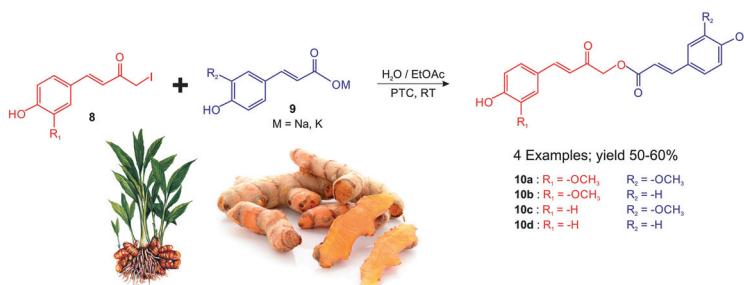


An Expeditious, Green and Protecting-Group-Free Synthesis of a Potent Secondary Metabolite Calebin-A and Its Analogues

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Abstract The synthesis of Calebin-A (**10a**) and analogues (**10b-d**), which are minor but pharmacologically active components (<0.01% of the oleoresin) of *Curcuma longa*, is described. This flexible synthesis is achieved under near neutral conditions in water/solvent biphasic medium at room temperature without involving any protecting groups.

Key words Calebin-A, *Curcuma longa*, ferulic acid ester, phase-transfer catalyst (PTC), green synthesis

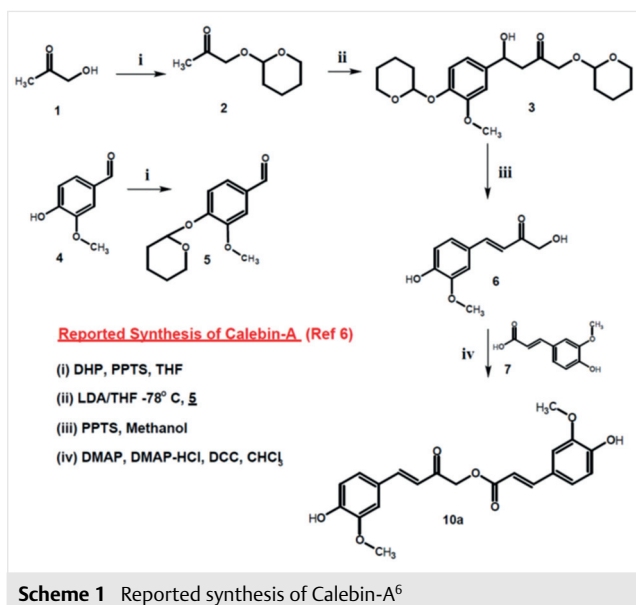
Curcuma longa has been a traditional remedy for several disease conditions and is described extensively in Indian (Ayurvedic) and traditional Chinese medicine. Several active constituents have been isolated from this plant but prime among them are the curcuminoids.¹ The high popularity of curcuminoids and their pleiotropic activity have relegated to practically obscurity several minor compounds that occur in *Curcuma longa*, which have not been considered to merit any detailed study.² Park and Kim³ first isolated and identified Calebin-A (**10a**) from *Curcuma longa* in 2001 through a tedious isolation. The isolation itself was performed by repeated normal column chromatography over silica gel followed by semipreparative HPLC. The content of Calebin-A (**10a**) is ca. 0.01% of the turmeric oleoresin. The structure of Calebin-A was characterized as an ester of ferulic acid, contrasting with the dynamic pair of ketoenol forms that characterize curcuminoid structures. Esters of ferulic acid are a ubiquitous structural type occurring in several diverse plant varieties.⁴

