Synthesis and Structure−Phytotoxicity Relationships of Acetylenic Phenols and Chromene Metabolites, and Their Analogues, from the Grapevine Pathogen *Eutypa lata*

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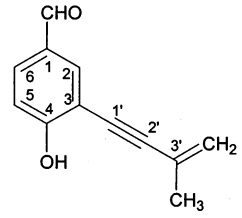
Eutypa lata, the fungus responsible for dying-arm disease in grapevines, produces a number of structurally related secondary metabolites, of which eutypine (**1**) has been implicated as the principal phytotoxin. However, analysis of an *E. lata* strain from California known to be pathogenic to grapevines showed that eutypine was not present, suggesting that other metabolites could be phytotoxic. Investigation of the relative phytotoxicities of individual metabolites has been limited by insufficient material and lack of a reliable bioassay. Metabolites of particular interest and their precursors were therefore synthesized, and a rapid, quantitative bioassay via topical application of individual compounds to disks of grape leaves and measurement of chlorophyll loss was developed to provide a relative measure of tissue damage. The recently reported metabolite eulatachromene (**2**) was found to have phytotoxicity greater than that of eutypine (**1**). The cyclization product, 5-formyl-2-methylvinyl[1]benzofuran (**3**), also showed significant activity, whereas the reduction product, eutypinol (**4**), was inactive, as was the quinol, siccayne (**5**). These results indicate that before strains of *Eutypa* are incriminated as pathogenic they must be analyzed for the presence or absence of specific constituents for which the phytotoxicity has been unequivocally established.

Eutyposis, or "dying-arm disease", is a progressive fungal disease that has resulted in worldwide economic losses in the grape industry and also infects numerous types of fruit trees.[1−4] In California alone, losses of $260 million per annum have been estimated as a consequence of reduced yields, shortened life-span of the vines, and management costs.[5] The causative agent, the ascomycete *Eutypa lata*, produces necrosis in the wood close to the point of infection, stunting of new shoots, and small, deformed, chlorotic leaves. The ultimate result is dieback of cordons and reduced fruit production. Eutypine (**1**), a phenolic aldehyde possessing an unusual five-carbon acetylenic side chain, was isolated and identified by Renaud et al.[6,7] from fungal cultures of an unspecified strain of *E. lata*, together with structurally related metabolites and reported to be the principal phytotoxin on the basis of assays performed on excised leaves and leaf protoplasts.[8]

A comparative study in our laboratory[9] of metabolites produced by two strains of *E. lata* obtained from grapevines in California and Italy, respectively, showed that **1** was not the primary constituent and led to the isolation and identification of eulatachromene [6-hydroxymethyl-2,2-dimethyl-2*H*-chromene] (**2**) from the Italian *Eutypa* culture. In addition, **1** was found to be readily cyclized to the benzofuran (**3**) in the presence of traces of acid, and the corresponding alcohol, eutypinol (**4**), was the major metabolite produced under most culture conditions. The quinol, siccayne (**5**), initially isolated from *Helminthosporium siccans*,[10] was also present in moderate amounts in some strains of *E. lata*.[9] These results suggested that the phytotoxicity of *E. lata* might be due to a suite of structurally related compounds each exhibiting a different degree of activity, rather than **1** alone, and that **2**, **3**, and **5** could also contribute to phytotoxicity. To examine this possibility, the metabolites were synthesized in sufficient quantity for evaluation, together with the chromene analogues (**6**−**8**), and a quantitative bioassay for phytotoxicity to grape leaves was developed.



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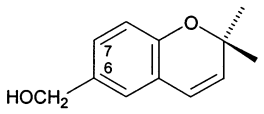

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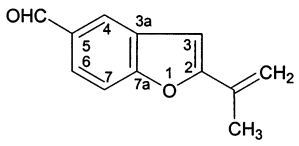

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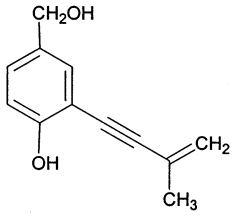

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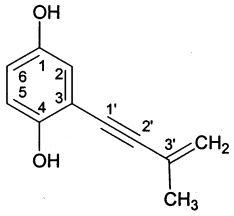

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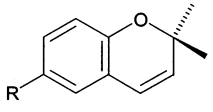

6: R = -CO_2CH_3

7: R = -CHO

8: R = -CO_2H

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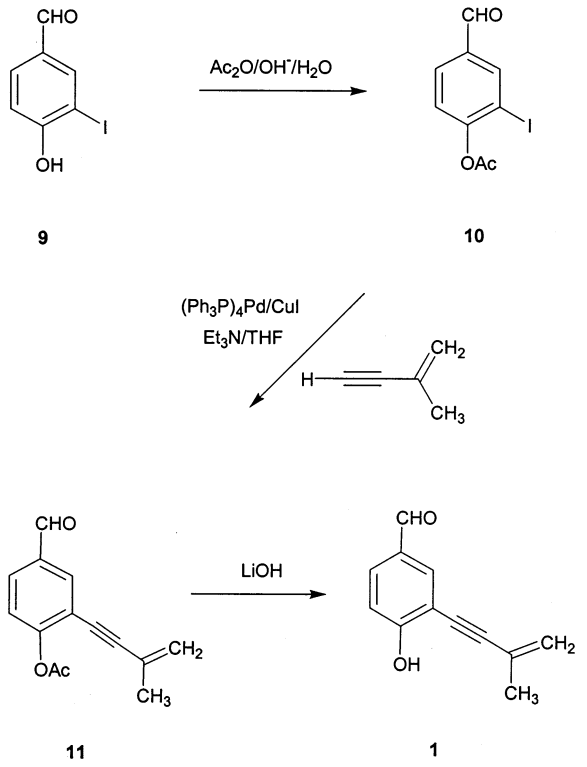

Figure 1. Synthetic route to eutypine (**1**).

Results and Discussion

A number of syntheses of eutypine (**1**) and certain of its congeners have been developed.[11−13] However, **1** was most conveniently prepared in satisfactory yield by a modification, summarized in Figure 1, of the method of Bates et al.[12] 4-Hydroxy-3-iodobenzaldehyde (**9**) was prepared by the method of Defranq et al.,[11] then treated with acetic anhydride and aqueous sodium hydroxide to give the acetate (**10**).[14] This was more convenient and gave better yields than preparation by formylation of 2,4-diiodoacetoxybenzene, which gave rise to a mixture of regioisomers.[12] The iodoaldehyde acetate (**10**) was coupled with 2-methyl-1-buten-3-yne in the presence of cuprous iodide and tetrakis(triphenylphosphine)palladium(0), in triethylamine/THF, to give **11**,[12,15] which was hydrolyzed by lithium hydroxide in aqueous THF[12] to give **1**, identical to the natural product in all respects, including GC−MS retention time and fragmentation pattern.[9] HPLC cleanup of compound **11** was preferable, and HPLC purification of **1** was essential to prevent its cyclization to **3**, which occurred rapidly if impurities were not removed. This was in contrast to the report[12] that none of the cyclization product was detectable on hydrolysis with LiOH, whereas the only isolable product was the benzofuran under other mildly basic conditions. Although **1** obtained after HPLC cleanup showed no traces of impurity by spectroscopic techniques and GC−MS, the melting point varied slightly from batch to batch, and similar discrepancies were noted in the literature values.[11,12] It is possible that partial thermal cyclization to the benzofuran occurs during the melting point determination. The benzofuran (**3**) was obtained in sufficient quantity for bioassays as a byproduct of the eutypine HPLC purification and fully characterized by spectroscopic methods, since earlier reports of its occurrence in fungal filtrates[7] and formation by cyclization of eutypine[6,12] had not provided details of its physical properties or structural elucidation.

