using Me_4Si as a reference and the indicated solvent at ambient temperatures. Although ir and NMR data are reported only where considered significant, these spectra were determined for all reported compounds and were considered consistent with the assigned structures. Mass spectral data were obtained on a Hitachi Perkin-Elmer RMU-6E mass spectrometer.
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N-Hydroxylation of 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane by Rabbit Liver Microsomes

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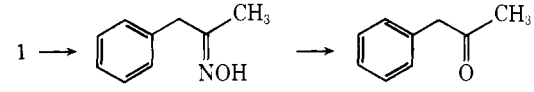
Metabolic N-hydroxylation of the potent psychotomimetic amine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (**5**) by rabbit liver microsomal preparations has been investigated. Synthetic hydroxylamine **8** was obtained by sequential reduction of the corresponding nitropropene **10** with sodium borohydride followed by zinc reduction of the resulting nitropropane **11**. Compound **8** in water (pH 7.4) was rapidly air oxidized to oxime **12**; this oxidation was completely blocked by rabbit liver microsomes. Microsomal incubations of amine **5** or its bis(methoxy-d_3)hexadeuterio analog **5**-d_6 resulted in the formation of **8** and **8**-d_6, respectively, identified as their bis(trifluoroacetyl) derivatives by GLC–MS. Quantitative estimations of metabolite formation employing selected ion monitoring with the aid of an accelerating voltage alternator were accomplished by stable isotope dilution analyses with **5**-d_6 as substrate and **8**-d_0 as internal standard. Similar analyses starting with "pseudoracemates" (*R*)-**5**-d_0:(*S*)-**5**-d_6 or (*R*)-**5**-d_6:(*S*)-**5**-d_0 as substrates established metabolite **8** to be enriched with its *R* enantiomer.

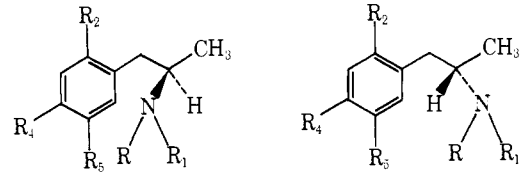
The metabolic N-hydroxylation of aromatic amines, a reaction thought to be involved in the carcinogenic and other toxic effects of some aromatic amines, has been investigated extensively.[1] Recently, interest in metabolic N-hydroxylation has been extended to aliphatic amines.[2] The in vitro[2a,b,g] and in vivo[2f] N-hydroxylation of amphetamine (**1**) has been studied by several groups.[2b,c,g] It has been suggested[3] that phenyl-2-propanone (**3**), a major urinary metabolite[4] of amphetamine in man and several other species, may arise, in part, via the oxime **2** (eq 1). However, the



2 3 (1)

mode of formation of oxime **2** is a matter of controversy. Beckett and his coworkers have suggested that **2** arises from hydroxylamine **4** (a metabolite of **1**) via nonenzymatic oxidation, while Hucker and his group have proposed[2g,3b] other pathways to oxime **2**. Indeed, it has been claimed that[2g,3b] no *N*-hydroxyamphetamine (**4**) is formed in incubations of **1** with rabbit liver microsomes under conditions where others[2b,c] report N-hydroxylation.

These inconsistencies may be partly due to the instability of aliphatic *N*-hydroxy compounds, especially when subjected to basic conditions in the presence of oxygen[2a,5] (see below). Metabolic studies[2b] by Beckett and Al-Sarraj on the influence of the chiral center of amphetamines on N-hydroxylation have shown that (*R*)-(−)-amphetamine [(*R*)-**1**] is about eight times better as a substrate for N-hydroxylation than the *S* enantiomer (*S*)-**1**. This metabolic stereoselectivity, although described without detailed evidence, is interesting in view of the greater CNS stimulant activity of (*S*)-(+)-amphetamine.[6] Benington and his coworkers found[7] that replacement of the amino group in a series of 1-phenyl-2-aminopropanes by the hydroxylamino group decreases but does not abolish the central stimulant



