A comparison of the strength of the hypocholesterolemic effect of cholesterol oxidase and the atherosclerotic preparation miscleron in the case of their brief administration shows that cholesterol oxidase surpasses miscleron in effectiveness in the experiments. This provides a basis for considering the cholesterol oxidase produced by Act. lavendulae as a potential hypocholesterolemic agent.

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SYNTHESIS AND STUDY OF α-PIPERIDINOACETANILIDES AS MONOAMINE OXIDASE INHIBITORS

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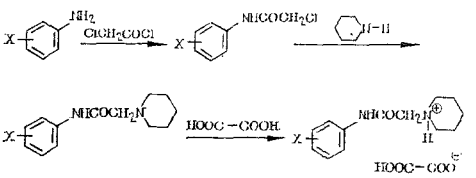
In studies on monoamine oxidase [MAO; monoamine: oxygen – oxidoreductase (deaminating), EC 1.4.3.4] and its active center, reversible competing inhibitors are very important [1]. However, only MAO inhibitors of other types have been studied in detail: quantitative structure–activity relationships have been established in individual classes [2, 3] and among different classes [3, 4]. A similar analysis with reversible MAO inhibitors is very difficult, because this type of inhibitors has been less studied, and data available in the literature refer to different test systems.

We have already found [5] that certain α-piperidinoacetanilides are inhibitors of the MAO enzyme. In the present work, it has been shown on several examples that these are of the reversible competing type (see Fig. 1).

To obtain the most active MAO inhibitor in this series, we varied in a predetermined way the substituent X, using Topliss schemes [6, 7]. The synthesis was carried out by the following general scheme:



α-Piperidinoacetanilides were isolated and purified in the form of oxalates. We synthesized 19 compounds, and found that the substituents for influencing the activity are X = 4-OC_6H_5, 4-$OCH_2C_6H_5$, 4-cyclo-C_6H_{11}, 3,4-Cl_2, 2,4-Cl_2 (see Table 1).

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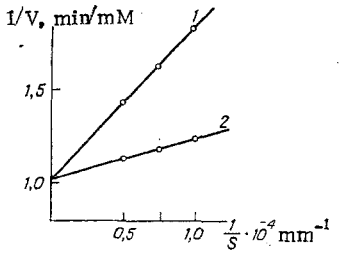

Fig. 1. Lineweaver–Burk plot of the enzymatic reaction in the presence of α-piperidino-3-chloroacetanilide inhibitor (1) and in its absence (2).

TABLE 1. Degree of Inhibition of Deamination Reaction of Tyramine by α-Piperidinoacetanilides and Physicochemical Parameters of Substituents X

Compound	X	α, % 10^{-3} M	α, % 10^{-4} M	α, % 10^{-5} M	β, 10^{-3}	π	MR	F_p	R_p	F_m	R_m
I	H	15,0	12,0	1,0	—1,73	0,00	30,55	0,00	0,00	0,00	0,00
II	4-CH_3	45,3	20,5	2,0	—0,19	0,56	35,17	—0,04	—0,13	0,00	0,00
III	4-Cl	36,5	22,0	11,0	—0,53	0,71	35,55	0,41	—0,15	0,00	0,00
IV	4-OCH_3	27,5	19,0	9,5	—0,94	—0,02	37,39	0,26	—0,51	0,00	0,00
V	3,4-Cl_2	70,0	21,0	1,0	0,85	1,25	40,55	0,41	—0,15	0,41	—0,15
VI	4-NO_2	30,0	12,0	1,0	—0,85	—0,28	36,88	0,67	0,16	0,00	0,00
VII	4-I	48,5	20,0	2,0	—0,06	—1,12	43,46	0,40	—0,19	0,00	0,00
VIII	4-Br	45,0	20,0	14,0	—0,20	0,86	38,40	0,44	—0,17	0,00	0,00
IX	4-OC_6H_5	88,0	66,0	26,0	1,99	2,08	57,20	0,34	—0,35	0,00	0,00
X	4-OC_4H_9	51,0	27,0	10,0	0,04	1,55	51,18	0,25	—0,55	0,00	0,00
XI	4-C_6H_5	24,0	14,0	8,0	—1,15	1,96	54,88	0,08	—0,08	0,00	0,00
XII	3-I	36,0	9,5	1,0	—0,58	1,12	43,46	0,00	0,00	0,40	—0,19
XIII	3-Br	42,0	24,0	7,0	—0,32	0,86	38,40	0,00	0,00	0,44	—0,17
XIV	3-OCH_3	45,0	18,0	4,0	—0,2	—0,02	37,39	0,00	0,00	0,26	—0,51
XV	3-Cl	32,0	15,0	7,0	—0,75	0,71	35,55	0,00	0,00	0,41	—0,15
XVI	4-$OCH_2C_6H_5$	68,0	34,0	18,0	0,75	1,66	61,71				
XVII	4-cC_6H_{11}	73,0	34,0	13,0	0,99	2,15	56,21				
XVIII	2,4-Cl_2	66,0	25,0	19,0	0,66						
XIX	2,6-Cl_2	25,0	10,0	6,0	—1,10						

