SYNTHESIS AND MECHANISM OF BIOCHEMICAL ACTION OF DICARBOXYLIC ACID BENZYLHYDRAZIDES

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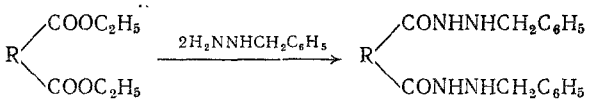
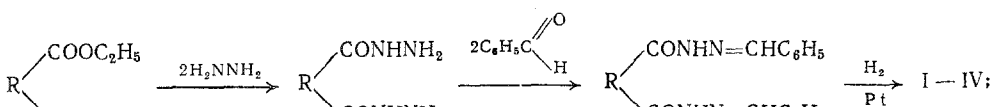
In an attempt to prepare new low-toxicity inhibitors of the enzymatic activity of monoamine oxidase (MAO) (deaminating amine-oxygen oxyreductase, E.C. 1.4.3.4), and in connection with a proposal for the expansion of the pharmacotherapeutic aspect of the study of this group of compounds [1, 2], we studied the basic physicochemical and biochemical properties of a series of di-N-2-benzylhydrazides of dibasic carboxylic acids prepared from the natural metabolites malonic, succinic, glutaric, and malic acids (I-IV).

The synthesis is most conveniently carried out in one step, which consists in the condensation of benzylhydrazine with the diethyl ester of the dicarboxylic acid (malonic, succinic, glutaric, and DL-malic acids):



$R = CH_2$(I), CH_2CH_2 (II), $CH_2CH_2CH_2$ (III), $CHOHCH_2$ (IV).

To prove that the benzyl group is attached to the N-2 carbon atom of the hydrazine in the reaction product, we conducted the alternative synthesis shown in the scheme:



$R = CH_2$ (V), CH_2CH_2 (VI), $CH_2CH_2CH_2$ (VII), $CHOHCH_2$ (VIII).

In all cases, the acid hydrazides prepared were identical to those prepared from the ester and benzylhydrazine.

The synthesized N-2-substituted hydrazides were colorless crystalline compounds, easily forming salts with mineral acids (Table 1). In their IR spectra, absorptions were observed in several bands corresponding to valence vibrations of the N–H bonds in the 3225-3315 cm^{-1} range, and also the amide (I) and (II) bands at 1632-1652 cm^{-1} and 1525-1545 cm^{-1}, respectively. The presence of the benzyl substituent on the N-2 atom was shown by the PMR data (Table 2).

In the compilation of the values of antiserotonin deaminase activity produced by (I-III) (Table 3), it is seen that the degree of blocking of active MAO centers depends upon the number of methylene groups in the acyl radical, i.e., on the length of the middle lipophilic portion which binds to the active structure of the attacking molecule of the agent.

It is known from examination of the mechanism of the interaction of inhibitors on enzymes that besides the lipophilic factor, a special significance is derived from the steric conformity of the molecule of attacking agent, which guarantees blocking by means of electrostatic interactions or complex formation of the inhibitor with the active center of the enzyme [3]. Literature data [3] also show that the structure of the active center of MAO which participates in the interaction of the enzyme with its inhibitor may involve the nucleophilic N-10 of the iso-alloxazine portion of FAD, an electrophilic ion of a divalent metal (Fe^{++}), or a hydrophobic contact region [4, 5].

In view of the above, we attempted, with the help of Dreiding stereomodels, to evaluate the significance of structural parameters on the formation of stable intermolecular bonds

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TABLE 1. Dicarboxylic Acid Di-N-2-benzylhydrazides and Di-2-benzalhydrazides

Compound	mp, °C	Found, %			Empirical formula	Calculated, %		
		C	H	N		C	H	N
I	108—110	65,02	6,12	17,60	$C_{17}H_{20}N_4O_2$	65,37	6,45	17,94
II	153—5	66,15	6,57	17,53	$C_{18}H_{22}N_4O_2$	66,23	6,80	17,17
III	144—6	66,87	7,51	16,34	$C_{19}H_{24}N_4O_2$	67,04	7,11	16,46
IV	158—9	62,97	6,36	16,46	$C_{18}H_{22}N_4O_3$	63,14	6,48	16,36
V	226	66,44	5,23	18,96	$C_{17}H_{16}N_4O_2$	66,22	5,23	18,17
VI	234	67,08	5,56	17,36	$C_{18}H_{18}N_4O_2$	67,06	5,63	17,38
VII	245	67,63	5,77	17,10	$C_{19}H_{20}N_4O_2$	67,84	5,99	16,66
VIII	195	63,43	5,25	16,31	$C_{18}H_{18}N_4O_3$	63,89	5,36	16,56

between the compounds studied and the active center of the enzyme, thus promoting blockage. On the basis of the information obtained in this manner, it can be suggested that blockage by the hydrazine derivatives of the FAD α-isoalloxazine structure in the MAO is brought about initially by the mobility of the protons attached to the N-2 and the N-1 atoms in the hydrazine group, and their participation in the formation of hydrogen bonds with the nucleophilic N-10 atom and the carbonyl oxygen at C-4, respectively.

