Properties of the System for the Mixed Function Oxidation of Kaurene and Kaurene Derivatives in Microsomes of the Immature Seed of *Marah macrocarpus*

**ELECTRON TRANSFER COMPONENTS**

**ABSTRACT**

Cytochrome P-450 and cytochrome b2 at levels of approximately 0.10 and 0.60 nanomole per milligram of microsomal protein were detected by spectral measurements in microsomes prepared from endosperm tissue of immature *Marah macrocarpus* seeds. TPNH-cytochrome c reductase, DPNH-cytochrome c reductase, and DPNH-cytochrome b2 reductase activities were also present in these microsomes at levels of approximately 0.060, 0.22, and 0.52 unit per milligram of microsomal protein, respectively. (One unit of reductase is the amount of enzyme catalyzing the reduction of 1 micromole of electron acceptor per minute.) Treatments of microsomes with stearpin or trypsin were not effective in solubilizing any of these electron transport components in detectable form. However, treatment of a microsomal suspension in 25% glycerol with 1% sodium deoxycholate led to the release of about 60% of the protein and each of the above hemoproteins and electron transfer activities to the fraction which was not pelleted after centrifugation for 2 hours at 105,000g. Some ent-kaur-16-ene oxidase activity could be detected in the solubilized fraction after removal of the detergent. Cytochrome b5 and DPNH-cytochrome b2 reductase activity were largely separated from one another and from an overlapping mixture of TPNH-cytochrome c reductase and DPNH-cytochrome c reductase when the sodium deoxycholate-solubilized fraction was chromatographed on a DEAE-cellulose column. No cytochrome P-450 or cytochrome P-420 was detected in the column fractions and no ent-kaur-16-ene oxidase activity was detected when the column fractions were tested singly or in combination.

The possible participation of these components in the mixed function oxidation of ent-kaur-16-ene and a number of its oxidized derivatives catalyzed by these microsomes is discussed in relation to the model which has been developed to explain the function of analogous components in mixed function oxidase reactions in mammalian liver microsomes.

**MATERIALS AND METHODS**

**Microsomal Preparations.** The microsomal fraction was prepared from a homogenate of endosperm wild cucumber (A. macrocarpa) Greene. The latter name has been used in earlier publications from our laboratory dealing with the biochemical properties of immature seed of this plant.

**Figure 1.**

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1 This work was supported in part by National Institutes of Health Grant GM 07065 from the National Institute of General Medical Sciences.

2 See Figure 1 of Hasson and West (9) for the structure of this compound.

3 *Marah macrocarpus* (Greene) Greene was earlier classified as *Echinocystis macrocarpa* Greene. The latter name has been used in earlier publications from our laboratory dealing with the biochemical properties of immature seed of this plant.


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cumber (*M. macrocarpus*) seeds as described previously by Hasson and West (8). The microsomal pellets were routinely resuspended at the level of 1 to 3 mg microsomal protein/ml 0.1 M tris-maleate, pH 7.3, which contained 25% glycerol (by volume) under an atmosphere of N₂.

**Kaurene Oxidase Activity.** Kaurene oxidase was measured as previously described (8). In a typical assay [¹⁴C]kaurene (10,000 cpm; 0.3 nmol), TPNH (0.5–1.0 μmol), and FAD (0.05 μmol) were incubated with the enzyme in a total volume of 1 ml for 45 min at 30 C in a water bath with shaking. The products were extracted into acetone-benzene and the amounts of radioactivity associated with kaurene, kaurenol, kaurenal, kaurenone, acid, and 7β-hydroxykaurenoic acid were determined after chromatography of the extract on a silica gel thin layer as a basis for determining the relative oxidation values (see ref. 9).

