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SYNTHESES OF CARBON-14 AND TRITIUM LABELLED FORMS OF BUPROPION HYDROCHLORIDE – A NOVEL ANTIDEPRESSANT

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SUMMARY

Bupropion hydrochloride 1 (2-*tert*-butylamino-3'-chloropropiophenone hydrochloride) was synthesized in the [^{14}C]-labelled form with specific activity 36.5 mCi/mmol suitable for drug metabolism, distribution and pharmacokinetic studies. The drug was synthesized in the [^{3}H]-labelled form with specific activity 20.5 Ci/mmol suitable for development of a radioimmunoassay procedure.

Key Words: [^{14}C]-bupropion, [^{3}H]-bupropion, BW A323U, WELLBUTRIN®, antidepressant, radioimmunoassay.

INTRODUCTION

The structurally novel antidepressant agent bupropion hydrochloride (1, 2-*tert*-butylamino-3'-chloropropiophenone hydrochloride, WELLBUTRIN®) (1) has neurochemical properties different from those of the commonly used tricyclic antidepressants (2). Bupropion appears to have an excellent side-effect profile; it is non-sedating, does not cause anticholinergic effects, and is devoid of cardiovascular effects (3). Further, it does not produce weight gain (4), and appears to have a very low propensity for inducing adverse sexual side-effects (5).

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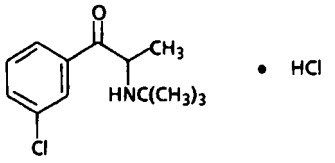

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Elucidation of the metabolic fate, tissue distribution and pharmacokinetic profile of the drug required the preparation of [^{14}C]-labelled 1, with the label either in the aromatic ring or on the metabolically stable (*in vivo*) C-1 position of the propiophenone side-chain.

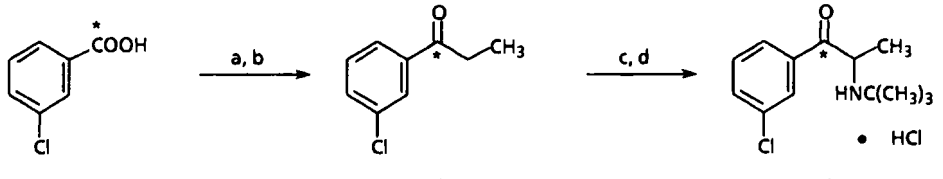
Subsequent studies made necessary the development of a radioimmunoassay procedure for analysis of clinical trial samples, and required a high specific activity tritium labelled form of 1. These studies demanded that the [^{3}H]-1 should be chemically stable, should have a high chemical and radiochemical purity, and a minimum specific activity of 15 Ci/mmol.

This paper describes the preparation of [^{14}C]-labelled 1 with specific activity 36.5 mCi/mmol, and of [^{3}H]-labelled 1 with specific activity 20.5 Ci/mmol.

RESULTS AND DISCUSSION

The observation that the carbonyl position of the propiophenone side-chain of bupropion 1 was metabolically stable *in vivo* (6), coupled with the availability of [carboxyl-^{14}C]-3-chlorobenzoic acid 2, prompted the development of a synthesis of [^{14}C]-labelled bupropion 4 as shown in Scheme I.

Scheme I



2 3 4

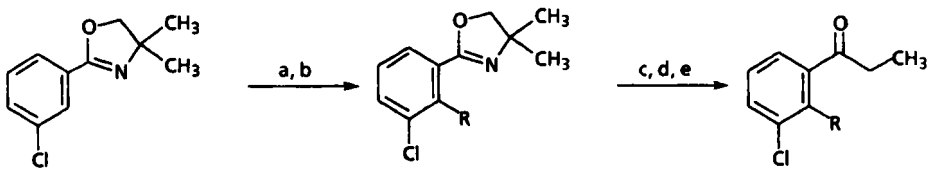
a, $SOCl_2$, reflux 3 h; b, $LiCuEt_2$, Et_2O, -78°C; c, Br_2, CH_2Cl_2; d, excess $(CH_3)_3CNH_2$, MeCN.

