Design and Synthesis of a Hapten for the Radioimmunoassay of Bupropion

NARIMAN B. MEHTA[x] AND DAVID L. MUSSO

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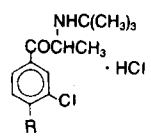
Abstract □ The synthesis of a hapten useful in the radioimmunoassay of bupropion is described. Since bupropion has no functional group that can be easily derivatized, a hydroxypropyl group was incorporated into the molecule. Studies in cross-reactivity with possible metabolites required the synthesis of the 4′-hydroxy analogue of bupropion. This synthesis is also described.

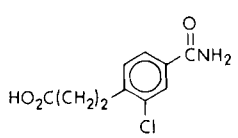
Bupropion (Wellbutrin, 1) a non tricyclic antidepressant[1a] is presently being clinically evaluated.[1b] An analytical method that could quantitate the drug at picogram and nanogram levels in unprocessed serum and other biological materials was desired. Since radioimmunoassay (RIA) has been employed to measure such low drug levels in plasma and tissue, the development of an RIA for bupropion was investigated.[2]

Bupropion does not have a functional group that can directly conjugate with the carrier protein for immunogen preparation to elicit an antibody response. Therefore, it was necessary to design and synthesize a specific hapten. Two important factors should be considered in the design of such a hapten. First, in order to minimize cross-reactivity with metabolites, the drug receptor sites should not be too close to the conjugation site. Second, the chemical moiety incorporated into the drug should terminate with a derivatizable functional group such as an OH, COOH, or NH_2 and, preferably should be a minimum of four atoms in chain length.

Initial studies were therefore directed toward the synthesis of the 4′-ß-propanoic acid derivative of bupropion-compound 2. This was a carefully planned synthesis in view of the inherent instability of the aminoketone base. Although 2 could not be obtained from the reaction sequence chosen, the 4′-γ-hydroxypropyl analogue of bupropion 3 was prepared. The conjugate of bovine serum albumin and the succinoyl derivative of 3 resulted in production of antisera which are not only sensitive, but have low cross-reactivity with known side-chain metabolites of bupropion.[2] The cross-reactivity of the antisera produced from 3 with the 4′-hydroxy analogue of bupropion was also studied.



1, R = H
2, R = $(CH_2)_2CO_2H$
3, R = $(CH_2)_3OH$



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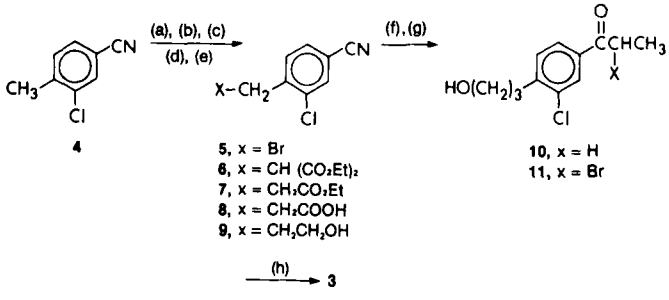
Discussion

The 4′-γ-hydroxypropyl analogue of bupropion 3 was synthesized by the route outlined in *Scheme I*. Bromination of 3-chloro-4-methylbenzonitrile 4 with N-bromosuccinimide was carried out as described by Gogolimska[4] to give α-bromotoluene 5. Alkylation of 5 with diethyl malonate was accomplished using sodium ethoxide in ethanol or sodium hydride in dimethylformamide[7] to give 6. Neither method offered any advantage. The decarbalkoxylation of 6 was effected with NaCl, H_2O, and Me_2SO according to the procedure of Krapcho and Lovey[5] to give the monoester 7. Hydrolysis of 7 with potassium hydroxide in 95% ethanol gave the corresponding acid 8. This hydrolysis was done using less than one equivalent of potassium hydroxide. If excess potassium hydroxide was used, it appeared that some hydrolysis of the cyano group to the primary amide 12 occurred.



Scheme I

The acid 8 was reduced with diborane to the alcohol 9 which was converted to the propiophenone 10 by reaction with ethylmagnesium bromide. Bromination of 10 with bromine in methanol, followed by amination with *tert*-butylamine, gave the desired hapten 3.

