TABLE 1. Nicotino- and Muscarinomimetic Activity of Compounds IIa-d and IIIa-d

Compound	N-Mimetic activity		M-Mimetic activity	
	EK_{50}, moles/liter	activity ratio*	EK_{50}, moles/liter	activity ratio*
IIa	$2{,}0\cdot10^{-4}$	44	$6{,}0\cdot10^{-4}$	3
IIIa	$4{,}5\cdot10^{-6}$		$2{,}0\cdot10^{-4}$	
IIb	$1{,}3\cdot10^{-4}$		$1{,}0\cdot10^{-4}$	100
IIIb	$1{,}1\cdot10^{-6}$	118	$1{,}0\cdot10^{-6}$	
IIc	$2{,}8\cdot10^{-4}$	175	$2{,}0\cdot10^{-4}$	10
IIIc	$1{,}6\cdot10^{-6}$		$2{,}0\cdot10^{-5}$	
IId	$6{,}3\cdot10^{-4}$	315	$3{,}0\cdot10^{-4}$	60
IIId	$2{,}0\cdot10^{-6}$		$5{,}0\cdot10^{-6}$	

*The activity ratio of the circular compound IIIa-d to the activity of the linear compound IIa-d with the same acyl radicals.

The conformational influence on the muscarinomimetic activity is a maximum at R = Me; in the sequence from compound IIa to compound IIIa there is a 100-fold increase in the muscarinomimetic activity.

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β-AMINO KETONES — α-AMINO ACID DERIVATIVES.

IV. AMINOMETHYL DERIVATIVES OF ACETOPHENONES AND THEIR BIOLOGICAL ACTIVITY

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As we have previously shown [1], many β-amino ketones — derivatives of α-amino acids — display antiinflammatory, antipyretic, and bactericidal properties. In a continuation of our research on the synthesis and study of the biological activity of amino ketones — α-amino acid derivatives [2-4] — we have obtained a number of β-amino ketones, viz., serine, threonine, valine, and leucine derivatives, and have studied their antibacterial, local-anesthetic,and antiinflammatory properties.

The hydrochlorides of N-[β(p-substituted benzoyl)ethyl] amino acids (I-VI) and ethyl esters (VII, VIII) were obtained via the Mannich reaction [2, 4].

$$n\text{-}RC_6H_4COCH_2CH_2A$$

I—VIII

I: R = H; K = NHCH(COOH)$CH(CH_3)_2$; II: R = CH_3O; A = = NHCH(COOH)$CH(CH_3)_2$; III: R = H; NHCH(COOH)$CH_2CH(CH_3)_2$; IV: R = CH_3O;

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TABLE 1. Properties of p-$RC_6H_4COCH_2CH_2A \cdot HCl$ (I-VIII)

Compound	Optical form	Yield, %	mp, °C	Found, %				Empirical formula	Calculated, %				R_f
				C	H	N	Cl		C	H	N	Cl	
I*	DL	65,0	178—180	59,06	6,82	5,13	12,00	$C_{14}H_{20}ClNO_3$	58,84	7,05	4,90	12,41	0,72
II	DL	36,5	172—174	56,85	7,20	4,60	11,51	$C_{15}H_{22}ClNO_4$	57,05	7,02	4,44	11,23	0,71
III	L †	22,0	196—198	59,58	7,58	4,60	12,01	$C_{15}H_{22}ClNO_3$	60,09	7,40	4,67	11,83	0,84
IV	L †	29,7	170—172	57,80	7,00	4,75	11,20	$C_{16}H_{24}ClNO_4$	58,26	7,33	4,25	10,75	0,80
V	—	32,6	149—152	54,44	6,49	4,52	12,00	$C_{13}H_{18}ClNO_4$	54,26	6,31	4,87	12,32	0,67
VI	—	38,0	124—126	55,81	6,60	4,47	11,93	$C_{14}H_{20}ClNO_4$	55,72	6,68	4,64	11,75	0,71
VII	DL	27,0	158—160	50,17	5,45	4,25	20,91	$C_{14}H_{19}Cl_2NO_4$	50,01	5,70	4,17	21,09	0,74
VIII	DL	40,5	130—133	55,29	6,50	4,30	20,10	$C_{16}H_{23}Cl_2NO_3$	55,18	6,66	4,02	20,36	0,67

*UV spectrum : λ^1 = 246 nm (E_1 = 1.16), λ^2 = 284 nm (E_2 = 0.10).
†The angles of rotation were +40.0° for III and +10° for IV.

