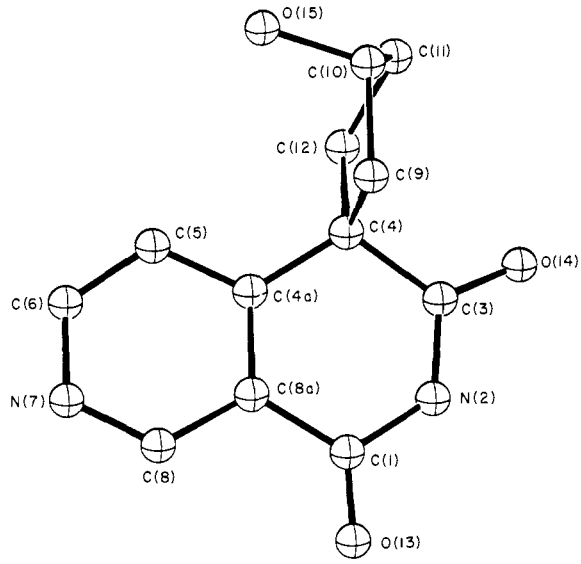

Figure 1. A computer-generated drawing of sesbanine. Hydrogens are omitted for clarity and no absolute configuration is implied.

Both independent molecules have the same configuration and a computer-generated perspective drawing of one of them is shown in Figure 1. During least-squares refinements, there was a marked correlation between pesudosymmetry related coordinates and, with the exception of C(10), C(11), and O(15), all final atomic positions are related within ~0.2 Å by the rule ($x' = \frac{3}{2} - x$, $y' = 1 - y$, $z' = 1 - z$). The X-ray experiment defines only the relative configuration of sesbanine as C(4) (R^*) and C(10) (R^*).

Bond lengths in the planar heterocyclic nucleus of **1** are in excellent agreement with generally accepted values. The spirocyclopentane fragment is less well behaved. The cyclopentane ring is best described as having the envelope conformation with C(11) serving as the flap and the three bonds between C(9), C(10) and C(11), and C(12) are all several hundredths of an Å shorter than expected. It is not clear whether this is due to the quality of the diffraction data, the breakdown of the pseudosymmetry in this region of the molecule, or some disordering process in the cyclopentane ring. Curiously, the two independent molecules do not make any close contacts with each other and all hydrogen bonds are formed between a molecule and its symmetry related mates. The hydrogen bond lengths follow: O(15) H–O(13), 2.82; O(15′) H–O(13′), 2.82; N(2) H–N(7), 2.87; N(2′) H–N(7′), 2.91 Å. There are no other abnormally short intermolecular contacts. The supplementary material described at the end of this paper contains further crystallographic details.

We propose the trivial name sesbanine (**1**) for this metabolite.[8] There are no reports of closely related compounds in the literature and further work in our laboratory will explore the chemistry of sesbanine and related metabolites. The isolation of sesbanine was originally guided by both in vivo (P388 leukemia) and in vitro (KB cell culture) bioassays[9] which showed parallel results. The scarcity of material precluded reliance on the in vivo assay in the latter stages of the isolation and pure sesbanine was isolated by the in vitro cell culture.

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Supplementary Material Available: Tables of fractional coordinates, bond distances, bond angles, and structure factors for sesbanine (12 pages). Ordering information is given on any current masthead page.

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(2) The initial stages of the isolation were carried out essentially as described in ref 1 except that conditions were manipulated so as to obtain the active principle in fraction D (Scheme I) instead of fraction C.
(3) The high-resolution chemical ionization–mass spectrum of **1** was carried out with a Kratos MS-30 instrument using methane as the reacting gas. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over firms or similar products not mentioned.
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Stopped-Flow Circular Dichroism (SFCD) Spectroscopy. Implication of Significant Conformational Differences in the Redox Mechanism of Cytochrome *c*

Sir:

Recently we found preliminary evidence that cytochrome *c* (cyt *c*)[1] undergoes a considerable and rapid conformation change associated with the exchange of the sixth ligand of heme *c* during rapid reductions with some inorganic reductants.[2] This conclusion was based principally on the following observations from SFCD spectroscopy: (1) a remarkably enhanced CD absorption appeared at the early stages of the reaction between the alkaline form of cyt c^{III} and the reductant (dithionite), indicating rapid formation of a complex ($\tau < 5$ ms); (2) the subsequent rapid appearance of an intense transient peak ($\tau \sim 15$ ms) was followed by its relatively slow disappearance ($\tau \sim 40$ ms) leading to the final absorption of cyt c^{II}.

