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SYNTHESIS OF ^{3}H-TOLPERISONE

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Tolperisone (**2**) has been tritiated to 50 Ci/mmol specific activity in order to use this compound in the study of muscle relaxant binding. Of the two reaction pathways investigated, hydrogenolytic exchange of aromatic bromine is favored over hydrogenation of a double bond.

Keywords: tolperisone, tritiation, muscle relaxants

Introduction

Although centrally active muscle relaxants (MR) are intensively used in medicine, their mode of action is still largely unknown [1]. The drug tolperisone (**2**) is of special interest among the MRs because of its dual mode of action, as it is known to interact at both centrally and peripherally synapses. It has been shown to block voltage gated sodium channels [2] and to exhibit structural and functional similarities to lidocaine [3]. Tolperisone therefore is a preferred candidate for investigation of the neuropharmacology of centrally acting MRs by studying the location and the characteristics of MR binding. These experiments prompted us to synthesize a radioactively labeled tolperisone

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(^{3}H-tolperisone) that can be used in photoaffinity labeling experiments as well as in pharmacodynamic and pharmakokinetic investigations.

Results and Discussion

Two alternative routes for specific labeling of tolperisone with tritium are evident as outlined in Fig. 1. Here we describe the synthetic approach to both compounds and the final tritiation of the dibromo derivative **3**.

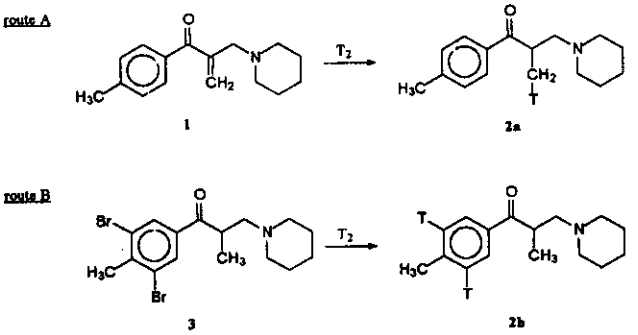

Fig. 1: Routes to ^{3}H-tolperisone

Investigation of route A

The unsaturated tolperisone derivative **1** can be synthesized by a sequence of Mannich and Aldol reactions without workup of the intermediate normethyl-tolperisone **5** hydrochloride using two equivalents of formaldehyde in acetic acid (see Fig. 2).

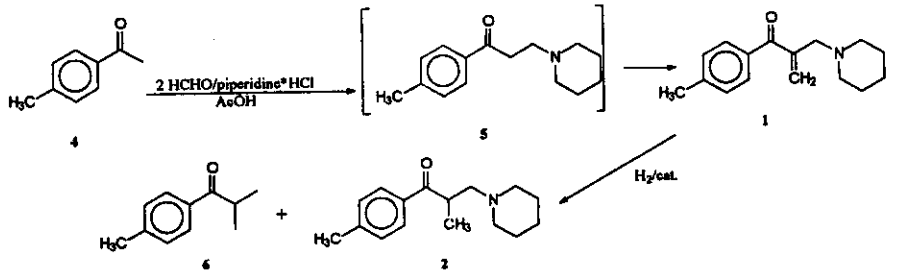

Fig. 2: Investigation of route A

Direct synthesis of the unsaturated derivative **1** was achieved in 80 % yield and this compound was then hydrogenated adapting a literature method described for a tolperisone derivative that lacked the aromatic methyl group [4]. For this procedure the hydrochloride of **1** was used employing a Pd/C catalyst in acetic acid. Although the reaction yields the desired product **2**, no complete turnover of starting material could be achieved but rather a developing decomposition to the deaminated compound **6** was observed on prolonged reaction times. In addition the chromatographic separation of product and starting material turned out to be unfavorable, as the remaining non-converted starting material **1** decomposed on the silica gel. On alumina no satisfying separation could be achieved. Use of different solvents (EtOH, H_2O, EtOAc, Et_2O, 1,4-dioxane, acetone) and catalysts (PtO_2, $(PPh_3)_3RhCl$) did not significantly change the results, except for the conversion rate of compound **1** to tolperisone (**2**) which varied between 40 and 90 %.