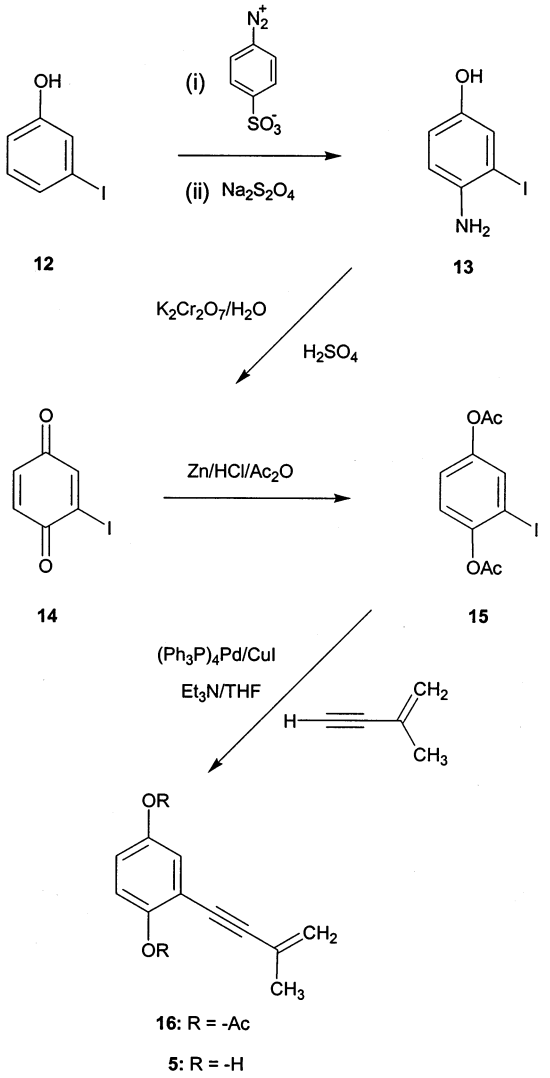

Figure 2. Synthetic route to siccayne (**5**).

The conversion of eutypine (**1**) into siccayne (**5**) by two five-step variants, with overall yields from 4-hydroxybenzaldeyde of 26% and 10%, respectively, has been reported by Defranq et al.[11] This appeared to be an inappropriate approach because it involved **1**, with its attendant lability, as an intermediate. Siccayne was therefore prepared by modification of the general approach of Pinault et al., reported as a six-step synthesis with an overall yield of 8.8%.[16] The route reported here, summarized in Figure 2, involved only five steps from the commercially available 3-iodophenol and approximately doubled the overall yield to 16.7%. 3-Iodophenol (**12**) was subjected to diazo coupling and reduced to the aminophenol (**13**), which was oxidized to the iodoquinone (**14**).[17] Reductive acetylation[18] gave the quinol diacetate (**15**), which was treated with 2-methyl-1-buten-3-yne in the presence of tetrakis(triphenylphosphine)palladium and copper(I) iodide to yield siccayne diacetate (**16**). Deacetylation to siccayne (**5**) was achieved by base hydrolysis with lithium hydroxide. The basic conditions gave a dark colored product which required purification by HPLC prior to crystallization. Subsequently, acidic hydrolysis conditions were found to give relatively little discoloration of the crude product, but purification was still necessary. Although both hydrolysis methods gave comparable yields, hydrolysis with acid appears to be preferable because residues bound to the HPLC column are

less, and more easily removed, than with the dark colored basic hydrolysate.

Eulatachromene (**2**) was isolated from an Italian culture of *Eutypa lata*, and its structure established as 2,2-dimethyl-6-hydroxymethylchromene by homo- and heteronuclear shift correlation NMR experiments.[9] Desert plants of the *Encelia* genus (Asteraceae) have been reported to produce a compound of this structure, but the ^{1}H NMR data[19] showed significantly different shifts from those that we observed for **2**. In particular, the resonances observed for the two aromatic ring protons adjacent to the hydroxymethyl group were approximately 0.5 ppm upfield relative to the literature values. It therefore appeared probable that the structure originally reported[19] for the *Encelia* chromene was incorrect and the regioisomeric 7-hydroxymethyl structure was more likely for this compound, since the structure of eulatachromene (**2**) was rigorously established by correlation experiments. The location of the hydroxymethyl group *para* to the chromene ring oxygen functionality in **2** was also consistent with the structures of other fungal metabolites occurring in *E. lata*, with **2** being an analogue of **4** in which the pentenyne chain is reduced and cyclized onto the phenolic group. Furthermore, the corresponding 6-hydroxymethylchromanone has recently been isolated from submerged cultures of a *Stereum* species,[20] and a glycoside of 6-hydroxy-2,2-dimethylchromene has been isolated from *S. hirsutum*,[21] a fungus associated with "esca", a trunk disease of older grapevines. Nevertheless, unequivocal confirmation of the structure required its synthesis, which would also provide sufficient material for evaluation of phytotoxicity.

The synthesis of 2,2-dimethyl-6-hydroxymethylchromene (**2**) as an intermediate in the preparation of chroman derivatives evaluated for their effects on platelet aggregation has been reported,[22] but details of the method of preparation and the physical properties of the compound were not described, and no spectroscopic data were provided in support of this structure. The compound was therefore synthesized *de novo*, based on methods used for other chromene derivatives, as shown in Figure 3. Alkylation of methyl 4-hydroxybenzoate (**17**) with 3-chloro-3-methyl-1-butyne[23] smoothly gave propynyloxy benzoate (**18**), which underwent rearrangement in refluxing diethylaniline[24,25] to give the 6-carbomethoxychromene (**6**) in excellent yield and purity. This compound has been previously reported as a constituent of the bark of *Piper hostmannianum*, and the spectroscopic properties were consistent with this structure,[26] although the natural product was reported as an oil whereas the synthetic compound was obtained as a low-melting solid. Finally, a routine LAH reduction gave **2** in 72% overall yield. Synthetic **2** was identical in all respects, including GC−MS and HPLC retention times, UV spectroscopic data, and ^{1}H and ^{13}C NMR shifts, with those of the natural product,[9] thus establishing the structure of the latter unequivocally; the structure of the chromene isolated from *Encelia* species[19] therefore needs to be reexamined.

