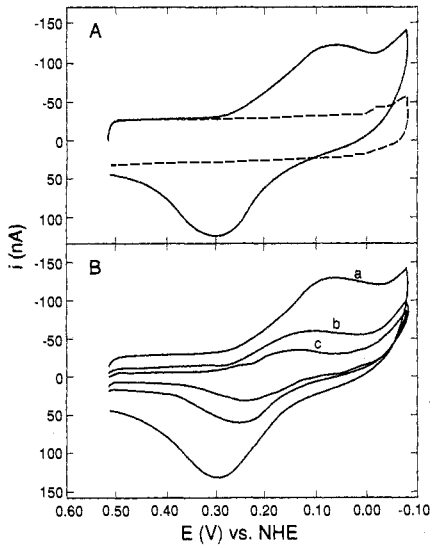

Figure 1. (A) Cyclic voltammograms acquired at a scan rate of 50 mV/s of an MHDA monolayer covered Au electrode (dashed) and the same electrode after adsorption of cyt *c* on the MHDA monolayer (solid). In both cases, the supporting electrolyte is 4 mM potassium phosphate buffered at pH 7. See text for details of cyt *c* adsorption procedure. (B) Cyclic voltammograms of cyt *c* adsorbed on an MHDA monolayer at scan rates of (a) 50, (b) 20, and (c) 10 mV/s. Solution conditions are the same as in part A. Electrode area = 0.32 cm^2.

ET reaction of adsorbed cyt *c*. Through synthetic manipulation, we expect that the self-assembled alkanethiol monolayer system will be very useful for controlling protein/monolayer surface interactions as well as the nonadiabaticity of protein ET reactions. Moreover, the monolayers may serve as useful analogues of biological membranes as suggested by the redox thermodynamic data given above.

Acknowledgment. We gratefully acknowledge Steve Choquette for synthesizing the MHDA, Maryanne Collinson for purification of cyt *c*, William Yap for use of his potentiostat, and Holden Thorp, Jim Willit, and Marc Porter for useful discussions. Support for this work by the National Science Foundation through CHE-8820832 (E.F.B.) is gratefully acknowledged.

Biosynthesis of Coronatine: Investigations of the Biosynthesis of Coronamic Acid

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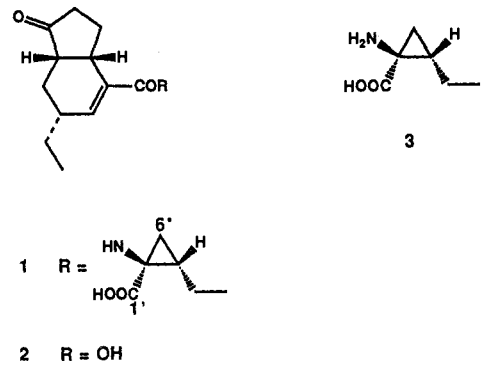
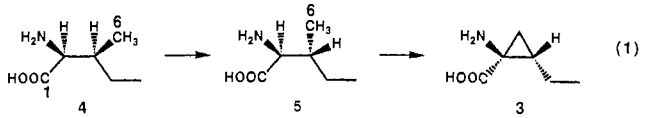
Received November 20, 1990

Coronatine (**1**) is a phytotoxin of novel structure isolated from liquid cultures of the plant pathogens *Pseudomonas syringae* pv. *atropurpurea*[1,3] and *Ps. syringae* pv. *glycinea*.[2,3] Infection of the host plants by these bacteria induces chlorosis on the leaves due to the production of coronatine.[1,2] The coronatine molecule is constructed from two moieties of distinct biosynthetic origin, coronafacic acid (**2**) and coronamic acid (**3**). Previous investigations in our laboratory have shown that coronafacic acid (**2**) is a polyketide derived from five acetate units and one pyruvate unit and that coronamic acid (**3**) is derived from isoleucine.[4] We now report the results of investigations that provide some insight into the mechanism of conversion of isoleucine into coronamic acid.



