Novel Synthesis of 2-Oxo-4-phenyl-3-butynoic Acid, a New Inhibitor and Alternate Substrate of Pyruvate Decarboxylase

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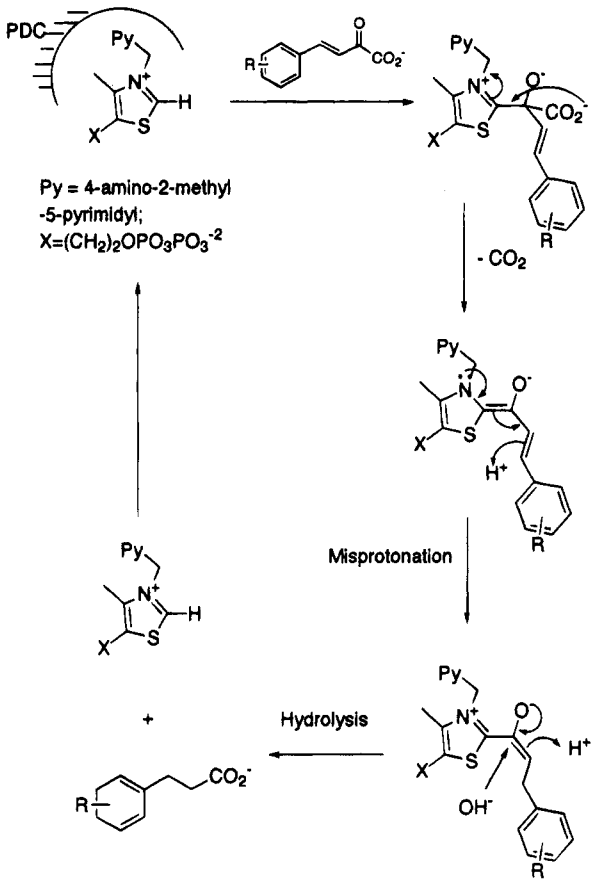
An improved method is reported for the synthesis of 2-oxo acids and is applied to the synthesis of 2-oxo-4-phenyl-3-butynoic acid. The compound is synthesized by reacting the *N*-methoxy-*N*-methylamide of monoethyloxalic acid with lithium phenylacetylide yielding ethyl 2-oxo-4-phenyl-3-butynoate (78% yield), followed by strictly pH-controlled hydrolysis to the free acid in nearly quantitative yield. The compound is shown to be a potent irreversible inhibitor of brewers' yeast pyruvate decarboxylase, in addition to producing both *cis*- and *trans*-cinnamic acids as products of turnover. The formation of these isomeric cinnamic acids can be rationalized if the thiamin diphosphate-bound α-carbanion/enamine intermediate resulting from decarboxylation is protonated at the side chain γ carbon to form two diastereomeric allenols, whose tautomerization and hydrolysis lead to the two products.

Introduction

It was demonstrated in this laboratory during the past few years that some pyruvic acid analogs that have ring-substituted styrenes in place of a methyl group, when exposed to the enzyme brewers' yeast pyruvate decarboxylase (PDC, EC 4.1.1.1), exhibit three characteristics of mechanistic interest: they are alternate substrates yielding products resulting from protonation at both the α (the expected *trans*-cinnamaldehydes) and γ (the product of "misprotonation" yielding dihydrocinnamic acids, see Scheme 1) positions of the intermediate α-carbanion/enamine; these intermediates can be observed by vis spectroscopy when enzyme-bound and are produced at rates consistent with enzymatic turnover; and these compounds (or, more likely their products upon decarboxylation) are effective covalent inhibitors of the enzyme.[1] In this paper we present synthesis and inhibitory action of 2-oxo-4-phenyl-3-butynoic acid, compound **1**, which carries a triple bond in place of the double bond found in our previous class of inhibitors. The synthetic methodology affords considerable improvement over previous methods used to synthesize 2-oxo-3-alkynoic acids, while the new inhibitor is one of the most potent ones that we have examined, whose turnover mechanism is readily explained by invoking the intermediacy of an allene-conjugated enamine/α-carbanion. Compound **1** is the first triple-bonded alternate substrate/inhibitor reported for thiamin diphosphate-dependent enzymes.

