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INFLUENCE OF CYCLOPROPYLETHYL-CONTAINING AMINES AND AMIDES OF THE ISOENZYME FORMS OF RAT LIVER ALDEHYDE DEHYDROGENASE

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Compounds whose structure includes cyclopropyl rings possess a broad spectrum of biological activity: insecticidal, herbicidal, analgesic, neuroleptic, bactericidal [7], and also antialcohol. Such activity is possessed by N^5-(1-hydroxycyclopropyl)-L-glutamine (coprin), isolated from the fungus *Coprinus atramentarius* [9, 10]. Its action, like that of a number of well-known drugs used for the treatment of chronic alcoholism [5], involves inhibition of the liver aldehyde dehydrogenase (AldDH, EC 1.2.1.3) activity and the appearance of a negative conditioned reflex to ethanol.

Biochemical investigations have been conducted on the action of coprin and its putative metabolites on isoforms of liver AldDH. It is suggested that coprin is metabolized in the organism to aminocyclopropanol, which is unstable in the form of the free base and undergoes a number of rapid conversions, forming reactive cyclopropanone and cyclopropanone hydrate [11, 14].

Coprin did not inhibit partially purified mouse liver AldDH *in vitro* [13, 14]. Aminocyclopropanol hydrochloride in a concentration of 0.1 mM, when preincubated for 10 min with the enzyme, virtually completely suppressed the enzyme activity (the residual activity was 5-8%) [13]. The interaction of this compound with rat liver AldDH is a two-step process: Reversible binding of the enzyme to the inhibitor with an inhibition constant 63 μM is followed by an irreversible conversion of the intermediate complex to a covalent complex with inactivation constant $2.8 \cdot 10^{-3}$ sec^{-1}. The presence of a cofactor of the reaction, NAD^+, promoted inactivation by cyclopropanol. The authors suggested that the binding of NAD^+, promotes electrophilic attack of aminocyclopropanol on thiol groups [11]. Cyclopropanone hydrate is a less effective inhibitor of AldDH, since the activity of the enzyme is decreased by only 33% after preincubation for 50 min [14].

Preliminary administration of coprin *in vivo* [13] in doses of 9, 27, and 81 mg/kg, followed by administration of ethanol in a dose of 1.5 g/kg after 24 h led to an increase in the acetaldehyde level in the blood by a factor of 12, 24, and 49, respectively. The AldDH activity with high affinity for the substrate was maximally decreased after 6 h, and inhibition came to 54, 86, and 92% for the indicated doses. 1-Aminocyclopropanol had a similar effect on the acetaldehyde metabolism, but a more rapid inhibition of the enzyme with high affinity for the substrate was observed.

To detect antialcohol activity, rats were given compounds structurally related to pargyline (N-methyl-N-propargylbenzylamine [12]) in which the propargyl group was substituted. Only the compound whose structure possessed a cyclopropyl ring was an effective inhibitor and inhibited the liver AldDH activity by 70% 2 h after administration.

In this work we synthesized cyclopropylethyl-containing amines and amides and studied their effects on one of the key enzymes of ethanol conversion — rat liver mitochondrial AldDH.

N-Benzyl- and N-phenyl-N-(1-cyclopropylethyl)amines were synthesized according to the procedure of [2] in two steps: production of Schiff's bases from methyl cyclopropyl ketone and benzylamine or aniline, followed by reduction by sodium in isopropanol:

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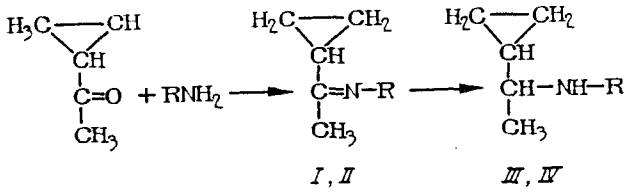
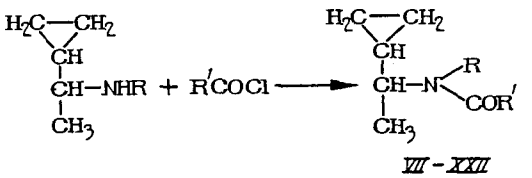


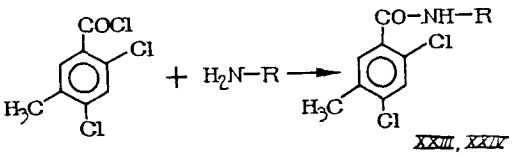
where R = $C_6H_5CH_2$ (I, III); C_6H_5 (II, IV).

