17. D. A. Holaday and R. England, Anesthesiology, 57, 246 (1982).
18. M. G. Horning, J. P. Thenot, O. Bouwsma, et al., in: Biochemical-Clinical Pharmacology, Oxford (1979), pp. 245-256.
19. J. Katz, Fed. Proc., 41, 123-128 (1982).
20. M. J. Kuhar, W. S. Young, J. M. Palacios, and J. K. Wamsley, in: Psychopharmacology and Biochemistry of Neurotransmitter Receptors, New York (1980), pp. 357-368.
21. N. Kurihara, T. Suzuki, and M. Nakajima, Pestic. Biochem. Physiol., 14, 41-49 (1980).
22. L. P. McCarty, R. S. Malek, and E. R. Larsen, Anesthesiology, 51, 106-110 (1979).
23. G. Meyniel, in: Colloque Medical Nucleaire Langue Francaise, 22eme Rapports, Toulouse (1981), pp. 1-40.
24. R. R. Minor, J. Biol. Chem., 257, 10400-10413 (1982).
25. A. L. Misra and R. B. Pontani, Drug Metab. Disposit. Biol. Fate Chem., 5, 556-563 (1977).
26. G. T. Miwa, J. A. Walsh, N. Harada, and A. Y. H. Lu, in: Synthesis and Application of Isotope Labeled Compounds, Amsterdam (1983), pp. 83-88.
27. J. L. Morgat, P. Fromageot, R. Micrhelot, and J. Clowinski, FEBS Lett., 111, 19-22 (1980).
28. S. D. Nelson, in: Synthesis and Application of Isotope Labeled Compounds, Amsterdam (1983), pp. 89-94.
29. W. Sacks, Sh. Sacks, A. Badalamenti, and A. Fleischer, J. Neurosci. Res., 7, 57-69 (1982).
30. A. Sapota and W. Draminski, in: Industrial and Environmental Xenobiotics, Berlin, (1981), pp. 231-237.
31. J. Schmid, A. Prox, H. Zipp, and F. W. Koss, Biomed. Mass Spectrom., 7, 560-564 (1980).
32. A. J. Siemens, O. L. Doyle, and G. T. Pryor, Life Sci., 24, 1261-1274 (1979).
33. I. G. Sipes, A. J. Gandolfi, L. R. Pohl, et al., J. Pharmacol. Exp. Ther., 214, 716-720 (1980).
34. P. E. Stokes, M. Okamoto, K. W. Lieberman, et al., Biol. Psychiat., 17, 413-421 (1982).
35. K. Tanaka, M. Nakajima, and N. Kurihara, Pestic. Biochem. Physiol., 16, 149-157 (1981).
36. I. W. Taylor, C. Ivannides, P. Sacra, et al., Biochem. Pharmacol., 32, 641-647 (1983).
37. J. H. Tinker, J. H. Milde, and C. R. Noback, Anesthesiology, 55, No. 3A, 3 (1981).
38. S. Wood, B. Wardley-Smith, M.-J. Halsey, and C. J. Green, Brit. J. Anaesth., 54, 387-391 (1982).
39. P. H. Yu, C. Kazakoff, B. A. Davis, and A. A. Boulton, Biochem. Pharmacol., 31, 3697-3698 (1982).

SYNTHESIS AND FEATURES OF THE PHARMACOKINETICS OF THE ^{14}C METABOLITE OF PEPTIDOBENZOPHENONES AND SOME PREPARATIONS OF THE BENZODIAZEPINE SERIES

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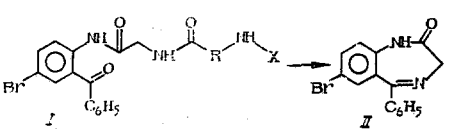
For some time past, the attention of investigators has been drawn to drug precursors possessing a series of advantages over the actual preparations. In our laboratory, the derivatives of peptidobenzophenones (I), which are converted to the corresponding 1,4-benzodiazepine (II) in the animal organism [1, 6], are being studied.



I II

(The residue of the α-aminoacid is R. The carboxybenzoxy or other protecting group is X; in the absence of the latter, it is the base of (I), i.e., the free terminal group.)

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Compound (II) possesses a broad spectrum of pharmacological activity [2] and is structurally close to nordiazepam and phenazepam.

