ported as cannabidiol, is more abundant in nature than previously thought; any previous classification of *C. sativa* L. based on cannabidiol, cannabinol, and (−)-Δ^9-*trans*-tetrahydrocannabinol must be questioned. Since it is known that synthetic cannabidiol antagonizes certain effects of (−)-Δ^9-*trans*-tetrahydrocannabinol, much work is now needed on variants of *Cannabis* containing various ratios of cannabidiol and cannabichromene in relation to (−)-Δ^9-*trans*-tetrahydrocannabinol.

REFERENCES

(1) E. A. Carlini, M. Santos, U. Claussen, D. Bieniek, and F. Korte, *Psychopharmacologia*, **18**, 82(1970).

(2) I. G. Karniol and E. A. Carlini, *J. Pharm. Pharmacol.*, **24**, 833(1972).

(3) I. G. Karniol and E. A. Carlini, *Psychopharmacologia*, **33**, 53(1973).

(4) L. A. Borgen and W. M. Davis, *Res. Commun. Chem. Pathol. Pharmacol.*, **7**, 663(1974).

(5) G. Jones and R. G. Pertwee, *Brit. J. Pharmacol.*, **45**, 375(1972).

(6) C. E. Turner and K. W. Hadley, A.A.A.S. and C.N.C.Y. Science and Man in the Americas: Symposium No. 12, Psychodysleptic Drugs and Addiction, Mexico City, Mexico, June 28, 1973.

(7) C. E. Turner and K. W. Hadley, 36th Annual Meeting of the Committee on Problems of Drug Dependence, Mexico City, Mexico, Mar. 10–14, 1974.

(8) C. E. Turner and K. W. Hadley, *J. Pharm. Sci.*, **62**, 251(1973).

(9) C. E. Turner, K. W. Hadley, and K. H. Davis, Jr., *Acta Pharm. Jugoslav.*, **23**, 89(1973).

(10) T. B. Vree, D. D. Breimer, C. A. M. van Ginneken, and J. M. van Rossum, *J. Pharm. Pharmacol.*, **24**, 7(1972).

(11) M. J. de Faubert Maunder, *J. Ass. Pub. Anal.*, **8**, 42(1970).

(12) Y. Gaoni and R. Mechoulam, *J. Amer. Chem. Soc.*, **93**, 217(1971).

(13) C. E. Turner and K. W. Hadley, *J. Pharm. Sci.*, **62**, 1083(1973).

(14) C. E. Turner, K. W. Hadley, J. Henry, and M. L. Mole, *ibid.*, **63**, 1872(1974).

(15) R. A. de Zeeuw, J. Wijsbeek, and T. M. Malingre, *J. Pharm. Pharmacol.*, **25**, 21(1973).

(16) E. Small and H. D. Beckstead, *Lloydia*, **36**, 144(1973).

(17) M. D. Willinsky, in "Marihuana Chemistry, Pharmacology, Metabolism and Clinical Effects," R. Mechoulam, Ed., Academic, New York, N.Y., 1973.

(18) C. E. Turner, K. W. Hadley, P. S. Fetterman, N. J. Doorenbos, M. W. Quimby, and C. Waller, *J. Pharm. Sci.*, **62**, 1601(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 31, 1973, from the *Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677*

Accepted for publication October 11, 1974.

Supported by Contract HSM-42-70-109 from the National Institute on Drug Abuse and by the Research Institute of Pharmaceutical Sciences.

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Electron-Capture GLC Determination of a New Antiarrhythmic Agent, α,α-Dimethyl-4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine, in Biological Fluids

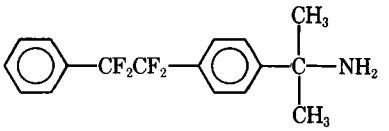
ANTHONY G. ZACCHEI [x] and LINDA WEIDNER

Abstract □ A highly specific and sensitive GLC method was developed for the analysis of α,α-dimethyl-4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine, a new orally active antiarrhythmic drug, in biological fluids. The procedure involves the addition of an internal standard, 4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine, to the plasma or urine samples followed by extraction of the drugs into benzene at pH 8. The extracted amines are converted to the trifluoroacetyl derivatives (characterized by GLC–mass spectrometry), chromatographed, and detected with a ^{63}Ni electron-capture detector. The sensitivity of the method is such that 10 ng of α,α-dimethyl-4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine/ml of plasma can be analyzed. These levels are suitable for the analysis of samples obtained following a therapeutic dose.

