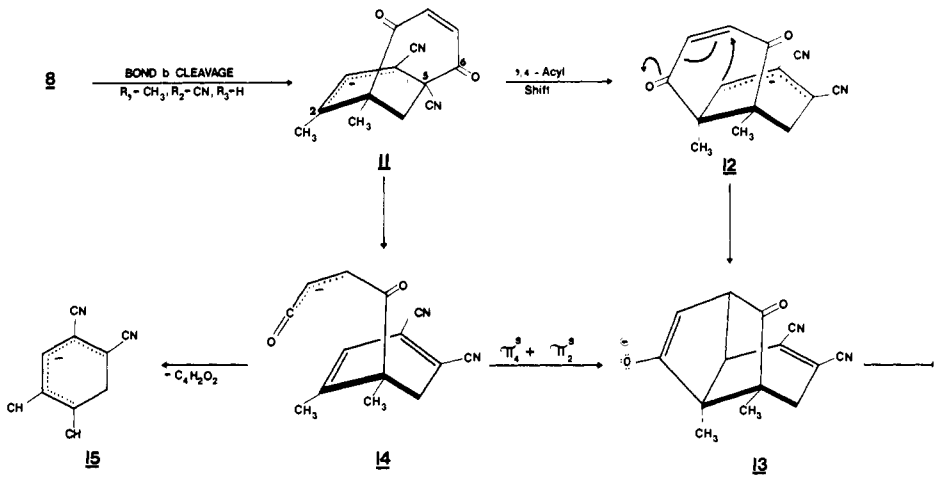
Scheme II



Michael addition as shown in the above reaction. Formation of diketone **5**, however, is impossible from **9** and requires an intervening isomerization of **9** into conformation **10** followed by the alternative intramolecular Michael addition shown. Similarly, a crossover closure from anion **10** to twistane **4** is geometrically prohibited. This scheme can be used to explain the **4**:**5** product ratio differences observed between substrates **3a** and **3b**, namely, that **3b** gives products of type **5** only and that **3a** gives both types.[7]

It appears that once again the dominant influence is the nature of R_2, in this case its steric effect. The simplest interpretation is that when R_2 = H (substrate **3b**) conformational equilibration of **9** and **10** is rapid relative to the rates of closure of **9** to **4** and **10** to **5**, but when R_2 = CH_3 (substrate **3a**), conformational equilibration is slower than closure. This effect stems from the fact that the equilibration necessarily involves eclipsing of the bridgehead R_2 groups. As a result, the **4**:**5** ratio for **3b** (R_2 = H) will be governed by the difference in the activation energies for the processes **9** → **4** and **10** → **5** (Curtin–Hammett principle).[8] Two factors lead to the clear prediction that the **10** → **5** closure should be favored as is observed experimentally. (1) The twistane ring system of **4** possesses a greater strain energy than the tricyclo[4.4.0.$0^{3,7}$]decane ring system of **5**,[9] and (2) bonding in **9** occurs between atoms which are not only further apart but more highly substituted (R_1 = CH_3) than in the case of **10**. In contrast, when equilibration between two conformers is *slower* than the rate of reaction of either, the product ratio depends on the relative conformer populations.[10] Thus, **3a** (R = CH_3) leads to twistane **4** via initially formed anion **9** before **9** can isomerize appreciably to **10**. As is observed experimentally,[7] this mechanism predicts larger amounts of product **5** at higher temperatures. We have observed similar methyl group eclipsing effects in some of our photochemical studies.[4]

Turning now to a discussion of the mechanism of the rearrangement of substrate **3c**, we suggest that the corresponding alkoxide undergoes preferential bond b cleavage to afford allyl anion **11** (Scheme II). There are several plausible mechanisms by which intermediate **11** can proceed on to final product **6**. One involves a symmetry-allowed[11] 1,4-sigmatropic acyl shift of **11** to give **12**. Carbanion **12** can in turn close to give **13** (the enolate of diketone **6**) via an internal Michael addition process exactly analogous to the conversion of **10** to **5** as previously shown. Anionic 1,4-sigmatropic rearrangements, while rare,[12] have been established for 2-alkoxypyridine *N*-oxides (migration from one oxygen atom to another).[13] Carbanion **11** could also rearrange to **12** via a nonconcerted process involving nucleophilic attack by C(2) on C(6) followed by C(5)–C(6) bond cleavage. A third possibility for the formation of **6** involves ring opening of **11** to give ketene enolate **14** followed by symmetry-allowed[11] [4 + 2] cycloaddition to afford, once again, enolate **13**. Intermolecular cycloadditions of allyl anions to olefins are well-established provided the product anion is resonance stabilized.[14] This condition is met in the conversion of **14** to **13**.

