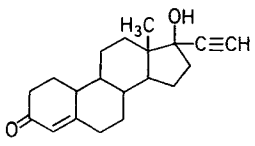
Suicidal Destruction of Cytochrome P-450 by Ethynyl Substituted Compounds

Ian N. H. White[1]

Abstract: Compounds containing a terminal carbon-carbon triple bond, ranging in structure from the 17α-ethynyl substituted contraceptive steroids to acetylene gas, when administered to rats cause a selective and rapid time dependent loss of up to 50% of hepatic cytochrome P-450. Cytochrome b_5 is not affected. Metabolic activation of the acetylenic substituent by the phenobarbital inducible, NADPH dependent, mixed function oxidases results in the formation of a reactive species which alkylates one of the tetrapyrrole nitrogen atoms of heme to form a 1 : 1 covalent adduct. Either cytochrome P-450 destruction or the formation of N-alkylated porphyrins (green pigments) have been used to assess factors affecting the extent of metabolic activation of the acetylenic group in rats, man, and other laboratory species. The chemical identity of a number of green pigments have been resolved. Those formed from 1-octyne consist of the protoporphyrin IX ring of heme substituted with the saturated 2-oxo-octyl group. In contrast, reactive metabolites of 1-octyne trapped with N-acetylcysteine contain the unsaturated 3-oxo-octenyl substituent. Two independent routes of activation of terminal acetylenes have been described to account for these results. Both pathways can lead to cytochrome P-450 loss but only one, probably involving an oxirene intermediate, leads to green pigment formation.

Compounds containing the carbon-carbon triple bond are not known to occur naturally in mammalian systems although they are quite widely distributed in the plant kingdom (1, 2). Until the late 1960s, the ethynyl substituent was regarded as non-toxic in biochemical systems. It was introduced into drugs to improve their biological properties. An example of this is seen in the contraceptive steroids. The naturally occurring hormones progesterone and estradiol are not particularly effective in humans when given orally due to their rapid turnover in the body. The introduction of an ethynyl substituent at C17 conferred oral activity on these compounds (3, 4), e.g. norethindrone (Fig. 1).



Norethindrone

Fig. 1 Chemical structure of norethindrone.

Endo et al. (5) were the first to demonstrate that an acetylenic substituted compound could be used as a specific site directed inhibitor of the bacterial enzyme, β-hydroxyacyl-thioester dehydratase. The use of ethynyl substituted compounds as suicidal inhibitors of mammalian cytochrome P-450 has only recently been explored.

[1] Toxicology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey, GB.

Activation of Acetylenes: Introduction

Although it had been noted by Skrinjaric-Spoljar et al. (6) that the administration of certain ethynyl-substituted insecticide synergists to mice resulted in a decreased cytochrome P-450 content in the liver, the studies of White & Muller-Eberhard (7) using the ethynyl substituted contraceptive steroids norethindrone and ethynylestradiol first showed the significance of the acetylenic group in the destruction of this cytochrome. Moreover, they demonstrated the necessity for the ethynyl substituent to undergo metabolic activation to bring about destruction of cytochrome P-450. Since that time a considerable number of ethynyl substituted steroidal and non-steroidal compounds, including acetylene gas have been shown to undergo metabolic activation and to cause destruction of cytochrome P-450 in this way (8–11).

As the form(s) of cytochrome P-450 which activate the ethynyl substitutent are destroyed by the active metabolite, the process has been termed suicidal destruction of this cytochrome (12).

Loss of cytochrome P-450 is due to the destruction of the heme moiety (8). Heme which is destroyed does not go to the normal breakdown product bilirubin, but is converted into abnormal green colored pigments (7–11, 13). Ortiz de Montellano et al. demonstrated that such green pigments represent a 1:1 covalent adduct between the activated acetylene and the protoporphyrin IX ring of heme (9). From their aetio-type optical absorption spectra and titration behavior with acids De Matteis et al. demonstrated the site of alkylation to be on one of the nitrogen atoms of the tetrapyrrole ring (14, 15).

Metabolic activation of acetylenes to cause the destruction of hepatic cytochrome P-450 and the formation of green pigments closely parallels that of certain allylic, allenic and vinyl substituted compounds (16–19), for review see ref. 20).

Effects of Acetylenes *in vivo*

Destruction of Cytochrome P-450

The contraceptive steroid norethindrone has been used for the majority of the studies carried out into the effects of ethynyl substituted compounds on cytochrome P-450 and its consequences *in vivo*. A single large dose (100 mg/kg) of this steroid given to male rats causes a rapid time-dependent loss of cytochrome P-450 from the liver. Minimum levels of this cytochrome are reached 2–4 h after dosing when approximately 40% has been destroyed. This is followed by a return to control values by 24 h (7) (Fig. 2). A 17α-ethyl substituted analog, the anabolic steroid norethandrolone, does not cause such destruction. The percentage fall in cytochrome P-450 levels in the liver is dependent on the compound employed, but

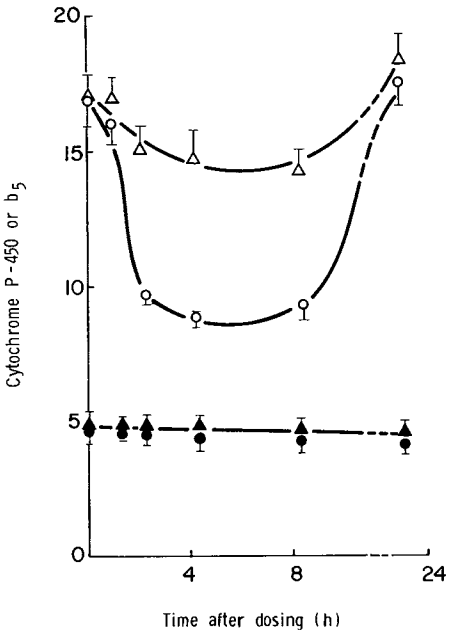


Fig. 2 Effects of a single dose of norethindrone (100 mg/kg body weight) on the hepatic cytochromes P-450 or b_5 with time. At various times as indicated after dosing, male rats were killed and cytochrome P-450 (open symbols) or cytochrome b_5 (closed symbols) content in the liver microsomal fraction determined.
○, ●, norethindrone; △, ▲, norethandrolone (Figure redrawn from ref. 7*).

