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Antiherbivore Prenylated Benzoic Acid Derivatives from *Piper kelleyi*

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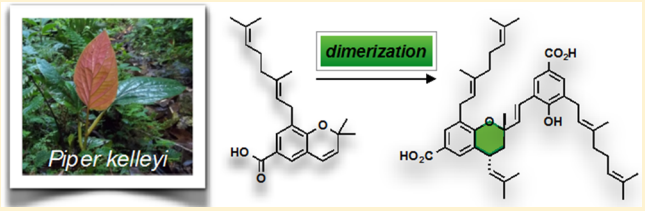
Supporting Information

ABSTRACT: The known prenylated benzoic acid derivative 3-geranyl-4-hydroxy-5-(3″,3″-dimethylallyl)benzoic acid (**1**) and two new chromane natural products were isolated from the methanolic extract of the leaves of *Piper kelleyi* Tepe (Piperaceae), a midcanopy tropical shrub that grows in lower montane rain forests in Ecuador and Peru. Structure determination using 1D and 2D NMR analysis led to the structure of the chromene **2** and to the reassignment of the structure of cumanensic acid as **4**, an isomeric chromene previously isolated from *Piper gaudichaudianum*. The structure and relative configuration of new chromane **3** was determined using 1D and 2D NMR spectroscopic analysis and was found to be racemic by ECD spectropolarimetry. The biological activity of **1**−**3** was evaluated against a lab colony of the generalist caterpillar *Spodoptera exigua* (Noctuidae), and low concentrations of **2** and **3** were found to significantly reduce fitness. Further consideration of the biosynthetic relationship of the three compounds led to the proposal that **1** is converted to **2** via an oxidative process, whereas **3** is produced through hetero-[4+2] dimerization of a quinone methide derived from the chromene **2**.



The genus *Piper* (Piperaceae) is a diverse pantropical plant genus that is a rich source of new biologically active natural products. These natural products include phenyl propanoids, amides, imides, lignans, neolignans, terpenoids, pyrones, and flavonoids, many of which have been established to have both ecological and medicinal relevance.[1] Investigations of the phytochemical mediation of plant−insect interactions have led to the isolation and characterization of three geranylated natural products from the recently described species *Piper kelleyi*, a midcanopy shrub that grows in lower montane rain forests in Ecuador and Peru.[2] Analysis of the methanolic extract of dried leaves resulted in the isolation and characterization of the new chromene **2** and its dimer **3**, along with a known prenylated benzoic acid derivative, 3-geranyl-4-hydroxy-5-(3″,3″-dimethylallyl)benzoic acid (**1**) (Figure 1). Herein, we report the isolation, structural elucidation, and biological activity of compounds **2** and **3** and the structural reassignment of cumanensic acid.

RESULTS AND DISCUSSION

The leaves of *P. kelleyi* were collected in a cloud forest habitat on the eastern slope of the Andes, Yanayacu Biological Station, Napo Province, Ecuador, 2100 m elevation, 77.90W, 00.60S. The field station is at the upper end of the elevational range of this plant, but densities were high at the collection site. The leaves were dried in an outdoor drying cabinet at ambient temperatures (mean temperature ∼27 °C) for 2−5 days. Ground leaves were extracted using MeOH with sonication, followed by vacuum filtration through a course frit and evaporation under reduced pressure to provide a crude oil. The crude oil was purified using RP-MPLC, providing baseline separation of three benzoic acid derivatives.

3-Geranyl-4-hydroxy-5-(3″,3″-dimethylallyl)benzoic acid (**1**) was obtained as a colorless oil from RP-MPLC. HRESIMS analysis revealed a molecular formula of $C_{22}H_{30}O_3$, and FT-IR provided evidence of a carboxylic acid [3270 (broad) and 1680 cm^{-1}]. Analysis of ^1H NMR data suggested the presence of a 1,2,3,5-tetrasubstituted aromatic ring and three prenyl units as indicated by the vinylic resonances and the two benzylic methylene resonances. ^{13}C NMR spectra indicated the presence of an aromatic carboxylic acid and a phenolic hydroxyl moiety, which was supportive of a prenylated hydroxybenzoic acid derivative. This compound was spectroscopically identical to 3-geranyl-4-hydroxy-5-(3″,3″-dimethylallyl)benzoic acid (**1**), an anti-inflammatory prenylated benzoic acid derivative isolated from *Myrisine* and *Rapanea* species.[3]