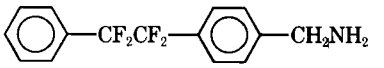
Keyphrases □ α,α-Dimethyl-4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine—electron-capture GLC determination in biological fluids □ Antiarrhythmic agents—electron-capture GLC determination of α,α-dimethyl-4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine in biological fluids □ GLC, electron capture—analysis, α,α-dimethyl-4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine in biological fluids

α,α-Dimethyl-4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine (I) is a new orally effective agent for the treatment of ventricular arrhythmias resulting from myocardial infarction (1). The compound is one of the more potent agents in preventing or modifying the ventricular arrhythmia produced in anesthetized dogs by the intracoronary injection of a sclerosing agent (2, 3). The drug was designed as a prophylactic agent and has advantages over other drugs currently employed. Studies on the physiological disposition of the compound (4) have not included a specific method of assay.

Preliminary quantitative experiments in this laboratory using colorimetric, fluorometric, and GLC (flame-ionization detection) methods lacked the sensitivity required to detect clinical levels of the drug. The use of electron-capture techniques for the assay of low levels of drugs, has been reported (5–15). How-



I



II

ever, many drugs, such as I, are not responsive to the electron-capture detector without prior derivatization to a compound that has electron-capturing properties. This report describes an extremely sensitive and specific GLC method for the determination of I using an internal standard (II), derivatization with trifluoroacetic anhydride, and an electron-capture detector.

EXPERIMENTAL

Reagents and Chemicals—The chemicals and reagents used were: α,α-dimethyl-4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine (I), the internal standard 4-($\alpha,\alpha,\beta,\beta$-tetrafluorophenethyl)benzylamine (II), pesticide grade benzene, trifluoroacetic anhydride[1], 5% ammonium hydroxide, and benzene containing 2.5% trifluoroacetic anhydride (freshly prepared).

Instrumentation—*GLC*—A gas chromatograph[2] equipped with a ^{63}Ni electron-capture detector and a 183 × 6-mm glass column containing 2% PPE-20 (polyphenyl ether) on 80–100-mesh Chromosorb W[3] (AW-DMCS) was employed for the analysis. The inlet and detector temperatures were 230° and the column temperature was 200°. Nitrogen was used as the carrier gas at a flow rate of 50 ml/min.

