(11) O. Hutzinger, W. D. Jamieson, S. Safe, L. Paulmann, and R. Ammon, *Nature (London)*, **252**, 698 (1974).
(12) S. Safe, D. Jones, and O. Hutzinger, *J. Chem. Soc. (Perkin I)*, **4**, 357 (1976).
(13) J. Kohli and S. Safe, *Chemosphere*, **5**, 433 (1976).
(14) H. Elias and H. T. Lotterhos, *Chem. Ber.*, **109**, 1580 (1976).
(15) J. E. Sinsheimer, T. Wang, S. Röder, and Y. Y. Shun, *Biochem. Biophys. Res. Commun.*, **83**, 281 (1978).
(16) B. N. Ames, J. McCann, and E. Yamasaki, *Mutat. Res.*, **31**, 347 (1975).
(17) B. S. Levine and S. D. Murphy, *Toxicol. Appl. Pharmacol.*, **40**, 393, (1977).
(18) W. H. Habig, M. J. Pabst, and W. B. Jakoby, *J. Biol. Chem.*, **249**, 7130 (1974).
(19) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
(20) G. M. Benke *et al.*, *Toxicol. Appl. Pharmacol.*, **28**, 97 (1974).
(21) U. A. H. Hadi, D. J. Malcolme-Lawes, and G. Oldham, *Int. J. Appl. Rad. Isotop.*, **28**, 747 (1978).
(22) Y. C. J. Wang, *J. Pharm. Sci.*, **69**, 671 (1980).
(23) J. W. Daly, D. M. Jerina, and B. Witkop, *Experientia*, **28**, 1129 (1972).
(24) D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, **23**, 420 (1958).
(25) H. G. Bray, S. P. James, and W. V. Thorpe, *Biochem. J.*, **67**, 607 (1957).
(26) M. J. Pabst, W. H. Habig, and W. B. Jakoby, *J. Biol. Chem.*, **249**, 7140 (1974).
(27) P. Kraus, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **291**, 79 (1975).
(28) F. Oesch and J. Daly, *Biochem. Biophys. Res. Commun.*, **46**, 1713 (1972).

ACKNOWLEDGMENTS

The authors would like to thank William Harrity and Amy Khan, National Science Foundation Undergraduate Research Participants, Grants No. SPI 7826914 and SPI 8026361, respectively, and Randy Olenyk for their laboratory assistance. This study was supported in part by a Training Grant 5-T32-GM07767 from the National Institutes of Health.

The authors are grateful to Mr. John Jones and The University of Michigan's Phoenix Memorial Laboratory for their cooperation in conducting our neutron-activation analyses.

Determination of Bupropion and Its Major Basic Metabolites in Plasma by Liquid Chromatography With Dual-Wavelength Ultraviolet Detection

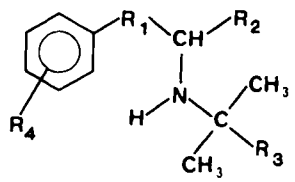
THOMAS B. COOPER ˣ, RAYMOND F. SUCKOW, and ALEXANDER GLASSMAN

Received March 11, 1983, from the *Analytical Psychopharmacology Laboratory, New York State Psychiatric Institute, New York, NY 10032.* Accepted for publication September 14, 1983.

Abstract □ A method for the determination of bupropion and its three major basic metabolites in plasma is described. Following an extraction from alkaline plasma into 1.5% v/v isoamyl alcohol in *n*-heptane, a portion of the acid-backwashed extract was injected onto a column packed with trimethylsilyl reverse-phase material and eluted with a phosphate buffer–acetonitrile (80:20) mobile phase containing an ion-pairing reagent and triethylamine. The compounds were detected with a dual-wavelength UV detector (214 and 254 nm) to optimize sensitivity and facilitate simultaneous detection. The method provides an absolute recovery of ~85% for bupropion and ~98% for the metabolites. Day-to-day reproducibility did not exceed 4.0% for all compounds. The detection limits were ~5 ng/mL for bupropion and 100 ng/mL for the major metabolites. The limit of 100 ng/mL for metabolite quantitation is imposed by the internal standard concentration selected for steady-state studies. In single-dose pharmacokinetic studies, 10% of the steady-state concentration of internal standard was used; this permitted a 10-ng/mL lower limit of detection. Steady-state plasma levels of bupropion and the metabolites from eight different patients are presented.