Investigation of route B

Tritiation of aromatic compounds by halogen exchange is a widely known method in radiochemistry using chlorine or bromine substituted aromatic compounds [5]. The necessary precursor **3** for the tritiation reaction can be synthesized by Mannich reaction from the corresponding brominated phenone **8**. Using excess $AlCl_3$ and bromine, propiophenone **7** can be converted directly to the bromide **8** in 58 % yield in a solid phase bromination reaction. Conventional Mannich reaction conditions (HCHO, piperidine hydrochloride) only gave moderate yields of the desired dibromotolperisone **3**, however, employing the preformed methylene-piperidiniumchloride as the reactive iminium salt [6] increased the yield to 88 %. Hydrogenolysis of this compound as the free base in ethanol/triethylamine using Pd/C as catalyst gave a 90 % yield of tolperisone (**2**) after 90 min. Additional increase of the yield to practically 100 % was achieved by optimizing the workup procedure, which involves conversion of the free base to the hydrochloride salt by use of a acetylchoride/methanol mixture as the crucial step.

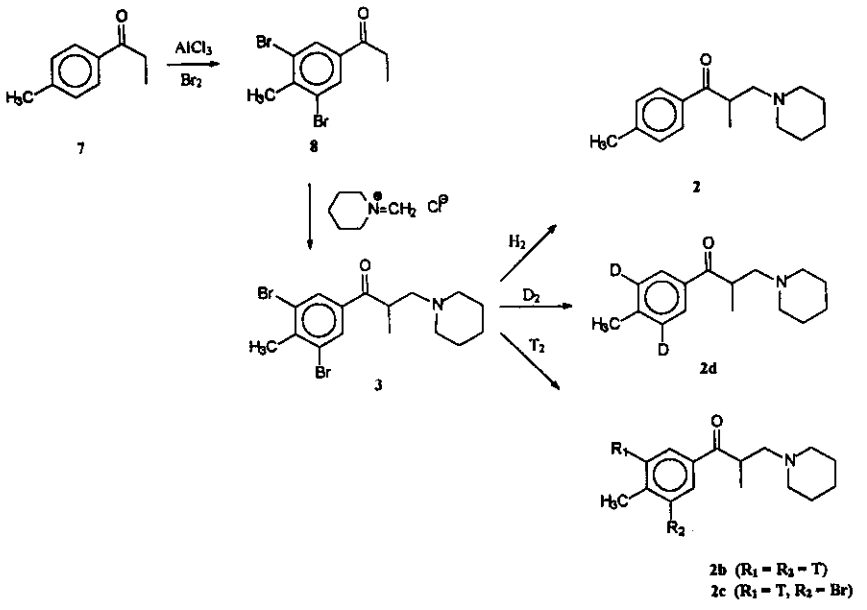

Fig. 3: Investigation of route B

In order to verify the hydrogenation step and to check for an isotope effect, deuterium gas was employed in the hydrogenolysis reaction yielding compound **2d** from dibromide **3**. NMR-analysis proved the different reaction products of the hydrogenation of compound **3** using hydrogen and deuterium, respectively (see Fig. 4). No significant change was observed with respect to the yield and the purity of the product.

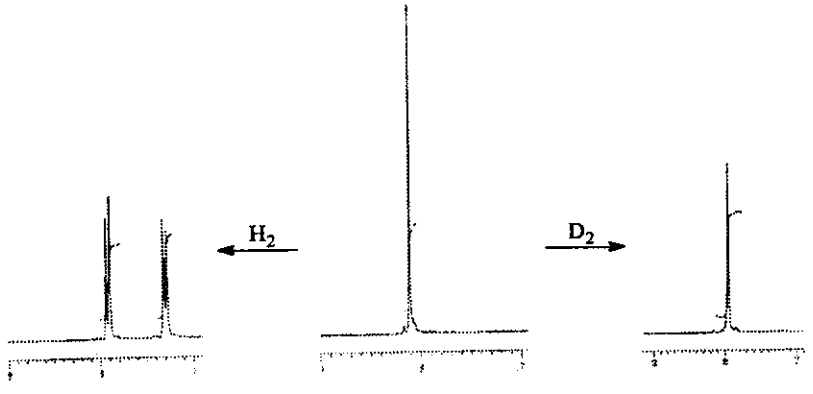

Fig. 4: ^{1}H-NMR (aromatic part) of the compounds involved in the halogen exchange reaction with hydrogen (left) and deuterium (right).

Tritiation of compound 3

Tritiation was carried out using 140 mg of the hydrochloride of dibromo-compound **3** with an excess of T_2-gas. Despite employing the exact reaction conditions as worked out for the hydrogenation and deuteration, tritiation of **3** resulted only in a mixture of the desired ^{3}H-tolperisone (**2b**) along with about 20 % of the monobromo-compound **2c**, which, however, could be removed by semipreparative HPLC. The specific activity of the purified ^{3}H-tolperisone was determined by LC/MS to be 51 Ci/mmol (see Table 1).

