fluoro-1,10-diiodo-1,10-bis(trimethylsilyl)-1,9-decadiene, 81388-12-7; (*E,Z*)-3,3,4,4,5,5,6,6,7,7,8,8-dodecafluoro-1,10-diiodo-1,10-bis(trimethylsilyl)-1,9-decadiene, 81388-13-8; (*E*)-3,3,4,4,5,5,6,6,7,7,8,8-dodecafluoro-1,8-diiodo-1-(trimethylsilyl)octene, 81388-14-9; (*Z*)-3,3,4,4,5,5,6,6,7,7,8,8-dodecafluoro-1,8-diiodo-1-(trimethylsilyl)octene, 81388-15-0; (*E,E*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-hexadecafluoro-1,12-diiodo-1,12-bis(trimethylsilyl)-1,11-dodecadiene, 81388-16-1; (*Z,Z*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-hexadecafluoro-1,12-diiodo-1,12-bis(trimethylsilyl)-1,11-dodecadiene, 81388-17-2; (*E,Z*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-hexadecafluoro-1,12-diiodo-1,12-bis(trimethylsilyl)-1,11-dodecadiene, 81388-18-3; (*E,E*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12-eicosafluoro-1,14-diiodo-1,14-bis(trimethylsilyl)-1,13-tetradecadiene, 81388-19-4; (*Z,Z*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12-eicosafluoro-1,14-diiodo-1,14-bis(trimethylsilyl)-1,13-tetradecadiene, 81388-20-7; (*E,Z*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12-eicosafluoro-1,14-diiodo-1,14-bis(trimethylsilyl)-1,13-tetradecadiene, 81388-21-8; (*E,E*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14-tetraeicosafluoro-1,16-diiodo-1,16-bis(trimethylsilyl)-1,15-hexadecadiene, 81388-22-9; (*Z,Z*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14-tetraeicosafluoro-1,16-diiodo-1,16-bis(trimethylsilyl)-1,15-hexadecadiene, 81388-23-0; (*E,Z*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14-tetraeicosafluoro-1,16-diiodo-1,16-bis(trimethylsilyl)-1,15-hexadecadiene, 81388-24-1; 1,8-diiodoperfluorooctane, 335-70-6; 1,10-diiodoperfluorodecane, 65975-18-0; 1,12-diiodoperfluorododecane, 72049-11-7; (*E*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-pentadecafluoro-1-iodo-1-(trimethylsilyl)nonene, 81388-25-2; (*Z*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-pentadecafluoro-1-iodo-1-(trimethylsilyl)-nonene, 81388-26-3; perfluoroheptyl iodide, 335-58-0; (*E*)-1,6-diiodo-1-(trimethylsilyl)-3,3,4,4,5,5,6,6-octafluorohexene, 81388-27-4; (*Z*)-1,6-diiodo-1-(trimethylsilyl)-3,3,4,4,5,5,6,6-octafluorohexene, 81388-28-5; (*E,E*)-3,3,4,4,5,5,6,6-octafluoro-1,8-diiodo-1,8-bis(trimethylsilyl)-1,7-octadiene, 81388-29-6; (*Z,Z*)-3,3,4,4,5,5,6,6-octafluoro-1,8-diiodo-1,8-bis(trimethylsilyl)-1,7-octadiene, 81388-30-9; (*E,Z*)-3,3,4,4,5,5,6,6-octafluoro-1,8-diiodo-1,8-bis(trimethylsilyl)-1,7-octadiene, 81388-31-0; tetrafluoro-1,2-diiodoethane, 354-65-4; (*E*)-3,3,4,4-tetrafluoro-1,4-diiodo-1-(trimethylsilyl)butene, 81388-32-1; (*Z*)-3,3,4,4-tetrafluoro-1,4-diiodo-1-(trimethylsilyl)butene, 81388-33-2; 3,3,4,4,5,5,6,6,7,7,8,8-dodecafluoro-1,10-diiodo-1,9-decadiene, 81388-34-3; 3,3,4,4,5,5,6,6,7,7,8,8-dodecafluoro-1,9-decadiyne, 81388-35-4; 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-hexadecafluoro-1,11-dodecadiyne, 81388-36-5; 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12-eicosafluoro-1,13-tetradecadiyne, 81388-37-6; 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11-,11,12,12,13,13,14,14-tetraeicosafluoro-1,15-hexadecadiyne, 81388-38-7; 3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-pentadecafluorononyne, 81388-39-8; phenylacetylene, 536-74-3; (*E*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-pentadecafluoro-1-iodo-1-phenylnonene, 81388-40-1; 1-phenylperfluorononyne, 52717-10-9; bis(trimethylsilyl)acetylene, 14630-40-1; (*E,E*)-3,3,4,4,5,5,6,6,7,7,8,8-dodecafluoro-2,9-bis[(iodomethyl)dimethylsilyl]-1,10-bis(trimethylsilyl)-1,9-decadiene, 81388-41-2; (*E*)-3,3,4,4,5,5,5-heptafluoro-2-[(iodomethyl)dimethylsilyl]-1-(trimethylsilyl)pentene, 81388-42-3; perfluoropropyl iodide, 754-34-7; (*E*)-3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-pentadecafluoro-2-[(iodomethyl)dimethylsilyl]-1-(trimethylsilyl)nonene, 81388-43-4; 1,12-bis(trimethylsilyl)perfluoro-1,11-dodecadiyne, 81388-44-5; 1-(trimethylsilyl)perfluorononyne, 81388-45-6; 1-(trimethylsilyl)perfluorooctyne, 81388-46-7; 1-(trimethylsilyl)perfluorodecyne, 81388-47-8; 1-(trimethylsilyl)perfluorododecyne, 81388-48-9; perfluorohexyl iodide, 355-43-1; perfluorooctyl iodide, 507-63-1; perfluorodecyl iodide, 423-62-1; 1,1-dimethoxy-3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-pentadecafluorononane, 81388-49-0; (*E*)-1-methoxy-3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-pentadecafluorononene, 81388-50-3; (*Z*)-1-methoxy-3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-pentadecafluorononene, 81388-51-4; 1,10-bis(trimethylsilyl)perfluoro-1,9-decadiyne, 81388-52-5.

