adrenoprolonging and antialternative properties of halogen preparations and natural compounds.

Thus, in certain phenolcarboxylic acids, gastroprotective properties have been established, as well as the ability to increase the resistance of stomach tissues to alteration. Especially active are IX and X, which is combined with their adrenoprolonging properties and can be explained by the presence of hydroxyl groups in the o-position to one another.

LITERATURE CITED

1. O. D. Barnaulov, O. A. Manicheva, and P. P. Denisenko, Farmakol. Toksikol., No. 4, 105-110 (1982).
2. O. D. Barnaulov, O. A. Manicheva, and G. G. Zapesochnaya, Khim.-farm. Zh., No. 3, 300-303 (1982).
3. O. D. Barnaulov, O. A. Manicheva, and N. F. Komissarenko, Khim.-farm. Zh., No. 8, 946-951 (1983).
4. O. D. Barnaulov, O. A. Manicheva, V. L. Shelyuto, et al., Khim.-farm. Zh., No. 8, 935-941 (1984).
5. O. D. Barnaulov, O. A. Manicheva, I. I. Chemesova, et al., Khim.-farm. Zh., No. 11, 1330-1333.
6. O. D. Barnaulov, O. A. Manicheva, R. K. Yasinov, et al., Rast. Resur., 21, No. 1, 85-90 (1985).
7. O. B. Maksimov, N. M. Rebachuk, and L. V. Boguslavskaya, Rast. Resur., No. 2, 216-220.
8. O. A. Manicheva and O. D. Barnaulov, The Use of Models of Pathological States in the Search for Biologically Active Preparations [in Russian], Part 1, Moscow (1983), pp. 102-103.
9. O. A. Manicheva, "The search for an experimental study of the pharmacological properties for the treatment of ulcer disease," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences, Leningrad (1984).
10. F. De Eds, The Pharmacology of Plant Phenolics, New York (1959), pp. 91-102.
11. K. Herrman, Fette, Seifen, Anstrichmittel, 75, 499-504 (1973).

SYNTHESIS AND ANTIBACTERIAL PROPERTIES OF AMINO ACID DERIVATIVES OF OXOLINIC ACID

T. S. Leonova, E. N. Nadeyskaya, and V. G. Yashunskii

UDC 615.281.541.831

We synthesized a number of amino acid derivatives of oxolinic acid (5-ethyl-8-oxo-5,8-dihydro-1,3-dioxolo [4,5-g]quinoline-7-carboxylic acid, I), which is an effective antibacterial agent, in order to make it hydrophilic.

Japanese investigators [3] recently described the synthesis of 1-carboxyethylamide 5-ethyl-8-oxo-5,8-dihydro-1,3-dioxolo[4,5-g]quinoline-7-carboxylic acid (II) by the activated esters method. However, the resultant yield of the substance was very low.

In order to work out a method to synthesize amino acid derivatives of I via the carboxyl group, we used a method of activating the amino group in amino acid by silylation. We obtained compounds II, IV-IX by reacting 5-ethyl-8-oxo-5,8-dihydro-1,3-dioxolo[4,5-g]quinoline-7-carboxylic acid chloroanhydride (III) with N,O-bistrimethylsilyl substituted glycine, α and β-alanine, γ-aminobutyric, and ε-aminocaproic acids.

Compound VIII was synthesized by using a method which employed the simultaneous introduction of amino acid, the silylating agent [$(CH_3)_3SiCl$], III, and triethylamine. This obviated the necessity of separating bistrimethylsilyl-γ-aminobutyric acid which easily cycles into 2-pyrrolidone [4].

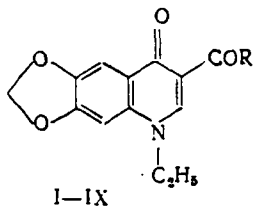
Institute of Biophysics, Ministry of Health of the USSR. S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow.

Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 21, No. 6, pp. 692-696, June, 1987. Original article submitted January 21, 1986.

