vibrational fine structure of detailed isolated $TCNQ^{-}\cdot$ in the solid state is reported here for the first time.

References and Notes

(1) Ritsko, J. J.; Nielsen, P.; Miller, J. S. *J. Chem. Phys.* **1977**, *67*, 687–690.
(2) Reis. A. H.; Preston, L. D.; Williams, J. M.; Peterson, S. W.; Candela, G. A.; Swartzendruber, L. J.; Miller, J. S.; Rice, M. J. *J. Am. Chem. Soc.* **1979**, *101*, 2756–2758.
(3) Candela, G. A.; Swartzendruber, L. J.; Miller. J. S.; Rice, M. J. *J. Am. Chem. Soc.* **1979**, *101*, 2755–2756.
(4) Boyd, R. H.; Phillips, W. D. *J. Chem. Phys.* **1965**, *43*, 2927–2929.
(5) (a) Suchanski, M. R.; Van Duyne, R. P. *J. Am. Chem. Soc.* **1976**, *98*, 250–252. (b) Jeanmarie, D. J.; Van Duyne, R. P. *Ibid.* **1976**, *98*, 4029–4034.
(6) Adam, E.; Rosenblum, M.; Sullivan, S.; Margulis, T. N. *J. Am. Chem. Soc.* **1967**, *89*, 4540–4542.
(7) Hanson, A. W. *Acta Crystallogr.* **1965**, *19*, 610–614.
(8) Braitsch, D. M. *J. Chem. Soc., Chem Commun.* **1974**, 460–461.
(9) Reis, A. H.; Gebert, E.; Miller, J. S., unpublished results.
(10) Jacobs, I. S.; Bray, J. W.; Hart, H. R., Jr.; Interrante, L. V.; Kasper, J. S.; Watkins, G. D.; Prober, D. E.; Bonner, J. C. *Phys. Rev. B* **1976**, *14*, 3036–3050.
(11) Mammano, N. J.; Zalkin, A.; Landers, A.; Reingold, A. L. *Inorg. Chem.* **1977**, *16*, 297–300.

(12) Work performed under the auspices of the Division of Physical Research of the U.S. Energy Research and Development Adminstration.

Joel S. Miller*
Occidental Research Corporation
Irvine, California 92713

Arthur H. Reis, Jr.,* E. Gebert
Chemistry Division, Argonne National Laboratory[12]
Argonne, Illinois 60439

J. J. Ritsko, W. R. Salaneck, L. Kovnat
Xerox Webster Research Center
Rochester, New York 14644

Thomas W. Cape, Richard P. Van Duyne
Department of Chemistry and Materials Research Center
Northwestern University, Evanston, Illinois 60201

Received December 4, 1978

Electrochemistry of Cytochrome c. Comparison of the Electron Transfer at a Surface-Modified Gold Electrode with That to Cytochrome Oxidase

Sir:

As we have recently shown,[1] rapid direct electron transfer between cytochrome *c* and a gold electrode takes place in the presence of 4,4′-bipyridyl and 1,2-bis(4-pyridyl)ethylene, which form an adsorbed layer on the electrode surface thus facilitating the electron-transfer reaction. We report here some striking analogies between the reaction of cytochrome *c* at this electrode and its reaction with cytochrome oxidase with respect to the effect of chemical modification of the cytochrome *c* lysine residues and the effect of poly-L-lysine on the electrode reaction. The results suggest that cytochrome *c* binds to the 4,4′-bipyridyl-modified gold electrode surface prior to electron transfer in a manner similar to its interaction with the oxidase.

The electron-transfer reaction between cytochrome *c* and cytochrome oxidase proceeds via a protein complex in which the ϵ-amino groups of the cytochrome *c* lysine residues are believed[2] to play an important role. Chemical modification of these lysines is well known[3] to affect the cytochrome *c*-oxidase electron-transfer reaction. The importance of lysine residues in the protein-protein interaction is further illustrated by the effect[4] of poly-L-lysine, a competitive inhibitor of the cytochrome *c*-oxidase reaction which acts by binding[5] to the oxidase.

