $H4_{endo}$, $H5_{endo}$), 1.71–1.79 (2H, m, $H5_{exo}$), 1.88–1.95 (2H, m, $H4_{exo}$), 1.98–2.05 (4H, m, $H2_{endo}$ and H3), 2.01 (2H, br s, $H2_{endo}$*), 2.02.02 (2H, d, $J_{3,\ 4exo}$ = 4.5, H3*), 2.53 (2H, ddd, ^2J = 16.9, $J_{2exo,\ 3}$ = 4.5, $J_{2exo,\ 4exo}$ = 3.2, $H2_{exo}$), 3.21 and 3.21 (6H for each, s, Me-13 and

Me-14), 3.66–3.72 (4H, m, H12), 3.72–3.83 (4H, m, H11), 4.83 (4H, s, H15), 7.84 (4H, s, H17 and H18). ^{13}C NMR: 188.59 s (C1), 135.12 d (C17 and C18), 131.71 s (C16), 68.95 t (C15), 65.20 t (C12), 55.41 t (C6), 51.48 q (C13 and C14), 48.50 s (C7), 47.18 t (C11), 45.30 d (C3), 45.21 d (C3*), 36.95 t (C2), 36.67 dt ($^1J_{C, D}$ = 19.7, CHD-2*), 33.09 t (C5), 28.04 t (C4), 28.01 t (C4*), 19.99 q (C9), 19.26 q (C10), 11.72 q (C8). (ESI): *m/z* $[M - Br^-]^+$ 627.394, (calculated for $C_{36}H_{60}N_4Br$) 627.400, $[M - 2Br^-]^{2+}$ 274.239, (calculated for $C_{36}H_{60}N_4$) 274.240.

(*R,R,E*)-*N,N*'-(1,3-Phenylenbis(methylene))bis-(*N,N*-dimethyl-2-((*E*)-((*1R,4R*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yliden)amino)ethanaminium) dibromide (XVI). *m*-Xylylene dibromide (0.26 g, 1 mmol) was added to a solution of compound (**XIV**) (0.5 g, 2 mmol) in dry acetonitrile, and the mixture was refluxed for 30 h. Compound (**XVI**) (0.27 g, 20%) was isolated as described above for compound (**XV**); mp 133–136°C. ^{1}H NMR: 0.77 (6H, s, Me-9), 0.93 (6H, s, Me-8), 0.95 (6H, s, Me-10), 1.25–1.37 (4H, m, $H4_{endo}$, $H5_{endo}$), 1.66–1.78 (2H, m, $H5_{exo}$), 1.83–1.93 (2H, m, $H4_{exo}$), 1.95–2.05 (4H, m, $H2_{endo}$ and H3), 2.51 (2H, ddd, ^2J = 16.9, $J_{2exo, 3}$ = 4.5, $J_{2exo, 4exo}$ = 3.2, $H2_{exo}$), 3.17 (6H, s, Me-13 and Me-14), 3.62–3.68 (4H, m, H12), 3.70–3.82 (4H, m, H11), 4.80 (4H, s, H15), 7.66–7.76 (1H, m, H18), 7.87–7.95 (2H, m, H17), 8.08 (1H, s, H19). ^{13}C NMR: 187.1 s (C1), 137.6 d (C17, 17'), 135.08 d (C18, 18'), 129.5 d (C19,19'), 128.6 s (C16, 16'), 67.4 t (C15, 15'), 63.7 t (C12), 53.8 s (C6), 49.8 q (C13 and C14), 45.50 s (C7), 47.18 t (C11), 43.6 d (C3), 35.5 t (C2), 31.5 t (C5), 29.04 t (C4), 18.5 q (C9, 9'), 17.8 q (C10, 10'), 10.2 q (C8, 8'). (ESI): *m/z* $[M - Br^-]^+$ 627.396, (calculated for $C_{36}H_{60}N_4Br$) 627.400, $[M - 2Br^-]^{2+}$ 274.239, (calculated for $C_{36}H_{60}N_4$) 274.240.