Examination of (II) as the most promising of its analogs for inhibition of MAO in this series of dibasic acid hydrazides is of interest in regard to the question of whether both or only one of the benzylhydrazine residues is involved in blocking the active center of the enzyme. Comparison of results obtained by biochemical study of the antimonoamine oxidase properties of the benzylhydrazine derivatives of succinic and of propionic acid ($C_6H_5CH_2$-$NHNHC(O)CH_2CH_3$, IX) shows (Table 3) that the two compounds are very similar in their inhibitory properties. Therefore, the retardation of MAO by compound (II), and also by its hydroxy derivative ($C_6H_5CH_2NHNHC(O)CH_2CH(OH)C(O)NHNHCH_2C_6H_5$, IV) may be considered to be accomplished by participation of only one benzylhydrazine residue. The separation between the N-2 atom and the bond to the hydroxy group on the β-carbon atom, determined for the maximum elongated conformation of molecule (IV) by Dreiding models is approximately 5 Å. This value corresponds to that suggested earlier [4] for the separation between the nucleophilic N-10 in the α-isoalloxazine structure of FAD and a metal cation situated in another region of the MAO active center (Scheme 1). The introduction of a nucleophilic hydroxy group into the succinyl residue (in compound IV) may increase the probability of contact of the inhibitor with the MAO active center — in particular with its electrophilic region. From this it also is inferred that with a "non-optimal" separation of both benzylhydrazine groups in the inhibitor molecule, one of them is not involved in the principle mechanism of inhibition, but is directed to the side of the electrophilic part of the enzyme (Me^{++}); the resulting tendency to electrostatic repulsion may even bring about a lowering of the expected hydrazide biochemical effect.

We have obtained information about the acyl structure optimal for the blocking of the active center of MAO by dicarboxylic acid benzylhydrazides, which may be used for the creation of new, highly effective inhibitors for that enzyme.

EXPERIMENTAL CHEMICAL

IR spectra were recorded on a UR-20 instrument (GDR) on Nujol suspensions (1500-1800 cm^{-1}) or in hexachlorobutadiene (3000-3600 cm^{-1} region). PMR spectra were obtained on a Bruker HX-90 instrument (FRG) with a working frequency of 90 MHz in deuterated DMSO and with hexamethyldisiloxane as internal standard.

Dibasic Carboxylic Acid Di-N^1-benzylhydrazides (I-IV). A solution of 0.02 mole of dibasic acid diethyl ester and 0.04 mole of benzylhydrazine in 50 ml of absolute ethanol was heated under reflux for 8 h. The solvent was removed under vacuum and the residue was crystallized from ethanol to give a yield of 70-80%. The hydrochlorides of compounds (I-IV) were prepared by treatment with equivalent quantities of alcoholic hydrogen chloride, followed by recrystallization from anhydrous methanol or ethanol.

Dihydrazides of Malonic, Succinic, Glutaric, and Malic Acids. To 0.1 mole of diethyl ester was added 50 ml of absolute ethanol and 0.23 mole of hydrazine hydrate, and the solution was heated for 5 min on a water bath. The precipitate obtained after cooling was washed with small portions of ethanol and dried.