**TPNH- and DPNH-Cytochrome c Reductase Activities.** These reductase activities were determined spectrophotometrically by a procedure based on that of Masters *et al.* (20). A reaction mixture contained in a total volume of 1 ml: 0.10 μmol either DPNH or TPNH, 0.05 μmol Cyt c, 50 μmol tris-maleate buffer, pH 7.3, and the enzyme source to be tested. Reaction was initiated by the addition of the reduced pyridine nucleotide and the change of absorbance at 554 nm was monitored in a Gilford recording spectrophotometer. The rate of Cyt c reduction was found to be linear over a period of at least 5 min and was proportional to the amounts of enzyme sample added in the concentration range in which activities were measured. One unit of reductase activity is the amount of enzyme catalyzing the reduction of 1 μmol Cyt c/min under these conditions.

**DPNH-Cytochrome b₅ Reductase Activity.** This activity was assayed according to Strittmatter (31) with potassium ferricyanide as the artificial electron acceptor. The incubation mixture contained in a volume of 1 ml: 0.10 μmol DPNH, 0.2 μmol potassium ferricyanide, 50 μmol tris-maleate, pH 7.3, and the enzyme to be tested. Reaction was initiated by the addition of DPNH and the change of absorbance at 420 nm was monitored in a Gilford recording spectrophotometer. The rate of ferricyanide reduction was found to be linear with time over a period of at least 3 min and was proportional to the amounts of enzyme sample added in the concentration range in which activities were measured. One unit of reductase is that amount of enzyme which will catalyze the reduction of 1 μmol ferricyanide/min under these conditions.

**Measurement of Hemoproteins.** The concentration of Cyt P-450 was determined by the method of Omura and Sato (25) from the CO-difference spectrum of a sample reduced by the addition of either sodium dithionite or TPNH. The absorbance difference between 450 nm and 490 nm was employed and an extinction coefficient of 91 mm⁻¹ cm⁻¹ was assumed. Cyt b₅ was estimated according to the method of Omura and Sato (23) from the absorbance differences between 424 nm and 409 nm in the reduced minus oxidized difference spectrum. An extinction coefficient of 185 mm⁻¹ cm⁻¹ was assumed. Spectra were recorded on either an Aminco Chance DW-2 dual wavelength spectrophotometer or a Cary model 15 recording spectrophotometer.

**RESULTS**

**Electron Transfer Components of Microsomes.** A CO-difference spectrum for a suspension of microsomes from *M. macrocarpus* endosperm reduced with sodium dithionite is shown in Figure 1 (curve A). Absorption maxima are evident at 450 nm and 423 nm. The 450 nm peak is believed due to Cyt P-450 while the peak at 423 nm may be attributable in part, if not entirely, to Cyt P-420, a denatured form of Cyt P-450.

The absorbance at 450 nm decreased while that at 423 nm increased in time during the course of spectral measurements. This supports the idea that the 423 nm absorbance is due to Cyt

![Fig. 1. CO-difference spectra of the *macrocarpus* microsomal suspensions. Curve A (solid line): microsomal suspensions (1 mg protein/ml) were treated with solid sodium dithionite as the reductant before the addition of CO into one cuvette. The difference spectrum is recorded for a series of reduced microsomes containing CO minus an equivalent suspension of reduced microsomes (base-line not shown). The absorbance difference on the ordinate arbitrarily refers to the most negative ΔA value in the difference spectrum as zero. Curve B (dashed line): conditions were as for curve A except that 5 × 10⁻⁴ M TPNH replaced sodium dithionite as the reductant.](http://www.plantphysiol.org/)

P-450 and indicates the instability of Cyt P-450 under these conditions. The presence of 0.11 nmol Cyt P-450 and 0.023 nmol Cyt P-420/mg protein can be calculated from the initial spectral measurements made on the suspension shown in Figure 1, curve A. The changes in the spectrum after 30 min standing at ambient temperature indicated that the same suspension contained 0.074 nmol Cyt P-450 and 0.034 nmol Cyt P-420/mg protein. After 60 min of standing the suspension contained only 0.037 nmol Cyt P-450 and 0.052 nmol Cyt P-450/mg protein. The ratio of 450 nm to 423 nm absorbance was increased if the microsomal suspension was prepared in 25% glycerol and maintained under an N₂ atmosphere. Also the 450 nm peak was stable over a longer period of time under these conditions. Similar effects of these conditions on the balance of Cyt P-450 and P-420 in liver microsomes were reported by Sato et al. (30). A similar CO-difference spectrum was observed with TPNH as the reductant rather than sodium dithionite (Fig. 1, curve B), but the specific absorbance of both the 450 nm and 423 nm peaks was much less.