1,1'-(1,4-Phenylene)bis(*N,N,N*-trimethylmethaneaminium) dibromide (XVII). 40% Trimethylamine (1 mL) was added to a solution of *p*-xylylene dibromide (0.5 g, 2 mmol) in methanol and the mixture was refluxed for 15 h. The precipitate was filtered off and dried in air to give compound (**XVII**) (0.32 g, 23%); mp >330°C. ^{1}H NMR: 3.04 (18H, s, Me-1, 2, 3, 1', 2', 3'), 4.48 (4H, s, H4, H4'), 7.62 (4H, s, H6, 7, 6', 7'). ^{13}C NMR: 133.10 d (C6, 7, 6', 7'), 129,5 s (C5, 5'), 68,4 t (C4, 4'), 52,3 q (C1, 2, 3, 1', 2', 3'). (ESI): *m/z* $[M - Br^-]^+$ 301.127, (calculated for $C_{14}H_{26}N_2Br$) 301.127.

***N,N*'-(1,4-Phenylenebis(methylene))bis(*N,N*-diethylethaneaminium) dibromide (XVIII).** Trimethylamine (0.8 mL, 6 mmol) was added to a solution of *p*-xylylene dibromide (0.5 g, 2 mmol) in dry acetonitrile and the mixture was refluxed for 8 h. The precipitate was filtered off and dried in air to give compound (**XVIII**) (0.71 g, 40%); mp 270°C. ^{1}H NMR: 1.43 (18H, t, *J* = 7.06, Me-4, 5, 6, 4', 5', 6'), 3.32 (12H, t, *J* = 7.2, H1, 2, 3, 1', 2', 3'), 4.55 (4H, s, 2H7, 2H7'), 7.70 (4H, s, H9, 10, 9', 10'). ^{13}C NMR: 133.07 d (C9, 10, 9', 10'), 129,7 s (C8, 8'), 58,9 t (C7, 7'), 52,3 t (C1, 2, 3, 1', 2', 3'), 6.6 q (C-4, 5, 6, 4', 5', 6'). (ESI): *m/z* $[M - Br^-]^+$ 385.219, (calculated for $C_{20}H_{38}N_2Br$) 385.221.

1,1'-(1,4-Phenylene)bis(*N,N,N*-trimethylmethaneaminium) diiodide (XIX). 40% Dimethylamine (0.5 mL) and K_2CO_3 (1 g) were added to a solution of *p*-xylylene dibromide (0.5 g, 2 mmol) in acetonitrile and the mixture was refluxed for 10 h. 25% NaOH was added, the mixture was extracted with diethyl ester (3 × 20 mL), the organic layer was dried with Na_2SO_4, and the solvent was evaporated. Methyl iodide (30 mmol) was added to a resulting amine solution in acetonitrile, and the mixture was kept for 4 days at room temperature. The precipitate was filtered off and dried in air to give compound (**XIX**) (0.5 g, 50%), mp 281°C. ^{1}H NMR: 3.14 (18H, s, Me-1, 2, 3, 1', 2', 3'), 4.58 (4H, s, 2H4, 2H4'), 7.71 (4H, s, H6, 7, 6', 7'). ^{13}C NMR: 135.7 d (C9, 10, 9', 10'), 132.1 s (C8, 8'), 71.0 t (C4, 4'), 54.8 q (C1, 2, 3, 1', 2', 3'). (ESI): *m/z* $[M - I^-]^+$ 349.112, (calculated for $C_{14}H_{26}N_2I$) 349.114, $[M - 2I^-]^{2+}$ 111.102 (calculated for $C_{14}H_{26}N_2$) 111.104.

1,1'-(1,3-Phenylene)bis(*N,N,N*-trimethylmethaneaminium) dibromide (XX). 40% Trimethylamine (1 mL) was added to a solution of *m*-xylylene dibromide (0.5 g, 2 mmol) in methanol and the mixture was refluxed for 20 h. The precipitate was filtered off and dried in air to give compound (**XX**) (0.26 g, 35%), mp 95°C. ^{1}H NMR: 3.18 (18H, s, Me-1, 2, 3, 1', 2', 3'), 4.68 (4H, s, 2H4, 2H4'), 7.61–8.04 (4H, m, H6, 7, 8, 6'). ^{13}C NMR: 136.7 d, 135.0 d, 130.0 d, 128.3 s, 68.7 t, 52.4 q. (ESI): *m/z* $[M - Br^-]^+$ 301.126, (calculated for $C_{14}H_{26}N_2Br$) 301.127, $[M - 2Br^-]^{2+}$ 111.102 (calculated for $C_{14}H_{26}N_2$) 111.104.