Table 1: Specific activity of ^{3}H-tolperisone (**2d**) as determined from LC/MS experiments

mass no.	% of occurrence	max. specific activity (Ci/mmol)
248	26.9	29
250	71.5	58
252	1.6	87
	100.0	50.7

The derived ^{3}H-tolperisone bears a comparatively high specific activity and hence will be a valuable tool for further investigation of the location and the mode of binding of this CNS active muscle relaxant.

Experimental Section

3-Piperidine-1-p-toluyl-propan-1-on (5)

13.41 g (0.1 mol) 4'-methylacetophenone (**4**), 5.04 g (0.17 mol) paraformaldehyde and 12.20 g (0.1 mol) piperidine were diluted with 17 ml ethanol and refluxed for 1 h. 0.2 Ml conc. HCl was added and the hot mixture was filtered. Cooling to room temperature resulted in precipitation of aminoketone **5**. Additional product was isolated from the remaining solution after concentration to afford a total of 1.51 g (70 %) of **5**.

Fp.: 178 °C (hydrochloride; Lit.: 175 °C [7]); ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.43 - 1.59 (m, 6H, 3 x CH_2), 2.39 (s, 3H, CH_3), 2.41 -2.44 (m, 4H, 2 x CH_2), 2.78 (t, J = 7.4 Hz, 2H, CH_2), 3.16 (t, J = 7.4 Hz, 2H, CH_2), 7.23 (d, J_{ortho} = 8.0 Hz, 2H, 2 x aromat. CH), 7.84 (d, J_{ortho} = 8.0 Hz, 2H, 2 x aromat. CH); ^{13}C-NMR (50 MHz, $CDCl_3$): δ = 21.88 (CH_3), 24.61 (CH_2), 26.27 (2 x CH_2), 36.47 (CH_2), 54.29 (CH_2), 54.87 (2 x CH_2), 128.45 (2 x aromat. CH), 129.53 (2 x aromat. CH), 134.80 (quart. aromat. C), 143.97 (quart. aromat. C), 199.09 (C=O); $C_{15}H_{22}NOCl$ (268) calc.: C 67.28 H 8.28 N 5.23, gef.: C 66.09 H 8.93 N 5.97; IR (KBr): $\tilde{\nu}$ = 2980, 2920, 2700, 2520, 1680, 1610, 1450, 1430, 1370, 1300, 1240 cm^{-1}.

2,3-Didehydro-tolperisone (1)

Method 1: 2.03 g (7.1 mmol) 3-piperidine-1-*p*-toluyl-propan-1-one (**5**) and 0.21 g (7.1 mmol) paraformaldehyde were diluted with 15 ml ethanol. 0.93 g (16.3 mmol) of potassium hydroxide in 1.2 ml water was added and the mixture was refluxed for 4 h. After addition of water, the reaction mixture was extracted with ether. The organic phase was dried with sodium sulfate and the solvent was removed in vacuo. Chromatographic separation on basic Al_2O_3 (PE / EE / NEt_3 100:10:2) and conversion into the hydrochloride with acetylchloride and methanol gave 1.51 g (70 %) of the unsaturated tolperisone **1**.

Method 2: 10.04 g (74.5 mmol) 4'-methyl-acetophenone (**4**), 4.51 g (0.15 mol) paraformaldehyde and 9.02 g (74.5 mol) piperidine-hydrochloride were dissolved in 100 ml acetic acid and refluxed for 4 h. After removing the solvent, 1N NaOH was added to the residue and the organics extracted twice with ether. Removal of the solvent, chromatographic separation on Al_2O_3 (PE / EE / NEt_3 100:10:2) and conversion into the hydrochloride with acetylchloride and methanol yielded 14.51 g (80 %) of the unsaturated tolperisone **1**.

