(11) **16b**: colorless crystals; IR ($CHCl_3$) ν 1785 (β-lactam), 1722 (ester) cm^{-1}; NMR ($CDCl_3$) δ 1.8 (br s, NH_2), 3.25 (s, OMe), 4.47–4.60 (C-2 and C-7 H, CH_2OMe), 4.97 (d, J = 4 Hz, C-6 H), 6.98 (s, $OCHPh_2$).

(12) **16c**: IR ($CHCl_3$) ν 1794 (β-lactam), 1723 (ester) cm^{-1}; NMR ($CDCl_3$) δ 1.88 (s, NH_2), 2.67 (s, C-Me), 4.25, 4.55 (AB q, J = 14 Hz, C-2 H), 4.52 (d, J = 4 Hz, C-7 H), 4.68 (s, CH_2-S), 5.00 (d, J = 4 Hz, C-6 H), 7.07 (s, $OCHPh_2$).

(13) **16d**: $[\alpha]^{22}_D$ −232.8 ± 7.6° (Me_2SO, c 0.360); UV (Me_2SO) λ_{max} 286 nm (ϵ 8700); IR ($CHCl_3$) ν 1790 (β-lactam), 1718 (ester) cm^{-1}; NMR ($CDCl_3$) δ 1.75 (br s, NH_2), 3.81 (s, N-Me), 4.28 (br s, C-2 H), 4.50 (d, J = 4 Hz, C-7 H), 4.64 (br s, CH_2-S), 4.98 (d, J = 4 Hz, C-6 H), 6.90 (s, $OCHPh_2$).

(14) An excellent procedure for 7α-methoxylation of cephalosporins was applied; see H. Yanagisawa, M. Fukushima, A. Ando, and H. Nakao, *Heterocycles*, **3**, 1130 (1975); *Tetrahedron Lett.*, 259 (1976).

(15) Oxidation with nickel peroxide in place of originally reported lead dioxide proceeded under milder conditions.

(16) **20**: mp 160–162 °C (dec); IR ($CHCl_3$) ν 1792 (β-lactam), 1724 (ester) cm^{-1}; NMR ($CDCl_3$) δ 2.00 (br s, NH_2), 3.38 (s, OMe), 3.87 (s, N-Me), 4.32 (s, C-2 H), 4.73 (s, CH_2-S), 4.92 (s, C-6 H), 7.00 (s, $OCHPh_2$).

(17) Prepared by alkylation of p-hydroxyphenylacetic acid (2.4 equiv of p-anisyl chloride, 2.4 equiv of NaI, and 3 equiv of K_2CO_3, acetone, 50–60 °C, 48 h) giving 4-methoxybenzyl 4-[(4-methoxybenzyl)oxy]phenylacetate and subsequent carboxylation of the corresponding anion produced by deprotonation with lithium diisopropylamide [1.2 equiv of $LiN(i\text{-}Pr)_2$, THF, −78 °C, 30 min].

(18) A novel method for deprotection of esters, developed in our laboratories, was applied; see T. Tsuji, M. Yoshioka, T. Kataoka, Y. Sendo, S. Hirai, T. Maeda, and W. Nagata, Belgium Patent 856444 (1977); *Chem. Abstr.*, **89**, 6100s (1978).

(19) Disodium salt of **5**: UV (H_2O) λ_{max} 270 nm (ϵ 12000); NMR (D_2O–external Me_4Si) δ 3.91, 3.98 (two s, OMe), 4.44, 4.46 (two s, N-Me), 4.55, 4.60, 4.63 (C-2 H), 4.91, 4.95 (two br s, CH_2-S, benzyl proton), 5.57, 5.58 (two s, C-6 H).

