Electron-Capture Sensitivity Comparison of Various Derivatives of Primary and Secondary Amines

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Abstract □ A comparative electron-capture sensitivity study was performed with various derivatives (*e.g.*, heptafluorobutyramide, pentafluorobenzamide, and pentafluorobenzylidine) of some clinically important primary and secondary amines. Generally the order of electron-capture sensitivity for primary amines was: pentafluorobenzamide > pentafluorobenzylidine > heptafluorobutyramide, and for pentafluorobenzamides the primary amines were greater than secondary amines. Reduction of the C=O or C=N group in pentafluorobenzamide and pentafluorobenzylidine, respectively, led to a diminished response. In general, the order of sensitivity was the same but the response using a tritium detector was 10–20-fold greater than with a nickel-63 detector. The data are consistent with the theory that an electron-deficient system is necessary for good electron-capture response and that this response is also sensitive to the structure of the amine. The results further illuminate the electron-capture mechanism and suggest a more rational approach to the choice of a derivatizing agent for the determination of a particular amine.

Keyphrases □ Amines, primary and secondary—comparative electron-capture sensitivity with various derivatives □ Electron-capture detection—comparison of various derivatives of primary and secondary amines □ GLC and electron-capture detection—analysis, microquantities of primary and secondary amines

Advances in the microquantitation of drugs in biological fluids have paralleled the development of extremely sensitive and selective methods of estimation, such as GLC coupled with an electron-capture detector. In general, compounds of biological interest do not show a large response in electron-capture detector. However, it is a common practice, particularly with primary and secondary amines, to derivatize them with agents containing one or more halogen atoms or nitro groups. Halogenated reagents have included chloroacetic, trichloroacetic, heptafluorobutyric, and pentafluoropropionic acid chlorides or anhydrides (1–6). Pentafluorobenzoyl chloride also has been advocated (7, 8), while 2,4-dinitrofluorobenzene has been used as a reagent in the determination of primary and secondary amines by electron-capture detection (9, 10).

Since clinically useful amines are generally administered in small doses and are only present in trace amounts in biological fluids, any analytical procedure that is both highly sensitive and selective is very desirable. In the present study, some common derivatives of amines were examined for their GLC behavior and electron-capture response. The findings led to further enlightenment of the electron-capture process and suggested a more rational approach toward the choice of a derivatizing agent for estimation of a primary and secondary amine in biological fluids.

EXPERIMENTAL

Reagents—Pentafluorobenzoyl chloride, heptafluorobutyric anhydride, pentafluorobenzyl bromide, pentafluorobenzaldehyde, and heptafluorobutylaldehyde ethylhemiacetal were used[1]. All solvents were spectroscopic grade and distilled when necessary.

Apparatus—The GLC work was performed on either a Varian 600-D gas chromatograph with a tritium foil electron-capture detector or on a 1200 Varian chromatograph with a nickel-63 electron-capture detector. Glass columns, 1.8 m., 0.3 cm. (6 ft., 0.125 in.) o.d., packed with either 3% OV-17 or OV-225 coated onto Chromosorb W AW DMCS HP (100–120 mesh), were used with a nitrogen flow of 30 ml./min. Columns were conditioned for 48 hr. at 280°, the first 24 hr. without nitrogen flow.

Measurement of Electron-Capture Response—The amount of each derivative injected onto the column was adjusted so that the response did not exceed 30% of the standing current. Owing to overlapping as well as widely varying retention times, quantitative mixtures of different derivatives were prepared. Because the response to amphetamine pentafluorobenzamide when chromatographed at various temperatures (165–200°) was found to differ by no more than 5%, it was employed as a reference standard in each mixture. Corrections could then be made for variations in the quantity of derivative injected. All derivatives examined gave symmetrical peaks. The area under a peak was calculated as the product of peak height and width at half peak height. By knowing the chart speed and the current for full-scale deflection at the amplifier sensitivity setting used, area measurements were converted to coulombs. Response was expressed as k (thousand) coulombs/mole injected.

Detector Temperature—The Varian 600-D detector is externally placed and heated with a constant-output heating pad. While the detector temperature was reasonably constant, the potential problem of variation with oven temperature was considered since electron-capture response of a compound could be temperature dependent (2). When monitored, by placing a thermistor into the tritium detector, it was found to change only from 180 to 195° when the oven temperature was raised from 150 to 230°. In the Varian 1200 instrument, the detector temperature is independently controlled and was maintained at 320°.