The second eluting fraction from the column was found to have the formula $C_{22}H_{28}O_3$ (HRESIMS), suggesting a closely related, but oxidized (−2H), derivative of the prenylated benzoic acid **1**. Aromatic doublets (δ_H 7.66 and 7.52, J = 2.2 Hz) in the ^1H NMR spectrum provided evidence of a 1,2,3,5-tetrasubstituted aromatic moiety (Table 1). Vinylic proton resonances at δ_H 5.28 (tq) and 5.0 (t sept), allylic methylene

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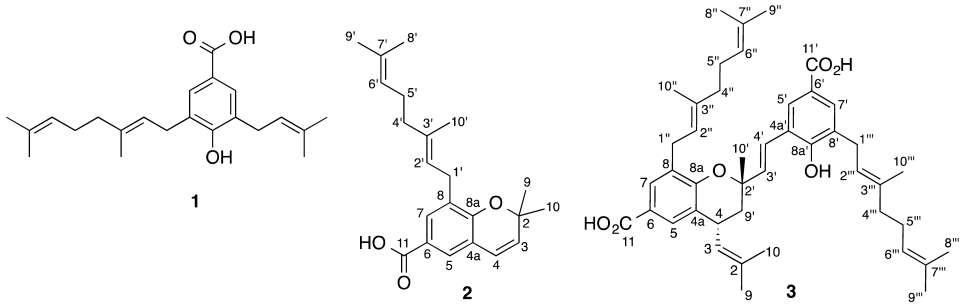

Figure 1. Structures of the known acid **1** and the new compounds **2** and **3**.

Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Chromene 2

position	δ_C, type	δ_H	HMBC[a]
2	77.6, C		
3	130.8, CH	5.65 d (9.8 Hz)	2, 4a, 9/10
4	122.2, CH	6.35 d (9.8 Hz)	2, 4a, 5, 8a, 9/10
4a	120.4, C		
5	126.8, CH	7.61 d (2.2 Hz)	7, 8, 8a, 11
6	121.0, C		
7	132.0, CH	7.76 d (2.2 Hz)	1′, 5, 8a, 11
8	129.5, C		
8a	155.8, C		
9	28.5, CH_3	1.45 s (3H)	3, 8a, 2
10	28.5, CH_3	1.45 s (3H)	3, 8a, 2
11	172.1, C		
1′	28.3, CH_2	3.3 d (7.3 Hz)	8a, 3′, 2′, 7, 8
2′	121.9, CH	5.3 tq (7.3, 1.0 Hz)	1′, 4′, 5′, 10′
3′	136.4, C		
4′	39.9, CH_2	2.06–2.01	2′, 3′, 5′, 6′, 10′
5′	26.8, CH_2	2.13–2.07	3′, 4′, 6′, 7′
6′	124.4, CH	5.11 tsept (7.0, 1.0 Hz)	4′, 5′, 8′, 9′
7′	131.6, C		
8′	17.8, CH_3	1.59 br s (1.0 Hz)	9′, 6′, 7′
9′	25.8, CH_3	1.67 br q (1.1 Hz)	8′, 6′, 7′
10′	16.3, CH_3	1.74 d (1.0 Hz)	4′, 2′, 3′

[a]HMBC correlations are from proton(s) stated to the indicated carbon.

