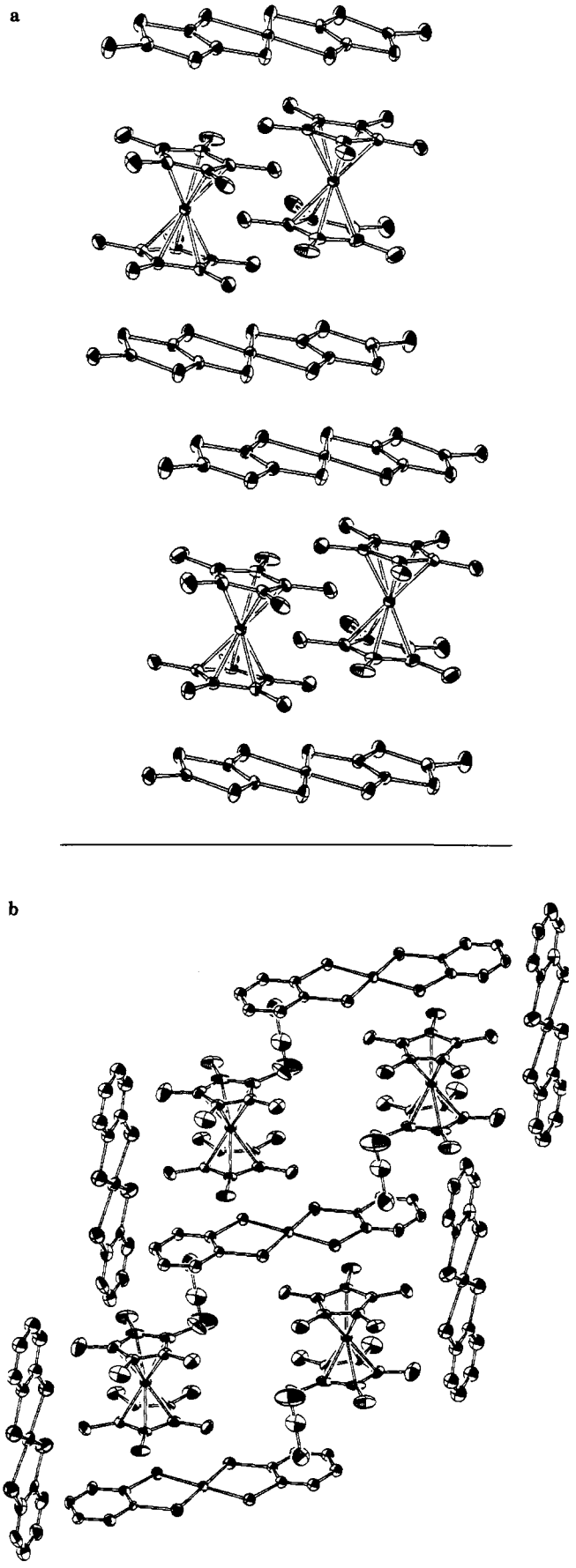


Figure 1. (a) Packing diagram of **1** showing a perspective view of a $..A^-D^+D^+A^-A^-D^+D^+A^-..$ stack. (b) Packing diagram of **2** showing a perspective view of a $..A^-D^+D^+A^-D^+D^+A^-..$ stack, portions of the flanking A^- sheets, and the CH_3CN of crystallization. (The plane of the paper corresponds roughly to the *ab* plane.)

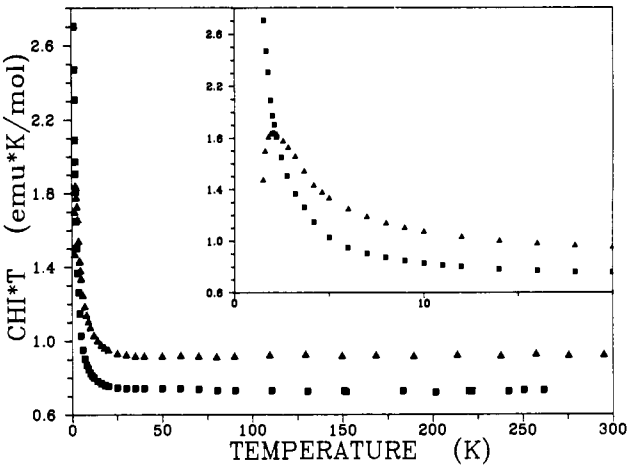


Figure 2. Temperature dependence of $\chi_M T$ for **1** (triangles) and **2** (squares). Inset: expansion of the 1.5–20 K region.

is sure to expand the range of structural types and magnetic properties exhibited by this class of compounds.[20]

Acknowledgment. This work has been supported by the Solid State Chemistry Program of the National Science Foundation (Grant DMR-8818599, B.M.H.) and by the Northwestern University Materials Research Center under the NSF-MRL program (DMR-8821571, B.M.H.).

