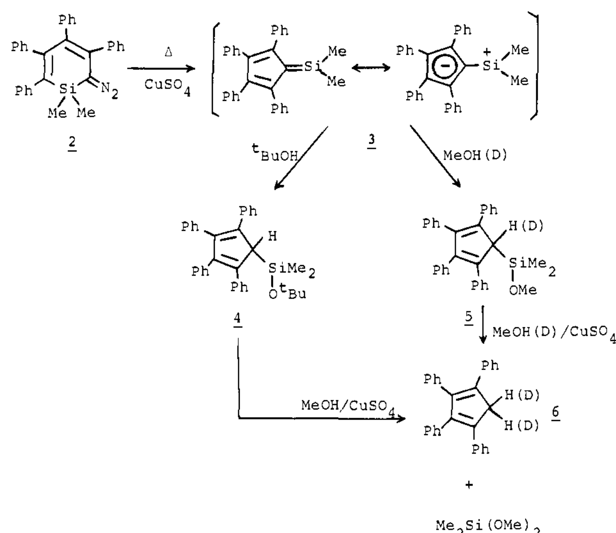
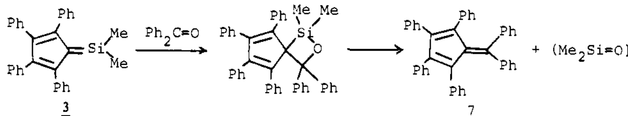


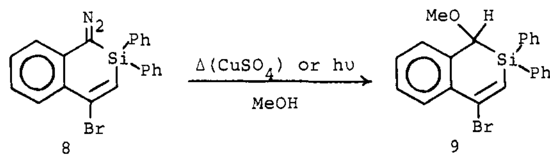
nolysis of the cyclopentadienylmethoxysilane **5**. The cyclopentadienyl-*tert*-butoxysilane **4** was found to give the desilylated cyclopentadiene **6** in 96% yield under the reaction conditions. The reaction of **2** with methanol-*d* led to the formation of 1,2,3,4-tetraphenyl-5,5-dideuterio-1,3-cyclopentadiene in 95% yield, mp 177–178 °C; mass spectrum, *m/e* 372 (M^+); and in its NMR spectrum the peak at δ 3.97, attributed to the methylene protons of the cyclopentadiene, was completely absent.

A benzene solution of **2** containing excess benzophenone was subjected to thermolysis at ca. 100 °C in the presence of anhydrous cupric sulfate to afford 1,2,3,4,6,6-hexaphenylfulvene **7**[9] in 66% yield as almost black crystals.[10] The formation of the fulvene



7 indicates 6-silafulvene **3** as an intermediate in the reaction of **2**. It is well documented that silicon–carbon double-bonded intermediates react with carbonyl compounds to give silaoxetanes which decompose to olefins and silanones.[11] Similar reaction of **2** with benzaldehyde produced 1,2,3,4,6-pentaphenylfulvene as rust-red crystals.[9] These results demonstrate that the reaction of **2** occurred only in the direction that forms the silafulvene **3** which was successfully trapped by alcohols and carbonyl compounds.

However, the reaction of silyl diazo compound **8**[12] was quite different from that of **2**. A simple OH insertion product **9**[13] was



obtained in 96% yield when benzene–methanol solution of **8** was refluxed at ca. 100 °C for 5 min in the presence of anhydrous cupric sulfate. The photochemical decomposition of **8** in methanol–benzene also produced the product **9**. Further investigation of the silafulvene as well as the chemistry of silabenzene is now in progress.

Acknowledgment. We gratefully acknowledge partial supports by the Ministry of Education, a grant for scientific research (447019), and the Shinetsu Chemical Co.

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(12) Silyl diazo compound **8** was prepared by the oxidation of the hydrazone from the corresponding silyl ketone with mercuric oxide, orange-yellow crystals, mp 129–130 °C; NMR (CCl_4, δ) 6.70–8.17 (m, ArH and BrC=CHSi); IR (KBr) 2030 cm^{-1} (N_2). Anal. Calcd for $C_{21}H_{15}N_2SiBr$: C, 62.53; H, 3.74; N, 6.94; Br, 19.81. Found: C, 62.47; H, 3.69; N, 7.05; Br, 20.10.
(13) NMR (CCl_4, δ) 3.27 (s, 3 H, OMe), 4.63 (s, 1 H, SiCHO), and 6.87–7.90 (m, 15 H, ArH and BrC=CHSi); mass spectrum, *m/e* 406 (M^+, ^{79}Br) and 408 (M^+, ^{81}Br).