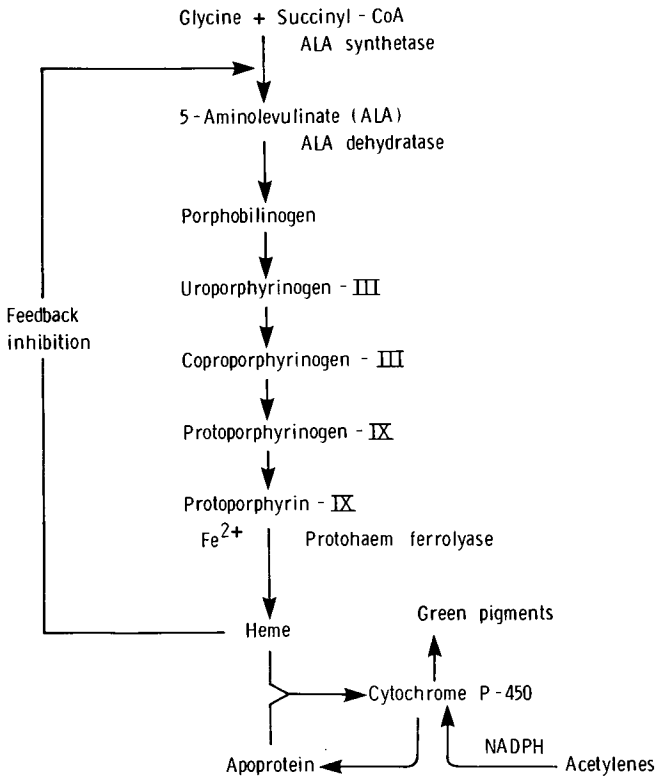


Fig. 3 The pathway for hepatic cytochrome P-450 synthesis and destruction by acetylenes.

even at the highest dose levels only up to half of the total cytochrome P-450 is generally lost from the liver (8, 10, 11). The concentration of other hepatic cytochromes, e.g., cytochrome b_5, is not affected over this time (7). No loss of cytochrome P-450 or the formation of green pigments have been reported in other organs, although this may only reflect the limited sensitivity of the present analytical procedures.

Interpretation of the effects that *low doses* of the ethynyl-substituted contraceptive steroids have on cytochrome P-450 and the drug metabolizing systems *in vivo* is difficult due to three factors:

1. Certain steroids, including the 17α-ethynyl substituted ones, are potent direct-acting inhibitors of some hepatic mixed function oxidase enzymes *in vitro* (21, 22). It is not easy to conclude therefore if the reduction in hepatic drug metabolizing activities caused by the administration of low doses of these steroids *in vivo* (23, 24) is due primarily to their direct inhibitory action or to the suicidal destruction of certain forms of cytochrome P-450.
2. Repeated administration of low doses of certain estrogenic steroids, e.g., ethynylestradiol, over a period of several days also results in a reduction in the concentration of hepatic cytochrome P-450 (25, 26). This appears due primarily to a cholestatic effect of these compounds (25), mechanistically not related to metabolic activation of the ethynyl substituent and the acute destruction of this cytochrome.
3. Longer term administration, ca. 30 days, of combined ethynyl estrogen/progesterone mixtures at contraceptive dose levels can result in an *induction* of hepatic cytochrome P-450 and liver monooxygenase activities (27).

* Reproduced with the permission of the Biochemical Journal.

Effects on the Hepatic Heme Biosynthesis Pathway

Because of the regulatory (repressive) action heme has over its own biosynthesis (28) (Fig. 3), destruction of heme in the liver by norethindrone leads to a compensatory time dependent increase in the activity of the rate limiting enzyme of heme biosynthesis, 5-aminolevulinic acid synthetase. This is followed by a 2–4 fold increase in the concentration of porphyrins in the liver (7). Concentrations of norethindrone used in such experiments are very much greater than the pharmacologically active dose used in the contraceptive pill. However, it has been suggested that the above mechanism may be involved in the photosensitization side effects which occur in some women taking these steroids (7).

Some N-alkylated porphyrins, e.g., N-methylprotoporphyrin IX also have a pronounced inhibitory action on the protoheme ferrolyase enzyme of heme biosynthesis causing the accumulation of porphyrins in the liver (29). This is not known to occur with the sterically more bulky green pigments formed from ethynyl-substituted compounds.

Hepatic Green Pigment Formation

Green pigments formed in the liver *in vivo* generally reflect cytochrome P-450 destruction in a time and dose dependent manner (13). With norethindrone in the rat, there is a good correlation between the amount of cytochrome P-450 lost and green pigment formed (13), but with other acetylenes, e.g. diisopropargylacetamide, there is much greater destruction of this cytochrome than production of green pigment (8).

Green pigments can be separated from heme in liver extracts by thin layer chromatography or high performance liquid chromatography (H.P.L.C.) (7–9, 13). These studies

show the chromatographic profiles of green pigments depend on the acetylene used. Three major green pigments can be separated in this way following the administration of norethindrone to male rats (Fig. 4). If heme in the liver is prelabeled with ^{14}C by giving [4-^{14}C]5-aminolaevulinic acid before the administration of norethindrone, radioactive label remains associated with all of the green pigment peaks (13). Similarly if [9,11^{3}H] norethindrone is used, green pigments contain the tritium label (9).

