ABSTRACT:

N relatively nonbasic are isolated or shown to exist only under very specific circumstances. arise mechanistically, carbinolamines are usually unstable, and they model systems (Baciocchi et al., 1998; Goto et al., 1998) have interpretations of very similar metabolic data and of data from 1996), or perhaps directly by hydrogen atom abstraction (Karki et al., 1983) showing that the oxygen in the carbinolamine comes from O2. The step(s) to the formation of the carbon-centered radical re- tains the related secondary amine substrates trifluoropropranolol (7), its O-methyl ether (23), and its N-trifluoroethyl-O-methyl ether analog (24) in the presence of rat liver microsomes and CYP1A2 were examined to determine whether these species were formed. The 19F NMR experiments showed the presence of carbinolamine and imine species from these primary amines and fluorinated carbonyl compounds in solution. Mass spectral experiments under atmospheric pressure chemical ionization and electrospray ionization trap conditions showed formation of imine metabolites (and/or oxazolidine from 7) as well as products of N-dealkylation and aromatic hydroxylation when the secondary amine substrates were incubated with rat liver microsomes or CYP1A2. In spite of mass spectral evidence for these imines as metabolites, we were unable to detect the carbinolamines under the conditions used in these studies. Their presence is inferred from the results of the 19F NMR experiments.

Carbinolamines, imines, and oxazolidines from fluorinated propranolol analogs. 19F NMR and mass spectral characterization and evidence for formation as intermediates in cytochrome P450-catalyzed N-dealkylation

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Cytochrome P450-catalyzed N-dealkylation of amines occurs through processes that involve formation of carbinolamine intermediates with ultimate decomposition of these intermediates to a carbonyl compound and an amine, e.g., from propranolol yielding acetone and desisopropylpropranolol (Fig. 1). The carbinolamine is generally thought to arise via oxygen rebound to a carbon-centered radical intermediate. This mechanism is supported by studies with 18O2 and/or H218O (McMahon et al., 1969; Shea et al., 1982; Kedderis et al., 1983) showing that the oxygen in the carbinolamine comes from O2. The step(s) to the formation of the carbon-centered radical remains a subject of conjecture. The carbinolamine may arise from an earlier intermediate nitrogen-centered cation radical via proton transfer and electron reorganization (Miwa et al., 1983; Guengerich et al., 1996), or perhaps directly by hydrogen atom abstraction (Karki et al., 1995; Karki and Dinnocenzo, 1995; Manchester et al., 1997). Different interpretations of very similar metabolic data and of data from model systems (Baciocchi et al., 1998; Goto et al., 1998) have generated conflicting views on their origin. Regardless of how they arise mechanistically, carbinolamines are usually unstable, and they are isolated or shown to exist only under very specific circumstances. Carbinolamines have been isolated as metabolites of well chosen relatively nonbasic N-alkyl-substituted compounds, such as N-alkyltriazenes (Jackson et al., 1991; Lang et al., 1996, 1997), arylamides (Fujimaki et al., 1995), and highly conjugated arylamines (Schwartz and Kolis, 1972; Gorrod and Temple, 1976; Shea et al., 1982; Kedderis et al., 1983). N-Hydroxymethylcarbazole from the microsomal metabolic oxidation of the arylamine N-methylcarbazole in the presence of 18O2 or H218O did not show the incorporation of oxygen from H2O (Shea et al., 1982; Kedderis et al., 1983), indicating not only that the source of the oxygen is O2 but also that this carbinolamine does not exchange hydroxide ion with the aqueous medium.

For most carbinolamines, the reversible loss of water or hydroxide ion to form imine or iminium ion intermediates competes with the loss of aldehyde or ketone from the carbinolamine (Fig. 1). Rehydration of the imine or iminium ion results in replacement of the oxygen atom arising via the enzyme-catalyzed activation of O2 with an oxygen atom from solvent water, e.g., from the tertiary amine sparteine (Ebner et al., 1991a,b). In spite of the dehydration-rehydration of these carbinolamine intermediates, 18O-labeled benzaldehyde formed as a result of microsomal N-dealkylation in the presence of 18O2 was successfully reduced to an 18O-alcohol without complete loss of the label (McMahon et al., 1969).