The presence of Cyt b₅ in the microsomes was indicated by the difference spectrum between a reduced and oxidized microsomal suspension. A prominent peak at 424 nm and a smaller one at 554 nm are typical of Cyt b₅ as reported by Omura and Sato (24). DPNH-Cyt c red. reductase, TPNH-Cyt c reductase, and DPNH-Cyt b₅ reductase activities were detected in microsomal suspensions by spectrophotometric assays as described under "Methods."

Table I summarizes the content of these hemoproteins and the reduced pyridine nucleotide-hemoprotein reductase activities in one preparation of *M. macrocarpus* endosperm microsomes. The results are typical of a series of determinations made on different microsomal preparations.

**Solubilization of Microsomal Electron Transfer Components.** Several procedures to extract the electron transfer components from the microsomes into the "soluble" fraction which would not pellet after centrifugation at high speed have been tested. Enzymic digestions of microsomes by steapsin and trypsin were attempted as described by Omura and Sato (24) for removal of Cyt P-450 from mammalian liver microsomes. These enzyme treatments failed to release any detectable electron transport components or kaurene oxidase activity to the supernatant fraction after high speed centrifugation. The three reductase activities and Cyt b₅ were still detectable in the pellet after treatment.
with the digestive enzymes. Most of the CO-binding Cyt was found in the 423 nm form in the pellet after digestion with enzymes, whereas Cyt P-450 was still evident in control microsomes not treated with enzyme. Also the overall kaurene oxidase activity was greatly reduced in the enzyme-treated pellets in comparison with the controls.

Solubilization by treatment with detergents was more successful. The approach utilized for solubilization of the activities and subsequent resolution of the components by ion exchange chromatography were based on those pioneered for hepatic microsomes by Lu and Coon (13). (See also a recent review on the resolution and reconstitution of the liver microsomal enzyme system [14].) The results of treatment with sodium deoxycholate are summarized in Table II. About 60% of the microsomal protein was present in the supernatant fraction after treatment with this detergent. Sodium deoxycholate treatment also released into the supernatant fraction in detectable form 43% of the Cyt b_{5}, 30% of the TPNH-Cyt c reductase, 49% of the DPNH-Cyt c reductase, and 46% of the DPNH-Cyt b_{5} reductase originally present in the microsomes. About 38% of the original Cyt P-450 plus Cyt P-420 was recovered in the supernatant fraction, but only one-third of this was in the form of P-450, whereas the original microsomal pellet had 70% of the total as P-450. The kaurene oxidase activity was completely inhibited by the levels of detergent used to effect solubilization. However, after the supernatant fraction was at least partially freed of detergent by a combination of Sephadex G-25 column chromatography and concentration of the void volume fraction on a Dianlo XM-50 membrane, it showed 24% of the kaurene oxidation activity initially observed in the microsomes.

Triton X-100 treatment of microsomes under similar conditions also solubilized a reasonable fraction of the microsomal protein (72%). Cyt b_{5} (47%), TPNH-Cyt c reductase (60%), and DPNH-Cyt b_{5} reductase (76%) were also detected in the supernatant fraction. However, neither Cyt P-450 nor DPNH-Cyt c reductase activity were detected in either the supernatant or pellet fractions after the Triton X-100 treatment. The absence of these two components may account for the fact that no kaurene oxidase activity could be found in either the pellet or supernatant fractions after detergent treatment, even after the fraction had been processed on a Sephadex G-25 column and Dianlo XM-50 membrane as described above.

