(Scheme I) that the isotope effects be the same for production of **1** and **2** is met.

The key observation that the isotope effects in the two experimental systems (Tables I and II)[13] are nearly identical fits the hypothesis that the low isotope effect in the mass spectrometer derives from the nature of the cation radical independent of its formative history. The correspondence draws one to the conclusion that 2-hexanone cation radical demands the same transition state for γ-hydrogen abstraction under vacuum as in the complex solvent medium of an electrochemical cell. The molecular cation radicals undergoing rearrangement in the mass spectrometer cannot be distinguished from thermal molecules in their discrimination for hydrogen over deuterium.[14,15]

References and Notes

(1) (a) P. J. Wagner, *Acc. Chem. Res.*, **4**, 168 (1971), discusses the nature of this photochemical process and gives leading references to the earlier literature. (b) J. Y. Becker, L. R. Byrd, L. L. Miller, and Y.-H. So, *J. Am. Chem. Soc.*, **97**, 853 (1975). (c) D. G. I. Kingston, J. T. Bursey, and M. M. Bursey, *Chem. Rev.*, **74**, 215 (1974), is a revlew of these observations.

(2) See ref 1a above and S. Meyerson and J. D. McCollum *Adv. Anal. Chem. Instrum.*, **2**, 184 (1963), and ref 1c above.

(3) D. R. Coulson and N. C. Yang, *J. Am. Chem. Soc.*, **88**, 4511 (1966); A. Padwa and W. Bergmark, *Tetrahedron Lett.*, 5795 (1968); F. D. Lewis, *J. Am. Chem. Soc.*, **92**, 5602 (1970).

(4) See ref 1c above and J. K. MacLeod and C. Djerassi, *J. Am. Chem. Soc.*, **89**, 5182 (1967).

(5) For a general treatment of these effects, see (a) K. B. Wiberg, *Chem. Rev.*, **55**, 713 (1955); (b) L. Melander, "Isotope Effects on Reaction Rates", Ronald Press, New York, 1960; (c) F. H. Westheimer, *Chem. Rev.*, **61**, 265 (1961); (d) R. P. Bell, *Chem. Soc. Rev.*, **3**(4), 513 (1974); (e) C. J. Collins and N. S. Bowman, *ACS Monogr.*, **No. 167** (1970).

(6) H. M. Rosenstock, M. B. Wallenstein, A. L. Wahrhaftig, and H. Eyring, *Proc. Natl. Acad. Sci. U.S.A.*, **38**, 667 (1952).

(7) D. H. Williams and I. Howe, "Principles of Organic Mass Spectrometry", McGraw-Hill, New York, 1972, Chapter 4; K. Levsen, "Fundamental Aspects of Organic Mass Spectrometry", Verlag Chemie, Weinheim/Bergstr., Germany, 1978.

(8) See K. B. Wiberg, in ref 5a herein.

(9) M. M. Green, J. M. Moldowan, M. W. Armstrong, T. L. Thompson, K. J. Sprague, A. J. Hass, and J. J. Artus, *J. Am. Chem. Soc.*, **98**, 849 (1976), and references therein.

(10) The NMR and mass spectra (MS) of **1** matched those of earlier report.[1b] **2** was isolated by extraction and GC collection on 20% SE-30 on Chrom W, flow 10 cm^3/15 s, column temperature 175 °C, retention time 8 min (colorless liquid). Yield was ~3:1 **1:2** under typical conditions.[1b] By high resolution the MS molecular weight of **2** corresponds to $C_8H_{13}NO$ with major fragments at *m/e* 97, 96, 82 (base peak). ^1H NMR on an FX-60Q spectrometer at 59.75 Hz: δ 1.22 (d, 3 H, J = 7 Hz, C_4 CH_3, irradiation of C-4 H at 4.35 collapses 1.22 to a singlet), 2.17 (s, 3 H, C_2 CH_3), 2.2 (br, overlapping 2.17, 3 H, C_7 CH_3), 2.6 (v br, 2 H, CH_2), 4.35 (br, 1 H, C_4 H), 4.9 (br, 1 H, C_6 H). ^{13}C NMR on a JEOL FX-60Q at 15.0 Hz including multiplicity from off-resonance data: δ 17.6 (q, 4-CH_3), 22.1 (q, 2-CH_3), 24.2 (q, 7-CH_3), 36.2 (t, C_5), 56.9 (d, C_4), 108.4 (d, C_8), 141 (v w, C_7), 167.4 (s, C_2).

