Biosynthesis of Sulfur Compounds. Investigations of the Biosynthesis of Asparagusic Acid[1]

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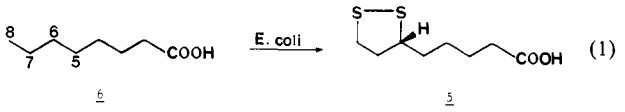
Abstract: Investigations of the biosynthesis of the naturally occurring 1,2-dithiolane asparagusic acid (**1**) in *Asparagus officinalis* have shown that the substance is derived from isobutyric acid via the intermediacy of methacrylic acid, 2-methyl-3-mercaptopropionic acid, and *S*-(2-carboxy-*n*-propyl)cysteine. The conversion of isobutyric acid to methacrylic acid in *Asparagus* has also been shown to proceed by oxidation of the 2-*pro*-*S* methyl group of isobutyrate. Finally, the absolute configuration of naturally occurring *S*-(2-carboxy-*n*-propyl)cysteine has been determined.

The naturally occurring 1,2-dithiolanes constitute a small group of natural products that have been isolated from a variety of living systems. Asparagusic acid (**1**) (Figure 1) has been obtained from both the roots and edible portions of *Asparagus officinalis*.[2-4] This dithiolane is a plant growth inhibitor exhibiting activity comparable to abscisic acid, and it also possesses potent nematicidal activity.[3,4] Other naturally occurring 1,2-dithiolanes include the alkaloids gerrardine (**2**) and brugine (**3**) which have been isolated from plants of the mangrove family (*Rhizophoraceae*) and the insecticidal 1,2-dithiolane nereistoxin (**4**) which occurs in a marine annelid.[6] The most widely distributed 1,2-dithiolane is probably α-(+)-lipoic acid (**5**) which functions as an essential coenzyme in many organisms.[7,8]

Until recently, very little has been known concerning the biosynthesis of the 1,2-dithiolanes. The first dithiolane to be carefully studied was lipoic acid, and the major features of the pathway leading to this compound have now been elucidated.[9] These will be summarized here in order to provide a background for our studies of asparagusic acid.

The biosynthesis of lipoic acid in *E. coli* has been found to proceed from octanoic acid (**6**) (eq 1). Furthermore, experiments with tritiated and deuterated forms of octanoate have revealed



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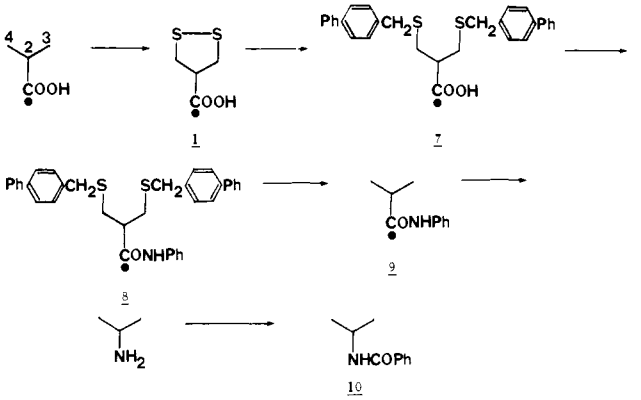
(a) that sulfur is introduced at C-6 and C-8 of the acid without apparent involvement of C-5 and C-7, (b) that functionalization proceeds by removal of one hydrogen atom from C-6 and one from C-8, and (c) that sulfur is introduced at C-6 of octanoic acid with overall inversion of configuration. Finally, evidence has been obtained which suggests that neither 6-hydroxy nor 8-hydroxyoctanoic acid is an intermediate in the sulfur introduction process but that 8-mercaptooctanoic acid may be involved.[9]

Results and Discussion

The initial stages of our investigation of the biosynthesis of asparagusic acid were based upon a presumed analogy with lipoic acid biosynthesis. Therefore, the first incorporation experiments were carried out with commercially available sodium [1-^{14}C]-isobutyrate which was administered to young *Asparagus officinalis* plants by the cottonwick method. Since asparagusic acid occurs in very low concentrations in *Asparagus*, the resulting labeled asparagusic acid was isolated by isotope dilution after 4 days. The asparagusic acid required for dilution purposes was obtained by modification of the Yanagawa synthesis[10] (see Experimental Section). The recovered asparagusic acid was derivatized as the bis(*p*-phenylbenzyl) thioether **7** (Scheme I). Purification of **7** by chromatography and repeated recrystallization gave the incorporation figure shown in Table I, experiment 1. Degradation of the thioether **7** was accomplished by conversion to the anilide **8** with DCC and aniline followed by Raney nickel desulfurization to give the anilide of isobutyric acid (**9**). Anilide **9** was purified by recrystallization and then hydrolyzed to yield isobutyric acid. The isobutyric acid was then degraded by the Schmidt reaction to yield isopropylamine, which was derivatized as the crystalline benzamide **10** for purposes of final purification. The amide **10** proved to be devoid of radioactivity (Table I, experiment 1), thereby showing that isobutyric acid had been specifically in-

Scheme I



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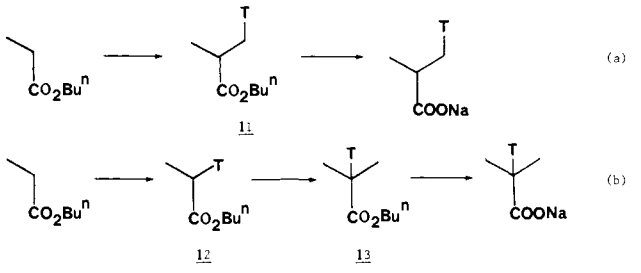
Scheme II



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(1) A portion of this work has appeared in preliminary form: (a) Parry, R. J.; Mizusawa, A. E; Riccardone, M. *J. Am. Chem. Soc.* **1982**, *104*, 1442. (b) Parry, R. J. *Tetrahedron* **1983**, *39*, 1215. (c) Parry, R. J.; Naidu, M. V. *Tetrahedron Lett.* **1983**, *24*, 1133.

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Table I. Precursor Incorporation Experiments with *Asparagus officinalis*

expt no.	precursor	precursor isotope ratio	% incorpn and/or isotope ratio in prod	labeling pattern or % isotope retent in prod
1	sodium [1-^{14}C]isobutyrate		1.2% into **1**	no label at C-2 to C-4 of **1**
2	sodium [3,4-^{3}H,1-^{14}C]isobutyrate	$^{3}H/^{14}C$ = 5.72	$^{3}H/^{14}C$ = 5.33 for **1**	96.7% ^{3}H retention in **1**
3	sodium [2-^{3}H,1-^{14}C]isobutyrate	$^{3}H/^{14}C$ = 6.36	$^{3}H/^{14}C$ = 0.16 for **1**	2.5% ^{3}H retention in **1**
4	sodium [1-^{14}C]methacrylate		0.38% into **1**	no label at C-2 to C-4 of **1**
5	(±)-sodium [2-^{3}H,1-^{14}C]-3-mercapto-2-methylpropanoate	$3H/^{14}C$ = 6.76	0.35% into **1**, $^{3}H/^{14}C$ = 0.154 for **1**	2.3% ^{3}H retention in **1**, no label at C-2 to C-4 of **1**
6	(±)-sodium [^{35}S,3(*R*,*S*)-^{3}H]-3-mercapto-2-methylpropanoate	$^{35}S/^{3}H$ = 0.341	1.1% into **1**, $^{35}S/^{3}H$ = 0.322 for **1**	94.4% ^{35}S retention in **1**
7	sodium [1-^{14}C]isobutyrate		0.04% into **20**	no label at C-2 to C-7 of **20**
8	[^{35}S,3(*R*,*S*)-^{3}H]-2(*RS*)-*S*-(2-carboxy-*n*-propyl)-L-cysteine	$^{35}S/^{3}H$ = 0.254	1.2% into **1**, $^{35}S/^{3}H$ = 0.256 for **1**	101% ^{35}S retention in **1**
9	potassium (*S*)-[3-^{3}H]isobutyrate		0.36% into **20**	4.1% label at C-4, 101% label at C-3
10	potassium (*R*)-[3-^{3}H]isobutyrate		0.32% into **20**	99.9% label at C-4

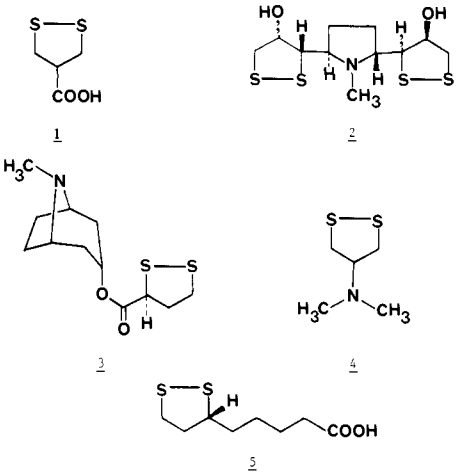
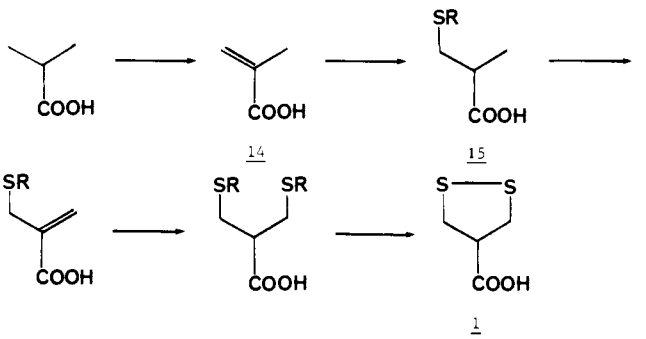


Figure 1.

corporated into asparagusic acid.

The specific incorporation of isobutyric acid into asparagusic acid having been established, experiments with tritiated forms of isobutyric acid were carried out to obtain clues to the mechanism of the introduction of sulfur into the isobutyrate molecule. Sodium [3,4-^{3}H]isobutyrate was synthesized as outlined in Scheme IIa. Propionic acid was converted to its *n*-butyl ester, which was relatively nonvolatile. This ester was treated with LDA in THF–HMPA[11] followed by alkylation with [^{3}H]methyl iodide to yield *n*-butyl [3,4-^{3}H]isobutyrate (**11**). Basic hydrolysis of **11** followed by acidification, steam distillation, and titration with alkali yielded the desired sodium [3,4-^{3}H]isobutyrate. Sodium [2-^{3}H]isobutyrate was synthesized in a similar fashion (Scheme IIb). The anion formed from *n*-butyl propionate and LDA was quenched with [^{3}H]trifluoroacetic acid to generate *n*-butyl [2-^{3}H]propionate (**12**). Deprotonation of **12** with LDA was then followed by alkylation with unlabeled methyl iodide to produce *n*-butyl [2-^{3}H]isobutyrate (**13**). The labeled ester **13** was finally converted into sodium [2-^{3}H]isobutyrate by hydrolysis, steam distillation, and titration. Each of the samples of specifically tritiated sodium isobutyrate was mixed with sodium [1-^{14}C]isobutyrate, and the tritium-to-carbon-14 ratios were checked directly and by derivatization of a portion of each mixture as the corresponding anilide. The two samples of doubly labeled sodium isobutyrate were then administered to *Asparagus*, and asparagusic acid was isolated and derivatized as already described. The results of these experiments appear in Table I (experiments 2 and 3). The incorporation of sodium [3,4-^{3}H]isobutyrate into asparagusic acid proceeded without tritium loss, within experimental error. The high degree of tritium retention observed in this experiment is presumably the consequence of a substantial tritium isotope

Scheme III



effect associated with the removal of a hydrogen atom from each of the methyl groups of isobutyrate. A similar degree of tritium retention accompanies the conversion of [8-^{3}H]octanoic acid into lipoic acid.[9] On the other hand, the incorporation of sodium [2-^{3}H]isobutyrate into asparagusate resulted in the loss of virtually all the tritium label. This result stands in complete contrast to the behavior observed during lipoic acid biosynthesis.[9] In the case of the latter 1,2-dithiolane, no tritium is lost from carbon atoms adjacent to the sites of sulfur introduction. It therefore appears that nature has at least two ways to create the 1,2-dithiolane ring system. A plausible explanation for the loss of tritium from sodium [2-^{3}H]isobutyrate would involve dehydrogenation to methacrylic acid (**14**) (Scheme III), a process that is reported to occur in animals and in microorganisms.[12,13] Consequently, sodium [1-^{14}C]methacrylate was synthesized by carboxylation of isopropenylmagnesium bromide with [^{14}C]carbon dioxide and administered to *Asparagus* plants. After 4 days radioactive asparagusic acid was obtained (Table I, experiment 4), and degradation in the usual manner (Scheme I) proved that the incorporation was specific.