A = $NHCH(COOH)CH_2CH(CH_3)_2$;
V: R = CH_3O; A = $N(CH_3)CH_2COOH$; VI: R = C_2H_5O; A = $N(CH_3)CH_2COOH$; VII: R = Cl; A = $NHCH(CH_2OH)COOC_2H_5$; VIII: R = Cl; A = $NHCH(COOC_2H_5)CH(CH_3)_2$.

The aminomethylation of p-substituted acetophenones has been studied in detail in the case of amino acids such as glycine, α-alanine, phenylalanine, etc. [2-4]. The optimum conditions for condensation involve the use of 10% formalin and an equimolar amount of hydrochloric acid with ethanol as the solvent. Proceeding from this, the aminomethylation of p-substituted acetophenones with valine, leucine, and sarcosine was carried out under the same conditions. Since the yields of products of aminomethylation of p-substituted acetophenones by the indicated amino acids are low (22-65%), and β-acylethyl derivatives of tryptophan and glyclylglycine could not be obtained by this method, we obtained a number of N-[β-(p-substituted benzoyl)ethyl]serines, -threonines, -valines, -leucines, -tryptophans, and -glycylglycines (X-XXII) via the following scheme [3]:

$$\underset{\text{IX}}{p\text{-}RC_6H_4COCH_2CH_2N(C_2H_5)_2} + HA \longrightarrow \underset{\text{X–XXIII}}{p\text{-}RC_6H_4COCH_2CH_2A}$$

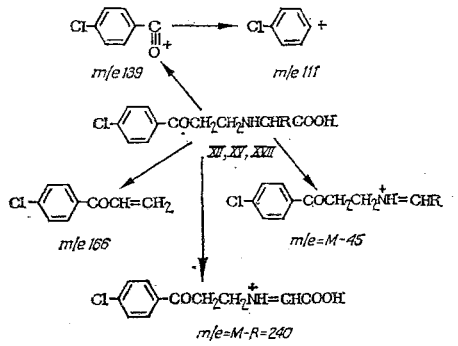
X: R = Cl; A = $NHCH(COOH)CH(CH_3)_2$; XI: R = H; A = $NHCH(COOH)CH_3CH(CH_3)_2$; XII: R = Cl; A = $NHCH(COOH)CH_2CH(CH_3)_2$(L); XIII: R = Cl; A = $NHCH(COOH)CH_2CH(CH_3)_2$(D); XIV; R = H; A = $NHCH(COOH)CH_2OH$; XV: R = Cl; A = $NHCH(COOH)CH_2OH$; XVI: R = H; A = $NHCH(COOH)CH(OH)CH_3$; XVII: R = Cl; A = $NHCH(COOH)CH(OH)CH_3$; XVIII: R = Cl; A = $N(CH_3)CH_2COOH$; XIX: R = H; A = $(NHCH_2CO)_2OH$; XX: R = Cl; A = $(NHCH_2CO)_2OH$; XXI: R = H; A = $NHCH(COOH)CH_2$-3-indolyl; XXII: R = Cl; A = $NHCH$-$(COOH)CH_2$-3(L)-indolyl; XXIII: R = Cl; A = $NHCH(COOH)CH_2$-3(D)-indolyl.

The structures of the synthesized compounds were confirmed by data from the IR, UV, and NMR spectra and, in a number of cases, by means of mass spectrometry.

An absorption band of a carbonyl group ($\nu_{C=O}$ 1680-1700 cm^{-1}) is observed in the IR spectra of β-amino ketones I-VIII and X-XXIII. Absorption bands of an ionized carboxy group [ν_{COO^-}(a) 1580-1590; ν_{COO^-}(s) 1405-1410 cm^{-1} are observed in the IR spectra of N-[β-(p-substituted benzoyl)ethyl]amino acids X-XXIII; absorption bands of carboxy and carbethoxy groups at 1730-1760 cm^{-1} are observed in the spectra of their hydrochlorides I-IV and the ethyl ester hydrochlorides VII and XV. Absorption bands of a hydroxy group at 3285 and 3235 cm^{-1}, respectively, are also observed in the spectra of serine derivatives XIV and XV and threonine derivatives XVI and XVII.

The data from the UV spectra of amino ketones I, XXI, and XXII are presented in Tables 1 and 2.

We also conducted a mass-spectrometric study of N-[β-(p-chlorobenzoyl)ethyl] amino acids XII, XVI, XVII. Despite the absence of a molecular peak, the fragmentation data may serve as evidence for the structures of these β-amino ketones.



