changed, indicating that IPA binds weakly to these HRP compounds, as has been found for native HRP.[41]

In summary, the present study on the incorporation of Mo=O- and W=O-substituted porphyrin complexes into apo-Mb and apo-HRP has revealed that Mb and HRP favor the lower and higher metal oxidation states, respectively. This appears to parallel the relative stabilities of higher oxidation states of native Mb and HRP. These oxometal-substituted hemoproteins are stable and structurally similar to compounds I and II in their heme environments, which allows us to study the substrate binding features of reactive intermediates of native hemoproteins.

Acknowledgment. This work was supported by grants from the Ministry of Education (61470079).

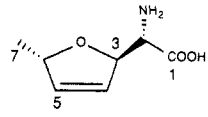
Communications to the Editor

The Biosynthesis of Furanomycin: On the Mechanism of Formation of the Ether Linkage

Ronald J. Parry,* Rajesh Turakhia, and Hanh Phuoc Buu

Department of Chemistry, Rice University
Houston, Texas 77251
Received January 29, 1988

The novel antibiotic furanomycin (**1**) was isolated in 1967 from *Streptomyces threomyceticus* (ATCC 15795).[1] The compound is a competitive antagonist of L-isoleucine, and it inhibits the growth of T-even coliphage. Furanomycin was synthesized in



1

1980 by Joullie and co-workers, who also revised the stereochemistry to that shown in **1**.[2] Previous experiments carried out in our laboratory have demonstrated that **1** is derived from two acetate units and one propionate unit, with the latter serving as the starter unit.[3] The specific incorporation of propionate into **1** requires the oxidation of C-2 of the propionate skeleton and formation of an ether linkage between C-2 of propionate and C-1 of the adjacent acetate unit. Our prior studies revealed that one hydrogen atom is removed from C-2 of propionate as the result of this oxidation and that lactate is not an intermediate. Experiments will now be outlined that provide additional insight into the mechanism of formation of the ether linkage.

The stereochemistry of hydrogen loss from C-2 of propionate was examined by means of incorporation experiments with (2*R*)- and (2*S*)-[2-^{3}H]propionate. Attempts to prepare the stereospecifically tritiated forms of propionic acid from D- and L-alanine utilizing a route reported for the synthesis of the chirally deuteriated compounds[4] failed due to the capricious nature of [^{3}H]lithium aluminum hydride. Consequently, we developed a new synthesis of chirally tritiated propionate in which the introduction of the isotopic label could be carried out with [^{3}H]-borohydride. The route devised (Scheme I) employs the vanillyl moiety as a carboxyl protecting group[5] and provides stereospecifically tritiated sodium propionate in ca. 10% overall yield. The optical purity and configuration of the labeled propionic acid obtained from this synthesis was determined by a Parker analysis[6] of the [2-^{2}H$_1$]propionic acid obtained from reduction of [*formyl*-^{2}H$_1$]-3-methoxy-4-mesyloxybenzaldehyde with R-Alpine Borane. The deuteriated propionate had the expected configuration (*S*) and an optical purity of ca. 90%. Administration of the (2*R*)- and (2*S*)-[2-^{3}H]propionate to *S. threomyceticus* in conjunction with [1-^{14}C]propionate yielded samples of radioactive furanomycin whose tritium to carbon-14 ratios revealed that propionic acid is converted into the antibiotic with loss of the 2 *pro*-R hydrogen atom (Table I, expt 1 and 2). Since the absolute stereochemistry at C-6 of furanomycin is *S*, it follows that the introduction of the ether oxygen atom at C-2 of propionate occurs with overall *inversion* of configuration.