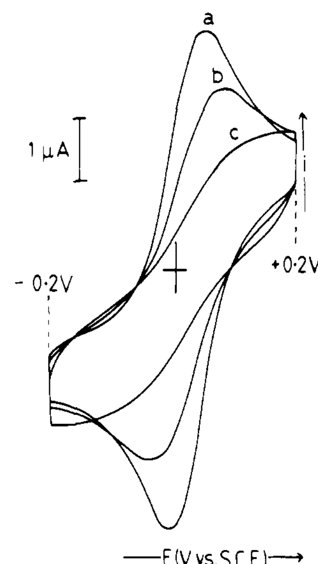


Figure 1. dc cyclic voltammogram of horse heart ferricytochrome *c*, 0.4 mM in 0.1 M $NaClO_4$, 0.02 M phosphate buffer, pH 7, saturated solution of 1,2-bis(4-pyridyl)ethylene in the potential range of +0.2 to −0.2 V vs. SCE with poly-L-lysine: (a) 0 mg/mL^{-1}, (b) 1 mg mL^{-1}, (c) 1.5 mg mL^{-1}. dc potential scan rate, 100 mV s^{-1}.

We have investigated the effect of acetimidilation,[6] guanidinilation,[7] trifluoracetylation,[8] and maleylation[9] of cytochrome *c* and the effect of poly-L-lysine on the electrode reaction studied by cyclic voltammetry as previously described,[1] and find the following: Both *N*-acetimidyllysyl[10] and *N*-guanidinyllysyl[11] horse heart cytochrome *c* are found to be electroactive[12] at the gold electrode in the presence of 4,4′-bipyridyl or 1,2-bis(4-pyridyl)ethylene, giving rise to quasi-reversible diffusion-controlled dc and ac voltammograms indistinguishable from those (Figure 1a) of the native protein,[1] with a half-wave potential, $E_{1/2}{}^{r}$ = 0.25 V vs. NHE. Similarly both are enzymatically active[5,6] in the cytochrome-oxidase system. However, the enzymatically inactive *N*-trifluoroacetyl[13] and *N*-maleyl[14] derivatives are both electroinactive.

Poly-L-lysine[15] is found to inhibit the electrode reaction of native horse heart cytochrome *c*, as shown by its effect on the dc voltammetry (Figure 1), again analogous to its inhibiting effect on the cytochrome *c*-oxidase reaction. The effect on the ac cyclic voltammetry peak current, i_p(ac), is more marked. The variation with poly-L-lysine concentration (Figure 2) is consistent with adsorption of poly-L-lysine onto the electrode surface, decreasing the effective free electrode area.

The electron-transfer reaction of the native protein has been studied by ac impedance measurements[16] and we find the heterogeneous electron-transfer rate to be as fast as that determined[17] for the ferri/ferrocyanide couple (0.3–3.0 × 10^{-4} m s^{-1}). The measured in-phase and quadrature-phase components of the ac current, treated as described[18] by De Levie and Pospisil, yield a plot of real, Z_F', against imaginary, Z_F'', component of faradaic impedance which is a straight line of unit slope, with an intercept on the real axis as expected for the quasi-reversible case. From this the heterogeneous rate constant, k_s, can be calculated[18] as 1.86 × 10^{-4} m s^{-1}. The cotangent of phase angle shows a linear relationship with the square root of ac modulation frequency, consistent[19] with the quasi-reversible nature of the reaction. The value of k_s = 1.4 × 10^{-4} m s^{-1} thus derived[19] is in good agreement with the value obtained above.