The success of this synthetic route permitted the preparation of sufficient quantities for biological evaluation of additional chromenes with structural correspondence to *Eutypa* metabolites. Thus, an identical route, starting with 4-hydroxybenzaldehyde, gave the chromene-6-carboxaldehyde (**7**), analogous to **1**, in 74% overall yield. The chromene-6-carboxylate (**8**), analogous to the minor metabolite, eutypine carboxylic acid, was obtained in 69% overall yield as a crystalline solid by hydrolysis of the ester (**6**). A previous synthesis, involving formylation of the parent 2,2-dimethylchromene, gave the aldehyde (**7**) in a yield of 40%, and this was subsequently oxidized to the acid (**8**) in only 24% overall yield;[27] another synthesis, via DDQ oxidation of 2,2,6-trimethylchromene, was reported to give a 70% yield.[28] Both the acid and the aldehyde have previously been isolated as natural products. The former compound (**8**) was first obtained from the plant *Anodendron affine* (Apocyanaceae) and named anofinic acid;[29] subsequently it has been found in the mushroom *Lactarius deliciosus* (Russulaceae),[30] the fungus *Curvularia fallax* (Hyphomycetes),[31] the wood of the southeast Asian tree *Bhesa paniculata* (Celastraceae),[32] and the herb *Gentiana algida* (Gentianaceae).[33] The physical and spectroscopic data for the synthetic material were completely consistent with those reported for the natural product.[29,30,32] Despite its occurrence in two genera of fungi, it has not been identified as a metabolite in the strains of *Eutypa lata* examined to date, even though the corresponding chromene alcohol, eulatachromene (**2**), has been isolated.[9] The chromene aldehyde (**6**) appears to be much less widely distributed in nature, reported only in the steam distillate of liquid cultures of the basidiomycete *Lentinellus cochleatus*.[34]

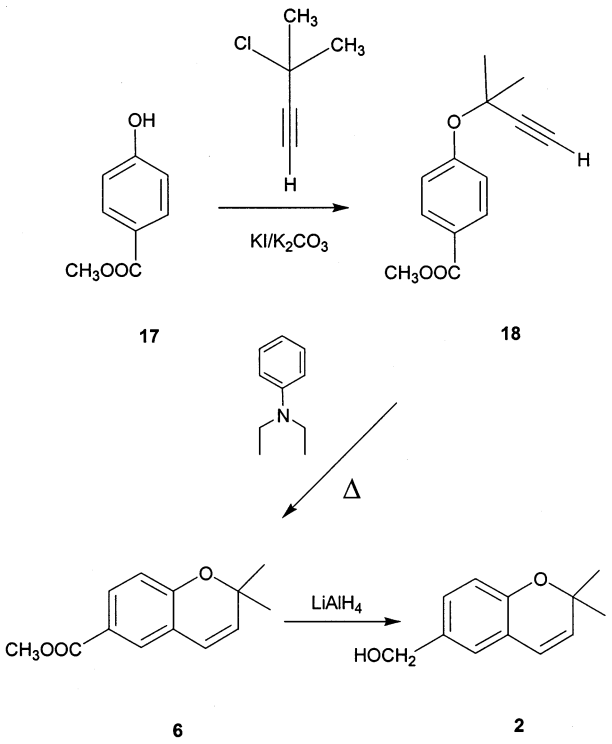

Figure 3. Synthetic route to eulatachromene (**2**).

Eulatachromene (**2**) and the three chromenes (**6**−**8**), together with eutypine (**1**), its cyclization product the benzofuran (**3**), eutypinol (**4**), and siccayne (**5**) provided a suite of compounds suitable for evaluation of their relative phytotoxicity. All of these compounds were obtained by synthesis, except for **4**, which was available in sufficient quantity from fungal cultures. The earliest signs of eutyposis in grapevines are manifested in young leaves, which redden and shrivel prior to complete dieback of the new shoots. In view of this sensitivity, a bioassay was developed that attempted to reproduce these symptoms without having to treat whole plants, which would have involved unacceptably large quantities of natural or synthetic compounds and attendant problems with respect to uptake and transport of the test materials through the root system. Tender leaves of Cabernet Sauvignon, a susceptible variety, were selected, and methanolic solutions of the metabolites or filtrate

extracts were applied in a spot to the surface of excised leaves with the stems immersed in water. Brown, circular areas of necrosis surrounding the point of application appeared, generally within 12 h, with some extracts and metabolites but not with others. In the case of some of the most active samples, signs of necrosis appeared within 1−2 h of their application. This qualitative assessment of phytotoxicity suggested that a more quantitative assay would be feasible and a method was devised to determine the extent of necrosis by measuring the loss of chlorophyll relative to control samples.

Leaf disks, 1 cm in diameter, were cut from Cabernet Sauvignon grape leaves and placed on wet filter paper in a Petri dish to maintain their viability. Aliquots of the selected metabolites in MeOH, or plain MeOH as a control, were applied to the center of the disks and the treated leaf samples kept under ambient light and temperature for 64 h. Leaf disks from each treatment were collected, and the chlorophyll was extracted with acetonitrile. The content of chlorophyll *a*+*b* was determined by measuring the absorbance at 646.8 and 663.8 nm relative to that at 750 nm, using the method of Porra et al.[35] It was immediately apparent that the extent of chlorophyll loss correlated closely with the visual assessment of necrosis for each metabolite and treatment; there was no visible necrosis in leaf disks treated only with MeOH. Chlorophyll loss was also concentration dependent, as shown in Figures 4A and 4B. Of the five metabolites tested, **4**, a common constituent of several *Eutypa* strains, and **5**[9] showed essentially no activity. Eutypine (**1**), the metabolite attributed as primarily responsible for phytotoxicity,[8] was only moderately active, whereas its cyclization product, **3**, showed significant activity. In view of the reported facile conversion of **1** into **3**,[7,12] and our own experience of this transformation, there has to be some concern as to whether such cyclization was occurring under the bioassay conditions; if so, the observed activity of **1** may in fact be due to the presence of the cyclized product. In an exploratory experiment, pure **1** was applied to grapeleaf disks, which were then extracted with acetone after 64 h. Analytical HPLC of the extract showed that small amounts of **3** were present (data not shown), indicating that a more detailed investigation of this question is warranted. It is noteworthy that the dihydro derivative of **3**, (2*S*)-fomannoxin, has been isolated from the wood rot fungus *Heterobasidion annosum* [*Fomes annosus*] and shown to be toxic to germinating seeds and young seedlings of Sitka spruce.[36,37] Eulatachromene (**2**) showed activity similar to that of **3** (Figure 4A), the chlorophyll level decreasing to ca. 60% of control. It is probable that further chlorophyll loss would occur with a longer bioassay period, but the 64 h time limit was selected as a convenient period to provide useful quantitative results and beyond which chlorophyll loss due to normal senescence of the leaf disks in the artificial Petri dish environment was likely to occur.

Comparison of eulatachromene (**2**) with three synthetic analogues, the corresponding methyl ester (**6**), aldehyde (**7**), and acid (**8**), respectively, showed that only **7** had analogous activity; **6** and **8** were inactive in the bioassay (Figure 4B). The reported biological activity of naturally occurring chromenes has been confined almost entirely to toxicity to insects and livestock and as photosensitizing antibiotics to certain fungi and bacteria.[38] However, it has recently been shown that the chromenes present in *Helianthella quinquenervis*, encecalin and demethylencecalin, inhibit radicle growth of *Amaranthus hypochondriacus* (Prince's feather) and *Echinochloa crusgalli* (barnyard grass)[39] and