(11) Z. Galus, "Fundamentals of Electrochemical Analysis", Ellis Horwood Ltd., Chichester, England, 1976, Chapter 15; "Organic Electrochemistry", M. M. Baizer, Ed., Marcel Dekker, New York, 1973, pp 99, 449; L. Meites in "Techniques of Chemistry", Vol. I, Part IIA, Wiley-Interscience, New York, 1971, Chapter IX.

(12) The k_H/K_O values in Table I are reasonable for a low primary isotope effect and would be remarkable for secondary isotope effects for monodeuterio substitution in varied classes of reactions. See D. E. Sunko and S. Borčić (Chapter 3) of ref 5e.

(13) We cannot account for the higher k_H/k_D values for 2-hexanone (Table II) compared with the values ranging from 1.0 to 1.2 for related molecules (2-hexanone was not measured) previously reported.[4] The invariance here within experimental error, of the results at 70 and 10 eV, argue against the scrambling mechanism which affected the earlier finding.[4] Moreover such scrambling processes[4] caused deviations in the opposite direction (k_H/k_O too low) from the results herein.

(14) The independence of temperature for k_H/k_D in both systems (Table I and II) is expected for low isotope effects and is consistent with recent results and predictions for bent transition states. See H. Kwart and T. J. George, *J. Org. Chem.* **44**, 162 (1979); H. Kwart, D. A. Benko, and M. E. Bromberg, *J. Am. Chem. Soc.*, **100**, 7093 (1978); H. Kwart, T. J. George, R. Louw, and W. Ultee, *ibid.*, **100**, 3927 (1978); M. E. Schneider and M. J. Stern, *ibid.*, **94**, 1517 (1972), and references therein. See also, S. B. Kaldor and W. H. Saunders, Jr., *ibid.*, **101**, 7594 (1979). A referee has suggested that the possibility that the k_H/k_O values reside substantially in a temperature-independent preexponential term would make the isotope effect a poor probe of the internal energy of the cation radical. Thus the present results, although demonstrating that electron impact is not prerequisite to the low isotope effects and that the transition states are comparable in the two systems (Tables I and II), could nevertheless allow that the cation radical in the mass spectrometer is vibrationally excited compared with its solution counterpart.

(15) See M. M. Green, T. J. Mangner, S. P. Turner, and F. J. Brown, *J. Am. Chem. Soc.*, **98**, 7082 (1976); M. M. Green, R. J. Giguere, and J. R. P. Nicholson, *ibid.*, **100**, 8020 (1978), and references therein to earlier papers in the series, strengthening the ties which bind mass spectrometry and free-radical chemistry.

(16) We are grateful to the National Institutes of Health, General Medical Sciences, and the donors of the Petroleum Research Fund, administered by the American Chemical Society, for the financial support for these studies. Direct correspondence to Mark M. Green at the Department of Chemistry, Polytechnic Institute of New York, 333 Jay Street, Brooklyn, New York 11201.

Mark M. Green*[16]
Department of Chemistry
Polytechnic Institute of New York
Brooklyn, New York 11201

G. J. Mayotte, Louis Meites
Department of Chemistry, Clarkson College
Potsdam, New York 13676

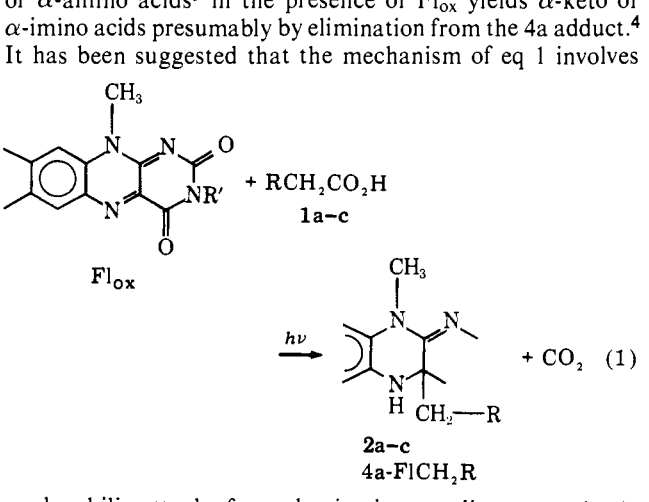
David Forsyth
Department of Chemistry, Northeastern University
Boston, Massachusetts 02115