The results of the aforementioned experiments suggest the biosynthetic pathway for asparagusic acid outlined in Scheme III. Dehydrogenation of isobutyric acid (or isobutyryl CoA) to methacrylic acid (**14**) could be followed by a Michael-type addition of an unknown sulfur nucleophile. A second dehydrogenation step would then lead to an unsaturated acid that could undergo addition of a second mole of the sulfur nucleophile to ultimately yield asparagusic acid. Additional incorporation experiments have been carried out that completely support this pathway.

Scheme III suggests that 2-methyl-3-mercaptopropanoic acid (**15**, R = H) may lie on the pathway from isobutyric acid to asparagusic acid. This possibility was evaluated by incorporation experiments with doubly labeled forms of **15** (R = H). Sodium [1-^{14}C]-2-methyl-3-mercaptopropanoate (**16**) was synthesized from [1-^{14}C]methacrylic acid by treatment with thiolacetic acid followed

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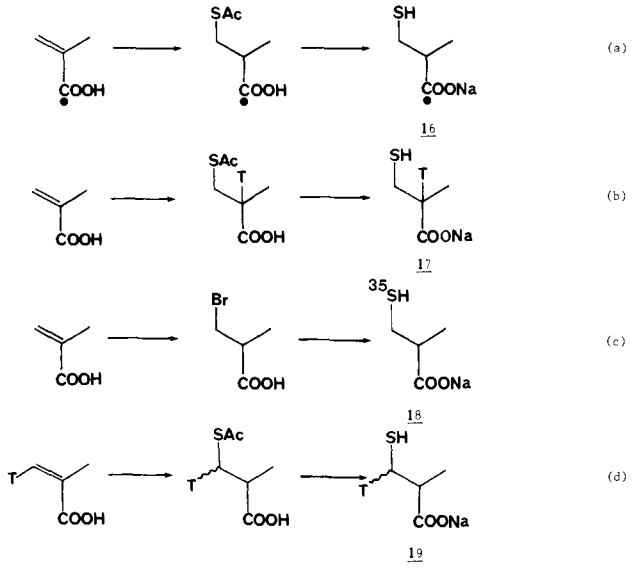
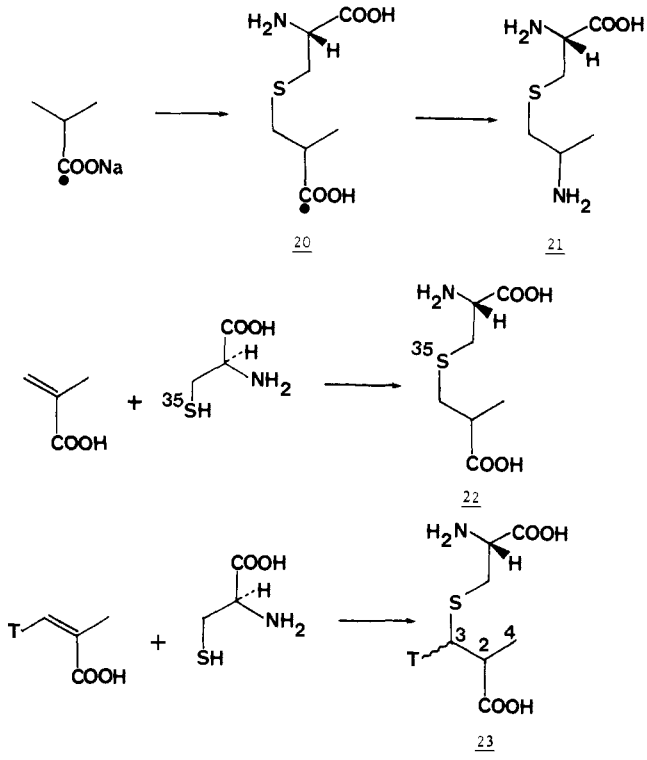
Scheme IV



Scheme V



by alkaline hydrolysis[14] (Scheme IVa). Similarly, sodium [2-^{3}H]-2-methyl-3-mercaptopropanoate (**17**) was prepared by Michael addition of thiolacetic acid to methacrylic acid in tritiated water with subsequent alkaline hydrolysis (Scheme IVb). [^{35}S]-2-Methyl-3-mercaptopropanoic acid (**18**) was obtained in two steps from methacrylic acid. Reaction of methacrylic acid with hydrogen bromide[15] gave 2-methyl-3-bromopropanoic acid which was then treated[15] with sodium [^{35}S]sulfide to yield **18** (Scheme IVc). Finally, sodium [3(*R*,*S*)-^{3}H]-2-methyl-3-mercaptopropanoate (**19**) was produced from (*Z*)-[3-^{3}H]-2-methylprop-2-enoic acid[16] by thiolacetic acid addition and alkaline hydrolysis (Scheme IVd). The labeled acids **16** and **17** were mixed to give [1-^{14}C,2-^{3}H]-2-methyl-3-mercaptopropanoate, and the labeled acids **18** and **19** were mixed to produce [^{35}S,3(*R*,*S*)-^{3}H]-2-methyl-3-mercaptopropanoate. The ratios of each doubly labeled precursor were measured directly and by derivatization of a portion of each mixture with *p*-bromophenacyl bromide. The two doubly labeled precursors were then administered to *Asparagus* plants to yield the results contained in Table I (experiments 5 and 6).

Three conclusions can be drawn from the data obtained in these experiments. First, it is clear that 2-methyl-3-mercaptopropanoic acid is a specific precursor of asparagusic acid. Second, asparagusic acid is formed from 2-methyl-3-mercaptopropanoate with complete retention of the sulfur atom present in the latter compound. This observation rules out the possibility that 2-methyl-3-mercaptopropanoate is incorporated into asparagusic by reversion to methacrylic acid. Finally, the incorporation of 2-methyl-3-mercaptopropanoic acid into asparagusic acid proceeds with the introduction of unsaturation between C-2 and C-4. The incorporation experiments with 2-methyl-3-mercaptopropanoic acid suggest that sulfide may be the sulfur donor in asparagusate biosynthesis. However, an alternative possibility is that cysteine is the immediate sulfur donor. This possibility arises from the fact that (−)-*S*-(2-carboxy-*n*-propyl)-L-cysteine (**15**, R = $CH_2CH(NH_2)CO_2H$, Scheme III) has been reported to occur in both onion and garlic plants.[17-19] Both of these plants are *Allium* species and are members of the *Liliaceae*. Since the genus *Asparagus* is also a member of the *Liliaceae*, it seemed probable that *S*-(2-carboxy-*n*-propyl)-L-cysteine might also occur in *A. officinalis*. This was shown to be the case by means of a trapping experiment. A mixture of the two diastereomeric forms of *S*-(2-carboxy-*n*-propyl)-L-cysteine was prepared by addition of L-cysteine to methacrylic acid.[20] Sodium [1-^{14}C]isobutyrate was then administered to *Asparagus* plants in the usual manner and the diastereomeric mixture of *S*-(2-carboxy-*n*-propyl)-L-cysteine added during workup after 4 days. The recovered *S*-(2-carboxy-*n*-propyl)-L-cysteine (**20**) proved to be radioactive (Table I, experiment 7), and degradation via a Schmidt reaction to the diamino acid **21** proved that the incorporation was specific (Scheme Va). Shortly, after this experiment had been performed, Kasai et al.[21] reported the isolation of (−)-*S*-(2-carboxy-*n*-propyl)-L-cysteine from *A. officinalis*, and they showed that the diastereomer present in *Asparagus* is the same as that present in *Allium* species.

The presence of (−)-*S*-(2-carboxy-*n*-propyl)-L-cysteine in *Asparagus* having been established, experiments were carried out to evaluate this substance as a precursor of asparagusic acid. For this purpose, [^{35}S]- and [^{3}H]-labeled forms of *S*-(2-carboxy-*n*-propyl)-L-cysteine (**22** and **23**) were synthesized as shown in Scheme Vb,c. The two labeled amino acids were then mixed to obtain [^{35}S,3(*R*,*S*)-^{3}H]-2(*R*,*S*)-*S*-(2-carboxy-*n*-propyl)-L-cysteine which was administered to *Asparagus* plants. The results of this experiment (Table I, experiment 8) reveal that the amino acid is specifically incorporated into asparagusic acid with retention of the sulfur atom derived from cysteine.

The combined data from experiments 5–8 (Table I) can be interpreted in two ways. One interpretation is that both (−)-*S*-(2-carboxy-*n*-propyl)-L-cysteine and 2-methyl-3-mercaptopropanoic acid lie on the pathway to asparagusic acid, with the former compound most likely preceding the latter. An alternative interpretation is that the two thio compounds are interconvertible in vivo (Scheme VI). If this is the case, then only one of the two sulfur compounds need lie on the pathway in order to explain the results. The interconversion between the two thio compounds could presumably be mediated by pyridoxal phosphate.

The aforementioned experiments suggest that (−)-*S*-(2-carboxy-*n*-propyl)-L-cysteine plays a significant role in the bio-

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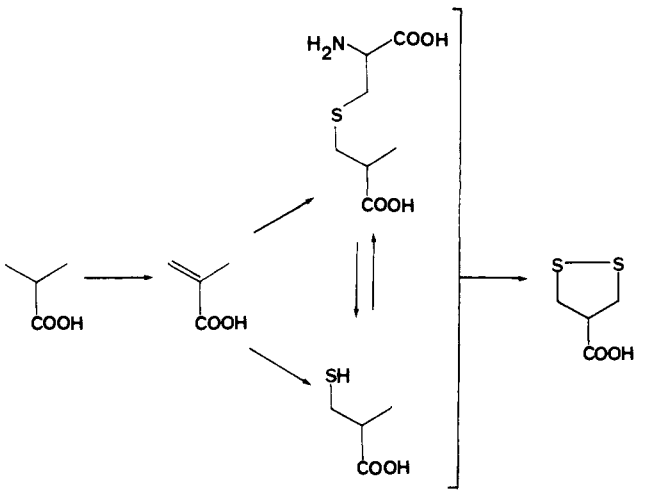
Scheme VI



synthesis of asparagusic acid. In addition, (−)-*S*-(2-carboxy-*n*-propyl)-L-cysteine has been shown[18,22] to be the precursor of *trans*-*S*-prop-1-enyl-L-cysteine sulfoxide in onions where the latter amino acid is in turn the progenitor of the lacrymatory principle.[23] For these reasons, it appeared desirable to determine the absolute configuration at C-2 of (−)-*S*-(2-carboxy-*n*-propyl)-L-cysteine. This was accomplished by asymmetric synthesis as outlined below.