Demethoxycalcebin-A1 (**10b**) and demethoxycalcebin-A2 (**10c**) have been isolated as an inseparable pair from *Curcuma longa* by Qiu and co-workers⁵ in 2007, again through a

laborious procedure using repeated chromatography over normal phase, reversed phase and preparative HPLC. The total content of isomeric demethoxycalcebin-A1 (**10b**) and demethoxycalcebin-A2 (**10c**) amounts to ca. 0.002% of the turmeric oleoresin. Calebin-A (**10a**) and demethoxycalcebin-A1 (**10b**) protect PC12 rat pheochromocytoma and IMR-32 human neuroblastoma cells from β -amyloid(25–35) damage in *in vitro* models.⁶ Calebin-A might be an effective compound for the treatment of human gastric and other MDR cancers.⁷ It induces cell cycle arrest in human colon cancer cells and xenografts in nude mice.⁸ Calebin-A has been shown to inhibit the growth of HepG2 cells better than curcumin at the same concentration.⁹ The antiobesity potential of Calebin-A has been patented by Majeed et al.^{10–13} It inhibits adipogenesis and hepatic steatosis in high-fat, diet-induced obesity through activation of AMPK signaling.¹⁴ Calebin-A and its compositions have been patented for their potential to prevent pathological damage to mammalian articular cartilage¹⁵ and for the treatment of hypercholesterolemia.¹⁶ Calebin A is nontoxic with a NOAEL determined as 100 mg kg⁻¹ in mammals.¹⁷ Calebin-A downregulates osteoclastogenesis through suppression of RANKL signaling.¹⁸ These extremely diverse beneficial biological activities¹⁹ of Calebin-A and its natural demethoxy analogues, combined with absence of any toxicity, forebode their prospects as emerging drug candidates and health-promoting nutrients. Given that the concentrations of Calebin-A (**10a**) and its natural demethoxy analogues are extremely low (<0.01%) in turmeric oleoresin, rendering isolation from natural sources a distant and costly possibility, the development of new synthetic routes and other sources are required to continue to explore their ever-increasing areas of biological activity.

In addition to this divergent biological activity reminiscent of curcuminoids, Calebin-A has an additional advantage of higher stability over a wide range of pH values, which is an advantageous metric not possessed by curcumin.²⁰

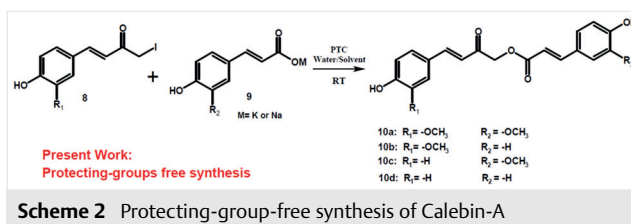
Kim and Kim synthesized⁶ Calebin-A through a circuitous route (five steps) starting from 1-hydroxyacetone (**1**; Scheme 1). The reported route involves protection of 1-hydroxyacetone (**1**) and vanillin (**4**) as their tetrahydropyranyl ethers (**2** and **5**). The tetrahydropyranyl ether of 1-hydroxyacetone (**2**) is treated with lithium diisopropylamide (LDA) at low temperature (-78°C) to generate the lithio anion at the α -methyl of the keto group. This lithio anion adds to the aldehyde group of the tetrahydropyranyl ether of vanillin (**5**) to give the β -hydroxy-ketone **3**. This is dehydrated and deprotected to feruloylmethanol (**6**), which is coupled to ferulic acid (**7**) in the presence of 4-(*N,N*-dimethylamino)pyridine (DMAP), DMAP-HCl and *N,N*-dicyclohexylcarbodiimide (DCC) to furnish Calebin-A (**10a**).



This synthesis suffers from the disadvantages that the starting material 1-hydroxyacetone (**1**) is expensive, the condensation of vanillin (**4**) with **1** involves protection of the hydroxyl groups as their tetrahydropyranyl ethers to avoid LDA reacting with them, and DMAP and DCC are also expensive. Overall, this synthesis involves a minimum of five steps, involving very low temperature (-78°C), and pyrophoric and moisture-sensitive reagents. All these factors conspire against adoption of this method for scale-up of Calebin-A or its analogues.

