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Disparate Reactions of Thioesters of (Methylenecyclopropyl)acetic and β-(Cyclopropylidene)propionic Acids with Lithium Diisopropylamide and with General Acyl Dehydrogenase from Pig Kidney

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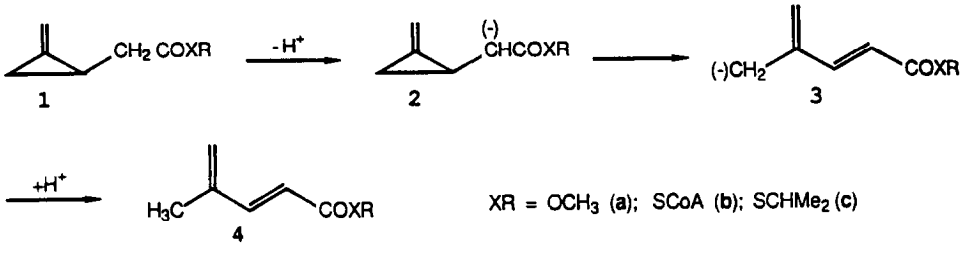
The anion generated from the isopropyl thioester of (methylenecyclopropyl)acetic acid may be quenched with water to give an unrearranged thioester and an acyclic isomer, isopropyl 4-methylpenta-2*E*,4-dienethioate; the coenzyme A thioester of (methylenecyclopropyl)acetic acid is a known inactivator of acyl dehydrogenases. The isopropyl thioester of β-(cyclopropylidene) propionic acid reacts with LDA followed by water to give only 3-(cyclopropyl)prop-2*E*-enethioate, and its coenzyme A thioester does not inactivate the acyl dehydrogenase from pig kidney (EC 1.3.99.3).

INTRODUCTION

In 1961 Ullman and Fanshawe reported (*1*) that 97 mg of methyl (methylenecyclopropyl)acetate **(1a)** heated at reflux with sodium hydride in cyclohexane containing a little methanol gave 3 mg of methyl 4-methylpenta-2,4-dienoate **(4a).** The structural identification rested on infrared and ultraviolet spectroscopic comparisons with an independently prepared sample of the ester; no NMR data were secured. The reaction was thought to occur through formation of the carbonyl-stabilized anion **2a,** cleavage of the three-membered ring to give the allylic anion **3a,** and protonation.



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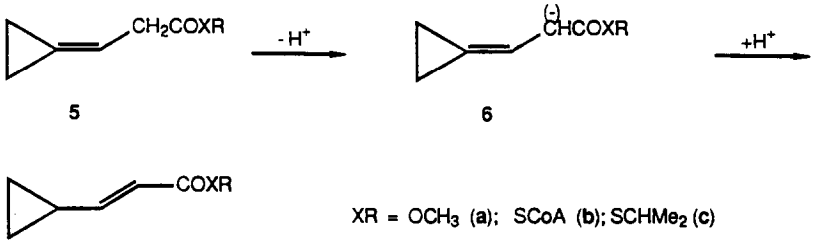
Ullman and Fanshawe (*1*) also found that the methyl ester of β-(cyclopropylidene)propionate **(5a)** under similar conditions was isomerized to methyl β-(cyclopropyl)acrylate **(7a);** the presumed intermediate **6a** did not lead to **3a** and thence to **4a,** according to gas chromatographic analyses (*1*).



Twenty years later, the isomerization of **1a** to **4a** was cited as a precedent for an important mechanistic conjecture: (methylenecyclopropyl)acetyl-CoA **(1b),** the potent inactivator of the general acyl dehydrogenase from pig kidney and other acyl dehydrogenases known to be responsible for the Jamacian vomiting sickness (*2*), might in the enzymatic active site lose a proton to form **2b** which then might isomerize to the allylic anion **3b;** this reactive intermediate might add to the flavin adenosine dinucleotide (FAD) cofactor of the enzyme to give covalently modified FAD derivatives and thus irreversibly inactivate the enzyme (*3*).