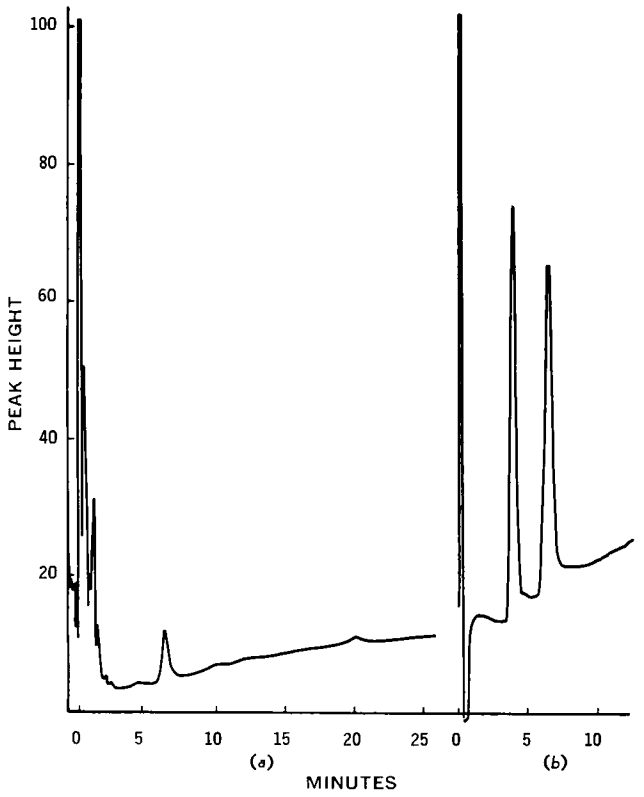


Figure 1—*Gas chromatograms of:* (a) *control dog plasma carried through extraction procedure, and* (b) *I and II as the trifluoroacetyl derivatives (500 pg of each compound). Five microliters was injected out of a total volume of 100 μl of benzene.*

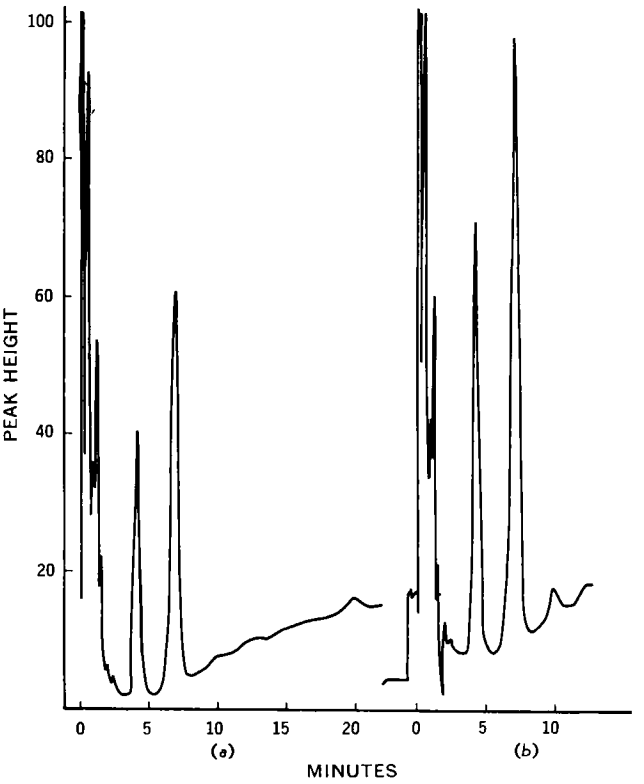


Figure 2—*Gas chromatograms of:* (a) *I and II added to control dog urine and carried through the extraction procedure, and* (b) *material obtained from a 0–24-hr dog urine sample following the administration of I. Compound II was added prior to extraction. Five microliters was injected in each case out of 100–500 μl of solvent.*

GLC–Mass Spectrometry—All mass spectra were obtained on a mass spectrometer[4] using the GLC inlet. A 183 × 5-mm glass column packed with 1% OV-17 on 80–100-mesh Supelcoport[3] and a 183 × 5-mm glass column packed with 2% PPE-20 were used. The gas chromatograph was operated isothermally at 190° with a helium flow rate of 30 ml/min. The mass spectrometer ionizing and accelerating potentials were 70 ev and 3.5 kv, respectively. The source, separator, and injection port temperatures were 270, 260, and 255°, respectively.

Measurement of I in Biological Samples—Compound I was determined in biological samples as follows. One milliliter of plasma or urine was added to a 50-ml glass-stoppered centrifuge tube containing 50 ng (calculated as the free base) of the internal standard as the hydrochloride in 1 ml of water, 1 ml of 0.2 *M* pH 8 phosphate buffer, and 25 ml of reagent grade benzene. The tube was shaken for 10 min and centrifuged, and at least 20 ml of the organic phase was transferred to a similar tube containing 5 ml of 0.1 *N* HCl. After shaking for 5 min, the tube was centrifuged and the organic phase was removed by aspiration. The pH of the aqueous phase was adjusted to greater than 8 by the addition of 0.5 ml of 1.25 *N* NaOH and 1 ml of 0.2 *M* pH 8 phosphate buffer.

