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Furanocoumarin with Phytotoxic Activity from the Leaves of *Amyris elemifera* (Rutaceae)

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ABSTRACT: Bioassay-guided fractionation of the ethyl acetate extract of *Amyris elemifera* leaves was carried out to identify phytotoxic and antifungal constituents. A novel phytotoxic furanocoumarin 8-(3-methylbut-2-enyloxy)-marmesin acetate (**1**) and its deacyl analog 8-(3-methylbut-2-enyloxy)-marmesin (**2**) were isolated. The X-ray crystal structure determination is reported for the first time for **1**. Both **1** and **2** have the S configuration at C-2′ based on X-ray crystallographic data. Both these compounds inhibited the growth of the dicot *Lactuca sativa* (lettuce) and the monocot *Agrostis stolonifera* with a more pronounced inhibitory effect on the monocots at 330 μM by **1**. In *Lemna paucicostata* Hegelm phytotoxicity bioassay, the IC_{50} value for **1** was 26 μM, whereas **2** had an IC_{50} value of 102 μM. Compounds **1** and **2** were weakly antifungal against *Colletotrichum fragariae* Brooks in TLC bioautography.

Amyris elemifera leaves →
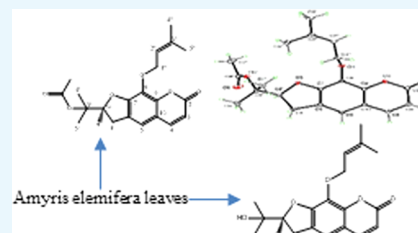
1. INTRODUCTION

Plant secondary metabolites are good sources or lead templates that can be used to develop and design agrochemicals and pharmaceuticals.[1−3] Most secondary metabolites in plants are produced as a result of coevolution with other living organisms such as other plants, insects, and microbes. These secondary metabolites of plants can have phytotoxic, antifungal, and insecticide properties as part of natural defense mechanism to survive in their ecological niche. Thus, evaluating plant extracts and their secondary metabolites is the rationale in our investigation. In search of natural product-based pesticides, the ethyl acetate extract of the leaves of *Amyris elemifera* was investigated via bioassay-guided fractionation. *A. elemifera* is a widely distributed plant in Florida in the United States, Central America, the Caribbean, and the northern part of South America. This plant is also known as sea torchwood and sea amyris and is a member of the Rutaceae family. Plants in the Rutaceae family are well known for diverse classes of biologically active natural products such as alkaloids, coumarins, oxazoles, quinolones, and essential oils.[4−7] There are numerous reports on isolating fungicidal, herbicidal, bactericidal, insecticidal, and algicidal compounds from Rutaceae plants.[4,8−12] In this paper, we describe isolation, structure elucidation, and biological activities of a novel furanocoumarin and its analog. The absolute configuration of the novel compound was determined by X-ray crystallography.

2. MATERIALS AND METHODS

2.1. Chemicals and Instrumentation. The organic solvents used in the experiments were of reagent grade and used without purification. Silica gel thin-layer chromatography

(TLC) was carried out on 250 μm thick silica gel plates with a GF fluorescent indicator (Analtech, Newark, DE). Anisaldehyde spray reagent, I_2 vapor, and UV light (254 and 365 nm) were used to visualize the compounds on TLC plates. Colum chromatography fractionation was carried out using a Biotage (Biotage Inc., Charlottesville, Virginia) flash chromatography system with an Isolera pump and a dual wavelength detector (254 and 280 nm) using silica gel SNAP flash columns (particle size 40−65 μm) with increasing ratios of ethyl acetate in hexane. Melting points were recorded by Optimelt melting point apparatus (Stanford Research System, Sunnyvale, California). NMR spectra were recorded on Bruker NMR spectrometers (Billerica, Massachusetts) operating at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR. Optical rotations were measured using Autopol IV Automatic Polarimeter model 589-546 (Rudolph Research Analytical, Hackettstown, New Jersey). High-resolution mass spectra were obtained using a Jeol Accu TOF 4G LC-plus atmospheric pressure ionization time-of-flight mass spectrometer (Jeol, Tokyo, Japan) fitted with a DART ion source (IonSense DART controller, Sangus, MA).

