Phenylacetylene and biphenylacetylene are oxidized by cytochrome P-450 to the corresponding arylacetic acids. The acetylenic hydrogen shifts to the adjacent carbon and one atom of molecular oxygen is incorporated into the carboxylic acid group in these transformations, which are subject to a large kinetic isotope effect when the acetylenic hydrogen is replaced by deuterium. The same products and isotope effects are observed when the two arylacetylenes are oxidized by m-chloroperbenzoic acid rather than by the enzyme. In contrast, the inactivation of cytochrome P-450 that occurs during the oxidation of phenylacetylene is insensitive to deuterium substitution. The partition ratio between metabolite formation and enzyme inactivation consequently changes from 26 to 15 in going from phenylacetylene to the deuterated analogue. Metabolite formation therefore diverges from heme alkylation very early in the catalytic process.

The epoxidation of olefins by cytochrome P-450 could involve simultaneous formation of bonds between the activated oxygen and the two carbons of the π-bond, or formation of the two bonds in discrete steps separated by an ionic or radical intermediate. Retention of the olefin stereochemistry in the epoxides favors a concerted epoxidation mechanism (1-4), but the fact that the prosthetic heme of cytochrome P-450 is alkylated during the oxidation of terminal olefins favors a nonconcerted mechanism. Heme alkylation, which also occurs with retention of the olefin stereochemistry (4), is observed when the activated oxygen is transferred to the internal carbon of the π-bonds (5-9). Additional support for a nonconcerted mechanism is provided by the reports that 1,2-chlorine migration precedes rather than follows epoxide formation from halogenated olefins (10, 11), that aldehydes and ketones are formed as trace metabolites from certain olefins in a process that does not involve the epoxide (11, 12), and that the oxidation of styrene is subject to a secondary isotope effect when deuterium is located on the internal but not the terminal carbon of the π-bond (13).

The cytochrome P-450-catalyzed oxidation of a carbon-carbon triple bond, a reaction formally related to olefin epoxidation, has only been unambiguously demonstrated in the metabolism of biphenylacetylenes (14-19) but is implied by the excretion of phenylacetic acid when animals are treated with phenylacetylene (20) and by rearrangements observed in the metabolism of 17-ethynyl sterols (9). The catalytic oxidation of biphenylacetylene yields biphenylacetic acid in a reaction subject to a kinetic isotope effect of 1.4 when the acetylenic hydrogen is replaced by deuterium (19). The possibility that oxygen is inserted into the acetylenic carbon-hydrogen bond, suggested by the isotope effect, is ruled out by quantitative shift of the acetylenic hydrogen to the vicinal carbon during the oxidation (17-19) and by the observation of a similar intramolecular hydrogen shift in the chemical oxidation of biphenylacetylene (17, 18). The enzymatic oxidation of acetylenes, like that of olefins, thus involves reaction of the activated oxygen with the π-bond. In agreement with this, the turnover of acetylenes by cytochrome P-450 results in alkylation of the prosthetic heme group (21-23) and the formation of heme adducts similar to those obtained with terminal olefins (7, 24, 25). We describe here a mechanistic investigation of the oxidation of π-bonds by cytochrome P-450 that exploits the hydrogen shift associated with triple bond oxidation as an experimental probe.

MATERIALS AND METHODS

RESULTS

Incorporation of Oxygen into the Biphenylacetylene Metabolite—Biphenylacetylene was incubated with hepatic microsomes from phenobarbital pretreated rats under an 18O2 atmosphere, and the resulting biphenylacetic acid was isolated and methylated with diazomethane. Mass spectrometric analysis (Fig. 1) of the esterified metabolite established that approximately 75% of one oxygen in the carboxylic acid moiety derived from labeled molecular oxygen. This fractional incorporation of label points to catalytic incorporation of one atom of molecular oxygen because some dilution of the label by oxygen not removed in the purging operations is unavoidable. The second oxygen in the carboxyl group, by implication, derives from the medium.