Received August 20, 1979

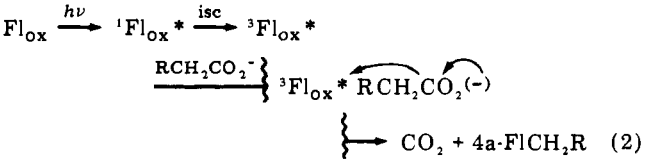
The Mechanism of Flavin 4a Substitution Which Accompanies Photolytic Decarboxylation of α-Substituted Acetic Acids. Carbanion vs. Radical Intermediates

Sir:

Flavin-mediated photodecarboxylation (PDC) of certain α-substituted acetic acids (eq 1) has been claimed to serve as a model for flavoenzyme-catalyzed dehydrogenations.[1,2] The reaction is facile only in cases in which a heteroatom (O, N, and S) is bonded directly or vinylogously to the methylene carbon of the substituted acetic acid (in eq 1, R is C_6H_5O- for **1a**, C_6H_5S- for **1b**, and 3-indolyl for **1c**). PDC of α-hydroxyl[2a] or α-amino acids[3] in the presence of Fl_{ox} yields α-keto or α-imino acids presumably by elimination from the 4a adduct.[4] It has been suggested that the mechanism of eq 1 involves



nucleophilic attack of a carbanion intermediate upon the 4a position of Fl_{ox} (eq 2).[1,2] However, PDC of the α-substituted



carboxylic acids **1a–c** by the triplet states of benzophenone, quinones and various quinoid dye molecules has been established (spin trapping, CIDNP, product analysis)[5] to be radical in nature. We are now able to show that a radical mechanism is involved in the $^3Fl_{ox}$*-mediated PDC reactions. This communication deals with the results of laser flash photolysis and

0002-7863/80/1502-1465$01.00/0

Table I. Radicals Observed during the Fl_{ox}-Mediated Photodecarboxylation of Carboxylic Acids **1a–c** in the Presence of NtB[a]

acid	radicals obsvd	hyperfine splittings, a_n in G[b] ($a_H^{CH_2}$)	g[c]
1a	$(t\text{-Bu})_2\dot{N}O$· (**4**)	15.5	2.0067
	$t\text{-BuN}(\dot{O})CH_2OC_6H_5$ (**5**)	13.3 (4.5)	2.0069
1b	$(t\text{-Bu})_2\dot{N}O$· (**4**)	15.5	2.0067
1c	$(t\text{-Bu})_2\dot{N}O$· (**4**)	15.5	2.0067
	3-indolyl$CH_2N(\dot{O})$-t-Bu (**6**)	15.5 (8.5)	2.0069

[a] Fl_{ox} (5×10^{-5} M), NtB (0.01 M), **1a** (0.5 M), **1b** (0.1 M), or **1c** (0.1 M) in degassed acetonitrile. Irradiation was accomplished using the Hg–Xe lamp and the 441-nm filter. ESR spectra will be included as Xerox copies with reprints. [b] ±0.1 G. [c] ±0.0002.

2-methyl-2-nitrosopropane (NtB) spin trapping[6] studies of the PDC of **1a–c** mediated by the triplet of 3-methyllumiflavin (R′ = CH_3, λ_{max} 442 nm).[7]

When Fl_{ox} alone in acetonitrile is flashed (430 nm), transients due to $^3Fl_{ox}$* (645 nm) and neutral flavin radical (FlH·, 560 nm) can be observed. The latter species results from $1e^-$ transfer accompanying $^3Fl_{ox}$* quenching by Fl_{ox} followed by H^+ transfer to Fl^{-}· by trace H_2O in the solvent.[9] All of the compounds investigated here were found to quench $^3Fl_{ox}$* by $1e^-$ donation to produce FlH· ($[^3Fl_{ox}*]_{initial} = [FlH\cdot]_{produced}$) [$k_q$, $M^{-1}\,s^{-1}$: 2.1×10^9 (NtB), 6.7×10^7 (**1a**), 3.7×10^9 (**1b**), 8.3×10^9 (**1c**), 3.6×10^8 (Fl_{ox})[10]]. In all instances the disappearance of FlH· followed the second-order rate law. The flashing of solutions of **1a–c** and Fl_{ox} also yields a transient (520–650 nm with λ_{max} 630 nm) which decays slowly by second-order kinetics ($t_{1/2}$ = 17 ms with **1c** and 12.5 ms with **1b**). The laser flash photolysis experiments are in accord with the reaction sequence of eq 3. The initially formed ion-radical pair (**I**) is rapidly converted by a thermodynamically favored proton transfer into the metastable species II whose components undergo decarboxylation and radical coupling (eq 3b) and disproportionation (eq 3c). Species II is presumed to be the slowly