Although iminium ions formed in the metabolism of tertiary amines have been trapped by reaction with cyanide, e.g., nicotine (Murphy, 1973), trapping of imines with cyanide has not been successful. This is probably due to the instability of the α-aminonitrile products of this reaction (Taillades and Commevras, 1974; Shetty and Nelson, 1985).

Many years ago, formation of acetone and desisopropylpropranolol in incubations of propranolol (1) in rat liver microsomes was demonstrated (Bakke et al., 1973). In the case of propranolol, cyclization of the imine to form an oxazolidine can occur (Fig. 2). Attempts
have been made in this laboratory to demonstrate the existence of the
cytochrome P450-catalyzed N-dealkylation of propranolol (I). Al-
though the imine (3) and oxazolidine (4) species were readily ob-
served by NMR spectroscopy in organic solution, neither was ob-
served in extracts from in vitro metabolic experiments, probably due
to the rapid decomposition of the carbinolamine (2) to primary amine
and acetone (Shetty and Nelson, 1985).

Carbinolamines can be stabilized by electron-withdrawing groups
attached to the tetrahedral carbon (Rosenberg et al., 1974; Sayer and
Jencks, 1977), similar to the stabilization of hydrates of aldehydes or
ketones by adjacent electron-withdrawing substituents (Bover and
Zuman, 1973; Greenzaid, 1973; Lamaty et al., 1986a,b). Electron-
withdrawing groups also stabilize related species, such as hemiacetals
(Crampton, 1975), oxazolidines (Alva Astudillo et al., 1985), and
cyanohydrins (Ching and Kallen, 1978). Addition of fluorine atoms to
the carbon atom α to the carbonyl stabilizes the hydrates and carbi-
nolamines of carbonyl compounds significantly (Szinai et al.,
1970a,b; Guthrie, 1975; Buschmann et al., 1980, 1982; Misпelaere
and Roques, 1999). The aldehyde-hydrate and aldehyde-hemiacetal
equilibria from trifluoroacetalddehyde favor hydrate and hemiacetal
formation to a greater extent than the related equilibria from triflu-
oroacetone (Guthrie, 1975).

It seemed possible that trifluoroopropranolol (6)-related carbino-
lanines, imines, or oxazolidines (7-9; Fig. 2) would be stable enough
to observe as metabolites. Here we present 19F NMR and mass
spectral data for trifluoromethyl carbinolamines, imines, and oxazo-
lidines and our assessment of their stability in organic and aqueous
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and Nelson, 1985).
Desisopropylpropranolol O-methyl ether (10). Desisopropylpropranolol (5) (1.02 g, 4.71 mmol) was dissolved in CH$_2$Cl$_2$ (20 ml). Di-t-butyl dicarbonate (1.13 g, 5.2 mmol) and triethylamine (2.60 g, 18.8 mmol) were added. The mixture was heated at reflux for 1.5 h, and the solvent and triethylamine was evaporated. Methylene chloride (30 ml) was added, and the mixture was washed with 2 x 20 ml of aqueous 0.5 N HCl and then with 20 ml of H$_2$O. The CH$_2$Cl$_2$ layer was dried over Na$_2$SO$_4$, and evaporation afforded 1.50 g (−100% yield) of the t-butyl carbamate ester of 10. This carbamate ester (1.50 g, 4.71 mmol) was dissolved in 50 ml of tetrahydrofuran, and 1.36 g (23.5 mmol) of powdered KOH was added. Methyl iodide (3.0 ml, 47 mmol) was then added, the flask was sealed, and the mixture was stirred at room temperature for approximately 24 h. After cooling, the reaction mixture was transferred into CH$_3$OH (5 ml). The mixture was heated at 40°C for 6 to 10 days resulted in more complete conversion to the oxazolidine (17) and 22. Isolation was accomplished by flash column chromatography on silica gel using CH$_2$Cl$_2$ as eluent. 1H NMR (CDCl$_3$): δ 8.23 (1H, m, H-8’), 7.80 (1H, m, H-5’), 7.51-7.43 (3H, m, H-7’,-6’,-4’), 7.37 (1H, dd, H-3’), 6.82 (1H, d, H-2’), 4.22 (2H, m, H-3), 3.85 (1H, m, H-2), 3.58 (3H, s, OCH$_3$), 3.26 (2H, qm, H-2’), 3.07 (2H, m, H-1). ESI-MS/MS [MH]+ 183 → [C$_4$H$_7$NO]$.^+$ 170.