# On the Mechanism of the Conversion of Dethiobiotin to Biotin in *E. coli*. Studies with Deuterated Precursors Using Tandem Mass Spectroscopic (MS-MS) Techniques

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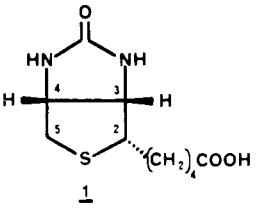
The last step of the biosynthesis of biotin (1), i.e., the conversion of dethiobiotin (2) into biotin has been studied in *E. coli* by starting from specifically deuterated precursors: [3-$^2H_{0;1}$,4-$^2H_{1;0}$]-, [5,5,5-$^2H_3$]-, and [6,6-$^2H_2$]-*dl*-dethiobiotins. Deuterium has been localized in the biosynthesized biotin by using MS–MS techniques. In each case, it is possible to conclude to the absence of deuterium migration: the two C–S bonds of biotin are formed with removal of only one hydrogen from C-2 and C-5 and without involvement of the adjacent positions.

Deuterium is now widely used instead of tritium for biosynthetic experiments, its localization by NMR avoiding degradation experiments. However, when very low amounts of material are produced, $^2H$ NMR is not sensitive enough. Isotope determination by mass spectrometry requires a highly purified product, not always easily available with small quantities. This problem can now be overcome by using MS–MS techniques,[2] which allow the localization of deuterium even on very small amounts of an impure sample.

We report here some studies on biotin biosynthesis which illustrate the advantages of this method.

The last step of the biosynthesis of biotin (1), i.e., the conversion of dethiobiotin (2) to biotin[3] has been intensively studied by using specifically tritiated dethiobiotins.

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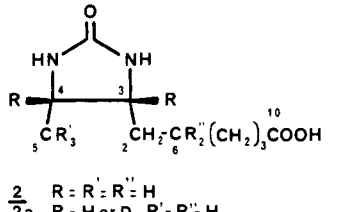


2 R = R' = R'' = H
2a R = H or D, R' = R'' = H
2b R' = D, R = R'' = H
2c R'' = D, R = R' = H

In our first investigation,[4] we have shown, using doubly labeled samples, that [10-$^{14}$C,3,4-$^3H_2$]dethiobiotin was converted into biotin by *E. coli*, with complete tritium retention. The same results have been obtained by Parry[5] with *Aspergillus niger* as the microorganism. In the same

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(2) F. W. Mc Lafferty, *Acc. Chem. Res.*, **13**, 33 (1980).
(3) M. A. Eisenberg, *Adv. Enzymol.*, **38**, 317 (1973).
(4) G. Guillerm, F. Frappier, M. Gaudry, and A. Marquet, *Biochimie*, **59**, 119, (1977).
(5) R. J. Parry and M. G. Kunitani, *J. Am. Chem. Soc.*, **98**, 4024 (1976).

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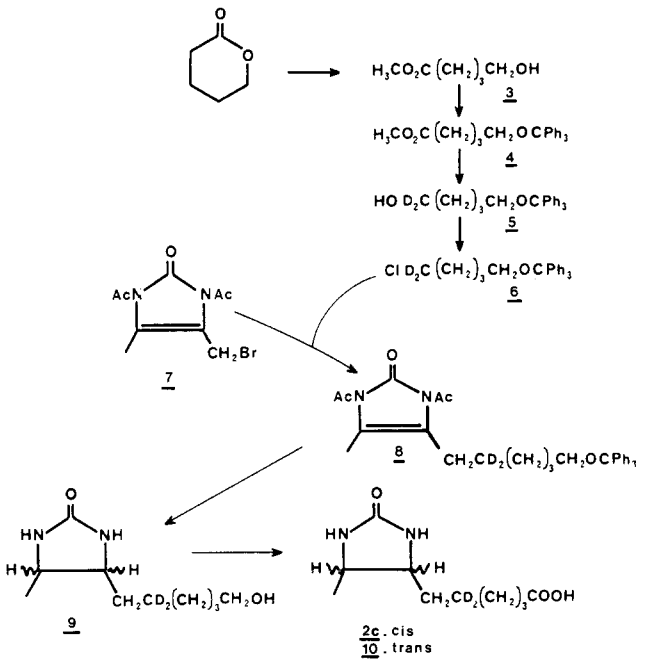
Scheme I



work, this author has shown that [2-$^3$H]- and [5-$^3$H]-dethiobiotins were incorporated into biotin respectively with about 50% and 30% loss of tritium. It has also been reported that [6-$^3$H]dethiobiotin is transformed into biotin without loss of tritium.[6]

In all these experiments, small amounts of biotin have been isolated, and the tritium atoms have not been localized: the posibility of hydrogen migrations during the biosynthesis cannot be rigorously ruled out. In the particular case of the incorporation of [5-$^3$H]-*dl*-dethiobiotin, the 30% tritium loss from the methyl group is consistent either with the removal of one hydrogen with no, or a small, isotope effect or with the removal of two hydrogen atoms with an isotope effect.

