

(90:4:6).
 (34) Unless otherwise stated, ir spectra were taken in CHCl_3 using a Perkin-Elmer Model 257 grating spectrophotometer. Nmr data (CDCl_3) were obtained with a Varian Associates Model A-60A and HA-100 spectrometer. Nmr shifts are expressed in parts per million downfield from internal tetramethylsilane ($\delta = 0$). Mass spectra were recorded using an AEI

MS-9 double focusing mass spectrometer at an ionizing voltage of 70 eV. All compounds which were submitted to mass spectrometric molecular-weight determination were of high purity as determined by nmr analysis and tlc. Uv spectra were taken on methanol solutions using a Cary Model 15 spectrophotometer. Carbon-hydrogen analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Asymmetric Total Synthesis of (–)-Prostaglandin E_1 and (–)-Prostaglandin E_2 ¹

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Abstract: A bioorganic asymmetric total synthesis of (–)-PGE₁ and (–)-PGE₂ has been accomplished *via* conjugate addition of organocuprates derived from *trans*-3(*S*)-hydroxy-1-iodo-1-octene (**3a**) to 2-(6-carbomethoxyhexyl)-4(*R*)-hydroxy-2-cyclopenten-1-one (**1a**) and 2-(6-carbomethoxy-*cis*-2-hexenyl)-4(*R*)-hydroxy-2-cyclopenten-1-one (**2a**), respectively. The chiral centers of these key synthons were introduced by the use of microbial enzymes. A variety of microbiological asymmetric oxidations and reductions were examined for the transformation of prochiral substrates into optically active prostaglandin synthons. Microbiological reactions are discussed and compared with the corresponding nonenzymatic reactions. The synthon **1a** was prepared from 2-(6-carbomethoxyhexyl)cyclopentane-1,3,4-trione (**4**) *via* the sequence: microbiological asymmetric reduction of the C-4 carbonyl in **4** yielding the *R* alcohol **5a** which may be converted into any number of suitable enolates **6** followed by reduction of the C-3 carbonyl and acid rearrangement. By an analogous sequence of reactions, **2a** was prepared from 2-(6-carbomethoxy-*cis*-2-hexenyl)cyclopentane-1,3,4-trione (**14**). The complementary octenyl synthon **3a** was synthesized *via* a modified hydroalumination method and *via* microbiological reduction of 1-iodo-1-*trans*-octen-3-one. A key step in the synthetic scheme for transformation of these synthons into prostaglandins involves the conjugate addition of octenylcopper reagents with cyclopentenones. The results of an extensive study of this key step are reported. The effects of reaction time and temperature on yield are delineated. The relative effectiveness and reactivities of various divinylcuprate, mixed cuprate, and vinylcopper reagents in the conjugate addition reaction are assessed.

In the accompanying paper,¹ details are given for the synthesis of (–)-prostaglandin E_1 (PGE₁) and related substances *via* the reaction of conjugated 2-alkylcyclopentenones with both unfunctionalized and oxygenated organocuprates. Further, the importance of the C-4 center (C-11 prostaglandin numbering) of **1a** in dictating the eventual stereochemistry of the substituents at C-12 and C-8 (prostaglandin numbering) of prostaglandins through steric interactions has been clearly demonstrated. We now report a bioorganic total synthesis of (–)-PGE₁ and (–)-PGE₂ wherein all the chiral centers are asymmetrically introduced.

Results and Discussion

Microbiological Induction of Asymmetry into the Cyclopentyl Synthon. Microbial enzymatic oxidation of unactivated (remote) centers in hydrocarbons is a well-known phenomenon. *A priori*, an asymmetric center might be introduced at any position in a prochiral substrate by, for example, microbial hydroxylation. Further, numerous microbial transformations occur at substrate centers which possess readily apparent activation toward the chemical reaction involved.

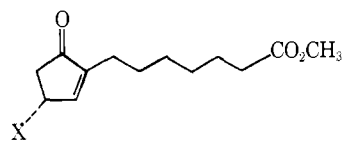
The allylic ring methylene of 2-(1-oxocyclopent-2-ene)heptanoic acid methyl ester (**1d**) is activated toward oxidation. In fact, the desired 4-hydroxy-2-cyclopenten-1-one **1a** has been prepared in racemic form from **1d** by chemical oxidation. Moreover, microbiological hydroxylation of 2-alkyl-1-oxocyclopent-2-enes such as cinerone to cinerolone is known.² However, the optical purity of the resulting cinerolone was only of the order of 60%. In addition, we have found that fungi containing hydroxylases have a general

proclivity toward degradation of the acid side chain of **1a**.³ We therefore turned our attention to the use of yeast reductases for asymmetric induction.

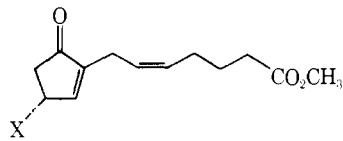
Cyclopent-2-ene-1,4-diones are activated toward reduction. These vinylogous α -diketones readily undergo selective monoreduction with a variety of reagents to yield cyclopent-2-en-4-ol-1-ones.⁴ Moreover, chemical reduction of 2,3-dialkylcyclopent-2-ene-1,4-diones often proceeds with very high regioselectivity for reduction of the least sterically hindered C=O bond. We therefore examined microbial reduction of 2-(1,4-dioxocyclopent-2-ene)heptanoic acid methyl ester (**1e**).

Initial experiments employing Baker's yeast for the reduction of **1e** indicated that reduction of the C=C bond accompanied C=O reduction. Saturation of α,β -unsaturated ketones is a very common microbiological transformation.⁵ On the assumption that C=C and C=O reductions were catalyzed by two independent enzymes, we performed the microbiological reduction of **1e** in the presence of excess 2-cyclohexenone or methyl vinyl ketone, in order to competitively inhibit the reduction of the double bond. This strategy succeeded. Remarkably, however, our first experiment, performed with Baker's yeast, yielded mostly 3-(1-oxo-4-hydroxycyclopent-2-ene)heptanoic acid, the product of regioselective monoreduction of the most sterically hindered C=O bond. This result poignantly reflects the unusual steric demands of enzymatic reactions in comparison with those of more common laboratory reagents.

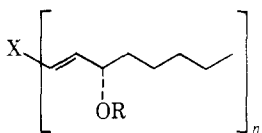
As most microorganisms provided only mixtures of the above product and the desired 2-(1-oxo-4-hydroxycyclopent-2-ene)heptanoic acid **1a** in low yields, we examined an alternate approach based on the observation that 2-(6-car-



- 1a, X = OH
 b, X = 2-tetrahydropyranyloxy
 c, X = OSi(CH₃)₂-*t*-Bu
 d, X = H
 e, X = O



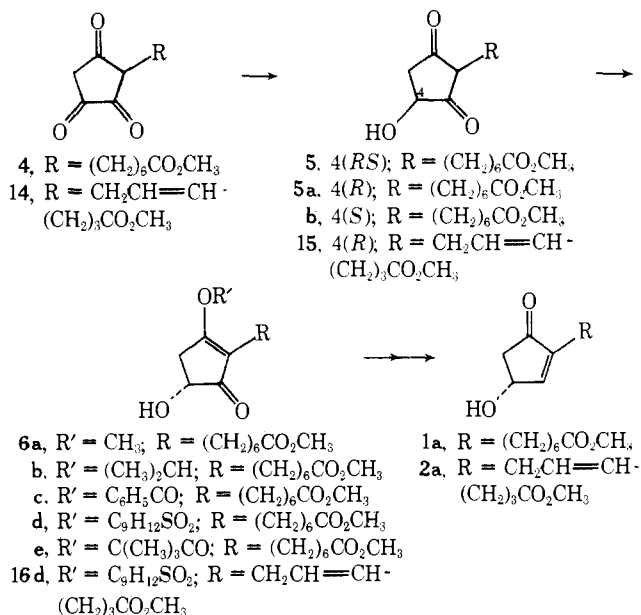
- 2a, X = OH
 b, X = 2-tetrahydropyranyloxy



- 3a, X = I; R = H; *n* = 1
 b, X = I; R = OCH(CH₃)OCH₂CH₃; *n* = 1
 c, X = Li; R = OCH(CH₃)OCH₂CH₃; *n* = 1
 d, X = Cu; R = OCH(CH₃)OCH₂CH₃; *n* = 2
 e, X = I; R = Si(CH₃)₂-*t*-Bu; *n* = 1
 f, X = Li; R = Si(CH₃)₂-*t*-Bu; *n* = 1
 g, X = Cu; R = Si(CH₃)₂-*t*-Bu; *n* = 2

bomethoxyhexyl)cyclopentane-1,3,4-trione (**4**) is readily chemically monoreduced.⁶ Moreover, microbiological monoreduction of α -diketones is a common reaction.⁷ We envisaged a pathway for the conversion of the readily available⁸ starting material **4** into the key intermediate **1a** via the sequence: (a) asymmetric reduction of the C-4 carbonyl to yield the *R* alcohol **5a**; (b) efficient conversion of **5a** into the enolate **6**; and (c) reduction of the C-3 carbonyl followed by acidic allylic rearrangement to yield **1a** (Scheme I).

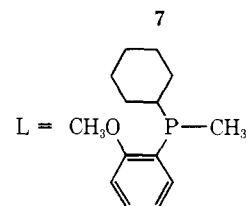
Scheme I



A variety of different types of alcohol dehydrogenases with varying degrees of chiralities are present among microorganisms; many of these not only possess broad substrate specificities but also catalyze reactions with high optical

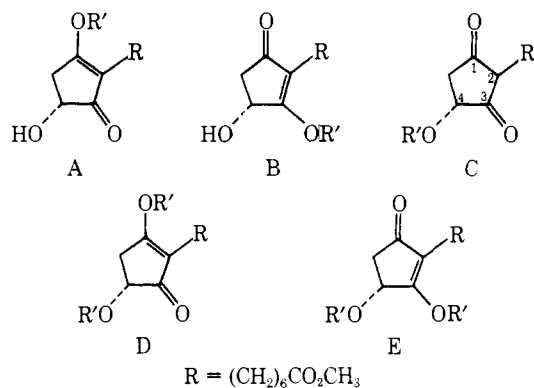
yields. Although many microorganisms were capable of catalyzing the reduction of **4** to **5**, most of these produced partially asymmetric products in low yields accompanied by side reactions such as partial cleavage of the methyl ester and acid side chain. However, two microorganisms were found to be uniquely suited for the introduction of this chiral center in **5**. *Dipodascus uninucleatus* catalyzed the completely asymmetric reduction of **4** to the 4(*R*) alcohol **5a** (75% yield) as manifested by its CD spectrum ($[\theta]_{281} -85,000$; $[\theta]_{262} +87,000$), whereas *Mucor rammanianus* gave the 4(*S*) alcohol **5b** (43% yield), characterized by virtue of an inverse CD spectrum ($[\theta]_{281} +89,000$; $[\theta]_{262} -82,000$).