Trifluoroethylpropranolol O-methyl ether (24). Desisopropylpropranolol O-methyl ether (10) (200 mg, 0.99 mmol) and trifluoroacetone (−200 µl, 2.2 mmol) were dissolved in CH$_2$Cl$_2$ (5 ml) and heated in a sealed vial to 70°C for 24 h. After cooling, the reaction mixture was transferred into CH$_3$OH (5 ml). Sodium cyanoborohydride (259 mg, 4.1 mmol) and acetic acid (−50 µl) were added, and the reaction mixture was allowed to stir for 4 h. The mixture was partitioned between CH$_2$Cl$_2$ (20 ml) and aqueous 1 N NaOH (20 ml). The organic layer was washed with H$_2$O (5 ml), dried over Na$_2$SO$_4$, and the solvent was evaporated. The crude product was purified by flash column chromatography on silica gel with a stepwise gradient of CH$_2$Cl$_2$ to 20% MeOH. 1H NMR (CDCl$_3$): δ 8.23 (1H, m, H-8’), 7.80 (1H, m, H-5’), 7.52-7.43 (3H, m, H-7’,-6’,-4’), 7.37 (1H, dd, H-3’), 6.83 (1H, d, H-2’), 4.23 (2H, m, H-3), 3.84 (1H, m, H-2), 3.58 (3H, s, OCH$_3$), 3.20 (1H, m, H-2), 2.60 (2H, m, H-1), 1.29, 1.26 (3H, 2d, 2H, s, CH$_3$). ESI-MS/MS [MH]+ 328 → [C$_4$H$_7$NOF$_3$]+ 184, [C$_4$H$_7$O]+ 183.

Trifluoroethyl imine and oxazoline of desisopropylpropranolol (17 and 22). Desisopropylpropranolol (5) (200 mg, 0.92 mmol) was dissolved in 10 ml of CH$_2$Cl$_2$ and 0.50 ml of trifluoroacetic acid methyl hemiacetal (0.62 g, 5.6 mmol) was added. The reaction vessel was sealed and heated at 60°C for approximately 24 h. Nearly complete conversion to diastereomeric oxazolidines was also observed in these samples. A further 24 h was added, and the reaction mixture was still almost complete. Isolation was accomplished by flash column chromatography on silica gel using CH$_2$Cl$_2$ as eluent. 1H NMR (CDCl$_3$): δ −71.46. In samples examined at earlier time points, diastereomeric carbinaldine 12 was present as indicated by 19F NMR (CDCl$_3$): δ −81.44, −81.46. Oxazolidine 22 was also observed in these samples. The reaction mixture for preparation of the imine (17) was heated at 40°C for 6 to 10 days resulted in more complete conversion to the oxazolidine (22). 19F NMR (CDCl$_3$): δ −79.65 and −79.67.

Trifluoropropyl imine and desisopropylpropranolol (8). Desisopropylpropranolol (5) (200 mg, 0.92 mmol) was dissolved in 10 ml of CH$_2$Cl$_2$ and 0.50 ml of trifluoroacetone (0.62 g, 5.6 mmol) was added. The reaction vessel was sealed and heated at 40°C for approximately 24 h. Nearly complete conversion to the imine was indicated by proton NMR in CDCl$_3$. The imine (8) was isolated by flash column chromatography on silica gel using CH$_2$Cl$_2$ as eluent. 1H NMR (CDCl$_3$): δ 8.22 (1H, m, H-8’), 7.80 (1H, m, H-5’), 7.53-7.44 (3H, m, H-7’,-6’,-4’), 7.37 (1H, dd, H-3’), 6.84 (1H, d, H-2’), 4.49 (1H, m, H-2), 4.25 (2H, m, H-3), 3.72 (2H, m, H-1), 2.05 (3H, s, 2CH$_3$). 19F NMR (CDCl$_3$): δ −73.71 (CDCl$_3$): δ −75.26. ESI-MS/MS [MH]+ 312 → [C$_3$H$_7$NOF$_3$]+ 183, [C$_7$H$_13$NOF$_3$]+ 168. In samples maintained at 25°C, significant amounts of the intermediate carbinaldine diastereoisomers 7 were present. 19F NMR (CDCl$_3$): δ −82.58, −82.88.

