of hypothermic and antihypoxic effects for this particular class of compounds has to do with the molecular mechanisms of action, which differ from those of aminazine, adenosine, and GABA. Thus, the modification of GABA by lipophilic radicals leads to a substantial change in the fundamental properties of the initial substance, and one can expect a wide spectrum of pharmacological activity from GABA amides.

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SYNTHESIS AND LOCAL ANESTHETIC ACTIVITY OF SUBSTITUTED ANILIDES OF 6-METHYLPIPECOLINIC ACID

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Extensive experience in the use of medical applications of local anesthetics of various chemical compound groups has demonstrated that with respect to pharmacotherapeutic characteristics, the greatest interest has been shown in preparations whose basic structure includes 2,6-xylidine, i.e., lidocaine, and particularly the long-acting preparation marcaine (bupivacaine) that induces all types of anesthesia [7, 8]. For the purpose of finding new improved local anesthetics of the 2,6-xylidine ring we synthesized and studied marcaine analogs, i.e., subtituted anilides of 6-methylpipecolinic acid.

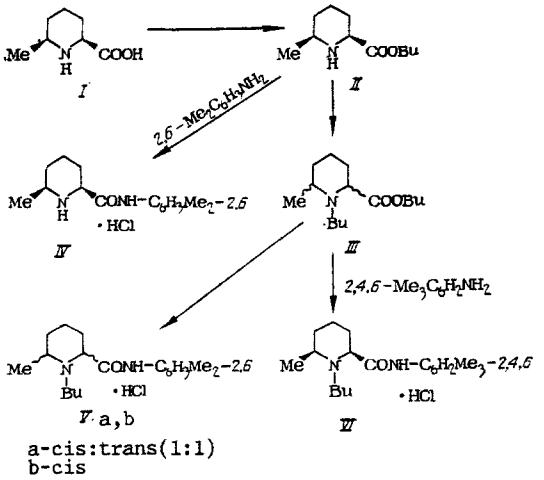

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TABLE 1. PMR Spectral Data for Compounds III-VI

Compound	Type of stereoisomer	Chemical shifts, δ, ppm						Coupling constants (J, Hz)					Relative isomer content, %
		2-H	6-H	6-CH_3	CH_3 of side chain	1-N-CH_2 of side chain	3, 4, 5-CH_2 and $(CH_2)_3$ of side chain	$^3J_{2\text{-H, }3\text{-H}}$	$^3J_{6\text{-H, }3\text{-H}'}$	$^3J_{6\text{-H, }5\text{-H}}$	$^3J_{6\text{-H, }5\text{-H}'}$	$^3J_{6\text{-H, }6\text{-H}_3}$	
III*	Cis	3,28 q	2,48 m	1,20 d	1,00 t	2,45—2,85 m	1,2—1,9m	10,8	3,0	$\Sigma\lvert^3J_{6\text{-H, }5\text{-H}}+{}^3J_{6\text{-H, }5\text{-H}'}\rvert$=13,0		6,2	70—80
	Trans	3,68 t	3,34 m	1,10 d	0,96 t	2,45—2,85 m	1,2—1,9 m	$\Sigma\lvert^3J_{2\text{-H, }3\text{-H}}+{}^3J_{2\text{-H, }3\text{-H}'}\rvert$=9,7	8,7	3,3		6,4	30—20
IV**	Cis	4,29 q	3,31 m	1,33 d			1,3—2,6m	12,0	3,5	12,0	~3	6,5	100
Vb***	Cis	4,65 q	3,60 m	1,42 d	1,01 t	3,10—3,40 m	1,25—2,00m 2,00 (3-Ha) 2,44 (3-He)	11,7	2,6	10,4	2,0	6,4	100
	Cis	4,80 q	3,85 m	1,46 d	0,97 t	3,05—3,50 m	1,25—2,00m ~2,00 (3-Ha) ~2,40 (3-Ha)	10,9	3,0	$\Sigma\lvert^3J_{6\text{-H, }5\text{-H}}+{}^3J_{6\text{-H, }5\text{-H}'}\rvert$=12		6,4	~50****
Va	Trans	4,69 br.d	4,37 m	1,45 d	0,99 t	3,05—3,50 m	2,35 (3-He) 2,57 (3-Ha)	5,0	~2,0	$\Sigma\lvert^3J_{6\text{-H, }5\text{-H}}+{}^3J_{6\text{-H, }5\text{-H}'}\rvert$=14		6,4	~50
VI	Cis	4,47 br.s with raising	3,55 br.s	1,45 d	0,98 t	3,15—3,45 m	1,25—2,00m 2,15 (3-Ha) 2,43 (3-He)	~12,0	~2,5	10,5	~2,0	6,4	100