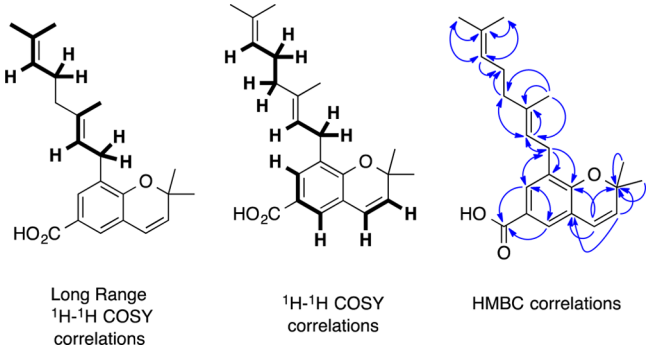

Figure 2. $^{1}H-^{1}H$ COSY and $^{1}H-^{13}C$ HMBC correlations for chromene **2**.

resonances (apparent t, δ_H 2.10 and 2.03), and methyl resonances at δ_H 1.74, 1.67, and 1.59 revealed substitution by a geranyl unit. Distinct doublets at δ_H 6.39 and 5.73 (J = 9.8 Hz) provided evidence of the presence of a chromene moiety. A literature search of prenylated chromenes with carboxylic acid functional groups indicated two isomeric chromene

Table 2. Comparisons of Reported ^{13}C and ^{1}H NMR Chemical Shifts of Cumanensic Acid (CA)[5] and Gaudichaudianic Acid (4),[4] with Experimental Values for the Chromene 2 Isolated from *Piper kelleyi* in $CDCl_3$

		^{13}C NMR $\Delta\delta$		^{1}H NMR $\Delta\delta$	
position (**2**)	equivalent position (**4**)	$\Delta\delta$ (δ**4** − δCA)	$\Delta\delta$ (δCA − δ**2**)	$\Delta\delta$ (δ**4** − δCA)	$\Delta\delta$ (δCA − δ**2**)
2	2	−0.1	**2.3**		
3	3	0.1	−1.3	−0.01	−0.07
4	4	−0.7	−0.3	−0.02	0.03
4a	4a	0.5	0.2		
5	5	0	−0.1	−0.02	−0.02
6	6	0	−0.2		
7	7	0	−0.2	−0.02	−0.01
8	8	0	−0.6		
8a	8a	−0.1	0		
9	9	−0.2	**−1.6**	−0.01	−0.04
10	4″	0.1	**−2.7**	−0.02	**0.28**
11	10	−0.2	0		
1′	1″	0	−0.1	−0.03	−0.02
2′	2″	0	0	−0.02	−0.02
3′	3″	0	**−3.8**		
4′	1′	0.5	2	0.03	**−0.25**
5′	2′	0	**−4.1**	**0.12**	**0.15**
6′	3′	0	−0.5	−0.01	−0.02
7′	4′	−0.1	0.2		
8′	5′	0.1	−0.2	−0.01	−0.03
9′	6′	0	−0.2	0.01	0
10′	5″	0	**6.4**	−0.02	−0.01
average deviation		0.12	**1.2**	0.01	**0.07**

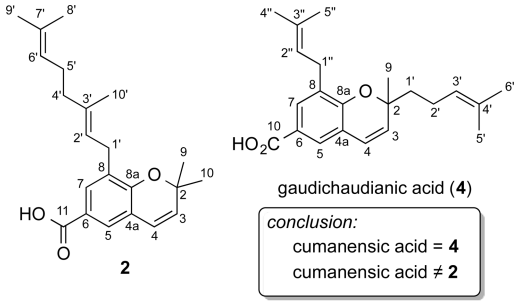

Figure 3. Summary of the structural reassignment of cumanensic acid as **4**.

derivatives that were previously isolated from *Piper* species.[4,5] $^{1}H-^{1}H$ COSY analysis provided evidence of a geranyl substituent with correlations between the H-1′ and C-4′ methylene resonances and HMBC correlations between H-2′

