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INHIBITION OF RABBIT NASAL AND HEPATIC CYTOCHROME P-450-DEPENDENT HEXAMETHYLPHOSPHORAMIDE (HMPA) *N*-DEMETHYLASE BY METHYLENEDIOXYPHENYL COMPOUNDS*

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Abstract—Eighteen methylenedioxyphenyl (MDP) compounds, including some commonly inhaled by people, were tested for the ability to inhibit rabbit nasal microsomal cytochrome P-450-dependent hexamethylphosphoramide (HMPA) *N*-demethylase. For comparison, liver microsomes were also used. Nasal cytochrome P-450 from rabbits metabolized MDP compounds to form cytochrome P-450–metabolite (P-450–MI) complexes as indicated by difference spectra in the Soret region. Several of the MDP compounds were potent inhibitors of nasal P-450-dependent *N*-demethylase. If inhibition of nasal P-450 also occurs *in vivo* after inhibiting MDP compounds are inhaled, the metabolism of concurrently or subsequently inhaled compounds may be altered.

The nose protects the lungs from airborne toxicants, in addition to its important olfactory function. Perhaps necessary for these functions, the nasal mucosa contains high concentrations of cytochrome P-450-dependent enzyme activities [1–3]. These activities are particularly high in the olfactory tissue, where they may be important in the removal of deposited odorants, thereby preventing confusion of the sense of smell by a high background of odorants. However, some compounds may increase in toxicity after oxidization by nasal cytochrome P-450. For example, nitropyrene has been noted to be rapidly oxidized to potentially toxic compounds by homogenates of nasal tissue [4], and a variety of inhalable compounds are metabolized by nasal microsomal cytochrome P-450 to formaldehyde [5]. The carcinogenicity of hexamethylphosphoramide (HMPA) has been described as possible due to the release of metabolic formaldehyde in the nasal cavity [2].

Because the nasal cytochrome P-450-dependent activities may be important in the metabolism of airborne toxicants and in the maintenance of acuity in the sense of smell, the inhibition of these activities could have important effects. In view of the potential effects of nasal cytochrome P-450 inhibition, we investigated methylenedioxyphenyl (MDP) compounds that are readily inhalable potential inhibitors of the nasal cytochromes P-450.

Methylenedioxyphenyl compounds have long been known to be insecticide synergists. One member in particular, piperonyl butoxide, is commonly used as a synergist with pyrethrins in household aerosol sprays. Other MDP compounds are found in pepper, nutmeg, sassafras oil, and a variety of other foods and spices. Examples are isosafrole, safrole, dihydrosafrole and piperonal, which occur in essential oils or are added (piperonal) to foods as flavoring agents. Isosafrole, safrole, and dihydrosafrole are weak carcinogens.

The MDP compounds are well-known inhibitors of liver P-450, but only one such compound, piperonal, has been reported to be an effective inhibitor of nasal P-450 [6]. Piperonal was not effective as an inhibitor of liver P-450 in that study, indicating that the inhibition of nasal P-450 by MDP compounds could not be accurately predicted from studies with liver microsomes.

The main purpose of these experiments was to identify effective inhibitors of nasal cytochrome P-450 *in vitro* so that these can be tested at a later time for *in vivo* effects.

MATERIALS AND METHODS

Chemicals. Eighteen MDP compounds (Table 1) were screened initially for spectrum formation with nasal microsomal cytochrome P-450. The effect of added carbon monoxide on the spectra was also examined. All of the tested chemicals were purchased from the Aldrich Chemical Co., Milwaukee, WI, with the exception of the following: piperonyl butoxide was purchased from the McClaughlin, Gormley, King Co., Minneapolis, MN; isosafrole was purchased from Eastman Kodak, Rochester, NY; dihydrosafrole was a gift from the ArChem Co.,

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Houston, TX; and U.S.P. grade metyrapone was a gift from the College of Pharmacy, University of New Mexico. Hexamethylphosphoramide (HMPA) was purchased from the Sigma Chemical Co., St. Louis, MO, as were all of the chemicals used for preparations of buffers and reagents in the demethylation assays. Sodium dithionite was purchased from the J. T. Baker Co., Phillipsburg, NJ. Unless noted otherwise, all chemicals were of the highest purity available from the supplying company.