Kinetic Isotope Effect in the Metabolism of Biphenylacetylene—The kinetic isotope effect for the oxidation of biphenylacetylene was remeasured to confirm the value reported earlier (kH/kD = 1.42) (19). Labeled and unlabeled biphenylacetylene were incubated with hepatic microsomes...
ylacetylene were incubated with hepatic microsomes from phenobarbital-induced rats and aliquots were taken from the incubation mixtures at 10-min intervals. Biphenylacetylene and biphynalactic acid were extracted into methylene chloride, and their concentrations were determined spectrophotometrically. This spectroscopic assay is feasible because the absorbance maximum of biphenylacetylene is at 273 nm whereas that of biphynalactic acid is at 254 nm, although direct spectroscopic analysis of the incubation mixture is not possible because an interfering chromophore is present in the assay mixture that is eliminated in the extraction procedure. Metabolite formation is limited to the first 10 min of the incubation because the same amount of metabolite is present at 30 as at 10 min. The isotope effect calculated from the data is $k_{H}/k_{D} = 1.38$ (Table 1), a value within experimental error of that obtained earlier by a gas chromatographic assay (19).

**Isotope Effects on the Destruction of Cytochrome P-450 by Arylacetylenes**—Biphynalactic acid causes NADPH- and time-dependent loss of cytochrome P-450 when incubated with hepatic microsomes from phenobarbital-pretreated rats (Table 2). The enzyme loss determined by spectroscopic quantitation of the ferrous-carbon monoxide complex, however, does not exceed 4–5% of the total microsomal enzyme. This loss occurs within the first 10 min and is not increased if the incubation is prolonged a further 20 min. Essentially identical results are obtained when the incubations are carried out with [1-$^2$H]biphynalactic acid (Table 2). The quantitative reliability of these values, however, is compromised by their small magnitude and by the fact that they reflect a 4% correction for the enzyme lost in the absence of substrates.

The completion of both metabolite formation and enzyme inactivation within the same 10 min period suggests that one isozyme, representing no more than 4–5% of the cytochrome P-450 in microsomes from phenobarbital-induced rats, is involved in both processes. This result, unexpected in view of the report that phenobarbital and 3-methylcholanthrene stimulate biphynalactic acid metabolism (14), led us to investigate whether the cytochrome P-450 loss could be amplified by pretreatment with clofibrate, an inducer of cytochrome P-450 enzymes involved in fatty acid hydroxylation (27–28), or Arochlor 1254, an inducer of multiple isozymes (29, 30). However, microsomal enzyme loss from Arochlor 1254-treated rats is the same as that from phenobarbital-treated rats while enzyme loss from clofibrate-treated rats is negligible (Table 2). These results suggest that biphynalactic acid is a specific substrate for a minor, phenobarbital-inducible, isozyme.

Phenylnacetylene was found earlier to cause time- and NADPH-dependent loss of cytochrome P-450 when incubated with hepatic microsomes from phenobarbital-pretreated rats (22). Losses of 18, 25, and 27% were observed after 10, 20, and 30 min of incubation with 10 mM phenylacetylene. A hepatic pigment with the absorption spectrum of an N-alkylprotoporphyrin IX derivative was furthermore isolated from the livers of rats injected with phenylacetylene (22). The larger amplitude of the cytochrome P-450 loss and the evidence that phenylacetylene alkylates the prosthetic heme of cytochrome P-450 led us to use it as an alternative probe of the oxidative mechanism. The rates of inactivation of cytochrome P-450 by phenylacetylene and [1-$^2$H]phenylacetylene appear, within experimental error, to be identical (Fig. 2).

**The Metabolism of Phenylacetylene**—No information is available on the metabolism of phenylacetylene except for the report that phenylacetic acid is excreted by rabbits injected with phenylacetylene (20). Phenylacetylene therefore was incubated with hepatic microsomes from phenobarbital-pretreated rats and the metabolites were isolated by extraction into diethyl ether. The diethyl ether fraction, after esterification with diazomethane, was analyzed by gas chromatography. The only quantitatively significant (>5%) metabolite detected in the extracts is the methyl ester of phenylacetic acid (Fig. 3). A specific search was made for acetophenone, but none was detected. The metabolite was identified as phenylacetic acid by direct gas chromatographic and mass spectrometric comparison with an authentic sample (not shown).

