On the other hand, consideration of the nature of the reductant, namely, that catechol is a weak acid, suggests an alternate formulation of the rate expression. If the actual reductant is catechol anion, HC^-, the pH dependence can be explained on the basis of increasing $[HC^-]$ with decreasing acidity. That is, if rate = k[cyt c(III)]^2[HC^-], incorporation of the equilibrium constant for catechol dissociation ($pK_a = 9.15$)[9] and mass balance in [catechol], $[HC^-] = [\text{catechol}]_{total} - [H_2C]$, gives

$$\text{rate} = k[\text{cyt } c(\text{III})]^2[\text{catechol}](1 + [H^+]/K_a)^{-1}$$

for which the pseudo-second-order rate constant is

$$k_{obsd} = k[\text{catechol}](1 + [H^+]/K_a)^{-1}$$

A plot of $k_{obsd}/[\text{catechol}]$ vs. $(1 + [H^+]/K_a)^{-1}$ should be linear with zero intercept (in the absence of contributions from catechol species other than HC^-) and slope = k. Such a plot is indeed linear, least-squares analysis giving slope = $k = (4.51 \pm 0.12) \times 10^7\ M^{-2}\ s^{-1}$ and intercept[10] = $(1.5 \pm 1.3) \times 10^4\ M^{-2}\ s^{-1}$ (errors are 1σ).

At least two mechanisms are consistent with the observed rate expression. The first involves a rapid equilibrium association of two cyt c(III) species, followed by two electron reduction of the associate by HC^-,

$$\text{cyt } c(\text{III}) + HC^- \rightleftharpoons \text{complex (rapid)}$$

$$\text{complex} + \text{cyt } c(\text{III}) \rightarrow 2\text{cyt } c(\text{II}) + P \text{ (rate determining)}$$

where P represents oxidized catechol (presumably *O*-diquinone).[11]

The second mechanism involves initial complexation of cyt c(III) by catechol anion, followed by attack of a second cyt c(III) species.

$$\text{cyt } c(\text{III}) + HC^- \rightleftharpoons \text{complex (rapid)}$$

$$\text{complex} + \text{cyt } c(\text{III}) \rightarrow 2\text{cyt } c(\text{II}) + P \text{ (rate determining)}$$

In either case, (1) the precursor equilibrium constants must be very small or (2) association or complexation must have no effect on the optical density of cyt c(III) to be consistent with the observed isosbestic behavior. Furthermore, the precursor equilibrium must be obtained fairly rapidly compared to the rate determining step else saturation of the pseudo-second-order rate constant would have been observed at high [catechol]. The present study does not provide sufficient information to discriminate between these possible mechanisms. The demonstrated ability of such ligands as azide, imidazole, and pyridine to bind cyt c(III) and the fairly rapid electron exchange rate in the cyt c(II)–cyt c(III) system[3] enhance the plausibility of the second mechanism.

Consideration of the nature of the reactants suggests that the kinetic behavior observed is not needlessly complex. Catechol is a two-electron reductant, whereas cyt c(III) is a one-electron oxidant. If cyt c(III) cannot oxidize catechol in a one-electron process (presumably to a semiquinone) then participation of an additional cyt c(III) as second electron acceptor site becomes necessary. The reduction of V(V), a one-electron oxidant, by the two-electron reductants ascorbic acid and catechol, appears to occur via analogous mechanisms. Thus the rate law for V(V) oxidation of ascorbic acid exhibits a first-order dependence on V(V),[12] and a radical intermediate is produced, whereas the rate expression for V(V) oxidation of catechol (a weaker reductant by ca. 0.3 V) exhibits a second-order dependence on V(V).[11]

The most striking feature of the present study is the evidence for a pair of cyt c(III) species in the encounter complex leading to products. Assuming first that catechol binds an iron center prior to cyt c(III) reduction, *direct* transfer of the second electron to the second iron center must be sterically impossible. On the other hand, catechol may transfer electrons to the metalloprotein via the exposed porphyrin edge. A pair of cationic cytochrome molecules can approach closely enough in solution for fairly rapid electron transfer.[3] An anionic HC^- species would be expected to stabilize such an interaction through formation of a 2:1 complex leading to subsequent transfer of one catechol electron to each cytochrome. In either case both electrons cannot be transferred to iron via direct transfer to the respective metal center. Further research in this area is required for the development of a clear and consistent picture of the mechanism of electron transfer to cytochrome c(III).

