12. H. O. Hoppe, E. B. Alexander, and L. C. Miller, J. Am. Pharm. Ass. Sci. Ed., 39, 147-151 (1950).
13. W. R. Jones, T. L. Kerley, and L. E. Wearer, J. Pharm. Sci., 54, 1680-1681 (1965).
14. P. P. Koelzer and K. H. Weher, Arnzneimittel-Forch., 8, 181-190 (1958).
15. C. Vassiliades, Bull. Soc. Chim. Fr., No. 6, 1131-1136 (1937).
16. W. Wemer, J. Org. Chem., 17, 523-528 (1952).

SYNTHESIS AND VASCULAR ACTIVITY EXAMINATION OF 3-DIMETHOXYPHOSPHORYL PROPYL ESTERS OF N-ACYL DERIVATIVES OF NEUROACTIVE MONOCARBOXYLIC AMINO ACIDS

G. V. Kovalev, A. I. Rakhimov, A. A. Ozerov, V. I. Petrov, V. A. Sazhin, and O. P. Buzinova

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Neuroactive monocarboxylic amino acids, particularly γ-amino butyric, β-alanine and glycine, acting as inhibitory mediators in the CNS, participate in the central regulation of blood circulation. However, these substances' low penetrability of the blood-brain barrier (BBB) constitutes a considerable limitation to the use of these amino acids for clinical purposes [6]. A number of structural analogs of the neuroactive inhibitor amino acids have been synthesized that are capable of penetrating the BBB and that can reproduce various effects of inhibitory mediators [4]. We know, for example, that the phosphorylated analogs of γ-aminobutyric acid are effective anticonvulsants [9].

For the purpose of finding new prototype drugs based on the inhibitory amino acids that can easily gain entry into the brain and that exhibit cardiovascular activity, we synthesized 3-dimethoxyphosphorylated esters of N-acyl derivatives of glycine (VII), β-alanine (VIII), and γ-aminobutyric acid (IX), and investigated their vascular activity. The indicated compounds were synthesized by alkylating the sodium or potassium salts of N-benzolglycine (I), N-acetyl-β-analine (II) and N-acetyl-γ-aminobutyric acid (III) with allyl bromide in an aqueous acetonitrile medium in the presence of interphase transfer catalysts followed by the homolytic phosphorylation of the resultant allyl esters by dimethylphosphite in the presence of a peroxide initiator:

$$\underset{\text{I—III}}{RCONH(CH_2)_nCOONa} \xrightarrow{CH_2=CHCH_2Br,\ \text{Crown ether}} \underset{\text{IV—VI}}{RCONH(CH_2)_nCOOCH_2CH=CH_2} \xrightarrow{HP(O)(OCH_3)_2,\ \text{peroxide}} RCONH(CH_2)_nCOOCH_2CH_2CH_2PO(OCH_3)_2\ (\text{VII—IX})$$

R = C_6H_5 (I, IV, VII), CH_3 (II, III, V, VI, VIII, IX); n = 1 (I, IV, VII), 2 (II, V, VIII), 3 (III, VI, IX).

Crown ethers are widely used in organic synthesis, including the synthesis of complex carboxylic acid esters [8]. However, the existing methods require the use of anhydrous salts and absolute solvents. We have devised a convenient preparatory method of obtaining the allyl ethers of N-acylated amino acids by alkylating the corresponding sodium or potassium salts with allyl bromide in the presence of crown ethers (15-crown-5, 18-crown-6, dibenzo-18-crown-6) at a molar ratio of acylamino acid salt:allyl bromide:crown ether corresponding to 1:(1-1.25):(0.02-0.05) at a temperature range of 20°C-80°C. The process takes place in an aqueous acetonitrile medium with an allowable water content of up to 20 wt. %. In accordance with this method an aqueous solution of a sodium or potassium salt of the N-acylated amino acid is added to the crown ether solution in acetonitrile and the resultant emulsion is treated with allyl bromide. This obviates the preparation of anhydrous salts and the use of absolute solvents. The highest yield of the allyl ester is observed in the case of N-benzolglycine (92.6%). However, when N-acetyl-β-alanine and N-acetyl-γ-aminobutyric acid are used as the substrates, the yield of the allyl esters is reduced to 43.8 and 34.6% respectively. This is probably due to the lower solubility of the indicated N-acyl amino acid salt complexes with the crown ethers