The cross-reactivity of the antisera produced from hapten 3 with the 4′-hydroxy analogue of bupropion 21 was studied. The sequence of reactions used to prepare 21 is shown in *Scheme II*. Reaction of 3-chloro-4-hydroxybenzoic acid (13) with either dimethyl sulfate and potassium carbonate in refluxing acetone or with potassium hydroxide in ethanol, followed by methyl iodide in acetone gave the methoxyester 14 (mp 91–93°C, lit.[8] mp 72–74°C). The dimethyl sulfate: potassium carbonate method gave better yields of 14.

Hydrolysis of this ester with potassium hydroxide in methanol gave the acid 15 (mp 215–218°C, lit.[8] mp 199–202°C). The benzamide 16 (mp 209–213°C, lit.[9] mp 192–193°C) was prepared from 15 using thionyl chloride, followed by aqueous ammonia. Treatment of 16 with thionyl chloride gave benzonitrile 17[10] (mp 104–106°C), which was converted to the corresponding propiophenone 18 (mp 87–88°C, lit.[6] mp 88–

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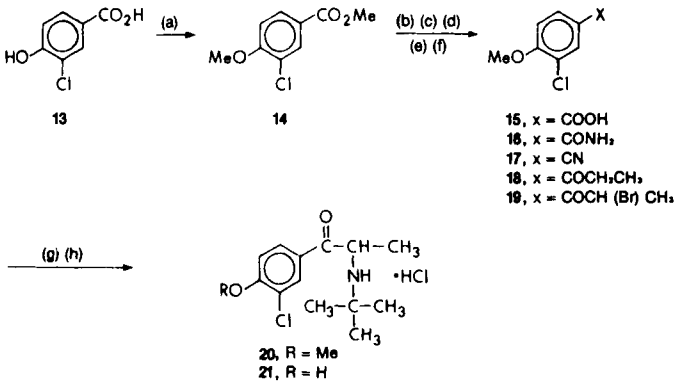
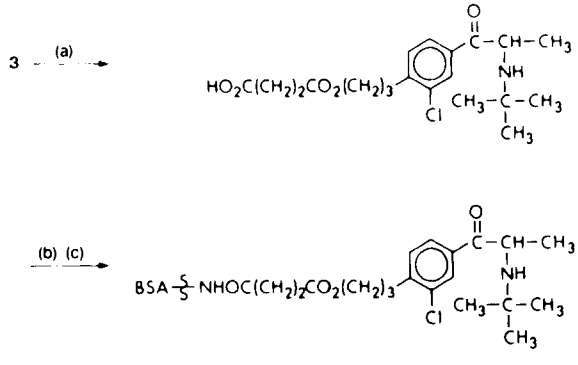


Scheme II

90°C) by treating **17** with ethylmagnesium bromide. Bromination of **18** with bromine in methanol, followed by amination with *tert*-butylamine gave the 4′-methoxy analogue of bupropion **20**. Reaction of **20** with boron tribromide in dichloromethane gave the desired 4′-hydroxy analogue **21**.

The immunogen synthesis shown in *Scheme III*, antisera production, and antisera specificities have been reported by Butz et al.[2] They report that antisera produced from the immunogen obtained from **3** show good sensitivity and specificity for determining bupropion in biological fluids using RIA techniques.



Scheme III

Experimental Section

Chemistry—Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by either Dr. Stuart Hurlburt and his associates at the Analytical/Organic Dept. of Burroughs Wellcome Co., Atlantic Microlab, Inc., or Integral Microanalytical Laboratories, Inc. Results are within ± 0.4% of the theoretical values. The NMR and IR spectra are consistent with the assigned structures. The ^{1}H NMR spectra were recorded on a Hitachi Perkin-Elmer R-24A or a Varian T-60 spectrometer with Me_4Si as the internal standard. The IR spectra were recorded on a Perkin-Elmer 267 or a Unicam SP1000 instrument. High vacuum distillations were performed on a previously described apparatus.[3] 3-Chloro-4-methylbenzonitrile (**4**) and 3-chloro-4-hydroxybenzoic acid (**13**) were purchased from Aldrich Chemical Co.