Attempts to reduce the prochiral compound **4** by hydrogenation in the presence of a soluble rhodium catalyst with chiral phosphine ligands, which was successfully applied to the synthesis of *L*-amino acids,⁹ were only partially successful. For example, when **4** was subjected to hydrogenation in the presence of (1,5-cyclooctadiene)bis(*O*-anisylcyclohexylmethylphosphine)rhodium(I) tetrafluoroborate (**7**), only partial asymmetric reduction of **4** was achieved as evidenced by the isolation of **5a** (75% yield) with an optical purity of 30%. Recrystallization of **5a** improved the optical purity of **5a** (34% yield) to 68%.



Synthesis of Chiral Enone 1a from Chiral Dione 5a. Having surmounted the chirality problem at C-4 in **5**, it was necessary to transform **5a** into **6**. It had been shown⁶ that when (+)-**5** was refluxed with 2,2-dimethoxypropane in the presence of an acid catalyst, two isomeric methyl enol ethers A and B were formed in a ratio of 40:60. Under these conditions however, (+)-**5a** afforded only totally racemic products. Since this asymmetric center appears relatively stable to mild base, it was decided to examine the effect of acylating and alkylating agents under mildly basic conditions, with a view to obtaining a more favorable ratio of the desired enolate A without racemization. Reaction of (+)-**5a** with excess acetyl chloride in the presence of triethylamine gave isomers D and E in a ratio of 75:25, whereas benzoyl chloride further improved the ratio to 90:10. By using 1 equiv of the acylating agent, the amount of diacylated products may be reduced considerably. Although acylation at C-4 proceeded slower than that of the enolates at C-1 and C-3, it was not possible to eliminate it entirely. By using a bulky acylating agent such as pivaloyl chloride, the desired enol acylate A was obtained almost exclusively, and no isomeric enol acylate B was detected. Similarly, the formation of isomeric enol ethers via reaction of (+)-**5a** with alkyl halides of varying sizes was examined. It was found that alkylation using methyl iodide gave a ratio of A to B of 40:60, but by increasing the size of the alkylating reagent such as 1-iodo-3-methylbutane, this ratio may be reversed to 70:30. However, of these, only the isopropyl enol ether A was crystalline. A similar trend was observed via the formation of enol sulfonates. While methanesulfonyl chloride gave a ratio of A:B of 40:60, mesitylenesulfonyl chloride afforded the isomeric mesitylene enol sulfonates in a ratio of 90:10 (Table I). These results clearly show that the enolate at C-1 is sterically less hindered than that at C-3, and that the size

of the acylating or alkylating agent plays an important role in determining the ratios of the resulting isomeric enolates.



No apparent racemization occurred when these experiments were repeated using (+)-**5a**, for the resulting isopropyl enol ether **6b**, the enol benzoate **6c**, and the mesitylene enol sulfonate **6d** were all found to be optically active.

Our attention was then focused on the conversion of **6** into **1a** via reduction of **6a**, followed by allylic rearrangement in acid. Although a variety of hydrides may be used, the most suitable one was sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al). When **6b** was reduced with an excess of Red-Al, at -78° followed by acid treatment, **1a** was obtained in yield of 60%. Using similar conditions, it was also possible to convert **6c** into **1a** (60% yield). However, because of the electron-withdrawing property of the enol benzoate **6c**, the acid-catalyzed allylic rearrangement was considerably slower than those of enol ethers. The reduction of **6e** gave the diol in poor yield (10%), which appeared to be due to the loss of the C(CH₃)₃CO group during reduction to give back **5a**. The acid-catalyzed rearrangement of the diol gave **1a** in very low yield. On the other hand, the mesitylene enol sulfonate **6d**, which was efficiently formed from (+)-**5a**, was readily reduced by Red-Al¹⁰ and was rapidly rearranged¹¹ to **1a** (overall yield 54% from **5a**).

Asymmetric Synthesis of the Octenyl Synthone. Several procedures may now be followed for the synthesis of (*S*)-iodovinylcarbinol (**3a**). In the accompanying paper,¹ (*S*)-1-octyn-3-ol was obtained by chemical resolution and converted to (*S*)-**3a** via the hydroalumination method. Alternatively, it may be prepared from (*S*)-1-octyn-3-ol via a borinate intermediate^{12,13} or via chemical resolution of (+)-**3a**, prepared from 1-chloro-*trans*-1-octen-3-one.^{14,15} We have now found that it is possible to introduce this chiral center with a high degree of stereoselectivity via microbial reduction of 1-iodo-1-octen-3-one.^{14,15}

As a rule α,β -unsaturated ketones do not yield allylic alcohols by enzymic reduction owing to resonance stabilization of the carbon-oxygen double bond.¹⁶ However, when an electronegative substituent such as a halogen is introduced into the α,β -unsaturated ketone on the double bond, there is interference with the usual α,β -unsaturated ketone resonance, and a net destabilization of the system and enzymic reduction to substituted allylic alcohols may be achieved.¹⁷ Based on this rationale, it was found that washed cells of *Penicillium decumbens* catalyzed the reduction of 1-iodo-1-*trans*-octen-3-one to the desired 3(*S*) alcohol **3a** (10% yield), $[\alpha]^{24}_D +7.51^\circ$,¹⁸ whereas *Asperigillus ustus* gave the 3(*R*) enantiomer of **3a** (12%), $[\alpha]^{24}_D -8.0^\circ$. Using growing cultures of these organisms, the yield of **3a** was reduced to ca. 3% as the majority of the substrate was incorporated by the microorganism.¹⁹

Conjugate Addition of Vinyl Synthones to Cyclopentenones. PGE₁. After protection of the (*S*)-**3a** as the ethoxy-

Table I. Effect of Acylating and Alkylating Agents on Isomer Distribution

Reagents	Yield of isomers, %				
	A	B	C	D	E
Me ₂ C(OMe) ₂ , MeOH, HCl	40	60			
CH ₃ COCl (excess), Et ₃ N				75	25
C ₆ H ₅ COCl (excess), Et ₃ N				90	10
C ₆ H ₅ COCl (1 equiv), Et ₃ N	78		22	<i>a</i>	
Me ₂ CHCOCl (1 equiv), Et ₃ N	85		<i>b</i>	15	
Me ₂ CCOCl (1 equiv), Et ₃ N	>95		<i>b</i>	Trace	
MeSO ₂ Cl	40	60			
C ₆ H ₅ SO ₂ Cl	85	15			
2-C ₁₀ H ₇ SO ₂ Cl	85	15			
Mesitylenesulfonyl chloride	90	10			
2-Iodobutane	40	60			
2-Iodopropane	60	40			
1-Iodo-2-methylpropane	70	30			
1-Iodo-3-methylbutane	70	30			

^a Formed in variable amounts (ca. 0–15%). ^b Acidic fraction not examined. The procedures for acylation and alkylation are described in the Experimental Section.

ethyl ether **3b**, it was found more convenient to generate the vinylolithium reagent **3c** by the use of 2 mol equiv of *tert*-butyllithium.²⁰ We have earlier¹ alluded to the lability of the vinylolithium species **3c** so that it must be used immediately for the subsequent conjugate addition upon generation. Moreover, the nature of ligands appeared to influence the stability and reactivity of the cuprate **3d** as demonstrated by the results of our studies on the conjugate addition of the divinylcuprate reagent **3d** to either **1b** or **1c**. The failure of others¹² to obtain significant yields of 1,4 adducts could be attributed to two main factors. It is likely that trimethyl phosphite is a poor complexing ligand for the *trans*-divinylcuprate, although it appears quite suitable for the *cis*-divinylcuprate.²¹ Further, in our experience at -78° , conjugate addition proceeds extremely slowly, which is consistent with their recovery of large quantities of unreacted starting material.¹² To obtain optimum yields of 1,4 adducts, several critical experimental factors must be kept in mind. While it is desirable to generate the vinylolithium species at -78° , the optimum temperature needed for conjugate addition with the *trans*-divinylcuprate lies between -15 and -20° for a period of 30 min, depending on the ligand and the size of the protecting group in **1a**.²² Because the resulting enolate may react further at higher temperatures, resulting in lower yields of desired products, it is preferable to quench the reaction at -15 to -20° prior to work-up.

The results of our conjugate addition experiments are summarized in Table II. We have consistently noted that highest yield of PGE₁ methyl ester (60%) was obtained by reaction of the *trans*-divinylcuprate **3g** with **1c**. However, the condition²⁰ used for the removal of dimethyl-*tert*-butylsilyl protecting group always resulted in significant quantities of PGA₁ methyl ester. Also, as only 1 mol of **3c** is consumed in the reaction, considerable amount of coupling of the vinylcuprates²³ was noted. Corey and Beams²⁰ proposed to overcome this disadvantage by the use of a mixed cuprate, comprised of 1-pentyne and **3c** complexed with 2 mol of hexamethylphosphoric triamide. We have been unable to demonstrate a significant advantage of this mixed cuprate over vinylcopper reagents. The yields of PGE₁ methyl ester obtained using the vinylcopper reagent (X₃P)_n(R)CuLi are of the order of 40–50%, which are comparable to the yields found for the reactions using the Corey mixed cuprate reagent. With the vinylcopper reagent, the time required for the reaction to go to completion was found to be dependent on the phosphine used. With tri-*n*-butylphosphine, the reac-

Table II. Yield of PGE₁ Methyl Ester from 1,4-Addition Reactions Using Organocuprates^a