### Table I. Levels of Electron Transfer Components Detected in M. macrocarpus Endosperm Microsomes and Microsomes from Other Sources

<table>
<thead>
<tr>
<th></th>
<th>M. macrocarpus</th>
<th>Sorghum seedlings</th>
<th>Rat liver-control</th>
<th>Rat liver-phenobarbital treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per mg protein</td>
<td>per 100 ml endosperm</td>
<td>per mg protein</td>
<td>per mg protein</td>
</tr>
<tr>
<td>Cytchrome P-450</td>
<td>0.11 &quot;d&quot;</td>
<td>2.4 &quot;d&quot;</td>
<td>0.10 &quot;d&quot;</td>
<td>0.24 &quot;d&quot;</td>
</tr>
<tr>
<td>Cytchrome b_{5}</td>
<td>0.61 &quot;d&quot;</td>
<td>13.5 &quot;d&quot;</td>
<td>0.55 &quot;d&quot;</td>
<td>0.34 &quot;d&quot;</td>
</tr>
<tr>
<td>TPNH-cyt c reductase</td>
<td>0.060 &quot;d&quot;</td>
<td>1.22 &quot;d&quot;</td>
<td>-</td>
<td>0.045 &quot;d&quot;</td>
</tr>
<tr>
<td>DPNH-cyt c reductase</td>
<td>0.215 &quot;e&quot;</td>
<td>4.75 &quot;e&quot;</td>
<td>-</td>
<td>1.26 &quot;e&quot;</td>
</tr>
<tr>
<td>DPNH-cyt b_{5} reductase</td>
<td>0.325 &quot;e&quot;</td>
<td>11.6 &quot;e&quot;</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*a* Determined in this study by the procedures described under "Materials and Methods."

*b* Data from Potts et al. (27).

*c* Data from Orrenius et al. (26). The results are expressed in different units from those originally presented. Cyt P-450 was converted to nmol mg protein^{-1} from \( \Delta E_{\text{450-660}} \times mg \) protein^{-1} by assuming an extinction coefficient of 91 mM^{-1} cm^{-1}. Cyt b_{5} was converted to nmol \( \times mg \) protein^{-1} from \( \Delta E_{\text{450-660}} \) by assuming an extinction coefficient of 185 mM^{-1} cm^{-1}. The TPNH-cyt c reductase and DPNH-cyt c reductase values were converted from \( \mu mol \) reduced pyridine nucleotide oxidized \( \times min^{-1} \times mg \) protein^{-1} to \( \mu mol \) Cyt c reduced \( \times min^{-1} \times mg \) protein^{-1} by multiplying by 2.

*d* Nmol.

*e* \( \mu mol \) electron acceptor reduced \( \times min^{-1} \).

### Table II. Localization of Electron Transfer Components after Treatment of Microsomes with Sodium Deoxycholate

Microsomes (25 mg protein derived from about 250 ml endosperm) were resuspended in 2.5 ml 0.05 M tris-chloride (pH 7.2) containing 25% glycerol and 1% sodium deoxycholate. The suspension was stirred continuously at 4 C under an \( N_2 \) atmosphere for 2 hr. After this treatment the suspension was centrifuged at 105,000g for 2 hr. The resulting supernatant and pellet fractions were analyzed for their contents of electron transfer components by the procedures described under "Materials and Methods." The amount of protein taken for assays for kaurene oxidase activity was in all cases 0.1 mg. The levels of the components in intact microsomes are presented for comparison.

<table>
<thead>
<tr>
<th></th>
<th>Microsomes</th>
<th>Pellet</th>
<th>Supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32 mg</td>
<td>9 mg</td>
<td>20 mg</td>
</tr>
<tr>
<td>Cytchrome P-450</td>
<td>0.073 mmol/mg protein</td>
<td>None detected</td>
<td>0.021 mmol/mg protein</td>
</tr>
<tr>
<td>Cytchrome P-420</td>
<td>0.031 mmol/mg protein</td>
<td>0.02 mmol/mg protein</td>
<td>0.042 mmol/mg protein</td>
</tr>
<tr>
<td>Cytchrome b_{5}</td>
<td>0.65 mmol/mg protein</td>
<td>0.13 mmol/mg protein</td>
<td>0.65 mmol/mg protein</td>
</tr>
<tr>
<td>TPNH-cyt c reductase</td>
<td>0.062 unit/mg protein</td>
<td>0.014 unit/mg protein</td>
<td>0.03 unit/mg protein</td>
</tr>
<tr>
<td>DPNH-cyt c reductase</td>
<td>0.23 unit/mg protein</td>
<td>0.025 unit/mg protein</td>
<td>0.18 unit/mg protein</td>
</tr>
<tr>
<td>DPNH-cyt b_{5} reductase</td>
<td>0.53 unit/mg protein</td>
<td>0.096 unit/mg protein</td>
<td>0.38 unit/mg protein</td>
</tr>
<tr>
<td>Kaurene oxidase activity</td>
<td>23% relative oxidation</td>
<td>162% relative oxidation</td>
<td>9% relative oxidation</td>
</tr>
</tbody>
</table>