*δR'; 4.3-4.1 (multiplet, $COCH_2$), 1.2-1.9 (multiplet, $3CH_2$), ~1.00 (triplet, CH_3).
**δR': 2.23 (singlet, $2CH_3$), 7.10 (multiplet, aromatic protons).
***δR': 2.24 (singlet, $2CH_3$), 7.13 (narrow multiplet, aromatic protons) ($CDCl_3$) 9.72 (broadened singlet, $^+_{NH}$), 10.65 (singlet, NHCO) (D-DMSO).
****In the media of $CDCl_3$ and $CDCl_3$ + — a small amount of CD_3OD, product V does not completely dissolve; the trans isomer content is ~4 times greater than the cis-isomer. In $CDCl_3$—CD_3OD (2:1) the product dissolved completely, and both spatial isomers are present in the solution at approximately equal ratios.
δR' trans ($CDCl_3$): 2.28 (singlet $2CH_3$), 7.06 (narrow multiplet, aromatic protons). 10.10 (singlet NHCO), 10.67 (broadened singlet, $^+_{NH}$).
δR'; 2.20 (singlet, $2CH_3$), 2.26 (singlet, CH_3), 6.68 (singlet, aromatic protons), ($CDCl_3$ + CD_3OD); 9.74 (broadened singlet, $^+_{NH}$), 10.69 (broadened singlet NHCO) (D-DMSO).

TABLE 2. Hydrochlorides of Substituted 6-Methylpipecolinic Acid Anilides

Hydrochlorides	Yield, %	mp, °C (from alcohol)	M^+	Empirical formula
IV	43	282—4	246	$C_{15}H_{22}N_2O \cdot HCl$
Va	28	234—6	302	$C_{19}H_{30}N_2O \cdot HCl$
Vb	14	270—2	302	$C_{19}H_{30}N_2O \cdot HCl$
VI	30	274—5	316	$C_{20}H_{32}N_2O \cdot HCl$

Cis-6-methylpipecolinic acid (I) [5], obtained by the stereospecific hydrogenation of 6-methylpipecolinic acid, was esterified by boiling with an n-butanol solution of HCl for 7 h. As in the previously described case in which we esterified piperidine carboxylic acids in the presence of cationites [2], the reaction proceeded without isomerization. However, the yield of the n-butyl ester of cis-6-methylpipercolinic acid (II) with a trans-isomer content of less than 2% was 95% in this case in comparison to the 66.3% yield which we obtained when we used the cationite KU-2×8 [1] as the catalyst. N-alkylation of the n-butyl ester of II was carried out with n-butylbromide in anhydrous toluene at 160°C. The process was accompanied by partial isomerization and the formation of a mixture of n-butyl esters of cis- and trans-1-n-butyl-6-methylpipercolinic acids (III) at a ratio of 70-80% to 30-20%. The stereochemical composition of this mixture was determined by the magnitudes of the geminal coupling constants of protons at C(2), C(3), and C(5), C(6) or by the sum of the geminal coupling constants of these protons in the NMR ^1H spectra (Table 1). The magnitudes $^3J_{6\text{-H},5\text{-H}}$, $^3J_{6\text{-H},5\text{-H}'}$ for both isomers were estimated by the spin-spin uncoupling from the 6-CH_3 group. An analysis of the geminal coupling constants values showed that in the predominant isomer the 2-H and 6-H protons were axially oriented relative to the ring and, consequently, 2-R' and 6-CH_3 in this isomer were positioned equatorially.

