LABELLING OF 1-BENZYL-1-(3'-DIMETHYLAMINOPROPOXY)-CYCLO-HEPTANE. FUMARATE WITH TRITIUM.

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SUMMARY

In order to complete our previous publications on pharmacokinetics, action mechanism and metabolism studies, the following new, tritium-labelled derivatives of Halidor have been prepared: 1-benzyl-1-(3'-dimethylaminopropoxy)-3,4,5,6-^{3}H-cycloheptane fumarate, 1-benzyl-1-(2'-^{3}H-3'-dimethylaminopropoxy)-cycloheptane fumarate, and 1-(benzyl-4-^{3}H)-1-(3'-dimethylaminopropoxy)-cycloheptane fumarate.

INTRODUCTION.

The radiocarbon-labelled isotopic isomers of 1-benzyl-1-(3'--dimethylaminopropoxy)-cycloheptane fumarate (Bencyclan^{+++}, Halidor^{++++}, Fludilat^{+++++}) have formerly been prepared[1] for radioisotope-indicated pharmacological tests[2]. The subsequent metabolism and transport studies have called for the preparation of compounds labelled with tritium in definite positions. The present report deals with the preparation of molecules labelled with tritium in the cycloheptyl ring, in the aromatic ring and in the

$^{+++}$ Generic name
$^{++++}$ Registered name in Hungary
$^{+++++}$ Registered name in G.F.R.

alkylene group of the side chain, respectively.

Special attention had to be paid to the proper selection of the method of introducing the tritium isotope into the cycloalkyl ring. A synthesis pathway had to be found where the position of introduced T atoms was exactly known and the interfering effect of $T \rightleftharpoons H$ exchange reaction could be avoided both with the endproduct and each intermediate. A rather complicated method, normally unusual in tritium-labelling technique, had to be applied to comply with this task (Figure 1). The olefinic bonds of ethyl muconate were saturated with tritium gas in the presence of Pd catalyst. The obtained ethyl adipate was converted first by means of $LiAlH_4$ into hexamethylene glycol, then the dibromo derivative of the latter was transformed to suberodinitrile by treatment with KCN. The reduction by the complex metal hydride was performed according to Nystrom and Brown[3], while the bromination reaction used the investigations of Stone[4]. The basic magnesium suberate, obtained by acidic hydrolysis of suberodinitrile, was converted by heating at 350° into 3,4,5,6-$^3H_4$-cycloheptanone, according to the method of Böeseken and Derx[5]. The 1-benzyl-1-(3'-dimethylaminopropoxy)-3,4,5,6-$^3H_4$-cycloheptane fumarate was prepared according to the method of Pallos and co-workers[6]. Labelling was performed in 10 mmolar scale and a 17 per cent radiochemical yield was obtained.

1-(4-Chlorobenzyl)-1-(3'-dimethylaminopropoxy)-cycloheptane, synthetized by Pallos and coworkers[6], was used as starting material for the preparation of the molecule labelled in the aromatic ring (Figure 2). The introduction of tritium was performed by catalytic dehalogenation in 0.01 mmolar scale in ethyl acetate solution, and using Halidor base for acid binding. The product was diluted with inactive Halidor before the final operation. The radiochemical yield was nearly quantitative.

The labelling with tritium of the alkylene group of the side chain was based on the radioactive key-intermediate of 1-chloro-2--bromo-2-^{3}H-propane (Figure 3). TBr was generated by the method of Brown and Groot[7], as modified by Bengsch[8] and TBr was reacted with allyl chloride in the presence of dibenzoyl peroxide, according to the method of Kharasch and Mayo[9]. The obtained chlorobromopropane was reacted with dimethylamine according to Marxer's