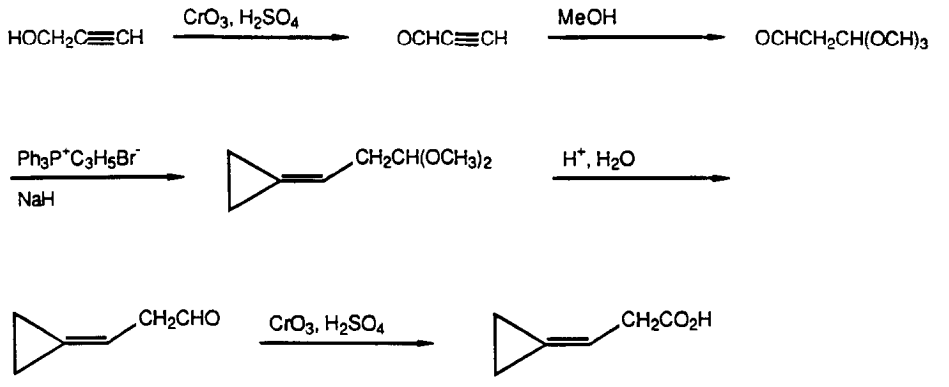
This possibility remains a standard speculation for the chemistry responsible for the inactivation of acyl dehydrogenases by **1b** (*4*); in light of the added significance thereby accruing to the base-catalyzed isomerization of methyl ester **1a** to **4a,** we have studied the base-catalyzed isomerizations of the isopropyl thioesters **1c** and **5c** and the *in situ* reactions of **1b** and **5b** with the general acyl dehydrogenase from pig kidney (EC 1.3.99.3).

RESULTS AND DISCUSSION

Syntheses. (Methylenecyclopropyl)acetic acid was available from an earlier synthesis (*5*). 4-Methylpenta-2*E*,4-dienoic acid was obtained from α-methacrolein and triethyl phosphonoacetate through a modified Wittig condensation, followed by hydrolysis (*6*). β-(Cyclopropylidene)propionic acid was prepared from propargyl alcohol in five steps (*7–10*), as outlined in Scheme I.

These three acids were converted to the corresponding acid chlorides with oxalyl chloride in DMF (*11*) and to the isopropyl thioesters **1c, 4c,** and **5c** (*12*). The coenzyme A thioesters **1b** and **4b** were prepared by way of the corresponding *N*-hydroxysuccinimide esters (*5*).

Reactions. When a dilute solution of anion (or the lithium enolate of) **2c,** prepared from **1c** with LDA in THF at −78°C, was allowed to warm to 0°C and quenched with water, analysis of the reaction mixture by GC–MS showed recovered starting material (37%), an isomeric product (16%), which proved to be **4c,** and some higher molecular weight by-products. The structural assignment of **4c**



SCHEME I. Synthesis of β-(cyclopropylidene)propionic acid.

was supported by NMR data and by direct chromatographic and other spectroscopic comparisons with an authentic, independently synthesized sample. When D_2O was used to quench the reaction mixture, both recovered starting material and isomeric product were monodeuterated. The recovered starting material then had been in the anionic form and had been protonated or deuterated when the reaction mixture was quenched. Thus anion or lithium enolate **2c** has a definite kinetic stability: this anion and its isomer **3c** are separated by a nontrivial activation barrier, and solutions of **2c** will show bimolecular chemistry in addition to the unimolecular ring-opening isomerization process.

The thioester **5c** shows the rearrangement seen previously for the oxygen ester **5a** (*1*): the base just brings the double bond into conjugation with the carbonyl function, and no trace of the isomeric thioester **4c** is detected by capillary gas chromatographic analyses under conditions known to distinguish clearly among **4c, 5c,** and **7c.** Under these chemical conditions, the isomerization of thioester **6c** to **3c** through cleavage of the three-membered ring does not take place to a detectable extent.

Nor does the coenzyme A thioester **5b** behave like its isomer **1b; 5b** does not inactivate the dehydrogenase. The rate of enzymatic oxidation of octanoyl-CoA is not diminished when four equivalents of **5b** are added: **5b** is not effective as an inactivator or as a competitive inhibitor of the octanoyl-CoA reaction.

