takes place (slightly positive ΔOD's were seen only after ~390 nm). These results are inconsistent with an 11-12-cis to 11-12-trans isomerization as assumed earlier.[1] TLC of either Hg lamp (5 min) or laser (15 pulses) irradiated samples did not show the presence of *all-trans*-RSB (the only definite spot corresponded to 11-*cis*-RSB). Therefore we propose that the ΔOD spectrum that was consistently observed corresponds to another isomerization process such as a trans to cis isomerization about another double bond with a very low $\phi_{PI}$.

In the case of protonated 11-*cis*-RSB in alkanes, positive ΔOD's were observed over the entire wavelength region (400-500 nm) studied (Figure 2). Identification of *all-trans*-RSB as the primary photoproduct was accomplished by TLC as above except 20% hexane-80% ether was used as the eluting solvent. The $\phi_{PI}$ values are listed in Table I.

It appears that in an alkane solvent, the absence of a proton not only prohibits the 11-12-cis to 11-12-trans isomerization but may promote another type of isomerization. The proton in this type of solvent (alkane) does play a role in directing the same type of isomerization that occurs in rhodopsin. However, in the case of the polar solvents, protonation exerts no influence on the type of isomerization already existing for the unprotonated RSB and little or no influence on the $\phi_{PI}$. This clearly indicates that protonation is not a general requirement for the 11-cis to all-trans isomerization in the Schiff base.

These results raise important questions regarding a restricted view of the proton as necessary for promoting the isomerization of rhodopsin and open the way for broader roles of the proton in the early steps of the visual process.

**Registry No.** 11-*cis*-Retinal, 564-87-4.

## Electron-Transfer Kinetics of Pentaammineruthenium(III)(histidine-33)-Ferricytochrome *c*. Measurement of the Rate of Intramolecular Electron Transfer between Redox Centers Separated by 15 Å in a Protein

Jay R. Winkler, Daniel G. Nocera, Kathryn M. Yocom, Emilio Bordignon,[1] and Harry B. Gray*

*Contribution No. 6637 from the Arthur Amos Noyes Laboratory California Institute of Technology Pasadena, California 91125*

*Received April 28, 1982*

A central problem in metalloprotein oxidation-reduction chemistry is the elucidation of the relationship between electron-transfer rate and redox-center separation.[2-4] The most direct way to address this problem is to make measurements of intramolecular electron-transfer rates between redox centers in proteins in cases where the separation distance is known. An attractive system in this regard is $Ru(NH_3)_5(His\text{-}33)^{3+}$-ferricytochrome *c* ($PFe^{III}$-$Ru^{III}$), whose redox centers are separated by 15 Å (Figure 1).[4b] This protein derivative is ideally suited for electron-transfer kinetic studies, because the $Ru(NH_3)_5(His\text{-}33)^{n+}$ unit is substitution inert in both $n = 3$ and $n = 2$ oxidation states and its reduction potential is very near that of the heme *c* ($PFe^{III}$-$Ru^{III}$: $E°Fe^{III}/Fe^{II}$) = 0.26 V vs. NHE; $E°(Ru^{III}/Ru^{II})$ = 0.15 V vs. NHE). Here we report experiments that show unambiguously that electron transfer from $Ru^{II}$ to $Fe^{III}$ in the "mixed-valence" $PFe^{III}$-$Ru^{II}$ ($d$ = 15 Å) species takes place at a significant rate.