The pattern of metabolites obtained with [1-$^2$H]phenylacetylene is the same as that obtained with the unlabeled substrate, but the molecular ion of the methyl phenylacetate metabolite is 1 mass unit higher (Fig. 4). The peak for the fragment obtained by decarboxylation of the molecular ion retains the difference of 1 mass unit. It is thus evident that the acetylenic hydrogen of phenylacetylene, like that of biphynalactic acid, shifts quantitatively to the vicinal carbon on oxidation of the triple bond.

**Kinetic Isotope Effect in the Metabolism of Phenylacetylene**—The formation of phenylacetic acid from phenylacetylene and [1-$^2$H]phenylacetylene in incubations with hepatic microsomes from phenobarbital-pretreated rats was quantitated as a function of time by gas chromatographic analysis (Fig. 2). Deuterium substitution significantly retards enzymatic oxidation of the triple bond and gives rise to a kinetic isotope effect $k_{H}/k_{D} = 1.80$ (Table 1).

The isotope effect for phenylacetylene metabolism was independently measured by incubating a 1:1 mixture of phenylacetylene and [1-$^2$H]phenylacetylene with hepatic microsomes and quantitating the ratio of the deuterated to undeuterated phenylacetic acid metabolites by gas chromatography–mass spectrometry. The kinetic isotope effect obtained by this internal competition method ($k_{H}/k_{D} = 1.60$) (Table 1) confirms that the oxidation of phenylacetylene by cytochrome P-450 is subject to a major isotope effect.

**Isotope Effects on the Chemical Oxidation of Arylacetylenes**—The oxidation of biphynalactic acid by m-chloroperoxybenzoic acid in methylene chloride with a trace of methanol yields methyl 2-biphynalacetate as the only detectable product. The ratio of the rates of reaction with biphynalactic acid and [1-$^2$H]biphynalactic acid reflects an isotope effect $k_{H}/k_{D} = 1.38$ (Table 1). Attempts to study the oxidation of phen-
Mechanism of $\pi$-Bond Oxidation

The oxidation of phenylacetylene to phenylacacetate (C) and [1-$^3$H]phenylacetylene to [2-$^3$H]phenylacetate (D) by m-chloroperbenzoic acid in anhydrous benzene.

The oxidation of phenylacetylene was therefore examined in anhydrous benzene in the absence of proton sources as described in an earlier study (31). A kinetic isotope effect $k_H/k_D = 1.73$ is obtained from the rates of product formation from phenylacetylene and [1-$^3$H]phenylacetylene under these conditions (Fig. 5). Addition of methanol to the reaction mixture altered the kinetics and attenuated the isotope effect, but these changes were not investigated.

**DISCUSSION**

The oxidation of [1-$^3$H]phenylacetylene by both cytochrome P-450 and m-chloroperbenzoic acid yields phenylacetic acid with the acetylenic deuterium atom shifted to the benzylic carbon. The oxidation of phenylacetylene thus proceeds with a 1,2-hydrogen shift identical to that shown to occur previously in the oxidation of biphenylacetylene (17, 18). The oxidation of carbon-carbon triple bonds with concomitant shift of the acetylenic hydrogen thus appears to be a general, if frequently only minor, metabolic process. The analogy between the enzymatic and chemical processes is strengthened by the incorporation of a labeled oxygen atom into the carboxylic acid function when biphenylacetylene is incubated under $^{18}$O because this labeling pattern is that expected if biphenylacetylene is oxidized to biphenylketene which then reacts with a molecule of water. The chemical oxidation of acetylenes is believed to proceed through such a ketene mechanism (32).