Trifluoropropyl oxazolidine of desisopropylpropranolol (9). Desisopropylpropranolol (5) (200 mg, 0.92 mmol) was dissolved in 10 ml of CH$_2$Cl$_2$ and 0.50 ml of trifluoroacetone (0.62 g, 5.6 mmol) was added. The reaction vessel was sealed and heated to 40°C for several days, resulting in nearly complete conversion to diastereomeric oxazolidines. Isolation was accomplished by column chromatography on silica gel using CH$_2$Cl$_2$ as eluent. 1H NMR
Substrate concentration 50 μM. Benzene was evaporated. 1H NMR (CDCl₃): 3.80, 3.60 (1H, 2 d, H-4); 8.00 (1H, d, H-3); 7.53, 7.47 (2H, m, H-2, H-3); 6.85, 6.67 (2H, d, H-2, H-3); 4.68, 4.50 (1H, d, H-2); 4.40, 4.19 (2H, 2 m, H-3, H-4); 3.57, 3.16 (2H, 2 m, H-5, H-6); 1.80, 1.60 (2H, 2 m, H-7, H-8); 0.81 (3H, s, 2 CH₃). Trifluoroethyl imine of desisopropylpropranolol O-methyl ether (18). Desisopropylpropranolol O-methyl ether (10) (500 mg, 2.17 mmol) was dissolved in 10 ml of CH₂Cl₂ or CDCl₃. Trifluoroacetaldehyde methyl hemiacetal (0.50 ml) (0.62 g, 5.6 mmol) was added, the reaction vessel sealed, and heated at 40°C for approximately 24 h. 19F NMR (CDCl₃): δ –71.56. Shorter reaction times showed the presence of the diastereomeric carbinoline 13. 19F NMR (CDCl₃): δ –81.57. Trifluoroethyl imine of desisopropylpropranolol O-methyl ether (10) (500 mg, 2.17 mmol) was dissolved in 10 ml of CH₂Cl₂ or CDCl₃. Trifluoroacetone (0.50 ml) (0.62 g, 5.6 mmol) was added, and the reaction vessel was sealed and heated at 40°C for approximately 24 h. The imine was isolated by column chromatography on silica gel eluting with CH₂Cl₂. 1H NMR (CDCl₃): δ 8.25 (1H, m, H-8); 7.80 (1H, m, H-5′); 7.53-7.44 (4H, m, H-7, H-8, H-3, H-4); 7.37 (1H, d, H-3); 6.84 (1H, d, H-2); 4.29 (2H, m, H-3); 4.11 (1H, m, H-2); 3.79 (2H, m, H-1); 3.58 (3H, s, OCH₃); 2.04 (3H, s, 2 CH₃). 19F NMR (CDCl₃): δ –73.19. 1H NMR: δ –75.29. ESI-MS/MS [MH]+ 326 – [C₁₃H₁₁O]+ 183, [C₁₃H₁₁NOF₃]+ 182.

Trifluoroethyl imine of 3-(1-naphthoxy)propylamine (19). 3-(1-Naphthoxy)propylamine (11) (400 mg, 2.00 mmol) and trifluoroacetaldehyde methyl hemiacetal (500 mg, 3.85 mmol) were dissolved in 5 ml of benzene, and the mixture flushed with dry argon and stirred at room temperature for 5 to 8 days. At the end of this time, the benzene was evaporated. 19F NMR (CDCl₃): δ –70.76 (d, 3 J HF = 2 Hz). Shorter reaction times showed the presence of carbinoline 14. 19F NMR (CDCl₃): δ –81.15 (d, 3 J HF = 2 Hz). Trifluoroethyl imine of 3-(1-naphthoxy)propylamine (21). 3-(1-Naphthoxy)propylamine (11) (400 mg, 2.00 mmol) and trifluoroacetone (500 mg, 4.5 mmol) were dissolved in 5 ml of benzene, and the mixture flushed with dry argon and stirred at room temperature for 7 days. At the end of this time, the benzene was evaporated. 1H NMR (CDCl₃): δ 8.32 (1H, m, H-8); 7.86 (1H, m, H-5'); 7.58-7.37 (4H, m, H-7, H-8, H-3, H-4); 6.85 (1H, d, H-2); 4.26 (2H, t, H-3); 3.77 (2H, t, H-1); 2.38 (2H, m, H-2); 2.03 (3H, s, 2 CH₃). 19F NMR (CDCl₃): δ –73.69. Shorter reaction times showed the presence of the intermediate carbinoline 16. 19F NMR (CDCl₃): δ –83.24.