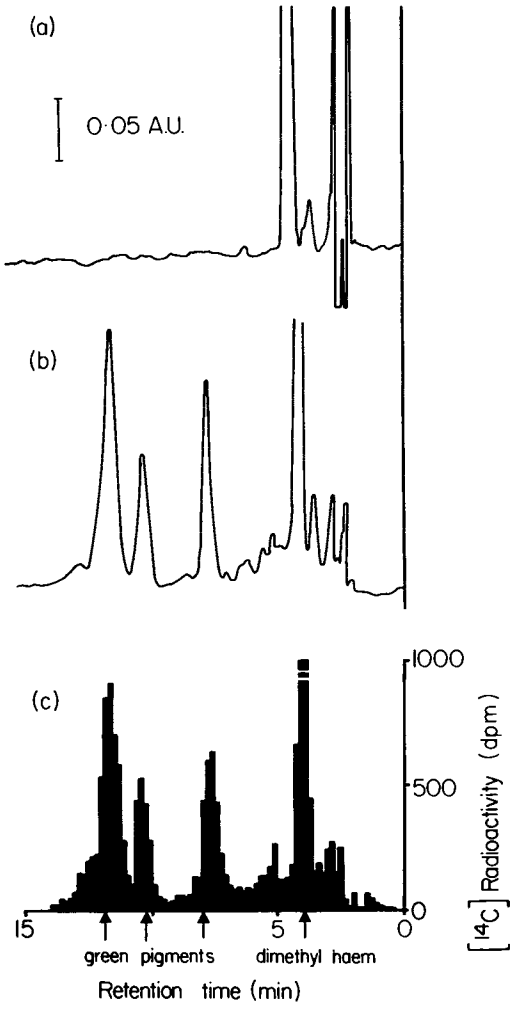


Fig. 4 Separation of heme and green pigments by HPLC Phenobarbitone pretreated rats with liver heme prelabeled with ^{14}C were dosed with norethindrone acetate (100 mg/kg body weight) (b) and (c) and killed 8 h later. Liver extracts were subjected to silica gel H.P.L.C. Trace (a) represents a liver extract from a control rat. The ordinates in (a) and (b) represent relative absorbance at 417 nm. (c) Represents ^{14}C radioactivity in fractions collected from the detector outlet from (b). Figure reproduced from Ref. 13*.

Green pigments isolated by conventional procedures as the dimethyl esters fluoresce red under long wave U.V. light, having lost the iron atom of heme. If such liver extracts are not exposed to low pH values, iron remains associated with the green pigments and they no longer show red fluorescence. As iron is only co-ordinated with three of the tetrapyrrole nitrogen atoms in such green pigments it is readily lost under the acidic workup conditions generally employed (13). The optical absorption spectra of the green pigments depend on whether they are metal free, contain iron, or other metals, e. g. zinc, which is readily chelated by the metal free pigments (9, 10, 13) (Fig. 5). All of the norethindrone green pigments have similar absorption spectra but have not been fully characterized. It has been suggested that the different components separated by H.P.L.C. may represent isomeric forms of the same adduct (9). However, the fact that only a single green pigment is produced by microsomal systems *in vitro* (13) and that the chromatographic profile of the norethindrone green pigments produced *in vivo* differs between species (30) suggests metabolic modification may be involved.

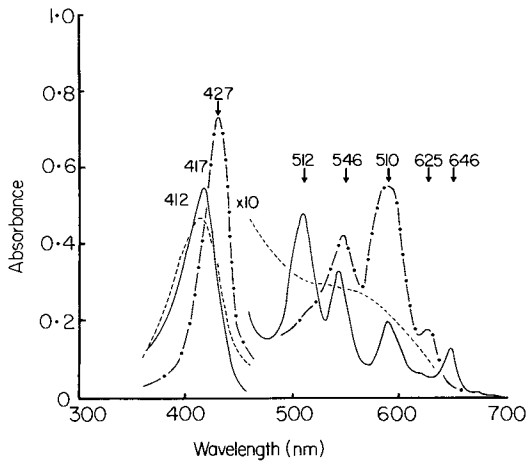


Fig. 5 Absorption spectra of purified green pigments formed from norethindrone. Neutral absorption spectra in $CHCl_3$ of norethindrone green pigments purified as the dimethyl esters. ——, Metal free pigment; –.–, zinc chelate; ---, iron chelate. Figure reproduced from Ref. 13*.

Green pigments formed in the liver following the administration of norethindrone are excreted into the bile. Traces are found in the plasma while none can be detected in the urine (31). In this respect green pigments behave in a similar manner to the heme breakdown product bilirubin. However, unlike bilirubin, if heme in the liver is prelabeled with ^{59}Fe prior to dosing with steroid, biliary green pigments purified under mild conditions of pH, still contain ^{59}Fe label (31).

The total amount of green pigment excreted into the bile in the 24 h after dosing with norethindrone is greater than the total concentration of cytochrome P-450 in the liver. This suggests a rapid turnover of the forms of cytochrome P-450 responsible for metabolic activation of this steroid. Some turnover is due to the synthesis of new cytochrome protein since green pigment excretion is reduced in rats pretreated with cycloheximide (31). However, there is evidence to suggest that heme, either from an endogenous *free heme pool* or from exogenously administered heme, can be incorporated into apocytochrome P-450 to yield an enzymically active holoenzyme capable of forming more green pigment (32).

Short term loss of up to half of the total liver cytochrome P-450 does not appear to have adverse physiological effects on the animal. Rapid regeneration of the holoenzyme from the *free heme pool* suggests that rather than this cytochrome being *suicidally* destroyed, the expendable cytochrome heme may

* Reproduced with the permission of the Biochemical Journal.

act as a trapping nucleophile for reactive metabolites formed in the endoplasmic reticulum in a manner analogous to the role reduced glutathione plays in the cytosol. Active metabolites of the ethynyl substituent of norethindrone, trapped as an adduct with heme are eliminated via the feces (31). They are not retained in the body by the extensive enterohepatic circulation experienced by the parent steroids (33, 34).