Supplementary Material Available: Tables of atomic positional parameters, anisotropic thermal parameters, and bond distances and angles (6 pages); table of observed and calculated structure factors for **1** and **2** (26 pages). Ordering information is given on any current masthead page.

(20) For example, we have observed ferromagnetic interactions in 1:1 decamethylferrocenium salts of $[Ni[Se_2C_2(CF_3)_2]_2]^-$ and $[Ni[S_2C_2(CF_3)_2]_2]^-$, and more extensive, independent investigations by the Dupont group show that the latter compound, at least, exhibits a different structural motif from either of those reported here (Miller, J. S., private communication).

Transient Binding of Photodissociated CO to Cu_B^+ of Eukaryotic Cytochrome Oxidase at Ambient Temperature. Direct Evidence from Time-Resolved Infrared Spectroscopy

R. Brian Dyer,*,‡ Ólöf Einarsdóttir,† Patrick M. Killough,†,§ Juan J. López-Garriga,† and William H. Woodruff*,†

Isotope and Structural Chemistry Group (INC-4, Mail Stop C-345) and Photochemistry and Photophysics Group (CLS-4, Mail Stop J-567) University of California Los Alamos National Laboratory Los Alamos, New Mexico 87545

Received February 23, 1989

The reactions of CO with the metal centers of cytochrome oxidase exemplify the mechanisms open to O_2 and other small-molecule ligands. In particular, the fast reactions following photodissociation of CO from cytochrome a_3 yield important information about the pathways available to ligands to and from the active site. FTIR studies have demonstrated that, below 180 K, photodissociated CO binds to Cu_B^+ both in mitochondrial preparations[1,2] and in the detergent-solubilized enzyme.[3,4] We

* To whom correspondence should be addressed at: INC-4, Mail Stop C-345, Los Alamos National Laboratory, Los Alamos, NM 87545.
† INC-4.
‡ CLS-4.
§ Present address: Shell Development Company, Westhollow Research Center, P.O. Box 1380, Houston TX 77001.

0002-7863/89/1511-7657$01.50/0

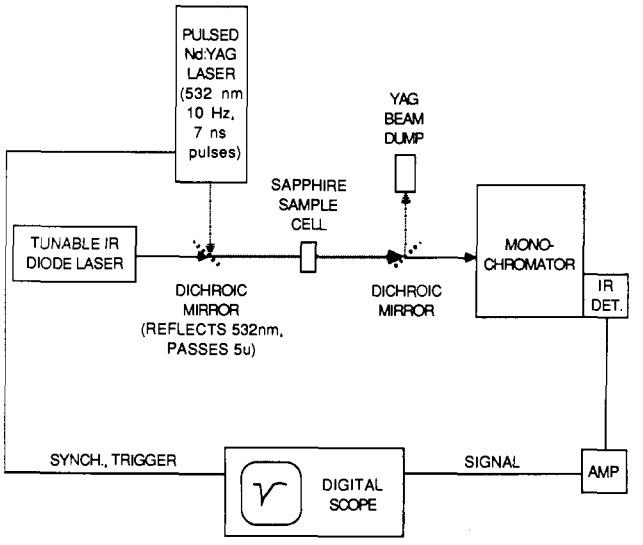


Figure 1. Schematic diagram of the time-resolved infrared setup.