2.2. Plant Material. Leaves of *A. elemifera* were collected in April 2009 in Miami-Dade County in Florida and identified by Dr. Charles Burandt of the School of Pharmacy of the
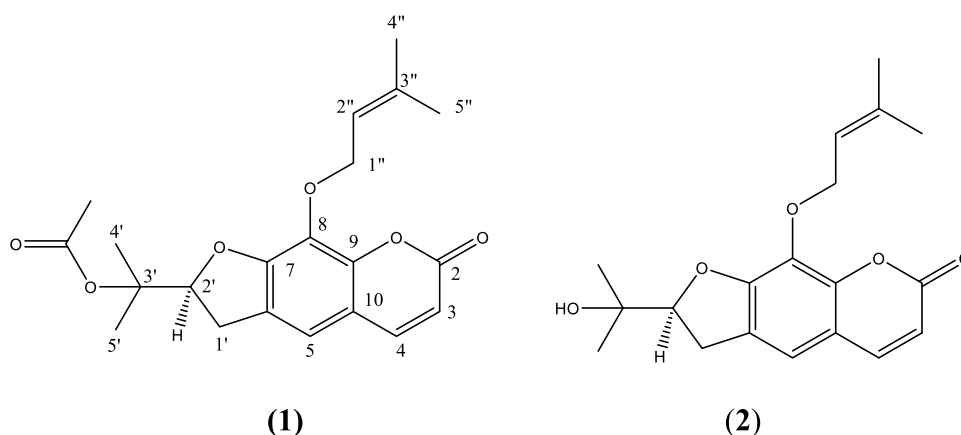
Figure 1. Structures of (**1**) and (**2**).

University of Mississippi. A voucher specimen is deposited in the University of Mississippi herbarium BUR 280703. The leaves were air-dried, ground, and stored in plastic bottles at 25 °C until use.

2.3. Extraction of Plant Material. Ground leaves (500 g) were successively extracted with each solvent (2 L × 2) using a mechanical stirrer for 12 h with hexane, ethyl acetate, and methanol at ambient temperature to afford 10, 49, and 64 g of extracts, respectively, after evaporating the solvent under reduced pressure at 40 °C. These extracts were tested for phytotoxicity, with only the ethyl acetate fraction testing positive for this activity.

2.4. Isolation of Phytotoxic Compounds. A portion of ethyl acetate extract (29 g) was fractionated using 340 SNAP Biotage silica gel column using ethyl acetate in hexane (0–100%) gradient elution over 10 column volumes. Fractions (200 mL per fraction) were assessed with TLC, and fractions with similar phytochemical profiles were combined according to TLC profile to obtain 28 fractions. These fractions were tested for phytotoxicity, and fractions 20 and 21 had the highest phytotoxicity. Fractions 20 and 21 were combined (6.7 g), further purified using 50 SNAP Biotage silica gel column using ethyl acetate in hexane (20–60%) gradient elution over eight column volumes, and the major constituent isolated was recrystallized three times with ethyl acetate:methylene chloride:hexane (1:1:4) to obtain white crystals (**1**) (2.2 g).

2.4.1. 8-(3-Methylbut-2-enyloxy)-marmesin acetate (1). High-resolution DART positive m/z 373.16511 $[M + H]^+$, calculated for $C_{21}H_{25}O_6$ 373.165115. $[\alpha]_D$ (−) 36.6 (c = 0.2, CHCl$_3$); m.p. 93.5 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.53 (s, 3H, 5′-CH$_3$), 1.59 (s, 3H, 4′-CH$_3$), 1.72 (brs, 3H, 5″-CH$_3$), 1.74 (brs, 3H, 4″-CH$_3$), 1.97 (s, 3H, OAc), 3.20 (ddd, J = 16, 7.72, 1.16 Hz, 1H, 1′b-CH$_2$), 3.27 (ddd, J = 15.9, 9.6, 1.3 Hz, 1H, 1a′-CH$_2$), 4.80–4.65 (m, 2H, 1″-CH$_2$), 5.11 (dd, J = 9.5, 7.7 Hz, 1H, 2′-CH), 5.56 (tp, J = 7.2, 1.4 Hz, 1H, 2″-CH), 6.20 (d, J = 9.5 Hz, 1H, 3-CH), 6.94 (brt, J = 1.2 Hz, 1H, 5-CH), and 7.57 (d, J = 9.5 Hz, 1H, 1H, 4-CH). ^{13}C NMR (100 MHz, CDCl$_3$) δ 18.3 (C-5′), 23.4 (C-4′), 21.2 (C(O)$\underline{C}$H$_3$), 23.4 (C-5″), 25.9 (C-4″), 30.3 (C-1′), 69.7 (C-1″), 82.3 (C-3′), 88.9 (C-2′), 112.7 (C-3), 113.9 C-10), 117.3 (C-5), 120.3 (C-2″), 125.2 (C-6), 130.3 (C-3″), 139.4 (C-8), 144.9 (C-4), 148.4 (C-9), 155 (C-7), 161 (C-2), and 170.3 (OAc) (Figure 1).

The crystal structure of **1** was determined from data collected at 90 K with Cu Kα (λ = 1.54184 Å) radiation on a Bruker KappaAPEX II DUO diffractometer equipped with a microfocus source.