The key step was the development of an efficient method for the preparation of [1-^{14}C]-3'-chloropropiophenone 3 from the carboxylic acid or a close relative. Of several methods (7,8) to effect this conversion, the coupling of lithium diethylcuprate and 3-chlorobenzoyl chloride proved to be the method of choice in our hands. Treatment of crude 3-chlorobenzoyl chloride with 6 equivalents of

lithium diethylcuprate in diethyl ether at -78°C routinely afforded 3'-chloropropiophenone in greater than 90% assayed yield based on gas chromatographic analysis of the product. Further optimization of the entire reaction scheme, without purification of intermediates, gave crude bupropion hydrochloride 1 in an overall yield of 60% from 3-chlorobenzoic acid. Although the product was of excellent purity by thin-layer chromatography and proton NMR, it was recrystallized from 2-butanol to give a crystalline solid in an overall yield of 43%. The [1-^{14}C]bupropion hydrochloride 4 was subsequently prepared by this method in similar yield and purity, and the details are included in the experimental section. The radiolabelled material was obtained at a specific activity of 36.5 mCi/mmol and was identical to an authentic sample of bupropion hydrochloride by thin-layer chromatography. Two minor impurities were detected but zonal analysis of the TLC plate followed by liquid scintillation counting verified that at least 96.6% of the activity was associated with bupropion hydrochloride.

Our approach to the synthesis of [^{3}H]-labelled bupropion 13 at high specific activity, for use in the development of a radioimmunoassay procedure (9), was based upon the preparation of tritium labelled 3'-chloropropiophenone by elaboration of 2-(3-chlorophenyl)-4,4-dimethyl-2-oxazoline 5 (Scheme II).

Scheme II

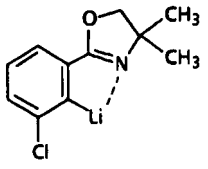


5 6 R = D; 8 R = CH_3 7

a, *tert*-BuLi, Et_2O, -78°C, 0.5 h; b, D_2O, -78→0°C, 83% from 5; c, FSO_3Me, C_6H_6, 4 h; d, EtMgCl-Et_2O, THF, 25°C, 1 h; e, 2 equivalents $(CO_2H)_2$, H_2O, reflux 1 h, 75% from 6.

Regiospecific metalation was expected due to the strong *ortho*-directing effect of the oxazoline (10) coupled with the inductive effect of the chlorine. This was confirmed by reaction of oxazoline 5 with *tert*-butyl lithium in diethyl ether or *n*-butyl lithium in tetrahydrofuran at -78°C followed by quenching with methyl iodide. In each case a single product was obtained, in over 96% isolated yield, whose proton NMR spectrum was consistent with that reported for 2-(3-chloro-2-methylphenyl)-4,4-dimethyl-2-oxazoline 8 (11). When the organometallic species 9 was quenched with excess D_2O at -78°C followed by warming to 0°C, oxazoline 6 was obtained in 83% yield after chromatography on silica gel

to remove a small amount of the amide 10. Apparently 10 was formed by lithium hydroxide induced hydrolysis of the oxazoline ring under the strongly basic conditions of the reaction.