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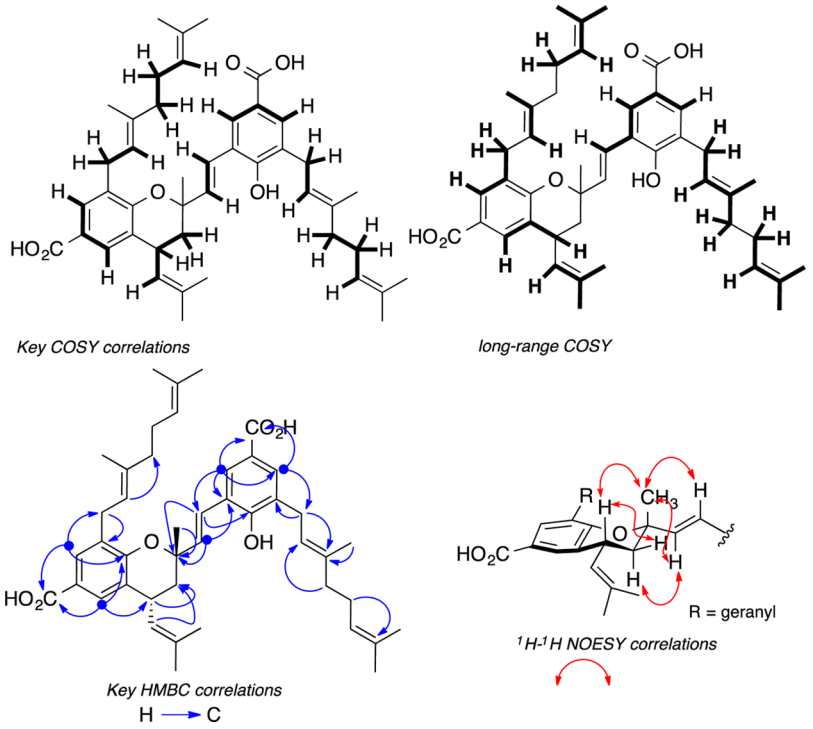

Figure 4. Key $^{1}H-^{1}H$ COSY, NOESY, and HMBC correlations for the dimeric benzopyran **3**.

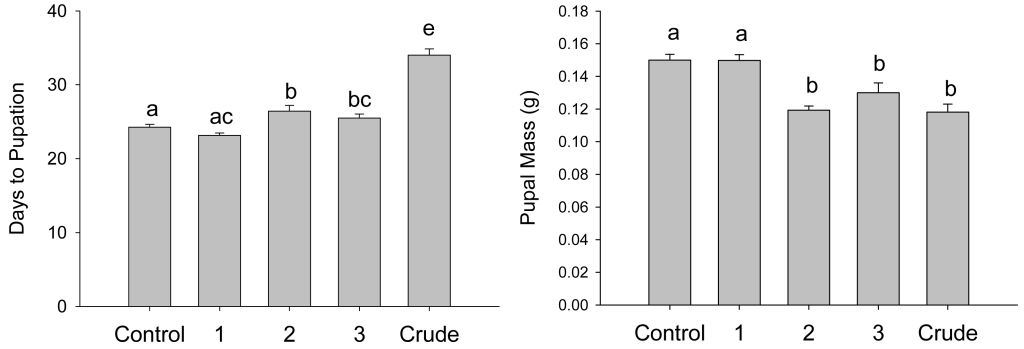

Figure 5. Bioassay data showing significant treatment effects of compounds **1**, **2**, and **3** and the mixture ("crude") on larval development and pupal mass (ANOVA, $F_{4,56}$ = 37.025 and 9.790, respectively, $p < 0.001$). Letters indicate significant differences between treatments (post hoc, LSD $p < 0.05$).

and C-4′. Key HMBC correlations between the chromene-ring methyl resonances (δ_H 1.45, 6H) and the quaternary carbon (δ_C 77.6) along with correlation between the C-1 benzylic methylene (δ_H 3.30) and C-8 (δ_C 129.5) and C-8a (δ_C 155.8) was consistent with the structure of the chromene that was previously reported for cumanensic acid, reported from *P. cumanense* (Figure 2).[5]

Comparison of the ^{1}H and ^{13}C NMR data highlighted numerous discrepancies between the spectroscopic data of **2** and cumanensic acid, revealing that the structure of cumanensic acid was incorrectly assigned (Table 2). Further analysis of the NMR data suggested that cumanensic acid was not **2**, but instead the isomeric chromene gaudichaudianic acid (**4**), a known compound isolated from *P. gaudichaudianum*.[4] Indeed, the reported ^{1}H and ^{13}C NMR data for cumanensic acid and **4** were identical and confirmed our assignment of **2** as a new chromene from *P. kelleyi* and established the reassignment of the structure cumanensic acid as gaudichaudianic acid (**4**) (Figure 3).