Methyl (*S*)-(+)-3-hydroxy-2-methylpropanoate (**24**)[24] (Scheme VII) was converted to methyl (*R*)-(+)-3-(acetylthio)-2-methylpropanoate (**25**) by treatment with thiolacetic acid in the presence of DEAD and triphenylphosphine.[25] Deacetylation of **25** with sodium ethoxide in ethanol yielded (*R*)-3-mercapto-2-methylpropanoate (**26**). The benzyl ester of (*S*)-aziridinecarboxylic acid was next prepared from L-serine by the method of Nakajima et al.[26] and acylated with ethyl chloroformate to yield the optically active aziridine **27**. Finally, the thiol ester **26** was reacted with the aziridine **27** in the presence of boron trifluoride etherate[27] to produce the adduct **28**. Adduct **28** was deprotected to yield the 2(*R*) diastereomer of *S*-(2-carboxy-*n*-propyl)-L-cysteine (**29**) by sequential treatment with (trimethylsilyl)iodide,[28] which removed the urethane and benzyl ester moieties, followed by 1 N potassium hydroxide, which hydrolyzed the methyl ester. The 2(*S*) diastereomer of *S*-(2-carboxy-*n*-propyl)-L-cysteine (**31**) was prepared in a similar manner from methyl (*S*)-(−)-3-(acetylthio)-2-methylpropanoate (**30**)[29] (Scheme VII). The 2(*R*) diastereomer **29** exhibited $[\alpha]^{25}_D$ +34.5° (*c* 0.04, H_2O) while the 2(*S*) diastereomer **31** gave $[\alpha]^{25}_D$ −58.3° (*c* 0.04, H_2O). *S*-(2-Carboxy-*n*-propyl)-L-cysteine isolated from hydrolysis of naturally occurring *S*-(2-carboxy-*n*-propyl)glutathione has been reported[17] to show $[\alpha]^{21}_D$ −50.1° (H_2O). Furthermore, Carson[30] resolved the two diastereomeric forms of *S*-(2-carboxy-*n*-propyl)-L-cysteine and reported rotations for the two isomers of $[\alpha]^{25}_D$ −66.1° (*c* 2.5, H_2O) and $[\alpha]^{25}_D$ +35.8° (*c* 1.25, H_2O). From these data, we conclude that naturally occurring (−)-*S*-(2-carboxy-*n*-propyl)-L-cysteine has the *S* configuration at C-2 and corresponds to structure **31** (Scheme VII).

The experiments summarized in Table I demonstrate that asparagusic acid is biosynthesized by oxidation of isobutyric acid to methacrylic acid followed by addition of a sulfur nucleophile. Since the two methyl groups of isobutyrate are enantiotopic, the oxidation process should be stereospecific and take place exclusively at either the 2-pro-*R* or the 2-pro-*S* methyl group. The experiments that will now be discussed prove that this is indeed the case.

In order to carry out the stereochemical analysis, potassium (2*S*)-[3-^{3}H]- and (2*R*)-[3-^{3}H]isobutyrate were synthesized by modification of the method of Aberhart.[31,32] The major modification was the use of the Sharpless chiral epoxidation[33] to synthesize (2*R*,3*R*)- and (2*S*,3*S*)-*trans*-2,3-epoxy-1-butanol. These chiral epoxy alcohols had been previously prepared via the resolution of *trans*-2,3-epoxybutanoic acid. The chiral epoxides were opened with [^{3}H]methyllithium[31] and the resulting diols oxidized with permanganate[32] to give (2*S*)- and (2*R*)-[3-^{3}H]-isobutyric acid, which was isolated as the potassium salt. In our hands, the salts proved to be impure, and so an additional purification step was introduced wherein the potassium salts were converted to their benzyl esters. The esters were purified chromatographically and then cleaved by catalytic hydrogenolysis to afford pure (2*S*)- and (2*R*)-[3-^{3}H]isobutyrate in ca. 4% overall yield from crotyl alcohol. The synthesis of the 2*S* isomer is summarized in Scheme VIII.

When the potassium salts of both (2*S*)- and (2*R*)-[3-^{3}H]isobutyrate were in hand, both were administered to *Asparagus* plants, and *S*-(2-carboxy-*n*-propyl)-L-cysteine was isolated after 4 days by isotope dilution. The labeled samples of amino acid were purified as their *N*-benzoyl derivatives, and the *N*-benzoyl-*S*-(2-carboxy-*n*-propyl)-L-cysteine (**32**) obtained from (2*S*)-[3-^{3}H]isobutyrate was degraded in two ways. The first degradation involved Kuhn–Roth oxidation to isolate C-2 and C-4 as *p*-bromophenacyl acetate. The *p*-bromophenacyl ester obtained in this fashion was nearly radioinactive (Table I, experiment 9). This result suggested that the label in *S*-(2-carboxy-*n*-propyl)-L-cysteine derived from (2*S*)-[3-^{3}H]isobutyrate was probably located at C-3 of the amino acid. This was shown to be the case by degradation of the amino acid by the method outlined in Scheme IX. Conversion of the *N*-benzoyl amino acid **32** to the dibenzyl ester **33** (83%) was followed by alkylation of the sulfur atom with methyl triflate and base-catalyzed elimination to yield benzyl 2-methyl-3-(methylthio)propanoate (**34**) (80%, two steps). Sequential treatment of **34** with methyl triflate and DBU then gave the benzyl ester of methacrylic acid (**35**) (67%, two steps). Osmate–periodate cleavage of **35** yielded radioactive formaldehyde which was trapped as its dimedone adduct. The resulting dimedone–formaldehyde contained 100% of the expected radioactivity (Table I, experiment 9), thereby proving that the conversion of isobutyrate to methacrylate in *Asparagus* plants proceeds by oxidation of the 2-*pro*-*S* methyl group. This conclusion was confirmed by Kuhn–Roth oxidation of the *S*-(2-carboxy-*n*-propyl)-L-cysteine biosynthesized from (2*R*)-[3-^{3}H]isobutyrate. In this instance, all the expected radioactivity was found to reside in the *p*-bromophenacyl acetate (Table I, experiment 10). It is of interest to note that the oxidation of isobutyric acid to methacrylic acid by *Pseudomonas putida* also takes place at the 2-*pro*-*S* methyl group.[32]

Conclusions

Three general conclusions can be derived from the investigations presented in this article. First, it has been shown that the biosynthesis of the 1,2-dithiolane asparagusic acid proceeds by a different mechanism than the biosynthesis of the 1,2-dithiolane lipoic acid. The introduction of both sulfur atoms of asparagusic acid appears to proceed via the addition of a sulfur nucleophile to an α,β-unsaturated acid. In contrast, lipoic acid appears to be biosynthesized by the direct introduction of sulfur at two saturated carbon atoms. Second, it has been demonstrated that cysteine can act as the donor for at least one of the sulfur atoms

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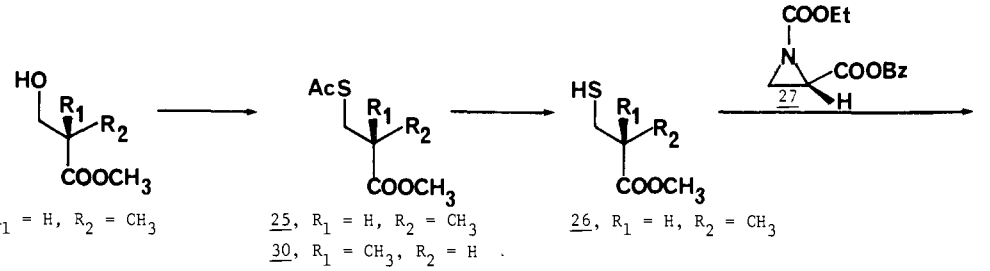
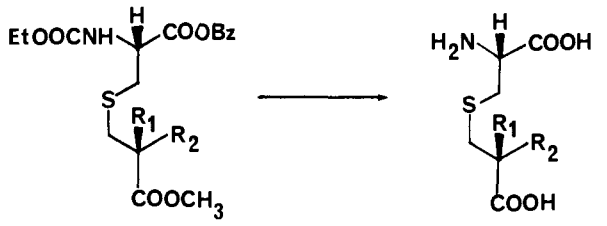
Scheme VII

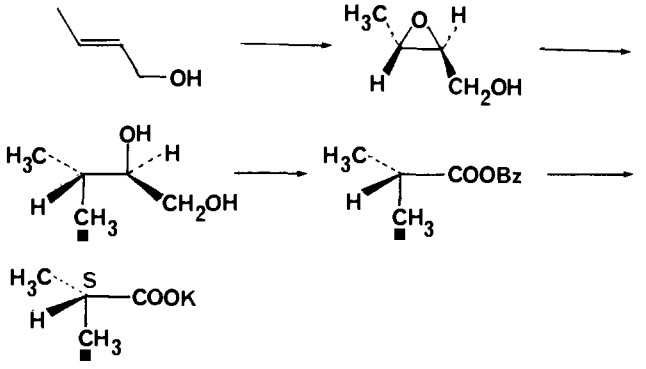


24, $R_1 = H$, $R_2 = CH_3$ 25, $R_1 = H$, $R_2 = CH_3$ 26, $R_1 = H$, $R_2 = CH_3$
30, $R_1 = CH_3$, $R_2 = H$

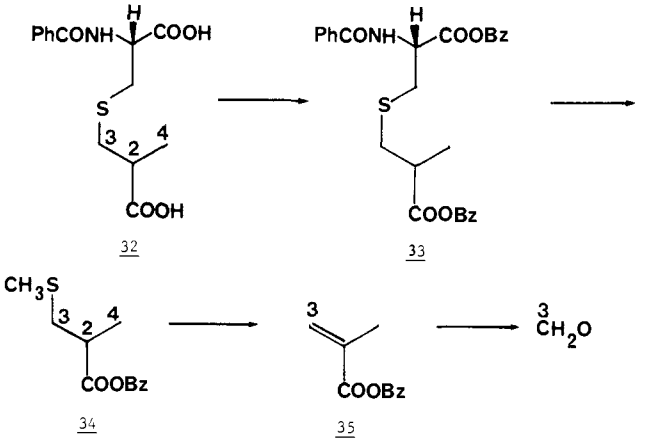


28, $R_1 = H$, $R_2 = CH_3$ 29, $R_1 = H$, $R_2 = CH_3$
31, $R_1 = CH_3$, $R_2 = H$

Scheme VIII



Scheme IX



of asparagusic acid. The nature of the sulfur donor(s) in lipoic acid biosynthesis is presently unclear. Finally, it has been established that the oxidation of isobutyric acid to methacrylic acid in *Asparagus*, a eucaryotic organism, involves the 2-pro-*S* methyl group; the same stereochemistry is exhibited in the oxidation of isobutyrate to methacrylate by the procaryote, *Pseudomonas putida*.