Scheme I

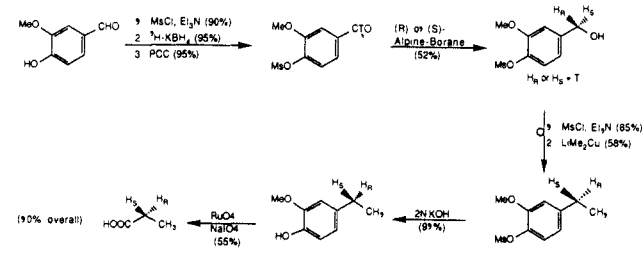


Table I. Incorporation of Labeled Propionic Acid into Furanomycin

expt no.	precursor (^{3}H/^{14}C)	^{3}H/^{14}C furanomycin	% ^{3}H retn
1	sodium [1-^{14}C,2(*S*)-2-^{3}H]propionate (4.80)	4.37	91[a]
2	sodium [1-^{14}C,2(*R*)-2-^{3}H]propionate (4.88)	0.97	21[b]

[a] The optical purity of the precursor was ca. 90%. [b] The optical purity of the precursor was ca. 80%.

The stereochemistry of carbon oxygen bond formation having been elucidated, the question of the origin of the ether oxygen atom was addressed. A priori, it appeared that the oxygen atom could be derived either from the carbonyl oxygen of the adjacent acetate unit or from molecular oxygen. Administration of sodium [$^{18}O_2$, 1-^{13}C]acetate[7] to the fermentation yielded furanomycin exhibiting ^{13}C enrichment at C-1 and C-3, but no oxygen-18 induced shift[8] was apparent for either of these carbon atoms. Appropriate conditions for the administration of ^{18}O-labeled molecular oxygen to the *S. threomyceticus* fermentation were therefore sought. The experiments utilized a closed system containing 1-L shake flasks through which molecular oxygen was circulated with a modified aquarium pump. The system also contained a concentrated aqueous potassium hydroxide solution to trap the expired carbon dioxide. All of the initial attempts to utilize this setup were unsuccessful due to an increase in the pH of the medium from 7 to ca. 9 during the course of the fermentation. Eventually, we discovered that this behavior was the consequence of the production of volatile, basic compounds (ammonia?), and so a 2 N sulfuric acid trap was introduced into the

(1) Katagiri, K.; Tori, K.; Kimura, Y.; Yoshida, T.; Nagasaki, T.; Minato, H. *J. Med. Chem.* **1967**, *10*, 1149.
(2) Semple, J. E.; Wang, P. C.; Lysenko, Z.; Jouillie, M. M. *J. Am. Chem. Soc.* **1980**, *102*, 7505.
(3) Parry, R. J.; Buu, H. P. *J. Am. Chem. Soc.* **1983**, *105*, 7446.
(4) Prescott, D. J.; Rabinowitz, J. L. *J. Biol. Chem.* **1968**, 1551.
(5) Battersby, A. R.; Sheldrake, P. W.; Staunton, J.; Williams, D. C. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1056.
(6) Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1983**, 83.
(7) Cane, D. E.; Liang, T.-C.; Hasler, H. *J. Am. Chem. Soc.* **1982**, *104*, 7274.
(8) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 252; **1980**, *102*, 4609, 6699. Vederas, J. C. *Ibid.* **1980**, *102*, 374. Vederas, J. C.; Nakashima, T. T. *J. Chem. Soc., Chem. Commun.* **1980**, 183.

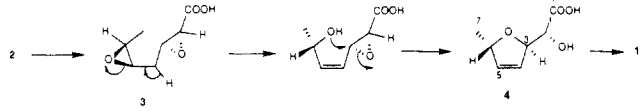
0002-7863/88/1510-4035$01.50/0

Table II. Incorporation of ($^{18}O_2$) into Furanomycin[a]

C	^{13}C shift (ppm)	% enrchmnt	$\Delta\delta^b$ (ppm)
3	86.46	25	0.024
6	86.39	25	0.031

[a] Carbon spectra were measured in D_2O at 75.47 mHz with Gaussian multiplication with LB = -1.0 Hz, GB = 0.40, SW = 20 000, 64 K data points. [b] $^{13}C^{18}O$ isotope shift.