TABLE 2. Regression Equations Found by the Hansch Method and by the Nonlinear Correlation Method

n	r	S	F_{exp}	F_{crit} R=0,05	a_0 (Δa_0)	a_1 (Δa_1)	a_2 (Δa_2)	a_3 (Δa_3)
$\beta=(a_0 \pm \Delta a_0) + (a_1 \pm \Delta a_1)\,\pi + (a_2 \pm \Delta a_2)\,F_p + (a_3 \pm \Delta a_3)\,R_m$								
15	0,57	0,74	6,12	4,67	—0,88 (0,42)	0,69 (0,28)		
15	0,64	0,72	4,12	3,89	—1,13 (0,60)	0,69 (0,27)	1,15 (0,86)	
15	0,74	0,66	4,4	3,59	—1,58 (0,69)	0,81 (0,26)	1,9 (0,88)	—2,6 (1,4)
$\beta=(a_0 \pm \Delta a_0) + (a_1 \pm \Delta a_1)\,\pi \cdot F_p + (a_2 \pm \Delta a_2)\,R_m \cdot F_m + (a_3 \pm \Delta a_3)\,\pi \cdot R_p$								
15	0,77	0,56	18,82	4,67	—0,77 (0,26)	2,59 (0,60)		
15	0,82	0,54	12,26	3,89	—0,98 (0,34)	2,85 (0,58)	—6,07 (3,6)	
15	0,84	0,53	9,1	3,59	—1,1 (0,51)	2,15 (0,8)	—7,2 (3,6)	—1,0 (0,8)

TABLE 3. Properties of α-Chloroacetanilides

Compound	X	Yield, %	mp, °C		Reference
			found	according to literature data	
XX	H	72	134—5	134—6	[12]
XXI	4-CH_3	82	162—5	165—7	[12]
XXII	4-Cl	98	168—70	170	[20]
XXIII	4-OCH_3	88	120	119,5	[21]
XXIV	3,4-Cl_2	98	103—5	106—7	[22]
XXV	4-NO_2	78	180—2	182—4	[23]
XXVI	4-I	86	190—3	191—4	[21]
XXVII	4-Br	83	180	178—80	[24]
XXVIII	4-OC_6H_5	84	100—3	99—101	[25]
XXIX	4-OC_4H_9	82	132	133	[17]
XXX	4-C_6H_5	57	175—80	175—8	[25]
XXXI	3-I	82	110—15	120—2	[26]
XXXII	3-Br	78	112—4	114	[27]
XXXIII	3-OCH_3	74	90—3	90—2	[21]
XXXIV	3-Cl	76	96—8	100—2	[18]
XXXV	4-$OCH_2C_6H_5$	75	145—6	149	[19]
XXXVI*	4-cC_6H_{11}	81	136—9	—	—
XXXVII	2,4-Cl_2	83	102—4	100—2	[26]
XXXVIII†	2,6-Cl_2	84	176—8	—	—

*Found, %: C 66.37; H 7.03; Cl 14.64; N 5.14. $C_{14}H_{18}ClNO$.
Calculated, %: C 66.80; H 7.16; Cl 14.12; N 5.57.
†Found, %: C 40.42; H 2.19; Cl 44.03; N 5.67. $C_8H_6Cl_3NO$.
Calculated, %: C 40.25; H 2.25; Cl 44.65; N 5.87.

TABLE 4. Properties of α-Piperidinoacetanilides

Compound	X	Yield, %	mp, °C		Reference	mp of oxalate, °C
			found	according to literature data		
I	H	85	95—7	95—6	[12]	170—2
II	4-CH_3	80	66—8	67—9	[12]	186—8
III	4-Cl	86	82—4	86—8	[13]	174—7
IV	4-OCH_3	78	31	34	[14]	170—2
V	3,4-Cl_2	77	70—2	69—74	[15]	202—4
VI	4-NO_2	97	140—2	141—3	[12]	222—4
VII	4-I	91	78—80	80	[13]	198—200
VIII	4-Br	84	86—8	85—8	[13]	180—3
IX	4-OC_6H_5	78	78—80	80	[16]	173—5
X*	4-OC_4H_9	77††	—	115	[17]	130—2
XI	4-C_6H_5	76	103—5	108—10	[13]	186—9
XII†	3-I	74††	—	—	—	180—2
XIII	3-Br	81	74—6	75	[18]	183—6
XIV	3-OCH_3	86	50—2	53	[18]	200—3
XV	3-Cl	72	74—6	76	[18]	178—9
XVI	4-$OCH_2C_6H_5$	89	154—6	156	[19]	170—2
XVII‡	4-cC_6H_{11}	88	119—21	—	—	193—6
XVIII**	2,4-Cl_2	83	90—4	—	—	198—200
XIX	2,6-Cl_2	86	128—30	132	[15]	192—5