Finally, turning to the formation of 4,5-dimethylphthalonitrile (**7**) which is produced in 5% yield along with diketone **6** in the reaction of **3c**, we suggest that it arises via protonation of anion **15** followed by aromatization during workup. Anion **14** (as well as anion **12**) represents a possible precursor of carbanion **15** via expulsion of the bis ketene fragment $C_4H_2O_2$. No products resulting from reaction of this interesting species could be isolated, however.

In summary, the results provide novel examples of the ways in which substituents can affect anionic rearrangement pathways. Particularly intriguing are the unusual mechanistic possibilities presented by the rearrangement of dinitrile **3c**. Further work aimed at differentiating among these possibilities is under way.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada for financial support.

(7) The ratio of **4**:**5** (R_1 = R_2 = R_3 = CH_3) was found[2] to be 8:1 at 83 °C (potassium hydride in refluxing dimethoxyethane) and 1:1 at 101 °C (potassium *tert*-butoxide in refluxing dioxane). The 1:1 ratio includes a side product derived from **4** in potassium *tert*-butoxide–dioxane. Compounds **4** and **5** did not interconvert under either set of reaction conditions.

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John R. Scheffer,* Yiu-Fai Wong

Department of Chemistry, University of British Columbia
Vancouver, British Columbia, Canada V6T 1Y6

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Biosynthesis of Lipoic Acid: Extent of Incorporation of Deuterated Hydroxy- and Thiooctanoic Acids into Lipoic Acid

Sir:

It has been demonstrated that lipoic acid can be biosynthesized in *Escherichia coli* from octanoic acid.[1,2] It has also been dem-

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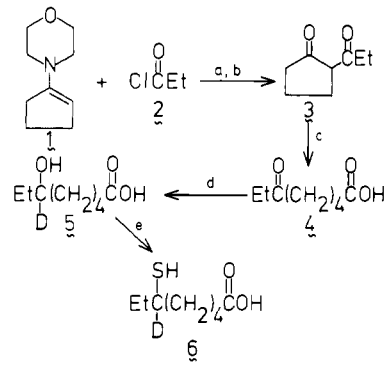
Table I. Incorporation of Deuterated Precursors into Lipoic Acid by *Escherichia coli*[a]

expt	precursor	amount added to culture, mg	% of lipoic acid derived from fed precursor[b]
1	[8,8-$^2H_2$]-6,8-dihydroxyoctanoic acid	55.6	<0.5
2a	[8,8-$^2H_2$]-8-hydroxyoctanoic acid	35.0	<0.5
2b	[8,8-$^2H_2$]-8-thiooctanoic acid	15.8	19.0
2c	[8,8-$^2H_2$]-8-thiooctanoic acid	30.0	27.9
3a	[6-^{2}H]-6-hydroxyoctanoic acid	35.0	<0.2
3b	[5,5,6,7,7-$^2H_5$]-6-thiooctanoic acid	~20.0	2.1
3c	[6-^{2}H]-6-thiooctanoic acid	32.3	5.1

[a] Cells were grown on 200 mL of a defined medium containing glucose and all of the amino acids, to which the indicated amounts of labeled precursors were added.[1,3] At the end of log-phase growth, the cells (2.3 g) were isolated by centrifugation, and the lipoic acid was extracted, derivatized, and assayed by gas chromatography–mass spectrometry for deuterium incorporation as previously described.[2] [b] The amount of lipoic acid produced in each culture was approximately 6.0 μg of lipoic acid per gram wet weight of cells.

Scheme I[a]



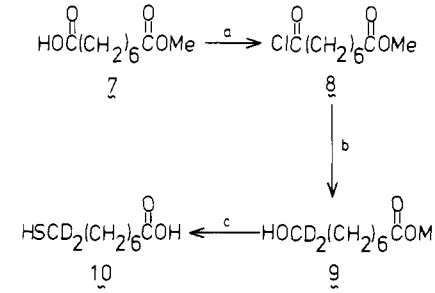
[a] (a) Et_3N in $CHCl_3$; (b) aqueous HCl; (c) aqueous NaOH; (d) $NaBD_4$; (e) $(NH_2)_2CS$ in 57% HI.

onstrated that each sulfur in the lipoic acid is inserted at each of the saturated carbons with the loss of only that hydrogen which each sulfur replaces. In addition, sulfur introduction at C-6 has been shown to involve an overall inversion of the stereochemistry at this carbon.[1,2] These observations are consistent with a pathway for lipoic acid biosynthesis which would involve hydroxylated octanoic acids, and this, in turn, is consistent with the known ability of bacteria to perform w and w-2 hydroxylations.[3,4]

