The antimutagenic action of the compounds was examined on the same specimens and by the same methods. The effect of the substances was tested on spontaneous mutation in the bacteria and on UV-induced mutations [7, 8].

Our results for the mutagenic activity of the compounds, summarized in Table 3, reveal that, when tested in high equimolar concentrations — 100 mmole (*E. coli*) and 200 mmole (*Act. rimosus*) — the amino acid derivatives generally had weak mutagenic activity on prolonged treatment of the specimens (120 min), except for some compounds that, depending on their structure, had either appreciable (IIa) or fairly marked mutagenic activity (IIj, k, *l*). In the majority of cases the test substances had a slight lethal effect; *E. coli* was more sensitive to the action of the compounds. Urethane, which as the starting substance, was examined under the same conditions, had a very weak mutagenic effect. Examination of the antimutagenic effect of IIIa, which has no mutagenic activity, revealed that it had a protective effect in small doses (25 mmole, 10 min treatment): it reduced the spontaneous mutation in *E. coli* by 38% on average and increased the survival rate of these cells by 49%. It also reduced the number of UV-induced mutations in the test cultures, by 55% on average, and protected 31% of the UV-irradiated bacterial cells from death.

Thus our results show that compounds that have biological activity to some extent can be found in each of the areas examined here. Consequently the search for biologically active agents among amino acid derivatives of sulfanilic acid is of interest, and it should be carried out on a large scale and in several areas.

LITERATURE CITED

1. N. M. Divanyan, S. A. Kazaryan, L. Kh. Galstyan, et al., Khim.-farm. Zh., No. 9, 45 (1978).
2. M. V. Rubtsov and A. G. Baichikov, Synthetic Preparations in Pharmaceutical Chemistry [in Russian], Moscow (1971), pp. 115-150.
3. J. Greenstein and M. Winitz, Chemistry of the Amino Acids, Wiley (1961).
4. R. Lender, Clin. Chim. Acta, 8, 351 (1963).
5. V. A. Chernov, in: Methods of Experimental Chemotherapy [in Russian], Moscow (1971), p. 357.
6. G. M. Paronikyan, L. G. Akopyan, G. A. Darbinyan, et al., Genetika, 13, No. 9, 1621 (1977).
7. G. M. Paronikyan, L. G. Akopyan, et al., Biol. Zh. Arm., 32, No. 11, 1146 (1979).
8. J. Miller, Experiments in Molecular Genetics, Cold Spring Harbor (1972).

SEARCHES FOR NONDEPOLARIZING SHORT-ACTION MYORELAXANTS

N. V. Martyushina, G. G. Solomatin,
D. Yu. Fedorov, G. M. Kheifets,
N. V. Khromov-Borisov, S. A. Shelkovnikov,
and V. A. Shigalevskii

UDC 615.216.5.07

Modern anesthesiology requires the creation of a rapid-acting myorelaxant, the effect of which would last for no more than 10 min. Such preparations may include ditilin and diadonium, but ditilin is a depolarizing myorelaxant [1], while diadonium is a nondepolarizing myorelaxant, but its activity and selectivity are low [2]. The short duration of the action of these preparations is associated with hydrolysis of the ester group by pseudocholinesterase [3]. Attempts to create short-action myorelaxants in the series of tetrazenes RN=NR (I) and (II) have not met with success.

Institute of Experimental Medicine, Academy of Medical Sciences of the USSR. I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. Rubezhnoe Branch of the Scientific-Research Institute of Organic Intermediates and Dyes. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 16, No. 7, pp. 801-806, July, 1982. Original article submitted June 23, 1981.