This observation indicates that an unstable (transient) cyt c^{II} having a markedly enhanced and distinct positive rotational strength was formed from other stable species; then it converted to native cyt c^{II} relatively slowly with a substantial conformation change ($k_1 = 17\ s^{-1}$, 28 °C). Previously reported SFCD spectroscopy[3] is restricted only to changes (at 222 nm) which do not afford direct information about conformational changes of an active site, but provide some indirect information.

Now we report interesting and significant results of the

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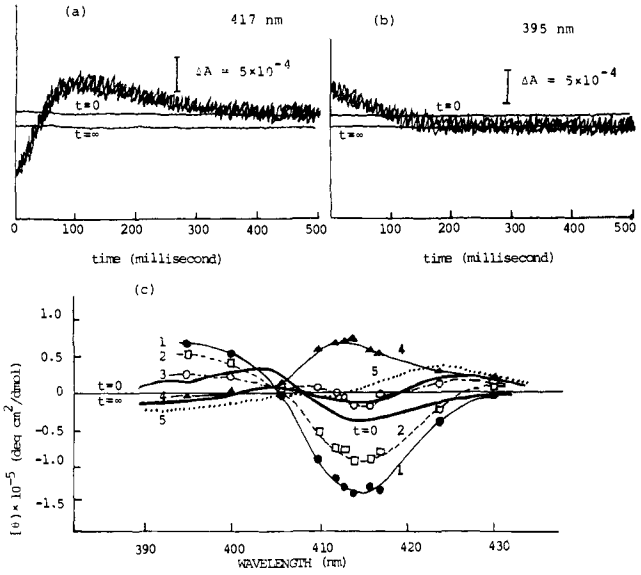


Figure 1. Stopped-flow circular dichroism spectra of the reduction of ferricytochrome *c* from horse heart by protoporphyrin-iron(II). (a and b): two or more independent traces are satisfactorily superimposed. Conditions: cyt c^{III}, 10 μM, protoporphyrin-iron(II), 26 μM, pH 7.0, 0.1 M phosphate buffer (5% MeOH), 28 °C; response, 55 s. Traces at $t = 0$ and $t = \infty$ are those treated by use of a computer-programmed averaging system. (c) At $t = 0$, ferricytochrome *c*: (1) 10 ms, (2) 20 ms, (3) 50 ms, (4) 100 ms, (5) ferrocytochrome *c*.

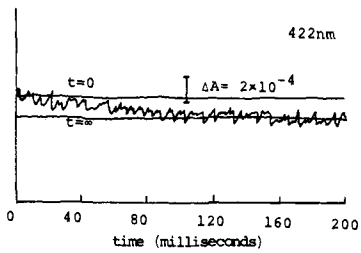
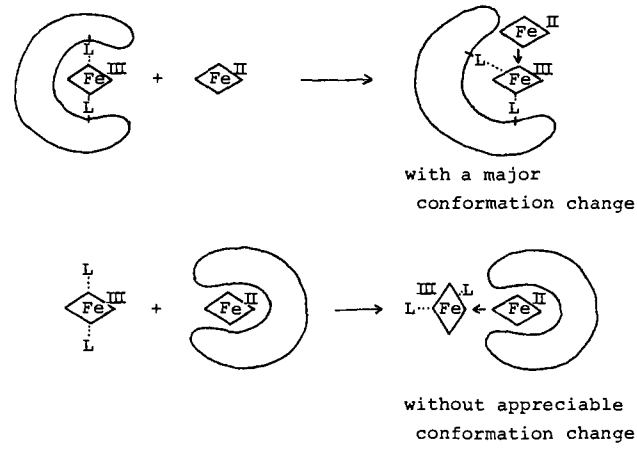


Figure 2. Stopped-flow circular dichroism spectrum of the oxidation of ferrocytochrome *c* from horse heart by protoporphyrin-iron(III). Traces of six independent runs were averaged by use of a computer-programmed averaging system. Conditions: Cyt c^{II}, 10 μM, protoporphyrin-iron(III), 26 μM, pH 7.0, 0.1 M phosphate buffer (5% MeOH), 28 °C.