EXPERIMENTAL CHEMISTRY

Thin-layer chromatography (TLC) was carried out on plates with a fixed layer of silica gel–gypsum by the method in [3]. The IR spectra were recorded with a UR-20 spectrometer (East Germany). The mass spectra were recorded with MKh-1320 spectrometer. The angles of rotation were determined with an SM polarimeter.

TABLE 2. Properties of Amines p-$RC_6H_4COCH_2CH_2A$ (X-XIII)

Compound	Optical form (angle of rotation, deg)	mp, °C	Yield, %	Found, %				Empirical formula	Calculated, %				R_f
				C	H	N	Cl		C	H	N	Cl	
X	DL	168—170	69,2	59,47	6,50	4,63	12,81	$C_{14}H_{19}ClNO_3$	59,26	6,39	4,94	12,50	0,81
XI	L (+25)	218,5—222,5	53,5	68,19	8,28	5,30	—	$C_{15}H_{21}NO_3$	68,41	8,04	5,32	—	0,76
XII	L (+30)	199—204	73,7	61,00	6,38	4,49	12,05	$C_{15}H_{20}ClNO_3$	60,50	6,77	4,70	11,91	0,71
XIII	DL (—30)	200—204	47,6	60,40	6,60	4,77	11,80	$C_{15}H_{20}ClNO_3$	60,50	6,77	4,70	11,91	0,71
XIV	DL	179—183	57,5	60,58	6,20	5,83	—	$C_{12}H_{15}NO_4$	60,75	6,37	5,90	—	0,63
XV	DL	192—197 deg	49,0	53,27	5,40	4,96	12,76	$C_{12}H_{14}ClNO_4$	53,05	5,19	5,16	13,05	0,61
XVI	DL	175—178	87,0	61,88	6,40	5,64	—	$C_{13}H_{17}NO_4$	62,14	6,82	5,57	—	0,75
XVII	DL	192—194	41,5	54,38	5,70	5,30	12,50	$C_{13}H_{16}ClNO_4$	54,65	5,64	4,90	12,41	0,76
XVIII	—	152—156	22,4	56,01	5,40	5,35	14,09	$C_{12}H_{14}ClNO_3$	56,37	5,52	5,48	13,87	0,70
XIX	—	175—178	40,5	59,60	6,46	10,55	—	$C_{13}H_{16}N_2O_4$	59,08	6,10	10,60	—	0,53
XX	—	110—112	39,5	52,01	5,20	9,32	12,40	$C_{13}H_{15}ClN_2O_4$	52,27	5,06	9,38	11,87	0,61
XXI*	D (—20)	193—195	84,5	70,95	6,10	8,16	—	$C_{20}H_{20}N_2O_3$	71,41	5,99	8,33	—	0,77
XXII*	L (+106)	175—178	96,2	64,48	5,50	7,70	9,45	$C_{20}H_{19}ClN_2O_3$	64,78	5,14	7,55	9,56	0,74
XIXII	D (—102)	172—174	84,5	64,40	5,49	8,00	9,30	$C_{20}H_{19}ClN_2O_3$	64,78	5,14	7,55	9,56	0,73

*UV spectra: XXI: λ_1 = 203 (E_1 = 1.0), λ_2 = 220 (E_2 = 1.6), λ_3 = 250 (E_3 = 0.4), λ_4 = 280 (E_4 = 0.22), λ_5 = 290 nm (E_5 = 0.18); XXII: λ_1 = 217 (E_1 = 1.1), λ_2 = 257 (E_2 = 0.58), λ_3 = 290 nm (E_3 = 0.18).

TABLE 3. Comparative Evaluation of the Acute Toxicity, Anesthetizing Activity, and Local-Irritating Action of the Synthesized Compounds