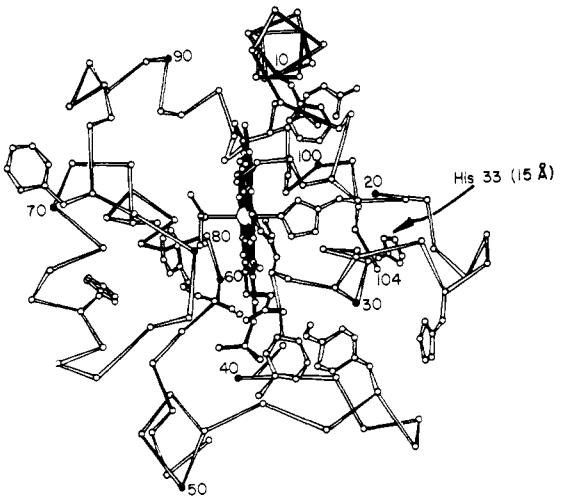



**Figure 1.** Molecular skeleton of horse heart cytochrome *c* illustrating the position of His-33 relative to the heme *c* group (the axial Fe ligands are Met-80 and His-18). The shortest (His-33)-(heme *c*) distance is 15 (1) Å (imidazole N3 to the closest aromatic C in the porphyrin is shorter than any of the imidazole(His-33)-imidazole(His-18) contacts).[4b]

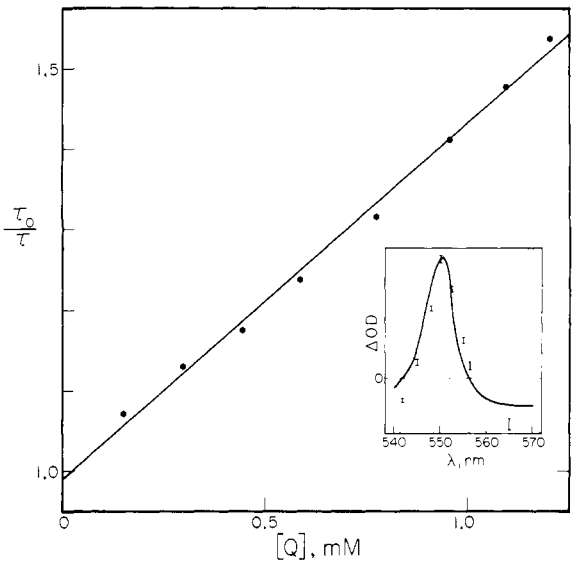



**Figure 2.** Stern-Volmer plot of luminescence quenching of $Ru(bpy)_3^{2+*}$ ($\tau_0$ = 570 ns) by horse heart $PFe^{III}$-$Ru^{III}$ in 0.1 $\mu$ (pH 7) phosphate buffer under nitrogen. Excitation source: second harmonic (532 nm), Nd:YAG laser (pulse duration, 8 ns fwhm). Inset: solid line, transient difference spectrum calculated from the absorption spectra of $PFe^{III}$ and $PFe^{II}$; points with error bars, transient difference spectrum for a $PFe^{III}/Ru(bpy)_3^{2+}$ solution.

The long-lived excited state of $Ru(bpy)_3^{2+}$ (bpy = 2,2'-bipyridine) is a powerful reducing agent,[5] and we have demonstrated[6] that it will transfer electrons to both $PFe^{III}$ and $Ru(NH_3)_5His^{3+}$. A Stern-Volmer plot of the luminescence quenching of $Ru(bpy)_3^{2+*}$ by $PFe^{III}$-$Ru^{III}$ (Figure 2) yields $k_q(PFe^{III}\text{-}Ru^{III}) = 7.8 \times 10^8\ M^{-1}\ s^{-1}$. The inset in Figure 2 is the transient absorption spectrum of a deaerated solution of $Ru(bpy)_3^{2+}$ and $PFe^{III}$ recorded

(1) Istituto di Chimica Generale ed Inorganica dell' Universitá, 30123 Venezia, Italy.

(2) (a) DeVault, D. *Q. Rev. Biophys.* **1980**, *13*, 387. (b) Hopfield, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3640.

(3) Mauk, A. G.; Scott, R. A. Gray, H. B. *J. Am. Chem. Soc.* **1980**, *102*, 4360.

