

(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)<sup>13</sup> 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  $\gamma$ -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.<sup>14,15</sup>

## References and Notes

- (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 review of these observations.
- See ref 1a above and S. Meyerson and J. D. McCollum *Adv. Anal. Chem. Instrum.*, **2**, 184 (1963), and ref 1c above.
- 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).
- See ref 1c above and J. K. MacLeod and C. Djerassi, *J. Am. Chem. Soc.*, **89**, 5182 (1967).
- 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).
- H. M. Rosenstock, M. B. Wallenstein, A. L. Wahrhaftig, and H. Eyring, *Proc. Natl. Acad. Sci. U.S.A.*, **38**, 667 (1952).
- 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.
- See K. B. Wiberg, in ref 5a herein.
- 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.
- The NMR and mass spectra (MS) of **1** matched those of earlier report.<sup>1b</sup> **2** was isolated by extraction and GC collection on 20% SE-30 on Chrom W, flow 10 cm<sup>3</sup>/15 s, column temperature 175 °C, retention time 8 min (colorless liquid). Yield was ~3:1 **1:2** under typical conditions.<sup>1b</sup> By high resolution the MS molecular weight of **2** corresponds to C<sub>8</sub>H<sub>13</sub>NO with major fragments at *m/e* 97, 96, 82 (base peak). <sup>1</sup>H NMR on an FX-60Q spectrometer at 59.75 Hz:  $\delta$  1.22 (d, 3 H, *J* = 7 Hz, C<sub>4</sub> CH<sub>3</sub>, irradiation of C-4 H at 4.35 collapses 1.22 to a singlet), 2.17 (s, 3 H, C<sub>2</sub> CH<sub>3</sub>), 2.2 (br, overlapping 2.17, 3 H, C<sub>7</sub> CH<sub>3</sub>), 2.6 (v br, 2 H, CH<sub>2</sub>), 4.35 (br, 1 H, C<sub>4</sub> H), 4.9 (br, 1 H, C<sub>6</sub> H). <sup>13</sup>C NMR on a JEOL FX-60Q at 15.0 Hz including multiplicity from off-resonance data:  $\delta$  17.6 (q, 4-CH<sub>3</sub>), 22.1 (q, 2-CH<sub>3</sub>), 24.2 (q, 7-CH<sub>3</sub>), 36.2 (t, C<sub>5</sub>), 56.9 (d, C<sub>4</sub>), 108.4 (d, C<sub>6</sub>), 141 (v w, C<sub>7</sub>), 167.4 (s, C<sub>2</sub>).
- 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. 1, Part II A, Wiley-Interscience, New York, 1971, Chapter IX.
- The *k<sub>H</sub>*/*k<sub>D</sub>* 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.
- We cannot account for the higher *k<sub>H</sub>*/*k<sub>D</sub>* 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.<sup>4</sup> The invariance here within experimental error, of the results at 70 and 10 eV, argue against the scrambling mechanism which affected the earlier finding.<sup>4</sup> Moreover such scrambling processes<sup>4</sup> caused deviations in the opposite direction (*k<sub>H</sub>*/*k<sub>D</sub>* too low) from the results herein.
- The independence of temperature for *k<sub>H</sub>*/*k<sub>D</sub>* 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<sub>H</sub>*/*k<sub>D</sub>* 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.
- 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.