Effects of Acetylenes *in vitro*

The Identity of the Activating Enzyme System

Incubation of many terminally substituted acetylenes with hepatic microsomes and an NADPH generating system, in air, leads to a time dependent reduction in the concentration of cytochrome P-450 and heme in the reaction mixture and the formation of green pigments (7–9, 11, 13). As with experiments carried out *in vivo,* the proportion of cytochrome P-450 destroyed varies depending on the acetylene used, concentration and time of incubation, but rarely exceeds 50% of the total (Table I). Destruction of cytochrome P-450 and particularly the formation of green pigment rapidly becomes nonlinear with time (1–2 min) due to the suicidal destruction of the cytochrome. In such microsomal systems, in the absence of cytosol, reactivation of the apocytochrome P-450 from noncytochrome heme pools does not appear to occur (13).

If microsomal incubations are carried out in an atmosphere enriched with ^{18}O, this isotope is incorporated into the green

Table I. Destruction of Cytochrome P-450 by acetylenic substituted compounds in microsomal systems in vitro compared with the ability to form green pigments when administered in vivo.
Microsomes from phenobarbitone pretreated male rats were incubated aerobically with the appropriate substrate at a saturating concentration together with a NADPH generating system for 10 mins at 37°C.

Compound	Cytochrome P-450 destroyed (percent)	Reference
a) Terminal acetylenes causing the loss of Cytochrome P-450 and green pigment formation.		
Dipropargylacetamide	45	(8)
1-Decyne	43	(11)
1-Octyne	33	(estimated from ref. 44)
Norethindrone	27	(7)
Phenylacetylene	18	(10)
b) Substrates causing loss of Cytochrome P-450 but no green pigment formation.		
2-Decyne	52	(11)
4-methyl-2-octyn-4-ol	35	(10)
2-Hexyne	27	(10)
c) Acetylenes causing no Cytochrome P-450 loss.		
5-Decyne	0	(11)
Tremorine	0	(11)
Pargylene	0	(11)

pigment adduct (35). Loss of cytochrome P-450 and green pigment formation is dependent on the presence of oxygen and NADPH and is prevented if incubations are carried out anaerobically or in the presence of carbon monoxide (7). These are typical properties of the microsomal mixed function oxidase enzymes. Loss of cytochrome P-450 or the formation of green pigments cannot be prevented by the inclusion of nucleophiles such as reduced glutathione in the incubation mixtures, suggesting the reactive species of the acetylenic group may have a very short half life (7).

The activating enzyme is induced by pretreatment of rats with phenobarbitone and to a lesser extent Arochlor 1254 (13). The steroidal inducer pregnenolone-16α-carbonitrile has little effect, while cytochrome P-448 induced by pretreatment of rats with 3-methylcholanthrene does not appear to be able to activate the ethynyl substituent (13). Similar results have been obtained using highly purified cytochrome systems (12).

Species variations in cytochrome P-450 destruction and in the formation of green pigments using norethindrone as a model acetylene showed the male rat is most active in this respect, followed by hamster > female rat > rabbit > mouse > marmoset > hen (30). Human liver microsomes have activities which are only 2 % those of male rats. Microsomal systems from the different species incubated with norethindrone for short periods of time form only one green pigment as judged by H.P.L.C. Additional cytosolic factors are required to form the different chromatographic profiles of green pigments produced *in vivo* (30).

Structural Requirements for Metabolic Activation of the Ethynyl Substituent

Structure activity studies using rat liver microsomal systems suggest criteria for metabolic activation leading to cytochrome P-450 destruction may differ in certain respects from those which lead to green pigment formation. Both green pigment formation and cytochrome P-450 destruction generally occur following metabolic activation of an ethynyl substituent in a terminal, sterically unhindered, position in the molecule, e. g. the sedative ethchlorvynol (36), or the model acetylene 1-decyne (11) (Table I). Activation does not occur, or only occurs to a limited extent, if preferential alternative routes of metabolism exist or if the compound is not sufficiently lipophilic to enter the hydrophobic environment of the microsomal mixed function oxidase activating system, e. g. the monoamine oxidase inhibitor pargylene (11). Metabolic activation also does not occur if the carbon-carbon triple bond is at a non-terminal location in the molecule not adjacent to the terminal carbon atom, e. g. tremorine or 5-decyne (11). In other instances, i. e. where the acetylenic bond is adjacent to the terminal carbon atom, e. g., 2-decyne, activation occurs but the active metabolites, while causing the loss of cytochrome P-450 do not give rise to green pigments (Table I).

There is some evidence to suggest that naturally occurring acetylenes, some of which meet the structural requirements needed to undergo metabolic activation, may cause the destruction of cytochrome P-450 when administered to rats (11). As far as is known, no studies have been carried out on the substrate specificities of plant or bacterial cytochrome P-450 in relation to the metabolic activation of acetylenes.

Routes of Metabolic Activation of the Ethynyl Substituent

Classical studies on the metabolism of acetylenes such as phenylacetylene showed its conversion to phenylacetic acid *in vivo*. An aldehyde intermediate was proposed in this reaction (37). Similarly, 4'-ethynyl biphenyl (38) and 4'-ethynyl-2-fluorobiphenyl (39) are metabolized to the corresponding biphenylacetic acids. A different mechanism has been proposed for the oxidation of these compounds involving: a) conversion of the ethynyl substituent to an oxirene intermediate, b) isomerization of the oxirene to a ketene, c) addition of water to the ketene to yield the acid (38, 40) (Fig. 6). Strong evidence for the existence of this pathway operating in the conversion of acetylenes to the corresponding carboxylic acids was obtained by Ortiz de Montellano and Kunze (41) using [1-^{2}H]ethynylbiphenyl. This group demonstrated retention of the deuterium label following microsomal metabolism and an intramolecular transfer of the ^{2}H atom to C2 of the biphenylacetic acid. These observations rule out possible alternative routes of conversion to the acid via a hydroxylated ethynyl intermediate.