In the trans isomer 2R' becomes axial and 2-H becomes equatorial which causes a significant reduction in the sum of the geminal coupling constants $\Sigma|^3J_{2\text{-H},3\text{-H}} + {}^3J_{2\text{-H},3\text{-H}'}$ (9.7 Hz) in comparison to the predominant cis-isomer (13.8 Hz). Inasmuch as the sum of the geminal coupling constants for 6-H is practically unchanged during the transition from the cis-isomer to the trans ($\Sigma|^3J_{6\text{-H},5\text{-H}} + {}^3J_{6\text{-H},5\text{-H}'}| = 13.0$ Hz), hence it follows that group III 6-CH_3 in the cis-isomer is primarily equatorially oriented relative to the plane of the ring.

The corresponding 2,6-dimethyl- and 2,4,6-trimethylsubstituted anilides of 6-methylpipecolinic (IV) and 1-butyl-6-methylpipecolinic acids (V, VI) were synthesized by reacting the n-butyl esters of I and II with xylidine or mesidine in anhydrous toluene in the presence of NaH after which the resultant bases were converted to hydrochlorides. Their characteristics are given in Table 2. The stereochemistry of the hydrochlorides IV, Va, b, and VI was found, as in the case of compound III, by analyzing the PMR spectra that are shown in Table 1. Judging from the values of the geminal coupling constants of protons at C(2) and C(6), IV, Vb, and VI turned out to be cis-isomers ($\Sigma|^3J_{2\text{-H},3\text{-H}} + {}^3J_{2\text{-H},3\text{-H}'}| \sim 13\text{-}15$ H; $\Sigma|^3J_{6\text{-H},5\text{-H}} + {}^3J_{6\text{-H},5\text{-H}'} \sim 12\text{-}13$ Hz). Compound Va constituted a mixture of steric isomers: 50% cis and 50% trans. The trans-isomers were characterized by a significant reduction in $\Sigma|^3J_{2\text{-H},3\text{-H}} + {}^3J_{2\text{-H},3\text{-H}'}$ ($\sim$7.0 Hz) and somewhat of an increased $\Sigma|^3J_{6\text{-H},5\text{-H}} + {}^3J_{6\text{-H},5\text{-H}'}$ ($\sim$14 Hz) in comparison to the cis-isomer Vb. This is associated with the change in the orientational change of the substituents at C(2) (2-R' axio, 2-H equat.) and the significant retention of the substituents' orientation at C(6) (6-CH_3 equat., 6-H axio). A similar situation was noted above for the trans-isomer III as well. Thus, a significant shift in the conformational equilibrium toward the isomer 2-R'(e), 6CH_3 (a) was observed for the trans-isomers of the examined derivatives of 6-methylpipecolinic acid. This situation differs from the one that took place for the trans-isomers of 2,6-piperidine carboxylic acids and their dimethyl salts [4].

In order to examine the thermodynamic equilibrium of the cis-isomer, the hydrochloride of VI was boiled in $CHCl_3$ for 6 h, although the PMR spectra of the treated and initial products were identical. This allowed us to conclude that the thermodynamic equilibrium for the compound VI under study was practically completely shifted toward the cis-isomer.

The hydrochlorides IV-VI constitute white crystalline substances. Hydrochloride IV is readily soluble in water, and the hydrochlorides V and VI dissolve in water up to concentration of 0.5% upon prolonged heating on a water bath. The results of the pharmacological study of the anesthetic activity and acute toxicity of the synthesized compounds in comparison to marcaine, lidocaine, and dicaine are given in Table 3.

As can be seen, the examined hydrochlorides of the substituted anilides of 6-methylpipecolinic acid IV-VI exhibit pronounced local anesthetic activity by surface, infiltration, and conductive anesthesia.

The most active compounds are those containing a butyl residue (V-VI) in position 1. In determining terminal anesthesia, the analgesic activity of 0.05-0.5% aq. solutions of these compounds was reliably higher than the reference preparations marcaine and dicaine. The anesthetic action lasted 60-90 min. In these experiments the most active compound was the hydrochloride Va which constitutes a 1:1 mixture of cis- and trans-isomers; the hydrochloride of the pure Vb compound was less active but less toxic.