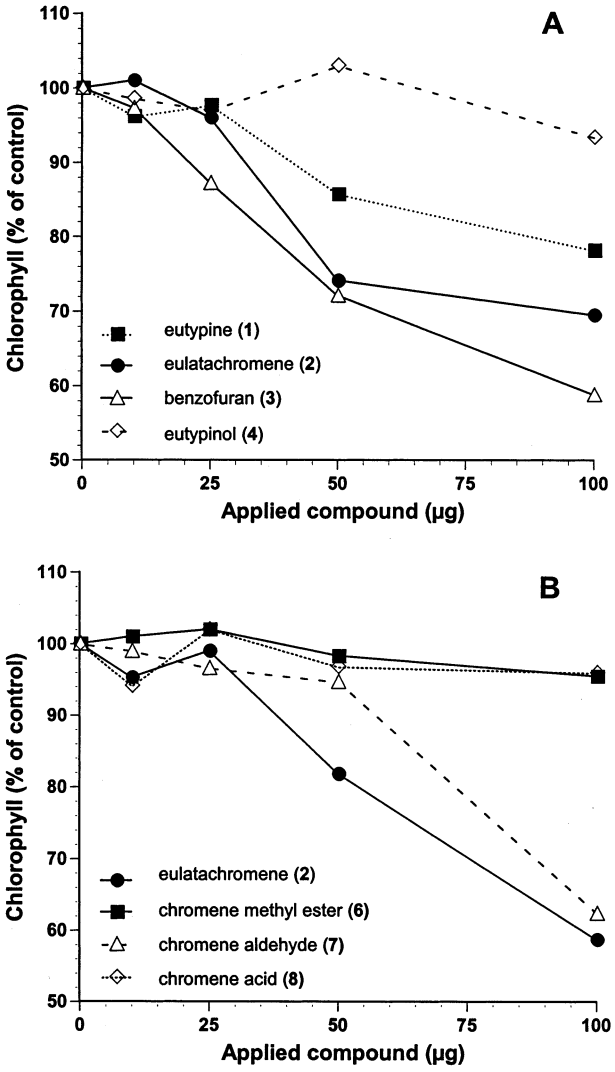

Figure 4. Phytotoxicity of *E. lata* metabolites and synthetic analogues measured as percent reduction in chlorophyll relative to control in the grapeleaf disk bioassay. (A) *E. lata* metabolites eutypine (**1**), eulatachromene (**2**), 5-formyl-2-(methylvinyl)[1]benzofuran (**3**), eutypinol (**4**), and siccayne (**5**). (B) Eulatachromene (**2**) and the synthetic analogues 6-carboxymethylchromene (**6**), 6-formylchromene (**7**), and 6-carboxychromene (**8**).

together with the structurally related and co-occurring benzofuran, euparin, affect photophosphorylation and electron transport in isolated spinach chloroplasts.[40] The syntheses of some halogenated chromenes as potential herbicides have also been reported in the patent literature, although specifics of the bioassay used were not described.[41,42] Our results show that some structurally simple chromenes, including a metabolite of *Eutypa lata*, are capable of producing necrosis in the grapeleaf disk bioassay. Moreover, the active chromenes **2** and **7**, and the corresponding benzofuran (**3**), exhibit greater activity than the *E. lata* metabolites **1** and **4**, with formyl and hydroxymethyl substituents, respectively, which possess an acetylenic side chain. The conjugated acetylenic moiety is therefore not an essential feature for bioactivity even though many of the metabolites isolated to date possess this functionality. As sufficient material becomes available, additional metabolites or their synthetic counterparts will be evaluated in an attempt to identify specific structural features that are essential for phytotoxicity and possibly establish the mode of action of such compounds. The synthetic strategies developed in this investigation should

prove adaptable to the synthesis of other *E. lata* metabolites and their structural analogues.

The fairly rapid bioassay provides a convenient system whereby filtrate extracts from various strains of pathogenic and nonpathogenic *E. lata* can be screened for the presence of phytotoxins and used for bioassay-directed fractionation of the most toxic metabolites. It is anticipated that with a sufficiently large selection of fungal strains, not only from the United States but also from other grape-growing areas of the world, a specific metabolite or group of metabolites can be selected as diagnostic for pathogenic strains and used to predict and control the spread of dying-arm disease in vineyards. Attempts are currently underway to correlate the presence of individual metabolites with DNA probes specific for pathogenic and nonpathogenic *E. lata* strains developed using PCR techniques.[43]

Experimental Section

General Experimental Procedures. Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra were obtained with a Nicolet Magna-IR 550 Series II spectrometer. High-resolution MS results were determined by the Mass Spectrometry Facility, College of Chemistry, University of California, Berkeley. NMR spectra were obtained at 298 K from samples dissolved in $CDCl_3$ with TMS as an internal standard on a Bruker ARX400 spectrometer at frequencies of 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C). A 90° pulse at a 7−8 s repetition rate was used for ^{1}H experiments, and a 30° pulse at a 2.3 s repetition rate was used for ^{13}C experiments. The number of attached protons for ^{13}C signals was determined from DEPT90 and DEPT135 assays.

Methyl 4-hydroxybenzoate, 3-chloro-3-methyl-1-butyne, 3-iodophenol, copper(I) iodide, tetrakis(triphenylphosphine)-palladium, and 2-methyl-1-but-3-yne were obtained from Sigma-Aldrich (Milwaukee, WI). Zinc powder (−100 mesh) was provided by Alfa Products (Danvers, MA).

When necessary, synthetic products were purified by preparative HPLC using a 250 × 21.4 mm i.d., 8 μm, C18 Dynamax column, and 50 mm guard column (Rainin), with isocratic elution by CH_3CN/H_2O (1:1) pumped (Beckman110B) at a flow rate of 10 mL/min and UV detection at 254 nm (Gilson 115). Purity was checked and compounds established as being identical to natural metabolites by GC−MS analysis of their TMS derivatives, prepared by suspension of the sample (ca. 0.5 mg) in dry pyridine (100 μL) in a 1.0 mL Reacti-Vial (Pierce, Rockford, IL), to which was added *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) (100 μL) (Pierce). The reaction mixture was then heated at ca. 60 °C for 1 h. Analyses were performed on a Hewlett-Packard 5890 Series II instrument equipped with a 5971 mass-selective detector (MSD) and a 60 m × 0.32 mm i.d., 0.25 μm, SE-30 fused Si capillary column (J&W Scientific). The column was held at an initial temperature of 105 °C for 0.2 min, ramped at 30 °C/min for 0.5 min, programmed from 120 °C to 300 °C at 10 °C/min, and held at the final temperature for 10 min. Helium was used as carrier gas with a head pressure of 60 psi. Derivatized samples (0.1−0.2 μL) were introduced through an SGE model OC1-3 on-column injector held at ambient temperature. The MSD was operated at 70 eV in the EI mode with scanning from 75 to 600 amu at a sampling rate of 1.5 scans/s. A postinjection delay of 7.0 min was set in order to avoid MS data acquisition during elution of the solvent and derivatization reagent.

4-Hydroxy-3-iodobenzaldehyde (9). This compound was prepared by the method of Defranq et al.,[12] except that the product was purified by recrystallization from H_2O (ca. 75 mL of H_2O per gram of crude product) rather than by column chromatography: GC-EIMS (monoTMS derivative) t_R 12.50 min; *m/z* 320 (M^+, 41), 305 (100), 185 (10), 178 (13), 177 (11), 150 (7), 149 (11).