 0091-150X/82/1607-0524$07.50

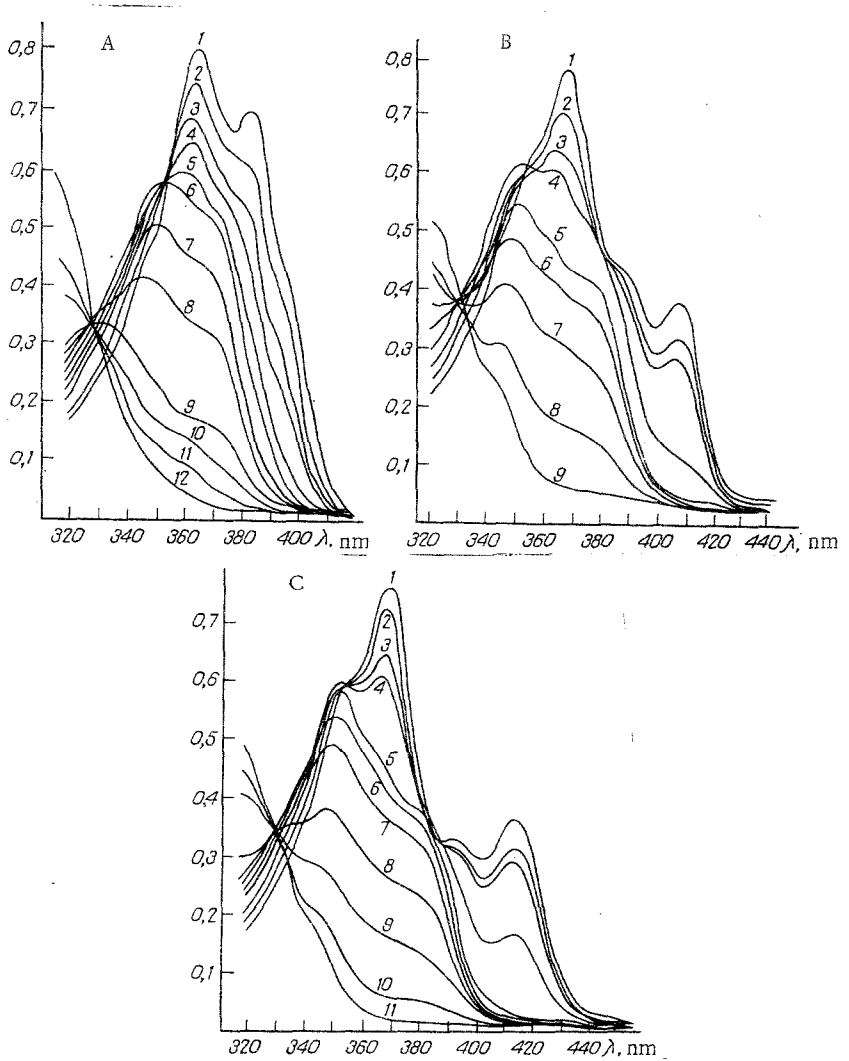
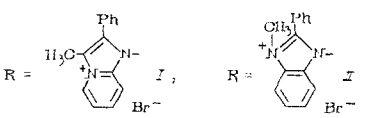


Fig. 1. UV spectra of diimides IV (A), V (B), and VI (C) 24 h after incubation in phosphate buffer solutions at 37°C. Numbers of curves (pH): A — 1 (3, 0), 2 (5, 5), 3 (5, 8), 4 (6, 3), 5 (6, 8), 6 (7, 25), 7 (8, 3), 8 (8, 4), 9 (8, 75, 10 (9, 10), 11 (10, 0) 12 (10, 7); B — 1 (3.0—4.0), 2 (5, 0), 3 (5, 8), 4 (6, 0), 5 (6, 8), 6 (7, 1), 7 (7, 54), 8 (8, 3), 9 (10, 85); C — 1 (4, 0), 2 (5, 3), 3 (5, 5), 4 (6, 01), 5 (6, 6), 6 (6, 8), 7 (7, 05), 8 (7, 54), 9 (8, 0), 10 (8, 75), 11 (10, 85).



Preparation I with duration of the myoparalytic effect 2-3 min in experiments on cats gave a value of the block of 36-37 min in clinical testing [4], which is evidently explained by the difference in the enzymatic activity of azoreductase of the microsomal cells of the liver. Preparation II is decomposed too rapidly under the action of blood plasma bicarbonate ions (lifetime 1-1.5 min) [5] and therefore has not achieved wide practical use.

The creation of a myorelaxant decomposed not enzymatically but under the action of ions of the blood plasma and intercellular fluid presents definite advantages, since inactivation occurs under the action of more stable factors, not associated with species and individual differences.