The base-catalyzed isomerizations reported by Ullman and Fanshawe (*1*) for **1a** and **5a** are seen as well with the related isopropyl thioesters, and the coenzyme A thioester **5c** is not an inactivator of the general acyl-CoA dehydrogenase from pig kidney. There is then an observed parallelism between the distinct differences shown by thioesters of **1** and **5** in base-promoted isomerization reactions and in reactions with the acyl dehydrogenase from pig kidney, but this fact is not readily translated into a definitive mechanistic insight. Whether the postulated isomerization steps **2b** to **3b** may actually be involved in the enzyme inactivation chemistry remains to be seen: structural and mechanistic studies in progress address this issue. What seems clear now is that if anion **3b** is the reactive intermediate in the active site which combines irreversibly with the flavin cofactor to inactivate the

enzyme, its precursor anion **2b** and the flavin cofactor must for some reason fail to react with one another prior to the kinetically languid unimolecular isomerization step.

EXPERIMENTAL SECTION

Proton NMR spectra were recorded for $CDCl_3$ solutions with Me_4Si as an internal standard on a Varian XL-100, a GE QE-300, or a Nicolet NT-360 NMR spectrometer. Analytical gas–liquid chromatographic analyses were done using 0.2-mm-i.d. 25-m crosslinked dimethyl silicone and phenyl methyl silicone fused silica capillary columns, a Hewlett–Packard 5790 gas chromatograph with both columns connected to a single injection port, and the two FID detectors connected to HP 3390A and 3392A reporting integrators. Preparative gas chromatographic separations were accomplished using a Varian Aerograph A-90P3 and a 0.6 × 366-cm 20% SE-30 on a Chromosorb-W HMDS 60/80 column. Mass spectra and GC/MS data were obtained with a HP 5970B mass selective detector interfaced to a 5890 series gas chromatograph and a 9336 computer. Ultraviolet spectra were recorded on HP 8450 or 8451A spectrophotometers. CH_2Cl_2 was dried over P_2O_5 and THF was distilled from the benzophenone ketyl radical anion just prior to use. E+R Microanalytical Laboratory (Corona, NY) provided the elemental analyses.

β-(Cyclopropylidene)propional. β-(Cyclopropylidene)propional was prepared from commercial propargyl alcohol through a four-step reaction sequence: oxidation with CrO_3 to give propynal (*7*), Michael addition of 2 eq of methanol in the presence of diisopropylethylamine to form 3,3-dimethoxypropanol (*8*), Wittig reaction of this aldehyde with cyclopropylidene triphenylphosphorane (*9*), and finally hydrolysis of 1,1-dimethoxy-3-(cyclopropylidene)propane with oxalic acid and silica gel in a mixture of water and methylene chloride (*10*). The aldehyde, bp 55–56°C (26 mm), was characterized by NMR spectroscopic comparisons with a published spectrum (*10*).

β-(Cyclopropylidene)propionic acid. A 250-ml three-necked round-bottomed flask was fitted with a low-temperature thermometer, mechanical stirrer, nitrogen inlet, and addition funnel. To the flask was added 1.48 g (15.4 mmol) of β-(cyclopropylidene)propional in 150 ml of acetone. The reaction mixture was cooled to −10°C and then 6 ml of Jones reagent was added over 30 min while the temperature was maintained between −12 and −7°C. The reaction mixture was stirred at this temperature for 2 h, the cooling bath was removed, and 2-propanol was added dropwise until the orange color changed to blue green. The mixture was then decanted into a separatory funnel containing ice and water, the solid in the flask was washed with acetone, and the washings were added to the funnel. Ether and more water were added to the funnel to obtain two layers; several milliliters of concentrated HCl was added. The aqueous acidic phase was extracted several times with ether; the ether extracts were combined and extracted with saturated aqueous sodium bicarbonate. The basic solution was placed in a beaker and acidified with concentrated HCl, and when it became acidic the aqueous solution was

extracted with ether. The ethereal solution was dried over $MgSO_4$ and decanted; concentration by rotary evaporation gave the crude acid. ^1H NMR: δ 1.05 (br s, 4H), 3.26 (d, 2H), 5.90 (m, 1H), 11.5 (br s, 1H).