Thus, we decided to check the results obtained with tritiated dethiobiotin using deuterated precursors. The bioconversions were carried out with *E. coli* C124, a mutant which does not synthesize dethiobiotin, thus avoiding the dilution problems.

Deuterium localization in the biosynthesized biotin samples requires a knowledge of the fragmentations of biotin and a choice of the fragment ions which can be used for isotope analysis. A complete mechanistic study, which will be published elsewhere,[7] has been carried out with biotin regioselectively labeled in different positions by chemical methods, and its conclusions will be used here.

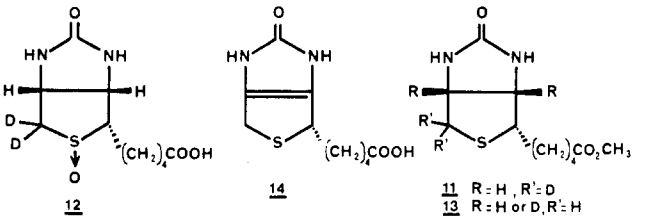
## Results and Discussion

**Syntheses of Labeled Compounds.** The preparation of [3-$^2H_{1;0}$,4-$^2H_{0;1}$]-*dl*-dethiobiotin (**2a**) has been previously described,[8] and the synthesis of [5,5,5-$^2H_3$]-*dl*-dethiobiotin (**2b**) will be published elsewhere.[9]

**[6,6-$^2H_2$]-*dl*-Dethiobiotin (2c)** has been synthesized according to Scheme I. The ester–alcohol **3** was obtained by acid-catalyzed methanolysis of δ-valerolactone. The corresponding trityl ether **4** was reduced by $LiAlD_4$ to the deutero alcohol **5** which was converted into the chloride **6** by $Ph_3P/CCl_4$. The Grignard derivative of **6** was coupled with the allylic bromide[10] **7** in the presence of CuBr.[11] Ionic hydrogenation[8] of **8** followed by saponification gave a mixture of *cis*- and *trans*-dethiobiotinols **9** which was oxidized with $O_2$ over $PtO_2$.[12] The mixture of *cis*- and *trans*-dethiobiotins **2c** and **10** was converted to the corresponding methyl esters and separated by HPLC.

After saponification, the [6,6-$^2H_2$]-*dl*-dethiobiotin was analyzed by mass spectrometry:[13] 97 ± 2% of the molecules were dideuterated.

**[5,5-$^2H_2$]-*d*-Biotin Methyl Ester (11).** In biotin sulfoxides, the C-5 methylene group can be selectively exchanged in basic medium.[14] In the equatorial sulfoxide, the two hydrogens at C-5 are exchanged with similar rates, and the dideuterated sulfoxide **12** is readily obtained. Reduction of the sulfoxide group by $TiCl_3$[15] and esterification with $CH_2N_2$ gave [5,5-$^2H_2$]-*d*-biotin methyl ester (**11**). The total deuterium content, determined by mass spectrometry,[7] was found to be 1.90 ± 0.05% deuterium atoms per molecule. The NMR spectrum shows that deuterium has been introduced exclusively at C-5 (the shape of the signal corresponding to H at C-2 is not altered by the exchange).



**[3-$^2H_{0;1}$,4-$^2H_{1;0}$]-*dl*-Biotin Methyl Ester (13).** Compound **13** has been obtained by ionic hydrogenation of 3-dehydrobiotin[17] (**14**) which when treated with $Et_3SiD$ in $CF_3COOH$[8] affords a 2:1 mixture of *dl*-biotin monodeuterated on C-3 or C-4 and a second product to which the *trans*-biotin structure has been assigned, according to the spectroscopic data. The total deuterium content of **13** was found to be 99 ± 1 atom %.

**Bioconversion Studies.** A small amount of [3,4-$^3H_2$]-*dl*-dethiobiotin (200 mCi/mmol)[8] was added, in order to follow the purification of the small amounts of biotin produced by *E. coli* C124 (150 ng/L of culture medium).

The total deuterium content in the recovered biotin has been determined by mass spectrometry, either under ammonia chemical ionization conditions [CI($NH_3$)], using the protonated molecular ion or under electron-impact conditions (EI, 70 eV), using the characteristic fragment ion $A^+$ (Scheme II) whose structure has been well demonstrated.[7] We have checked that no nonspecific exchange of hydrogen or deuterium occurs before the fragmentation of the molecular ion produced under EI conditions and tested the validity of the method by examining standard samples. The measurements in the CI and EI modes are in very good agreement.