Scheme II



system. When both the base and acid traps were present, and when the fermentation was carried out for 72 h under normal conditions before connection to the closed system, then significant production of furanomycin was observed. Administration of ^{18}O-labeled molecular oxygen (52.8 atom % ^{18}O) under these conditions yielded furanomycin whose ^{13}C NMR spectrum exhibited ^{18}O-induced shifts for both C-3 and C-6 (Table II). It can therefore be concluded that the oxygen atom comprising the ether linkage of furanomycin is derived from molecular oxygen.

On the basis of the findings described above, two mechanisms can be considered for the formation of the ether linkage of furanomycin. On the one hand, an oxygen atom could be introduced by hydroxylation of C-2 of the propionic acid moiety after its incorporation into the growing polyketide chain. Ether formation could then take place by attack of the oxygen-derived hydroxyl group upon the carbonyl carbon of the adjacent acetate unit. The plausibility of this mechanism is limited by the fact that hydroxylations at a saturated carbon atom generally take place with retention of configuration.[9] Alternatively, an oxygen could be introduced by epoxidation of a double-bond generated between C-5 and C-6 of the furanomycin skeleton as the result of a "processive" polyketide assembly process.[10] A likely substrate for this epoxidation would be *trans,cis*-2,3,5,6-heptadienoic acid (**2**) which could undergo epoxidation to give the diepoxide **3**. Ring-opening of the 5,6-epoxide followed by attack of the resulting hydroxyl group on the adjacent epoxide would then yield the α-hydroxy acid **4** that could be readily converted into furanomycin (Scheme II). This second mechanism for the formation of the ether linkage of furanomycin is of particular interest since it bears a close resemblance to that suggested for the formation of the ether linkages in the polyether antibiotics.[7] Support for the second mechanism is provided by studies of the allylic rearrangements catalyzed by β-hydroxydecanoylthioester dehydrase,[11] an enzyme involved in the anaerobic synthesis of unsaturated fatty acids. These studies suggest that the 5,6-double bond of **2** could arise by isomerization of a *trans*-2,3-pentenoate thiol ester intermediate to a *cis*-3,4-pentenoate thiol ester with loss of the 4 *pro*-R hydrogen. Since the 2 *pro*-R hydrogen of propionate corresponds to the 4 *pro*-R hydrogen atom of the putative 2,3-pentenoate thiol ester, the observed loss of the 2 *pro*-R hydrogen atom of propionate during furanomycin biosynthesis is consistent with such an isomerization and with the cyclization mechanism shown in Scheme II.

Acknowledgment. We are pleased to acknowledge financial support of this work by PHS Grant no. CA25142 awarded by the National Cancer Institute, DHHS, and by The Robert A. Welch Foundation (C-729). We also thank Drs. John Vederas and Steven Gould for helpful discussions concerning the administration of oxygen-18 to fermentations and the Arco Foundation for funds to purchase the IBM AF300 spectrometer used in this work.

Registry No. 1, 18455-25-9; O_2, 7782-44-7; propionic acid, 79-09-4; *trans,cis*-2,3,5,6-heptadienoic acid, 85110-95-8.

(9) Herbert, R. B. *The Biosynthesis of Secondary Metabolites*; Chapman and Hall: New York, 1981; p 9.
(10) Yue, S.; Duncan, J. S.; Yamomoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1987**, *109*, 1253.
(11) Schwab, J. M.; Klassen, J. B. *J. Am. Chem. Soc.* **1984**, *106*, 7217.

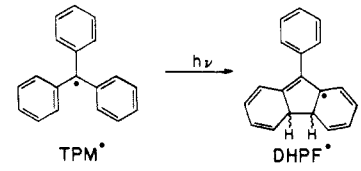
Evolution of Electronically Excited Triphenylmethyl Radical. Picosecond Preparation-Pump-Probe Spectroscopic Experiments

Jeffrey A. Schmidt and Edwin F. Hilinski*

Department of Chemistry, The Florida State University Tallahassee, Florida 32306-3006

