A Novel Chemicoenzymatic Rearrangement by Asymmetric Hydrolysis with Pig Liver Esterase†,[1]

Satomi Niwayama,*,[2a] Susumu Kobayashi,*,[2b] and Masaji Ohno[2c]

Contribution from the Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, 113, Japan

Received January 7, 1994®

Abstract: A new regio- and stereospecific asymmetric rearrangement was discovered during the asymmetric hydrolysis of the meso epoxy diesters dialkyl 5,6-epoxybicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate, **3a-c**, with pig liver esterase (PLE). The rearrangement quantitatively produces the optically active monoester 6-formyl-2-(alkoxycarbonyl)bicyclo-[3.1.0]hex-2-ene-1-carboxylic acid, **6a-c**. These rearranged products, **6a-c**, have the same skeleton as **10**, which was reported by Meinwald (*J. Am. Chem. Soc.* **1963**, *85*, 582). In contrast to the classical Meinwald rearrangement, this new rearrangement proceeds under slightly basic conditions efficiently. The reaction mechanism was investigated, and it is proposed to proceed via the α-lactone-type intermediate **24**, which was formed by intramolecular Michael addition of the carboxylate anion generated by the enzymatic hydrolysis.

Introduction

Enzymatic asymmetric hydrolysis has been a powerful tool for developing numerous elegant synthetic methodologies for natural products. We have reported a number of enantioselective total syntheses of biologically significant compounds, such as the carbocyclic nucleosides, (–)-aristeromycin, **1**, and (–)-neoplanocin A, **2**, using asymmetric hydrolysis with pig liver esterase (PLE).[3] (See Scheme 1.) For these cyclopentanoids, the dissymmetrization of the meso diesters of bicyclo[2.2.1]heptene systems with PLE is the pivotal stereo-differentiating reaction. This asymmetry enables unique stereochemical control at later stages, such as regiospecific decarboxylative ozonolysis through the ozonide intermediate **A**.[3]

Most enzymatic hydrolyses induce simple chemoselective conversion of a limited number of substituents. Further skeletal conversions induced by the enzymatic dissymmetrization are quite rare. The decarboxylative ozonolysis that is described above is one good example of the regiospecific reactions for the dissymmetric carboxyl ester. Although this reaction revealed the potential of enzymes toward regio- and stereospecific reactions, no other unusual chemicoenzymatic rearrangement has ever been reported.

Here, we report a novel, completely stereo- and regiospecific rearrangement of norbornene carboxylates **5a-c** induced by PLE enzymatic hydrolysis.[5,6] This rearrangement produces the bicyclo-[3.1.0]hexene derivatives **6a-c** in almost quantitative yields from the diesters **3a-c** (Scheme 2).

Meinwald et al. reported a similar stereospecific rearrangement of norbornadiene **7**[7,8] (Scheme 3). Thus, peracid oxidation of **7** leads to the rearranged product, bicyclo[3.1.0]hex-2-ene-*endo*-carbaldehyde **10**, without isolating the monoepoxide **8**. From a synthetic point of view, this reaction has been successfully applied to a series of prostaglandin syntheses by the Upjohn group.[9]

The mechanism of the Meinwald rearrangement has been proposed to proceed via the exomonoepoxide **8**, which is formed initially by the peracid oxidation. The rearrangement of unstable intermediate **8** is catalyzed by residual acid.

The rearranged products **6a-c** have the same skeleton as compound **10** in the Meinwald rearrangement. However, in contrast to the classical Meinwald rearrangement, the enzymatic hydrolysis is carried out under slightly basic conditions in pH 8 phosphate buffer solution instead of acidic conditions. In addition, the diesters **3** involve electron-deficient C=C, and therefore the initiation of Meinwald rearrangement for this alkene is highly deactivated unlike the epoxynorbornene, **8**. It does not seem likely that the electron-deficient diesters also cause a Meinwald-type rearrangement. It should be noted that the 7-oxa-analog **11** is hydrolyzed quantitatively with pig liver esterase to give the monoester **12** without any evidence of Meinwald rearrangement (Scheme 4). We applied **12** to the total synthesis of a series of nucleosides.[3] In fact, in a separate experiment, the monoester **4a** can be obtained as a quite stable crystalline without causing further conversion, as will be described later.

To our knowledge, the only example of the classical Meinwald rearrangement in the electron-deficient system was reported by Prinzbach et al.[11] The monoepoxide **3a** rearranges to produce **13** with acid or upon heating (Scheme 5), although the yield turned out to be quite low, as will be described later.

Therefore, we became interested in examining the reaction mechanism of our new rearrangement. Particularly, we focus on the following intriguing points: (1) why the diesters, **3a-c**, undergo the Meinwald rearrangement so smoothly under basic condition

* To whom correspondence and reprint requests should be addressed.

† Presented at the 207th National ACS Meeting, San Diego, CA, March 13–17, 1994; Abstract ORGN-439.

® Abstract published in *Advance ACS Abstracts,* March 15, 1994.

(1) Taken in part from the Ph.D. Thesis of S. Niwayama, University of Tokyo, 1989.

(2) Present address: (a) Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA, 90024. (b) Sagami Chemical Research Center, Nishi-Ohnuma, Sagamihara, Kanagawa, 229, Japan. (c) Eisai Co., Ltd., Tokodai, Tsukuba, Ibaraki, 300-26, Japan.

(3) (a) Ito, Y.; Shibata, T.; Arita, M.; Sawai, H.; Ohno, M. *J. Am. Chem. Soc.* **1981**, *103*, 6739. (b) Ohno, M.; Ito, Y.; Arita, M.; Shibata, T.; Adachi, K.; Sawai, H. *Tetrahedron, Symposia-in-Print*, **1984**, *40*, 145. (c) Arita, M.; Adachi, K.; Ito, Y.; Sawai, H.; Ohno, M. *J. Am. Chem. Soc.* **1983**, *105*, 4049.

(4) (a) Grimshaw, C. E.; Sogo, S. G.; Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 2699. (b) Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492 and references cited therein.

(5) Niwayama, S.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1988**, *29*, 6313.

(6) Niwayama, S.; Noguchi, H.; Ohno, M.; Kobayashi, S. *Tetrahedron Lett.* **1993**, *34*, 665.

(7) Meinwald, J.; Labana, S. S.; Chadha, M. S. *J. Am. Chem. Soc.* **1963**, *85*, 582.

(8) Meinwald, J.; Labana, S. S.; Labana, L. L.; Wahl, G. H., Jr. *Tetrahedron Lett.* **1965**, 1789.