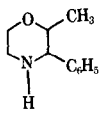
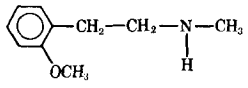
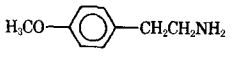
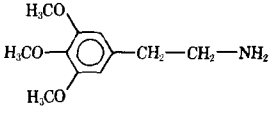
Synthesis of Derivatives—N-*Pentafluorobenzamides*—The hydrochloride or sulfate salt of the respective amine (0.5 g.) was dissolved in 25 ml. water, and 10% sodium hydroxide solution was added until the pH was raised to 10–12; the basic solution was extracted with 100 ml. methylene chloride. The organic layer was dried and evaporated to provide the amine. Pentafluorobenzoyl chloride (0.3–0.4 g.) was added to the amine (0.1–0.2 g.) in 10 ml. of 2.5 *N* NaOH and shaken vigorously for 5 min. The product, generally solid, was filtered and recrystallized from 90% ethanol. When the reaction product was initially an oil, it was extracted from the basic solution with methylene chloride. Upon evaporation of methylene chloride, the residue was dissolved in hot 90% ethanol which, on cooling, always yielded a crystalline product.

N-*Heptafluorobutyramide*—Heptafluorobutyric anhydride, 0.5 ml., was added to the amine (0.2–0.3 g.) and isolated by the general procedure already described; then the mixture was shaken. Usually the reaction proceeded vigorously; otherwise, it was heated for 10 min. on a steam bath. Sufficient 5% sodium hydroxide was then added to render the solution basic. Most derivatives of primary amines yielded a solid product, which was recrystallized from 90% ethanol. Amides of secondary amines were liquids and were vacuum distilled at 60–90° at 100–200 μ Hg.

N-*Pentafluorobenzylidine (Schiff Base)*—Pentafluorobenzaldehyde (0.2 ml.) in 1.0 ml. acetonitrile was added to a solution of amine (0.1–0.2 g.) in acetonitrile (1 ml.). This mixture was heated at 60° for 1 hr., and the acetonitrile was removed under reduced pressure. The reaction product was distilled under reduced pressure.

[1] Obtained from Pierce Chemical Co.

Table I—Physical Constants of the Heptafluorobutyramide and Pentafluorobenzamide Derivatives of Various Amines