Keyphrases □ Bupropion—metabolites, HPLC, dual-wavelength UV detection □ Antidepressants—bupropion and its metabolites, HPLC, dual-wavelength UV detection

Bupropion hydrochloride[1] [(±)-2-*tert*-butylamino-3′-chloropropiophenone hydrochloride], a chemically unique antidepressant currently undergoing clinical evaluation, appears to be free of any significant anticholinergic or cardiovascular effects (1–3). The assay of bupropion in biological fluids has been limited to RIA (4) and high-performance liquid chromatography (HPLC) (5). The RIA procedure appears to be sensitive (<1 ng/mL) and specific for bupropion, whereas the HPLC method is less sensitive (50 ng/mL). However, neither method quantitates metabolites.



I: R_1 = C=O, R_2 = R_3 = CH_3, R_4 = 3-Cl
II: R_1 = C=O, R_2 = CH_3, R_3 = CH_2OH, R_4 = 3-Cl
III: R_1 = CHOH, R_2 = R_3 = CH_3, R_4 = 3-Cl (*erythro*)
IV: R_1 = CHOH, R_2 = R_3 = CH_3, R_4 = 3-Cl (*threo*)
V: R_1 = CHOH, R_2 = CH_3, R_3 = CH_2OH, R_4 = 3-Cl (*erythro*)
VI: R_1 = C=O, R_2 = C_2H_5, R_3 = CH_3, R_4 = 4-F
VII: R_1 = C=O, R_2 = C_3H_7, R_3 = CH_3, R_4 = 4-F

Current interest in biologically active metabolites of psychotropic drugs led us to develop an HPLC procedure which separates and quantitates bupropion and its major basic metabolites in plasma with dual-wavelength UV detection.

EXPERIMENTAL SECTION

Apparatus—Chromatography was performed with a dual-piston solvent delivery pump[2] with either a manual injector[3] or an automatic sample processor[4]. The column was 25 cm × 4.6 mm i.d. packed with 5-μm particle size trimethylsilyl material[5]. The effluent was monitored at 254 and 214 nm by

[1] Wellbutrin.

[2] Model 6000A; Water Associates, Milford, Mass.
[3] U6K Injector; Waters Associates.
[4] Wisp 710B; Waters Associates.
[5] LC-1; Supelco, Bellafonte, Pa.