Rabbits and preparation of microsomes. Male New Zealand white rabbits were supplied by Bell Breeding Ranch, Clovis, NM. After shipment, the rabbits were quarantined for 2 weeks on a 12-hr light:dark cycle at 68–72°F and at 40–60% relative humidity. Water bottles were changed daily, and food (Purina Rabbit Chow, Allied Mills, Chicago, IL) was allowed *ad lib.* After the 2-week quarantine period, the rabbits were certified healthy by a veterinarian. They weighed 2.5 to 4 kg. The rabbits were killed, their nasal tissue and livers were removed, and microsomes were prepared as previously described [3]. The microsomes from the nasal tissues of fifty rabbits were pooled, placed in 20% glycerol/Tris buffer (pH 7.4), gassed with nitrogen, frozen in liquid nitrogen, and stored at −75°. The concentration of nasal cytochrome P-450 in the microsomes was about 5 nmoles cytochrome P-450/ml. Portions of the livers from the rabbits were pooled and microsomes were prepared and stored as described above for the nasal microsomes.

Spectra. Difference spectra were obtained using an Aminco DW-2A spectrophotometer. For initial screening, the nasal microsomal cytochrome P-450 concentration was 0.77 μM, as verified by the carbon monoxide–cytochrome P-450 spectrum using an extinction coefficient of 91 mM^{-1} cm^{-1}. Potential inhibitors to be tested were dissolved in ether to a concentration of 700 mM, with the following exceptions: piperine, 3,4-methylenedioxy aniline and 1,2-methylenedioxy-4-nitrobenzene were dissolved in 95% ethanol. Piperonyl nitrile, piperonylic acid, 1-piperonyl piperazine and 3,4-methylenedioxy cinnamic acid were not sufficiently soluble in ether, water, or ethanol for our purposes, so these materials were added neat to the cuvettes in estimated amounts of 1–3 mg each. For the soluble compounds, 10 μl of the 700 mM solution was added to the sample cuvette. An equal volume of solvent was added to the reference cuvette, and a ferric cytochrome P-450 difference spectrum was obtained. NADPH was added to both cuvettes to a concentration of 10 mM, and the spectrum was recorded repetitively until no further changes were observed (2–10 min). Approximately equal amounts of sodium dithionite were added to the sample and reference cuvettes, and a ferrous cytochrome P-450 difference spectrum was obtained. For each ligand both the reference and the sample cuvette were saturated with carbon monoxide. If the spectrum obtained included a trough at 450 nm, the magnitude was compared to the 450 nm peak for the carbon monoxide difference spectrum of the microsomes alone. The ratio was used as an indication of the ability to inhibit carbon monoxide binding [7].

Ligand concentration versus absorption. To obtain the spectra in panels A and B of Fig. 2, and similar spectra described in the text, 0.7, 3.5, 17.5, and 87.5 mM solutions of the appropriate ligands were dissolved in ether, and 5 μl of one of these solutions was added to fresh, untreated microsomes in the sample cuvette (5 μl of ether was added in the reference cuvette), and the ferric cytochrome P-450 difference spectrum was recorded. NADPH was then placed into each cuvette to a concentration of approximately 10 mM. The difference spectrum of the metabolizing microsomes was then scanned until the peaks at approximately 455 nm and 422 nm remained stable. Contents of both cuvettes were then treated with approximately equal amounts of dithionite, and the ferrous cytochrome P-450 difference spectrum was recorded.

Assays for inhibition of HMPA N-demethylase. The apparent best inhibitors of carbon monoxide binding (Table 1) were selected for further study, except that piperonyl butoxide was included because of its use as an insecticide synergist, whereas piperonyl isobutyrate, piperonylic acid, and 3,4-methylenedioxy cinnamic acid were excluded. The selected potential inhibitors are shown in Table 2. For each, ether solutions were prepared so that 5 μl added to 2 ml of buffer would give the concentrations listed in Table 2. The N-demethylation assay measured formaldehyde production [2]. An NADPH-generating system was not used. Instead, the suspension of microsomes was made 10 mM in initial NADPH concentration. The concentration of nasal cytochrome P-450 was 0.19 μM in 2 ml final assay volume. Microsomes, inhibitor and NADPH were incubated at 37° for 5 min before the addition of HMPA in 0.1 M Tris–HCl buffer, pH 7.4. For each assay, a control containing HMPA but no potential inhibitor and one containing the highest concentration of inhibitor but no HMPA were used. Inhibition was expressed as a percentage of the HMPA-only control value. Control activity was 43.65 ± 5.26 (S.D.) nmoles formaldehyde per nmole P-450/min. Incubation after addition of HMPA was for 20 min. Initial [HMPA] was 2 mM. Appropriate standards and blanks containing heat-denatured microsomes were used.