$$^3Fl_{ox}* + R\ddot{X}CH_2CO_2H \longrightarrow \underbrace{(Fl^{-}\cdot + R\overset{+\cdot}{X}CH_2CO_2H)}_{\mathbf{I}}$$

$$\longrightarrow \underbrace{(FlH\cdot + RXCH_2CO_2^{+\cdot})}_{\mathbf{II}} \quad (3a)$$

$$R\overset{+\cdot}{X}CH_2CO_2^- \xrightarrow{-CO_2} R\ddot{X}CH_2\cdot \xrightarrow{FlH\cdot} 4a\text{-}FlHCH_2XR \quad (3b)$$

$$2FlH\cdot \longrightarrow FlH_2 + Fl_{ox} \quad (3c)$$

decaying transient noted above, its decay being controlled by reactions 3b,c and its spectral properties being due to change-transfer interactions.

NtB very effectively prevents the formation of the 4a adducts **2** during PDC in the presence of flavin. For example, the initial rate of disappearance of Fl_{ox} in the presence of **1a** or **1b** is decreased by $>10^2$ when solutions of Fl_{ox} (7.5×10^{-5} M), **1a** or **1b** (0.005 M), and NtB (0.005 M) are irradiated at the Hg–Xe lamp with the 441-nm band pass. With **1c** the rate of disappearance of Fl_{ox} is decreased by >30-fold. When solutions of Fl_{ox} (5×10^{-5} M), **1a–c** (0.1–0.5 M), and NtB (0.01 M) are irradiated through the grid of the ESR cavity at 441 nm with the Hg–Xe lamp (~30-cm path length), a number of strong radical signals reach steady state within 10–15 min of the start of irradiation and remain at this concentration for at least 0.5 h. If illumination is discontinued, the signals decay very slowly. Table I provides a summary of the radicals which were observed in these experiments. The radical species were identified by comparison with authentic ones generated by literature procedures.[5a,11,12] The hydrogen hyperfine splitting constant, $a_H^{CH_2}$, for **6** is typical of those observed for similar radicals.[12] Nitroxides **5** and **6** could not be generated by irradiation of solutions of NtB and the respective acids at any wavelength between 300 and 700 nm if a triplet source (Fl_{ox}, benzophenone, etc.) was not present.

Irradiation (441 nm) of **1b** (0.002 M) and Fl_{ox} in O_2-free benzene-d_6 yields thioanisole as well as the expected 4a adduct. The thioanisole was identified by NMR and is present in 5–20% yield compared with 4a-$FlHCH_2SC_6H_5$. The presence of this material is best explained by a competition of radical pair collapse and H· transfer from FlH· to the thiophenoxymethyl radical[5] (eq 4). When acetonitrile solutions of benzophenone (0.1 M), **1b** (0.1 M), and NtB (0.05 M) were irradiated at 362 nm, it was possible to observe *tert*-butyl thiophenoxymethyl nitroxide as well as *tert*-butyl thiophenoxy nitroxide [$t\text{-BuN}(\dot{O})SC_6H_5$]. With white light (Hg–Xe lamp filtered to remove UV) these two radicals could no longer be observed even though significant amounts of **4** arose on photolysis of NtB. Apparently the thiyl radicals are not stable in the presence of significant quantities of **4**, or white light, or a combination of both. In fact, thiyl nitroxides are notoriously unstable.[6] Since the Fl_{ox} solutions fluoresce out to ~650 nm when irradiated at 441 nm, and **4** is present during these experiments, it is not surprising that the *tert*-butyl thiophenoxymethyl nitroxide ($t\text{-BuN}(\dot{O})CH_2SC_6H_5$) radical cannot be observed during the flavin-mediated photodecarboxylations.

