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The Mosaic of Rottlerin

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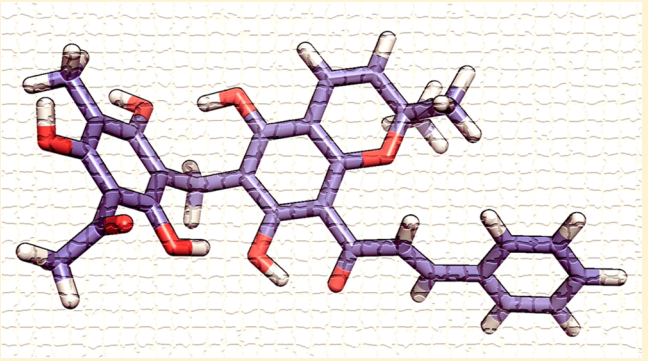
Supporting Information

ABSTRACT: The first total synthesis of rottlerin is described. The methodology allows the development of potential novel protein kinase C δ (PKCδ) analogues for better treatment of various diseases. Kamalachalcone A and dimeric rottlerin were synthesized in a very practical and economical way using $FeCl_3$ as a catalyst.



INTRODUCTION

Malloutus philippensis, a plant commonly found throughout Southeast Asia and Australia,[1] possesses a range of biological properties, including antioxidant, antiulcer, antitumor,[2] cytotoxic,[3,4] and antifungal activities.[5] The granular hairs on the surface of the fruits are covered with red exudates, called kamala, that have traditionally been used as a medicine and a dye.[6] Various complex flavonoids and other molecules, including the protein kinase C (PKC) inhibitor rottlerin, have been isolated from this plant. Among these complex molecules, kamalachalcones A, B, and E possess a unique pentacyclic ring system that is probably formed from intermolecular dimerization of a chalcone dihydropyran, also known as the "red compound".[6] Tanaka et al.[6] have hypothesized that kamalachalcone A could be synthesized via an acid-catalyzed reaction. In fact, the biosynthetic origin of kamalachalcone A and rottlerin appeared to be "red compound" related (Scheme 1).

The role of rottlerin as a specific PKCδ inhibitor has been questioned, as researchers have provided conflicting arguments. Despite the debate on the selectivity of PKCδ inhibition, rottlerin is still a compound that possesses a range of potent biological activities, including inhibition of micropinocytosism,[7] anticancer (pancreatic, prostate, breast, and lung), and antiangiogenic effects. Nevertheless, the synthesis of rottlerin has never been reported. This is perhaps due to the sensitivity of rottlerin toward both basic and acidic conditions,[8,13] which discourage the chemical synthesis. Therefore, it is desirable to develop an efficient and economical synthesis of rottlerin and its analogues and it is envisaged that the synthesis of rottlerin will bring social impact to a certain extent. This paper describes the first total synthesis of rottlerin and also the corresponding kamalachalcone dimers. We propose that the final step in the biosynthesis of kamalachalcone A involves the acid-catalyzed dimerization of a chalcone derivative.

RESULTS AND DISCUSSION

To evaluate the feasibility of this proposed biosynthetic pathway, we planned a short synthesis of kamalachalcone A employing an acid-catalyzed dimerization reaction as the final step (Scheme 2).

The synthesis of the "red compound" was prepared in six steps using the literature method;[9] a stepwise preparation from **1** to **3** using Vilsmeier−Haack formylation[10] followed by Clemmensen reduction appeared to have a higher percentage yield than the one-step methylation from **1** to **3** suggested by the literature.[9] Characterization data for all intermediates were consistent with those in the literature.[9,10] Although rottlerin resembles the "red compound" **7**, its synthesis is challenging due to its methylene bridge moiety. Although many phenolic natural products bearing a methylene bridge have been synthesized, they are symmetrical dimers and their syntheses employ a simple coupling using an aldehyde-containing substrate.[11,12] In fact, rottlerin-related structures such as mallotus B[13] and mallotochromene,[14] which are nonsymmetrical, have never been synthesized. Although Meikle and Stevens' synthesis of uliginosin A is an example of successful synthesis of unsymmetrical phloroglucinol,[15] it involves the synthesis of a symmetrical dimer with the presence of formaldehyde and phloroglucinol, followed by the convoluted so-called "rottlerone change" to give the unsymmetrical dimer via a disproportionation using NaH as a base.

Rottlerin's biosynthesis is believed to be an oxidation of the "red compound" **5** to form the highly reactive quinone methide intermediate **6** and subsequent Michael addition with the corresponding acetophenone **3** to form rottlerin (Scheme 3).[16]

