reaction of **2b** with dimethylallyl PP and with **4d** are the *R* isomers, **3c-PP** and **3e-PP**, respectively, since they show opposite behavior in ORD.

Consequently, it was concluded that the new artificial homologues (**2a** and **2b**) of isopentenyl PP (**1a**) could be substrates for farnesyl PP synthetase in place of **1a** to condense with the allylic substrates in the same stereochemical manner with that demonstrated for the natural substrates by Cornforth et al.[4] In other words, these homologues served as probes to distinguish the side of the double bond of **1a** from which the C–C bond is newly formed; namely, this is an example of the visualization of the stereospecificity latent in an enzyme-catalyzed prochiral process.

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(6) The reaction of **1c** with **4d** results in an exclusive formation of the *Z*-homologue of farnesyl PP.[3c]

(7) A Shimadzu-LKB GLC–mass spectrograph type 9000 was used with a 1-m column of 5% Carbowax 20M at linear programmed temperature at a rate of 4 °C/min from 130 to 220 °C.

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(9) The yield of 4,8-dimethylfarnesol, 4-methylgeraniol, and 4-methylfarnesol was 3.4, 0.8, and 7.6% on the basis of **2b**, respectively.

(10) Silica gel TLC in a system of benzene–ethyl acetate (4:1) in which the R_f for **3a** is 0.51.

(11) Yield, 3.5 mg; R_f, 0.48 on TLC; NMR (CCl_4) δ 1.00 (d, 3 H), 1.62 (s, 9 H), 1.68 (s, 3 H), 1.99 (br s, 7 H), 4.03 (d, 2 H), 5.02 (m, 2 H), and 5.34 ppm (t, 1 H).

(12) Optical activities were measured in hexane except for methylsuccinic acid (in water) with JASCO ORD Recorder Model ORD/UV-5 in a cuvette of 1-mm light path.

(13) Yield, 3.7 mg; R_f, 0.47 on TLC; NMR (CCl_4) δ 0.97 (d, 3 H), 1.03 (d, 3 H), 1.62 (s, 3 H), 1.66 (s, 6 H), 1.71 (s, 3 H), 2.07 (br s, 6 H), 4.08 (d, 2 H), 5.06 (m, 2 H), and 5.39 ppm (t, 1 H).

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Resonance Raman Spectra of Cytochrome $P450_{cam}$

Sir:

We present the first well-resolved resonance Raman spectra of the heme protein, cytochrome $P450_{cam}$. This protein, isolated from the bacterium, *Pseudomonas putida*, is instrumental in the methylene hydroxylation of D-(+)-camphor and belongs to a general class of hydroxylating heme proteins involved in detoxification, drug metabolism, carcinogenesis, and steroid biosynthesis. Cytochromes of the P450 type are identified by a unique Soret band (ca. 450 nm) of the reduced and carbon monoxide complexed heme. The P450 cytochromes share some properties of the heme iron with other proteins. The principal difference lies in their ability to serve both the oxygen binding role of heme proteins and the ferrous–ferric redox role of cytochromes, and also to bind selectively their carbon substrates. We limit this report to a discussion of the native (ferric) enzyme in the presence of a saturating level of substrate (camphor).

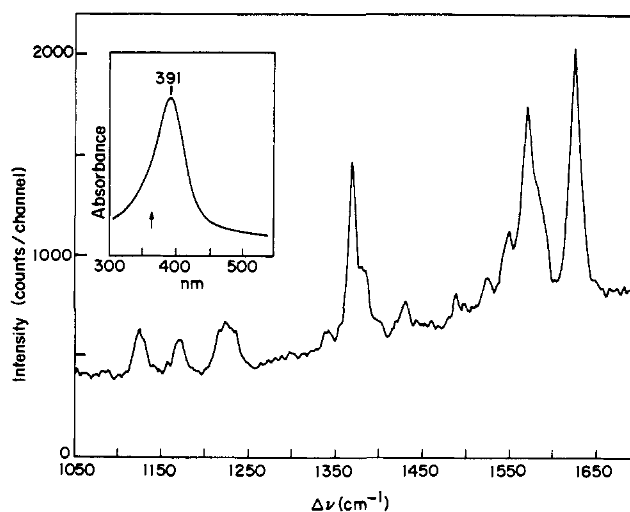

Figure 1. Resonance Raman spectrum of cytochrome $P450_{cam}$. The laser frequency is 3638 Å with 20 mW power at the sample. Slit widths are 4 cm^{-1} and counting time is 10 s/channel, 1 cm^{-1} step size. The insert shows the optical absorption spectrum of the reaction state under study (oxidized $P450_{cam}$ plus camphor). The arrow denotes the position of the laser excitation frequency.