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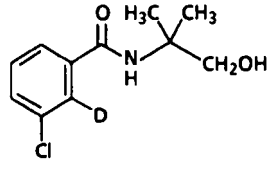

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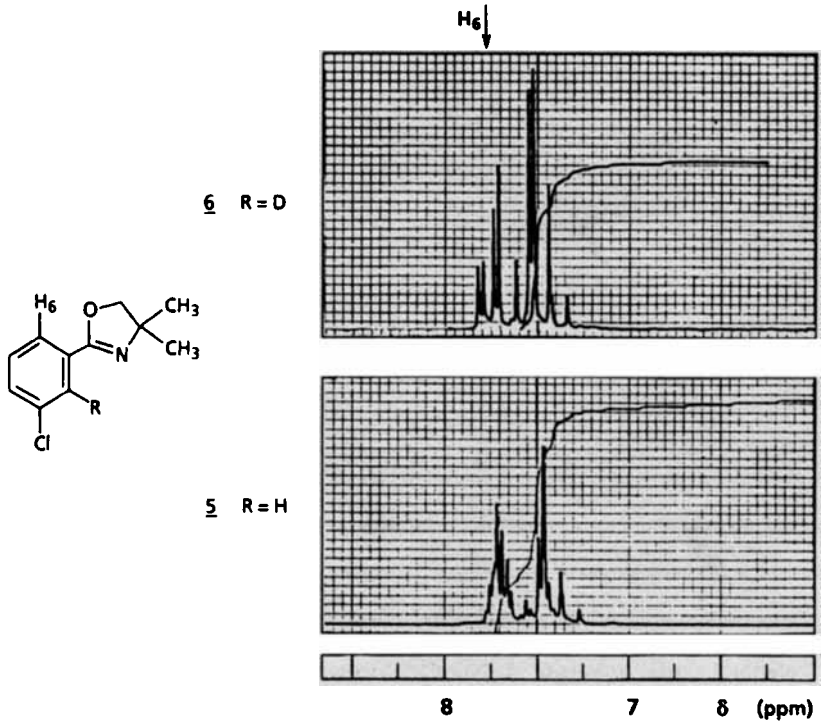
The aromatic region of the 80 MHz proton NMR spectrum (Figure 1) of the deuterated oxazoline 6 confirmed a high level of deuterium incorporation at the 2-position of the aromatic ring. The quasi first-order splitting pattern for H-6 near δ7.75 was also consistent with this assignment. Having verified the feasibility of the labelling scheme, we focused on the conversion of the oxazoline to 3'-chloropropiophenone.

Figure 1

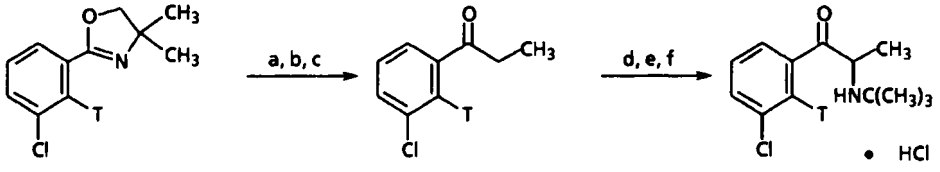


Activation of the oxazoline moiety towards addition of a Grignard reagent was achieved by quaternization (12). Although methylation with excess methyl iodide or trimethyloxonium fluoroborate

was slow and largely incomplete under forcing conditions, alkylation with a small excess of methyl fluorosulfonate in dry benzene was rapid and complete at room temperature as shown by TLC. The crystalline quaternary salt could be isolated but we found this to be unnecessary, and subsequently treated the product directly with ethyl magnesium chloride in Et_2O/THF. The crude *N*-methyloxazolidine was isolated and converted to 3'-chloropropiophenone by treatment with aqueous oxalic acid at reflux. The product was obtained as an oil which crystallized upon standing. It was identical by TLC and NMR to an authentic sample of 3'-chloropropiophenone and required no further purification.

Labelling of oxazoline 5 was subsequently performed by Amersham International plc using tritiated water of high isotopic abundance. Elaboration of the product 11 as described above provided tritiated bupropion hydrochloride 13, of specific activity 20.5 Ci/mmol, in an overall yield of 35% (Scheme III).

Scheme III



11 12 13

a, FSO_3Me, C_6H_6, 18 h; b, EtMgCl-Et_2O, THF, 25°C, 1 h; c, 1.7 equivalents $(CO_2H)_2$, H_2O, reflux 1 h, 77% from 11; d, Br_2, CH_2Cl_2; e, excess $(CH_3)_3CNH_2$, MeCN; f, recrystallization from 2-butanol, 46% from 12.