Fp.: 127 °C (hydrochloride); ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.41 - 1.61 (m, 6H, 3 x CH_2), 2.41 (s, 3H, Ar-CH_3), 2.31 - 2.55 (m, 4H, CH_2-N-CH_2), 3.36 (s, 2H, CH_2-N), 5.68 (d, 1H, C=CH_2, J = 0.8 Hz), 5.95 (d, 1H, C=CH_2, J = 0.8 Hz), 7.23 (d, 2H, Ar-H, J = 8.2 Hz), 7.72 (d, 2H, Ar-H, J = 8.2 Hz); ^{13}C-NMR (50 MHz,

$CDCl_3$): δ = 22.17 (q, Ar-CH_3), 22.22 (t, CH_2), 23.17 (t, 2 x CH_2), 46.16 (t, CH_2-N-CH_2), 53.25 (t, CH_2), 55.87 (t, =CH_2), 56.00 (s, =C), 129, 45 (d, 2 x C_{arom}), 130.43 (d, 2 x C_{arom}), 132.11 (s, C_{arom}), 146.15 (s, C_{arom}), 197.22 (s, C=O); $C_{16}H_{21}NO$ (243.162314) fnd. (HRMS): 243.16157; IR (KBr): $\tilde{\nu}$ = 2930, 2650, 2540, 1680, 1650, 1600, 1450, 1420, 1310, 1240, 1200 cm^{-1}.

Tolperisone (2) from 1

To a solution (solvent s. text) of 0.10 g (0.41 mmol) didehydro-tolperisone (**1**) the catalyst (s. text) was added and the reaction flask was purged with hydrogen. The hydrogen atmosphere was maintained using a balloon and the mixture hydrogenated for 4 h. The catalyst was removed using Celite and the solution was concentrated. 1N NaOH and ether were added, the organic phase was separated and dried over sodium sulfate. Evaporation of the solvent gave a mixture of starting material **1**, tolperison (**2**) and **6**. Chromatographic seperation on silica gel led to decompositon of **1**, preventing further purfication of the material. Employment of Al_2O_3 was not successful to seperate **1** and **2**.

3',5'-Dibromo-4'-methyl-propiophenone (8)

To 90.73 g (0.67 mol) mechanically stirred $AlCl_3$ 20 ml (0.14 mol) of 4'-methylpropiophenone was added slowly to keep the temperature below 120 °C. The resulting mixture was stirred for 1 h at 80 °C followed by addition of 16.7 ml (0.32 mol) bromine while keeping the temperature below 160 °C. After stirring for 2 h at 110 °C the mixture was poured onto ice / conc. HCl (1:1) and vigorously stirred. The aqueous phase was extracted three times with ether and thoroughly washed with sodium sulfite and water and dried over sodium sulfate. Removal of the solvent and distillation afforded 19.42 g (46 %) of dibromo-propiophenone **8** as a colorless solid.

Fp.: 54 °C; ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.20 (t, 3H, CH_3, J = 7.1 Hz), 2.58 (s, 3H, Ar-CH_3), 2.92 (q, 2H, CH_2, J = 7.1 Hz), 8.02 (s, 2H, 2 x Ar-H); ^{13}C-NMR (50 MHz, $CDCl_3$): δ = 8.41 (q, Ar-CH_3), 24.47 (q,. CH_3), 32.23 (t, CH_2), 126.00 (s, CH_3-C_{arom}), 131.55 (d, C_{arom}), 137.00 (s, C_{arom}), 141.83 (s, Br-C_{arom}.), 198.08 (s,

C=O); GC-MS (70 eV): m/z (%) = 306 (M^+), 276 (31), 248 (17), 207 (12), 169 (29), 89 (100), 73 (27), 63 (54), 57 (45); $C_{10}H_{10}Br_2O$ (303.909837) fnd.: 303.91012; IR (KBr): $\tilde{\nu}$ = 3120, 2920, 1780, 1690, 1580, 1390, 1260, 1150 cm^{-1}.

3',5'-Dibromo-tolperisone (3)

To a solution of 0.90 g methylenepiperidiniumchloride (6.70 mmol) in 15 ml abs. CH_2Cl_2 a solution of 2.05 g (6.70 mmol) 3',5'-dibromo-4'-methyl-propiophenone (**8**) in 5 ml CH_2Cl_2 was added under an argon atmosphere. The solution was refluxed for 2 h followed by concentration and addition of dilute HCl (1N, 50 ml). After extraction with ether, dilute NaOH was added and the aqueous phase was again extracted with ether. The organic phase was dried over magnesium sulfate, concentrated and dried in vacuo. Conversion into the hydochloride with acetylchloride and methanol gave 2.60 g (88 %) of **3**.