We accomplished the synthesis of 7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepam-2-one-(2-^{14}C) (II) and its potential metabolites to determine the contribution of (II) to the pharmacological activity of (I) and to study the secondary metabolites. Data on the kinetics of the content of the initial compound and its metabolites in the mouse brain are presented. The optimal conditions for the kinetics of extraction of the investigated biological samples and the quantitative determination of (II) and its metabolites were studied.

EXPERIMENTAL (CHEMICAL)

Glycine-(I-^{14}C) of specific radioactivity 1 Ci/mole was taken as the initial compound for the synthesis of (II). Radiochromatographic analysis was performed in the 7:3 system of propanol–water on plates of Silufol UV-254 (R_f of glycine was 0.3-0.35); it permitted the detection of 88.1 ± 1.5% of the total amount of radioactivity. The content of ^{14}C material which corresponded to glycine according to the R_f value comprised 93.9 ± 0.8% of the radioactivity determined on the chromatogram (82.7 ± 1.3% of the applied amount).

The radiochromatographic analysis of the synthesized sample (2-^{14}C-(II) was first purified by the method of column chromatography) was performed on plates of Silufol UV-254 in the 70:30:0.01 system of chloroform–acetone–aqueous ammonia. We detected 97.9 ± 3.4% of the total radioactivity. For this, 91.2 ± 4.2% of the ^{14}C product corresponded in the R_f value to (II) (89.3 ± 4.2% of the applied amount).

Hydrochloride of Glycine-(1-^{14}C) Acid Chloride. The suspension of 0.52 g (0.007 mole) of glycine-(1-^{14}C) with the specific activity of 1 Ci/mole (first ground to a powder and dried at 110°C for 2 h) in 10 ml of dry chloroform is saturated with dry HCl at 20°C for 15 min. After this, 1.45 g (0.007 mole) of phosphorus pentachloride is added, and the mixture is stirred for at least 20 h at 20°C. The reaction mixture is filtered, and the residue is washed with 10 ml of dry CCl_4. The hydrochloride of glycine-(1-^{14}C) acid chloride has the mp in the range of 110-120°C [11].

7-Bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one-2-^{14}C (II). To the solution of 1.38 g (0.005 mole) of 5-bromo-2-aminobenzophenone in 30 ml of dry chloroform is added the hydrochloride of glycine-(1-^{14}C) acid chloride. The reaction mixture is boiled with stirring until the cessation of the separation of HCl (3 h). After cooling the mixture to room temperature, 5 ml of water is added; aqueous ammonia is gradually added with stirring until the ascertainment of a stable weakly alkaline reaction. After the separation of the aqueous layer, the chloroform solution is washed once with 3 ml of water. It is then concentrated *in vacuo*. To the remaining mass is added 10 ml of toluene, and the solution is evaporated until the complete removal of the solvent at atmospheric pressure. The residue is again dissolved in toluene prior to filtration. After crystallization from the toluene solution, we obtain 0.92 g of (II) with the specific activity of 0.79 Ci/mole. The radiochemical yield is 38.4%; mp 220-221°C. Found, %: C 57.4, H 3.8, and N 9.1. $C_{15}H_{11}BrN_2O$. Calculated, %: C 57.1, H 3.5, and N 8.9.

5-Brom-2-aminobenzophenone-α-oxime. This is obtained by the method of [13] from 5-bromo-2-aminobenzophenone with a yield of 76%, mp 192-193°C. Found, %: C 53.9, H 4.0, and N 9.8. $C_{13}H_{11}BrN_2O$. Calculated, %: C 53.6, H 3.8, and N 9.6.

5-Bromo-2-chloroacetamidobenzophenone-α-oxime. This is obtained by the method of [13] from 5-bromo-2-aminobenzophenone-α-oxime and chloroacetyl chloride with a yield of 80%, mp 178-180°C. Found, %: C 49.0, H 3.3, and N 7.6. $C_{15}H_{12}BrClN_2O_2$. Calculated, %: C 49.4, H 3.6, and N 7.9.

7-Bromo-5-phenyl-3-hydroxy-1,2-dihydro-3H-1,4-benzodiazepin-2-one (III). The solution of 1.47 g (0.004 mole) of 5-bromo-2-chloroacetamidobenzophenone-α-oxime mixed with 20 ml of ethanol and 1.5 ml of an 8% solution of NaOH is stirred for 2.5 h at 18°C. The resulting residue of the sodium salt is separated prior to dissolving it in 150 ml of 66% aqueous ethanol. The solution obtained is acidified to pH 2.0, whereby a precipitate of white color comes out of the solution. After the recrystallization from toluene, we obtain 80% of (III) with mp 190-191°C. Found, %: C 54.5, H 3.5, and N 8.6. $C_{16}H_{14}N_2O_2$. Calculated, %: C 54.5, H 3.3, and N 8.4.