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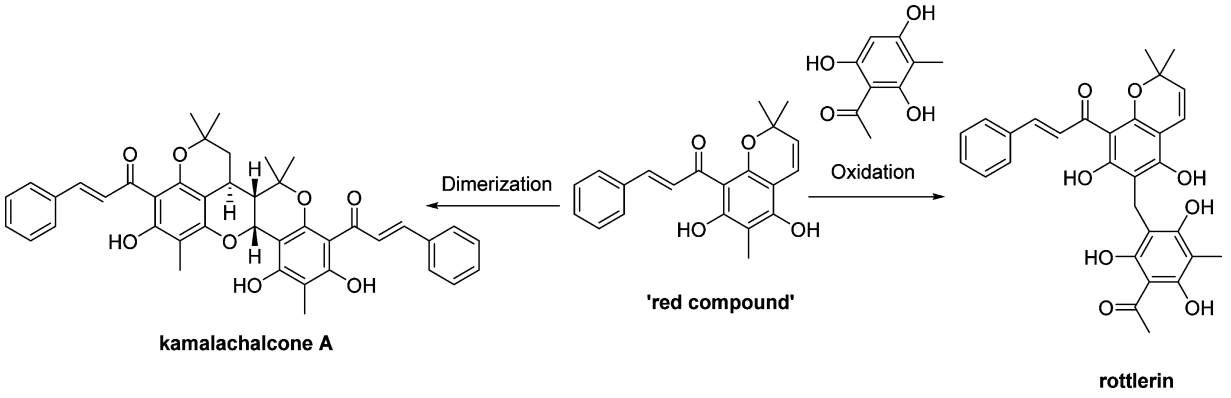
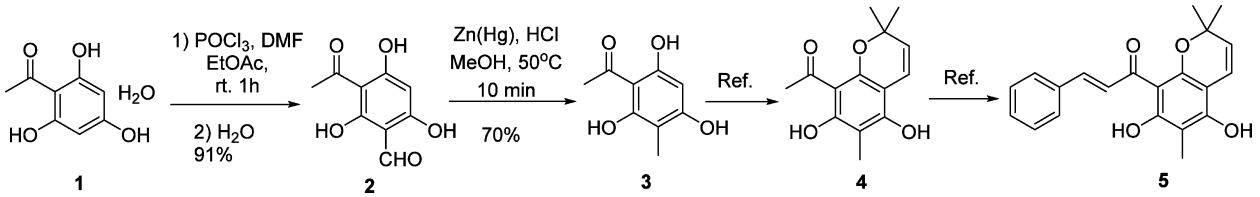
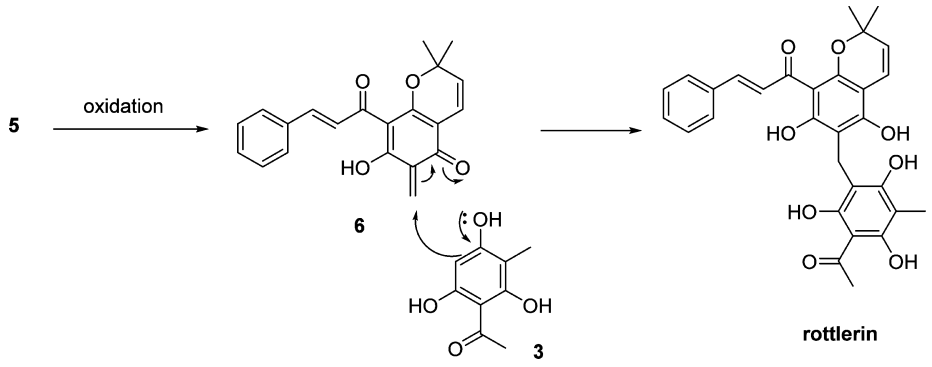
Scheme 1. Proposed Biogenesis of Kamalachalcone A and Rottlerin



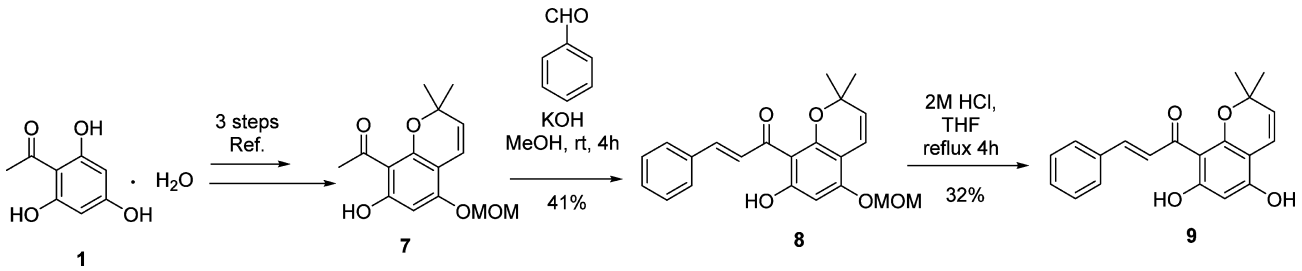
Scheme 2. Preparation of the "Red Compound" 5



Scheme 3. Proposed Biosynthesis of Rottlerin



Scheme 4. Synthesis of Chromene 9



Our initial screening of the reaction between chromene **7** and acetophenone **3** in the presence of an oxidizing agent (Ag_2O, $AgNO_3$, DDQ) failed to give rottlerin. Therefore, an alternative approach to generate an *o*-quinone methide via a nonoxidative pathway was taken.

Monoprotected chromene **8** was prepared according to the literature procedure.[17] Subsequent aldol condensation gave chromene **8** in moderate yield (Scheme 4). Finally the deprotection afforded the 5-hydroxyl chromene **9** in 32% yield similar to the literature report. Spence's synthesis of penilactone A[18] gave inspiration for the synthesis of a quinone methide precursor using formaldehyde, acetic acid, and sodium acetate. Its synthesis has the advantage of avoiding the use of protecting groups and is therefore neat and economical. However, the desired target **10** was not achieved in our case (Scheme 5); instead, rottlerone was formed preferentially from a homodimerization of **9**.