The deuterium distributions have been determined by using the fragment ions $B^+$, $C^{+\cdot}$,$C'^{+\cdot}$,$D^{+\cdot}$, and $D'^{+\cdot}$ produced

(6) R. J. Parry and M. V. Naidu, *Tetrahedron Lett.*, 4783 (1980).
(7) D. Fraisse, F. Frappier, M. Jouany, A. Marquet, and J.-C. Tabet, *Biomed. Mass Spectrom.*, in press.
(8) G. Guillerm, F. Frappier, J.-C. Tabet, and A. Marquet, *J. Org. Chem.*, **42**, 3776 (1977).
(9) Results to be submitted for publication.
(10) R. Duschinsky and L. A. Dolan, *J. Am. Chem. Soc.*, **70**, 657 (1948).
(11) J.-F. Normant, *Synthesis*, **11**, 63 (1972).
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(14) R. Lett, S. Bory, B. Moreau, and A. Marquet, *Tetrahedron Lett.*, 2851 (1971); R. Lett, unpublished results.
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(16) R. Lett and A. Marquet, *Tetrahedron Lett.*, 2855 (1971).
(17) J. Vasilevskis, J. A. Gualtieri, S. D. Hutchings, R. C. West, J. W. Scott, D. R. Parrish, F. T. Bizzaro, and G. F. Field, *J. Am. Chem. Soc.*, **100**, 7423 (1978).

Scheme II. Specific Unimolecular Decompositions in the Second FFR of Molecular Ion $M^{+}\cdot(m/z\ 258)$ and Fragment Ion $C^{+}\cdot(m/z\ 166)$

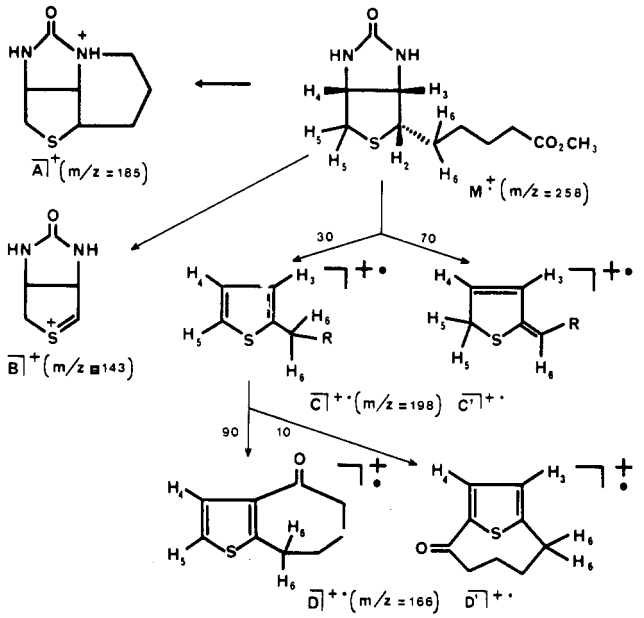



Table I. Relative Intensities of Peak at $m/z$ 215 ($d_0$) to 218 ($d_3$) and at $m/z$ 259 ($d_0$) to 261 ($d_2$), Corresponding to the Protonated Molecular Ion of Dethiobiotin and Biotin in CI($NH_3$) Spectra

| | precursor [5,5,5-$^2H_3$]-dethiobiotin (**2b**)[a] | biosynthesized biotin | |
|---|---|---|---|
| | | measd | calcd[b] |
| $d_0$ | 10.0 ± 0.3 | 14 ± 1 | 10 |
| $d_1$ | | 16 ± 1 | 16.5 |
| $d_2$ | 25.0 ± 0.5 | 71 ± 1 | 73.5 |
| $d_3$ | 65.0 ± 0.5 | | |

[a] After dilution with [$^3$H]dethiobiotin. [b] Calculated from the initial deuterium content of **2b** after the loss of one deuterium, without isotopic effect.

under EI conditions whose structures and mechanisms of formation have been deduced from the study of [3-$^2H_{1;0}$,4-$^2H_{0;1}$]-, [3,4-$^2H_2$]-, and [5,5-$^2H_2$]biotin methyl esters.[7] Evidence for the transitions $M^{+}\cdot \rightarrow B^{+}$, $M^{+}\cdot \rightarrow C^{+}\cdot + C'^{+}\cdot$, and $C^{+}\cdot \rightarrow D^{+}\cdot + D'^{+}\cdot$ was obtained by the MS–MS techniques [mass-analyzed ion kinetic energy (MIKE)[18] into the second field-free region (FFR) and linked scan $B^2/E$[19] into the first FFR (Scheme II)].

**Conversion of [5,5,5-$^2H_3$]-*dl*-Dethiobiotin (2b) into Biotin.** The CI($NH_3$) mass spectrum of the biosynthesized biotin methyl ester contains three peaks at $m/z$ 259, 260, and 261, the relative intensities of which are given in Table I. These results are consistent with the removal of one hydrogen atom from the methyl group by a process which exhibits no isotope effect.

In the above-cited work,[7] we have studied the fragmentations of the [5,5-$^2H_2$]biotin methyl ester **11** under EI (70 eV) conditions. The MIKE spectrum of the molecular ion $M^{+}\cdot$ ($m/z$ 260) contains two peaks at $m/z$ 199 and 200 in the ratio 30:70 corresponding, respectively, to the isomer ions $C^{+}\cdot$ and $C'^{+}\cdot$ (Scheme II). The MIKE spectrum of the fragment ion $C^{+}\cdot$ ($m/z$ 199) shows two signals at $m/z$ 166 and 167 in the ratio 10:90. Analysis of the biosynthesized [5,5-$^2H_2$]biotin gives exactly the same pattern (Table II).