Diethyl 2-(2-chloro-4-cyanobenzyl)malonate (6)—Diethyl malonate (15.7g, 0.098 mol) was added in a dropwise manner over a 5-min. period to a solution of sodium ethoxide prepared from 2.0 g (0.089 g.-atom) of Na in 120 mL of ethanol. Then 20.0 g (0.087 mol) of 2-chloro-4-cyanobenzylbromide (**5**)[4] was added. Tne resulting mixture was refluxed for 3.5 h, cooled, and allowed to stand at room temperature overnight. The mixture was evaporated to dryness under reduced pressure. Water (120 mL) and conc. HCl (3.6 mL) were added to the residue. The resulting layers were separated and the organic layer was distilled to give 12.5 g (46%) of **6**, bp 140–142°C (100 μm), which solidified on standing to a white solid; mp 50–53°C IR (film): 2230 (C≡N) and 1730 (C=O) cm^{-1} ^{1}H NMR ($CDCl_3$): δ 1.21 (t, 6, CH_3), 3.36 (m, 2, ArCH_2), 3.80 (m, 1, CH), 4.16 (q, 4, OCH_2CH_3), and 7.25–7.66 ppm (m, 3, ArH). Anal. ($C_{15}H_{16}ClNO_4$) C, H, N.

Ethyl 3-(2-chloro-4-cyanophenyl)propanoate (7)—This procedure was based on the decarbalkoxylations described by Krapcho and Lovey.[5] A mixture of 9.0 g (0.029 mol) of **6**, 2.12 g (0.036 mol) of NaCl, and 0.86 g (0.107 mol) of H_2O in 30 mL of Me_2SO was heated to 135°C, after which the temperature was slowly raised to 170°C over a 3 h period. After standing at room temperature overnight, water was added and the organic phase was separated. The aqueous phase was extracted with ether, and the combined organic phase was dried over magnesium sulfate. Evaporation and distillation of the residue afforded 5.8 g (84%) of **7** as a yellow oil; bp 138–140°C (100 μm) IR (film); 2230 (C≡N) and 1735 (C=O) cm^{-1}: ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3, CH_3), 2.56 (m, 2, ArCH_2CH_2CO), 3.07 (m, 2, ArCH_2CH_2CO), 4.10 (q, 2, OCH_2), and 7.23–7.68 ppm (m, 3, ArH). Anal. ($C_{12}H_{12}ClNO_2$) C, H, N.

3-(2-Chloro-4-cyanophenyl)propanoic Acid (8)—Compound **7** (33.0 g, 0.139 mol) was added to a solution of 6.27 g (0.112 mol) of KOH in 109 mL of 95% ethanol. The mixture was kept at 40°C for 1 h. After cooling to room temperature, the mixture was neutralized to pH 7 with 10% HCl and concentrated under reduced pressure. The residue was taken up in 5% aqueous $NaHCO_3$ solution and washed with ethyl acetate. The aqueous bicarbonate layer was acidified with conc. HCl and the precipitate was collected, washed with water, and dried to give 18.4 g (63%) of **8**; mp 127–129°C. A sample was recrystallized from benzene:hexane to give analytically pure **8**; mp 132–134°C; ^{1}H NMR ($CDCl_3$): δ 2.75 (m, 2, ArCH_2CH_2CO), 3.17 (m, 2, ArCH_2CH_2CO), 7.1–8.1 (m, 3, ArH), and 10.6 ppm (s, 1, COOH). Anal. ($C_{10}H_8ClNO_2$) C, H, N.

3-Chloro-4-(3-hydroxypropyl)benzonitrile (9)—A 0.94 M borane–tetrahydrofuran solution (72 mL, 0.068 mol) was added in a dropwise manner to a solution of 14.3 g (0.068 mol) of **8** in 70 mL of tetrahydrofuran at −18°C under a N_2 atmosphere. The mixture was allowed to equilibrate to room temperature overnight, after which it was chilled to 0°C and hydrolyzed with 50 mL of H_2O. After the addition of 20.0 g of potassium carbonate, the organic phase was separated and the aqueous phase was extracted with ether. The combined organic phase was dried over magnesium sulfate and concentrated under reduced pressure. Distillation of the residue gave 11.0 g (83%) of **9**; bp 55–60°C (5 μm); ^{1}H NMR ($CDCl_3$): δ 1.88 (m, 2, Ar$CH_2CH_2CH_2$OH), 2.08 (s, 1, OH), 2.92 (m, 2, Ar$CH_2CH_2CH_2$OH), 3.72 (t, 2, Ar$CH_2CH_2CH_2$OH), and 7.45 ppm (m, 3, ArH). Anal. ($C_{10}H_{10}ClNO$) C, H, N.