2.4.2. 8-(3-Methylbut-2-enyloxy)-marmesin (2). Further purification of fraction 24 (56 mg) by a short florisil column (8 cm × 1.8 cm id) using 30% ethyl acetate in hexane to rid of chlorophyll followed by Biotage flash chromatography using a 10 g silica gel column with gradient elution (3:7 ethyl acetate in hexane to 8:2 of the same) afforded 38 mg of **2** as a colorless gum. High-resolution DART positive m/z 331.1545 $[M + H]^+$, calculated for $C_{19}H_{23}O_5$ 331.154550 $[\alpha]_D$ −16.5 (c = 0.2, CHCl$_3$). ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.25 (s, 3H, 5′-CH$_3$), 1.38 (s, 3H, 4′-CH$_3$), 1.70 (brs, 3H, 5″-CH$_3$), 1.74 (brs, 3H, 4″-CH$_3$), 3.23 (qdd, J = 15.8, 5.8, 1.4 Hz, 2H, 1′-CH$_2$), 4.73–4.78 (m, 2H, 1″-CH$_2$), 5.55 (tp, J = 7.2, 1.4 Hz, 1H, 2″-CH), 6.18 (d, J = 9.5 Hz, 1H, 3-CH), 6.94 (brt, J = 1.2 Hz, 1H, 5-CH), and 7.56 (d, J = 9.5 Hz, 1H, 4-CH). ^{13}C NMR (100 MHz, CDCl$_3$) δ 18.1, 24.5, 25.8, 25.9, 29.9, 69.5, 71.6, 91.4, 112.3, 113.7, 117.4, 120.1, 125.7, 129.9, 139.3, 143.9, 148.1, 154.8, and 160.9. NMR data are in agreement with those reported in the literature but not the specific rotation value[13] (Figure 1).

2.4.3. Base Hydrolysis of 8-(3-Methylbut-2-enyloxy)-marmesin Acetate (1). To a solution containing **1** (200 mg, 537 mmol) in methanol (10 mL) was added 10% aqueous NaOH (20 mL), and the mixture was stirred at room temperature for 4 h. The solution was diluted with water (50 mL), acidified with 1 N HCl and extracted with CH$_2$Cl$_2$ (75 mL × 2), washed with water (50 mL) followed by saturated brine (50 mL) and dried over anhydrous Na$_2$SO$_4$, and the solvent was evaporated to afford a viscous gum. The product was purified by Biotage flash chromatography (10 g SNAP column using 20–80% ethyl acetate in hexane) to obtain a gum. Optical rotation and NMR data were in agreement with those for **2**.

2.5. Quantification of 1 in the Ethyl Acetate Extract by HPLC. Chromatography was carried out on an Agilent 1260 Infinity HPLC system equipped with a quaternary pump and a diode array detector using LUNA C-18 (2) 100A column (250 × 4.6 mm, 10μ; Phenomenex) fitted with a silica guard column (10 × 3 mm) at 25 °C. The mobile phase was a gradient from 5 to 95% acetonitrile in 0.1% formic acid in water over 20 min, followed by column wash and re-equilibration at a flow rate of 1.0 mL/min. UV wavelengths 254 and 280 were used to monitor the signals. The injection volume was 5 μL. A standard solution of purified **1** was used to construct the standard curve (R^2 = 0.99) with 1, 0.5. 0.25,

0.125, 0.0625, and 0.0312 mg/mL standard solutions. Ethyl acetate extract of *A. elemifera* 1.0 and 0.5 mg/mL solutions were used for the quantification of **1** in the extract.

2.6. Bioassay against *Lactuca sativa* and *Agrostis stolonifera*. Plant extracts, silica gel column chromatographic fractions, and isolated pure compounds were tested on *L. sativa* and *A. stolonifera* seeds according to Dayan et al. to check for phytotoxic activity.[14] *L. sativa* (Iceberg A Crisphead from Burpee Seeds) and *A. stolonifera* seeds [Penncross variety (Creeping Bentgrass species) obtained from Turf-Seed, Inc. of Hubbard, Oregon] were surface sterilized prior to use in bioassay by mixing with a 0.5−1% sodium hypochlorite solution for approximately 10 min. Seeds were thoroughly rinsed with sterile deionized water and air-dried in a sterile environment.

In a 24-well multiwell plate (Corning Inc., Corning, NY) *A. solonifera* seeds (10 mg) and *L. sativa* (5 seeds) were placed in each well on a filter paper (Whatmann no. 1). The test compounds and fractions were dissolved in acetone and DI water such that the final concentration of acetone was 10%. To each well-containing seeds, 250 μL of test solution was added while the control wells were treated only with 250 μL of acetone and DI water. Atrazine 1000 μM solution was used as the positive control. The plate was covered and sealed using Parafilm and incubated in a Percival Scientific CU-36L5 incubator under continuous light conditions at 26 °C and 120 μmol s^{-1} m^{-2} average photosynthetically active radiation (PAR). The phytotoxic activity was qualitatively evaluated visually by comparing the extent of germination of the seeds in each well after 7 days for *L. sativa* and after 10 days for *A. stolonifera* using a rating scale of 0−5, where 0 represented no effect (all of the seeds germinated) and 5 represented no germination of seeds. Each experiment was carried out in triplicate.