In order to test for the possible involvement of hydroxylated octanoic acids and for the possible intermediacy of thiooctanoic acids in lipoic acid biosynthesis, the compounds shown in Table I were prepared and tested for their incorporation into lipoic acid. The compounds were synthesized with deuterium so that their incorporation into lipoic acid could be measured by gas chromatography–mass spectrometry as previously outlined.[2]

The [8,8-$^2H_2$]-6,8-dihydroxyoctanoic acid (98% $^2H_2$) was prepared by saponification of the 1,6-lactone of [8,8-$^2H_2$]-6-hydroxy-8-acetoxyoctanoic acid. This lactone was prepared from ethyl 2-oxocyclohexaneacetate as outlined by Segre et al.[5] with

Scheme II[a]



[a] (a) $SOCl_2$; (b) $NaBD_4$ in monoglyme; (c) $(NH_2)_2CS$ in 57% HI.

the following modifications: the ethylene ketal of ethyl 2-oxocyclohexaneacetate was reduced with lithium aluminum deuteride instead of lithium aluminum hydride, and the intermediate compounds at each step of the synthesis were purified by column chromatography instead of vacuum distillation.

[6-^{2}H]Hydroxyoctanoic acid and [6-^{2}H]-6-thiooctanoic acid were prepared as shown in Scheme I. 1-Morpholino-1-cyclopentene (**1**) was condensed in dry chloroform with propionyl chloride (**2**) in the presence of triethylamine to generate the ketoenamine adduct of 2-propionyl-1-cyclopentanone.[7] Acid hydrolysis of this adduct followed by alkaline cleavage of the released 2-propionyl-1-cyclopentanone (**3**) led to the generation of 6-ketooctanoic acid (**4**), mp 53–54 °C (lit.[8] mp 52–53 °C). Reduction of this compound in water with sodium borodeuteride gave [6-^{2}H]-6-hydroxyoctanoic acid (95.2% $^2H_1$) (**5**). Alternatively, the base-catalyzed exchange of the 6-ketooctanoic acid **4** in D_2O prior to borodeuteride reduction led to the isolation of [5,5,6,7,7-$^2H_5$]-6-hydroxyoctanoic acid (87.9% $^2H_5$). Treatment of these deuterated 6-hydroxyoctanoic acids with thiourea and hydroiodic acid followed by hydrolysis of the resulting thiuronium salts with sodium hydroxide gave the deuterated 6-thiooctanoic acids.[5,6]

The syntheses of the 8-hydroxy- and 8-thiooctanoic acids were performed as outlined in Scheme II. Suberic acid monomethyl ester (**7**) was reacted with thionyl chloride to give the acid chloride **8** which was reduced with sodium borodeuteride to monoglyme[9] to give [8,8-$^2H_2$]-8-hydroxyoctanoic acid (**9**), mp 56–57 °C (lit.[10] mp 56–57 °C) (87% $^2H_2$). The deuterated 8-hydroxyoctanoic acid was converted to the 8-thiol derivative **10** by the method described above for the preparation of **6**.

From the incorporation data reported in Table I, it is clear that the thiooctanoic acids are much more efficiently incorporated into lipoic acid than are the hydroxyoctanoic acids. The lack of incorporation of the hydroxyoctanoic acids could be due to the inability of the cells to take up the compounds, or it could be due to the rapid metabolism of hydroxyoctanoic acid into products not convertible into lipoic acid. In order to test these possibilities, a stable isotope dilution analysis was developed for the analysis of the distribution of [8,8-$^2H_2$]-8-hydroxyoctanoic acid at the end of a feeding experiment, i.e., 2a in Table I. The results of these analyses showed that almost all of the [8,8-$^2H_2$]-8-hydroxyoctanoic acid (35.0 ± 0.1 mg) was recovered unchanged in the medium. The cells, however, did contain 26.5 μg of [8,8-$^2H_2$]-8-hydroxyoctanoic acid per gram weight of cells, of which ~90% was present in a nonesterified form. This represents only a small amount of the material fed to the culture, but if only 1% had been converted into lipoic acid by the cells, then ~10% of the lipoic acid in the cells would have been derived from this labeled compound. Clearly, this amount of incorporation would have been easily detected by the methods used in this paper.

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These findings tend to discredit the idea that hydroxylated intermediates are involved in the biosynthesis of lipoic acid and support the idea that sulfur is introduced directly at the saturated carbons of octanoic acid as has been previously discussed.[2] This apparent lack of hydroxylated intermediates has been previously demonstrated in both biotin[11] and penicillin biosyntheses,[12] both being further examples of sulfur insertion at saturated carbons.