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David L. Toppen
Department of Chemistry
California State University, Northridge
Northridge, California 91324

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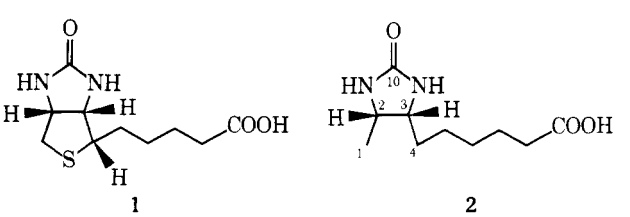
Biotin Biosynthesis. 1. The Incorporation of Specifically Tritiated Dethiobiotin into Biotin

Sir:

The vitamin (+)-biotin (**1**) is widely distributed in plant and animal tissues where it functions as the cofactor for a variety of enzymic carboxylation reactions.[1] A number of fungi and bacteria synthesize biotin from pimelic acid via a metabolic



1 2

Table I. Incorporation of Specifically Tritiated Dethiobiotin into Biotin

Expt no.	Precursor	$^3H/^{14}C$ for precursor	$^3H/^{14}C$ for biotin sulfone methyl ester	% ^3H retention
1	[2,3-^{3}H;10-^{14}C]-(±)-**2**[a]	6.05	5.74	95
2	[3-^{3}H;10-^{14}C]-(±)-**2**[b]	2.89	3.04	105
3	[1-^{3}H;10-^{14}C]-(±)-**2**	6.88	4.81	70
4	[4(*RS*)-^{3}H;10-^{14}C]-(±)-**2**	5.88	3.10	53

[a] Precursor had 58% ^3H at C-2, 42% at C-3. [b] Precursor had 17% ^3H at C-2, 83% at C-3.

pathway whose last step is the conversion of (+)-dethiobiotin (**2**) into (+)-biotin.[2] This final stage in the biosynthesis of **1** is of great interest because of the unusual nature of the transformation: the formation of the thiophane ring system is without any close biochemical parallel.

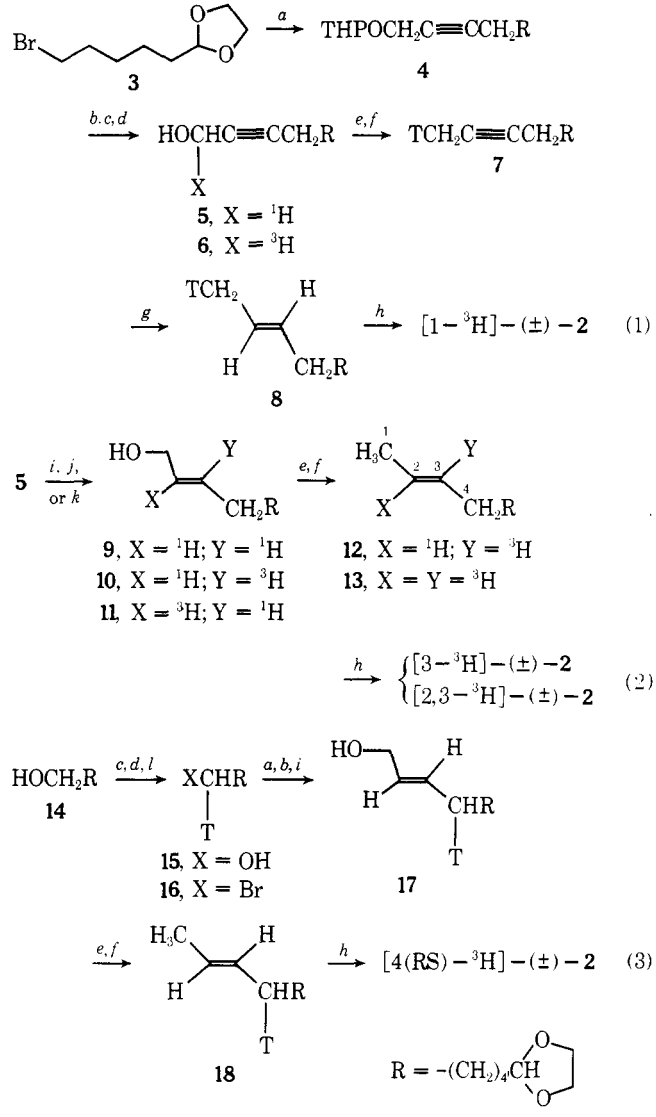
The only evidence bearing directly on the mechanism of the conversion of **2** into **1** comes from work of Li, McCormick, and Wright.[3] These authors administered a mixture of carbon-14 labeled dethiobiotin and "randomly" tritiated dethiobiotin to *Aspergillus niger*. The tritium to carbon-14 ratio in the biotin formed from the doubly labeled dethiobiotin led the authors to suggest that approximately four hydrogen atoms are removed from carbon atoms 1–4 of dethiobiotin during its conversion to biotin; it was also suggested that the reaction may proceed with the introduction of unsaturation at C-2 and/or C-3 of **2**. Unfortunately, these experimental results are attended by considerable uncertainty due to the use of "randomly" tritiated dethiobiotin. We have chosen to reexamine the mechanism of the conversion of dethiobiotin into biotin by using specifically labeled forms of **2**.

