TRITIUM LABELLED COMPOUNDS OF HIGH SPECIFIC ACTIVITY I. TOFIZOPAM

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SUMMARY

The possibilities of tritium labelling of tofizopam (1) at high specific activity were investigated. Two methods, one by isotopic exchange, and one by catalysed halogen-tritium replacement were developed. The latter was found to be more rational, and 1 specifically labelled with tritium at a specific activity of 12 Ci/mmole was prepared by applying this route. An attempted labelling by catalysed multiple bond reduction with tritium is also described.

Key words: Tritium labelling, 5H-2,3-Benzodiazepine, Isotopic exchange, Catalysed halogen-tritium replacement.

INTRODUCTION

Tofizopam [1-(3,4-dimethoxyphenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine] (1), the first member of 5H-2,3-benzodiazepines synthesized by Kőrösi and Láng (1), has been used in therapy as minor tranquillant, possessing the advantage of no muscle relaxant side-effect. In an earlier paper (2) we described the preparation of 1 labelled with ^{14}C for pharmacological and metabolism studies. In order to investigate its localization in the brain, an analogue of high specific activity specifically labelled

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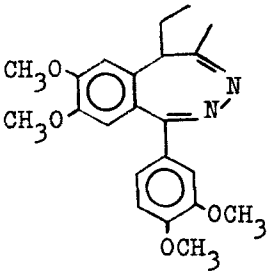
with tritium at a site of the molecule, which is not affected by metabolism was required. Here we report on labelling by isotopic exchange and synthesis.

RESULTS AND DISCUSSION



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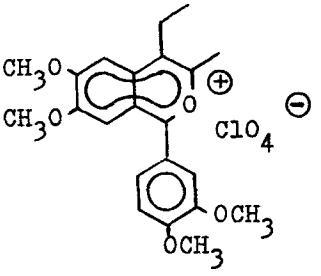

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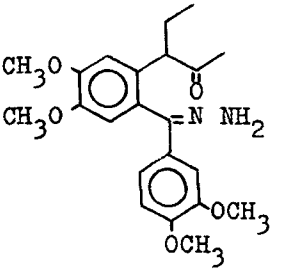

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The exchange reactions were investigated by means of deuterated solvents. On treatment of 1 with CH_3OD under neutral, basic and acidic conditions, incorporation of deuterium could be detected by mass spectrometry only in the last case. Considering the fragmentation of 1 (3), deuterium distribution was calculated from the molecular ion, and the m/e 326 fragment ion which does not contain the C-4 methyl group. The results (see Table 1) proved that only the protons of C-4-methyl group underwent exchange reaction, consequently all the other protons of the molecule are suitable for labelling, except those of the methoxy groups which interfere with the metabolism. The fact that the precursor isobenzpyrylium salt (2) has no proton in the position which becomes position 5 of the benzodiazepine ring, prompted us to carry out the conversion of 2 with deuterated hydrazine in CH_3OD. As it had been expected we managed to introduce more than 90 % deuterium into position 5 of 1, (the position of deuterium was verified later by ^{1}H-NMR spectra), but an isotopic exchange occured in the C-4 methyl group as well.

Then we attempted to find out the optimum of introducing the hydrogen isotope. The best result was achieved by adding 2 in a

solution of dimethylformamide to the mixture of deuterium oxide and hydrazine hydrate at 100 C° (Table 1, experiment I.c). In this way the amount of deuterium incorporated is close to the calculated one.

Experiment	M +• 382					m/e 326		
	d_o	d_1	d_2	d_3	d_4	d_o	d_1	d_2
I./a 1 + CH_3OD + AcOH	39.5	36.3	19.1	5.1	-	100	-	-
I./b 2 + D_2N-ND_2 . D_2O in CH_3OD	6.8	54.3	28.6	8.9	1.1	7.5	89.7	1.7
I./c 2 + H_2N-NH_2.H_2O + D_2O molar ratio: 1:2.5:10	22.5	47.5	23	6	-	43.5	56.5 calc: 57.1	-
washed with MeOH + AcOH	38	58	4	-	-	43.5	56.5	-
I./d 2 + H_2N-NH_2.H_2O + D_2O molar ratio: 1:2.5:50	24	43.5	26.5	5.5	0.5	48.5	51.5 calc: 87.0	-
washed with MeOH + AcOH	33.5	53.5	11	2	-	48.5	51.5	-
I./e 3 + CH_3OD	100	-	-	-	-	100	-	-

Table 1. Content (in %) and distribution of deuterium in 1 by mass spectrometry. (Solvent and conditions are described in the experimental part.)

