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STRUCTURE–ACTIVITY RELATIONSHIPS IN THE DESTRUCTION OF CYTOCHROME P-450 MEDIATED BY CERTAIN ETHYNYL-SUBSTITUTED COMPOUNDS IN RATS

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Abstract—A number of acetylenic compounds have been examined for their ability to cause the destruction of cytochrome P-450 in a liver microsomal test system *in vitro*. Certain water-soluble drugs were inactive in this system although water solubility alone was not sufficient to account for the absence of metabolic activation. Lipophilic compounds where the ethynyl substituent was sterically hindered in a non-terminal position in the molecule, e.g. tremorine or 5-decyne, were also inactive in this test system. In contrast, 1-heptyne, 1-decyne and 1-tridecyne readily caused the destruction of cytochrome P-450 in the presence of NADPH. However, both shorter chain length (1-pentyne) and longer (1-octadecyne) caused little or no loss of this cytochrome. Destruction of cytochrome P-450 in the liver *in vivo* occurred following the administration of naturally occurring products extracted from the avocado fruit. Attempts to isolate the active principles involved have not yet been successful.

Ethynyl substituted (—C≡CH) compounds, including some contraceptive steroids, undergo metabolic activation in the liver by a NADPH-dependent enzyme system having many of the characteristics of the mixed function oxidases, to give derivatives which cause the destruction of the haem moiety of cytochrome P-450 [1, 2]. Covalent binding of the active metabolite of ethynyl-substituent to the porphyrin ring of haem results in the formation of abnormal green pigments [3]. Metabolic cleavage of the ethynyl group occurs in monkeys [4] while in rodents, oxidation to the corresponding carboxylic acid takes place [5, 6]. It is not known if intermediates from either of these reactions are involved in the destruction of hepatic haem. This paper reports on a study of the substrate specificity of the enzyme system responsible for the metabolic activation of the ethynyl-substituent and describes some model compounds where the metabolism of this group can be studied. Evidence is presented which suggests that some naturally occurring acetylenes such as those found in extracts of avocado fruit may undergo metabolic activation in the liver in a similar manner to synthetically produced ones.

MATERIALS AND METHODS

1-Alkynes (>98% purity) were from Fluka A.G., Buchs, Switzerland; 2,3 and 5-decyne were from ICN Pharmaceuticals, N.Y., U.S.A. Male Fischer F.344 rats (150–180 g) were given 0.1% (w/v) phenobarbitone sodium in the drinking water for 7 days before use. Washed liver microsomal fractions were prepared as described previously [2]. Incubation mixtures of 3 ml volume contained the ethynyl-substituted substrate nominal concn 2 mM added in 30 μl propylene glycol together with $MgCl_2$, 5 mM: EDTA, 2 mM; NADP, 0.65 mM; glucose-6-phosphate, 8 mM; glucose-6-phosphate dehydrogenase, 0.67 U/ml and sodium phosphate buffer pH 7.5, 100 mM. Reactions were started with the addition of 0.2 ml rat liver microsomes containing 50 nmoles cytochrome P-450/ml. Incubations were for 10 min at 37° in air in 25 ml conical flasks. Where alkynes were used, flasks were sealed with teflon septa. At the end of the incubation, flasks were placed on a salt–ice mixture and cytochrome P-450 was determined from the CO-reduced versus reduced difference spectrum [8]. Results were expressed relative to controls incubated for the same length of times in the absence of substrate. In controls, substrate was added immediately before the determination of cytochrome P-450. Avocado fruit were divided into mesocarp and stone fractions (approx. 200 g) and were extracted ×3 with 300 ml diethylether in a Waring blender. The ether extracts were dried (anhyd. Na_2SO_4), passed through a 25 × 2.5 cm column of neutral alumina to remove chlorophylls and then rotary evaporated to give a pale yellow oil. Phenobarbitone pretreated rats were given 20 ml/kg, i.p. (mesocarp extract) or 1 ml/kg, i.p. (stone extract). Controls received a similar volume of triocctanoin. At various times after dosing, animals were killed and the concentrations of the liver microsomal cytochrome P-450 estimated as described previously [2].

RESULTS AND DISCUSSION

The results shown in Table 1, group A indicate that a number of ethynyl-substituted water soluble drugs were without effect on hepatic microsomal cytochrome P-450 in a test system *in vitro*. These drugs included the monoamine oxidase inhibitor pargylene (*N*-methyl-*N*-2-propynylbenzylamine) (Fig. 1, I) and the sedative ethinamate (1-ethynylcyclohexanol carbamate) (Fig. 1, II). Similar negative effects were obtained when these compounds were administered to rats (1 mmole/kg, i.p.) *in vitro* (I.

White, unpublished results). All the drugs in group A are water-soluble and this may reduce their ability to enter the hydrophobic environment of the microsomal mixed function oxidase activating system [1]. However, water solubility alone does not preclude metabolic activation; other factors must also be involved. For example while ethinamate was inactive in this system, the closely related highly water-soluble 1-ethynyl cyclohexanol is able to cause the destruction of cytochrome P-450 [3].

Table 1 group B shows results obtained with compounds where the ethynyl substituent was not in a terminal position in the molecule. Metabolic activation in these examples appeared to be relatively easily prevented by steric hindrance of the ethynyl substituent, e.g. by a chloromethyl group in the herbicide barban (4-chloro-but-2-ynyl-3-chlorocarbanilate) (Fig. 1, III) or a relatively long hydrocarbon chain as in 5-decyne (Fig. 1, IV). Similarly, 3-decyne was only poorly metabolically activated. In contrast, both 2-decyne and 1-decyne were much more effective in causing the loss of cytochrome P-450. Somewhat unexpectedly, 2-decyne was rather more effective than was 1-decyne in this respect (Table 1, group C). In this complex microsomal system, the formation of active metabolites can not be directly determined but only indirectly estimated by the destruction of cytochrome P-450. Even if metabolic activation of the ethynyl-substituent occurs, other factors including the structure of the remainder of the molecule may affect the extent of cytochrome P-450 destruction. The nature of the active metabolites involved are not yet known.