The third, least polar fraction was determined to have the formula $C_{44}H_{56}O_6$ by HRESIMS analysis, exactly double that of **2** and indicating that **3** is a dimer of the chromene **2**. Analysis of the ^{1}H NMR spectrum revealed similarities to the ^{1}H NMR spectrum of **1** and **2**, but with four distinct aromatic resonances corresponding to two 1,2,3,5-tetrasubstituted aromatic rings. Doublets at δ_H 7.21 and 6.37 (J = 16.1 Hz) provided evidence of a conjugated disubstituted *E*-olefinic moiety. A ddd at δ_H 3.68 (J = 9.4, 9.4, 6.4 Hz) indicated a benzylic methine proton that was coupled to two diastereotopic methylene protons (δ_H 1.67 and 1.61) and the vinylic proton (δ_H 5.10) of a prenyl group via $^{1}H-^{1}H$ COSY analysis. The ^{13}C NMR spectrum provided evidence of two carboxylic acid moieties (δ_C 173.1 and 172.6) and two oxygenated aromatic carbon atoms (δ_C 157.6 and 156.6). Long-range $^{1}H-^{1}H$-COSY coupling between the C-5 aryl proton and the C-4 methine proton and additional coupling between the *E*-vinylic resonances and C-5′ confirmed the 1,2,3,5-substitution patterns for both aromatic rings. These structural assignments were further confirmed by HMBC

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Table 3. ^{1}H and ^{13}C NMR Data of Dimeric Benzopyran (3)

position	δ_H (J in Hz)	δ_C, type	HMBC[b]
1			
2		133.6, C	
3	5.10 m[a]	127.6, CH	9, 10
4	3.68 ddd (6.7, 6.7, 9.2 Hz)	32.3, CH	2, 3, 4a, 9′
4a		124.6, C	
5	8.18 d (2.1)	130.6, CH	4, 8a, 7, 11
6		122.2, C	
7	8.23 d (2.1)	130.6, CH	5, 8a, 11, 1″
8		132.2, C	
8a		156.6, C	
9	1.69 d (1.4)	25.8, CH_3	10, 3, 2
10	1.61 d (1.4)	17.8, CH_3	9, 3, 2
11		173.1, C	
1′			
2′		77.5, C	
3′	6.37 d (16.1)	137.4, CH	2′, 4a′, 10′
4′	3.67	121.8, CH_2	8a′, 3′, 5′, 2′
4a′		125.4, C	
5′	8.48 d (1.7)	128.6, CH	11′, 8a′, 7′, 4′
6′		128.7, C	
7′	7.99 d (2.0)	131.9, CH	11′, 8a′, 5, 1‴
8′		127.2, C	
8a′		156.5, C	
9′	1.62, 1.68 m[a] overlapped	39.1, CH_2	
10′	1.28 s	24.6, CH_3	9′, 2′, 3′
11′		172.6, C	
1″	3.63 dd (15.4, 7.1), 3.56 dd (15.4, 7.2)	29.2, CH_2	8a, 3″, 5, 7, 2″
2″	5.6 br t (7.1 Hz)	122.8, CH	10″, 1″, 4″
3″		136.5, C	
4″	2.14 br t (7.6 Hz)	40.3, CH_2	10″, 5″, 3″, 6″, 2″
5″	2.22 br q (7.7 Hz)	27.2, CH_2	4″, 3″, 7″, 6″
6″	5.26 t sept (7.0, 1.3 Hz)	125, CH	8″, 9″
7″		131.2, C	
8″	1.57 s	17.9, CH_3	9″, 6″, 7″
9″	1.7 s	25.9, CH_3	8″, 6″, 7″
10″	1.75 s	16.4, CH_3	4″, 2″, 3″
1‴	3.03 d (7.2 Hz)	30.4, CH_2	2‴, 3‴, 8′, 2‴
2‴	5.12 m[a]	121.3, CH	1‴, 4‴, 10‴
3‴		140.1, C	
4‴	1.93 br t (7.3 Hz)	39.8, CH_2	10‴, 2‴, 3‴, 5‴
5‴	2.05 br q (7.3 Hz)	26.6, CH_2	4‴, 3‴, 6‴, 7‴
6‴	5.08 m[a]	124.1, CH	8‴, 9‴
7‴		132.2, C	
8‴	1.53, s	17.8, CH_3	9‴, 6‴, 7‴
9‴	1.72, s	25.9, CH_3	8‴, 6‴, 7‴
10‴	1.49, s	16, CH_3	4‴, 2‴, 3‴

[a]Signal is partially obscured. [b]HMBC correlations are from proton(s) stated to the indicated carbon.

