The structure of the B anion differs from that of A (Figure 2a) by the introduction of face sharing between two of the MoO_6 octahedra. This arrangement requires 25 oxygen atoms, as opposed to 24 in the A structure, and consequently A and B are, strictly speaking, not isomers. The metal–oxygen bond lengths in the new structure fall in the expected ranges[8] for the types of bridging or terminal oxygen involved, *with one exception*—the bonds to O(15), one of the atoms in the shared face, are anomalously long, 2.393 (14) and 2.478 (15) Å.[9] We therefore believe that a bridging water molecule occupies this site. If O(15) is removed from the structure, only a slight rotation of the previously face-shared dimolydate group is necessary to produce the A structure. Conversely, A could be transformed to B if a water molecule is introduced adjacent to any two of the oxygen atoms of a $PhAsO_3$ group.[10]

Although the B ("hydrated") structure is the predominant solute species in aqueous solution, the equilibrium, $B \rightleftharpoons A + H_2O$, can be displaced to the right by rigorous removal of water from an acetonitrile solution. The pure B salt $(Bu_4N)_4[(PhAs)_2Mo_6O_{25}H_2]$ can be obtained by precipitation from aqueous solution and drying at 110 °C. Recrystallization of this salt from MeCN yields mixtures of A and B anions according to IR spectra. The 90-MHz ^1H FT-NMR spectrum of a recrystallized sample (predominantly B) in CD_3CN shows two phenyl multiplets centered at 8.26 (2 H) and 7.49 (3 H) ppm,[12] and the H_2O resonance (exchange peak between anion and traces of water in solvent) at 3.38 ppm (at 26 °C).[13] The chemical shifts of the phenyl resonances are unchanged between −20 and 90 °C, but, if the solution is kept at 90 °C (sealed tube) for several hours, the phenyl resonances then appear at 8.10 and 7.52 ppm, the H_2O peak has disappeared, and two broad peaks have developed at 5.7 and 6.3 ppm. Addition of a drop of water to the cooled solution immediately restores the original phenyl spectrum (8.22 and 7.48 ppm). Parallel treatment of a solution in MeCN, monitored by infrared spectroscopy, confirms that the above changes reflect the dehydration and subsequent rehydration

$$B \xrightarrow{-H_2O} A \xrightarrow{+H_2O} B$$

During the heating, water present in the solution from dissociation of B reacts with the solvent to produce acetamide. The acetamide accounts for the NMR peaks at 5.7 and 6.3 ppm, and also for IR bands at 3485, 3375, 1682, and 1670 cm^{-1} that appear in the heated solutions.[14,15] Further NMR studies of the A–B interconversion are in progress.

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(6) At the higher end of the pH range, absorption bands due to small amounts of MoO_4^{2-} and $RAsO_3^{2-}$ are also observed.
(7) Submitted to *Bull. Chem. Soc. Jpn.*
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(9) The Mo–O bond lengths to O(13) are 1.952 (13) and 1.908 (14) Å and to O(14) (triply bridging) are 2.255 (16) and 2.314 (14) Å. The As–O(15) bond length is 1.707 Å.
(10) We note that Day et al.[3] have recently speculated about the lability of the $RAsO_3^{2-}$ groups in the A structure.
(11) The IR spectrum of this salt was unchanged after 9 h in vacuo at 125 °C.
(12) The corresponding resonances in a solution $(Bu_4N)_2PhAsO_3$ in CD_3CN appear at 7.87 and 7.61 ppm, respectively.
(13) The chemical shift of this resonance is strongly temperature dependent and varies from 4.34 (−20 °C) to 2.43 ppm (85 °C).
(14) All the NMR and IR features that develop in the heated solutions could be reproduced by dissolving acetamide in a fresh solution of the B anion.
(15) Filowitz and Klemperer[5] deduced the A structure from the ^{17}O NMR spectrum of the Bu_4N^+ salt in MeCN. The spectrum was accumulated at 80 °C, i.e., under conditions where B is dehydrated by the process described here.