R *S*

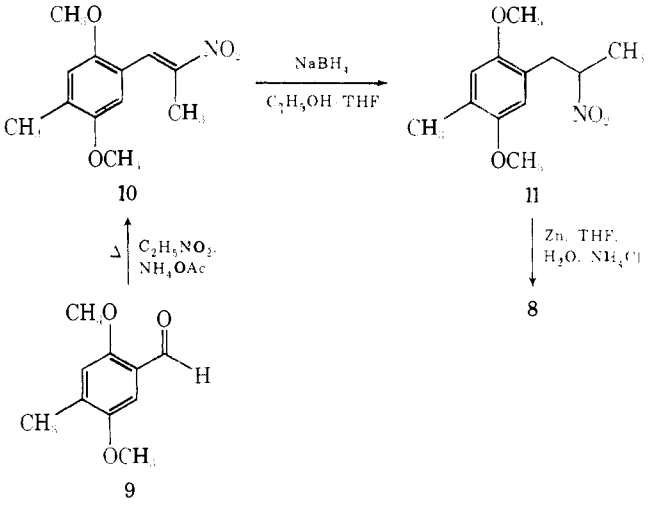
	R	R_1	R_2	R_4	R_5
1	H	H	H	H	H
4	H	OH	H	H	H
5	H	H	OCH_3	CH_3	OCH_3
5-d_3	H	H	OCH_3	CD_3	OCH_3
5-d_6	H	H	OCD_3	CH_3	OCD_3
5-TFA	H	$COCF_3$	OCH_3	CH_3	OCH_3
5-d_6-TFA	H	$COCF_3$	OCD_3	CH_3	OCD_3
6	H	H	OH	CH_3	OCH_3
7	H	H	OCH_3	CH_3	OH
8	H	OH	OCH_3	CH_3	OCH_3
8-TFA	$COCF_3$	$OCOCF_3$	OCH_3	CH_3	OCH_3
8-d_6	H	OH	OCD_3	CH_3	OCD_3
8-d_6-TFA	$COCF_3$	$OCOCF_3$	OCD_3	CH_3	OCD_3

effects of this series of compounds. However, this activity may be due to the parent amine which may be formed in vivo by the reduction of the *N*-hydroxy compounds.[2a]

Our interest in the relationship between metabolism and psychotomimetic activity of 1-phenyl-2-aminopropane derivatives has led us to investigate the in vitro[8,9] and in vivo[9,10] metabolism of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (**5**), a potent psychotomimetic amphetamine derivative.[11] Metabolites resulting from oxidative attack at the aromatic methyl group,[9,10] O-demethylation,[8] and oxidative deamination[9,10] have been found. The factors governing the steric course of the metabolism of amine **5** have been under investigation, since it has been reported[12] that (*R*)-**5** but not (*S*)-**5** is the psychoactive species. We have found that (*R*)-**5** is excreted in the urine of rabbits treated with *rac*-**5** to a greater extent than is (*S*)-**5**[10] and that (*S*)-**5** is metabolized by rabbit liver 10,000g supernatant fractions incubated with *rac*-**5** to a greater extent than its antipode.[9] In addition, incubation of *rac*-**5** with the above rabbit liver preparations leads to the stereoselective formation of the two O-demethylated metabolites **6** and **7**, both enriched in their *S* enantiomer.[8] In order to further characterize the oxidative metabolic pathways of **5** we have examined the in vitro formation of the two enantiomeric N-oxidation products, (*R*)-**8** and (*S*)-**8**, in rabbit liver microsomal preparations.

Chemistry. A synthetic route leading to hydroxylamine **8** has been described in the literature.[13] The procedure involves the condensation of 2,5-dimethoxy-4-methylbenzaldehyde (**9**) with nitroethane, followed by catalytic hydrogenation of the resulting nitropropene **10**. The last step in this sequence gave a complex product mixture and a low yield (8%) of the desired compound **8**. An alternative route to **8** would be the lithium aluminum hydride reduction of **10**, but this reaction is expected to give a substantial amount of the primary amine **5** as side product.[14,†] We now wish to report an alternative synthesis of **8** (Scheme I) which is applicable to a variety of 1-phenyl-2-hydroxyaminoalkanes[15a] and which gives reasonable yields of **8** free of side products.

Scheme I. Synthesis of **8**



The reduction of nitroolefin **10** to nitropropane **11** was patterned after procedures reported in the literature.[15] Such reductions of nitroolefins using sodium borohydride often lead to a mixture of products arising from several possible side reactions.[15b,c,d,16] However, by carefully controlling the reaction conditions (see Experimental Section) the side reactions can be minimized,[15b,17] and in our hands the reduction of **10** proceeded smoothly to give a good yield of **11**. Reduction of **11** to the desired hydroxylamine **8** was accomplished using zinc powder in aqueous tetrahydrofuran (THF) containing ammonium chloride.[18] Since zinc powder can reduce hydroxylamines to amines,[19] it is important to control the reaction conditions (ratio of reactants, reaction time) to avoid formation of side product **5**. By means of a gas chromatographic (GLC) analysis, it was possible to monitor the reduction of **11** (see Analytical Methods) and to optimize the yield of hydroxylamine **8**. The melting point of **8**·HCl and the electron ionization mass spectrum (EIMS) of **8** agreed with those published.[13] The purity of **8** thus prepared was shown by GLC to be >99%. Hydroxylamine **8** hydrochloride could be stored in the cold (0–5°) for long periods without decomposition.