Published syntheses of (±)-dethiobiotin[4] proved unsatisfactory when applied to the problem of synthesizing **2** labeled specifically with tritium at C-1, -2, -3, or -4. Consequently, a new, stereospecific synthesis of (±)-dethiobiotin that is useful for labeling purposes was developed.[5] We describe here the modifications of this synthesis which allow the preparation of four specifically tritiated forms of dethiobiotin and the results which are obtained when these labeled forms of **2** are converted into (+)-biotin by *A. niger*.

Alkylation of the tetrahydropyranyl ether of propargyl alcohol[6] with the previously prepared[5] bromoacetal **3** yielded the acetylenic acetal **4** (62%) (Scheme I).[7] Removal of the tetrahydropyranyl group from **4** by room temperature treatment with a 3:1:1 mixture of acetic acid, water, and ethylene glycol[8] afforded the propargylic alcohol **5** (80%): δ 2.55 (1 H, s, exchanges with D_2O, *HO*–), 3.87 (4 H, m, –OC*H*$_2$C*H*$_2$O–), 4.20 (2 H, t, J = 2 Hz, HOC*H*$_2$C≡), 4.81 (1 H, t, J = 4 Hz, acetal H). Collins oxidation[9] of the acetal **5** followed by reduction of the resulting propargyl aldehyde with potassium [^{3}H]borohydride gave the [1-^{3}H]propargylic alcohol **6** (53%). Treatment of **6** with methanesulfonyl chloride and triethylamine in THF generated the primary mesylate which was not isolated but immediately reduced at −78° with lithium aluminum hydride to the [1-^{3}H]acetylenic acetal **7** (76%). Compound **7** had been previously synthesized in unlabeled form.[5] Reduction of **7** with sodium in liquid ammonia proceeded without loss of label to yield trans acetal olefin[5] bearing a tritium label at C-1 (**8**) (84%). The 1-^{3}H trans acetal olefin (**8**) was converted into [1-^{3}H]-(±)-dethiobiotin using methods previously described.[5]

Reduction of the propargyl alcohol **5** with lithium aluminum hydride in THF (Scheme I) gave the anticipated[10] trans allylic alcohol **9** (86%): δ 3.22 (1 H, s, *HO*–), 3.83 (4 H, m, –OC*H*$_2$C*H*$_2$O–), 3.98 (2 H, m, HOC*H*$_2$CH=), 4.80 (1 H, t, J = 4 Hz, acetal H), 5.66 (2 H, m, –C*H*=C*H*–); ν_{max} 970 cm^{-1} (trans C=C). When the reduction of **5** was carried out with unlabeled lithium aluminum hydride and the reaction quenched with tritiated water, the 3-^{3}H allylic alcohol **10** was obtained, as expected.[11] It was also expected that reduction of **5** with lithium aluminum [^{3}H]hydride followed by workup with unlabeled water would give the 2-^{3}H allylic alcohol **11**, but the labeling pattern in **11** proved to be much less specific than in alcohol **10** (vide infra). The alcohols **10** and **11** were converted to the corresponding labeled acetal olefins **12** and **13** by generation of the mesylates and reduction at low temperature with lithium aluminum hydride. Preliminary experiments with the unlabeled alcohol **9** demonstrated that no isomerization of the trans double bond occurred during the removal of the alcohol function in this fashion. The precise

Scheme I



[a] THPOCH$_2$C≡CH, NaNH$_2$. [b] HOAc, H$_2$O, HOCH$_2$CH$_2$OH. [c] CrO$_3$, C$_5$H$_5$N. [d] [^{3}H]-KBH$_4$. [e] CH$_3$SO$_2$Cl, Et$_3$N. [f] LiAlH$_4$. [g] Na, NH$_3$. [h] Reactions in ref 5. [i] LiAlH$_4$, H$_2$O. [j] LiAlH$_4$, [^{3}H]-H$_2$O. [k] [^{3}H]-LiAlH$_4$, H$_2$O. [l] Ph$_3$P, CBr$_4$.

distribution of the tritium label between C-2 and C-3 in **12** and **13** was determined by ozonolysis of the labeled olefins to give acetaldehyde and the ethylene glycol ester[12] of 6-formylhexanoic acid. The two labeled aldehydes obtained from each olefin were derivatized as thiosemicarbazones and recrystallized to constant specific activity. In this way, it was established that the labeled olefin **12** carried 17% of the tritium label at C-2 and 83% at C-3 while the labeled olefin **13** carried 58% of its tritium at C-2 and 42% at C-3. The labeling pattern in **12** and **13** having been defined, each of the labeled olefins was converted into the corresponding labeled form of (±)-dethiobiotin by established methods.[5]