EXPERIMENTAL (PHARMACOLOGIC)

The determination of the optimal conditions of the extraction of (II) and its derivatives from biological samples was performed taking into account the preceding investigations for the optimization of the mono- and biphasic extraction of phenazepam [3, 8] and tetrahydrophenazepam [7].

The kinetics of the extraction of the ^{14}C material from the biological samples (0.25-0.4 g of mouse brain, ground with anhydrous sodium sulfate) by sequential extractions with chloroform (10 ml) are determined by the equation:

$$\ln [S_n] = A - Bn \pm (m_A + nm_B), \quad (1)$$

where $\ln[S_n]$ is the logarithm of the geometric mean of the content of the total radioactivity determined in the chloroform extracts; A and B are constants of regression; m_A and m_B are their mean square deviations of the mean; n is the number of the extraction.

The value of the magnitude of the coefficient of regression B does not correlate with the value of the experimental time (Table 1) and with the value of the magnitude of A on changing the ratio of the content of (II) and its 3-hydroxy metabolite (III). This is characteristic of the monophasic extraction and permits the determination of the given degree of the completeness of extraction of (II) and its ^{14}C metabolites on introducing different doses of the compound.

The extraction with a given degree of completeness ($\Sigma[S_n] = 0.95\ \Sigma[S_\infty]$ and $0.99\ \Sigma[S_\infty]$) is provided by two to three sequential extractions of the samples (see Table 1). The corresponding algorithms are presented in the preceding investigations [3, 7, 8].

The radiochromatographic analysis of the chloroform extracts of the mouse brain was accomplished by methods analogous to those presented in the works [3, 4, 9-11].

It was noted that together with the initial compound is present its 3-hydroxy metabolite; the respective R_f values are 0.5-0.7 and 0.25-0.3. The products of the aromatic hydroxylation of (II) ($R_f < 0.25$) were not detected in significant amounts (Fig. 1). In contrast to the kinetics of the content of phenazepam and its metabolites in the mouse brain [4, 9, 10], where the level of the initial compound exceeded the content of the metabolites, (II) was detected in significant amounts in the interval of 15-120 min, and its maximal content was noted 30 min after application, the level of (II) decreased intensively in the interval of the experiment (Fig. 2). The main metabolite of (II) — the 3-hydroxy derivative — was detected in the chloroform extracts of the mouse brain. The content of (III) exceeds the level of (II) in the interval of 60-240 min, and intensively increases in the experimental interval of 15-60 min (see Fig. 2).

The character of the interdependence of the action of (II) and its content in the animal brain was investigated by the capacity to weaken the spasmodic effect of corazol [12], and by the simultaneous registration of the minimal effective doses inducing clonic spasms (DCS) and tonic extension (DTE) [9].

The reliable antispasmodic effect, on the level of the application of (II) (1.4 mg/kg), was registered in the experimental time interval of 15-240 min for the tonic indicator; it was registered in the experimental time interval of 15-120 min for the clonic indicator (Table 2). The maximal antispasmodic action of (II) was noted in the interval of 30-120 min and was more marked according to the indicator of the prevention of the tonic extension of the animals (see the values of t_d in Table 2). The character of the interaction of corazol and (II) in forming the pharmacological response was investigated after 60 min (Fig. 3).

The dependence of the content of ^{14}C material in the animal brain on the applied dose of (II) (in the interval of 0.35-5.6 mg/kg) was determined as linear:

$$C_i = 0.14 + 3.79D_i \pm (0.0058 + 0.0018D_i^2)^{0.5}, \quad (2)$$

where C_i is the content of the ^{14}C products (in counts$\cdot 10^3 \cdot min^{-1} \cdot g^{-1}$) in the mouse brain at 60 min after the ip application of (II) at the dose D_i (in mg/kg) (Fig. 4).

