H), 1.98 (t, J = 7 Hz, 2 H), 1.37 (s, 6 H).

8,8-Dimethyl-10-(ethoxycarbonyl)-$\Delta^{1(9),3}$-2-hexalone (33).[43] A related procedure was adapted.[44] A solution of 1.50 g (6.00 mmol) of **32**,[32] 1.60 g (14.4 mmol) of SeO_2, and 6 drops of pyridine in 120 mL of t-BuOH was stirred at reflux for 31 h, filtered, and evaporated to afford 3.15 g of a reddish oil which slowly solidified. This was refluxed with two 15-mL portions of cyclohexane which were filtered and evaporated to leave 1.19 g (80%) of crude **33** as a yellowish solid which was used without further purification. It had: IR (KBr) 1725, 1640 (br) cm^{-1}; UV λ_{max} (EtOH) 250 nm (ϵ 10 900); ^1H NMR δ 6.64 and 6.28 (AB, J = 9 Hz, 2 H), 6.38 (s, 1 H), 4.09 (q, J = 7 Hz, 2 H), 1.23 (s, 3 H), 1.17 (t, J = 7 Hz, 3 H), 1.07 (s, 3 H).

4,4-Dimethyl-6-tetralol (34). A. Hydrogenolysis of 29a. A mixture of 176 mg (0.926 mmol) of pure **29a**, 47 mg of 5% Pd/C, and 3 drops of conc. H_2SO_4 in 25 mL of absolute EtOH was hydrogenated for 36 h, filtered through Celite which was washed with Et_2O, and evaporated. Dissolution in Et_2O, washing with H_2O, extraction into 5% NaOH, and acidification with HCl followed by isolation B ($CHCl_3$) gave 130 mg (80%) of crude **34** as an oil which was crystallized from hexane to mp 103.5–104.5 °C. It was identical by IR, ^1H NMR, and mixture melting point with the product from route B.

B. Saponification of 33. A solution of 566 mg (2.28 mmol) of crude **33** and 1.76 g (31.4 mmol) of KOH in 115 mL of 2:1 H_2O–MeOH was refluxed for 90 h and extracted with Et_2O which was extracted with 5% NaOH. Acidification (HCl) and isolation B (Et_2O) gave 181 mg (45%) of crude **34** which recrystallized from hexane: mp 104–105 °C; IR ($CHCl_3$) 3610, 1603, 1575 cm^{-1}; UV λ_{max} (EtOH) 279 nm (ϵ 1470), (base) 294 nm (ϵ 1800); ^1H NMR δ 6.75 (d, J = 9 Hz, 1 H), 6.66 (d, J = 3 Hz, 1 H), 6.41 (dd, J = 9 and 3 Hz, 1 H), 4.82 (br s, 1 H), 2.63 (br t, J = 6 Hz, 2 H), 1.8–1.6 (m, 4 H), 1.26 (s, 6 H). Anal. Calcd for $C_{12}H_{16}O$: C, 81.78; H, 9.14. Found: C, 81.88; H, 9.28.

4,4-Dimethyl-6-methoxytetralin (31). A. Hydrogenolysis of 30a. A mixture of 119 mg (0.583 mmol) of **30a** and 21 mg of 5% Pd/C in 10 mL of absolute EtOH was hydrogenated until absorption ceased (20 h), filtered, and taken to dryness. Dissolution in Et_2O and isolation A (5% $NaHCO_3$ wash) gave 102 mg (92%) of **31** as a yellowish oil: IR ($CHCl_3$) 1595, 1555 cm^{-1}; ^1H NMR δ 6.82 (d, J = 8 Hz, 1 H), 6.73 (d, J = 2.5 Hz, 1 H), 6.50 (dd, J = 8 and 2.5 Hz, 1 H), 3.70 (s, 3 H), 2.65 (br t, J = 5.5 Hz, 2 H), 1.8–1.6 (m, 4 H), 1.27 (s, 6 H).

B. Methylation of 34. Methylation of **34** (85 mg, 0.48 mmol, prepared from **33**) was conducted like methylation of **29a**, using 1.8 g of K_2CO_3 and 0.2 mL of Me_2SO_4 in 10 mL of Me_2CO. Isolation A (5% NH_4OH wash) gave 85 mg (77%) of crude **31** which was chromatographed on 6 g of activity III Al_2O_3 with 1:1 petroleum ether–$CHCl_3$ to provide 84 mg (76%) of **31** as an oil with the same IR and ^1H NMR spectra as the product from route A.

Acknowledgment. We are grateful to the National Science Foundation, the National Aeronautics and Space Administration, and the Phillips Petroleum Company for fellowship support (T.E.G.), and the University of Arkansas Research Reserve Fund for partial financial support of this work. W.L.M. expresses special thanks to Prof. D. Seebach, Laboratory for Organic Chemistry, Swiss Federal Institute of Technology (ETH), Zurich, for providing facilities and warm hospitality during the period when most of this paper was written.