We have developed a simple, economical, and scalable green process for Calebin-A and its analogues. This is eventually a single-step process starting from hydroxycinnam-

oylmethyl iodide. Our general synthesis scheme for the preparation of Calebin-A (**10a**) and its demethoxy analogues (**10b–d**) is described in Scheme 2; wherein, protection of phenolic hydroxyls is unnecessary. We simply utilized the large difference between the $\text{p}K_{\text{a}}$ values of $-\text{COOH}$ protons and phenolic $-\text{OH}$ protons ($\Delta\text{p}K_{\text{a}} > 5$) and the resultant higher nucleophilicity of the carboxylate anion for a subsequent nucleophilic substitution reaction. This single-step process involves the reaction of substituted hydroxycinnamoylmethyl iodide with the sodium or potassium salt of the appropriately substituted hydroxycinnamic acid (prepared in situ) to give Calebin-A and its analogues. This is a biphasic reaction in water/organic solvent that takes place in the presence of a phase-transfer catalyst (PTC) at ambient temperature. The process obviates the use of protection/deprotection sequences, in addition avoiding reaction steps requiring low temperatures or rigorously anhydrous conditions, which were the hallmarks of the earlier synthesis.⁶



The method is easily adapted to the synthesis of analogues of Calebin-A. Feruloylmethyl iodide (**8a**; $\text{R}_1 = \text{OCH}_3$) and 4-hydroxycinnamoylmethyl iodide (**8b**; $\text{R}_1 = \text{H}$) were prepared according to the reported method.²¹ Demethoxycalcebin-A2 (**10c**) and bisdemethoxycalcebin-A (**10d**) have been synthesized for the first time. Bisdemethoxycalcebin-A (**10d**) is not yet detected or isolated from *Curcuma longa*, possibly because of its very low concentration when compared with demethoxycalcebin-A1 (**10b**) and demethoxycalcebin-A2 (**10c**).²²

The solvents were of bulk quality, used in our scale-up facilities. The starting materials were purchased from commercial sources in bulk. Melting points were determined by capillary method with a LABIN-DIA MR-VIS apparatus and are uncorrected. FTIR spectra were recorded with a PerkinElmer Spectrum Two spectrophotometer by HATR sampling technique on ZnSe. ^1H and ^{13}C NMR spectra were recorded with a Varian FT spectrometer (300 and 75 MHz, respectively) in $\text{DMSO}-d_6$ or acetone- d_6 solutions, relative to residual solvent signal [DMSO : $\delta = 2.50$ ppm (^1H), DMSO : $\delta = 39.51$ ppm (^{13}C); acetone- d_6 : $\delta = 2.05$ ppm (^1H), acetone- d_6 : $\delta = 29.92$ ppm (^{13}C)]. Chemical shifts and coupling constants are recorded in units of parts per million and hertz, respectively. LC-MS were recorded with a Finnigan LCQ Advantage Max of Thermo Electron Corporation. Elemental analyses were carried out with an elemental Vario EL III automatic CHNS analyzer.

Synthesis of Calebin-A (10a) and Its Analogues; General Procedure

Feruloyl or 4-hydroxycinnamoylmethyl iodide (**8**; 0.045 mol) dissolved in EtOAc or THF was mixed with an aqueous solution of the sodium or potassium salt of ferulic acid or 4-hydroxycinnamic acid (**9**; 1.0–2.0 mole equivalent) and the mixture was stirred at ambient temperature in the presence of a phase-transfer catalyst (5–10 mole percent with respect to the iodide) such as tetrabutylammonium bromide, tetrabutylammonium hydrogensulfate, benzyltributylammonium chloride, benzyltriethylammonium chloride, or benzyltrimethylammonium chloride. The reaction mixture was stirred vigorously for 24–72 h, then the organic layer was separated, washed with saturated aq. sodium hydrogen carbonate, dried over anhydrous sodium sulfate, filtered, the solvent stripped off under vacuum, and the crude product crystallized from EtOAc or EtOH to isolate Calebin-A (**10a**) or its demethoxy analogues as pale-yellow crystalline solids in 50–60% yield.

Calebin-A (Feruloylmethyl Ferulate, 10a)

The general synthetic procedure was followed for the preparation of Calebin-A (**10a**); wherein feruloylmethyl iodide (**8a**; $R_1 = \text{OMe}$) was reacted with the sodium or potassium salt of ferulic acid (**9a**; $R_2 = \text{OMe}$). The yield of the product was 10.5 g (61%). We have successfully scaled up this product to 25 kg per batch in a pilot plant.

Pale-yellow solid; m.p. 138–140 °C.