EXPERIMENTAL

2-([2-^{3}H]-3-Chlorophenyl)-4,4-dimethyl-2-oxazoline was obtained from Amersham International plc and was prepared according to a procedure furnished by Burroughs Wellcome Co. [1-^{14}C]-3-Chlorobenzoic acid was kindly provided by Dr. W.G. Duncombe, Wellcome Research Laboratories, Beckenham, U.K. Deuterium oxide was purchased from Merck Isotopes. Ethyl lithium was kindly provided by Professor R.K. Hill, University of Georgia. *n*-Butyl lithium, *tert*-butyl lithium, methyl fluorosulfonate, and *tert*-butylamine were purchased from Aldrich Chemical Company. Ethyl magnesium chloride was purchased from Alfa Products. All other solvents and reagents were of reagent purity and were obtained from readily available commercial sources. Benzene, diethyl ether and

tetrahydrofuran were dried over sodium metal or calcium hydride. Acetonitrile was dried over Type 4A Molecular Sieves. Thionyl chloride was redistilled under nitrogen prior to use.

Thin layer chromatography was performed on 5 x 20 cm glass plates pre-coated with 0.25 mm silica gel 60 (Merck). Column chromatography was performed on silica gel 60 (230-400 mesh) as supplied by Merck. Proton NMR spectra were obtained on a Varian CFT-20 instrument in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as internal standard.

Radiochemical purity was determined by radiochromatogram scanning of TLC plates using a Vangard VS940 Scanner.

Specific activities were determined on accurately weighed samples by liquid scintillation counting.

[1-^{14}C]-3-Chlorobenzoyl Chloride

A stirred mixture of [carboxyl-^{14}C]-3-chlorobenzoic acid 2 (135.0 mg) with a specific activity of ~53 mCi/mmol, unlabelled 2 (65.0 mg), and freshly distilled thionyl chloride (35 mL) was heated at reflux under dry nitrogen atmosphere for 3 h. The solution was cooled and evaporated almost to dryness under reduced pressure at ~20°C. Dry benzene (30 mL) was added, the solution was re-evaporated almost to dryness under reduced pressure at ~20°C, and the process was repeated twice, to give [carboxyl-^{14}C]-3-chlorobenzoyl chloride as a brown oil which was used without further purification.

[1-^{14}C]-3'-Chloropropiophenone 3

To a suspension of thoroughly dried cuprous iodide (1.707 g; 8.96 mmol) in dry ether (25 mL) stirred at -30 to -35°C under a stream of dry nitrogen was added 0.95*M* ethyl lithium in benzene (16.1 mL; 551 mg; 15.3 mmol) dropwise via syringe during 19 min. After 30 min at -30 to -35°C, the black mixture was cooled to -75 to -78°C and a solution of the crude [carboxyl-^{14}C]-3-chlorobenzoyl chloride in dry ether (10 mL) was added dropwise via syringe during 22 min, followed by dry ether washings (total 18 mL) during 11 min. The mixture was stirred at -78°C for 30 min, ethanol (2 mL) was added dropwise, and the cooling bath was warmed up to room temperature during 1.75 h. The reaction mixture was poured into saturated aqueous NH_4Cl solution (100 mL), and transferred to a separatory funnel together with saturated NH_4Cl solution (50 mL), water (50 mL) and ether (200 mL). After separation, the blue aqueous layer was extracted with ether (100 mL). The combined ethereal solutions were washed with water (100 mL), dilute Na_2CO_3 solution (100 mL), water (100 mL), dried, and evaporated to dryness under reduced pressure to give crude [1-^{14}C]-3'-chloropropiophenone as a yellow oil (219.0 mg; 101.7%

overall weight yield). The oil crystallized on standing at room temperature overnight, and was used without further purification in the next step.