The best inhibitors of nasal cytochrome P-450-dependent HMPA *N*-demethylase (Table 2) were tested with liver microsomes in a manner identical to that used with the nasal microsomes, except that the liver cytochrome P-450 concentration for each assay was 1.57 μM. Results are included in Table 2. The control rate of formaldehyde production from HMPA for liver microsomes was 6.58 ± 0.57 (S.D.) nmoles of formaldehyde per nmole P-450/min.

Determination of 50% inhibition (I_{50}) values. The best of the MDP nasal enzyme inhibitors (Table 2) were further tested to obtain accurate I_{50} values. A double-reciprocal plot used to obtain I_{50} is illustrated in Fig. 2 for isosafrole. For the remaining MDP compounds, similar plots were made after assays with inhibitor concentrations at 2, 4, 8, and 12 μM. In all cases, solutions were made in ethanol so that 5 μl of the appropriate solution added to 2 ml final volume would give the desired concentration. Linear regression analyses were carried out on the double-reciprocal plots. The correlation coefficients (r) and

Table 1. Rabbit nasal microsomal cytochrome P-450–MDP and –MDP metabolite spectra and carbon monoxide (CO) binding inhibition by MDP metabolites*

Methylenedioxyphenyl (MDP) compounds	Ferric P-450–MDP spectrum†	Ferrous P-450–MDP metabolite spectrum†	Inhibition of CO binding (%)
1,3-Benzodioxole‡	I	III	23
Dihydrosafrole‡	I	III	45
Isosafrole‡	I	III	43
3,4-Methylenedioxy aniline	None§	III (small)‖	0
3,4-Methylenedioxy cinnamic acid	I	III	24
3,4-Methylenedioxyphenyl acetonitrile‡	I	III	41
1,2-Methylenedioxy-4-nitrobenzene	I (small)‖	III (small)‖	0
Piperine	Peak at 350 nm	None§	12
Piperonal‡	I (small)	III	33
Piperonyl alcohol‡	II-A	III	27
Piperonyl amine	II	Denatured?	
Piperonyl butoxide‡	Mix¶	III	13
Piperonyl isobutyrate	I	III	32
Piperonyl nitrile	I	I	8
1-Piperonyl piperazine	II	III	0
Piperonylic acid	I	III	18
Safrole‡	I	III	25
Sesamol	I	P-450–CO spectrum**	

* See Materials and Methods for experimental details.

† Spectra types have the following approximate maxima (max) and minima (min); I, 385–390 nm (max) and 420 nm (min); II, 420–435 nm (max) and 390–410 nm (min); II-A, 420 nm (max) and 395–590 nm (min); III, 430 and 455 nm (max).

‡ Compounds selected for further study.

§ None detected.

‖ $\Delta A < 5\%$ of P-450–CO spectrum at 450 nm.

¶ Neither clear type I nor type II.

** An apparent type III on NADPH addition changed to P-450–CO spectrum on dithionite addition.

slopes of the line, as well as I_{50} values, are reported in Table 3.

Studies of types of inhibition. To obtain the data in Fig. 3, incubation mixtures of 0.133 μM in cytochrome P-450 in 2 ml (final assay volume) of 0.1 M Tris buffer (pH 7.4) were made 5 μM in isosafrole and incubated for 5 min at 37°. Solutions of HMPA in the same buffer were added so that final HMPA concentrations of 0.5, 1, 2, 3, 5, or 10 μM were attained. The assay mixture was then incubated with

Table 2. Inhibition of rabbit nasal and liver cytochrome P-450-dependent HMPA *N*-demethylase by selected MDP compounds, and metyrapone through wide concentration ranges (numbers in parentheses are for liver P-450)