4-Acetoxy-3-iodobenzaldehyde (10). 4-Hydroxy-3-iodobenzaldehyde (4.02 g, 16.2 mmol) was dissolved in 15 mL of 7.5% aqueous NaOH with magnetic stirring to give a clear, light yellow solution. The solution was cooled in an ice-bath, and Ac_2O (7.5 mL, 79 mmol) was added to the rapidly stirred solution. After 6 min, the mixture was diluted with Et_2O (60 mL) and water (20 mL). The Et_2O layer was separated and washed with 5% aqueous $NaHCO_3$ (3 × 50 mL), then dried over Na_2SO_4. The solvent was removed on a rotary evaporator, and the residue was subjected to a vacuum of 0.5 Torr, with gentle warming, until Ac_2O was no longer detectable by IR. The product was a very viscous, almost colorless, clear oil, which slowly crystallized to a white mass, mp 47−49 °C. Yield was 4.36 g (15.0 mmol, 93%). GC-EIMS: t_R 11.80 min; *m/z* 290 (M^+, 23), 248 (100), 247 (60), 219 (4), 191 (5), 127 (4), 119 (5), 92 (13). Spectroscopic data agreed with that reported in the literature,[11] except that the latter reference did not include the ^{13}C NMR signal at δ 123.7, corresponding to one of the aromatic carbon atoms.

4-Acetoxy-3-(3′-methylbut-3-en-1-ynyl)benzaldehyde (11). This compound was prepared by a modification of the method of Bates et al.,[12] based also on a related procedure described by Brisbois et al.[15] (see Supporting Information). The product (**11**) was obtained in 63% yield as a yellow, crystalline material, mp 47−49 °C. Spectroscopic data agreed with that of Bates et al.;[12] complete assignments of the ^{1}H NMR spectrum not previously reported are as follows: ^{1}H NMR ($CDCl_3$) δ 1.99 (3 H, dd, J = 0.8, 1.6 Hz, $-CH_3$), 2.36 (3H, s, $-OCOCH_3$), 5.37 (1H, appearing as a "pentuplet", J = 1.6 Hz, $=CH_2$), 5.43 (1H, dq, appearing as a "sextet", J = 0.8 and 1.6 Hz, $=CH_2$), 7.26 (1H, d, J = 8.4 Hz, H-5), 7.83 (1H, dd, J = 8.4 Hz, 2 Hz, H-6), 7.98 (1H, dd, J = 2 Hz, 0.4 Hz, H-2), 9.95 (1H, d, J = 0.4 Hz, −CHO); ^{13}C NMR ($CDCl_3$) δ 20.7 ($-OCOCH_3$), 21.2 (CH_3), 82.1 (C-1′), 96.9 (C-2′), 118.8 (C-3), 123.2 (C-5), 123.4 ($=CH_2$), 126.2 (C-3′), 130.1 (C-6), 134.2 (C-1), 134.6 (C-2), 155.9 (C-4), 168.0 ($-OCOCH_3$), 190.2 (−CHO).

Eutypine (1). This compound was prepared from 4-acetoxy-3-(3′-methylbut-3-en-1-ynyl)benzaldehyde (**11**) by deacetylation with LiOH in aqueous THF using the method of Bates et al.[12] Crude material rapidly (<1 day) isomerized to **3** if not promptly processed to remove impurities. HPLC purification gave a stable, cream-colored, crystalline solid. Despite consistently clean NMR spectra, melting ranges varied, for example, 70.5−72.5 °C for one batch, 67.5−71 °C for another (lit. 76−77 °C;[11] 67−69 °C[12]). Spectroscopic properties were in agreement with those reported by Defranq et al.[11] and Bates et al.:[12] GC-EIMS (monoTMS derivative) t_R 13.75 min; *m/z* 258 (M^+, 100), 243 (82), 227 (9), 203 (22), 199 (43), 189 (15), 185 (28), 141 (12), 128 (12), 115 (15), consistent with that of the natural product;[9] ^{1}H NMR ($CDCl_3$) δ 2.03 (3H, dd, J = 1.6 Hz, 0.8 Hz), 5.41 (1H, p, J = 1.6 Hz), 5.49 (1H, dq, appearing as a "sextet", J = 1.6 Hz, 0.8 Hz), 6.37 (1H, br s), 7.07 (1H, d, J = 8.4 Hz), 7.79 (1H, dd, J = 8.4 Hz, 2 Hz), 7.89 (1H, d, J = 2 Hz), 9.84 (1H, s); ^{13}C NMR ($CDCl_3$) δ 23.3, 80.5, 98.9, 110.7, 115.5, 123.9, 125.8, 129.9, 131.8, 134.4, 161.2, 190.1.

5-Formyl-2-(methylvinyl)-1-benzofuran (3). This compound was isolated by HPLC in varying yields as a crystalline byproduct, mp 58−59 °C, from preparations of **1**: GC-EIMS t_R 11.94 min; *m/z* 186 (M^+, 100), 185 (79), 157 (26), 128 (12), 115 (5), 92 (5); IR ν_{max} (NaCl, thin film) cm^{-1} 3125, 3096, 2955, 2914, 2725, 1694, 1606, 1560, 1444, 1336, 1277, 1115, 887, 807; UV (MeOH) λ_{max} nm (log ϵ) 204 (3.84), 256 (4.62); ^{1}H NMR ($CDCl_3$) δ 2.14 (3H, dd, J = 1.4, 1.0 Hz, $-CH_3$), 5.25 (1H, appearing as a "pentuplet", J = 1.4 Hz, $=CH_2$), 5.85 (1H, m, $=CH_2$), 6.72 (1H, s, H-3), 7.54 (1H, d, J = 8.8 Hz, H-7), 7.83 (1H, dd, J = 8.6, 1.8 Hz, H-6), 8.06 (1H, d, J = 1.6 Hz, H-4), 10.04 (1H, −CHO); ^{13}C NMR ($CDCl_3$) δ 19.2 ($-CH_3$), 103.0 (C-3), 111.7 (C-7), 114.7 ($=CH_2$), 124.0 (C-4), 126.3 (C-6), 129.7, 132.3, 132.4 (C-3a, C-5, $C=CH_2$) 158.2, 158.8 (C-2, C-7a), 191.7 (−CHO); HREIMS *m/z* 186.0681 (calcd for $C_{12}H_{10}O_2$, 186.0681).

4-Amino-3-iodophenol (13). This compound was prepared by the method of Kvalnes,[17] except that the extraction with ether was omitted; the product (**13**) was recrystallized directly from the reaction solvent as a light-colored, fibrous solid, in 50% yield, mp 134.5−136.5 °C (dec); lit. darkening at 135 °C, melting at 140 °C with charring:[17] ^{1}H NMR (DMSO-d_6) δ 4.51 (2H, br s), 6.58 (1H, dd, J = 8.6, 2.6 Hz), 6.38 (1H, d, J = 8.6

Hz), 7.01 (1H, d, J = 2.4 Hz), 8.70 (1H, s); ^{13}C NMR (DMSO-d_6) δ 83.6, 115.2, 116.6, 124.1, 140.9, 149.0.

2-Iodoquinone (14). This compound was prepared by the method of Kvalnes[17] using saturated aqueous $K_2Cr_2O_7$, instead of $Na_2Cr_2O_7$ solution. 4-Amino-3-iodophenol (5.12 g; 21.8 mmol) was dissolved in concentrated H_2SO_4 (38 mL) and H_2O (110 mL) at 50 °C. The mixture was cooled in an ice bath to 10 °C, and saturated aqueous $K_2Cr_2O_7$ (165 mL) added in portions over 15 min at 14−17 °C. After stirring for an additional 5 min, the ice bath was removed and stirring continued for a further 10 min. The slurry was filtered and the solid washed with H_2O (4 × 10 mL) to give 3.73 g (15.9 mmol, 73%) of **14**: mp 61−63 °C; lit. 62 °C, after sublimation;[17] IR ν_{max} (NaCl, thin film) cm^{-1} 3308, 3073, 3046, 1667, 1651, 1619, 1570, 1271, 1096, 961, 917, 822; ^{1}H NMR ($CDCl_3$) δ 6.84 (1H, dd, J = 10, 2.4 Hz), 7.00 (1H, d, J = 10 Hz), 7.68 (1H, d, J = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 119.5, 134.6, 136.7, 146.2, 180.2, 184.0.

