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Synthesis of Novel Polyphenols Consisted of Ferulic and Gallic Acids, and Their Inhibitory Effects on Phorbol Ester-Induced Epstein–Barr Virus Activation and Superoxide Generation

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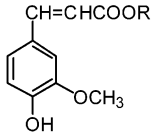
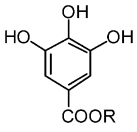
Abstract—We prepared novel polyphenols which were esters composed of two naturally occurring products, ferulic and gallic acids, and investigated their inhibitory effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein–Barr virus (EBV) activation and superoxide (O_2^-) generation. Most of these compounds exhibited significant EBV activation suppression at a concentration of 20 μM and in particular, the ester **5f** having 2-metyl-1-butyl group showed high activity. The suppressive effects on O_2^- generation were also observed in most of the esters.

Introduction

Ferulic acid **1a** is a phenolic compound and some authors have recently developed a mass production method of **1a** from rice bran.[1,2] The acid **1a** is known to form various derivatives having a variety of biological functions by combining with other compounds. For example, chlorogenic acids are natural products containing ferulic acid skeleton and they have the potential for anti-carcinogenesis.[3,4] Although there are many natural compounds which have biological activities, it is difficult to utilize practically as chemopreventive agents due to the presence of small quantities of these compounds in plants. Therefore, interest in the design of the synthetic compounds with enhanced chemopreventive activities has intensified in recent years.[5,6] The studies along with this line were performed by some authors. Alkyl ferulates have a high anti-carcinogenic potential.[7] A ferulic acid derivative in which geranyl group is attached to the phenolic hydroxyl group of ethyl ferulate exhibits colon anti-carcinogenesis in rodent models.[2,8,9] The ester compounds consisting of ferulic acid and mio-inositol exert the inhibitory effect on superoxide generation.[10]





1 a: (R=H)
b: (R=alkyl)

2 a: (R=H)
b: (R=methyl)

We now prepared novel polyphenols which were composed of two naturally occurring products, ferulic acid and gallic acid, and assessed their anti-caricinogenic potential. It was found that these polyphenols were obtained in quantity and had higher activity as an anti-carcinogen than the original phytochemicals. Gallic acid **2a** is a component of plant tannin and exhibits a variety of chemical and biological properties that include pharmacological activity.[11,12] In this paper, we describe the condensation of the two molecules and investigations of inhibitory effects of the conjugates on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein–Barr virus (EBV) activation and superoxide generation in vitro.[13,14]