2.7. Bioassays with *Lemna paucicostata*. *L. paucicostata* stocks were grown from a single colony consisting of a mother and two daughter fronds in a beaker in Hoagland's No. 2 Basal Salt Mixture (Sigma H2395) (1.6 g/L) with added iron (1 mL of 1000× FeEDTA solution to 1 L of Hoagland media). The pH of the media was adjusted to 5.5 with 1 N NaOH and was filter sterilized using a 0.2 μm filter. The *L. paucicostata* stocks were grown in approximately 100 mL of media in sterile jars with vented lids in a Percival Scientific CU-36L5 incubator under continuous light conditions at 26 °C and 120 μmol s^{-1} m^{-2} average PAR. Plant doubling time was approximately 24−36 h. Assays were conducted using nonpyrogenic polystyrene sterile six-well plates (CoStar 3506, Corning Incorporated). Each well contained 4950 μL of the Hoagland's media and 50 μL of water, or the solvent, or the compound dissolved in the appropriate solvent (at a concentration of 100×). The final concentration of the solvent was therefore approximately 1% by volume. A graphic template of the six-well plates was used for LemnaTec (LemnaTec, Würselen, Germany) image analysis software. Each well was inoculated with two three-frond plants of the same age (4−5 day old) and approximate size. All six-well plates were incubated in the Percival incubator as described above at 26 °C and 120 μmol s^{-1} m^{-2} average PAR.

The plant areas were measured at day 0 and 7 and varied days in between using LemnaTec image analysis methodology, and the results are saved as csv files.[15,16] These files show frond number, total frond area as well as color classes that indicated chlorotic and necrotic effects.

2.8. Cellular Leakage Test. Cellular leakage test was carried out according to a modification of the method of Duke and Kenyon.[17] *Cucumis sativus* seeds were grown in flats with Miracle Grow potting soil in a Conviron growth chamber under 173 μmol s^{-1} m^{-2} continuous PAR at 26 °C for 6 days. Disks 4 mm in diameter were cut from cotyledons of 6-day-old plants with a cork borer under dim green light. Fifty disks were placed in polystyrene Petri dishes (6 cm in diameter) with 5 mL of 1 mM MES buffer [2-(4-morpholino)ethane sulfonic acid] supplemented with 2% sucrose (by weight) and were adjusted to pH 6.5 with 1 N NaOH. The test compounds were dissolved in acetone and were added directly to the buffer. Compounds were tested at concentrations of 10, 100, and 1000 μM. Electrical conductivity readings of the solutions were taken with a dip cell and a model 1056 digital conductivity meter (VWR Scientific) to measure cellular leakage. Conductivity measurements were taken at an initial 0 h time and also at 1, 2, 4, 6, and 8 h after exposure to the chemical in the dark at 25 °C. Dishes were then covered with aluminum foil and placed in the darkness for a total of 18 h after exposure to the chemical. Another reading was taken in the dark before placing the dishes in the light (200 μmol s^{-1} m^{-2} PAR). Readings were taken at 0, 1, 2, 4, 6, and 8 h, and the results were plotted as changes in conductivity. The experiment was performed in triplicate. A maximum potential leakage reading was taken for the solution with the same number of cucumber disks boiled for 8 min in MES buffer.

2.9. Bioautography against *Colletotrichum* Species. Isolates of *Colletotrichum fragariae* Brooks were obtained from Barbara J. Smith, USDA, ARS Poplarville MS and were maintained at USDA, ARS University, MS. Fungi were grown on potato dextrose agar (PDA, Difco, Detroit MI) and incubated at 24 °C under fluorescent light (55 μmol/m^2/s) with a 12 h photocycle. Conidia were harvested according to previously published methods.[18] The crude extracts and isolated pure compounds were eluted in a suitable mixture of ethyl acetate in hexane on silica gel TLC plates, and the plates were dried in a fume hood for 15 min. The plates were sprayed with a spore suspension (10^6 spores/mL) of the *C. fragariae* spores and incubated for 4 days in a moisture saturated transparent box at 26 °C under fluorescent light with a 12 h photoperiod. Clear zones of inhibition on the TLC plate indicated the antifungal constituents. Both compounds **1** and **2** were tested with TLC bioautography for fungitoxicity. Only compound **1** was antifungal against *C. fragariae* at 1000 μM, and this low level of activity did not warrant further study with micro bioassays to determine IC$_{50}$.