2-Propyl β-(cyclopropylidene)thiopropionate **(5c).** To a portion of the acid prepared immediately above (56 mg, 0.5 mmol) in 1 ml of dry CH_2Cl_2 in a flame-derived vial with a magnetic stirring bar under N_2 was added oxalyl chloride (85 μl, 0.98 mmol at 0°C and then 4–5 μl of dry DMF (*11*). The reaction mixture was stirred at 0°C for 15 min and then allowed to warm to room temperature. Stirring was continued until no further gas evolution was observed. Concentration under reduced pressure to remove excess oxalyl chloride gave the crude acid chloride which was dissolved in more CH_2Cl_2 and combined (*12*) with an excess of 2-propanethiol (~5 mmol). This reaction mixture was allowed to stir overnight at room temperature, and the isopropyl thioester product was isolated and purified by preparative GC. NMR: δ 5.93–5.87 (m, 1H), 3.63 (septuplet, 1H, J = 7.16 Hz), 3.38 (br d, 2H, J = 6.7 Hz), 1.30 (d, 6H, J = 7.16 Hz), 1.21–1.04 (m, 4H); mass spectrum: *m/e* 170 (M+ 0.2%), 142 (10.8), 128 (8.2), 127 (5.8), 113 (8.9), 100 (11.6), 99 (18.7), 67 (48.9), 65 (12.2), 43 (100), 41 (70.3), 39 (42.3); uv: λ_{max} 236 nm.

*Anal.*Calcd. for $C_9H_{14}OS$: C, 63.49; H, 8.29. Found: C, 63.35; H, 8.24.

2-Propyl (methylenecyclopropyl)thioacetate **(1c).** 2-Propyl (methylenecyclopropyl)thioacetate was prepared from (methylenecyclopropyl) acetic acid (*5*) through the same procedure and was purified by GC. NMR: δ 5.53 (br s, 1H), 5.42 (br s, 1H), 3.67 (septuplet, 1H, J = 6.83 Hz), 2.50 (d, 2H, J = 7.21 Hz), 1.77–1.71 (m, 1H), 1.43–1.36 (m, 1H), 1.31 (d, 6H, J = 6.83 Hz), 0.95–0.89 (m, 1H); mass spectrum: *m/e* 170 (M+ 0.1%), 128 (15.4), 103 (11.6), 100 (32.2), 95 (13.7), 68 (23.2), 67 (61.2), 65 (16.7), 43 (100), 41 (97.8), 39 (69.4); uv: λ_{max} = 234 nm.

Anal. Calcd for $C_9H_{14}OS$: C, 63.49; H, 8.29. Found: C, 63.56; H, 8.34.

2-Propyl 4-methylpenta-2E,4-dienethioate **(4c).** Unrecrystallized 4-methylpenta-2*E*, 4-dienoic acid, mp 59–60°C (lit. (*6*) mp 58–59°C; sample obtained after several recrystallizations from hexane had reported (*6*) mp 64–65°C), was prepared through a condensation of α-methacrolein with triethyl phosphonoacetate followed by hydrolysis (*6*). This acid was converted to the corresponding acid chloride and 2-propyl thioester as described for the isomeric acids above. NMR: δ 7.25 and 6.10 (two d, 2H, J = 15.67 Hz), 5.40 (br s, 2H), 3.75 (septuplet, 1H, J = 6.9 Hz), 1.88 (s, 3H), 1.34 (d, 6H, J = 6.83 Hz); mass spectrum: *m/e* 170 (M+ 4.0%), 142 (6), 99 (11.3), 98 (10.8), 96 (7.3), 95 (100), 67 (74.4), 65 (14.9), 43 (8.1), 41 (48.7), 39 (29.2); uv: λ_{max} = 270 nm (compare to the corresponding *O*-methyl ester (*1*), λ_{max} = 250 nm).

Anal. Calcd for $C_9H_{14}OS$: C, 63.49; H, 8.29. Found: C, 63.48; H, 8.34.

Base-promoted isomerization of 2-propyl (methylenecyclopropyl)thioacetate **(1c).** To a solution of 0.66 mmol of LDA in 60 ml of THF in a flame-dried 100-ml three-necked flask at −78°C was added dropwise thioester **1c** (23 mg, 0.14 mmol) in 2 ml of THF containing decane as an internal standard. The stirred pale yellow reaction mixture was kept at −78°C for 1 min and then at 0°C for 1.5 min: water (100 μl) was added, and the quenched reaction mixture was poured into a separatory funnel. Water (15 ml) was added and the aqueous THF solution was extracted with ether (2× 30 ml). The combined ether extracts were dried over $MgSO_4$,

filtered, and concentrated. Analysis by GC–MS at this stage revealed 37% of unreacted starting material and 16% of an isomeric product. This product was separated by preparative GC and identified as 2-propyl 4-methylpenta-2*E*,4-dienethioate **(4c)** through direct chromatographic (under the capillary GC conditions employed, the retention times of **1c, 4c,** and **7c,** on the dimethyl silicone column, were 4.64, 5.58, and 6.26 min, respectively), mass spectromeric, and ^{1}H NMR and ultraviolet spectroscopic comparisons with an authentic sample.

