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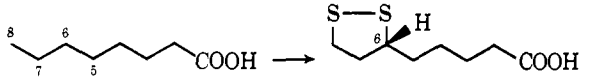
Biosynthesis of Lipoic Acid. 2. Stereochemistry of Sulfur Introduction at C-6 of Octanoic Acid

Sir:

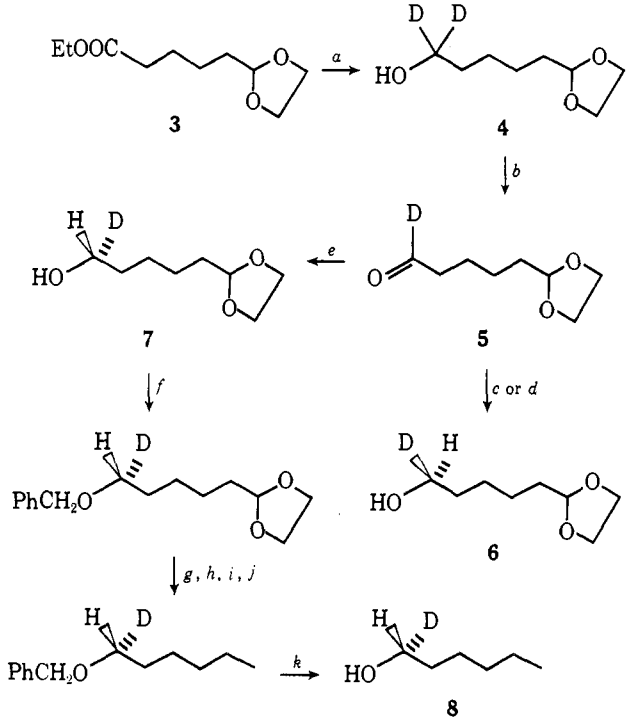
α-(+)-Lipoic acid (**1**) is an essential coenzyme for all systems of α-keto acid dehydrogenase complexes that have been investigated.[1] We recently reported experiments which establish that the biosynthesis of **1** in *Escherichia coli* proceeds



2 **1**

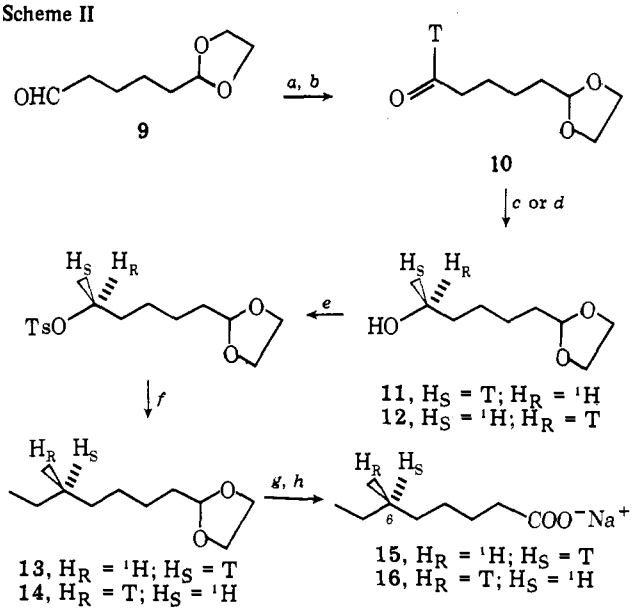
from octanoic acid (**2**) via the introduction of sulfur at C-6 and C-8 of **2** without apparent involvement of C-5 and C-7.[2] Earlier investigations demonstrated the operation of similar processes in the conversion of (+)-dethiobiotin to (+)-biotin.[3] Since the nature of reactions involved in the introduction of sulfur at saturated carbon atoms is currently unknown, we decided to investigate the stereochemistry of the sulfur introduction process. We now report the results of experiments which elucidate the stereochemistry of the introduction of sulfur at C-6 of octanoic acid.

Scheme I



[a] $LiAlD_4$. [b] $C_5H_5NH^+CrO_3Cl^-$. [c] Horse-liver alcohol dehydrogenase, NADH, cyclohexanol. [d] (+)-α-Pinene-9-BBN. [e] (−)-α-Pinene-9-BBN. [f] NaH, $PhCH_2Br$. [g] H_3O^+. [h] $LiAlH_4$. [i] Ph_3P, CBr_4. [j] $LiBEt_3H$. [k] H_2, Pd/C.