	% Inhibition			
	Concentration* (μM)			
Compounds	5	50	500	5000
MDP compounds				
Isosafrole	67 (1)	94 (34)	98 (80)	
Piperonal	64 (21)	80 (40)	94 (58)	94
Dihydrosafrole	57 (5)	88 (22)	99 (63)	99
Piperonyl butoxide	53 (11)	74 (32)	88 (64)	94
Safrole	48† (15)	91 (38)	99 (83)	100
3,4-MDP acetonitrile	40† (13)	81	91 (48)	93
1,3-Benzodioxole	20†	51	90	95
Piperonyl alcohol	18	58	85	99
Metyrapone	25	65	93	91†

* Cytochrome P-450 concentrations were 0.19 and 1.57 μM for nasal and liver enzymes respectively. Initial [HMPA] was 2 mM. See Methods for additional experimental details. Standard deviations (S.D.) were $< 5\%$ (N = 2) except where noted.

† $5\% < \text{S.D.} < 10\%$ (N = 2).

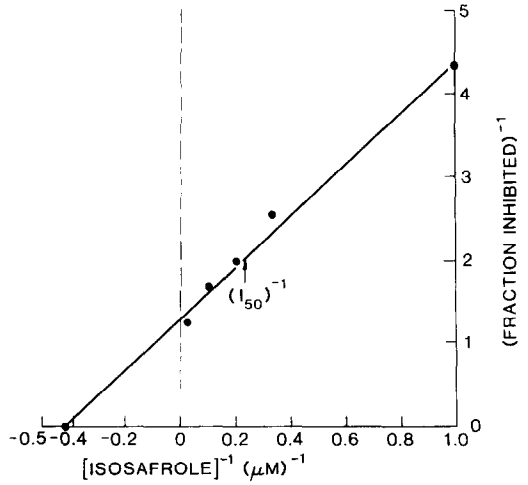

Fig. 1. Double-reciprocal plot used to determine the isosafrole 50% inhibition concentration (I_{50}) of rabbit nasal microsomal HMPA *N*-demethylase. [P-450] was 0.19 μM. Initial [HMPA] was 2 mM. See Materials and Methods for additional experimental details. Line parameters are: r = 0.993; m = 3.1.

shaking for 20 min at 37°. Controls containing no isosafrole were also run. Linear regression analyses, assuming a combination of two straight lines, were used to determine fits for the lines shown in these figures.

RESULTS

Table 1 contains a summary of spectral data with nasal cytochrome P-450 and MDP compounds.

Table 3. I_{50} values of rabbit nasal microsomal cytochrome P-450-dependent HMPA *N*-demethylase for selected inhibitors and associated parameters for the double-reciprocal plots*

Inhibitor	I_{50}† (μM)	Reciprocal plot parameters	
		r	Slope
Piperonal	2.4	0.998	1.5
Isosafrole	4.4	0.993	3.1
Dihydrosafrole	6.2	0.972	6.7
MDP acetonitrile	6.4	0.995	8.5
Safrole	7.0	0.999	5.7
Piperonyl butoxide	10.6	0.991	13.8

* See Methods for experimental details.

† The I_{50} values are the concentration of inhibitor which inhibits HMPA *N*-demethylase 50% as calculated from the double-reciprocal plots (1/fraction inhibited vs 1/[I]) (cf. Fig. 1).

Twelve of the eighteen MDP compounds tested formed metabolite inhibitor (MI) complexes [8] after incubation with NADPH, which appeared to inhibit more than 10% of the carbon monoxide binding. The ferric cytochrome P-450 spectra of the MDP compounds were generally those expected from examination of the functional groups present. For dithionite-reduced ferrous cytochrome P-450-inhibitor metabolite spectra, all but one of the twelve best inhibitors of carbon monoxide binding formed type III spectra. The exception was the pepper constituent, piperine. Conversely, all but one of the twelve compounds that formed large type III spectra also were good inhibitors of carbon monoxide binding.