After several screenings, we discovered that Eschenmoser's salt provided the desired quinone methide precursor in a mild and high-yield fashion, similar to the synthesis of arzanol.[19] When chromene **9** was heated with Eschenmoser's salt in $CHCl_3$ at reflux, a full conversion of starting materials into the

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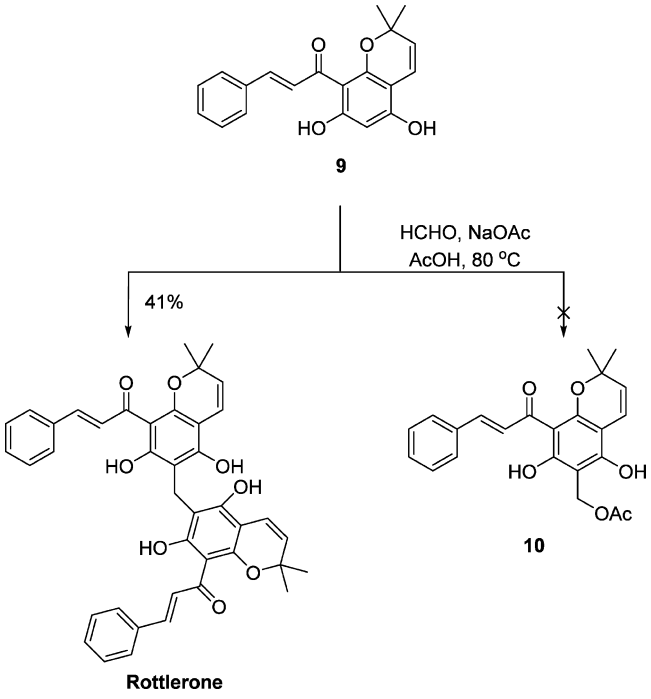
Scheme 5. Attempted Synthesis of Quinone Methide Precursor 10



tertiary amine **11** was observed. Dimethylamine serves as a good leaving group under thermal conditions to convert tertiary amine **11** into the *o*-quinone methide. When acetophenone **3** was heated with chromene **11** in toluene at reflux, rottlerin was produced effectively in 63% yield (Scheme 6).

Comparison of retention times of a rottlerin standard from Sigma-Aldrich and the synthetic rottlerin using preparative HPLC resulted in a close match (see the Supporting Information). Both ^{1}H and ^{13}C NMR spectra provided a close match with literature values.[13,20]

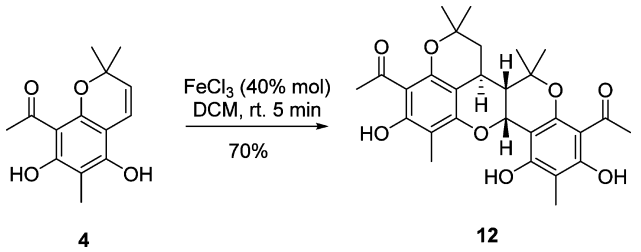
To demonstrate that "red compound" **5** could be dimerized to give kamalachalcone A, chromene **4** was used as a model compound to determine the optimal conditions for the dimerization reaction. When chromene **4** was treated with concentrated hydrochloric acid, dimer **12** was isolated as a white solid by filtration after 1 h.

The ^{1}H NMR spectrum showed the disappearance of the two doublet protons from **4**. With the rise of four new aliphatic and methylene protons between 1.90 and 2.65 ppm along with a doublet proton at 4.7 ppm (d, J = 4.7 Hz), it was believed that dimerization had occurred. A single crystal was also grown using aqueous ethanol as solvent, and X-ray crystallography confirmed the structure of **12** (see the Supporting Information).

Other protic acids, including trifluoroacetic acid and glacial acetic acid, were explored. None of these showed the formation of **12** with prolonged reaction time. However, when a trace amount of water was present, product **12** was observed. However, addition of water to glacial acetic acid did not provide any product. This is perhaps due to the glacial acetic acid being too weak to drive the reaction. Moreover, to explore the importance of water to the dimerization reaction, an experiment used HCl gas pumped into the reaction mixture in anhydrous methanol. Both NMR and TLC showed no product formation in 24 h. However, when water was present, precipitation happened after a short period of time and the solid was determined to be dimer **12**.

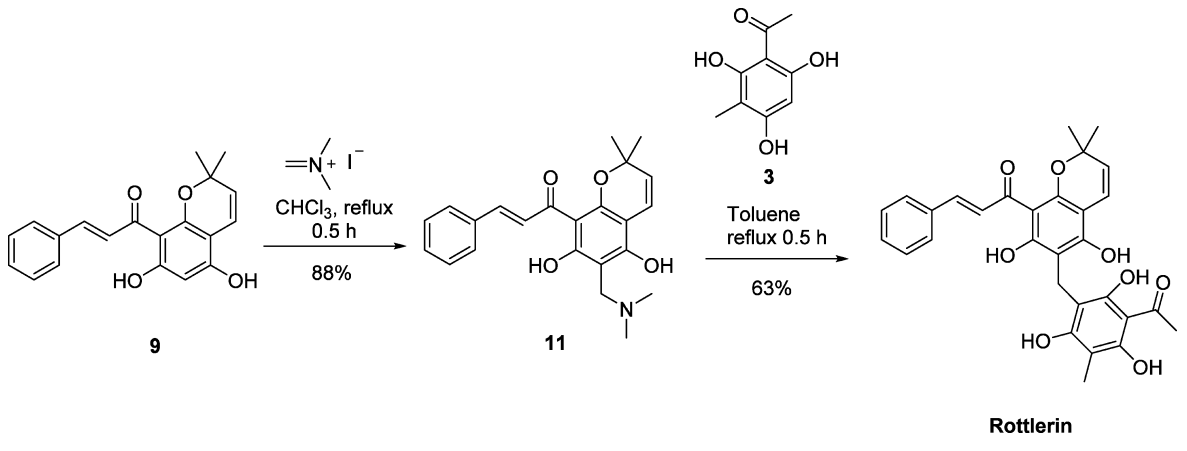
To confirm that concentrated HCl acts as a catalyst, a stoichiometric amount of acid was added and no significant change of product yield was observed. Some Lewis acids were also screened for their effectiveness, as Huan Sun and Schaus[21] demonstrated that $FeCl_3{\cdot}6H_2O$ can catalyze the homodimerization of flavenes to form dependensin and other dimeric flavonoids. The results (Scheme 7) showed that, without any water or hydronium ion, the reaction can still proceed in the presence of anhydrous $FeCl_3$ (40 mol %).