Compound	Structure	Molecular Formula	*N*-Heptafluorobutyramide: Melting Point	Analysis, % Calc.	Found	RT[a], OV-17	*N*-Pentafluorobenzamide: Melting Point	Analysis, % Calc.	Found	RT[b] OV-17	RT[b] OV-225
Amphetamine	$C_6H_5—CH_2—\underset{\displaystyle CH_3}{\underset{\vert}{CH}}—NH_2$	$C_9H_{13}N$	73–74°	C 47.12 H 3.62 N 4.22	47.17 3.84 4.01	1.62(1.00[c])	112–113°	C 58.40 H 3.60 N 4.30	58.21 3.66 4.18	6.4(1.00[c])	6.9(1.00[c])
β-Phenethylamine	$C_6H_5—CH_2—CH_2—NH_2$	$C_8H_{11}N$	56–57°	C 45.42 H 3.15 N 4.41	45.81 2.98 4.42	1.75(1.07)	109–111°	C 57.10 H 3.20 N 4.40	57.17 3.46 4.24	7.3(1.15)	8.7(1.25)
Methamphetamine	$C_6H_5—CH_2—\underset{\displaystyle CH_3}{\underset{\vert}{CH}}—\underset{\displaystyle H}{\underset{\vert}{N}}—CH_3$	$C_{10}H_{15}N$	—[d]	C 48.69 H 4.08 N 4.05	48.75 4.14 3.90	2.75(1.69)	96–97°	C 59.5 H 4.1 N 4.1	59.46 4.17 3.99	6.4(1.0)	5.8(0.83)
α-Methylbenzylamine	$C_6H_5—\underset{\displaystyle CH_3}{\underset{\vert}{CH}}—NH_2$	$C_8H_{11}N$	89–90°	C 45.42 H 3.15 N 4.41	45.59 3.10 4.59	1.06(0.65)	93–94°	C 57.1 H 3.2 N 4.4	57.16 3.41 4.33	5.1(0.80)	5.0(0.72)
Phentermine	$C_6H_5—CH_2—\underset{\displaystyle CH_3}{\underset{\vert}{\overset{\displaystyle CH_3}{\overset{\vert}{C}}}}—NH_2$	$C_{10}H_{15}N$	—[d]	C 48.7 H 4.08 N 4.06	48.52 4.09 3.98	1.39(0.86)	120–121°	C 59.5 H 4.1 N 4.1	59.65 4.17 4.12	5.9(0.92)	—
Mephentermine	$C_6H_5—CH_2—\underset{\displaystyle H_3C_2}{\underset{\vert}{\overset{\displaystyle CH_3}{\overset{\vert}{C}}}}—\underset{\displaystyle H}{\underset{\vert}{N}}—CH_3$	$C_{11}H_{17}N$	—[d]	C 50.14 H 4.48 N 3.9	50.02 4.52 3.72	1.69(1.04)	94–96°	C 60.5 H 4.4 N 3.9	60.63 4.76 3.75	7.8(1.22)	—
Phenmetrazine		$C_{11}H_{17}N$	—[d]	C 48.26 H 3.78 N 3.75	48.05 3.90 3.57	5.91(3.65)	138–139°	C 58.2 H 3.8 N 3.8	58.50 4.02 3.80	15.1(2.37)	19.1(2.75)
Methoxyphenamine		$C_{11}H_{17}NO$	—[d]	C 48.0 H 4.3 N 3.73	47.88 4.41 3.86	6.06(3.73)	101–104°	C 57.9 H 4.3 N 3.7	57.83 4.12 3.86	11.0(1.72)	11.0(1.58)
p-Methoxyphenethylamine		$C_9H_{13}NO$	75–77°	C 44.95 H 3.45 N 4.03	45.25 3.34 4.12	5.56(3.4)	134–135°	C 55.7 H 3.5 N 4.0	55.68 3.62 4.26	18.7(2.88)	—
Mescaline		$C_{11}H_{17}NO_3$	86–88°	C 44.22 H 3.93 N 3.43	44.46 3.80 3.60	25.50(15.7)	108–109°	C 53.5 H 4.0 N 3.4	54.29 4.53 3.39	37.2(5.34)	37.2(5.38)

[a] Retention time (minutes); oven temperature 164°. [b] Retention time (minutes); oven temperature 200°. [c] Figures in parentheses refer to relative retention time compared to the corresponding amphetamine derivative. [d] Liquid at room temperature.

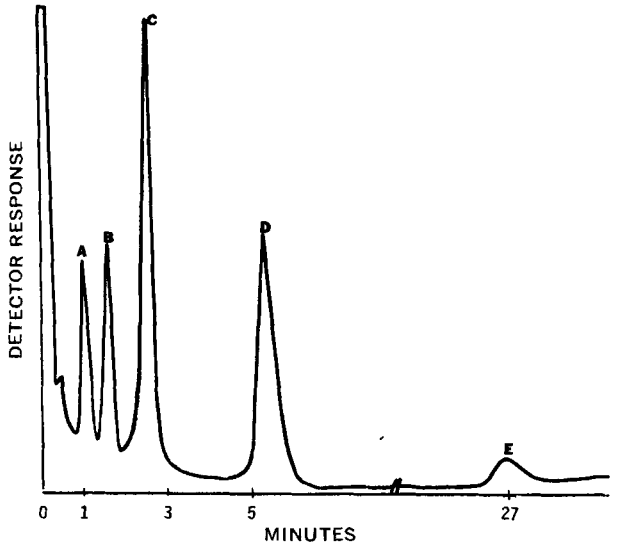


Figure 1—*Chromatogram showing comparative electron-capture sensitivity of α-methylbenzylamine heptafluorobutyramide (A, 0.6 ng.), amphetamine heptafluorobutyramide (B, 1.0 ng.), methamphetamine heptafluorobutyramide (C, 1.5 ng.), methoxyphenamine heptafluorobutyramide (D, 5 ng.), and α-methylbenzylamine pentafluorobenzamide (E, 0.01 ng.). Column temperature, 164°; detector sensitivity, 1 × 16; nitrogen flow, 25 ml./min.; and column packing, 3% OV-17.*

N-*Pentafluorobenzyl Amphetamine*—Pentafluorobenzyl bromide was added to amphetamine in benzene. The solution was briefly heated on a steam bath and then shaken for 5 min. with 0.5 *N* NaOH (2 ml.). A white precipitate was filtered and recrystallized from 95% ethanol.