Registry No. **10b**, 98-53-3; **10c**, 4255-62-3; **10d**, 54531-74-7; **10d** (2,4-dinitrophenylhydrazone), 94250-45-0; **11a**, 823-45-0; **11b**, 22252-96-6; **11c**, 64230-02-0; **11d**, 94250-46-1; **11e**, 94250-53-0; **12a**, 94250-47-2; **12b**, 94250-49-4; **12c**, 77630-12-7; **12d**, 94250-51-8; **12e**, 94250-52-9; **13a** (diastereomer 1), 94250-57-4; **13a** (diastereomer 2), 94250-58-5; **13a** (enol acetate diastereomer 1), 94250-60-9; **13a** (enol acetate diastereomer 2), 94250-61-0; **13b** (diastereomer 1), 94250-62-1; **13b** (diastereomer 2), 94250-63-2; **13c** (diastereomer 1), 94250-64-3; **13c** (diastereomer 2), 94250-65-4; **13d** (diastereomer 1), 94250-66-5; **13d** (diastereomer 2), 94250-67-6; **14a**, 94250-71-2; **14b**, 94250-74-5; **15a**, 94250-72-3; **16a**, 94250-48-3; **16b**, 94250-50-7; **19a**, 1694-31-1; **19b**, 94250-54-1; **19c**, 5396-89-4; **19d**, 94250-56-3; **20a**, 94250-59-6; **23a**, 94250-75-6; **23b**, 94250-76-7; **24a**, 94250-68-7; **24a** (enol acetate), 94250-69-8; **24b**, 94250-70-1; **25**, 94250-73-4; **27**, 94250-83-6; **28a**, 94250-77-8; **28b**, 94250-78-9; **29a**, 28204-62-8; **29b**, 94250-79-0; **30a**, 23203-51-2; **30b**, 94250-80-3; **31**, 23203-50-1; **32**, 1146-13-0; **33**, 94250-81-4; **34**, 94250-82-5; $(CH_2SH)_2$, 540-63-6; HCO_2Et, 109-94-4; i-PrCOCl, 79-30-1; i-PrCOCH(Ac)CO_2-t-Bu, 94250-55-2; i-PrCOCH$_2$CO$_2$Et, 7152-15-0; $PhCH_2OH$, 100-51-6; 4,4-dimethyl-2-cyclohexenone, 1073-13-8; 4,4-[1,2-ethanediylbis(thio)]cyclohexyl benzoate, 54531-77-0; 4-oxocyclohexyl benzoate, 23510-95-4; 4,4-[1,2-ethanediylbis(thio)]cyclohexanol, 22428-86-0; 2,4-dinitrophenylhydrazine, 119-26-6; 4-*tert*-butylcyclohexanol, 98-52-2.

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Synthesis and Interconversion of the Four Isomeric 6-Oxo-2,4-heptadienoic Acids[1]

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Received April 12, 1984

The synthesis of the structural isomers of 6-oxo-2,4-heptadienoic acid was undertaken as a means of providing analogues to investigate the structural requirements of maleylacetone cis–trans isomerase. 6-Oxo-2(Z),4(E)-heptadienoic acid and the methyl esters of 6-oxo-2(E),4(Z)-, 6-oxo-2(Z),4(Z)-, and 6-oxo-2(E),4(E)-heptadienoic acid were synthesized. Base-catalyzed hyrolysis of these esters furnished the corresponding acids except in the case of the 2Z,4Z isomer, which yielded instead the 2Z,4E acid. A mechanism for isomerization is suggested. Photocatalyzed isomerization of the acids and esters as a possible way of generating the ZZ acid was studied. The properties of the acids, their interaction with the enzyme, and what this suggests about the interaction of substrate maleylacetone with the enzyme is discussed.

Studies in this laboratory have been concerned with the mechanism of the enzyme-catalyzed cis–trans isomerization of maleylacetone (1) to fumarylacetone (4-hydroxy-6-oxo-2(E),4-heptadienoic acid, **2**; eq 1).[2] The enzyme requires glutathione as a coenzyme for this reaction. Maleylacetone has been shown to be an approximately 1:1 mixture of diketo (4,6-dioxo-2(Z)-heptenoic, **1b**) and ketoenol (4-hydroxy-6-oxo-2(Z),4(E)-heptadienoic, **1a**, and/or

(1) This work was supported by a grant from the National Institute of Health (GM 27636) and by the Office of Basic Research, Department of Energy.

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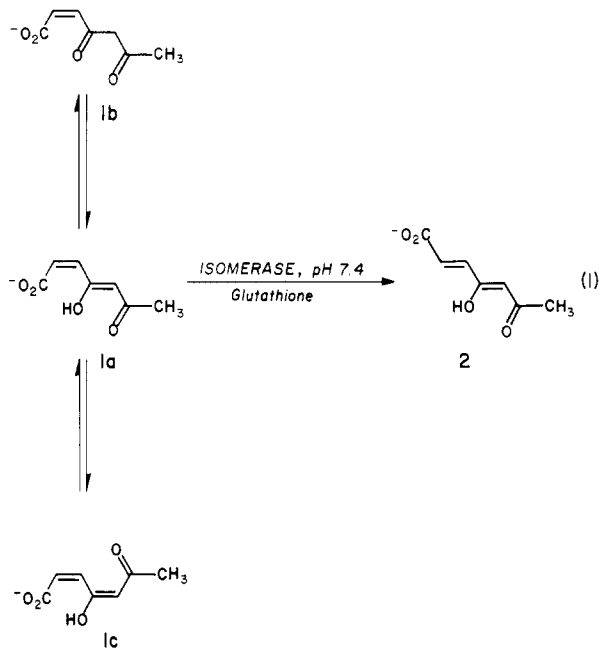

4-hydroxy-6-oxo-2(*Z*),4(*Z*)-heptadienoic, **1c**) acids in aqueous solution.[3] Interconversion among isomers is rapid; however, it is sufficiently slow on the NMR time scale to observe two methyl resonances, one from **1b** and the other from **1a** and/or **1c**.[3] Interconversion between **1a** and **1c** is expected to be much faster than between **1a** and **1b**.[3] At present there are no indications whether or not **1c** is present in significant amounts. With at least two isomers, however, and possibly a third, the question arises as to which, if any, structure is preferred by the enzyme.