Table 4. Percent Survival of *Spodoptera exigua* on Diets (±SE)

treatment	survival (%)
control	94 (±4.3)
1	88 (±12.8)
2	88 (±12.8)
3	88 (±12.8)
crude extract	63 (±18.3)

analysis, showing correlations of H-9′, H-3′, H-4′, and H-10′ with C-2′; H-3 with C-9′; H-4 with C-9′; H-5 with C-4, C-8, and C-11; H-5′ with C-4′, C-11′, C-7′, C-8a′, and C-4a′; H-7 with C-1″, C-8a, and C-11; and H-7′ with C-11′, C-1‴, and C-8a′ (Figure 3). Furthermore, NOESY correlations between the axial H-4 and the axial C-10′ methyl group verified the relative configuration of the chromane derivative (Figure 4). The low specific rotation (−8, *c* 0.4) and the absence of a Cotton effect in the ECD spectrum revealed that **3** occurs as a racemic mixture. A literature search revealed that **3** is a new compound, and there are only three other examples of naturally occurring dimeric chromanes with a similar *E*-olefinic moiety, two of which occur as racemic mixtures.[6−8]

Evaluation of Antiherbivore Activity of Benzoic Acid Derivatives 1−3. The antiherbivore activity of the benzoic acid derivatives **1**−**3** was tested against a lab colony of the generalist caterpillar *Spodoptera exigua* (Noctuidae), using established methods.[9] Although other species of *Spodoptera* consume *Piper* leaf tissues in the wild, *S. exigua* does not and was utilized as a naïve (nonadapted) generalist herbivore assay. Briefly, second instar larvae were placed on both control and experimental diets that were treated with relatively low natural concentrations. Survival, development time, growth, and pupal mass were measured, all of which are correlated with fitness. Compounds **2** and **3** and the crude mixture significantly increased larval development time and reduced pupal mass, which is an indicator of reduced adult fecundity (Figure 5). Larval survival rates were lower on experimental diets, although not significantly (Table 4). Overall results indicated lower fitness on diets with individual compounds and with the mixture, and the magnitude of these effects was biologically significant and similar to those found for other important antiherbivore compounds in *Piper* species.[9]

Dimerization of secondary metabolites is a common pathway that can occur via biotic and abiotic pathways through various reaction types, including the Diels−Alder reaction and oxidative aryl-O bond formation.[10] The interesting biological activity, lack of optical activity, and unique structure of **3** led us to consider its biosynthetic relationship to its presumed chromene monomeric precursor **2**. Retrosynthetic analysis of the pyran ring through a hetero Diels−Alder dissection provides *ortho*-quinone methide heterodiene **6** along with an isoprenylated dienophilic component, **8** (Scheme 1). *Ortho*-quinone methide intermediates have been established to undergo facile hetero-[4+2] cycloaddition reactions with trisubstituted alkenes to provide chromanes.[11] Padwa and co-workers established that natural chromene **5** readily undergoes a photochemical retro-6π electrocyclization to generate the reactive *ortho*-quinone methide intermediate **7**.[12] The photogenerated *ortho*-quinone methide **7** tautomerized to **9** (analogous to **8**) upon irradiation in benzene or acetone and undergoes solvolysis at the electrophilic quinone-methide β-carbon when irradiated in methanol, providing **11**. Trauner and co-workers have recently demonstrated that a quinone methide intermediate, generated by *in situ* oxidation of *ortho*-prenylated phenols, provided a benzopyran dimer similar to **3** in 10% yield.[13] Given this evidence, we propose that **3** is the result of an abiotic [4+2] heterodimerization of quinone methide **6** and butadienyl dienophile **8**, which are generated by photoinduced retro-electrocyclization of the chromene **2** or by eliminative pathways from benzylic oxidized prenylated phenol **10**. Studies of the role, variation, diastereoselectivity, and mechanism of this dimerization are currently under way.