Preliminary investigations[20] using the methods of double-potential-step chronocoulometry, rotating disk and ac rotating

0002-7863/79/1501-7113$01.00/0 © 1979 American Chemical Society

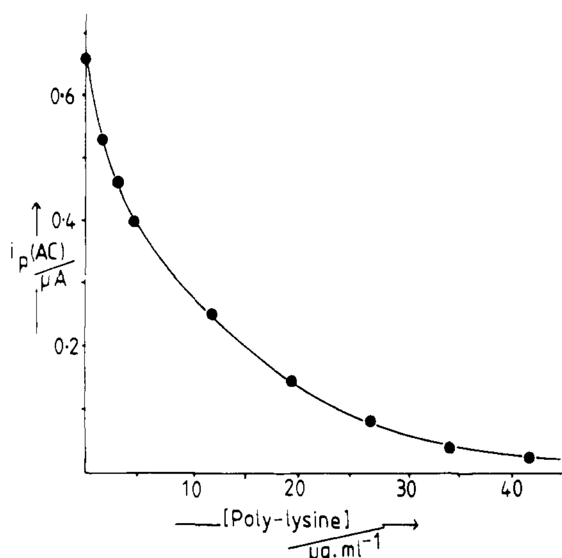


Figure 2. ac cyclic voltammetry peak current i_p(ac) against poly-L-lysine concentration, solution as in Figure 1. ac modulation frequency, 479 Hz; modulation amplitude, 8 mV.

ring disk indicate that the native cytochrome binds to the 4,4′-bipyridyl-modified gold electrode surface prior to electron transfer. Thus it appears that 4,4′-bipyridyl acts by forming a suitable surface at the electrode-solution interface to which the cytochrome can bind. The binding of the protein to the electrode may therefore be similar in kind to that observed in the cytochrome *c*-oxidase reaction, involving the ϵ-amino groups of lysine residues in the vicinity of the cytochrome *c* heme crevice. The observed similarities to the cytochrome *c*-oxidase reaction may result from a preferred orientation of the cytochrome when bound at the electrode surface or to cytochrome oxidase such that the exposed heme edge is adjacent to the electrode surface or the cytochrome oxidase thereby enabling rapid electron transfer to occur.

Acknowledgments. We thank Drs. R. E. Offord and D. E. Harris, Molecular Biophysics, University of Oxford, for the kind gift of modified cytochromes and Professor W. J. Albery and Mr. A. E. G. Cass for useful discussion. This is a contribution from the Oxford Imperial Energy Group. We are grateful to the Science Research Council and the National Research Development Corporation for support.

References and Notes

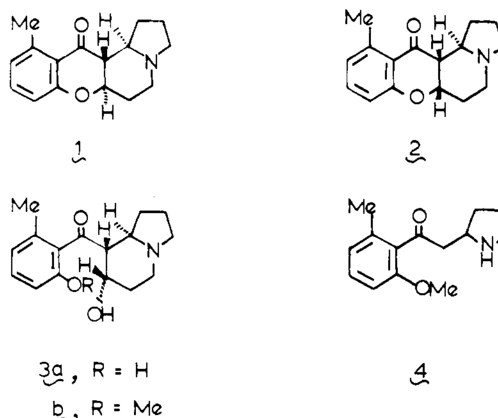
(1) Eddowes, M. J.; Hill, H. A. O. *J. Am. Chem. Soc.* **1979**, *101*, 4461.
(2) Ferguson-Miller, S.; Brautigan, D. L.; Margoliash, E. *J. Biol. Chem.* **1978**, *253*, 149.
(3) Takemori, S.; Wada, K.; Ando, K.; Hosokawa, M.; Sekuzu, I.; Okunuki, K. *J. Biochem. (Tokyo)* **1962**, *52*, 28.
(4) Mochan, B. S.; Elliott, W. B.; Nicholls, P. *J. Bioenerg.* **1973**, *4*, 329.
(5) Seiter, C. H. A.; Margalit, R.; Perreault, R. A. *Biochem. Biophys. Res. Commun.* **1976**, *68*, 807.
(6) Wallace, C. J. A.; Offord, R. E. *Biochem. J.* **1979**, *179*, 169.
(7) Hettinger, T. P.; Harbury, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1964**, *52*, 1469.
(8) Fanger, M. W.; Harbury, H. A. *Biochemistry*, **1965** *4*, 2541.
(9) Pettigrew, G. W.; Aviram, I.; Schejter, A. *Biochem. Biophys. Res. Commun.* **1976**, *68*, 807.
(10) Prepared by the method of Wallace and Offord.[6]
(11) Prepared by the method of Hettinger and Harbury.[7]
(12) dc and ac cyclic voltammetry was carried out as previously described.[1]
(13) Prepared by the method of Fanger and Harbury.[8]
(14) Prepared by the method of Pettigrew et al.[9]
(15) Poly-L-lysine Type II, mol wt 3000, obtained from the Sigma Chemical Co. Ltd.
(16) The electrochemical measurements were made on 0.357 mM solutions of ferricytochrome *c* (Sigma, grade VI) in buffer (K_2HPO_4, 0.02 M; $NaClO_4$, 0.1 M) in a cell (5 cm^3) containing three electrodes: working, a gold wire (diameter, 5×10^{-4} m, length 5×10^{-3} m); counter, a cylindrical platinum net surrounding the working electrode; reference, an SCE, separated from the main compartment by a Luggin capillary. A Princeton Applied Research 173 potentiostat with a 179 coulometer was used. The amplitude of the ac modulation sine wave was 5 mV and the current responses in-phase and quadrature phase of the applied ac was measured by an Ortec Brookdeal 9503 lock-in amplifier. The impedence measurements were carried out at OV (vs SCE), the position of maximum ac current, and at 20–1000 Hz.
(17) Marecek, V.; Samec, Z.; Weber, J., *J. Electroanal. Chem.* **1978**, *94*, 169.
(18) De Levie, R.; Pospisil, L. *J. Electroanal. Chem.* **1969**, *22*, 277.
(19) Pospisil, L.; Mittal, M. L.; Kuta, J.; Koryta, J. *J. Electroanal. Chem.* **1973**, *46*, 203.
(20) Eddowes, M. J.; Hill, H. A. O.; Uosaki, K., work in progress.