Scheme 7. Optimized Conditions for Dimerization of Chromene 4



Dichloromethane was the most suitable solvent, as it provided easy workup and gave fewer side products. Moreover, the reaction was completed within 5 min, as indicated by both

Scheme 6. Successful Synthesis of Rottlerin



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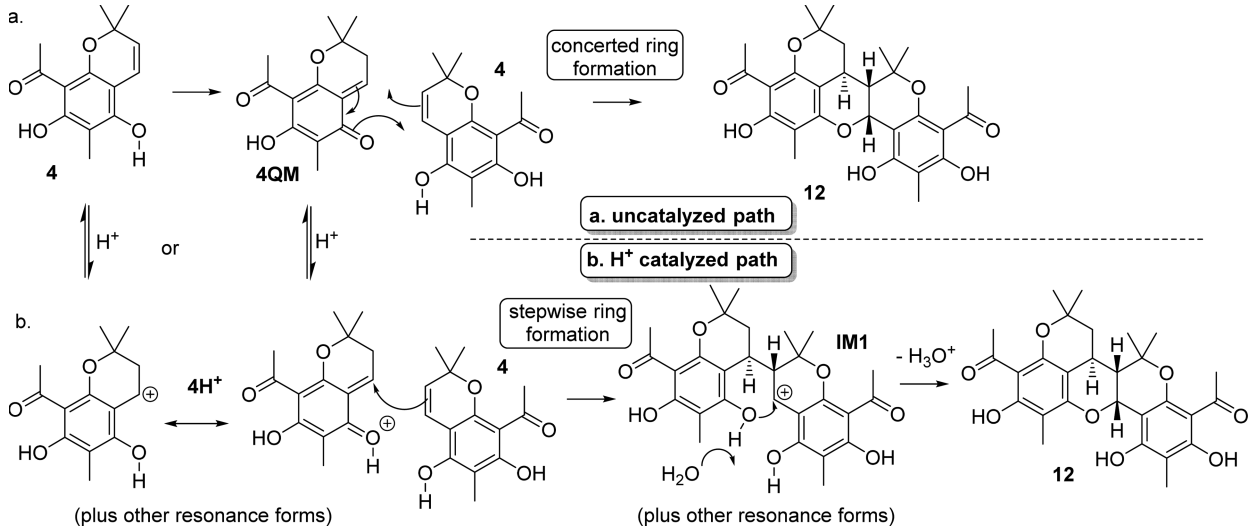
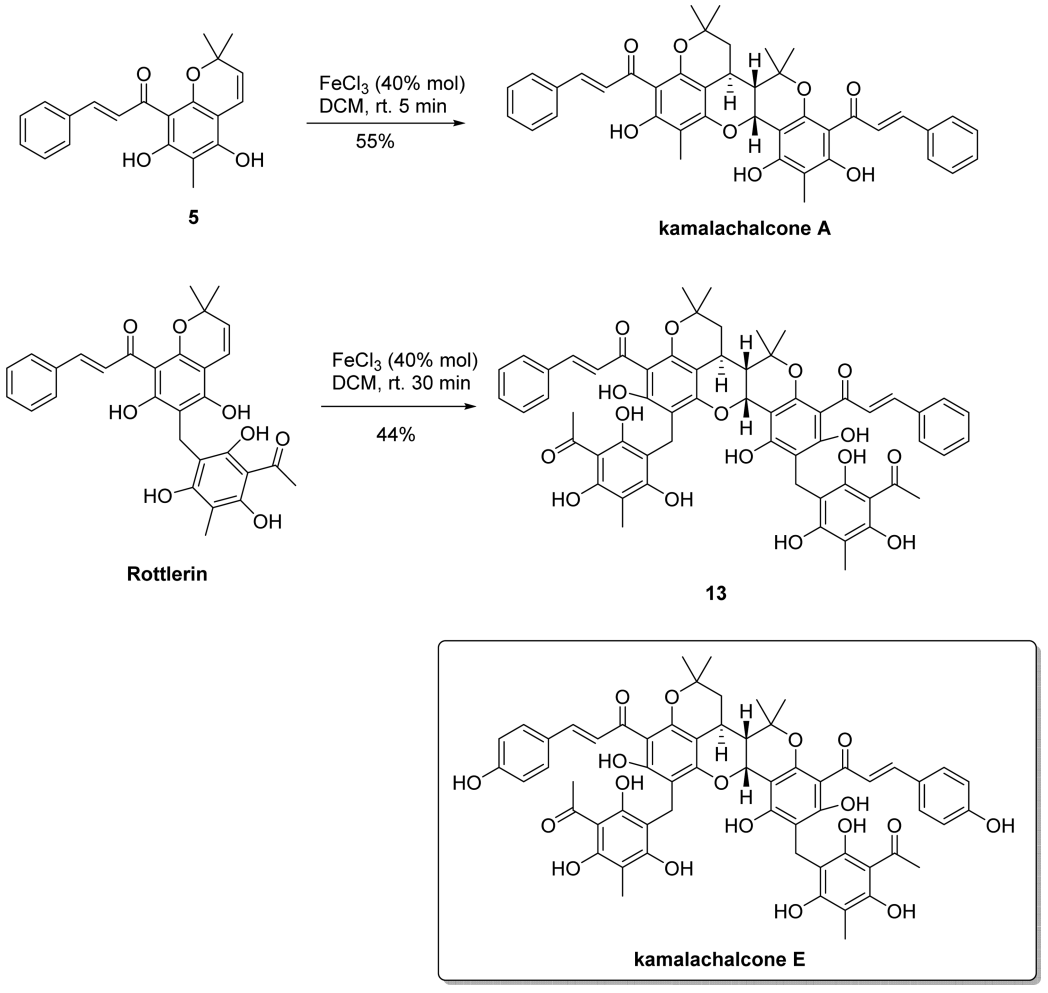
Scheme 8. Mechanistic Possibilities for Conversion of 4 to 12