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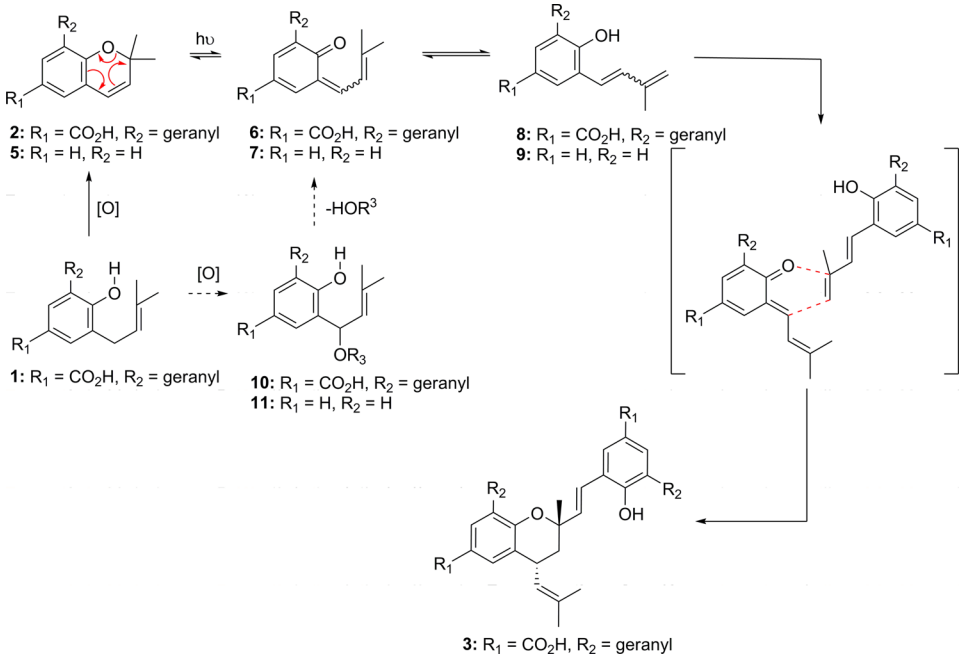
Scheme 1. Proposed Biosynthetic Hypothesis for the Formation of Dimeric Benzopyran 3 from Chromene 2



EXPERIMENTAL SECTION

General Experimental Procedures. Methanol used for the extraction of leaf material and for MPLC separation was used without further purification. TLC was performed on Silicycle glass 60 F254 plates visualized using UV light (254 nm) and developed by staining with $KMnO_4$ or ceric ammonium molybdate. ECD spectra were recorded with a Jasco J-715 spectropolarimeter. Optical rotations were recorded using a Jasco P-2000 polarimeter. ^{1}H NMR spectra were recorded on a Varian 500 (500 MHz) spectrometer, and chemical shifts are reported in ppm and coupling constant(s) in Hz, using TMS as an internal standard in $CDCl_3$ or the solvent peak (δ_H 7.16) in benzene-d_6. ^{13}C NMR spectra were recorded on the V500 spectrometer and reported in ppm using solvent as an internal standard ($CDCl_3$ at δ_C 77.16 or benzene-d_6 δ_C at 128.06). IR spectra were recorded on a Nicolet 6700 FT-IR with a diamond ATR, and data are reported as cm^{-1} (br = broad, s = strong). HRESIMS data were obtained using an Agilent 6230 TOF LC/MS with purine and HP-0921 as internal calibrants.

Plant Material. Samples of *Piper kelleyi* were collected in 2012 at the Yanayacu Biological Station, Napo Province, Ecuador (0°36′ S, 77°53′ W, 2080 m). Plant material was identified by Eric Tepe, and voucher specimens were deposited at the Herbario Nacional del Ecuador, Quito, Ecuador (QCNE), and the Missouri Botanical Gardens, USA. Newly emerging, recently expanded, and 2−5-year-old leaves were collected from understory shrubs in the late afternoon. The leaves were combined, pressed, field dried in a drying cabinet heated by four 60 W light bulbs at 27 °C, and transported to the University of Nevada, Reno, for chemical analysis.