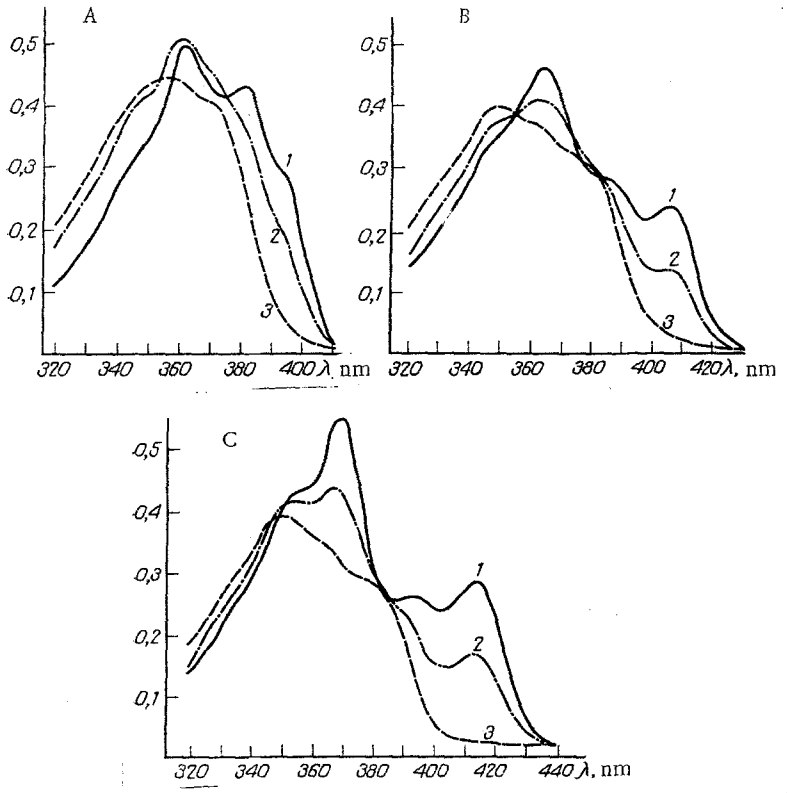
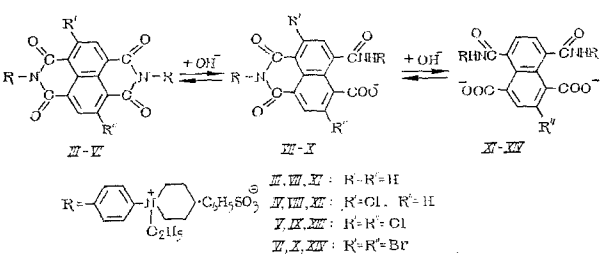


Fig. 2. UV spectra of diimides IV (A), V (B), and VI (C) in water at pH 3.0 after 24 h incubation at 37°C in phosphate buffer solutions and potentiometric titration. 1) Initial spectrum; 2) after addition of 1/2 equivalent of alkali or incubation at pH 6.8 (IV), 5.8 (V), and 6.01 (VI); 3) after addition of an equivalent of alkali or incubation at pH 7.5 (IV), 6.8 (V), and 6.8 (VI).

Among the nondepolarizing myorelaxants, such an ability is possessed by ritetronium (III) — a derivative of the diimide of naphthalene-1,4,5,8-tetracarboxylic acid (NTCA) [6]. It was found that this myorelaxant is hydrolyzed at the imide bond in physiological media (37°C, pH 7.4) to a relatively inactive imidoamide (VII) under the action of hydroxyl, phosphate, and bicarbonate ions with a half-conversion time 20 min [6].



We suggested that with all other conditions equal, by decreasing the lifetime of the myorelaxant, we can decrease the time of the myoparalytic block. For this purpose we synthesized three analogs of ritetronium — compounds IV-VI. Fearing to lose myorelaxant activity in the attempt to increase the lability of the imide bond, we attempted to introduce the least changes into the structure of the molecule; therefore activating groups — halogen atoms — were introduced into the naphthalene ring. Compounds IV-IV were produced by condensation of the corresponding dianhydrides of NTCA with N-p-aminophenylpiperidine, followed by alkylation with the ethyl ester of benzenesulfonic acid (EBS).