The oxygen atom is bound to the terminal carbon in the arylacetylene metabolites but to the internal carbon in the heme adducts (Scheme 1) (7, 24, 25). The terminal carbon in the heme adducts is the site to which the protoporphyrin IX prosthetic group is bound. The reaction regiochemistry observed in the metabolites is favored by rate-determining oxygen addition to the $\pi$-bond because any electron deficiency that develops in the transition state can be stabilized by conjugation with the phenyl ring. The absence of acetophenone among the metabolites in the chemical and enzymatic reactions supports the view that metabolites stem from delivery of the oxygen to the terminal carbon. The fact that hydrogen migration is coupled to metabolite formation but not heme alkylation makes the observation of differential isotope effects highly informative. Olefins are not suitable for such studies because the regiochemistry of oxygen addition, which is masked in the epoxide products, can only be extracted by kinetic studies of secondary isotope effects. The isotope effects measured here for conversion of biphenylacetylene to biphenylacetic acid ($k_H/k_D = 1.38$) and phenylacetylene to 2-phenylacetic acid ($k_H/k_D = 1.80$), on the other hand, establish that enzymatic triple bond oxidation is subject to quite large primary isotope effects. The observed isotope effects approximate the maximum possible values for transition states in which the hydrogen moves in a sidewise rather than linear fashion (see Scheme 3) between the donor and acceptor atoms. Theoretical calculations suggest that the maximum isotope effect, barring tunneling effects, for a linear transition state (C–H–C angle = 180°) is approximately $k_H/k_D = 7.9$ whereas the corresponding values for angles of 120°, 90°, and 60° are 3.0, 1.7, and 0.9, respectively (33). Experimental isotope effects for reactions known to involve bent transition states conform to these theoretical predictions (e.g., pinacol rearrangement ($k_H/k_D = 2.7–3.3$) (34), amine oxide pyrolysis ($k_H/k_D = 2.7–3.2$) (35), insertion of carbenes into C–H bonds ($k_H/k_D = 0.9–2.5$) (36, 37), 1,2-shift of a hydrogen to a vicinal carbene center ($k_H/k_D = 1.7$) (38), and epoxide ring cleavage concerted with 1,2-shift of a hydrogen ($k_H/k_D = 1.59$) (39)). The secondary isotope effects on a reaction in which the carbons undergo sp to sp$^2$ rehybridization are, on the contrary, expected to be inverse and of much smaller magnitude (40).

The primary isotope effects observed in this study are therefore, if anything, slightly larger than the cited values if allowance is made for secondary isotope effects. Proportionately large intrinsic isotope effects have been demonstrated for cytochrome P-450-catalyzed carbon hydroxylations by experiments which measure the competition between deuterated and undeuterated sites in the same molecule (43, 44), but the present $V_{max}$ isotope effects are among the largest (relative to the theoretical maximum) so far observed for cytochrome P-450.

Oxidation of the biphenylacetylene triple bond with m-chloroperbenzoic acid parallels the biological reaction in all respects including, as shown here, the magnitude of the kinetic isotope effect associated with replacement of the acetylenic hydrogen by deuterium. The isotope effect for the chemical oxidation of biphenylacetylene ($k_H/k_D = 1.82$) is larger than...
that for the enzymatic oxidation ($k_H/k_D = 1.38$) but is com-
parable to that for the enzymatic ($k_H/k_D = 1.30$) and chemical
($k_H/k_D = 1.75$) oxidations of phenylacetylene. The isotope effect
in the chemical reaction requires that oxygen transfer from
the peracid to the triple bond occur in concert with
hydrogen migration. The alternative explanation, formation of a
quasi-stable oxirene intermediate that decomposes to the
ketene product in a second, rate-limiting, step, is incompatible
with the failure to detect (much less isolate) oxirene inter-
mediates even in frozen matrices at cryogenic temperatures
(32, 43, 44). The isotope effect data essentially rule out the
formation of oxirenes in the perbenzoic acid reaction and
consequently resolve the long standing question of whether
oxirenes are formed in the chemical oxidation (32). The data
substantiate the theoretically predicted asymmetry of the
transition state for the oxidation of acetylene by peroxyformic
acid (45). The parallel reaction course and isotope effects for
the chemical and enzymatic reactions strongly argue, in turn,
that oxirenes are also not intermediates in the enzymatic
oxidation of acetylenes. Oxirene intermediates would, if any-
thing, be destabilized by the interactions available within the
cytochrome P-450 active site (e.g. metal coordination, hydro-
gen bonding) and would therefore be even less likely to
accumulate prior to a rate-limiting step coupled to hydrogen
migration.