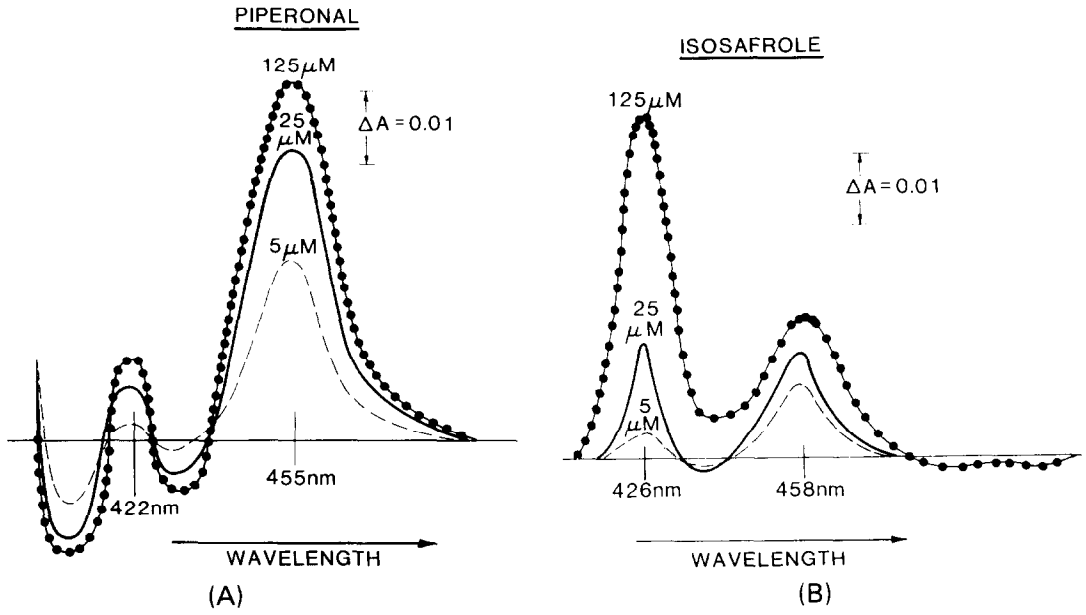

Fig. 2. (A) Rabbit nasal cytochrome P-450-MI difference spectrum for piperonal. (B) Rabbit nasal cytochrome P-450-MI difference spectrum for isosafrole. Dithionite was added after incubation with NADPH. Concentrations refer to concentrations of MDP compounds. [P-450] was 0.77 μM.

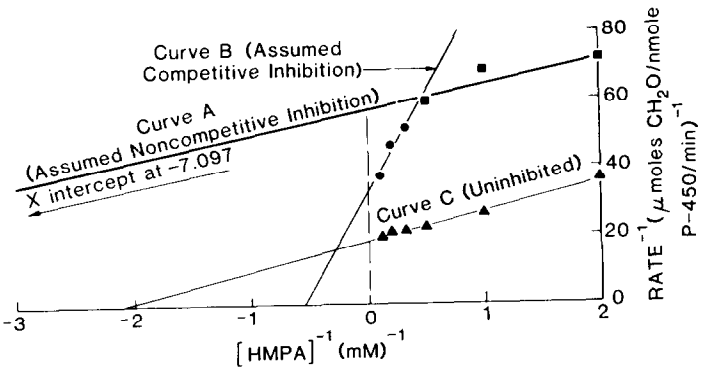

Fig. 3. Double-reciprocal plots used to examine the effect of HMPA concentration on the inhibition of rabbit nasal HMPA *N*-demethylase in the presence of 5 μM isosafrole. [P-450] was 0.133 μM. See Materials and Methods for additional experimental details. Line parameters are: curve A, $r = 0.895$, $m = 5.5$; curve B, $r = 0.971$, $m = 43.1$; and curve C, $r = 0.993$, $m = 6.5$.

The exception, 1-piperonyl piperazine, formed a type III spectrum but did not inhibit carbon monoxide binding. Of the eleven compounds forming MI complexes that were not displaceable by carbon monoxide, eight were chosen for further study.

Sesamol is unique among the compounds studied. On the addition of NADPH to nasal microsomes in the presence of sesamol, an apparent type III spectrum formed. This is not what occurs with liver microsomes where an oxidized minus reduced type spectrum has been observed [9], which has been explained as possibly the result of benzoquinone formation [7]. As with liver microsomes, a spectrum typical of the carbon monoxide–P-450 complex formed when dithionite was added.