	Protecting group on 1a	Protecting group on 3a	Type of copper reagent	Reaction time, min	Percentage yield ^b
(a) Divinylcuprate Reagents					
1	DMTBS ^c	DMTBS	<i>n</i> -Bu ₃ P·CuI	90	65–70
2	DMTBS	DMTBS	<i>n</i> -Bu ₃ P·CuI	60	62
3	DMTBS	DMTBS	(Me ₂ N) ₃ P·CuI	60	37 ^d
4	DMTBS	DMTBS	(Me ₂ N) ₃ P·CuI	90	55
5	THP	(1-EtO)Et ^e	<i>n</i> -Bu ₃ P·CuI	60	55–60
6	THP	(1-EtO)Et	<i>n</i> -Bu ₃ P·CuI	30	50
7	THP	(1-EtO)Et	(Me ₂ N) ₃ P·CuI	60	47
8	DMIS ^f	(1-EtO)Et	<i>n</i> -Bu ₃ P·CuI	90	44
9	THP	(1-EtO)Et	X(CH ₃) ₃ N·CuI ^g	30	35.5
10	THP	(1-EtO)Et	2Bu ₂ S·CuI	40	30
11	THP	(1-EtO)Et	4 <i>i</i> -Pr ₂ S·CuI	40	30
(b) Mixed Cuprate Reagents					
12	DMTBS	(1-EtO)Et	HMPCu–pentyne (Corey's reagent)	90	38
13	THP	(1-EtO)Et	HMPCu–pentyne (Corey's reagent)	90	38
14	THP	(1-EtO)Et	HMPCu–pentyne (Corey's reagent)	60	50
15	THP	(1-EtO)Et	<i>t</i> -Bu-O-Cu	90	0
16	THP	(1-EtO)Et	Ph-S-Cu	60	61
17	THP	(1-EtO)Et	(Me ₃ Si) ₂ N-Cu	70	27
18	(a) THP	(1-EtO)Et	Ph ₂ P·Cu ^h	60	54
	(b) THP	(1-EtO)Et	Ph ₂ P·Cu ⁱ	60	50
(c) Vinylcopper Reagents					
19	DMTBS	DMTBS	(Me ₂ N) ₃ P·CuI	135	46
20	THP	(1-EtO)Et	(Me ₂ N) ₃ P·CuI	135	40
21	THP	(1-EtO)Et	2(Me ₂ N) ₃ P·CuI	135	48
22	THP	(1-EtO)Et	2(Me ₂ N) ₃ P·CuI	135 ^j	45
23	THP	(1-EtO)Et	<i>n</i> -Bu ₃ P·CuI	60	42
24	THP	(1-EtO)Et	<i>n</i> -Bu ₃ P·CuI	35	43
25	THP	(1-EtO)Et	2(<i>n</i> -Bu ₃ P)·CuI	60	45
26	THP	(1-EtO)Et	2(<i>n</i> -Bu ₃ P)·CuI	35	50
PGE ₂ Methyl Ester					
27	DMTBS	DMTBS	<i>n</i> -Bu ₃ P·CuI	90	55
28	THP	(1-EtO)Et	<i>n</i> -Bu ₃ P·CuI	60	50

^a All reactions were carried out in diethyl ether at -22 to -18° , unless otherwise stated. ^b Yields are based on protected 1a. ^c Dimethyl-*tert*-butylsilyl. ^d Unreacted starting material (33%). ^e (1-Ethoxy)ethyl. ^f Dimethylisopropylsilyl. ^g The composition of this reagent is not known. ^h Prepared by treating diphenylphosphine with a molar equivalent of *n*-butyllithium at 0° to give lithium diphenylphosphide (Ph₂PLi), followed, after 60 min stirring at 0° , by reaction with tri-*n*-butylphosphine–copper iodide complex. ⁱ In this reaction, lithium diphenylphosphide was prepared by the reaction of chlorodiphenylphosphine and lithium wire. Reaction with tri-*n*-butylphosphine–copper iodide complex as in *h* gave the required copper reagent. ^j Temperature of reaction was -20 to -10° .

tion was complete after 30 min, whereas with hexamethylphosphoric triamide,²⁴ the reaction took longer, *viz.*, 135 min. Further, the addition of a second equivalent of phosphine appeared to stabilize the vinylcopper reagent at -20° and gave slightly better yields, *viz.*, 45–50% *vs.* 40–42%. When 1 equiv of *n*-Bu₃P was used, the color of the reaction mixture at -20° turned brown after 10–15 min and black after 20–35 min. With (Me₂N)₃P, the color was brown after 60 min and black after 90–100 min. When 2 equiv of phosphine was used, the color remained yellow throughout the reaction.

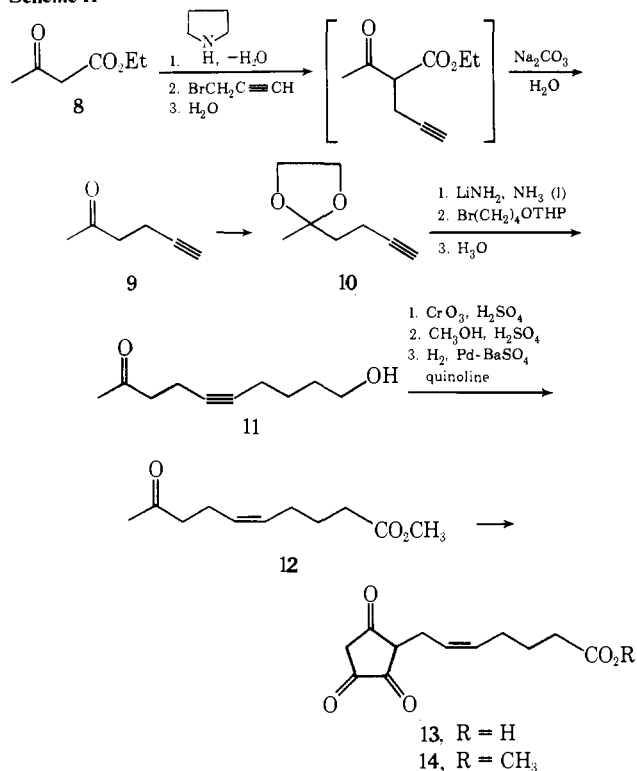
In addition to the Corey reagent, other mixed cuprates²⁵ (prepared from the corresponding heterocuprate species and 3c) such as PhS(R)CuLi and (Ph)₂P(R)CuLi may be used efficiently for conjugate addition to 1b with yields in the range of 50–60% (Table II). However, nitrogen-containing mixed cuprates such as [(CH₃)₃Si]₂N(R)CuLi are inferior and gave much lower yields (25–30%). Oxygen-containing mixed cuprates such as *t*-BuO(R)CuLi gave no 1,4-addition product. As a rule, the mixed cuprates and the vinylcopper reagents gave fewer reaction products than the divinyl cuprates making separation somewhat easier and cleaner and in the former two cases improving the yield based on vinyl synthon. The synthesis of PGE₁ was successfully completed by hydrolysis of the ester grouping with *Rhizopus oryzae*. The product was optically pure and crystalline and identical with the natural product.

Asymmetric Synthesis of PGE₂. Reaction of 1,4-butane-

diol with dihydropyran gave 1-(2-tetrahydropyranyloxy)butan-4-ol in 77% yield,²⁶ which was converted into 1-bromo-4-(2-tetrahydropyranyloxy)butane²⁷ (77% yield) *via* mesylation and treatment of the mesylate with LiBr in acetone. The pyrrolidine enamine²⁸ of acetoacetic ester (8) was condensed with propargyl bromide^{29,30} which after alkaline hydrolysis and decarboxylation afforded 1-hexyn-5-one^{30,31} (9) in 70% yield. After conversion of 9 into the cycloethylene ketal³² 10 (89% yield) in the usual manner, 10 was treated with lithium amide in liquid NH₃ and reacted with 1-bromo-4-(2-tetrahydropyranyloxy)butane using THF as cosolvent. Acidic hydrolysis of the resultant ethylene ketal-THP ether gave 1-hydroxy-5-decyn-9-one (11) in 89% yield from 10. Jones oxidation³³ of 11, followed by acid-catalyzed esterification and hydrogenation over Lindlar's catalyst, afforded methyl 9-keto-*cis*-5-decenoate (12) in 60% yield overall (Scheme II). Condensation of 12 with diethyl oxalate,⁸ followed by acid hydrolysis and methylation, gave 2-(6-carbomethoxy-*cis*-2-hexenyl)cyclopentane-1,3,4-trione (14) in 50% yield. The acid 13 was only reduced very slowly by *D. uniuucleatus*; in contrast, the methyl ester 14 was rapidly converted to the 4(*R*) alcohol 15 (48%) with an optical purity of 100%, which was converted into 2a in a sequence of reactions analogous to those of 1a (Scheme I).

Reaction of 15 with 1.2 equiv of 2-mesitylenesulfonyl chloride and triethylamine at -10° afforded two isomeric enol sulfonates (16d and 16e) in a ratio of 9:1. This mixture was directly reduced with an excess of sodium bis(2-

Scheme II



methoxyethoxy)aluminum hydride in toluene at -70° , followed by acid-catalyzed rearrangement and elimination to afford **2a** as an oil in 50% yield from **14**.

After protection of **2a** as the tetrahydropyranyl ether **2b**, it was treated with 1.2 equiv of the cuprate **3d** (-20° , 1 hr). Upon acidic hydrolysis of the protecting groups and chromatography (–)-PGE₂ methyl ester was obtained in 50% yield from **2a**. Again, *Rhizopus oryzae* was used for hydrolysis of the methyl ester to yield (–)-PGE₂.

Experimental Section³⁴

Asymmetric Hydrogenation of 2-(6-Carbomethoxyhexyl)cyclopentane-1,3,4-trione (4). To 5 g of **4**, dissolved in 35 ml of methanol, were added 96 mg of the catalyst $\text{L}_2\text{Rh}^+\text{CODBF}_4^-$ (available from Monsanto Co., St. Louis, Mo., under the identification CP 71327 where L indicates *o*-anisylcyclohexylmethylphosphine and COD represents 1,5-cyclooctadiene) and 2.78 ml of triethylamine, and the mixture was subjected to hydrogenation at 1 atm. After 92.6% of the theoretical quantity of hydrogen had been consumed, the reaction was terminated by pouring the hydrogenation reaction mixture into $\text{HCl-H}_2\text{O}$ (about pH 2). The resulting mixture was extracted three times with ethyl acetate, the organic layers were separated and washed with sodium bicarbonate solution (5% solution) until no color appeared in the aqueous phase. The combined bicarbonate extracts were extracted with ethyl acetate, and the resulting yellow aqueous layer was carefully acidified to pH 2.0 with hydrochloric acid and extracted with ethyl acetate. The organic layer was separated, dried over Na_2SO_4 , and evaporated to dryness. The dried material was dissolved in and then crystallized from ethyl acetate giving 1.7 g of product (34% yield): $\text{uv } \lambda_{\text{max}} 272 \text{ nm}$ ($\epsilon 24,000$); $\text{CD } \lambda 281 \text{ nm}$ ($\theta = -58.3 \times 10^3$); $\theta = +60 \times 10^3$ at $\lambda 262 \text{ nm}$ (68% optical purity). A second crop (1.6%) showed no optical activity, filtrate almost no optical activity.

Microbiological Synthesis of 2-(6-Carbomethoxyhexyl)-4(R)-hydroxycyclopentane-1,3-dione (5a). The surface growth from a 1 week old agar slant of *Dipodascus uninucleatus* was suspended in 5 ml of saline (0.85%) solution. Portions (2 ml) of this suspension were used to inoculate 50 ml of the soybean-dextrose medium (soybean meal, 5 g; glucose, 20 g; yeast extract, 5 g; K_2HPO_4 , 5 g; NaCl , 5 g; distilled water to 1 l.; pH adjusted to 6.5 with HCl) held in 250-ml erlenmeyer flasks (F-1 stage). The flasks were incubated at 25° on a rotary shaker (250 cycles/min; 2-in. radius) for

24 hr, after which a 10% by volume transfer was made to each of four 2 l. erlenmeyer flasks (F-2 stage), containing 500 ml of the soybean-dextrose medium. After 24 hr of incubation on a rotary shaker, 250 mg of **4**, dissolved in 2 ml of dimethylformamide, was added to each flask. The F-2 stage flasks were then incubated for an additional 24 hr under the condition used in the incubation of the F-1 stage flasks.