0091-150X/87/2106-0430$12.50

TABLE 1. Properties of Compound I Derivatives

Compound	Yield, %	mp, °C (solvent)	Found, %			Empirical formula	Calculated, %		
			C	H	N		C	H	N
II	52	250—2(Methanol)	57,7	4,81	8,4	$C_{16}H_{16}N_6O_6$	57,8	4,8	8,4
IV	60	295—7 (DMFA)	56,1	4,4	9,0	$C_{15}H_{14}N_2O_6$	56,5	4,4	8,9
V	46	210—11 (Isopropanol)	59,3	5,3	8,1	$C_{17}H_{18}N_2O_6$	59,0	5,2	8,1
VI	70	287—8 (Acetic acid)	56,1	4,8	8,1	$C_{16}H_{16}N_2O_6 \cdot 0{,}5H_2O$	56,3	5,0	8,2
VII	50	257—8 (Ethanol)	57,2	5,7	7,7	$C_{17}H_{18}N_2O_6 \cdot 0{,}5H_2O$	57,5	5,4	7,9
VIII	35	218—20 (Isopropanol)	59,0	5,2	8,1	$C_{17}H_{18}N_2O_4$	59,0	5,2	8,1
X	79	295—6 Toluene	70,0	6,8	7,0	$C_{23}H_{26}N_2O_4$	70,0	6,6	7,1



I—IX

R = OH (I); $NHCH(CH_3)COOH$ (II); Cl (III); $NHCH_2COOH$ (IV); $NHCH_2COOC_2H_5$ (V); $NHCH_2CH_2COOH$ (VI); $NHCH(C_2H_5)COOH$ (VII); $NH(CH_2)_3COOH$ (VIII); $NH(CH_2)_5COOH$ (IX); NH-adamantyl-1 (X).

The end products obtained after heating with ethanol were converted to the corresponding acids of II, IV-IX.

For the sake of comparison we synthesized the N-adamantyl derivative (X), which has lipophilic properties.

The structure of the amides II, V, and X was confirmed by PMR spectra which had the following common proton signals: 1.36 (CH_3, triplet) ppm, 4.41 (N—CH_2, triplet), ppm, 6.24 (O—CH_2—O, singlet) ppm; 7.64, 7.94 (4-H, 9-H, singlet) ppm, 8.86 (6-H, singlet) ppm; 10.49 (NH, singlet or triplet) ppm. In addition, for compound II: 1.28 (CH_3, triplet, J = 9.3 Hz) ppm, 1.41 (CH, doublet, J = 8.3 Hz), ppm; V: 1.21 (CH_3, triplet) ppm, 4.12 (CH_2, doublet) ppm; 4.44 (CH_2), quartet) ppm, X: 1.67 singlet, 2.05 singlet (1-ad) ppm.

EXPERIMENTAL

PMR spectra were read on a Brucker-WM-250 spectrometer in DMSO with HMDS as the internal standard.

Oxolinic chloroanhydride was obtained by the method in [2].

5-Ethyl-8-oxo-5,8-dihydro-1,3-dioxolo[4,5-g]quinoline-7-carboxylic 1-Carboxyethylamide (II). A 4-g (0.017 mole) portion of bistrimethylsilylalanine in 10 ml of dry methylene chloride was added to a suspension of 4 g (0.015 mole) of III in 50 ml of dry methylene chloride in a nitrogen atmosphere. This resulted in the complete dissolution of the residue. The reaction mixture was then heated for 3 h and cooled to room temperature. The resultant precipitate was filtered off in a nitrogen stream. The yield was 3.34 g of colorless substance. It was heated in 150 ml of ethanol which resulted in a yield of 2.6 g of compound II.

The substances presented in Table 1 were obtained in the same way.

5-Ethyl-8-oxo-5,8,-dihydro-1,3-dioxolo-[4,5-g]quinoline-7-carboxylic Ethoxycarbonylmethylamide (V). A solution of 5.5 g (0.035 mole) of N-trimethylsilylglycine ethyl ester in 10 ml of dry methylene chloride was added to a suspension of 8.5 g (0.033 mole) of III in 50 ml of dry methylene chloride. The mixture was heated at 40°C for 3 h and left overnight in a refrigerator. The residue was filtered off, resulting in 5 g of the ester V.