3. RESULTS AND DISCUSSION

3.1. Structure Elucidation of 1. The compound **1** was obtained as a white crystalline solid. The HR-DART positive mode mass spectrometric analysis exhibited the *pseudo*-molecular ion [M + H]$^+$ at *m/z* 373.16511, which corresponds to the molecular formula C$_{21}$H$_{24}$O$_6$. The UV spectrum of **1** in MeOH showed a λ_{max} of 331 nm typical for a coumarin. It was also visualized as a blue fluorescent and as a dark quenching spot on TLC at 365 and 254 nm, respectively. The ^{1}H (400 MHz) NMR spectrum revealed the AB doublets (*J* = 9.5 Hz) at δ 6.20 and 7.57 characteristic for a coumarin ring. ^{13}C and DEPT 135 spectrum showed the presence of five CH$_3$ groups, two CH$_2$ groups, five CH groups, and nine quaternary carbons, including two carbonyl groups at 170.3 and 161 ppm. The carbon resonance at 170.3 ppm was assigned to the OAc

carbonyl carbon, and the resonance at 161 ppm was assigned to the coumarin ring carbonyl based on HMBC correlations. HSQC spectrum indicated only one aromatic proton at δ 6.94 (brt, J = 1.2 Hz) other than the two coumarin ring protons, suggesting the presence of a trisubstituted aromatic ring. HMBC correlations of the proton at δ 6.94 (C-5 proton) with 144.9 (C-4), 148.4 (C-9), and 155 (C-7) ppm confirm that the single aromatic proton is at C-5. Also the HMBC correlation of 155 (C-7) with 6.94 (brt, J = 1.2 Hz), 5.11 (dd, J = 9.5, 7.7 Hz, 1H, 2′-CH), 3.27 (ddd, J = 15.9, 9.6, 1.3 Hz, 1H, 1a′-CH$_2$), and 3.20 (ddd, J = 16, 7.72, 1.16 Hz, 1H, 1′b-CH$_2$) confirms that the furan ring is fused at C-6 and C-7. Two vinylic methyl singlets together with AB$_2$ spin system is attributed to 3-methylbut-2-enyloxy moiety at C-8. The main difference between **1** and **2** is the presence of an OAc group at C-3′. Analysis and comparison of proton, DQFCOSY, HSQC, and HMBC spectra of **1** and **2** suggest the structure of **1** as shown. To determine the absolute stereochemistry at C2′ of **1**, X-ray crystallographic data were obtained.

Crystal data of (**1**): C$_{21}$H$_{24}$O$_6$, orthorhombic space group $P2_12_12_1$; a = 7.5977 (12) Å; b = 12.622 (2) Å; c = 19.483 (3) Å; V = 1868.5(5) Å^3; Z = 4; μ = 0.80 mm^{-1}. Refinement using 3467 reflections with θ = 3.5−69.3° and 250 parameters yielded R = 0.028. The absolute configuration was determined from Flack parameter x = 0.05(3) based on 1446 quotients. A higher-resolution refinement was also done, based on a data set to θ_{max} = 39.3° with Mo Kα radiation. Results of both refinements have been deposited with the Cambridge Crystallographic Data Centre, with the deposition numbers CCDC 1866085−1866086 (Figure 2).
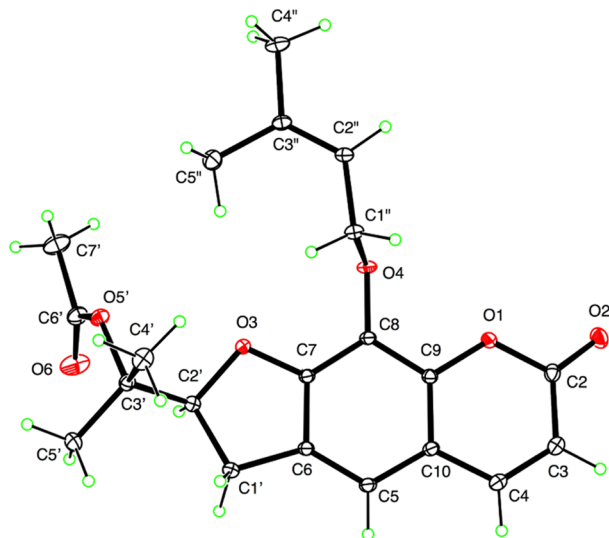

Figure 2. Crystal structure of (**1**) obtained from X-ray crystallography indication S configuration at C-2′.

X-ray crystallographic data indicated the absolute stereochemistry at C2′ of **1** as S, thus confirmed the structure as shown in Figure 1.

3.2. Phytotoxicity. The ethyl acetate extract of *A. elemifera* had the higher phytotoxic activity than the hexane and MeOH extracts at 1 mg/mL with rankings of 4 and 3 for *A. stolonifera* (monocot) and *L. sativa* (dicot), respectively (Table 1). Therefore, the ethyl acetate extract was subjected to bioassay-guided fractionation using the Biotage silica gel flash chromatography system using increasing amounts of ethyl

Table 1. Phytotoxicity Ranking of *A. elemifera* Ethyl Acetate Extract and Silica Gel Column Fractions (Fractions 19−21) of the Same at 1 mg/mL at 7 and 10 Days After Treatment for *L. sativa* and *A. stolonifera* Seeds, Respectively[a]

sample ID	lettuce	agrostis
A. elemifera EtOAc extract	3	4
A. elemifera EtOAc frac 19	2	2
A. elemifera EtOAc frac 20	3	4
A. elemifera EtOAc frac 21	3	4