Table I. Resonance Raman Peak Positions of $P450_{cam}$

Δν (I, pol)[a]	Δν (I, pol)	Δν (I, pol)	Δν (I, pol)
318 (w, u)	785 (w, u)	1224 (m, u)	1525 (m, p)
351 (m, p)	797 (w, u)	1339 (w, u)	1549 (m, u)
377 (w, u)	822 (w, u)	1368 (s, p)	1570 (s, p)
675 (w, u)	978 (w, u)	1379 (m, u)	1584 (m, p)
691 (w, u)	1003 (w, u)	1398 (w, u)	1623 (s, dp)
720 (w, u)	1125 (m, u)	1429 (m, u)	
754 (m, dp)	1170 (m, u)	1488 (m, p)	

[a] Δν is Stokes shift in cm^{-1} (±2 cm^{-1}) relative to the excitation wavelength (3637.8 Å); I corresponds to relative intensity of peaks: s = strong, m = medium, w = weak; pol refers to the polarization of peaks: p = polarized ($I_{\perp}/I_{\parallel} \lesssim 1/8$), dp = depolarized ($I_{\perp}/I_{\parallel} \simeq 3/4$), u = undetermined.

Three main experimental findings arise from this preliminary study: (1) the optical absorption on the high energy side of the Soret band (Soret maximum at 391 nm) is strongly coupled to low frequency modes (<500 cm^{-1}) normally associated with iron-nitrogen vibrations; (2) a large number of Raman peaks appear in the region 650–850 cm^{-1}, possibly reflecting the excitation of a ligand vibrational mode; (3) peak positions in the region 1050–1700 cm^{-1} are rather unusual and indicate a weakening of prophyrin ring bond strengths.

The Raman spectra are obtained using an excitation wavelength of 3637.8 Å from a Coherent Radiation Model CR12 argon ion laser. The power at the sample in all runs is close to 20 mW. The quartz sample container allows accumulation of both (right angle) Raman scattering and optical absorption data. The Raman data are collected by means of a Spex 1401 double monochromator equipped with photon counting apparatus and digital data storage. The spectra are averaged to reduce statistical fluctuations and plotted using a small computer. The optical absorption measurements are performed routinely before and after each run using a Cary 14 spectrophotometer. During the Raman runs the sample is kept at approximately 4 °C by means of a thermoelectric cooling unit. The polarization of the Raman scattered light is determined with a polarization filter and a polarization scrambler is used to ensure equal response of the monochromator to both polarizations.

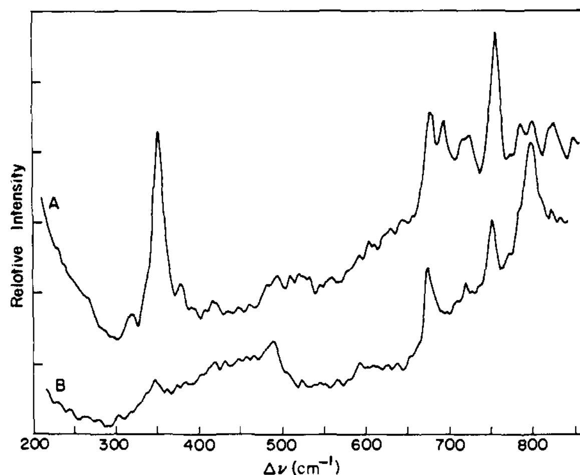

Figure 2. Resonance Raman spectra of the low frequency region, instrumental conditions as in Figure 1: (A) oxidized $P450_{cam}$ plus camphor, (B) oxidized P420 plus camphor. The large peak at 785 cm^{-1} in B is due to acetone. The broad hump around 500 cm^{-1} is caused by weak scattering from the quartz sample cell. The intensity scale for 2A is 600–1800 counts/channel and 2B is 800–2400 counts/channel.

Cytochrome $P450_{cam}$ is prepared as discussed previously.[1] A typical sample consists of 0.1 mM $P450_{cam}$ in pH 7.0, 0.05 M potassium phosphate buffer containing 0.2 mM camphor. Inactive $P450_{cam}$ (commonly referred to as P420 because of the normal position of the Soret band in the carbonmonoxy complex) is prepared by treating an aliquot of $P450_{cam}$ with 30%, V/V, acetone for 1 h at room temperature.[2]

In Figure 1, we display an unpolarized Raman spectrum of $P450_{cam}$ in the range of high energy vibrations (1050–1700 cm^{-1}). In this region porphyrin ring modes are expected and the vibrational frequencies are sensitive to the spin and oxidation state of the heme iron.[3] The Raman peak positions of $P450_{cam}$ can be found in Table I. Three bands of immediate interest are the markers A, E, and F, discussed by Spiro.[3] The A band, at 1368 cm^{-1},[4] is anomalously low. The E band (1488 cm^{-1}) falls between the high- and low-spin ferric classifications[3] as does the F band at 1623 cm^{-1}. These line positions are similar to chloroperoxidase,[5,6] another heme protein with P450 characteristics.[7,8]

In a recent article,[9] Spiro has reversed his previous classification scheme,[3] thus spectra once considered "anomalous" for ferric systems are now considered "normal" high-spin ferric spectra and vice versa. We feel that it is still premature to make such distinctions, since high-spin ferric heme proteins apparently have a rather broad range of porphyrin configurations.

