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Supplementary Material Available: Synthesis and spectral data for **1**, **3**, and **3a** (1 page). Ordering information is given on any current masthead page.

Biosynthesis of Coronatine, a Novel Polyketide

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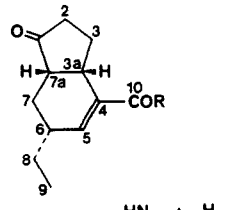
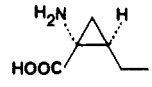
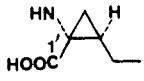
Coronatine (**1**) is a novel phytotoxin isolated from liquid cultures of the plant pathogens *Pseudomonas syringae* pv. *atropurpurea*,[1,3] which infects Italian ryegrass, and *Ps. syringae* pv. *glycinea*,[2,3] which infects soybean plants. Infection of the host plants by these bacteria induces chlorosis on the leaves due to the production of coronatine.[1,2] The importance of coronatine and its unique structure have prompted us to carry out the biosynthetic investigations reported here.

The structure of coronatine (**1**) poses two major biosynthetic problems. One concerns the formation of coronafacic acid (**2**),



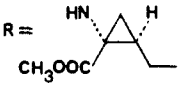


3

1 R = 

2 R = OH

4 R = OCH_3

5 R = 

while the other involves the formation of coronamic acid (**3**). We have examined both of these problems by administration of labeled precursors to liquid cultures of *Ps. syringae* pv. *glycinea* PDDCC 4182.[3] Investigations of coronafacic acid **2** were conducted by isolation of **2** from the culture broth as its methyl ester (**4**) after treatment with diazomethane. The complete assignment of the ^{13}C NMR spectrum of **4** (Table I) was accomplished by using several techniques. Treatment of **4** with D_2O/DCl led to the disappearance of the ^{13}C NMR signal of C-2 from the noise-decoupled spectrum. Reduction of **4** with lithium tri-*tert*-butoxyaluminum hydride yielded a single alcohol,[4] in which the ^{13}C NMR signals for C-2 and C-7a were shifted upfield to 31.05 and 42.28 ppm, respectively.

Biosynthetic studies also proved an invaluable aid to assignment. The structure of coronafacic acid suggested that the molecule might be a polyketide. Accordingly, sodium (1-^{13}C)- and (2-^{13}C)acetate were administered to *Ps. syringae* cultures. The results of these experiments (Table II, experiments 1, 2) clearly indicated that five molecules of acetate are incorporated into **4**. The positions of the labels and their connectivities were unequivocally

Table I. Carbon-13 Chemical Shifts for Methyl Coronafacate (**4**)

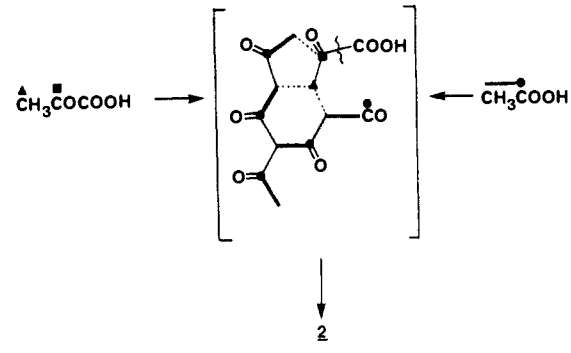
carbon atom	δ^a	carbon atom	δ^a
1	220.5	7	25.8
2	38.1	7a	46.6
3	28.1	8	27.8
3a	36.2	9	11.2
4	131.3	10	167.3
5	144.2	11	51.7
6	37.7		

[a] Shifts were measured in deuteriochloroform at 75.47 MHz.