Amphetamine N-*Heptafluorobutyrylidine*—Heptafluorobutyraldehyde ethylhemiacetal was added dropwise to polyphosphoric acid preheated to 170–180°. The aldehyde so generated was distilled and collected in dry benzene. This solution was mixed and refluxed with a benzene solution of amphetamine. The azeotrope was trapped in a Dean-Stark tube. After completion of the reaction, benzene was removed under reduced pressure and the Schiff base was distilled at 150 μ Hg at 70–75°.

N-*Heptafluoropentyl Amphetamine*—The above-mentioned Schiff base was reduced by aluminum hydride to the corresponding alkyl compound. The fluorinated alkylamine was distilled at 45° and 200 μ Hg.

Amphetamine N-p-*Nitrobenzamide*—The procedure for the preparation of pentafluorobenzamides was employed, except that *p*-nitrobenzoyl chloride was used. The white solid precipitate so formed was recrystallized from ethanol.

Amphetamine N-*2,4-Dinitrobenzene*—2,4-Dinitrofluorobenzene, in 5 ml. ether, was mixed with 135 mg. of amphetamine in 5 ml. ether. A yellow crystalline solid, obtained after 15 min. at room temperature, was recrystallized from petroleum ether.

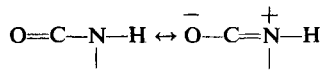
IR—Absorption frequencies were measured using 2% solutions in carbon tetrachloride. However, due to solubility limitations, only 0.5% solutions were used for ring methoxy compounds.

RESULTS AND DISCUSSION

The elemental analyses and retention times of the pentafluorobenzamide and heptafluorobutyramide derivatives of various amines are listed in Table I.

GLC Performance—The GLC performance of the derivatives was tested on OV-17, and some were also examined on OV-225 (Table I). All derivatives exhibited excellent GC properties (Figs. 1 and 2). These phases separated most derivatives; *e.g.*, amphetamine and methamphetamine pentafluorobenzamides gave the same retention time on OV-17 but were completely resolved on OV-225 (Table I). These phases can be operated at high temperatures with very low bleed and produce stable baselines even at high sensitivity settings. As little as 5 picograms of amphetamine pentafluorobenzamide could be reproducibly measured. Walle (11) proposed Carbowax 20M as an alternative stationary phase. The retention times of heptafluorobutyramides, at a 30° lower oven temperature, were comparable to the corresponding pentafluorobenzamide. With a high molecular weight amine, formation of the former derivative may avoid use of the nickel-63 detector which, while possessing a higher operating temperature than the tritium detector, is less sensitive and has a shorter linear dynamic response range. Alternatively, the retention time of a derivative may be sufficiently reduced, by increasing carrier gas flow or decreasing the liquid phase, to enable oven temperatures to be used compatible with the tritium detector.

Structure and Electron-Capture Response—*Significance of C=O Group*—To explain differences in the electron-capture response of haloacetates, Landowne and Lipsky (12) proposed that the carbonyl carbon is responsible for the initial electron capture. In contrast, Clarke *et al.* (1), observing that the trifluoroacetamides possess very much lower sensitivity than the corresponding heptafluorobutyramides, proposed that electron capture occurred in the perfluoroalkyl chain. To improve understanding of the electron-capture mechanism, several derivatives of amphetamine, some lacking an amido group, were prepared and examined (Table II). Reduction of the carbonyl group of amphetamine heptafluorobutyramide to the corresponding *N*-heptafluorobutyl derivative resulted in a markedly lower response, while formation of the Schiff base, which contains the polarizable C=N group, produced little loss of sensitivity. Similar trends are seen in the perfluorobenzyl derivatives. Also, amphetamine *N-p*-nitrobenzamide is fourfold more sensitive than the 2,4-dinitrophenyl derivative. These observations tend to favor the hypothesis that electron capture occurs primarily at the carbonyl group rather than the side chain. Moreover, any amide group, in which C=O and C=N can be present simultaneously due to the resonance forms (Scheme I), provides a good-to-excellent electron-



Scheme I

capture region (electrophore). However, the final electron-capture response of the molecule is determined by secondary stabilizing processes after the initial electron capture.