The strong analogy between the enzymatic and m-chloro-
perbenzoic acid reactions contrasts with the reaction of acety-
lenes with hydroxyl radicals. Hydroxyl radicals readily add
to terminal acetylenes, including acetylene, propargyl alcohol,
and 3-hydroxy-3-methyl-I-butyne, but the corresponding
products are not obtained and products compatible with hydrogen
migration are not observed (46). Products analogous to those
obtained in the enzymatic reaction are not obtained even if
Cu$^{2+}$ or Fe$^{3+}$ is added to the Fenton system. The Fenton
oxidation of phenylacetylene differs in that it gives a trace of
phenylacetic acid (Fig. 6), but is generally consistent with the
earlier work in that phenylacetic acid is but a very minor
component of a complex mixture of products. Clearly,
the two-electron oxidation of acetylenes mediated by m-chloro-
perbenzoic acid, which exclusively yields the product obtained
in the enzymatic reaction, better models cytochrome P-450
metabolism than the corresponding reaction with hydroxyl
radicals.

The sharp contrast between the large isotope effects on
metabolite formation and the absence of a detectable isotope
effect on cytochrome P-450 destruction requires the oxidative
trajectories that result in metabolite formation and enzyme
inactivation to diverge prior to the point where the hydrogen
shifts in the former pathway. This conclusion specifically
rules out destruction of the enzyme by the ketene or any other
species subsequent to migration of the hydrogen. The differ-
ential isotope effects are consistent with the fact that the
oxygen finishes on the terminal carbon in the metabolite and
the internal carbon in the heme adduct (7, 24, 25).

The oxidation of asymmetrically substituted acetylenes can,
in principle, yield two $\alpha$-ketocarbenes that are formally re-
lated to a common oxirene (Scheme 2) (32). If the $\alpha$-ketocar-
benes are not interconvertible via the oxirene, one $\alpha$-ketocar-
bene isomer could give rise to the ketene metabolite and the
other could alkylate the prosthetic heme. The $\alpha$-ketocarbenes,
however, can only be involved in the enzymatic reaction if $\alpha$
ketocarbone formation is followed by a rate-determining hy-
drogen shift in the pathway to metabolites. The required
formation of a relatively stable carbene intermediate is feasi-
ble in view of the chemical synthesis of stable carbone-iron
complexes (47–50) and of the fact that such structures have
been postulated for the stable complexes formed in the re-
actions of cytochrome P-450 with methyleneoxophenyl com-
ounds and halocarbons (51–53). Carbene complexes are read-
tly detected in microsomal incubations because they have a
characteristic absorption in the 440–490 nm range. We have
recently demonstrated that fairly stable complexes with an
absorption maximum at 445 nm are formed in the reactions
of cytochrome P-450 with ethyl diazoacetate and diazoacet-
ophenone. The carbene from diazoacetophenone is identical
to that expected if a carbene intermediate is generated by
oxygen addition to the internal carbon of phenylacetylene.
The microsomal oxidation of phenylacetylene, however, is
accompanied by the detectable formation of a species that
absorbs in the 445 nm region. The absence of such a chro-
mophore, in view of the fact that the chromophore is readily
detected when the expected carbene is generated by an alter-
native procedure (from the diazoketone), makes the interven-
tion of $\alpha$-ketocarbone intermediates unlikely.