$$FlH\cdot + C_6H_5SCH_2\cdot \longrightarrow Fl_{ox} + C_6H_5SCH_3 \quad (4)$$
$$\longrightarrow 4a\text{-}FlHCH_2SC_6H_5$$

Generation of the nitroxide **4** occurs by reaction of some trace impurity in the acetonitrile solvent with $^3Fl_{ox}$*. Thus, a very small amount of **4** is generated on irradiation of Fl_{ox} in the presence of NtB and the radical decays in a period of 4–5 min after illumination was initiated. The radical could not be regenerated upon reirradiation of the solution even though visible spectroscopy showed that the flavin and NtB were still present in essentially the same concentrations as before the experiment. Whatever the trace impurity in the acetonitrile may be, it is likely to provide FlH_2 on irradiation with Fl_{ox}. Thus, solutions of FlH_2 (5×10^{-5} M), Fl_{ox} (5×10^{-5} M), and NtB (0.01 M) give rise to a steady-state concentration of **4** when irradiated at 441 nm. Whatever the mechanism of the formation of **4**, its detection during the experiments involving the acids **1a–c** does indicate the presence of FlH_2 or FlH·. Thus, after completion of the formation of 4a adduct,[13] upon irradiation (441 nm) of Fl_{ox} (7.5×10^{-5} M) and any of the acids **1a–c** (0.005 M), admittance of O_2 (dark) regenerates immediately 4–6% original Fl_{ox}. The adducts **2** are stable to O_2 in the dark[1,2] and N-5 adducts are known to react rather slowly with O_2, giving rise to colored intermediate radical products.[2b,c] The presence of FlH_2 would readily explain the rapid reappearance of Fl_{ox} upon admission of O_2.[14] The formation of FlH· and FlH_2 is accounted for in the proposed mechanism of eq 3. Control experiments showed that no FlH_2 was formed when solutions of Fl_{ox} (7.5×10^{-4} M) in degassed acetonitrile were irradiated in the absence of acids **1a–c.** The presence of FlH_2 is inconsistent with the decarboxylation mechanism proposed by earlier workers (eq 2) but is expected from the radical mechanism of eq 3.

No ESR signals are observed and no Fl_{ox} is formed when the 4a adduct **2a**[15] (5×10^{-5} M) is irradiated at 441 nm in the presence of **1a** (0.50 M) and NtB (0.01 M). In addition solutions of Fl_{ox} (3.7×10^{-5} M) and **2a** (3.7×10^{-5} M) are stable in the presence of NtB (0.025 M) when irradiated at 441 nm. Irradiation at 441 nm of solutions containing Fl_{ox} (5×10^{-5}

M), NtB (0.01 M), and the nonreactive carboxylic acids[1] acetic acid (0.1 M), adipic acid (3.2×10^{-2} M), and benzoic acid (0.1 M) gave results identical with those of the blank experiment (Fl_{ox} and NtB).

The origins of the nitroxides **5** and **6** are most easily explained on the basis of trapping by NtB of the intermediate substituted methyl radical formed by decarboxylation of an initially formed substrate cation radical (eq 3). It might be argued that decrease in the rate of disappearance of Fl_{ox} caused by the presence of NtB is simply due to the competition between NtB and **1a–c** for $^3Fl_{ox}$* and that the formation of spin-trapped radicals results from only a minor component of the reaction. From the second-order quenching constants (k_q) given above it may be concluded that this could only be a partial factor with **1a** and would be inconsistent for **1b** and **1c**.

We believe that the results of these experiments provide firm evidence for the radical nature of the flavin-mediated photodecarboxylation reactions. The contention of Hemmerich and his associates[1,2] that these reactions proceed via a nucleophilic addition to $^3Fl_{ox}$* (eq 2) appears to be wrong as is their suggestion[1,2,16] that these reactions provide support for a mechanism of flavoenzyme-catalyzed dehydrogenation involving nucleophilic addition of carbanion to the ground-state flavin. These results also seriously call into question the suggested intermediacy[1,2] of covalent adducts in the PDC of α-amino and α-hydroxy acids since the observed products can easily be accounted for through radical mechanisms.[17] This study represents the second successful application of radical-trapping techniques in the study of the mechanisms of reactions of flavin model systems.[18] We believe that further applications of these methods in this field will prove quite fruitful.