It is remarkable that when carrying out the conversion by adding hydrazine hydrate to the mixture of 2 and D_2O at 0 C° (experiment I.d), a moderate isotope incorporation was observed. Probably the formation of the monooxo intermediate 3 occurs immediately and one of the protons of hydrazine migrates to the α-position,

before the isotopic exchange between deuterated water and hydrazine takes place completly. We assumed that the role of the enolization could not be significant, otherwise a greater extent of deuterium incorporation would have been observed. Indeed, in the next experiment (I.e) when 3 was isolated and the ring closure was carried out in deuterated methanol, no deuterium was found in the product. The results are in agreement with the mechanism of deuterium incorporation outlined in Figure 1.

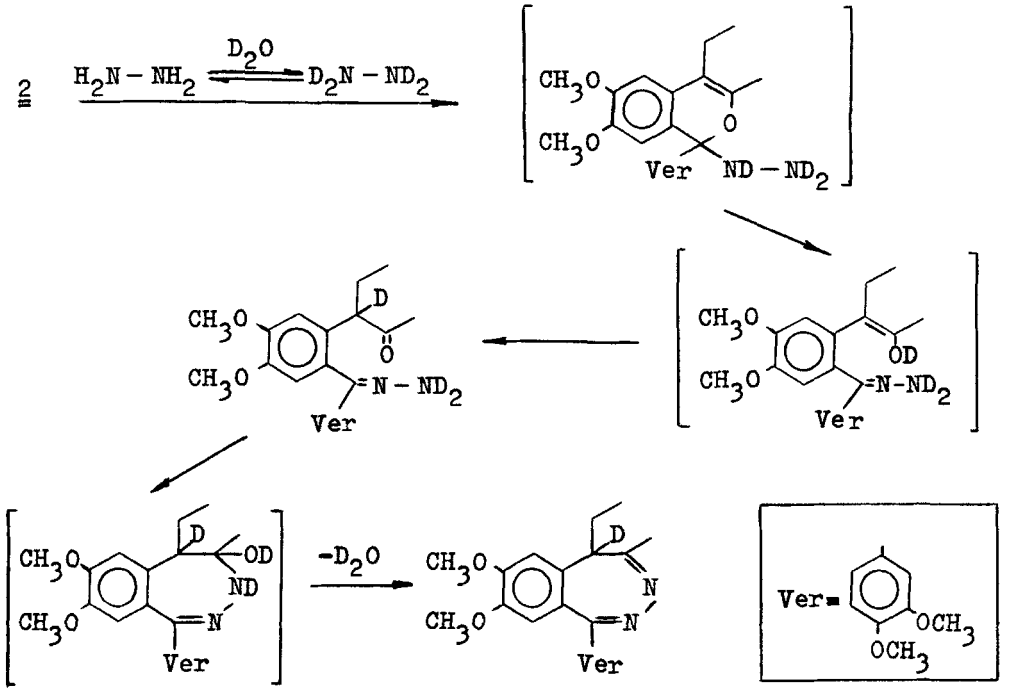
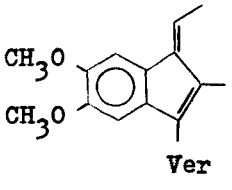


Figure 1. Mechanism of deuterium incorporation.

Comparison of the ^{1}H-NMR spectra of the labelled and unlabelled compounds showed that the method developed is suitable for preparation of 1 labelled in a specified position. The labile deuterium being incorporated into the C-4 methyl group can be removed on treatment with MeOH in the presence of acetic acid.

As for the tritium labelling, it must be taken into consideration that only 2.8 % of the isotope applied can be utilized, and the specific activity is merely one quarter of that of the original tritium oxide. Varying the molar ratios, both the utilization

of isotope and the specific activity can be improved, but only to the detriment of each other. So it would be reasonable to prepare the tritiated compound only if 1 of high specific activity would not be storable, and the process should be repeated several times in order to supply the requirements of the biological research. Since over a period of six months practically no change in the isotopic purity took place when 1 with a specific activity of 12 Ci/mmole was stored in solution (EtOH, 0.001 M) it seemed to be more rational to introduce the tritium by synthesis. For this purpose 4, prepared from 7 by refluxing in formic acid according to Lempert-Sréter (4), appeared to be a suitable precursor.