2-Iodohydroquinone Diacetate (15). This compound was prepared by reductive acetylation[18] of 2-iodoquinone. A mixture of 2-iodoquinone (0.946 g; 4.04 mmol) and Zn powder (1.91 g; 29.2 mmol) was chilled in an ice/water bath and then treated with an ice-cold solution of concentrated aqueous HCl (3 mL) in Ac_2O (20 mL) with vigorous stirring. The dark quinone color disappeared completely in less than 90 s; after 3 min, the ice bath was removed, and stirring continued for 7 min. The mixture was diluted with Et_2O (60 mL), filtered through cotton wool to remove unreacted Zn, and then washed with H_2O (2 × 20 mL) followed by saturated aqueous NaCl (2 × 20 mL). The ethereal solution was dried over Na_2SO_4 and concentrated (rotary evaporator, then ca. 1 Torr) to give a pale amber oil, which crystallized under vacuum (1.24 g; 3.88 mmol, 96%): mp 82−84.5 °C; lit. 86−87 °C recrystallized from aqueous EtOH;[17] IR ν_{max} (NaCl, thin film) cm^{-1} 3090, 2936, 1763, 1586, 1476, 1370, 1203, 1170, 1011, 923; ^{1}H NMR ($CDCl_3$) δ 2.27 (3H, s), 2.35 (3H, s), 7.08 (1H, dd, J = 8.4 and 0.4 Hz), 7.11 (1H, dd, J = 8.8, 2.4 Hz), 7.57 (1H, dd, J = 2.4, 0.4 Hz); ^{13}C NMR ($CDCl_3$) δ 21.0, 21.1, 90.0, 122.6, 123.0, 132.2, 148.4, 148.9, 168.4, 168.8.

Siccayne Diacetate (16). To a 100 mL three-necked flask, flushed with N_2, was added 2-iodohydroquinone diacetate (1.833 g; 5.72 mmol), CuI (0.208 g; 1.09 mmol), and tetrakis-(triphenylphosphine)palladium (0.373 g; 0.323 mmol). Triethylamine (17 mL) was added with stirring, followed by anhydrous THF (10 mL). After a few minutes a clear, light amber solution was produced, to which 2-methyl-1-buten-3-yne (4.5 mL; 47 mmol) was added. A momentary lightening in color was followed by a gradual (1−2 min) darkening, and within a few minutes, turbidity was followed by formation of a granular precipitate. The flask was covered with aluminum foil and stirring continued at room temperature for 25 h. The mixture was then diluted with hexane/ether (9:1) (70 mL). After stirring and settling, the supernatant was filtered through cotton wool into a separatory funnel and the cream-colored solid remaining in the flask rinsed with 4 × 15 mL portions of the solvent mixture. The combined organic phase was washed with saturated aqueous NH_4Cl (60 mL), 5% aqueous $NaHCO_3$ (60 mL), and saturated aqueous NaCl (60 mL). After drying over anhydrous Na_2SO_4, the solution was passed through a short column of silica gel (5.0 g), eluted with an additional 100 mL of solvent. Concentration on a rotary evaporator gave a pale crystalline solid admixed with an orange oil. The solid was washed with hexane (3 × 5 mL), then dried at ca. 1 Torr to give a pale yellow solid (0.998 g), mp 66−67.5 °C. Concentration of the hexane washes gave additional product (0.053 g), mp 64−67 °C. Total yield: 1.051 g (4.07 mmol, 71%); GC-EIMS t_R 14.33 min; m/z 258 (M$^+$, 6), 216 (20), 174 (100), 159 (9), 147 (5), 127 (5); IR ν_{max} (NaCl, thin film) cm^{-1} 3072, 2978, 2925, 1768, 1759, 1614, 1577, 1486, 1371, 1218, 1170, 910; ^{1}H NMR ($CDCl_3$) δ 1.96 (3H, dd, J = 1.6, 1 Hz, −CH_3), 2.28 (3H, s, −OCOCH_3), 2.32 (3H, s, −OCOCH_3), 5.32 (1H, m, J = 1.8 Hz, =CH_2), 5.38 (1H, dq, appearing as a "sextet", J = 2, 1 Hz, =CH_2), 7.05 (1H, dd, J = 8.8, 2.4 Hz, H-5), 7.08 (1H, dd, J = 8.8, 0.8 Hz, H-6), 7.22 (1H, dd, J = 2.4, 0.8 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 20.7 (−OCOCH_3), 21.0 (−OCOCH_3), 23.2 (−CH_3), 82.5 (−C≡), 96.2 (−C≡), 118.5 (C-3), 122.5 (C-6), 122.9 (=CH_2 [C-4′]), 123.0 (C-5), 125.7 (C-2), 126.4 (C-3′); 147.9, 149.0 (C-1 and C-4); 168.6 (−OCOCH$_3$), 169.0 (−OCOCH$_3$); HREIMS m/z 258.0894 (calcd for $C_{15}H_{14}O_4$, 258.0892).

Siccayne (5). (a) Via base hydrolysis. LiOH·H_2O (0.154 g; 3.67 mmol) in a 60 mL flask was cooled in an ice bath and H_2O (2.0 mL) added to dissolve the solid. A solution of siccayne diacetate (**16**) (0.233 g; 0.903 mmol) in THF (1.5 mL) was added with rapid stirring. After 30 min, the dark mixture was diluted with Et_2O (50 mL) and acidified by addition of 10% aqueous HCl (5 mL). After mixing and allowing to settle, the aqueous layer was removed by pipet. The red-amber Et_2O layer was washed with saturated aqueous NaCl (2 × 5 mL), then dried over anhydrous Na_2SO_4. Solvent removal (rotary evaporator, then ca. 1 Torr) gave a molasses-colored, very viscous oil, from which siccayne was isolated by HPLC (two passes). The yield of purified siccayne was 0.106 g (0.609 mmol, 67%): white solid, mp 114.5−116 °C; lit.114−116 °C;[16] GC-EIMS (as di-TMS derivative) t_R 13.60 min; m/z 318 (M$^+$, 100), 304 (13), 303 (42), 287 (8), 263 (8); IR ν_{max} (NaCl, thin film) cm^{-1} 3500−3100, 2955, 2920, 2206, 1616, 1450, 1232; ^{1}H NMR ($CDCl_3$) δ 2.00 (3H, dd, J = 1.6, 1.2 Hz, −CH_3), 4.69 (1H, br s, −OH), 5.35 (1H, "pentuplet", J = 1.6 Hz, =CH_2), 5.42 (1H, br s, −OH), 5.43 (1H, dq, appearing as a "sextet", J = 1.6, 1 Hz, =CH_2), 6.74 (1H, dd, J = 8.8, 3 Hz, H-6), 6.80−6.83 (2H, m, H-2 and H-5); ^{13}C NMR ($CDCl_3$) δ 23.4 (−CH_3); 81.9 (−C≡), 97.6 (−C≡), 110.0 (C-3); 115.5, 117.4 (C-2, C-6); 118.0 (C-5), 123.1 (=CH_2 [C-4′]), 126.1 (C-3′); 148.7, 150.8 (C-1, C-4). (b) Via acid hydrolysis. Siccayne diacetate (**16**) (0.051 g; 0.198 mmol) in THF (5.0 mL) was treated with 6 M aqueous HCl (1.0 mL) and the clear, almost colorless solution stirred in the closed flask at room temperature for 51 h. The mixture was diluted with Et_2O (50 mL) and the small lower layer which separated removed by pipet. The Et_2O solution was then washed with saturated aqueous NaCl (4 × 5 mL) and dried over Na_2SO_4. Removal of solvent (rotary evaporator, then ca. 1 Torr) gave a yellow oil, from which **5** was isolated by HPLC (one pass). Duplicate experiments gave 20 mg (0.115 mmol; 58%) and 23 mg (0.132 mmol; 67%), respectively, of **5**, mp 113−115 °C. IR and NMR spectra matched those of material obtained from base hydrolysis.