Acknowledgment. This work was supported by grants from the National Institutes of Health and M.N. gratefully acknowledges support as an NIH Fellow.

References and Notes

(1) W. R. Knappe and P. Hemmerich, *Justus Liebigs Ann. Chem.*, 2037 (1976).
(2) (a) W. Haas and P. Hemmerich, *Z. Naturforsch., B*, **27**, 1035 (1972); (b) P. Hemmerich, V. Massey and G. Weber, *Nature (London)*, **213**, 728 (1967); (c) W. H. Walker and P. Hemmerich, *Eur. J. Biochem.*, **13**, 248 (1970).
(3) P. Byrom and J. H. Turnbull, *Photochem. Photobiol.*, **6**, 125 (1967); G. R. Penzer and G. K. Radda, *Biochem. J.*, **109**, 259 (1968).
(4) D. Clerin and T. C. Bruice, *J. Am. Chem. Soc.*, **96**, 5571 (1974).
(5) (a) P. R. Bowers, K. A. McLauchlan, and R. C. Sealy, *J. Chem. Soc., Perkin Trans. 2*, 915 (1976); (b) M. Weinstein, K. A. Muszkat, and J. Dobkin, *J. Chem. Soc., Chem. Commun.*, 68 (1975); (c) D. R. G. Brimage, R. S. Davidson, and P. R. Steiner, *J. Chem. Soc., Perkin Trans. 1*, 526 (1973); (d) R. S. Davidson and P. R. Steiner, *J. Chem. Soc., Perkin Trans. 2*, 1357 (1972).
(6) E. G. Janzen, *Acc. Chem. Res.*, **4**, 31 (1971).
(7) **1a–c** were recrystallized to constant melting points. Acetonitrile (stored under N_2) was purified by stirring with CaH_2 (24 h), distillation from P_2O_5 under N_2 followed by five freeze–evacuate–thaw cycles. All solutions were prepared under anaerobic conditions. The 3-methyllumiflavin was available from a previous study and was recrystallized from $CHCl_3$–acetone prior to use. The 2-methyl-2-nitrosopropane was obtained commercially and was used without further purification. The light sources used were either a 300-W tungsten lamp or a 75-W Hg–Xe lamp in an Oriel 6137 lamp housing equipped with a spherical collimating mirror, focusing lens, and IR filter. Interference filters were as follows: Oriel G-572-4416 interference filter, 441-nm band-pass maximum (55% T) with a band width of 22 nm at 1% of maximum transmittance; Balzers B-40 interference filter, 651-nm band-pass maximum (47.5% T) with a band width of 60 nm at 1% of maximum transmittance. A band pass at ~360 nm was constructed from an Oriel G-774-3550 colored glass filter, a 2-mm Pyrex glass filter, and 1 cm of a 0.3 M solution of $CuSO_4$ in a circular UV cell. This gave a band pass with a maximum transmittance of 45% at 362 nm with a band width of 80 nm at 1% of the maximum transmittance. A Varian E4-ESR spectrometer was used to record the spectra of the observed radicals. Laser flash photolysis was performed as previously described.[8]
(8) G. Tollin, R. Chan, T. R. Malefyt, and T. C. Bruice, *Photochem. Photobiol.*, **29**, 233 (1979).
(9) S. G. Ballard, D. C. Mauzerall, and G. Tollin, *J. Phys. Chem.*, **80**, 341 (1976).
(10) The value of 3.6×10^8 M^{-1} s^{-1} obtained for $^3Fl_{ox}$* quenching by Fl_{ox} in acetonitrile is in excellent agreement with the value of 3.7×10^8 M^{-1} s^{-1} previously determined in water: S. P. Vaish and G. Tollin, *J. Bioenerg.*, **1**, 181 (1970).
(11) H. Lemaire, A. Rassat, P. Serrox-Gavin, and G. Barthier, *J. Chim. Phys.*, **59**, 1247 (1962).
(12) J. C. Baird and J. R. Thomas, *J. Chem. Phys.*, **35**, 1507 (1961); G. Chapelet-Letourneux, H. Lemaire, and A. Rassat, *Bull. Soc. Chem. Fr.*, 3283 (1965); I. H. Leaver and G. C. Ramsay, *Tetrahedron*, **25**, 5669 (1969).
(13) The λ_{max} and apparent extinction coefficients of the final product spectra are as follows: for **1a**, 354 nm (ϵ 6300 M^{-1} cm^{-1}); for **1b**, 356 nm (ϵ 5800 M^{-1} cm^{-1}); for **1c**, 360 nm (ϵ 6100 M^{-1} cm^{-1}).
(14) C. Kemal, T. W. Chan, and T. C. Bruice, *J. Am. Chem. Soc.*, **99**, 7272 (1977), and references therein.
(15) This 4a adduct was prepared by the method of Knappe and Hemmerich.[1] The final purification was brought about by recrystallization from diethyl ether–hexane. Spectral data: NMR (100 MHz) ($CDCl_3$) δ 7.3–6.5 (m, 7 H), 4.77 (s, 1 H), 4.22 (d, J = 8.9 Hz, 1 H), 4.03 (d, J = 8.9 Hz, 1 H), 3.61 (s, 3 H), 3.29 (s, 3 H), 2.21 (s, 6 H); UV–vis (in acetonitrile) 354 nm (ϵ 6400 M^{-1} cm^{-1}).
(16) P. Hemmerich, *Prog. Chem. Org. Nat. Prod.*, **33**, 451 (1976).
(17) T. W. Chan and T. C. Bruice, *Biochemistry*, **17**, 4284 (1978).
(18) M. Novak and T. C. Bruice, *J. Am. Chem. Soc.*, **99**, 8079 (1977).