Experimental Section

General Considerations. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Nuclear magnetic resonance spectra were obtained by using Varian EM-390 and Jeol FX-90Q NMR spectrometers. Chemical shifts are recorded in parts per million (δ) downfield from tetramethylsilane. Mass spectra were measured with a Finnigan 3300 and a CEC 1110 21-110B mass spectrometer. Infrared spectra were recorded on a Beckmann 4230 spectrophotometer. Gas chromatography was accomplished with Perkin-Elmer 3920 (6-ft × $^1/_8$-in., 10% UCW-96 on Chromosorb) and Hewlett-Packard 5710A (6-ft × 2-mm, 10% UCW-98 on Chromosorb) gas chromatographs.

The radioactivity of labeled compounds was measured by using a Beckmann LS 100-C liquid scintillation counter with a toluene-based scintillation cocktail or Aquasol, an aqueous scintillation cocktail purchased from New England Nuclear Corp. [^{3}H]- and [^{14}C]Toluene as well as [^{35}S]dioctyl sulfide were used for internal standardization of the radioactive samples. Radioisotopes for standardization, synthesis, and incorporation experiments were purchased from New England Nuclear Corp., Amersham-Searle Corp., and ICN. A Varian 6000-1 radiochromatogram scanner was employed to measure the radiochemical purity of radioactive samples.

Asparagus roots ("Mary Washington") were purchased from the W. Atlee Burpee Co., and the plants were maintained in a greenhouse until 2 weeks prior to an incorporation experiment, at which time they were transferred to a LAB-LINE Biotronette Mark III environmental chamber.

Preparative thin-layer chromatography was accomplished by using 750-nm layers of Merck silica gel 60 PF-254 and analytical TLC utilized Merck Polygram SIL 6/UV_{259} (0.25 mm) plates. Column chromatography was performed by using either Grace silica gel (grade H), Mallinckrodt SilicAR CC-4, or Merck microcrystalline cellulose EM-2331. Visualization was accomplished by short- or long-wave UV, phosphomolybdic acid, bromocresol green, iodine, or ninhydrin.

Feeding Experiments. Administration of Labeled Compounds to *Asparagus* and Workup of Plant Material to Isolate Asparagusic Acid. Whole *Asparagus* plants 1–2 ft high growing in an environmental chamber with a 12-hr day cycle were fed tracer solution via the cotton-wick method, and the plants were allowed to metabolize the precursor for 4 days. The plants were harvested and homogenized in 95% ethanol in a commercial Waring blender. Synthetic asparagusic acid (ca. 250 mg) was added to the resulting slurry which was then poured into a large chromatographic column. Ethanol was percolated slowly through the plant material until about 12 L had been collected. The ethanol was removed in vacuo and the residue partitioned between ether and aqueous bicarbonate (300 mL). The aqueous phase was acidified with concentrated hydrochloric acid to pH 3 and extracted repeatedly with ethyl acetate. The combined organic extracts were dried and evaporated to give crude asparagusic acid.

Derivatization of Asparagusic Acid as *S,S'*-Bis(*p*-phenylbenzyl)dihydroasparagusate (7). Liquid ammonia (15 mL) was distilled from sodium into a three-necked flask equipped with a drying tube and a dry-ice condenser. Crude asparagusic acid (ca. 250 mg, 1.7 mmol) reisolated from a precursor incorporation experiment was dissolved in dry THF (2 mL) and added alternately with small pieces of sodium metal while stirring until a permanent blue color was obtained. The blue color was then quenched by the addition of a small quantity of synthetic asparagusic acid. Chromatographically pure *p*-(chloromethyl)biphenyl (0.68 g, 3.4 mmol) was added with stirring and the mixture refluxed at −33 °C for 1 h. The condenser was then removed and the ammonia allowed to evaporate. Residual solvent was removed in vacuo, and water (25 mL) was added to the residue. The mixture was acidified to pH 2 and extracted with chloroform (3 × 25 mL). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to give a residue that was purified by preparative TLC (silica PF-254, 1:10 ethyl acetate–benzene) to yield the purified derivative which was recrystallized from benzene–cyclohexane to give white crystals (120 mg, 15%): mp 135–136 °C; ^1H NMR ($CDCl_3$) δ 2.78 (5 H, m), 3.73 (4 H, s), 7.0–8.1 (18 H, m): MS (EI), *m/e* 317.0671 (317.066 985 calcd for $C_{17}H_{17}O_2S_2$, M − 167), 199.0583 (199.058 145 calcd for $C_{13}H_{11}S$, M − 285), 167.0858 (167.086 075 calcd for $C_{13}H_{11}$, M − 317).

S,S'-Bis(*p*-phenylbenzyl)dihydroasparagusic Acid Anilide (8). Freshly distilled oxalyl chloride (0.24 mL, 2.85 mmol) was added to *S,S'*-bis(*p*-phenylbenzyl)dihydroasparagusic acid (130 mg, 0.27 mmol) in dry benzene (4.7 mL), at room temperature under nitrogen. The solution was refluxed at 80 °C for 15 min and allowed to stand for 2 h at room temperature. The benzene and excess oxalyl chloride were removed in vacuo, and the yellow residue was repeatedly dissolved in benzene and evaporated to remove all traces of the oxalyl chloride. The residue, dissolved in benzene, was cooled in an ice–water bath, and aniline (0.26 mL, 2.9 mmol) was added to it. The resulting solution was stirred overnight. The reaction mixture was extracted with 6% hydrochloric acid (3 × 15 mL) and water (3 × 15 mL). The organic layer was dried over anhydrous magnesium sulfate and the solvent removed. The solid residue was recrystallized from benzene–cyclohexane to yield *S,S'*-bis(*p*-phenylbenzyl)dihydroasparagusic acid anilide (103 mg, 67% yield): mp 167–168 °C; ^1H NMR ($CDCl_3$) δ 2.66 (5 H, m), 3.70 (4 H, s), 7,40 (23 H, m); IR ($CHCl_3$) 3450, 1710 cm^{-1}; MS (EI), *m/e* 392.1139 (392.114 270 calcd for $C_{23}H_{22}OS_2N$, M − 167), 167.0858 (167.086 075 calcd for $C_{13}H_{11}$, M − 392).

Raney Nickel Desulfurization of S,S'-Bis(*p*-phenylbenzyl)dihydroasparagusic Acid Anilide (8). Raney nickel catalyst[34] (3 g) was added to *S,S'*-bis(*p*-phenylbenzyl)dihydroasparagusic acid anilide (113.8 mg, 0.20 mmol) in 100% ethanol (10 mL). The mixture was heated for 7 h at 90 °C and the catalyst filtered off. The solution was evaporated and the crude product chromatographed on preparative silica TLC plates (1:4 ethyl acetate–toluene). Isobutyric acid anilide (9) was obtained (27 mg, 85% yield, mp 106–107 °C (lit[35] mp 105 °C)).

Hydrolysis of Isobutyric Acid Anilide (9). Isobutyric acid anilide (65 mg, 0.4 mmol) was dissolved in 20% hydrochloric acid (4 mL) and heated at 95 °C for 5 h. The resulting solution was allowed to cool, the pH was adjusted to 11 with 0.5 N sodium hydroxide, and the basic solution was extracted with ether to remove aniline. The aqueous phase was taken to dryness in vacuo and the residue extracted with 3 mL of 100% ethanol. The ethanol extract was filtered and evaporated to yield crude sodium isobutyrate (50 mg, 113%, indicating the presence of NaOH).

Schmidt Degradation of Sodium Isobutyrate. Crude sodium isobutyrate (50 mg, ca. 0.45 mmol) obtained by hydrolysis of isobutyric acid anilide was placed in a 5-mL round-bottom flask and 100% sulfuric acid (0.27 mL) added while cooling the flask in an ice bath. Sodium azide (50 mg, 0.77 mmol) was then added, and the reaction mixture was heated to 70 °C and held there for 1 h. The reaction mixture was then cooled in ice and the pH carefully adjusted to 12 with 5 N sodium hydroxide. Benzoyl chloride (0.12 mL, 1 mmol) was added and the solution stirred at room temperature for 30 min. Additional benzoyl chloride (0.12 mL, 1 mmol) was added and the pH readjusted to 12. After 15 min, the mixture was extracted with chloroform. The organic layer was washed with aqueous bicarbonate and with water and then dried ($MgSO_4$). The residue obtained by removal of the chloroform was purified by preparative TLC on silica plates (1:10 ethyl acetate–benzene) to give *N*-isopropylbenzamide (**10**) (20 mg, 27%): mp 104–105 °C (lit[36] mp 99 °C).

Synthesis of Asparagusic Acid. A. Ethyl Bis(hydroxymethyl)malonate. Ethyl bis(hydroxymethyl)malonate was prepared by the procedure of Gault and Roesch.[37]

B. β,β'-Diiodoisobutyric Acid. Attempts to prepare β,β'-diiodobutyric acid directly from bis(hydroxymethyl)malonate by the procedure of Yanagawa et al.[10] led to mixtures of β,β'-diiodoisobutyric acid and α-(iodomethyl)acrylic acid which were formed in poor yield. Pure β,β-diiodoisobutyric acid was obtained in two steps by modification of the procedures of Welch[38] and Corse.[39]

(a) α-(Iodomethyl)acrylic Acid Plus β,β'-Diiodoisobutyric Acid. Ethyl bis(hydroxymethyl)malonate (60 g, 0.3 M) was dissolved in 243 g (1.1 M) 57% hydroiodic acid to form a clear, yellow solution. This reaction mixture was gradually heated to 120 °C and held at this temperature for 45 min during which time an evolution of gas was noted and the solution turned a dark reddish-brown. Volatile materials were removed directly into the hood as they interfered with the reaction when contained by use of a condenser. After the solution was cooled at room temperature for 8–16 h, yellow crystals were deposited from the solution. These were collected on a sintered glass funnel. After the crystals are washed with a minimum volume of chilled H_2O, the resulting slightly yellow material is dissolved in an excess of hot carbon tetrachloride, dried over magnesium sulfate, and concentrated in vacuo until crystals just appeared. Crystallization was allowed to proceed at −5 °C before collecting 17.07 g white powder whose ^1H NMR ($CDCl_3$) indicated it to be a mixture of α-(iodomethyl)acrylic acid and β,β'-diiodoisobutyric acid: ^1H NMR ($CDCl_3$) δ 2.88–3.22 (1 H, quintet), 3.50–3.70 (4 H, d), 4.18 (2 H, s), 6.15 (1 H, s), 6.46 (1 H, s).

(b) β,β'-Diiodoisobutyric Acid. The mixture of α-(iodomethyl)acrylic acid and β,β'-diiodoisobutyric acid (25.7 g) obtained in step a was ground in a motar and pestle with potassium iodide (32 g, 0.19 M) and cold 95% phosphoric acid (57.6 g, 0.56 M) added to make a smooth yellow paste. This mixture was heated for 6 h at 100 °C during which time the separation of crystals and a red oil were noted. The reaction mixture was cooled to room temperature and then poured into ice–water. The chunks of yellow–brown solid which separated were triturated and collected on a sintered glass funnel. The product was washed with a solution of 0.5 g of sodium thiosulfate in 25 mL of water, and the resulting orange product was dried over P_2O_5. The crude product (31.9 g) was dissolved in excess ethyl acetate and filtered to remove insoluble matter. The filtrate was concentrated in vacuo and cold petroleum ether added to induce crystallization. The product separated as colorless plates (17 g, 36% yield): mp 128–130 °C (lit[34] mp 128–129 °C); NMR ($CDCl_3$) δ 2.88–3.22 (1 H, quintet), 3.52–3.70 (4 H, d).