Methyl 4-(1,1-Dimethyl-2-propynyloxy)benzoate (18). A mixture of KI (6.0 g), K_2CO_3 (6.0 g), methyl 4-hydroxybenzoate (3.05 g, 25.0 mmol), acetone (30 mL), and 3-chloro-3-methyl-1-butyne (6.8 mL, 60.5 mmol) was heated with stirring and exclusion of moisture at 49−53 °C. After 48 h, the mixture was allowed to cool to room temperature, then partitioned between Et_2O (100 mL) and 1.0 M aqueous NaOH (100 mL) in a separatory funnel. The organic fraction was washed with additional 1.0 M NaOH (2 × 100 mL) and dried over anhydrous $MgSO_4$. Removal of solvent (rotary evaporator, then ca. 1 Torr) gave a clear yellow oil (yield 4.29 g, 19.7 mmol, 79%), which rapidly crystallized: mp 49−53 °C; GC-EIMS m/z 218 (M$^+$, 6), 203 (66), 152 (67), 121 (100); IR ν_{max} (NaCl, thin film) cm^{-1} 3292, 2991, 2951, 2111, 1720, 1604, 1507, 1435, 1280, 1249, 1139, 1114; ^{1}H NMR ($CDCl_3$) δ 1.69 (6H, s), 2.62 (1H, s), 3.88 (3H, s), 7.24 (2H, d, J = 9 Hz), 7.97 (2H, d, J = 9 Hz); ^{13}C NMR ($CDCl_3$) δ 29.6, 51.9, 72.4, 74.7, 85.3, 119.5, 123.9, 131.0, 159.8, 166.9; HREIMS m/z 218.0941 (calcd for $C_{13}H_{14}O_3$, 218.0943).

6-Methoxycarbonyl-2,2-dimethyl-2*H*-chromene (6). Methyl 4-(1,1-dimethyl-2-propynyloxy)benzoate (**18**) (3.22 g, 14.8 mmol) and *N*,*N*-diethylaniline (15 mL) were stirred magnetically in a 25 mL flask, until almost all of the solid dissolved, giving a yellow solution. The mixture was brought to reflux (210−220 °C), at which point the remaining solid had dissolved. After 75 min the reaction was allowed to cool to room temperature. The mixture was diluted with Et_2O (75 mL), washed (caution; exothermic) with 6 M aqueous HCl (4 × 30 mL) followed by saturated aqueous NaCl (2 × 10 mL), and dried over anhydrous Na_2SO_4. Solvent removal (rotary evaporator, then 0.25 Torr) gave an amber oil, which soon crystallized into prisms and rosettes; yield 3.09 g (14.2 mmol, 96%). The product could be further purified by sublimation under vacuum, which gave ca. 96% recovery of an almost white crystalline solid: mp 43−45 °C; GC-EIMS t_R 12.16 min; m/z

218 (M^+, 8), 203 (100), 144 (11), 115 (8); IR ν^{melt}_{max} cm^{-1} 3044, 2977, 2951, 1720, 1641, 1609, 1576, 1490, 1441, 1367, 1291, 1274, 1196, 1166, 1096, 960, 766; UV (MeOH) λ_{max} nm (log ϵ) 240 (4.60), 282 (3.64); ^{1}H NMR ($CDCl_3$) δ 1.44 (6H, s), 3.86 (3H, s), 5.63 (1H, d, J = 10 Hz), 6.33 (1H, d, J = 10), 6.77 (1H, dd, J = 8.4 hz, 0.4 Hz), 7.67 (1H, dd, J = 2 Hz, 0.4 Hz), 7.80 (1H, dd, J = 8.4 Hz, 2 Hz); ^{13}C NMR ($CDCl_3$) δ 28.3, 51.8, 77.4, 116.2, 120.7, 121.7, 122.6, 128.1, 131.0, 131.1, 157.2, 166.8; HREIMS *m/z* 218.0946 (calcd for $C_{13}H_{14}O_3$, 218.0943).

6-Hydroxymethyl-2,2-dimethyl-2*H*-chromene; Eulatachromene (2). 6-Methoxycarbonyl-2,2-dimethyl-2*H*-chromene (**5**) (1.247 g, 5.72 mmol) was reduced by stirring with $LiAlH_4$ (0.722 g, 19 mmol) in anhydrous Et_2O (50 mL) with gentle reflux, under an atmosphere of N_2. After 30 min, the reaction mixture was chilled in an ice bath, and 15% aqueous NaOH (5 mL) was added dropwise over a 10 min period with vigorous stirring. Partway through the quenching process, Et_2O (25 mL) was added. After all the NaOH solution had been added, a clear colorless supernatant was obtained, over white semisolid clumps. The supernatant was decanted, the solid was washed with Et_2O (3 × 10 mL), and the combined Et_2O phase was dried over anhydrous Na_2SO_4. Solvent removal gave a clear, colorless, viscous oil: yield 1.066 g (5.61 mmol, 98%); GC-EIMS (mono-TMS derivative) t_R 12.05 min; *m/z* 262 (M^+, 8), 247 (100), 173 (13), 158 (5), consistent with that of the natural product;[9] IR ν_{max} (NaCl, thin film) cm^{-1} 3600−3150, 3039, 2975, 2933, 2872, 1638, 1614, 1490, 1361, 1261, 1212, 1151; UV (MeOH) λ_{max} nm (log ϵ) 224 (4.51), 264 (3.56), 312 (3.44); ^{1}H NMR ($CDCl_3$) δ 1.42 (6H, s), 1.80 (1H, s), 4.54 (2H, s), 5.60 (1H, d, J = 9.6 Hz), 6.30 (1H, d, J = 9.6 Hz), 6.74 (1H, d, J = 8 Hz), 6.96 (1H, d, J = 2 Hz), 7.07 (1H, dd, J = 8, 2 Hz); ^{13}C NMR ($CDCl_3$) δ 28.0, 65.1, 76.3, 116.4, 121.3, 122.2, 125.4, 128.1, 131.1, 133.2, 152.6; HREIMS *m/z* 190.0993 (calcd for $C_{12}H_{14}O_2$, 190.0994).