In an attempt to gain some information about these questions it was decided to synthesize 6-oxo-2(*Z*),4(*E*)- and 6-oxo-2(*Z*),4(*Z*)-heptadienoic acids and test these for inhibitory action. These previously unknown analogues, lacking an hydroxyl group at C4 would be expected to be configurationally stable about their C4–C5 bonds. The present report describes the syntheses, properties, and some reactions of these compounds and the other configurational isomers of 6-oxo-2,4-heptadienoates.

Experimental Section

General Data. Reactions requiring anhydrous conditions were conducted under a positive pressure of argon in predried glassware. THF was distilled from sodium benzophenone ketyl immediately prior to use. Furfural and 2-methylfuran were distilled prior to use. Other reagents, except where noted, were used without further purification. TLC was performed on fluorescent silica gel plates (E. Merck) and visualized with a UV source or by moistening with a (2,4-dinitrophenyl)hydrazine solution. Preparation-scale separations used the technique of flash chromatography.[4] Analytical HPLC was carried out on a 10 μm Lichrosorb RP-18 (E. Merck) column using a flow detector at 254 nm.

Routine ^1H and ^{13}C NMR were measured on a Varian CFT-20. Observed integrals of individual NMR resonances were within ±10% of the expected values. ^1H NMR at 300.1 MHz were measured on a Nicolet instrument. UV spectra were measured on a Cary 14. Microanalyses were carried out by Schwarzkoff Laboratories, Woodside, NY. High-resolution mass spectra were obtained with an AEI Model 30 mass spectrometer.

5-Hydroxy-2(5*H*)-furanone (3a) was prepared by a modification of a method published by Doerr and Willette[5] which utilizes the Rose Bengal photosensitized oxygenation of 2-furfural in methanol: NMR (80 MHz, $CDCl_3$, internal Me_4Si) δ 7.32 (dd, J = 5.8, 1.1 Hz, H-4), 6.23 (cm, J = 1.1 Hz, H-3 and H-5), 4.97 (b s, OH).

6-Oxo-2(*Z*),4(*E*)-heptadienoic Acid (4). 5-Hydroxy-2-(5*H*)-furanone (**3a**) (506 mg, 5.05 mol) in dry THF (5 mL) was added dropwise to an ice-cold suspension of sodium hydride (273 mg, 50% w/w in oil, 5.69 mmol) in dry THF (6 mL). The mixture was homogeneous except for the excess sodium hydride. The ice bath was removed after the addition was completed, and the reaction was allowed to continue for an additional few minutes. The solution was then transferred dropwise to an incompletely dissolved mixture of triphenylphosphoranylidene-2-propanone (2.09 g, 6.56 mmol) in dry THF (30 mL). The reaction mixture gradually became homogeneous, and the color darkened. After 2.5 h at ambient temperature the reaction mixture was cooled to 0 °C and quenched with 50 mL of ice water. The mixture was extracted with ether (2 × 50 mL) to remove most of the triphenylphosphine oxide. The alkaline, red-brown aqueous phase was mixed with an equal volume of ether and then acidified to pH 2 by the addition of 6 N HCl under vigorous stirring. The tan aqueous layer was separated from the ether layer and was further extracted with ether (3 × 50 mL). The combined extracts were concentrated, diluted with CH_2Cl_2, dried with sodium sulfate, and evaporated to a brown-black solid. Flash chromatography ($CHCl_3$ saturated with formic acid) afforded an unresolved mixture of *ZE* acid and its butenoilide as a light yellow solid (368 mg, 52%).

The crude product mixture was dissolved in 1 N NaOH (10 mL). After 3 min, the orange-brown solution was mixed with ether (10 mL) and acidified to pH 2 with 6 N HCl. The bright yellow aqueous phase was further extracted with ether (3 × 10 mL), and the combined ether extracts were concentrated, diluted with CH_2Cl_2, dried with sodium sulfate, and evaporated to a yellow solid (309 mg, 44%). The *ZE* acid so produced was homogeneous by NMR and HPLC; however, a sample was recrystallized twice from THF–hexanes for analysis. ^1H NMR (80 MHz, $CDCl_3$, internal Me_4Si chemical shifts and coupling constants (Hz) calculated by the use of LAOCOON III, root-mean-square error 0.046) δ 11.25 (b s, carboxyl), 8.28 (H-4), 6.80 (H-3), 6.34 (H-5), 6.05 (H-2), 2.39 (s, methyl), J_{23} = 11.4, J_{24} = 0.8, J_{25} = 0.8, J_{34} = 11.4, J_{35} = 0.7, J_{45} = 16.0; ^{13}C NMR ($CDCl_3$, internal Me_4Si) δ 199.2, 170.5, 143.5, 138.0, 137.1, 124.3, 27.2; UV (ethanol) λ_{max} 269 nm (log ε 4.36); MS (Finnigan 5100, EI mode, solids probe), m/e (relative intensity) 79 (5), 81 (6), 82 (43), 83 (89), 84 (14), 85 (7), 95 (5), 97 (88), 98 (91), 99 (6), 111 (5), 112 (13), 125 (16), 140 M (100), 141 (11), 142 (1). Anal. Calcd for $C_7H_8O_3$: C, 59.99; H, 5.75. Found: C, 59.59; H, 5.72.