*a* After detergent removal with a Sephadex G-25 column. No activity was detected before detergent removal.

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Partial Resolution of Electron Transfer Components. A column of DEAE-cellulose was employed to resolve partially the electron transfer components which were released to the supernatant fraction by sodium deoxycholate treatment. A supernatant fraction from treatment of 25 mg of microsomal protein was passed through a Sephadex G-25 column to remove detergent and the void volume fraction was concentrated to the volume of the original suspension on a Diaflo XM-50 membrane. The sample was transferred to a column of DEAE-cellulose (1.5 x 20 cm) which had been previously equilibrated with 0.05 M K-phosphate, pH 6.7. The column was eluted with a stepwise gradient of 0.1 to 0.5 M KCl and the resulting fractions were analyzed for Cyt b5, DPNH-Cyt b5 reductase, TPNH-Cyt c reductase, and DPNH-Cyt c reductase (Fig 2). Cyt b5 was not retained by the column. DPNH-Cyt b5 reductase was eluted completely by 0.1 M and 0.2 M KCl along with the major portion of protein. DPNH and TPNH-Cyt c reductase activities overlapped in the fractions eluted with 0.3 M and 0.4 M KCl. Neither Cyt P-450 nor Cyt P-420 were detectable in any of the column fractions.

Kaurene oxidase activity could not be detected in any of the column fractions tested singly or in combination. The addition of phosphatidylserine or phosphatidylcholine (in amounts ranging from 0.01 mg to 1 mg/reaction mixture) did not restore kaurene oxidase activity in these reconstitution experiments. However, a control suspension of microsomes which was maintained at 4 C for the period of time required for solubilization, removal of detergent, and chromatography had very low kaurene oxidase activity itself at the end of this period. It is possible that the failure to recover kaurene oxidase activity was at least in part the result of the inherent instability of the system under the conditions employed.

![Graph](image.png)

**Fig. 2.** Ion exchange chromatography of electron transfer components solubilized from *M. macrocarpus* microsomes by sodium deoxycholate treatment. The supernatant fraction derived from 25 mg microsomal protein by sodium deoxycholate treatment as described in Table II was passed through a Sephadex G-25 column (1 x 15 cm) which had been pre-equilibrated with 0.05 M K-phosphate, pH 6.7, containing 25% glycerol. The column was eluted with this same buffer at room temperature and the pooled void volume fractions were concentrated on a Diaflo XM-50 membrane filter. This fraction was then introduced onto a DEAE-cellulose column (1.5 x 20 cm) which had been previously equilibrated with 0.05 M K-phosphate, pH 6.7, containing 25% glycerol. The column was eluted with successive 8-ml portions of the same buffer containing 0, 0.1, 0.2, 0.3, 0.4, and 0.5 M KCl. Two-ml fractions were collected and each was analyzed for Cyt b5 (C), DPNH-Cyt b5 reductase (Δ), DPNH-Cyt c reductase (Δ), TPNH-Cyt c reductase (C), and protein (○) as described under "Methods." Results are expressed in terms of ΔA min⁻¹ 0.1 ml of fraction⁻¹ for the reductases and A 440 nm-410 nm 0.1 ml of fraction⁻¹ for Cyt b5. Where no symbol is shown for a fraction, the activity or amount of component detected in that fraction was zero.