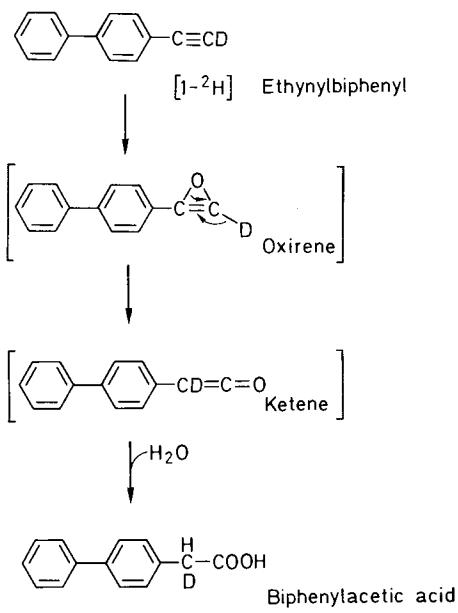


Fig. 6 Proposed pathway for the metabolism of 4-ethynyl biphenyl to the carboxylic acid via an oxirene intermediate (after Refs. 38, 41).

Metabolism of the ethynyl substituent of the contraceptive steroid ethynylestradiol via an oxirene intermediate has also been proposed to account for a novel pathway involving D-ring enlargement (D-homoannulation) found following the administration of this compound to rabbits or monkeys (42, 43). De-ethynylation of [20,21^{14}C]ethynylestradiol via a possible aldehyde intermediate in this mechanism is accompanied by the formation of $^{14}CO_2$. It is not known if such an intermediate could be involved in the destruction of cytochrome P-450; certain aldehydes do cause loss of this cytochrome in microsomal systems *in vitro* (44).

Using 1-octyne as a model acetylene, an alternative route for the metabolic activation of terminal acetylenes, not involving an oxirene intermediate has recently been described (45).

1-Octyne like other terminally substituted acetylenes causes a time dependent loss of cytochrome P-450 from microsomal systems *in vitro* (44). Using [1,2^{14}C]octyne, some ^{14}C label, in addition to becoming covalently bound to heme to give green pigments, also becomes covalently bound to microsomal protein, or to DNA if the latter is added to the incubation mixtures (45). The non-linearity of the time course of covalent binding to protein is similar to that of covalent binding to heme, suggesting the same forms of cytochrome P-450 are catalyzing both reactions. However, covalent binding to protein but not to heme can be prevented by the inclusion of various nucleophiles, e. g. N-acetylcysteine, in the reaction mixture.

Fig. 7 shows the proposed activation pathway. Cytochrome P-450 mixed function oxidases hydroxylate 1-octyne at a position α to the carbon-carbon triple bond to yield octyn-3-ol.

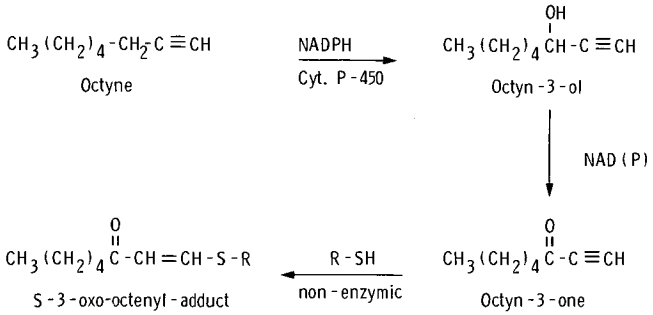


Fig. 7 Proposed pathway for the metabolic activation of 1-octyne via a 3-oxo intermediate (Ref. 45).

Octyn-3-ol can be further oxidized by NAD(P)-dependent microsomal dehydrogenases to yield the reactive intermediate 1-octyn-3-one. The 3-oxo intermediate prepared chemically reacts non-enzymically with microsomal thiols or trapping nucleophiles such as N-acetylcysteine to form the unsaturated S-3-oxo-octenyl adducts (45). Octyn-3-one can also cause the destruction of cytochrome P-450 by reacting with the cytochrome protein (I.N.H. White, unpublished results). Octyn-3-one does not, however, react with the heme of this cytochrome and no green pigments are formed in microsomal systems *in vitro* (44). The 3-oxo pathway can only operate where the carbon atom adjacent to the triple bond is not fully substituted, e. g. certain propargyl substituted insecticide synergists may be activated in this way (46, 47). The 3-oxo pathway cannot take place with the ethynyl-substituted contraceptive steroids since C17 is fully substituted. The extent to which this mechanism competes with alternative routes of metabolism of the ethynyl substituent, e. g. oxirene formation, has not been defined. The presence of this alternative route of activation which does not lead to green pigment formation may account for the disproportionately large loss of cytochrome P-450 relative to green pigment formation which occurs following the administration of certain propargyl substituted compounds, e. g., diisopropargylacetamide, to rats (8).

It is suggested that a mechanism analogous to the 3-oxo pathway may also occur during the metabolic activation of 2-acetylenes, e. g. 2-decyne, where cytochrome P-450 loss takes place but no green pigment is formed (cf. Table I). Microsomal hydroxylation of the *terminal* carbon atom would yield, after oxidation, highly reactive propargyl aldehyde intermediates. Certain unsaturated aldehydes, e. g. acrolein, when formed *in vivo* cause the loss of cytochrome P-450 by an analogous mechanism (48), presumably by reacting with the cytochrome protein thiol groups.

