SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF AMIRIDINE ANALOGS

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Amiridine (9-amino-2,3,5,6,7,8-hexahydro-1-cyclopenta[b]quinoline hydrochloride, hydrate) (I) is a new domestic drug which exhibits a complex of important pharmacological properties. It restores and stimulates learning and memory processes, it stimulates neuromuscular transfer, it restores excitation in the peripheral nervous system when it is blocked by various factors, and it enhances smooth muscle contractions in response to all agonists except KCl. The authors believe that a combination of two molecular mechanisms in a biologically suitable proportion underlies all of the amiridine effects, i.e., blockage of a membrane's calcium permeability and cholinesterase inhibition [3].

In that connection we felt it would be of interest to study the pharmacological activity of several amiridine derivatives. The amiridine base (Ia) was used as the starting compound for that synthesis [6]. Inasmuch as the primary chemical properties of Ia are determined by the presence of a 4-aminopyridine fragment in its structure, we investigated the reaction between the base Ia and several electrophilic reagents. Moreover, an essential question to be answered was through which reaction centers, the endocyclic nitrogen atom or the amino group nitrogen atom, do these reactions take place.

According to the literature data the endocyclic nitrogen atom in aminopyridines is more basic than the amino group nitrogen and the protonation reaction takes place on the nitrogen. The pK_a of compound Ia as determined by potentiometric titration in 50% ethyl alcohol is 9.54 and 9.12 in 4-aminopyridine [10]. The closeness of the pK_a values for both compounds indicates that the protonation of Ia also occurs on the nitrogen of the ring. The somewhat higher basicity of Ia is probably due to the donor effect of the polymethylene substituents.

Steric factors have a significant effect on the direction of reactions with halogen alkyls, such as the volume of the reagent and the spatial accessibility of the reaction center. Thus, if the quaternary salts of pyridine or quinoline are the usual products obtained by the alkylation of 4-aminopyridine and 4-aminoquinoline derivatives in a neutral medium [11, 15], then the presence of steric substituents (e.g., methyl groups) in the a,a position to the ring nitrogen atom results in a partial or complete transfer of the reaction center and the formation of 4-alkyl-amino derivatives [8]. Inasmuch as compound Ia can be viewed as a tetra-alkylsubstituted 4-aminopyridine, we felt it would be of interest to test the effect that steric factors have on the reaction center.

A slow addition of MeI to a solution of Ia in MeOH results in a quantitative yield of the hydroiodide (II) which is formed by alkylation of the nitrogen on the ring.

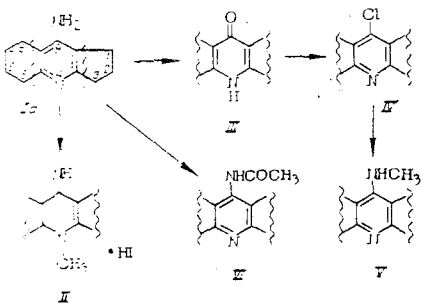

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TABLE 1. Toxicity of Amiridine Derivatives, Cholinesterase Inhibition, and Potassium Flow Block in Nerve Membrane

Compound	LD_{50}, subcutaneous injection to mice, mg/kg	TD_{50}, mg/kg	K_i acetylcholinesterase inhibition, M	IC_{50} potassium flow block, M
V	17 (14,6÷19,7)	3 (2,83÷3,18)	$2{,}98 \cdot 10^{-7}$	$2 \cdot 10^{-3}$
II	75 (65,7÷85,5)	35 (31,2÷9,2)	$7{,}1 \cdot 10^{-5}$	At a concentration M at 20%
VI	600 (512,8÷702)	—	$7 \cdot 10^{-5}$	0
III	150 (134÷168)	—	$1 \cdot 10^{-4}$	0
Ia	52 (48,6÷55,6)	30 (26÷34,5)	$1{,}47 \cdot 10^{-7}$	$5 \cdot 10^{-5}$

Note. LD_{50} is the toxic dose causing tremor in 50% of the animals; IC_{50} is the inhibitor concentration which blocks 50% of enzyme activity.

The proposed structure is suggested by the high basicity of compound II and the pK_a value which is greater than 12 when measured by potentiometric titration. High basicity is generally characteristic of the 4-iminopyridines. Thus, for example, the pK_a of 1-methyl-4-iminopyridine is 17.87 while the pK_a for the corresponding 4-amino derivatives ranges between 9 and 10 [9]. Thus, both the alkylation of Ia by MeI and the protonation reaction take place along the same reaction center, i.e., the endocyclic nitrogen atom.