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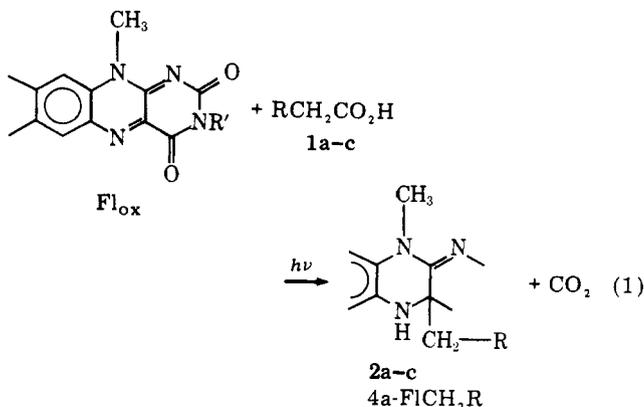
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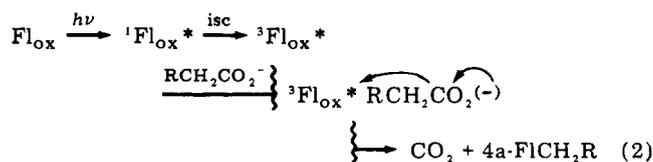
## The Mechanism of Flavin 4a Substitution Which Accompanies Photolytic Decarboxylation of $\alpha$ -Substituted Acetic Acids. Carbanion vs. Radical Intermediates

Sir:

Flavin-mediated photodecarboxylation (PDC) of certain  $\alpha$ -substituted acetic acids (eq 1) has been claimed to serve as a model for flavoenzyme-catalyzed dehydrogenations.<sup>1,2</sup> 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<sub>6</sub>H<sub>5</sub>O- for **1a**, C<sub>6</sub>H<sub>5</sub>S- for **1b**, and 3-indolyl for **1c**). PDC of  $\alpha$ -hydroxy<sup>2a</sup> or  $\alpha$ -amino acids<sup>3</sup> in the presence of Fl<sub>ox</sub> yields  $\alpha$ -keto or  $\alpha$ -imino acids presumably by elimination from the 4a adduct.<sup>4</sup> It has been suggested that the mechanism of eq 1 involves



nucleophilic attack of a carbanion intermediate upon the 4a position of Fl<sub>ox</sub> (eq 2).<sup>1,2</sup> However, PDC of the  $\alpha$ -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)<sup>5</sup> to be radical in nature. We are now able to show that a radical mechanism is involved in the <sup>3</sup>Fl<sub>ox</sub>\*-mediated PDC reactions. This communication deals with the results of laser flash photolysis and



M), NtB (0.01 M), and the nonreactive carboxylic acids<sup>1</sup> acetic acid (0.1 M), adipic acid ( $3.2 \times 10^{-2}$  M), and benzoic acid (0.1 M) gave results identical with those of the blank experiment ( $\text{Fl}_{\text{ox}}$  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  $\text{Fl}_{\text{ox}}$  caused by the presence of NtB is simply due to the competition between NtB and **1a-c** for  $^3\text{Fl}_{\text{ox}}^*$  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<sup>1,2</sup> that these reactions proceed via a nucleophilic addition to  $^3\text{Fl}_{\text{ox}}^*$  (eq 2) appears to be wrong as is their suggestion<sup>1,2,16</sup> 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<sup>1,2</sup> of covalent adducts in the PDC of  $\alpha$ -amino and  $\alpha$ -hydroxy acids since the observed products can easily be accounted for through radical mechanisms.<sup>17</sup> This study represents the second successful application of radical-trapping techniques in the study of the mechanisms of reactions of flavin model systems.<sup>18</sup> We believe that further applications of these methods in this field will prove quite fruitful.

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## References and Notes

- W. R. Knappe and P. Hemmerich, *Justus Liebigs Ann. Chem.*, 2037 (1976).
- (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).
- P. Byrom and J. H. Turnbull, *Photochem. Photobiol.*, **6**, 125 (1967); G. R. Penzer and G. K. Radda, *Biochem. J.*, **109**, 259 (1968).
- D. Clerin and T. C. Bruice, *J. Am. Chem. Soc.*, **96**, 5571 (1974).
- (a) P. R. Bowers, K. A. McLaughlan, 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).
- E. G. Janzen, *Acc. Chem. Res.*, **4**, 31 (1971).
- 1a-c** were recrystallized to constant melting points. Acetonitrile (stored under  $\text{N}_2$ ) was purified by stirring with  $\text{CaH}_2$  (24 h), distillation from  $\text{P}_2\text{O}_5$  under  $\text{N}_2$  followed by five freeze-evacuate-thaw cycles. All solutions were prepared under anaerobic conditions. The 3-methylumiflavin was available from a previous study and was recrystallized from  $\text{CHCl}_3$ -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  $\sim 360$  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  $\text{CuSO}_4$  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.<sup>8</sup>
- G. Tollin, R. Chan, T. R. Malefyt, and T. C. Bruice, *Photochem. Photobiol.*, **29**, 233 (1979).
- S. G. Ballard, D. C. Mauzerall, and G. Tollin, *J. Phys. Chem.*, **80**, 341 (1976).
- The value of  $3.6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  obtained for  $^3\text{Fl}_{\text{ox}}^*$  quenching by  $\text{Fl}_{\text{ox}}$  in acetonitrile is in excellent agreement with the value of  $3.7 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  previously determined in water: S. P. Vaish and G. Tollin, *J. Bioenerg.*, **1**, 181 (1970).