[a]The solvent used was 10% acetone in deionized water. Ranking based on a scale of 0−5, where 0 = no effect and 5 = no growth.

acetate in hexane (5−100% ethyl acetate). Similar fractions according to the TLC profile were combined to obtain 28 fractions. Fractions 20 and 21 showed the highest phytotoxicity activity with the ranking of 4 and 3 for *A. stolonifera* and *L. sativa*, respectively (Table 1). Upon further purification of combined fractions of 20 and 21 using Biotage flash chromatography followed by repeated crystallization using ethyl acetate:methylene chloride:hexane (1:1:4) solvent mixture to afford white crystals. This crystalline compound was tested on *A. stolonifera* and *L. sativa* (Table 1). Upon further purification of fraction 24 of the fractionation of ethyl acetate extract, hydroxyl analog **2** was obtained as a colorless gum.

The effects of **1** and **2** on the growth of *L. sativa* germinating seeds were evaluated in a dose-dependent manner (Table 2). Compound **1** is more phytotoxic than **2**, perhaps because its higher lipophilicity allows better movement across cell membranes.

Table 2. Phytotoxicity Ranking of 1 and 2 at Varying Molar Concentrations at 7 and 12 Days After Treatment for *L. sativa* and *A. stolonifera* Seeds, Respectively[a]

	concentration (μM)	lettuce	agrostis
	sample (**1**)		
	3	0	0
	10	0	1
	33	0	2
	100	0	3
	330	0	4
	1000	2	4
atrazine	1000	5	5
	sample (**2**)		
	0	0	0
	10	0	0
	33	0	0
	100	1	0
	333	2	2
	1000	2	2
atrazine	1000	5	5

[a]The solvent used was 10% acetone in deionized water.

A cellular leakage bioassay with **1** was conducted to see if its activity was related to plasma membrane function or was light dependent. *C. sativus* leaf disks were treated with **1** in a dose-response manner, and conductivity of the bathing solution was measured. If the test compound disrupts the cell membrane, releasing electrolyte metabolites, the conductivity of the solution will increase (Figure 3). Acifluorofen, a known
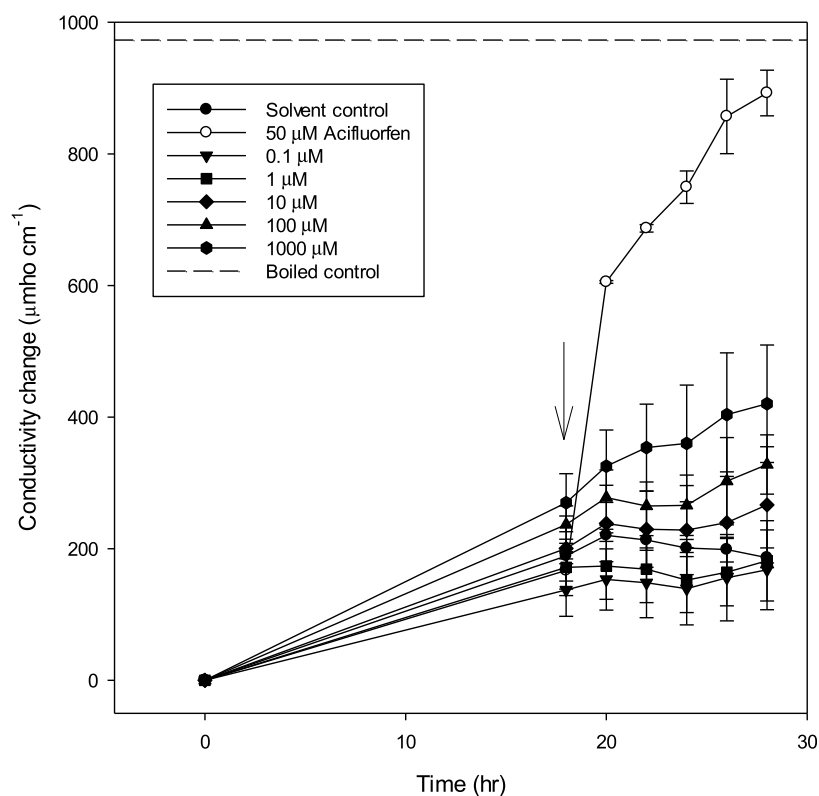
Figure 3. Conductivity changes of the solution of the cumber cotyledon disks treated with (**1**) at varying concentrations (0.1, 1, 10, 100, and 1000 μM) in the dark and after exposing to light (arrow) at 18 h. The dotted line represents the maximum possible leakage value determined by boiled leaf disks in MES buffer. Each treatment was carried out in triplicate. Error bars are ± 1 standard error of the mean.
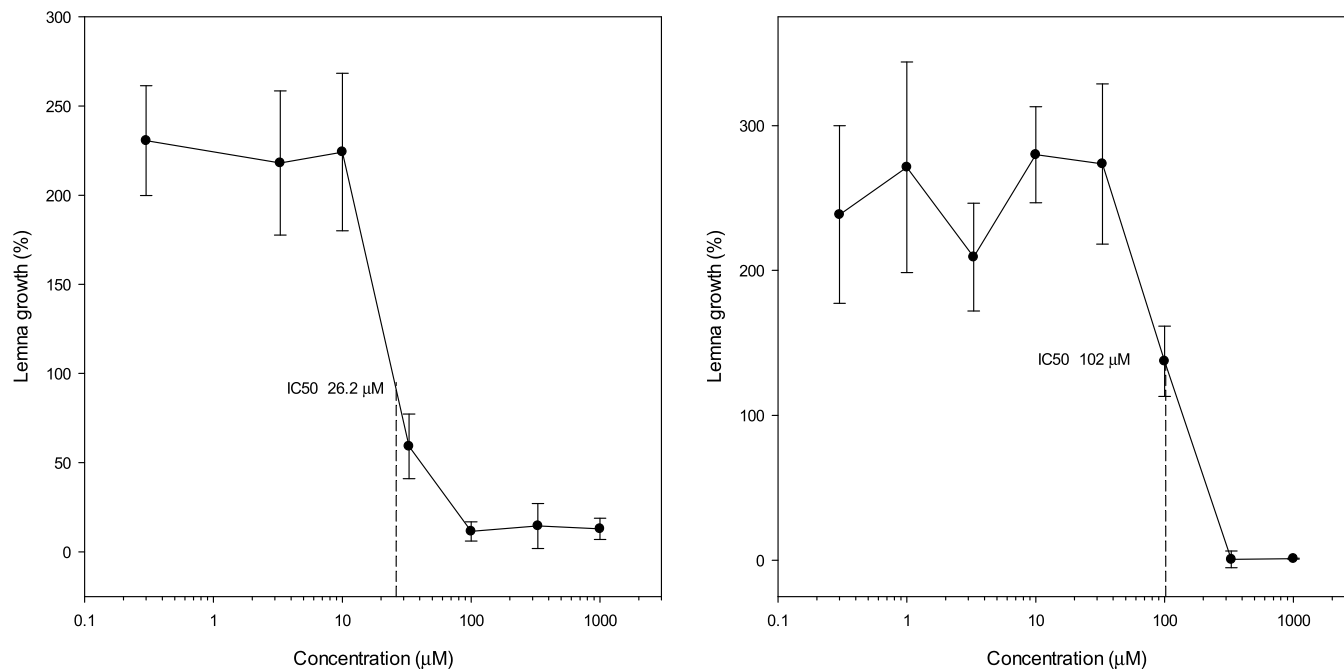