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TABLE 1. Physicochemical Properties of Allyl Esters of N-Acyl Derivatives of Neuroactive Amino Acids

Compound	Yield, %	b.p., °C (mm Hg)	n_D^{20}	d_4^{20}	MR_D found	MR_D calculated	Empirical formula
I	92,6	176—178 (4)	1,5422	1,1642	59,279	59,063	$C_{12}H_{13}NO_3$
II	43,8	128—132 (4)	1,4710	1,0849	44,107	44,192	$C_8H_{13}NO_3$
III	34,6	160—164 (4)	1,4742	1,0732	48,552	48,810	$C_9H_{15}NO_3$

TABLE 2. Chemical Shifts in the PMR Spectra of Allyl (IV-VI) and 3-Dimethoxyphosphorylpropyl (VII-IX) Esters of N-Acyl Derivatives of Neuroactive Amino Acids

Compound	Chemical shifts, ppm
IV	4,08 d (5 Hz 2 H, N—CH_2—CO); 4,52 d (6 Hz, 2 H, O—CH_2—); 5,06—5,37 m (2 H, =CH_2); 5,62—6,06 m (6 Hz 1 H, —CH=); 7,20—7,48 s (3 H, Phenyl); 7,70—7,96 m (2 H, phenyl)
V	2,00 s (3 H, CO—CH_3); 2,58 t (6 Hz, 2 H, —CH_2—CO); 3,48 q (6 Hz, 2 H, N—CH_2—); 4,56 d (5 Hz, 2 H, O—CH_2—); 5,12—5,43 m (2 H, =CH_2); 5,70—6,14 m (5 Hz, 1 H, —CH=)
VI	1,56—2,05 m (7 Hz, 2 H, —CH_2—); 1,87 s (3 H, CO—CH_3); 2,31 t (7 Hz 2 H, — CH_2—CO); 3,19 q (6 Hz, 2 H, N—CH_2—); 4,49 d (5 Hz, 2 H, O—CH_2—); 5,06—5,38 m (2 H, =CH_2); 5,65—6,05 m (6 Hz, 1 H, —CH=).
VII	1,50—1,95 m (4 H, —CH_2—CH_2—P); 3,70 d (11 Hz, 6 H, P—O—CH_3); 4,00—4,15 m (4 H, N—CH_2—CO, O—CH_2—); 7,20—7,48 m (3 H, phenyl); 7,70—7,96 m (2 H phenyl)
VIII	1,50—1,95 m (4 H, —CH_2—CH_2—P); 2,00 s (3 H, CO—CH_3); 2,54 t (6 Hz, 2 H, —CH_2—CO); 3,40 q (6 Hz, 2 H, N—CH_2—); 3,68 d (11 Hz 6 H, P—O—CH_3); 4,04 t (6 Hz, 2 H, O—CH_2)
IX	1,50—2,00 m (6 H, —CH_2—CH_2—P, —CH_2—); 1,86 s (3 H, CO—CH_3); 2,26 t (7 Hz, 2 H, —CH_2—CO); 3,20 q (6 Hz, 2 H, N—CH_2—); 3,64 d (11 Hz, 6 H, P—O—CH_3); 4,01 t (6 Hz, 2 H, O—CH_2—)

Note: PMR spectra were recorded on a Tesla BS-567 A spectrometer (100 MHz) in a FT system, 10-15% solutions in deuterochloroform, temperature of samples 20-25°C, internal standard HMDS.

TABLE 3. Physicochemical Properties of 3-Dimethoxyphosphorylpropyl Esters of N-Acyl Amino Acid Derivatives

Compound	Yield, %	n_D^{20}	Empirical formula
VII	94,4	1,5212	$C_{14}H_{20}NO_6P$
VIII	93,4	1,4676	$C_{10}H_{20}NO_6P$
IX	96,8	1,4766	$C_{11}H_{22}NO_6P$

TABLE 4. Effect of 3-Dimethoxyphosphorylpropyl Esters of N-Acyl Derivatives of Neuroactive Amino Acids on Blood Circulation in Experiments on Anesthetized Cats (dose 50 mg/kg).