Scheme I



application of SFCD spectroscopy to conformational studies, especially around the active site, in the cross reaction between cyt *c* from horse heart and iron protoporphyrins.

$$\text{cyt } c^{III} + \text{P·Fe}^{II} \rightleftharpoons \text{cyt } c^{II} + \text{P·Fe}^{III}$$

A SFCD instrument (250-W Xe light source, mirror-collimated double monochrometer, 18-kHz photoelastic birefringence modulator, and phase-sensitive heterodyne lock in amplification)[4a] was modified to improve signal sensitivity. The instrument, which was used in conjunction with an improved flow cell of better optical and flow design,[4b] has a satisfactory time response, as fast as $^1/_{13}$ ms.[5]

Figure 1a and 1b show the displayed SFCD data, without further treatment, after the rapid 1:1 mixing of 20 μM cyt c^{III} from horse heart[6] with 52 μM iron(II) protoporphyrin,[7] each in a 0.1 M phosphate buffer at pH 7.0 (5% MeOH). SFCD spectra ($t = 0$ and $t = \infty$)[8] obtained in control experiments of rapid 1:1 mixing of cyt *c* (III and II, respectively) with buffer solution are also shown in Figure 1a and 1b, after treatment by use of a computer programmed integrator. Apparently artifacts are not detected under the conditions of measurement (even before integration). The rapid change of the CD spectrum during the reduction is shown in Figure 1c.

Judging from the signal level for cyt c^{III} ($t = 0$) at each wavelength monitored (Figure 1a and 1b), a remarkable jump or drop of the CD signal was observed at the early stages of the reduction, which is attributed to the very rapid complex formation between cyt c^{III} and the iron(II) protoporphyrin, because λ_{max} in the electronic spectrum did not show any detectable change and the CD absorption maximum (~395 nm) was close to that of the starting cyt c^{III} (402 nm).[9] The subsequent rapid decrease at 395 nm and concurrent increase at 417 nm in CD signals refer to the electron transfer which is essentially complete within ~100 ms. Therefore, the biphasic CD signal change that was generally observable in a wavelength range of 410–425 nm clearly demonstrates the presence of transient (conformationally unstable) cyt c^{II} which relatively slowly ($k_1 = 7.1 \pm 0.3$ s^{-1}, 28 °C) converts to native cyt c^{II} with a marked conformation change around the active site of the protein. The conformation of the unstable cyt c^{II} is concluded to be a distinctive one which directly affects the Soret transition to enhance its rotational strength (spectrum 4) remarkably. This conformation change seems to be due to the exchange of a 6th ligand from Lys to Met. However, for the reverse process, the oxidation of cyt c^{II} by iron(II) protoporphyrin, no such remarkable and characteristic CD signal change was observed (Figure 2) at any wavelength in the Soret region. The very rapid but monotonous decay of the CD signal at 422 nm (Figure 2) corresponds to a straightforward conversion of cyt c^{II} (spectrum 5, Figure 1c) to an equilibrium mixture of 4:6 cyt c^{III}–cyt c^{II} (spectrum $t = \infty$, Figure 1c); i.e., no CD active intermediate was detectable during the oxidation. This may be interpreted by a mechanism in which the electron transfer from iron(II) protoporphyrin to cyt c^{III} is not accompanied by a significant conformational change in proximity to the active site.

One plausible mechanism to interpret this microscopically irreversible phenominum is depicted in Scheme I, where a strict axial electron acceptance and rather adaptable electron release of heme[10] is assumed. The considerable conformational change in proximity to heme *c* (the active site) which directly affects the Soret rotational strength[11] at 417 nm suggests that, between two nearly degenerate in-plane transitions (417 and 390–400 nm) of heme *c*, the one at longer wavelength seems to experience a larger change in the magnetic and/or electric dipole character.

To provide further detailed information, extensive SFCD experiments involving redox reactions of modified cytochromes *c* are now underway.

In conclusion, the present results provide for the first time concrete evidence for a marked difference in conformation between the oxidation and reduction of cyt *c* with other iron porphyrins, suggesting that microscopic irreversibility in the

conformational change of the protein is one of the requisites of biological electron transport to be performed "one direction" by using a series of cytochromes, oxidases,[13] and reductases.[14] More importantly, the present significant information can be obtained only by the use of SFCD. Neither static CD nor electronic spectroscopy will suffice, since this abnormally enhanced CD comes from the rapid conformation change of the active site and/or the dynamic magnetic polarization induced chemically. This point is discussed in detail in a forthcoming paper.

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(5) Much faster than that in the SFCD techniques reported in the literature (<1 ms).[3]
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Pulsed Infrared Laser Induced Visible Luminescence

Sir:

Triboluminescence (TL) can be excited by fracturing or grinding a crystal, or by subjecting it to thermal shock.[1-3] We have been interested in whether comparable phenomena could be made to occur using a high energy laser pulse as the stress inducing agent. We report here on three cases in which there is luminescence induced by a 20-ns, 1060-nm pulse from a Nd glass laser system,[4] the pulse energies ranging from 0.5 to 4 J cm^{-2} (~200-MW peak power). The crystalline materials were doped saccharin (see immediately below), coumarin, sodium chloride, and potassium chloride. Individual crystals were used in each case; they were single crystal in appearance and several mm in each dimension. All were studied at room temperature, ~25 °C.

