METABOLISM OF 7-DIFLUOROMETHYLTHIO-5-PHENYL-1,2-PHENYL-1,2-DIHYDRO-3H-1,4-BENZODIAZEPIN-2-ONE

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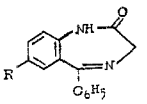
We have previously [1] described the synthesis of 1,2-dihydro-3H-1,4-benzodiazepin-2-ones with fluorine-containing substituents in position 7, including 7-difluoromethylthio- and 7-difluoromethylsulfonyl-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-ones (Ia and b).

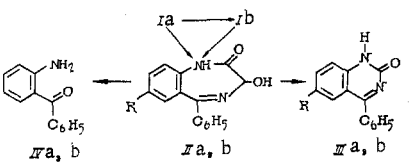


Ia: R = $SCHF_2$; Ib: R = SO_2CHF_2; Ic: R = Cl.

On the basis of known correlations [2] it could have been expected that substance (Ia) would be similar in pharmacological properties to (Ic), since in its electronic influence the difluoromethylthio group is an analog of the halogen atoms [1]. However, a pharmacological study of substance (Ia) has shown that, in contrast to the 7-halo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-ones, this substance exhibits no antispasmodic action even in a dose of 100 mg/kg in the corazole [pentylenetetrazole] antagonism test nor does it possess a sedative and myorelaxant action (Table 1).

The reason for this anomaly could be a peculiar metabolism and pharmacokinetics of this benzodiazepine. The present investigation was undertaken in order to test this hypothesis.

To study the metabolism of substance (I) and its distribution and those of its biotransformation products in the organism of white rats we have synthesized potential metabolites of this substance. Taking into account the main pathways of the metabolism of the 1,2-dihydro-3H-1,4-benzodiazepin-2-ones [3] and of sulfur-containing organic compounds [4], it might have been expected that the metabolites of substance (Ia) would be the products of its oxidation at position 3 and at the sulfur atom of the difluoromethylthio group – 7-substituted 3-hydroxy-7-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-ones (IIa, b), and also the products of their further transformations – 4-phenylquinazolin-2-ones (IIIa, b) and 2-aminobenzophenones (IVa, b).



IIa, b IIa, b IIIa, b

a) R = $SCHF_2$
b) R = SO_2CHF_2

Compounds IIIa and b and IVa and b (R = $SCHF_2$, SO_2CHF_2) were obtained by methods described previously [1, 5]. Then, by known schemes [6, 7], compounds (IIa and b) were synthesized by a series of transformations from the aminobenzophenones (Table 2).

The individualities of all the compounds were established by TLC, and their structures were confirmed by IR and UV spectroscopy.

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TABLE 1. Pharmacological Activities of Compounds (Ia-c)

Compound	Antagonism to corazole	Disturbance of orientation reactions	Disturbance of motor coordination
	% effect		
Ia	100—0	100—0	100—10
Ib	100—0	100—0	100—10
Ic	0,35 (0,23—0,53)	3,7 (2,46—5,5)	14,0 (9,6—20,3)

Note. The ED (in mg/kg) and the confidence intervals are given for compound (Ic).

TABLE 2. Properties of the Compounds Synthesized

Compound	Yield, %	mp, °C	Found, %				Empirical formula	Calculated, %			
			C	H	F	N		C	H	F	N
IIa	76	196—8	57,5	3,6	11,3	8,4	$C_{16}H_{12}F_2N_2O_2S$	57,6	3,6	11,4	8,4
IIb	83	256—7	52,4	3,4	10,6	7,5	$C_{16}H_{12}F_2N_2O_4S$	52,5	3,3	10,4	7,7
Va	46	127—8	57,2	4,2	12,9	9,4	$C_{14}H_{12}F_2N_2OS$	57,1	4,1	12,9	9, 5
Vb	39	187—8	51,4	3,6	11,9	8,5	$C_{14}H_{12}F_2N_2O_3S$	51,5	3,7	11,7	8,6
VIa	53	120—1	54,3	3,0	—	8,1	$C_{16}H_{11}ClF_2N_2OS$	54,5	3,1	—	8,0
VIb	44	179—81	49,8	2,7	—	7,2	$C_{16}H_{11}ClF_2N_2O_3S$	50,0	2,9	—	7,0
VIIa	62	204—6	57,3	3,8	11,3	8,5	$C_{16}H_{12}F_2N_2O_2S$	57,5	3,6	11,4	8,4
VIIb	71	271—3	52,3	3,2	10,5	7,8	$C_{16}H_{12}F_2N_2O_4S$	52,5	3,3	10,4	7,7
VIIIa	85	154—5	57,2	3,6	10,2	7,6	$C_{18}H_{14}F_2N_2O_3S$	57,4	3,7	10,1	7,4
VIIIb	79	238—9	52,8	3,3	9,2	7,1	$C_{18}H_{14}F_2N_2O_5S$	53,0	3,4	9,1	7,0