For the description of the characteristics of the action of (II) under the conditions of the spasmodic attack induced by the application of corazol, the following dependence was utilized:

TABLE 1. Content ($\ln[S_n]$) of the ^{14}C Products in the Sequential Chloroform Extracts of Mouse Brain after the ip Application of (II) (1.4 mg/kg)

No. of extraction	Time of experiment, min									
	15		30		60		120		240	
	$\lvert\ln S_n\rvert$		$\lvert\ln S_n\rvert$		$\lvert\ln S_n\rvert$		$\lvert\ln S_n\rvert$		$\lvert\ln S_n\rvert$	
	found	calculated	found	calculated	found	calculated	found	calculated	found	calculated
1	7,219±0,028	7,211	7,498±0,024	7,485	7,620±0,022	7,599	7,229±0,027	7,247	7,404±0,025	7,399
2	5,293±0,078	5,390	5,768±0,060	5,881	5,861±0,057	6,131	5,565±0,067	5,323	6,031±0,051	6,059
3	3,638±0,240	3,569	4,357±0,141	4,285	5,024±0,092	4,663	4,431±0,135	3,399	4,804±0,106	4,739
4	2,773±0,481	1,748	3,401±0,287	2,685	3,135±0,357	3,195	2,773±0,481	1,475	3,496±0,267	3,409
Indicators of regression										
$A \pm m_A$	9,032±0,069		9,085±0,084		9,067±0,099		9,171±0,179		8,729±,,018	
$B \pm m_B$	1,821±0,051		1,600±0,065		1,468±0,074		1,924±0,140		1,330±0,012	
$n_{0,95}$	1,65		1,87		2,04		1,56		2,25	
$n_{0,99}$	2,53		2,88		3,14		2,39		3,46	

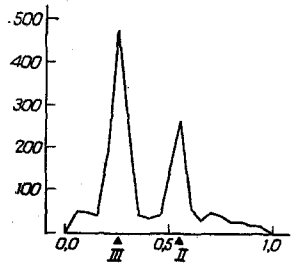

Fig. 1

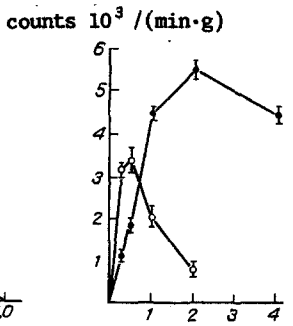

Fig. 2

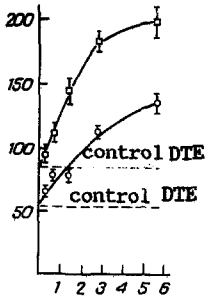

Fig. 3

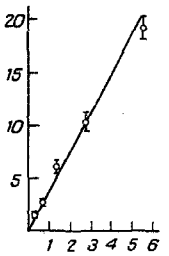

Fig. 4

Fig. 1. The radiochromatogram of the chloroform extract of the mouse brain in the 30:70:0.1 system of acetone—chloroform—ammonia. The time of the experiment is 1 h. The peak of radioactivity II (R_f 0.53) is due to the initial compound; the peak of radioactivity III (R_f 0.25) is due to the 3-hydroxy metabolite. The time (in hours) is on the X-axis; the activity (in counts•min) is on the Y-axis.

Fig. 2. The content of (II) (open circles) and its 3-hydroxy metabolite (solid circles) in the mouse brain after the ip application of the ^{14}C compound at the dose of 1.4 mg/kg. Time (in hours) is on the X-axis; activity [in counts•10^3/(min•g)] is on the Y-axis.

Fig. 3. The dependence of the dynamics of the antispasmodic action of (II) on the dose of the compound applied. The circles show the values of the minimal effective doses of corazol inducing clonic-tonic spasms; the squares show the values for tonic extension. The dose of (I) (in mg/kg) is on the X-axis; the dose of corazol (in mg/kg) is on the Y-axis.

Fig. 4. The dependence of the content of the ^{14}C products in the mouse brain on the applied dose of (II). The dose of (II) (in mg/kg) is on the X-axis; the activity [in counts•10^3/(min•g)] is on the Y-axis.

TABLE 2. Change of DCS DTE (in mg/kg) and of the Content of the ^{14}C Products in the Mouse Brain after the ip Application of ^{14}C-(II) at a Dose of 1.4 mg/kg (M ± m; n = 6)

Time of experiment, min	DCS		DTE		Content of ^{14}C products in the brain, in counts 10^3/min·g
	M ± m	t_d	M ± m	t_d	
15	68,8±3,95	2,72*	120,4±9,26	4,26**	4,99±0,395
30	84,3±5,24	4,59**	149,2±9,17	7,21***	5,50±0,614
60	73,0±3,75	3,48**	137,4±5,96	8,54***	6,05±0,547
120	78,0±4,97	3,79**	131,0±4,27	9,50***	5,60±0,511
240	55,1±2,11	0,57	102,6±3,30	5,05***	5,42±0,368
Control	52,2±4,64		77,8±3,64		