Michael Novak, Audrey Miller, Thomas C. Bruice*
Department of Chemistry, University of California
Santa Barbara, California 93106

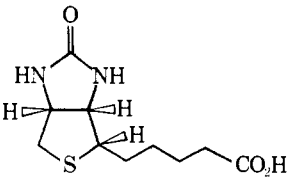
Gordon Tollin
Department of Biochemistry, University of Arizona
Tucson, Arizona 87521

Received April 25, 1979

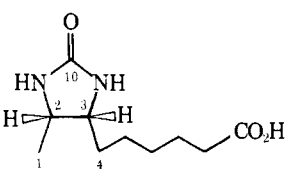
Biotin Biosynthesis. 2. Stereochemistry of Sulfur Introduction at C-4 of Dethiobiotin

Sir:

The vitamin (+)-biotin (**1**) is widely distributed in plant and animal tissues where it functions as the cofactor for a variety of enzymatic carboxylation reactions.[1] A number of fungi and bacteria synthesize biotin from pimelic acid via a metabolic pathway whose last step is the conversion of (+)-dethiobiotin (**2**) into (+)-biotin.[2] We recently reported experiments which



1



2

establish that the biosynthesis of biotin in *Aspergillus niger* proceeds via the introduction of sulfur at C-1 and C-4 of dethiobiotin without apparent involvement of C-2 or C-3.[3] A similar situation has since been shown to obtain in *Escherichia coli*.[4] Since the nature of the reactions involved in the introduction of sulfur at saturated carbon atoms is presently unknown, we decided to investigate the stereochemistry of the sulfur introduction process in *A. niger*. We now report the results of experiments that elucidate the stereochemistry of the introduction of sulfur at C-4 of dethiobiotin.

The elucidation of the stereochemistry of sulfur introduction was accomplished by means of precursor incorporation experiments with [4(R)-^{3}H]dethiobiotin (**3**) and [4(S)-^{3}H]-dethiobiotin (**4**). These chirally labeled forms of dethiobiotin were synthesized from the [(1S)-^{3}H]- and [(1R)-^{3}H]tosylates **5** and **6**, which had been previously prepared in our laboratories (Scheme I).[5] The tosylates **5** and **6** were treated with the lithio derivative of the THP ether of propargyl alcohol according to the method of Corey et al.[6] On the basis of the assumption that this reaction proceeds with inversion of configuration, the products of the alkylation are the [(1R)-^{3}H] and [(1S)-^{3}H] acetylenic acetals **7** and **8**, respectively. These chirally tritiated acetylenes were transformed into [(4R)-^{3}H]- and [(4S)-

0002-7863/80/1502-1467$01.00/0