Twenty-four hours after the addition of **4**, the cells were removed by centrifugation. The supernatant was adjusted to pH 2.5 with 6 N HCl and was exhaustively extracted with 1.5 l. of ethyl acetate three times. The ethyl acetate was dried over Na_2SO_4 and evaporated to afford an oily residue. This residue was dissolved in 5 ml of benzene-ethyl acetate (1:1) and added onto a column (32 \times 2.5 cm) of silicic acid-Celite (85:15). The column was eluted with a gradient system consisting of 500 ml of 50% ethyl acetate-benzene in the mixing chamber and 500 ml of pure ethyl acetate in the reservoir, and 7-ml fractions were collected. Fractions 25–104 containing the desired product were pooled and concentrated to dryness yielding 1.2 g of crystalline residue. Recrystallization from ethyl acetate-petroleum ether afforded 750 mg of **5a**: mp $89-91^\circ$; $[\alpha]_D^{23} +16.2$ ($c 1.02$, CHCl_3), $+7.22^\circ$ ($c 0.54$, CH_3OH); $\text{uv } \lambda_{\text{max}} 272 \text{ nm}$ ($\epsilon 23,500$); $\text{CD } [\theta] \times 10^{-3} = -85^\circ$ at $\lambda 281 \text{ nm}$ and $[\theta] \times 10^{-3} = 87^\circ$ at $\lambda 262 \text{ nm}$; $\text{nmr } \delta 3.68$ (s, 3, OCH_3) and 4.75 ppm (broad m, 1, C-4 H); $\text{ir} (\text{CHCl}_3) 3400, 1725, \text{ and } 1605 \text{ cm}^{-1}$. *Anal.* Calcd for $\text{C}_{13}\text{H}_{20}\text{O}_5$: C, 60.92; H, 7.87. Found: C, 61.16; H, 7.95.

2-(6-Carbomethoxyhexyl)-4(S)-hydroxycyclopentane-1,3-dione (5b). The fermentation conditions are the same as those described previously, except *Mucor rammanianus* was substituted for *Dipodascus uninucleatus*.

Twenty-four hours after the addition of **4**, the mycelia were removed by filtration through cheesecloth. The supernatant was adjusted to pH 2.5 and was exhaustively extracted with two esters of ethyl acetate three times. The ethyl acetate layer was dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed over a silicic acid-Celite (85:15) column (2.5 \times 30 cm). The column was eluted with a gradient system consisting of 350 ml of benzene-ethyl acetate (1:1) in the mixing flask and 350 ml of ethyl acetate in the reservoir flask, and 7-ml fractions were collected. Fractions 38–59 containing the desired product were pooled and concentrated to dryness yielding 430 mg of crystalline **5b**. Recrystallization from ethyl acetate-hexane afforded pure **5b**: mp $92-94^\circ$; $[\alpha]_D^{24} -16.4^\circ$ ($c 0.82$, CHCl_3); $\text{uv } \lambda_{\text{max}} 272 \text{ nm}$ ($\epsilon 22,500$); $\text{nmr } \delta 3.68$ (s, 3, OCH_3) and 4.75 ppm (broad m, 1, C-4 H); $\text{ir} (\text{CHCl}_3) 3450, 1730, \text{ and } 1605 \text{ cm}^{-1}$. *Anal.* Calcd for $\text{C}_{13}\text{H}_{20}\text{O}_5$: C, 60.92; H, 7.87. Found: C, 61.24; H, 7.83.

1-Isopropoxy-2-(6-carbomethoxyhexyl)-4(R)-hydroxy-1-cyclopenten-3-one (6b). To 514 mg of **5a** (2.0 mmol), were added 546 mg of K_2CO_3 (4 mmol) and 5 ml (8.5 g, 50 mmol) of isopropyl iodide in 25 ml of dry acetone. The mixture was heated under reflux (N_2 atmosphere) for 24 hr. After cooling, the reaction mixture was diluted with Et_2O , and the ethereal layer was washed successively with water, NaHCO_3 , water, and saturated NaCl . The solvent layer was then dried over Na_2SO_4 and evaporated to dryness to yield 560 mg of an oily residue. The residue was dissolved in isopropyl ether to yield 316 mg (53%) of **6b**: mp $60-62^\circ$; $\text{uv } \lambda_{\text{max}} 259 \text{ nm}$ ($\epsilon 20,000$); $\text{nmr} (\text{CDCl}_3) \delta 1.35$ (d, 6, $\text{HC}(\text{CH}_3)_2$), 3.65 (s, 3, COOCH_3), 4.32 (q, 1, $>\text{CHOH}$), and 4.67 ppm (s, 1, $\text{CH}(\text{CH}_3)_2$); $[\alpha]_D^{24} +35.1^\circ$ ($c 1.02$, MeOH); $\text{ir} (\text{CCl}_4) 1745, 1700, \text{ and } 1625 \text{ cm}^{-1}$. *Anal.* Calcd for $\text{C}_{16}\text{H}_{26}\text{O}_5$: C, 64.40; H, 8.78. Found: C, 64.73; H, 9.03.

1-Benzoyloxy-2-(6-carbomethoxyhexyl)-4(R)-hydroxy-1-cyclopenten-3-one (6c). To a stirred cooled (-15°) solution of **5a** (512 mg, 2 mmol) and triethylamine (0.6 ml, ca. 4 mmol) (distilled over CaH_2) in dry THF (8 ml) was added benzoyl chloride (0.23 ml, 2 mmol) over 5 min. A nitrogen atmosphere was maintained at all times. The resulting solution was stirred at -15 to -10° for 1 hr. Methanol (4 ml) was then added and the solution allowed to warm to room temperature. After being poured into water (70 ml), the resulting solution was extracted with ethyl acetate (4 \times 50 ml). The combined extract was washed successively with saturated NaHCO_3 (2 \times 50 ml) and NaCl (50 ml) solutions, dried (MgSO_4), and evaporated to afford a yellow oil. The nmr spectrum of this residue was shown to consist mainly of the enol benzoate **16c** and two isomeric dibenzoates, isomers D and E. (An approximate ratio of isomers A, D, and E in the reaction mixture

may be obtained by comparing the signals for their C-4 hydrogens at δ 4.44, 5.57, and 6.47, respectively. The ratio of A:D:E was found to be 16:2.5:1.) None of the isomeric monoenoil benzoate **B** could be detected. The oily residue was dissolved in benzene and chromatographed over a silicic acid-Celite (85:15) column (2.5 \times 23 cm). The column was eluted with a gradient system consisting of 300 ml of benzene in the mixing chamber and 300 ml of benzene-ethyl acetate (7:3) in the reservoir, and 7-ml fractions were collected. Fractions 39-46 were pooled to give 81 mg of the two isomeric dibenzoates **D** and **E**: nmr δ 3.58 (s, 3, OCH₃), 5.57 (dd, 1, $J_{4,5} = 6$, $J_{4,5} = 3$ Hz, C-4 H), 6.47 (dd, trace, isomeric benzoate **E**, C-4 H), 7.5 (m, 3, phenyl C-3, C-4, and C-5 H), and 8.08 ppm (m, 2, phenyl C-2 and C-6 H); ir (CHCl₃) 1740, 1720, 1650, and 1590 cm⁻¹; molecular ion at *m/e* 464.1834 (theory for C₂₇H₂₈O₇, 464.1818). Fractions 55-66 were combined to yield 450 mg of **6c**: nmr (CDCl₃) δ 3.58 (s, 3, OCH₃), 4.44 (dd, 1, $J = 6$, $J = 3$ Hz, C-4 H), 5.06 (broad s, 1, OH), 7.5 (m, 3, phenyl C-3, C-4, and C-5 H), and 8.08 ppm (m, 2, phenyl C-2 and C-6 H); $[\alpha]^{24D} +35^\circ$ (*c* 2.0, CHCl₃); uv λ_{max} 241 nm (ϵ 20,100); ir (CHCl₃) 1742, 1735, 1720, 1650, and 1590 cm⁻¹. Peak match (*M* - 18) at *m/e* 342.1466 (theory for C₂₀H₂₂O₅, 342.1463).

The pH of the NaHCO₃ washings was adjusted with 1 *N* HCl to 2.5, and the resulting solution was extracted with ethyl acetate (3 \times 50 ml). The combined extract was washed with a saturated NaCl solution (50 ml), dried (MgSO₄), and evaporated to give an orange-yellow oily solid, which was shown by nmr to consist mainly of the *O*-benzoate. This residue was chromatographed over a silicic acid-Celite (85:15) column (2.5 \times 18 cm). The column was eluted with a gradient system consisting of 300 ml of benzene-ethyl acetate (95:5) in the mixing flask and 300 ml of benzene-ethyl acetate (7:3) in the reservoir, and 7-ml fractions were collected. Fractions 38-55 were combined to yield 114 mg of 2-(6-carbomethoxyhexyl)-4(*R*)-benzoyloxycyclopentane-1,3-dione: mp 114-116°; $[\alpha]^{24D} +185.3^\circ$ (*c* 0.80, CHCl₃); uv λ_{max} 228 nm (ϵ 16,200), 273 (26,000); ir (CHCl₃) 1740, 1730, 1690, 1660, 1603, and 1590 cm⁻¹; nmr (CD₃COCD₃) δ 3.58 (s, 1, OCH₃), 5.86 (dd, 1, C-4 H), 7.50 (m, 3, phenyl C-3, C-4, and C-5 H), and 8.06 ppm (m, 2, phenyl C-2 and C-6 H). *Anal.* Calcd for C₂₀H₂₄O₆: C, 66.65; H, 6.71. Found: C, 66.46; H, 7.10.

1-Benzoyloxy-2-(6-carbomethoxyhexyl)-(3*E*,4*R*)-dihydroxy-1-cyclopentene. A 1.5 *M* Red-Al (Aldrich) solution in toluene (2.66 ml, 4 mmol) was added dropwise to a cooled (-78°) stirred solution of monoenoil benzoate (**6c**) (360 mg, 1 mmol) in dry THF (75 ml) over 15 min under a nitrogen atmosphere, and the resulting solution was stirred at the same temperature for 35 min. A glacial acetic acid-THF (1:1) solution (5 ml) was slowly added over 15 min. The resulting solution was then allowed to warm to room temperature, poured into 100 ml of water, and extracted with ether (4 \times 100 ml). The combined ethereal extract was washed with a saturated NaCl solution, dried (MgSO₄), and evaporated to yield a light yellow solid. The residue was chromatographed over a silicic acid-Celite (85:15) column (1.8 \times 33 cm). The column was eluted with a gradient system comprised of 300 ml of benzene-ethyl acetate (7:3) in the mixing flask and 300 ml of benzene-ethyl acetate (3:7) in the reservoir flask, and 5-ml fractions were collected. Fractions 28-37 were combined to yield 252 mg of the desired diol: mp 92-93°; $[\alpha]^{24D} -44.5^\circ$ (*c* 0.51, CH₃OH); ir (CHCl₃) 3610, 3460, 1740, 1736, and 1600 cm⁻¹; nmr δ 3.63 (s, 3, OCH₃), 4.42 (broad m, 1, C-4 H), 4.63 (broad m, 1, C-3 H), 7.60 (m, 3, phenyl C-3, C-4, and C-5 H), and 8.16 ppm (m, 2, phenyl C-2 and C-6 H); peak match for (*M* - 18) at *m/e* 344.1623 (theory for C₂₀H₂₄O₅, 344.1628).