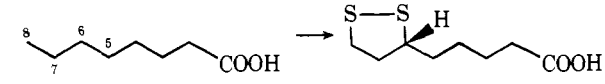
Wonsuk Kwak, Ljudmila M. Rajković
Michael T. Pope,* Carl O. Quicksall
Department of Chemistry, Georgetown University
Washington, D.C. 20057

Kazuko Y. Matsumoto, Yukiyoshi Sasaki*
Department of Chemistry, University of Tokyo
Bunkyo-ku, Tokyo 113
Received June 13, 1977

Biosynthesis of Lipoic Acid. 1. Incorporation of Specifically Tritiated Octanoic Acid into Lipoic Acid

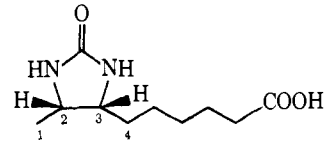
Sir:

α-(+)-Lipoic (6,8-thioctic) acid (**1**) is an essential coenzyme for all systems of α-keto acid dehydrogenase complexes that have been investigated.[1] Although considerable information is available concerning the mechanism of action of lipoic acid, little is known about the biosynthesis of this important compound. In 1964, Reed reported[2] that octanoic acid (**2**) serves as a specific precursor of **1** in *Escherichia coli*. This is an in-



2 **1**

triguing observation since it suggests that the dithiolane ring system of **1** may be generated via the introduction of sulfur at two saturated carbon atoms. The biosynthesis of lipoic acid may therefore resemble the biosynthesis of the vitamin (+)-biotin (**3**) from (+)-dethiobiotin (**4**), a transformation that has



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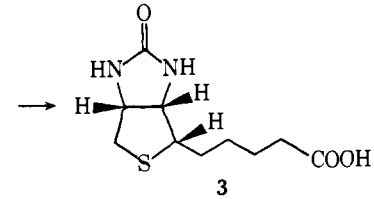

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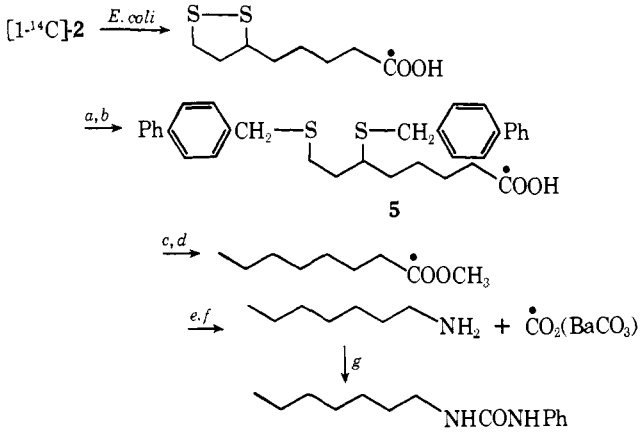
recently been investigated in our laboratories.[3] We now report experiments that confirm Reed's unpublished observation and demonstrate that the mode of sulfur introduction in lipoate biosynthesis does indeed bear some resemblance to the mechanism of sulfur introduction in biotin biosynthesis.

The first stage of our investigation required that we verify the observation that [1-^{14}C]octanoic acid is specifically incorporated into lipoic acid. Accordingly, sodium [1-^{14}C]octanoate was administered to shake cultures of *E. coli* (Crookes strain, ATCC 8739) and the cells harvested by centrifugation after 16 h at 32–34 °C. The cells were sonicated, radioinactive lipoic acid was added as carrier, and the mixture was then

Table I. Incorporation of Specifically Tritiated Octanoic Acid into Lipoic Acid

Expt	Precursor	$^3H/^{14}C$ for precursor	% incorpn	$^3H/^{14}C$ for lipoic acid	% ^3H retention
1	[1-^{14}C-5(*RS*)-^{3}H]-**2**	4.05	0.18	4.11	102
2	[1-^{14}C-7(*RS*)-^{3}H]-**2**	3.95	0.21	3.81	96.5
3	[1-^{14}C-8-^{3}H]-**2**	5.02	0.11	4.81	95.8
4	[1-^{14}C-6(*RS*)-^{3}H]-**2**	5.08	0.10	2.53	49.8