We should also remark that frozen solutions of ferric $P450_{cam}$ in the presence of substrate are mixtures of high- and low-spin states, and tend toward low spin as the temperature is lowered.[10] This fact is perhaps related to the intermediate positions of the E and F bands. The temperature dependent spin transition phenomenon is not presently well understood, but new progress is expected since we can now probe both the frozen and liquid state of a sample using Raman spectroscopy.[11,12]

The anomalous position of the A band is perhaps even more interesting regarding the electronic structure of the active site. Spiro[3] has suggested that the A band is due primarily to breathing of the outer porphyrin ring with the position influenced by the amount of Π back-donation from iron to porphyrin. As the Π* antibonding orbitals are filled, the bond strength tends to weaken, and the frequencies are shifted to lower values. This effect is observed in cytochrome *c*, for example, when the iron accepts another electron and goes from Fe^{3+} (1374 cm^{-1}) to Fe^{2+} (1362 cm^{-1}). Thus, the low frequencies found for the A bands of both $P450_{cam}$ and chloroperoxidase suggest a large amount of Π back-donation in these proteins. This quite possibly is caused by an electron rich axial ligand donor, strongly interacting with the iron-porphyrin system. (A mercaptide sulfur ligand is a strong candidate for this role.[13-15])

In Figure 2a we present the low frequency region of the $P450_{cam}$ Raman spectrum. Two important features should be noted. First, a dominant peak at 351 cm^{-1} indicative of a strongly enhanced Fe–N vibrational mode.[16,17] Second, a wealth of structure in the 650–850 cm^{-1} region that, after a careful check of midrange vibrational modes in other heme proteins,[16,18-20] indicates an "extra" peak might be present at 691 cm^{-1}.[21]

Let us (urn first to the implications of the strongly enhanced Fe–N vibration at 351 cm^{-1}.[22] Asher and Sauer[17] have recently outlined the theory of charge transfer enhancement of low frequency vibrational modes in porphyrin systems. The crucial point is that resonance Raman bands enhanced by excitation within a charge transfer absorption correspond to vibrations associated with the central metal. Thus, when vibrations below 500 cm^{-1} appear with high intensity, one may reasonably assume that there is a charge transfer band (involving the iron atom) in the neighborhood of the excitation wavelength (in this case 3638 Å). Indeed, the hypothesis of a charge transfer transition has been put forward to explain the unusual hyperporphyrin absorption spectrum of CO-$P450_{cam}$.[13] This theory, while not directly applicable to the high-spin ferric case, may contain the necessary ingredients (i.e., a low lying mercaptide sulfur orbital coupled to *both* the iron and porphyrin electronic states) to explain the enhancement of the $P450_{cam}$ low frequency Raman modes. In any case, more detailed Raman experiments will certainly help clear up this very intriguing problem.

In order to gain insight into the nature of the peak at 691 cm^{-1}, we converted a sample of $P450_{cam}$ into the P420 form. The Raman spectrum of the P420 low frequency region is presented in Figure 2b. Notice that the 691-cm^{-1} peak has disappeared along with the peak at 351 cm^{-1}. With the exception of the A band, which shifts to 1372 cm^{-1}, the dramatic disappearance of two low frequency lines appears as the most significant change to occur upon conversion of $P450_{cam}$ into the P420 form.[23] These results immediately bring to mind the reversal experiments with formation of P450 from P420 by treatment with sulfhydryl reagents[2] as well as recent model compound studies[15,24] that suggest P450-type proteins contain a cysteine axial ligand. Free cysteine has one strong Raman active mode, the C–S stretching vibration at 683 cm^{-1}.[25] We feel that this mode, enhanced by charge transfer absorption, might well be responsible for the peak at 691 cm^{-1}, especially in light of the recent report[9] of enhanced axial ligand vibrations in heme model compounds. The 691-cm^{-1} peak could alternatively be assigned to an overtone of the 351-cm^{-1} mode and, thus, nicely account for the simultaneous disappearance of both peaks in Figure 2b. Further experiments should discriminate between these two possibilities.[26]