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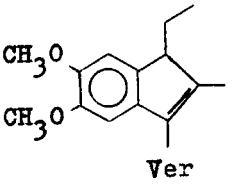

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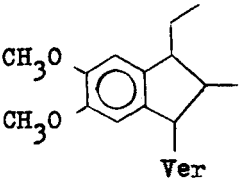

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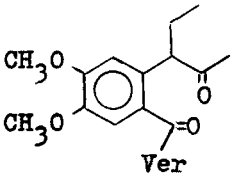

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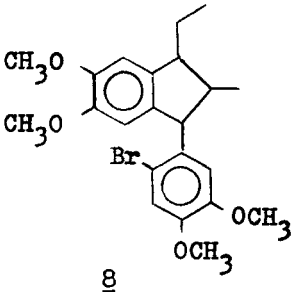

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Catalytic reduction of 4 in benzene solution over Pd catalyst afforded 5, which can be oxidized to 7 in a yield of 90 %. If the reduction was performed similarly but in methanol instead of benzene, according to the data published (4), 2 moles of hydrogen was taken up resulting in 6. This can also be oxidized to diketone 7 but under less mild conditions, and the yield in our experiments was as low as 40 % in a scale of 0.65 mmole.

Unfortunately we could not utilize the aforementioned selectivity, because the reduction of 4 with tritium, independently of

the solvent used, gave the compound 6, which can not be oxidized under mild conditions (see Figure 2). When the oxidation was carried out under less mild conditions we did obtain labelled 7, the specific activity of the tofizopam prepared from this compound however was only a negligible proportion of the calculated one.

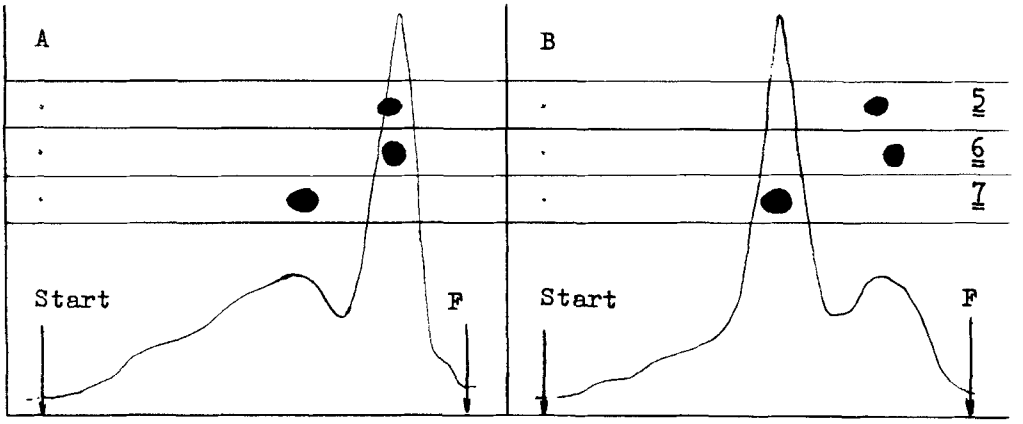

Figure 2. Radio-TLC of tritium-labelled 7. Solvent system: benzene-ethyl acetate = 4:1. The compound obtained from 4 by catalytic reduction with tritium was oxidized as 5 (A) and as 6 (B) respectively.

Therefore we looked for a halogenated precursor suitable for catalysed halogen-tritium replacement. 8 can be prepared from 6 (6) but unfortunately the oxidation of this compound did not give the 1,5-diketone derivative (7). So the strategy of introducing the tritium in the last step had to be given up.

8 was reduced with tritium over Pd catalyst in the presence of triethylamine, and the compound obtained was oxidized to 7, from which the labelled 1 was prepared via 2 (isobenzpyrylium salt). The specific activity of the tritiated 6 was 26 Ci/mmole = 962 GBq/mmole, nearly the calculated one, which was diluted during the manipulation to 12 Ci/mmole = 444 GBq/mmole. The stability of the labelled tofizopam was investigated by checking the isotopic purity by radio-TLC during a period of six months.

The results are shown in Table 2.

Stored	Isotopic purity (%)	
	original	after six months
as solid	> 98	~70
in 0.1 M EtOH sol.	> 98	>94
in 0.001 M EtOH sol.	> 98	>97

Table 2. Change in isotopic purity of 1 during storage.

EXPERIMENTAL

The melting points were determined on a Boëtius hot stage, and are uncorrected. The content and distribution of deuterium were determined by Varian MAT SM 1 mass spectrometer. Radioactivity was measured with a Packard TRI CARB liquid scintillation spectrometer. TLC was carried out on silica gel HF_{254} (Merck) and a Berthold TLC scanner was used for evaluation. All evaporations were carried out under reduced pressure.