Scheme II



[a] [^{3}H]-KBH_4. [b] $C_5H_5NH^+CrO_3Cl^-$. [c] (+)-α-Pinene-9-BBN. [d] (−)-α-Pinene-9-BBN. [e] $C_7H_7SO_2Cl$, C_5H_5N. [f] Et_2CuLi. [g] O_3, CH_3OH. [h] NaOH.

The elucidation of the stereochemistry of sulfur introduction was accomplished by means of precursor incorporation experiments with sodium [(6*S*)-6-^{3}H]- and [(6*R*)-6-^{3}H]octanoate. The synthesis of the chirally tritiated forms of octanoic acid was achieved as follows. The acetal ester **3**[4] (Scheme I) was reduced with lithium aluminum deuteride to the deuterated acetal alcohol **4** (93%). Oxidation of **4** with pyridinium chlorochromate[5] yielded the deuterated aldehyde **5** (78%). Reduction of the deuterated aldehyde with horse-liver alcohol dehydrogenase, NADH, and cyclohexanol[6] yielded the chirally deuterated alcohol **6**. On the basis of the stereochemistry observed when a wide variety of aldehydes are reduced by liver alcohol dehydrogenase,[7] it was expected that the alcohol **6** would possess the *S* configuration. Derivatization of **6** with (−)-camphanoyl chloride and examination of the NMR spectrum of the camphanate ester[8] in the presence of $Eu(dpm)_3$ supported this prediction: the diastereotopic hydrogen atom at C-1 of the camphanate ester of **6** resonated at higher field, as anticipated.[9] However, derivatization of **6** with *p*-bromophenyl isocyanate and mass spectral analysis of the derivative revealed that the chirally deuterated alcohol contained ~20% of dideuterio alcohol with no detectable quantity of undeuterated alcohol being present. The dideuterated alcohol presumably arises via a dismutation reaction which is known to be catalyzed by horse-liver alcohol dehydrogenase.[10] A more suitable preparation of the *S* alcohol **6** proved to be the reduction of the deuterated aldehyde **5** to **6** (28%) with the adduct of (+)-α-pinene (81% optical purity) and 9-BBN.[11] NMR analysis of the camphanate ester of alcohol **6** obtained from the (+)-α-pinene-9-BBN reduction indicated that the reaction had proceeded to give the *S* alcohol with an optical purity of ~80%. Similarly, reduction of aldehyde **5** with the adduct of (−)-α-pinene (74% optical purity) and 9-BBN yielded (28%) the *R* alcohol **7** (~72% optical purity). As an additional check of the chirality assigned to **7**, this alcohol was degraded in the manner shown (Scheme I) to [(1*R*)-1-^{2}H$_1$]hexanol (**8**) (28% yield from **7**) whose chirality was verified using the camphanate method.

The chirally tritiated alcohols **11** and **12** were then prepared using the same technique (Scheme II). Reduction of the aldehyde **9** with potassium borotritiide (94%) and oxidation of the resulting labeled alcohol with pyridinium chlorochromate gave (81%) the tritiated aldehyde **10**. Reduction of **10** with the

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Table I. Incorporation of Chirally Tritiated Octanoate into Lipoate

expt	precursor	$^3H/^{14}C$ for precursor	$^3H/^{14}C$ for lipoate	% ^3H retention
1	[6(*S*)-6-^{3}H-1-^{14}C]-**2**	4.13	3.47	84
2	[6(*R*)-6-^{3}H-1-^{14}C]-**2**	4.40	0.48	10