The amines were extracted into 10 ml of pesticide-grade benzene. The benzene phase was transferred to a 25-ml glass-stoppered tube containing 1 ml of benzene (2.5% trifluoroacetic anhydride). The tube was then placed in a 65° warm water bath for 15 min. The tube was cooled and the contents were shaken with 1 ml of water (vortex, 1 min) and 1 ml of 5% NH_4OH. Following centrifugation, the benzene phase was transferred to a 13-ml tube for careful evaporation under nitrogen to about 100–500 μl (evaporation of the samples to dryness resulted in greater than 50% loss of detection response). Appropriate aliquots, usually 2–5 μl, were in-

[1] Aldrich Chemical Co.
[2] Packard model 7400.
[3] Supelco, Inc.
[4] LKB-9000S.

Table I—Recovery of I from Dog Plasma and Water

Amount Added, ng		Amount Recovered from Water[a]	Amount Recovered from Plasma[a]						Mean ± *SD* of Plasma Recovery
100	A	87.0	93.8	99.4	—[b]	105.6	99.4	109.2	101.5 ± 6.0
	B	87	94	99	—	106	99	109	
80	A	72.9	78.3	76.1	81.6	91.2	81.6	79.6	81.4 ± 5.2
	B	91	98	95	102	114	102	99	
60	A	60.1	59.4	66.5	70.6	77.5	68.6	—	68.5 ± 6.6
	B	100	99	111	118	129	114	—	
50	A	50.3	47.3	56.2	59.0	43.9	51.4	47.2	50.8 ± 5.8
	B	101	95	112	118	88	103	94	
30	A	30.6	32.9	35.7	35.0	37.7	35.0	—	35.3 ± 1.7
	B	102	110	119	117	125	117	—	
25	A	28.1	25	28.8	21.9	24.7	25.4	25.4	25.2 ± 0.9
	B	112	100	115	88	99	102	102	
15	A	16.6	16.6	13.7	18.5	15.1	15.8	11.9	15.3 ± 2.3
	B	111	111	91	123	101	105	79	
10	A	11.6	10.6	9.6	11.7	9.6	10.3	7.8	9.9 ± 1.3
	B	116	106	96	117	96	103	78	
5	A	10.1	3.7	3.4	5.5	5.5	4.8	3.5	4.4 ± 1.0
	B	202	74	69	110	110	96	70	

[a] Values in the A rows represent nanograms recovered; values in the B rows represent percent recovery. [b] No sample analyzed.

jected into the column. The retention times of I and II as the trifluoroacetyl derivatives were 4.0 and 6.6 min, respectively.

Standard water, urine, and plasma curves were constructed by plotting the peak height ratios (I/II) *versus* the weight ratios (I/II). The peak height ratio obtained from an unknown was then used to determine the amount of I present. The standard samples were run concurrently with the unknown samples as previously described.