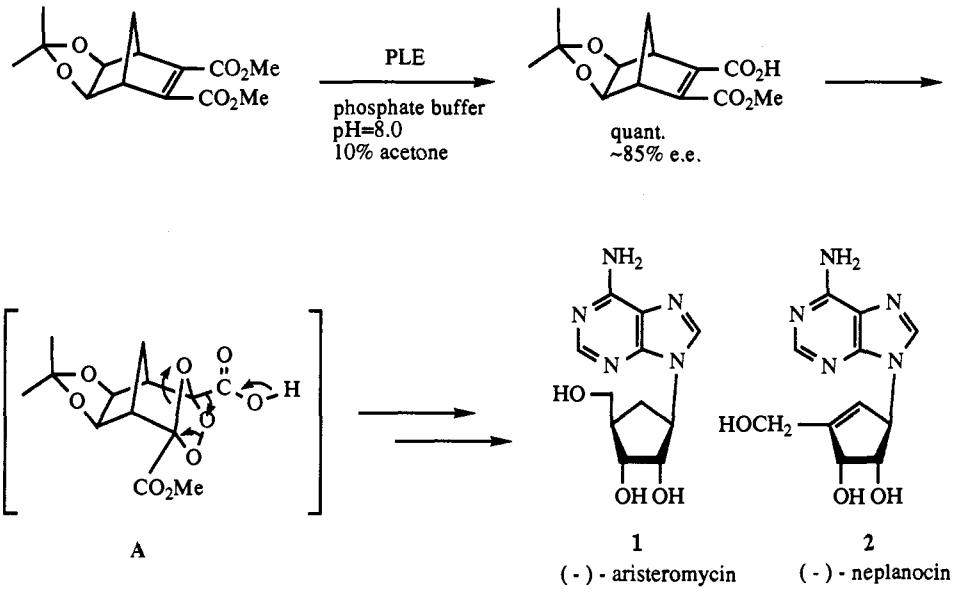
(9) (a) Axen, U.; Lincoln, F. H.; Thompson, J. L. *J. Chem. Soc., Chem. Commun.* **1969**, 303. (b) Kelly, R. C.; VanRheenen, V.; Schletter, I.; Pillai, M. D. *J. Am. Chem. Soc.* **1973**, *95*, 2746. (c) White, D. R. *Tetrahedron Lett.* **1976**, 1753 and references cited herein.

(10) This selective reduction did not afford **4b** in good yield, since 1,4-reduction preferentially occurred under this condition.

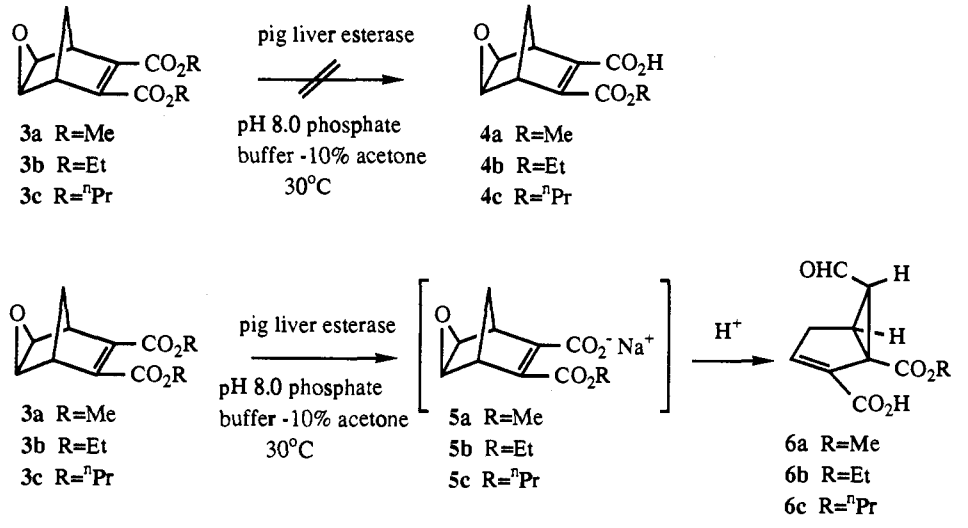
(11) (a) Prinzbach, H.; Klaus, M. *Angew. Chem. Int. Ed. Engl.* **1969**, *8*, 276. (b) Klaus, M.; Prinzbach, H.; Achenbach, H. *Ibid.* **1969**, *8*, 800.

0002-7863/94/1516-3290$04.50/0

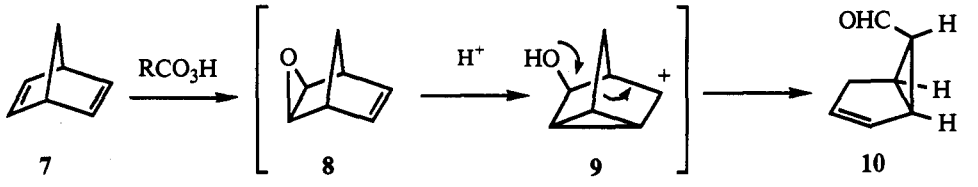
Scheme 1



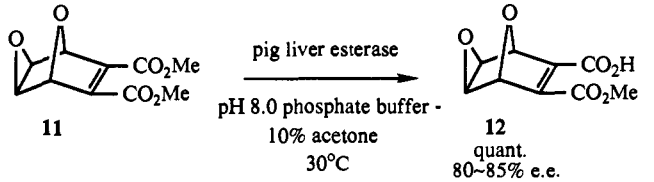
Scheme 2



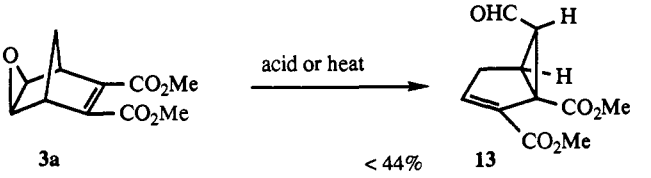
Scheme 3



Scheme 4



Scheme 5



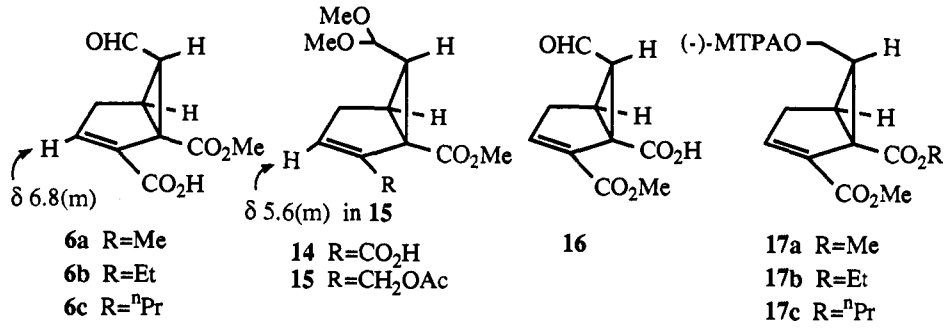
despite the electron-deficient systems; (2) why the rearrangement in the electron-deficient diesters, **3a-c**, is so regiospecific; and (3) why the 7-oxa-analog, **11**, does not undergo this rearrangement.