C. Dihydroasparagusic Acid. This compound was prepared from β,β-diiodoisobutyric acid by using the procedure of Yanagawa et al.[10] The yield was 59%, and the compound could not readily be obtained in crystalline form contrary to the previous report;[10] NMR ($CDCl_3$) δ 1.49 (t, 2 H, exchanging with D_2O), 2.80–2.90 (5 H, overlapping doublet and quintet).

D. Asparagusic Acid. Asparagusic acid was prepared from dihydroasparagusic acid by heating in Me_2SO using the procedure of Yanagawa et al.[10] The yield of compound, (mp 72–76 °C (lit[10] 75.7–76.5 °C)) was 60%: NMR ($CDCl_3$) δ 2.52–2.82 (5 H, m).

Sodium [3,4-^{3}H]Isobutyrate. A. *n*-Butyl Propionate. Propionyl chloride (9.25 g, 0.1 M) dissolved in methylene chloride (10.7 mL) was slowly added over 1 h to a chilled solution of pyridine (8.06 mL, 0.1 M), *n*-butyl alcohol (9.15 mL, 0.1 M), and methylene chloride (10.7 mL). The solution was stirred in an ice bath for 4 h and then overnight at room temperature. The reaction mixture was extracted with water, the organic phase was dried by using anhydrous magnesium sulfate, and the solvent was removed. The resulting liquid was distilled to yield *n*-butyl propionate (12.8 g, 99% yield, bp 140–150 °C at 360 mm): NMR ($CDCl_3$) δ 0.77–1.80 (10 H, m), 2.27 (2 H, q), 4.10 (2 H, t). Anal. Calcd for $C_7H_{14}O_2$: C, 64.58; H, 10.84. Found: C, 64.31; H, 10.99.

B. *n*-Butyl [3,4-^{3}H]Propionate (11). [^{3}H]Methyl iodide (100 mCi, Amersham) was vacuum transferred into a flask containing nonradioactive methyl iodide (0.15 mL, 2.4 mmol) and HMPA (0.1 mL).

n-Butyl propionate was alkylated by following the procedure of Schlessinger et al.[11] LDA was prepared by addition of butyllithium (1.23 mL, 2 mmol) to diisopropylamine (0.28 mL, 2 mmol) in THF (1.7 mL) at −78 °C under nitrogen. The solution was warmed to 0 °C over 30 min to allow complete formation of the LDA, and it was then recooled to −78 °C. *n*-Butyl propionate (0.3 mL, 2 mmol) was added, and the solution was stirred for 1 h at −78 °C. The previously prepared tritiated methyl iodide/HMPA solution was then syringed into the reaction flask at −78 °C to quench the anion. The mixture was stirred for 30 min while allowing the solution to warm to room temperature. The THF was

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removed, and the residue was dissolved in ether. The organic layer was washed with 10% hydrochloric acid, sodium bicarbonate, and saturated sodium chloride and dried over anhydrous magnesium sulfate. The ether was removed to yield *n*-butyl [3-^{3}H]isobutyrate (194 mg, 67% yield) which was checked for purity by gas chromatography. The ester was hydrolyzed by refluxing it for 7.5 h with dilute sodium hydroxide. The solution was acidified to pH 1 with sulfuric acid and subjected to steam distillation. The distillate (300 mL) was brought to pH 8 with a dilute solution of sodium hydroxide. The water was removed in vacuo, and the resulting sodium [3,4-^{3}H]isobutyrate was shown to be pure by using analytical TLC (cellulose, 3:1 isopropyl alcohol–2 N ammonium hydroxide) with visualization by bromocresol green and radiochromatogram scanning. The yield was 70 mg (48%, sp activity = 0.24 mCi/mg; radiochemical yield = 17%).

Sodium [2-^{3}H]Isobutyrate. *n*-Butyl propionate was treated with lithium diisopropylamide (LDA) following the procedure of Schlessinger et al.[11] Diisopropylamine (0.56 mL, 4 mmol) in a mixture of THF (10 mL) and HMPA (0.1 mL) was stirred at −78 °C under nitrogen for 15 min. LDA was generated in situ by addition of butyllithium (2.5 mL, 4 mmol) at −78 °C. The solution was warmed to 0 °C over 30 min to allow complete formation of LDA and was then recooled to −78 °C. *n*-Butyl propionate (0.6 mL, 4 mmol) was added, and the solution was stirred for 1 h at −78 °C. The resulting anion was quenched at this temperature with [^{3}H]trifluoroacetic acid (0.47 mL, 6 mmol, 30 mCi) prepared from trifluoroacetic anhydride and tritiated water.[40] The solution was stirred for 30 min at −78 °C and at room temperature for 1 h, and the THF was then removed by bulb-to-bulb distillation. The yellow residue dissolved in ether was washed with 5% hydrochloric acid, water, sodium bicarbonate, and brine and dried over anhydrous magnesium sulfate. The ether was removed, and the resulting liquid (340 mg) was purified by silica gel column chromatography (1:1 benzene–hexanes). The fractions were monitored for *n*-butyl [2-^{3}H]propionate by gas chromatography. After purification the tritiated ester (64 mg, 12% yield) was diluted with nonradioactive *n*-butyl propionate (62.75 mg).

Alkylation of the *n*-butyl [2-^{3}H]propionate with methyl iodide was carried out in the same manner as the alkylation of unlabeled *n*-butyl propionate with [^{3}H]methyl iodide. The resulting *n*-butyl [2-^{3}H]isobutyrate was hydrolyzed with base and the free acid isolated by steam distillation. After conversion to the sodium salt, 89 mg of sodium [2-^{3}H]isobutyrate was obtained (83% yield, sp activity = 0.015 mCi/mg, 4.4% radiochemical yield). The salt found to be pure by analytical TLC.

Potassium [1-^{14}C]Methacrylate. 2-Bromopropene (8.89 mL, 0.1 M) in dry ether (36 mL) was added dropwise over 1 h to a flask containing magnesium metal (26.7 g, 1.1 M) in dry ether (36 mL). The solution was refluxed at 45 °C for 1 h, and it was then decanted from the excess magnesium turnings. The molarity of the solution containing the Grignard reagent in ether was determined by titration with *s*-butyl alcohol in the presence of 1,10-phenanthroline.[41] It was found to be 0.6 M. [^{14}C]Carbon dioxide, which was generated by addition of concentrated sulfuric acid to barium [^{14}C]carbonate (52.7 mg, 0.27 mmol, 38 mCi/mmol), was vacuum-transferred into a solution of the Grignard reagent (4.4 mmol) in ether. Unlabeled carbon dioxide was generated in the same way from barium carbonate (148.3 mg, 0.75 mmol) and vacuum-transferred to consume the unreacted Grignard reagent. The ether was evaporated from the reaction mixture and the solution acidified to pH 3 with sulfuric acid. Hydroquinone (1 mg) was added, and the reaction mixture was subjected to steam distillation. The distillate was titrated with potassium hydroxide to pH 8. Water was removed and potassium [^{14}C]methacrylate (19.5 mg, 16% yield, sp activity = 0.028 mCi/mg, 5.4% radiochemical yield) was obtained. Analytical TLC (cellulose, 3:1 isopropylalcohol–ammonium hydroxide) indicated that the labeled acid was pure.

Sodium [1-^{14}C]-2-Methyl-3-mercaptopropanoate (16). The synthesis of [1-^{14}C]-2-methyl-3-mercaptopropanoate was based upon the procedure of Larsson.[14]

Potassium [1-^{14}C]methacrylate (25 mg, 0.2 mmol, 0.5 mCi) was dissolved in water (50 μL) and placed in a 1-mL Reactivial. Thiolacetic acid (75 μL, 1.05 mmol) and 1.45 N sulfuric acid (50 μL) were added, and the vial was sealed and heated for 3 h at 80–85 °C. An additional amount of thiolacetic acid (40 μL, 0.56 mmol) was added and the solution was heated again for 3 h. To hydrolyze the thioester, 5 N sodium hydroxide (0.5 mL, 0.25 mmol) was added to the vial, and the mixture was heated at 90–95 °C for 3 h. It was then cooled and acidified to pH 4, and the [1-^{14}C]-2-methyl-3-mercaptopropanoic acid was extracted into ether. The crude product was purified by preparative thin-layer chromatography (silica, 1:1 benzene–ethyl acetate). The acid (21.6 mg, 0.18 mmol) was converted to the sodium salt by titration with a dilute sodium hydroxide solution to give sodium [1-^{14}C]-2-methyl-3-mercaptopropanoic acid (24.3 mg, 85% yield, sp activity = 0.017 mCi/mg, 83% radiochemical yield).

Sodium [2-^{3}H]-2-Methyl-3-mercaptopropanoate (17). Sodium [2-^{3}H]-2-methyl-3-mercaptopropanoate was prepared by heating a mixture of methacrylic acid (0.12 mL, 1.4 mmol) and thiolacetic acid (0.1 mL, 1.4 mmol) under nitrogen in the presence of tritiated water (20 μL, 0.1 Ci, 1 mmol) for 4 h at 80–90 °C. The resulting thioester was hydrolyzed by addition of aqueous sodium hydroxide (2 equiv) to the reaction mixture, followed by heating under argon for 6 h at 90–95 °C. The solution was acidified to pH 4 with sulfuric acid and extracted with ether. The ether was dried and evaporated and the residue was purified by silica gel chromatography (1:1 benzene–ethyl acetate). The acid was titrated with sodium hydroxide to give the sodium salt (62 mg, 31% yield, 3% radiochemical yield, sp activity = 0.05 mCi/mg).

When the same procedure was carried out using 20 μL of D_2O, the resulting product was shown to be labeled at C-2 with deuterium by a significant decrease in the intensity of the multiplet at δ 2.80.

Sodium [^{35}S]-2-Methyl-3-mercaptopropanoate (18). [^{35}S]-Labeled 2-methyl-3-mercaptopropanoic acid was obtained by modification of a procedure of Larsson.[15]

[^{35}S]Hydrogen sulfide (8.9 mCi, Amersham), that had been vacuum-transferred into a 3.75 N solution of sodium hydroxide (0.2 mL), was added to 2-methyl-3-bromopropanoic acid[15] (0.208 g, 1.25 mmol) dissolved in a 1.67 M sodium hydroxide solution (0.38 mL, 0.63 mmol). The mixture was heated to 95 °C for 90 min. An additional amount of 3.75 N sodium hydroxide solution (0.25 mL) saturated with unlabeled hydrogen sulfide was added, and heating was continued for another 90 min. The reaction flask was cooled, and the solution was acidified to pH 1 with dilute sulfuric acid. It was then extracted with ether, and the ether was dried over anhydrous sodium sulfate. The solvent was evaporated and the crude product (159 mg) was purified by bulb-to-bulb distillation. The distillate was further purified by silica gel chromatography (1:1 ethyl acetate–benzene) to yield [^{35}S]-2-methyl-3-mercaptopropanoic acid (20.6 mg, 14% yield) which was titrated with sodium hydroxide to give sodium [^{35}S]-2-methyl-3-mercaptopropanoate (24.1 mg, sp activity = 0.015 mCi/mg, 4.1% radiochemical yield).

Sodium [3-^{3}H]-2-Methyl-3-mercaptopropanoate. (*E*)-[3-^{3}H]-3-Bromo-2-methylprop-2-enoic acid was prepared by the methods used[13] to synthesize the corresponding deuterated compound. The tritiated acid (22.9 mCi) was reduced with sodium amalgam[16] to give (*Z*)-[3-^{3}H]-2-methylprop-2-enoic acid mixed with [3-^{3}H]-2-methylpropanoic acid in a ratio of ca. 3:1 as determined by NMR analysis. This mixture was used directly for the synthesis of [3-^{3}H]-2-methyl-3-mercaptopropanoate as described below.