Amides of N-substituted N-(1-cyclopropylethyl)amines were synthesized by acylation of the amines by acid chlorides in indifferent solvents in the presence of a hydrogen chloride acceptor:



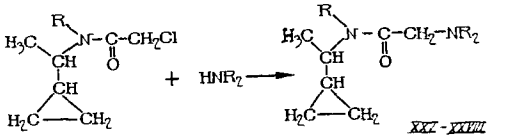
where R = $C_6H_5CH_2$ (VII, XII-XVI), C_6H_5 (VIII-XI, XVII, XVIII), $C_6H_4OCH_3$-4 (XIX, XX), H (XXI, XXII); R′ = CH_3CH_2 (VII, VIII), p-$H_2NC_6H_4$ (IX), p-ClC_6H_4 (X), 3-pyridyl (XI), p-FC_6H_4 (XII), o-$CH_3OC_6H_4$ (XIII), m-FC_6H_4 (XIV), $C_6H_3Cl_2$-2,4 (XV), $C_6H_2Cl_2$-2,4-CH_3-5 (XVI, XVII, XIX, XXI), $C_6H_2Cl_2$-2,5-CH_3-4 (XVIII, XX, XXII).

To study the influence of individual fragments of the amides synthesized as inhibitors, the corresponding amides were produced by the reaction of 2,4-dichloro-5-methylbenzoyl chloride with ammonia and with benzylamine:



where R = H (XXIII), $CH_2C_6H_5$ (XXIV).

N-(1-Cyclopropylethyl)-amides of 2-substituted acetic acid were produced by the reaction of the corresponding amides of chloroacetic acid with piperidine and morpholine.



where R = $C_6H_5CH_2$ (XXV, XXVI), H (XXVII, XXVIII); NR_2 = piperidino (XXV, XXVII), morpholino (XXVI, XXVIII).

The characteristics of the compounds synthesized are presented in Table 1. The structure of all the compounds synthesized was confirmed by the data of elementary analysis and IR spectroscopy. The IR spectra of the compounds obtained had characteristic absorption bands 1640-1690 cm^{-1} (CO), as well as 1020-1030 cm^{-1}, belonging to the three-membered ring of cyclopropyl-containing compounds. The degree of purity of the compounds synthesized is 98-99% according to the data of gas–liquid chromatography and thin-layer chromatography.

EXPERIMENTAL (CHEMICAL)

N-Benzyl- and N-Phenyl-N-(1-cyclopropylethylidene)amines (I, II). To a solution of 1 mole of methyl cyclopropyl ketone in 250 ml of toluene we added 1 mole of benzylamine or aniline. The reaction mixture was boiled with azeotropic

TABLE 1. Yields and Constants of the Compounds Synthesized

Compound	Yield, %	mp, °C, bp °C/Pa	Gross formula
I	71	94—5/266	$C_{12}H_{15}N$
II	70	87—9/266	$C_{11}H_{13}N$
III	92	89—90/266	$C_{12}H_{17}N$
IV	80	86—7/266	$C_{11}H_{15}N$
V	99	157—8	$C_{12}H_{18}ClN$
VI	99	163—5	$C_{11}H_{16}ClN$
VII	89	122—3/67	$C_{15}H_{21}NO$
VIII	81	97—97,5/67	$C_{14}H_{19}NO$
IX	97	124—5	$C_{18}H_{20}N_2O$
X	97	95—6	$C_{18}H_{18}ClNO$
XI	51	73—4	$C_{17}H_{18}N_2O$
XII	93	192—3/133	$C_{19}H_{20}FNO$
XIII	76	202—3/133	$C_{20}H_{23}NO_2$
XIV	87	176—7/133	$C_{19}H_{20}FNO$
XV	91	77—9	$C_{19}H_{19}Cl_2NO$
XVI	73	64—5	$C_{20}H_{21}Cl_2NO$
XVII	84	90—1	$C_{19}H_{19}Cl_2NO$
XVIII	89	139—40	$C_{19}H_{19}Cl_2NO$
XIX	97	31—3	$C_{20}H_{21}Cl_2NO$
XX	91	34—5,5	$C_{20}H_{21}Cl_2NO$
XXI	91	162—3	$C_{13}H_{15}Cl_2NO$
XXII	93	160—2	$C_{13}H_{15}Cl_2NO$
XXIII	99	190—1	$C_8H_7Cl_2NO$
XXIV	98	166—166,5	$C_{15}H_{13}Cl_2NO$
XXV	99	172—4/133	$C_{19}H_{28}N_2O$
XXVI	73	185—6/133	$C_{18}H_{26}N_2O_2$
XXVII	81	36—7	$C_{12}H_{22}N_2O$
XXVIII	82	72—3	$C_{11}H_{20}N_2O_2$