Mark J. Eddowes, H. Allen O. Hill,* K. Uosaki
Inorganic Chemistry Laboratory, South Parks Road
Oxford OX1 3QR, England
Received June 22, 1979

Elaeocarpus Alkaloids. Synthesis Using Nitrones

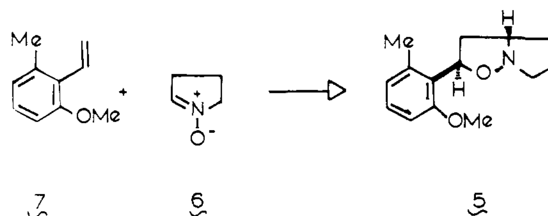
Sir:

The *Elaeocarpus* alkaloids comprise a relatively new and sizable group of natural products derived from the plant family Elaeocarpaceae, comprising several large, spreading trees found in New Guinea and India.[1,2] The leaves of these trees are rich in *dl*-elaeocarpine (**1**) and *dl*-isoelaeocarpine (**2**) as well as numerous other related indolizidine alkaloids. We report herein an extremely facile and pointedly direct synthesis[3] of two major (i.e., *dl*-elaeocarpine and *dl*-isoelaeocarpine) and one minor member (i.e., *dl*-isoelaeocarpicine, **3a**) of the *Elaeocarpus* family of alkaloids.



Our approach derives from the recognition that, as β-amino ketones, elaeocarpine, isoelaeocarpine, and isoelaeocarpicine would, in principle, be derivable from a route which incorporates a methodology involving nitrones.[4] Hence, we selected β-amino ketone **4** as our initial target which we envisioned could be transformed efficiently into the desired alkaloids.

While we considered that **4** could be obtainable from the isoxazolidine **5** derived from 1-pyrroline 1-oxide (**6**) and 6-methoxy-2-methylstyrene (**7**), the steric encumbrances in-



volved in such a cycloaddition were of some concern. Fortunately, we recognized that the steric crowding centered about the developing carbon-oxygen bond, while the nonsynchronous transition states probably involved in these $[_\pi4_s + _\pi2_s]$ cycloadditions[5,6] appear to involve more extensive formation of

 © 1979 American Chemical Society