Scheme 1. Proposed Mechanism for the Reaction of Pyruvate Decarboxylase with Derivatives of (*E*)-4-Aryl-2-oxo-3-butenoic Acid



Results and Discussion

Numerous methods have been reported for the synthesis of a variety of 2-oxo acids.[2] Among those methods, direct coupling between phenylacetylene and derivatives of oxalic ester followed by hydrolysis were initially thought to be most attractive for our purposes, e.g., Scheme 2.[3] However, the limited availability of the required Grignard starting material, and the low yields reported using this methodology, discouraged us from applying such methods to our target. Instead, we devel-

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(2) For a review of the chemistry of 2-oxo acids, see: (a) Copper, A. J. L.; Ginos, J. Z.; Meister, A. *Chem. Rev.* **1983**, *83*, 321. (b) Brown, J. M. In *Comp. Org. Chem.*; Barton, D. H. R., Ollis, W. D., Eds.; Pergamon Press: Oxford, 1979; Vol. 2, p 799. Current progress on the synthesis of 2-oxo acids: (a) Barton, D. H. R.; Jaszberenyi, J. C.; Shinada, T. *Tetrahedron Lett.* **1993**, *34*, 7191. (b) Enders, D.; Dyker, H.; Raabe, G. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 421. (c) Boeyken, M.; Kimpe, N. D. *Synth. Commun.* **1992**, *22*, 3285. (d) Chen, C.; Crich, D. *J. Chem. Soc., Chem. Commun.* **1991**, 1289. (e) Ramage, R.; MacLeod, A. M.; Rose, G. W. *Tetrahedron* **1991**, *47*, 5625. (f) Dondoni, A.; Merino, P. *J. Org. Chem.* **1991**, *56*, 5294. (g) Blanchot, V.; Fetizon, M.; Hanna, I. *Synthesis* **1990**, 755. (h) Taipia, I.; Alcazar, N.; Moran, J. R.; Caballero, C.; Grande, M. *Chem. Lett.* **1990**, 697. (i) Pade, P. C. B. *Tetrahedron Lett.* **1986**, *27*, 1947. (j) Wasserman, H. H.; Ives, J. L. *J. Org. Chem.* **1985**, *50*, 3573.

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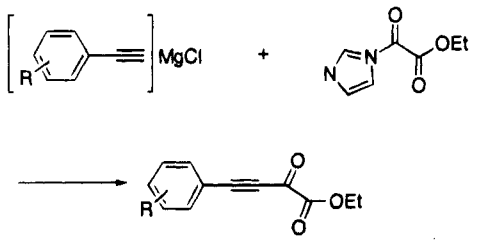
Scheme 2. Direct Coupling Reaction between an Alkynyl Grignard Reagent and an Oxalyl Electrophile



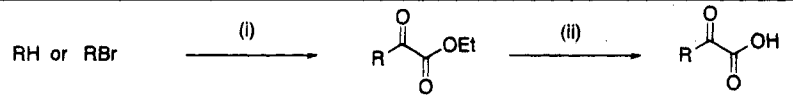
oped a modified procedure, replacing the Grignard reagent by a lithium reagent and using a new oxalic ester **2**, a derivative of the "Weinreb amide", as the electrophile.

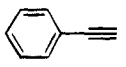
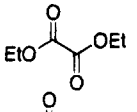
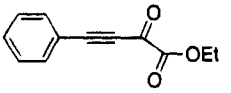
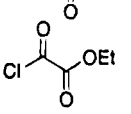
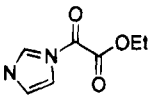
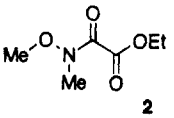
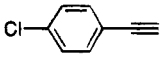
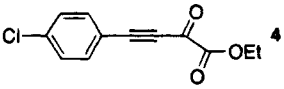
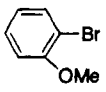
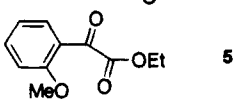
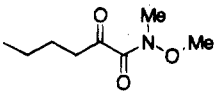
Results of the synthesis are summarized in Table 1. The electrophiles used in entries 1–4 were either commercially available or were synthesized according to literature procedures.[3] The 2-oxo esters were synthesized in very low yields using those electrophiles. The yield improved (37%) once the lithium salt was replaced by the cuprous salt (entry 4). Because of these unsatisfactory yields, a new electrophile **2** was proposed and synthesized. The literature procedure[4] was modified because of the solubility of **2** in water. The procedure increased the yield of **2** to 78%, with only a small quantity of a side product being formed. The low temperature (−78 °C) and 1 equiv of nucleophile used (not the usual conditions used for ketone synthesis when utilizing the Weinreb amide) were crucial for the success of the reaction.[5,6] This procedure is generally applicable to the lithium bases derived from acetylene and phenyl derivatives as exemplified by entries 6 and 7.