Bifunctional Chiral Synthons via Microbiological Methods. 1. Optically Active 2,4-Dimethylglutaric Acid Monomethyl Esters

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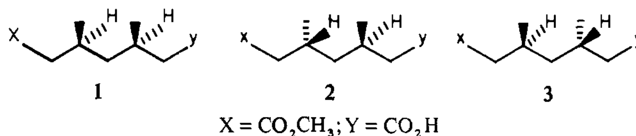
In recent years, the "polyoxo" macrolide aglycons[1,2] and other multifunctional macrocyclic compounds such as the maytansines,[3,4] ansa antibiotics,[5,6] and ionophores[7-9] have attracted the attention of many synthetic chemists. Most of the approaches used in the construction of acyclic chains with multiple chiral centers entailed the successive assembly of a series of prefabricated optically active building blocks containing the desired stereochemical functional features of the intended synthetic targets. For example, (*S*)-β-hydroxyisobutyric acid[10] has been used extensively as a starter chiral unit, which has greatly facilitated the design and execution of several total syntheses.[6-8,11] Herein, we report the preparation of three bifunctional chiral synthons, **1–3** representing partial



X = CO_2CH_3; Y = CO_2H

structural units, commonly encountered in macrolide[1,2] and polyether[7-9] antibiotics.

We had previously demonstrated the enantiotopic specificity of pig liver esterase[12] (PLE), which catalyzed the stereospecific hydrolysis of the pro-*R* ester grouping in dimethyl β-hydroxy-β-methylglutarate. Chemoselective reduction of either the acid or ester functionality in the resulting product afforded (*R*)- and (*S*)-mevalonic acids, respectively. Because PLE has a relatively

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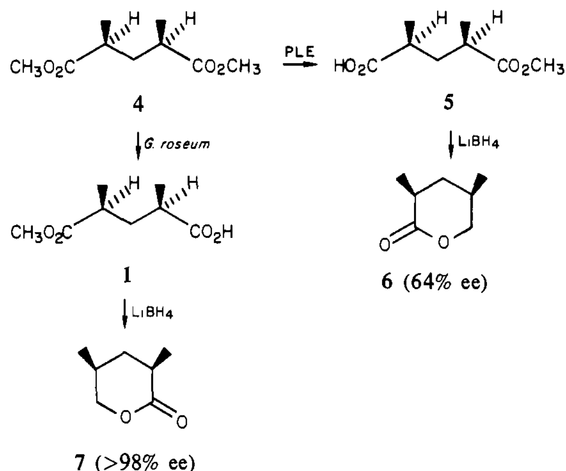
Table I. Chirality and Enantiotopic Specificity of Microbial Esterases

microorganism[a]	attack site	yield, %	enantiomeric excess[b] (ee), %
Gliocladium roseum	pro-*R*	80	>98
Helminthosporium sp.	pro-*R*	91	84
Penicillium citrinum	pro-*R*	50	30
Scopulariopsis constantini	pro-*R*	86	9
Cladosarum olivaceum	pro-*S*	72	50
Zygorhynchus moelleri	pro-*S*	50	75

[a] Each of the microorganisms was incubated[18] with 2 g/L of 4 for 22 h. [b] Calculated from the $[\alpha]_D$ values of the half-esters.

relaxed substrate binding requirement, we decided to further explore its enantiotopic specificity. When 200 mg of dimethyl *cis*-2,4-dimethylglutarate (4)[13] in 20 mL of 0.1 M phosphate buffer, pH 8.0, was incubated with 100 units of PLE (Sigma) for 60 min, the half-ester 5, $[\alpha]^{25}_D$ −2.8°,[14] was isolated in 85% yield.



Its absolute configuration was established by reduction of 5 with $LiBH_4$ to yield the known lactone (2*S*,4*R*)-6, $[\alpha]^{25}_D$ +25.1° (lit.[15] +39.1°). Since $LiBH_4$[16] selectively reduces carboxylic acid esters and not carboxylic acids, it follows that PLE preferentially cleaved the pro-*S* ester grouping of 4. Unfortunately, the optical purity of 6 (confirmed by ^1H NMR spectroscopy using $Eu(hfc)_3$[17]) was only 64% ee.