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Cyclopropylamines as Suicide Substrates for Cytochromes P-450

Sir:

There is considerable current interest in enzyme inhibitors of the k_{cat} or suicide substrate variety.[1] Because the action of such inhibitors is intimately related to the enzymatic mechanism, knowledge of the latter often provides an excellent starting point for the rational design of highly specific and effective inhibitors. Conversely, the discovery of such inhibitors for an enzyme whose mechanism and active site are not well characterized should, in principle, provde an equally specific and effective probe of catalytic mechanism and active-site structure for that enzyme. The cytochrome P-450 mixed-function oxidases constitute an important family of enzymes whose mechanism and active sites remain incompletely characterized despite more than a decade of intense effort. A recent report[2] that allylisopropylacetamide (AIA), long known for its ability to deplete cytochrome P-450 in vivo, undergoes metabolic activation in vivo, leading to its covalent attachment to the heme group of P-450, now prompts us to report our own work with cyclopropylamines as suicide substrates for cytochrome P-450.

Table I. Inhibition of in Vitro Aminopyrine Demethylation by Para-Substituted *N*-Cyclopropyl- and *N*-Isopropylbenzylamines (p-$XC_6H_4CH_2NHR$)[a]

X	inhibitor, % inhibn R = c-Pr	R = *i*-Pr
CH_3O	1a, 54 ± 1	1b, 0 ± 2
CH_3	2a, 77 ± 2	2b, 2 ± 4
H	3a, 61 ± 1	3b, 9 ± 6
Cl	4a, 79 ± 4	4b, 25 ± 3
Br	5a, 79 ± 1	5b, 27 ± 4

[a] The inhibitor (1 mM) and aminopyrine (3 mM) were each added at the start of the assay. Incubations were carried out in triplicate for a total of 12 min at 33 °C under air, followed by quenching with $ZnSO_4$ and $Ba(OH)_2$; formaldehyde was determined colorimetrically with Nash reagent.[4]

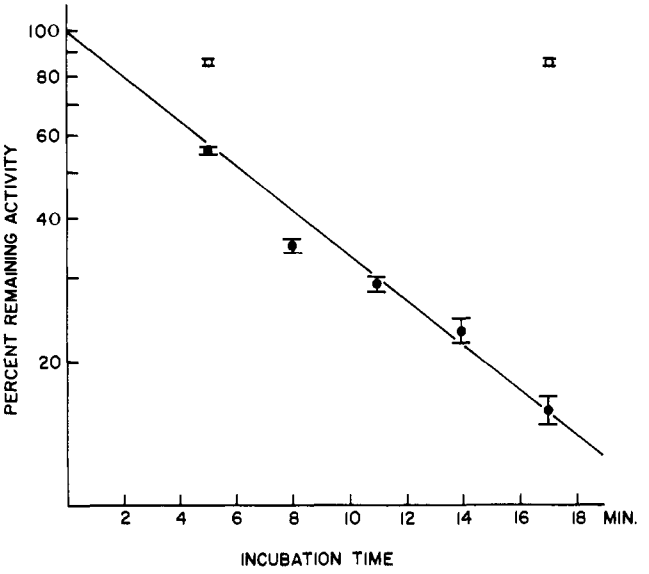

Figure 1. Kinetics of aminopyrine demethylase inhibition by **2a** (closed circles) and **2b** (open circles). Assays were carried out under conditions described in Tables I and II. The points represent the mean ± SE of four experiments.