0022-3549/84/0800-1104$01.00/0
© 1984, American Pharmaceutical Association

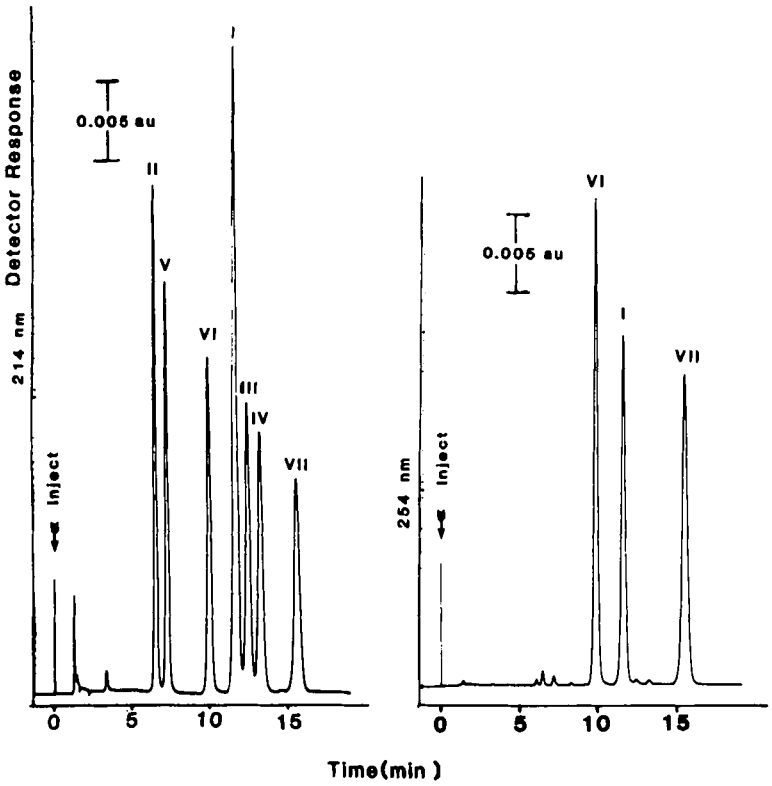


Figure 1—*Dual chromatogram of a standard aqueous solution containing 400 ng each of I, its major metabolites (II, III, and IV), a possible urinary metabolite (V), and internal standards (VI and VII). The compounds were detected at 214 and 254 nm simultaneously.*

a dual (in series) UV detector system[6], a signal recorder[7], and a laboratory data acquisition system[8]. The column temperature was controlled with an aluminum block devised to fit this column and with a circulating water pump[9].

Reagents—Phosphoric acid[10], monobasic potassium phosphate[10], sodium carbonate[10], and sodium bicarbonate[10] were all analytical reagent grade. Sodium heptanesulfonate[11] and triethylamine[12] were used as received. UV-grade acetonitrile[13] and *n*-heptane[10] were used without further purification. Isoamyl alcohol[14] was distilled before use. Distilled water was passed through a water purification system[15] before use.

Standards—Bupropion hydrochloride (I), 3'-chloro-2-(2-hydroxy-1,1-dimethylethylamino)propiophenone hydrochloride (II), (+)(*R**,*S**)-2-(*tert*-butylamino)-1-(3-chlorophenyl)propanol (III), (*R**,*R**)-2-(*tert*-butylamino)-1-(3-chlorophenyl)propanol (IV), (±)(*R**,*S**)-1-(3-chlorophenyl)-2-(2-hydroxy-1,1-dimethylethylamino)propanol (V), 2-(*tert*-butylamino)-4'-fluorobutyrophenone hydrochloride (VI), and 2-(*tert*-butylamino)-4'-fluorovalerophenone hydrochloride (VII) stock solutions (1 mg/mL) were prepared in 0.01 M HCl. Working solutions were further diluted to 1 ng/μL in 0.01 M HCl for I and V, and to 10 ng/μL in 0.01 M HCl for II, III, IV, VI, and VII. The structures and activities of these metabolites have been described elsewhere (3).

Extraction—To 1.0 mL of plasma, 800 ng of internal standard VI and 150 ng of internal standard VII were added to 1.0 mL of 0.6 M carbonate buffer (pH 9.5) and 10 mL of 1.5% v/v isoamyl alcohol in *n*-heptane. The mixture was shaken for 15 min and centrifuged at 1500×*g* for 10 min. The organic layer was then transferred to a 15-mL tube containing 250 μL of 0.1 M HCl. After mixing for 10 min and centrifuging at 1500×*g* for 10 min, the organic layer was removed by aspiration. Suitable aliquots of the aqueous phase were injected for chromatographic separation.

Chromatographic Conditions—The mobile phase consisted of 0.05 M monobasic potassium phosphate–acetonitrile (80:20), with 0.007 M sodium heptanesulfonate and 0.01 M triethylamine added. The final pH of the aqueous portion was brought to 3.0 by the addition of phosphoric acid. The mixture was filtered and deaerated prior to use. The flow rate was 2.3 mL/min, and the column temperature was set at 30°C. The effluent was monitored with a dual UV detector system at 254 and 214 nm in series.