I. Isotopic exchange

a./ 1 (10 mg) was dissolved in CH_3OD (1 ml) and heated for 10 minutes at boiling point. After standing overnight the solution was evaporated, and the residue was stirred in hot water (1 ml) for one hour. After cooling the solid was filtered off, washed with water, and dried. A yellowish crystalline product (8.8 mg) was obtained, m.p. 154-155 C^{o}. This experiment was repeated in the presence of 0.1 ml of acetic acid and 0.1 ml of 1 N NaOH respectively.

b./ To hydrazine hydrate (250 µl, 5 mmole) D_2O (1 ml) was added, and the water was distilled off at 100 C^{o} in N_2 atmosphere. The residue was added to a suspension of 2 (470 mg, 1 mmole) in CH_3OD (2 ml). The solution was boiled for 5 minutes, and evaporated. The residue was stirred in hot water (10 ml) for one hour. After cooling the solid was filtered off, washed

with water, and dried to give 316 mg (83.0 %) of crystalline product. M.p. 152-154 C°.

c./ To a mixture of hydrazine hydrate (250 µl, 5 mmole) and D_2O (36 µl, 2 mmole), 2 (94 mg, 0.2 mmole) in DMF (0.5 ml) was added at 100 C°. After cooling, the solvent was removed by freeze-drying and the residue was treated with hot water as described above to give 59.5 mg (77.9 %) of crystalline product. M.p. 152-154 C°.

10 mg of this compound was dissolved in MeOH (3 ml), and acetic acid (0.1 ml) was added to the solution. It was evaporated and the residue was reevaporated three times with MeOH (3x3 ml). The adduct was decomposed in hot water to give 9.1 mg of crystalline product. M.p. 152-154 C°.

d./ Hydrazine hydrate (25 µl, 0.5 mmole) was added at 0 C° to a mixture of D_2O (180 µl, 10 mmole), DMF (2 ml) and 2 (94 mg, 0.2 mmole). After standing one hour at 0 C° the mixture was gradually heated to 100 C°, then cooled and the solvent was removed by freeze-drying. The residue was treated with hot water, cooled and filtered off to give 61 mg (79.8 %) of crystalline product. M.p. 152-154 C°. 10 mg of this compound was treated with a mixture of MeOH and acetic acid as described above giving 8.9 mg of crystalline material. M.p. 152-154 C°.

e./ To a solution of 1-(3,4-dimethoxyphenyl)-3-methyl-4-ethyl--6,7-dimethoxy-isobenzpyrylium sulfate (466 mg, 1 mmole) in water (10 ml), hydrazine hydrate (120 µl, 24 mmole) was added at 0 C°. The precipitated 3 was filtered off, and dried. Yield: 340 mg (82.5 %).

80 mg (0.2 mmole) of this compound was dissolved in CH_3OD (1 ml) and warmed on a steam bath for 10 minutes. The solution was evaporated and the residue was decomposed in hot water as described previously. Yellowish crystals, yield: 57 mg, m.p. 149-152 C°.

II. Reduction of 4

a./ 352 mg (1 mmole) of 4 in MeOH (10 ml) was hydrogenated over 5 % Pd/C catalyst. 43 ml of hydrogen were absorbed during 30 minutes. The solution was filtered and evaporated. Recrystallization of the residue from MeOH gave white crystals, (286 mg, 80.4 %). M.p. 103-104 C°.

231 mg (0.65 mmole) of this compound was dissolved in acetic acid (1 ml) and water (0.4 ml) was added. After cooling to 0 C°, a solution of CrO_3 (130 mg, 1.3 mmole) in acetic acid (1 ml) and water (0.6 ml) was added dropwise to the stirred mixture. After being stirred at 0 C° for an hour and at 25-30 C° for 1.5 hours, water (13 ml) was added to the solution and it was left to stand overnight. The precipitated material was filtered off, washed with water and dissolved in acetic acid (1 ml). To the solution 70 % perchloric acid (80 µl) was added, and the mixture was warmed for 3 minutes on a steam bath. After cooling to 0 C°, ethyl acetate (6 ml) and diethyl ether (8 ml) was added, and the precipitated isobenzpyrylium salt was filtered off, washed with ethyl acetate and dried. Yield: 100.6 mg (0.21 mmole, 32.9 %), m.p. 221-233 C°, dec.

b./ A solution of 4 (352 mg, 1 mmole) in benzene (10 ml) was hydrogenated over 5 % Pd/C catalyst. In one hour 24 ml of hydrogen was absorbed. The solution was filtered, evaporated, and recrystallization of the residue from 50 % acetone gave a crystalline material (286 mg, 0.21 mmole, 80.8 %), m.p. 107-108 C°.