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Results and Discussion

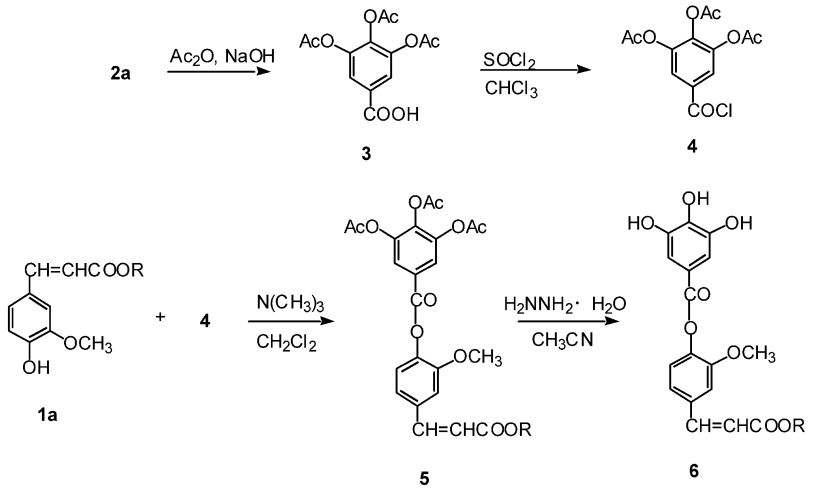
Chemistry

Two types of esters can be prepared by the esterification of ferulic acid with gallic acid. One is a family that galloyl group introduced into the hydroxyl group of ferulic acid. The other is a family that feruloyl group introduced into the hydroxyl groups of gallic acid. The latter family has five possible kinds of esters as feruloyl group can be stayed in the three hydroxyl groups of gallic acid. We chose compounds of the former family because it was easy to prepare and structurally simple. Various types of alkyl ferulates **1b** were chosen to the condensation with gallic acid because some authors reported previously that **1b** were more effective chemopreventive agents than **1a**.[7] The reaction was performed by the reaction of alkyl ferulates with *O*-acetyl galloyl chloride, followed by deacetylation as shown in Scheme 1. *O*-acetyl gallic acid was prepared by the reaction of gallic acid with acetic anhydride in aqueous NaOH in high yield and then converted to the acid chloride using thionyl chloride in a quantitative yield. The esterification was performed in dichloromethane in the presence of triethylamine at room temperature to afford corresponding esters **5a**–**g** in moderate or high yields (67–86%). The removal of acetyl groups without cleaving ester bond was carried out using hydrazine monohydrate at room temperature to yield **6a**–**g**. The compounds which we prepared are listed in Table 1. The esters **6a**–**g** are target polyphenols to estimate biological activities. The acetylated compounds **5a**–**g** might exhibit the biological activities because naturally occurring product such as 1′-actoxychavicol acetate showed the inhibition of a tumor promotion.[15] Therefore, we used the esters **5a**–**g** and **6a**–**g** to assess their anti-caricinogenic potential.

The ester **6f** was recrystallized from methanol to give prism crystals which included methanol molecules inside the crystals. The crystal structure of **6f** was determined by a single crystal X-ray analysis (Fig. 1). Two methanol molecules are captured in the crystal lattice and form hydrogen bonds with two hydroxyl groups of galloyl group. The distances of O(9)···O(2) and O(10)···O(3) are 2.795(5) and 2.845(6) Å, respectively. The rest of the hydroxyl group of galloyl group forms a hydrogen bond with the ester oxygen O(7) of neighboring molecule and the distance is 2.722(5) Å.

Biological activity

For the ester compounds prepared, we investigated their suppressive effects on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced EBV activation in vitro in order to estimate their anti-carcinogenic potential. This assay system was established for the evaluation of anti-tumor-promoting activities in vitro.[13,14] The results were shown in Figure 2. The compounds **5a**–**g** are OH-protected hydrophobic molecules and **6a**–**g** are molecules carrying three OH groups. As shown in Figure 2A, **1a** was inactive at a concentration of 100 μM [6] and methyl gallate **2b**, which is a control compound, showed moderate suppression (inhibitory rate of 64%) of the EBV activation. The esters **5a** and **6a** showed marked suppression of the EBV activation (inhibitory rates of 100%) without notable cytotoxicity. On the other hand, while **5b**–**g** and **6b**–**g** exhibited high suppression of the EBV activation, substantial cytotoxicity (cell viability = CV < 25%) to Raji cells was observed at a concentration of 100 μM. Figure 2B shows the activities for **5b**–**g** and **6b**–**g** at a concentration of 20 μM. All compounds tested still exhibited high suppression (inhibitory rates of 70–100%) and low cytotoxicity (CV = 60–81%) at this concentration. The suppressive effects of alkyl ferulates **1b** at a concentration of 20 μM were previously reported.[7] The ferulates **1b** carrying straight chains (carbon atom number: C5–C12) showed the activation (inhibitory rates of 3.3–67.5%) and only **1b** having 2-methyl-1-hexyl group exhibited high-suppression (inhibitory rate of 100%). We then examined the activities for **5a**–**g** and **6a**–**g** at a lower concentration of 4 μM as shown in Figure 2C. The compounds **5a**–**b** and **6a**–**b** showed weak suppressive activity (inhibitory rates of 13–30%), and **5c**–**e** and **6c**–**e** exhibited low or no activities at 4 μM. Moreover, **5f**–**g** and **6f**–**g**, having branched alkyl groups, showed weak or high activity (inhibitory rates of 10–83%) and it was found that **5f** and **6f** were higher potent suppressor (inhibitory rates of



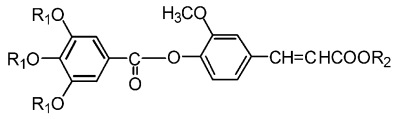
Scheme 1.

62 and 83%, respectively) among the compounds evaluated.

The suppressive effects on the NADPH oxidase system responsible for TPA-induced O_2^- generation were examined using DMSO-differentiated HL-60 cells, a neutrophil model. The suppressive effects for **5a–g** and **6a–g** at 20 μM are shown in Figure 3. The compounds **5a–c**, **5f–g**, **6a–c**, and **6g** exhibited high suppressive activities on O_2^- generation (inhibitory rates of 53–100%), however, and **5d–e** and **6d–f** were less potent (inhibitory rates of 13–40%). It was noteworthy that **5c** and **5f** suppressed O_2^- generations by 100% and **6f** was less active (inhibitory rates of 13%) in contrast to the data shown in the EBV activation test. The structural difference between **5f** and **6f** is whether phenolic hydroxyl groups are protected or not.