Heptafluorobutyramides—The electron-capture response of heptafluorobutyramides differed considerably (Table III), confirming the observation of Walle (11). A comparison of secondary and tertiary heptafluorobutyramides formed from primary and secondary amines, respectively, indicates that *N*-methylation enhances the

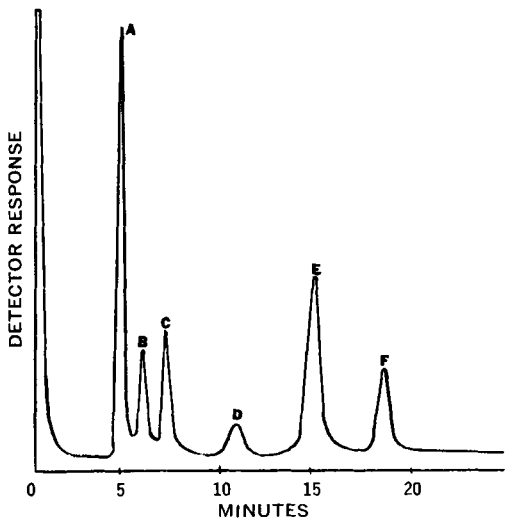
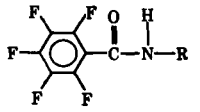
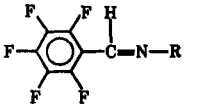
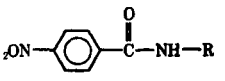
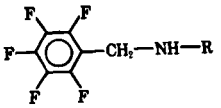
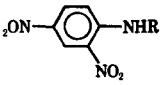
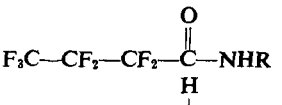
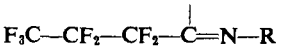
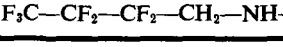


Figure 2—*Chromatogram showing comparative electron-capture sensitivity of pentafluorobenzamides of α-methylbenzylamine (A, 166 picograms), methamphetamine (B, 5 ng.), mephentermine (C, 5 ng.), methoxyphenamine (D, 5 ng.), phenmetrazine (E, 5 ng.), and p-methoxyphenethylamine (F, 5 ng.). Column temperature, 200°; detector sensitivity, 1 × 16; nitrogen flow rate, 25 ml./min.; and column packing, 3% OV-17.*

Table II—Electron-Capture Sensitivity and Retention Times of Various Amphetamine Derivatives

Compound	Structure[a]	Response, Coulombs × 10^3/mole	Retention Time[b], min.	Column Temperature
N-Pentafluorobenzamide		225	6.4	200°
N-Pentafluorobenzylidine		43	2.32	160°
N-*p*-Nitrobenzamide		11	30.0	205°
N-Pentafluorobenzylamine		3.7	3.76	160°
N-2,4-Dinitroaniline		2.8	16	200°
N-Heptafluorobutyramide		2.2	1.62	164°
N-Heptafluorobutyrylidine		2.1	0.6	116°
N-Heptafluorobutylamine		0.41	0.75	116°

[a] R = $C_6H_5CH_2CH(CH_3)$. [b] Chromatographed on OV-17.

response—*viz.*, methamphetamine > amphetamine and mephentermine > phentermine (Table III). Similar increases after *N*-alkylation were noted by Bruce and Maynard (3) and Walle (11). These findings agree with the proposal since tertiary amides, which possess a higher contribution from resonating forms than secondary amides (13, 14), can provide a better area for electron attachment (*cf.*, forms 3 and 4 of Scheme II). Furthermore, it is expected that any steric crowding around the amide group will, through reduced O—C—N coplanarity, decrease resonance and thereby diminish response. The increasing response with decreasing carbonyl frequency, reflecting greater resonance of the amide group, supports this hypothesis (Table III).