Scheme 9. Synthesis of Kamalachalcone A and Dimeric Rottlerin 15



NMR analysis of an aliquot and TLC analysis. These experiments demonstrated the formation of the unique pentacyclic ring system possibly formed via a hetero-Diels–Alder pathway.

The mechanism of the dimerization of compound **4** was investigated computationally using DFT at the B3LYP/6-31G(d), B3LYP/6-311+G(2df,2p)//B3LYP/6-31G(d), and M06-2X/6-31+G(d)//B3LYP/6-31G(d) levels of theory, the last method being selected, as previously it has proved reliable in 6-π-electron cyclization reactions.[22] Continuum model methanol solvent using the SMD method was employed.[23] Using H^+ as a model (Lewis) acid catalyst, the activation barrier

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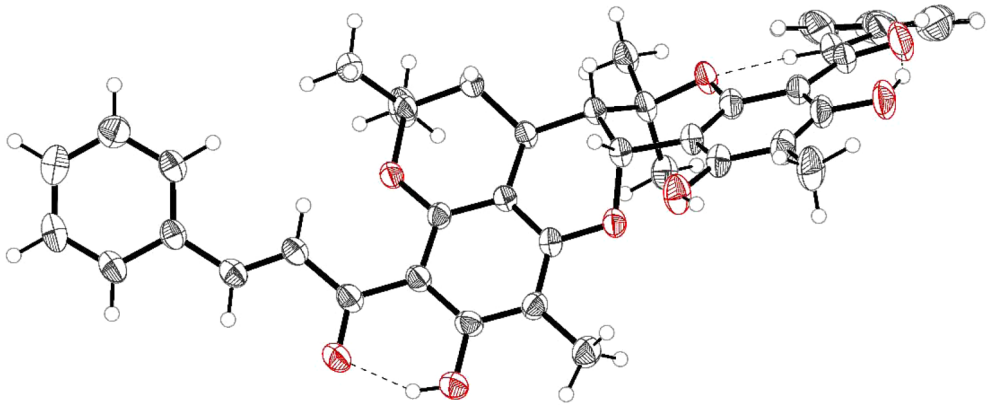

Figure 1. ORTEP diagram of kamalachalcone A. Thermal ellipsoids are drawn at the 50% probability level.

for the key dimerization step was found to be lower in the case of proceeding via the protonated intermediate **4H**$^+$ ($\Delta G^{\ddagger}$ = 110 kJ mol^{-1} (M06-2X; Scheme 8b) in comparison with proceeding via an uncatalyzed path involving the neutral *o*-quinone methide intermediate **4QM** ($\Delta G^{\ddagger}$ = 133 kJ mol^{-1}; Scheme 8a). The overall lower free energy barrier for the path shown in Scheme 8b suggests that H$^+$ interacting with the oxygen atom of the quinone methide may indeed catalyze the dimerization step of the reaction. We note that the errors in the calculated solvation energies, and by extension the reaction energies, for the sequential pathway containing changed species (Scheme 8b) are likely to be larger than for the concerted pathway containing only neutral species (Scheme 8a).[23] Since the reaction leading to the formation of **12** is stereoselective, in that only the isomer in which the newly formed ring with a cis fusion is observed, this suggests that a concerted formation of **12**, such as the reaction of a preformed quinone methide intermediate, **4QM**, with a second molecule of the starting compound **4**, found in Scheme 8a is more likely. However, a sequential pathway such as that in Scheme 8b, in which an intermediate **IM1** was found using the computational methodology employed, may still be possible. The intermediate **IM1** is formed via formation of only the C−C bond in the dimerization step rather than a concerted formation of C−C and C−O bonds. We note that the barrier to the synchronous deprotonation (using water as the base) and ring closure of **IM1** to form the C−O bond in the final product **12** is calculated to be very low ($\Delta G^{\ddagger}$ = 24 kJ mol^{-1} relative to **IM1**), significantly less than the barrier to rotation around the newly formed C−C bond in **IM1** ($\Delta G^{\ddagger}$ = 60 kJ mol^{-1} relative to **IM1**).

With the success of dimerization of compound **4**, kamalachalcone A was synthesized using the optimized $FeCl_3$ dimerization strategy and was obtained in 55% yield. Characterization of ^{1}H and ^{13}C NMR showed a close match with the literature values[6] (see the Supporting Information). Dimerization of rottlerin also gave the dimeric product **13**, similar to kamalachalcone E, in 44% yield (Scheme 9).

To give further structural confirmation of the product, single crystals of kamalachalcone A were grown in THF and an X-ray crystal structure was determined (Figure 1).

CONCLUSIONS

In conclusion, rottlerin was synthesized for the first time using a biomimetic approach which is practical and inexpensive. The synthetic methodology allows potential rottlerin analogues to be developed and provide a wider range of novel protein kinase C delta inhibitors. An optimized dimerization method using $FeCl_3$ also provided kamalachalcone A and dimeric rottlerin **13**. The biological activities of rottlerin analogues and other dimeric compounds will be reported in the future.