distillation of water until its evolution ceased. Then the toluene was distilled off, and the residue was redistilled at reduced pressure. In the production of compound II, a catalytic amount of anhydrous zinc chloride was added to the reaction mixture.

N-Benzyl- and N-Phenyl-N-(1-cyclopropylethyl)amines (III, IV). To a solution of 0.2 mole of the Schiff base I or II in 300 ml of isopropanol, metallic sodium was added in small portions with mixing and heating to boiling, in amounts exceeding the theoretical by a factor of 4-5. After the sodium was completely dissolved, the reaction mixture was poured out into a fourfold amount of water, the product was extracted with hexane, the hexane extract was dried with potash, the hexane was distilled off, and the product was isolated by fractionation at reduced pressure.

Hydrochlorides of N-Benzyl- and N-Phenyl-N-(1-cyclopropylethyl)amines (V, VI). Dry hydrogen chloride was passed into a solution of 0.01 mole of the amine (III, IV) in 20 ml of diethyl ether with mixing. The precipitate formed was filtered off and recrystallized from ethanol.

N-Benzyl- and N-Phenyl-N-(1-cyclopropylethyl)amides (VII, VIII, X-XIV). To a solution of 0.05 mole of the amine (III or IV) and 0.05 mole triethylamine in 30 ml of hexane, a solution of 0.05 mole of the corresponding acid chloride in 30 ml of hexane was added dropwise with vigorous mixing. After the end of the addition of the acid chloride, the reaction mixture was mixed for another 30-40 min at 55-60°C, cooled to room temperature, and the triethylamine hydrochloride was removed by filtration. Hexane was distilled off from the filtrate. The product was isolated by redistillation at reduced pressure or by crystallization.

N-Phenyl-N-(1-cyclopropylethyl)amide of p-Aminobenzoic Acid (IX). To a solution of 0.05 mole of the N-phenyl-N-(1-cyclopropylethyl)amide of p-nitrobenzoic acid, produced according to the procedure described above, in 150 ml of ethanol we added 2.5 ml of concentrated hydrochloric acid. The mixture was heated to boiling, and 10.7 g of powdered iron was added in portions with vigorous mixing, then the mixture was boiled with mixing for 2.5 h. The reaction mass was cooled, the precipitate was filtered off, and it was washed on the filter with 50 ml of hot ethanol. Alcohol was distilled off from the filtrate, and the product was isolated by crystallization from hexane.

N-(1-Cyclopropylethyl)-N-benzylamide of 2,4-Dichlorobenzoic and 2,4-Dichloro-5-methylbenzoic Acids (XV, XVI). To a solution of 0.028 mole of N-(1-cyclopropylethyl)-N-benzylamine in 20 ml of hexane we added 0.028 mole of triethylamine, and a solution of 0.028 mole of 2,4-dichlorobenzoyl chloride or 2,4-dichloro-5-methylbenzoyl chloride in 10 ml of hexane was added with vigorous mixing, then the mixture was mixed for 0.5 h and triethylamine hydrochloride was filtered off; hexane was distilled off from the filtrate, and the residue was redistilled at reduced pressure and crystallized from hexane.