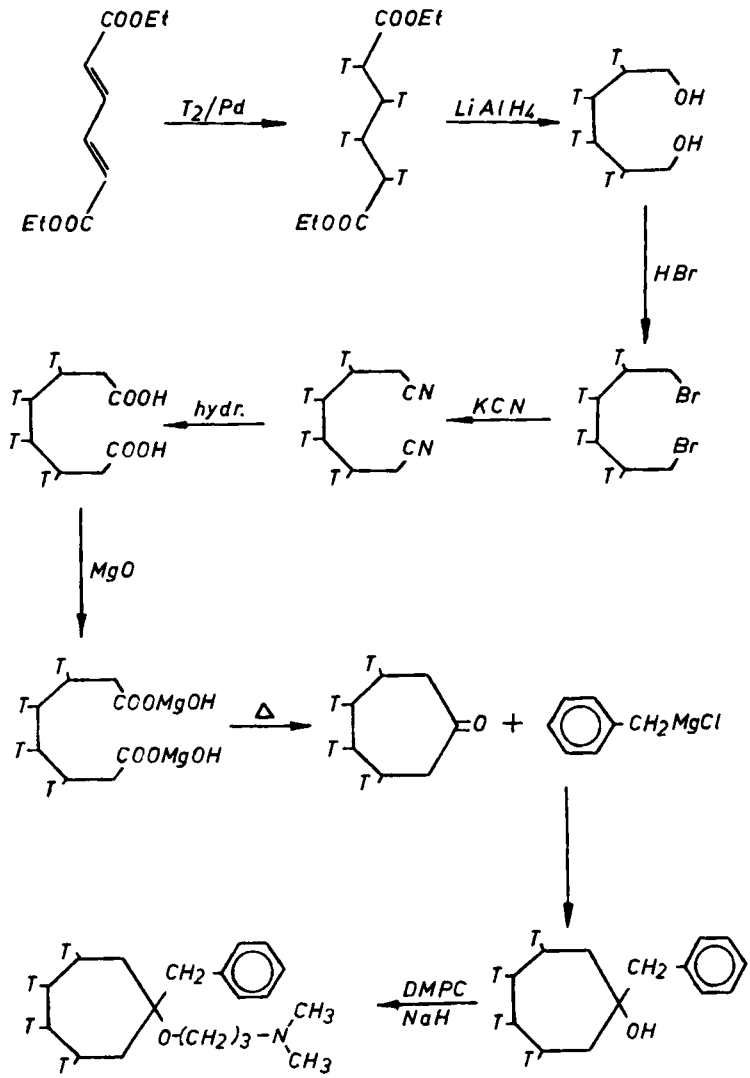
COOEt
EtOOC
T2/Pd
LiAlH4
OH
HBr
Br
KCN
CN
hydr.
COOH
MgO
COOMgOH
Δ
O
CH2MgCl
CH2
O-(CH2)3-N
CH3
DMPC
NaH

Fig. 1.

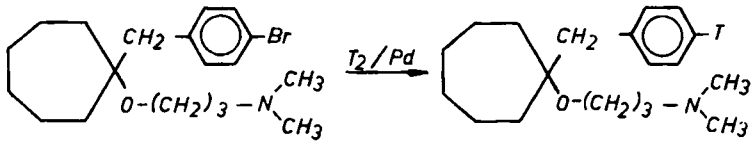
CH2
Br
T2 / Pd
CH2
T
O-(CH2)3 – N
CH3
CH3
O-(CH2)3 – N
CH3
CH3

Fig. 2.

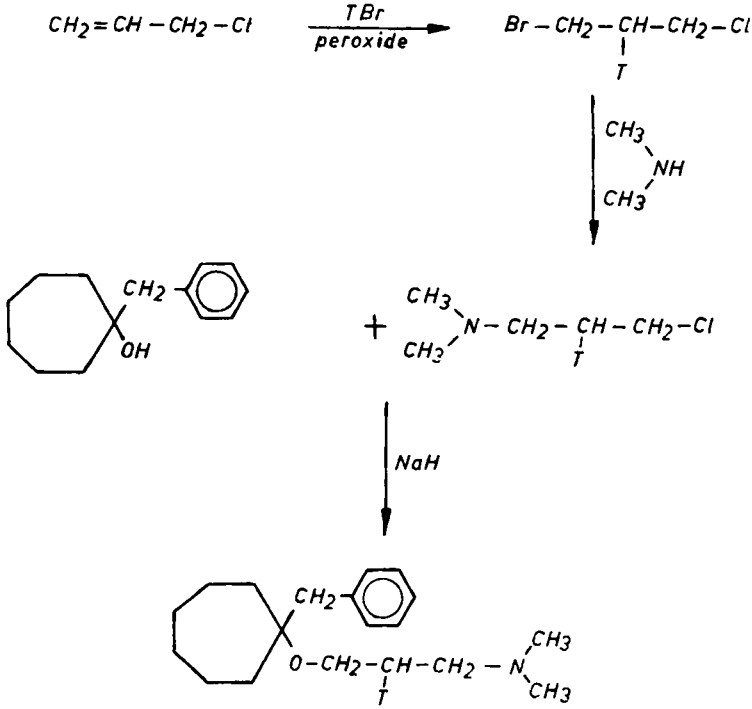
CH2=CH – CH2 – Cl
TBr
peroxide
Br – CH2 – CH – CH2 – Cl
T
CH3
NH
CH3
CH2
OH
+
CH3
N – CH2 – CH – CH2 – Cl
CH3
T
NaH
CH2
O – CH2 – CH – CH2 – N
T
CH3
CH3