4-Acetonyl-2-butenolide was obtained in pure form by allowing a solution of 6-oxo-2(*Z*),4(*E*)-heptadienoic acid in chloroform to react with a catalytic amount of formic acid for 18 h at ambient temperature: ^1H NMR ($CDCl_3$, internal Me_4Si) δ 7.60 (dd, H-3, J = 5.7, 1.5 Hz), 6.14 (dd, H-2, J = 5.7, 2.0 Hz), 5.43 (dddd, H-4, J = 7.5, 6.4, 2.0, 1.5 Hz), 3.10 (dd, H-5a, J = 17.5, 6.4 Hz), 2.66 (dd, H-5b, J = 17.5, 7.5 Hz), 2.23 (s, methyl).

2-Methyl-2,5-dimethoxy-2,5-dihydrofuran (5) was prepared by treating 2-methylfuran in methanol with bromine according to the literature procedure for the corresponding reaction with furan.[6] NMR ($CDCl_3$, internal Me_4Si, mixture of isomers, chemical shift of major isomer given first) δ 6.05–5.8 (both, cm, vinyl), 5.48 and 5.77 (cm, H-5), 3.50 and 3.42 (s, methoxy), 3.20 and 3.12 (s, methoxy), 1.51 and 1.57 (s, C2-methyl).

4-Oxo-2(*Z*)-pentenal (6). A mixture of 2-methyl-2,5-dimethoxy-2,5-dihydrofuran (1.44 g, 10 mmol), 10 mL of 0.01 N HCl, and 50 mL of THF was allowed to react at ambient temperature for 2 h. The colorless solution turned yellow. The reaction was diluted with 10 mL of brine and was extracted with ether (4 × 50 mL). The combined extracts were concentrated, diluted with CH_2Cl_2, dried with sodium sulfate, and evaported to provide 4-oxo-2(*Z*)-pentenal (1.0 g) as a bright yellow labile liquid which was used in the next reaction without further purification:[7] NMR

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($CDCl_3$, internal Me_4Si) δ 10.22 (d, CHO, J = 7.0 Hz), 6.96 (d, H-3, J = 11.8 Hz), 6.17 (dd, H-2, J = 11.8, 7.0 Hz), 2.39 (s, CH_3).

Methyl 6-Oxo-2(*E*),4(*Z*)-heptadienoate (7) and Methyl 6-Oxo-2(*Z*),4(*Z*)-heptadienoate (8). The above crude 4-oxo-2(*Z*)-pentenal was dissolved in dry THF (9 mL) and then added rapidly dropwise via cannula to a solution of methyl (triphenylphosphoranylidene)acetate (2.7 g, 8.1 mmol) in dry THF (40 mL). After 45 min, the yellow solution was quenched with 8 mL of brine and 4 mL of water and extracted with ether (3 × 40 mL). The combined extracts were concentrated, diluted with methylene chloride, dried with sodium sulfate, and evaporated to a yellow liquid. The residue was triturated with hexanes–THF (3:1) to precipitate most of the triphenylphosphine oxide. Using a step gradient of 10% and then 15% THF in hexanes, flash chromatography of the yellow liquid provided the following compounds: (a) **methyl 6-oxo-2(*Z*),4(*Z*)-heptadienoate (8)** (yellow oil, 178 mg, 12%), (b) **methyl 6-oxo-2(*Z*),4(*E*)-heptadienoate** (ester of 4) (impure oil, 13 mg, 1%), (c) **methyl 6-oxo-2(*E*),4(*Z*)-heptadienoate (7)** (fluffy yellow solid contaminated with 5% of the 2(*E*),4(*E*) ester, 696 mg, 45%).

The 2*Z*,4*Z* ester proved to be quite labile; it isomerized slowly in alcohol: NMR (300 MHz, $CDCl_3$, internal Me_4Si, LAOCOON III, root-mean-mean error 0.232) δ 7.761 (H-4), 7.712 (H-3), 6.325 (H-2), 5.992 (H-5), 3.758 (s, OCH_3), 2.289 (s, CH_3), J_{23} = 11.51, J_{24} = −0.73, J_{25} = 1.70, J_{34} = 11.24, J_{35} = −0.83, J_{45} = 11.53 Hz; UV (ethanol) λ_{max} 273 nm (log ϵ 4.29).

An analytical sample of the 2*E*,4*Z* ester was obtained by recrystallization from THF–hexanes: UV (ethanol) λ_{max} 273.5 nm (log ϵ 4.42); NMR (300 MHz, $CDCl_3$, internal Me_4Si LAOCOON III, root-mean-square 0.035) δ 8.271 (H-3), 6.466 (H-4), 6.300 (H-5), 6.116 (H-2), 3.776 (s, OCH_3), 2.292 (s, CH_3), J_{23} = 15.58, J_{24} = 0.77, J_{25} = 0.80, J_{34} = 11.59, J_{35} = 0.76, J_{45} = 11.39. Anal. Calcd for $C_8H_{10}O_3$: C, 62.32; H, 6.54. Found: C, 62.48; H, 6.68.