[1-^{14}C]-2-Bromo-3'-chloropropiophenone

A solution of crude [1-^{14}C]-3'-chloropropiophenone 3 (219.0 mg; 1.3 mmol) in CH_2Cl_2 (12 mL) was stirred in a water-bath at 35-38°C, and a solution made up from CH_2Cl_2 (6 mL) and 4.19 mL of a standardized bromine/CH_2Cl_2 solution containing 357.7 mg Br_2 (1.75 equivalents) was added dropwise during 3 h, followed by CH_2Cl_2 washings (3 mL). The mixture was stirred at 35-38°C for another 30 min, heated at reflux for 75 min, then cooled and evaporated almost to dryness under reduced pressure at 20°C. Methanol (25 mL) was added, the solution was re-evaporated almost to dryness under reduced pressure at 20°C, and the process was repeated twice to give crude [1-^{14}C]-2-bromo-3'-chloropropiophenone (324.1 mg; 102.5% overall weight yield), as a dark brown oil which was used in the final step without further purification.

[1-^{14}C]-2-*tert*-Butylamino-3'-chloropropiophenone Hydrochloride 4

To a solution of the crude [1-^{14}C]-2-bromo-3'-chloropropiophenone (324.1 mg) in dry acetonitrile (16 mL) stirred in a water-bath at 35-36°C under a stream of dry nitrogen, was added a solution of *tert*-butylamine (6.69 g) in acetonitrile (17 mL) during 4 min. The yellow solution was stirred at 35-38° for 4 h, then at room temperature for 3 days. The solution was evaporated to dryness under reduced pressure at <40°C, and the residue was transferred to a separatory funnel in ether (100 mL) and water (50 mL). After separation, the aqueous layer was extracted with ether (50 mL). The combined ethereal solutions were then extracted with ice-cold dilute HCl (4 x 50 mL), each portion being made up from conc. HCl (17 mL) and water/ice (33 mL). The combined aqueous solutions were washed with ether (50 mL), then evaporated to dryness under reduced pressure. Methanol (50 mL) was added, and the solution was evaporated to dryness under reduced pressure to give a yellow mainly-crystalline solid (250.0 mg; 70.8% overall weight yield) that was shown to be mainly 4 by TLC in $CHCl_3/Et_3N$ (19/1, v/v). The crude 4 dissolved in methanol (10 mL) was treated with activated charcoal (Darco G-60), filtered through Celite 545, and the solution evaporated to dryness under reduced pressure to give a yellow solid (225.5 mg). 2-Butanol (7.0 mL) was added, the mixture heated until complete solution was attained, then evaporated under reduced pressure to a volume of ~1.5 mL, and allowed to stand for 2 h. The mixture was cooled at

0°C overnight, the crystals were filtered, washed with ice-cold acetone, and dried *in vacuo* at 78°C overnight. The yield of off-white solid 4 was 152.8 mg (20.2 mCi; 43% overall) with specific activity 36.5 mCi/mmol.

TLC using $CHCl_3/Et_3N$ (19/1, v/v) showed single-spot material under UV 254 nm light at R_f = 0.61 corresponding to authentic 1. Radioactive chromatogram scanning and autoradiography showed, however, the presence of trace impurities at R_f = 0 and R_f = 0.68. Zonal scraping of the TLC plate followed by liquid scintillation counting indicated a minimum radiochemical purity of 96.6%.

Co-crystallization of the mother liquors with unlabelled 1 (142 mg) from 2-butanol gave a second crop of 4 (140.7 mg; 3.62 mCi) with specific activity 7.1 mCi/mmol, and of 95.2% minimum radiochemical purity.