Quantitation—All determinations were performed by calculating the peak height and/or area ratios of each compound to the internal standard. A linear regression analysis for each of the standard curves was performed with an on-line data acquisition system resulting in the computation of the slope, *x*-intercept, correlation coefficient, and standard error. Compound I was quantitated at 254 nm with internal standard VII. Metabolites II, III, and IV were quantitated at 214 nm with internal standard VI.

RESULTS AND DISCUSSION

Bupropion (I) and its major metabolites in plasma were separated and quantitated by HPLC with a dual-wavelength UV detector system. Although all compounds absorb at 214 nm, only I and the two internal standards (VI and VII) absorb at 254 nm (Fig. 1). Since it was found that the steady-state plasma concentration of I was low in comparison to its metabolites, simultaneous quantitation was not possible for all compounds at 214 nm. The major metabolite of I (*i.e.*, II) is usually found at concentrations 10–100 times that of bupropion. Thus, I was quantitated at 254 nm with internal standard VII.

All compounds were adequately resolved and eluted within a 20-min period (Fig. 1). An actual spiked plasma sample is shown in Fig. 2. A drug-free patient plasma sample did not show any interfering endogenous peaks at either wavelength (Fig. 3). Although V is reported to be a urinary metabolite (6), no detectable levels were found in steady-state plasma samples obtained from patients (Fig. 4). Therefore, this metabolite was not included in the precision and accuracy tests in plasma. Figure 2 illustrates that V could be coextracted, separated, and quantitated, thus making this method possibly useful in metabolite studies in urine.

In general, it was found that the addition of triethylamine enhanced peak symmetry. Other amine modifiers such as *n*-butylamine or nonylamine did not afford the resolution needed. Other columns such as an octyldecylsilyl and an octylsilyl-bonded column resulted in longer retention times and inadequate resolution between III and IV.

The detection limit for I is ~5 ng/mL of plasma, and the detector response remained linear at least up to 400 ng. The detection limit for II, III, and IV in this assay was 100 ng/mL, and the detector response showed linearity from 100 to at least 3000 ng.

The limit of 100 ng/mL for metabolite quantitation is imposed by the internal standard concentration selected for steady-state studies. In single-dose pharmacokinetic studies, 10% of the concentration of internal standard used for steady state was utilized; this permitted a 10-ng/mL lower limit of detection.

[6] Model 440 (254 nm) with extended wavelength module (214 nm); Waters Associates.
[7] Houston Omniscribe model B5217B-2; Houston Instruments, Austin, Tex.
[8] PDP 11/34 "Peak II" System; Digital Equipment Co., Maynard, Mass.
[9] Model FE; Haake Co., Saddlebrook, N.J.
[10] Fisher Scientific Co., Fair Lawn, N.J.
[11] Eastman Kodak Co., Rochester, N.Y.
[12] Aldrich Chemical Co., Milwaukee, Wis.
[13] Burdick and Jackson Laboratories, Muskegon, Mich.
[14] Sigma Chemical Co., St. Louis, Mo.
[15] Milli-Q; Millipore Corp., Bedford, Mass.