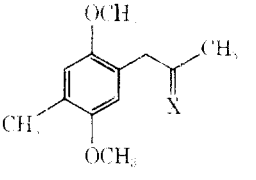
Table I. Gas Chromatographic Characteristics of 8-TFA and Related Compounds

Compound	13	14	8-TFA	5-TFA	11
Retention time, min	3.8[a]	11.5[a]	4.9,[a] 2.6[b]	4.3[b]	5.0[b]

[a] Conditions 3% OV-25; temperature program, initial 128°, increase 2°/min, N_2 30 ml/min. [b] Conditions 3% OV-1, isothermal 130°, N_2 flow 30 ml/min.

Analytical Methods. Hydroxylamines have been identified and quantitatively determined using a variety of analytical techniques.[1] In keeping with our efforts,[8,9,20] to apply stable-isotope labeling in conjunction with GLC separation and mass spectrometry to the study of drug metabolism, we decided to use these techniques in our investigation of the N-hydroxylation of **5**. *N*-Hydroxy compounds have been subjected to GLC analysis as their acetyl[21] or trimethylsilyl[21,22] derivatives. Some *N*-hydroxy compounds have been analyzed by GLC directly, without derivatization.[2b,e,23] However, this practice is questionable, since underivatized *N*-hydroxy compounds may decompose when injected into the gas chromatograph,[24] often to compounds which are themselves potential metabolites.

Derivatization of **8** with trifluoroacetic anhydride[‡] (TFAA) produced the bis(trifluoroacetyl) derivative, **8**-TFA. This compound is stable in dry benzene or dichloromethane, or in excess TFAA, but slowly undergoes decomposition in ether or ethyl acetate. Compound **8**-TFA can be conveniently gas chromatographed on a variety of columns (OV-1, OV-17, Dexsyl 300). Table I shows the GLC characteristics of **8**-TFA together with data on nitropropane **11** and amine **5**, compounds of importance in the preparation of **8** (see above). In addition, Table I includes the GLC characteristics of the TFA derivative **13** of oxime **12**,[13] and of ketone **14**.[10] Compounds **12**[13] and **14**[10] are potential me-



12, X = NOH
13, X = NOCOCF$_3$
14, X = O

† In fact, reduction of nitropropene **10** using lithium aluminum deuteride with the purpose of preparing d_2-labeled **5** gave a product containing ca. 1.5% of d_2-labeled **8** as determined by GC–MS.

‡ Derivatization of **8** with pentafluoropropionic anhydride (PFPA) gave, in addition to the expected *N,O*-bis(pentafluoropropionyl) derivative, the hydroxylamine **8** acylated with one pentafluoropropionyl and one *trifluoroacetyl* group (determined by GC–MS). This is due, apparently, to trifluoroacetic anhydride impurity in PFPA. Similar observations have been made by others.[25]

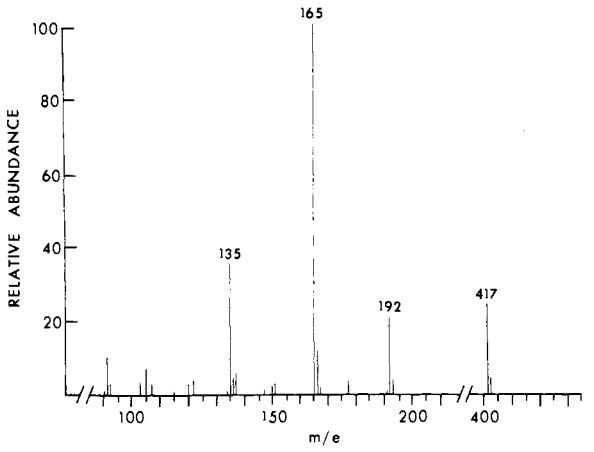
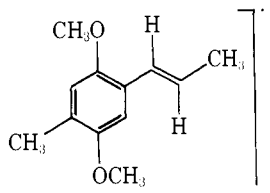
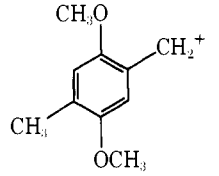
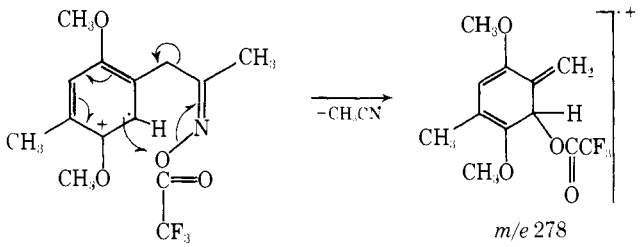