Reaction of 5a with Pivaloyl Chloride. To a solution of **5a** (256 mg, 1 mmol) and triethylamine (0.3 ml, 2 mmol) in dry THF (8 ml) cooled to -20° under a nitrogen atmosphere was added pivaloyl chloride (0.12 ml, 1 mmol) over 5 min with stirring. The resulting solution was stirred at -20 to -10° for 3 hr. Methanol (2.5 ml) was added, and the solution was allowed to warm to room temperature and poured into a water saturated NaCl solution (20 ml). This solution was extracted with ethyl acetate (4 \times 20 ml), and the combined extracts were washed with saturated NaHCO₃ (2 \times 50 ml) and saturated NaCl (50 ml) solutions, dried (MgSO₄), and evaporated to yield an orange oil, which was shown by nmr to consist mainly of the desired enol pivaloate along with a small quantity of the dipivaloate isomers **D** and **E**. (An approximate ratio of these three compounds can be derived from their C-4 nmr signals

at δ 4.42, 5.21, and 6.1 ppm, respectively. The ratio for A:D:E was 85:13.5:1.5.) None of the isomeric enol pivaloate (isomer **B**) could be detected. The residue was chromatographed over a silicic acid-Celite (85:15) column (2.5 \times 21 cm), and the column was eluted with a gradient system consisting of 300 ml of benzene-ethyl acetate (7:3) in the reservoir; 7-ml fractions were collected. Fractions 25-36 were combined to yield 87 mg of the dipivaloates (isomers **D** and **E**): ir (Nujol) 1770, 1730, and 1660 cm⁻¹; nmr δ 1.32 (s, 18, C(CH₃)₃), 3.66 (s, 3, OCH₃), 5.21 (dd, 1, $J_{4,5} = 6$, $J_{4,5} = 3$ Hz, C-4 H), and 6.10 ppm (dd, trace, isomeric dipivaloate **E**, C-4 H). *Anal.* Calcd for C₂₃H₃₆O₇: C, 65.07; H, 8.55. Found: C, 65.62; H, 8.39. Fractions 55-73 were combined and evaporated to give 161 mg of **6e**: ir (Nujol) 1770, 1740, 1725, and 1657 cm⁻¹; nmr δ 1.34 (s, 9, C(CH₃)₃), 3.66 (s, 3, OCH₃), 4.42 (dd, 1, $J_{4,5} = 6$, $J_{4,5} = 3$ Hz, C-4 H), and 5.83 ppm (broad s, 1, OH); uv λ_{max} 242 nm (ϵ 12,000); $[\alpha]^{24D} +38.2^\circ$ (*c* 0.5, CHCl₃). *Anal.* Calcd for C₁₈H₂₈O₆: C, 63.51; H, 8.29. Found: C, 63.40; H, 8.01.

Transformation of 5a into 1a via the Mesitylene Enol Sulfonate 6d. To a solution of **5a** (3.94 g, 15.4 mmol) and triethylamine (4.4 ml, 31.28 mmol) in dry THF (50 ml), cooled to -10° under a nitrogen atmosphere was added dropwise a solution of 2-mesitylensulfonyl chloride (3.6 g, 16.4 mmol) in dry THF (30 ml) over 20 min with stirring. A solid precipitated out after a few milliliters of the latter solution had been added. The resulting mixture was stirred at -10 to 0° for 20 min and then at room temperature for 45 min. This solution was then filtered through glass wool into a pressure-equalizing dropping funnel, being rinsed well with dry THF (30 ml), and was added dropwise over 30 min to a 1.5 *M* Red-Al solution in toluene (45 ml, 67.5 mmol) in dry THF (80 ml), kept at -70° during the addition by a Dry Ice-acetone bath and by the rate of addition. The resulting solution was stirred at -76° for 50 min. The reaction was quenched by the addition of a glacial acetic acid-water (1:1) solution (80 ml) over a 20-min period. This solution was added very slowly at first, because of extensive frothing, but more rapidly later. After warming to room temperature, the solution was poured into 400 ml of water and extracted with ether (3 \times 350 ml). The combined ethereal extract was washed with water (2 \times 400 ml), saturated NaHCO₃ (250 ml), and saturated NaCl (100 ml) solutions, dried (MgSO₄), and evaporated to afford a yellow oil, which was dissolved in chloroform (200 ml). To this solution was added sodium oxalate (6 g, 44.8 mmol) and oxalic acid (3 g, 33.3 mmol). The resulting reaction mixture was stirred at room temperature for 2 hr. The solution was then filtered, the solid was washed well with chloroform, and the chloroform filtrate and washings were evaporated to dryness. The resulting oily solid was dissolved in ether, washed with saturated NaHCO₃ (50 ml) and NaCl (50 ml) solutions, dried (MgSO₄), and concentrated to yield a yellow oil (4.5 g). This oil was chromatographed over a silicic acid-Celite (85:15) column (3.2 \times 31 cm). The column was eluted with a gradient system consisting of 400 ml of benzene-ethyl acetate (90:10) in the mixing flask and 400 ml of benzene-ethyl acetate (60:40) in the reservoir flask, and 8-ml fractions were collected. Fractions 75-105 containing the desired product were pooled and concentrated to dryness yielding 2.0 g (54%) of **1a**; two recrystallizations from acetone-petroleum ether gave **1a**; mp 60-61°; $[\alpha]^{24D} +17.2^\circ$ [*c* 0.57, MeOH].

Reaction of 5 with Mesitylensulfonyl Chloride. To a mixture of (\pm)-(6-carbomethoxyhexyl)-4-hydroxycyclopentane-1,3-dione (**5**) (256 mg, 1 mmol), triethylamine (0.3 ml, 2.13 mmol), and dry THF (5 ml) stirred under nitrogen at -18° was added dropwise a solution of mesitylensulfonyl chloride (241 mg, 1.1 mmol) in dry THF (5 ml) over 20 min. The resulting solution was stirred at -18 to -10° for 20 min and then at room temperature for 45 min. The reaction was quenched with methanol (2 ml), and then poured into water and saturated with NaCl. The mixture was then extracted with ether (5 \times 30 ml), and the combined ethereal extracts were washed with saturated NaHCO₃ (30 ml) and saturated NaCl (30 ml) solutions, dried (MgSO₄), and concentrated to yield a reddish oil (450 mg). This oily residue was chromatographed over a silicic acid-Celite (85:15) column (1.5 \times 22.5 cm). The column was eluted with a gradient system consisting of 300 ml of benzene-ethyl acetate (90:10) in the mixing chamber and 300 ml of benzene-ethyl acetate (60:40) in the reservoir flask, and 5-ml fractions were collected. Fractions 36-60 were pooled and concentrated to afford 350 mg of (\pm)-mesitylene enol sulfonate: mp 71-71.5° (two recrystallizations from ethyl acetate-pentane); uv λ_{max} 240 nm (ϵ

21,400), (sh) 278 (2750), (sh) 287 (2300); nmr δ 2.37 (s, 3, phenyl C-3 CH₃), 2.68 (s, 6, phenyl C-2 and C-6 CH₃), 3.68 (s, 3, OCH₃), 4.00 (broad s, 1, OH), 4.35 (dd, 1, $J_{4,5} = 6$, $J_{4,5} = 3$ Hz, C-4 H), and 7.12 ppm (s, 2, phenyl C-3 and C-5 H). *Anal.* Calcd for C₂₂H₃₀O₇S: C, 60.25; H, 6.89. Found: C, 60.26; H, 6.95.

Conversion of 6c into 1a. To a cooled (-78°), stirred solution of 50 mg (0.147 mmol) of monoenoil benzoate (6c) in 10 ml of dry tetrahydrofuran under N₂, Red-Al solution (1.5 M in toluene) was added in four 0.38-ml aliquots over 15 min. The resulting solution was stirred at -78° for a further 30 min. Two milliliters of glacial acetic acid was added, and the solution was allowed to warm to room temperature. The solution was poured into 40 ml of water and extracted with ethyl acetate (4 \times 30 ml). The ethyl acetate layer was washed with 10 ml each of saturated sodium bicarbonate and saturated NaCl solutions and dried over MgSO₄. Evaporation gave an oil which was dissolved in 10 ml of acetic acid-water (75:25) solution. The resulting solution was stirred at room temperature for 24 hr. The acetic acid-water was evaporated off under reduced pressure. The resulting oil was dissolved in ethyl acetate (10 ml), washed with saturated sodium bicarbonate and saturated sodium chloride solutions, and dried over MgSO₄. Evaporation of the solvent gave a yellow oil (30 mg). Crystallization from ethyl acetate-Skelly B afforded 20 mg of 1a: mp 60–61°; $[\alpha]^{24}_D +17.82^\circ$ (*c* 0.49, MeOH); nmr (CDCl₃) δ 2.80 (m, 2, C-5 H), 3.61 (s, 3, COOCH₃), 5.08 (m, 1, HCOH), and 7.21 ppm (m, 1, vinyl H); uv λ_{max} 222 nm (ϵ 8200); CD 231 nm $\theta = -9.9^\circ \times 10^{-3}$ (MeOH); ir (CHCl₃) 3448, 1748, and 1675 cm⁻¹. *Anal.* Calcd for C₁₃H₂₀O₄: C, 64.98; H, 8.39. Found: C, 65.15; H, 8.41.

Transformation of 6b into 1a. To 123 mg of the isopropyl enol ether 6b (0.412 mmol) in 10 ml of THF, stirred under N₂ at -78° , four 1.1-ml aliquots of a solution of Red-Al in benzene (1.5 M) were added dropwise over a 7-min period. The reaction mixture was stirred at -78° for 45 min, and 2 ml of acetic acid was then added. The reaction mixture was allowed to warm to room temperature and evaporated to dryness. Ten milliliters of 75% acetic acid solution was added, and the reaction mixture was stirred for 24 hr. The acetic acid-water was evaporated off under pressure. The resulting oil was dissolved in ethyl acetate (25 ml), washed with saturated NaHCO₃ and NaCl solutions, and then dried over MgSO₄. Crystallization of the residue from ethyl acetate-Skelly B afforded 1a (60 mg), which was identified by the following characteristics: mp 60–61°; $[\alpha]^{24}_D +17.60^\circ$ (*c* 0.71, MeOH); uv λ_{max} 222 nm (ϵ 8400).