TABLE 3. Local Anesthetic Activity and Acute Toxicity of 6-Methylpipecolinic Acid Derivatives in Comparison to Marcaine, Lidocaine, and Dicaine

Hydrochlorides	Surface anesthesia (Regnier index; M ± m, n = 7)			Infiltration anesthesia index (M ± m; n = 8)	Conduction anesthesia (extinction time of frog reflexes, min; M ± m; n = 5)			LD_{50} mg/kg (iv)
	concentration of solutions							
	0,05 %	0,1 %	0,5 %	0,1 %	0,1 %	0,5 %	1 %	
IV	39±12	55±18	164±38	23±2	7,4±0,8	4,2±0,5	...	35 (30,2—40,6)
Va	874±68	1075±58	1240±65	33±1	3,2±0,9	1,4±0,5	...	6,8 (5,7—8,1)
Vb	669±52	968±47	1057±103	27±3	5,6±0,5	1,6±0,6	...	11,8 (10,2—13,7)
VI	604±71	814±110	...	32±3	...	...	...	14 (10,2—17,8)
Mercaine	552±150	628±60	1004±60	32±2	3,8±0,6	...	...	10,2 (9,3—11,2)
Lidocaine	...	...	...	...	6,5±2,5	...	2±0,7	37 (28,9—45,0)
Dicaine	...	673±48	1127±85	35±1	2,7±0,2	...	...	10,5

*Average values with confidence limits (p = 0.05).

The same activity characteristics of the examined compounds were retained when they were tested on infiltration and conduction anesthesia models. The most active compound, hydrochloride Va, exceeded marcaine activity and approached that of dicaine. The introduction of an additional methyl group into the para-position of the phenyl ring of the Vb cis-isomer did not significantly affect activity or toxicity.

The 6-methylpipecolinic acid derivatives did not cause any noticeable reaction by the mucous membrane of the eye in the form of hyperemia or lacrimation upon the application of 0.05-0.5% aq. solutions. A relatively high degree of toxicity constitutes a negative property of the tested compounds. The LD_{50} on white mice was 6.8 mg/kg when the most active substance was administered iv, i.e., hydrochloride Va. This was almost double the toxicity of marcaine and dicaine and significantly less than for lidocaine (37 g/kg). The toxicity of the pure cis-isomers Vb and VI was comparable to that of marcaine and dicaine. Their poor water solubility was another significant disadvantage of these compounds.

However, the high degree of local anesthetic activity in the substituted anilides of 6-methylpipecolinic acid warrants further examination of this series of compounds.

EXPERIMENTAL (CHEMICAL)

The PMR spectra were recorded on a XL-100 (200 MHz, Varian) spectrometer. $CDCl_3$ and $CDCl_3$ + CD_3OD were used as the solvent. TMS was the internal standard. The ratios of the stereoisomers in the examined samples were measured as integral intensity ratios of the least overlapping signals that respond to the various isomers.

Mass spectra were obtained on the MAT-112 (Varian) spectrometer upon the direct input of the sample into the ion source. TLC was performed in a $CHCl_3$—ethanol—ammonia (15:5:0.1) system on Silufol UV-254 plates. Detection by UV-irradiation. The values found for element analyses corresponded to the calculated values.

n-Butyl cis-6-Methylpipecolinate (II). A mixture of 50 g of cis-6-methylpipecolinic acid (I) and 500 ml of anhydrous n-butanol, saturated with dry HCl gas, was boiled for 7 h. After vacuum evaporation, 200 ml of water was added to the residue which was then made alkaline by a 50% solution of KOH, followed by extraction with $CHCl_3$ (3 × 100 ml). The extract was dried with $MgSO_4$ and vacuum evaporated. The yield was 66.0 g (95%) of the cis-n-butyl ester II. A colorless liquid with bp 94-96°C/3 mmHg [2]. According to the PMR-data the trans-isomer content was less than 2%.

n-Butyl 1-Butyl-6-methylpipecolinate (III). A mixture of 8.6 g (43.2 mmole) of cis-n-butyl ester II, 15 ml (19.03 g, 138.9 mmole) of n-butylbromide, 12 g (113.2 mmole) of anhydrous Na_2CO_3, and 40 ml of anhydrous toluene was heated for 28 h at 160°C in a stainless steel cylinder. After cooling, the cylinder was opened and the resultant precipitate was filtered off and washed with 100 ml of toluene. The combined filtrates were then vacuum evaporated.