have also observed Cu_B^+–CO in the *room temperature* FTIR spectrum of cytochrome ba_3 from *Thermus thermophilus*.[3] However, despite previous inferences,[5-7] direct evidence for CO binding to Cu_B^+ at room temperature has been lacking for the eukaryotic enzyme. The question of the possible existence and lifetime of Cu_B^+–CO at room temperature is of critical importance in cytochrome oxidase dynamics. Such a species (if present) might suggest that Cu_B, in addition to its established redox function, plays a role as a "ligand shuttle" to cytochrome a_3 in the functional dynamics of the enzyme. It might also profoundly affect the results of experiments which exploit the photolability of the aa_3–CO complex to initiate redox reactivity with O_2.[8-12] In this report we present the first direct, ambient temperature evidence that CO does indeed bind to Cu_B^+. This conclusion is based upon the observation of the transient infrared absorbance due to the C–O stretching vibration of Cu_B^+–CO, following photodissociation of CO from heme a_3. We observe that the transient Cu_B^+–CO complex equilibrates with its surroundings on a short time scale, losing CO into solution with a half-life of 1.5 μs.

Cytochrome oxidase was isolated from fresh bovine heart muscle by the method of Yoshikawa et al.[13] with modifications to be described elsewhere.[4] The final enzyme solution in 10 mM sodium phosphate, pH 7.4, 0.1% lauryl maltoside, was 1.0 mM in cytochrome oxidase. Solutions approximately 50% v/v in glycerol were also examined. Deoxygenated enzyme solutions were reduced by a small excess of dithionite. Incubation under 1 atm CO gave the fully reduced CO-bound complex, which was transferred to a sapphire infrared cell (l = 0.05 mm). The formation and integrity of the CO complex were verified by UV–vis absorption spectra before and after the infrared measurements.

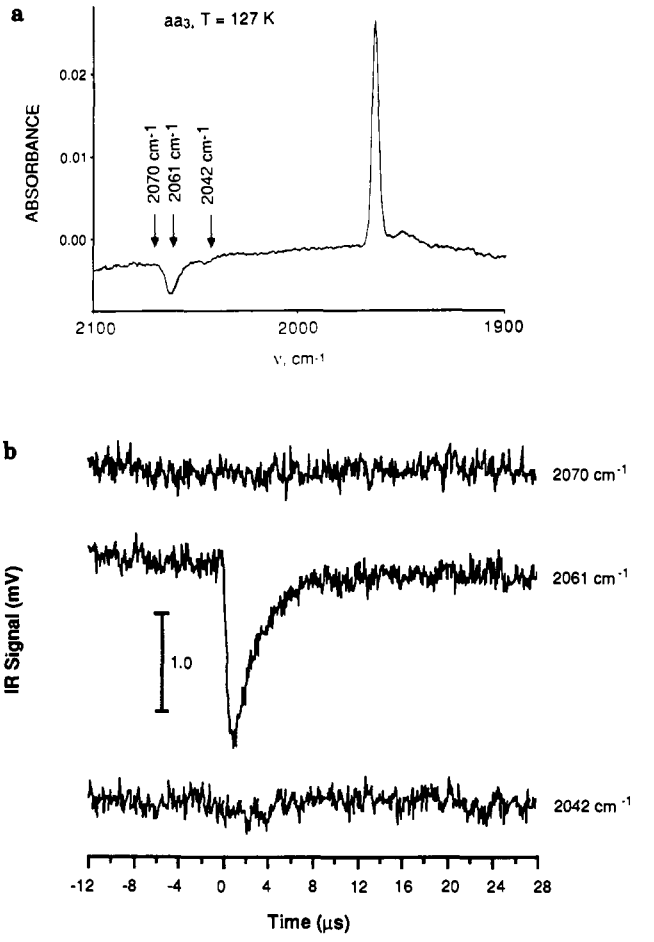
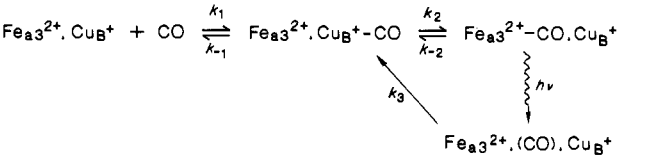


Figure 2. (A) Fourier transform infrared absorbance difference spectrum (dark minus light) of carbonmonoxy cytochrome aa_3 at 127 K, showing the Cu_B^+–CO peak at 2062 cm^{-1} and the observation frequencies used for the control transient infrared experiments at 2070 and 2042 cm^{-1}. The dark and light spectra were recorded before and after photolysis, respectively. (B) The room temperature infrared transients of cytochrome aa_3–CO following photodissociation of the CO from the heme a_3.