We present herein the full details of our mechanistic investigation of this new rearrangement.

Results and Discussion

1. Asymmetric Hydrolysis with Pig Liver Esterase. Dimethyl ester **3a** was hydrolyzed with pig liver esterase under previously reported conditions (pH 8.0 phosphate buffer containing 10% acetone at 30 °C for 5 h).[3,5,6] Usual workup afforded a single optically active monoester in a quantitative yield with the optical rotation +72.3° (c = 0.96, MeOH). However, the product was not the expected monoester, **4a**. The ^{1}H-NMR spectrum showed the presence of a new formyl group at δ 9.36 and an olefinic proton from δ 6.8, which is characteristic of the β-proton of the α,β-unsaturated carbonyl moiety. The characteristic AB pattern of the methylene protons in bicyclo[2.2.1]heptanes (or heptenes)

Scheme 6



6a R=Me
6b R=Et
6c R=^{n}Pr

14 R=CO_2H
15 R=CH_2OAc

16

17a R=Me
17b R=Et
17c R=^{n}Pr

was not observed. After converting this aldehyde to dimethyl acetal, we observed quite a large J^{1}_{CH} value of ~165 Hz for two tertiary carbons in the sp^3 region in the gated decoupled ^{13}C-NMR spectrum, which implies the existence of a cyclopropane ring. We also observed the unusually shielded acetal proton resonance at δ 3.9, which also suggests that it is attached to the cyclopropane ring. On the basis of these spectroscopic analyses along with the close serial proton decoupling experiment from the acetal proton, the structure of 1,2-dialkoxycarbonyl-6-formylbicyclo[3.1.0]hex-2-ene was elucidated.

The regiochemistry in the enoic moiety was assigned by reducing the carboxyl group selectively followed by acetylation of the resultant primary alcohol ((1) ClCOOEt, NEt_3/THF, 0 °C; (2) $NaBH_4$/THF–H_2O, 0 °C; (3) Ac_2O, Py/CH_2Cl_2, room temperature).[10] This selective reduction shifted the enoic proton resonance from δ 6.8 up to δ 5.6, which indicates that the proton resonance at δ 6.8 is the one in the α,β-unsaturated carboxylic acid instead of the isomeric unsaturated ester as in **16**. On the basis of these data, the structure of this rearranged product was unambiguously determined to be 6-formyl-2-(methoxycarbonyl)-bicyclo[3.1.0]hex-2-ene-1-carboxylic acid, **6a**. As mentioned above, the regioisomer, **16**, where the two substituents are switched, was not detected at all.

The enantiomeric excess of **6a** was determined to be 47% ee by ^{19}F-NMR spectra after converting **6a** to the (–)-MTPA ester derivative **17a** ((1) CH_2N_2/Et_2O, (2) $NaBH_4$/THF, (3) (–)-MTPACl, Py/CCl_4].

The determination of the absolute configuration of **6** was tentatively made as depicted in Scheme 6, referring to the analogy developed for the synthesis of nucleoside derivatives.[3]

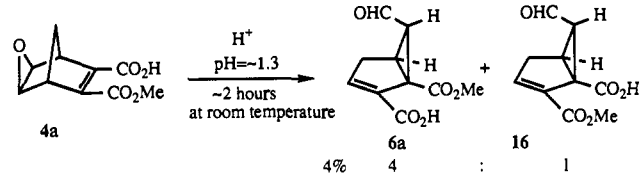
The diethyl ester **3b** and the di-*n*-propyl esters **3c** were also submitted to the same pig liver asymmetric hydrolysis. In the case of **3b**, the rearranged product **6b** was obtained quantitatively, and the enantiomeric excess was determined to be 65% ee by a similar manner. The *n*-propyl ester **3c** gave the monoester **6c** in 75% yield (28% ee) after 3 days of incubation, with the starting diester **3c** recovered.

2. Thermal and Acid-Catalyzed Rearrangement of Diester 3a. Since Prinzbach et al. did not report the yield of the rearranged product **13** (Scheme 5), we reexamined this rearrangement in an electron-deficient system. In our experiments, treatment of **3a** with BF_3·OEt_2 in CH_2Cl_2 for 5 min at room temperature and in AcOH for 8 h at room temperature and then refluxing for 26 h afforded the rearranged product **13** in 8% and 31%, respectively, accompanying decomposed mixture. Silica gel treatment in CH_2Cl_2 at room temperature caused the very slow rearrangement (6 days) to form the rearranged product **13** in 44% yield. Simple reflux of **3a** in ethyl acetate afforded only 23% of the rearranged product, **13**.

The rearranged structure **13** was confirmed to be identical with the methyl ester of **6a** in ^1H-NMR, which was obtained from the treatment of **3a** with ethereal diazomethane.

3. Mechanistic Study. First, in order to examine the reaction sequence, the dimethyl ester **3a** was stirred for 5 h in the same phosphate buffer solution (pH 8, containing 10% acetone) without

Scheme 7



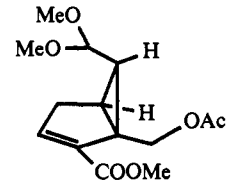
4a → **6a** : **16**
4% 4 : 1

pig liver esterase. However, the formation of the rearranged diester **13** was not observed at all, and the starting diester **3a** was recovered quantitatively. This result shows that the rearranged product **6a** is formed via the intermediary monocarboxylate, **5**. Furthermore, this result excludes the alternative sequence, namely, the initial rearrangement of the diester, followed by the enzymatic hydrolysis of the ester group attached to the olefinic carbon.

In the second step, we examined whether the acid plays a pivotal role in initiating this rearrangement for the electron-deficient epoxide **4**, as is the case with the classical Meinwald rearrangement. The racemic epoxy monoester **4a** was prepared separately in crystalline form and exposed to acidic solution. In the pig liver esterase asymmetric hydrolysis, the reaction mixture must be treated with mineral acid, such as aqueous hydrochloric acid, for extraction of the carboxylic acid. This is the only stage where the substrate is exposed to acid, which is normally only for a few minutes. Therefore, the racemic epoxide was stirred in diluted hydrochloric acid solution containing 10% acetone at room temperature. However, after this acid treatment for a short period, no reaction was observed. Upon continuing the acid treatment for 2 h, two rearranged regioisomers, **6a** and **16**, were formed, where the formation ratio is 4:1 in only a 4% yield altogether (Scheme 7).

