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.

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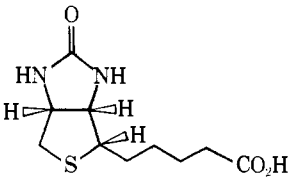
Received April 25, 1979

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



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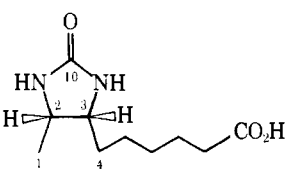

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establish that the biosynthesis of biotin in *Aspergillus niger* proceeds via the introduction of sulfur at C-1 and C-4 of dethiobiotin without apparent involvement of C-2 or C-3.[3] A similar situation has since been shown to obtain in *Escherichia 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)-

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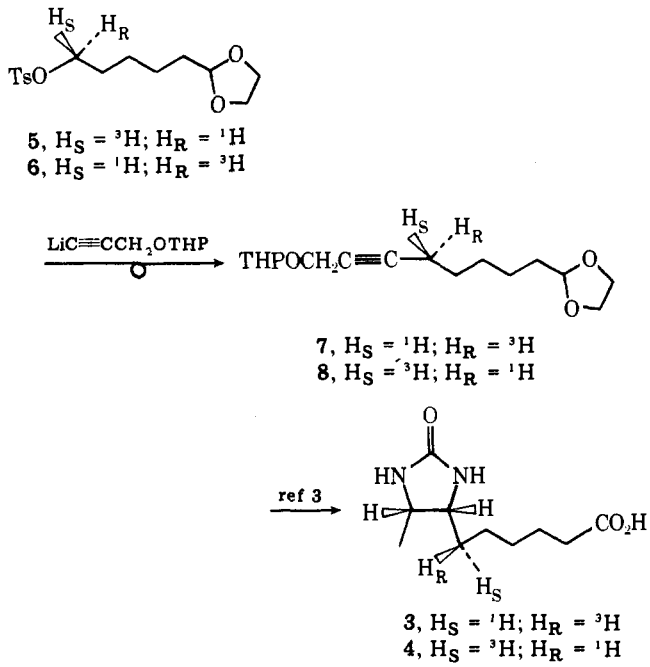
Scheme I



^3H]-(±)-dethiobiotin (**3** and **4**) using methods previously developed in our laboratories.[3]

The samples of chirally tritiated (±)-dethiobiotin[7] were each mixed with [10-^{14}C]-(±)-**2** and the doubly labeled dethiobiotin recrystallized to constant specific activity and constant tritium to carbon-14 ratio. The double labeled precursors were administered to cultures of *A. niger* (ATCC 1004).[8] The precursors were not resolved since only (+)-dethiobiotin appears to serve as a biotin precursor.[9] After an incubation period of 5 days, the biotin produced from each doubly labeled precursor was isolated[8] as (+)-biotin sulfone and converted into biotin sulfone methyl ester. The samples of methyl ester were recrystallized to constant specific activity and constant tritium to carbon-14 ratio. The results of the experiments are summarized in Table I.

The tritium to carbon-14 ratios of the biotin sulfone methyl ester isolated in these experiments clearly demonstrate that sulfur is introduced at C-4 of dethiobiotin with loss of the 4-*pro S* hydrogen atom. Since the absolute configuration of (+)-biotin at C-4 is known[10] to be *S*, it follows that sulfur is introduced at C-4 of dethiobiotin with *retention* of configuration at that prochiral center. This result can be compared with that of the two other examples of sulfur introduction whose stereochemistry has been elucidated. It has been established that sulfur is introduced at C-6 of octanoic acid during lipoic acid biosynthesis with *inversion* of configuration,[5] while the introduction of sulfur at C-3 of valine during penicillin biosynthesis has been found to occur with *retention* of configuration.[11] At the present time, it is not clear whether the biotin and penicillin cases proceed by a mechanism that is fundamentally different from that involved in lipoate biosynthesis, or whether the mechanism is the same in all three cases with the stereochemical outcome being dictated by other factors.[12]

Table I. Incorporation of Chirally Tritiated Dethiobiotin into Biotin

expt	precursor	$^3H/^{14}C$ for precursor	$^3H/^{14}C$ for biotin sulfone methyl ester	% ^3H retention
1	[4(*R*)-4-^{3}H-10-^{14}C]-**2**	3.37	3.07	91
2	[4(*S*)-4-^{3}H-10-^{14}C]-**2**	6.00	0.42	7

Acknowledgment. We thank the National Science Foundation (Grant CHE78-21738) for partial support of this research.

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An Explanation of Unusual Properties of Spin-Crossover Ferric Complexes

Sir:

The biochemical importance of spin-state equilibria (both $S = 5/2 \rightleftharpoons S = 1/2$ and $S = 5/2 \rightleftharpoons S = 3/2$) and the $S = 3/2$ intermediate-spin state for Fe(III)-containing proteins has been noted.[1,2] A number of model ferric complexes have been reported[3,4] which exhibit the $S = 5/2$ to $S = 1/2$ spin crossover. *Solid* samples of several of these compounds exhibit unusual magnetic susceptibility characteristics. In particular, the decrease in μ_{eff}/Fe with decreasing temperature does not reflect a simple Boltzmann distribution over electronic states[4] and in some cases μ_{eff}/Fe does not decrease to a value appropriate for a low-spin ($S = 1/2$) state at low temperature.[3,5] In these latter complexes, the spin-crossover phase transition appears to be incomplete and below a certain temperature μ_{eff}/Fe remains relatively constant at a value intermediate between high spin and low spin. A "plateau" in the μ_{eff}/Fe vs. temperature curve is seen. In the case of the dichloromethane solvate of tris(4-morpholinecarbodithioato-*S*,*S*')iron(III), for example, the μ_{eff}/Fe vs. *T* curve plateaus at a value of 3.7–3.9 μ_B below ~60 K. An intermediate-spin ($S = 3/2$) $^4T_1$ ground state was proposed[8] for this FeS_6 complex.

Interesting observations have been made in our laboratories recently that shed light on the nature of the incomplete (i.e., plateau in μ_{eff}/Fe) spin-crossover phase transitions observed for solid samples of ferric complexes. A series of complexes with the composition $[Fe^{III}(X\text{-SalEen})_2]Y$, where X-SalEen is the monoanion of the condensation product of salicylaldehyde (or a methoxy-substituted salicylaldehyde) and *N*-ethylethylenediamine, and Y is variously NO_3^-, PF_6^-, or BPh_4^-, was prepared. These compounds were precipitated as microcrystalline solids from methanol. No evidence of solvation

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