Scheme I



A schematic of the transient infrared apparatus is shown in Figure 1. The sample was illuminated by the CW output of an infrared diode laser. The tuning range of the diode contained the Cu_B^+–CO stretching frequencies suggested by the low-temperature results.[1-4] Photodissociation was at the Nd:YAG second harmonic (532 nm, 20 mJ/pulse, 7 ns duration pulses). A repetition rate of 10 Hz allowed reformation of the aa_3–CO complex between laser pulses. The photodissociation and IR beams were combined and passed colinearly through the sample. The IR beam was then monochromated, and the transient IR absorbance signal was detected by a fast InSb detector. The effective detector/amplifier risetime was approximately 200 ns. This signal was extensively averaged with a digital oscilloscope and a microcomputer. Low-temperature (nontransient) CO-FTIR spectra were recorded with a FTIR spectrometer equipped with a closed-cycle helium cryostat.

The binding of photodissociated CO to Cu_B^+ of eukaryotic

(1) Alben, J. O.; Moh, P. P.; Fiamingo, F. G.; Altschuld, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 234–237.
(2) Fiamingo, F. G.; Altschuld, R. A.; Moh, P. P.; Alben, J. O. *J. Biol. Chem.* **1982**, *257*, 1639–1650.
(3) Einarsdóttir, Ó.; Killough, P. M.; Fee, J. A.; Woodruff, W. H. *J. Biol. Chem.* **1989**, *264*, 2405–2408.
(4) Einarsdóttir, Ó.; Killough, P. M.; López-Garriga, J. J.; Dyer, R. B.; Atherton, S. J.; Hubig, S. M.; Palmer, G.; Woodruff, W. H., unpublished results.
(5) Sharrock, M.; Yonetani, T. *Biochim. Biophys. Acta* **1977**, *462*, 718–730.
(6) Findsen, E. W.; Ondrias, M. R. *J. Am. Chem. Soc.* **1984**, *106*, 5736–5738.
(7) Findsen, E. W.; Centeno, J.; Babcock, G. T.; Ondrias, M. R. *J. Am. Chem. Soc.* **1987**, *109*, 5367–5372.
(8) Gibson, Q. H.; Greenwood, C. *Biochem. J.* **1963**, *86*, 541–554.
(9) Greenwood, C.; Gibson, Q. H. *J. Biol. Chem.* **1967**, *242*, 1782–1787.
(10) Chance, B.; Saronio, C.; Leigh, Jr., J. S. *J. Biol. Chem.* **1975**, *250*, 9226–9237.
(11) Babcock, G. T.; Jean, J. M.; Johnston, L. N.; Palmer, G. W.; Woodruff, W. H. *J. Am. Chem. Soc.* **1984**, *106*, 8305–8306.
(12) Hill, B. C.; Greenwood, C.; Nicholls, P. *Biochim. Biophys. Acta* **1986**, *853*, 91–113.
(13) Yoshikawa, S.; Choc, M. G.; O'Toole, M. C.; Caughey, W. S. *J. Biol. Chem.* **1977**, *252*, 5498–5508.
(14) Martin, J.-L.; Migus, A.; Poyart, C.; Lecarpentier, Y.; Astier, R.; Antonetti, A. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 173–178.

cytochrome oxidase can be observed directly by CO-FTIR at $T \leq 180$ K.[2,4] Above this temperature Cu_B^+–CO has not been observed by conventional FTIR measurements, because the thermodynamically stable Fe_{a3}^{2+}–CO reforms too rapidly. Figure 2A shows the CO-FTIR absorbance difference spectrum (*before* minus *after* photodissociation) recorded at 127 K. The positive peaks represent CO bound to the heme a_3 (1963 and ca. 1949 cm^{-1}), and the negative peak at 2061 cm^{-1} represents CO bound to Cu_B^+. A minor Cu–CO peak, which decreases in intensity with increasing temperature,[2,4] is observed at ~2045 cm^{-1} at 127 K. The temperature invariance of the 2061 cm^{-1} frequency between 20 and 180 K[2,4] suggests that the frequency of the Cu_B^+–CO absorption at ambient temperature is close to this value. Accordingly, the time-resolved infrared absorption was followed at 2061 cm^{-1}, with control experiments at 2070 and 2042 cm^{-1} where the complex should not absorb (Figure 2B). The observation of the infrared transient at 2061 cm^{-1} and its absence at the higher and lower frequencies clearly demonstrate the room temperature binding of CO to Cu_B^+, following photodissociation of CO from the heme a_3. The Cu_B^+–CO intermediate subsequently decays to form the unliganded oxidase and free CO.

