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SYNTHESIS OF ^{14}C-PHENAZEPAM AND ITS POTENTIAL METABOLITES

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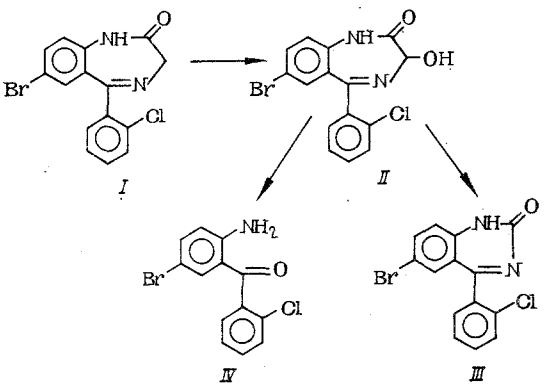
In the preceding papers [1-3], we showed that phenazepam [7-bromo-5-(o-chlorophenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-one] (I) has a high physiological activity, and in several tests exceeds certain preparations of the 1,4-benzdiazepine series. To study the metabolism and pharmacokinetics, and to clarify certain aspects of the action mechanism of I, we synthesized I tagged with ^{14}C isotope at position 2. We used the available data [4-6], and also synthesized the potential metabolites of I: the 3-hydroxy derivative (II), quinazolin-2-one (III), and the corresponding benzophenone (IV).



The total radioactivity of synthesized I was determined in a solution of a toluene–alcoholic scintillator on a liquid scintillation photometer SL made by the firm "Intertechnique" (France).

The radioactivity of the sample was studied at intervals of 0.014-0.14 and 0.7-70 μg. In all cases, we observed a linear dependence between the recorded radioactivity and the amount of the substance. The specific radioactivity of I was 1 mCi/mole.

To find the purity of I and to separate it from potential metabolites, compounds I-IV were deposited on a chromatographic plate in the form of a line 1 cm long. For this purpose, we used chromatographic 150 × 150 mm plates "Silufol UN-254." We chromatographed the samples in the solvent system chloroform–acetone–30% aq. ammonia (75 : 25 : 0.5). Under these conditions, compounds I-IV separate on the plates with different R_f values. The distribution of the starting compound and its metabolites on the chromatograms in their extraction from biological media was controlled by comparing their R_f values and the color in UV light (λ 254 nm) with similar parameters of the synthesized compounds.

The quantitative determination of the tagged compound and its metabolites usually involves its elution from the chromatographic plate and finding the degree of extractability from both the chromatograms and their biological samples. Figure 1 shows that after chromatographic isolation of compound I, followed by repeated extraction in a toluene–alcoholic scintillator, the specific radioactivity of the samples studied decreased. To find the reason for the decrease in the specific radioactivity of I, we determined the radioactivity of different

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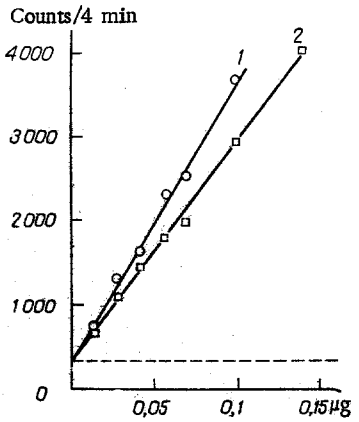

Fig. 1

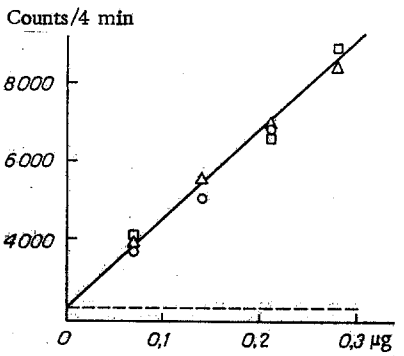

Fig. 2

Fig. 1. Radioactivity of samples of compound I. 1) Total radioactivity of compound determined at the range of 0.01-0.15 μg; 2) radioactivity of extracts from chromatographic plate (R_f 0.5-0.6) after chromatographing I.

Fig. 2. Radioactivity of I extracted from liver (triangles), brain (circles), and blood plasma (squares) homogenates after chromatography.

sections of the chromatograms (see Table 1). We found that 83.36 ± 1.52% of the compound studied, or 98.75% of the total amount of sample deposited on the plate, can be reported by the radiochromatographic method. These results indicate the high purity of the synthesized compound.