Repetition of this isomerization procedure, but using D_2O to quench the reaction mixture, gave recovered **1c**–d_1 and product **4c**–d_1, according to GC–MS and mass spectrometric ion intensity ratios at *m/e* 170 and 171.

Base-promoted isomerization of 2-propyl β-(cyclopropylidene)thiopropionate **(5c).** To 0.1 mmol of LDA in 9 ml of THF in a flame-dried 25-ml three-necked flask at −78°C was added dropwise thioester **5c** (3.9 mg, 0.023 mmol) in 1 ml of THF containing decane as an internal standard. The reaction mixture was stirred at −78°C for 3 min and then at 0°C for 3 min. The very faint yellow reaction mixture was quenched with 1 ml of water, poured into a separatory funnel, and extracted with ether (2× 5 ml). The combined ether extracts were dried over $MgSO_4$, filtered, and concentrated. Analysis by GC at this stage revealed a quantitative conversion to another compound, and GC–MS revealed this compound to be an isomer. This product was isolated by preparative GC and and its structure was assigned as **7c** on the basis of ^{1}H NMR and mass spectral data. NMR: δ 6.38–6.30 (m, 1H), 6.20–6.15 (m, 1H), 3.72 (septuplet, 1H, J = 7.15 Hz), 1.61–1.49 (m, 1H), 1.33 (d, 6H, J = 7.15 Hz), 1.01–0.94 (m, 2H), 0.69–0.64 (m, 2H); mass spectrum: *m/e* 170 (M+ 3.7%), 142 (4.8), 100 (6.7), 96 (6.7), 95 (100), 67 (83.1), 66 (7.8), 65 (19.9), 43 (11.5), 41 (57.0), 40 (49.2), 39 (44.3).

Repetition of this isomerization, but using D_2O to quench the reaction mixture, gave 2-propyl 3-(1-deuteriocyclopropyl)prop-2*E*-enethioate. NMR: 6.32 (br d, 1H, J = 15.08 Hz), 6.16 (d, 1M, J = 15.08 Hz), 3.70 (septuplet, 1H, J = 7.09 Hz), 1.32 (d, 6H, J = 7.09 Hz), 0.97–0.93 (m, 2H), 0.66–0.63 (m, 2H); mass spectrum: *m/e* 171 (M+ 4.8%), 97 (7.5), 96 (100), 68 (82.7), 67 (13.8), 66 (12.1), 43 (9.8), 42 (28.2), 41 (30.7), 40 (20.9), 39 (24.4); uv: λ_{max} = 268 nm.

Reactions of the coenzyme A esters of (methylenecyclopropyl)acetic acid and β-(cyclopropylidene)propionic acid with the general acyl dehydrogenase from pig kidney. The CoA esters were prepared by first converting the acids to the corresponding *N*-hydroxysuccinimide esters which upon treatment with coenzyme A gave the desired CoA esters (*5*). Inactivation of the dehydrogenase by **1b** has been reported (*3, 5*). To 100 μl of a solution of the enzyme (*13*) (13 μM active enzyme (*14*)) in 0.1 M potassium phosphate buffer at pH 7.6 was added 100 μl of an aqueous solution of **5b** (49 μM (*15*)); seven 10-μl aliquots were removed periodically over 29 min and immediately added to a buffered mixture of 2,6-dichlorophenolindophenol, phenazine methosulfate, and octanoyl-CoA in a ultraviolet spectrophotometer cuvette (*14*): the rate of oxidation of octanoyl-CoA was monitored for each sample at 600 nm and compared with samples containing only buffered enzyme at the same concentration. The average relative activity of the seven inactivation kinetic samples was 99 ± 5% of the average activity of four enzyme control samples.

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