Figure 4. Effects of **1** and **2** on the growth (% of initial frond area) of *L. pausicostata* at varying concentrations after 7 days of exposure. Each treatment was carried out in triplicate. Error bars are ± 1 standard error of the mean. IC_{50} values are marked for each compound with a dotted line.

herbicidal compound that causes pronounced cellular leakage in the light, was used as the positive control. The bathing solution with boiled *C. sativus* leaf disks was used to measure the maximum possible conductivity reading caused by cellular leakage. Compound **1** causes low levels of dose-dependent

leakage in the dark. The leakage rate decreases temporarily when exposed to light after 18 h in the dark, except at 1000 μM, in which case the leakage rate remained the same. Thus, the phytotoxicity is not light dependent and is unlikely to be related to a plasma membrane effect.
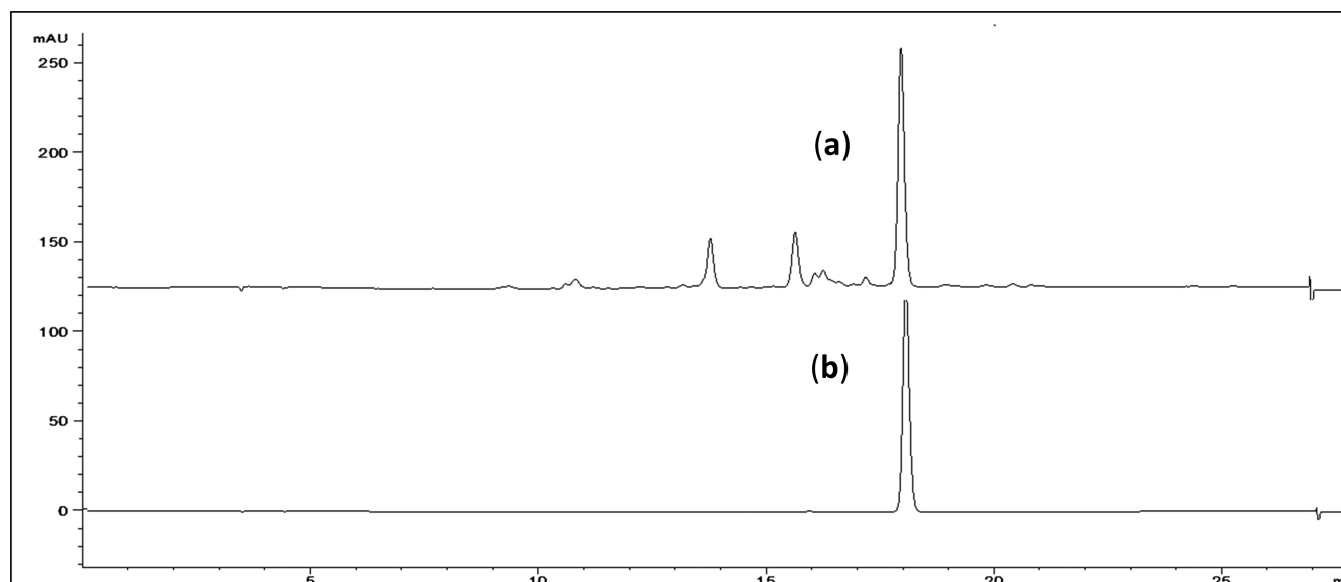
Figure 5. HPLC profiles of ethyl acetate extract of *A. elemifera* (a) and **1** (b) at 0.5 mg/mL monitored at 254 nm. The injection volume was 5 μL, and the flow rate was 1.0 mL/min.