In determining the extent of inhibition of HMPA *N*-demethylase activity over a wide range of concentrations (Table 2), virtually all of the compounds examined were effective inhibitors at 5000 μM. This is possibly due to competitive inhibition, since 5000 μM exceeded the concentration of the HMPA. At 5 μM, the MDP compounds were of two types, six good inhibitors (≥ 40% inhibition) and two poor inhibitors (≤ 20% inhibition). For purposes of comparison, a known nasal microsomal cytochrome P-450 inhibitor, metyrapone [10], was tested under the conditions used to obtain the data in Table 2. At 5 μM it inhibited nasal HMPA *N*-demethylase by 25%.

In Table 2 are compared nasal HMPA *N*-demethylase inhibition and liver HMPA *N*-demethylase inhibition by the most potent nasal inhibitors. For all the compounds studied, the nasal enzyme activity was inhibited more than was that of liver. This greater susceptibility was particularly noticeable at lower concentrations of inhibitors.

The I_{50} values for rabbit nasal microsomal HMPA *N*-demethylase (Table 3) were determined from plots like those in Fig. 1 for the six best inhibitors from Table 2. Such plots were used because the usual plots of inhibition versus log of the inhibitor concentration gave lines with steep slopes not amenable to accurate measurement of I_{50}. Moreover, since inhibition depended on prior metabolism of the inhibitor, it seemed reasonable to plot inhibitor (substrate) concentration as the reciprocal, thereby taking advantage of the ease of computation of the 50% effective concentration offered by such plots. The correlation coefficients were good for such lines (Table 3), possibly indicating the appropriateness of this method at the low concentrations of inhibitor for which it was used. The I_{50} values for the MDP compounds were all less than 10 μM, except for piperonyl butoxide (10.55 μM).

Two of the spectral types encountered in this study are illustrated in Fig.2 panels a and b. Although both are type III spectra, there are differences in the ratios of the shorter wavelength peak intensity to the longer wavelength peak intensity. Thus, isosafrole formed an intense short wavelength peak, whereas piperonal had a more intense long wavelength peak.

In Fig. 3 are shown reciprocal plots for uninhibited nasal HMPA *N*-demethylase activity and for activity inhibited by 5 μM isosafrole. The typical "curvilinearity" previously reported for MDP compounds [11] is observed in this plot. At low HMPA concentrations, the isosafrole appears to inhibit by a noncompetitive mechanism. At high HMPA concentrations, a competitive mechanism may be operative. This may indicate that, at low HMPA concentrations, effective displacement of the MI complex formed from isosafrole does not occur, but, as the HMPA concentration is increased, displacement occurs and metabolism rate returns to the level for the uninhibited enzyme.

DISCUSSION

The MDP compounds are known inhibitors of hepatic microsomal enzymes. With liver microsomes, I_{50} values of less than 100 μM are uncommon [9], and we are unaware of any reported I_{50} values less than 10 μM. Methylenedioxyphenyl compounds are common insecticide synergists and microsomes from flies often are inhibited by lower concentrations of MDP compounds than are those from the mammalian liver [11]. In many cases, the reported I_{50} values for fly microsomes were similar to the values we report here for nasal microsomes.

Curvilinearity of double-reciprocal plots, such as

shown for isosafrole in Fig. 3, is typical for inhibition by MDP compounds [11, 12]. We interpret this observation as a combination of noncompetitive inhibition at low HMPA concentrations and competitive inhibition at high HMPA concentrations. This interpretation implies that high HMPA concentrations can displace the isosafrole MI complex. Displacement of such complexes from liver P-450 has been reported [13, 14].

Although the MDP compounds studied here produced type III spectra upon metabolism by nasal microsomes, there was wide variation in shape, as indicated in panels A and B of Fig. 2. Isosafrole produced a much larger short wavelength peak than did piperonal. This may be due to the greater lipophilicity of isosafrole caused by the presence of a propenyl group. Increased lipophilicity correlates with increased MI complex stability for MDP compounds [15]. The shorter wavelength peak of the type III spectrum has been postulated to indicate binding of a ligand to both the heme iron and a lipophilic site on the protein [7]. A large short wavelength peak would be expected, then, to indicate both increased lipophilicity and increased MI complex stability. Our observations support the former expectation, but not the latter, since piperonal appears to form a very stable MI complex, as indicated by its low I_{50} value.