Scheme I

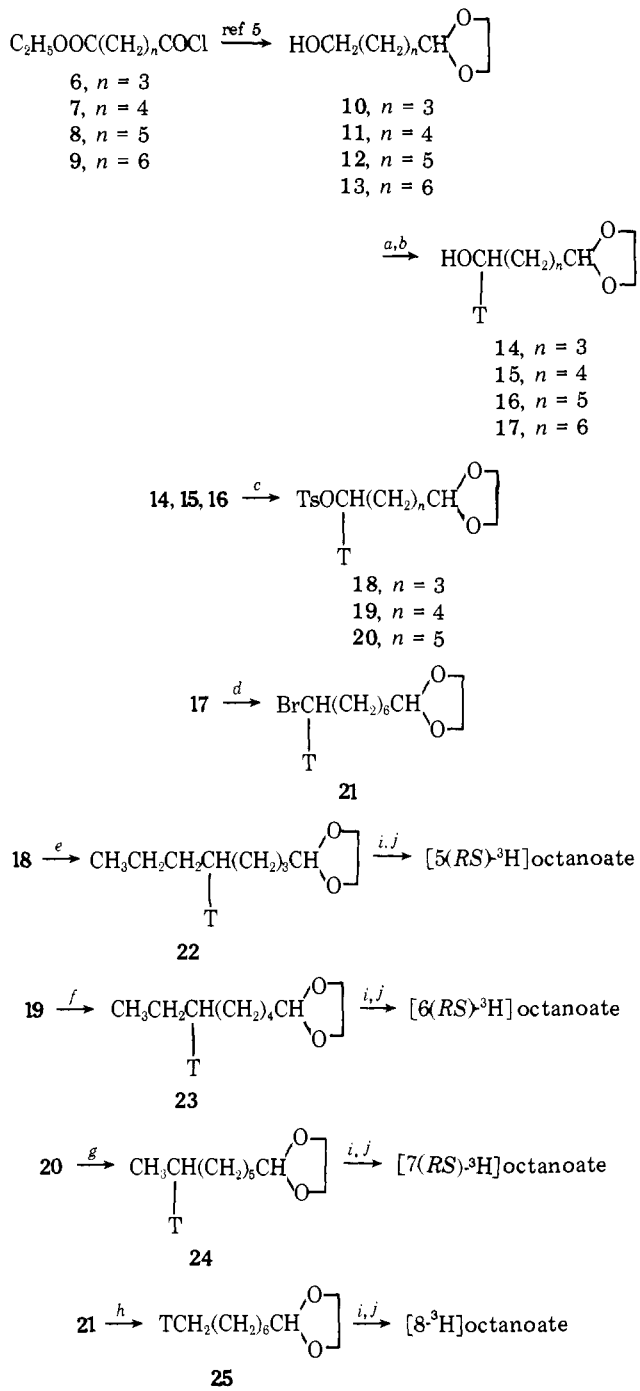


[a] Na, NH_3. [b] *p*-$PhC_6H_4CH_2Cl$. [c] CH_3OH, H^+. [d] Raney Ni. [e] NaOH. [f] HN_3, H_2SO_4. [g] PhNCO.

autoclaved in 6 N sulfuric acid at 120 °C for 2 h in order to liberate protein-bound lipoic acid. The crude lipoic acid recovered by benzene extraction of the autoclaved mixture was derivatized by reduction with sodium in liquid ammonia followed by treatment with *p*-phenylbenzyl chloride to give the bis(*p*-phenylbenzyl) derivative **5** (Scheme I).[4] This derivative was recrystallized to constant activity to give an incorporation figure of 0.17%. The purified derivative was converted to the corresponding methyl ester which was recrystallized and then desulfurized with Raney nickel to yield methyl octanoate. Hydrolysis of the methyl octanoate and Schmidt degradation of the resulting octanoic acid then gave carbon dioxide, trapped as barium carbonate, and *n*-heptylamine, which was derivatized with phenyl isocyanate (Scheme I). The barium carbonate carried 90% of the radioactivity of the lipoic acid and the *n*-heptylamine was radioinactive. The specific incorporation of [1-^{14}C]octanoic acid into lipoic acid was therefore confirmed.