Fig. 3.

method[10] and dimethylaminopropyl chloride (DMPC) was obtained. By condensing the radioactive DMPC with 1-benzyl-cycloheptan-1-ol in the presence of sodium hydride, 1-benzyl-1-(2'-^{3}H-3'-dimethylaminopropoxy)-cycloheptane was obtained which was converted into fumarate salt and recrystallized as described in the former experiment. Labelling was performed in a 10 mmolar scale and a 22 per cent yield was obtained, calculated with reference to the initial quantity of HTO.

The chemical and radiochemical purity of the intermediates and end-products were controlled by TLC and autoradiography. The radioactivity of the substances was determined with a TRI-CARB type liquid scintillation beta-spectrometer.

EXPERIMENTAL.

2,3,4,5-$^3H_4$-hexane-1,6-diol

2 mg (0.01 mmole) of ethyl muconate in 0.5 ml of abs. ether is saturated at 600 mmHg pressure with carrier-free tritium gas, in the presence of Pd-C catalyst, in a microhydrogenation apparatus (Calculated T_2 consumption: 4.5 ml), and at the end of the reaction, 2.022 g (10 mmoles) of inactive ethyl adipate dissolved in 30 ml of abs. ether are added. The obtained mixture is filtered and the filtrate is added dropwise to the suspension of 1.14 g (30 mmoles) of $LiAlH_4$ in 20 ml of ether. The mixture is refluxed for 3 hours, then 20 ml of 20 % hydrochloric acid is added dropwise under constant cooling in an ice-bath. The mixture is extracted for 15 hours in a continuous extraction apparatus, the separated ethereal phase is dried by filtration through $MgSO_4$ and evaporated to dryness. Yield: 1.182 g, 1274 mCi (10 mmoles, 127.4 mCi/mmole), 100 %.

1,6-Dibromo-2,3,4,5-$^3H_4$-hexane

Sulphur dioxide gas is introduced into the mixture of 1.53 ml (4.8 g, 30 mmoles) of bromine and 5.2 g of ice, until the colour of bromine disappears. The obtained solution is mixed with the formerly obtained 1.182 g (10 mmoles) of hexane diol. The mixture is set aside at 20°C overnight and then kept at 100°C for 1 hour more. After cooling, the mixture is extracted 3 times with 20 ml portions

of benzene. The benzene extract is washed first with 10 ml of 85 % sulphuric acid, subsequently with 5 % sodium carbonate solution and water, then dried with $CaCl_2$ and evaporated to dryness.
Yield: 2.36 g (9.66 mmoles), 96.6 %.

2,3,4,5-$^3H_4$-hexane dinitrile

2.36 g (9.66 mmoles) of dibromohexane in 8 ml of 75 % ethanol is reacted with 1.26 g (19.32 mmoles) of 75 % ethanol is reacted with 1.26 g (19.32 mmoles) of potassium cyanide, under boiling for 2 hours. After cooling, the mixture is diluted with 18 ml of water and extracted 3 times with 20 ml portions of ether. The ethereal extract is dried over $CaCl_2$ and evaporated to dryness.
Yield: 1.165 g (8.55 mmoles), 88.5 %.