Microbiological Reduction of 3-Keto-1-iodo-1-trans-octene. (a) *Penicillium decumbens* was grown in 500 ml of soybean-dextrose medium in a 2-l. erlenmeyer flask, which was incubated at 27° on a rotary shaker (270 rpm, 1-in. stroke) for 24 hr. The cells were harvested by centrifugation and suspended in 500 ml of 0.033 M borate buffer, pH 8.5. 3-Keto-1-iodo-1-trans-octene (250 mg), dissolved in 10 ml of acetone, was added to this cell suspension in a 2-l. erlenmeyer flask, which was incubated for 19 hr on a rotary shaker (290 rpm) at 27°. A total of 1 g of the substrate was divided among four flasks, then removed by centrifugation, and the supernatant was extracted with three 750-ml portions of chloroform. The collected cells were also exhaustively extracted with ethyl acetate, and the resulting emulsion was broken by filtration through a Celite pad. After evaporation of the combined chloroform and ethyl acetate extracts, the residues were combined and chromatographed over a neutral alumina (Woelm) column (2 \times 21 cm). The column was eluted with a gradient system consisting of 300 ml of 10% ethyl acetate-benzene in the mixing flask and 300 ml of 30% ethyl acetate-benzene in the reservoir flask, and 4.8 ml fractions were collected. Fractions 9–17 were pooled to yield 156 mg of unreacted 3-keto-1-iodo-1-trans-octene. Fractions 42–102 were collected to afford 104 mg of 3a, $[\alpha]^{24}_D +7.51^\circ$ [*c* 0.37, MeOH]. (b) *Aspergillus ustus* was incubated with 1 g of 3-keto-1-iodo-1-trans-octene under these same experimental conditions. After chromatography, 140 mg of the unreacted iodo ketone was recovered, and 118 mg of 3(R)-hydroxy-1-iodo-1-trans-octene, $[\alpha]^{24}_D -8.04^\circ$ (*c* 0.60, MeOH) was obtained.

(-)-Prostaglandin E₁ Methyl Ester. (a) **Divinylcuprate.** *tert*-Butyllithium (1.6 ml, 1.3 N in pentane, 2.08 mmol) was rapidly added to a solution of 343 mg (1.1 mmol) of 3b in 7 ml of dry diethyl ether at -78° (Dry Ice-acetone bath) under a blanket of argon, and the resulting solution was stirred at -78° for 2 hr. Then a solution of 178 mg (0.45 mmol) of tri-*n*-butylphosphine-

copper(I) iodide complex in 5 ml of dry ether was slowly added to the vinylolithium solution. The resulting yellow solution was stirred at -78° for 50 min, whereupon a solution of 140 mg (0.43 mmol) of 1b in 6 ml of dry ether was added dropwise to the divinylcuprate solution. After stirring at -78° for 15 min, the reaction mixture was allowed to warm to -23° (ice-methanol bath) and stirred at -23 to -18° for 30 min. Then 10 ml of cold 20% aqueous (NH₄)₂SO₄ was added and this mixture poured into a mixture of 15 ml of ether and 10 ml of the cold 20% (NH₄)₂SO₄ in a separatory funnel and shaken until the color of the organic layer disappeared, and a blue aqueous layer was formed. The organic layer was shaken with an additional 10-ml portion of 20% (NH₄)₂SO₄. The combined aqueous solution was extracted three times with ether, and the combined organic extract was washed once with a saturated NaCl solution, dried (MgSO₄), and concentrated to yield a yellow oil (484 mg). The protecting groups were removed by treating this oil with a solution of 8 ml of acetic acid-water (65:35) and 0.8 ml THF at 31–32° for 6.5 hr. Then this solution was diluted with ether and washed with a saturated NaHCO₃ solution until the washings were basic, washed with a saturated NaCl solution, dried (MgSO₄), and evaporated to yield a yellow oil (405 mg).

This oil was chromatographed over a silicic acid-Celite (85:15) column (1.8 \times 20 cm). The column was eluted with a gradient system consisting of 350 ml of benzene-ethyl acetate (80:20) in the mixing flask and 350 ml of benzene-ethyl acetate (30:70) in the reservoir flask and then with a further 300 ml of benzene-ethyl acetate (30:70) when the above 700 ml was eluted; 6.5-ml fractions were collected. Fractions 52–120 were pooled and concentrated yielding 91 mg (57%) of PGE₁ methyl ester: tlc mobility,³⁴ R_f 0.36; nmr (CDCl₃) δ 3.69 (s, 3, OCH₃), 3.95–4.27 (m, 2, C-11 and C-15 H), 5.55–5.73 ppm (m, 2, olefinic). *Anal.* Calcd for C₂₁H₃₆O₅: C, 68.44; H, 9.85. Found: C, 68.18; H, 9.37.

(b) **Corey's Reagent.** *tert*-Butyllithium (0.58 ml, 1.3 N in pentane, 0.754 mmol) was rapidly added to a solution of 123 mg (0.38 mmol) of 3b in 3 ml of dry diethyl ether at -78° (Dry Ice-acetone bath) under an argon atmosphere, and the resulting solution was stirred at -78° for 2 hr. A slurry of 46 mg (0.35 mmol) of dry pentynylcopper in 1 ml of dry ether (under argon) was treated with 0.13 ml (0.7 mmol) of dry hexamethylphosphoric triamide and the mixture stirred at room temperature for 5 min. (A clear solution was obtained after 2 min.) This solution was then added to the vinylolithium solution, the flask being washed out a number of times with ether. The mixed cuprate solution was stirred at -78° for 60 min, whereupon a solution of 104 mg (0.32 mmol) of 1b in 4 ml of dry ether was slowly added to the mixed cuprate solution. After stirring at -78° for 15 min, the reaction mixture was allowed to warm to -23° (ice-methanol bath) and stirred at -23 to -18° C for 60 min. Ten milliliters of cold 20% (NH₄)₂SO₄ solution was added and this solution poured into a mixture of 15 ml of ether and 10 ml of 20% (NH₄)₂SO₄ solution in a separatory funnel and shaken until the color of the organic layer disappeared, and a blue aqueous layer was formed. The organic layer was shaken with an additional 10-ml portion of cold 10% (NH₄)₂SO₄ solution. The combined aqueous solution was extracted three times with ether. The combined organic layer and ethereal extract were washed twice with cold 2% H₂SO₄ acid (v/v), once each with saturated NaHCO₃ and saturated NaCl solutions, dried (MgSO₄), and evaporated to give a yellow oil (196 mg). The protecting groups were removed by treating this oil with a solution of 4 ml of acetic acid-water (65:35) and 0.4 ml of THF at 31° for 3.5 hr. This solution was then diluted with ether and washed with a saturated NaHCO₃ solution until the washings were basic, washed with a saturated NaCl solution, dried (MgSO₄), and concentrated to yield a yellow oil (147 mg). This oil was chromatographed over a silicic acid-Celite (85:15) column (1.8 \times 22 cm). The column was eluted with 350 ml of benzene-ethyl acetate (80:20) in the mixing flask and benzene-ethyl acetate (80:20) in the reservoir flask and benzene-ethyl acetate (30:70) in the reservoir flask and then with an additional 300 ml of benzene-ethyl acetate (30:70) after the above 700 ml was eluted. Fractions 64–129 were pooled and concentrated yielding 59 mg (50.5%) of PGE₁ methyl ester.

(c) **Vinylcopper Reagent.** *tert*-Butyllithium (1 ml, 1.3 N in pentane, 1.3 mmol) was rapidly added to a solution of 210 mg (0.644 mmol) of 3b in 5 ml of dry diethyl ether at -78° (Dry Ice-acetone) under a blanket of argon. The resulting solution was stirred

at -78° for 2 hr. A solution of 222 mg (0.59 mmol) of tri-*n*-butylcopper(I)-iodide complex in 2 ml of dry ether was treated with 0.14 ml (0.583 mmol) of tri-*n*-butylphosphine at room temperature, under argon, and the resulting solution was added to the vinyl lithium solution, the flask being washed out a number of times with ether. The resulting yellow vinylcopper solution was stirred at -78° for 50 min, whereupon a solution of 176 mg (0.54 mmol) of **1b** in 8 ml of dry ether was added dropwise to the former solution. The reaction mixture was stirred at -78° for 20 min and was then allowed to warm to -23° (ice-methanol) and stirred at -23 to -18° C for 34 min. Ten milliliters of cold 20% $(\text{NH}_4)_2\text{SO}_4$ solution was added and this solution poured into a mixture of 15 ml of ether and 10 ml of cold 20% $(\text{NH}_4)_2\text{SO}_4$ in a separatory funnel and shaken well to give a light yellow organic layer and a blue aqueous layer. The organic layer was shaken with an additional 10-ml portion of cold 20% $(\text{NH}_4)_2\text{SO}_4$ solution. The combined blue $(\text{NH}_4)_2\text{SO}_4$ layer was extracted three times with ether, and the combined organic layer and ethereal extract were washed with a saturated NaCl solution, dried (MgSO_4), and concentrated to give a yellow oil (688 mg). The protecting groups were removed by stirring this oil with a solution of 10 ml of acetic acid-water (65:35) and 1 ml of THF at 30 – 32° for 18 hr. This solution was diluted with ether and shaken with a saturated NaHCO_3 solution until the washings were basic. The organic layer was washed with a saturated NaCl solution, dried (MgSO_4), and evaporated to afford a yellow oil (651 mg), which was chromatographed over silicic acid-Celite (85:15) column (1.8×21 cm). The column was eluted with a gradient system consisting of 350 ml of benzene-ethyl acetate (80:20) in the mixing chamber and 350 ml of benzene-ethyl acetate (30:70) in the reservoir chamber and then with an additional 300 ml of benzene-ethyl acetate (30:70) when the first 700 ml had been eluted; 7-ml fractions were collected. Fractions 54–116 were pooled and concentrated to yield 99 mg (50%) of PGE₁ methyl ester.