The Identity of the Reactive Species Responsible for Green Pigment Formation

Helton and Goldzieher (49) suggested that the ethynyl substituent of the contraceptive steroids could undergo metabolic activation via an oxirene intermediate to yield a carbonium ion as the reactive alkylating species. Carbonium ions formed as a result of metabolic activation of, for example, diethylnitrosamine are known to give rise to green pigments in the liver (50). However, the most important evidence for the nature of the reactive species has come from the work of Ortiz de Montellano et al. who have elucidated the chemical identity of a number of green pigments formed from ethynyl-substituted compounds (10, 40, 51) and particularly that formed from 1-octyne (35). The octyne green pigment represents protoporphyrin IX substituted with the saturated 2-oxo-octyl group on one of the tetrapyrrole nitrogen atoms (Fig. 8). Apart from acetylene gas which alkylates two nitrogen atoms of the protoporphyrin IX ring (35), comparable results have been obtained with other ethynyl-substituted compounds (10, 35, 51). Sophisticated chemical studies into the regiospecificity of heme alkylation at the cytochrome P-450 active site, show the tetrapyrrole nitrogen atoms are not randomly alkylated but only pyrrole ring A is attacked in this way (35). The mechanisms for such an attack is proposed to occur via the formation of an oxirene intermediate (35, 39). The oxirene forming an oxygen-iron bond before alkylating the pyrrole nitrogen atom (Fig. 8).

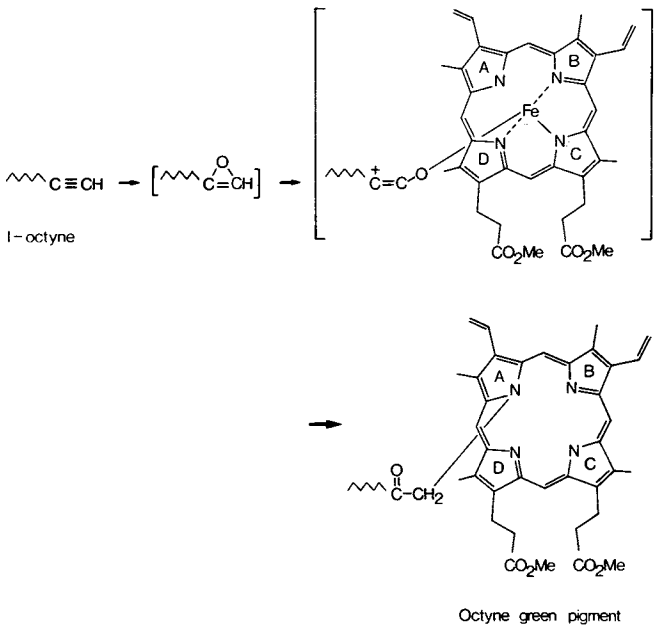


Fig. 8 Mechanism of activation of 1-octyne via an oxirene intermediate and the structure of the green pigment formed from this compound (after Refs. 10, 35).

Oxirenes are chemically too unstable to be isolated (52) and tested directly for their ability to cause the destruction of cytochrome P-450. There is some evidence to suggest that an

oxirene intermediate may not be directly involved in the alkylation of heme. (i) Neither phenylacetylene or 4'-ethynylbiphenyl, where a major metabolic pathway is conversion to the corresponding carboxylic acid via an oxirene intermediate, are particularly effective in giving rise to green pigments in rats (cf. Table I, Ref. 44). In contrast, no conversion of 1-octyne to octanoic acid can be demonstrated (45). As far as is known, there are no reports of the conversion of the 17α-ethynyl substituent of the contraceptive steroids to the corresponding carboxylic acid. (ii) Involvement of an *oxirane* in the metabolic activation of the carbon-carbon double bond to derivatives which form green pigments has been specifically excluded (53).

Activation of the ethynyl substituent through a number of alternative mechanisms have been excluded. These include (i) an initial reductive step resulting in the formation of a vinyl substituent (8). (ii) Rearrangement of the acetylenic bond into an allenic one (19) and isomerization of an oxirene to form monosubstituted ketene (44).

Oxidation of acetylenes leading to intermediates involved in either heme alkylation or subsequent oxirene formation may proceed via independent mechanisms from a common precursor in a manner analogous to that proposed for the activation of olefins (53, 54).

Metabolic Activation of the Ethynyl Substituent: Chronic Effects

The majority of studies carried out on the actions of the active metabolites of the ethynyl substituent have been on the short term alkylation of the heme of cytochrome P-450. The administration of radiolabeled ethynyl-substituted contraceptive steroids to rats also results in radiolabel becoming covalently bound to liver proteins (55, 56) and, in the case of ethynyl estradiol, to DNA (57).

Binding may at least in part be due to active metabolites of the 17α-ethynyl substituent (cf 1-octyne), although classically it has always been attributed to the activation of the ring A of the steroid (55, 56). The involvement of the ethynyl group in the genotoxic effects (58, 59) or the vascular changes (60, 61) which are associated with the long term use of the contraceptive steroids has not yet been defined.

Conclusions: Relevance to Human Exposure

Terminally-substituted acetylenes, previously regarded as biochemically unreactive, can undergo metabolic activation in the liver by the cytochrome P-450 dependent mixed function oxidases. At least two species of reactive intermediate may be produced. With the 3-oxo-activated acetylenes (Fig. 7), some information on the chemical reactivity and potential for covalent binding to protein or to DNA is available (45). Possible human exposure to compounds activated by this pathway appears limited. Examples may include certain synthetic insecticides synergists and some naturally occurring acetylenes. The alternative route of activation of this group involves the proposed oxirene intermediate (Fig. 8). Such derivatives are chemically too unstable to be isolated and direct studies made on their sites of interaction. Only covalent binding of this species to the heme of cytochrome P-450 has been established (9, 10, 35, 51). Human exposure to compounds activated by this pathway is more widespread, for example, the ethynyl-substituted contraceptive steroids. Activation of this substituent by human liver microsomal preparations, when expressed on a *per mg of microsomal protein basis* is fortunately low compared with many rodent species (30). Short term destruction of the heme of cytochrome P-450 does not appear to have any harmful effects, the holoenzyme being regenerated from endogenous heme pools (32). However, epidemiological studies indicate chronic exposure of women to the contraceptive steroids is associated with a number of undesirable side effects (58–61). It remains to be established the role, if any, that active metabolites of the ethynyl substituent plays in these side effects.