In contrast to the alkylation reaction, we know that the acetylation of aminopyridines takes place on the amino group with the formation of 4-acetylaminopyridines whose formation is a possible consequence of the rearrangement of the intermediate N-acetylamidopyridine adduct. The 9-acetamido derivative (VI) is obtained when compound Ia is boiled in an excess of Ac_2O for 6 h. It is interesting that under more moderate conditions the acetylation of 9-aminoacridine results in the formation of 9,9-diacetaminoacridine. The structure of compound VI was confirmed by element analysis and spectral data. Thus, along with a molecular ion $M^{+\bullet}$230 the mass spectrum of VI exhibits a metastable peak with m/z 162 which is formed as a result of the rupture of the $NHCOCH_3$ fragment. The IR-spectrum of compound VI has characteristic absorption bands in the 1660 cm^{-1} ($\nu_{C=O\ amide}$) and 3240 cm^{-1} (ν_{NH}) region.

Thus, compound Ia can react with electrophilic reagents to form reaction products along both reaction centers of the molecule.

A closely related homolog of amiridine is the 9-methylamino derivative (V) which was synthesized by the following pattern: The oxo derivative (III) obtained by the diazotization of Ia with $NaNO_2$ in 10% H_2SO_4 [6] was converted to the corresponding 9-chloro derivative (VI) by heating III in a $POCl_3$ medium in the presence of $Et_3N \cdot HCl$ followed by the decomposition of the dichlorophosphate adduct in an aq. alkaline medium. However, the chlorine atom in compound IV turned out to be inactive so that we could replace it by a methyl group only by heating IV in a 25% aq. $MeNH_2$ medium in an autoclave at 210°C for 18 h. This is probably due primarily to the steric difficulties at the $C_{(9)}$ atom in the transfer state inasmuch the reaction between 2,6-dimethyl-4-chloropyridine and the primary amines proceeded at 150°C for 3 h [16]. The pK_a value for the 9-methylamino derivative V was 9.52, i.e., the introduction of a methyl substituent into the aromatic amino group did not significantly alter the basic properties of V in comparison to compound Ia. At the same time the big difference in the pK_a values for the isomer compounds II and V clearly confirms the structure of II as a product resulting from the alkylation of the endocyclic nitrogen atom.

EXPERIMENTAL (CHEMICAL)

IR-spectra were recorded on a Perkin-Elmer (Sweden) spectrometer in KBr pellets. The pK_a values were measured on a Radiometer type (Denmark) instrument. Mass spectra were recorded on a LKV-2091 chromatographic mass spectrometer with a direct input system and calculated on a computer on the basis of a RDR 11/34 using the MSP (Sweden) program. The found element analysis values corresponded to the calculated ones.

4-Methyl-9-amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]quinoline Iodide (II). A 1.3 ml (10 mmoles) portion of MeI was added dropwise over a three-hour period to a solution of 1 g (5.3 mmole) of Ia in 2.5 ml of MeOH at 40°C. The reaction mixture was cooled and the resultant precipitate II was filtered off. Yield was 1.6 g (92%), mp 275–276°C (from alcohol). Mass spectrum: $M^{+\cdot}$202 (base). IR-spectrum, ν_{max}, cm^{-1}: 1635 (C=N), 3170, 3285, 3320 (NH_2), $C_{13}H_{19}N_2I$.

TABLE 2. Certain Pharmacological Effects of Amiridine Derivatives

Compound	Concentration, M	Change in effect, % of control			
		motor orientation activity	duration of hexenal sleep	pain threshold	duration of arecoline hyperkinesis
V	1,7	+45±4*	+38±3,5*	+22±2,5*	+30±1,2*
	3,4	−25±2,5*	+56±2,5*	+45±0,8*	+42±1,6*
	5,1	−44±1,8*	+60±2,8*	+42±0,5*	+55±0,9*
II	17,5	+52±5*	−21±4,2	−7±0,9	+14±0,8
	15	+65±9,5*	−18±1,5	−11±0,5	+18±0,9*
VI	120,0	+12±1,8	−12±1,5	−7±0,9	+25±3,5*
III	220,0	+31±4,5*	−15±0,8	−15±0,8*	−28±1,5
Ia	2,6	+22±1,8*	−29±0,8*	+19±0,3*	+65±1,9*
	5,2	+32±2,5*	−25±1,5*	+20±0,8*	+70±2,8*

*Difference from control is statistically reliable.

TABLE 3. Effect of Amiridine Derivatives on Neuromuscular Transfer and Contractile Responses of Smooth Muscle Organs

Compound	Concentration, M	Change in effect, % of control					
		frog abdominal rectus		guinea pig intestine		rat uterus	
		contraction amplitude (agonist – acetylcholine)	duration	contraction amplitude (agonist – acetylcholine)	duration	contraction amplitude (agonist – serotonin)	duration
V	$1\cdot10^{-4}$	+35±3,5*	52±5*	30±5*	52±1,8*	55±4*	68±5,6*
	$1\cdot10^{-6}$	18±0,9*	29±2,6*	10±2,1	38±1,5*	26±2*	44±2,8*
II	$1\cdot10^{-4}$	10±1,8	15±0,9	12±2,5	15±2,2	12±1,5	8±0,7
VI	$1\cdot10^{-4}$	0	0	0	0	10±0,8	0
III	$1\cdot10^{-4}$	0	0	0	0	0	0
Ia	$1\cdot10^{-4}$	85±4,5*	180±11*	29±6,9*	95±10*	112±11*	125±12*
	$1\cdot10^{-6}$	40±2,5*	70±8*	50±3,8*	50±4*	43±3*	60±3,5*

*Difference from control is statistically reliable.

