SYNTHESIS AND ^3H NMR OF ^3H-ALAPROCLATE OF HIGH SPECIFIC ACTIVITY

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SUMMARY

The antidepressant alaproclate (1) is a selective inhibitor of neuronal serotonin reuptake with low affinity for other receptor sites. ^3H-Alaproclate (3, 2-([2,5-$^3H_2$]-4-chlorophenyl)-2-methyl-2-propyl 2-aminopropanoate) with high specific activity (38 Ci/mmol) was prepared by hydrogenolysis of its 2,5-dibromo analogue 2 with tritium gas. The compound 2 was prepared in a seven step synthesis from 4-chlorotoluene. The position of the tritium incorporation was verified by ^3H NMR.

Key-words: Alaproclate, Antidepressant, Tritium Labelling, Tritium NMR.

INTRODUCTION

The antidepressant alaproclate (1) was developed on basis of the serotonin hypothesis.[1] The agent is a selective inhibitor of the neuronal serotonin reuptake.[2] Its low affinity for other receptor sites prompted the synthesis of ^3H-labelled alaproclate for binding studies.[2] Material with an activity of more than 35 Ci/mmol was required, i.e. a minimum of two tritium atoms per molecule. Since it was desirable to lable the aromatic nucleus, a convenient way of introducing the tritium would be via selective hydrogenolysis. Thus, the dibromo analogue 2 was regarded as a suitable precursor since it would enable this transformation to be carried out in the last step.

Alaproclate (1) has been prepared via the alcohol 4,[1] which is easily obtained by a Grignard reaction of 4-chlorobenzylmagnesium chloride and acetone, and we decided to adopt a similar approach in the synthesis of the key intermediate 5.

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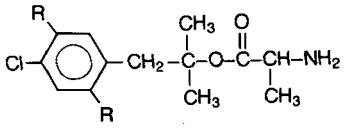
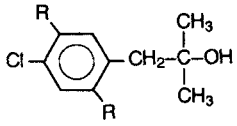