- H. Lemaire, A. Rassat, P. Serroix-Gavin, and G. Barthier, *J. Chim. Phys.*, **59**, 1247 (1962).
- 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).
- The  $\lambda_{\text{max}}$  and apparent extinction coefficients of the final product spectra are as follows: for **1a**, 354 nm ( $\epsilon$  6300  $\text{M}^{-1} \text{ cm}^{-1}$ ); for **1b**, 356 nm ( $\epsilon$  5800  $\text{M}^{-1} \text{ cm}^{-1}$ ); for **1c**, 360 nm ( $\epsilon$  6100  $\text{M}^{-1} \text{ cm}^{-1}$ ).
- C. Kemal, T. W. Chan, and T. C. Bruice, *J. Am. Chem. Soc.*, **99**, 7272 (1977), and references therein.
- This **4a** adduct was prepared by the method of Knappe and Hemmerich.<sup>1</sup> The final purification was brought about by recrystallization from diethyl ether-hexane. Spectral data: NMR (100 MHz) ( $\text{CDCl}_3$ )  $\delta$  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 ( $\epsilon$  6400  $\text{M}^{-1} \text{ cm}^{-1}$ ).
- P. Hemmerich, *Prog. Chem. Org. Nat. Prod.*, **33**, 451 (1976).
- T. W. Chan and T. C. Bruice, *Biochemistry*, **17**, 4284 (1978).
- M. Novak and T. C. Bruice, *J. Am. Chem. Soc.*, **99**, 8079 (1977).

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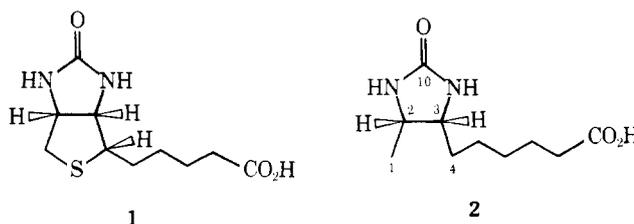
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## Biotin Biosynthesis. 2. Stereochemistry of Sulfur Introduction at C-4 of Dethiobiotin

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.<sup>1</sup> 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.<sup>2</sup> We recently reported experiments which



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.<sup>3</sup> A similar situation has since been shown to obtain in *Escherichia coli*.<sup>4</sup> 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*)-<sup>3</sup>H]dethiobiotin (**3**) and [4(*S*)-<sup>3</sup>H]-dethiobiotin (**4**). These chirally labeled forms of dethiobiotin were synthesized from the [(1*S*)-<sup>3</sup>H]- and [(1*R*)-<sup>3</sup>H]tosylates **5** and **6**, which had been previously prepared in our laboratories (Scheme 1).<sup>5</sup> 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.<sup>6</sup> On the basis of the assumption that this reaction proceeds with inversion of configuration, the products of the alkylation are the [(1*R*)-<sup>3</sup>H] and [(1*S*)-<sup>3</sup>H] acetylenic acetals **7** and **8**, respectively. These chirally tritiated acetylenes were transformed into [(4*R*)-<sup>3</sup>H]- and [(4*S*)-