The role of octanoic acid as a lipoate precursor having been established, samples of octanoic acid specifically tritiated at C-5, C-6, C-7, and C-8 were synthesized. It was anticipated that the degree of tritium loss associated with the incorporation of each of the specifically tritiated forms of **2** into **1** would provide some insight into the mechanism of sulfur introduction. The synthesis of the tritiated octanoic acids is summarized in Scheme II. The chloroformyl ethyl esters **6–9**[5–7] were transformed into the corresponding acetal alcohols[4] **10–13** in a manner previously described[5] for the preparation of **11**. The tritiated alcohols **14–17** were obtained by Collins oxidation of the unlabeled alcohols to the aldehydes and reduction with potassium [^{3}H]borohydride. The tritiated alcohols **14–16** were then converted into the tosylates **18–20** (~95% yield) and each of the tosylates[4] was treated with the appropriate lithium dialkylcuprate to yield[8] (60–80%) the tritiated octanal derivatives **22–24**.[4] The tritiated octanal derivative **25** was obtained from the tritiated alcohol **17** by conversion of **17** to the bromide **21**[4] (90%) and reduction[9] of the bromide with lithium triethylborohydride (60%). Each of the labeled octanal derivatives **22–25** was then transformed into a specifically labeled form of sodium octanoate by ozonolysis[5,10] followed by alkaline

Scheme II



[a] CrO_3, C_5H_5N. [b] [^{3}H]-KBH_4. [c] *p*-TsCl, C_5H_5N. [d] Ph_3P, CBr_4. [e] *n*-Pr_2CuLi. [f] Et_2CuLi. [g] Me_2CuLi. [h] $LiEt_3BH$. [i] O_3. [j] NaOH.

hydrolysis of the resulting ethylene glycol esters. The radiochemical purity of the intermediates in these syntheses was checked by thin-layer chromatography or paper chromatography followed by radiochromatogram scanning. Each of the samples of tritiated sodium octanoate was mixed with sodium

[1-^{14}C]octanoate and the tritium to carbon-14 ratio determined directly and by derivatization of a portion of the mixture with *p*-bromophenacyl bromide. The *p*-bromophenacyl esters derived from each mixture were recrystallized to constant activity and constant ratio. In each case, the tritium to carbon-14 ratios obtained directly were identical within experimental error with the ratios obtained by recrystallization of the *p*-bromophenacyl esters. The four samples of doubly labeled octanoate were then administered to *E. coli* and lipoic acid isolated as described above. The lipoic acid obtained from each experiment was derivatized with *p*-phenylbenzyl chloride and the derivatives recrystallized to constant activity and constant tritium to carbon-14 ratio. The bis(*p*-phenylbenzyl) acids were then converted into the corresponding methyl esters which were purified by chromatography and by recrystallization to constant activity and constant ratio. The results of these experiments are shown in Table I.