[2'-^{3}H]-3'-Chloropropiophenone 12

A stirred solution of 2-([2-^{3}H]-3-chlorophenyl)-4,4-dimethyl-2-oxazoline 11 (285.7 mg with specific activity ~27 Ci/mmol) in dry benzene (3 mL) was treated with methyl fluorosulfonate (0.37 mL; 525 mg), and the mixture was stirred under dry nitrogen for 18 h. The solvent was removed and the dark solid was washed with benzene (2 x 5 mL) and ether (2 x 5 mL), and the crude *N*-methyl-2-oxazolinium salt was suspended in dry THF (5 mL) under dry nitrogen. Ethyl magnesium chloride (3.0 mM in ether) (0.98 mL; 2.17 equivalents) was added carefully to the stirred mixture and, after the exotherm had subsided, the dark yellow solution was stirred 1 h. The reaction was quenched in stirred ice-H_2O (50 mL) and extracted with ether (50 mL and 25 mL). The combined organic extracts were washed with H_2O (2 x 30 mL) and saturated aqueous NaCl (30 mL), dried and the solvents evaporated *in vacuo* to give crude 2-([2-^{3}H]-3-chlorophenyl)-2-ethyl-3,4,4-trimethyloxazolidine as a yellow oil.

The crude oxazolidine was refluxed with oxalic acid (213.4 mg; 1.74 equivalents) in H_2O (10 mL) for 1 h. The cooled mixture was extracted with ether (20 mL and 15 mL). The combined organic extracts were washed with 5% aqueous $KHCO_3$ (2 x 15 mL), 0.5*M* aqueous HCl (2 x 15 mL) and H_2O (2 x 15 mL), dried and the solvent evaporated *in vacuo* to give a yellow oil (242.4 mg).

Column chromatography on SiO_2 (12 g) in benzene followed by TLC monitoring of fractions and removal of the solvent under high vacuum gave the desired propiophenone 12 (177.5 mg) as a light yellow oil in 77.2% yield, and which solidified upon standing. TLC (SiO_2, benzene) showed single-spot material at R_f = 0.54 corresponding to authentic 3'-chloropropiophenone. Plate scanning confirmed the identity and purity.

[2'-^{3}H]-2-Bromo-3'-chloropropiophenone

Prepared essentially as for the [1-^{14}C]-compound above using 1.75 equivalents of bromine in CH_2Cl_2, to give crude [2'-^{3}H]-2-bromo-3'-chloropropiophenone (276.1 mg; 106.4% weight yield), as an orange-red oil which was used in the final step without further purification.

[2'-^{3}H]-2-*tert*-Butylamino-3'-chloropropiophenone Hydrochloride 13

To a solution of the crude [2'-^{3}H]-2-bromo-3'-chloropropiophenone (276.1 mg) in dry CH_3CN (10 mL) stirred at 35-36°C under dry argon was added a solution of *tert*-butylamine (5.54 g) in CH_3CN (11 mL) during 3 min. The yellow solution was stirred at 35-38°C for 4 h, then at room temperature for 41 h. After work-up similar to that for the [^{14}C]-labelled 4 above, a yellowish-white crystalline solid (256.3 mg; 88.6% yield from 12) was obtained that was shown to be almost pure 13 by TLC in $CHCl_3/Et_3N$ (19/1, v/v) followed by plate scanning. 2-Butanol (5.0 mL) was added, the mixture heated until complete solution was attained, then evaporated under reduced pressure to a volume of ~1.5 mL, and allowed to stand for 2 h. The mixture was cooled at 0°C overnight, the crystals were filtered, washed with ice-cold 2-butanol (1 mL) and acetone (2 mL), and dried *in vacuo* at 56°C overnight. The yield of off-white solid 13 was 131.7 mg (9.72 Ci; 45.5% yield from 12) with specific activity 20.53 Ci/mmol.

TLC using $CHCl_3/Et_3N$ (19/1, v/v) showed single-spot material with $R_f = 0.64$ corresponding to authentic bupropion hydrochloride. Plate scanning showed no detectable impurities, indicating a minimum radiochemical purity of 98%.

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