(4) (a) Yocom, K. M.; Shelton, J. B.; Shelton, J. R.; Schroeder, W. E.; Worosila, G.; Isied, S. S.; Bordignon, E.; Gray, H. B. *Proc. Natl. Acad. Sci. U.S.A.*, in press. (b) Yocom, K. M. Ph.D. Thesis, California Institute of Technology, Pasadena, CA, 1982.

(5) (a) Sutin, N.; Creutz, C. *Adv. Chem. Ser.* **1978**, *No. 168*, 1. (b) English, A. M.; Lum, V. R.; DeLaive, P. J.; Gray, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 870.

(6) The rate constants for luminescence quenching of $Ru(bpy)_3^{2+*}$ under a nitrogen atmosphere in 0.1 $\mu$ (pH 7) phosphate buffer by purified horse heart $PFe^{III}$ and $Ru(NH_3)_5His^{3+}$ are $2.5 \times 10^8$ and $1.2 \times 10^9\ M^{-1}\ s^{-1}$, respectively. Quenching of $Ru(bpy)_3^{2+*}$ by $PFe^{III}$ has been studied previously (Sutin, N. *Adv. Chem. Ser.* **1977**, *No. 162*, 156. McLendon, G.; Lum, V. R.; English, A. M. Gray, H. B., unpublished results).

0002-7863/82/1504-5798$01.25/0 

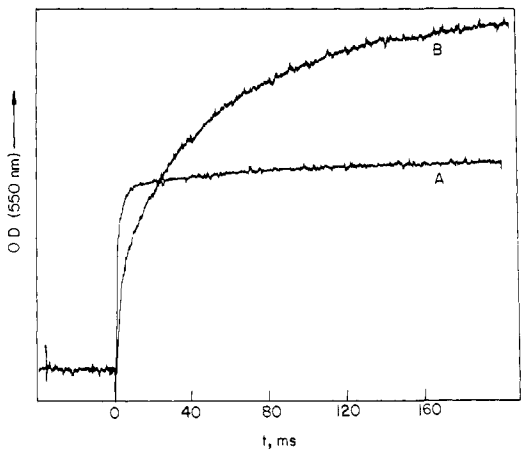



**Figure 3.** Traces of the change in 550-nm optical density resulting from flash photolysis of (A) $PFe^{III}/Ru(bpy)_3^{2+}$/EDTA and (B) $PFe^{III}$-$Ru^{III}/Ru(bpy)_3^{2+}$/EDTA solutions at 22 °C. The vertical axis refers to light intensity at the detector and $t = 0$ on the horizontal axis coincides with the flash pulse. The intensities of the two traces have been normalized to reflect the differences in quenching rate constants.

100 μs after a 532-nm laser pulse. This transient spectrum correlates well with that predicted for the production of $PFe^{II}$. Solutions of $Ru(bpy)_3^{2+}$ with $PFe^{III}$ and $PFe^{III}$–$Ru^{III}$ exhibit transient absorptions maximizing near 550 nm, but the ratio $\Delta OD(PFe)/\Delta OD(PFe–Ru)$ measured immediately after the laser pulse is 2.6 for equivalent concentration conditions. That this ratio is greater than 1 while the ratio of quenching rate constants is ~0.3 clearly demonstrates that the electrons transferred to the modified protein are partitioned between the iron and ruthenium centers *and* that intramolecular electron transfer ($Ru^{II} \rightarrow Fe^{III}$) is much slower than the back reaction between the reduced protein and $Ru(bpy)_3^{3+}$. Furthermore, this ratio of transient absorption intensity has allowed us to estimate that the rates of electron transfer from $Ru(bpy)_3^{2+*}$ to the iron and ruthenium centers in the modified protein are respectively $1.2 \times 10^8$ and $6.6 \times 10^8$ $M^{-1}$ $s^{-1}$. We conclude, therefore, that electron transfer from $Ru(bpy)_3^{2+*}$ to $PFe^{III}$–$Ru^{III}$ produces $PFe^{III}$–$Ru^{II}$ in a 5-fold molar excess over the thermodynamic product, $PFe^{II}$–$Ru^{III}$.