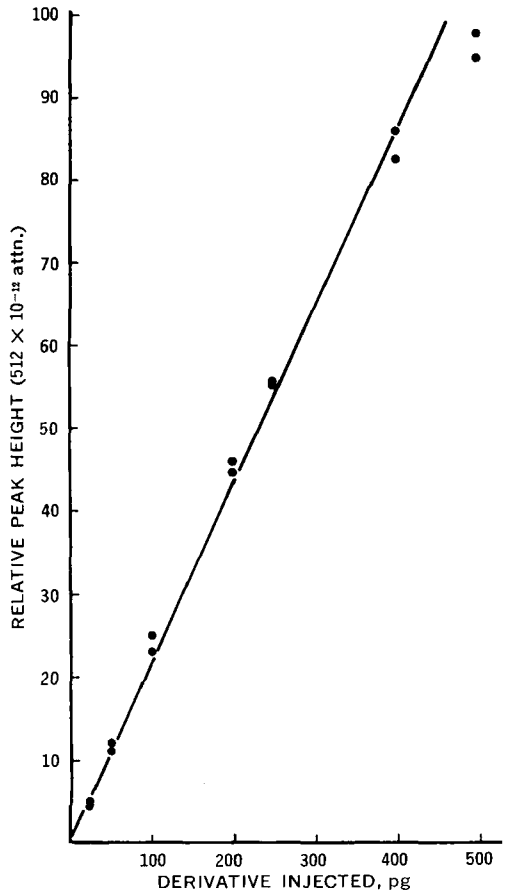


Figure 3—*Relationship between peak height and amount injected on a 2% PPE-20 column operated at 200°. Nitrogen flow rate was 50 ml/min. Five microliters was injected in duplicate in each instance.*

Absorption Studies—Four beagle dogs (7.0–10.3 kg) were administered a therapeutic dose of I (5 mg/kg) orally in a water solution. Blood samples were collected in heparinized tubes, the plasma was separated, and aliquots were removed for assay. Urine specimens were immediately frozen upon collection and remained frozen until assayed.

RESULTS AND DISCUSSION

A number of reagents (trifluoroacetic anhydride, heptafluorobutyric anhydride, *N*-trifluoroacetylimidazole, and pentafluoropropionic anhydride) were examined as potential derivatizing agents to act as electron-capturing groups to facilitate compound detection. Exploratory studies with I and three related analogs, 4-

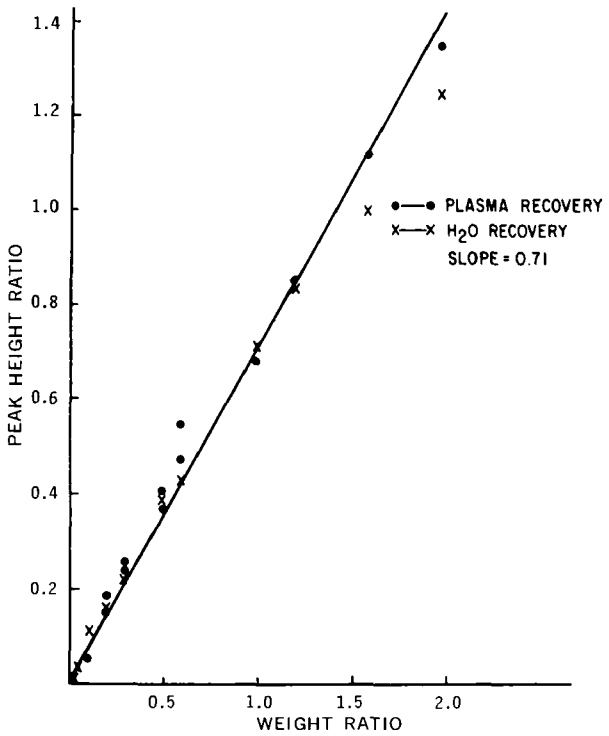


Figure 4—*Relationship between peak height ratio of the derivatives (I/II) and weight ratio (I/II). Five microliters was injected in each case out of 100–500 μl following appropriate recovery (water or plasma).*

Table II—Recovery of I from Human Urine

Amount Added, ng		Amount Recovered[a]							Mean ± *SD*
400	A	410	378	375	365	363	401	395	383 ± 18
	B	102	95	94	91	91	100	99	
200	A	212	228	208	202	201	—	196	207 ± 11
	B	106	114	104	101	101	—	98	
100	A	91	88	83	104	100	99	109	96 ± 9
	B	91	88	83	104	100	99	109	
50	A	43	47	54	57	54	39	60	50 ± 7
	B	86	94	108	114	108	78	120	
25	A	17	24	19	22	19	10	19	19 ± 4
	B	86	96	76	88	76	40	76	
12.5	A	10	13	9	10	11	14	11	11 ± 2
	B	80	104	72	80	88	112	88	
6.2	A	6	4	3	5	6	2	4	4 ± 1
	B	96	64	48	80	96	32	64	

[a] Values in the A rows represent nanograms recovered; values in the B rows represent percent recovery. Each value represents a separate recovery analysis.