TABLE 1. Half-Conversion Times of Diimides of NTCA in 0.05 M Phosphate Buffer Solutions at 37°C

Diimides	Half-conversion time, min	
	pH 7,4	pH 6,0
III	20	500
IV	13	320
V	7	170
VI	9	200

The investigation showed that haloderivatives, like ritetronium itself, are hydrolyzed to the corresponding imidoamides (VIII-X) and diamides (XII-XIV) (on the scheme the compounds are represented by one of the possible structural isomers) or a mixture of them, depending on the pH. Within the range of pH 5.0-10.0, hydrolysis is at least 80-90% reversible. From an analysis of the UV spectra (Fig. 1) it follows that at pH 7.4 in phosphate buffer after incubation at 37°C for 24 h, compound IV is entirely converted to the corresponding imidoamide, while compounds V and IV represent a mixture of the imidoamide and diamide in a 3:2 ratio, respectively. Under analogous conditions ritetronium is converted to a mixture of the diamide XI and the imidoamide VII in a 1:7 ratio [6]. Just as in the case of ritetronium, the conversion of the diimides IV-VI to the corresponding imidoamides is accompanied by consumption of an equivalent of alkali (potentiometric titration at 37°C). The composition of the solutions at various pH values according to the data of UV spectra coincided with the composition calculated according to the data of potentiometric titration (Fig. 2).

From Table 1, which presents the half-conversion times of the diimides III-VI in phosphate mixtures, it can be seen that the lability of the imide bond increases even when one chlorine atom is introduced. The large difference in the rates of hydrolysis at pH 6.0 and 7.4 is due to a difference in the concentrations not only of hydroxyl but also of phosphate ions (HPO_4^{2-}) [6].

In experiments on an isolated rat phrenico-diaphragm preparation, haloderivates of ritetronium, like the preparation itself, produced a nondepolarizing-type reversible block of myoneural conduction. The activity of these compounds and the corresponding imidoamides is presented in Table 2.

The myoparalytic activity (EC_{50}) of compound III was determined under conditions of III-VII equilibrium [6] and calculated from the ratio I/EC_{50} (sum) = $A_{III}/EC_{50}III + A_{VII}/EC_{50}VII$, where $EC_{50}III$, $EC_{50}VII$, and EC_{50} (sum) are the molar concentrations of the diimide III, the imidoamide VII, and their sum, respectively; A_{III} and A_{VII} are the fractions of the diimide III and the imidoamide VII.

For compounds IV-VI the activities were determined at pH 6.0, when the rate of conversion of the diimide to the imidoamide and back can be neglected (see Table 1).

From Table 2 it is evident that the introduction of chlorine atoms into the 2- and 6-positions of the naphthalene ring of compound III practically does not decrease the myoparalytic activity, while the introduction of a bromine atom produces little decrease in it. In this case the duration of the myoparalytic block in haloderivatives is approximately the same and is half the time of ritetronium block or less. The activity of their hydrolysis products (imidoamides) is 5-10 times lower than the activity of the investigated diimides IV-VI, respectively.

Thus, the initial premise is evidently correct: with increasing lability of the imide bond (see Table 1), the time of the myoparalytic block decreased while the ratio of the activities of the corresponding diimides and their hydrolysis products was preserved. However, although there is a difference in the specific rates of hydrolysis in phosphate buffer solutions for haloderivatives, this difference was equalized in a pharmacological experiment. The times of myoparalytic block obtained — 7-8 min (ED_{50} for cats) are still large and to some degree commensurate with the value of the block induced by a number of myorelaxants of medium duration of action, incapable of inactivation. However, it can be assumed that for structures of the ritetronium type, this time barrier will be overcome if the lability of the imide bond can be increased to an even greater degree with all other conditions equal.

TABLE 2. Effective Concentrations and Doses Blocking Contraction of an Isolated Rat Diaphragm (ED_{50}) of Ritetronium and Its Haloderivatives and Products of Their Hydrolysis; Time of Myoparalytic Action

Compound	Rat diaphragm, EC_{50} · 10^5 M	Cat muscle ED_{50}, μM /kg	t, min
III diimide	1,7±0,3 (5)	0,17±0,01 (5)	18±2
VII imidoamide	18±3	1,8±0,2 (6)	—
IV diimide	1,1±0,2 (4)	0,27±0,07 (5)	7±0,6
VII imidoamide	10±2	—	—
V diimide	3,1±0,4 (6)	0,2±0,03 (3)	7±0,5
IX imidoamide	30±5	—	—
VI diimide	6,0±0,5 (6)	0,3±0,06 (5)	8±1
X imidoamide	30±4	—	—

Note. The number of experiments is indicated in parentheses; t is the time from introduction of the preparation up to complete restoration of muscle contractions.