Microsecond flash photolysis of $PFe^{III}$ and $PFe^{III}$–$Ru^{III}$ with $Ru(bpy)_3^{2+}$ in the absence of EDTA results in a small but rapid increase in absorbance at 550 nm that remains constant for several hundred milliseconds.[7] Addition of excess EDTA to $PFe^{III}/Ru(bpy)_3^{2+}$ ([Fe] ~$5 \times 10^{-6}$ M, [EDTA] ~$5 \times 10^{-3}$ M) leads to a large increase in absorption at 550 nm upon flashing (trace A, Figure 3). The rise time for this signal is about 15 ms, indicating that some $PFe^{II}$ is being produced *after* the primary photochemical event of electron transfer from $Ru(bpy)_3^{2+*}$.[8] This secondary production of $PFe^{II}$, however, is essentially complete 15 ms after the flash, leading to a constant increased absorbance at 550 nm. Flash photolysis of $PFe^{III}$–$Ru^{III}/Ru(bpy)_3^{2+}$/EDTA solutions produces trace B in Figure 3 (these experiments were performed at three temperatures: 0.5, 22, and 37 °C). Again the optical density at 550 nm significantly increases upon flashing, but the rise time for this signal is much longer than for the other traces. The magnitude of the transient signal decreases as the temperature is lowered and as the $PFe^{III}$–$Ru^{III}$ concentration is decreased by repeated flashing. We attribute this slow increase

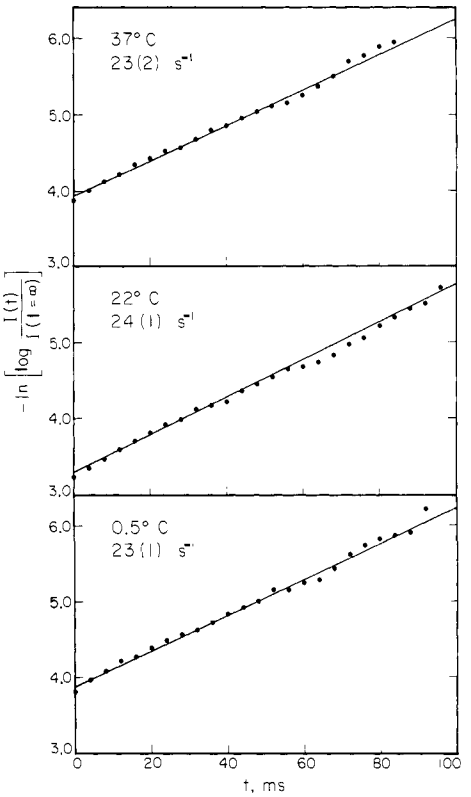



**Figure 4.** First-order plots of the production of $PFe^{II}$–$Ru^{III}$ from P-$Fe^{III}$–$Ru^{II}$ at 0.5, 22, and 37 °C. $I(t)$ is the 550-nm light intensity at the detector where $t = 0$ is chosen to be ~15 ms after the flash pulse. The solid lines are least-squares fits to the data generated independently of the values of $I(t_\infty)$.[9]

in absorbance at 550 nm to the intramolecular electron transfer reaction

$$PFe^{III}–Ru^{II} \xrightarrow{k_{et}} PFe^{II}–Ru^{III}$$

The rate constant for the intramolecular electron transfer in $PFe^{III}$–$Ru^{II}$ is $(2.0 \pm 0.5) \times 10^1$ $s^{-1}$ and, within the error limits of the experiment, does not depend on temperature between 0 and 37 °C (Figure 4).[9,10]