Introduction of either an *ortho*- or *para*-methoxy group into the aromatic ring decreases electron-capture response—*viz.*, methamphetamine > methoxyphenamine and β-phenethylamine > *p*-methoxyphenethylamine. Similar effects were observed by Walle (11). Evidently *ortho*- and *para*-methoxy substitution produces negatively charged centers in the molecule *via* resonance, which decreases the capacity of the derivative to capture electrons. However, 3,4,5-trimethoxy-β-phenethylamine (mescaline) shows a response

Table III—Electron-Capture Response of Various Heptafluorobutyramides

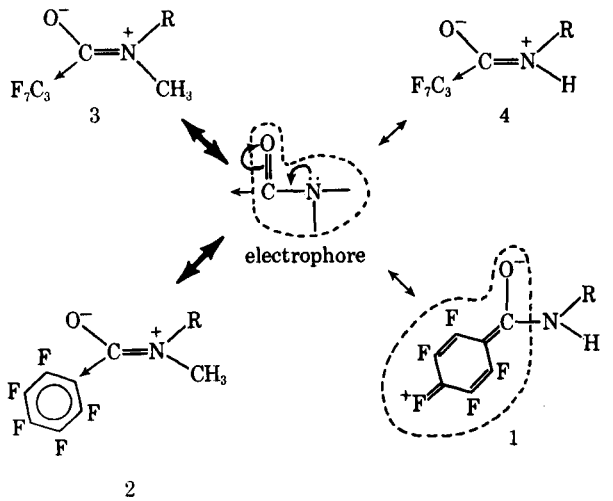
Amine	Response, Coulombs × 10^3/mole	$\nu_{C=O}$, $cm.^{-1}$	Comments
β-Phenethylamine	2.0	1755	Lower contribution from form 4 (Scheme II); reduced steric crowding around amide nitrogen allows greater rotation around C—N amide bond
Amphetamine	2.2	1748	Increased contribution from form 4 (Scheme II); increased steric crowding around amide nitrogen reduces rotation around C—N amide bond
α-Methylbenzylamine	2.5	1740	Highest contribution from form 4 (Scheme II) favored by rigid crowding around amide nitrogen
p-Methoxyphenethylamine	1.9	1750	See *Results and Discussion*
Mescaline	3.7	1748	See *Results and Discussion*
Phentermine	1.1	1750	Lower contribution from form 4 (Scheme II); high energy required to form (C=N) planar structure
Phenmetrazine	8.8	1650	Higher contribution from form 3 (Scheme II); *N*-alkylation combined with formation of ring structure around amide bond
Mephentermine	2.2	1700	Lower contribution from form 3 (Scheme II) due to steric Interaction between *N*-methyl and α-methyl groups
Methamphetamine	4.0	1690	Substantial contribution from form 3 (Scheme II) due to electron-donating effect of *N*-methyl group, although somewhat reduced by steric crowding around amide bond
Methoxyphenamine	1.6	1695	See *Results and Discussion*

Table IV—Electron-Capture Response of Various Pentafluorobenzamides

Amine	Response, Coulombs × 10^3/mole	$\nu_{C=O}$, cm.$^{-1}$	Comments
β-Phenethylamine	300.0	1710	Increasing contribution from form 1 (Scheme II); coplanarity between C=O and pentafluorobenzene ring decreases amide resonance
Amphetamine	225.0	1700	Decreasing contribution from form 1 (Scheme II); α-substitution sterically hinders coplanarity between C=O and pentafluorobenzene ring
α-Methylbenzylamine	180.0	1700	Further decrease in contribution from form 1 (Scheme II); further steric hindrance aids amide resonance
p-Methoxyphenethylamine	135.0	1705	See *Results and Discussion*
Mescaline	155.0	1750	See *Results and Discussion*
Phentermine	29.0	1700	Steric crowding prohibits attainment of planar structures, form 1 or 2 (Scheme II); probability of electron capture and secondary stabilization is reduced
Phenmetrazine	28.0	1680	Higher contribution from form 2 (Scheme II), but *N*-alkylation combined with the formation of ring structure around amide bond reduces secondary stabilization
Mephentermine	9.2	1690	Even greater contribution from form 2 (Scheme II) due to steric interactions between *N*-methyl and α-methyl groups
Methamphetamine	6.1	1680	Form 2 (Scheme II) favored; similar effect as in mephentermine but with higher amide resonance (less steric crowding)
Methoxyphenamine	3.2	1675	See *Results and Discussion*