**DISCUSSION**

Extensive investigations of the mixed function oxidations of steroids, drugs, and other substrates in mammalian hepatic microsomes have led to the formulation of an associated electron transfer scheme as shown in Figure 3 (adapted from Estabrook et al. [5]). A major feature of this scheme is the participation of two one-electron reduction steps in the cycle of Cyt P-450-dependent oxidation of the substrate, one after the oxidized form of P-450 has bound the substrate and the second after molecular O₂ has been bound to the reduced P-450-substrate complex. The first electron is most efficiently provided by TPNAH by a flavoprotein, fₚ₋, which is pictured as transferring electrons through a proposed, but not identified, carrier "X." The second electron can also originate from TPNAH, but is more efficiently donated from DPNH by a separate flavoprotein, fₚₒ, and Cyt b₅. The evidence strongly favors the direct participation of DPNH-Cyt b₅ reductase and Cyt b₅ when electrons are supplied from DPNH to Cyt P-450 (12, 18, 29, 33). However, there is less certainty about the participation of Cyt b₅ when TPNAH is serving as the source of electrons for Cyt P-450-dependent oxidations. Some investigations support the idea that Cyt b₅ may function as an intermediate carrier in at least some types of TPNAH-dependent oxidations (1, 15, 29), whereas other work indicates that Cyt b₅ is not an obligatory participant with TPNAH as the sole electron donor (16). The precise situation in vivo remains unclear. The activity of fₚ₋ can be measured as a TPNAH-Cyt c reductase with Cyt c serving as an artificial electron acceptor (20). Similarly the fₚₒ-Cyt b₅ reductase can be measured as a DPNH-Cyt c reductase with added Cyt c as an artificial electron acceptor (32). The fₚₒ activity itself can be measured with TPNAH as a donor and ferricyanide as an artificial acceptor (termed DPNH-Cyt b₅ reductase [31]).

The present work confirms the presence of Cyt P-450 and Cyt P-420-like pigments in the endosperm microsomal preparations as originally proposed by Murphy and West (22). Other electron transfer components and activities analogous to those found in hepatic microsomes were also detected. These included Cyt b₅, TPNAH-Cyt c reductase, TPNAH-Cyt P-450 reductase, DPNH-Cyt c reductase, and DPNH-Cyt b₅ reductase. The presence of these components supports the idea that similar electron transfer pathways may participate in mixed function oxidations in this higher plant microsomal system and in hepatic microsomes. Table I summarizes the concentrations and activities of these components in a typical endosperm microsome preparation. For comparison, the values of some of these same components reported for sorghum endosperms (27) and for hepatic microsomes and hepatic microsomes induced by phenobarbital treatment (26),
are also presented in Table I. It can be seen that much higher concentrations of Cyt P-450 are present in hepatic microsomes, especially after drug induction. Furthermore, 100 g liver yields 400 to 500 mg microsomal protein, whereas 100 ml endosperm (derived from an average of 500 immature seeds) contains only 15 to 20 mg microsomal protein. There are no known means of increasing the concentration of Cyt P-450 or other electron transport components in the plant microsomes comparable to the inductions by drugs in liver. These facts have complicated the task of adequately characterizing the electron transfer components in the plant system and of demonstrating their role in the mixed function oxidation reactions.

Although it has been possible to solubilize and fractionate the electron transfer components, the reconstitution of an active system in a reproducible manner, even in the presence of exogenous phospholipids, has not been achieved. Had this approach worked, it could have provided direct evidence for the participation of the individual components in the overall process. The failure to obtain an active system may well have been due to loss of Cyt P-450 during the manipulations required for solubilization and fractionation. The Cyt P-450 detectable spectrally after application of these procedures was either greatly reduced or absent. Even control microsomal suspensions not treated with detergents, but held at 4°C in 25% glycerol under an N₂ atmosphere for 30 hr, had lost almost all of their capacity for kaurene oxidation. It should be recalled that the initial concentration of Cyt P-450 in these preparations is also rather low. Of course, it is possible that other required components had been inactivated by the detergent treatment as well.