The alkynes appear to be a good model for the study factors affecting metabolic activation. The effect of chain length on metabolic activation of some terminally substituted alkynes is shown in Fig. 2. 1-Decyne was one of the most effective in causing the destruction of cytochrome P-450; both shorter and longer chain lengths were less effective. Acetylene gas can also cause loss of cytochrome P-450 *in vivo* and *in vitro* [2].

Table 1. Effect on liver microsomal cytochrome P-450 of various ethynyl-substituted compounds *in vitro*

	Cytochrome P-450 remaining (per cent)*
Group A. Water-soluble drugs	
Pargylene	99.4 ± 1.3
4-Amino hex-5-ynoic acid	104.3 ± 2.3
Ethinamate	102.4 ± 2.7
Group B. Compounds with a non-terminal ethynyl substituent	
Barban	108.8 ± 4.2
Tremorine	110.7 ± 4.5
5-Decyne	97.7 ± 1.2
3-Decyne	90.6 ± 1.5
2-Decyne	48.1 ± 2.1
Group C. Terminally-substituted ethynyl compounds	
1-Decyne	56.9 ± 1.3
Norethindrone	74.8 ± 2.6

* Data represent the mean ± S.E. of 4 experiments.

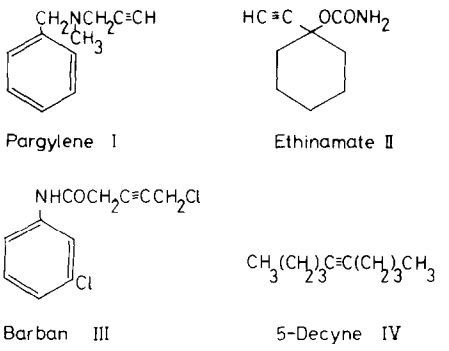

Fig. 1. Chemical structures of some ethynyl-substituted compounds.

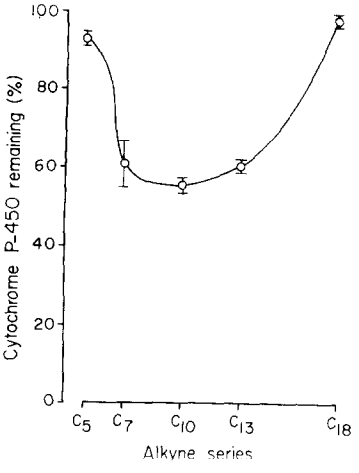

Fig. 2. Effects of various 1-alkynes of different chain lengths on microsomal cytochrome P-450 *in vitro*. Results represent the mean ± S.E. of 4 experiments. C_5 = 1-pentyne; C_7 = 1-heptyne; C_{10} = 1-decyne; C_{13} = tridecyne; C_{18} = 1-octadecyne.

Acetylenic substituted compounds are found in some plant species (reviewed in ref. 9). The fruit of the avocado (*Persia* sp.) contains a number of lipophilic compounds with terminal acetylenic substituents [7]. Extracts of avocado stone or mesocarp when given in large doses to rats caused a time dependent loss of hepatic cytochrome P-450. There was no significant effect on cytochrome b_5 (Fig. 3). A similar selective action on cytochrome P-450 has been reported with other ethynyl-substituted compounds [1, 2]. Rats given avocado stone extracts and killed at times 2 hr after dosing had dark congested livers and clear ascites in the peritoneal cavity. Such toxic effects may have contributed to the more prolonged depression of hepatic cytochrome P-450 in these animals. Rats given mesocarp extract were not affected in this way. When extracts from the livers of rats killed 4 hr after dosing with avocado stone (but not mesocarp) preparations were separated on silica gel thin layers plates [2], characteristic green pigments which fluoresced red under u.v. light were observed. Attempts to isolate the active principles involved in these effects have not yet been successful.

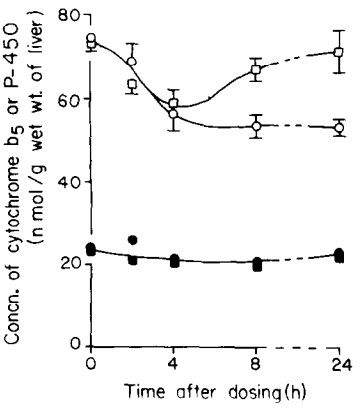

Fig. 3. Effects on liver cytochromes P-450 and b_5 concentrations of extracts of avocado fruit. At various times after dosing with avocado extracts, animals were killed and the concentrations of the liver microsomal cytochrome P-450 (open symbols) or b_5 (closed symbols) estimated. □, ■ mesocarp extract, ○, ● stone extract. Results represent the mean ± S.E. of 4 experiments.

Whether the loss of cytochrome P-450 is due to acetylenic-substituted compounds, or to other components in the crude extract such as allylic or thiono-compounds known to exert similar effects [10] is not yet clear. The formation of green pigments in the liver, however, suggests that a specific effect is involved rather than a general suppression of the concentration of these cytochromes. At present, only ethynyl, vinyl or allylic substituted compounds are known to be activated to derivatives which can cause the formation of green pigments from haem in the liver [10]. These results also suggest that destruction of cytochrome P-450 in the liver may occur following the ingestion of such compounds in the diet.

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