To verify the possibility of using the radioactive preparation obtained to study metabolism and the distribution in the experimental animals of phenazepam and its metabolites from biological media, we used the liver, brain (1 : 5), and blood plasma homogenates of white rats. A given amount of I in 0.05 ml of ethanol was introduced into 1 ml of the corresponding media. After incubation of the samples for 1 h at 37°C, the pH of the probes was adjusted to pH 8.0, and the mixture was twice extracted by a 5-fold volume of chloroform. The extracts were evaporated to dryness in concentrators and the residue dissolved in ethanol. The alcoholic solutions (0.1-0.2 ml) were deposited on a chromatographic plate. Part of the coextracted compounds were removed from the plate by a method already described [7]. We chromatographed the compound given as above. Figure 2 shows that the degree of extraction of I was independent of the nature of biological substrate. In all the procedures which were carried out, we detected 80.9 ± 2.6% of the total radioactivity introduced into the biological media. If we correct for the extractability of the radioactive sample from the chromatographic plates (see Fig. 1), we can conclude that during a twice-repeated extraction of I by chloroform from the liver and brain homogenates and also from the blood plasma, more than 97% of the total radioactivity is extracted.

By combining the methods of extraction of I and its metabolites from blood plasma of white rats, to which the compound was administered intraperitoneally in a dose of 14 mg/kg, chromatographic separation, and qualitative determination, we showed that the metabolism of I can be studied in vivo (Fig. 3). In this case, the radioactivity was determined on a chromatographic plate with an interval of 0.05 of the R_f value. We noted that, together with the initial compound I, its principal metabolite II is present in blood plasma.

EXPERIMENTAL

Glycyl-(1-^{14}C) Chloride Hydrochloride. A suspension of 0.47 g (0.0062 mole) of glycine-(1-^{14}C) with a specific activity of 30.5 mCi/mole (preliminarily ground to powder, and dried to 110°C for 2 h) in 7 ml of dry chloroform is saturated with dry hydrogen chloride at 20°C for 10-15 min, and then 1.29 g (0.0061 mole) of phosphorus pentachloride is added. The reaction mixture is stirred for not less than 20 h at 20°C. The reaction mixture is then filtered, and the precipitate is washed with 10 ml of dry carbon tetrachloride. Glycyl-(1-^{14}C) chloride hydrochloride melts between 110 and 120°C.

TABLE 1. Amount of Radioactive Material Detected at Given Sections of Chromatographic Plate during Chromatography of I in Chloroform–Acetone–30% aq. Ammonia System (75 : 25 : 0.5) (M ± m, n = 6).

R_f	Radioactivity detected	
	% of amount of I introduced	% of total amount of I found on plate
0—0,1	0,70±0,06	0,83±0,08
0,1—0,25	0,14±0,01	0,17±0,03
0,25—0,40	0,15±0,03	0,19±0,04
0,40—0,60	82,32±1,42	98,75±2,47
0,60—1,0	0,03±0,005	0,3±0,006
0—1	83,36±1,51	100,00±2,48

7-Bromo-5-(o-chlorophenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-one-(2-^{14}C) (I). Glycyl-(1-^{14}C) chloride hydrochloride is added to a solution of 1.07 g (0.0035 mole) of 5-bromo-2'-chloro-2-aminobenzophenone [2] in 5 ml of dry chloroform. The reaction mixture is boiled, with stirring, until evolution of hydrogen chloride ceases (3.5 h). The mixture is cooled to room temperature, 2 ml of water is added, and aqueous ammonia is gradually added, with stirring, to a persistent slightly alkaline reaction. The aqueous layer is separated, the chloroform solution is once again washed with 3 ml of water, and then evaporated in vacuo. A 4-ml portion of toluene is added to the residue, and the solution is evaporated to complete removal of the solvent at atmospheric pressure. The precipitate is redissolved in toluene and filtered.

By crystallization from a toluene solution, 0.5 g of 7-bromo-5-(o-chlorophenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-one(2-^{14}C) with specific activity of 1 mCi/mole is obtained; radiochemical yield 98.75%, mp 220-222°C. Found %: C 51.6; H 2.6; N 8.1. $C_{15}H_{10}BrClN_2O$. Calculated, %: C 51.5; H 2.9, N 8.0.