Compound	Acute toxicity (LD_{50}, mg/kg)	EC_{50},† mg/10 ml	Local-irritating effect
Novocain	192 (176,6—208,7)	5 (3,6—6,8)	—
I	580 (479,3—701,8)	17,5 (10,3—29,7)	++++
II	262 (222,03—309,2)	57 (34—92)	+++
III	278 (227,8—339,2)	0**	+++
IV	520 (452,2—598)	47,5 (29,7—76)	++++
V	597 (485,3—734,3)	0	++++
VI	1240 (1137,6—1351,6)	0	++++
VIII	1000 (1018,5—1188,1)	14,5 (8,7—2,4)	++++
X	310 (227,9—421,6)	21 (16,2—27,3)	—
XI	484 (400—585,6)	0	—
XII	840 (672—1050)	47 (27,6—79,9)	—
XIII	310 (268,4—359,1)	0	—
XIV	650 (526,3—802,7)	49 (28,8—83,3)	—
XV	410 (310,6—541,2)	44 (25,8—74,8)	—
XVI	1580 (1874,0—1959,2)	0	—
XVIII	618 (490,5—478,7)	24 (18,2—31,7)	—
XIX	520 (481,8—500,6)	0	—
XX	1015 (930,07—1098,2)	13 (10—16,9)	—
XXI	650 (500—845)	17 (11,5—24,5)	—
XXII	550 (423—715)	33 (17—60)	—
XXIII	800 (666,6—960)	0	—

*Slight reddening is indicated by +, spot necrosis is denoted by +++, and pronounced necrosis is indicated by ++++.
†The absence of activity is indicated by 0.
Note: The range of variations is indicated in parentheses.

TABLE 4. Antibacterial Activity of N-[β-(p-Substituted benzoyl)ethyl]-α-amino Acids X-XXIII

Compound	Minimal suppressing concentration, μg/ml	
	Staph. aureus 209P	Sh. dysenteriae Flexnori 6858
X	610	610
XI	5000	5000
XII	70	310
XIII	310	610
XIV	2500	2500
XV	35	150
XVI	2500	2500
XVII	35	150
XVIII	310	310
XIX	1250	1250
XX	610	610
XXI	5000	5000
XXII	1250	1250
XXIII	2500	2500

N-[β-(p-Substituted benzoyl)ethyl]-DL-valine, L-Leucine, and Sarcosine Hydrochlorides. These compounds were obtained by the method in [3].

Ethyl-N-[β-(p-chlorobenzoyl)ethyl]-DL-serine and DL-Valine Hydrochlorides (VII, VIII). These compounds were synthesized by the method in [2].

N-[β-(p-Substituted benzoyl)ethyl]-DL-Valines, D(L)-Leucines, DL-Serines, DL-Threonines, DL-Glycylglycinates, D(L)-Tryptophans, and Sarcosine (X-XXIII). A mixture of 0.01 mole of the amino acid and 0.01 mole of IX in 25 ml of water was heated with stirring on a boiling-water bath for 2 h, after which the mixture was cooled, and the crystalline product was removed by filtration and washed on the filter with acetone. Evaporation of the mother liquor to half the volume of the added water gave up to 20% overall yield of the amount of the amino ketone.

The constants of the synthesized compounds are presented in Table 2.

EXPERIMENTAL PHARMACOLOGY

The acute toxicity of the compounds in the case of intraperitoneal administration was determined with respect to white male mice with masses ranging from 18 to 22 g. The results of the experiments were taken into account after 24 h in an alternative form of taking into account the reaction from the number of perished animals. The statistical treatment (here and subsequently) was carried out by the method of Litchfield and Wilcoxon [6]. The data obtained are presented in Table 3.

The local toxicity was studied with respect to white guinea pigs with masses ranging from 250 to 300 g [11]. The 0.25-2% test solutions, which were prepared in an isotonic solution of sodium chloride, was administered subcutaneously in a volume of 0.2 ml in the spinal region of the animal. We ascertained a relationship between the chemical structures of the tested compounds and the existence of local-irritating effect. Thus N-[β-(p-substituted benzoyl)ethyl]amino acid hydrochlorides (I-VI) has a pronounced local-irritating effect, whereas N-β-(p-substituted benzoyl)ethyl]amino acids X-XXIII do not have this property (see Table 3).

The pharmacological evaluation of the synthesized compounds was realized by means of anesthesiometric and analgesiometric tests; their antagonistic activity with respect to narcotic analgesics was evaluated.

The study of the anesthetic activity involving a model of conductor anesthesia *in vitro* was carried out on the isolated nerves of frogs [7]. The concentration of the compound that gives rise to 50% blocking (EC_{50}) of the potential of the nerve action was taken as the standard. The results of the experiments showed that, of the tested substances, I, VIII, and XX were most effective; however, even they are somewhat inferior to the control preparation novocain (see Table 3). It should be noted that I-VIII and X-XXIII are significantly less toxic than novocain (another control preparation, viz., dicain, is more toxic than novocain by a factor of 10).