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Robert H. White

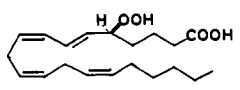
Department of Biochemistry and Nutrition
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

Received March 10, 1980

Simple Synthesis of the 11,12-Oxido and 14,15-Oxido Analogues of Leukotriene A and the Corresponding Conjugates with Glutathione and Cysteinylglycine, Analogues of Leukotrienes C and D

Sir:

Recent studies have led to the identification and synthesis of four of the naturally occurring "slow-reacting substances" (SRSs),[1,2] leukotriene C (**3**, RS = *S*-glutathionyl),[3,4] 11-*trans*-leukotriene C,[5] leukotriene D (**3**, RS = *S*-cysteinylglycyl), and leukotriene E (**3**, RS = cysteinyl).[3,5,6] These biologically active



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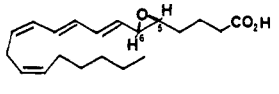

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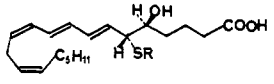

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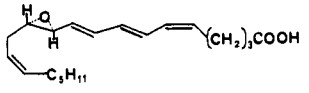

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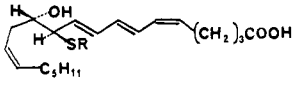

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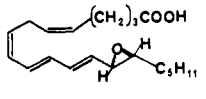

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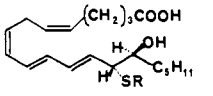
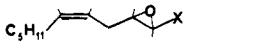


7



8 X = CHO
9 X = $(CH{=}CH)_2CHO$

eicosanoids are of unusual interest in a number of areas of experimental medicine since they appear to be major agonists in various forms of immediate hypersensitivity, including asthma, allergic rhinitis, and urticaria.[7] They are formed biosynthetically from the precursor leukotriene A (**2**),[2] which in turn arises from (5*S*)-HPETE (**1**).[8,9] Since both (12*S*)-HPETE and (15*S*)-HPETE are known and can be generated enzymatically and since it is reasonable that these could be predecessors of epoxyeicosatetraenoic acids (EPETEs) analogous to leukotriene A (**2**), i.e., compounds **4** and **6**, respectively, it is obvious that two new families of sulfur-linked peptide conjugates (**5** and **7**) are in principle possible.[10] In view of the possibility that the EPETEs **4** and **6** and the peptide conjugates **5** and **7** might be naturally occurring, biologically active eicosanoids, we have undertaken the synthesis of these substances. The successful completion of this endeavor has made these compounds available both for biological study and for comparison with yet unidentified natural agonists. Such studies could also shed light on the biological role of naturally occurring HPETEs.[11]

The synthesis of the racemic methyl ester of the 11,12-oxido analogue (11,12-EPETE, **4**) of leukotriene A (**2**) started from undeca-2,5-diyn-1-ol[12] which was converted to the aldehyde **8** by the following sequence: (1) selective reduction of the 2,3 triple bond by using excess lithium aluminum hydride in ether at 23 °C[13] for 3 h to afford undeca-5-yn-*trans*-2-en-1-ol (96% yield), (2) epoxidation of the double bond by using 1.1 equiv of *m*-chloroperbenzoic acid in methylene chloride containing powdered sodium carbonate (98% yield), (3) cis hydrogenation of the triple bond by using 5% palladium-on-calcium carbonate catalyst and 10 equiv of triethylamine in tetrahydrofuran (THF) at 23 °C with 1 atmosphere of hydrogen (100% yield), and (4) oxidation of alcohol to aldehyde **8** (93%) by using in situ generated CrO_3·2Pyr in methylene chloride at 23 °C for 15 min.[14] The aldehyde **8**[14] was converted to the epoxy trienal **9** (88% yield) as described for the synthesis of **2**[3] with 1-lithio-4-ethoxybutadiene[15] as reagent, and finally **9** was converted to the methyl ester of **4** by sequential reaction in THF–hexamethylphosphoramide and dimethyl sulfoxide with the ylide from 5-(triphenylphosphonio)pentanoic acid[16] (at −78 to 0 °C over 2.5 h) followed by sodium bicarbonate and excess dimethyl sulfate at 23 °C for 1 h. After extractive isolation and chromatography on silica gel in the presence of triethylamine (1:1 hexane–ether solvent), (±)-**4**[14] [UV_{max} 269, 277, 288 nm (ε at 277 nm 40000)] was obtained in 70% yield as the methyl ester[17]

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