This disappointingly low level of asymmetric induction by PLE prompted us to examine enantiotopically selective transformations of 4 by microorganisms, which are prodigious sources of esteratic enzymes. Although numerous microbes cleaved the ester groupings of 4, most of these preferentially attacked the pro-*R* ester grouping, yielding monoesters of varying degrees of optical purities. A representative list of microbial actions on 4 is tabulated in Table I. After an exhaustive study, only one microorganism, *Gliocladium roseum*, was found to catalyze the stereospecific hydrolysis of the pro-*R* ester grouping of 4 in high yield. In a typical experiment, when 2 g of 4 was incubated with *G. roseum* in 1 L of soybean dextrose medium[18] for 22 h, 1.6 g of 1, $[\alpha]^{25}_D$ +4.0°, was isolated. Reduction of 1 with $LiBH_4$ gave 7 (75%), $[\alpha]^{25}_D$ −41.1°; enantiomeric excess (ee) determination[17] revealed 7 to be greater than 98% ee.

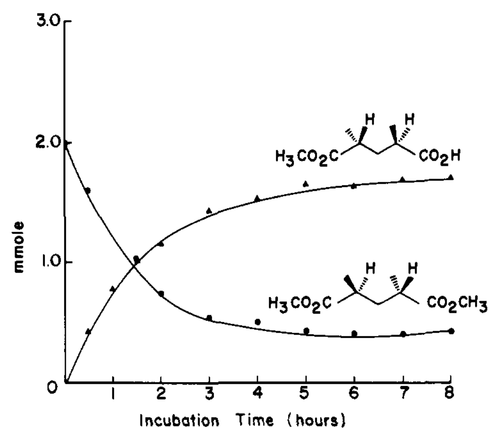
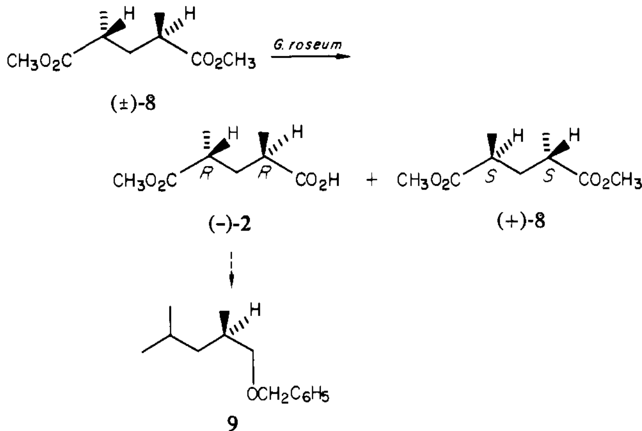


Figure 1. Kinetics of hydrolysis of (±)-8 by *Gliocladium roseum*. The microorganism was incubated[18] with 2 g/L of (±)-8. At the indicated time intervals, an aliquot of the incubation mixture was removed, extracted with ethyl acetate, and analyzed by GLC (1.5% OV-101 on Chromosorb GHP column, 5 ft) at a column temperature of 125–140 °C.

Since 2 and 3 are useful chiral precursors to calcimycin[8] and narasin,[7] respectively, a racemic mixture of dimethyl *trans*-2,4-dimethylglutarate (8)[13] was incubated with *G. roseum*.[18] We



envisaged that this organism would likewise preferentially attack the *R*,*R* isomer resulting in a kinetic resolution of (±)-8. The rate of hydrolysis of (±)-8 by *G. roseum* is shown in Figure 1. By terminating the reaction at 90 min, 2, $[\alpha]^{25}_D$ −21.1° (68% ee),[19] was isolated in 95% yield. Its absolute configuration was correlated by converting[20] 2 to (+)-(2*R*)-2,4-dimethylpentanyl benzyl ether (9), $[\alpha]^{25}_D$ +0.68°, and compared with (−)-(2*S*)-2,4-dimethylpentanyl benzyl ether,[21] $[\alpha]^{25}_D$ −0.62° (64% ee), thereby confirming that the *R*,*R* isomer was indeed preferentially hydrolyzed. To obtain 2 of high optical purity, we subjected 2 (68% ee) to recycling by first treating 2 with diazomethane, and the resulting diester (330 mg) was then reincubated with *G. roseum* for 60 min. The ee[19] of the recycled 2 (148 mg), $[\alpha]^{25}_D$ −31.8°, was established to be >98%.