Our observations can be understood in the context of the following kinetics model of the mechanism of CO photodissociation and rebinding. In Scheme I pulsed illumination of the stable aa_3–CO complex yields the geminate photodissociated species, probably on subpicosecond time scale.[14] The k_3 and k_{-1} processes, which represent the formation of Cu_B^+–CO and the subsequent loss of CO into solution, respectively, are responsible for the infrared transient in Figure 2B. We have established in other work that the competing pathway (k_2) for decay of Cu_B^+–CO, the transfer of CO from Cu_B^+ to Fe_{a3}^{2+}, occurs on a much slower time scale with a half-life of 1 ms.[4] The apparent risetime of the Cu_B^+–CO transient, $t_{1/2} = 220$ ns, is equal to the effective time constant of our instrumentation. Hence, we cannot measure k_3 by using the present approach. Subsequent to its appearance Cu_B^+–CO decays with a first-order rate constant (k_{-1}) of $(4.7 \pm 0.6) \times 10^5$ s^{-1}, corresponding to a half-life of 1.5 ± 0.2 μs. The results obtained for glycerol- and non-glycerol-containing enzyme solutions were experimentally the same.

We emphasize the importance of the time-resolved infrared approach in establishing the precise chemical nature of the kinetics. Our measurements were made by following the infrared transient absorbance associated with a specific structural feature of the system, namely the C–O stretching frequency of the Cu_B^+–CO complex. Accordingly, there is no ambiguity in the assignment of the transient to the molecular phenomenon at issue, as there often is in kinetic UV–vis spectrophotometry.

These results provide the first direct evidence for a Cu_B^+–CO intermediate prior to the formation of the thermodynamically stable Fe_{a3}^{2+}–CO complex in eukaryotic cytochrome oxidase at room temperature. Details of the above reaction scheme will be reported elsewhere.[4] The lifetime of the Cu_B^+–CO complex is comparable to the time scale attributed to the formation of the Fe_{a3}^{2+}–O_2 intermediate in the flow-flash kinetics,[12] hence it is possible that the loss of CO by Cu_B^+ is the rate-determining step in the formation of the heme–O_2 adduct. Alternatively, the facts may indicate that the lifetime of Cu_B^+–CO is too short to interfere directly with the reaction of the oxidase with O_2. Even in the latter case, indirect effects upon the O_2 kinetics are possible. The room temperature observation of Cu_B^+–CO in the present study as well as in cytochrome ba_3[3] suggests the possibility that the binding of incoming ligands to Cu_B, and the "ligand shuttle" function, may be general mechanistic features of cytochrome oxidase reactivity.

Acknowledgment. This work was supported by National Institutes of Health Grant DK36263 (WHW) and performed at The University of California, Los Alamos National Laboratory under the auspices of the U.S. Department of Energy. We gratefully acknowledge Dr. Stephen Buelow of CLS-4 for technical assistance.

Registry No. Cu, 7440-50-8; CO, 630-08-0; cytochrome oxidase, 9001-16-5.

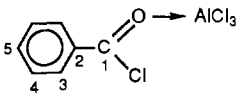
Exceptionally Stable Phenyldichlorocarbenium Ion as a Friedel–Crafts Intermediate: A High-Field Multinuclear NMR Study

Uday S. Racherla,*,† Thomas Daniel, P. R. Rajamohanan, and Nagaraj R. Ayyangar*,‡

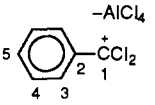
National Chemical Laboratory
Pune-411 008, India