EXPERIMENTAL CHEMICAL PART

The PMR spectra were measured on an EM-360 spectrometer in H_2O and DMSO-d_6; internal standard: tertiary butyl alcohol. The UV spectra were recorded on an SF-8 spectrophotometer. An SF-16 spectrophotometer was used for the kinetic measurements. The IR spectra in liquid petrolatum were recorded on an IKS-24 instrument. Potentiometric measurements were performed with a pH-340 pH meter in a thermostatically controlled cell at 37°C. Within the interval pH 5.8-8.0 we used phosphate buffer mixtures, at pH more than 8.0 Na_2HPO_4–NaOH; at pH less than 5.0 we used acidified water. The ritetronium (III) used was analytically pure [7].

Haloderivatives of ritetronium IV-VI were produced by condensation of 2-chloro- [8], 2,6-dichloro- [9], and 2,6-dibromo- [10] substituted dianhydrides of NTCA with N-(p-aminophenyl)-piperidine and subsequent quaternization.

The N,N'-bis(4-piperidinophenyl)diimide of 2-chloro-1,4,5,8-NTCA (XV) was produced by heating the dianhydride of 2-chloro-NTCA with N-(p-aminophenyl)-piperidine in acetic acid medium for 2 h and purified by crystallization from nitrobenzene; yield 60%. Found, %: C 66.0; H 4.3; Cl 5.7; N 9.1. $C_{36}H_{31}ClN_4O_4$. Calculated, %: C 66.2; H 4.7; Cl 5.7; N 9.0. IR spectrum, $\nu_{C=O}$, cm^{-1}: 1715, 1678 sh, 1669.

The N,N'-bis(4-piperidinophenyl)diimide of 2,6-dichloro-1,4,5,8-NTCA (XVI) was produced and purified analogously with a 76% yield. Found, %: C 65.6; H 4.2; Cl 9.7; N 8.7. C_{36}· $H_{30}Cl_2N_4O_4$. Calculated, %: C 66.2; H 4.6; Cl 10.8; N 8.55. IR spectrum, $\nu_{C=O}$, cm^{-1}: 1719, 1672.

The N,N'-bis(4-piperidinophenyldiimide of 2,6-dibromo-1,4,5,8-NTCA (XVII) was produced and purified analogously with a yield of 74%. Found, %: C 57.8; H 4.0; Br 21.0; N 7.6. $C_{36}H_{30}Br_2N_4O_4$. Calculated, %: C 58.2; H 4.1; Br 21.5; N 7.55. IR spectrum, $\nu_{C=O}$, cm^{-1}: 1721, 1675.

Dibenzenesulfonate of N,N'-Bis[4-(ethylpiperidino)phenyl]-diimide of 2-Chloro-1,4,5,8-NTCA (IV). A 1 g portion of the base in 25 ml ethyl benzenesulfonate was heated with mixing for 1 h at 160°C. After cooling the precipitate formed was filtered off, washed with EBS and with acetone, and dried. Yield 1.47 g (95% of the theoretical). It was crystallized from a mixture of DMFA–acetone (10:1). Found, %: C 61.7; H 5.1; Cl 3.5; S 6.4. $C_{52}H_{51}ClN_4$· $O_{10}S_2$·H_2O. Calculated, %: C 61.75; H 5.15; Cl 3.6; S 6.45; PMR spectrum, δ, protons of the naphthalene ring 9.0, 875 ppm (2:1); IR spectrum, $\nu_{C=O}$, cm^{-1}: 1714, 1682 sh., 1677.

Dibenzenesulfonate of N,N'-bis[4-(ethylpiperidino)phenyl]-diimide of 2,6-Dichloro-1,4,5,8-NTCA (V). A 0.7 g portion of the base in 25 ml EBS is heated with mixing for 15 min at 180°C and exposed for 30 min at 160-170°C. The cooled mass is diluted with diethyl ether and filtered off. The precipitated product is dissolved in boiling absolute alcohol, boiled with charcoal, and filtered. A small quantity of an ether–acetone mixture is added to the warm filtrate and cooled. The precipitate formed is filtered off and dried. Yield 0.6 g (55% of theoretical). Found, %: C 60.1; H 4.8; Cl 6.1; S 6.2. $C_{52}H_{50}Cl_2N_4O_{10}S_2$. Calculated, %: C 60.7; H 4.9; Cl 6.9; S 6.2. PMR, δ, protons of the naphthalene ring 9.1 ppm. IR spectrum, $\nu_{C=O}$, cm^{-1}: 1717, 1677.