Extraction and Isolation. The air-dried and ground leaves (2.0 g) were extracted with HPLC grade MeOH (2 × 10 mL) at room temperature with sonication (10 min per extract). The MeOH was evaporated under reduced pressure at room temperature, and the crude oil was subjected to preparative RP-MPLC chromatography (120 g, KP-C18-HS, 4.5 cm × 16 cm) eluting with a gradient of MeOH/H_2O (0.01% TFA buffer, pH = 6.5, 50 mL/min) and UV detection (210 and 254 nm). Separation via this method afforded the three components **1** (33.5 mg, t_R = 46 min), **2** (40.9 mg, t_R = 51 min), and **3** (23.2 mg, t_R = 56 min).

*3-Geranyl-4-hydroxy-5-(3″,3″-dimethylallyl)benzoic acid (**1**):* light yellow oil (33.5 mg, 1.7%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (s, 2H), 5.43−5.24 (m, 2H), 5.08 (ddq, J = 6.8, 5.3, 1.5 Hz, 1H), 3.43−3.38 (m, 2H), 2.19−2.04 (m, 5H), 1.78 (s, 3H), 1.69 (d, J = 1.4 Hz, 3H), 1.61 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 158.1, 139.2, 135.1, 132.1, 130.6, 130.6, 127.6, 127.1, 123.9, 121.5, 121.3, 121.2, 39.8, 29.8, 29.0, 26.6, 26.0, 25.8, 18.1, 17.9, and 16.4 ppm; HRESIMS m/z 365.2080 [M + Na]$^+$, requires 365.2087.

*8-[(2E)-3,7-Dimethyl-2,6-octadienyl]-2,2-dimethyl-2H-chromene-6-carboxylic acid (**2**):* light yellow oil (40.9 mg, 2.0%); FT-IR (neat) 3269, 2973, 2918, 1680, 1598, 1191, 1023 cm^{-1}; see Table 1 for ^{1}H and ^{13}C NMR data; HRESIMS m/z 341.2104 [M + H]$^+$, requires 341.2111; HRESIMS m/z 363.1926 [M + Na]$^+$, requires 363.1931.

*2-[(E)-2-{3-[(2E)-3,7-Dimethyl-2,6-octadienyl]-5-carboxy-2-hydroxyphenyl}ethenyl]-8-[(2E)-3,7-dimethyl-2,6-octadienyl]-2-methyl-4-(2-methyl-1-propenyl)-6-chromancarboxylic acid (**3**):* light yellow oil (40.9 mg, 1.2%); $[\alpha]_D^{20}$ −8 (c 0.4); FT-IR (neat) 3413, 2967, 2921, 2853, 1680, 1597, 1209 cm^{-1}; see Table 3 for ^{1}H and ^{13}C NMR data; HRESIMS m/z = 681.4132 [M + H]$^+$, requires 681.4150.

Herbivore Assay. An artificial diet was prepared from 81 g of powdered fall armyworm diet (Southland Products, Inc. Lake Village, AR, USA) and 465 mL of distilled H_2O. The bulk armyworm diet was weighed into portions for the control and experimental diets immediately after being mixed and prior to forming a gel. Experimental diets were prepared by mixing a solution of compounds **1**−**3** in EtOH (0.3 mL), 2.5% dry weight of **1**, 1.55% dry weight of **2**, 0.8% of **3**, and 3.75% dry weight of the crude extract into the partitioned diet; this yielded diet concentrations similar to leaf concentrations found in the field. A control diet was prepared by mixing EtOH (0.3 mL) into the appropriate control diet. Each treatment included 60 larvae, and response variables were recorded daily until all larvae had either died or pupated.

ASSOCIATED CONTENT

Supporting Information

Copies of spectroscopic data and ^{1}H and ^{13}C NMR chemical shift data for CA and **4** are included in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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