5-Bromo-2'-chloro-2-bromoacetamidobenzophenone is obtained by the procedure given in [8] from 5-bromo-2'-chloro-2-amino-benzophenone (IV) in a yield of 90%, mp 157-158°C. Found, %: C 41.6; H 2.5; N 3.5. $C_{15}H_{10}Br_2ClNO_2$. Calculated, %: C 41.7; H 2.3; N 3.2.

5-Bromo-2'-chloro-2-iodoacetamidobenzophenone is obtained by the procedure given in [9] from 5-bromo-2'-chloro-2-bromoacetamido-benzophenone in a yield of 80.5%, mp 114-116°C. Found, %: C 37.2; H 2.2; N 3.2. $C_{15}H_{10}BrClNO_2$. Calculated, %: C 37.6; H 2.1; N 2.9.

5-Bromo-2'-chloro-2-(2-hydroxyaminoacetamido)benzophenone is obtained by the procedure given in [9] from 5-bromo-2'-chloro-2-iodoacetamidobenzophenone in a yield of 99%, mp 171-172°C. Found, %: C 47.0; H 3.3; N 7.5. $C_{15}H_{12}BrClN_2O_3$. Calculated, %: C 46.9; H 3.1; N 7.3.

7-Bromo-5-(o-chlorophenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-one-4-oxide is obtained by the procedure given in [10] from 5-bromo-2'-chloro-2-(2-hydroxyaminoacetamido)benzophenone in a yield of 66%, mp 245-247°C. Found, %: C 49.6; H 2.9; N 8.0. $C_{15}H_{10}BrClN_2O_2$. Calculated, %: C 49.2; H 2.7; N 7.7.

7-Bromo-3-acetoxy-5-(o-chlorophenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-one is obtained by the method given in [10] in a yield of 70%, mp 280-282°C. Found, %: C 49.8; H 3.1; N 7.0. $C_{17}H_{12}BrClN_2O_3$. Calculated, %: C 50.1; H 2.9; N 6.9.

7-Bromo-3-hydroxy-5-(o-chlorophenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-one (II). A 30 ml portion of 4 N sodium hydroxide is added to a suspension of 2.1 g (0.0051 mole) of 7-bromo-3-acetoxy-5-(o-chlorophenyl)-1,2-dihydro-3H-1,4-benzdiazepin-2-one in 40 ml of alcohol. The sodium salt of II precipitates after stirring in the cold. A 50-ml portion of water is added to the suspension, and the reaction mixture is acidified with acetic acid to a neutral reaction. Partial precipitation occurs. The precipitate is filtered and water is added to the solution to precipitate the remainder of the product. Recrystallization from ethanol gives 63% of II, mp 160-162°C. Found, %: C 49.2; H 2.9; N 7.9. $C_{15}H_{10}BrClN_2O_2$. Calculated, %: C 49.2; H 2.7; N 7.7.

6-Bromo-4-(o-chlorophenyl)quinazolin-2-one (III). A mixture of 2.17 g (0.007 mole) of 5-bromo-2'-chloro-2-aminobenzophenone and 0.72 g (0.012 mole) of urea is heated on an oil bath for 2 h at 180-200°C. Then another 0.36 g (0.006 mole) of urea is added, and heating is continued for another 1.5 h. The cold solid precipitate is dissolved in chloroform and water. The chloroform solution is evaporated, and the residue recrystallized from toluene. Yield 89%, mp 350°C (decomp.). Found, %: C 51.0; H 2.3; N 8.2. $C_{14}H_8BrClN_2O$. Calculated, %: C 50.1; H 2.4; N 8.3.

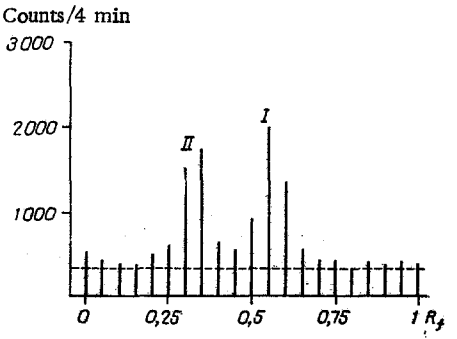

Fig. 3. Radiochromatogram of a blood plasma extract of white rats 180 min after intraperitoneal administration of I in a dose of 14 mg/kg.

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