In previous studies, a mixture of (1-^{13}C)-DL-isoleucine and (1-^{13}C)-DL-alloisoleucine was found to label coronatine specifically at C-1′. This experiment provided no information on the stereochemistry of the amino acids that serve as precursors of coronamate. The absolute configuration of coronamic acid[5] suggests that the compound should be derived from L-isoleucine (**4**) via L-alloisoleucine (**5**) (eq 1). This hypothesis was evaluated by



(1)

administration of commercially available (1-^{13}C)-L-isoleucine and (1-^{13}C)-L-alloisoleucine to *Ps. syringae* pv. *glycinea* and isolation of coronatine as its methyl ester. The results (Table I, experiments 1 and 2) show that both of these amino acids are specifically incorporated into coronamic acid, but that L-alloisoleucine is a much more efficient precursor. Since the cyclization of the amino acid L-alloisoleucine to a cyclopropane derivative is a highly unusual process, we decided to verify that the methyl group of the amino acid is indeed the source of the cyclopropane bridge. This was accomplished by administration of a mixture of (6-^{13}C)-DL-isoleucine and (6-^{13}C)-DL-alloisoleucine[6] to *Ps. syringae*. The results (Table I, experiment 3) clearly show that the cyclopropane bridge is derived from the methyl group of L-alloisoleucine. These observations set the stage for a mechanistic probe of the cyclization mechanism. A mixture of (6-^{13}C,6-^{2}H$_3$)-DL-isoleucine and (6-^{13}C,6-^{2}H$_3$)-DL-alloisoleucine was synthesized[7] and administered to *Ps. syringae*. Examination of the carbon-13 NMR spectrum of the coronatine methyl ester obtained from this fermentation while carrying out simultaneous ^{1}H and ^{2}H decoupling revealed the presence of a new ^{13}C resonance shifted upfield by 0.59 ppm relative to the signal for C-6′ of coronatine. The magnitude of the upfield shift indicates that two deuterium atoms are located at C-6′.[8] The conversion of L-alloisoleucine to coronamic acid therefore proceeds with the removal of one hydrogen atom from C-6 of the precursor. It is also noteworthy that the level of incorporation observed in this experiment (Table I, experiment 4) was dramatically lower than that observed when no deuterium was present at C-6 (Table I, experiment 3). This difference in incorporation efficiency is probably due to the operation of a substantial deuterium isotope effect.

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(6) The precursor was synthesized by deprotonation of *n*-butyl butyrate with LDA followed by treatment with (^{13}C)methyl iodide, reduction of the alkylated ester to the corresponding aldehyde with DIBAL, and conversion of the labeled aldehyde to the amino acid using the Strecker synthesis.
(7) The synthesis was accomplished as indicated in ref 6, using (^{13}C,^{2}H$_3$)methyl iodide.
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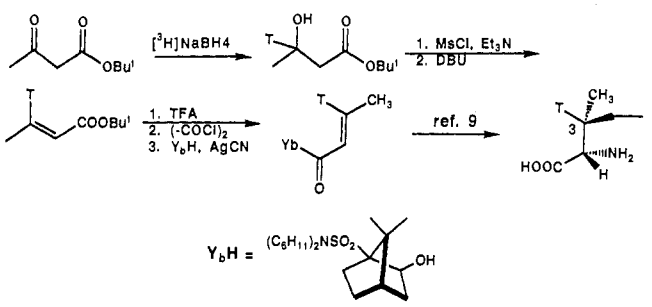
0002-7863/91/1513-1849$02.50/0

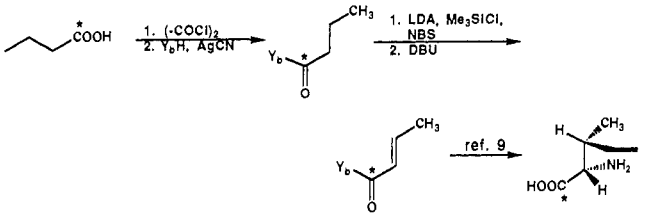
Table I. Incorporation of Labeled Precursors into Coronatine Methyl Ester

expt	precursor ($^3H/^{14}C$)	% enrichmnt or incorporatn	labeling pattern
1	(1-^{13}C)-L-isoleucine	9	C-1′
2	(1-^{13}C)-L-alloisoleucine	60	C-1′
3	(6-^{13}C)-DL-isoleucine + (6-^{13}C)-DL-alloisoleucine	31	C-6′
4	(6-^{13}C,6-$^{2}H_3$)-DL-isoleucine + (6-^{13}C,6-$^{2}H_3$)-DL-alloisoleucine	0.5	^{13}C and 2 D at C-6′
5	[1-^{14}C,3-^{3}H]-L-alloisoleucine (3.89)	1.8	$^3H/^{14}C$ = 3.67 (94% ^{3}H retention)