(d) **Thiophenoxide Mixed Cuprate**. *tert*-Butyllithium (0.58 ml, 1.3 *N* in pentane, 0.75 mmol) was added rapidly to a solution of 122 mg (0.38 mmol) of **3b** in 3 ml of dry diethyl ether at -78° (Dry Ice-acetone) under a blanket of argon. The resulting solution was stirred at -78° for 2 hr. *n*-Butyllithium (0.145 ml, 2.2 *N* in hexane, 0.32 mmol) was added dropwise to a stirred solution of 35 mg (0.32 mmol) of thiophenol in 1 ml of dry ether at 0° (ice bath), and the mixture was stirred at 0° for 10 min and then at -78° for 1 hr. Then a solution of 125.7 mg (0.32 mmol) of tri-*n*-butylphosphinecopper(I)-iodide complex in 3 ml of dry ether was added dropwise to the lithium thiophenoxide solution. After stirring at -78° for 15 min, this solution was added slowly to the vinyl lithium solution at -78° for 45 min. A solution of 96 mg (0.30 mmol) of **1b** in 4 ml of dry ether was then added to the mixed cuprate solution and stirred at -78° for 15 min. After warming to -23° (ice-methanol), the reaction mixture was stirred at -23 to -18° for 1 hr. Ten milliliters of cold 20% $(\text{NH}_4)_2\text{SO}_4$ solution was added and the solution poured into a mixture of 15 ml of ether and 10 ml of cold 20% $(\text{NH}_4)_2\text{SO}_4$ solution in a separatory funnel and shaken well. The organic layer was shaken with an additional 10 ml of the 20% $(\text{NH}_4)_2\text{SO}_4$ solution. The blue $(\text{NH}_4)_2\text{SO}_4$ solution was extracted three times with ether, and the combined organic layer and ethereal extract were washed with a saturated NaCl solution, dried (MgSO_4), and evaporated to give a yellow oil (284 mg). The protecting groups were removed by stirring this oil with a solution of 6 ml of acetic acid-water (65:35) and 0.6 ml of THF at 30° for 6 hr. This solution was then diluted with ether and shaken with a saturated NaHCO_3 solution until the washings were basic. After the solution was washed with a saturated NaCl solution and was dried (MgSO_4), evaporation of the solvent yielded a yellow oil (257 mg) which was chromatographed over a silicic acid-Celite (85:15) column (1.8×21 cm). This column was eluted with a gradient system consisting of 350 ml of benzene-ethyl acetate (80:20) in the mixing chamber and 350 ml of benzene-ethyl acetate (30:70) in the reservoir chamber followed by 350 ml of benzene-ethyl acetate (30:70) after elution of the first 700 ml; 6-ml fractions were collected. Fractions 52–124 were pooled and concentrated to yield 66 mg (61%) of PGE₁ methyl ester.

Microbiological Hydrolysis of (-)-PGE₁ Methyl Ester. *Rhizopus oryzae* was grown in the soybean-dextrose medium for 24 hr. The cells were then harvested by filtering through cheesecloth; approximately 56 mg of wet mycelia were obtained from 500 ml of

medium in a 2-l. erlenmeyer flask. After the cells were washed twice with 0.1 *M* phosphate buffer, pH 7.0, the mycelia were suspended in 500 ml of the same phosphate buffer. (-)-PGE₁ methyl ester (500 mg) dissolved in 5 ml of ethanol was added to the flask which was incubated on a rotary shaker (280 rpm) at 26° . After 24 hr, the mycelia were removed by filtration, and the filtrate was extracted twice with ether and then acidified to pH 2.5 with 6 *N* HCl. The acidified aqueous layer was then extracted three times with ethyl acetate, and the combined ethyl acetate extracts were washed with water and evaporated to dryness. Crystallization of the residue from ethyl acetate-hexane afforded 298 mg of (-)-PGE₁, mp 115 – 116° , $[\alpha]_D^{25} -54^{\circ}$ (*c* 0.7, THF); its infrared, nmr, and mass spectra were identical with an authentic sample of (-)-PGE₁.¹ A second crop of crystals (120 mg), mp 110 – 112° , was obtained from the mother liquor.

1-(2-Tetrahydropyranyloxy)butan-4-ol. A mixture of 168 g (2 mol) of dihydropyran, 360 g (4 mol) of 1,4-butanediol, and 2 drops of concentrated HCl was stirred at room temperature overnight. The mixture was then stirred a few minutes with solid K_2CO_3 , diluted with ether, and washed three times with saturated NaCl solution. The ether layer was dried (Na_2SO_4), concentrated, and distilled to give 195 g (77% yield based on dihydropyran) of the monotetrahydropyranyl ether,²⁶ bp 85 – 88° (0.5 mm).

1-Bromo-4-(2-tetrahydropyranyloxy)butane. Methanesulfonyl chloride (112 ml, 1.92 mol) was added dropwise over a period of 0.5 hr to a cold (*ca.* 0°) solution of 167 g (0.96 mol) of 1-(2-tetrahydropyranyloxy)butan-4-ol and 320 ml of triethylamine in 1 l. of THF maintained at 0° with an ice-water bath and stirring under N_2 . Stirring was continued for 1 hr at 0° , and the mixture was then diluted with water and extracted with ether. The organic layer was washed with water, dried (Na_2SO_4), and evaporated to a yellow oil.

The crude mesylate was mixed with 2 l. of acetone and 120 g of anhydrous LiBr, stirred at room temperature for 2 hr and heated with refluxing for 1 hr. The cooled mixture was concentrated under reduced pressure, diluted with water, and extracted with ether. The ether extracts were washed with water and saturated NaCl solution, dried (Na_2SO_4), and concentrated. The residue was distilled to give the bromide (164 g, 77% yield), bp 85 – 87° (2 mm) [lit.²⁷ bp 82 – 84° (2 mm)].

Ethyl 3-(1-Pyrrolidyl)-2-butenate. A mixture of 254 ml (260 g, 2 mol) of ethyl acetoacetate and 2 l. of benzene was treated with 190 ml (162 g, 2.3 mol) of pyrrolidine. After mixing, the solution was allowed to stand as an exotherm developed, and the temperature rose to *ca.* 50° . When the temperature began to drop, the mixture was heated with refluxing through a Dean-Stark trap until no more water was collected. Benzene was removed on a rotary evaporator, and the residue was distilled to give 360 g (98%) of the oily enamine, bp 114 – 116° (0.6 mm) [lit.²⁸ bp 200° (40 mm)].

1-Hexyn-5-one (9).^{29,30} Ethyl 3-(1-pyrrolidyl)-2-butenate (360 g, 1.97 mol) and propargyl bromide (240 g, 2.02 mol) were mixed, placed in a room temperature water bath, and allowed to stand overnight. The viscous oil was then dissolved in 400 ml of water heated on a steam bath for 10 min. The mixture was cooled and extracted with ether, and the ether extracts were washed successively with water (two times), 1 *N* HCl solution, water, and saturated NaCl solution. The ether extracts were concentrated, and the residue was added dropwise over 3 hr to a slowly distilling mixture of 600 g of Na_2CO_3 and 4.25 l. of water.³¹ Distillation was continued until the distillate was no longer cloudy. The distillate was extracted with ether, and the ether extracts were washed with water, 5% NaHCO_3 solution, water, and saturated NaCl solution. After drying (Na_2SO_4) and concentrating, the residue was distilled to give 139.5 g (74%) of 1-hexyn-5-one, bp 50 – 52° (15 mm) [lit.^{28,30} bp 62 – 64° (12 mm)].

2-Methyl-2-(3-butynyl)-1,3-dioxolane (10). A mixture of 138 g (1.43 mol) of 1-hexyn-5-one, 150 ml of ethylene glycol, 600 ml of benzene, and 100 mg of *p*-toluenesulfonic acid was heated with refluxing through a Dean-Stark trap until no more water separated. The mixture was cooled, poured into 5% NaHCO_3 , and extracted with ether. The ether extracts were washed with water (three times) and saturated NaCl solution. The extract was dried (Na_2SO_4), concentrated, and distilled to give 177 g (89%) of the ketal **10**, bp 73 – 76° (16 mm) [lit.³² bp 75 – 76° (15 mm)].

1-Hydroxy-5-decyn-9-one (11). A solution of 97.6 g (0.70 mol) of **10** in 100 ml of THF was added dropwise over a 20-min period

to a suspension of 18.32 g (0.8 mol) of lithium amide in *ca.* 800 ml of liquid NH_3 , stirred with refluxing under a solid CO_2 -acetone cold finger condenser. After stirring for 1 hr, a solution of 166 g (0.7 mol) of 1-bromo-4-(2-tetrahydropyranyloxy)butane in 200 ml of THF was added dropwise over 45 min. Stirring was continued under reflux for 2 hr, and the ammonia was allowed to evaporate overnight. Methanol (800 ml), concentrated HCl (160 ml to bring to pH 1–2), and water (300 ml) were added, and the homogeneous mixture was allowed to stand for 4 hr. Most of the methanol was then evaporated under reduced pressure. The residue was diluted with water and extracted three times with ethyl acetate. The combined extracts were washed with water and saturated NaCl solution, dried (Na_2SO_4), and evaporated to 104 g (89%) of crude keto alcohol **11** which was used in the next reaction without purification. In a separate run, a portion of the product was distilled to give pure **11**: bp 87–90° (0.04 mm); ir (film) 3410, 2200, and 1710 cm^{-1} ; nmr (CDCl_3) δ 1.57 (4, m), 2.14 (3, s, $-\text{COCH}_3$), 2.00–2.79 (6, m), and 3.60 ppm (2, m, $-\text{CH}_2\text{OH}$). *Anal.* Calcd for $\text{C}_{10}\text{H}_{10}\text{O}_2$: C, 71.39; H, 9.59. Found: C, 71.45; H, 9.80.

Methyl 9-Keto-5-decyanoate. Crude keto alcohol **11** (104 g) was dissolved in 1 l. of acetone, cooled to 0° in the ice-water bath, and stirred during the dropwise addition of 300 ml of Jones reagent.³³ After the addition was completed, the mixture was stirred an additional 0.5 hr at 0°, and 2-propanol was then added to destroy the excess oxidant. The mixture was diluted with water to dissolve the inorganic salts and was extracted with saturated NaHCO_3 solution which was carefully acidified with HCl solution and extracted with ether. The acidic ether extracts were washed with water, dried (Na_2SO_4), and concentrated to 83 g of keto acid. This crude acid was esterified by stirring overnight with 500 ml of 3% methanolic HCl. The mixture was then concentrated under reduced pressure, diluted with water, and extracted with ether. The ether extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was distilled to afford 75 g (62% yield based on crude **11**) of methyl 9-keto-5-decyanoate: bp 94–96° (0.03 mm); ir (film) 2200, 1735, and 1718 cm^{-1} ; nmr (CDCl_3) δ 1.80 (2, m), 2.14 (3, s, $-\text{COCH}_3$), 2.06–2.80 (8, m), and 3.67 ppm (3, s, $-\text{CO}_2\text{CH}_3$). *Anal.* Calcd for $\text{C}_{11}\text{H}_{16}\text{O}_3$: C, 67.32; H, 8.22. Found: C, 67.16; H, 8.32.