Table II. Administration of Labeled Precursors to *Ps. syringae*

expt	precursor	compd isolated	labeling pattern
1	sodium (1-^{13}C)acetate	**4**	C-1, C-5, C-7, C-8, C-10
2	sodium (2-^{13}C)acetate	**4**	C-2, C-4, C-6, C-7a, C-9
3	sodium (1,2-$^{13}C_2$)acetate	**4**	connectivities between C-1, C-2; C-4, C-10; C-5, C-6; C-7, C-7a; C-8, C-9
4	sodium (1-^{13}C)butyrate	**4**	no enrichment
5	(1-^{13}C)glycine	**4**	no enrichment
6	(2-^{13}C)glycine	**4**	no enrichment
7	(1,3-$^{13}C_2$)glycerol	**4**	all carbons except OCH_3 enriched
8	sodium (1-^{13}C)pyruvate	**4**	no enrichment
9	sodium (2-^{13}C)pyruvate	**4**	C-3; C-1, C-5, C-7 C-8, C-10
10	sodium (3-^{13}C)pyruvate	**4**	C-3a; C-2, C-4, C-6, C-7a, C-9
11	sodium (2,3-$^{13}C_2$)pyruvate	**4**	connectivities between C-3, C-3a; C-1, C-2; C-4, C-10; C-5, C-6; C-7, C-7a; C-8, C-9
12	(1-^{13}C)-DL-isoleucine plus (1-^{13}C)-DL-alloisoleucine	**5**	C-1′

Scheme I



established by administration of sodium (1,2-$^{13}C_2$)acetate followed by analysis of the resulting ester by using a COSYX (^{13}C COSY) experiment (Table II, experiment 3).[5,6] The presence of an ethyl group in **2** suggested that butyrate might also be a specific precusor. However, administration of (1-^{13}C)butyrate yielded unlabeled ester (experiment 4).

The incorporation experiments with labeled acetate demonstrated that two carbon atoms of coronafacic acid, C-3 and C-3a, are *not* acetate derived. It therefore appeared that **2** is a polyketide with a novel starter unit. A number of compounds were evaluated as potential starter units. These included glycine (experiments 5, 6) and glycerol (experiment 7). However, neither of these substances were specific precursors. Pyruvic acid was then evaluated. Administration of pyruvate labeled with ^{13}C at C-1, C-2, and C-3 revealed the surprising fact that C-3 and C-3a of coronafacate are derived from C-2 and C-3 of pyruvate, respectively (experiments 8–10). In these experiments, the remaining

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(2) Mitchell, R. E.; Young, H. *Phytochemistry* **1978**, *17*, 2028.
(3) Mitchell, R. E. *Physiol Plant Pathol.* **1982**, *20*, 83.
(4) The hydroxy group in the reduction product is presumed to be trans to the ethyl group due to approach of the reagent from the less-hindered side.
(5) Aue, W. P.; Bartholdt, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229. Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542.
(6) Spectra were taken in deuteriochloroform on an IBM AF300 spectrometer.

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carbon atoms of **4** exhibited a labeling pattern consistent with partial degradation of pyruvate to acetate. The specific incorporation of C-2 and C-3 of pyruvate into **4** was confirmed by administration of (2,3-$^{13}C_2$)pyruvate and analysis of the labeled **4** by a COSYX experiment (experiment 11). The COSYX revealed connectivity between C-3 and C-3a as well as the connectivities expected from degradation of the precursor to doubly labeled acetate. Coronafacic acid therefore appears to be biosynthesized from a branched polyketide with pyruvate serving as the starter unit for one of the polyketide chains (Scheme I). The mechanism for formation of the C–C single bonds between C-3 of pyruvate and C-2 of two acetate units is presently obscure.

Preliminary investigations of coronamic acid (**3**) biosynthesis have also been carried out. The resemblance between **3** and isoleucine suggested a possible biosynthetic relationship. Mitchell has recently reported[7] that administration of [U-^{14}C]-L-isoleucine to *Ps. syringae* pv. *atropurpurea* yielded radioactive coronatine that carried most of the radioactivity in the coronamic acid moiety. We have obtained more rigorous evidence for the role of isoleucine as a precursor by administration of a mixture of (1-^{13}C)-DL-isoleucine and (1-^{13}C)-DL-alloisoleucine[8] to *Ps. syringae*. The ^{13}C NMR spectrum of the coronatine methyl ester (**5**) obtained from this experiment displayed a high level of enrichment at C-1′ (171.4 ppm) (experiment 12). Elucidation of the mechanism of the novel cyclization of isoleucine to **3** will require further study.

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(8) The labeled amino acids were prepared from 2-methylbutanal and (^{13}C)cyanide by the Strecker synthesis.