Interestingly, when the method was applied to an aliphatic base, such as *n*-BuLi, a 2-oxo amide resulted in 52% yield as the major product, with only 8% of the 2-oxo ester being produced. When 2 equiv of base was added, the α-diketone was the major product (32%).

The second step was unexpectedly troublesome, even though facile hydrolysis of the 2-oxo ester was reported in the literature on treatment with a mild base such as Et_3N. Prolonged standing in alkaline solution leads to decomposition of **1**. For example, when **3** was treated with pH 10 buffer, a total of three products was isolated, including **1** and phenylpropynoic acid. It was found that the hydrolysis was completed in a few minutes. This exceptionally fast hydrolytic rate may be attributed to a neighboring effect, perhaps via an α-lactone intermediate (since we could demonstrate that alkyl esters of pyruvic acid are also hydrolyzed at similarly fast rates). Such α-lactones have been proposed as intermediates in a

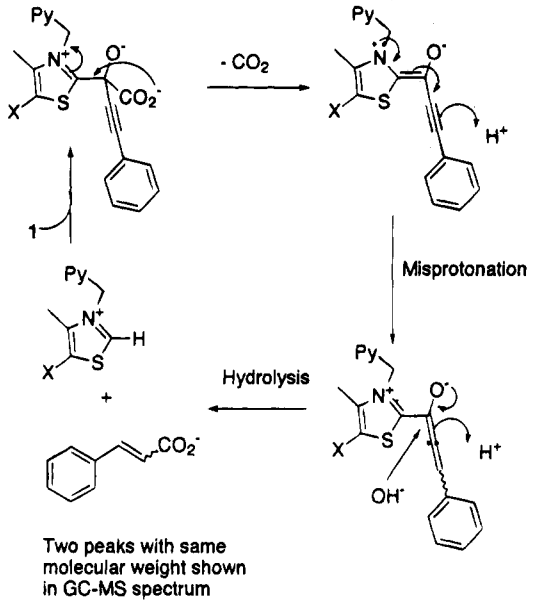
Table 1. Direct Coupling Reaction between Lithium Reagents and Derivatives of Oxalyl Electrophiles[a]



Entry	R or RBr	Electrophile	Catalyst	Product	Yield
1			No	 **3**	0
2			No	**3**	trace
3			No	**3**	10
4			CuCl	**3**	37
5		 **2**	No	**3**	78
6		**2**	No	 **4**	66
7		**2**	No	 **5**	73
8		**2**	No	 **6**	58

[a] Key: (i) *n*-BuLi, −78 °C, THF; **2**, −78 °C; (ii) THF, MeOH, H_2O (1:1:2), adding 0.1 M NaOH dropwise not exceeding pH 8; H_3O^+.

Scheme 3. Proposed Mechanism for the Reaction of Pyruvate Decarboxylase with 2-Oxo-4-phenyl-3-butynoic Acid (1)



number of reactions[7] and have also been detected under special conditions at very low temperatures.[8] In order to minimize side reactions, hydrolysis was conducted while closely controlling the pH. The pH of the solution was never allowed to exceed 8, so as to prevent side reactions. This strict pH control resulted in the successful synthesis of 2-oxo acids from the corresponding esters within a few minutes with quantitative yields. The success of this hydrolysis method enabled us to use oxalic ester derived from commercially available ethyl oxalyl chloride, rather than *tert*-butyl oxalyl chloride which has to be synthesized.

After recrystallization, **1** gave yellow crystalline needles, which slowly decomposed at 0 °C in the dark. There is a low-field proton magnetic resonance in the spectrum of the acid at δ 7.9 in $CDCl_3$ which can be attributed to COO*H* since its integral is diminished by addition of D_2O. This acid **1** or ester **3** after hydrogenation with Lindlar catalyst in a variety of solvents did not yield *cis* olefin product ($J \approx 12$ Hz) exclusively; rather, the mixture consisted mostly of the saturated aliphatic acid and *trans* acid or ester ($J = 16$ Hz). It was known in the literature that the reduction of triple bonds attached directly to carbonyl carbons gives unsatisfactory results.