Figure 1A shows the laser induced luminescence spectrum for saccharin; it is the same within our resolution as the TL and photoluminescence (PL) spectra. The emission is actually from *p*-toluenesulfonamide impurity normally present in saccharin; such doped saccharin crystals are among the more intensely triboluminescent materials observed in our laboratory,[5] hence this initial choice. The laser induced emission (LIE) showed

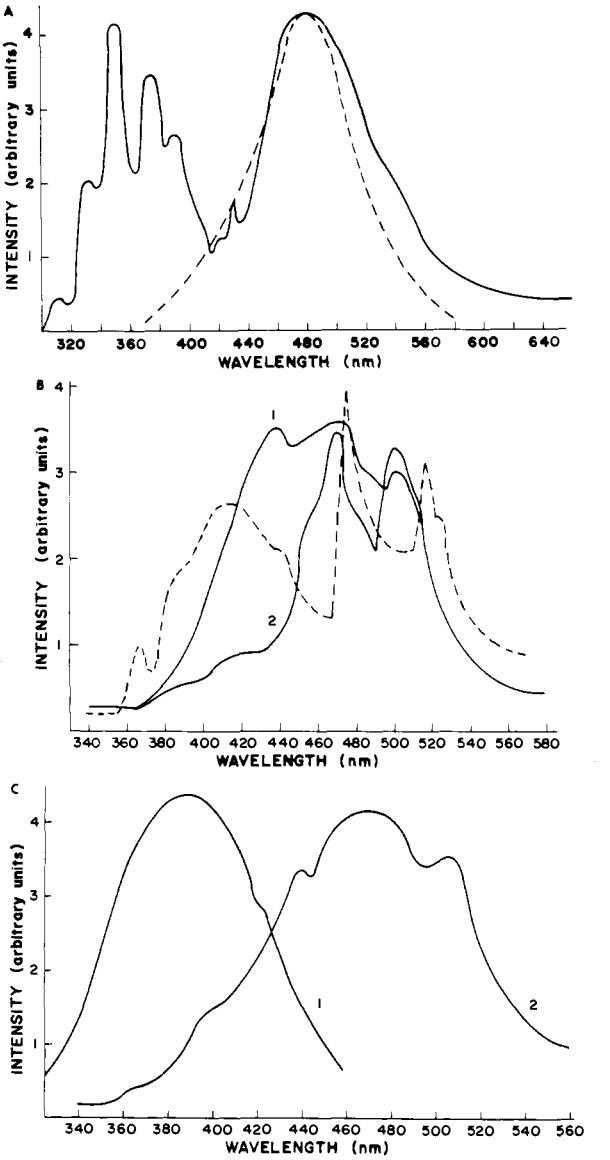

Figure 1. Spectra. (A) *p*-Toluenesulfonamide doped saccharin: full line, triboluminescence spectrum (the short wavelength, structured emission is due to excited state N_2);[6] dashed line, laser induced emission. (Both measurements at room temperature; the 480 nm peaks are normalized.) (B) Coumarin: full lines, laser induced emission, curve 1 with no delay, and curve 2 with 80-ns delay; dashed line, 353-nm photoexcited emission at 77 K (at room temperature, only the 420 nm centered emission is observed). (The intensity scale is arbitrary, and the spectra are roughly normalized; they are uncorrected.) (C) Laser induced emissions from NaCl, curve 1, and KCl, curve 2.

two time regimes, as followed at 480 nm: a prompt emission and a longer-lived one whose decay fit an exponential of ~300-ns lifetime. From separate optical excitation experiments, the 480-nm emission has a lifetime of 57 ps at 77 K. The prompt component of the laser induced emission could represent ordinarily decaying excited states, but the 300-ns component must have some other explanation, as discussed further below. The intensity of the 300-ns emission was proportional to the laser pulse energy over about a tenfold range.

Substantial differences exist between the laser induced emission and TL spectra of coumarin. The LIE spectrum shown in Figure 1B consists of three peaks at 430, 475, and 505 nm. At room temperature, the PL and TL spectra of coumarin consist of a broad band centered at ~400 nm, which has been assigned as a $\pi \leftarrow \pi^*$ fluorescence.[7,8] At 77 K, the PL spectrum consists of the fluorescence and a highly structured phosphorescence. The LIE of coumarin most closely resembles

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