Compound	Ratio of LD_{50} dose	Change in index as % of initial value: systemic arterial pressure	cardiac contraction frequency	minute blood volume	total peripheral resistance	Left ventricle function	Duration of effect, min
VII	1/50	+12,5±2,5	+9,8±2,1	+76,8±19,8	—40,6±2,2	+55,4±7,0	5—30
VIII	1/35	—27,6±14,7	+5,5±2,1	—3,9±1,5	—10,1±2,5	—16,4±4,2	5—15
		+18,8±10,4	+10,2±3,5	—13,7±3,2	+42,0±8,3	+7,6±3,4	15—30
IX	1/40	—10,7±1,4	—6,6±2,6	—27,3±12,5	+43,7±13,1	—21,9±11,9	5—30

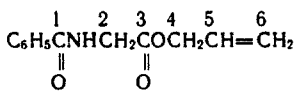
Note: Compound VIII exhibited a dual phase action.

in the aqueous-acetonitrile phase. The structure of the allyl esters of the N-acyl amino acid derivatives was confirmed by PMR- and NMR ^{13}C-spectroscopy and molecular refraction. The physicochemical properties of the allyl esters IV-VI are given in Table 1 and the spectral characteristics are given in Table 2.

The addition of dimethylphosphite to the resultant allyl esters on the double carbon—carbon bond was accomplished by the method suggested for the allyl and vinyl esters of carboxylic acids [1, 2] whereby the mixture of the reagents was heated at 135-140°C in a nitrogen atmosphere at an allyl ether: dimethylphosphite reaction of di-tert-butylperoxide 1:(3-4):(0.02-0.05). The physicochemical and spectral properties of 3-dimethoxyphosphorylpropyl esters of N-acyl amino acid derivatives VII-IX are shown in Tables 2 and 3. The element analysis data satisfied the calculated values.

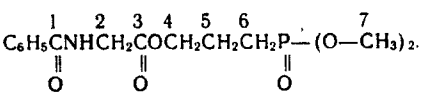
EXPERIMENTAL (CHEMICAL PART)

Allyl N-Benzoylglycinate (IV). A 0.45 g (0.002 mole) portion of 15-crown-5 in 250 ml acetonitrile was placed into a reaction vessel fitted with an effective stirrer, a reflux condenser, and a drop funnel. Then a solution of 17.9 g (0.1 mole) of N-benzoylglycine and 4.5 g (0.1 mole) of 90% granulated NaOH in 50 ml of water was added upon intensive stirring at 20°C for 5 min. Upon intensive stirring 8.7 ml (0.1 mole) of freshly distilled allyl bromide was added at a temperature of 20-25°C over a period of 10 min after which the mixture was stirred at the same temperature for 8 h. The reaction mass was then filtered and the organic layer separated. The solvent was distilled off and the remainder of the solvent was vacuumed off to yield 20.3 g of a viscous yellow product containing no less than 98% (according to TLC data) of allyl N-benzoylglycine, a yield of 92.6%. The product was subsequently purified by vacuum-distillation, bp 176-178°C (4 mm Hg), R_f 0.13 (chloroform), 0.81 (chloroform: ethanol, 4:1).



NMR ^{13}C-spectrum, δ, ppm: 40.033 (C^2); 64.238 (C^4); 117.123 (C^6); 130.046 (C^5); 125; 414; 126.834; 129.822; 131.988 (phenyl); 166.049 (C^1); 168.141 (C^3).

3-Dimethoxyphosphorylpropyl N-Benzoylglycinate (VII). A mixture of 5.5 g (0.025 mole) of allyl N-benzoylglycinate, 7.0 ml (0.075 mole) of freshly distilled dimethylphosphite and 0.1 ml (0.0005 mole) of di-tert-butylperoxide was heated in a glass tube in a nitrogen atmosphere at 135-140°C for 6 h. The excess dimethylphosphite was distilled off on a boiling water bath at a residual pressure of 4 mm Hg. The residue was then dissolved in 100 ml of chloroform and stirred with 5 g of activated charcoal for 24 h at room temperature. The mixture was then filtered and the solvent was removed to yield 7.8 g of a viscous light-yellow product at a yield of 94.4%.



NMR ^{13}C-spectrum, δ, ppm: 17.554 d (60.1 Hz, C^6); 19.938 d (5.5 Hz, C^5); 39.654 (C^2); 50.486 d (7.5 Hz, C^7); 62.791 d (16.8 Hz, C^4); 125.325; 126.594; 129.730; 131.677 (phenyl); 166.121 (C^1); 168.259 (C^3).