EXPERIMENTAL SECTION

All reactions requiring anhydrous conditions were performed in oven-dried glassware under an argon atmosphere unless otherwise stated. All other reagents were purchased from commercial sources and used without further purification. Reactions were monitored using thin-layer chromatography (TLC) using aluminum plates coated with silica gel GF_{254}. Compounds were detected by short- and long-wavelength ultraviolet light. Gravity column chromatography was carried out using 40−63 μm silica gel. Flash column chromatography was carried out using 6−35 μm silica gel. Preparative reversed phase HPLC was performed using a PDA detector (254 nm) and C18 column (150 mm × 19 nm) on a gradient elution of 1−100% over 60 min with a flow rate of 5 mL/min. Formic acid (0.1%)/Milli-Q water was used as eluent A, and formic acid (0.1%)/acetonitrile was used as eluent B. Rottlerin standard was purchased from Sigma-Aldrich to compare the retention time with the synthetic rottlerin using preparative HPLC. NMR spectra were obtained in the designated solvents at 298 K on a 300, 400, or 600 MHz spectrometer as designated. Chemical shifts (δ) are in parts per million and are internally referenced relative to the solvent nuclei. ^{1}H NMR spectral data are reported as follows: chemical shift measured in parts per million (ppm) downfield from TMS (δ); multiplicity; observed coupling constant (J) in hertz (Hz); proton count; assignment. Multiplicities are assigned as singlet (s), doublet (d), doublet of doublets (dd), doublet of triplets (dt), triplet, (t), quartet (q), quintet (p), doublet of doublets of doublets (ddd), multiplet (m), and broad singlet (bs) where appropriate. ^{13}C NMR spectra were recorded in the designated solvents, and chemical shifts are reported in ppm downfield from TMS. Melting points were measured using a Mel-Temp melting point apparatus and are uncorrected. Infrared spectra were recorded using an attenuated total reflection FTIR spectrometer. High-resolution mass spectra were measured at 70 eV using a quadrupole analyzer and are reported with ion mass/charge (m/z) ratios as values in atomic mass units.

1-(2,4,6-Trihydroxy-3-methylphenyl)ethan-1-one (3).[9] *(1). Preparation of Zinc Amalgam.* Mercuric chloride (1.0 g) was dissolved in a mixture of concentrated hydrochloric acid (3 mL) and water (50 mL). Zinc powder (15.0 g) was added, and the mixture was stirred vigorously for 5 min. During the stirring the zinc wool was broken into small shiny pieces. The aqueous layer was decanted. The amalgamated zinc was used immediately for the reduction.

(2). Synthesis of Acetophenone ***3****.* Concentrated hydrochloric acid (30 mL) and water (20 mL) were added to a solution of acetophenone **2** (2.5 g, 12.7 mmol) in methanol (50 mL) in a 250 mL conical flask (*note*: the mixture existed as a suspension). The mixture was warmed to 50 °C, and amalgamated zinc (prepared in part 1) was added in one

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lot. The suspension dissolved immediately and formed a homogeneous solution. This reaction mixture was stirred vigorously for 5 min under open-flask conditions (*note*: bubbling and exothermic) and then quickly filtered through a filter funnel with cotton employed to remove the amalgamated zinc. The residual compound was transferred without loss using methanol. The solution was then evaporated in vacuo to form a yellow solid. The solid was washed with water and filtered to give **3** as a yellow solid (1.63 g, 70%): mp 208−212 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.97 (s, 1H), 10.54 (s, 1H), 10.31 (s, 1H), 6.00 (s, 1H), 2.54 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 204.3, 165.2, 164.5, 161.9, 105.6, 103.1, 95.7, 34.4, 9.2.

(*E*)-1-(7-Hydroxy-5-(methoxymethoxy)-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-one (8). To a solution of chromene **7** (1.0 g, 3.59 mmol) in MeOH (15 mL) were added benzaldehyde (0.37 mL, 3.59 mmol) and KOH pellets (0.60 g, 10.77 mmol). The mixture was stirred for 10 h, during which time the solution gradually changed from orange to red and eventually precipitate formed. After completion, water (100 mL) was added and the mixture extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with brine, dried using Na_2SO_4, and evaporated in vacuo. Flash chromatography on silica gel using hexane/EtOAc (9/1) afforded a bright orange red solid. The solid was washed with hexane (3 × 5 mL) to give (*note*: hexane wash to remove excess benzaldehyde) a fine red-orange powder of **8** (0.54 g, 41%): mp 100−104 °C; IR ν_{max} 3066, 1630, 1582, 1340, 1148, 1062, 921 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) δ 13.96 (s, 1H), 8.19 (d, J = 16 Hz, 1H), 7.81 (d, J = 16 Hz, 1H), 7.74−7.78 (m, 2H), 7.47−7.53 (m, 3H), 6.65 (d, J = 10 Hz, 1H), 6.26 (s, 1H), 5.65 (d, J = 10 Hz, 1H), 5.35 (s, 2H), 3.50 (s, 3H), 1.60 (s, 6H); ^{13}C NMR (100 MHz, acetone-d_6) δ 193.7, 167.7, 156.7, 143.2, 136.3, 131.3, 130.0, 129.2, 128.2, 126.2, 117.2, 107.4, 96.0, 95.3, 79.1, 56.7, 28.1; HRMS (ESI) m/z calcd for $C_{22}H_{22}O_5$ (M + Na)$^+$ 389.1359, found 389.1358.