The rate of electron transfer across 15 Å of protein is relatively rapid for redox centers whose reduction potentials are closely matched. Indeed, the rate constant is in the same ballpark as the turnover numbers for several multisite redox enzymes (e.g., cytochrome *c* oxidase),[11] indicating that such enzymes conceivably could function effectively in conformations with relatively large (10–15 Å) site-to-site separation distances. Perhaps the most significant aspect of this type of intramolecular fixed-site experiment is that as more data are gathered it will provide a means of critically assessing the factors that control the rates of biological electron-transfer processes. With the results now in hand, for example, it is apparent that the inner-sphere reorganizational barrier associated with heme *c* electron-transfer reactions is

(7) So that intramolecular electron transfer in photochemically generated $PFe^{III}$–$Ru^{II}$ can be observed, the $Ru(bpy)_3^{3+}$ must be scavenged by a reagent that reacts rapidly with $Ru(bpy)_3^{3+}$ but not with $Ru(bpy)_3^{2+*}$ or the protein and that does not inhibit electron transfer to $PFe^{III}$–$Ru^{III}$. The disodium salt of ethylenediaminetetraacetic acid (EDTA) satisfies those requirements (Whitten, D. G. *Acc. Chem. Res.* **1980**, *13*, 83). The laser flash experiments also suggested that intramolecular electron transfer in $PFe^{III}$–$Ru^{II}$ is slow enough to be observed in a microsecond flash photolysis experiment. Our instrument utilizes a xenon flashlamp (5-μs duration) and a 15-cm path length for monitoring the change in optical density at 550 nm of very dilute $Ru(bpy)_3^{2+}$/protein solutions ([Fe] ~$5 \times 10^{-6}$ M).

(8) This production of $PFe^{II}$ is presumably the result of reaction between $PFe^{III}$ and reactive organic fragments from the oxidation of EDTA.

(9) The vertical axis in the traces in Figure 3 corresponds to light intensity, $I$, at the detector. For a first-order reaction, a plot of $\ln \{\log I(t)/I(t_\infty)\}$ vs. time yields a straight line with a slope equal to the rate constant, $k$. A value for $k$ can be determined by plotting $\ln \{\log I(t)/I(t+\tau)\}$ vs. $t$ ($\tau$ = constant). The value of $k$ evaluated from this plot can then be used to determine an optimum value for $I(t_\infty)$. Finally, with these values of $I(t_\infty)$ and $k$ the three first-order decay plots shown in Figure 4 were generated.

(10) Three pieces of evidence demonstrate that the rate constant corresponds to an intramolecular process rather than intermolecular electron transfer between $PFe^{III}$–$Ru^{II}$ and $PFe^{III}$–$Ru^{III}$: (a) the latter reaction should exhibit a significant temperature dependence (as does the quenching); (b) the rate constant for the reaction between $Ru(NH_3)_5His^{2+}$ and $PFe^{III}$ ($k \sim 10^4$ $M^{-1}$ $s^{-1}$)[4b] gives an upper limit for the pseudo-first-order rate constant $k' = k[Fe^{III}] \sim 10^{-2}$ $s^{-1}$ for the intermolecular process; and, conclusively, (c) the rate constant ($2 \times 10^1$ $s^{-1}$) does not change with a 4-fold variation in the $PFe^{III}$–$Ru^{III}$ concentration.

(11) Malmström, B. G. *Ann. Rev. Biochem.* **1982**, *51*, 21.

negligibly small. A key role for electron tunneling[2] in the Ru(II) → Fe(III) electron transfer is indicated, and we intend to explore appropriate theoretical descriptions of this process in subsequent work.

**Acknowledgment.** We thank Bob Austin, Dick Dickerson, John Hopfield, Stephan Isied, Bo Malmström, Rudy Marcus, George McLendon, Tom Meyer, Norman Sutin, and Henry Taube for their encouragement and for several helpful discussions. This research was supported by National Science Foundation Grant CHE80-24863.