A number of conclusions can be drawn from the data in Table I. Experiments 1 and 2 clearly show that the introduction of sulfur at C-6 and C-8 of octanoic acid takes place without loss of hydrogen from C-5 or C-7. It therefore seems unlikely that unsaturation is introduced at C-5 or C-7 during the biosynthesis of (+)-lipoic acid from octanoic acid; however, the possiblility of enzymatic removal of hydrogen from C-5 or C-7 followed by replacement of the hydrogen without exchange cannot be excluded. Similar results have been obtained in investigations of the mechanism of sulfur introduction during biotin biosynthesis: the conversion of dethiobiotin (**4**) into biotin (**3**) has been found to proceed without hydrogen loss from C-2 or C-3.[3,11] Experiment 3 shows that the incorporation of sodium [8-^{3}H]octanoate into lipoate proceeds without tritium loss, within experimental error. This result is presumably the consequence of a substantial tritium isotope effect associated with the removal of a hydrogen atom from C-8 of octanoate. In contrast, the incorporation of [1-^{3}H]dethiobiotin into biotin has been found to proceed with little or no isotope effect.[3] Since the nature of the reaction(s) associated with the oxidation of the methyl groups in octanoic acid and dethiobiotin is unknown, it is not possible at present to account for these differences. Experiment 4 reveals that sodium [6(*RS*)-^{3}H]octanoate is incorporated into lipoic acid with about 50% tritium loss. This figure is precisely that expected for the stereospecific removal of one hydrogen atom from C-6 of octanoic acid as a consequence of sulfur introduction, and it parallels the results obtained when [4(*RS*)-^{3}H]dethiobiotin is transformed into biotin.[3] Work is in progress to determine the stereochemistry of the reactions associated with the introduction of sulfur at C-6 and C-8 of **2**.

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Ronald J. Parry[12]
Edison-Lecks Laboratories, Department of Chemistry
Brandeis University, Waltham, Massachusetts 02154
Received May 13, 1977

Book Reviews

Advances in Carbohydrate Chemistry and Biochemistry. Edited by R. STUART TIPSON and DEREK HORTON. Academic Press, New York, N.Y. 1976. Vol. 32: x + 439 pp. $42.00. Vol. 33: x + 464 pp. $44.00.

As expected of this series, these two volumes contain several excellent and definitive reviews of widely scattered literature. There is a reasonable proportion of material for the synthetic chemist and biochemist, a continuing bibliography of crystallographic data, and reminiscences on the lives of W. Z. Hassid and A. Gottschalk. Volume 32 contains articles on Dithioacetals of Sugars, J. D. Wander and D. Horton; Utilization of Sugars by Yeasts, J. A. Barnett; Noncytotoxic Antitumor Polysaccharides, R. L. Whistler, A. A. Bushway, P. P. Singh, W. Nakahara, and R. Tokuzen; Hemicellulases, R. F. H. Decker and G. N. Richards; Crystal Structures of Carbohydrates, Nucleosides and Nucleotides 1974, G. A. Jeffrey and M. Sundaralingham. Volume 33 contains Relative Reactivities of Carbohydrate Hydroxyl Groups, A. H. Haines; Synthesis of C Nucleosides, Analogs and Precursors, S. Hanessian and A. G. Permet; Reactions of D-Glucofuranurono-6,3-lactone, K. Dax and H. Weidmann; Chemistry of Sucrose, R. Khan; Pneumococcal Polysaccharides, O. Larm and B. Lindberg; Pectic Enzymes, L. Rexová-Benková and O. Markovic; Crystal Structures of Polysaccharides 1967–1974, R. H. Marchessault and P. R. Sundarajan.

This wide distribution of subject matter accurately reflects the changing character of carbohydrate research as well as the change in title of the series (in 1969) to include biochemistry. There is today less compartmentalized research on structure proof and transformations, per se, on carbohydrates as food and fiber components. The carbohydrate chemist now asks himself how his discipline advances knowledge on a wider front: the increasingly appreciated role of carbohydrates in information carrying, self and foreign recognition, and growth control mechanisms, in genetics, immunity, allergy, cell surface structure, metabolism, and other areas.

The chapters on crystallography, enzymes, and parts of other chapters call to mind that classic structure proof methods are now greatly supported if not superceded by biochemical, spectrometric, and crystallographic techniques. The specific examples of Larm and Lindberg and the chapter on antitumor polysaccharides emphasize that there are entire classes of important substrates that still await structural clarification. The synthetic chapters emphasize weaknesses and strengths of modern synthetic methodology and the difficult synthetic problems still presented by important natural carbohydrate structures. A review of the relative reactivity of hydroxyls indicates how nonspecific most direct methods of blocking hydroxyl groups still are. The few examples of specific reactions within metal complexes should excite young scientists to explore systematically the reactions