Scheme I



Scheme II



The loss of only one hydrogen atom from C-6 of L-alloisoleucine during coronamate formation rules out the intermediacy of species with a C-6 oxidation level higher than that of an alcohol. Putative intermediates with the appropriate oxidation level would therefore include 3-methylene-L-norvaline and 6-hydroxy-L-alloisoleucine. The possible intermediacy of the former amino acid was evaluated by means of a precursor incorporation experiment with [1-^{14}C,3-^{3}H]-L-alloisoleucine. [3-^{3}H]-L-Alloisoleucine was synthesized as shown in Scheme I, while [1-^{14}C]-L-alloisoleucine was prepared as outlined in Scheme II. Administration of a mixture of these two amino acids to *Ps. syringae* gave doubly labeled coronatine methyl ester, which was purified chromatographically and then reduced to the corresponding alcohol with lithium tri-*tert*-butoxyaluminum hydride. The alcohol was further chromatographed to remove any lingering radiochemical impurities. The results of this experiment (Table I, experiment 5) demonstrate that L-alloisoleucine is converted to coronamic acid without loss of the hydrogen atom present at C-3. 3-Methylene-L-norvaline is thereby ruled out as an intermediate. The potential intermediacy of the 6-hydroxy-L-alloisoleucine was examined by administration of a mixture of (1-^{13}C)-6-hydroxy-DL-isoleucine and (1-^{13}C)-6-hydroxy-DL-alloisoleucine to *Ps. syringae*.[10] No incorporation into coronatine methyl ester was observed.

The findings just described limit the number of mechanisms that can be envisioned for the conversion of L-alloisoleucine to coronamic acid. At the present time it is tempting to conclude that the mechanism may represent a diversion of an enzymatic hydroxylation reaction into an oxidative cyclization. Such processes have recently been uncovered in the biosynthesis of clavaminic acid,[11] isopenicillin N,[12] and deacetoxycephalosporin C.[12] The mechanism of coronamic acid biosynthesis may also be related to the sulfur insertion reactions involved in biotin and lipoic acid biosynthesis.[13]

Acknowledgment. We thank the National Institutes of Health (GM 26569) and The Robert A. Welch Foundation (C-729) for financial support and Dr. Alan M. Kook for the ^{1}H, ^{2}H decoupling experiment.

Registry No. 1, 62251-96-1; **3**, 63393-56-6; **5**, 1509-34-8.

(10) The synthesis of these compounds will be described elsewhere.

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Intramolecular Enyne Metathesis Reaction. Route to Bridged Bicycles with Bridgehead Olefins

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Received October 26, 1990

Ever since the discovery of the remarkable olefin metathesis, this fundamentally new process has generated great excitement as to its applicability to other unsaturated substrates and its mechanism.[1] While it has been extended to alkynes,[2] to our knowledge, no examples of a crossed metathesis involving an olefin with an acetylene have been recorded. Further, while early mechanistic speculation centered on the possible involvement of four-membered carbocyclic rings, such interpretations were not supported by subsequent studies.[1] We report the development of a catalyst that converts a mechanistic curiosity into a preparatively useful crossed enyne metathesis that likely proceeds via a four-membered carbocyclic intermediate.[3] This new method provides a very simple route to bridged bicycles possessing bridgehead olefins, interesting molecular structures that also may be useful building blocks for the synthesis of natural products (cf. taxanes,[4] shikodomedin,[5] etc.).

The reaction of enyne **1a** with a catalyst derived from 2,3,4,5-tetrakis(methoxycarbonyl)palladacyclopentadiene (TCPC, **4**, R = CH_3) gave a 2.8:1 ratio of the cyclorearrangement product **2a** to the "Alder ene type" product **3a** in 68% yield (eq 1).[3,6]

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(6) Reinvestigation of this reaction revealed the presence of the Alder ene type product **3** in addition to the cyclorearrangement product **2a**.

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