(13) Condensation of 2-butanone and methyl methacrylate afforded a 3:1 mixture of *cis*- and *trans*-2,4-dimethylcyclohexane-1,5-dione, which was carefully separated by silica gel chromatography. Periodate cleavage of the diones followed by methylation gave 4 and (±)-8. Stork, G.; Nair, V. *J. Am. Chem. Soc.* **1979**, *101*, 1315.

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(19) After treatment of 2 with CH_2N_2, the enantiomeric excess of the diester was determined by ^1H NMR spectroscopy (CCl_4) in the presence of the chiral shift reagent $Eu(hfc)_3$ (0.3–0.4 equiv).

(20) $LiAlH_4$ reduction, monobenzylation (1.1 equiv of $PhCH_2Br/NaH$), tosylation, and $LiAlH_4$ reduction transformed 2 → 9.

(21) The following sequence of reactions was employed for the transformation of (+)-6 into (−)-(2*S*)-2,4-dimethylpentanyl benzyl ether: (a) $Et_3N/MeOH$; (b) $ClCH_2OCH_3/EtN(i\text{-}Pr)_2$; (c) $LiAlH_4/Et_2O$; (d) NaH/$PhCH_2Br$; (e) concentrated HCl/MeOH; (f) TsCl/Py; (g) $LiAlH_4$/THF.

The optical purities[19] and theoretical yields of the residual (+)-(*S*,*S*) diester (**8**) were analyzed at the following time intervals: 90 min (68% ee; 95%); 150 min (95% ee; 64%); 210 min (>98% ee; 54%). Consequently, it is evident that to secure (+)-**8** of high optical purity, it is necessary to conduct the incubation for about 210 min to ensure the complete hydrolysis of (−)-**8**. The conversion of (+)-**8** into **3** may be achieved by selective chemical hydrolysis, because **8** possesses a C_2 axis of symmetry. However, in our experience, this transformation can be more advantageously effected in quantitative yields by exposure of (+)-**8** to PLE.

The esterase of *G. roseum* provides the synthetic organic chemist with a powerful chiral reagent for the preparation of the aforementioned valuable synthons in quantities sufficient for chiral syntheses. We are currently probing the enantiotopic specificity of this enzyme on structural units of the type $XCH(CH_3)CHOHCH(CH_3)X$. The results of these investigations will be forthcoming.

Acknowledgment. We are indebted to Professor J. B. Jones for helpful discussions. This investigation was supported in part by Grant HL25772 of the National Institutes of Health.

Cleavage of DNA by the 1,10-Phenanthroline-Copper Ion Complex. Superoxide Mediates the Reaction Dependent on NADH and Hydrogen Peroxide

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The 2:1 1,10-phenanthroline–cuprous complex $[(OP)_2Cu^+]$ cleaves double-stranded DNA in a reaction that requires hydrogen peroxide.[1,2] One-electron reductants such as superoxide anion and thiol have previously been used to produce $(OP)_2Cu^+$ in situ from the 1,10-phenanthroline–cupric complex $[(OP)_2Cu^{2+}]$.[2,3] The ability of NADH, an important intracellular two-electron reductant, to sustain the $(OP)_2Cu^+$-mediated cleavage has now been investigated, because we are interested in determining if this reaction shares the same useful cytotoxic properties as the DNA scission reaction of bleomycin and neocarzinostatin.[4-6] In this communication we wish to report that 0.1 mM NADH effectively facilitates the DNA cleavage reaction in the presence of 0.5 mM hydrogen peroxide. We further report that superoxide plays a central role in the chemistry by which the NADH maintains a kinetically significant concentration of $(OP)_2Cu^+$ for the DNA cleavage reaction.