(*E*)-1-(5,7-Dihydroxy-2,2-dimethyl-2H-chromen-8-yl)-3-phenylprop-2-en-1-one (9). Chromene **8** (0.50 g, 1.36 mmol) was dissolved in 20 mL of a MeOH/THF mixture (1/1) and was heated at reflux for 4 h. The reaction was monitored by TLC. After completion, water (100 mL) was added and the mixture extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried using Na_2SO_4, and evaporated in vacuo. Flash chromatography on silica gel using hexane/EtOAc (8/2) gave **9** (0.14 g, 32%) as a red oil: IR ν_{max} 3273, 1627, 1598, 1342, 1156, 1087, 975 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.23 (s, 1H), 8.11 (d, J = 16 Hz, 1H), 7.76 (d, J = 16 Hz, 1H), 7.58−7.62 (m, 2H), 7.24−7.43 (m, 3H), 6.59 (d, J = 10 Hz, 1H), 6.01 (s, 1H), 5.48 (d, J = 10 Hz, 1H), 1.55 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.2, 166.5, 158.8, 156.9, 142.6, 135.7, 130.3, 129.1, 128.4, 127.5, 124.9, 116.7, 106.6, 102.7, 96.5, 78.4, 28.1; HRMS (ESI) m/z calcd for $C_{20}H_{18}O_4$ (M + H)$^+$ 323.1278, found 323.1276.

(*E*)-1-(6-((Dimethylamino)methyl)-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-one (11). To a solution of chromene **9** (0.10 g, 0.31 mmol) in $CHCl_3$ was added Eschenomoser's salt (0.057 g, 0.31 mmol). The mixture was heated at reflux for 0.5 h. After completion, water (50 mL) was added and the mixture extracted with $CHCl_3$ (3 × 50 mL). The combined organic extracts were washed with brine, dried using Na_2SO_4, and evaporated in vacuo. A small amount of acetonitrile was added and orange precipitate was formed, which was filtered and dried to give **11** (0.10 g, 88%) as an orange powder: mp 110−114 °C; IR ν_{max} 2966, 2925, 1624, 1590, 1340, 1130, 972 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.14 (s, 1H), 8.11 (d, J = 15.6 Hz, 1H), 7.81 (d, J = 15.6 Hz, 1H), 7.59−7.63 (m, 2H), 7.39−7.46 (m, 3H), 6.80 (d, J = 10 Hz, 1H), 5.51 (d, J = 10 Hz, 1H), 4.21 (s, 2H), 2.86 (s, 6H), 1.56 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.3, 166.4, 160.1, 158.2, 143.5, 135.5, 130.6, 129.2, 128.5, 127.1, 125.1, 117.5, 106.0, 105.4, 99.0, 79.1, 51.0, 42.1, 28.5; HRMS (ESI) m/z calcd for $C_{23}H_{25}NO_4$ (M + H)$^+$ 380.1856, found 380.1858.

Rottlerin.[13,19] A mixture of chromene **11** (0.060 g, 0.16 mmol) and acetophenone **3** (0.029 g, 0.16 mmol) in toluene was heated at reflux for 0.5 h. The reaction was monitored by TLC. After completion of the reaction, toluene was evaporated in vacuo. The remaining crude product was purified by flash column chromatography on silica gel using hexane/EtOAc (8/2) to give rottlerin (0.052 g, 63%) as a red-orange solid: mp 210−214 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 16.51 (s. 1H), 15.60 (bs, 1H), 9.55 (bs, 1H), 8.19 (d, J = 15.6 Hz, 1H), 7.84 (d, J = 15.6 Hz, 1H), 7.59−7.63 (m, 2H), 7.39−7.45 (m, 3H), 6.66 (d, J = 10.1 Hz, 1H), 5.49 (d, J = 10.1 Hz, 1H), 3.81 (s, 2H), 2.71 (s, 3H), 2.08 (s, 3H), 1.53 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 204.0, 193.1, 163.1, 160.9, 159.7, 159.0, 156.7, 155.6, 143.4, 135.6, 130.5, 129.1, 128.9, 128.4, 126.8 125.1, 117.2, 106.6, 105.5, 104.5, 104.0, 102.8, 102.0, 78.2, 32.6, 28.0, 16.0, 7.6; HRMS (ESI) m/z calcd for $C_{30}H_{28}O_8$ (M + H)$^+$ 517.1857, found 517.1851.

1,1′-(5,8,10-Trihydroxy-2,2,6,9,13,13-hexamethyl-1,7a,13a,13b-tetrahydro-2*H*,13*H*-pyrano[3,2-*c*:4,5,6-*d*′*e*′]-dichromene-4,11-diyl)bis(ethan-1-one) (12). To a solution of **4** (0.020 g, 0.081 mmol) in DCM (1 mL) was added anhydrous $FeCl_3$ (0.005 g, 0.032 mmol), and the mixture was stirred at room temperature for 5 min. After completion of reaction as indicated by TLC or NMR (over 90% conversion of **4**), the reaction mixture was washed with brine (30 mL) and extracted with DCM (3 × 20 mL), dried over Na_2SO_4, and evaporated in vacuo. The solid was washed with MeOH (3 × 5 mL) to give spectroscopically pure compound **12** (0.028 g, 70%) as a white solid: mp 260−264 °C; IR ν_{max} 3589, 3385, 1598, 1422, 1367, 1159, 1126, 790 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 14.19 (s, 1H), 13.85 (s, 1H), 6.82 (s, 1H), 4.68 (d, J =5.2 Hz, 1H), 2.66 (s, 3H), 2.65 (s, 3H), 2.53−2.60 (m, 1H), 2.15−2.19 (m, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.05−2.10 (m, 1H), 1.83−1.92 (m, 1H), 1.59 (s, 3H), 1.54 (s, 3H), 1.47 (s, 3H), 1.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.9, 203.3, 165.8, 162.5, 160.5, 158.5, 155.0, 154.3, 107.3, 105.7, 105.6, 100.7, 98.0, 78.6, 77.7, 77.4, 70.3, 47.5, 42.3, 33.7, 33.5, 30.3, 28.3, 27.1, 24.3, 20.6, 7.4, 7.3; HRMS (ESI) m/z calcd for $C_{28}H_{32}O_8$ (M + H)$^+$ 497.2170, found 497.2166.