2,3,4,5-$^3H_4$-suberic acid

1.165 g (8.55 mmoles) of hexane dinitrile is refluxed with 10 ml of 50 % sulphuric acid for 6 hours. After cooling, the mixture is diluted with 10 ml of water; the precipitated crystals are collected by filtration and rinsed with a little water. The obtained substance is dissolved in 10 % sodium hydroxide solution: the solution is clarified by activated carbon, filtered and acidified with concentrated hydrochloric acid to pH 1. The precipitated material is collected on a filter, washed twice with 1 ml of water and dried.
Yield: 1.220 g (7.01 mmoles), 83 %.

2,3,4,5-$^3H_4$-Cycloheptanone

The suspension of 1.220 g (7.01 mmoles) of suberic acid and 0.85 g of magnesium oxide in 30 ml of water is agitated at 100°C for 2 hours; the hot mixture is filtered and the filtrate is evaporated to dryness. The obtained substance (1.644 g) is heated in nitrogen stream, with such a rate as to reach 350°C in 2 1/2 hours, and this final temperature is maintained for 1 hour more. The distillate is collected in a receiver cooled by liquid nitrogen, then dissolved in 50 ml of abs. ether and dried over $MgSO_4$.

1-Benzyl-3,4,5,6-$^{3}H_4$-cycloheptan-1-ol

A Grignard reagent is prepared from 1.266 g (10 mmoles) of benzyl chloride and 0.243 g (10 mmoles) of magnesium turings in 15 ml of abs. ether and the previously obtained ethereal solution of radioactive cycloheptanone is added dropwise. The mixture is refluxed for 1 hour, then the complex is decomposed with ammonium chloride solution; the ethereal layer is separated and evaporated to dryness. The residual oil is dissolved in 1 ml of light petroleum, then crystallized at -50°C; the crystals are collected on a filter and rinsed with 0.2 ml of light petroleum of -50°C.
Yield: 0.52 g (2.79 mmoles), 43 %.

0.204 g (1 mmole) of inactive benzylcycloheptanone are dissolved in the mother liquor of the above reaction, then the substance crystallized at -50°C is collected on a filter (0.19 g, 0.93 mmoles) and added to the previously obtained product.
Yield: 0.260 g (3.72 mmoles).

1-Benzyl-1-(3'-dimethylaminopropoxy)-3,4,5,6-$^{3}H_4$-cycloheptane fumarate

0.76 g (3.72 mmoles) of the above carbinol is dissolved in 10 ml of anhydrous benzene and 0.196 g (4.09 mmoles) of 50 % sodium hydride is added. The mixture is refluxed for 3 hours, then a 50 % benzene solution of 1.09 g of dimethylaminopropyl chloride is added dropwise; the mixture is refluxed for 6 hours further, then 10 ml of water is added. The benzene layer is separated, washed with water, dried over $MgSO_4$, and evaporated to dryness. The residual oily liquid is dissolved in 2 ml of abs. ethanol and a hot solution of 0.430 g of fumaric acid in 6 ml of water is added. The mixture is allowed to crystallize overnight, then the precipitated crystals are collected on a filter, rinsed with 5 ml of light petroleum, and dissolved in 5 ml of 25 % aqueous alcohol; the solution is clarified with activated carbon, filtered and allowed to crystallize in a refrigerator overnight. The separated white, crystalline substance is collected on a filter, rinsed with a little water and dried (m.p. 131° to 133°C).
Yield: 0.856 g (2.11 mmoles), 56.7 % (216 mCi, 102.4 mCi/mmole,

253 mCi/g).

The light petroleum solution is evaporated to dryness, dissolved in 0.5 ml of petroleum ether and crystallized at -50°C: an additional yield of 0.143 g, 71.2 mCi (0.70 mmoles, 101.7 mCi/mmole) of carbinol is obtained.