To evaluate the quantitative value of phytotoxic activity, **1** and **2** were tested on the growth of *L. pausicostata* at varying concentrations. At 30, 100, 300, and 1000 μM concentrations of **1**, growth of *L. pausicostata* plants was significantly inhibited (Figure 4) and bleaching or chlorosis of the plants was observed. At 100 μM and higher concentrations, complete inhibition of growth of *L. pausicostata* plants occurred. At or below 10 μM of **1**, the inhibitory effect on growth of *L. pausicostata* was not observed. At the same concentrations, the inhibitory effect of **2** on the growth of *L. pausicostata* was less effective (Figure 4). Complete inhibition of *L. pausicostata* with **2** was observed only at or above 330 μM. The IC_{50} values for **1** and **2** were calculated using the plots (Figure 4) to be 26.2 and 102 μM, respectively. Some herbicidal compounds can cause cellular leakage and chlorosis (bleaching of leaves due to inhibition of chlorophyll production).[17]

We have isolated a novel furanocoumarin (**1**) and its deacetyl analog (**2**). The furanocoumarin (**1**) is the major constituent in the ethyl acetate extract. HPLC analysis data indicated that the concentration of **1** was around 338 mg/g of the ethyl acetate extract (Figure 5). X-ray data of furanocoumarin (**1**) indicated the absolute stereochemistry of **1** at C-2′ to have the S configuration (Figure 2). Optical rotation measurements showed the $[\alpha]_D$ (−) 36.6 (*c* = 0.2, CHCl$_3$) for **1**. The KOH hydrolyzed product of **1** showed the $[\alpha]_D$ (−)16.5 (*c* = 0.2, CHCl$_3$) that was the same value as that of the specific rotation for the naturally occurring **2**. NMR data for the KOH hydrolyzed product of **1** are in full agreement with those for **2**. During base hydrolysis of **1**, the stereochemistry at C-2′ remains unchanged; thus, we can conclude that **2** and KOH hydrolyzed product of **1** to be identical compounds. A compound with identical NMR data of **2** has been previously isolated from *Microcybe multiflora* Turcz., another plant in the Rutaceae family.[13] However, the specific rotation for this compound is reported as $[\alpha]_D$ (+) 126 (*c* = 0.2, CHCl$_3$), not the identical negative value for that was observed for **2**. It is not very common that two members of the Rutaceae family would produce the same compound with opposite stereochemistry in the two species. So, the specific rotation for the isomer isolated from *M. multiflora* needs further reinvestigation. Another furanocoumarin isomer of **2** with a different fused ring system has been isolated from *Chorilaena quercifolia*, a plant in the Rutaceae family.[19] This compound was not detected in our extract, and neither the specific rotation nor the absolute stereochemistry at the stereogenic center is reported.

In summary, a novel phytotoxic compound was isolated from the ethyl acetate extract of *A. elemifera* leaves. The structure was elucidated by spectroscopic methods, and the stereochemistry was determined by X-ray crystallography. These data could be extrapolated to determine the stereochemistry of the deacetyl compound, which was isolated from the same extract. This novel compound or analogs can be used as templates in developing more potent phytotoxic compounds that can be useful in agriculture. The mechanism of phytotoxicity is not obvious, but more experiments need to be carried out. We have previously isolated biologically active compound from members in the Rutaceae family.[8−12] The work presented in this paper adds novel information to the ecological importance of secondary metabolites produced by plants in the Rutaceae family.

■ ASSOCIATED CONTENT

ⓈI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04778.

> ^{1}H NMR spectrum, ^{13}C NMR spectrum, DEPT 135 NMR spectrum, and DQFCOSY NMR spectrum of (**1**) (PDF)

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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