6-Oxo-2(*E*),4(*Z*)-heptadienoic Acid (Acid of 7). A solution of the 2*E*,4*Z* methyl ester 7 (99 mg, 0.64 mmol, 96% isomerically pure by HPLC) in 1 mL of *tert*-butyl alcohol was treated with 10 mL of ice-cold 1 N sodium hydroxide. The reaction flask was immediately plunged into an ice bath. After 4 min the mixture was acidified with 1.7 mL of 6 N HCl and extracted with ether (4 × 10 mL). The combined extracts were concentrated, diluted with methylene chloride, dried with sodium sulfate, and evaporated to provide the 2*E*,4*Z* acid as a light yellow solid (85.3 mg, 95% yield, 95% isomeric purity by HPLC). A small portion was recrystallized from THF–hexanes to provide yellow feathers (97% isomerically pure): NMR (300 MHz, $CDCl_3$, internal Me_4Si LAOCOON III, root-mean-square error 0.032) δ 8.359 (H-3), 6.338 (H-5), 6.492 (H-4), 6.124 (H-2), 2.306 (CH_3), J_{23} = 15.53 Hz, J_{24} = 0.75, J_{25} = 0.77, J_{34} = 11.60, J_{35} = 0.70, J_{45} = 11.38 Hz; UV (ethanol) λ_{max} 272 nm (log ϵ 4.36). Anal. Calcd for $C_7H_8O_3$: C, 59.99; H, 5.75. Found: C, 59.75, H, 5.91.

Methyl 6-Oxo-2(*E*),4(*E*)-heptadienoate (9). A solution of methyl 6-oxo-2(*E*),4(*Z*)-heptadienoate (7) (139 mg) in dry benzene (7 mL), in which a small crystal of iodine was dissolved, was refluxed for 4 h. After being cooled, the solution was washed with 0.19 M aqueous sodium thiosulfate (4 mL), dried with sodium sulfate, and evaporated to an orange-yellow solid. Flash chromatography of this residue (15% THF in hexanes) afforded the 2(*E*),4(*E*) ester as a pale yellow fluffy solid (132 mg, 95%):[8] NMR (300 MHz, $CDCl_3$, internal Me_4Si, LAOCOON III root-mean-square error 0.061) δ 7.331 (H-4), 7.146 (H-3), 6.424 (H-2), 6.250 (H-5), 3.793 (s, methoxy), 2.335 (s, CH_3), J_{23} = 15.63, J_{24} = 0.39, J_{25} = 0.61, J_{34} = 11.30, J_{35} = 0.42, J_{45} = 15.41 Hz, reported[8] δ 7.50–7.06 (m, 2 H), 6.53–6.20 (m 2 H), 3.77 (s, 3 H), 2.32 (s, 3 H); UV (ethanol) λ_{max} 270.0 nm (log ϵ 4.52), reported[8] 233 (log ϵ 3.05), 266 nm (log ϵ 3.15).

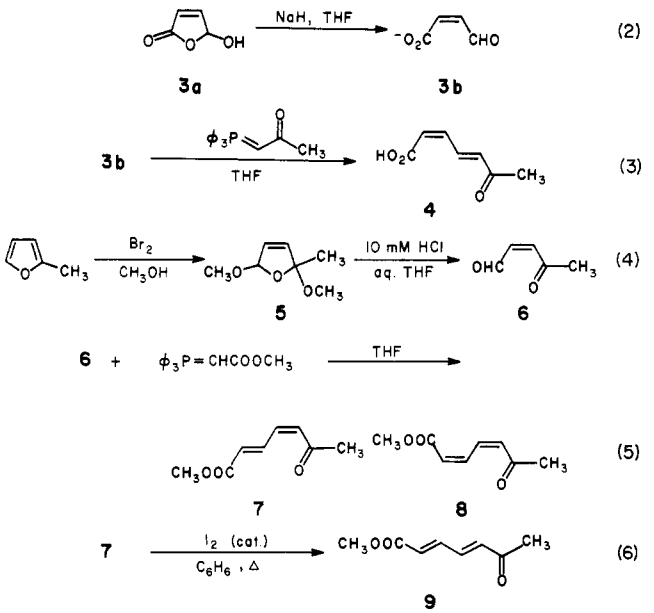
6-Oxo-2(*E*),4(*E*)-heptadienoic Acid (Acid of 9). The above ester (30.1 mg, 0.195 mmol), dissolved in a minimum amount of *tert*-butyl alcohol (0.7 mL), was mixed with 1.5 mL of 1 N aqueous sodium hydroxide. The reaction mixture darkened. After 10 min the mixture was acidified with 6 N HCl and extracted with ether (3 × 1.5 mL). The combined extracts were concentrated, diluted with methylene chloride, dried with sodium sulfate and evaporated to provide the 2(*E*),4(*E*) acid as an off-white powder (26.3 mg, 96%) which was homogeneous by NMR: NMR ($CDCl_3$, internal Me_4Si, 80 MHz, LAOCOON III, root-mean-square error 0.205) δ 8.4 (b s, CO_2H), 7.42 (H-4), 7.17 (H-3), 6.47 (H-2), 6.27 (H-5), 2.35 (s, CH_3), J_{23} = 15.6, J_{24} = −0.6, J_{25} = 0.8, J_{34} = 11.3, J_{35} = −0.6, J_{45} = 15.5; reported[8] δ 10.05 (b s), 7.45–7.03 (cm), 6.54–6.11 (cm), 2.38 (s); UV (ethanol) λ_{max} 270 nm (log ϵ 4.5) deviates from Beer's Law.