Figure 1.

tabolites of **5**, and **12** is also a chemical (air) oxidation product of hydroxylamine **8** (see below). Gas chromatography–mass spectrometry (GC–MS) of 8-TFA gave a simple mass spectrum shown in Figure 1. The spectrum is interpreted as follows.

m/e	structure
417	molecular ion
192	
165	
135	$[165 - CH_2O]^+$

The EIMS of **13** is shown in Figure 2. The molecular ion is not discernible, and the ion of highest *m/e* value is at 278, arising most likely via transfer of the trifluoroacetoxy group to the aromatic ring (eq 2). An analogous rearrange-



(2)

ment of a hydrogen from nitrogen to the phenyl ring with cleavage of the side chain has frequently been observed in the spectra of 2-phenylethylamines and related compounds.[26] The ion of *m/e* 278 then fragments as shown in Scheme II (* refers to metastables observed).

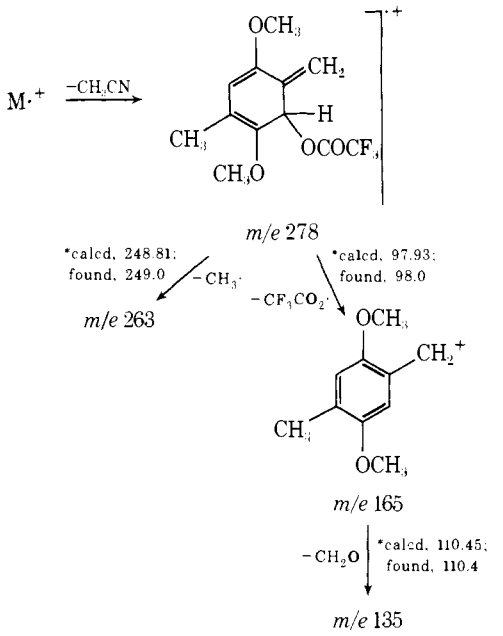
Attempts to obtain a chemical ionization[27] mass spectrum (CIMS) of 8-TFA were frustrated by a reductive N–O bond cleavage occurring under CI conditions. We have found[28] that this process seriously interferes with the CIMS analysis of *N*-hydroxy compounds.

Table II. Stability of 8 under Various Incubation Conditions[a]

Run no.	Microsomes	NADPH	Atmosphere	Compd recovered[b]
1		No	Air	Oxime
2		Yes	Air	Oxime
3		No	Argon	Hydroxylamine[c]
4	Viable	No	Air	Hydroxylamine
5	Boiled	No	Air	Hydroxylamine[d]

[a] See Experimental Section for details of incubation. [b] Oxime = 12, hydroxylamine = 8. [c] Ca. 15% oxime was formed, probably from residual dissolved oxygen. [d] Ca. 5% oxime formed.

Scheme II. Interpretation of the EIMS of **13**



Metabolism. The behavior of 8 under conditions of incubation and work-up was first examined since there are scattered reports[2a,e,5] in the literature on the instability of aliphatic hydroxylamines. The results of these experiments are shown in Table II. It was found that the *N*-hydroxy compound 8 was cleanly and completely converted to the corresponding oxime **12** by shaking under air at 37° in a pH 7.4 phosphate buffer (Table II, run no. 1). The oxime was identified by comparison of the GLC retention time (Table I) and EIMS (Figure 2) and CIMS of its TFA derivative **13** to those of an authentic sample. This oxidation also occurs in the presence of NADPH (run no. 2) but not in the absence of air (run no. 3). Surprisingly, the presence of rabbit liver microsomes completely abolishes this oxidation (run no. 4). Even microsomes deactivated by boiling provided 95% protection (run no. 5). In an attempt to demonstrate the chemical conversion of *N*-hydroxyamphetamine (**4**) to phenyl-2-propanone oxime (**2**) Beckett and Al-Sarraj found[5] that **4** was oxidized to **2** when shaken in air for 20 hr in aqueous solution at pH 12 in the presence of liver microsomal preparations. These conditions are much more drastic than those used for normal incubations. In fact, while 8 is stable at pH 7.4 in the presence of rabbit liver microsomes, when shaken at pH 12 for 20 hr in the presence of microsomes 8 was found to have undergone extensive decomposition. Among the products identified by GC–MS were oxime **12** and ketone **14**. Admittedly, the stability of 8