Compounds V, VI, VIII, and IX were synthesized in a similar fashion.

The effect of the 3-dimethoxyphosphorylpropyl esters of N-acyl derivatives of neuroactive monocarboxylic amino acids VII-IX on blood circulation factors was examined in experiments on anesthetized (nembutal, 50 mg/kg ip) cats weighing 2.0-4.4 kg. Minute blood volume (MBV) was measured by the thermodilution method [3]. Cardiac contraction frequency was measured on an electrocardiogram recorded in a II standard lead. Stroke volume (SV), total peripheral resistance (TPR) and left ventricle function (LVF) were measured by computation [7]. Acute toxicity of the tested compounds was examined on white non-pedigree mice upon ip administration. LD_{50} was computed by the V. B. Prozorovskii method [5]. Data are given in Table 4.

At a dose of 50 mg/kg (1/50 of the LD_{50}) compound VII slightly increased systemic arterial pressure (SAP) by 12.5 ± 2.5% over a period of 5-30 min in which case a pronounced intensification of cardiac left ventricle effort and an increase in MBV was accompanied by a significant decrease of TPR by 40.6 ± 2.2%.

Compound VIII at a dose of 50 mg/kg (1/35 of the LD_{50}) had a two-phase effect on SAP: During the first 5-15 min SAP was observed to fall abruptly by 27.6 ± 14.1% due to lowered left ventricular function and reduced TPR.

This was followed by a rise in SAP by 18.8 ± 10.4% over the next 15-30 min due to the abrupt increase in TPR by 42.0 ± 8.3%.

In most of the experiments compound IX at doses of 50-100 mg/kg (1/40-1/20 of the LD_{50}) reduced SAP by 10.7 ± 1.4% during the first 5-30 min.

The substances of the series under examination were only slightly toxic: The LD_{50} of compounds VII, VIII, and IX upon ip administration to white mice was 2630, 1700, and 1950 mg/kg respectively.

The results we obtained indicate that among the 3-dimethoxyphosphorylpropyl esters of N-acyl derivatives of inhibitor amino acids, acute toxicity increases as the hypotensive properties of the substances increase. We presume that after penetration of the BBB, compounds VIII and IX undergo metabolic conversion in the brain as a result of which the dimethoxyphosphorylpropyl and acetyl fragments split off the initial structure. The resultant γ-aminobutyric acid and β-alanine bond with the specific receptors and exhibit a sympatho-inhibitory action, thereby reducing the SAP. After the dimethoxyphosphorylpropyl component is split off, the compound VII fragment, i.e., N-benzoylglycine, most probably does not break up any further and exerts a hypertensive and cardiotonic effect.

Compound VII is of considerable interest as a cardiotonic substance with a nonglycoside structure. Compounds VIII and IX exhibit moderate hypotensive activity. A further modification of their structure might offer a promising way for finding hypotensive agents among derivatives of monocarboxylic amino acids.

LITERATURE CITED

1. A. K. Brel', L. M. Filimonova, and A. I. Rakhimov, Inventor's Certificate No. 707,920, Otkryt., No. 1 (1980).
2. A. K. Brel', I. N. Tyurenkov, G. V. Strel'tsova, et al., Khim. Farm. Zh., No. 2, 167-174 (1988).
3. M. I. Gurevich, S. D. A. Bershtein, and D. A. Golov, Fiziol. Zh., **53**, No. 3, 350-354 (1967).
4. V. M. Kopelevich, Usp. Khim., **48**, No. 7, 1273-1296 (1979).
5. V. B. Prozorovskii, Farmakol. Toksikol., No. 4, 497-502 (1978).
6. K. S. Raevskii and V. P. Georgiev, Mediator Amino Acids [in Russian], Moscow (1986).
7. S. A. Seleznev, S. M. Vashetina, and G. S. Mazurkevich, Comprehensive Evaluation of Blood Circulation in Experimental Pathology [in Russian], Leningrad (1976).
8. M. Khiraoka, Crown Compounds [in Russian], Moscow (1986).
9. L. A. Cates, V.-S. Li, C. C. Yakshe, et al., J. Med. Chem., **27**, No. 5, 654-659 (1984).