Kamalachalcone A.[6] To a solution of **5** (0.060 g, 0.18 mmol) in DCM (1 mL) was added anhydrous $FeCl_3$ (0.011 g, 0.07 mmol), and the mixture was stirred at room temperature for 5 min (*note*: the solution turned from red to brown and eventually black). After completion of reaction as indicated by TLC or NMR (over 95% conversion of **5**), the reaction mixture was washed with brine (30 mL) and extracted with DCM (3 × 20 mL), dried over Na_2SO_4, and evaporated in vacuo. The solid was washed with MeOH (3 × 5 mL) to give spectroscopically pure kamalachalcone A (0.067 g, 55%) as a yellow solid: mp 274−280 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.40 (s, 1H), 13.95 (s, 1H), 7.99 (d, J = 15.7 Hz, 2H), 7.77 (d, J = 15.7 Hz, 2H), 7.55−7.68 (m, 4H), 7.35−7.48 (m, 6H), 6.90 (s, 1H), 4.75 (d, J = 5.19 Hz, 1H), 2.57−2.68 (m, 1H), 2.25 (t, J = 4.66 Hz, 1H), 2.08−2.17 (m, 1H), 2.15 (s, 3H), 2.13 (s, 3H), 1.94 (t, J = 12.89 Hz, 1H), 1.67 (s, 3H), 1.60 (s, 3H), 1.51 (s, 3H), 1.49 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.6, 193.1, 166.5, 163.0, 160.6, 158.7, 154.5, 153.9, 142.2, 142.1, 135.7, 135.7, 130.3, 130.2, 129.1, 129.0, 128.4, 128.3, 127.9, 127.8, 107.8, 106.1, 106.0, 105.5, 101.1, 98.3, 78.7, 77.8, 77.4, 70.5, 47.5, 42.3, 30.5, 28.6, 27.1, 24.5, 20.6, 7.6, 7.4; HRMS (ESI) m/z calcd for $C_{42}H_{40}O_8$ (M + Na)$^+$ 695.2615, found 695.2610.

(2*E*,2′*E*)-1,1′-(6,9-Bis(3-acetyl-2,4,6-trihydroxy-5-methylbenzyl)-5,8,10-trihydroxy-2,2,13,13-tetramethyl-1,7a,13a,13b-tetrahydro-2*H*,13*H*-pyrano[3,2-*c*:4,5,6-*d*′*e*′]dichromene-4,11-diyl)bis(3-phenylprop-2-en-1-one) (13). To a solution of rottlerin (0.050 g, 0.097 mmol) in DCM (3 mL) was added anhydrous $FeCl_3$ (0.006 g, 0.039 mmol), and the mixture was stirred at room temperature for 30 min. The reaction mixture was washed with brine (30 mL) and extracted with DCM (3 × 20 mL), dried over Na_2SO_4, and evaporated in vacuo. The remaining crude product was purified by flash column chromatography on silica gel using hexane/EtOAc (7/3) to give dimer **13** (0.044 g, 44%) as an orange solid: mp 246−250 °C; IR ν_{max} 3328, 2973, 2929, 1602, 1425, 1356, 1159, 1126, 1051, 969 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 16.25 (s, 1H), 14.61 (s, 1H), 12.88 (s, 1H), 12.03 (s, 1H), 8.05 (d, J = 15.7 Hz, 1H), 7.98 (d, J = 15.7 Hz, 1H), 7.68−7.76 (m, 6H), 7.43−7.50 (m, 6H), 4.84 (d, J = 4.37 Hz, 1H), 3.70−3.92 (m, 4H), 2.58−2.63 (m, 1H), 2.45 (s, 3H), 2.26−2.28 (m, 1H), 2.25 (s, 3H), 2.05−2.11 (m, 1H), 1.92−1.95 (m, 1H) 1.89 (s, 3H), 1.81 (s, 3H), 1.59 (s, 3H), 1.50 (s, 3H), 1.47 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 203.5, 203.3, 193.1, 164.1, 160.4, 160.4, 160.3, 160.0, 160.0, 159.8, 158.4, 157.3,

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155.5, 155.0, 142.6, 141.8, 135.0, 134.8, 130.7, 130.5, 129.3, 129.2, 128.4, 128.3, 127.2, 127.0, 108.2, 107.7, 106.6, 106.1, 105.2, 105.0, 104.9, 103.3, 102.9, 102.6, 100.5, 79.2, 79.1, 79.0, 78.9, 78.8, 70.4, 45.5, 32.3, 32.3, 29.9, 27.9, 26.7, 24.1, 20.3, 16.4, 15.9, 8.3, 8.0; HRMS (ESI) m/z calcd for $C_{60}H_{56}O_{16}$ $(M + Na)^+$ 1055.3461, found 1055.3445.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01827. CCDC-1406109 contains supplementary crystallographic data for kamalachalcone A. CCDC-1406110 contains supplementary crystallographic data for compound **12**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

^{1}H NMR and ^{13}C NMR spectra for **3**, **8**, **9**, **11**−**13**, rottlerin, and kamalachalcone A and crystallographic and computational data (PDF)
Crystallographic data (CIF)
Crystallographic data (CIF)

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Notes

The authors declare no competing financial interest.

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