TABLE 3. Dynamics of the Distribution of (Ia) and Its Metabolites (IIa) and (IIIa) in the Liver, Blood, and Brain of White Rats (M± m)

Object of investigation	Compound	Time after administration, min			
		5	15	30	120
		amount of the substance after administration, mg/kg			
Liver	Ia	3,07±0,32	14,32±0,66	9,91±0,25	16,83±0,53
	IIa	3,87±0,38	7,76±1,10	5,60±0,47	7,04±0,93
	IIIa	2,05±0,10	2,63±0,25	1,76±0,35	6,27±0,22
Plasma	Ia	3,80±0,17	5,84±0,32	7,90±0,78	14,25±0,42
	IIa	2,92±0,56	3,24±0,43	4,75±0,73	2,80±0,89
	IIIa	1,63±0,3	3,17±0,65	1,73±0,29	3,91±0,49
Brain	Ia	4,05±0,26	5,69±0,59	8,75±0,54	12,91±0,5
	IIa	2,60±0,36	3,81±0,77	5,61±0,98	6,06±0,40
	IIIa	1,19±0,18	2,74±0,63	1,77±0,35	1,79±0,29

EXPERIMENTAL (PHARMACOLOGICAL)

The pharmacological properties of substances (I-III) were studied as described as in [2], and their metabolism and distribution in the organism of white rats was given in [3]. Compound (Ia) in a dose of 20 mg/kg was administered intraperitoneally in the form of a suspension in Tween-80. The structures of the metabolites were established by comparison with authentic samples of substances (II–IV). It was shown by TLC and UV spectroscopy that the main metabolites of (Ia) were 7-difluoromethylthio-3-hydroxy-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one (IIa) and 6-difluoromethylthio-4-phenylquinazolin-2-one (IIIa). In addition, another metabolite was detected the structure of which it has not been possible to establish by the methods indicated above.

Compounds (Ia), (IIa), and (IIIa) were detected in the brain, liver, and blood plasma (Table 3). In the blood plasma the concentrations of the initial compounds increased with time, reaching a maximum at the end of the investigation. The maximum amount of the 3-hydroxy metabolite (IIa) appeared at the 30-minute investigation, and the distribution of the quinazoline (IIIa) had a two-phase nature with maxima at the 25th and 125th minutes of investigation. A two-phase nature of the distribution for the initial (Ia) and its metabolites was observed in the livers of the animals. In this respect, compound (Ia) differed sharply from other 7-substituted 1,4-benzodiazepin-2-ones [3]. Practically all the metabolites penetrated to the brain uniformly and reached their maximum concentration there at the end of the investigation.

The results obtained indicate that the absence or low level of activity in the above-mentioned tests is not due to the pharmacokinetic characteristics of (Ia) and its metabolite. In the light of information that has recently appeared on the influence of steric factors on the affinity of 1,4-benzodiazepine derivatives for the corresponding receptors [8], it may be assumed that the reduction of the activity of substance (I) is due to the larger volume of the difluoromethylthio and difluoromethylsulfonyl groups as compared with halogen atoms and the nitro group.

EXPERIMENTAL (CHEMICAL)

IR spectra were taken on a IKS-14a instrument in CCl_4, and UV spectra on a Specord UV-VIS (GDR) spectrophotometer in ethanolic solution.

2-Amino-5-difluoromethylthiobenzophenone α-Oxime (Va). At 35°C, with stirring, 6 ml of a solution of 3.6 g of caustic soda was added to a mixture of 5.58 g (0.02 mole) of 2-amino-5-difluoromethylthiobenzophenone, 2.63 g (0.02 mole) of hydroxylamine sulfate, and 32 ml of ethanol, the mixture was heated at 80°C for 30 min and was then cooled, and a 3% solution of hydrochloric acid was added to pH 7.0. The precipitate that deposited was crystallized from toluene.

2-Amino-5-difluoromethylsulfonylbenzophenone α-Oxime (Vb). This was obtained similarly.

IR spectrum, cm^{-1}: 3611-3535 (OH), 3412-3386 (NH), 1609 (C=N).

2-Chloromethyl-6-difluoromethylthio-4-phenylquinazoline 3-Oxide (VIa). A solution of 1.5 g (0.005 mole) of (Va) in 6.4 ml of glacial acetic acid and 0.7 ml (0.0052 mole) of chloroacetyl chloride was stirred at 20°C for 48 h while being periodically saturated with hydrogen chloride, and it was then concentrated in vacuum. The resulting precipitate was dissolved in methylene chloride, the solution was washed with sodium carbonate solution and with water, and the (VIa) was precipitated with petroleum ether and recrystallized from ethanol.