The mechanism for enzymatic carbon-carbon triple bond
oxidation must adhere to the following constraints: (a) con-
current ketene formation and heme alkylation, (b) quantita-
tive shift of the acetylenic hydrogen in the ketene pathway,
(c) large primary isotope effects on metabolite formation but
not heme alkylation, (d) incorporation of one atom of molec-
ular oxygen into the metabolite, (e) location of the oxygen on
the terminal carbon in metabolites but on the internal carbon
in heme adducts, and (f) strong parallels in the reactions of
acetylenes with cytochrome P-450 and peracids but not hy-
droxyl radicals. These results require heme alkylation and
metabolite formation to diverge prior to (or during) transfer
of the oxygen to the terminal carbon for metabolite formation
and the internal carbon for heme alkylation. The preference
for oxygen transfer to the terminal carbon is shown by the
absence of products from oxygen addition to the internal
carbon in the analogous oxidation by m-chloroperbenzoic
acid. The substrate could, however, be bound a fraction of
the time in an orientation that forces oxygen transfer to the
internal carbon. The relatively loose binding of substrates by
cytochrome P-450 required for the observation of high intra-
molecular isotope effects (41, 42) suggests that a more com-
licated mechanism may be involved. One alternative is for
enzymatic electron transfer from the $\pi$-bond to precede car-
bon-oxygen bond formation in the heme alkylation pathway
(Scheme 3, upper mechanism). The partitioning of substrates
between metabolism and heme alkylation would be deter-
mined by the ratio of electron transfer to direct oxygen
addition. A second alternative is for the iron-oxo complex to
add to the phenylacetylene $\pi$-bond to form the two possible
metallooxocyclobutene isomers (Scheme 3, lower mechanism).
Rearrangement of the isomer with the phenyl vicinal to the
iron would result in ketene formation whereas internal ligand
transfer in the isomer with the phenyl vicinal to the oxygen
would result in heme alkylation. The isotope effect on metab-
olite formation requires, however, reversible formation of the

\[ \text{Scheme 2. The theoretical relationship between the two carbenes and the oxirene that could result from oxidation of an arylacetylene.} \]

\[ \text{P. R. Ortiz de Montellano and E. A. Komives, unpublished results.} \]

\[ \text{A search for long wavelength absorption in incubations of phenyl-
acetylene with liver microsomes from phenobarbital-induced rats has been fruitless.} \]
mechanism in which heme alkylation results from initial electron transfer and metabolite formation from direct oxygen transfer. In the bottom half is a mechanism involving the reversible formation of isomeric metallooxocyclobutene.

metallooxocyclobutene isomers followed by rate-determining hydrogen migration in the pathway to metabolites. Metallooxycyclobutane intermediates, first proposed by Sharpless et al. (54) to explain the oxidation of olefins by chromyl chloride, have been observed by NMR in the reaction of osmium tetroxide with 1,1-diphenylethylene (55) and have been invoked by Collman et al. (56) to explain olefin oxidation by an oxo-manganese complex. No precedent is yet available, however, for the formation of metallooxocyclobutene intermediates with iron porphyrins or from acetylenes. The factors that control the regiochemistry of oxygen addition thus remain to be defined.

Acknowledgment—We thank Dr. Norbert Reich for helpful criticisms and suggestions relative to interpretation of the isotope effects.

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SUPPLEMENTARY MATERIAL TO

BRANCHPOINT FOR THE ALTRIBUTION AND METABOLITE FORMATION IN THE OXIDATION OF ARYL ACETYLENES BY CYTOKINE 3-45H 

Paul A. Ottis de Montellano and Elizabeth A. Kusnet

3-Metabolite Products

Hijlpllusteine was synthesized from deuterated 4-acetylbiphenyl as described earlier (15,16). Phenylisopropylmethylnitrite, phenylisopropylmethylnitrite, and phenylacetamide were synthesized from Airlift II. Phenylacetamide was hydrolyzed with 1 M sulfuric acid to produce phenylacetate, which was then deuterated with sodium and deuterium oxide. The deuterated product was purified by column chromatography on silica gel (15,16).