The synthesis of [4(*RS*)-^{3}H]-(±)-dethiobiotin was accomplished as outlined in Scheme I. The known[5] acetal alcohol **14** was oxidized to the corresponding aldehyde using Collins reagent, and the aldehyde was then reduced with tritiated potassium borohydride to the 1-^{3}H acetal alcohol **15.** The labeled bromide **16** obtained from the labeled alcohol **15** by treatment with triphenylphosphine and carbon tetrabromide[5] was converted into [4(*RS*)-^{3}H]-(±)-dethiobiotin via the trans-allylic alcohol **17** and the trans-acetal olefin **18.**

The samples of tritiated (±)-dethiobiotin prepared as described were each mixed with [10-^{14}C]-(±)-**2** and the doubly labeled precursors were then administered to cultures of *A. niger* (ATCC 1004) using previously reported methods.[3] The precursors were not resolved since only (+)-dethiobiotin appears to serve as a biotin precursor.[13] After an incubation period of 5 or 6 days, the biotin produced from each doubly labeled precursor was isolated as (+)-biotin sulfone,[3] and converted to biotin sulfone methyl ester. The methyl esters were purified by chromatography and then recrystallized to constant activity and constant tritium to carbon-14 ratio. The results of these experiments are summarized in Table I.

A number of conclusions can be drawn from the data in Table I. Experiments 1 and 2 clearly demonstrate that the introduction of sulfur at C-1 and C-4 of (+)-dethiobiotin takes place without the loss of hydrogen from C-2 or C-3. It therefore seems unlikely that unsaturation is introduced at C-2 or C-3 during the biosynthesis of (+)-biotin from (+)-dethiobiotin; however, the possibility of enzymatic removal of hydrogen from C-2 or C-3 followed by replacement of the hydrogen without exchange cannot be excluded. Experiment 3 shows that the incorporation of [1-^{3}H]-(±)-**2** into biotin proceeds with about 30% tritium loss. The nature of the reaction associated with the oxidation of the methyl group of (+)-dethiobiotin is unknown, but the tritium loss observed in experiment 3 is consistent with the removal of one hydrogen atom from the methyl group of **2** by a process which exhibits little or no isotope effect. Experiment 4 reveals that [4(*RS*)-^{3}H]-(±)-dethiobiotin is incorporated into (+)-biotin with about 47% tritium loss. This figure is within experimental error of that expected (50%) for the stereospecific removal of one hydrogen atom from C-4 of **2** during the formation of biotin. Thus, it appears that two hydrogen atoms are removed from (+)-dethiobiotin (**2**) as the result of its conversion to (+)-biotin (**1**). Work is now in progress to determine the stereochemistry of hydrogen loss from C-4 of **2** and to ascertain the order of functionalization of C-1 and C-4 in this precursor.

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Ronald J. Parry,*[14] Michael G. Kunitani
Edison-Lecks Laboratories, Department of Chemistry
Brandeis University
Waltham, Massachusetts 02154
Received April 5, 1976

Book Reviews

Ion-Selective Electrodes. By JIRI KORYTA (Charles University, Prague). Cambridge University Press, New York, N.Y. 1975. 206 pp. $29.95.

This small volume (number two in Cambridge Monographs in Physical Chemistry series) is a survey of the field of ion-selective electrodes up to the beginning of 1973, based on published literature. There are eight chapters. The first is a brief history of the concepts leading to the theoretical basis of ion-selective electrodes. This is followed by chapters on the theory of membrane potentials, basic properties of ion-selective electrodes, analytical applications, electrodes with fixed membrane sites, electrodes with liquid membranes, electrodes with electroneutral macrocyclic ion-carriers, and the relatively new field of enzyme electrodes. There is an extensive list of references (over 1150) and a short index of subject matter.

An informative section on the construction of various types of ion-selective electrodes and several tables showing the applications of certain ion-selective electrodes to various types of analysis make the book useful for the practicing chemist.

This book contains a wealth of information and is recommended for all professional analytical and clinical chemists.

R. H. Lansing, *Eastman Kodak Company*

Topics in Current Chemistry. Volume 52. Medicinal Chemistry. Edited by F. BOSCHKE. Springer-Verlag, Berlin—Heidelberg—New York. 1974. 233 pp. $25.50.

This volume of "Topics in Current Chemistry" contains five chapters that range from general approaches in the design of active compounds to very specific and limited topics. Taken separately, the chapters are well written and clearly of importance to their specific area. However, the lack of a general theme or direction throughout the entire volume is distracting.

The first chapter, by E. J. Ariens and A. M. Simonis, deals with the design of bioactive compounds with emphasis on a rational and