Compound **1** was processed as a substrate by PDC. The major product of the enzyme-catalyzed reaction was shown to be cinnamic acid according to GC–MS and NMR. Significantly, we suspect that both *cis* and *trans* isomers of cinnamic acid are produced, since there are two GC–MS peaks with identical molecular weights corresponding to cinnamic acid. This finding suggests the mechanism of turnover shown in Scheme 3 and is totally analogous to the "misprotonation" leading to dihydrocinnamic acids from the double-bonded inhibitors

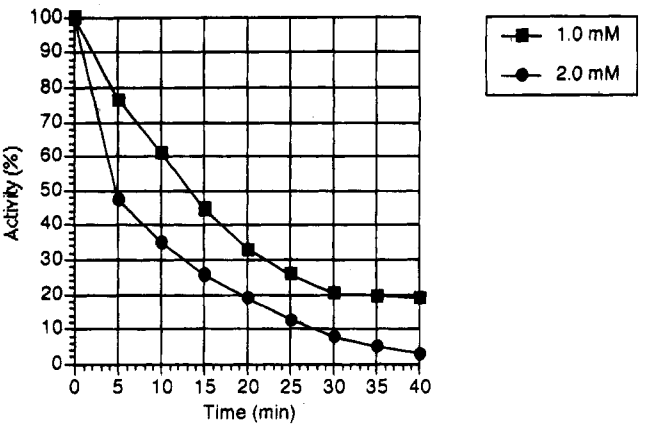


Figure 1. Time course of inactivation of PDC with compound **1**. See experimental details in the Experimental Section.

revealed by the work of Zeng.[1b,c] Such a rearrangement via an allene has precedent in other α-carbanions located between a carbonyl group and a triple bond.[9]

Acid **1** gave strong inhibition of PDC as shown on Figure 1. The enzyme activity decreased to less than 50% of its initial value in 5 min at a 2.0 mM concentration of **1**. In 1 h almost quantitative irreversible inhibition resulted. We believe that the inhibition very likely takes place at the regulatory Cys221 of the enzyme[10ab] by a Michael addition of the CysSH to either the 2-oxo acid starting material or to the cinnamic acid product.[11] The extent and the $t_{1/2}$ for inactivation achieved by 2 mM of **1** place this compound among our most potent inactivators of PDC found to date.[12]

Experimental Section

General. Solutions were concentrated in vacuo with a rotary evaporator, and organic layers were dried over $MgSO_4$.

Synthesis. Monoethyloxalic Acid-*N*-Methoxy-*N*-methylamide (2). To a solution of *N,O*-dimethylhydroxylamine hydrochloride (3.00 g, 30.9 mmol) and ethyl oxalyl chloride (1.2 equiv, 5.07 g, 37.1 mmol) in CH_2Cl_2 (60 mL) was added Et_3N (2.0 equiv, 6.21 g) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 0.5 h, and methanol (10 mL) was added to quench the reaction. The reaction mixture was concentrated to dryness, and THF (20 mL) was added to facilitate the precipitation. The white salt that formed was filtered and washed with THF (10 mL). The combined THF layer was concentrated to a yellow oil which was purified by vacuum distillation to furnish 4.08 g (82%) of **2** as a colorless liquid: bp 49–50 °C (0.05 mmHg); ^1H NMR ($CDCl_3$/TMS) δ 1.34 (t, 3, $J = 7$ Hz), 3.48 (s, 3), 3.65 (s, 3), 4.31 (q, 2, $J = 7$ Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 31.4, 62.1, 62.3, 162.2, 162.6.

Ethyl 2-Oxo-4-phenyl-3-butynoate (3). A solution of phenylacetylene (204 mg, 2.0 mmol) in THF (5 mL) at −78 °C was treated dropwise with *n*-BuLi (1.51 M, 1.33 mL) over 10 min. The mixture was stirred for 30 min and then was added to a solution of amide **2** (322 mg, 2.0 mmol) in THF (10 mL) that had been cooled to −78 °C via a cannula over 30 min. After 15 min, the reaction mixture was poured into a mixture of ice (10 g) and 20% H_3PO_4/ether (20 mL/40 mL). The aqueous layer was separated and extracted with ether. The combined ether layer was washed with 10% H_3PO_4 and water and then dried and concentrated. Flash chromatography (petroleum ether/ethyl acetate, 93:7) provided the 2-oxo ester (315 mg, 78%