In the EBV activation assay, an important structural factor seems to be not the presence of the OH group but the nature of alkyl chain. On the other hand, in attenuating O_2^- generation, the presence of the OH groups and the nature of alkyl chain affect the activities. The OH group and alkyl groups in bioactive phytochemicals are supposed to be effective or ineffective depending on assay system or permeability of cellular membrane. For example, alkyl ferulates having a longer or branched alkyl chain are highly active in above both assay systems.[7] It was assumed that their potent suppressors of O_2^- generation were correlated to hydrophobicity of the molecules to incorporate into cells, which were demonstrated by the cellular uptake experiments.

Table 1. The esters consisting of farulic and gallic acids



Compound	R_1	R_2	Yield (%)
5a	Acetyl	Ethyl	83
6a	H	Ethyl	60
5b	Acetyl	Butyl	86
6b	H	Butyl	70
5c	Acetyl	Hexyl	84
6c	H	Hexyl	78
5d	Acetyl	Decyl	73
6d	H	Decyl	83
5e	Acetyl	Undecyl	67
6e	H	Undecyl	73
5f	Acetyl	2-Methyl-1-butyl	67
6f	H	2-Methyl-1-butyl	71
5g	Acetyl	2-Ethyl-1-hexyl	81
6g	H	2-Ethyl-1-hexyl	42

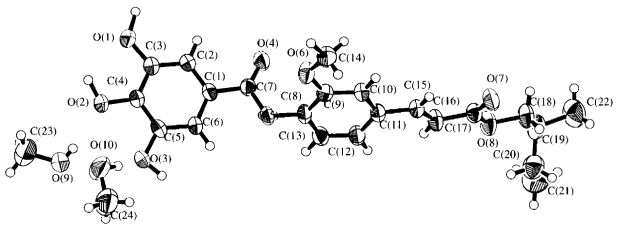


Figure 1. ORTEP drawing of **6f**.

Conclusion

The novel polyphenols consisting of two naturally occurring compounds, ferulic and gallic acids, were prepared. Most of these compounds exhibited their inhibitory abilities on TPA-induced EBV activation and O_2^- generation in vitro. In particular, **5f** could be the most promising chemopreventive agents among the compounds evaluated in the present study. Mass production method of ferulic acid from rice bran was developed in recent years[1,2] and the production of gallic acid was already on a commercial basis. Therefore, it is valuable to obtain more effective anti-carcinogenic agents as derivatives from natural compounds for practical use. While the present study provided the anti-carcinogenic capability of the conjugates in the first stage

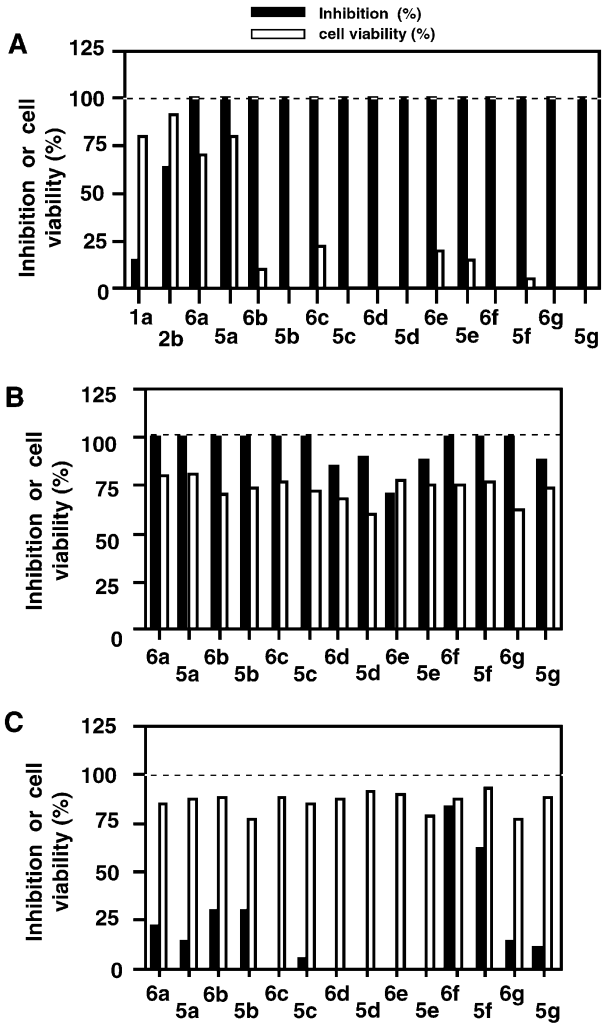


Figure 2. Suppressive effects of the polyphenols on TPA-induced EBV activation in Raji cells. Cells were incubated with *n*-BA (3 mM), TPA (50 nM), and test compounds (0, 4 (panel C), 20 (panel B), or 100 (panel A) μM). EBV activation was measured by detecting EA-induction. Open and closed bars represent cell viability (%) and EBV activation suppression (%), respectively. Data are shown as mean values from duplicate experiments.

of the assay systems, the studies of these compounds in rodent model in the future will serve to elucidate the efficacies.