1 R=H
2 R=Br
3 R=^{3}H

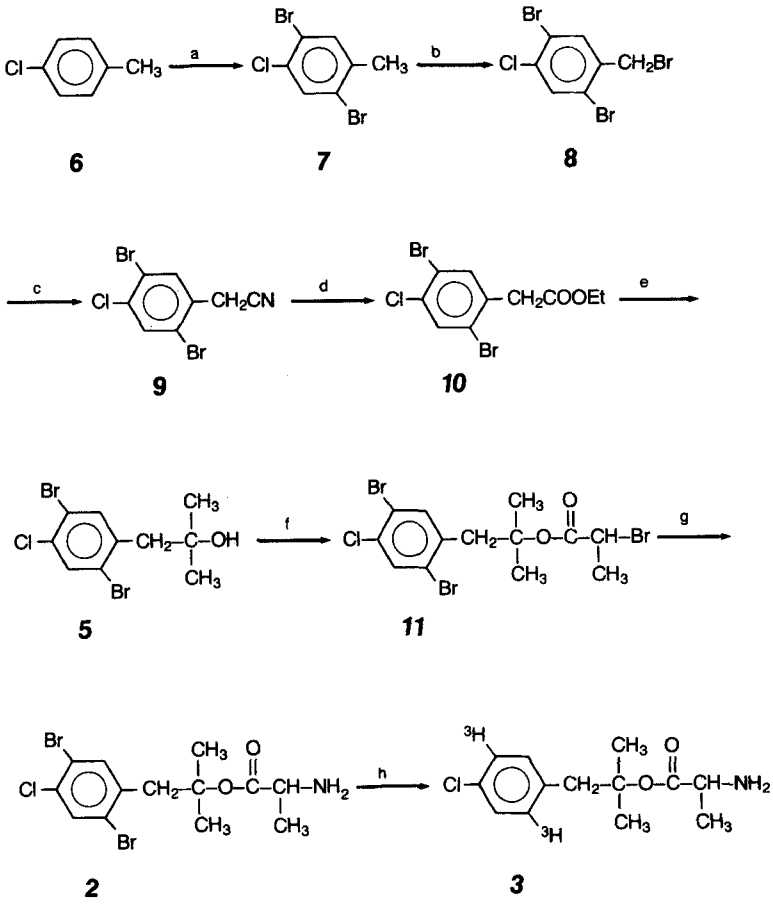


4 R=H
5 R=Br

RESULTS AND DISCUSSION

Attempts to selectively brominate 4-chlorobenzyl chloride in the aromatic ring failed. Accordingly, bromination had to be carried out prior to functionalizing the methyl group. The iron catalysed bromination of 4-chlorotoluene (6) required a concentrated reaction medium (Scheme 1). The following α-bromination took place in a modest yield due to further bromination of the product 8. Since a direct Grignard reaction of the benzyl bromide 8 to the alcohol 5 failed, 8 was converted via the nitrile 9 and ester 10 (Scheme 1). In the reaction of 8 with cyanide we observed that the presence of the three halogens in the product 9 renders the α-hydrogens surprisingly acidic. Thus, the nitrile 9 was alkylated with the benzyl bromide 8 to a considerable degree during various standard procedures. This side-reaction was suppressed by reacting 8 with an excess of tetrabutylammonium cyanide in dichloromethane. The ethanolysis of 9 was very slow compared to less substituted analogues, again showing the influence of the bromo substituents. The ester 10 was obtained in 48% yield from 8. The following reaction with methylmagnesium iodide afforded 59% of alcohol 5, which was acylated with 2-bromopropionyl bromide. Compared to the corresponding acylation of 4,[1] the dibromo analogue 5 required a longer reaction time and the use of 4-dimethylaminopyridine as catalyst to give 11. This labile compound was aminated directly to give pure 2 in 23% yield from 5.

Scheme 1



a: Fe (cat.), Br_2, $CHCl_3$; b: Br_2, Na_2CO_3, $CHCl_3$; c: $(C_4H_9)_4NCN$, CH_2Cl_2; d: conc. H_2SO_4, EtOH, CH_2Cl_2; e: CH_3MgI, Et_2O, THF; f: $CH_3CHBrCOBr$, DMAP, CH_2Cl_2; g: liq. NH_3, EtOH; h: $^3H_2$, Pd/C, Et_3N, DMF.

In order to ascertain selective hydrogenolysis of the two bromine atoms in the presence of a chlorine, the proper conditions were elucidated from cold runs on 2. We found the choice of solvent and the ratio of added amine to substrate 2 to be critical. The reaction was preferably performed in dimethylformamide with 1.6 equivalents of triethylamine and 5% Pd/C as catalyst. Under these conditions ^3H-alaproclate (3) with a specific activity of 38 Ci/mmol was obtained by reaction of 2 with tritium gas.