(α,α,β,β-tetrafluorophenethyl)benzylamine, α,α-dimethyl-*N*-methyl-4-(α,α,β,β-tetrafluorophenethyl)benzylamine, and α-methyl-4-(α,α,β,β-tetrafluorophenethyl)benzylamine, led to the selection of trifluoroacetic anhydride and heptafluorobutyric anhydride as the best derivatizing reagents and 4-(α,α,β,β-tetrafluorophenethyl)benzylamine (II) as the best internal standard. Trial runs with each of the selected derivatives on a series of 183 × 6-mm columns packed with 1% SE-30, 1% OV-17, 1% QF-1, or 2% PPE-20 showed that the 2% PPE-20 column gave the highest resolution and separation of the I derivative from the internal standard (II) derivative and interfering peaks. The formation of each trifluoroacetyl derivative was confirmed by GLC–mass spectrometry.

Figure 1 presents gas chromatograms of the following samples: *(a)* control dog plasma carried through the procedure, and *(b)* I and II as the trifluoroacetyl derivatives (~500 pg of each compound injected directly into column). Figure 2 presents gas chromatograms of: *(a)* I and II added to control dog urine and carried through the extraction procedure, and *(b)* material obtained from a 0–24-hr dog urine sample. Compound II was added prior to extraction. As stated previously, the trifluoroacetyl derivatives of I and II had retention times of 4.0 and 6.6 min, respectively. As indicated in Fig. 1*a*, at a column temperature of 200°, the control plasma sample did give a very small peak which eluted (6.2 min) just prior to the internal standard peak (6.6 min). This peak did not present any problem in the analysis and could be separated from the peak of II by a slight decrease (4–5°) in column temperature. Several plasma and urine samples were adjusted to pH 6 prior to the initial extraction to remove or minimize endogenous peaks present. A linear relationship is observed when peak height is plotted *versus* amount injected (Fig. 3). Figure 4 presents a standard ratio curve obtained after various amounts of I (5–100 ng) and internal standard (50 ng) were carried through the entire procedure.

A summary of the recovery results of I obtained with dog plasma is presented in Table I. A similar set of recovery data from human urine is presented in Table II. Recoveries of I added to dog plasma