Photochemistry of Pd–Pd σ-Bonds: Electron-Transfer Reactions of Photogenerated $Pd(CNMe)_3^{\bullet+}$ Radicals

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We report the first direct observation of electron transfer from a photogenerated organometallic radical of the platinum group. The photochemistry of metal–metal σ-bonds has been an area of intense interest.[1] Photochemical metal–metal bond homolysis,[3,4,6,9] disproportionation,[7] metal–ligand bond dissociation,[3-5] and atom-transfer[3,8,9] reactions are now all familiar. It has been recognized that photogenerated $^{\bullet}ML_n$ radicals are both potentially stronger oxidants and reductants than their parent ground-state, metal–metal bonded L_nM–ML_n complexes.[2] Curiously, however, there have been few reports of electron-transfer reactivity of photogenerated organometallic radicals.[1,2]

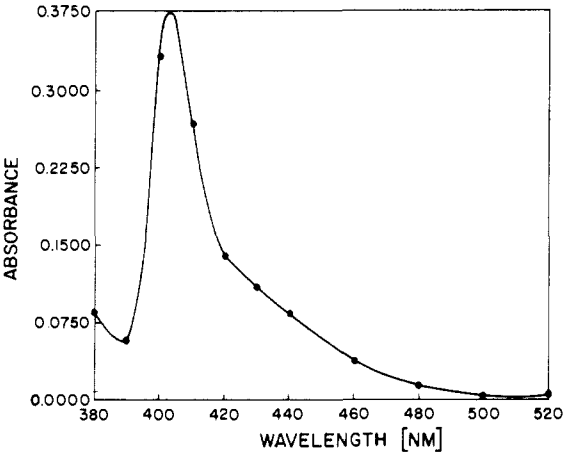
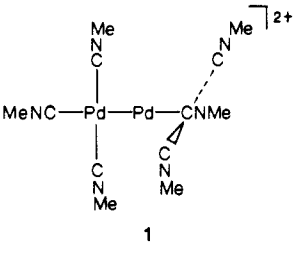


Figure 1. Transient absorbance spectrum of photogenerated $Pd(CNMe)_3^+$ radicals obtained by measuring ΔA as a function of $\lambda_{monitor}$. Each data point represents ΔA for a single flash on a fresh solution sample.[10b]

The Pd–Pd σ-bonded complex $[Pd_2(CNMe)_6][PF_6]_2$ (**1**) previously was shown to exhibit photochemistry characteristic of Pd–Pd bond homolysis.[9] We therefore sought to identify the properties and chemical reactivity of the transients formed upon σ–σ^* excitation of **1**. Flash photolysis of acetonitrile solutions of **1** was found to give rise to an intense transient absorbance with $\lambda_{max} \sim 405$ nm.[10] In contrast, 10^{-4} M acetonitrile solutions of **1** exhibit no significant absorbance in the 380–520-nm region.[9] The transient absorbance spectrum is shown in Figure 1.[10b] The observed transient decayed by a second-order process, assigned to recombination of photogenerated $Pd(CNMe)_3^+$ radicals, eq 1, 2. The rate expression for second order recombination, $2k_r/\epsilon_{405}$,



1

$$[Pd_2(CNMe)_6]^{2+} \xrightarrow{h\nu} 2Pd(CNMe)_3^{\bullet+} \quad (1)$$
1

$$2Pd(CNMe)_3^{\bullet+} \xrightarrow{k_r} [Pd_2(CNMe)_6]^{2+} \quad (2)$$
1

is 4×10^4 cm s^{-1} by flash photolysis. We have determined the extinction coefficient, ϵ_{405}, and with it the rate of recombination, k_r, of photogenerated $Pd(CNMe)_3^+$ radicals by examining their quantitative reduction of benzylviologen (BV^{2+}) to $BV^{\bullet+}$, eq 3 (vide infra). The extinction coefficient, ϵ_{603}, of $BV^{\bullet+}$ has been determined by several workers to be 14 000 M^{-1} cm^{-1} in acetonitrile.[11] The disappearing $Pd(CNMe)_3^+$ radical therefore is characterized

$$Pd^{I}(CNMe)_3^{\bullet+} + BV^{2+} \xrightarrow[\text{solvent}]{k_e} Pd^{II}(CNMe)_3(solvent)^{2+} + BV^{\bullet+} \quad (3)$$

$\epsilon_{405}(Pd^+) = 50\,000 M^{-1}$ cm^{-1} $\quad \epsilon_{603}(BV^+) = 14\,000$ M^{-1} cm^{-1}

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