2,2-Dimethyl-2*H*-chromene-6-carboxylic Acid; Anofinic Acid (8). 6-Methoxycarbonyl-2,2-dimethyl-2*H*-chromene (**5**) (0.102 g, 0.468 mmol) was hydrolyzed by stirring with NaOH (0.34 g, 8.5 mmol) dissolved in absolute EtOH (2.5 mL) and H_2O (0.5 mL) at room temperature for 4.5 h. The mixture was diluted with Et_2O (15 mL), then acidified with 6 M aqueous HCl (5 mL). After separation, the Et_2O layer was dried over anhydrous Na_2SO_4, then evaporated to give the carboxylic acid (**7**): mp 159−162 °C; lit. 158.5−160 °C,[29] 150−156 °C;[30] yield 0.083 g (0.407 mmol, 87%); GC-EIMS (mono-TMS derivative) t_R 13.71 min; *m/z* 276 (M^+, 6), 262 (20), 261 (100), 189 (5), 144 (8), 115 (7); IR ν_{max} (NaCl, thin film) cm^{-1} 3300−2400 (br), 2975, 1674, 1606, 1575, 1446, 1412, 1299, 1276, 1200, 1123, 954, 768; UV (MeOH) λ_{max} nm (log ϵ) 240 (4.58), 282 (3.60); ^{1}H NMR ($CDCl_3$) δ 1.46 (6H, s), 5.65 (1H, d, J = 10 Hz), 6.36 (1H, d, J = 10 Hz), 6.80 (1H, d, J = 8.4 Hz), 7.74 (1H, d, J = 2 Hz), 7.88 (1H, dd, J = 8.4, 2 Hz), 10.5 (1H, v br); ^{13}C NMR ($CDCl_3$) δ 28.4, 77.6, 116.3, 120.7, 121.61, 121.63, 128.8, 131.1, 131.9, 158.0, 171.8; HREIMS *m/z* 204.0783 (calcd for $C_{12}H_{12}O_3$, 204.0786).

4-(1,1-Dimethyl-2-propynyloxy)benzaldehyde. A mixture of 4-hydroxybenzaldehyde (1.26 g, 10.3 mmol) and 3-chloro-3-methyl-1-butyne (5.0 mL, 44.5 mmol) was heated under reflux over KI (3.0 g) and K_2CO_3 (3.0 g) in dry acetone (15 mL) for 47 h, with stirring and exclusion of moisture. The reaction was cooled to room temperature, diluted with ether (50 mL), washed with 1 M aqueous NaOH (3 × 50 mL), and dried over anhydrous $MgSO_4$. After solvent removal, the sample was dissolved in hexane/ether (9:1) (ca. 20 mL) then passed through silica gel (5 g), using 150 mL more solvent to complete elution. This gave the 4-(1,1-dimethyl-2-propynyloxy)benzaldehyde as a light amber oil: yield 1.74 g (9.26 mmol; 90%); GC-EIMS t_R 9.48 min; EIMS *m/z* 188 (M^+, 5), 173 (31), 159 (11), 122 (78), 121 (100), 115 (10), 93 (11); IR ν_{max} (NaCl, thin film) cm^{-1} 3290, 2991, 2939, 2828, 2738, 2111, 1698, 1601, 1577, 1505, 1248, 1220, 1138; ^{1}H NMR ($CDCl_3$) δ 1.72 (6H, s), 2.66 (1H, s), 7.33 and 7.81 (each 2H, AB multiplets), 9.90 (1H, s); ^{13}C NMR ($CDCl_3$) δ 29.6, 72.5, 75.0, 85.0, 119.6, 130.7, 131.3, 161.2, 190.9; HREIMS *m/z* 188.0834 (calcd for $C_{12}H_{12}O_2$, 188.0837).

2,2-Dimethyl-2*H*-chromene-6-carboxaldehyde (7). 4-(1,1-Dimethyl-2-propynyloxy)benzaldehyde (0.40 g, 2.13 mmol) in *N,N*-diethylaniline (4.0 mL) was heated under reflux (205−210 °C) in a 5 mL flask under anhydrous conditions, with stirring. The initially yellow solution rapidly became dark amber in color. After 5 h, IR of a sample of the reaction mixture showed no alkyne C−H band. The reaction mixture was diluted with Et_2O (25 mL), then washed with 6 M aqueous HCl (4 × 10 mL), followed by H_2O (10 mL). Drying over anhydrous Na_2SO_4, followed by solvent removal, gave an oil, which was dissolved in hexane/ether (9:1) and passed through 5 g of silica gel, eluting with 150 mL of the same solvent. Removal of the solvent gave the chromene carboxaldehyde (**7**) as an oil: yield 0.33 g (1.76 mmol, 82%); GC-EIMS t_R 10.65 min; *m/z* 188 (M^+, 9), 174 (12), 173 (100), 144 (7), 115 (13); IR ν_{max} (NaCl, thin film) cm^{-1} 3046, 2977, 2934, 2820, 2734, 1690, 1639, 1601, 1572, 1486, 1364, 1278, 1146, 1119, 963; UV (MeOH) λ_{max} nm (log ϵ) 252 (4.46), 298 (3.75); ^{1}H NMR ($CDCl_3$) δ 1.47 (6H, s), 5.68 (1H, d, J = 10 Hz), 6.36 (1H, d, J = 10 Hz), 6.85 (1H, dm, J = 8.4 Hz), 7.50 (1H, dd, J = 2, 0.4 Hz), 7.63 (1H, dd, J = 8.4, 2 Hz), 9.82 (1H, s); ^{13}C NMR ($CDCl_3$) δ 28.5, 77.9, 116.8, 121.2, 121.4, 127.8, 130.0, 131.5, 131.9, 158.7, 190.6; HREIMS *m/z* 188.0834 (calcd for $C_{12}H_{12}O_2$, 188.0837).

Phytotoxicity Bioassay with Grapeleaf Disks. Leaf disks (1 cm diameter) were cut from greenhouse grown Cabernet Sauvignon grape leaves (4−5 cm at the widest point) with a cork borer with the major veins being avoided. Ten leaves were used for each concentration such that each of the tested compounds and its associated control had one leaf disk from each of the same 10 leaves. The 10 leaf disks for each sample were arranged around the perimeter of filter paper (7.0 cm Whatman #3) saturated with H_2O (2 mL) and placed in a Petri dish (100 × 15 mm). Compounds to be tested were dissolved in MeOH, and a volume of 5 μL was applied as a single spot at the center of the surface of each leaf disk; the control disks were similarly treated with 5 μL of MeOH. The Petri dishes were covered with lids and the test samples exposed at room temperature to ambient lighting on a laboratory bench; this consisted of approximately 12 h of mixed natural and artificial light and 12 h of darkness. An additional volume of H_2O (1.5 mL) was added to each Petri dish after 24 h. The degree of leaf disk browning was measured after 64 h by comparing the chlorophyll content of the treated leaf disks to the control disks. All 10 leaf disks for a given compound and concentration were placed in DMF (10 mL) and shaken in the dark for 4 h at 250 rpm. Chlorophyll concentrations were calculated according to the method of Porra et al.[35] using the formula

$$\text{total chlorophyll } a + b \ (\mu\text{g/mL}) = 17.67A^{646.8} + 7.12A^{663.8}$$

where $A^{646.8}$ = absorbance at 646.8 nm − 750 nm and $A^{663.8}$ = absorbance at 663.8 nm − 750 nm. Results were plotted as remaining chlorophyll (percent of control) versus amount of applied sample (μg) and are shown in Figure 4.

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Supporting Information Available: Procedure for synthesis of compound **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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