1-Benzyl-(4-^{3}H)-1-(3'-dimethylaminopropoxy)-cycloheptane fumarate

The mixture of 3.3 mg (0.01 mmole) of 1-(4-Chlorobenzyl)-1--3'-dimethylaminopropoxy)-cycloheptane and 29 mg (0.1 mmole) of Halidor base are dissolved in 0.5 ml of ethyl acetate and reacted at 600 mmHg pressure, in a microhydrogenation apparatus, with tritium gas in the presence of Pd-C catalyst. After the calculated volume (2.25 ml) of tritium gas has been taken up, the solution is mixed with 579 mg (2 mmoles) of Halidor base dissolved in 10 ml of ethyl acetate, 1 drop of alcoholic ammonia solution is added; then the mixture is filtered and evaporated to dryness. The yellow, oily substance (614 mg) is dissolved in 2 ml of abs. ethanol and a hot solution of 244 mg (2.1 mmoles) of fumaric acid in 6 ml of water is added. The mixture is allowed to crystallize overnight then the separated crystals are collected on a filter, rinsed 3 times with 1 ml of water, and dried (m.p. 131° to 133°C).
Yield: 822 mg, 312 mCi (2.03 mmoles, 157.5 mCi/mmole, 390 mCi/g), 96 %.

1-Benzyl-1-(2'-^{3}H-3'-dimethylaminopropoxy)-cycloheptane fumarate

Hydrogen bromide is generated from 5 ml of benzoyl bromide and 0.135 ml (7.5 mmoles) of tritiated water (1.2 Ci, 160 mCi/mmole) and the obtained gas is frozen on the mixture of 0.760 g (10 mmoles) of allyl chloride and 0.05 g of benzoyl peroxide in a bomb tube which then is sealed. The mixture is set aside at -75°C for 2 hours, then the bomb tube is opened and its content is dissolved in 5 ml of benzene; this solution is washed first with water, subsequently with 5 % sodium bicarbonate solution and again with water; the solution is dried over $CaCl_2$ and 10 ml of 20 % dimethylamine solution in benzene are added, and the mixture is kept standing at 0°C overnight, then the reaction is completed at 50°C for 3 hours, under

agitation. After cooling, the separated salt is collected on a filter and the filtrate is added to the sodium salt prepared from 2.04 g (10 mmoles) of benzylcycloheptanone and 0.59 g (11 mmoles) of 50 % sodium hydride, using anhydrous benzene as medium. The mixture is agitated at 80°C for 6 hours, then 25 ml of water is added. The benzene phase is separated, washed with water, dried over $MgSO_4$ and evaporated to dryness. The obtained light-yellow, oily liquid (2.62 g) is dissolved in 6 ml of ethanol and mixed with the hot solution of 1.16 g of fumaric acid in 18 ml of water. The mixture is allowed to crystallize in a refrigerator overnight, then the separated crystals are collected on a filter and washed with 10 ml of petroleum ether. The white, crystalline substance is dissolved in 20 ml of water; the solution is made alkaline by adding 20 ml of 1N sodium hydroxide and the separated oily substance is extracted twice with 10 ml of petroleum ether. Upon evaporating the solvent, 0.870 g of yellow oily liquid are obtained; the liquid is dissolved in 2.5 ml of ethanol and mixed with the hot solution of 0.348 g (3 mmoles) of fumaric acid in 7.5 ml of water. The mixture is allowed to crystallize overnight, the separated crystals are collected on a filter and washed with petroleum ether. The obtained crystalline product is recrystallized from 5 ml of 25 % alcohol (m.p. 131° to 133°C).

Yield: 0.891 g, 170.5 mCi (2.2 mmoles, 77.5 mCi/mmole, 192 mCi/g), 22 %.

Chromatographic tests

The chemical and radiochemical purity of all the three isotopic isomers was controlled by TLC.

Sorbent: Kieselgel G.

Solvent: ethyl acetate:ethanol:dimethylformamide:triethylamine = = 60:30:5:5

R_f: 0.65

Chemical detection: spraying with Draggendorf's reagent. Contact autoradiograms of the thin layer chromatograms were prepared to control the radiochemical purity; 10 uCi of sample was applied from each product; the above mixture of solvents was used for development, and after careful drying, the thin layer chromatograms

were sprayed with a 2 % anthracene solution in chloroform. The plates were kept in a dark place for 24 hours, then exposed on FORTE X-ray film for 72 hours.

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