Several properties of the endosperm microsomal oxidations indicate that the electron transfer components are serving in a manner similar to those proposed for analogous components in mammalian microsomes shown in Figure 3. (a) The involvement of Cyt P-450 was deduced in earlier work from the characteristics of inhibition of the oxidation reactions, especially the inhibition by CO and the maximal photo-reversal of this inhibition by 450 nm light (22). (b) Cyt c has been shown to inhibit drug oxidations in liver microsomes (6), presumably by diverting the flow of electrons from TPNH to Cyt P-450 at the level of f₃P and possibly also from DPNH to Cyt P-450 at the level of Cyt b₂. Cyt c had a similar inhibitory effect on kaurene oxidation in the endosperm microsomes. (c) The synergistic stimulation of the rates of kaurene oxidation by added DPNH in the presence of low levels of TPNH reported in the accompanying paper (9) is very reminiscent of the situation in hepatic microsomes (3). This was a major factor in prompting Estabrook et al. to propose the microsomal electron transport model with two reduction steps in P-450-dependent oxidations pictured in Figure 3. (d) The synergistic stimulation of the rates of kaurene oxidation by added FMN in the presence of low levels of FAD reported in the accompanying paper (9) can also be rationalized in terms of this model if it is assumed that both FMN and FAD have a role in the associated reductases as they do in mammalian microsomes.

The DPNH-Cyt c reductase and the TPNH-Cyt c reductase are probably different enzymes just as they are in animal microsomes even though they eluted in overlapping positions from the DEAE-cellulose column used for the fractionation of deoxycholate-solubilized microsomes. For example, the TPNH-Cyt c reductase activity was little affected by treatment with Triton X-100, while the DPNH-Cyt c reductase was totally inactivated after exposure to this detergent. One feature of the DPNH-Cyt c reductase activity from the present work does seem anomalous. The mammalian microsomal DPNH-Cyt c reductase require both f₃P and Cyt b₂ for activity. However, the DEAE-cellulose column fractionation of deoxycholate-treated microsomes (Fig. 2) seems to have resolved both Cyt b₂ and DPNH-Cyt b₂ reductase activity (f₃P) from the DPNH-Cyt c reductase. Either the detergent treatment only partially resolved the components and somehow the DPNH-Cyt c reductase retains some Cyt b₂ and DPNH-Cyt b₂ reductase which are not revealed by assays for those individual components or DPNH-Cyt c reductase is of a different nature in this case.

The report of Pedersen (ed. 27) is the only one dealing substantially with the properties of a Cyt P-450-dependent oxidase system from a higher plant source. With respect to the microsomal system from sorghum seedlings which catalyzes the 4-hydroxylation of trans-cinnamic acid, they report levels of Cyt P-450 and Cyt b₂ very similar to those found in the endosperm microsomes. Their evidence for CO-inhibition and its photoreversal clearly implicates the Cyt P-450 in the hydroxylation reaction. They find that TPNH is utilized more efficiently than DPNH as the reduced pyridine nucleotide electron donor in much the same way as is true of kaurene oxidation in microsomes (9). A stimulatory effect on the hydroxylation rate is seen when 1 mm DPNH and 1 mm TPNH are included together in comparison with either reduced pyridine nucleotide alone under conditions such that the overall rate of hydroxylation is inhibited by the addition of catechol. This result is interpreted as support for a stoichiometric that two electron transfer pathways are operating as proposed by Hildebrandt and Estabrook (11). The effects of a number of inhibitors or potential inhibitors are also generally in accord with the types of electron transfer pathways proposed for the mammalian microsomal systems. Although it is not possible to compare in detail the results of their work with the properties for the M. macrocarpus microsomal oxidations presented in this paper because of the somewhat different experimental approaches made, it seems that these two systems have many features of their electron transport systems in common.

Clearly there is much yet to be learned about the characteristics of Cyt P-450-dependent mixed function oxidations in higher plant systems. But at the level at which it has been possible to make comparisons to date, it seems they have many features in common with the hepatic microsomal Cyt P-450-dependent oxidase.

**LITERATURE CITED**


1331-1332.