In the course of some mechanistic studies aimed at developing effective inhibitors of first-pass N-dealkylation reactions, a series of para-substituted *N*-cyclopropyl- and *N*-isopropylbenzylamines was prepared[3] and evaluated in vitro[4] for inhibition of aminopyrine demethylation using rat liver microsomes. The cyclopropylamines were consistently found to be significantly more inhibitory than the corresponding isopropylamines (Table I).[5] During attempts to determine the kinetics of inhibition of aminopyrine metabolism by the *N*-cyclopropylbenzylamines, it was noticed that the degree of inhibition appeared to be both time and concentration dependent. In addition, among numerous individual batches of microsomes, those with the highest overall cytochromes P-450 activity were the most susceptible to inhibition. When the time dependence was specifically investigated, it was found that loss of enzyme activity was kinetically a first-order process with a rather short half-life (e.g., 6 min with **2a** in Figure 1). In contrast, inhibition due to **2b**, a very weak inhibitor with a high pK_a, and *N*-benzylmorpholine (8), a good inhibitor with a low pK_a, was time independent (Figure 1 and Table II).[6] Similar results (not shown) were obtained with **3a** and **3b**. Since control experiments indicated that the cyclopropylamines did not inhibit NADPH–cytochrome P-450 reductase nor interfere with cofactor regeneration, it appeared that the cyclopropylamines might be undergoing metabolic activation to a

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Table II. Dependence of Loss of Microsomal Aminopyrine Demethylase Activity on Preincubation Time and Conditions[a]

preincubation conditions[c]	% inhibn obsd[b] without preincubation	% inhibn obsd[b] after 12-min preincubation
control	0	0[d]
+3a	29	70
+3b	9	9
+8	27	34
+3a, omit cofactor		13
+3a, +GSH	17	62
+3a, CO/O_2 (80:20)[e]		24
+MMI	25	57

[a] General incubation conditions and assay procedures are as described for Table I and in ref 4. [b] Average of three to six determinations with several different microsome preparations. [c] Preincubation conditions also obtain during the subsequent 5-min assay period, except for a slight volume change upon addition of aminopyrine (3.0 mM) and a second aliquot of cofactor-generating system. Glutathione (GSH) or other compounds (1.0 mM) were added at the start of the preincubation. [d] Loss of activity due to preincubation of microsomes and NADPH alone was variable but always less than 7–8%. [e] CO was replaced by air at the start of the assay period. Separate controls showed that CO inhibition was readily reversed by this procedure.

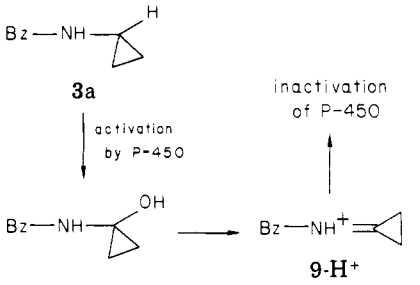
species which in turn inactivates cytochromes P-450. Further results which support this suggestion and implicate cytochromes P-450 in the activation process are reported in Table II.

The time-dependent inactivation of cytochromes P-450 by **3a** is seen to require NADPH. This would be true whether metabolism of **3a** to an active species were effected by cytochromes P-450 or the FAD-containing monooxygenase isolated and described by Ziegler and coworkers.[8] However, CO very effectively prevents the inactivation of cytochromes P-450, while methimazole (MMI),[9] an alternate-substrate inhibitor of the flavoprotein amine oxidase which does not significantly inhibit P-450, fails to do so. These observations suggest that cytochromes P-450 activity is required for generation of the cytochromes P-450 inactivating species, i.e., that secondary cyclopropylamines such as **1a**–**5a** appear to be true suicide substrates for cytochromes P-450.

In most cases suicide inactivation of enzymes involves formation of a covalent bond between an electrophilic metabolite and a nucleophilic functional group on the enzyme, usually at the catalytic site.[1] Similar processes are thought to be involved in the production of cytotoxic and mutagenic effects of a wide variety of chemicals and/or their metabolites. Thus, one generally observes that addition of nucleophilic species to the system protects against these effects. In the present case, however, semicarbazide (normally added[4] to capture CH_2O from N-demethylation of aminopyrine) did not prevent the inactivation nor did glutathione, a sulfur nucleophile well known for scavenging electrophilic metabolites.