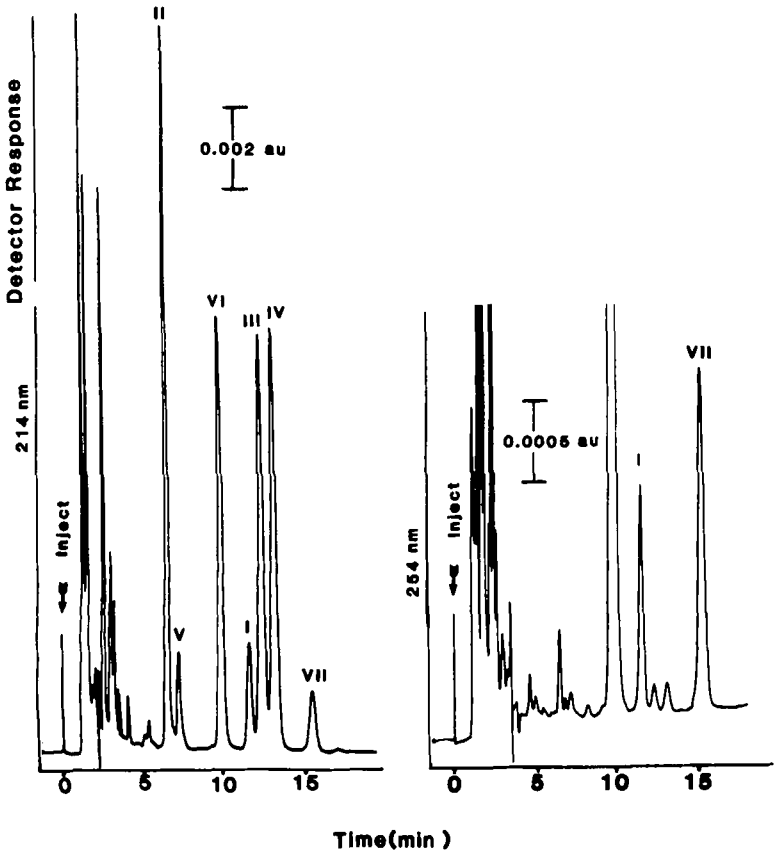


Figure 2—*Dual chromatogram of a 1-mL spiked plasma extract containing 100 ng of I and metabolite V and 1000 ng of II, III, and IV. Fifty microliters of the acid extract was injected. Compounds VI and VII are the internal standards.*

In preliminary single-dose studies, the major metabolites (II and IV) were present at 30 min postdose, reaching a maximum of 2–3 times that of bupropion. Furthermore, inspection of these data clearly indicates that the metabolites have markedly different (longer) half-lives of elimination[16].

The absolute recovery was determined by preparing a solution of known

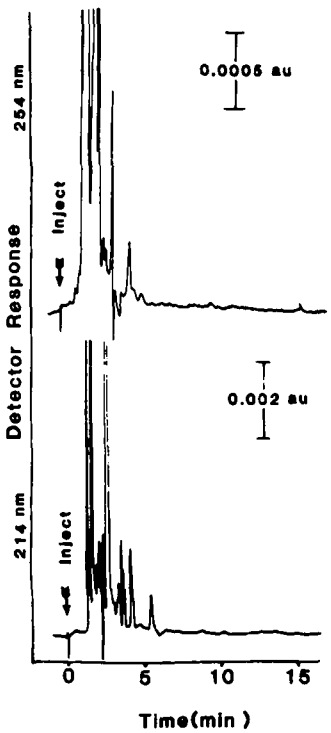


Figure 3—*Dual chromatogram of a 1-mL plasma extract from a drug-free patient. The volume injected was 100 μL.*

[16] Unpublished results.

Table I—Recovery of I and Metabolites from 1 mL of Plasma

Compound	Recovery, %[a] 3000 ng/mL	1000 ng/mL	400 ng/mL	100 ng/mL	12.5 ng/mL
I	—	—	82 ± 3.2	87 ± 2.9	85 ± 10.4
II	95 ± 2.2	96 ± 3.8	—	97 ± 10.0	—
III	98 ± 2.4	102 ± 2.8	—	97 ± 9.3	—
IV	99 ± 2.4	104 ± 3.0	—	97 ± 9.7	—

[a] Mean ± *RSD*; $n = 8$.

Table II—Precision of Assay for I and Metabolites Based on Peak Height Ratios[a]

Concentration, ng/mL	*RSD*, % I	II	III	IV
3000		±0.8	±1.1	±1.2
1500		±2.3	±2.6	±2.7
1000		±1.3	±1.1	±1.2
500		±3.3	±3.7	±4.0
400	±2.9			
250		±4.0	±3.6	±4.4
200	±2.0			
100	±4.7	±5.0	±8.2	±3.9
50	±4.3			
25	±2.5			
10	±6.0			

[a] $n = 8$.