Table II. MIKE Spectral Data[a] for Molecular Ion $M^{+}\cdot(m/z\ 260)$ and Fragment Ion $\bar{C}^{+}\cdot(m/z\ 199)$ of [5,5-$^2H_2$]Biotin Methyl Ester **11** and [5,5-$^2H_2$]Biotin Methyl Ester Biosynthesized

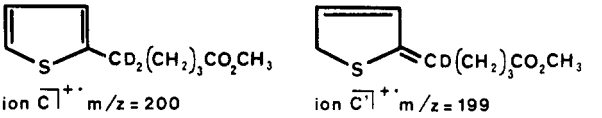
| | MIKE spectral data[a] of | | | |
|---|---|---|---|---|
| | $M^{+}\cdot(m/z\ 260)$ | | $\bar{C}^{+}\cdot(m/z\ 199)$ | |
| compd | 199[b] | 200[b] | 166[b] | 167[b] |
| biosynthesized biotin-$d_2$ | 30 ± 1 | 70 ± 1 | 10.0 ± 0.5 | 90 ± 1 |
| **11** | 29 ± 1 | 71 ± 1 | 9.0 ± 0.5 | 91 ± 1 |

[a] Relative intensities. [b] Observed peaks, $m/z$.

These data clearly demonstrate that the two deuterium atoms are identically located in the two molecules. So, we can conclude that the transformation of dethiobiotin into biotin occurs in *E. coli* with removal of only one hydrogen atom from the methyl group and without migration of the other two.

**Conversion of [6,6-$^2H_2$]-*dl*-Dethiobiotin (2c) into Biotin.** The isotopic distribution of the starting [6,6-$^2H_2$]-*dl*-dethiobiotin after dilution with [3,4-$^3H_2$]dethiobiotin was 88% $d_2$ and 12% $d_0$ and that of the recovered biotin was 84% $d_2$ and 14% $d_0$ as determined by ammonia chemical-ionization mass spectrometry. It is clear that no deuterium is lost during the conversion.

In the MIKE spectrum of the molecular ion $M^{+}\cdot$ ($m/z$ 260) produced under EI conditions the peak corresponding to ion $B^{+}$ remains unchanged compared to biotin-$d_0$ ester and appears at $m/z$ 143 (Scheme II). This proves that the two deuterium atoms are not located on the heterocycle but on the side chain of biotin. The same spectrum contains the peaks at $m/z$ 199 and 200 corresponding to isomer ions $C^{+}\cdot$ and $C'^{+}\cdot$. If we assume that the two deuteriums are located on C-6, the ions $C^{+}\cdot$ and $C'^{+}\cdot$ should appear at $m/z$ 200 and 199 in the ratio 30:70. These values correspond exactly with experimental measurements.




We can conclude that the conversion of dethiobiotin into biotin occurs in *E. coli* with total retention of the two hydrogens initially on carbon 6.

**Conversion of [3-$^2H_{1;0}$,4-$^2H_{0;1}$]-*dl*-Dethiobiotin (2a) into Biotin.** CI and EI mass spectra of the biosynthesized biotin methyl ester confirm our previous results obtained with tritiated dethiobiotin:[4] there is a complete deuterium retention during the reaction. To determine the deuterium distribution, we have compared the biosynthesized sample to [3-$^2H_{1;0}$,4-$^2H_{0;1}$]biotin **13**, where the deuterium is located exclusively on C-3 and C-4.

The distribution between these two carbons has been evaluated, in **13**, from the MIKE spectra of the molecular ion $M^{+}\cdot$ and of the ion $C^{+}\cdot$: measurements of the relative intensities of the two peaks at $m/z$ 198 and 199 on the one hand and $m/z$ 166 and 167 on the other hand allow us, taking in account the competitive formation of the isomer ions $C^{+}\cdot$, $C'^{+}\cdot$ and $D^{+}\cdot$, $D'^{+}\cdot$, to estimate the distribution of one deuterium atom between C-3, C-4, and C-5.[7] Using this method, we located the deuterium in **13** as 86 ± 2% on C-4 and 14 ± 2% on C-3. This result is in good agreement with the NMR estimation 80 ± 5% and 20 ± 5%, respectively).

Thus, it happens (Table III) that the synthetic biotin-*d* **13** and dethiobiotin-*d* **2a** which have been obtained in the

(18) R. J. Cooks and J. H. Beynon, *J. Chem. Educ.*, **51**, 437 (1974).
(19) C. J. Porter, J. H. Beynon, and T. Ast, *Org. Mass Spectrom.*, **14**, 313 (1979); A. P. Bovins, K. R. Jennings, and S. Evans, *Int. J. Mass Spectrom. Ion Physics*, **26**, 395 (1978).

Table III

| compd | MIKE spectral data[a] of $m/z$ 199 | | deuterium distribution (%) between C-3 and C-4 atoms | |
|---|---|---|---|---|
| | $m/z$ 166 | $m/z$ 167 | C-3 | C-4 |
| **13** | 12.0 ± 0.5 | 88 ± 1 | 14 ± 2 | 86 ± 2 |
| biosynthetized biotin-*d* | 10.0 ± 0.5 | 90 ± 1 | 11 ± 2 | 89 ± 2 |
| **2a** | | | 15 ± 1 | 85 ± 1 |

[a] Relative abundances of ions $D^{+\cdot}$ and $D'^{+\cdot}$.

same way (ionic hydrogenation of the corresponding unsaturated 3,4-derivatives) have the same deuterium distribution between positions 3 and 4.