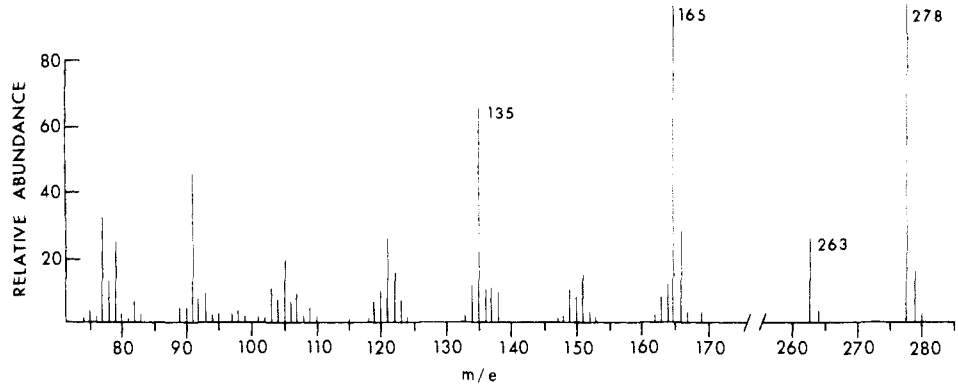


Figure 2.

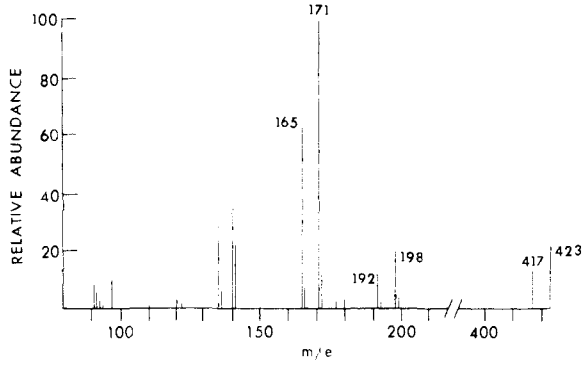


Figure 3.

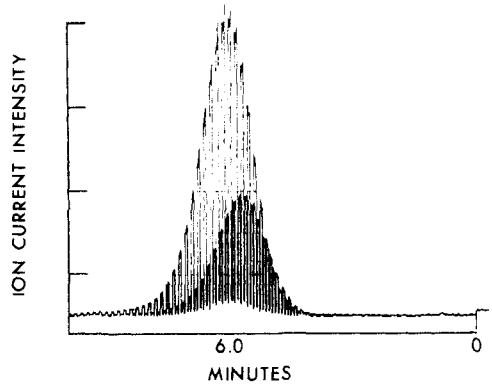


Figure 4.

is not necessarily similar to that of *N*-hydroxyamphetamine (**4**) and therefore no direct comparison should be made. However, our experiments do suggest that the stability of *N*-hydroxyamphetamines is pH dependent, and conclusions[2a,c,5a] regarding the stability of these compounds in microsomal incubation mixtures at pH 7.4 based on stability studies[5a] at pH 12 should be viewed with caution.

Incubations of **5** were carried out using rabbit liver microsomes fortified with magnesium chloride and NADPH in phosphate buffer at pH 7.4. TFAA-derivatized extracts from incubations with viable liver preparations showed a GLC peak with the retention time identical with that of authentic 8-TFA (Table I). The peak was absent when extracts from boiled microsomal incubations were analyzed. In order to confirm the identity of the metabolite, GC–MS analysis was performed. The mass spectrum for the GLC peak with retention time of 8-TFA was identical with that of an authentic sample (Figure 1). Incubation of **5**-d_6,[9] a hexadeuterio analog of **5**, followed by the addition of authentic unlabeled synthetic **8** as internal standard after 1 hr of incubation, and the usual work-up, gave the EIMS shown in Figure 3. The spectrum shows the presence of both 8-TFA (internal standard) and 8-d_6-TFA. The spectrum obtained from incubation with boiled microsomes showed only the presence of 8-TFA, confirming the enzymatic nature of the N-hydroxylation reaction. By scanning the mass spectrum several times during the emergence of the GLC peak composed of a mixture of 8-TFA and 8-d_6-TFA, it was found that the composition of the GLC effluent stream was changing continuously, indicating in fact that the deuterium-labeled compound 8-d_6-TFA had a slightly shorter retention time than the nonlabeled analog 8-TFA. Such isotope fractionation was also observed for the isotopic pair 5-TFA and 5-d_6-TFA. The ability of GLC columns to partially resolve some isotopically labeled compounds from their nonlabeled analogs has been amply demonstrated.[29] This phenomenon precludes the determination of the composition of the GLC effluent of an isotopic mixture by a single mass spectrum and has resulted in the introduction of selected ion monitoring methods[30] in GC–MS. By using a modified accelerating voltage alternator (AVA) system developed for our AEI MS 12 mass spectrometer,[31] we were able to measure the ion currents at two selected *m/e* values. This is accomplished by rapidly switching the accelerating voltage between two fixed values while holding the magnetic field constant so as to bring the desired ions into focus.