**NMR Spectroscopy.** NMR spectra were acquired on a Varian VXR 300 spectrometer (Varian Inc., Palo Alto, CA) or a Bruker AF-300 spectrometer (Bruker Instruments Inc., Billerica, MA). 19F NMR spectra were calibrated using neat trifluoroethanol (Aldrich, Milwaukee, WI) as an external reference, δ = –77.8 ppm relative to CFCl₃ (Everett, 1995). Reactions of 4 to 10 mg (20–50 μmol) of primary amine and one to two equivalents of trifluoroacetone (Aldrich) or trifluoroacetaldehyde methyl hemiacetal (PCR, Gainesville, FL) were carried out in deuterated solvents (total volume approximately 1 ml) in 5-mm NMR tubes.

**Mass Spectrometry.** ESI mass spectra were obtained using a Finnigan LCQ quadrupole ion trap mass spectrometer (Finnigan/Thermoquest, San Jose, CA) equipped with a capillary electrospray ion source modification (Wang and Hackett, 1998) of the Finnigan API interface. Collision-induced dissociation was carried out in the mass analyzer on an ion selected from the mass spectrum, using the helium gas present in the trap. The samples, either synthetic standards or metabolic product mixtures in methanol, were infused directly via a syringe pump at a flow rate of 300 nl/min. The heated capillary was maintained at 160°C and the source voltage at 1.7 kV. APCI mass spectra were acquired using a Micromass Qtrap mass spectrometer equipped with the Micromass Peptorpin ion source (Micromass Ltd., Manchester, UK). MS/MS spectra were produced by collision-induced dissociation with argon in the collision cell. Samples were dissolved in EtOAc or CH₃OH. The 5- to 25-μl injections of sample were infused directly into the mass spectrometer with hexane at a rate of 200 μl/min.

**Metabolism. Incubations with rat liver microsomes.** Substrates were incubated at 37°C for 20 to 60 min in the presence of 3-methylcholanthrene-induced rat liver microsomes. The typical incubation volume was 250 μl, substrate concentration 50 μM, microsomal protein concentration 1 mg/ml, NADPH concentration 1.0 mM, in 100 mM sodium phosphate buffer, pH 7.4. After incubation, the samples were made alkaline by the addition of aqueous saturated sodium carbonate (100 μl). Samples were extracted into EtOAc (5 ml). The EtOAc solution was dried over sodium sulfate, then the solvent was evaporated. The residue was dissolved in 200 μl of CH₃OH. This CH₃OH solution was infused into the mass spectrometer.

**Results.** Characterization of the synthesized carbinolamines, imines, and oxazolidines and detection of them as metabolites required development of analytical methods. 19F NMR proved to be very useful in monitoring the formation and stability of carbinolamines, imines, and other species produced in the reactions of primary amines with fluorinated carbonyl compounds. Because we were unable to achieve sufficient sensitivity by NMR analysis to demonstrate the presence of carbinoline, imine, or oxazolidine metabolites from incubations of trifluoromethyl substrates with P450s, other analytical methods were sought.

Attempts to adapt standard gas chromatography/MS methods for propranolol metabolite assays that involve derivatizations, e.g., trialkylsilyl ether formation or trifluoroacetylation (Shetty and Nelson, 1985), were not successful due to low sensitivity of the assay and instability of the carbinoline species. Although imine 8 generated an N-trifluoroacetyl derivative via isomerization of the imine to an enamine, we could not attain complete conversion due to significant decomposition.

Attempts to develop a liquid chromatography/MS method to separate the metabolites were unsuccessful. Under reverse phase conditions, elution of the trifluoroacetylpropranolol-related substrates from the column required low pH. The intermediates were not stable to these acidic conditions. Under normal phase high-performance liquid chromatography conditions, separation of imine 8, oxazolidine 9, and trifluoroacetylpropranolol (6) on a CN column was successful. However, elution of the N-dealkylation product 5 from the column required a steep hexane/ethanol gradient with a relatively high concentration of triethylamine (4% by volume), conditions that suppressed ionization of these compounds in the mass spectrometer.

APCI ionization is amenable to nonaqueous conditions. Small volumes of sample in EtOAc or CH₃OH solution were infused into the mass spectrometer with hexane flowing at a rate of 100 to 200 μl/min. Under these unusual conditions, mass spectra of fluorinated imines and oxazolidines were obtained; however, nonfluorinated components of compound mixtures, e.g., N-dealkylation products 5 and 10, ionized very poorly under these conditions.