* $P \leqslant 0,05$.
** $P \leqslant 0,01$.
*** $P \leqslant 0,001$.

$$E_i \cdot E_{max}^{-1} = C_i^u \left(C_i^u + K\right)^{-1}, \quad (3)$$

where E_i is the effect observed in the presence of the concentration C_i, and of the concentration or dose of the antagonist proceeding from (2) in the given experiment; E_{max} is the maximum possible effect; u and K are constants. The given dependence is linear in dual inverse coordinates (u = 1). The indicators of regression are presented in Table 3. It can be concluded from the results of the analysis that the application of (II) exerts an antispasmodic effect. The action equal to 0.5 of the maximal thereby develops with the application of 4.25 ± 2.06 to

TABLE 3. Values of the Parameters of the Regressional Analysis of the Dose-Effect Dependence of the Antispasmodic Action of (II) at 60 min after the ip Application (0.35-5.6 mg/kg)

Indicator of regression	DCS	DTE
$\varepsilon_{max}=m$	0,0073±0,0031	0,0042±0,0018
$\varepsilon_{max}K=m$	0,031±0,0074	0,018±0,0048
σ^2_{exp}	1,094	1,075
α	1,0	1,0
$K=m$	4,25±2,065	4,29±2,14
$\varepsilon_{max}-\varepsilon_{contr.}$	137,0±58,36	238,1±102,1

4.29 ± 2.14 mg/kg of the compound; this exceeds the antispasmodic activity of tetrahydrophenazepam, chlordiazepoxide, and diazepam [5].

Therefore, the methods presented in the present work for the determination of ^{14}C-(II), its metabolites, and the total radioactivity in the biological samples, as well as the antispasmodic effect dependent on the presence of the compound and its derivatives in the animal brain, render possible the parallel study of the pharmacokinetics and pharmacodynamics of (II).

LITERATURE CITED

1. A. V. Bogatskii, N. Ya. Golovenko, O. P. Rudenko, et al., Pharmacology of Derivatives of Gamma-aminobutyric Acids [in Russian], Tartu (1983), p. 22.
2. Yu. I. Vikhlyaev, T. A. Klygul', and A. V. Bogatskii, Physiologically Active Substances [in Russian], Kiev (1971), pp. 265-579.
3. N. Ya. Golovenko, V. G. Zin'kovskii, and O. V. Zhuk, Khim.-farm. Zh., No. 12, 14-18 (1980).
4. N. Ya. Golovenko, V. G. Zin'kovskii, and S. B. Seredenin, Khim.-farm. Zh., No. 2, 151-157 (1982).
5. N. Ya. Golovenko, V. G. Zin'kovskii, O. V. Zhuk, and V. A. Sozinov, Pharmacology of Derivatives of Gamma-aminobutyric Acid [in Russian], Tartu (1983), pp. 37-38.
6. N. Ya. Golovenko, O. P. Rudenko, and M. Yu. Totrova, 6th All-Union Symposium on the Purposeful Investigation of Physiologically Active Substances. Summaries of Reports [in Russian], Riga (1984), pp. 301-302.
7. O. V. Zhuk, V. G. Zin'kovskii, N. Ya. Golovenko, et al., Methods of the Individualization and Optimization of the Application of Drugs Based on the Study of Their Pharmacokinetics [in Russian], Moscow (1982), Sec. 1, pp. 153-156.
8. O. V. Zhuk, V. G. Zin'kovskii, and N. Ya. Golovenko, Khim.-farm. Zh., No. 3, 361-366 (1983).
9. V. G. Zin'kovskii, N. Ya. Golovenko, and A. V. Bogatskii, Byull. Éksp. Biol., No. 1, 40-42 (1981).
10. S. B. Seredenin, V. G. Zin'kovskii, N. Ya. Golovenko, et al., Khim.-farm. Zh., No. 9, 23-26 (1981).
11. L. N. Yakubovskii, A. V. Bogatskii, and S. A. Andronati, Khim.-farm. Zh., No. 2, 85-89 (1979).
12. M. Orloff, P. Williams, and C. Pfeilffer, Proc. Soc. Exp. Biol., 70, 254-258 (1949).
13. L. H. Sternbach, J. Org. Chem., 27, 3788 (1962).