The MIKE spectra of the fragment ions $m/z$ 199 obtained under 70-eV EI conditions from **13** and from the biotin-*d* biosynthesized were then compared.

In the two metastable spectra, the peaks at $m/z$ 166 and 167, which allow the determination of the deuterium repartition of one deuterium atom between C-3 and C-4, appear in a quite similar ratio (Table III). So we can assume that the deuterium repartitions are identical, within experimental error, in the two biotin-*d* esters. This result was corroborated by the deuterium determination by using the same method as for **13** (see above): the distribution was estimated as 89 ± 2% on C-4, 11 ± 2% on C-5, and, as expected, no deuterium on C-5 (Table III).

If we compare the isotope distribution of the biosynthesized biotin-*d* to the starting dethiobiotin-*d* **2a**, it is clear that the conversion of dethiobiotin into biotin occurs with total retention of the hydrogens on both the C-3 and C-4 atoms.

The data obtained in this work are in agreement with those obtained from tritiated dethiobiotins. They yield, however, further information: deuterium has been localized in biotin produced from dethiobiotins which were regiospecifically labeled in different positions. It is possible to conclude, in each case, that there is an absence of deuterium migration. The ambiguity remaining about the oxydation level of carbon 5 of dethiobiotin during its functionalization has been removed: only one hydrogen is lost. That is also the case for position 2.[5] Thus the two C–S bonds are formed with removal of only one hydrogen and without involvement of the adjacent positions.

As we have shown in another investigation[20] that an intermediate hydroxylation either at C-5 or C-2 is very unlikely, a direct sulfur introduction has to be considered for both positions. We are continuing the investigation of this mechanism.

## Experimental Section

Melting points were determined on a Kofler apparatus. NMR spectra were recorded on a Varian HA-100 or Varian XL-100 spectrometers with $Me_4Si$ as an internal standard.

Conventional mass spectra were obtained on a Vg 70-70 spectrometer coupled with a PDP8 computer. EI (70 eV) conditions were as follows: acceleration voltage, 4 kV; trap current, 100 μA. CI conditions were as follows: pressure in the source, 0.8 torr; plasma of protonating gas, $NH_3^{+\cdot}$ (3–5%), $NH_4^+$ (90%), $N_2H_7^+$ (5–7%); trap current, 500 μA; ion repeller zero at 50 eV. The samples were introduced directly (source temperature 180 °C). Spectra were repeated 10 times in order to obtain reliable data (error ±1%). The metastable transitions produced in the second field-free region were measured by using the MIKE method on a ZAB 2F Vg micromass spectrometer (inversed geometry) coupled with a PDP 11 computer: acceleration voltage, 8 kV; trap current, 100 μA.

**Methyl 5-[(Triphenylmethyl)oxy]pentanoate (4).** To a solution of 20 g of δ-valerolactone in 200 mL of MeOH was added 50 mg of *p*-toluenesulfonic acid. After being refluxed over night, the mixture was neutralized by $NH_3$ and filtered, and the solvent was evaporated to afford 25 g (95%) of methyl 5-hydroxypentanoate (**3**): IR ($CHCl_3$) 1730, 3400 $cm^{-1}$; NMR δ 2.29 (t, 2 H, $J$ = 8 Hz, $CH_2COOCH_3$), 3.60 (t, 2 H, $CH_2OH$), 3.64 (s, 3 H, $CH_3O$).

To a solution of 20 g of **3** in 20 mL of pyridine was added dropwise a solution of 50 g of triphenylmethyl chloride in 90 mL of pyridine. The mixture was stirred for 24 h and extracted with dichloromethane. The organic layer was dried and evaporated, and the residue, dissolved in hexane–ethyl acetate (9:1), was purified by filtration on silica gel: 22.5 g (40%); IR ($CHCl_3$) 1730; NMR ($CDCl_3$) δ 1.60–1.74 (m, 4 H, $(CH_2)_2$), 2.22 (m, 2 H, $CH_2CO_2CH_3$), 3.02 (m, 2 H, $CH_2OCPh_3$), 3.60 (s, 3 H, $OCH_3$), 7.14–7.44 (m, 15 H, $CPh_3$).

**[1,1-$^2H_2$]-1-Hydroxy-5-[(triphenylmethyl)oxy]pentane (5).** A solution of 22.5 g of **4** in 250 mL of anhydrous ether was treated with 2 g of $LiAlD_4$ for 1 h to afford 17.7 g (85%) of **5**: NMR δ 3.02 (m, 2 H, $CH_2OCPh_3$), 7.14–7.50 (m, 15 H, $CPh_3$); MS $m/z$ 348 ($M^{+\cdot}$).

**[1,1-$^2H_2$]-1-Chloro-5-[(triphenylmethyl)oxy]pentane (6).** To a solution of 17.7 g of **5** in 600 mL of $CCl_4$ was added 15 g of triphenylphosphine. The mixture was refluxed for 60 h, and the organic layer was filtered and evaporated. After purification by column chromatography on silica gel (hexane–ethyl acetate, 98:2), crystallization from hexane gave **6**: 7.3 g (40%); mp 69–71 °C; NMR ($CDCl_3$) δ 1.2–1.9 (m, 6 H), 3.02 (t, 2 H, $J$ = 7 Hz, $CH_2OCPh_3$), 7.1–7.6 (m, 15 H, $CPh_3$), no signal at 3.4 (t, 2 H, $J$ = 7 Hz corresponding to $CH_2Cl$ in the nonlabeled compound.