$(OP)_2Cu^{2+}$ in the presence of NADH and hydrogen peroxide readily forms acid-soluble products from [^{3}H]poly[d(A-T)] (Figure 1). The cleavage reaction observed in the presence of NADH and hydrogen peroxide shares important properties with the reaction observed in the presence of thiol and hydrogen peroxide.[2] These include the production of inhibitors of *E. coli* DNA polymerase I and the inhibition of the cleavage reaction by intercalating agent(s) (e.g., ethidium bromide) and 2,9-dimethyl-1,10-phenanthroline, a cuprous ion chelating agent whose

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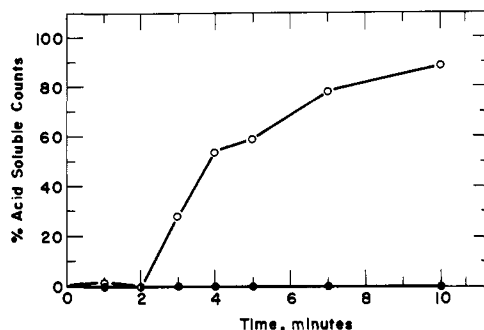


Figure 1. DNA cleavage by the 1,10-phenanthroline–copper complex facilitated by NADH and H_2O_2. [^{3}H]Poly[d(A-T)] (10 μg/mL) was incubated at 37 °C with 100 μM NADH, 20 μM OP, 2 μM cupric acetate, and 500 μM H_2O_2 in a Hepes buffer, pH 7.10. Acid-soluble counts were measured as previously described.[2] No other addition (○); 160 units/mL bovine erythrocyte superoxide dismutase (Worthington) (●).

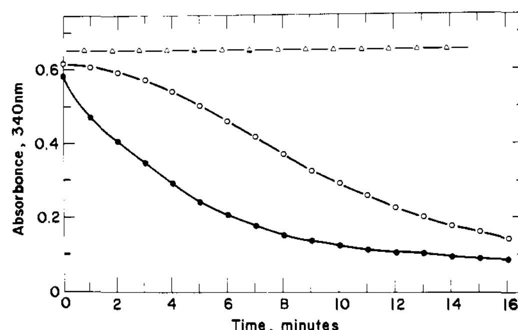
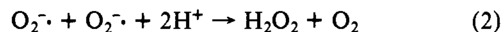


Figure 2. Oxidation of NADH by H_2O_2 catalyzed by $(OP)_2Cu^{2+}$. Hydrogen peroxide was added to a solution of NADH (100 μM), OP (20 μM), and cupric acetate (2 μM). Final concentration of H_2O_2, 500 μM. Total volume, 1 mL; pH 7.10 Hepes buffer; *T*, 21 °C. No other components (○); 140 μM 3-mercaptopropionic acid (●); 160 units bovine erythrocyte superoxide dismutase (△).

chelate does not degrade DNA. The important difference between them is that the reaction caused by NADH and hydrogen peroxide is inhibited by superoxide dismutase (Figure 1) whereas that observed with thiol and hydrogen peroxide is not.[2,3]

We and others have previously shown that diffusible superoxide anion produced by xanthine and xanthine oxidase does not cleave DNA on the time scale relevant here (Figure 1) in the absence of OP and cupric ion.[2,3,7,8] The sensitivity of the DNA cleavage reaction to superoxide dismutase therefore cannot be due to NADH, hydrogen peroxide, OP, and copper ion serving as a superoxide generator. It can, however, result if superoxide is important in mediating the formation of $(OP)_2Cu^+$, an essential coreactant in the cleavage reaction. Precedent for superoxide serving this function is the demonstration that the superoxide generating system, xanthine and xanthine oxidase, promotes an efficient catalase-sensitive DNA cleavage, because it increases the concentration of $(OP)_2Cu^+$ and hydrogen peroxide via the one-electron transfer reactions summarized in eq 1 and 2, respectively.[3] We propose that superoxide serves an analogous role

$$(OP)_2Cu^{2+} + O_2^{-}\cdot \rightarrow (OP)_2Cu^+ + O_2 \quad (1)$$



$$O_2^{-}\cdot + O_2^{-}\cdot + 2H^+ \rightarrow H_2O_2 + O_2 \quad (2)$$

in the NADH potentiated cleavage. This hypothesis is based on parallel kinetic studies of the DNA cleavage reaction and the oxidation of NADH by hydrogen peroxide catalyzed by $(OP)_2Cu^{2+}$.

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