2-Chloromethyl-6-difluoromethylsulfonyl-4-phenylquinazoline 3-Oxide (VIb). This was synthesized similarly from (Vb).

7-Difluoromethylthio-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one 4-Oxide (VIIa). To a solution of 1 g of caustic soda in 15.3 ml of 85% ethanol was added 0.9 g (0.0025 mole) of (VIa), and then the mixture was stirred for 20 min and it was diluted with 15 ml of water and acidified with dilute HCl. The precipiate that deposited was crystallized from ethanol.

IR spectrum, cm^{-1}: 3416 (NH), 1710 (C=O), 1619 (C=N), 1286 (N→O).

7-Difluoromethylsulfonyl-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one 4-Oxide (VIIb). This was obtained from (VIb) similarly.

3-Acetoxy-7-difluoromethylthio-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one (VIIIa). A suspension of 1.15 g (0.00035 mole) of (VIIa) in 10 ml of acetic acid was stirred with heating in the water bath until dissolution was complete (20-30 min). After cooling, the (VIIIa) was separated off and it was recrystallized from ethanol.

IR spectrum, cm^{-1}: 1745 (ester C=O), 1697 (amide C=O).

3-Acetoxy-7-difluoromethylsulfonyl-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one (VIIIb). This was synthesized from (VIIb) similarly.

7-Difluoromethyl-3-hydroxy-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one (IIa). A suspension of 1.9 g (0.0051 mole) of (VIIIa) in 40 ml of ethanol was treated with 3 ml of 4 N caustic soda, and then 40 ml of water and acetic acid to pH 4.0-5.0 were added, and the (IIa) that deposited was crystallized from ethanol.

IR spectrum, cm^{-1}: 3614-3480 (OH), 3390-3200 (NH), 1685 (C=O).

UV spectrum, λ_{max}, nm (log ε): 205 (4.2), 240 (4.4).

(IIb) was obtained similarly.

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STUDIES ON THE MUTAGENIC EFFECTS OF TOMIZINE

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Tomizine (4-methoxy-6-aminopyrimido[4,5-b](1,4)-thiazine chlorohydrate) differs from other antineoplastic agents that act as folic acid antagonists in its mechanism of action [1]. Tomizine inhibits dehydrofolatereductase and the aminopterin-inactivating enzyme. While tomizine is as effective as methotraxate as an antineoplastic agent, it is less toxic and accumulates to a lesser extent. Since no information is currently available on the mutagenicity of tomizine, we tested it for such effect on a number of test objects.

EXPERIMENTAL

The Ames Salmonella/microsome test was employed to test for the ability of tomizine* to induce genetic mutations in bacteria [2]. The techniques have been described in [3, 4]. The strains of Salmonella typhimurium used were TA 1535 and TA 1538, and their isogenic strains TA 100 and TA 98 containing plasmid pKM 101 [5, 6]. Use of these strains favors detections of various types of mutations. Mutagenesis was followed in terms of induction of revertants from auxotrophy for histidine to prototrophy. Variations were introduced which made it possible to follow the mutagenicity of tomizine without (NMA) and with metabolic activation (WMA) [4]. For positive controls in the NMA experiments nitrosomethylurea was used on TA 1535 and TA 100, and DDDTDP [7] on TA 1538 and TA 98. For positive controls in the WMA experiments nitrosomorpholine II was used on TA 1535 and TA 100. The results are presented in Table 1. A mutagenic effect is indicated when the number of revertants on the experimental dishes exceeds by 2.5-times the number on pure control (negative control) dishes. There was not a single result that would indicate tomizine mutagenicity on the basis of this criterion. However, the agents used in the positive controls were efficient in inducing mutations in these strains. Therefore, this test showed that tomizine did not induce genetic mutations in bacteria.

The standard Meller-5 method was used to test tomizine for ability to induce genetic mutations in drosophila [8]. D-32 males were treated with three concentrations of tomizine (in 5% sugar) for three days on glass filters. The results are given in Table 2.

Table 2 indicates that tomizine induces a statistically significant increase in the incidence of lethal recessive mutations at every concentration tested.

Standard methods were also employed in the evaluation of tomizine-induced dominant lethal mutations in mouse embryonal cells based on the selective susceptibility of the different stages of spermatogenesis [9]; the five-week experiment was performed with outbred mice. The dosages employed were 3 mg/kg (human therapeutic dose), and 14, 45, and 90 mg/kg (0.5 LD_{50} for mice). Induced mortality was determined only on the basis of postimplantation death. The data were treated according to the nonparametric criteria of Wilcoxon [10].

* The tomizine sample was kindly provided by Prof. T. O. Safonova, chief of the Laboratory for the Synthesis of Antineoplastic Agents, S. Ordzhonikidze All-Union Scientific-Research Institute of Chemical Pharmacology.

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