Dibenzenesulfonate of N,N'-Bis[4(ethylpiperidino)phenyl]-diimide of 2,6-Dibromo-1,4,5,8-NTCA (VI). A 0.7 g portion of the base in 25 ml EBS is heated with mixing for 20 min at 170°C until dissolution and for 40 min at 160°C. After cooling, the fine-crystalline precipitate formed is filtered off, washed with EBS and ether. Yield 0.96 g (92% of the theoretical). It is crystallized from alcohol. Found, %: C 55.9; H 4.6; S 5.6. $C_{52}H_{50}Br_2N_4O_{10}S_2$. Calculated, %: C 55.2; H 4.5; S 5.7. PMR, δ, protons of the naphthalene ring 8.9 ppm. IR spectrum, $\nu_{C=O}$, cm^{-1}: 1720, 1675.

Solutions of the imidoamides VII-X in concentrations of 10 and 50% were prepared by titrating the corresponding diimides at 37°C with an equivalent and a half-equivalent of an alkali, respectively; final concentration of substrates $2 \cdot 10^{-3}$ M. Alkali was added at a rate such that the pH value of the solution did not exceed the value in the pH region where the imidoamide exists, established spectrophotometrically. In all cases, after preparation the solutions were monitored with the UV spectra. Kinetic measurements in 0.05 M phosphate buffer solutions at 37 ± 0.1°C were performed spectrophotometrically according to the decrease in the absorption at 382 nm for the diimide III, 392 nm for the diimide IV, 407 nm for the diimide V, and 415 nm for the diimide VI. The measurement procedure and calculation of the data were described earlier [6].

EXPERIMENTAL PHARMACOLOGICAL PART

The myoparalytic action of ritetronium and its analogs was determined in experiments on cats according to the usual procedure and on an isolated rat phrenico-diaphragm preparation [11, 12]. The concentration of the myorelaxant blocking isometric contractions of the rat diaphragm induced by stimulation of the nerve by 50% (EC_{50}) were determined.

In experimental series I we determined the action of haloderivatives of ritetronium on the diaphragm muscle in Krebs solution, pH 7.4. Concentrated solutions of the substrates ($2 \cdot 10^{-3}$ M), containing 100% diimides, had pH 3.0. Inhibition of neuromuscular transmission, induced by these myorelaxants, was restored without washing out the preparations. Such a restoration of neuromuscular transmission was never observed in an investigation of nondegradable myorelaxants — tubocurarine, pancuronium, flaxedil, tercuronium.

In the second series of experiments, analogous solutions of diimides were investigated in Krebs solution pH 6.0. The muscle was washed free of the preparation with Krebs solution pH 7.4. Control experiments showed that the activity of tubocurarine and tercuronium at pH 6.0 was practically the same as at pH 7.4. The activity of the investigated ritetronium analogs at pH 6.0 practically did not differ from their activity at pH 7.4. However, although the effect of haloderivatives of ritetronium at pH 7.4 disappeared without washing out the preparation, no spontaneous restoration of interrupted neuromuscular transmission was observed at pH 6.0.

In the third experimental series, concentrated solutions contained 100% imidoamides and were tested at pH 6.0.

In the fourth series of experiments concentrated solutions represented mixtures of diimides and the corresponding imidoamides in a 1:1 ratio and were tested in Krebs solution pH 6.0. Just as we should have expected, the blocking concentration of the mixtures was an average of half as great as in the case of the corresponding diimides.

In experiments on cats the ability of myorelaxants to halve the evoked contractions of the tibialis anterior muscle (ED_{50}) after stimulation of the motor nerve by single supramaximal current pulses with a frequency of 0.5 Hz and a duration 0.1 msec (ESL-2 electrostimulator) was evaluated. The substances were injected intravenously in a volume of 0.2 ml. The solutions had pH 3.0.

LITERATURE CITED

1. R. D. Dripps, in: Neuromuscular Junction, E. Zaimis, ed., New York (1976), pp. 583-592; S. E. Smith, ibid., pp. 593-660; J. King and D. Griffin, Br. J. Anaesthesiol., 45, 450-454 (1973); M. O. Mkheidze, in: Materials of the Second All-Union Congress of Anesthiologists and Reanimatologists [in Russian], Tashkent (1977), pp. 469-472.
2. D. A. Kharkevich, Farmakol. Toksikol., No. 5, 531-536 (1970); D. A. Kharkevich, in: Advances in the Creation of New Drugs [in Russian], Moscow (1973), pp. 179-183.