Mass spectra of the fluorinated imines and oxazolidines were successfully obtained using a capillary ESI source and ion trap mass spectrometer. Methanol served as the proton source because in the presence of water only the primary amine decomposition product was observed. Ionization did not occur in ethyl acetate and acetonitrile solutions of samples. The capillary ESI source had the advantage over APCI that both fluorinated and nonfluorinated compounds ionized and produced satisfactory mass spectra under the same conditions.

**NMR Spectra.** By 19F NMR spectroscopy, formation of carbo-
laines, imines, and in some cases oxazolidines from the reaction of primary amines 5, 10, and 11 with trifluoroacetone or trifluorocetalddehyde in organic solution was observed (Fig. 3). $^{19}$F NMR chemical shifts of the trifluoromethyl groups from the carbinolamine and imines from the three amines and the two fluorinated carbonyl compounds obtained in solution are given in Table 1. Besides carbinolamines and imines, resonances of the trifluoromethyl groups of other species were observed, e.g., oxazolidines and aminals. Where possible, the compound identities were confirmed by $^1$H and $^{13}$C NMR spectra and/or mass spectra (under Materials and Methods; Table 2). $^{19}$F Resonances for the trifluoromethyl groups of the imines are the farthest downfield and consistently downfield from those of the carbinolamines by ca. 10 ppm (Figs. 5-7; Table 1). Carbinolamine trifluoromethyl group resonances are downfield from those of the hydrates of the carbonyl compounds by about 3 ppm.

The formation of the diastereomeric carbinolamine 7, imine 8, and oxazolidine 9 from desisopropylpropranolol (5) and trifluoroacetone in benzene is shown in Fig. 5. Signals for the carbinolamine appear rapidly, with slower appearance of imine 8. In benzene conversion of imine to oxazolidine is very slow, but we observed more rapid oxazolidine formation in chloroform. Imine 8 and the oxazolidine 9 were isolated by column chromatography and their identities confirmed by $^{1}$H and $^{13}$C NMR and mass spectrometry; for comparison, samples were analyzed in methanol solution, methanol adducts were commonly observed (Table 2). In some cases, NMR samples containing predominantly imine but also other species, e.g., primary amines and aminals, were used to acquire mass spectra. Molecular ions of desisopropylpropranolol (5) were isolated by column chromatography and their identities confirmed by $^{1}$H and $^{13}$C NMR and mass spectrometry. Where possible, the compound identities were confirmed by $^{1}$H and $^{13}$C NMR spectra and/or mass spectra (under Materials and Methods; Table 2). $^{19}$F Resonances for the trifluoromethyl groups of the imines are the farthest downfield and consistently downfield from those of the carbinolamines by ca. 10 ppm (Figs. 5-7; Table 1). Carbinolamine trifluoromethyl group resonances are downfield from those of the hydrates of the carbonyl compounds by about 3 ppm.

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<table>
<thead>
<tr>
<th>Primary Amines</th>
<th>Carboxyl Compound</th>
<th>Carbinolamine</th>
<th>Imine</th>
<th>Other</th>
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<tbody>
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|$^{19}$F Chemical shifts (ppm) of trifluoromethyl groups of products from primary amines and trifluoroacetaldehyde$^a,b$ and trifluoroacetone$^c$;

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The relative amount of hydrolysis of imine 8 to primary amine 5 was large, whereas oxazolidine 9 produced only a small amount of 5 (Table 2). Ions for methanol adducts to imine 8 or oxazolidine 9 were not observed.

FIG. 5. Reaction of desisopropylpropranolol (5) with trifluoroacetone in benzene at 60°C monitored by $^{19}$F NMR.

(a) formation of diastereomeric carbinolamine 7 ($\delta = 73.2$ ppm) at 3 h, 60°C. 8, 24 h, 60°C. Imine 8 ($\delta = 73.1$ ppm) is beginning to form, b, after heating, 9 is the major product. c, mixture of oxazolidine diastereoisomer standards (9). e, imine 8 in acetoni
trile/water (4:1), 19 h f, oxazolidine 9 in acetoni/trile/water (4:1), 19 h. g, hydrolysis of 8 occurs after addition of one equivalent of HCl.