IR: 3182, 2918, 2850, 2127, 1678, 1626, 1592, 1514, 1468, 1451, 1434, 1383, 1349, 1274, 1251, 1240, 1204, 1185, 1163, 1139, 1124, 1078, 1031, 975, 926, 867, 843, 815, 781, 741, 727, 655, 620, 602 cm^{-1} .

^1H NMR (acetone- d_6 , 300 MHz): $\delta = 3.902$ (s, 3 H), 3.929 (s, 3 H), 5.106 (s, 2 H), 6.518 (d, $J = 15.9$ Hz, 1 H), 6.856 (d, $J = 16.2$ Hz, 1 H), 6.886 (d, $J = 8.4$ Hz, 1 H), 6.892 (d, $J = 8.4$ Hz, 1 H), 7.180 (dd, $J = 8.4$ Hz, 1.8 Hz, 1 H), 7.214 (dd, $J = 8.4$ Hz, 1.8 Hz, 1 H), 7.375 (d, $J = 1.8$ Hz, 1 H), 7.386 (d, $J = 1.8$ Hz, 1 H), 7.666 (d, $J = 16.2$ Hz, 1 H), 7.671 (d, $J = 15.9$ Hz, 1 H), 8.246 (s, 1 H), 8.309 (s, 1 H).

^{13}C NMR (acetone- d_6 , 75 MHz): $\delta = 56.27, 56.30, 67.93, 111.29, 111.52, 116.08, 116.20, 120.32, 124.16, 124.49, 127.35, 127.41, 144.39, 146.50, 148.75, 150.21, 150.50, 166.90, 192.96$.

^1H NMR (300 MHz, acetone- d_6) and ^{13}C NMR (75 MHz, acetone- d_6) spectral data are completely in consonance with the values reported by Kim³ for natural Calebin-A.

LC-MS (+APCI): m/z 385 [M + H]⁺; LC-MS (–APCI): m/z 383 [M – H][–].

Anal. Calcd for $\text{C}_{21}\text{H}_{20}\text{O}_7$: C, 65.62; H, 5.24. Found: C, 65.54; H, 5.21.

Demethoxycalcebin-A1 (Feruloylmethyl 4-Hydroxycinnamate, 10b)

The general synthetic procedure was followed; wherein feruloylmethyl iodide (**8a**; $R_1 = \text{OMe}$) was reacted with the sodium or potassium salt of 4-hydroxycinnamic acid (**9b**; $R_2 = \text{H}$). The yield of the product was 8.5 g (53%), pale-yellow solid; m.p. 181.4 – 183.4 °C;

IR: 3236, 1709, 1649, 1621, 1597, 1517, 1464, 1439, 1427, 1380, 1326, 1293, 1282, 1244, 1199, 1182, 1171, 1153, 1126, 1103, 1053, 1028, 982, 967, 937, 851, 830, 807, 751, 721, 693, 622, 606 cm^{-1} .

^1H NMR (DMSO- d_6 , 300 MHz): $\delta = 3.81$ (s, 3 H), 5.132 (s, 2 H), 6.508 (d, $J = 16.2$ Hz, 1 H), 6.813 (d, $J = 8.4$ Hz, 2 H), 6.825 (d, $J = 8.4$ Hz, 1 H), 6.842 (d, $J = 16.8$ Hz, 1 H), 7.173 (dd, $J = 8.4$ Hz, 1.5 Hz, 1 H), 7.336 (d, $J = 1.5$ Hz, 1 H), 7.592 (d, $J = 8.4$ Hz, 2 H), 7.623 (d, $J = 16.8$ Hz, 1 H), 7.626 (d, $J = 16.2$ Hz, 1 H), 9.814 (s, 1 H), 10.135 (s, 1 H).

^{13}C NMR (DMSO- d_6 , 75 MHz): $\delta = 55.76, 55.79, 67.26, 111.43, 113.66, 115.78, 115.96, 119.54, 123.84, 125.16, 125.76, 130.64, 143.96, 145.64, 148.11, 149.92, 160.12, 166.19, 192.75$.

LC-MS (+APCI): m/z 355 [M + H]⁺; LC-MS (–APCI): m/z 353 [M – H][–].