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(21) Although not reported in ref 20, a preliminary study in our laboratory has resolved a peak at 690 cm^{-1} in native horseradish peroxidase.
(22) An analogous peak is present in chloroperoxidase, but with lowered relative intensity. Horseradish peroxidase, another high-spin ferric heme protein, shows some very weak low frequency structure but essentially no enhancement at 351 cm^{-1}.
(23) The fact that the resonance Raman intensities are sensitive to the absorption envelope does complicate matters. We should point out that a substantial change in the overall absorption spectrum accompanies the conversion of $P450_{cam}$ to the P420 form.[2] In the region of the Raman excitation (3638 Å), however, there is little change in the total absorbance and we feel that this small difference, by itself, cannot explain the selective deenhancement of the modes at 351 and 691 cm^{-1}.
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(26) The display of a peak near 690 cm^{-1} by native horseradish peroxidase tends to cloud both possibilities: first, because horseradish peroxidase is thought not to have a cysteine axial ligand and second, because this protein does not show appreciable enhancement of the 351 cm^{-1} mode. The appearance of a 690-cm^{-1} vibration in horseradish peroxidase could be coincidental and unrelated to the P450 problem; if, however, it arises from the same source as the 691 cm^{-1} $P450_{cam}$ vibration, we must look for yet another explanation for the results presented in Figure 2.
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The Raman Spectrum of Adsorbed Iodine on a Platinum Electrode Surface

Sir:

Laser Raman spectroscopy has been employed in a number of recent investigations of chemical processes at working electrode surfaces.[1-4] In the present investigation, Raman spectroscopy has been employed to examine the nature of adsorbed iodine on platinum electrodes in an acidic medium. The essential electrode processes involved in this investigation have been identified by electrochemical and radiochemical techniques.[5]

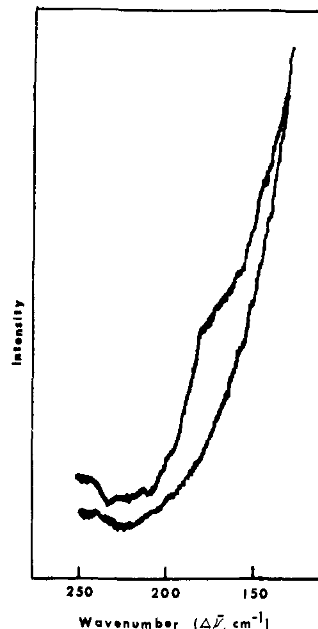

Figure 1. The Raman spectra from the surface of a platinized Pt electrode in 2×10^{-3} M KI, 0.1 M H_2SO_4 at +500 mV (upper trace) and +200 mV (lower trace). Exciting line: 514 nm Ar^+ (130 mW). Intensity of the 174-cm^{-1} line after background correction is ca. 250 counts s^{-1}.

Raman spectra were recorded using a modified Coderg T800 spectrometer coupled to a Spectra Physics 170 argon ion laser. A slit width of 6 cm^{-1} was used. The photometric system of the spectrometer consisted of an EMI 9789B S-11 phototube and a Brookdeal Ortec 5C1 photon counter. The signal-to-noise ratio was enhanced, where necessary, using a Hi-Tek PA1 signal averager. The electrochemical equipment including the spectroscopic cell has been described previously.[2] Potentials in the present study were measured relative to a saturated calomel reference electrode (SCE).

For Raman work it is usually essential that the electrode have a high surface area and be relatively reflecting. The gray-bronze colored platinized platinum electrodes used here were prepared by electrolysis of a lead-free chloroplatinic acid solution.[6,7] The electrodes were cycled in 1 M H_2SO_4 between hydrogen and oxygen evolution for about 15 min prior to commencing the spectroscopic experiments. The final pretreatment cycle was terminated cathodically, and weighed quantities of solid KI were added to the solution in the cell. The working solution (2×10^{-3} M KI, 1 M H_2SO_4) was purged with N_2 before and during the spectroscopic experiments to prevent oxidation of iodide to iodine. The profile of the cyclic voltammograms recorded using the platinized electrodes closely resembled (except for the quantity of current flowing) those recorded using smooth electrodes and also those in the literature.[5]

As the potential of the working electrode was adjusted from hydrogen evolution to +400 mV (vs. saturated calomel electrode) no potential sensitive features were observed in the Raman spectrum between $\Delta\bar{\nu} = 90$ and 350 cm^{-1}. According to Johnson,[5] inter alia, the surface in this potential region holds adsorbed iodide ions. At an applied potential of +500 mV a band appeared at $\Delta\bar{\nu} = 174$ cm^{-1} which was not detectable at potentials of ≥700 mV. The potential at which this line was observed coincides with the voltammetric prewave attributed to the oxidation of I^- from the bulk solution at the electrode surface and the subsequent adsorption of the I_2 produced.[5] Similar results to these have been obtained for neutral 0.1 M