TABLE 2. Influence of Cyclopropylethyl-Containing Amines and Amides on the Activity of Various Forms of Mitochondrial AldDH (in nmoles of NADH per mg protein per min; M ± m; n = 6)

Compound	AldDH-I activity			AldDH-II activity		
	without inhibitor	with inhibitor	% inhibition	without inhibitor	with inhibitor	% inhibition
III	8,7±0,5	8,7±1,0	0	19,6±2,0	21,7±1,1	0
IV	8,7±0,5	8,7±1,4	0	19,9±2,0	20,3±1,7	0
V	8,7±0,5	9,1±0,9	0	19,9±2,0	21,2±1,8	0
VI	8,7±0,5	9,4±1,1	0	19,9±2,0	19,8±1,7	0
VII	8,7±0,5	9,2±1,1	0	19,9±2,0	18,9±1,8	5
VIII	8,7±0,5	10,4±1,3	0	19,9±2,0	17,9±3,1	10
IX	6,0±0,8	5,7±0,5	5	30,0±2,8	26,8±2,6	3
X	10,61±0,67	7,82±0,82	25	33,88±1,98	32,27±1,71	8
XI	6,0±0,8	4,5±0,5	30	30,0±2,8	28,3±2,5	6
XII	10,61±0,67	7,70±1,35	27	33,88±1,98	32,91±1,94	8
XIII	10,61±0,67	6,28±1,03*	41	33,88±2,01	29,04±2,44	14
XIV	10,61±0,67	6,4±1,18*	40	33,88±2,01	33,88±2,44	0
XV	7,02±0,83	3,87±0,68*	45	20,33±2,01	13,88±1,71	32
XVI	7,47±1,01	2,53±1,29*	66	19,08±2,78	10,97±1,05*	40
XVII	7,47±1,01	3,50±0,91*	53	18,09±2,78	10,62±1,03*	≤1
XVIII	7,47±1,01	2,77±1,48*	63	18,08±2,78	11,69±1,42*	34
XIX	7,47±1,01	2,18±0,57*	71	18,08±2,78	10,13±2,36*	44
XX	7,47±1,01	2,77±0,93*	62	18,08±2,78	10,91±2,43*	40
XXI	7,47±1,01	5,75±0,91	23	18,08±2,78	12,05±1,44	33,3
XXII	7,47±1,01	6,14±0,82	18	18,08±2,78	13,26±1,73	27
XXIII	8,6±0,9	7,22±0,8	2	14,75±2,1	14,2±1,8	4
XXIV	8,6±0,9	8,6±1,0	0	14,75±2,1	15,65±3,30	11
XXV	10,61±0,67	5,42±0,64*	49	33,88±2,01	29,04±1,12	14
XXVI	10,61±0,67	6,05±0,64*	43	33,88±2,01	30,33±4,23	10
XXVII	10,61±0,67	3,80±1,26*	64	33,88±2,01	32,27±1,96	5
XXVIII	10,61±0,67	6,87±0,65*	35	33,88±2,01	30,33±1,98	10

*The differences from the corresponding control are significant at $p \leq 0.05$.

N-(1-Cyclopropylethyl)-N-phenylamides of 2,4-Dichloro-5-methyl- and 2,5-Dichloro-4-methylbenzoic Acids (XVII, XVIII). They were produced analogously from N-(1-cyclopropylethyl)-N-phenylamine and 2,4-dichloro-5-methyl- and 2,5-dichloro-4-methylbenzoyl chlorides.

N-(1-Cyclopropylethyl)-N-(4-methoxyphenyl)amide of 2,4-Dichloro-5-methylbenzoic Acid (XIX). To a solution of 0.025 mole of N-(1-cyclopropylethyl)-N-(4-methoxyphenyl)amine in 20 ml of hexane we added 0.025 ml of triethylamine, and a solution of 0.025 mole of 2,4-dichloro-5-methylbenzoyl chloride in 10 ml of hexane was added with vigorous mixing. After the end of the addition of the entire amount of the acid chloride, the reaction mixture was mixed for 0.5 h at 50°C, then cooled, the triethylamine hydrochloride was filtered off, hexane was distilled off from the filtrate, and the residue was redistilled at reduced pressure and crystallized from hexane.

N-(1-Cyclopropylethyl)-N-(4-methoxyphenyl)amide of 2,5-Dichloro-4-methylbenzoic Acid (XX). It was produced analogously from N-(1-cyclopropylethyl)-N-(4-methoxyphenyl)amine and 2,5-dichloro-4-methylbenzoyl chloride.