adduct of (+)-α-pinene (81% optical purity) and 9-BBN yielded the *S* alcohol **11** whose optical purity was presumed to be ~80%. Reduction of **10** with the adduct of (−)-α-pinene (optical purity 74%) generated the *R* alcohol **12** with an optical purity presumed to be ~72%. The two chirally tritiated alcohols **11** and **12** were converted to their tosylates and the latter compounds treated with lithium diethylcopper.[2] Since the reaction of dialkylcuprates with tosylates proceeds with inversion of configuration at the sulfonate bearing carbon atom,[12] this reaction sequence transforms the *S* alcohol **11** into the *S* acetal **13** and the *R* alcohol **12** into the *R* acetal **14**.[13] Ozonolysis of the chirally tritiated acetals and base-catalyzed hydrolysis of the resulting ethylene glycol esters[2] then produced sodium [(6*S*)-6-^{3}H]- and [(6*R*)-6-^{3}H]octanoate (**15** and **16**, respectively). The two samples of chirally tritiated sodium octanoate were each mixed with sodium [1-^{14}C]octanoate and the tritium to carbon-14 ratios of each doubly labeled mixture were determined in the manner previously described.[2] The two doubly labeled precursors were then administered to shake cultures of *E. coli* and the lipoic acid isolated as the *S,S'*-bis(*p*-phenylbenzyl) derivative.[2] The derivative from each experiment was recrystallized, converted to its methyl ester, chromatographed, and then recrystallized to constant specific radioactivity and constant tritium to carbon-14 ratio. The results of these experiments are summarized in Table I.

The tritium to carbon-14 ratios of the lipoic acid derivatives isolated in these experiments clearly demonstrate that sulfur is introduced at C-6 of octanoic acid with loss of the 6-*pro R* hydrogen atom. Since the absolute configuration of lipoic acid at C-6 is known to be *R*,[14] it follows that sulfur is introduced at C-6 of octanoic acid with *inversion* of configuration at that prochiral center. This observation suggests that the mechanism of sulfur introduction involves a multistep process. An obvious possibility which would account for the observed stereochemistry is hydroxylation at C-6 of octanoate with retention of configuration,[15] activation of the hydroxyl group, and displacement of the activated group by a sulfur nucleophile. In view of the apparent similarity between the modes of sulfur introduction in the biosynthesis of lipoic acid[2] and biotin,[3] one would predict that the introduction of sulfur at C-4 of dethiobiotin should also proceed with inversion of configuration. Experiments designed to test this prediction are in progress.

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Time-Resolved Electron Paramagnetic Resonance and Flow Fourier Transform Nuclear Magnetic Resonance Study in Radiation Chemistry. An Example of Overhauser CIDNP[1]

Sir:

Parallel study of CIDEP and CIDNP[2a-c] in radiation chemistry[2d] has allowed observation of a straightforward example of polarization transfer between electron and nuclear spin systems by electron–nuclear cross relaxation. While this Overhauser CIDNP can be observed in many related systems, we will discuss the aqueous acetate radiolysis, since we have made extensive study of both CIDEP and CIDNP in this system.

The experiment is schematically outlined as follows:

$$e_{aq}^- \xrightarrow{[e_{aq}^-\cdot R]} \underset{\text{CIDEP}}{e_{aq}^-(\downarrow)} \xrightarrow{ClCH_2CO_2^-} \underset{\text{CIDEP}}{\cdot CH_2CO_2^-(\downarrow)} \rightarrow \underset{\text{CIDNP}}{(CH_2CO_2^-)_2(\downarrow)} \quad (1)$$

The hydrated electron is one of the primary radicals in aqueous radiolysis.[2d] Dissociative electron capture by haloacetates yields the acetate radical; in turn, this radical can undergo bimolecular reaction yielding succinate.

The hydrated electron can be prepared with its electron spin polarization in emission (↓) as shown by the CIDEP observed using time-resolved EPR. The electron spin polarization is transferred to the acetate radical (↓) (CIDEP observed by time-resolved EPR) and subsequently the nuclear spin of the (diamagnetic) product succinate can be observed in emission (↓) as well (CIDNP using flow FT NMR). We will discuss each of these steps in turn.

Emission in e_{aq}^-. Fessenden has observed that several radicals (from carbonate (CO_3^-), phosphite ($\cdot PO_3^{2-}$), phenol (phenoxyl), etc.) yield emissive CIDEP in e_{aq}^-.[3] Since the electron *g* factor is the smallest of all these radicals, this unusual polarization has been rationalized by proposing that the radical pair $\overline{e_{aq}^-\cdot R}$ (where R is one of the aforementioned radicals) reacts into the triplet state of the products. (This amounts to an "inverse" *g*-factor effect.) This somewhat unusual mechanism of radical-pair CIDEP has yet to be substantiated. Our recent CIDNP studies in these systems have provided further indications that a *g*-factor effect is involved in reactions of e_{aq}^- with the $CO_3^-\cdot$ and phenoxy radicals.[7]

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