*Found, %: C 60.62; H 7.24; N 7.38. $C_{19}H_{28}N_2O_6$.
Calculated, %: C 60.00; H 7.37; N 7.37.
†Found, %: C 41.47; H 4.38; I 29.26; N 6.45. $C_{15}H_{19}IN_2O_5$.
Calculated, %: C 41.83; 4.49; I 18.83; N 6.91.
‡Found, %: C 64.62; H 7.18; N 7.18. $C_{21}H_{30}N_2O_5$.
Calculated, %: C 64.39; H 7.89; N 7.80.
**Found, %: C 47.63; H 4.94; Cl 18.57; N 7.79. $C_{15}H_{18}Cl_2N_2O_5$.
Calculated, %: C 47.75; H 4.77; Cl 18.83; N 7.43.
††Yield calculated for oxalate.

To clarify the interrelationship between the structure and the biological activity of compounds, we used the correlation method of Hansch [8] and also the approach developed by Schwarz and Barth [9].

To analyze the structure–biological activity interrelationship, the latter was expressed in β-units proportional to a change in the free energy in the inhibition process: $\beta_i = -(100\%/\alpha_i - 1)$, where α is the degree of inhibition of the deamination reaction of tyramine.

To estimate the effect of substituent X, we used the physicochemical parameters, π (hydrophobic parameter), MR (molar refraction), as well as electronic parameters R_m, R_p (resonance effect), and F_m, F_p (field effect) [10]) (see Table 1).

The results of the Hansch correlational method are listed in Table 2.

Since the biological activity can be correlated nonlinearly with the parameters of the substituents, and we did not have any specific suggestions on the analytical form of this dependence, we searched for regression equations using the concept of the perturbation effect of the substituent on the properties of the compounds [9] in which terms of a Taylor series to a second power, inclusively, with respect to the above parameters are used. In both approaches stepwise linear regression analysis [11] was used to derive the regression equations.

Table 2 shows that the inhibiting power of the compounds increases with increase in the hydrophobicity and electron acceptor properties of the substituent. Since with increase in one factor another usually decreases, it can be assumed that the molecules of the inhibitor react with at least two receptors of the enzyme molecule, hydrophobic and nucleophilic, respectively. The last assumption follows from the fact that the introduction of optimal substituents, such as X = 3,4-Cl_2, 2,4-Cl_2, 2,6-Cl_6, 4-OC_6H_5, 4-$OCH_2C_6H_5$, leads to a decrease in the electron density on the nitrogen atoms.

EXPERIMENTAL

Preparation of α-Chloroacetanilides. A 0.1-mole portion of substituted aniline is dissolved in 50-100 ml of anhydrous dioxane, and 0.1 mole of sodium bicarbonate is added. The mixture is cooled by cold water, and 0.13 mole of chloroacetyl chloride is added slowly. During the reaction the precipitate of bicarbonate dissolves and sodium chloride is formed. The mixture is stirred for 30 min and poured into 250-300 ml of water. The precipitate is filtered and recrystallized from dilute alcohol (Table 3).

Preparation of α-Piperidinoacetanilides. A 0.1-mole portion of the corresponding α-chloroacetanilide is dissolved in 80-100 ml of anhydrous acetone, and 0.3 mole of piperidine is added. The mixture is boiled for 2 h. Piperidine hydrochloride is filtered, and the filtrate is poured into 200 ml of water. Solid α-piperidinoacetanilides are filtered, and the oily products extracted with ether; the extract is dried over anhydrous sodium sulfate and ether distilled.

From the α-piperidinoacetanilides obtained, without further purification, oxalates are prepared by mixing an ether solution of anhydrous oxalic acid with an ether solution of the corresponding α-piperidinoacetanilide (Table 4).

The anti-MAO activity of the inhibitors was determined under in vitro conditions by the method already described in [28] using mitochondria from swine liver as the enzyme preparation, tyramine hydrochloride at a concentration of 10^{-2} M as substrate, and final concentrations of the inhibitors of 10^{-3}, 10^{-4}, and 10^{-5} M. The rate of the enzymatic reaction was determined from the amount of ammonia evolved, and the activity of the inhibitors was judged from the degree of inhibition of the deamination reaction of tyramine $\alpha = [(V - V_1)/V] \cdot 100\%$, where V_1 is rate of the deamination reaction in the presence of inhibitor, and V is the rate of deamination in the absence of inhibitor.

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