3′-Chloro-4′-(3-hydroxypropyl)propiophenone (10)—A solution of 3 M ethylmagnesium bromide in ether (100 mL, 0.30 mol) was added in a dropwise manner to a solution of 18.0 g (0.092 mol) of **9** in dry ether at 0°C. The mixture was heated on a steam bath for 4 h, chilled in an ice bath, and hydrolyzed with 150 mL of 15% HCl. After heating on a steam bath overnight, conc. HCl was added, and the mixture was heated for 0.5 h. The mixture was cooled and extracted with ethyl acetate. The organic phase was concentrated and the residue was crystallized from ether:pentane to give 12.4 g (60%) of **10**; mp 42–44°C; IR (nujol): 3300 (OH) and 1680 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3, CH_3), 1.90 (m, 2, Ar$CH_2CH_2CH_2$OH), 2.42 (s, 1, OH), 2.95 (m, 4, 2 CH_2's), 3.73 (t, 2, Ar$CH_2CH_2CH_2$OH), and 7.25–8.05 ppm (m, 3, ArH). A sample was recrystallized from ether:pentane to give analytically pure **10**, mp 46–48°C. Anal. ($C_{12}H_{15}ClO_2$) C, H.

(±)-2-*tert*-Butylamino-3′-chloro-4′-(3-hydroxypropyl)propiophenone Hydrochloride (3)—A solution of 5.7 g (0.036 mol) of bromine in 50 mL of methanol was added to a mixture of 7.7 g (0.034 mol) of **10** and 1 mL of conc. HCl in 50 mL of methanol at 0°C. The mixture was stirred overnight at room temperature, concentrated under reduced pressure, and the residue was distilled to give 9.6 g (93%) of **11**; bp 102–105°C (5 μm). Anal. ($C_{12}H_{14}BrClO_2$) C, H.

An excess of *tert*-butylamine was added to a solution of 2.9 g (0.0095 mol) of **11** in 30 mL of acetonitrile at 0°C, and the mixture was kept at room temperature for 4 h. The mixture was filtered and ether was added to the filtrate. The mixture was filtered, the solution was concentrated under reduced pressure, and then fresh ether was added to the residue, and the mixture was filtered again. The filtrate

was chilled and acidified with ethereal HCl. The hygroscopic salt was removed by filtration, washed repeatedly with ether, and triturated with boiling acetone to give 1.25 g (38%) of **3**; mp 203–205°C dec.; ^{1}H NMR (Me_2SO-d_6): δ 1.33 (s, 9, $C(CH_3)_3$), 1.55 (d, 3, CH_3), ~1.82 (m, 2, $ArCH_2CH_2CH_2OH$), 2.83 (m, 2, $ArCH_2CH_2CH_2OH$), 3.48 (t, 2, $ArCH_2CH_2CH_2OH$), 5.28 (br, 1, CH), 7.53–8.30 (m, 3, ArH), and 8.67 and 9.72 ppm (br, 2, NH and HCl). Anal. ($C_{16}H_{24}ClNO_2 \cdot HCl \cdot 3/4\ H_2O$) C, H, N.

(±)-2-*tert*-Butylamino-3′-chloro-4′-methoxypropiophenone Hydrochloride (20)—Bromine in methanol (6.24 g, 0.039 mol) was added in a dropwise manner to 5.18 g (0.026 mol) of 3-chloro-4-methoxypropiophenone (**18**), mp 87–88°C (lit.[6] mp 88–90°C) in 200 mL of methanol containing 4 mL of conc. HCl at 0°C. The mixture was stirred at room temperature overnight, and then was concentrated to give the α-bromoketone (**19**). Twenty milliliters of *tert*-butylamine was added to 7.0 g of the aforementioned **19** in 100 mL of acetonitrile at 0°C. The mixture was stirred overnight at room temperature, and then was concentrated under reduced pressure, and the residue was triturated with ether. The *tert*-butylamine hydrobromide was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was dissolved in ether and acidified with ethereal HCl. The product was removed by filtration and then recrystallized twice from ethanol: ether to give 3.26 g (43%) of **20**; mp 250°C dec.; ^{1}H NMR (Me_2SO-d_6): δ 1.37 (s, 9, $C(CH_3)_3$), 1.58 (d, 3, CH_3), 4.03 (s, 3, OCH_3), 5.30 (br, 1, CH), 7.30–7.47 and 8.18–8.42 (2 m, 3, ArH), and 8.55 and 10.07 ppm (br, 2, NH and HCl). Anal. ($C_{14}H_{20}ClNO_2 \cdot HCl$) C, H, N.

(±)-2-(*tert*-Butylamino)-3′-chloro-4′-hydroxypropiophenone (21)—This compound was prepared as described by Butz et al.[2]

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