TABLE 2. Proton Chemical Shifts for Dicarboxylic Acid Dibenzylhydrazides

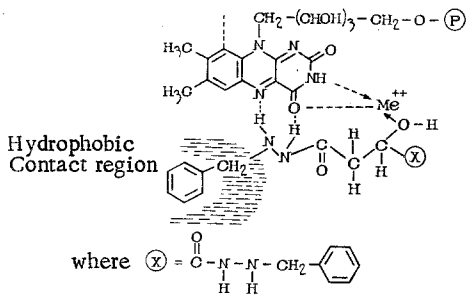
Compound	^1NH	^2NH	$N-CH_2-C_6H_5$	C_6H_5	R
I	9,33 (s, 2H)	5,13 (s, 2H)	3,78 (s, 4H)	7,23 (s, 10H)	2,85 (s, 2H, CH_2)
II	8,74 (s, 2H)	4,73 (s, 2H)	3,82 (s, 4H)	7,29 (s, 10H)	2,31 (s, 4H, CH_2)
III	9,15 (s, 2H)	4,78 (s, 2H)	3,82 (s, 4H)	7,28 (s, 10H)	1,55—2,25 (m, 6H, CH_2)
IV	9,23 (s, 1H)	4,94 (m, 2H)	3,85 (s, 4H)	7,27 (s, 10H)	5,50 (d, 1H, J-6Hz, OH)
	8,96 (s, 1H)				4,32 (m 1H, CH)
					2,25—2,47 (m, 2H,CH_2)

TABLE 3. Inhibition of Serotonin Deaminase Activity of Mitochondrial MAO by Dibasic Acid Alkylhydrazides

Compound	Concentration, M	Rat liver		Ox brain	
		nmoles of NH_3 in 1 ml albumin per min	inhibition, %	nmoles of NH_3 in 1 ml albumin per min	inhibition, %
I·2HCl	10^{-4}	0,5 (0,2—0,8)	92	1,4 (0,9—1,9)	68
	5.10^{-5}	2,3 (1,8—2,8)	64	2,6 (2,2—3,0)	42
	10^{-5}	4,9 (4,4—5,4)	24	3,8 (3,4—4,2)	15
II·2HCl	5.10^{-5}	1,1 (0,8—1,4)	83	1,4 (1,0—1,8)	69
	10^{-5}	3,4 (3,1—3,7)	47	3,0 (2,6—3,4)	34
	5.10^{-6}	4,5 (4,1—4,9)	30	3,9 (3,4—4,4)	13
III·2HCl	10^{-4}	0	100	1,0 (0,6—1,4)	80
	5.10^{-5}	1,3 (0,9—1,7)	79	2,2 (1,8—2,6)	52
	10^{-5}	3,6 (3,1—4,1)	44	2,6 (2,1—3,1)	26
IV·2HCl	10^{-5}	0	100	1,6 (1,3—1,9)	64
	5.10^{-6}	2,6 (2,2—3,0)	59	2,4 (2,1—2,7)	47
	10^{-6}	3,8 (3,3—4,3)	41	3,3 (2,9—3,7)	27
IX	5.10^{-5}	1,1 (0,8—1,4)	83	1,1 (0,7—1,5)	75
	5.10^{-6}	4,3 (4,0—4,6)	33	3,6 (3,3—3,9)	19
Benzylhydrazine·HCl	10^{-6}	2,9 (2,5—3,3)	55	0,9 (0,4—1,4)	80
	5.10^{-7}	4,1 (3,7—4,5)	36	2,2 (1,8—2,6)	52
Control	—	6,4 (5,9—6,9)	—	4,5 (4,1—4,9)	—

Note. Number of parallel determinations for each point is 6-7. The ranges of variation are given in parentheses.

Scheme 1



Dicarboxylic Acid Di-N^2-benzalhydrazides (V-VIII). The diacid dihydrazides (0.037 mole) were dissolved in 350 ml of absolute methanol and 0.076 mole of benzaldehyde was added. The solutions were heated to boiling, and then about 250 ml of methanol was distilled. The resulting precipitates were filtered off and crystallized from methanol.

Hydrogenation of Dicarboxylic Acid Di-N^2-benzalhydrazides (V-VIII). This was carried out in alcohol in the presence of hydrogen chloride and catalyzed by platinum dioxide [6]. After taking up the calculated amount of hydrogen, the reaction mixture was concentrated to the consistency of syrup and neutralized with sodium carbonate. The resulting (I-IV) were crystallized from ethanol.

EXPERIMENTAL (BIOCHEMICAL)

Determination of Antiserotonin Deaminase Activity of the Preparations. Mitochondria from ox brain were isolated by the method of Brody and Bain [7], and from rat livers by the method of Schneider and Hogeboom [8]. A suspension was prepared from 4 mg (calculated) of mitochondrial protein in 1 ml of 0.2 M phosphate buffer (pH 7.4). The duration of preincubation of the suspension with the preparation and also the incubation of the MAO sources with serotonin (in the form of creatine sulfate, 10^{-3} M) was 30 min. The NH_3 liberated by deamination of serotonin (in control experiments and under the influence of the preparations) was determined by isothermal distillation with subsequent photocolorimetric determination by the Nessler reaction [9].

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