9-Chloro-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]quinoline (IV). A mixture of 1 g (5.3 mmoles) of III and 0.5 g of $Et_3N\cdot HCl$ in 10 ml of $POCl_3$ was stirred at 60°C for 40 min. The excess $POCl_3$ was vacuum filtered from the mixture and 100 ml of an aq. solution of $NaHCO_3$ was added to the residue upon cooling. The resultant solution was extracted with $CHCl_3$ and the combined chloroform extracts were evaporated, after drying with Na_2SO_4, to dryness. The residue was titrated with water and the resultant precipitate IV was filtered off to yield 1.0 g (91%) of IV, mp 38-39°C (from a 1:1 water—alcohol mixture). Mass spectrum: $M^{+\cdot}$207.5. IR-spectrum, ν_{max}, cm^{-1}: 1590, 1655, (arom. C=C, C=N), $C_{12}H_{14}NCl$.

9-Methylamino-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]quinoline (V). A 1.6 g (7.7 mmoles) portion of III was kept at 210°C in an autoclave in a mixture of 5 ml of alcohol and 25 ml of 25% aq. $MeNH_2$ for 18 h. The alcohol was distilled off the reaction mixture and the precipitate V was filtered off to yield 1.5 g (97%) of compound V, mp 202-203°C (from i = PrOH). Mass spectrum: $M^{+\cdot}$202. IR-spectrum ν_{max}, cm^{-1}: 1570, 1610 (arom. C=C, C=N), 3720 (NH), $C_{13}H_{18}N_2$.

9-Acetamido-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]quinoline (VI). A 0.96 g (5 mmoles) portion of compound I was boiled in 2 ml of Ac_2O for 6 h. After cooling, 20 ml of water and a 20% aq. alkaline solution was added to the reaction mixture to bring it to pH 10. The aq. layer was extracted with $CHCl_3$. After drying with Na_2SO_4 the chloroform was evaporated to dryness. Yield of VI was 0.6 g (52%), mp 241-242°C (from ethylacetate). Mass spectrum: $M^{+\cdot}$ 230. IR-spectrum, ν_{max}, cm^{-1}: 1570, 1600 (arom. C=C, C=N), 1660 (C=O), 3240 (NH), $C_{14}H_{18}N_2O$.

The synthesized analogs of amiridine possessed the same type of activity as amiridine itself but with a lesser degree of activity in all of the tests. The most active derivative was 9-methylamino-2,3,5,6,7,8-hexahydro-1H-cyclopenta[b]quinoline (V) although it was more toxic than amiridine (Tables 1-3). Its sedative and analgesic

components were more prominent in its spectrum of activity. This was manifested in the ability to reduce the motor orientation of mice activity, to prolong hexenal sleep, and to increase the pain threshold of mice. In small doses (5-10 mg/kg) the substance induced signs of m-cholinoreactive system stimulation in the form of tremors and diarrhea. This was also indicative of the substance's high toxicity and the degree of its side and m-cholinomimetic effects. This might be associated with its effect on cholinesterase ($K_i = 2.98 \cdot 10^{-7}$). It resembles amiridine Ia with respect to this property.

EXPERIMENTAL (PHARMACOLOGICAL)

Acute toxicity of the compounds was tested by a single subcutaneous injection to the mice by method [14]. A set of test [4, 2] was used to study the effect the substances had on animal behavior as well as the effects of central mediator system stimuli. The effect on neuromuscular transfer was examined on frog abdominal rectus muscle and on an isolated neuromuscular mice preparation (*n. peroneus—m. extensor digitorum longus*).

Muscle contraction was recorded on a KSP-4 automatic recording device, employing tension sensors, a TU-4M tensor amplifier and a LPU-01 transformer device [5, 7].

The effect the substances had on isolated smooth muscle objects was tested on strips of uterine tissue, rat seminal vesicles, and guinea pig ilium by the standard method. Muscle contractions were recorded with a 6M × 2B mechanotron. Acetylcholine, adrenalin, serotonin, and histamine were used as the agonists.

The substances' effect on human blood acetylcholinesterase activity was tested by method [12]. The principle of this method is that an enzyme from a modified substrate of acetylcholine actuates the release of thiocholine which upon reacting with a chromogene of dithiobisnitrobenzoic acid (DTNB) yields a yellow-colored 2-nitro-5-mercaptobenzoate. At $\lambda = 412$ nm, the optical density increase is proportional to the formation of the enzyme reaction product. The kinetic constants of the enzyme (Michaelis constant and maximum enzyme reaction rate) were measured by the double inverse coordinates method, and the constants for enzyme inhibition by chemical substances were measured by the Deacon method [1].

The effect of the substances on ionic flow in the node of Ranvier membrane of the frog sciatic nerve was studied by the potential fixation method [13].

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