Methyl 9-Keto-cis-5-decenoate (12). The acetylenic keto ester **11** (55.4 g, 0.28 mol) was mixed with 1.75 g of 5% palladium on BaSO_4 , 9.4 ml of synthetic quinoline, and 550 ml of benzene, and the mixture was stirred under a hydrogen atmosphere (1 atm) until the theoretical amount of hydrogen (6.8 l.) was absorbed. At this point, the stirring was stopped, the catalyst removed by filtration, and the solvent evaporated. The residue was distilled to give 56 g (100%) of keto ester **12**: bp 85–87° (0.03 mm); ir (film) 1740 and 1718 cm^{-1} ; nmr (CDCl_3) δ 1.72 (2, m), 2.10 (3, s, $-\text{COCH}_3$), 1.98–2.64 (8, m), 3.67 (3, s, $-\text{CO}_2\text{CH}_3$), and 5.38 ppm (2, "t," $J = 5$ Hz). *Anal.* Calcd for $\text{C}_{11}\text{H}_{18}\text{O}_3$: C, 66.63; H, 9.15. Found: C, 66.73; H, 9.15.

2-(6-Carbomethoxy-cis-2-hexenyl)cyclopentane-1,3,4-trione (14). Into a 500-ml three-necked flask was placed 100 ml of anhydrous ethanol under a stream of nitrogen. Sodium ethoxide was prepared by the addition of 1.95 g (85 mmol) of sodium metal. After the resulting sodium ethoxide solution was cooled to 0°, 7.5 ml (10 g) of diethyl oxalate was added over a 2-min period (the clear solution turned yellow) followed by the dropwise addition of 5 g (25 mmol) of methyl 9-keto-cis-5-decenoate (**12**) over a period of 1 hr. After the solution was allowed to stir overnight at room temperature and then refluxed for 1 hr (turned brown), 200 ml of 2 *N* HCl was added, and the reaction mixture was again refluxed for 5 hr; during reflux, the ethanol was distilled off. After cooling, the reaction mixture was extracted with ethyl acetate (3 × 150 ml), dried (MgSO_4), and evaporated to dryness to give 5.1 g of solid. This solid was dissolved in 50 ml of methanol, containing 1 ml of concentrated HCl, and allowed to stand at room temperature overnight under nitrogen. It was then refluxed for 1 hr, poured into ice water, and allowed to stand for 2 hr. The product was extracted from the aqueous layer using ethyl acetate (3 × 70 ml), dried (MgSO_4), and evaporated to give 2.8 g of **14** as an oil: ir (CHCl_3) 1732, 1720, 1697, 1687, 1655, and 725 cm^{-1} ; nmr (CDCl_3) δ 1.74 (2, m), 2.30 (4, m), 2.94 (2, s, C-10), 3.07 (3, s, OCH_3), 3.13 (2, d, $J = 5$ Hz, C-7), and 5.43 ppm (s, m, $\text{HC}=\text{CH}$); uv λ_{max} 233 nm (ϵ 11,700) and 325 (11,400). *Anal.* Calcd for $\text{C}_{13}\text{H}_{16}\text{O}_5$: C, 61.89; H, 6.39. Found: C, 61.72; H, 6.48.

Microbiological Reduction of 14. The fermentation conditions are the same as those described previously (four 2-l. erlenmeyer flasks), except 1 g of **14** was incubated with *Dipodascus uniuiculus* instead.

Twenty-four hours after the addition of **14**, the cells were removed by centrifugation. After the usual work-up, the oily residue was chromatographed over a silicic acid-Celite (85:15) column (35 × 2.5 cm). The column was eluted with a gradient system consisting of 500 ml of ethyl acetate-benzene (1:1) in the mixing chamber and 500 ml of ethyl acetate in the reservoir, and 7-ml fractions were collected. Fractions 42–65 were pooled to yield 0.48 g of **15**: mp 57.5–59°; $[\alpha]_D^{23} +19.0^\circ$ (1.34, CHCl_3); uv λ_{max} (95% ETOH) 272 nm (ϵ 21,000); nmr (CDCl_3) δ 1.2–3.2 (10 H, complex), 3.63 (3 H, s, $-\text{CO}_2\text{CH}_3$), 4.66 (1 H, m, $\text{CH}-\text{OH}$), 5.38 (2 H, m, $\text{CH}=\text{CH}$), 8.07 ppm (2 H, br. OH and enol OH); ir (CHCl_3) 3600–2400 (OH, enol OH), 1720 (CO_2CH_3), 1625 cm^{-1} ($\text{C}=\text{O}$). *Anal.* Calcd for $\text{C}_{13}\text{H}_{18}\text{O}_5$: C, 61.40; H, 7.13. Found: C, 61.20; H, 7.26.

2-(6-Carbomethoxy-cis-2-hexenyl)-4(R)-hydroxy-2-cyclopentene-1-one (2a). A solution of 1.21 g (5.5 mmol) of mesitylenesulfonyl chloride in 10 ml of THF was added dropwise over a 25-min period, with stirring under nitrogen, to a cold (–10°; ice-methanol bath) solution of 1.17 g (4.6 mmol) of **15** and 1.3 ml of triethylamine in 20 ml of THF. After being stirred an additional 30 min at –10° and 45 min at room temperature, the mixture was filtered into a pressure-equalizing addition funnel, rinsing with 20 ml of fresh THF. The filtered solution was then added dropwise, with stirring under nitrogen, to a solution of 12 ml of bis(2-methoxyethoxy)aluminum hydride (1.5 *M* in toluene) and 20 ml of THF, maintaining a temperature of –60° or lower with a solid CO_2 -acetone cooling bath. After the addition was completed, the mixture was stirred an additional 45 min at –70°. Glacial acetic acid (12 ml) was then added cautiously, and the mixture was allowed to warm to room temperature. The reaction mixture was diluted with water and extracted with ether. The ether extracts were washed successively with water (two times), saturated NaHCO_3 , water, and saturated NaCl. The ether solution was dried over Na_2SO_4 and evaporated to an oily residue which was dissolved in 50 ml of CHCl_3 and stirred for 1.5 hr with *ca.* 2 g each of sodium oxalate and oxalic acid. The mixture was filtered, evaporated to a brownish oil, and chromatographed on a silicic acid-Celite column (benzene-ethyl acetate gradient) to give 526 mg (50% yield based on **15**) of oily **2a**: uv λ_{max} 220 nm (ϵ 8000); nmr (CDCl_3) δ 1.08–3.06 (10 H, complex), 3.63 (3 H, s, $-\text{CO}_2\text{CH}_3$), 4.92 (1 H, m, $\text{CH}-\text{OH}$), 5.50 (2 H, m, $\text{CH}=\text{CH}$), 7.15 ppm (1 H, m, $\text{CO}-\text{C}=\text{CH}$); $[\alpha]_D^{23} +12.4^\circ$ (*c* 0.91, CH_3OH); ir (CHCl_3) 3450 (OH), 1740 ($-\text{CO}_2\text{CH}_3$), 1710 cm^{-1} ($\text{C}=\text{O}$). Peak match for (*M* – 18) at *m/e* 220.10989 (theory for $\text{C}_{13}\text{H}_{16}\text{O}_3$, 220.10968).

(–)-Prostaglandin E_2 Methyl Ester. *tert*-Butyllithium (3.3 ml, 1.4 *N* in pentane, 4.6 mmol) was rapidly added to a solution of 750 mg (2.3 mmol) of **3b** in 20 ml of dry ether at –78° (Dry Ice-acetone) under a blanket of argon. After the mixture was stirred at –78° for 2 hr, a solution of 393 mg (1 mmol) of tri-*n*-butylphosphine-copper(I)-iodide complex in 10 ml of dry ether was slowly added to the vinyl lithium solution, and the resulting yellow solution was stirred at –78° for 50 min. Then a solution of 309 mg (0.96 mmol) of **2b** in 10 ml of dry ether was slowly added to the divinylcuprate solution. After being stirred at –78° for 20 min, the solution was allowed to warm to –23° (methanol-ice) and was stirred at –23 to –15° for 60 min. Fifteen milliliters of cold 20% $(\text{NH}_4)_2\text{SO}_4$ solution was added and this solution poured into 10 ml of cold 20% $(\text{NH}_4)_2\text{SO}_4$ solution in a separatory funnel and shaken well. The organic layer was shaken with a further 15-ml portion of cold 20% $(\text{NH}_4)_2\text{SO}_4$. The combined $(\text{NH}_4)_2\text{SO}_4$ layers were extracted three times with ether and then the combined organic layer and ether extract washed with a saturated NaCl solution, dried (MgSO_4), and evaporated to yield a yellow oil (1.08 g). A mixture of this oil and 16 ml of acetic acid-water (65:35) and 1.6 ml of THF were stirred at 31° for 6.5 hr. This solution was then diluted with ether and shaken with a saturated NaHCO_3 solution until the washings were basic. The organic layer was shaken with a saturated NaCl solution, dried (MgSO_4), and concentrated to give a yellow oil (900 mg). This oil was chromatographed over a silicic acid-Celite (85:15) column (2 × 24 cm). The column was eluted with a gradient system consisting of 300 ml of 20% ethyl acetate-benzene in the reservoir flask: 5-ml fractions were collected. Fractions 86–

142 were pooled and concentrated to yield 175 mg (50%) of PGE₂ methyl ester; tlc mobility,³⁴ *R*_F 0.36; nmr (CDCl₃) δ 3.70 (s, 3, OCH₃), 3.90–4.30 (m, 2, C-11 and C-15 H), 5.45 (m, 2, olefinic), 5.70 ppm (m, 2, olefinic); ir 3390, 1740, and 965 cm⁻¹.

Hydrolysis of (–)-PGE₂ Methyl Ester. Frozen cells of *Rhizopus oryzae* (35 mg wet weight) were suspended in 200 ml of 0.1 M phosphate buffer, pH 7.0, in a 500-ml erlenmeyer flask. (–)PGE₂ methyl ester (100 mg), dissolved in 2 ml of ethanol, was added to the flask, which was incubated on a rotary shaker (280 rpm) at 26°. After 29 hr, the mycelia were removed by filtration, and the filtrate was extracted twice with diethyl ether and then acidified to pH 2.5 with 5 N HCl. The acidified aqueous layer was then extracted three times with ethyl acetate, and the combined ethyl acetate extracts were washed with water and evaporated to dryness. The semisolid residue was chromatographed over a silicic acid–Celite (85:15) column (0.75 × 9 in.). The column was eluted with a gradient system consisting of 350 ml of benzene–ethyl acetate (75:25) in the mixing flask and 350 ml of ethyl acetate in the reservoir flask, and 5-ml fractions were collected. Fractions 31–45 were combined to give 28 mg of unreacted (–)-PGE₂ methyl ester. Fractions 51–96 were pooled to yield 57 mg of (–)-PGE₂. Two crystallizations from ethyl acetate–hexane gave a sample, mp 62–64°, [α]_D²⁴ –52° (c 1.15, THF), identical (infrared nmr and mass spectra) with a biosynthetic specimen.

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- (11) The overall quantity of acid present in CDCl₃ was apparently sufficient to catalyze the allylic rearrangement of the reduction product of **6d**. Since sulfonic acid is produced, it is necessary to carry out the acid rearrangement in a buffered medium to prevent destruction of **1a** by the strong acid produced.
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