The surface-anesthetizing activity of the compounds was evaluated with respect to the cornea of rabbits by the Regnier method [13]. A 0.2% solution of dicain was used as the control preparation. The tested compounds did not display surface-anesthetizing activity.

In a study of the analgesic activity of the narcotic type with respect to a model of mechanical irritation of rat tails [10] it was established that only the N-[β-(p-substituted benzoyl)ethyl]amino acids hydrochlorides in a dose of 10 mg/kg had a weak central anesthetizing effect. In this group XXI was more active.

The antagonistic (with respect to opiates) activity (with respect to a model involving the suppression of the analgesic activity of morphine in a dose of 5 mg/kg, ED_{99} in the case of mechanical irritation of rat tails) was investigated. The data obtained demonstrated that XIV and XVI have an antagonistic effect, suppressing morphine analgesia by 31.4 and 67.6%, respectively, in a dose of 10 mg/kg administered subcutaneously, whereas the control antagonist naloxone in a dose of 3 mg/kg displayed 99% activity.

We also studied the antiinflammatory analgesic (of the non-narcotic type) [5, 12], and antipyretic [8] properties of the synthesized compounds. The antiinflammatory and analgesic activity was investigated in the case of internal administration in doses of 1 and 10 mg/kg, whereas the antipyretic activity was investigated in a dose of 5 mg/kg. The results of these experiments showed that all of the investigated compounds are inactive.

The bacteriological activity of I-VIII and X-XXIII was determined by the method of twofold serial culturing [9] with respect to test microbes *Staph. aureus* 209 P and *Sh. dysenteriae* Flexneri 6858. The inoculation dose of the culture was $2 \cdot 10^6$ microbe cells per milliliter. The activity was evaluated from the minimal suppressing concentration (MSC) with respect to the growth of test microbes. The experiments were repeated three times.

The β-amino ketone hydrochlorides — α-amino acid derivatives (I-VI) — did not display antibacterial activity (the MSC of these compounds does not exceed 2500 μg/ml). Their LD_{50} in the case of intraperitoneal administration to white mice ranges from 192 to 597 mg/kg (depending on the character of the compound) (see Table 3). Compound VI, the MSC of which is 610 μg/ml vis-a-vis an LD_{50} of 1240 mg/kg, constitutes an exception.

The activity, upon the whole, increases on passing to the free N-[β-(p-substituted benzoyl)ethyl]amino acids (X-XXIII); a decrease in toxicity is also observed. For the majority

of compounds of this series the MSC is equal to or less than 2500 μg/ml (Table 4), and the LD_{50} ranges from 310 to 840 mg/kg (see Table 3). All of the p-chloro derivatives of the amino ketones (X, XII, XIII, XV, XVII, XVIII, XX, and XXII) are more active than the unsubstituted analogs (compare XI and XII, XIV and XV, XVI and XVII, XIX and XX, and XXI and XXIII); this cannot be ascribed to their toxicity. In particular, N-[β-(p-chlorobenzoyl)ethyl]-L-leucine (XII) and L-glycylglycine (XX) (LD_{50} 840 and 1015 kg/kg, respectively) are less toxic than NO(β-benzoylethyl) derivatives XI and XIX, which have LD_{50} 484 and 520 kg/kg. The β-amino ketones that are derivatives of DL-threonine (XVII), DL-serine (XV), and D-leucine (XII) have pronounced antibacterial activity with respect to *Staphylococcus aureus*: Their MSC ranges from 35 to 70 μg/ml (see Table 4). In a study of the antimicrobial activity of these compounds with respect to *B. megatherium*, *Pseudomonas aeruginosa*, and *Enterobact aerogenes* we also discovered activity. The MSC of these compounds range from 30 to 80 μg/ml.

As regards the hydrochlorides of the ethyl esters of N-[β-(p-chlorobenzoyl)ethyl]-DL-serine and -DL-valine (VII, VIII), their MSC are 310 and 610 μg/ml, respectively.

In a comparison of the biological properties of the optical isomers in the case of leucine derivatives XII and XIII and tryptophan derivatives XXII and XXIII it was shown that the L forms of the amino ketones are more active than the D forms both with respect to local-anesthetizing and antibacterial activity (see Tables 3 and 4).

Thus among N-[β-(p-substituted benzoyl)ethyl] amino acids we have discovered low-toxicity compounds that have antibacterial, local-anesthetizing, and opiate-antagonistic properties. The results obtained provide evidence for the promising character of further study of β-amino ketones that are amino acid derivatives as compounds that have antibacterial and local-anesthetizing activity.

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