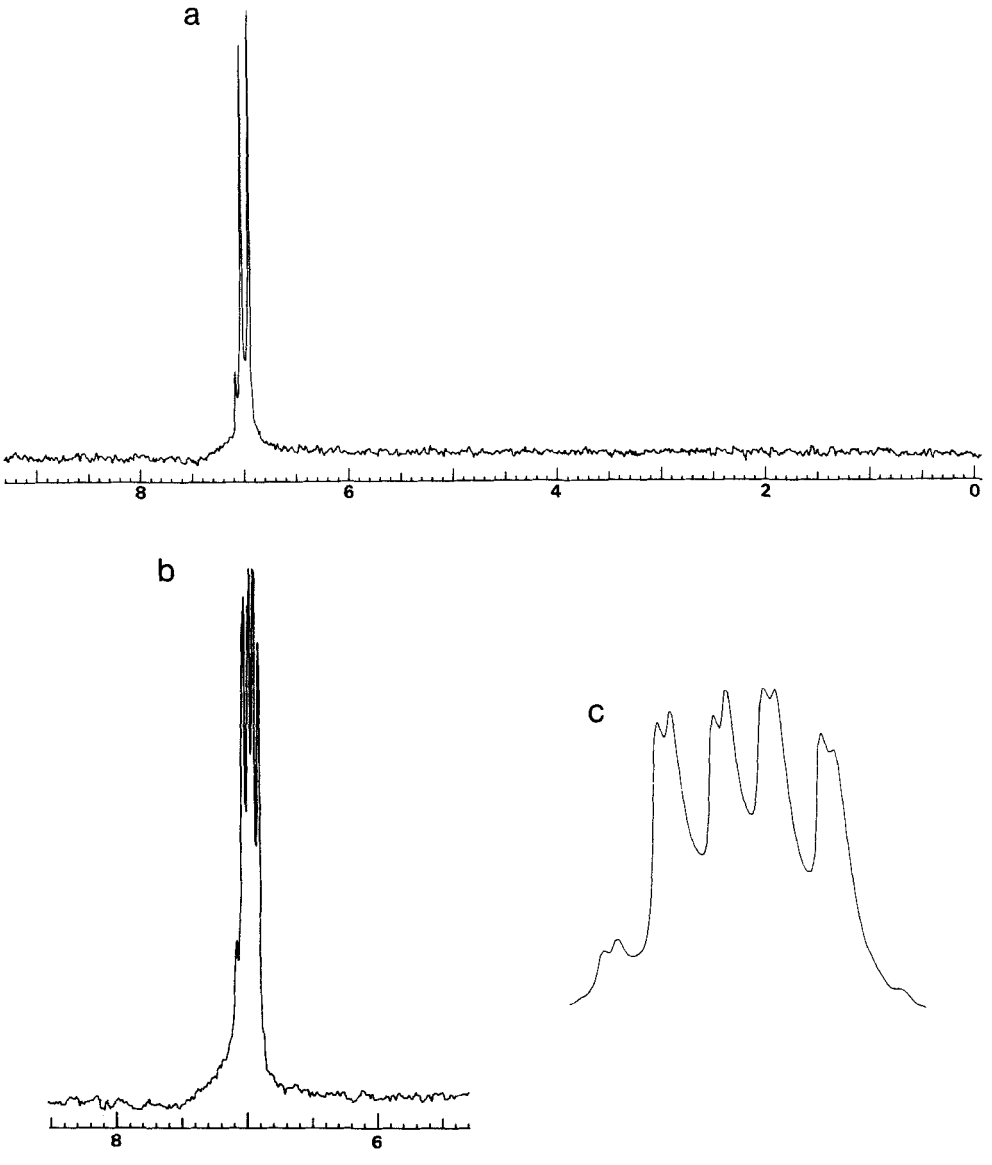


Fig. 1. ^{3}H NMR spectra of ^{3}H-alaproclate (3, 40 mCi) obtained after 3000 scans at 212.8 MHz; a: proton decoupled spectrum, b: proton coupled spectrum and c: expanded proton coupled spectrum.

Tritium NMR spectra confirmed the exclusive incorporation of tritium in the phenyl ring of compound 3. In the proton decoupled spectrum, the expected para substitution of the tritium atoms is supported by the presence of two uncoupled peaks of equal intensity (Fig. 1a). The fully coupled spectrum is consistent with this tritiation pattern (ortho and meta ^1H-^3H couplings, Figs 1b and 1c).

EXPERIMENTAL

Melting points were obtained on a Mettler FP 61 apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian EM 360A or a JEOL FX 200 spectrometer and ^{13}C NMR on a JEOL FX 200 spectrometer in $CDCl_3$ using Me_4Si as internal standard. ^3H NMR spectra were recorded in CD_3OD at 212.8 MHz using a JEOL FX 200 spectrometer. Me_4Si was used as a ghost-reference.[3] The NMR sample was prepared as described in reference 3. Mass spectra (EI, 70 eV) were recorded on an LKB 9000 instrument (only the lowest isotope is indicated). Elemental analysis were performed by Analytische Laboratorium, Elbach, W. Germany and are within ±0.4% of the theoretical values. Tritium gas (98%) was purchased from Amersham International plc , Amersham, Bucks, England. Radiochemical purity was determined from TLC plates using a Berthold LB 283 TLC Linear Analyzer. HPLC was performed on a 0.6 x 25 cm Whatman Partisil PXS 10/25 ODS column eluted with n-hexane/isopropanol/conc NH_3 (1000:30:1.5). Radioactivity was determined in a Packard liquid scintillator counter (Model 3320) using Bioflour (New England Nuclear) as the counting medium.

2,5-Dibromo-4-chlorotoluene (7).

A mixture of 4-chlorotoluene (6, 50.6 g, 0.40 mol), a catalytic amount (about 2 g) of iron powder and 80 ml $CHCl_3$ was heated to 30 °C in a 1 liter flask. Bromine (41.2 ml, 0.80 mol) was added with stirring at such a rate that a controlled evolution of HBr was maintained. The mixture was stirred for 1 h at

45 °C, cooled, diluted with $CHCl_3$ and washed twice with water. Drying (Na_2SO_4) and evaporation in vacuo gave 119 g of a product contaminated with a monobromo compound. Recrystallization from ethanol/water gave 69.5 g (61%) of pure 7, mp 92.5-93.5 °C.