Additionally, during the enzymatic hydrolysis of the meso diester **3a**, the mixture of the monoester **4a** and **3a** was observed upon termination of the reaction by acid treatment after about 30 min from the incubation.

The structure of this minor regioisomer **16** was confirmed by the same manner as the major isomer **3a**, converting to acetate **18**.

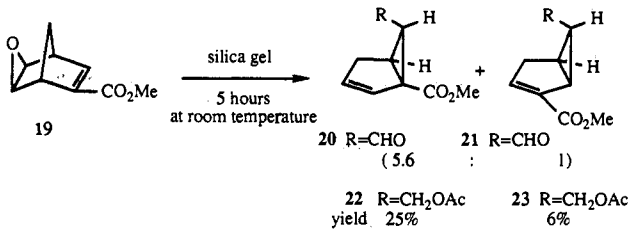


18

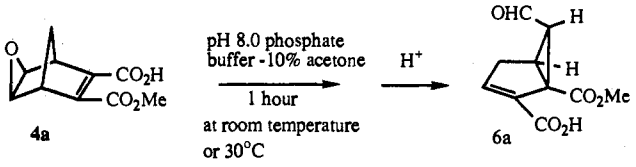
In addition to this acid treatment, this racemic monoester, **4a**, was gradually warmed in ethyl acetate to the reflux temperature. Neither the reaction rate acceleration nor improvement of the yield of the rearranged products was observed, and eventually the starting compound, **4a**, completely decomposed upon refluxing.

One could point out several important aspects from these simple experiments for investigating this reaction mechanism, First of

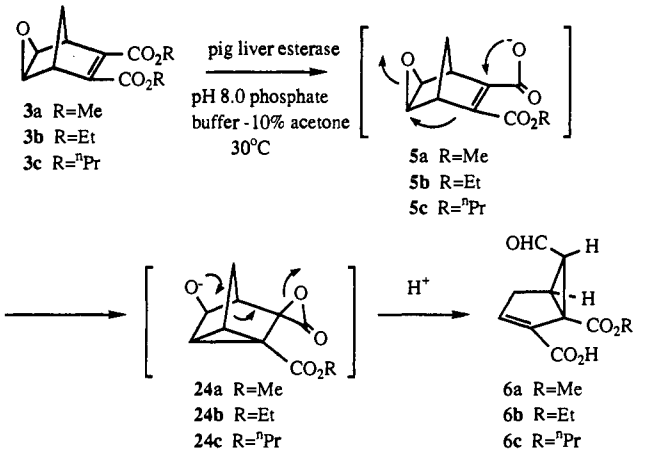
Scheme 8



Scheme 9



Scheme 10



all, it is essentially an unfavorable process for the electron-deficient olefins to cause this Meinwald rearrangement even though the starting expoxides are highly strained. Furthermore, the acid cannot be the trigger which initiates this rearrangement nor induces such regiospecificity, since carbomethoxy and carboxyl groups have essentially almost the same electron-withdrawing character. It is also unreasonable that carbocationic species are generated next to the electron-deficient groups under the acid-catalyzed mechanism. This rather unexpected regioselectivity, which was observed for the monoester **4a** that favors **6a** over **16**, is rationalized on the basis of the ^{13}C-NMR chemical shifts of both sp^2 carbons of the monoepoxide **4a**. We have discussed this issue in an earlier paper.[6]

Regarding this regiochemical problem, another experiment of acid treatment for the epoxide **19**, which has only one electron-acceptor and is more reactive than **4a**, confirmed the weak regioselectivity of this electron-deficient olefin. Although in the epoxide **19**, electrons are more localized on the carbon attached to COOMe, the treatment of **19** with silica gel in CH_2Cl_2 produced a mixture of the two rearranged products, **20** and **21**, in almost the same formation ratio as the rearrangement of monoester **4a** (Scheme 8). The formation ratio of the two aldehydes was determined by converting them to the acetates, **22** and **23**, due to the instability of the aldehydes, **20** and **21**.

However, in contrast to these acid-catalyzed reactions, when the racemic monoester was stirred in phosphate buffer solution (pH 8.0) containing 10% acetone at room temperature to 30 °C, the rearrangement was completed smoothly in 1 h to afford the single, pure rearranged product, **6a**, in quantitative yield without accompanying regioisomer **16**! (See Scheme 9.)

From these observations, it is concluded that an additional contribution, which pushes electrons exclusively from the "COOMe side", facilitates this rearrangement. We propose the intermediate, the α-lactone **24**, which was formed by an intramolecular Michael addition of the carboxylate anion generated by PLE asymmetric hydrolysis (Scheme 10). This reaction pathway can also explain the regiospecificity of this rearrangement quite unequivocally, which could not be elucidated from the same mechanism as the classical acid-catalyzed Meinwald rearrangement in Scheme 3.

The α-lactones have been invoked as highly reactive intermediates in a variety of reactions, such as nucleophilic displacements,[12] free radical processes,[13] thermal eliminations,[14] and photochemical reactions.[15] Historically, Ingold et al. proposed the α-lactone species first in order to explain the retention of configuration during the deamination of optically active amino acids by aqueous nitrous acid.[16] Chapman et al. characterized several disubstituted α-lactones by low-temperature IR spectra in photodecarbonylation of disubstituted malonyl peroxide.[17] The carbonyl stretching frequencies of the α-lactones were observed at 1895–1935 cm^{-1} at −196 °C. They demonstrated that these highly unstable species are spectroscopically detectable only below −100 °C, even though the reaction proceeds at room temperature.

We examined this rearrangement further in basic media using nonaqueous solvents. However, no condition facilitated this rearrangement. For instance, dissolving **4a** in $CDCl_3$ with 1 equiv of triethylamine very gradually caused this rearrangement at room temperature. It was extremely slow, and less than 20% of the rearranged product was observed in the ^{1}H-NMR spectrum. When **4a** was treated with *n*-BuLi in THF and stirred at room temperature, the starting monoester was recovered quantitatively. The same treatment of **4a** with *t*-BuOK in THF did not cause the rearrangement at room temperature and resulted in decomposition upon heating. Consequently, for the authorized method for enzymatic hydrolysis, pH 8 phosphate buffer solution containing 10% acetone turned out to be the best reaction media for this rearrangement. This is probably because ionic and cationic charges are quite localized and carboxylate anion and counter cations (Li, K) are almost covalently bonded in organic solvents, which would hinder the intramolecular Michael addition of the carboxylate anion. In addition, we assume that the formation of the α-lactone, **24**, is accelerated by the electrostatic interaction between a proton and the epoxide oxygen, which could ease the cleavage of the epoxide.