Using the GC–MS system in the AVA mode the ratio of 8-TFA/8-d_6-TFA could be determined by monitoring the GLC effluent for the parent ions at *m/e* 417 and 423. A typical output of such an analysis is shown in Figure 4. The difference in retention time between the deuterium-labeled and unlabeled compound is clearly visible in Figure 4. The peak heights[§] were used to calculate the ratio 8-TFA/8-d_6-TFA. This technique was used to quantify the hydroxylamine metabolite 8-d_6 formed in incubations of **5**-d_6 with rabbit liver microsomes.

The amount of **5**-d_6 metabolized was determined by adding **5** as internal standard at the end of the incubation and comparing the parent ion intensities of **5** and **5**-d_6 in the CIMS. The results are shown in Table III. When equal amounts of **5** and **5**-d_6 were incubated no apparent isotope effect was found in the formation of *N*-hydroxy metabolites **8** and 8-d_6.

In order to examine the steric course of the N-hydroxylation of **5** rabbit liver microsomes were incubated with mixtures of (*S*)-**5** and (*R*)-**5**-d_6 and alternatively with (*R*)-**5** and (*S*)-**5**-d_6.[9,10] The difference in the isotopic content of the enantiomers in each mixture permits the use of the GC–MS–AVA system to determine the enantiomeric com-

[§] Since synthetic 8-d_6 was not available no standard curve was obtained, and the values obtained in these experiments (Tables III and IV) are accurate to only about ±10%.

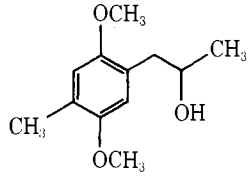
Table III. Quantification of N-Hydroxylation of 5-d_6

Run	Concn of 5-d_6, μM[a]	% 5-d_6 metabolized[b,d]	% metabolized 5-d_6 appearing as 8-d_6[c,e]
1	179	82	5.9
2	176	98	7.9

[a] On three times the scale described in the Experimental Section. [b] No metabolism occurred when boiled microsomes were used. [c] No 8-d_6 was found when using boiled microsomes. [d] Internal standard 5·HCl was added in amounts equivalent to original substrate 5-d_6. [e] Internal standard 8·HCl (10 mol % of original substrate 5) added.

position of the *N*-hydroxy metabolite. These results are summarized in Table IV. More of the *N*-hydroxy compound derived from *R* substrate is seen in all cases. Whether this is the result of faster N-hydroxylation of the *R* substrate and/or of preferential further metabolism of the *S* metabolite cannot be determined from the data presented.

Oxime **12** was not detected by GLC in these incubations. This is consistent with the observation[10] that **12** is not found in the urine of rabbits treated with amine **5**, and with the report[9] that **12** is not formed in incubations of rabbit liver 10,000g supernatant fraction with **5**. On the other hand, ketone **14** and its reduction product, the carbinol **15**,



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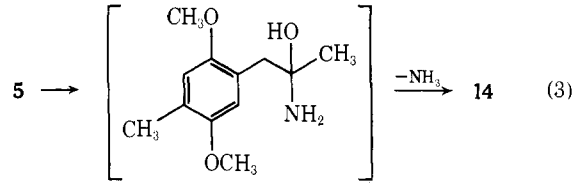
have been identified in incubations of **5** with rabbit liver microsomal[32] or 10,000g supernatant[9] fractions. The experimental results summarized in Table II show that oxime **12** is not *chemically* converted to ketone **14** under our incubation conditions. Enzymatic conversion of the oxime **12** or of the hydroxylamine **8** to the ketone **14** is yet to be demonstrated. Hucker et al.[3b] have reported that phenyl-2-propanone oxime (**2**) is extensively metabolized by rabbit liver 9000g supernatant preparations to phenyl-2-propanone (**3**) and its reduction product, 1-phenyl-2-propanol. These products were not found when boiled liver preparations were used. Alternatively, ketone **14** could arise, at least in part, by "α-hydroxylation" (eq 3) as has been suggested[2c,33]



(3)

by some authors for the metabolic formation of phenyl-2-propanone from amphetamine.