higher than β-phenethylamine. In mescaline, the close proximity of the three methoxy groups prevents the *para*-methoxy group from becoming coplanar with the aromatic ring, thereby diminishing electron donation into the aromatic ring *via* resonance. In addition, the inductive effect of the two *meta*-methoxy groups may allow the aromatic ring to act as another site for electron capture. An additional site for electron capture can also explain the high electron-capture response of fenfluramine heptafluorobutyramide observed by Bruce and Maynard (3).

According to the foregoing explanations, removal of the nitrogen atom of the amide group out of the plane of the carbonyl group decreases resonance and electron-capture response. However, the very low electron-capture sensitivity of trifluoroacetamide and the very high electron-capture response of the trifluoroacetates require additional explanation. Bellamy and Williams (15) reported that in the α-halogenated amides, the most stable steric arrangement of the amide group in the gaseous state is determined by the electrostatic repulsion between the halogen and the carbonyl oxygen. In trifluoroacetamides, this electrostatic repulsion cannot be reduced in any of the *gauche* forms. Consequently, the amide group will not resonate, resulting in an associated diminished electron-captur activity. Furthermore, if an electron did enter into the C=O group, the resultant charged form would be very unstable due to the electrostatic repulsion. The situation with trifluoroacetamides no longer prevails in the pentafluoropropionamides or the heptafluorobutyramides where the carbonyl carbon can exist *gauche* to the two α-halogens and *cis* to the relatively positive carbon (14), thus allowing resonance to occur which leads to the increased electron-capture activity. The very high response of the trifluoroacetates could readily result from the formation of a trifluoroacetate ion which promotes the secondary stabilization process.



Scheme II—Diagram summarizing the influence of resonance on electron-capture response. A heavier double-headed arrow indicates greater resonance between resonating forms. It is assumed that the carbonyl carbon acts as the initial site of electron acceptance and that subsequent stabilization determines the final electron-capture response. Generally, electron-capture sensitivity increases from 1 > 2 > 3 > 4. See text and tables for further explanation.

Pentafluorobenzamides—If electron capture occurs at the carbonyl carbon of a resonating amide group, then replacement of the nonresonating heptafluorobutyl side chain by a very highly electron-withdrawing resonating moiety, such as the pentafluorophenyl group, should enhance the electron-capture response. Generally, the results confirmed the predictions.

Pentafluorobenzamides always exhibited greater electron-capture sensitivity than the corresponding heptafluorobutyramide (Tables III and IV) when derived from primary amines. This increase was 60–200-fold (Table IV). While the initial electron attachment at the carbonyl group remains the same in the two derivatives, the highly electronegative pentafluorophenyl ring can resonate with the carbonyl group to provide a coplanar, highly electron-delocalized system, which acts as an excellent electrophore (form 1, Scheme II). Also, once accepted, an electron can be stabilized in the pentafluorobenzene ring or by the molecule as a whole in the excited state through a nondissociative electron-capture process (16, 17). Such a stabilization is far less possible in the heptafluorobutyl group. This line of argument for stabilization is supported by the data of Pettitt *et al.* (2); *i.e.*, aniline derivatives follow a nondissociative electron-capture mechanism, whereas the corresponding cyclohexylamine derivatives follow a dissociative mechanism. However,

Table V—Comparative Electron-Capture Response (Coulomb × 10^3/mole) of Heptafluorobutyryl Derivatives Using Tritium and Nickel-63 Detector

Amine	Tritium	Nickel-63	Tritium/ Nickel-63
β-Phenethylamine	2.0	0.17	11.76
Amphetamine	2.2	0.10	22.0
p-Methoxyphenethylamine	1.9	0.10	19.0
Mescaline	3.7	0.26	14.23
Phentermine	1.1	0.07	15.71
Phenmetrazine	8.8	0.40	22.0
Mephentermine	2.2	0.10	22.0
Methamphetamine	4.0	0.14	28.57

Table VI—Comparative Electron-Capture Response (Coulomb × 10^3/mole) of Various Pentafluorobenzamides Using Tritium and Nickel-63 Detector