Of major interest, of course, is the identity of the reactive metabolite being produced and its mode of interaction with cytochromes P-450. *N*-Benzylcyclopropylamine (**3a**) covalently inactivates monoamine oxidase,[10] apparently by alkylating the flavin moiety *after* undergoing enzymatic conversion to *N*-benzyliminocyclopropane (**9**). Related cyclopropyl derivatives (e.g., cyclopropanone hydrate, its ethyl hemiketal, and the mushroom toxic coprine) have been shown[11,12] to inactivate aldehyde oxidase, presumably by forming a very stable hemithioketal linkage with a sulfhydryl group at the active site of this enzyme. While

Scheme I



the metabolite of **3a** which inactivates cytochromes P-450 is not yet known, the above precedents permit speculation that a species such as **9** or **9**-H^+, formed by a typical P-450-mediated N-dealkylation process as shown in Scheme I, might be sufficiently electrophilic to inactivate cytochrome P-450.

Present work in our laboratory is aimed at investigating the chemistry of cytochromes P-450 inactivation by cyclopropylamines, as well as the generality of this property of cyclopropane derivatives and the selectivity of this inactivation among various types of cytochromes P-450 catalyzed reactions.

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References and Notes

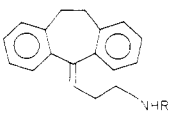
(1) For recent reviews, see (a) R. R. Rando, *Science*, **185**, 320 (1974); (b) R. H. Abeles and A. L. Maycock, *Acc. Chem. Res.*, **9**, 313 (1976); (c) M. J. Jung, *Annu. Rep. Med. Chem.*, **13**, 249 (1978); (d) R. R. Rando, *Acc. Chem. Res.*, **8**, 281 (1975).

(2) P. R. Ortiz deMontellano, B. A. Mico, and G. S. Yost, *Biochem. Biophys. Res. Commun.*, **83**, 132 (1978).

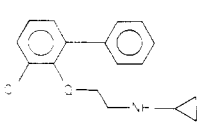
(3) The amines listed in Table I were prepared by reduction of the corresponding benzamides with BH_3·THF at room temperature. All new compounds gave satisfactory analytical and spectral data. Hydrochloride salts were prepared by addition of HCl gas to an ether solution of the amine.

(4) P. Mazel, in "Fundamentals of Drug Metabolism and Drug Disposition", B. N. LaDu, H. G. Mandel, and E. L. Way, Eds., Williams & Wilkins, Baltimore, Md., 1972, pp 546–550.

(5) While cyclopropylamines are well known as inhibitors of monoamine oxidase (MAO), a search of the literature revealed only a single report of their inhibitory action on cytochrome P-450. Thus, R. E. McMahon, H. W. Culp, and J. Mills, *J. Med. Chem.*, **13**, 986 (1970), found that the cyclopropylamines i and iii were significantly stronger



i, R = c-Pr
ii, R = *i*-Pr



iii

inhibitors than related *N*-alkylamines.[6] It was speculated that this effect was too large to be attributed simply to steric or pK_a effects, but no mechanistic studies were reported. In our hands compounds i and ii (0.1 mM) inhibited aminopyrine demethylation (Table I and ref 4) by 92 and 55%, respectively. We thank Dr. Robert McMahon for furnishing us with samples of these compounds.

(6) It has been shown that only the *unprotonated* amine interacts as a substrate or inhibitor with cytochromes P-450. A. K. Cho and G. T. Miwa, *Drug Metab. Disp.*, **2**, 477 (1973).

(7) Since the assay time was 5 min, significant enzyme loss was occurring during the assay. Thus, activity was plotted against the *total* time that the microsomes were incubated with inhibitor and cofactor, i.e., variable preincubation times

plus the 5-min assay time after addition of aminopyrine and more cofactor.
(8) D. M. Ziegler and C. H. Mitchell, *Arch. Biochem. Biophys.*, **150** 116 (1972).
(9) R. A. Prough and D. M. Ziegler, *Arch. Biochem. Biophys.*, **180**, 363 (1977).
(10) R. B. Silverman and S. J. Hoffman, "Abstracts of Papers", 177th National Meeting of the American Chemical Society, Honolulu, HI, April 1–6, 1979, BIOL-48.
(11) J. S. Wiseman and R. H. Abeles, *Biochemistry*, 18, 427 (1979).
(12) O. Tottmar and P. Lindberg, *Acta Pharmacol. Toxicol.*, **40**, 476–481 (1977).