The resultant product (10.0 g) which, according to the PMR data contained 78% of cis-III and 22% of trans-III, was converted without further treatment into the corresponding substituted anilides.

cis-6-Methylpipecolinic 2,6-Dimethylanilide HCl (IV). A 6 g portion (0.05 mole) of 2,6-xylidine followed by 12 g (0.06 mole) of II was added to a suspension of 6.0 g (0.25 mole) of NaH in 50 ml of anhydrous toluene. The mass was heated at 115-120°C for 18 h (TLC control). The mass was cooled to room temperature after which 10 ml of ethyl alcohol and 50 ml of water was added, followed by stirring for 10 min. The organic layer was separated and the aq. layer was extracted with toluene (3 × 50 ml). The toluene was evaporated to 2/3 volume and an alcohol solution of HCl was added to bring the pH to 1.0. The resultant precipitate of the IV hydrochloride was filtered off and recrystallized from ethanol. The yield was 6.1 g (43%) of substance IV with a mp of 282-284°C. According to PMR data, it contained no less than 2% of the trans-isomer IV.

1-Butyl-6-methylpipecolinic cis- and trans-2,6-dimethylanilide hydrochlorides Va, b were obtained in the same manner from 5 g (0.20 mole) of NaH, 5 g (0.04 mole) of 2,6-xylidine, and 15 g of III, containing 78% cis- and 22% trans-isomers during which the reaction proceeded for 18 h at 115-120°C in 50 ml of anhydrous toluene. After an addition of 5 ml of ethanol and 50 ml of water to the reaction mixture and further extraction with toluene (2 × 50 ml) and treatment of the dried toluene solution with an alcohol HCl solution, the resultant precipitate was 5.9 g of the hydrochloride mixture of cis- and trans-isomers of 2',6'-dimethylanilide of 1-butyl-6-methylpipecolinic acid with an mp of 234-240°C. The mixture was then boiled for 10 min with 100 ml $CHCl_3$.

The insoluble precipitate constituted a mixture of cis- and trans-isomer hydrochlorides at a 1:1 ratio and was filtered off. The yield was 4 g of Va, mp 234-235°C.

A 1.9 g portion of the pure cis-isomer Vb with mp 270-272°C was separated from the filtrate by partial evaporation.

1-Butyl-cis-6-methylpipecolinic 2',4',6'-trimethylanilide HCl (VI) was obtained in the same manner from 1.0 g (0.042 mole) of NaH, 2 g (0.015 mole) of mesidine, and 6.4 g of III (containing 78% cis- and 22% trans-isomers). The reaction proceeded for 30 h at 115-120°C in 20 ml of toluene followed by the addition of 5 ml of ethanol and 20 ml of water, extraction with toluene (3 × 20 ml), separation of the toluene solution, partial evaporation to 1/3 of the original volume, treatment with an alcohol HCl solution to pH 1.0, and recrystallization of the precipitated HCl VI from ethanol. Yield was 1.6 g of the pure VI hydrochloride with mp 274-275°C.

EXPERIMENTAL (PHARMACOLOGICAL)

Local anesthetic, local irritation, and acute toxicity of the substituted anilides of 6-methylpipecolinic acid was compared to marcaine, lidocaine, and dicaine.

Surface anesthetic activity was tested on rabbit cornea. The Regnier index was used to evaluate their activity [3]. Infiltration anesthesia was tested on guinea pigs by method [6], i.e., by subcutaneous injection of the preparations to determine the anesthetic index: a count was made of the number of painful skin irritations remaining without a reaction within a 30-min period. Conduction anesthesia was tested on frogs by method [6].