(*Z*)-[3-^{3}H]-2-Methylprop-2-enoic acid (145 mg containing 36 mg of saturated acid; 1.3 mmol) was treated with thiolacetic acid (200 μL, 2.8 mmol) under nitrogen at 80–90 °C for 4 h. The resulting thioester was hydrolyzed by addition of 2 equiv of aqueous sodium hydroxide followed by heating under argon at 90–95 °C for 6 h. The solution was then acidified to pH 4 with sulfuric acid and extracted with ether. The ether was evaporated and the residue purified by silica gel chromatography to give the free acid which was titrated with sodium hydroxide to give sodium [3-^{3}H]-2-methyl-3-mercaptopropanoate (24.7 mg, 7% yield, sp activity = 0.05 mCi/mg, 5.4% radiochemical yield from (*E*)-[3-^{3}H]-3-bromo-2-methylprop-2-enoic acid).

Administration of Labeled Isobutyric Acid to Asparagus and Isolation of *S*-(2-Carboxy-*n*-propyl)-L-cysteine. The experiments that involved the incorporation of labeled forms of isobuytric acid into *S*-(2-carboxy-*n*-propyl)-L-cysteine utilized a different workup procedure which is summarized below.

After a 4-day incorporation period, the *Asparagus* plants were macerated in 95% ethanol, and ca. 500 mg of synthetic *S*-(2-carboxy-*n*-propyl)-L-cysteine[2] was added as carrier. The mixture was poured into a large glass column and about 8 L of 95% ethanol percolated slowly through the plant material. The eluant was taken to dryness in vacuo and the residue dissolved in water and extracted successively with chloroform (3 × 200 mL) and with ether (3 × 200 mL). The yellowish aqueous solution was concentrated in vacuo to a small volume and applied to a column of Amberlite IR-120(plus) (H^+, 200 mL). The resin was washed with water (500 mL), and the amino acids were then eluted with 2 N NH_4OH (400 mL). The basic eluant was taken to dryness and the residue applied in water to a 50 cm × 2 cm column of Merck microcrystalline cellulose. Elution with *n*-propyl alcohol–ammonium hydroxide (3:1) under pressure (10 lb/in^2) yielded *S*-(2-carboxy-*n*-propyl)-L-cysteine contaminated with two additional amino acids. The partially purified amino acid was applied to a second cellulose column of the same

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dimensions and eluted with *n*-butyl alcohol–acetic acid–water (12:5:5). The fractions containing the pure amino acid were combined and stripped, and the residue was recrystallized from water–acetone to yield 137 mg of *S*-(2-carboxy-*n*-propyl)-L-cysteine, mp 192–193 °C (lit[20] mp 194 °C) (27% recovery).

Schmidt Degradation of *S*-(2-Carboxy-*n*-propyl)-L-cysteine to Amino Acid 21. *S*-(2-Carboxy-*n*-propyl)-L-cysteine (95 mg, 0.46 mmol) was chilled to 0 °C in a 5-mL round-bottom flask, concentrated sulfuric acid (0.17 mL) was added, and the mixture was stirred until the diacid dissolved. Sodium azide (35 mg, 0.54 mmol) was added, and the reaction mixture was stirred for 30 min at room temperature and then heated on a steam bath for 3 h. The mixture was then cooled in ice and the pH adjusted to 10 with concentrated aqueous sodium hydroxide. Benzoyl chloride (0.1 mL, 0.86 mmol) and benzene (6 mL) were then added, and the solution was stirred for 4 h at 95 °C. The benzene was evaporated, and the reaction mixture was dissolved in ether. The ether solution was extracted with water, dried over anhydrous magnesium sulfate, and evaporated. The crude *N,N'*-dibenzoyl derivative was purified by preparative TLC on silica (1:2 ethyl acetate–hexane) and then heated with 1 N sodium hydroxide (3 mL) for 7 h at 95 °C. The solution was cooled, acidified to pH 1 with dilute HCl, and extracted with ethyl acetate (3 × 5 mL). The pH of the aqueous phase was adjusted to 5 and the water removed in vacuo. The residue was purified by column chromatography on microcrystalline cellulose (12:5:5 *n*-butyl alcohol–acetic acid–water). The resulting *S*-(2-amino-*n*-propyl)-L-cysteine (**21**) was a white crystalline solid (16 mg, 20% yield): mp 220 °C dec; MS (EI), *m/e* 178.0779 (178.0779 (178.077 580 calcd for $C_6H_{14}N_2O_2SM^{+\cdot}$).

[^{35}S]-*S*-(2-Carboxy-*n*-propyl)-D-cysteine (22). A mixture of [^{35}S]-L-cysteine hydrochloride (26 mg, 0.16 mmol, 0.5 mCi, Amersham) and methacrylic acid (40 mg, 0.47 mmol) was stirred under a nitrogen atmosphere while 2 N sodium hydroxide was added to bring the pH to 8. The resulting solution was heated to 95 °C for 8 h, cooled, and brought to pH 2 with glacial acetic acid and the solution taken to dryness in vacuo. The residue was purified by column chromatography on microcrystalline cellulose (12:5:5 *n*-butyl alcohol–acetic acid–water) to yield [^{35}S]-*S*-(2-carboxy-2-propyl)-L-cysteine (**22**) (26.5 mg, 93% yield, sp activity = 0.015 mCi/mg, 80% radiochemical yield, mp 195 °C (lit[20] mp 194 °C)).

[3-^{3}H]-*S*-(2-Carboxy-*n*-propyl)-L-cysteine (23). A 3:1 mixture of (*Z*)-[3-^{3}H]-2-methylpropen-2-oic acid and [3-^{3}H]-2-methylpropanoic acid (100 mg, 0.87 mmol, 0.44 mCi) obtained by sodium amalgam reduction of (*E*)-[3-^{3}H]-3-bromo-2-methylprop-2-enoic acid was dissolved in 2 N sodium hydroxide (1 mL), and L-cysteine hydrochloride (140 mg, 0.89 mmol) was added. The solution was stirred under argon and heated to 95 °C for 4.5 h. Glacial acetic acid was added to the cooled reaction mixture to bring the pH to 4. Water was then removed and the residue purified by column chromatography on microcrystalline cellulose (12:5:5 *n*-butyl alcohol–acetic acid–water) followed by paper chromatography by using the same solvent system to yield [3-^{3}H]-*S*-(2-carboxy-*n*-propyl)-L-cysteine (**23**) (24 mg, 15% yield, sp activity = 0.002 mCi/mg, mp 193 °C (lit[20] mp 194 °C)).

***O,S*-Bis(*p*-bromophenacyl)-3-mercapto-2-methylpropanoic Acid.** 3-Mercapto-2-methylpropanoic acid (0.73 g, 6.1 mmol) in acetonitrile (20 mL) was stirred with potassium carbonate (0.84 g, 6.1 mmol) for 30 min. Dibenzo-18-crown-6 (0.21 g, 0.59 mmol) and *p*-bromophenacyl bromide (2.42 g, 12.2 mmol) were added to the mixture which was then heated at 60 °C overnight. The solution was filtered, the acetonitrile was evaporated, and the resulting residue was purified by silica gel chromatography (2:1 toluene–hexane). The derivative was recrystallized from chloroform–hexane (695 mg, 22% yield, mp 96.5–97.5 °C): NMR ($CDCl_3$) δ 1.28 (3 H, d), 2.78 (3 H, m), 3.76 (2 H, s), 5.20 (2 H, s), 8.60 (8 H, two A_2X_2 systems); MS (EI), *m/e* 511.9293 (511.929 160 calcd for $C_{20}H_{18}O_4Br_2S,M^{+\cdot}$, 513.9258 (513.927 160 calcd for $C_{20}H_{18}O_4{}^{81}Br^{79}BrS$, M + 2).

Methyl (*R*)-(+)-3-(Acetylthio)-2-methylpropanoate (25). Diethyl azodicarboxylate (3.1 mL, 19.5 mmol) was added to a stirred solution of triphenylphosphine (5.1 g, 19.5 mmol) in dry THF (40 mL). The mixture was stirred for 30 min during which time a heavy precipitate formed. A mixture of thiolacetic acid (1.4 mL, 19.5 mmol) and methyl (*S*)-(+)-3-hydroxy-2-methylpropanoate (1.15 g, 9.7 mmol) in THF (5 mL) was added dropwise with stirring at 0 °C and the mixture stirred at this temperature for 1 h. The mixture was then stirred overnight at room temperature. Removal of the solvent gave a residue which was chromatographed on silica gel by using ethyl acetate–hexane to give methyl (*R*)-(+)-3-(acetylthio)-2-methylpropanoate (**25**) (1.4 g, 82% yield): $[\alpha s]^{25}_D$ +59.8° (*c* 0.16, EtOH); NMR ($CDCl_3$) δ 1.11 (3 H, d), 2.10 (3 H, s), 2.58 (1 H, m), 2.97 (2 H, d), 3.58 (3 H, s).

Methyl (*R*)-3-Mercapto-2-methylpropanoate (26). A flask containing dry methanol (10 mL) was flushed with nitrogen and sodium metal (157 mg, 6.8 mmol) added slowly with stirring. To the resulting solution of sodium methoxide, methyl (*R*)-3-(acetylthio)-2-methylpropanoate (1.2 g, 6.8 mmol) was added. The solution was stirred for 15 min at room temperature, and the pH was then adjusted to 5 with glacial acetic acid. The solvents were removed in vacuo, and water (20 mL) was added to the residue. The aqueous solution was extracted with ether (3 × 25 mL), the extract was dried ($MgSO_4$), and the solvent was removed to yield methyl (*R*)-3-mercapto-2-methylpropanoate (**26**) (0.85 g, 93% yield): NMR ($CDCl_3$) δ 1.18 (3 H, d), 2.40–2.90 (3 H, m), 3.64 (3 H, s).

Benzyl (*S*)-*N*-Carbethoxyaziridinecarboxylate (27). Benzyl (*S*)-aziridinecarboxylate[26] (104 mg, 0.59 mmol) and triethylamine (89 mg, 0.88 mmol, 0.11 mL) were dissolved in anhydrous ether (15 mL), and the solution was cooled to −10 °C in an ice–salt bath. Ethyl chloroformate (95 mg, 0.88 mmol, 0.07 mL) was added and the resulting solution stirred for 1 h. The reaction mixture was washed with water, dried ($MgSO_4$), and stripped to give benzyl (*S*)-*N*-carbethoxyaziridinecarboxylate (**27**) as a colorless oil (142 mg, 96% yield): NMR ($CDCl_3$) δ 1.20 (3 H, t), 2.45 (1 H, dd), 2.65 (1 H, dd), 4.04 (2 H, q), 5.08 (2 H, s), 7.35 (5 H, s); MS (EI) *m/e* 249.2682 (249.268 832 calcd for $C_{13}H_{15}NO_4$, $M^{+\cdot}$).