The in vivo formation of the *N*-hydroxy compound **8** from **5** and its relationship to the psychotomimetic activity of **5** are yet to be studied. The pharmacological and toxicological implications of the metabolic N-hydroxylation of aliphatic amines are beginning to attract attention.[34]

Experimental Section

Melting points are uncorrected. NMR spectra were taken on a Varian Associates A-60A spectrometer and chemical shifts are reported in parts per million (δ) downfield relative to Me_4Si as internal standard. EIMS were obtained on an AEI MS 12 spectrometer at 50 eV. GC–MS analysis was carried out with the same mass spectrometer interfaced with a Biemann-Watson molecular separator to an Infotronics Model 2400 gas chromatograph using a U-shaped 6 ft × 0.25 in. × 2 mm i.d. glass column packed with 2% Dexyl 300 on 80–100 Chromosorb G H.P. The retention times of 8-TFA and of **13** on this system are 6.0 and 3.5 min, respectively, at 160°, N_2 30 ml/min. AVA analyses were obtained with the above GC–MS system modified for AVA operation.[31] CIMS were obtained on an AEI MS 902 instrument modified for CIMS and using isobutane (0.7 Torr) as reactant gas. GLC analyses were performed either on a Varian Aerograph 2100 Life Sciences gas chromatograph equipped with a hydrogen flame ionization detector and using 6 ft × 0.25 in. × 2 mm i.d. glass columns packed with 3% OV 25 on acid washed DMCS-treated Chromosorb W, 100–120, or on a F and M Model 402 gas chromatograph equipped with a hydrogen flame ionization detector and using a U-shaped 4 ft × 0.25 in. × 2 mm i.d. glass column packed with 3% OV 1 on acid-washed, DMCS-treated Chromosorb W, 100–120. Incubations were carried out on a Dubnoff metabolic shaking incubator at 37° shaking 120 per minute. Elemental analyses were performed by the Microanalytical Laboratory of the University of California, Berkeley.

1-(2,5-Dimethoxy-4-methylphenyl)-2-nitropropane (11). Sodium borohydride (1.5 g, 39.7 mmol) was dissolved in ethanol (50 ml) and the solution placed in a single-neck, round-bottom 500-ml flask. The solution was cooled in ice, stirred magnetically, and kept under nitrogen while 1-(2,5-dimethoxy-4-methylphenyl)-2-nitro-1-propene[13] (**10**, 4.25 g, 17.8 mmol) dissolved in a mixture of ethanol (50 ml) and THF (50 ml) was added slowly dropwise over 4 hr. The flask was then stored overnight in a refrigerator. The flask was then cooled in an ice bath while a solution of urea (6 g) in acetic acid (13 ml) and water (20 ml) was added dropwise to the magnetically stirred solution. Water (25 ml) was added and the solution extracted with hexane (6 × 50 ml); the combined hexane extracts were washed with 5% $NaHCO_3$ (50 ml) and with water (5 × 50 ml), dried (Na_2SO_4), and evaporated (rotary evaporation). The residue was sublimed (bath 50°, 0.5 mm) to give 3.70 g (87.0%) of **11**: mp 56–58°; NMR ($CDCl_3$) δ 1.53 (d, 3, J = 6 Hz, $CHCH_3$), 2.22 (s, 3, $ArCH_3$), the AB portion of an ABX pattern with ν_A = 3.07, ν_B = 3.27, J_{AB} = 13.5 Hz, $J_{AX} = J_{BX}$ = 7 Hz ($ArCH_2$), 3.82 (s, 3, OCH_3), 3.85 (s, OCH_3), 3.98 (sx, 1, CH_3CH), 6.73 (s, 1, ArH), 6.82 (s, 1, ArH); EIMS *m/e* (rel intensity) 239 (60, M), 208 (6.2), 193 (85), 192 (100), 178 (59), 177 (29), 165 (58), 163 (10), 149 (18), 135 (43), 91 (35). An analytical sample was prepared by resublimation. Anal. ($C_{12}H_{17}NO_4$) C, H, N.