Received September 14, 1988

Halogenated carbocations play an important role as intermediates in a variety of organic reactions.[1-3] Nearly two decades ago, Olah and co-workers demonstrated that stable and long-lived halogenated alkylcarbenium ions could be obtained in strongly acidic media such as FSO_3H–SbF_5–SO_2, HF–SbF_5–SO_2ClF, and SbF_5–SO_2 at low temperatures ranging from −60 to −120 °C.[4-6] They have shown further that the halogenated arylcarbenium ions could also be directly observed by NMR spectroscopy under similar conditions at −30 to −80 °C.[4b,7] Interestingly, although many of the commercially important Friedel–Crafts syntheses[8] might actually involve the intermediacy of the halogenated arylcarbenium ions, their existence has never been proven under such conditions. Thus, nothing is known regarding the existence and stability of these ions is common organic solvents at 25 °C. We report here, for the first time, the intermediacy of an exceptionally stable phenyldichlorocarbenium tetrachloroaluminate complex (**4**) in the Friedel–Crafts reaction of acetanilide with benzotrichloride and $AlCl_3$ in ethylene dichloride at 25 °C and its characterization by the high field ^1H, ^{13}C, and ^{27}Al NMR spectroscopy.



2



4

Methyl (5-benzoylbenzimidazol-2-yl)carbamate (known as Mebendazole) is an important human and veterinary broad spectrum anthelmintic drug.[9] As part of our research on the synthesis of potential anthelmintic drugs which are structurally analogous to Mebendazole, we needed to prepare 4-acetamidobenzophenone.[10] In this connection, we observed that while the

* To whom all the correspondence must be addressed. Present address: Department of Chemistry, Purdue University, West Lafayette, IN 47906.
† Present address: Department of Chemistry, Purdue University, West Lafayette, IN 47906.
‡ NCL Communication No. 4541.

(1) Olah, G. A.; Kuhn, S. J.; Flood, S. H. *J. Am. Chem. Soc.* **1961**, *83*, 4581.
(2) Chambers, R. D.; Mobbs, R. H. *Advances in Fluorine Chemistry*; Butterworths: London, 1965; Vol. 4.
(3) Hart, H.; Hartlage, J. A.; Fish, R. W.; Ratos, R. *J. Org. Chem.* **1966**, *31*, 2244.
(4) (a) Olah, G. A.; Chambers, R. D.; Comisarow, M. B. *J. Am. Chem. Soc.* **1967**, *89*, 1268. (b) Olah, G. A.; Comisarow, M. B. *Ibid.* **1969**, *91*, 2955. Olah, G. A.; Halpern, Y.; Mo, Y. K. *Ibid.* **1972**, *94*, 3551.
(5) Olah, G. A.; Mo, Y. K. *J. Org. Chem.* **1972**, *37*, 1029.
(6) (a) Olah, G. A.; Liang, G.; Mo, Y. K. *J. Am. Chem. Soc.* **1972**, *94*, 3544. (b) Olah, et al. *J. Org. Chem.* **1974**, *39*, 2394.
(7) (a) Volz, H. *Tetrahedron Lett.* **1963**, *38*, 3413. (b) Olah, G. A.; Mo, Y. K. *J. Org. Chem.* **1973**, *38*, 2686. (c) Olah, G. A.; Westerman, P. W.; Forsyth, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 3419.
(8) (a) Eiglmeir, K. Ger. Offen. 2,451,037 (Cl.C07C49/76), 1976. (b) Hoerhold, H. H.; Raabe, D.; Bader, C. Ger. (East). 143,901 (Cl.C07C17/32), 1980. (c) Liu, K. C. Eur. Pat. Appl. EP. 154,092 (Cl.C07C49/83), 1983. (d) Knapp, L. et al. Hung. Teljes. HU 37,588 (Cl.C07C17/14), 1986.
(9) Bosche, H. V.-D.; Rochette, F.; Horig, C. *Adv. Pharmacol. Chemother.* **1982**, *19*, 67.
(10) (a) Theilacker, W.; Blumeneron, H. M. V. *Chem. Ber.* **1956**, *89*, 984. (b) Yasue, M.; Itaya, M.; Takai, Y. *Yakugaku Zasshi* **1961**, *81*, 458. (c) Alper, H.; Madhuban, G. *J. Organomet. Chem.* **1981**, *219*, 125. (d) Rai, S. D. et al. Indian IN 157,725 (Cl.C07C49/00), 1986. (e) Tsekhanski, R. S.; Zobova, N. W.; Ushenina, V. R. *Izv. Vysshikh. Uehebn. Zavadenii, Khim. i. Khim. Tekhnol.* **1961**, *4*, 985.

0002-7863/89/1511-7659$01.50/0