Thiocyanate-Catalyzed Isomerization of 6-Oxo-2(*E*),4(*Z*)-heptadienoic Acid (Acid of 7). The 2(*E*),4(*Z*) acid (11.4 mM) and potassium thiocyanate (0.105 M) in phosphate buffer (8 mM, pH 7.4) were allowed to react at ambient temperature. Aliquots were analyzed at 0, 4, 5, 7, and 98 days, by HPLC (C-18 column, 5:1:94 acetonitrile/acetic acid/water). Detection was at 254 nm. Integration was by the cut-and-weigh method, and areas were corrected for differences in response factors.

Photoisomerization of 6-Oxo-2,4-heptadienoic Acids and Esters. Analytical photolyses were carried out with monochromatic light from a Bausch and Lomb (B&L) 200-W super high-pressure mercury source coupled to a B&L grating monochromator. In early experiments ethanolic solutions of *ZE* acid (0.4 mM), and in later experiments separate solutions of *ZE* acid (0.6 mM), *EZ* ester (0.44 mM), and *ZZ* ester (0.41 mM) in acetonitrile were photolyzed for 1.5 h at ambient temperature. Product distribution was determined by HPLC on a C-18 column; detection was at 254 nm. A 5:1:94 acetonitrile/acetic acid/water solution served as eluent for the acids while a 12:88 acetonitrile/water solution was used as eluent for the esters. Similar experiments at 255 nm were carried out at extended times to determine the phtosatationary compound distribution of the acids. Photoisomerization of higher concentrations of *ZE* acid (16.1 mg) in ethanol (10 mL) was carried out in a Rayonet photochemical reactor emitting 35 W at 254 nm. Aliquots were removed at 30, 60, and 90 min and examined by HPLC.

Results and Discussion

The four isomeric skeleta of 6-oxo-2,4-heptadienoic acid were prepared by the reactions shown in eq 2–6 which are



fully described in the Experimental Section. The parent acid of each structural isomer was desired for examination of its interaction with the cis–trans isomerase. Therfore conditions for hydrolysis of the esters with accompanying minimal isomerization were sought. This is particularly important under conditions of catalyzed hydrolysis by base since hydroxide is sufficiently nucloephilic to catalyze cis–trans isomerization effectively.[9] The esters are insoluble in water. Consequently treatment with 1 N NaOH alone increases the propensity for isomerization since

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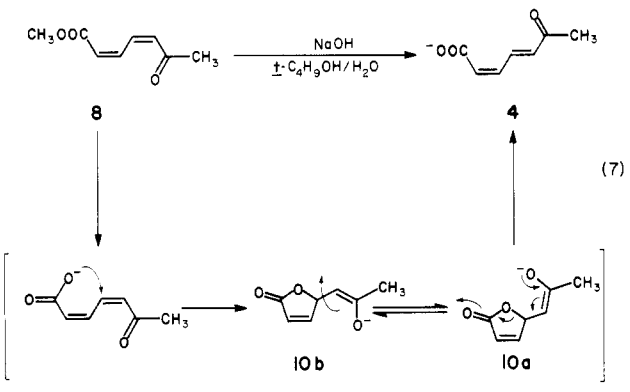
contact with the ester for hydrolysis is relatively low while the contact time with the anion of the carboxylic acid is high. Methanol was first used to increase the rate of ester hydrolysis. A solution of methanol and aqueous sodium hydroxide, however, provides a substantial concentration of methoxide ion which is a stronger nucleophile than hydroxide ion.[10] Thus the use of methanol to solubilize the ester and increase the rate of ester hydrolysis actually resulted in substantial isomerization. A less nucleophilic solubilizing agent was required. Solution of the ester in *tert*-butyl alcohol and then rapid mixing with 1 N NaOH to give a homogeneous solution led essentially to complete hydrolysis of the ester within minutes without cis–trans isomerization of the *EZ* ester **7** or of its corresponding acid.

Hydrolysis of the corresponding *ZZ* ester **8** by this and other methods have so far failed in our hands to yield the corresponding *ZZ* acid. Short reaction times of the *ZZ* ester with the *tert*-butyl alcohol/NaOH combination have only yielded the 2*Z*,4*E* acid and recovered *ZZ* ester. Reaction in the same system for longer times yielded, in addition, the 2*E*,4*E*. It would appear then that isomerization is primarily through the carboxylate anion suggesting the intermediacy of a butenolide anion (**10**) which allows rotation about the C4–C5 bond (eq 7). If this is true one might expect to find 4-acetonyl-2-butenolide under conditions where the base-catalyzed hydrolysis of the butenolide is not very rapid; indeed this was observed in one experiment. 4-Acetonyl-2-butenolide could be prepared from 6-oxo-2(*Z*),4(*E*)-heptadienoic acid by acid-catalyzed intramolecular conjugate addition. Base-catalyzed ring opening only provided the *ZE* acid **4** and no apparent *ZZ* acid. It is interesting that under conditions of slow ring opening (i.e., sodium borate buffer, pH 9) of the butenolide in D_2O, no deuterium incorporation at C-5 of **4** could be found as determined by NMR. This means that ring opening of the butenolide anion **10** is more rapid than reprotonation, lending support to the suggestion that **10** is the intermediate for cis–trans isomerization (eq 7).