References

(1) Jones, E. R. H., Thaller, V. (1978) in The Chemistry of the Carbon-Carbon Triple Bond (part 2) (Patai, S. ed.) pp. 621–633. John Wiley and Sons, New York.
(2) Bohlmann, E., Burkhart, R., Zdero, C. (1973) in Naturally Occurring Acetylenes, Academic Press, London and New York.
(3) Drill, V. A. (1977) J. Toxicol. Env. Hlth. 3, 133–138.
(4) Murad, F., Haynes, R. C. (1980) in The Pharmacological Basis of Therapeutics (6th edition) (Goodman Gilman, A., Goodman, L. S., Gilman, A., eds.) pp. 1420–1447, Macmillan Publishing Co. Inc., New York.
(5) Endo, K., Helmkamp, G. M., Bloch, K. (1970) J. Biol. Chem. 245, 4293–4296.
(6) Skrinjaric-Spoljar, M., Matthews, H. B., Engel, J. L., Casida, J. E. (1971) Biochem. Pharmac. 20, 1607–1618.
(7) White, I. N. N., Muller-Eberhard, U. (1977) Biochem. J. 166, 57–64.
(8) White, I. N. H. (1978) Biochem. J. 174, 853–861.
(9) Ortiz de Montellano, P. R., Kunze, K. L., Yost, G. S., Mico, B. A. (1979) Proc. Natl. Acad. Sci USA 76, 746–749.
(10) Ortiz de Montellano, P. R., Kunze, K. L. (1980) J. Biol. Chem. 255, 5578–5585.
(11) White, I. N. H. (1980) Biochem. Pharmac. 29, 3253–3255.
(12) Ortiz de Montellano, P. R., Mico, B. A., Mathews, J. M., Kunze, K. L., Miwa, G. T., Lu, A. Y. H. (1981) Arch. Biochem. Biophys. 210, 717–728.
(13) White, I. N. H. (1981) Biochem. J. 196, 575–583.
(14) De Matteis, F., Cantoni, L. (1979) Biochem. J. 183, 99–103.
(15) De Matteis, F., Gibbs, A. H., Cantoni, L., Francis, J. (1980) in Environmental Chemical Enzyme Function and Human Disease: Ciba Found. Symp. 76, 119–139.
(16) De Matteis, F. (1971) Biochem. J. 124, 767–777.
(17) De Matteis, F. (1978) Pharmac. Ther. A. 2, 693–725.
(18) Ziman, M. R., Bradshaw, J. J., Ivanetich, K. M. (1980) Biochem. J. 190, 571–580.
(19) Ortiz de Montellano, P. R., Kunze, K. L. (1980) Biochem. Biophys. Res. Comm. 94, 443–449.
(20) Testa, B., Jenner, P. (1981) Drug Metab. Rev. 12, 1–117.
(21) Judiau, M. R., Fouts, J. R. (1966) Biochem. Pharmac. 15, 891–898.
(22) Tuttenberg, K. H., Huthwohl, B., Kahl, R., Kahl, G. F. (1974) Biochem. Pharmac. 23, 2037–2043.
(23) Bellward, G. D., Morgan, R. G., Szonibathy, V. H. (1974) Can. J. Physiol. Pharmacol. 52, 28–38.
(24) Field, B., Lu, G., Hepner, G. W. (1979) Clin. Pharmacol. Thera. 25, 196–198.
(25) Mackinnon, M., Simon, F. (1975) Biochem. Pharmac. 24, 749–794.
(26) Sweeney, G. D., Cole, F. M. (1980) Lab. Invest. 42, 231–235.
(27) Jori, A., Salmona, M., Cantoni, L., Guiso, G. (1977) in Pharmacology of Steroid Contraceptive Drugs (Garattini, S., Berendes, W. H. eds.) pp. 313–325, Raven Press, New York.
(28) Granick, S. (1966) J. Biol. Chem. 241, 1359–1375.
(29) De Matteis, F., Gibbs, A. H., Smith, A. G. (1980) Biochem. J. 189, 645–648.