One purpose in studying inhibition *in vitro* was to lay the groundwork for later *in vivo* studies on the effects of these compounds on nasal metabolism. Predictions of *in vivo* effects from *in vitro* data are necessarily tentative, but a general correlation has been established for MDP compounds [13, 16]. The MI complexes of isosafrole and piperonyl butoxide formed *in vivo* can be detected as the isolated enzyme [17] or as the microsomal complex [18] from the livers of treated animals. Whether or not such stable complexes form *in vivo* in the nose is not known and will be the subject of a later investigation. Also the subject of later experiments will be the effectiveness of prolonged inhalation of MDP compound on the induction of nasal cytochrome P-450. Induction of liver cytochrome P-450 by MDP compounds is a well-known phenomenon for which the methylene carbon is a necessary structural component [19].

Calculations* show that, at an air concentration of 25 ppm, sufficient quantities of the six MDP compounds listed in Table 3 would be deposited in a rat's nose with each breath to inhibit 50% of the nasal *N*-demethylase. The percentage of MDP compound that would actually bind the cytochrome P-450 *in vivo* is not known, so the accuracy of this calculation needs to be tested. However, it is clear that concentrations sufficient to bring about extensive inhibition of nasal P-450 *in vivo* should be easily obtainable with these compounds.

In summary, we have identified six potent inhibitors of nasal microsomal HMPA *N*-demethylase. Except for the much greater potency of the compounds studied with regard to nasal cytochrome P-450, the interactions of the MDP compounds with nasal P-450 were similar to interactions with liver P-450.

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* Assuming 2 ml tidal volume 100% nasal deposition, 1 atm and 25° ambient conditions, 0.4 ml fluid volume of the nasal mucosa, and $I_{50} = 5\ \mu M$.

REFERENCES

1. W. M. Hadley and A. R. Dahl, *Toxic. Lett.* **10**, 417 (1982).
2. A. R. Dahl, W. M. Hadley, F. F. Hahn, J. M. Benson and R. O. McClellan, *Science* **216**, 57 (1982).
3. W. M. Hadley and A. R. Dahl, *Drug Metab. Dispos.* **11**, 275 (1983).
4. J. A. Bond, *Mutation Res.* **124**, 315 (1983).
5. A. R. Dahl and W. M. Hadley, *Toxic. appl. Pharmac.* **67**, 200 (1983).
6. A. R. Dahl, *Drug Metab. Dispos.* **10**, 553 (1982).
7. A. R. Dahl and E. Hodgson, *Chem. Biol. Interact.* **27**, 163, (1979).
8. M. R. Franklin, *Pharmac. Ther. (A)* **2**, 227 (1977).
9. E. Hodgson and R. M. Philpot, *Drug Metab.* **3**, 231 (1974).
10. R. W. Rickard and P. J. Gilles, *Toxicologist* **2**, 45 (1982).
11. S. E. Lewis, C. F. Wilkinson and J. W. Ray, *Biochem. Pharmac.* **16**, 1195 (1967).
12. M. R. Franklin, *Biochem. Pharmac.* **21**, 3287 (1972).
13. C. R. Elcombe, J. W. Bridges, T. J. B. Gray, R. H. Nimmo-Smith and K. J. Netter, *Biochem. Pharmac.* **24**, 1427 (1975).
14. M. Dickins, C. R. Elcombe, S. J. Moloney, K. J. Netter and J. W. Bridges, *Biochem. Pharmac.* **28**, 231 (1979).
15. M. Murray, C. F. Wilkinson, C. Marcus and C. E. Dube, *Molec. Pharmac.* **24**, 129 (1983).
16. B. Testa and P. Jenner, *Drug Metab. Rev.* **12**, 1 (1981).
17. D. E. Ryan, P. E. Thomas and W. Levin, *J. biol. Chem.* **255**, 7941 (1980).
18. R. M. Philpot and E. Hodgson, *Chem. Biol. Interact.* **4**, 185 (1971/1972).
19. J. C. Cook and E. Hodgson, *Toxic. appl. Pharmac.* **68**, 131 (1983).