^1H NMR (60 MHz) δ 2.35 (s, 3), 7.51 (s, 1), 7.65 (s, 1); MS, m/z (rel. int.): 282 (M, 80), 247 (M - Cl, 2), 203 (M - Br, 100), 123 (M - HBr - Br, 4). Anal. Calcd for $C_7H_5Br_2Cl$: C, 29.56; H, 1.77; Br, 56.20; Cl, 12.46. Found: C, 29.37; H, 1.75; Br, 56.35; Cl, 12.41.

2,5-Dibromo-4-chlorobenzyl bromide (8).

A mixture of 7 (68.3 g, 0.24 mol), Br_2 (27 ml, 0.52 mol) and Na_2CO_3 (58 g, 0.55 mol) in 600 ml $CHCl_3$ was refluxed for 3 h. The mixture was washed with water, dried (Na_2SO_4) and evaporated in vacuo. The crude product also containing starting material and 2,5-dibromo-4-chlorobenzal bromide was recrystallized from ethanol/water to give 45.6 g (52%) of pure 8, mp 65.5-66.5 °C.

^1H NMR (60 MHz) δ 4.52 (s, 2), 7.72 and 7.76 (two s, 2); Anal. Calcd for $C_7H_4Br_3Cl$: C, 23.14; Br, 65.99; Cl, 9.76. Found: C, 23.18; Br, 65.95; Cl, 9.94.

2,5-Dibromo-4-chlorophenylacetonitrile (9).

Tetrabutylammonium cyanide was prepared according to Brändström[4] by stirring tetrabutylammonium hydrogensulfate (109 g, 0.32 mol) in 350 ml CH_2Cl_2 and 32 ml 10 M NaOH at 0 °C. A solution of NaCN (18.6 g, 0.38 mol) dissolved in 50 ml water was added. After stirring for 10 min the organic layer was decanted, the residue extracted twice with CH_2Cl_2 and the combined organic phases dried (Na_2SO_4). To this solution of tetrabutylammonium cyanide a solution of 8 (29.0 g, 0.080 mol) in CH_2Cl_2 was added with stirring. The reaction was completed within 15 min. Water was added and the mixture was made acidic with aqueous HCl. The organic layer was washed with water, dried and evaporated in vacuo, leaving 23.8 g of a crude product. An analytical sample was recrystallized from ethanol/water, mp 106-108 °C.

^1H NMR (60 MHz) δ 3.80 (s, 2), 7.75 and 7.82 (two s, 2); Anal. Calcd for $C_8H_4Br_2ClN$: C, 31.04; Br, 51.63; N, 4.53. Found: C, 30.95; Br, 51.45; N, 4.49; MS, m/z (rel. int.): 307 (M, 59), 228 (M - Br, 100), 148 (M - Br - HBr, 35), 114 (35), 87 (22), 64 (25).

Ethyl 2,5-dibromo-4-chlorophenylacetate (10).

A solution of crude 9 (22 g), conc H_2SO_4 (14 g, 0.14 mol), 140 ml anhydrous ethanol and 45 ml CH_2Cl_2 was refluxed for 4 days. Aqueous NaOH and CH_2Cl_2 were added and the alkaline organic layer was washed with water, dried (Na_2SO_4) and evaporated in vacuo. The residue was flash chromatographed on SiO_2 with isooctane/iPr_2O 3:1 to give 10 (12.7 g, 48% from 8), mp 63-64.5 °C. ^1H NMR (60 MHz) δ 1.27 (t, 3), 3.74 (s, 3), 4.22 (q, 2), 7.61 and 7.72 (two s, 2). Anal. Calcd for $C_{10}H_9Br_2ClO_2$: C, 33.70; H, 2.54; Br, 44.84; Cl, 9.95. Found: C, 33.71; H, 2.56; Br, 44.78; Cl, 9.91.

1-(2,5-Dibromo-4-chlorophenyl)-2-methyl-2-propanol (5).