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Articles

Mapping the Dopamine Receptor. 1. Features Derived from Modifications in Ring E of the Neuroleptic Butaclamol

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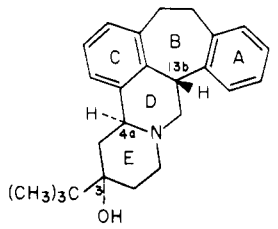
Several analogues of the neuroleptic agent butaclamol, having modifications in the ring E region of the molecule, have been synthesized. Pharmacological evaluation identified two of the analogues as being equipotent to butaclamol, namely, anhydrobutaclamol (**8**) and deoxybutaclamol (**9a**). The molecular structures of both the active and inactive analogues were analyzed and the results have been used for mapping the central dopamine receptor. The existence of a previously proposed lipophilic *accessory binding site* on the receptor macromolecule has been confirmed. Its minimum dimensions, as well as its locus with respect to the *primary binding sites*, have been defined. A receptor model incorporating the above features is proposed.

Previous communications from these laboratories[1-5] have described the syntheses and psychopharmacological activities of various benzocycloheptapyridoisoquinolines related to the clinically active[6-11] antipsychotic agent butaclamol (1). It has been shown that in this series



1

neuroleptic activity is associated with 4a,13b-trans and 3(OH),13b(H)-trans relative configurations[1,4,12] and that only the enantiomers having 3*S*,4a*S*,13b*S* absolute configurations are active.[4,5,12] We have described the qualitative and quantitative changes in neuroleptic activity which result from altering the bulky substituent at position 3,[1,2] by introducing a chlorine substitutent at various positions on rings A and C[3], and, in the following paper of this issue,[13] from making molecular modifications in the rings A/B region.

It was previously proposed[4] that the extended phenethylamine moiety of butaclamol constitutes the pharmacophore which confers neuroleptic activity on this molecule. It was further suggested that the phenyl ring and the nitrogen atom of the pharmacophore interact with primary binding sites[4] on the dopamine receptor. In the following paper in this issue[13] the nitrogen primary binding site has been redefined as a two-point system comprised of a *nitrogen location site* and a complementary *hydrogen bond donor site*. Also, the minimum dimensions of the phenyl ring primary binding site have been defined and its location relative to the above-mentioned sites has been specified.[13]

The dopamine receptor, genetically evolved to bind the endogeneous neurotransmitter dopamine, also binds butaclamol with high affinity.[14,15] Since the molecular dimensions of butaclamol are much larger than those of dopamine, we have suggested that certain structural entities of the butaclamol molecule, other than the pharmacophore, contribute to the observed affinity between ligand and receptor by interacting with accessory binding sites[4] on the dopamine receptor. Such accessory binding sites were proposed for the *tert*-butyl and the hydroxyl groups of butaclamol.

In the present study, a number of molecular modifications have been made in ring E of butaclamol involving the aforementioned groups. The potential neuroleptic activities of the resultant compounds have been determined and the results have been interpreted in terms of some detailed topographical features of the central dopamine receptor.

Chemistry. The compounds investigated fall into two groups. The first group shown in Scheme I, of which **1**[1] and **12**[16] have previously been described, is derived from the amino ketones **2** and **3**.[16]

Reduction of **2** with sodium borohydride gave a 73% yield of the secondary alcohol **4**, while reduction with lithium tri-*sec*-butylborohydride[17] afforded 66% of the epimeric alcohol **5**. The configurational assignments for

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