**1,3-Diacetyl-4-[ω-[(triphenylmethyl)oxy]amyl]-5-methyl-2-imidazolone (8).** To 1.6 g of dry magnesium were added 1 mL of THF, 1 drop of dibromoethane, and then, under reflux, a solution of 7.3 g of **6** in 20 mL of dry THF. The mixture was refluxed, under argon, for 1.5 h and used directly.

To a solution of 7.35 g of **7** in 90 mL of dry THF was added 0.85 g of CuBr. The mixture was cooled (−70 °C), and the previously obtained Grignard reagent solution was added dropwise. The reaction mixture was stirred overnight at −30 °C. After addition of ether, the organic layer was washed with 5% $NH_4Cl$, $H_2O$, and 5% $NaCO_3H$, dried ($MgSO_4$), and evaporated to dryness. The crude extract was purified by silica gel column chromatography (hexane–ethyl acetate, 88:12) to afford (10%) of **8** as an oil: NMR ($CDCl_3$) δ 1.20–1.70 [m, 6 H, $(CH_2)_3$], 2.04 (s, 2 H, $CH_2$—C=), 2.18 (s, 3 H, $CH_3C$=), 2.56 (s, 6 H, $COCH_3$), 3.02 (m, 2 H, $CH_2OCPh_3$) 7.14–7.45 (m, 15 H, $CPh_3$); MS $m/z$ 528 ($M^{+\cdot}$), 486, 444.

***cis*- and *trans*-[6,6-$^2H_2$]Dethiobiotinols (9).** To a solution of 0.95 g of **8** in 5 mL of $CF_3COOH$ was added gradually 1 mL of $Et_3SiH$. The mixture was stirred at 45 °C for 60 h and then evaporated to dryness. After treatment by MeOH, the $CH(C_6H_5)_3$ produced was filtered off and the solvent evaporated to afford 0.48 g of a mixture of *cis*- and *trans*-dethiobiotinols and their mono- and diacetyl derivatives.

A 0.32-g sample of this mixture was treated with 10 mL of 1 N NaOH in MeOH for 1.5 h. After neutralization by HCl, the solution was filtered to give **9**: 0.17 g (70%); NMR ($CF_3COOH$) δ 1.32 (d, $J$ = 6 Hz, 0.3 H, $CH_3CH$–CH cis), 1.35 (d, $J$ = 6 Hz, 0.7 H, $CH_3CH$–CH trans), 3.90–4.40 (m, 2 H, NCH–CHN), 4.46 (m, 2 H, $CH_2OH$), MS $m/z$ 202 ($M^+$), 187, 172, 99.

**[6,6-$^2H_2$]Dethiobiotin (2c).** To 25 mg of **9** and 5.5 mg of $NaHCO_3$ was added a suspension of 18 mg of Adam's catalyst previously reduced under a hydrogen atmosphere for 30 min in 2 mL of $H_2O$. The reaction mixture was stirred for 60 h under an oxygen atmosphere. Then, the catalyst was eliminated, and the supernatant was poured on a Dowex Ag 1-X2 column (formate form). Elution with formic acid (0.05 N) afforded 17 mg (65%) of a mixture of *cis*- and *trans*-dethiobiotins **2c** and **10**. For **10**: NMR ($CD_3OD$) δ 1.13 (d, $J$ = 6.5 Hz, 3 H, $CH_3CH$), 3.62 (m, 1 H, $NCHCH_3$). For **2c**: NMR δ 1.10 (d, $J$ = 6 Hz, 3 H, $CH_3CH$), 3.84 (m, 1 H, $NCHCH_3$).

Compound **2c** (35%) was separated from its trans isomer by semipreparative HPLC of the corresponding methyl esters

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(column μ-Bondapack, $C_{18}$, 10 μm, Waters; solvent $H_2O$–MeOH, 60:40). Spectral data compared to those of a reference sample obtained from biotin[21] confirm the structure of **2c**. The deuterium content of [6,6-$^2H_2$]dethiobiotin was determined by mass spectrometry on the purified acid: MS (CI, quasimolecular ion) $m/z$ (relative intensity) 217 ($d_2$, 100), 216 ($d_1$, 3.5), 215 ($d_0$, 5.2).

**[5,5-$^2H_2$]-*d*-Biotin Methyl Ester (11).** A solution of 100 mg of biotin-*d* sulfoxide[22] in 2.5 mL of 0.93 N NaOD/$D_2O$ was kept at room temperature for 9 days. After neutralization by HCl, the water was evaporated, the residue was dissolved in 7.5 mL of MeOH–$CHCl_3$ (2:1), and 1 mL of $TiCl_3$ was added. The mixture was refluxed for 6 h and, and then the solvent was evaporated. The residue dissolved in 2 mL of MeOH was treated with 2 mL of 1 N NaOH for 2 h. After neutralization by HCl, the solution was poured over a Dowex Ag 1-X2 column (formate form). Biotin-$d_2$ (49 mg, 53%) was eluted with 0.05 N formic acid. The methyl ester **11** was obtained by treatment with diazomethane: mp 128–130 °C; NMR ($CDCl_3$) δ 3.16 (m, 1 H, SCH), 4.25 (m, 1 H, H at C-3), 4.45 (d, 1 H, $J$ = 7 Hz, H at C-4), no signal corresponding to the two hydrogens at C-5 (δ 2.66 and 2.90);[16] MS (EI) $m/z$ 145, 146, 167, 187, 199, 200, 229, 260 ($M^+$·); MS (CI, quasimolecular ion) $m/z$ (relative intensity) 262 (1), 261 (100), 260 (13), 259 (2).