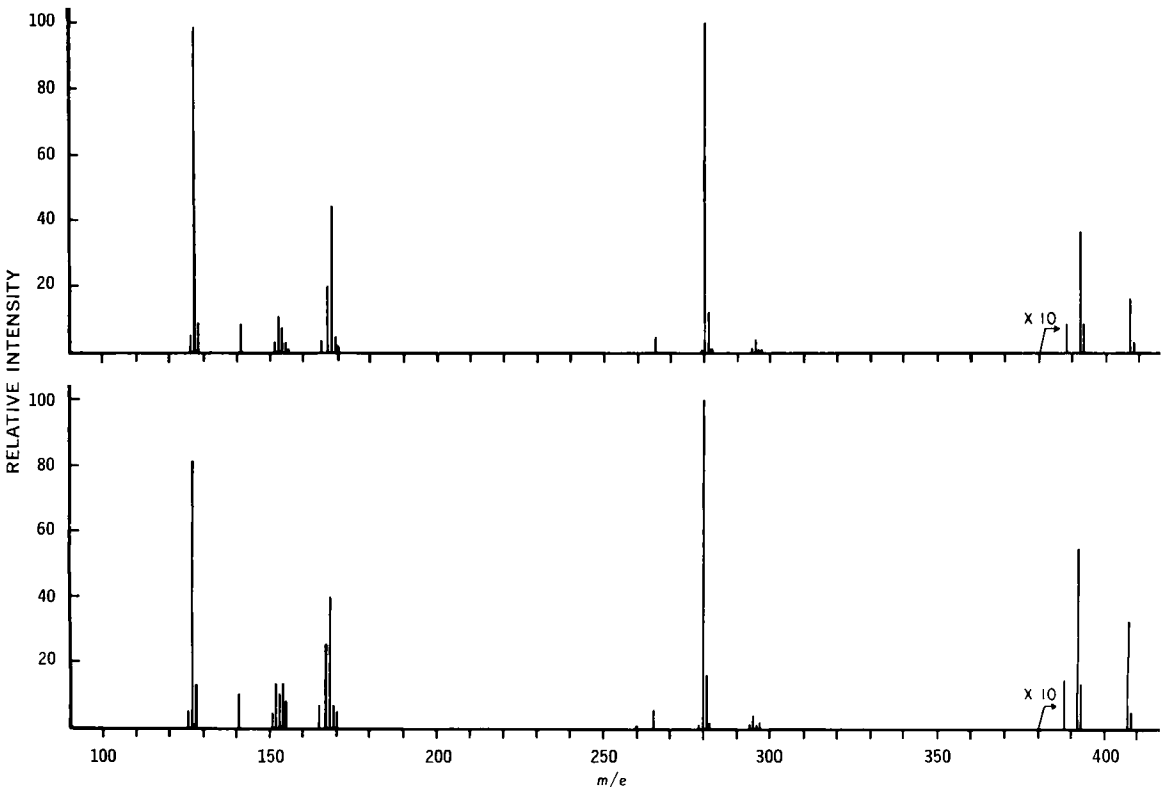


Figure 5—*Mass spectra of the trifluoroacetyl derivative of I (top) and the pooled material obtained from human plasma (bottom).*

Table III—Plasma Levels of I following Administration of I to Dogs at a Dose of 5 mg/kg

Hours	Nanograms of I per Milliliter of Plasma			
	Dog 1	Dog 2	Dog 3	Dog 4
0.25	40	10	26	23
0.5	81	21	61	37
0.75	103	30	87	65
1.0	108	33	88	93
1.5	99	38	96	85
2	98	58	95	86
3	81	60	96	78
4	78	58	82	66
6	59	55	83	57
24	28	18	23	23
30	12	12	15	17
48	—[a]	12	12	—[a]

[a] Less than 10 ng of I/ml.

in amounts of 5–100 ng ranged from 70 to 125% for analyses performed over several months. The mean recovery was 102.5 ± 13.5% over the entire concentration range. Recoveries of I added to human urine in amounts of 12.5–400 ng ranged from 72 to 114% for analyses performed over several months. The mean recovery was 93.7 ± 14.7% over the entire concentration range. Similar results were obtained when 100 ng of II was employed in the typical recovery studies. As evident, the GLC procedure for I in biological fluids is extremely sensitive and exhibits a high degree of accuracy and precision.

Typical I levels in dog plasma are seen in Table III. The plasma half-life of I in dogs has been shown to be 12–18 hr from these data and other data generated in these laboratories.

Confirmation of specificity of analysis was obtained when a pooled representative sample of several biological specimens was analyzed by combined GLC–mass spectrometry using the described technique. Figure 5 presents a comparison of the mass spectrum of the standard trifluoroacetyl derivative of I carried through the procedure with the material isolated from biological origin. The mass spectra were essentially identical in all respects for masses above 100. Below mass 100, small noncharacteristic peaks were seen at most masses in the spectrum of the isolated material.

A summary of the fragmentation pattern is presented in Table IV. The molecular ion (*m/e* 407) of the trifluoroacetyl derivative of I represents ~2% of the intensity of the base peak (*m/e* 280), which results from cleavage of the CF_2—CF_2 bond.

Studies have shown that caution must be exercised when the samples are evaporated. Samples should not be allowed to go to dryness since great losses (>50%) are encountered when this occurs. It is essential that an internal standard be carried through the entire procedure. There was no observable degradation of the trifluoroacetyl derivatives of either I or II when kept in solution (50–200 ng/ml) over a 2-week period.