FIG. 6. Reaction of O-methyl desisopropylpropranolol (10) with trifluoroacetone in benzene monitored by $^{19}$F NMR.

(a) initial formation of carbinolamine 15 at 2 h (room temperature). Imine 20 ($\delta = 73.1$ ppm) is beginning to form. b, after heating, 20 is the major product. c, trifluoroacetone and its hydrate in benzene. d, imine 20 in the presence of acetoni
trile/water (4:1), after 20 h. e, addition of approximately one equivalent of HCl to 20 in acetonitrile/water (4:1) affords trifluoroacetone hydrate. f, trifluoroacetone in acetonitrile/water (4:1) affords trifluoroacetone hydrate.

FIG. 7. Reaction of 10 with trifluoroacetaldehyde methyl hemiacetal in benzene was monitored by $^{19}$F NMR spectroscopy.

a, diastereomeric carbinolamine (13, $\delta = 81.5$ ppm), hemiacetal, and the hydrate of trifluoroacetaldehyde. b, heating the mixture catalyzed formation of imine 18. c, control spectrum of trifluoroacetaldehyde methyl hemiacetal in benzene. d, rehy-
dration of 18 in a mixture of acetonitrile and water. Carbinolamine 13 is stable for many hours under these conditions. e, addition of approximately one equivalent of HCl to 13 in acetonitrile/water. f, trifluoroacetaldehyde methyl hemiacetal in acetoni/trile/water with approximately one equivalent of acid.

Similar results for trifluoroacetone imines 21 and 20 (Table 2) were obtained. In addition to the molecular ions of 21 and 20 (m/z 296 and 326, respectively), ions from the primary amines 11 and 10 (m/z 202 and 232, respectively) were significant, as were the ions of the
products from the addition of methanol (m/z 328 and 358, respectively). Major ions observed from a sample of the trifluoroethylamine of 3-(1-naphthoxy)propylamine (19) are the [MH]$^+$ ions for the product of methanol addition (m/z 314), imine 19 (m/z 282), primary amine 11 (m/z 202), and the aminal addition product of 11 to 18 (m/z 483).

Characteristic product ions from the MS/MS spectra of imines 8, 21, and 20, and oxazolidine 9 appear in Table 3. Proposed structures of these ions are consistent with previous results (Upthagrove et al., 1999a,b). MS/MS spectra of 8 and 9 were virtually identical, with major fragment ions with the same relative intensity: m/z 183, 168 (side chain ion), and its fragment at m/z 126 (Fig. 8; Table 3). For comparison, data from standards of trifluoropropranolol (6) and its 4'-hydroxylated derivative 25 are also included in Table 3. The MS/MS spectra of the [MH]$^+$ ion of imine 21 showed similar fragment ions at m/z 183, 152 (side chain ion), and 126 (fragment of side chain ion). The [MH]$^+$ ion of imine 20 showed fragment ions at m/z 183, 182, and 126.

Metabolic Experiments. Three fluorinated propranolol analogs were incubated with 3-methylcholanthrene-induced rat liver microsomes and analyzed for the formation of carbinolamines and imines: trifluoropropranolol (6), trifluoroethylpropranolol O-methyl ether (24), and trifluoroethylpropranolol O-methyl ether (23). These samples were analyzed under the APCI-MS conditions described above. Trifluoropropranolol (6) was also incubated with recombinant human CYP1A2 and analyzed using the ESI-MS method, giving very similar results to those obtained from incubations with rat liver microsomes. In addition to the N-dealkylation products, imine and ring-hydroxylated metabolites were formed from the three substrates 6, 23, and 24. Mass spectral evidence for these metabolites is shown in Figs. 8 and 9.

MS/MS spectra of trifluoropropranolol (6) and two metabolites formed in incubations with recombinant human CYP1A2 are shown in Fig. 8. Ion trap experiments on extracted metabolites showed a product (or products) of addition of oxygen (m/z 330).

The MS/MS data from m/z 330 (Fig. 8b) showed major fragment ions at m/z 199 and 170 (side chain ion). The presence of the ion at m/z 170 indicates that the side chain is not oxygenated and the m/z 199 ion indicates that the hydroxylation occurred in the aromatic ring, a result very similar to the MS/MS spectrum of the 4'-hydroxytrifluoropropranolol standard 25 (Table 3) and to MS/MS spectra of 4', 5', and 7'-hydroxypropranolol (Upthagrove et al., 1999a). Trifluoropropranolol (6), 4'- and 5'-hydroxylated metabolites, were later identified in the presence of CYP1A2. In the MS/MS spectrum of the substrate 6, the analogous nonoxygenated ion appears at m/z 183 (Fig. 8a).