Amine	Tritium	Nickel-63	Tritium/ Nickel-63
β-Phenethylamine	300	24	12.5
Amphetamine	225	16	14.06
α-Methylbenzylamine	180	26	6.92
Mescaline	155	8.5	18.23
Mephentermine	9.2	0.7	13.14
Methamphetamine	6.1	0.34	17.94
Methoxyphenamine	3.2	0.21	15.24
Amphetamine Schiff base	42.6	5.3	7.93

the almost 30-fold lower sensitivity of *N*-pentafluorobenzyl amphetamine compared to either amphetamine pentafluorobenzamide or *N*-pentafluorobenzylidine (Table II) still emphasizes the need for a polarizable carbon (*e.g.*, C=O or C=N) for high electron-capture sensitivity.

As anticipated, pentafluorobenzamides of primary amines exhibited greater sensitivity than those of secondary amines; *e.g.*, amphetamine pentafluorobenzamide was 30 times more sensitive than methamphetamine pentafluorobenzamide (Table IV). Due to steric hindrance, coplanarity and hence resonance between the pentafluorophenyl ring and carbonyl group are lower in tertiary amides (derived from secondary amines) than in secondary amides (derived from primary amines) (form 2, Scheme II). This is supported by the carbonyl frequency data where a decreasing response is reflected by a decreasing frequency and contribution from the more electron-capture active form 1. The predominance of form 2 (Scheme II) with tertiary amides is anticipated because the +R and −I effects are acting against each other. Even so, the stronger inductive effect of the pentafluorophenyl ring still renders the carbonyl carbon sufficiently positive to act as a better electron-capturing region in the resonating tertiary amide group (form 2) than the corresponding heptafluorobutyramide (form 3).

As mentioned with heptafluorobutyramides, electron acceptance is suppressed by electron-donating substituents in the aromatic ring of the amine. A comparison of methamphetamine with methoxyphenamine pentafluorobenzamide and *p*-methoxyphenethylamine with β-phenethylamine pentafluorobenzamide (Table IV) suggests a similar mechanism. The higher sensitivity of 3,4,5-trimethoxyphenethylamine (mescaline) over *p*-methoxyphenethylamine pentafluorobenzamide probably results from a lower overall electron density in the molecule which increases its electron-capturing capacity, a factor that essentially governs the final response in a nondissociative electron-capture mechanism.

Tritium versus *Nickel-63 Detector*—High molecular weight derivatives require higher column temperatures; if above 220°, the tritium detector cannot be used and the nickel-63 detector, stable to 400°, is employed. However, the latter detector is generally considered less sensitive and possesses a shorter linear dynamic range. To examine this avenue, the electron-capture sensitivity of representative compounds was also measured using a nickel-63 detector. With all derivatives tested, the tritium detector was 10–20-fold more sensitive than the nickel-63 detector (Tables V and VI). Differences were also noted by Wilkinson (8). However, although the magnitude varies, the relative order of the sensitivity remains the same in both detectors. Evidently, steric and chemical factors affecting the electron-capture sensitivity in the tritium detector are operative to the same extent in the nickel-63 detector.

CONCLUSION

The sensitive and selective analysis of amines can be greatly enhanced by formation of an appropriately halogenated derivative. Formation of an amide or Schiff base, *i.e.*, presence of a C=O or C=N group, to which is attached a highly electronegative group capable of undergoing the electron-capture mechanisms proposed is desirable. Pentafluorobenzoyl chloride appears to be a suitable reagent for primary and some secondary amines. The reaction is facile, and the resultant pentafluorobenzamide is generally solid, easy to purify and characterize, and often sufficiently volatile to enable use of the tritium detector. Removal of excess reagent is readily accomplished with dilute base. When determining a secondary amine in the presence of an interfering primary amine, it might be preferable to prepare the heptafluorobutyramide. Other reagents (*e.g.*, pentafluorobenzaldehyde and trichloroacetyl chloride) can be used, but ease of formation, stability of product, and facility of removal of excess reagent must always be kept in mind. Furthermore, in the rational selection of a reagent, the entire structure of the derivative must be considered since the reagent only confers the electron-capture property while the final steric and electronic relationships determine the overall electron-capture sensitivity.

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