This reaction mechanism could also be used to rationalize why the 7-oxa-analog **12** does not cause the rearrangement that we found. Qualitatively, the α-lactone intermediate for the 7-oxa-analog would be destabilized by the repulsive interaction between the lone pair electrons of oxygen at the 7-position and either the carboxylate oxygen or alkoxide oxygen.

As for this mechanism, ab initio single point calculations with the STO-3G basis set (Gaussian 80)[18] on the AM1[19] fully optimized geometries were carried out for the carboxylates in the ground state. The most stable conformer turned out to be the one where the carbonyl of COOMe is parallel to C=C and COO$^-$

(12) (a) Boldwell, F. G.; Knipe, A. C. *J. Org. Chem.* **1970**, *35*, 2956. (b) Weinstein, S.; Lucas, H. J. *J. Am Chem. Soc.* **1939**, *61*, 1576.
(13) (a) Walling, C.; Savas, E. S. *J. Am Chem. Soc.* **1960**, *82*, 1738. (b) Bartlett, P. D.; Gortler, L. B. *J. Am Chem. Soc.* **1963**, *85*, 1864. (c) Gortler, L. B.; Satzman. M. D. *J. Org. Chem.* **1966**, *31*, 3821. (d) Leffler, J. E.; Zepp, R. G. *J. Am Chem. Soc.* **1970**, *92*, 3713.
(14) Ballard, D. G. H.; Tighe, B. J. *J. Chem. Soc. B.* **1967**, 702.
(15) Adam, W.; Rucktaeschel, R. *J. Am Chem. Soc.* **1971**, *93*, 557.
(16) Cowdrey, W. A.; Hughes, E. D.; Ingold, C. K. *J. Chem. Soc.* **1937**, 1208.
(17) Chapman, O. L.; Wojtkowski, P. W.; Adam, W.; Rodriquez, O.; Rucktaeschel, R. *J. Am Chem. Soc.* **1972**, *94*. 1365.
(18) Binkley, J. S.; Whiteside, R. A.; Krishnan, R.; Seeger, R., DeFrees, D. J.; Schlegel, H. B.; Topiol, S.; Kahn, L. R.; Pople, J. A. *QCPE* **1981**, *13*, 406.
(19) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *Ibid.* **1985**, *107*, 3902.

is perpendicular to C=C.[20] Although this is the result in the ground state, this calculation result can be interpreted to be consistent with the mechanism we proposed since this alignment would favorably enable the intramolecular Michael addition.

Concluding Remarks

We have discovered the first facile, completely regio- and stereospecific asymmetric rearrangement caused by chemicoenzymatic hydrolysis. The Meinwald-type rearrangement does take place smoothly and quantitatively even in the electron-deficient system. In contrast to the classical Meinwald rearrangement, this new rearrangement that we have found proceeds in an aqueous basic media such as in pH 8.0 phosphate buffer solution. We have proposed the reaction pathway shown in Scheme 10, that is, starting from carboxylate anion **5**, proceeding through the α-lactone-type intermediate **24** formed by an intramolecular Michael addition of a carboxylate anion, producing the rearranged product. This α-lactone-type intermediate **24** could explain not only the regio- and stereospecificity of this rearrangement but also the reason why the 7-oxa-analog **11** does not cause this rearrangement under the same conditions.

Although, at this point, the rearrangement does not afford very high optical purity from a synthetic point of view, this regio- and stereospecific rearrangement could generate a potential chiral synthon since it can introduce chiral quaternary and tertiary carbons, and three different functional groups simultaneously in quantitative yield.

Experimental Section

Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on a JEOL FX-100 (100 MHz) and GX-400 (400 MHz) spectrometer in $CDCl_3$, and chemical shifts are expressed in parts per million downfield from Me_4Si. Infrared (IR) spectra were recorded on a JASCO A-102 spectrometer. Optical rotations were measured with a JASCO DIP-104 digital polarimeter. Mass spectra were obtained on a JEOL JMS-01 SG-2 mass spectrometer.

(1) Preparation of Diesters 3a-c and Monoesters 4a and 19. Dimethyl 5,6-*exo*-Epoxybicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate (3a). Dimethyl bicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate[21] (689 mg, 3.31 mmol) was dissolved in CH_2Cl_2 under an Ar atmosphere. *m*-CPBA(70%, 954 mg, 3.87 mmol) and $NaHCO_3$ (333 mg, 3.97 mmol) were added to this solution at 0 °C, and the mixture was stirred for 5 h. Purification by silica gel column chromatography afforded oily material **3a**. Yield: 534 mg (72%). ^{1}H-NMR (90 MHz): 3.65 (6H, s), 3.56 (2H, br s), 3.38 (2H, br s), 1.80 (1H, br d, J = 10.0, AB system), 1.55 (1H, br d, J = 10.0, AB system). ^{13}C-NMR (25 MHz): 164.7 (s), 149.7 (s), 58.1 (s), 52.2 (q), 47.0 (d), 39.2 (t). IR ($CHCl_3$): 1719, 1285. MS: 224 (M^+), 194 ($M^+ - 2 \times CH_3$).

The diesters **3b** and **3c** were also prepared by the same procedure from corresponding diethyl ester and di-*n*-propyl ester, respectively.

Diethyl 5,6-*exo*-epoxybicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate (3b): yield, 72%. ^{1}H-NMR (90 MHz): 4.26 (4H, q, J = 8.1), 3.66 (2H, br s), 3.38 (2H, br s), 1.82 (1H, br d, J = 10.0, AB system), 1.60 (1H, br d, J = 10.0, AB system), 1.33 (6H, t, J = 8.1). IR ($CHCl_3$): 1711, 1284. MS: 254 (M^+), 222 ($M^+ - 2 \times CH_3$).

Di-*n*-propyl 5,6-*exo*-epoxybicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate (3c): yield, 90%. ^{1}H-NMR (90 MHz): 4.21 (4H, t, J = 7.2), 3.70 (2H, br s), 3.40 (2H, br s), 1.85 (1H, br d, J = 10.0, AB system), 1.75 (4H, sextet, J = 7.2), 1.60 (1H, br d, J = 10.0, AB system), 1.05 (6H, t, J = 7.2). IR ($CHCl_3$): 1719, 1285. MS: 280 (M^+), 250 ($M^+ - 2 \times CH_3$).