3. V. P. Whittaker and S. Wijesundera, Biochem. J., 51, 475-479 (1952); V. P. Whittaker and S. Wijesundera, ibid., 348-351; O. T. Fraser, Br. J. Pharmacol., 9, 429 (1954); G. Levy, J. Pharm. Sci., 56, 1687-1688 (1967); G. Levy, Anesthesiology, 32, 551-552 (1970).
4. R. T. Brittain and M. B. Tyers, Br. J. Pharmacol., 45, 158 (1972); C. E. Blogg, T. M. Savege, G. C. Simpson, et al., Proc. R. Soc. Med., 66, 1023-1026; A. J. Coleman, et al., Anaesthesia, 28, 262-267 (1973); Y. Hashimoto, T. Shima, S. Matsukowa, et al., ibid., 34, 10-13 (1979).
5. C. E. Blogg, R. T. Brittain, B. R. Simpson, et al., Brit. J. Pharmacol., 53, 446 (1975); R. T. Brittain, D. Gack, and M. B. Tyers, ibid., 61, 47-55 (1977).
6. N. V. Martyushina, G. M. Kheifets, N. V. Khromov-Borisov, et al., Khim.-farm. Zh., No. 8, 15-20 (1980).
7. G. M. Kheifets, N. V. Martyushina, T. A. Mikhailova, et al., Zh. Org. Khim., 13, 1262-1269 (1977).
8. Yu. E. Gerasimenko, V. A. Shigalevskii, E. F. Bondarenko, et al., Zh. Org. Khim., 8, 623-626 (1972).
9. H. Vollman, H. Becker, M. Corell, et al., Justus Liebigs Ann. Chem., 531, 1-159 (1937).
10. G. G. Solomatin, M. P. Filippov, and V. A. Shigalevskii, Zh. Org. Khim., 17, 877-879 (1981).
11. Pharmacological Experiments on Isolated Preparations, Edinburgh (1970), pp. 30-37.
12. Pharmacological Experiments on Intact Preparations, Edinburgh (1970), pp. 37-48.

ORGANOPHOSPHORUS DERIVATIVES OF 2-AMINO-1,4-NAPHTHOQUINONE CHROMOPHORE, A HYDROLYSIS PRODUCT OF RIFAMYCIN S ANTIBIOTIC

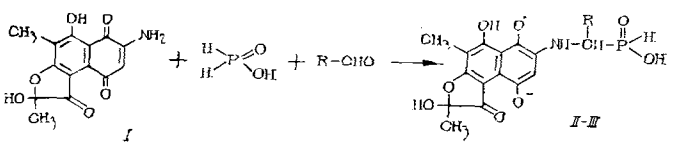
V. I. Yudelevich, A. P. Fetter, L. B. Sokolov, V. I. Ionin, L. G. Myasnikova, E. V. Komarov, G. G. Ivanov, M. I. Lifshits, and L. M. Elagin*

UDC 615.332:547.241].012.1

Known organophosphorus antibiotics of both natural origin (phosphomycin [1], macarbomycin [2], diumycin [3], etc.) and semisynthetic origin (derivatives of penicillin [4], lincomycin [5], aspergin [6], etc.) were obtained by one of the most productive methods for obtaining medicinal preparations, the chemical modification of biosynthetic products [7].

We used this method in the synthesis of new organophosphorus derivatives based on 2-amino-1,4-naphthoquinone chromophore (I), a known product of the hydrolysis of the rifamycin S antibiotic [8].

We found that in the reaction of I with hypophosphorus acid and aldehydes, N-substituted 1-aminoaryl-(heteryl)methanephosphonous acids are formed with an unpaired electron at the oxygen atom in the 1-position of the aromatic ring, as confirmed by EPR spectra (the g-factor for acid II is 2.0049, and for III 2.0052).



II: R = NO_2-(furan)-CH=CH-; III: R = 4-BrC_6H_4-

The PMR spectra of the modified antibiotic contain not only the signals characteristic of the hydrolysis products of rifamycin S, but also two doublets with different spin–spin coupling constants (J_{PH} 547 Hz for acid II, 552 Hz for III); J_{PCH} 12 Hz for II, 12 Hz for

*Deceased.

All-Union Scientific-Research Technological Institute of Antibiotics and Enzymes for Medical Applications, Leningrad. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 16, No. 7, pp. 806-808, July, 1982. Original article submitted January 8, 1982.

 0091-150X/82/1607-0530$07.50