5-Ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-g]quinoline-7-carboxylic 3-Carboxypropylamide (VIII). A solution of 12 ml of trimethylchlorosilane in 20 ml of dry methylene chloride was slowly added to a suspension of 4 g (0.056 mole) of γ-aminobutyric acid, 9.2 g (0.035 mole) of III, and 13.5 ml of triethylamine in 100 ml of dry methylene chloride. The mixture was heated for 1 h at 30-40°C. The reaction mixture was then evaporated to dryness and the

TABLE 2. Activity of Amino Acid Derivatives of I in in vitro Experiments

Compound	Maximum tolerable dose		
	E. coli, S. typhi, Sh. dysenteriae, Kl. pneumoniae	Pr. aeruginosa, Pr. vulgaris	Staph. aureus
I	⩽0,5—1	125—500	7,8—31,2
II	1,95—7,8	⩾500	250—500
IV	31,2—125	>500	⩾500
VI	15,6—62,5	>500	250
VII	15,6—31,2	>500	250—500
VIII	3,9—7,8	125—⩾500	125—500
VIII, N-methylglucamine salt of X	7,8—15,6	>500	—
	500	>500	⩾500

Note: Four strains are listed in the staphylococcus tests (see research method)

residue was boiled in 300 ml of ethanol. After filtering, the yield was 3.6 g of the acid VIII.

5-Ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-g]quinoline-3-carboxylic 1-Adamantylamide (X). A suspension of 2.65 g (0.01 mole) of III, 2 g (0.01 mole) of 1-aminoadamantane chlorohydrate, and 10 ml of dry pyridine in 10 ml of benzene was heated at 80-90°C for 3 h. The solution was evaporated to dryness, triturated with a 10% solution of sodium hydroxide, filtered, and the residue was washed with water up to the neutral point. The yield was 3 g of compound X.

A solution of the glucoamine salt of compound VIII was obtained by dissolving a 0.4066 g portion of VIII in 10.67 ml of a methylglucamine solution (0.15 mole). The solution obtained a 39% excess of glucamine, and the pH of the salt solution was 9.4.

EXPERIMENTAL BIOLOGICAL PART

The antimicrobial activity of compounds II, IV, VI-VIII, and X was studied in relation to gram-negative and gram-positive bacteria in in vitro tests by the series double dilution method in liquid nutrient media [1]. The following microorganisms and strains were used: *E. coli* M-17, *S. typhi* 4446, *Sh. dysentheriae* 644, *Kl. pneumoniae* 444 *Ps. aeruginosa* 165, *Proteus vulgaris* I, *Staph. aureus* Zhaev, 18^b, 178, 191 (the Zhaev strain is sensitive to known chemotherapeutic preparations, strains 18^b, 178, and 191 are polyresistant, and 178 and 191 are resistant to oxacillin and methycillin). The strains were obtained from the L. A. Tarasevich State Scientific-Research Institute for Selection and Cultivation and the microbiology laboratory of the All-Union Scientific-Research Center for Mother and Child Care. Twenty-four hour agar cultures were used in the experiments and the seed culture was prepared from a suspension of bacteria in an isotonic sodium chloride solution. The seed culture dose was 1×10^6 microbial cells per 1 ml of medium. Results were recorded after 24 h and 48 h after incubation at 37°C.

All six substances were tested in vivo on white mongrel mice weighing 15-16 g in simulated generalized infections induced by ip inoculation of *S. typhi* 446 and *E. coli* M-17. The infectious doses (ID) were 1×10^7 and 5×10^8 microbial cells respectively and were administered in a mixture of aqueous "nutritional" agar [1]. In addition, compounds II and VIII were tested in vivo with *Bacillus pyocyaneus* and *Staphylococcus* (ID 5×10^6 and 8×10^8 microbial cells). The experimental animals' tolerance of the examined substances was preliminarily tested by a single per os injection at doses of up to 500 mg/kg. Tolerance to the N-methylglucamine salt of VIII was also tested by subcutaneous injection. The substances' activity during experimental infections was evaluated at does of 12.5 to 400 mg/kg given once per os or subcutaneously 30 min after infection. The animals were kept under observation for 10 days. The therapeutic effect of the test substances was evaluated by the survival rate and longevity of the mice.