Fp.: 231 °C (hydrochloride); ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.16 (d, 3H, CH_3), 1.38 - 1.55 (m, 6H, 3 x CH_2), 2.32 - 2.44 (m, 4H, CH_2-N-CH_2), 2.63 (s, 3H, CH_3), 2.71 - 2.82 (m, 2H, CH-CH_2), 3.47 - 3.62 (m, 1H, CH), 8.11 (s, 2H, Ar-H); ^{13}C-NMR (50 MHz, $CDCl_3$): δ = 16.84 (q, Ar-CH_3), 24.47 (q, CH_3), 24.64 (t, CH_2), 26.40 (t, 2 x CH_2), 39.80 (d, CH), 55.44 (t, CH_2-N-CH_2), 63.16 (CH-CH_2), 125.96 (s, CH_3-C_{arom}), 132.09 (d, C_{arom}), 137.43 (s, C_{arom}), 142.67 (s, Br-C_{arom}), 201.99 (C=O); MS (70 eV, CI^+): m/z (%) = 403 (M^+), 388 (2), 336 (5), 324 (8), 279 (7), 239 (20), 178 (35), 156 (45), 98 (33), 86 (100); IR (KBr): $\tilde{\nu}$ = 2980, 2960, 2940, 2650, 2530, 1680, 1520, 1450, 1230, 1200, 1000 cm^{-1}.

3',5'-Dideutero-tolperisone (2d)

To a solution of 0.10 g (0.25 mmol) dibromo-tolperisone **3** in 15 ml ethyl acetate 10 mg Pd/C (10 %) were added and the reaction flask was purged with deuterium. The deuterium atmosphere was maintained using a balloon and the mixture hydrogenated for 2 h. The catalyst was removed using Celite and the solution was concentrated. 1N NaOH and ether were added and the organic phase was separated and dried over sodium sulfate. Evaporation of the solvent, dilution of the amine in abs. ether and conversion into the hydrochloride with acetylchloride and methanol afforded 70 mg (quant.) of deuterated tolperisone (**2d**).

Fp.: 191 °C; ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 1.22 (d, 3H, CH_3), 1.30 - 1.72 (m, 6H, 3 x CH_2), 2.20 -2.48 (m, 4H, 2 x CH_2), 2.48 (s, 3H, CH_3), 2.49 und 2.85 (d und AB-Spektrum, J = 7.2 Hz, $J_{A,B}$ = 12,6 Hz, 2H, CH_2), 3.78 (m, 1H, CH), 7.95 (s, 2H, Ar-H); ^{13}C-NMR (50 MHz, $CDCl_3$): δ = 19.21 (q, Ar-CH_3), 22.02 (q, CH_3), 22.20 (t, CH_2), 22.95 (t, CH_2), 37.32 (d, CH), 52.53 (t, 2 x CH_2), 58.60 (CH_2), 129.33 (d, C_{arom}), 130.32 (d, $C_{arom.}$), 132.15 (s, $C_{arom.}$), 145.55 (s, $C_{arom.}$), 201.02 (C=O); $C_{16}H_{22}ClD_2NO$ (247.190517) fnd. (HRMS): 247.19067; IR (KBr): $\tilde{\nu}$ = 2950, 2640, 2510, 1680, 1510, 1450, 1400, 1190 cm^{-1}.

*3',5'-Tritiated-tolperisone (**2b**)*

To 0.14 g (0.32 mmol) 3',5'-dibromo-tolperisone hydrochloride (**3**) 20 ml ether were added and mixed with 20 ml 1N NaOH. The organic phase was separated and the aqueous phase extracted twice with ether. The combined organic phases were dried over magnesium sulfate and the solvent was evaporated. The amine was dissolved in ether and transferred to the reaction flask and dried in vacuo. After addition of 10 ml ethyl acetate, 4 ml triethylamine and 15 mg Pd/C (10 %) the flask was attached to the tritiation device. After evacuating the apparatus, excess tritium gas was added and the reaction was continued until the pressure remained constant for 2 h. The solvent was removed by lyophilization and labile tritium was removed by two washing cycles with methanol. The residue was dissolved in 20 ml methanol and filtered through a millipore filter. After removing the solvent the residue was dissolved in a mixture of ether (20 ml) and 1N NaOH (20 ml) and the organic phase was dried over magnesium sulfate after separation. Evaporation of the solvent, dilution of the amine in abs. ether and conversion into the hydrochloride with acetylchloride (100 µl) and methanol (60 µl) afforded 11.4 Ci of product. For purification semi-preparative HPLC (solvent methanol / water + 0.1 % 1N HCl; gradient 50/50 - 70/30) was used yielding ^{3}H-tolperisone (**2b**) with a specific activity of 50.7 Ci/mmol (LC/MS analysis).

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