**Registry No.** $Ru(bpy)_3^{2+}$, 15158-62-0.

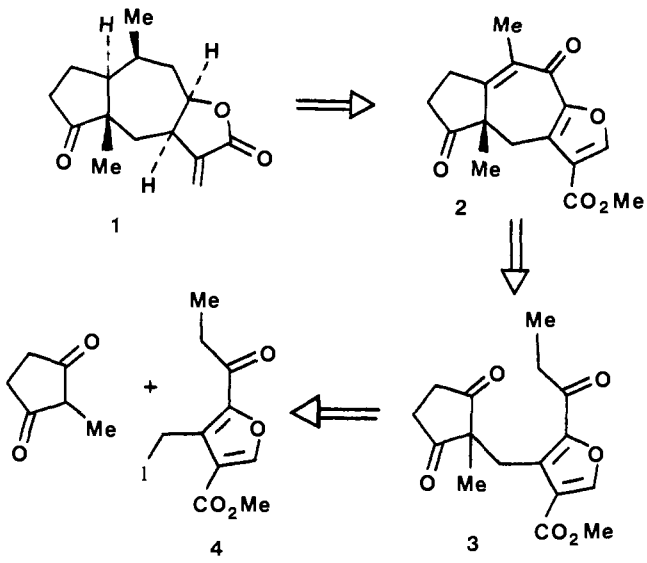
## New Approach to Pseudoguaianolide Sesquiterpene Lactone Construction. Total Synthesis of *dl*-Confertin†

Arthur G. Schultz* and Linda A. Motyka

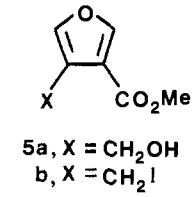
*Department of Chemistry, Rensselaer Polytechnic Institute Troy, New York 12181*

*Received July 7, 1982*

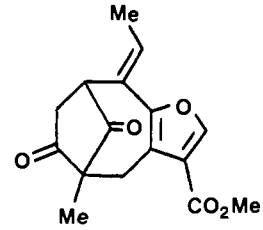
The prodigious effort directed at synthesis of the pseudoguaianolide sesquiterpene lactones[1] has been stimulated in part by reports of cytotoxic and antitumor activity for several of these compounds.[2] Our interest in pseudoguaiane synthesis has evolved from a desire to develop annelation reagents for highly convergent sesquiterpene lactone construction.[3] A total synthesis of *dl*-confertin (**1**), reported herein, demonstrates new seven-membered-ring annelation chemistry (e.g., **4** → **3** → **2**). It is noteworthy that the annelation reagent **4** and 2-methyl-1,3-cyclopentanedione provide all the carbon atoms required for a ten-step synthesis of tricyclic lactone **12**, an advanced intermediate in both the Schlessinger[4] and Heathcock[1] syntheses of confertin. This work should provide an efficient, new strategy for total synthesis of highly complex pseudoguaianes such as the fastigilins (**13**).[5]



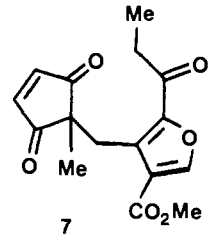

Furan **4** is prepared from methyl 4-(hydroxymethyl)furan-3-carboxylate (**5a**)[6] by reaction with phosphorous triiodide in ether



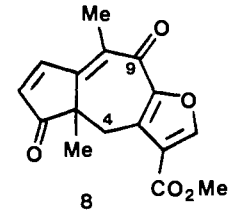

5a, X = $CH_2OH$
b, X = $CH_2I$




6




7




8

to give **5b** (64% yield), followed by acylation with propionic anhydride and boron trifluoride etherate (93%). The ester function in **5** not only provides a directing influence during preparation of **4** but also engenders stability to the furan ring during elaboration of **4** to the perhydroazulene **10** (vide infra); after this service, the ester function is discarded.