230 mg (0.65 mmole) of this compound was dissolved in a mixture of acetic acid (1 ml) and water (0.4 ml). CrO_3 (100 mg, 1 mmole) dissolved in acetic acid (1 ml) and water (0.6 ml) was added at 0 C°, and the solution was stirred for 30 minutes, then diluted with water (13 ml). The precipitated solid was filtered off, washed with water, dissolved in acetic acid

(1 ml), and 70 % perchloric acid (80 µl) was added. After warming on a steam bath for three minutes the mixture was cooled and the crystalline 2 was precipitated by adding ethyl acetate (6 ml) and diethyl ether (8 ml). The yellow crystals were filtered off, washed with ethyl acetate and dried. Yield: 235 mg (0.5 mmole, 76.9 %), m.p. 219-221 C°, dec.

c./ 35.2 mg (0.1 mmole) of 4 dissolved in benzene (1 ml) was reduced with carrier-free tritium over 5 % Pd/C catalyst on a vacuum manifold (8).

The amount of tritium absorbed was 4.1 N ml (10 Ci = 370 GBq). The solution was evaporated, the residue dissolved in acetic acid (10 ml) and reevaporated. The residue was mixed with 5 (195 mg, 0.55 mmole), dissolved in acetic acid (1 ml), and oxidized as described at II/b. The product obtained was the yellow isobenzpyrylium salt, 212 mg (0.45 mmole, 69 %), m.p. 219-222 C°, dec. This material was suspended in hot methanol (3 ml) and hydrazine hydrate (100 µl, 2 mmole) was added resulting in a pale yellow solution. The solution was treated with charcoal, filtered, and evaporated. To the residue 0.1 ml of acetic acid was added, and stirred in hot water for one hour. After cooling the crystalline material was filtered off, washed with water and dried to give 147 mg (0.38 mmole, 85.3 %) of labelled 1, m.p. 154-155 C°, specific activity: 140 mCi/mmole = 5.18 GBq/mmole.

This experiment was repeated under the same conditions, with the only exception that the oxidation was carried out as described at II/a. The yield of isobenzpyrylium salt obtained was 100 mg (0.21 mmole, 32.7 %), m.p. 219-222 C°, dec. This compound was reacted with hydrazine hydrate as described above to give 71 mg (0.19 mmole, 89.7 %) of crystalline 1 labelled with tritium. M.p. 153-155 C°, specific activity: 391 mCi/mmole = 14.47 GBq/mmole.

III. 1-(3,4-dimethoxyphenyl-6-^{3}H)-4-methyl-5-ethyl-7,8-dimethoxy--2,3-5H-benzodiazepine

130 mg (0.3 mmole) of 8 was dissolved in a mixture of ethyl acetate (3 ml) and triethylamine (50 µl). This solution was reduced with carrier-free tritium over 5 % Pd/C catalyst on a vacuum manifold. The absorbed tritium was 6.7 N ml (16.8 Ci = 622 GBq). The solution was then filtered, evaporated, and the residue was taken up in benzene (10 ml). The solution was shaken with 10 ml of 1 N HCl, the organic phase was evaporated, and the residue was reevaporated three times with 10 ml portions of ethanol. The semi-solid material obtained was 102 mg, 7.69 Ci = 284 GBq had a specific activity of 26.7 Ci/mmole = 988 GBq/mmole.

This material was mixed with 130 mg (0.36 mmole) of 6, dissolved in acetic acid (1 ml) and oxidized as described at II/a. The yield of isobenzpyrylium salt was 103 mg (0.22 mmole, 33.8 %) m.p. 220-222 C°, dec.

This material was converted into 1 with hydrazine hydrate to give 72.4 mg (0.19 mmole, 85.9 %) yellowish crystalline product, m.p. 154-155 C°. Activity: 2.22 Ci = 82 GBq, at a specific activity of 11.74 Ci/mmole = 434 GBq/mmole, 30.71 Ci/g = 1136 GBq/g. The isotopic purity checked by TLC (benzene-cyclohexane-ethanol = 5:4:1) was higher than 98 %.

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