1-(2,5-Dimethoxy-4-methylphenyl)-2-hydroxyaminopropane (8) Hydrochloride. A mixture of nitropropane **11** (2.4 g, 10 mmol), ammonium chloride (1.0 g, 19 mmol), THF (13 ml), and H_2O (13 ml) was stirred magnetically under nitrogen and cooled in ice while zinc powder (1.5 g, 22 mmol) was added in small portions over 20 min. The reaction was monitored by GLC as follows. A 0.1-ml aliquot of the reaction mixture was extracted with benzene (0.1 ml) in a small vial. The benzene layer was concentrated to half its volume, TFAA (50 μl) was added, and the solution analyzed by GLC under conditions described in the Analytical Section. The contents of the flask, cooled in an ice bath, were stirred under nitrogen for 40 min after the last portion of zinc had been added. Benzene (35 ml) was then added, the mixture stirred for 5 min,

Table IV. Steric Course of N-Hydroxylation of 5 and 5-d_6

Substrates (concn, μM)	% substrate metabolized[c,d]	Enantiomeric *R*/*S* ratio of *N*-hydroxy metabolite[e]
(*S*)-5-d_6 (88.0)[a]	96	6.3
(*R*)-5-d_0 (95.5)[a]	93	
(*S*)-5-d_6 (64.8)[b]	100	4.5
(*R*)-5-d_0 (66.6)[b]	70	
(*S*)-5-d_0 (92.8)[a]	95	2.4
(*R*)-5-d_6 (91.0)[a]	68	

[a] On three times the scale described in the Experimental Section. [b] On five times the scale described in the Experimental Section. [c] 5-d_3[8] (~100 mol % of original substrate) internal standard. [d] No metabolism occurred when boiled microsomes were used. [e] No N-hydroxylated metabolites found when using boiled microsomes.

and the mixture filtered. The solids obtained were washed with benzene (3 × 10 ml) and the washings added to the filtrate. The filtrate, consisting of an organic and an aqueous phase, was separated and the aqueous layer washed with benzene (2 × 10 ml). The combined organic layers were washed with water (2 × 20 ml), dried (Na_2SO_4), and evaporated. The residue was taken up in benzene (100 ml) and dry hydrogen chloride was bubbled into the solution. The precipitated 8 hydrochloride (1.6 g, 58%) was shown by GLC to contain ca. 3% of **5** hydrochloride. Recrystallization from ethanol–ether gave a first crop (0.9 g, 33%) of 8 hydrochloride of >99% purity (GLC): mp 126–128° (lit.[13] mp 121–123°). A second (150 mg) and third (210 mg) crop contained 2–5% of **5** hydrochloride.

Derivatization with TFAA. The compound to be derivatized was dissolved in excess TFAA and the solution allowed to stand for 10 min at room temperature before GLC analysis was performed. Excess TFAA served as solvent.

Microsomal Preparations. One male 6-month-old Dutch rabbit was used for each experiment in Tables III and IV. The animal was killed by blows to the neck. The liver was removed and washed in cold isotonic KCl. Liver tissue (10 g) was minced in 30 ml of cold isotonic KCl solution and homogenized using a Potter-Elvehjem Teflon pestle homogenizer. The homogenate was centrifuged at 10,000g in a refrigerated centrifuge at 3° for 20 min. The resulting supernatant was centrifuged at 3° at 94,000g for 1 hr. The microsomal pellet obtained was suspended in ice-cold isotonic KCl to make a total volume of 10 ml. Each milliliter of this suspension contained 12–20 mg of protein as determined by the Lowry[35] method.

Incubations. Incubation mixtures contained, for each milliliter of microsomal suspension from above, 1 ml of 37.5 m*M* $MgCl_2$ solution, 1 ml of 0.6 *M* potassium phosphate buffer (pH 7.4), and 0.3–0.6 μmol of substrate, for a total volume of 3 ml. NADPH (12 mg) was added in three 4-mg portions at time = 0, 20, and 40 min during the incubations. In order to obtain a sufficient amount of metabolites, incubations were run on a larger scale than described above, as indicated in Tables III and IV. After 1 hr of incubation the flasks were cooled in ice, the internal standards were added (see Tables III and IV), and the mixture was extracted with twice the volume of benzene. The benzene extracts were evaporated in a nitrogen stream at room temperature, and the residue was treated with 2 ml of TFAA. The solution was again evaporated to dryness and 50–100 μl of TFAA was added, followed by GLC or GC–MS analysis. In order to determine the amount of substrate recovered the aqueous phase from the above extraction was made strongly basic (pH 12) and the solution extracted with ether. The ether solution was evaporated and the residue subjected to CIMS analysis. Values of ion intensity ratios obtained from AVA or CIMS analysis (Tables III and IV) were the average of at least three scans.

Stability Studies on 8. Hydroxylamine (8) hydrochloride (500 μg), 2 ml of 37.5 m*M* $MgCl_2$ solution, potassium phosphate buffer (0.6 *M*, 1 ml, pH 7.4), and either 1.15% KCl solution (2 ml) or microsomal suspension (2 ml, see Table II) were incubated for 1 hr at 37° under the atmosphere given in Table II. NADPH (10 mg) was added in some cases. At the end of the incubation, the mixtures were worked up as described above and analyzed by GLC or GC–MS.

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