Alkylation of 2-methyl-1,3-cyclopentanedione with **4** (1 equiv) in refluxing *tert*-butyl alcohol with potassium *tert*-butoxide gives furan trione **3** (54%; mp 121–122 °C).[7] We note that with these reaction conditions only a trace of O-alkylated product is formed and very little alkoxide-induced cleavage of the nonenolizable β-dicarbonyl functionality in **3** occurs.[8]

We have not been able to directly convert **3** to **2** by base- or acid-catalyzed cyclodehydration. Nucleophilic bases result in β-dicarbonyl cleavage and acidic conditions promote bicyclization to give **6** (88% from *p*-toluenesulfonic acid in refluxing benzene solution; mp 158–159 °C). Apparently, enolization in **3** occurs preferentially within the cyclopentanedione ring. In accord with this supposition, **3** is converted to the yellow enetrione **7** (100%, mp 131–132 °C) on treatment with phenyltrimethylammonium tribromide in dry THF solution.[9] Cyclodehydration of **7** is catalyzed by *p*-toluenesulfonic acid in refluxing benzene solution to give the pale yellow dienedione **8** in 60% isolated yield (mp 186–187 °C).

Selective hydrogenation of **8** with 5% Pt on carbon in ethyl acetate at atmospheric pressure gives **2** (mp 176–177 °C). This can be converted to the bright yellow diene alcohol **9** (mp 125–126 °C) by treatment with sodium borohydride in methanol at room temperature followed by 1 N hydrochloric acid. Control experiments demonstrate that dehydration of an intermediate enediol occurs after addition of the hydrochloric acid.

The hydroxyl and angular methyl substituents in **9** would be expected to direct reagents to the α face of the diene system; in fact, hydrogenation of **9** with 5% Pd on carbon in absolute ethanol at atmospheric pressure gives only the furan alcohol **10a** (mp 112–113 °C) in ~60% overall yield from **8**.

† Dedicated to Professor Gilbert Stork on the occasion of his 60th birthday.

(1) For recent pseudoguaianolide synthetic work, see: Heathcock, C. H.; DelMar, E. G.; Graham, S. L. *J. Am. Chem. Soc.* **1982**, *104*, 1907 and references cited therein.

(2) Kupchan, S. M.; Eakin, M. A.; Thomas, A. M. *J. Med. Chem.* **1971**, *101*, 1147.

(3) Schultz, A. G.; Godfrey, J. D. *J. Am. Chem. Soc.* **1980**, *102*, 2414.

(4) Quallich, G. J.; Schlessinger, R. H. *J. Am. Chem. Soc.* **1979**, *101*, 7627.

(5) Herz, W.; Rajappa, S.; Roy, S. K.; Schmid, J. J.; Mirrington, R. N. *Tetrahedron* **1966**, *22*, 1907. The fastigilins have not as yet yielded to synthesis.

(6) Commerically available furan-3,4-dicarboxylic acid esters are converted to **5a** by monosaponification in methanolic solution and $BH_3$ reduction of the resulting carboxylic acid; see: Corey, E. J.; Crouse, D. N.; Anderson, J. E. *J. Org. Chem.* **1975**, *40*, 2140.

(7) Compounds **2**, **3**, **6–9**, **10a**, and **10b** gave satisfactory combustion analyses.

(8) Furan **4** is reminiscent of the 3,4-disubstituted 4-(halomethyl)isoxazole annelation reagents developed by Stork and co-workers for the construction of cyclohexenone rings; e.g.: Stork, G.; Danishefsky, S.; Ohashi, M. *J. Am. Chem. Soc.* **1967**, *89*, 5459. It is noteworthy that C-alkylation of β-dicarbonyl systems with **4** is possible despite the potential for electronic and steric deactivation of **4** by the C(3) and C(5) acyl substituents.

(9) The conversion of **3** into **7** is thought to occur by cyclopentanedione ring bromination followed by in situ dehydrobomination.

0002-7863/82/1504-5800$01.25/0 