5,6-*exo*-Epoxy-3-(methoxycarbonyl)bicyclo[2.2.1]hept-2-ene-2-carboxylic Acid (4a). Monoester 3-(methoxycarbonyl)bicyclo[2.2.1]hept-2-ene-2-carboxylic acid was prepared by basic hydrolysis (0.25 N NaOH, at 0 °C) or by PLE hydrolysis of the corresponding diester (dimethyl bicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate). The monoester (5.05 g, 26.03 mmol) formed by this hydrolysis was dissolved in CH_2Cl_2 (1200 mL), and the mixture was treated with 80% *m*-CPBA (24.3 g, 112.65 mmol, 4.33 equiv) at room temperature and stirred for 2 days. This mixture was saturated with NaCl and extracted with ethyl acetate prior to drying over Na_2SO_4. Concentration and purification by silica gel column chromatography (solvent: hexane:AcOEt = 2:1, AcOEt:AcOH = 100:1) afforded epoxide **4a** followed by recrystallization from hexane–CH_2Cl_2. Yield: 2.26 g (41%). ^{1}H-NMR (90 MHz): 3.95 (3H, s), 3.8–3.5 (4H, m), 1.83 (1H, br d, J = 9.6, AB system), 1.56 (1H, br d, J = 9.6, AB system). ^{13}C-NMR (25 MHz): 167.8 (s), 161.9 (s), 158.9 (s), 147.9 (s), 57.7 (d), 57.7 (d), 54.0 (q), 48.0 (d), 47.1 (d), 38.5 (t). IR ($CHCl_3$): 2500–3000, 1725, 1680, 1590, 1440, 1290, 1270. Mp: 91–92 °C (from hexane–CH_2Cl_2). MS: 210 (M^+).

5,6-*exo*-Epoxy-3-(methoxycarbonyl)bicyclo[2.2.1]hept-2-ene (19). Methyl bicyclo[2.2.1]hepta-2,5-diene-2-carboxylate (1.348 g, 8.99 mmol), which was prepared on the basis of the literature,[22] was dissolved in 380 mL of CH_2Cl_2 and $NaHCO_3$ (2.265 g, 26.97 mmol); 80% *m*-CPBA (2.521 g, 11.68 mmol) was added under an Ar atmosphere, and the mixture was stirred for 9 h at 0 °C. This mixture was saturated with NaCl and extracted with CH_2Cl_2 prior to drying over Na_2SO_4. Concentration and purification by Florisil column chromatography (solvent: hexane:AcOEt = 5:1) afforded the epoxide **19**. Yield: 699 mg (48%). ^{1}H-NMR (90 MHz): 7.41 (1H, d, J = 3.0), 3.81 (3H, s), 3.80 (1H, br s), 3.57 (1H, br d, J = 4.5), 3.47 (1H, br d, J = 4.5), 3.15 (1H, m), 1.80 (1H, br d, J = 9.0), 1.45 (1H, br d, J = 9.0). IR ($CHCl_3$): 1725, 1285. MS: 166 (M^+).

(2) Asymmetric Hydrolysis with Pig Liver Esterase (PLE). 6-Formyl-2-(methoxycarbonyl)bicyclo[3.1.0]hex-2-ene-1-carboxylic Acid (6a). The diester **3a** (249 mg, 1.22 mmol) was dissolved in 38.9 mL of phosphate buffer (pH 8.0) containing 4.33 mL of acetone, then 189 units of PLE was added, and the mixture was incubated at 30 °C for 6 h. The reaction mixture was acidified with 2 N hydrochloric acid, extracted with ethyl acetate, and then dried over $MgSO_4$. The evaporation under reduced pressure afforded rearranged monoester **6a**. Yield: 230 mg (~100%). ^{1}H-NMR (100 MHz): 9.36 (1H, d, J = 4.0), 8.7 (1H, br), 6.8 (1H, m), 3.76 (3H, s), 2.4–3.1 (4H, m). ^{13}C-NMR (25 MHz): 198.2 (d, $^1J_{CH}$ = 180.0), 170.4 (s), 168.9 (s), 147.8 (d, $^1J_{CH}$ = 168.0), 132.5 (s), 53.3 (q; $^1J_{CH}$ = 148.0), 45.1 (s), 35.5 (d, $^1J_{CH}$ = ~165), 35.5 (d, $^1J_{CH}$ = ~165), 34.1 (t, $^1J_{CH}$ = ~141.6); two of the 35.5 ppm signals were observed well separated in C_6D_6. $[\alpha]^{20}_D$: +72.3° (c 0.96, MeOH). IR ($CHCl_3$): 2500–3000, 1735, 1720, 1710, 1265. MS was obtained as MTPA ester **17a**.

The rearranged products **6b** and **6c** were obtained by the same procedure.

6-Formyl-2-(ethoxycarbonyl)bicyclo[3.1.0]hex-2-ene-1-carboxylic acid (6b): yield, ~100%. ^{1}H-NMR (100 MHz): 9.28 (1H, d, J = 4.0), 8.9 (1H, br), 6.8 (1H, m), 4.30 (4H, dq, J = 10.8, 7.2), 4.16 (1H, dq, J = 10.8, 7.2), 2.5–3.1 (4H, m), 1.20 (3H, q, J = 7.2). ^{13}C-NMR (25 MHz): 197.6 (d), 168.9 (s), 168.0 (s), 146.7 (d), 132.1 (s), 64.1 (t), 44.3 (s), 34.4 (d), 34.4 (d), 33.1 (t), 13.4 (q), $[\alpha]^{20}_D$: +70.5° (c 2.00, MeOH). IR ($CHCl_3$): 2500–3000, 1735, 1720, 1710, 1260. MS: 224 (M^+), 216 ($M^+ - H_2O$).

6-Formyl-2-(*n*-propoxycarbonyl)bicyclo[3.1.0]hex-2-ene-1-carboxylic acid (6c): yield, 75%. ^{1}H-NMR (100 MHz): 9.30 (1H, d, J = 4.0), 9.3 (1H, br), 6.8 (1H, m), 4.15 (4H, dt, J = 10.5, 7.8), 3.99 (1H, dt, J = 10.5, 7.8), 2.3–3.0 (4H, m), 1.50 (2H, sextet, J = 7.8), 0.85 (3H, q, J = 7.8). ^{13}C-NMR (25 MHz): 197.0 (d), 168.8 (s), 167.6 (s), 146.2 (d), 131.7 (s), 66.9 (t), 44.4 (s), 34.4 (d), 33.0 (t), 21.4 (t), 9.9 (q). $[\alpha]^{20}_D$: +49.8° (c 1.14, MeOH). IR ($CHCl_3$): 2500–3000, 1735, 1720, 1260. MS: 238 (M^+), 220 ($M^+ - H_2O$).