Table III—Reproducibility of Assay Based on Slopes of the Linear Regression Curves[a]

Compound	Slope, ng/mL	*RSD*, %
I	147.8	±3.3
II	627.4	±3.1
III	1074.8	±2.7
IV	1066.4	±4.0

[a] Data computed from five consecutive standard curves.

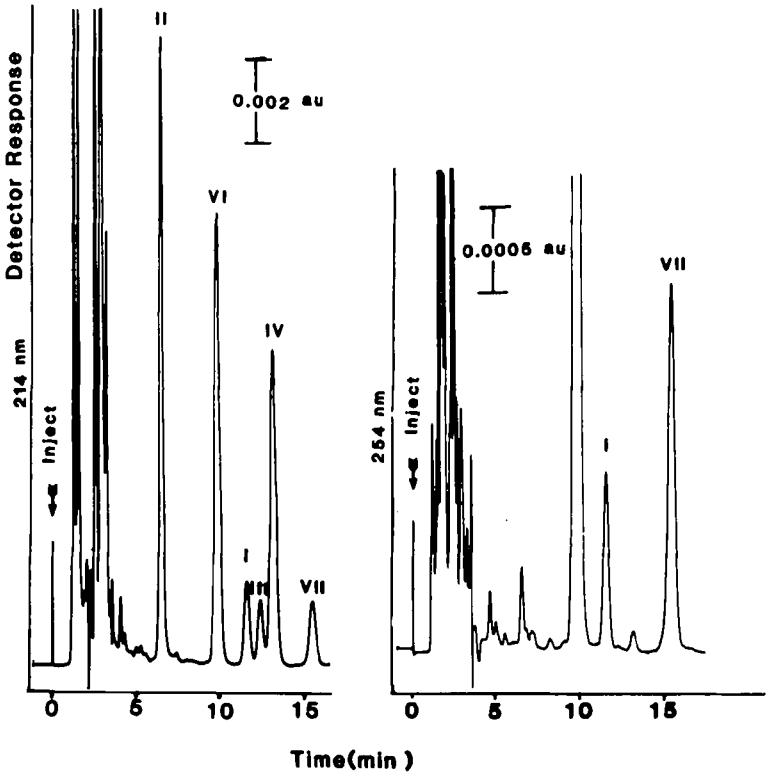


Figure 4—*Dual chromatogram of a 1-mL plasma extract from a patient receiving bupropion. Fifty microliters of the acid extract was injected. The plasma levels were calculated to be 72 ng of bupropion, 831 ng of II, 151 ng of III, and 743 ng of IV. Compounds VI and VII are the internal standards.*

concentration of all compounds. The internal standards were added to each solution, and the sample was injected into the chromatograph. The same standards were added to 1 mL of plasma and processed routinely, except for the internal standards. To 100 μL of the final acid extract, 100 μL of internal standard solution containing twice the usual concentration was added. The tube was capped, thoroughly mixed, and then injected. The difference between the ratios of the standards to internal standards in the processed samples *versus* direct injection samples gave a measure of overall recovery (Table I).

The precision of the method was determined by spiking eight standards; the sample was processed as described above. The percent relative standard deviations for various concentrations are reported in Table II.

A measure of the stability of the assay was demonstrated by the variability in the slope of the linear regression curves on 5 consecutive days (Table III). Accuracy was further assessed by comparing plasma concentrations of bupropion from two single-dose pharmacokinetic studies determined by this method and an RIA procedure (4). Twenty-eight samples (range, 5–130 ng/mL) were analyzed. Least-squares linear regression analysis resulted in the following: slope, 0.93; x-intercept, 2.2 ng/mL; and correlation coefficient, 0.987, where y and x were the bupropion concentrations by HPLC and RIA, respectively. Metabolite concentrations could not be compared since no other method has, as of now, been established.