Phenylacetate, a 2 M aliquot of a commercial solution of (0.01 M, pH 7.4) water, was added to 15 ml of deuterated water (99.5% D) to prepare a 5 M solution. The reaction mixture was allowed to stir for 30 min, and then the pH was adjusted to 7.4. The reaction was quenched with 15 ml of deuterated water, and the mixture was filtered to remove solid materials. The filtrate was then diluted with 200 ml of deuterated water, and the solution was filtered again. The resulting solution was then analyzed by high-performance liquid chromatography (HPLC) to determine the incorporation of deuterium into phenylacetate.

Preparation of Phenylacetate: Phenylacetate was synthesized from 4-acetylbiphenyl as described above (15,16). Phenylacetate was added to 2 M aliquots of a commercial solution of (0.01 M, pH 7.4) water, and the reaction mixture was allowed to stir for 30 min. The pH was then adjusted to 7.4, and the reaction was quenched with 15 ml of deuterated water. The mixture was filtered to remove solid materials, and the filtrate was then diluted with 200 ml of deuterated water. The solution was then filtered again, and the resulting solution was analyzed by HPLC to determine the incorporation of deuterium into phenylacetate.

Effect of Biphenylacetate on Phenylacetate Oxidation: Phenylacetate was added to the reaction mixture, and the reaction was allowed to proceed for 30 min. The pH was then adjusted to 7.4, and the reaction was quenched with 15 ml of deuterated water. The mixture was filtered to remove solid materials, and the filtrate was then diluted with 200 ml of deuterated water. The solution was then filtered again, and the resulting solution was analyzed by HPLC to determine the incorporation of deuterium into phenylacetate.

Table 1: Water and metal element effects on the role of bicarbonate oxidation of acetate.

<table>
<thead>
<tr>
<th>Water Type</th>
<th>Element</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized</td>
<td>Carbon</td>
<td>0.01 M</td>
</tr>
<tr>
<td>Deionized</td>
<td>Oxygen</td>
<td>0.01 M</td>
</tr>
<tr>
<td>Deionized</td>
<td>Nitrogen</td>
<td>0.01 M</td>
</tr>
</tbody>
</table>

Mechanism of p-Oxidation
Table 2. Destruction of cytochrome P-450 in incubations of microsomes with styryl acetones

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Induction</th>
<th>Loss of cytochrome P-450 (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
<td>20 min</td>
</tr>
<tr>
<td>Biphenylacetylene</td>
<td>phosphoribital</td>
<td>4.5 6.4</td>
</tr>
<tr>
<td>Apomorphine</td>
<td></td>
<td>3.2 3.5</td>
</tr>
<tr>
<td>clofibrate</td>
<td>MD</td>
<td>50</td>
</tr>
<tr>
<td>[1-\textsuperscript{2}H]_]</td>
<td>phosphoribital</td>
<td>4.2 7.9</td>
</tr>
<tr>
<td>Biphenylacetylene</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The values reflect a correction of up to 4% for the loss of cytochrome P-450 observed in incubations without substrate. The standard deviations for the individual values do not exceed ±1.

Figure 1. Gas-liquid chromatographic analysis of the extract from an incubation of phenylecetone with microsomes from phosphoribital-induced rats. Methyle phenylecetone (b), methyl benenate (c), internal standard (a), and the internal standard (d) are identified by the corresponding letter* in the chromatogram.

Figure 4. Mass spectra of methyl phenylecetone obtained from microsomal incubations of (a) phenylecetone and (b) [1-\textsuperscript{2}H]_] phenylecetone. The lower mass regions are not shown.

Figure 6. Analysis by gas-liquid chromatography of the methylated organic fraction from the Fauston blood of phenylecetone. The total mass spectrometric ion current is presented in the diagram. Methyl phenylecetone is identified by an arrow. Other tentatively identified (retention time, mass spectrometry peaks a (a) 1-phenyl-1,2-ethanediol, (b) phenylecetone, (c) methyl benenate, (d) acetophenone, (e) phenylocetone, (f) benzaldehyde, and (g) phenylecetone.