Anal. Calcd for $\text{C}_{20}\text{H}_{18}\text{O}_6$: C, 67.78; H, 5.12. Found: C, 67.38; H, 5.17.

Demethoxycalcebin-A2 (4-Hydroxycinnamoylmethyl Ferulate, 10c)

The general synthetic procedure was followed wherein 4-hydroxycinnamoylmethyl iodide (**8b**; $R_1 = \text{H}$) was reacted with **9a** ($R_2 = \text{OMe}$). The yield of the product was 8.2 g (51%).

Creamish solid; m.p. 175.7–177.7 °C.

IR: 3395, 3336, 2919, 2121, 1702, 1656, 1626, 1594, 1517, 1463, 1436, 1384, 1330, 1289, 1266, 1247, 1217, 1170, 1161, 1136, 1105, 1082, 1036, 1009, 985, 967, 934, 876, 866, 851, 825, 818, 716, 631, 606 cm^{-1} .

^1H NMR (DMSO- d_6 , 300 MHz): $\delta = 3.819$ (s, 3 H), 5.127 (s, 2 H), 6.591 (d, $J = 16.2$ Hz, 1 H), 6.773 (d, $J = 16.2$ Hz, 1 H), 6.807 (d, $J = 8.4$ Hz, 1 H), 6.825 (d, $J = 8.4$ Hz, 2 H), 7.157 (dd, $J = 8.4$ Hz, 1.8 Hz, 1 H), 7.357 (d, $J = 1.8$ Hz, 1 H), 7.589 (d, $J = 8.4$ Hz, 2 H), 7.614 (d, $J = 16.2$ Hz, 1 H), 7.631 (d, $J = 16.2$ Hz, 1 H), 9.731 (s, 1 H), 10.207 (s, 1 H).

^{13}C NMR (DMSO- d_6 , 75 MHz): $\delta = 55.79, 55.81, 67.25, 111.37, 113.93, 115.63, 116.04, 119.21, 123.45, 125.24, 125.63, 130.83, 143.60, 145.94, 148.06, 149.60, 160.37, 166.20, 192.77$.

LC-MS (+APCI): m/z 355 [M + H]⁺; LC-MS (–APCI): m/z 353 [M – H][–].

Anal. Calcd for $\text{C}_{20}\text{H}_{18}\text{O}_6$: C, 67.78; H, 5.12. Found: C, 67.38; H, 5.17.

Bisdemethoxycalcebin-A (4-Hydroxycinnamoylmethyl 4-Hydroxycinnamate, 10d)

The general synthetic procedure was followed; wherein 4-hydroxycinnamoylmethyl iodide (**8b**; $R_1 = \text{H}$) was reacted with **9b** ($R_2 = \text{H}$). The yield of the product was 8.0 g (55%).

Pale-yellow solid; m.p. 289–291 °C.

IR: 3363, 2962, 2916, 2849, 2355, 1692, 1672, 1631, 1598, 1577, 1513, 1439, 1367, 1329, 1259, 1200, 1160, 1099, 1041, 1019, 975, 939, 854, 800, 631, 602 cm^{-1} .

^1H NMR (DMSO- d_6 , 300 MHz): $\delta = 5.120$ (s, 2 H), 6.507 (d, $J = 15.9$ Hz, 1 H), 6.771 (d, $J = 15.9$ Hz, 1 H), 6.813 (d, $J = 8.4$ Hz, 2 H), 6.825 (d, $J = 8.4$ Hz, 2 H), 7.587 (d, $J = 8.4$ Hz, 4 H), 7.626 (d, $J = 15.9$ Hz, 2 H), 10.140 (s, 1 H), 10.201 (s, 1 H).

^{13}C NMR (DMSO- d_6 , 75 MHz): $\delta = 67.29, 113.65, 115.98, 116.09, 119.22, 125.18, 125.26, 130.64, 130.88, 143.64, 145.66, 160.14, 160.41, 166.20, 192.82$.

LC-MS (+APCI): m/z 325 [M + H]⁺; LC-MS (–APCI): m/z 323 [M – H][–].

HR-LC MS: m/z [M + H]⁺ calcd for $\text{C}_{19}\text{H}_{17}\text{O}_5^+$: 325.10760; found: 325.10713.

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