CONCLUSION

A sensitive and accurate GLC method is presented for the detection of low levels of the new antiarrhythmic agent using derivatization and electron-capture techniques. The use of an internal standard eliminates any errors in quantitation as a result of sample manipulation (16). Although the electron-capture technique has proved to be extremely sensitive (can detect 10 pg of the trifluoroacetyl derivative of I when injected directly into the column), the amount of substance required for analysis is many times greater than the minimal detectable quantity since only a small portion of the total sample is usually injected into the chromatograph for analysis. The sensitivity of this method is such that 5–10 ng of material can be detected in 1 ml of plasma. GLC–mass spectrometric results from various pooled samples confirmed the specificity and identity of I.

Table IV—Summary of GLC–Mass Spectrometry Data on I and the Trifluoroacetyl Derivative

Fragmentation	Characteristic *m/e* Values	
	I	I-Derivative
M^+	—	407
$M^+ - 1$ $(-H)$	310	—
$M^+ - 15$ $(-CH_3)$	296[a]	392
$M^+ - 19$ $(-F)$	292	388
$M^+ - 34$ $(-CH_3; -F)$	277	—
$M^+ - 69$ $(-CF_3)$	—	338
$M^+ - 127$ $(-C_6H_5CF_2)$	184	280[a]
$M^+ - 127 - 15$ $(-C_6H_5CF_2; -CH_3)$	169	265
$M^+ - 184$ $[-CF_2C_6H_5C(CH_3)_2NH_2]$	127	—
$M^+ - 112$ $(-NHCOCF_3)$	—	295

[a] Base peak; retention time for I was 3.3 min and retention time for the derivative was 4.0 min under the GC–mass spectrometry operating conditions (refer to *Experimental* section).

REFERENCES

(1) M. E. Christy, C. D. Colton, M. Mackay, W. Staas, J. Wong, and E. Englehardt, 167th National American Chemical Society Meeting, Los Angeles, Calif., Apr. 1–5, 1974, Medicinal Chemistry Division Paper No. 40.

(2) M. L. Torchiana and C. A. Stone, *Fed. Proc.*, **33,** 475(1974).

(3) M. L. Torchiana and C. A. Stone, *Pharmacologist*, **12,** 304(1970).

(4) A. G. Zacchei and L. Weidner, *Fed. Proc.*, **33,** 475(1974).

(5) J. W. Blake, R. Huffman, J. Noonan, and R. Ray, *Amer. Lab.*, **5,** 63(1973).

(6) S. H. Curry, *Anal. Chem.*, **40,** 1251(1968).

(7) H. Ehrsson and B. Mellstrom, *Acta Pharm. Suecica*, **9,** 107(1972).

(8) P. Hartvig, H. Sundin, and J. Vessman, *ibid.*, **9,** 269(1972).

(9) T. Walle and H. Ehrsson, *ibid.*, **8,** 27(1971).

(10) G. P. Beharrell, D. M. Hailey, and M. K. McLaurin, *J. Chromatogr.*, **70,** 45(1972).

(11) I. A. Zingales, *ibid.*, **61,** 237(1971).

(12) C. P. Talley, N. R. Trenner, G. V. Downing, Jr., and W. J. A. VandenHeuvel, *Anal. Chem.*, **43,** 1379(1971).

(13) A. Brachet-Liermain, L. Ferrus, and J. Caroff, *J. Chromatogr. Sci.*, **9,** 49(1971).

(14) A. C. Moffat and E. C. Horning, *Anal. Lett.*, **3,** 205(1970).

(15) D. J. Edwards and K. Blau, *Anal. Biochem.*, **45,** 387(1972).

(16) H. M. McNair and E. J. Bonelli, "Basic Gas Chromatograph," 5th ed., Varian Aerograph, Palo Alto, Calif., 1969, pp. 150, 151.

ACKNOWLEDGMENTS AND ADDRESSES

Received May 1, 1974, from the *Merck Institute for Therapeutic Research, West Point, PA 19486*

Accepted for publication November 8, 1974.

The authors thank Dr. Marcia Christy for supplies of several of the analogs of α,α-dimethyl-4-(α,α,β,β-tetrafluorophenethyl)-benzylamine.

[x] To whom inquiries should be directed.