Several psychotropic drugs and their metabolites were tested to determine potential interference in the assay. All commonly used tricyclic antidepressant compounds (imipramine, desipramine, amitriptyline, nortriptyline, doxepin, and desmethyldoxepin) and the new antidepressant drugs maprotiline, trazodone, mianserin, amoxapine, and chlorimipramine have much longer retention times than VII. Similarly, the major neuroleptic compounds such as chlorpromazine, thioridazine, loxapine, fluphenazine, perphenazine, and haloperidol do not interfere.

Compounds that may interfere are the hydroxy metabolites of amitriptyline, chlordiazepoxide and its *N*-demethyl metabolite, and nordiazepam.

Until now, there has been no reported human steady-state plasma level data for bupropion or its metabolites. The pharmacokinetics of bupropion has been reported (7), but metabolite determinations were not performed. Table IV is a summary of plasma concentration of bupropion and its major metabolites in eight different patients being treated for depression. The dose varied from patient to patient (100–450 mg/d). Although bupropion plasma concentrations averaged 50 ng/mL, the major metabolite (II) was found to be ~50 times higher (2500 ng/mL). The two diastereoisomers (III and IV) were also found in considerably higher concentrations (the *threo* isomer was always present in amounts 3–5 times greater than the *erythro* isomer).

Table IV—Typical Steady-State Plasma Concentrations of I and Metabolites in Eight Depressed Patients [a]

Patient	Dose, mg/24 h	Concentration, ng/mL I	II	III	IV
1	450	68	2784	103	453
2	350	43	3111	542	2226
3	100	24	1006	123	483
4	450	66	2931	368	1715
5	450	51	1931	379	1959
6	300	41	2801	348	1146
7	300	33	1512	174	552
8	350	35	2581	218	997

[a] All samples were collected immediately prior to morning dose. Dosage range, 100–450 mg/d.

REFERENCES

(1) H. J. Leighton and R. A. Maxwell, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **37**, 481 (1978).

(2) J. P. Feighner, *J. Clin. Psychiat.*, **44**, 5(2), 49 (1983).

(3) R. A. Maxwell, W. B. Mehta, W. E. Tucker, Jr., D. H. Schroeder, and W. C. Stern in "Pharmacological and Biochemical Properties of Substances," vol. 3, *Am. Pharmaceutical Assoc.*, Washington, D.C., 1981, pp. 1–55.

(4) R. F. Butz, D. H. Schroeder, R. M. Welch, N. B. Mehta, A. P. Phillips, and J. W. A. Findlay, *J. Pharmacol. Exp. Ther.*, **217**, 602 (1981).

(5) D. H. Schroeder, M. L. Hinton, P. G. Smith, C. A. Nichol, and R. M. Welch, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **37**, 691 (1978).

(6) D. H. Schroeder, J. L. Eiseman, N. B. Mehta, D. A. Brent, F. E. Soroko, and R. M. Welch, *Pharmacologist*, **16**, 191 (1979).

(7) J. W. A. Findlay, J. Vanwyck Fleet, P. G. Smith, R. F. Butz, M. L. Hinton, M. R. Blum, and D. H. Schroeder, *Eur. J. Clin. Pharmacol.*, **21**, 127 (1981).

ACKNOWLEDGMENTS

This work was made possible by a grant-in-aid to T.B.C. by Burroughs-Wellcome Co., Research Triangle Park, NC 27709. Help from the following Burroughs-Wellcome senior staff scientists is especially acknowledged: Dr. Warren C. Stern for generously supplying bupropion, metabolites, and the compounds used as internal standards and for his continuing advice and support; and Drs. A. A. Lai and D. H. Schroeder for supplying the plasma from bupropion-treated depressed patients and single-dose bupropion samples from normal volunteers.