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yield) as a yellow oil which solidified upon overnight storage in the refrigerator: bp 122–123 °C (0.05 mmHg) [lit.[13] 124–125 °C, (0.05 mmHg)]; ^{1}H NMR ($CDCl_3$/TMS) δ 7.31–7.61 (m, 5), 4.30 (q, 2, J = 7 Hz), 1.35 (t, 3, J = 7 Hz); IR (neat) 3400, 2201, 1745, 1675, 1250, 1090 cm^{-1}. Anal. Calcd for $C_{12}H_{10}O_3$: C, 71.28; H, 4.98. Found: C, 71.01; H, 5.04.

2-Oxo-4-phenyl-3-butynoic Acid (1). To the solution of ethyl 2-oxo-4-phenyl-3-butynoate (200 mg, 0.99 mmol) in a solvent system (10 mL) containing a 1:1:2 ratio of THF, methanol, and water cooled to 0 °C (pH = 3.0 on pH meter, pH = 7.0 on pH paper) was added aqueous NaOH (0.5 M) dropwise with stirring at room temperature, never exceeding pH 8 on the pH meter. The addition was stopped after the decrease of the pH reading became very slow (a little less than a stoichiometric amount of base had been added). The organic solvents were removed in vacuo, and the water layer was extracted with ether (5 mL). The water layer was titrated with concd H_2SO_4 to pH 1 and was extracted with ether (2 × 20 mL). The combined organic layer was dried and concentrated to a crude product which was purified by recrystallization from toluene to afford 159 mg of **1** (95%) as yellow needles: mp 80–81 °C; ^{1}H NMR ($CDCl_3$/TMS) δ 7.31–7.61 (m, 5); ^{13}C NMR ($CDCl_3$) δ 86.6, 102.0, 118.8, 129.5, 132.9, 134.4, 159.6, 170.2; IR (neat) 3400, 2201, 1745, 1675,1250, 1090 cm^{-1}. Anal. Calcd for $C_{10}H_6O_3$: C, 68.97; H, 3.47. Found: C, 68.74; H, 3.47.

Ethyl 4-(*p*-chlorophenyl)-2-oxo-3-butynoate (4) was synthesized using the same methods as for **3**, in 66% yield as a brown solid: mp 70–71 °C; ^{1}H NMR ($CDCl_3$/TMS) δ 7.53 (d, 2, J = 8 Hz), 7.34 (d, 2, J = 8 Hz), 4.35 (q, 2, J = 7 Hz), 1.35 (t, 3, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 14.2, 63.7, 88.1, 96.5, 117.8, 129.6, 135.2, 138.8, 159.2, 179.7; MS 236 (M, 1), 163 (100).

Isolation and Characterization of the Product of the Reaction of 1 with Pyruvate Decarboxylase. To 30 mL of 0.1 M citrate, pH 6.0, containing 0.1 mM each of thiamin diphosphate and $MgCl_2$ at 25 °C were added 3 mmol of **1** and 2000 units of PDC. After 3 h of reaction, 12 g of ammonium sulfate was added to precipitate the PDC, and then the solution was centrifuged at 12 000 rpm for 30 min. Next, the supernatant was acidified to pH 1–2 by the addition of concd H_2SO_4. The solution was centrifuged at 12 000 rpm for 30 min, and the supernatant was extracted with ether (2 × 30 mL). The combined ether layer was dried and then concentrated to 1 mL which was applied to the GC–MS to give three major peaks. Two of them are derived from cinnamic acid: MS (*m*/*z*, rel intensity) 149 (M + 1, 5), 148 (74), 147 (100), 131 (18), 103 (42), 91 (24), 77 (30).

Time-Dependent Inactivation Kinetics. In a typical experiment 30 units of holo-PDC was incubated with variable amounts of compound **1** in 0.1 M citrate, pH 6.0 also containing 1 mM each of thiamin diphosphate and $MgCl_2$ at 25 °C in a total volume of 3 mL. At the indicated times aliquots were removed and the activity was determined. Details of the enzyme assay and the enzyme purification were reported elsewhere.[14]

Acknowledgment. The authors are grateful for grants from NSF-DMB-87-0958, NIH-GM-50380, and the Rutgers University Busch Biomedical Fund for financial support.

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