The irritating action of the preparations was judged visually in response to the substances dropped into the conjunctival sac of rabbits.

Acute toxicity was measured on white mice weighing 16-18 g upon iv administration (rate of 1 ml/min). The LD_{50} was calculated by the Litchfield–Wilcoxon method [1].

The Student criteria were used for statistical processing and the arithmetic mean error was found at $P = 0.05$.

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BIOLOGICALLY ACTIVE SUBSTANCES IN HYDRAZIDE DERIVATIVES OF SUCCINIC HETERYLAMIDES

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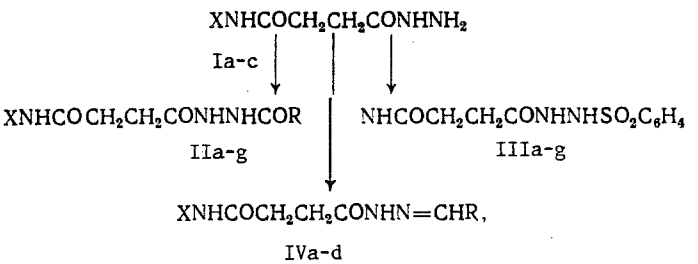
The present study was undertaken as a result of the results of our own observations [12-14] as well as the data from descriptor-statistical analysis [10] which indicated that the hydrazide derivatives of succinic heterylamides represent a promising series of substances that exhibit antiinflammatory, hydroglycemic, antimicrobial, and other pharmacological effects.

The purpose of the present study was to work out methods for the synthesis of acyl- (arensulfo- and ilidene-) hydrazides of succinic heterylamides, to examine their pharmacological properties, and identify any possible structure-pharmacological action relationships, i.e., for obtaining a theoretical basis for further research on biologically active substances.

The synthesis of the described groups of compounds was performed in accordance with the following patern:



where X = 2-(1,3,4-thiadiazolyl) (Ia, IIIa-d, IV, a, b); 5-n-propyl-2-(1,3,4-thiadiazolyl) (Ib, IIa-d); thiazolyl-2 (Ic, IId-g, IIId-h, IVc, d); R = C_2H_5 (IIa); C_3H_7(IIb, e); CH_2CH_2-COOH (IIc, f); 2-$CH_3C_6H_4$ (IId, g); 4-OCH_3 (IIIa, e); 4-$NHCOOCH_3$ (IIIb, f); 4-Cl (IIIc, g); 2-NO_2 (IIId); C_6H_5 (IVa, c); 4-OHC_6H_4 (IVb, d).

The starting hydrazides (Ia-c) were obtained by methods [4, 6]. Acylation of the hydrazides by carboxylic anhydrides and chloroanhydrides in dioxane as well as by arensulfochlorides in a pyridine medium was accompanied by the formation of the corresponding acyl- and arensulfohydrazides (IIa-g and IIIa-g, respectively). The ilidene hydrazides (IVa-d), obtained by reacting the hydrazides I and aldehydes in boiling DMFA, turned out to be the purest end products with the highest yield.

Structural and purity confirmation for the synthesized compounds was obtained by element analysis, counter synthesis, IR-spectroscopy, and chromatographic constants (Table 1).

The IR-spectra of compounds I-IV exhibited absorption bands in the 1759-1638 cm^{-1} region which corresponded to the stretching vibrations of the C=O ($\nu C{=}O$), and at 1646-1527 cm^{-1}, corresponding to the deformation vibrations of the NH (δNH) group. The bands of the asymmetric and symmetric stretching vibrations of the CH_2 group were in the 2974-2912 cm^{-1} ($\nu^{as}_{CH_2}$) range and in the 2890-2830 cm^{-1} ($\nu^{s}_{CH_2}$), whereas the deformation vibrations of the indicated group were in the 1435-1407 cm^{-1} (ρ_{CH_2}). Group C–H is characterized by shell vibrations in the 1226-1110 cm^{-1} (ν_{C-H}) range. The arensulfohydrazides IIIa-g exhibit intensive bands corresponding

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