**[3-$^2H_{1;0}$,4-$^2H_{0;1}$]-*dl*-Biotin Methyl Ester (13).** $Et_3SiD$ was prepared by $LiAlD_4$ reduction of chlorotriethylsilane.[23] Its isotopic purity (>95%) was estimated by NMR (no signal at δ 3.28 ($C_6H_6$ internal reference). To 50 mg (0.2 mmol) of 3-dehydrobiotin[17] **14** dissolved in 1 mL of $CF_3COOH$ were added 50 μL (0.2 mmol) of $Et_3SiD$. The mixture was stirred for 15 min at 20 °C. After removal of the solvent, the crude material was esterified by diazomethane to give a mixture of two compounds. After separation by silica gel column chromatography, we obtained the following.

**13**: 28 mg (60%); mp 128–130 °C; NMR ($CDCl_3$)[16] δ 4.25–4.63 (m, 0.8 H, H at C-3), 4.02–4.25 (m, 0.2 H, H at C-4); MS (EI, 70 eV) $m/z$ 85, 98, 144, 145,167, 186, 199, 228, 259 ($M^+$·); MS (CI, quasimolecular ion) $m/z$ (relative intensity) 260 (100), 259 (1); MIKE spectrum on $m/z$ 259, $m/z$ (relative intensities) 198 (2), 199 (98), 144 (37), 145 (62).

*trans*-[3-$^2H_{1;0}$, 4-$^2H_{0;1}$]Biotin methyl ester (noncrystallized): 14 mg (30%); NMR ($CDCl_3$) δ 1.35 (m, 8 H), 2.04 (m, 2 H, $CH_2CO_2CH_3$), 2.22–2.88 (m, 3 H, HCS), 3.26 (s, 3 H, $OCH_3$), 3.48 (m, 0.8 H, CN–CH), 3.90 (m, 0.2 H, NCH), 4.71 (m, 1 H, NH), 4.91 (m, 1 H, NH); mass spectrum, identical with that for **13**.

**Conversion of Dethiobiotin to Biotin.** The conversion of dethiobiotin to biotin was performed by using *E. coli* C124 His$^-$, bio A$^{-24}$ as previously described.[4] The culture medium was inoculated with 5 μg/L of deuterated *dl*-dethiobiotin diluted with [3,4-$^3H_2$]-*dl*-dethiobiotin[8] (200 mCi/mmol) in an approximate ratio of 9:1.

The bound biotin was extracted[4] and purified[25] according to the described techniques by using solvent of the highest purity available. Starting from 24 L of culture medium, we obtained 4 μg of crude biotin, yielding 700 ng of biotin methyl ester after purification by silica gel column chromatography (eluent MeOH–ACOEt, 1:4).

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**Registry No.** **1**, 22377-59-9; **2**, 636-20-4; **2a**-*d* (isomer 1), 81477-33-0; **2a**-*d* (isomer 2), 81477-34-1; **2b**, 81477-35-2; **2c**, 81477-36-3; **3**, 14273-92-8; **4**, 81477-37-4; **5**, 81496-93-7; **6**, 81477-38-5; **7**, 81477-39-6; **8**, 81477-40-9; *cis*-**9**, 81477-41-0; *trans*-**9**, 81496-84-6; **10**, 81477-42-1; **11**, 81496-94-8; **12**, 81477-43-2; **13**-*d* (isomer 1), 81477-44-3; **13**-*d* (isomer 2), 81477-45-4; **14**, 61379-20-2; δ-valerolactone, 542-28-9; triphenylmethyl chloride, 76-83-5.

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# Polar Effect in Addition Rates of Substituted Benzenethiyl Radicals to α-Methylstyrene Determined by Flash Photolysis[1]

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The absolute rate constants for the reversible addition reactions of the para-substituted benzenethiyl radicals ($p$-$XC_6H_4S$·) to α-methylstyrene have been determined by flash photolysis. The equilibrium constants have been estimated as the relative values. From the Hammett plots vs. $\sigma^+$, the reaction constant ($\rho^+$) for the reactivities was estimated to be 1.72 ($r$ = 0.9851). Since the polar effect was also found for the equilibrium constants ($\rho^+ \approx 1.4$), a portion of the polar effect on the reactivities is determined by the polar effect on the stabilities of $p$-$XC_6H_4S$· in terms of a linear free-energy relationship. The remaining positive portion of the polar effect on the reactivities may be attributed to the contribution of a polar resonance structure such as [$p$-$XC_6H_4S^-$,$CH_2$=$C(CH_3)Ph^+$·] to the transition state.

Negative Hammett reaction constants ($\rho$ or $\rho^+$) have been estimated from the Hammett-type linear free-energy treatments in the thiyl radical addition reactions toward substituted α-methylstyrene on the basis of the measurements of the relative reactivities.[2-4] The negative $\rho^+$ value has been confirmed by the Hammett plots of the absolute rate constants determined by the flash photolysis method.[5] The negative $\rho^+$ values suggest the polar nature of the transition state as follows:

$$[RS\cdots CH_2{=}C(CH_3)C_6H_4Y] \leftrightarrow [RS^-,CH_2{=}C(CH_3)C_6H_4Y^+\cdot]$$

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