N-(1-Cyclopropylethyl)amides of 2,4-Dichloro-5-methyl- and 2,5-Dichloro-4-methylbenzoic Acids (XXI, XXII). To a solution of 0.023 mole of 1-cyclopropylethylamine in 40 ml of water we added an equimolar amount of NaOH, and 0.023 mole of the corresponding acid chloride in 40 ml of benzene was slowly added dropwise with vigorous mixing to the mixture obtained; then the reaction mixture was mixed for 0.5 h at 50-55°C, cooled, and the residue was filtered off; the benzene layer was separated from the filtrate, washed with water to a neutral pH, and dried over K_2CO_3; the drying agent was filtered off, and the filtrate was evaporated. The residue was purified by crystallization from hexane.

2,4-Dichloro-5-methylbenzylamide (XXIII). It was produced by treatment of 2,4-dichloro-5-methylbenzoyl chloride with a 25% aqueous solution of ammonia. The residue was filtered off and crystallized from ethanol.

N-Benzylamide of 2,4-Dichloro-5-methylbenzoic Acid (XXIV). To 0.02 mole of benzylamine in 30 ml of hexane we added 0.02 mole of triethylamine, and a solution of 0.02 mole of 2,4-dichloro-5-methylbenzoic acid in 20 ml of hexane was added with vigorous mixing. Then the mixture was mixed for 0.5 h at 50°C, then cooled; the residue was filtered off and washed on the filter with water to remove triethylamine hydrochloride; the undissolved residue was crystallized from ethanol.

N-(1-Cyclopropylethyl)amides of 2-Substituted Acetic Acid (XXV-XXVIII). To a solution of 0.05 mole of the corresponding N-(1-cyclopropylethyl)amide of chloroacetic acid, produced according to the procedure described earlier, in 30

ml of diethyl ether, 0.1 mole of piperidine or morpholine was added, and the mixture was allowed to stand for 24 h at room temperature. The crystals of piperidine hydrochloride or morpholine that separated out were filtered off. After the ether was distilled off from the filtrate, the product was isolated by redistillation at reduced pressure.

EXPERIMENTAL (BIOLOGICAL)

The experiments were conducted on noninbred white rats weighing 200-250 g, kept on the vivarium diet. The mitochondrial fraction was isolated according to [4]. The fractions were treated with Na-deoxycholate (0.25 mg per mg protein) to detect latent AldDH activity and produce transparent solutions. The protein concentration was measured according to [8]. The activity of isoforms with high (AldDH-I) and low (AldDH-II) affinity for acetaldehyde was determined spectrophotometrically, as described in [6] and was calculated in nanomoles of NADH per mg protein per min. The investigated substances were dissolved in ethanol with a volume fraction of 96% and introduced into the incubation medium in a final concentration of 100 μM. The results were treated statistically, using the Student criteria [3], and were considered significant at $p \leq 0.05$. Most of the investigated compounds inhibited the activity of both forms of AldDH (Table 2).

The substances that did not affect the activity of the isoenzymes under consideration include amines and their hydrochlorides III-VI, as well as acetic acid amides VII and VIII.

Amides of monosubstituted benzoic acids IX-XIV inhibited AldDH-I by 10-30%, and AldDH-II by up to 10%. The appearance of a second substituent in the benzoic acid molecule in compound XV caused an increase in the inhibiting effect both for AldDH-I and for AldDH-II. Compounds XXI and XXII exhibit stronger inhibiton for AldDH-II. This fact is interesting, since usually the isoform with a high affinity for acetaldehyde was more sensitive to the action of inhibitors. But for most of the substituted amides of the compounds mentioned above, XVI-XX, an increase in the inhibition was observed for AldDH-I up to 50-70%, whereas for AldDH-II the changes were negligible.

To determine the role of the cyclopropyl ring in the manifestation of biological activity, we investigated the compounds XXIII and XXIV, which do not contain this residue. It was found that they did not exhibit any inhibiting effect. Consequently, the compounds studied are active as a whole.

The last group of compounds examined, XXV-XXVIII, contained piperidine and morpholine residues. In contrast to the groups indicated above, the inhibiting effect of these compounds is unchanged when the hydrogen atom is replaced by benzyl.

Thus, our investigations showed that, in contrast to coprin, the compounds under consideration are active *in vivo* as a whole. Compounds XV-XX significantly inhibit the activity of both isoforms of AldDH and are of interest for testing for the presence of antialcohol activity.

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