Hydrolysis of the *ZZ* ester with less than 1 equiv of base was also studied. The thought was that the acid generated would neutralize the base and thereby decrease the concentration of the carboxylate ion and perhaps allow the stabilization of the *ZZ* acid. This too gave only the *ZE* acid and unreacted ester. In this context it is interesting to note that hydroxide ion catalyzed hydrolysis of the half acid ester of *ZZ* muconic acid followed by acidification yields only the *ZZ* muconic acid.[11] That is to say that

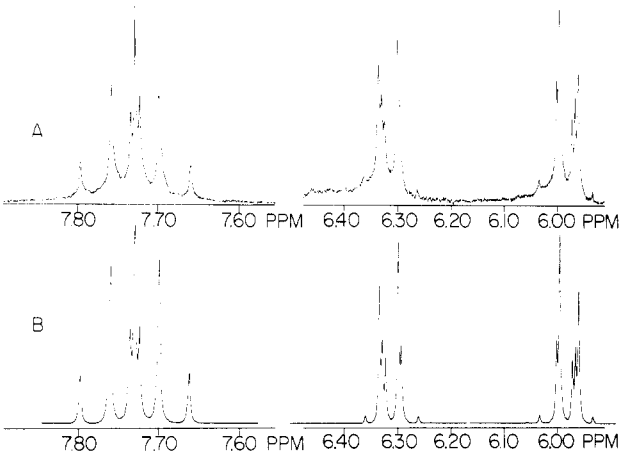


Figure 1. ^1H NMR spectra of methyl 6-oxo-2*Z*,4*Z*-heptadienoate in $CDCl_3$ at 300 MHz: (A) observed spectrum. (B) spectrum in the vinyl region simulated with the use of the program SIMEQ[13] from chemical shifts and coupling constants determined with the use of the program LAOCOON III.[12] See text.

isomerization via a butenolide anion as suggested in eq 7 does not occur presumably because of the lower electrophilicity of C_β in the muconic ester compared to that found in **8**. The synthetic routes and the ^1H NMR vinyl vicinal coupling constants establish the isomeric structures of the esters and acids.

NMR. The separation of the ^1H NMR spectra into its component parts in the vinyl region of this series of compounds was problematic because of the near chemical shift equivalences in each compound. In each of these ABCD systems two sets of similar protons: H3/H_4 and H2/H5 occur which cause the complexity. Most complex in this series turned out to be methyl 6-oxo-2(*Z*),4(*Z*)-heptadienoate (**8**). Chemical shifts and coupling constants were fitted to observed spectra with the use of the program LAOCOON III.[12] Spectra were recorded at 300 MHz to reduce the complexity and simplify the calculation but this was not sufficient for **8** whose 300-MHz ^1H NMR spectrum is shown in Figure 1A;; its spectrum simulated by the program SIMEQ,[13] from the chemical shifts and coupling constants obtained from LAOCOON III, is shown in Figure 1B. In this particular case the spectrum was still too complex even with spin decoupling to extract an intial guess for *J*'s and δ's to afford a proper convergent solution with LAOCOON III which resembled the observed spectrum. To overcome this dilemma, spectra from **8** were taken at 80 MHz in the presence of increasing amounts of Eu(fod)$_3$.[14] Consistent with the *Z* geometry of the double bonds,[15] the reagent induced larger downfield shifts in protons γ to the carbonyl groups (i.e., H3 and H4) than in the H2 and H5 protons. After eight additions of the shift reagent, the resonances in the vinyl region were sufficiently separated to obtain reliable initial values for the six coupling constants and four chemical shifts at the concentration of shift reagent. Calculations were performed on this spectrum to obtain the best fit, and the coupling constants so obtained were used as the initial values for the spectrum of the seventh Eu(fod)$_3$ addition.

(10) Hydroxide ion is a weaker nucleophile than aniline in aqueous medium while methoxide is a stronger nucleophile than aniline in methanol. See: Lowry, T. H.; Richardson, K. S. "Mechanisms and Theory in Organic Chemistry"; Harper and Row: New York, 1976; pp 186–187.

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Table I. Product Distribution upon Photolysis of 6-Oxo-2(*Z*),4(*E*)-heptadienoic Acid[a]

λ, nm	solvent	h	percents[b] *EE*	*ZE*	*EZ*	*ZZ*
240	ethanol	1.0	16	70	14	*c*
255	ethanol	1.5	21	58	21	*c*
270	ethanol	1.5	36	38	26	*c*
285	ethanol	1.5	45	28	27	*c*
254	acetonitrile	1.5	36	9	55	*c*
270	acetonitrile	1.5	49	9	42	*c*

[a] Light from a Bausch and Lomb 200-W high-pressure mercury lamp diffracted by a B&L grating monochromator, served as the irradiation source. Irradiations were carried out at ambient temperature. [b] Product distribution by HPLC corrected for relative response factors. [c] A fourth isomer was not detected.

This procedure was continued, until the spectrum with no $Eu(fod)_3$ present was solved. This is shown in Figure 1B. This method worked relatively well and is suggested in future cases of considerable complexity.