An ion at m/z 312 indicating loss of 2 amu from the substrate was also observed. Like the substrate 6, the MS/MS spectrum of m/z 312 (Fig. 8c) gave ions at aromatic ring-related ions at m/z 183 and 157 (Upthagrove et al., 1999a). An ion at m/z 168 was also observed, consistent with loss of 2 amu from the side chain and the imine (8) and/or oxazolidine (9) structure.

Figure 9 shows the mass spectra obtained under APCI conditions
made to determine the equilibrium ratios of imine to carbinolamine because the focus of the work was on determining whether they were produced metabolically. Because the equilibrium for hydration of acetaldehyde favors hydration to a much greater extent than for acetone (Buschmann et al., 1980) and for trifluoroacetaldehyde versus trifluoroaceton (Guthrie, 1975), it seemed that this might be true for their respective imines, i.e., more carbinolamine versus imine from the trifluoroacetaldehyde-derived imines. However, the situation is much more complex because equilibrium conditions were not assured, and the conditions were not carefully controlled for pH, solvent composition, or temperature, important factors that affect the composition of imine-oxazolidine-carbinolamine mixtures (Kurono et al., 1994). In addition, the process of hydration of the ketone or aldehyde competes under these conditions. These studies do provide evidence that the carbinolamines are produced and slowly dehydrate to form imines in organic solutions. The carbinolamines derived from trifluoroacetaldehyde formed from dehydration of their corresponding imines, suggesting that at equilibrium in neutral aqueous solution, these carbinolamines would be present.

Our attempts to use \textsuperscript{19}F NMR to demonstrate the presence of carbinolamine, imine, or oxazolidine metabolites from incubations of trifluoromethyl substrates with rat liver microsomes or recombinant human CYP1A2 expressed in insect cells were unsuccessful. We believe this was due to our inability to optimize the NMR instrument to detect small concentrations of the metabolites in situ, and the instability of the carbinolamines to methods for concentrating the samples. A \textsuperscript{19}F NMR method to detect and quantify low micromolar concentrations of more stable metabolites from P450 incubations has been reported (Vervoort et al., 1990), but these investigators used an instrument dedicated to \textsuperscript{19}F NMR on biological samples.

The mass spectral experiments under APCI and ESI-ion trap conditions clearly demonstrate imine and/or oxazolidine metabolite(s) and metabolic products of N-dealkylation and aromatic hydroxylation from fluorinated propranolol analogs in the presence of rat liver microsomes and CYP1A2. The structure of the metabolic product shown in Fig. 8c from trifluoropropranolol (6) could be imine 8 and/or oxazolidine 9. Since the NMR experiments indicated that imine 8 was relatively stable in a neutral aqueous environment, it is the more likely structure, though the oxazolidine 9 cannot be ruled out completely. The MS/MS spectra unambiguously showed that the imines 20 and 21 were formed as metabolites of substrates 23 and 24, respectively.

In spite of mass spectral evidence for the presence of imines in the metabolism of some of these trifluorinated propranolol analogs, we were unable to detect their expected carbinolamine precursors. These carbinolamines do not appear sufficiently stable to determine under the mass spectral conditions used. In the APCI experiments, even though nonaqueous solvents like hexane can be used for the sample infusion, they were not detected. In the less energetic ESI process, hydroxylic solvents are needed as a proton source for ionization. Methanol used in these experiments may be detrimental to the stability of the carbinolamines, as shown in the decomposition of imines under the ESI-ion trap conditions.

In conclusion, the addition of fluorines on the \(\beta\)-carbon in these aliphatic amines, stabilized imines, and carbinolamines to the extent that they could be detected by \textsuperscript{19}F NMR in solution. Direct determination of the carbinolamines’ mass spectrally was unsuccessful. Their presence in microsomal incubations is inferred from the demonstration that the imine or oxazolidine is a metabolite from trifluoropropranolol (6) and imine metabolites of the other related secondary amines with \(\beta\)-trifluoromethyl groups are formed.

**Acknowledgments.** This work was supported in part by National...