Our study of the antimicrobial activity of six amino acid derivatives of oxolinic acid (II, IV, VI-VIII, and X) has shown that five of them (II, IV, VI, VII,

TABLE 3. Activity of Amino Acid Derivatives in a Generalized Typhoid *S. typhi* Ty_2 4446 Infection Model in Mice

Compound (route of administration	Dose, mg/kg	Survival rate for 10 days		Longevity (total)	
		abs.	%	abs.	%
I	200	30/30	100	300/300	100
	100	27/30	90	285/300	95
	50	19/30	63	207/300	69
	25	7/30	23	73/300	24
II (per os)	400	17/20	85	175/200	87
	200	21/30	70	217/300	72
	100	4/20	20	42/200	21
VIII (per os)	400	19/20	95	191/200	95
	200	14/30	46	158/300	52
	100	6/20	30	103/200	51
VIII, Methylglucamine salt (subcutaneously)	400	10/20	50	108/200	54
	200	2/20	10	54/200	27
	100	0	0/10	1/100	1
Control	—	1/90	1	27/900	3

Note. Here and in Table 4 the numerator represents the number of surviving animals (or the total number of days lived) during the ten-day observation period, and the denominator represents the number of animals in the group (or maximum number of possible days lived during the observation period).

TABLE 4. Activity of Amino Acid Derivatives of I in a Generalized *E. coli* M-17 Typhoid Infection Model in Mice

Compound (route of administration)	Dose, mg/kg	Survival rate for 10 days		Longevity (total)	
		abs.	%	abs.	%
I	200	12/30	42	186/300	62
	100	18/30	60	203/300	68
	50	13/30	43	174/300	58
	25	8/30	26	108/300	31
II (per os)	400	13/20	65	135/200	67
	200	13/30	40	130/300	43
	100	9/30	30	102/300	33
VIII (per os)	400	14/20	70	146/200	73
	200	18/30	60	193/300	64
	100	11/30	33	126/300	42
Control	—	1/60	1,6	24/600	4

VI, VII, and VIII) in in vitro experiments are primarily active against gram-negative bacteria (Table 2), i.e., *Escherichia, Salmonella, Shigella,* and *Klebsiella.* Moreover, II and VIII turned out to be highly active (maximum tolerable dose was within the range of 1.95-7.8 mg/kg). The substance's activity was markedly reduced in experiments with *Proteus, Pseudomonadaceae,* and *Staphylococcus* (MPK 125-500 μg/ml). Compound X did not exhibit any activity against the tested microorganisms in vitro at concentrations of up to 500 μg/ml.

All of the investigated compounds have a low degree of toxicity and were well tolerated by the mice when administered orally at doses of up to 500 mg/kg. The methylglucamine salt of VIII was well tolerated when administered parenterally (subcutaneously) at a dose of up to 400 mg/kg.

Compounds II and VIII were found to have a highly effective chemotherapeutic activity in *S. typhi* and *E. coli* infections in mice when administered one time per os 30 min after

inoculation. The parenteral administration of the soluble N-methylglucamine salt of VIII was found to be chemotherapeutically effective in the model of typhoid septicemia (see Table 3).

Compounds IV, VI, and VII were inactive against *E. coli* and typhoid sepsis in the mice. No chemotherapeutic effect was exhibited by compounds II and VII against *Bacillus pyocyaneus* and *Staphylococcus* when administered one time per os in doses up to 400 mg/kg.

Compounds II, IV, and VI-VIII were found to be less active than oxolinic acid in the in vitro experiments, and compounds II and VII were less so in the typhoid septicemia model experiments. *E. coli* approached the activity of oxolinic acid in the mice experiments (with respect to longevity during the ten-day observation period), but did not exhibit any chemotherapeutic activity against *Staphylococcus* and *Bacillus pyocyaneus*. The N-adamantyl derivative of X was practically inactive in the animal experiments.

LITERATURE CITED

1. Methods of Experimental Therapy [in Russian], G. N. Pershin, ed., Moscow (1971).
2. Patent No. 3524858, USA (1970).
3. Patent No. 53679, Japan (1981), Chem. Abstr. 95, 187666-P (1982).
4. K. Ruhlmann, Chem. Ber., 94, 1876-1881 (1961).