A solution of 10 (12.5 g, 0.035 mol) in 65 ml dry Et_2O and 7 ml dry THF was added to a Grignard reagent, prepared from iodomethane (15.6 g, 0.110 mol) and Mg (2.43 g, 0.100 mol) in 70 ml dry Et_2O, with stirring at room temperature. After stirring for another 90 min the mixture was poured into 150 ml H_2O and 10 ml conc HCl. The organic layer was washed twice with water, dried (Na_2SO_4) and evaporated in vacuo. Flash chromatography on SiO_2 with hexane/iPr_2O 2:1 gave 7.1 g (59%) of alcohol 5, mp 85-86 °C.

^1H NMR (60 MHz) δ 2.24 (s, 6), 3.82 (s, 2), 7.49 and 7.70 (two s, 2). MS, m/z (rel. int.): 325 (M - CH_3, 1), 282 (M - Me_2CO, 3), 245 (M - Me_2CO - Cl, 1), 203 (M - Me_2CO - Br, 2), 123 (M - Me_2CO - Br - HBr, 4), 59 (Me_2COH^+, 100). Anal. Calcd for $C_{10}H_{11}Br_2ClO$: C, 35.07; H, 3.24; O, 4.67. Found: C, 35.09; H, 3.07; O, 4.68.

1-(2,5-Dibromo-4-chlorophenyl)-2-methyl-2-propyl 2-bromopropanoate (11).

2-Bromopropionyl bromide (4.9 g, 24 mmol) was treated with 5 (4.1 g, 12 mmol) and 4-dimethylaminopyridine (2.2 g, 18 mmol) in 20 ml dry CH_2Cl_2

under N_2 at room temperature. TLC and GLC showed complete conversion after 3 days. The reaction mixture was washed with water, aqueous NaOH and aqueous HCl. Drying (Na_2SO_4) and evaporation in vacuo gave 5.8 g crude 11 which was used directly in the next step.

^{1}H NMR (60 MHz) δ 1.38 (s, 6), 1.81 (d, 3), 3.22 (s, 2), 4.31 (q, 1), 4.31 (q, 1), 7.70 (s, 2).

1-(2,5-Dibromo-4-chlorophenyl)-2-methyl-2-propyl 2-aminopropanoate (2). Crude 11 (5.8 g) dissolved in 25 ml ethanol was added to 15 ml liquid ammonia dissolved in 125 ml ethanol at -40 °C in an autoclave. The reaction mixture was heated to 40 °C for 24 h. After evaporation Et_2O and aqueous HCl were added. The aqueous phase was separated, alkalized and extracted with Et_2O. Drying (Na_2SO_4) and evaporation in vacuo afforded 2.23 g of 2 (23% from 5).

^{1}H NMR (200 MHz) δ 1.31 (d, 3), 1.52 (d, 6), 1.60 (broad s), 3.21 (s, 2), 3.48 (q, 1), 7.55 (s, 1), 7.68 (s, 1); ^{13}C NMR (50 MHz) δ 20.9, 25.7, 26.0, 44.9, 50.9, 82.8, 121.0, 124.8, 133.7, 133.8, 136.6, 137.4, 176.1. MS, m/z (rel. int.): 411 (M, 0.1), 322 (M - alanine, 1), 281 ($C_6H_2Br_2ClCH_2$, 2), 44 ($CH_3CH{=}NH_2$, 100).

The hydrochloride was prepared and recrystallized from ethanol/acetone, mp 177-177.5 °C. Anal. Calcd for $C_{13}H_{17}Br_2Cl_2NO_2$: C, 34.70; H, 3.81; N, 3.11; O, 7.11. Found: C, 34.84; H, 3.85; N, 3.03; O, 7.28.

1-(4-Chloro[2,5-$^3H_2$]phenyl)-2-methyl-2-propyl 2-aminopropanoate (3). A solution of the hydrochloride of 2 (4.2 mg, 9 μmol) and triethylamine (2 μl, 14 μmol) in 0.5 ml dimethylformamide was stirred under an atmosphere of 5 Ci tritium gas in the presence of 2 mg 5% Pd/C. After 15 h the solvent was evaporated. In order to remove labile tritium the catalyst-product mixture was three times treated with ethanol followed by evaporation to dryness.

The resulting residue was taken up in 300 µl of ethanol and filtered from the catalyst and concentrated to 100 µl. Purification by HPLC gave 98 mCi of 3 with the specific activity of 38 Ci/mmol as measured by UV spectroscopy. The radiochemical purity was 96% as determined by TLC (SiO_2, $CHCl_3$/ethanol/conc NH_3 95:5:0.2).

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