(2*R*)-*O*-Benzyl-*N*-carbethoxy-*S*-(2-carbethoxy-*n*-propyl)-L-cysteine (28). Freshly distillated boron trifluoride etherate (1.24 mL) was added to a cold solution of benzyl (*S*)-*N*-carbethoxyaziridinecarboxylate (1.58 g, 6.3 mmol) and methyl (*R*)-3-mercapto-2-methylpropanoate (0.85 g, 6.3 mmol) in methylene chloride (27 mL) and the mixture stirred for 36 h at room temperature. The reaction mixture was then poured into an excess of cold aqueous sodium bicarbonate solution and the organic layer separated. The aqueous phase was extracted with methylene chloride (2 × 20 mL) and the combined organic extracts dried ($MgSO_4$) and taken to dryness in vacuo. The residue was dissolved in benzene and applied to a silica column. Preliminary elution with benzene removed a number of impurities while elution with ether gave the desired ring-opened product **28** (860 mg, 36% yield): ($CDCl_3$) δ 1.20 (6 H, t, d) 2.61 (3 H, m), 2.98 (2 H, d), 3.60 (3 H, s), 4.04 (2 H, q), 5.06 (2 H, s), 5.44 (1 H, br d), 7.24 (5 H, s); MS (EI), *m/e* 383.4664 (383.466 75 calcd for $C_{18}H_{25}NO_6S,M^{+\cdot}$).

(2*R*)-(+)-*S*-(2-Carboxy-*n*-propyl)-L-cysteine (29). (2*R*)-*O*-Benzyl-*N*-carbethoxy-*S*-(2-carboxy-*n*-propyl)-L-cysteine (**28**) (285 mg, 0.74 mmol) was dissolved in dry acetonitrile (5 mL), and trimethylsilyl iodide (386 mg, 0.275 mL, 1.9 mmol) was added under nitrogen with stirring. The resulting mixture was stirred at 45–50 °C for 20 h. At the end of this time, methanol (2 mL) was added to the reaction and the solution taken to dryness after stirring for 0.5 h. Dilute acetic acid (30%; 15 mL) was added to the residue, and the aqueous solution was extracted with ether (2 × 5 mL). The aqueous solution was then taken to dryness in vacuo to give 100 mg of residue. This residue was dissolved in 2 N potassium hydroxide (1 mL) and the solution obtained was stirred at room temperature for ca. 12 h. The solution was then acidified to pH 5 with 1 N hydrochloric acid and the water removed in vacuo. The residue was dissolved in water and applied to a column of Dowex 1 (acetate form, 25 mL, 20–50 mesh), the column was washed with water (25 mL), and the amino acid was eluted with 1 N acetic acid. The fractions containing the amine acid were combined, the solvent was stripped off, and the remaining solid was crystallized from water–acetone to give (2*R*)-(+)-*S*-(2-carboxy-*n*-propyl)-L-cysteine (**29**) (15 mg, 10% yield); $[\alpha]^{25}_D$ +34.5 °C (*c* 0.04, H_2O).

(*S*)-(−)-*S*-(2-Carboxy-*n*-propyl)-L-cysteine (31). This diastereomer of *S*-(2-carboxy-*n*-propyl)-L-cysteine was prepared from (*S*)-(−)-3-(acetylthio)-2-methylpropanoic acid by using the procedures outlined for the preparation of the 2*R* diastereomer. The recrystallized (2*S*)amino acid exhibited $[\alpha]^{25}_D$ −58.3° (*c* 0.04, H_2O).

Potassium (*R*)-[3-^{3}H]Isobutyrate. A. (2*R*,3*R*)-*trans*-2,3-Epoxy-1-butanol. Treatment of *trans*-crotyl alcohol (1.44 g, 20 mmol) with titanium(IV) isopropoxide (5.08 g, 20 mmol), (−)-diisopropyl-*O*-tartrate (4.91 g, 21 mmol) and anhydrous *tert*-butyl hydroperoxide (3.6 g, 40 mmol) in dichloromethane according to the procedure of Sharpless et al.[33] gave the title compound which was purified by column chromatography (silica, 1:1 ethyl acetate–hexane) and bulb-to-bulb distillation to give a colorless oil (0.57 g, 32% yield): $[\alpha]^{25}_D$ +54.9° (*c* 0.14, benzene); NMR ($CDCl_3$) δ 1.34 (3 H, d), 2.20 (1 H, br s), 2.84–3.16 (2 H, m), 3.52–4.02 (2 H, m).

B. [^{3}H]Methyllithium. [^{3}H]Methyl iodide (25 mCi, Amersham) was diluted with unlabeled methyl iodide (0.31 mL, 0.71 g, 5 mmol) in dry hexane (3 mL) and then treated with 1.3 M *n*-butyllithium in hexane (3.84 mL, 5 mmol) according to the procedure of Aberhart and Lin[31] to give a solution of [^{3}H]methyllithium in anhydrous ether (0.85 M, 4 mL, 64% yield).

C. (2*S*,3*R*)-*trans*-[3-^{3}H]-3-Methyl-1,2-butanediol. Following the procedure of Aberhart and Lin,[31] (2*S*,3*R*)-*trans*-[3-^{3}H]-3-methyl-1,2-butanediol was prepared by treatment of *trans*-(2*R*,3*R*)-2,3-epoxy-1-butanol (273 mg, 3.1 mmol) sequentially with unlabeled methyllithium

(1.5 M, 2.0 mL, 3.1 mmol) in anhydrous ether and [^{3}H]methyllithium (0.85 M, 3.64 mL, 3.1 mmol). The NMR spectrum ($CDCl_3$) of the crude reaction product suggested contamination with 1,3-butanediol. This contaminant could not be removed by chromatography or distillation.[31]

D. Potassium (*R*)-[3-^{3}H]Isobutyrate. (*R*)-[3-^{3}H]Isobutyric acid was prepared by oxidation of crude (2*S*,3*R*)-*trans*-[3-^{3}H]-3-methyl-1,2-butanediol with sodium periodate and potassium permanganate.[31] Titration of the crude isobutyric acid with dilute potassium carbonate solution to the phenolphthalein endpoint gave potassium (*R*)-[3-^{3}H]isobutyrate. The NMR spectrum (D_2O) of this salt showed the presence of considerable quantities of unknown impurities.

Purification of the potassium (*R*)-[3-^{3}H]isobutyrate was accomplished via the benzyl ester. The crude salt derived from 155 mg of (2*S*,3*R*)-[3-^{3}H]-3-methyl-1,2-butanediol was suspended in dry acetonitrile (15 mL) and benzyl bromide (280 mg, 1.64 mmol) added along with a catalytic quantity of dibenzo-18-crown-6 (54 mg, 0.16 mmol). The mixture was stirred and refluxed for 12 h. After cooling, the reaction mixture was filtered and the filtrate taken to dryness in vacuo to give a residue that was purified by column chromatography (silica, 5:95 ethyl acetate–hexane) to give (*R*)-benzyl[3-^{3}H]isobutyrate (115 mg, 52% yield from crude 3-methyl-1,2-butanediol): NMR ($CDCl_3$) δ 1.18 (6 H, d), 2.60 (1 H, quintet), 5.10 (2 H, s), 7.32 (5 H, s).

The purified benzyl ester (100 mg, 0.46 mmol) was dissolved in absolute ethanol (10 mL), and hydrogenolysis was carried out for 1 h at atmospheric pressure in the presence of 10% palladium on carbon (15 mg) as catalyst. The catalyst was removed by filtration and aqueous potassium bicarbonate solution (10 mL containing 41 mg of $KHCO_3$, 0.41 mmol) added to the alcoholic solution. After 10 min of stirring, the ethanol was removed in vacuo and the remaining aqueous solution was washed with ether (2 × 10 mL). Evaporation of the aqueous phase in vacuo gave potassium (*R*)-[3-^{3}H]isobutyrate (53 mg, 98% based upon $KHCO_3$) as a hygroscopic white solid: NMR (D_2O) δ 1.22 (6 H, d), 2.60 (1 H, quintet); sp activity = 2.2×10^{-2} mCi/mg (4.7% radiochemical yield from [^{3}H]methyl iodide).

Potassium (*S*)-[3-^{3}H]Isobutyrate. The *S* stereoisomer was prepared by using procedures identical with those described for the preparation of the *R* stereoisomer except that (+)-diisopropyl L-tartrate was employed in the Sharpless epoxidation reaction.

***N*-Benzoyl-*S*-(2-carboxy-*n*-propyl)-L-cysteine (32).** *S*-(2-Carboxy-*n*-propyl)-L-cysteine (41 mg, 0.19 mmol) was dissolved in 1 N sodium hydroxide (0.95 mL, 0.95 mmol), and a solution of benzoyl chloride (0.05 mL, 0.43 mmol) in methylene chloride (2 mL) was added. The mixture was stirred overnight. The aqueous phase was then separated and acidified with dilute HCl to pH 4. The resulting milky white suspension was extracted with ethyl acetate (3 × 5 mL), and the combined organic extracts were dried ($MgSO_4$) and evaporated to give a residue that was chromatographed (silica, 1% acetic acid in ethyl acetate) to afford the *N*-benzoyl derivative **32** as a white solid which was recrystallized from acetonitrile (45 mg, 76% yield): mp 176–177 °C; NMR (D_2O, NaOD) δ 1.12 (3 H, d), 2.36–3.32 (5 H, m), 4.56 (1 H, t), 7.44–7.96 (5 H, m); MS (EI), *m/e* 311.0830 (311.082 715 calcd for $C_{14}H_{17}NO_5S$, $M^{+\cdot}$).

Kuhn–Roth Oxidation of *N*-Benzoyl-*S*-(2-carboxy-*n*-propyl)-L-cysteine (32). *N*-Benzoyl-*S*-(2-carboxy-*n*-propyl)-L-cysteine (41 mg, 0.13 mmol) and 2 N H_2SO_4 (2 mL) were placed in a 25-mL three-necked flask equipped with a separatory funnel and two Kjeldahl traps placed one on top of the other. The upper Kjeldahl trap was connected via a distillation head to a water-cooled condensor. A solution of chromium trioxide (0.9 g, 9 mmol) in 2 N H_2SO_4 (4 mL) was added to the flask, and the contents were heated to boiling. Water was distilled from the flask while the volume was maintained at 8–10 mL by addition of water from the separatory funnel. After ca. 30 mL of distillate had been collected, the distillate was titrated to the phenolphthalein endpoint with aqueous potassium carbonate. Removal of the water gave crude potassium acetate.

The crude potassium acetate was suspended in dry acetonitrile (5 mL) and *p*-bromophenacyl bromide (54 mg, 0.19 mmol) and a catalytic amount of dibenzo-18-crown-6 (7 mg, 0.02 mmol) were added. The mixture was refluxed for 1 h, the solution was cooled and filtered, and the filtrate was taken to dryness in vacuo. The residue was chromatographed (silica, 10% ethyl acetate in hexane) to afford *p*-bromophenacyl acetate which was recrystallized from hexane (16 mg, 48% yileld): mp 85–86 °C; NMR ($CDCl_3$) δ 3.20 (3 H, s), 5.26 (2 H, s), 7.70 (4 H, A_2X_2 q).