Photoisomerization. An earlier attempt to generate the 2*Z*,4*Z* acid was made, when only the 2*Z*,4*E* acid was available to use, by photolyzing a solution of the 2*Z*,4*E* acid. Initial experiments were carried out in ethanol at different wavelengths. Product distribution was determined by HPLC. The results are shown in Table I. At prolonged irradiation times at 255 nm a photostationary state mixture of 43% *EE* acid, 9% *ZE* acid, and 48% *EZ* acid was formed. Although photolysis led to what appeared to be a mixture of three isomers by HPLC, there was concern that a fourth isomer, presumably the *ZZ* acid, if formed, might coelute with one of the other three. Therefore a preparative photolysis at 254 nm was carried out in a Rayonet reactor and the concentrated photolysate treated with diazomethane. The methyl esters were separated by a combination of HPLC and flash chromatography and examined by NMR. Evidence for only the three isomers was found, and these were later verified by comparison with authentic samples.

At a later time it became apparent that the *ZZ* acid is highly unstable in aqueous media and suggested the possibility that the *ZZ* acid is formed in photolysis but undergoes rapid isomerization to the *ZE* acid in ethanol by the mechanism shown in eq 7. To investigate this possiblity, photolyses of the *ZE* acid and the methyl esters of the *EZ* and the *ZZ* acids in acetonitrile were carried out. As can be seen in Table I, a fourth isomer could not be detected in the photolysis of the *ZE* acid in acetonitrile. In the case of the esters (Table II), however, a substantial amount of the *ZZ* ester is present at the photostationary state supporting our hypothesis that the *ZZ* acid forms photolytically but undergoes rapid chemcial isomerization.

Relative Stabilities. The interaction of the enzyme with these isomeric substrate analogues sheds some light on the relative stabilities of their carboxylate anions at pH 7. It has been shown that the enzyme processes both *EZ* and *ZE* acids.[16] Amazingly, the enzyme carries out a catalyzed cis–trans isomerization of two double bonds at one time; it interconverts *EZ* and *ZE* acids, reversibly in one step![16] In the process the *ZE* and *EZ* acid anion

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Table II. Product Distribution upon Photolysis of the 2*Z*,4*Z* and 2*E*,4*Z* Isomers of Methyl 6-Oxo-2,4-heptadienoate in Acetonitrile[a]

initial isomer	λ, nm	h	percents[b] *EE*	*EZ* + *ZE*[c]	*ZZ*
EZ	254	1.5	45	49	6
ZZ	254	1.5	45	49	6
EZ	270	1.5	59	37	4
ZZ	270	1.5	55	40	5

[a] See Experimental Section for conditions. [b] Percent composition is expressed as raw peak areas, uncorrected for relative response factors. [c] These isomers are unresolved under conditions of the analysis.

concentrations appear to approach a ratio of about 16 to 1. If this is a reasonable reflection of the equilibrium between these two, this ratio would suggest that the *ZE* acid anion is about 1.5 kcal/mol more stable than the *EZ* analogue. Concurrent with this interconversion is the essentially irreversible isomerization of each to the *EE* isomer. A parallel reaction, the thiocyanate-catalyzed isomerization of the *EZ* acid at pH 7, leads to a 99:1 ratio of *EE* acid to *EZ* acid and indicates that the *EE* acid is 2.5 kcal/mol more stable than the *EZ* acid. That the *ZZ* acid anion apparently once formed from its ester hydrolysis rapidly goes to the *ZE* acid without establishing detectable *ZZ* acid concentration suggests that the *ZZ* acid anion is by far the least stable of the four. Moreover, the inability to generate any *ZZ* acid during photocatlyzed isomerization of the *ZE* acid in either ethanol or acetonitrile also suggests that the *ZZ* acid in these solvents is highly unstable with respect to the other three. As discussed above, the apparent inability to form *ZZ* acid by a photochemical process results not from a zero probability of the excited state decaying to the *ZZ* acid but rather from a rapid thermal isomerization of the *ZZ* acid to the *ZE* acid by a mechanism similar to that shown in eq 7.

With respect to the enzyme-catalyzed reaction and the ketoenol forms of maleylacetone, the properties of the 6-oxo-2,4-heptadienoic acids which we describe above suggest that **1a** should be more stable than **1c**; the latter isomer may be present in relatively low concentrations. Moreover, since the enzyme catalyzes the cis–trans isomerization of the *ZE* and *EZ* isomers of 6-oxo-2,4-heptadienoic acid, it appears highly likely that the ketoenol form **1a** of maleylacetone is processed directly by the enzyme. Whether **1b** is also acted upon directly by the enzyme remains to be answered.

Acknowledgment. We gratefully acknowledge the 300-MHz ^1H NMR spectra obtained from Mr. Simon Chu at SUNY, Stony Brook, on an instrument purchased with funds from the National Science Foundation (CHE 81-14412). We also than Dr. Charles Iden, SUNY, Stony Brook, for the high-resolution mass spectrum.

Registry No. **3a**, 14032-66-7; **3b**, 1575-59-3; **4**, 89999-79-1; **4** methyl ester, 93714-85-3; **5**, 22414-24-0; **6**, 34218-22-9; **7**, 89999-82-6; **7** free acid, 89999-80-4; **8**, 89999-81-5; **8** free acid, 93714-86-4; **9**, 41967-71-9; **9** free acid, 52999-76-5; triphenylphosphoranylidene-2-propanone, 1439-36-7; methyl (triphenylphosphoranylidene)acetate, 2605-67-6; maleylacetone cis–trans isomerase, 9023-75-0; 2-furfural, 98-01-1; 4-acetonyl-2-butenolide, 93714-87-5; 2-methylfuran, 534-22-5.