(3) Determination of Regiochemistry of the Rearranged Product. The rearranged product **6a** (11 mg, 0.052 mmol) was dissolved in 0.1 mL of MeOH along with a catalytic amount of *p*-TsOH at 0 °C under an Ar atmosphere. After 20 min of stirring, the reaction mixture was passed through a silica gel column (solvent: AcOEt:AcOH = 100:1). To the THF (0.4 mL) solution of the resulting dimethyl acetal **14** were added at 0 °C under an Ar atmosphere Et_3N (8 mg, 0.079 mmol, 1.5 equiv) and ethyl chloroformate (8 mg, 0.076 mmol, 1.5 equiv), and the mixture was stirred for 1 h. After Celite filtration, concentrated filtrate was dissolved in THF (0.2 mL) and added dropwise to $NaBH_4$ (10 mg, 0.26 mmol, 5.1 equiv) in a water solution at 0 °C. This mixture was acidified after 1 h, followed by extraction with ethyl acetate prior to drying over Na_2SO_4. After concentrated in vacuo, the residue was dissolved in CH_2Cl_2 and treated with anhydrous acetic acid (0.2 mL) and pyridine (0.2 mL) for 1 h at room temperature. Ether was added, and then the mixture was washed with diluted hydrochloric acid, saturated sodium bicarbonate solution, and brine; this mixture was dried (Na_2SO_4) and concentrated

(20) This extensive theoretical study will be described in a separate manuscript.

(21) (a) *Organic Synthesis*; Wiley: New York, 1963; Collect. Vol. 4, p 329. (b) Nelson, W. L.; Freeman, D. S.; Sanker, R. *J. Org. Chem.* **1975**, *40*, 3658.

(22) Fienemann, H.; Hoffmann, H. M. R. *J. Org. Chem.* **1979**, *44*, 2802.

in vacuo. The residue was purified by column chromatography on silica gel (solvent: CH_2Cl_2:AcOEt = 4:1) to afford **15**. Yield: 1 mg (7%, four steps). ^{1}H-NMR (100 MHz): 5.6 (1H, m), 4.92 (2H, m), 4.02 (1H, dd, J = 6.0, 2.0), 3.70 (3H, s), 3.35 (3H, s), 3.32 (3H, s), 2.2–2.4 (4H, m), 2.08 (3H, s). IR: 1730, 1720, 1260, 1240. MS: 284 (M^+).

The regiochemistry of the regioisomer **16** was determined by the same manner by converting to **18**. **18**: ^{1}H-NMR (400 MHz) 6.66 (1H, m), 4.92 (1H, d, J = 11.7), 4.01 (1H, d, J = 11.7), 3.84 (1H, d, J = 7.3), 3.77 (3H, s), 3.34 (3H, s), 3.28 (3H, s), 2.77 (1H, ddd, J = ~7.7, ~2.5, ~20.5), 2.45 (1H, br d, J = ~20.5), 1.88 (1H, br t, J = ~8), 1.46 (1H, br t, J = ~8). IR ($CHCl_3$): 1725, 1250. MS: 284 (M^+).

(4) Determination of the Optical Purity of the Rearranged Products. The rearranged product **6a** was treated with ethereal diazomethane. The 42 mg (0.188 mmol) of resulting dimethyl ester in THF (0.4 mL) was reduced with $NaBH_4$ (7 mg, 0.18 mmol, 1.0 equiv) at 0 °C under an Ar atmosphere for 15 min. This reaction mixture was acidified with diluted hydrochloric acid, extracted with ethyl acetate, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (solvent: CH_2Cl_2:AcOEt = 2:3) to afford primary alcohol **17a**. This alcohol was submitted to Mosher's method [(–)-MTPACl, Py, CCl_4]. **17a**: yield, 42 mg (42%, 3 steps). MS: 442 (M^+).

The other rearranged products **6b** and **6c** were also submitted to Mosher's method to afford **17b** and **17c**, respectively. **17b**: MS, 456 (M^+). **17c**: MS, 470 (M^+).

(5) Treatment of Dimethyl 5,6-*exo*-Epoxybicyclo[2.2.1]hept-2-ene-2,3-dicarboxylate (3a) with Acid or Heat (Reexamination of Prinzbach's Results). (a) BF_3·EtO_2 (1 drop) was added to the solution of diester **3a** (11 mg, 0.049 mmol) in 1.5 mL of CH_2Cl_2 under an Ar atmosphere at room temperature for 5 min. This mixture was diluted with additional CH_2Cl_2 (~15 mL) and washed with saturated $NaHCO_3$ solution and brine, followed by drying over Na_2SO_4 prior to concentrating in vacuo. The residue was passed through silica gel column chromatography (solvent: CH_2Cl_2:AcOEt = 6:1) to afford 2.5 mg of the rearranged product **13**. Yield: 2.5 mg (23%). ^{1}H-NMR (90 MHz): 9.48 (1H, d, J = 4.0), 6.7 (1H, m), 3.80 (3H, s), 3.72 (3H, s), 2.5–3.0 (4H, m). IR: 1730, 1720, 1270. MS: 224 (M^+).

(b) The diester **3a** (12 mg, 0.056 mmol) was dissolved in 5 mL of ethyl acetate containing 5 drops of acetic acid under an Ar atmosphere at room temperature. After the reaction mixture stirred for 8 h, the solvent was removed under reduced pressure. The residue was submitted to silica gel column chromatography (solvent; hexane:AcOEt = 3:1) to afford 1 mg of the rearranged product **13** (8%).

(c) The diester **3a** (16 mg, 0.071 mmol) was dissolved in the same solvent (1.6 mL) as above and refluxed for 26 h under an Ar atmosphere. The solvent was evaporated in vacuo, and then the residue was submitted to silica gel column chromatography (the same eluting solvent) to afford 5 mg of the rearranged product **13** (31%).

(d) To the solution of diester **3a** (35 mg, 0.156 mmol) in CH_2Cl_2 (2 mL) was added under an Ar atmosphere at room temperature 700 mg of predried silica gel (BW-300), and the mixture was stirred for 6 days. After the silica gel was filtered off, the filtrate was concentrated in vacuo, followed by silica gel column chromatography to afford 15 mg of the rearranged product **13** (44%).