***N,N*'-(1,3-Phenylene bis(methylene))bis(*N,N*-diethylethaneaminium) dibromide (XXI).** Triethylamine (0.8 mL, 6 mmol) was added to a solution of *m*-xylylene dibromide (0.5 g, 2 mmol) in dry acetonitrile and the mixture was refluxed for 12 h. The precipitate was filtered off and dried in air to give compound (**XXI**) (0.5 g, 50%).^{1}H NMR: 1.51 (18H, t, Me-4, 5, 6, 4', 5', 6'), 3.39 (12H, q, H1, 2, 3, 1', 2', 3'), 4.68 (4H, s, H7, 7'), 7.69–7.85 (4H, m, H9, 9', 10, 11). ^{13}C NMR:136.35 d, 134.4 d, 134.39 d, 128.5 s, 59.2 t, 52.4 t, 6.8 q. (ESI): *m/z* $[M - Br^-]^+$ 385.222, (calculated for $C_{20}H_{38}N_2Br$) 385.221, $[M - 2Br^-]^{2+}$ 153.152 (calculated for $C_{20}H_{38}N_2$) 153.151.

1,1'-(1,3-Phenylene)bis(*N,N,N*-trimethylmethaneaminium) diiodide (XXII). 40% Dimethylamine (0.5 mL) was added to a solution of *m*-xylylene dibromide (0.5 g, 2 mmol) in acetonitrile and the mixture was refluxed for 10 h in the presence of K_2CO_3 (1 g). The mixture was treated with 25% NaOH and extracted with diethyl ester (3 × 20 mL). The organic layer was dried, and the solvent was evaporated. The precipitate was filtered off, the resulting tertiary amine (0.4 g) was dissolved in dry acetonitrile, and methyl iodide (20 mmol) was added. The mixture was kept for 2 days at room temperature. The precipitate was fil-

tered off and dried in air to give the target compound (**XXII**) (0.6 g, 60%), mp 215°C. ^{1}H NMR: 3.15 (18H, s, Me-1, 2, 3, 1', 2', 3'), 4.60 (4H, s, H4,4'), 7.68–7.82 (4H, m, H6, 7, 6', 8). ^{13}C NMR: 136.7 d, 135.0 d, 130.0 d, 128.3 s, 68.7 t, 52.4 q. (ESI): m/z $[M - I^-]^+$ 349.111, (calculated for $C_{14}H_{26}N_2I$) 349.114.

Biology

Primary experiments on the evaluation of the biological activity of the compounds tested were performed on male outbred mice 25–30 g in weight. The compounds were administered as aqueous solutions at doses 5 to 20 mg/kg. Median lethal doses, LD_{50}, were taken as toxicity indices (experimental time of 72 h); the inability of animals to remain on the rotating rod and inclined plate, as indices of the myorelaxant activity [19]. Each group had 6 to 8 animals. Control animals were intra-abdominally administered physiological solution, 0.1 mL/10 g body weight.

The rota-rod test in various modifications is widely used for both studies of animal neuroblocking activity and violation of coordination of movements [20, 21]. The mice were sorted out, which could remain on the rotating rod for at least 1 min. In 2, 12, and 22 min after the administration of the tested compound or physiological solution the mice were placed for 120 s on a horizontal rod rotating at a rate of 6.6 rpm. The latency of the first fall from the rod was recorded.

The "inclined plate" test in various modifications is used for the evaluation of muscular relaxation and ataxia in mice and rats [22–24]. During 30 min after the compound injection, the animals were placed several times on a close-meshed plate located at an angle of 60° relative to a horizontal surface. The inability of animals to remain on the plate was a criterion of the neuromuscular block. In each group the percent of animals with the neuromuscular block was calculated.

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Translated by E. Shirokova