Dibenzyl *N*-Benzoyl-*S*-(2-carboxy-*n*-propyl)-L-cysteine (33). A mixture of *N*-benzoyl-*S*-(2-carboxy-*n*-propyl)-L-cysteine (**32**) (400 mg, 1.28 mmol), benzyl alcohol (553 mg, 5.12 mmol), and *p*-toluenesulfonic acid hydrate (24 mg, 0.13 mmol) in benzene (25 mL) was refluxed under nitrogen in a flask equipped with a Dean–Stark apparatus topped by a condenser. After 12 h, the cooled reaction mixture was washed with aqueous $NaHCO_3$ (2 × 20 mL), dried ($MgSO_4$), and taken to dryness. The excess benzyl alcohol was removed from the residue by bulb-to-bulb distillation (120 °C, 0.2 mm), and the remaining yellow oil was purified by chromatography (silica, 3:1 ethyl acetate–hexane) to give the dibenzyl ester **33** (502 mg, 74% yield); NMR ($CDCl_3$) δ 1.18 (3 H, d), 2.44–2.92 (4 H, m), 3.12 (2 H, d), 4.96–5.32 (1 H, m), 5.10 (2 H, d), 5.20 (2 H, d), 7.06 (1 H, br d), 7.20–8.00 (15 H, m); MS (EI), *m/e* 491.1772 (491.176 615 calcd for $C_{28}H_{29}NO_5S$, $M^{+\cdot}$), 493.1728 (493.172 405 calcd for $C_{28}H_{29}NO_5{}^{34}S$, M + 2).

Conversion of Dibenzyl Ester 33 to Benzyl 2-Methyl-3-(methylthio)propanoate (34). A solution of dibenzyl ester **33** (502 mg, 0.94 mmol) and methyl trifluoromethanesulfonate (185 mg, 0.13 mL, 1.13 mmol) in methylene chloride (20 mL) was stirred overnight under nitrogen. The solvent was removed and the gummy residue extracted with hexane. The hexane insoluble product was dissolved in ethyl acetate (10 mL) and washed with aqueous potassium carbonate (1 × 5 mL). The aqueous wash was back-extracted with ethyl acetate (5 mL) and the combined organic layer dried ($MgSO_4$) and evaporated. The residue was chromatographed (silica, 15% ethyl acetate in hexane) to yield the desired ester **34** as a colorless liquid (169 mg, 80% yield): NMR ($CDCl_3$) δ 1.28 (3 H, d), 2.10 (3 H, s), 2.48–2.98 (3 H, m), 5.16 (2 H, s), 7.36 (5 H, s).

The byproduct, benzyl *N*-benzoyldehydroalanine, was isolated in 90% yield: NMR ($CDCl_3$) δ 5.10 (2 H, s), 5.83 (1 H, s), 6.58 (1 H, s), 7.10–7.30 (8 H, m), 7.50–7.67 (2 H, m), 8.28 (1 H, br s).

Conversion of Benzyl 2-Methyl-3-(methylthio)propanoate (34) to Benzyl 2-Methylpropenoate (35). A solution of benzyl 2-methyl-3-(methylthio)propanoate (**34**) (169 mg, 0.75 mmol) and methyl trifluoromethanesulfonate (147 mg, 0.10 mL, 0.90 mmol) in methylene chloride (15 mL) was stirred under nitrogen overnight. The solvent was removed and the residual oil extracted with hexane. The hexane insoluble product was dissolved in ethyl acetate (20 mL) and treated with 1,5-diazabicyclo[5.4.0]undec-5-ene (122 mg, 0.12 mL, 0.8 mmol) at room temperature for 1 h. The solvent was removed and the residue chromatographed (silica, 1:9 ethyl acetate–hexane) to afford benzyl methacrylate (86 mg, 65% yield) as a colorless liquid: NMR ($CDCl_3$) δ 1.96 (3 H, m), 5.20 (2 H, s), 5.56–5.62 (1 H, m), 6.16 (1 H, br s), 7.36 (5 H, s).

Degradation of Benzyl 2-Methylpropenoate (35). Water (6 mL) was added to a solution of benzyl 2-methylpropenoate (48 mg, 0.27 mmol) in *tert*-butyl alcohol (6 mL, freshly distilled from Na), and a solution of osmium tetroxide in *tert*-butyl alcohol (0.028 m, 1.07 mL, 0.03 mmol) was then added with stirring. Finely powdered sodium periodate (173 mg, 0.81 mmol) was added with stirring in several portions over a 40-min period, and stirring was then continued overnight. The resulting white suspension was poured into saturated aqueous arsenic trioxide solution (64 mL), and the resulting mixture was extracted with ether (3 × 20 mL). The pH of the aqueous phase was adjusted to 9–10 with saturated aqueous potassium carbonate, and 5,5-dimethyl-1,3-cyclohexanedione (dimedone) (350 mg, 2.51 mmol) was added. The solution was stirred for 10 min and the pH readjusted to 6 with concentrated HCl. The resulting mixture was stirred overnight. The reaction mixture was then repeatedly extracted with chloroform (3 × 20 mL). The combined extracts were dried ($MgSO_4$) and evaporated to yield a residue that was chromatographed (silica, 1:9 ethyl acetate–hexane) to yield crystalline dimedone–formaldehyde (59 mg, 75% yield). Recrystallization from ethanol gave colorless needles, mp 193 °C (lit[42] mp 191.5–192 °C).

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Registry No. 1, 2224-02-4; **7**, 94751-35-6; **8**, 94751-36-7; **9**, 4406-41-1; **10**, 5440-69-7; **11**, 94751-37-8; **12**, 94751-38-9; **13**, 94751-39-0; **15** (R = H), 26473-47-2; **16**, 94751-40-3; **17**, 94751-41-4; **18**, 94751-42-5; **19**, 94751-43-6; **20**, 6852-42-2; **21**, 94751-44-7; **22**, 94751-45-8; **23**, 94781-12-1; **24**, 80657-57-4; **25**, 86961-07-1; **26**, 86961-09-3; **27**, 86961-11-7; **28**, 86961-12-8; **29**, 66512-76-3; **30**, 86961-08-2; **31**, 66512-75-2; **32**, 94751-46-9; **33**, 94751-47-0; **34**, 94751-48-1; **35**, 2495-37-6; ethyl bis(hydroxymethyl)malonate, 20605-01-0; α-(iodomethyl)acrylic acid, 56750-62-0; dihydroasparagusic acid, 7634-96-0; sodium [3,4-^{3}H]isobutyrate, 80631-54-5; butyl propanoate, 590-01-2; sodium [2-^{3}H]isobutyrate, 80631-55-6; potassium [1-^{14}C]methacrylate, 94751-49-2; (*Z*)-[3-^{3}H]-2-methylprop-2-enoic acid, 94751-50-5; 0,5-bis(*p*-bromophenacyl)-3-mercapto-2-methylpropanoic acid, 94751-51-6; benzyl

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(S)-aziridinecarboxylate, 67413-26-7; (S)-(−)-3-(acetylthio)-2-methylpropanoic acid, 76497-39-7; (2R,3R)-*trans*-2,3-epoxy-1-butanol, 58845-50-4; [^{3}H]methyllithium, 94751-52-7; [3-^{3}H]-3-methyl-1,2-butanediol, 94751-53-8; potassium (R)-[3-^{3}H]isobutyrate, 94751-54-9; potassium (S)-[3-^{3}H]isobutyrate, 94751-55-0; methyl trifluoromethanesulfonate, 333-27-7; benzyl *N*-benzoyldehydroalanine, 94751-56-1; sodium [1-^{14}C]methacrylate, 80631-56-7; sodium [1-^{14}C]isobutyrate, 6917-21-1; sodium [3,4-^{3}H,1-^{14}C]isobutyrate, 94751-57-2; sodium [2-^{3}H,1-^{14}C]isobutyrate, 94751-58-3; (±)-sodium [2-^{3}H,1-^{14}C]-3-mercapto-2-methylpropanoate, 94781-13-2; (±)-sodium [^{35}S,3(R,S)-^{3}H]-3-mercapto-2-methylpropanoate, 94781-14-3; [^{35}S,3(R,S)-^{3}H]-2(R,S)-S-(2-carboxy-*n*-propyl)-L-cysteine, 94751-59-4; [^{35}S]-L-cysteine hydrochloride, 24321-13-9; (E)-[3-^{3}H]-3-bromo-2-methylprop-2-enoic acid, 94751-60-7; β,β′-diiodoisobutyric acid, 50891-94-6.

Enzymes in Organic Synthesis. 34.[1] Preparations of Enantiomerically Pure Exo- and Endo-Bridged Bicyclic [2.2.1] and [2.2.2] Chiral Lactones via Stereospecific Horse Liver Alcohol Dehydrogenase Catalyzed Oxidations of Meso Diols[2]

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Abstract: Preparative-scale horse liver alcohol dehydrogenase catalyzed oxidations of saturated and unsaturated exo- and endo-bridged bicyclic [2.2.1] and [2.2.2] meso diols proceed with complete enantiotopic specificity to give high (64–87%) yields of the corresponding chiral lactones of ≥97% ee. As for previous meso diol oxidations, the stereochemical course of each oxidation (*S*-center CH_2OH oxidation in all cases) is as predicted by the cubic-space model of the active site. An illustration of the asymmetric synthetic value of these chiral lactones is provided by the conversion of one of them into a prostaglandin precursor.

The viability of enzymes as practical chiral catalysts is now well established.[3] In particular, their ability to discriminate between enantiotopic groups of symmetrical substrates such as meso compounds is being exploited to an ever increasing extent in asymmetric synthesis. Horse liver alcohol dehydrogenase (HLADH[4]), a commercially available NAD-dependent alcohol dehydrogenase that catalyzes CH(OH) ⇌ C=O oxidoreductions of a wide range of substrates of organic chemical interest, is one of the most versatile enzymes in this regard. In its oxidative mode, it has been shown to operate stereospecifically on only one of the enantiotopic hydroxyl groups of meso diols possessing acyclic, monocyclic, and bicyclic structures.[5] We have now discovered that the enzyme's remarkable tolerance of structural variations in meso substrates extends to bridged bicyclic compounds. In this paper we report that preparative-scale HLADH-catalyzed oxidations of exo- and endo-bridged bicyclic [2.2.1] and [2.2.2] meso diols proceed with complete enantiotopic specificity to produce enantiomerically pure chiral lactones of asymmetric synthetic value.

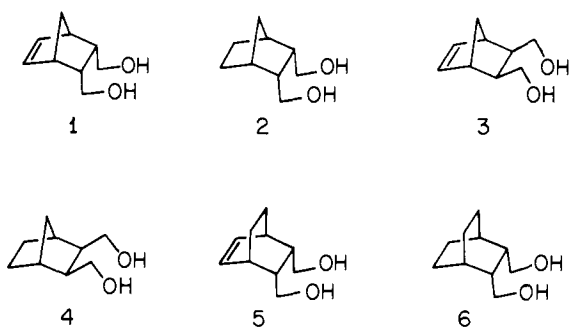
Table I. Relative Rates[a] of HLADH-Catalyzed Oxidations of Diols **1–6**

substrate	rel rate	substrate	rel rate
cyclohexanol	100	**4**	80
1	21	**5**	31
2	22	**6**	25
3	70		

[a] Oxidation rates were measured spectrophotometrically at 25 °C in 0.1 M NaOH–glycine buffer (pH 9) with [S] = 10^{-4} M and [NAD] = 5×10^{-4} M.

Results

Synthesis of Substrates. The substrates evaluated were the meso diols **1–6**. They were prepared by unexceptional routes that are described in full in the Experimental Section.



HLADH-Catalyzed Oxidations of 1–6. The rates of HLADH-catalyzed oxidations of **1–6** relative to that of the standard reference substrate cyclohexanol under the same conditions are recorded in Table I. All of the diols are seen to be good to excellent substrates for which preparative-scale reactions

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(4) Abbreviations: HLADH, horse liver alcohol dehydrogenase; NAD, nicotinamide adenine dinucleotide, oxidized form; FMN, flavin mononucleotide (riboflavin phosphate); $Eu(tfc)_3$, tris[((trifluoromethyl)hydroxymethylene)-(−)-camphorato]europium(III).

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