(6) Treatment of 5,6-*exo*-Epoxy-3-(methoxycarbonyl)bicyclo[2.2.1]hept-2-ene-2-carboxylic Acid (4a) with Acid and Base. A. Acid Treatment. (a) The monoester **3a** (220 mg, 1.05 mmol) was dissolved in 4.07 mL of acetone and treated with 36.60 mL of diluted hydrochloric acid (pH 1.29) at room temperature. After 2 h of stirring, the reaction mixture was saturated with NaCl and then extracted with ethyl acetate prior to drying over Na_2SO_4. Concentration and purification by silica gel column chromatography (solvent: AcOEt:AcOH = 100:1) afforded 9 mg of the mixture of the rearranged products **6a** and **16** (4%). Two formyl doublets were observed in a 4:1 ratio in C_6D_6.

(b) The monoester **4a** (2 mg, 0.01 mmol) was dissolved in 5 mL of ethyl acetate containing 5 drops of acetic acid under an Ar atmosphere at room temperature. After the reaction mixture stirred for 3 days, starting monoester **2a** was recovered quantitatively by concentration in vacuo.

B. Treatment with Base. (a) The monoester **4a** (42 mg, 0.20 mmol) was dissolved in 7.0 mL of phosphate buffer (pH 8.0) containing 0.778 mL of acetone at 30 °C, and the mixture was stirred for 3 h. The reaction was monitored by TLC and confirmed the consumption of starting monoester **4a**. This mixture was then acidified with diluted hydrochloric acid and extracted with ethyl acetate prior to drying over Na_2SO_4. Concentration in vacuo afforded the rearranged product **6a** quantitatively. Yield: 42 mg (~100%).

(b) The monoester **4a** (70 mg, 0.33 mmol) was dissolved in ~1 mL of chloroform. After addition of 0.05 mL of triethylamine, the mixture was concentrated in vacuo. The residue was dissolved in $CDCl_3$ in an NMR tube, and the ^{1}H-NMR was observed with the elapse of time.

(c) The monoester **4a** (32 mg, 0.15 mmol) was dissolved in ~1 mL of THF, and 1 equiv of *n*-BuLi was added under an Ar atmosphere at –78 °C. The reaction mixture was stirred at room temperature for 20 h. After addition of diluted HCl, the reaction mixture was extracted with ethyl acetate prior to drying over Na_2SO_4. Concentration in vacuo afforded the starting monoester **4a** quantitatively.

(d) The monoester **4a** (32 mg, 0.15 mmol) was dissolved in ~1 mL of THF, and 34 mg (0.30 mmol) of *t*-BuOK was added. The reaction mixture was stirred at room temperature for 15 h and then at 65 °C for 4 h. After addition of diluted HCl, the reaction mixture was extracted with ethyl acetate prior to drying over Na_2SO_4. Concentration in vacuo afforded the complex mixture.

(7) Treatment of 5,6-*exo*-Epoxy-3-(methoxycarbonyl)bicyclo[2.2.1]hept-2-ene (19) with Acid. The epoxide **19** (182 mg, 1.11 mmol) was dissolved in CH_2Cl_2 (12 mL), 4 g of predried silica gel (BW-300) was added under an Ar atmosphere at room temperature, and the mixture was stirred for 5 h. After the silica gel was filtered off, the mixture was concentrated in vacuo. The formation ratio of **20** and **21** (5.6:1) was determined by the ^{1}H-NMR spectrum in C_6D_6.

The mixture of **20** and **21** was dissolved in THF (10 mL), $NaBH_4$ (182 mg, 4.81 mmol) was added, and the mixture was stirred under Ar for 1 h at 0 °C. The mixture was acidified, followed by the extraction with ethyl acetate prior to drying over Na_2SO_4. After concentrated in vacuo, the residue was dissolved in CH_2Cl_2 and treated with anhydrous acetic acid (0.2 mL) and pyridine (0.2 mL) for 1 h at room temperature. Ether was added, and the mixture was then washed with diluted hydrochloric acid, saturated sodium bicarbonate solution, and brine; the mixture was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (solvent: CH_2Cl_2:AcOEt = 4:1) to afford **22** and **23**. **22**: yield, 59 mg (25%, three steps). ^{1}H-NMR (400 MHz): 5.89 (1H, ddd, J = 5.9, 2.2, 2.2), 5.7 (1H, m), 3.99 (1H, dd, J = 11.7, 6.2), 3.87 (1H, dd, J = 11.7, 8.1), 3.72 (3H, s), 2.66 (1H, dddd, J = 18.3, 2.2, 7.3, 2.2), 2.20 (1H, ddd, J = 18.3, 2.2, 2.8), 2.05 (3H, s), 2.33 (1H, ddd, J = 2.8, 7.3, 8.0), 2.3 (1H, ddddd, J = 6.2, 8.1, 8.0, 2.2, ~1.0). ^{13}C-NMR (25 MHz): 172.1 (s), 170.9 (s), 132.2 (d), 126.3 (s), 58.8 (t), 51.8 (q), 42.5 (s), 31.9 (t), 31.3 (d), 28.4 (d), 20.9 (q). IR ($CHCl_3$): 1725, 1240, 1030. MS: 210 (M^+). **23**: yield, 13 mg (6%, three steps). ^{1}H-NMR (400 MHz): 6.60 (1H, m), 3.92 (1H, dd, J = 14.3, 7.7), 3.88 (1H, dd, J = 14.3, 8.1), 3.75 (3H, s), 2.72 (1H, ddd, J = 20.5, 2.6, 7.7), 2.48 (1H, m), 2.40 (1H, br dd, J = 20.5, ~1.2), 2.03 (3H, s), 1.87 (1H, dddd, J = 7.7, ~8.0, ~8.0, ~1.2), 1.44 (1H, dddd, J = 7.7, 8.1, ~8.0, ~8.0). ^{13}C-NMR (25 MHz): 171.3 (s), 164.9 (s), 142.3 (d), 134.7 (s), 59.4 (t), 51.5 (q), 32.6 (t), 27.5 (d), 20.9 (q), 20.1 (d), 17.8 (d). IR ($CHCl_3$): 1720, 1255, 1100. MS: 210 (M^+).

Acknowledgment. We are grateful for the financial support of Grand-in-Aid (No. 63616005) for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture, Japan. The authors thank Professor Hiroshi Noguchi, University of Tokyo, for measuring 2-D NMR spectra. We also thank Professors Akiko Itai and Nobuo Tomioka, University of Tokyo, for their thoughtful encouragement during this research. This manuscript was prepared after S. Niwayama moved to the University of California, Los Angeles, as a Research Associate to Professor K. N. Houk. S. Niwayama thanks Professor Houk for his financial support. S. Niwayama also thanks Professor David C. Myles and Dr. Sandra I. Lamb at the University of California, Los Angeles, for their helpful discussions.