(30) White, I. N. H., Boobis, A. R., Davies, D. S. (1984) Biochem. Pharmac. 33, 459–464.
(31) White, I. N. H. (1982) Biochem. Pharmac. 31, 1337–1342.
(32) Correia, M. A., Farrell, G. C., Olson, S., Wong, J. S., Schmid, R., Ortiz de Montellano, P. R., Beilan, H. S., Kunze, K. L., Mico, B. A. (1981) J. Biol. Chem. 256, 5466–5470.
(33) Back, D. J., Breckenridge, A. M., Crawford, F. E., Orme, M. L'E., Rowe, P. H., Smith, E. (1978) J. Pharmacol. Exp. Thera. 207, 555–565.
(34) Back, D. J., Breckenridge, A. M., Challiner, M., Crawford, F. E., Orme, M. L'E., Rowe, P. H., Smith, E. (1978) J. Steroid. Biochem. 9, 527–531.
(35) Kunze, K. L., Mangold, B. L. K., Wheeler, C., Beilan, H. S., Ortiz de Montellano, P. R. (1983) J. Biol. Chem. 258, 4202–4207.
(36) Ortiz de Montellano, P. R., Beilan, H. S., Matthews, J. M. (1982) J. Med. Chem., 25, 1174–1179.
(37) El-Masri, A. M., Smith, J. N., Williams, R. T. (1958) Biochem. J. 68, 199–204.
(38) Wade, A., Symons, A. M., Martin, L., Parke, D. V. (1979) Biochem. J. 184, 509–517.
(39) Sullivan, H. R., Roffey, P., McMahon, R. E. (1979) Drug Metab. Dispos. 7, 76–80.
(40) McMahon, R. E., Turner, J. C., Whitaker, G. W., Sullivan, H. R. (1981) Biochem. Biophys. Res. Commun. 99, 662–667.
(41) Ortiz de Montellano, P. R., Kunze, K. L. (1980) J. Am. Chem. Soc. 102, 7375–7377.
(42) Abdel-Aziz, M., Williams, K. (1969) Steroids, 13, 809–820.
(43) Schmid, S. E., Au, W. Y. W., Hill, D. E., Kadlubar, F. F., Slikker, W. (1983) Drug Metab. Dispos. 11, 531–536.
(44) White, I. N. H. (1982) Chem. Biol. Interact. 39, 231–243.
(45) White, I. N. H., Campbell, J. B., Farmer, P. B., Bailey, E., Nam, N.-H., Thang, D. C. (1984) Biochem. J. 220, 85–94.
(46) Sacher, R. M., Metcalf, R. L., Fukuto, T. R. (1968) J. Agr. Food Chem. 16, 779–786.
(47) Fellig, J., Barnes, J. R., Rachlin, A. I., O'Brien, J. P., Focella, A. (1970) J. Agr. Food. Chem. 18, 78–80.
(48) Marinello, A. J., Gurtoo, H. L., Struck, R. F., Paul, B. (1978) Biochem. Biophys. Res. Comm. 83, 1347–1353.
(49) Helton, E. D., Goldzieher, J. W. (1977) J. Toxicol. Env. Hlth. 3, 231–241.
(50) White, I. N. H., Smith, A. G., Farmer, P. B. (1983) Biochem. J. 212, 599–608.
(51) Ortiz de Montellano, P. R., Kunze, K. L. (1981) Biochemistry 20, 7266–7271.
(52) Lewars, E. G. (1983) Chem. Rev. 83, 519–534.
(53) Oritz de Montellano, P. R., Marigold, B. L. K., Wheeler, C., Kunze, K. L., Reich, N. O. (1983) J. Biol. Chem. 258, 4208–4213.
(54) Liebler, D. C., Guengerich, F. P. (1983) Biochemistry, 22, 4582–4589.
(55) Kappus, H., Bolt, H. M., Remmer, H. (1973) Steroids 22, 203–225.
(56) Maggs, J. L., Grabowski, P. S., Park, B. K. (1983) Biochem. Pharmac. 32, 2793–2800.
(57) Jaggi, W., Lutz, W. K., Schlatter, C. (1978) Chem. Biol. Interact. 23, 13–18.
(58) Committee on the Safety of Medicines (1972) Carcinogenicity Tests of Oral Contraceptives, London, HMSO.
(59) Sherlock, S. (1978) in Primary Liver Tumors (Remmer, H., Bolt, H. M., Bannasch, P., Popper, H. eds), Falk Symposium number 25, pp. 201–216. MTP Press, Lancaster, U.K.
(60) Vessey, M. P., Doll, R. (1969) Br. Med. J. 2, 651–657.
(61) Mann, J. I., Inman, W. H. W. (1975) Br. Med. J. 2, 245–248.

Skin Structure and Metabolism: Relevance to the Design of Cutaneous Therapeutics

Daniel A. W. Bucks[1]

Abstract: The outer layer of the epidermis or stratum corneum is the major barrier to percutaneous absorption. It has been shown that there are numerous enzyme systems beneath the stratum corneum in the viable epidermis capable of metabolizing drugs. A number of prodrug and soft drug topical therapeutic agents have been designed. After these agents penetrate the stratum corneum, they are metabolized by the cutaneous esterase systems to the desired metabolites.

Interest in drug metabolism by cutaneous tissues is increasing as a result of pharmacokinetic, pharmacological, therapeutic, and toxicological considerations. The ability of the skin to metabolize xenobiotics is largely unexplored. Most current knowledge on cutaneous biotransformation is restricted to steroids and polycyclic aromatic hydrocarbons. This lack of knowledge hinders a proper understanding of the fate of topically applied drugs and limits our ability to design rational cutaneous therapies. To fully appreciate the problems involved in studying the metabolism of drugs by skin, it is sensible to first consider the structure and function of skin.

[1] Departments of Pharmacy and Pharmaceutical Chemistry, School of Pharmacy and Department of Dermatology, School of Medicine, University of California, San Francisco, CA 94143

Structure and Function of Skin

Mammalian skin consists of two distinct tissue components, the epidermis and dermis, that are derived from different germinative layers (Fig. 1). The thinner, outer, stratified squamous epithelium, epidermis, is derived from ectoderm. The thicker underlying dermis consists mainly of connective tissue of mesodermal origin. In addition, there are eccrine and apocrine sweat glands, sebaceous glands, and hair follicles in skin. These structures are located primarily in the dermis, although each is derived embryologically from ectoderm.

The epidermis, approximately 0.12 mm thick, contains no blood or lymph vessels. The epidermis consists of three components: the basal layer (stratum basale), the viable layer (stratum lucidum, stratum granulosum, and stratum spinosum), and the horny or barrier layer (stratum corneum). All cells of the epidermis originate from the basal layer by the process of epidermal differentiation or keratinization.

The mechanism controlling epidermal cell proliferation and differentiation is unclear. Recent studies have suggested that cyclic nucleotide (1–3), prostaglandin (4–6) and polyamine (7, 8) metabolism may be critical determinants of normal epidermal proliferation. Epidermal growth factor (EGF) is critical to growth and maturation of the epidermis. *In vitro* studies have