Received January 20, 1988
Revised Manuscript Received April 1, 1988

Investigations of the photochemistry and photophysics of excited states of short-lived organic species in solution are increasing in number as experimental methods are developed which permit the study of these intermediates.[1] Recent ns-resolved fluorescence and absorption studies of arylmethyl radicals revealed that the lifetime of the excited-state diphenylmethyl (DPM) radical in solution at room temperature is ~250 ns.[10-13,16] Itoh and co-workers[17] published the emission spectrum of benzyl radical (Bz$^\bullet$) in room temperature hexane solution and reported a fluorescence lifetime of ~1 ns. Meisel and co-workers[10,12,16] reported that excitation of the triphenylmethyl (TPM) radical in solution resulted in no detectable emission on the ns time scale at room temperature. For excitation of TPM$^\bullet$ (eq 1),[10,12] an absorption band at 490 nm that appeared within the 10-ns excitation pulse was assigned to the 4a,4b-dihydro-9-phenylfluorenyl (DHPF) radical which persisted with a lifetime greater than 100 μs.



(1)

We have performed a series of novel three-pulse ps-resolved absorption experiments which provide information about the excited-state TPM radical prior to the formation of DHPF$^\bullet$. For these experiments, three 30-ps pulses of light are generated from a single 1064-nm pulse emitted from an actively-passively mode-locked Nd:YAG laser.[21] The time of arrival of each of

(1) For example, see ref 2-20 and references cited therein.
(2) Kelley, D. F.; Milton, S. V.; Huppert, D.; Rentzepis, P. M. *J. Phys. Chem.* **1983**, *87*, 1842.
(3) Sitzmann, E. V.; Wang, Y.; Eisenthal, K. B. *J. Phys. Chem.* **1983**, *87*, 2283.
(4) Itoh, M.; Adachi, T.; Tokumura, K. *J. Am. Chem. Soc.* **1983**, *105*, 4828.
(5) Tobita, S.; Tanaka, I. *Chem. Phys. Lett.* **1983**, *96*, 517.
(6) Nagarajan, V.; Fessenden, R. W. *Chem. Phys. Lett.* **1984**, *112*, 207.
(7) Baumann, H.; Merckel, C.; Timpe, H.-J.; Graness, A.; Kleinschmidt, J.; Gould, I. R.; Turro, N. J. *Chem. Phys. Lett.* **1984**, *103*, 497.
(8) Itoh, M.; Adachi, T.; Tokumura, K. *J. Am. Chem. Soc.* **1984**, *106*, 850.
(9) Hilinski, E. F.; Huppert, D.; Kelley, D. F.; Milton, S. V.; Rentzepis, P. M. *J. Am. Chem. Soc.* **1984**, *106*, 1951.
(10) Bromberg, A.; Schmidt, K. H.; Meisel, D. *J. Am. Chem. Soc.* **1984**, *106*, 3056.
(11) Bromberg, A.; Meisel, D. *J. Phys. Chem.* **1985**, *89*, 2507.
(12) Bromberg, A.; Schmidt, K. H.; Meisel, D. *J. Am. Chem. Soc.* **1985**, *107*, 83.
(13) Scaiano, J. C.; Tanner, M.; Weir, D. *J. Am. Chem. Soc.* **1985**, *107*, 4396.
(14) Itoh, M.; Yoshida, N.; Takashima, M. *J. Am. Chem. Soc.* **1985**, *107*, 4819.
(15) Johnston, L. J.; Scaiano, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 6368.
(16) Meisel, D.; Das, P. K.; Hug, G. L.; Bhattacharyya, K.; Fessenden, R. W. *J. Am. Chem. Soc.* **1986**, *108*, 4706.
(17) Tokumura, K.; Udagawa, M.; Ozaki, T.; Itoh, M. *Chem. Phys. Lett.* **1987**, *141*, 558.
(18) Wilson, R. M.; Hannemann, K.; Peters, K.; Peters, E. M. *J. Am. Chem. Soc.* **1987**, *109*, 4741.
(19) Wilson, R. M.; Hannemann, K.; Heineman, W. R.; Kirchoff, J. R. *J. Am. Chem. Soc.* **1987**, *109*, 4743.

0002-7863/88/1510-4036$01.50/0