Experimental

General

Ferulic acid and alkyl ferulate were generously supplied by Tsuno Rice Fine Chemicals Co., Ltd. (Wakayama, Japan). Gallic acid and methyl gallate were kindly supplied by Fuji Chemical Co., Ltd. (Wakayama, Japan). Anhydrous CH_2Cl_2 was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and used without further purification. Other solvents and reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and used without further purification. Using acetic anhydride in aqueous NaOH was performed acetylation for hydroxyl groups of gallic acid. *O*-acetylgallic acid was converted to the acid chloride by using thionyl chloride in the presence of pyridine and a small amount of DMF. Melting points were determined by a Yanaco micro melting point apparatus and are uncorrected. Elemental analyses were performed on a Perkin–Elmer 2400II. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity-plus 400 spectrometer using a residual solvent as an internal standard. FT-IR spectra were obtained on a Shimadzu FT-IR 8200D spectrometer using a diffuse reflectance cell.

Chemicals and cells for bioassay

TPA was obtained from Research Biochemicals International, Natick, MA. DMEM and RPMI 1640 media, and fetal bovine serum (FBS) were purchased from Gibco BRL, NY. FITC-labeled anti-human IgG was obtained from Dako, (Glostrup, Denmark). Cytochrome *c* was obtained from Sigma, MO. Other chemicals were purchased from Wako Pure Chemical Industries, Ltd., unless specified otherwise. Human B-lymphoblastoid Raji cells and high-titer EBV-early antigen (EA)-positive sera from naso-pharyngeal carcinoma patients were kindly provided by. Dr. Ohsato (Health Sciences University of Hokkaido, Japan).

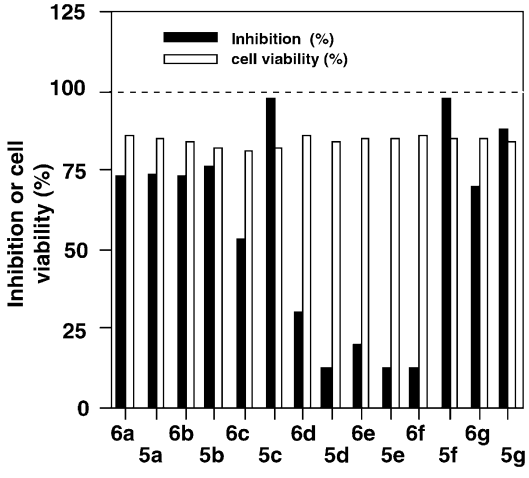


Figure 3. Suppressive effects of the polyphenols on TPA-induced O_2^- generation in differentiated HL-60 cells. O_2^- generation was induced by TPA (50 nM) and detected by cytochrome *c*. Sample concentrations were 0 or 20 μM). Open and closed bars represent cell viability (%) and O_2^- generation suppression (%), respectively. Data are shown as mean values from duplicate experiments.

EBV activation

An EBV activation inhibitory test was performed as previously reported.[16] Raji cells were incubated in 1 mL of RPMI 1640 medium (supplemented with 10% FBS) containing sodium *n*-butyric acid (BA) (3 mM), TPA (50 nM), and the test compound (4, 20, and 100 μM) at 37 °C under a 5% CO_2 atmosphere for 48 h. EBV activation was evaluated by the detection of EA, stained by an indirect immunofluorescence method with high-titer EA-positive sera from nasopharyngeal carcinoma patients, followed by flourescein isothiocyanate (FITC)-labeled IgG. The rate of EA-induced cells was compared with the rate obtained in a control experiment using only BA, TPA, and DMSO [0.5% (v/v)], in which the rate of EA-induced cells was ordinarily around 40%. Inhibitory rates were calculated by the following formula: inhibitory rate (%) = {1− [(sample,%EA-positive cells)/(TPA + *n*-BA,%EA-positive cells)]} × 100. Cell viability (CV) was determined by a Trypan Blue dye exclusion test. Each experiment was done in duplicate, and the mean values are shown.

O_2^- generation

Inhibitory tests of TPA-induced O_2^- generation were performed as previously reported.[13] HL-60 cells (5×10^5 cells/mL) were incubated in 1.3% dimethylsulfoxide in RPMI medium (supplemented with 10% FBS) for 6 days to induce differentiation into granulocyte-mimic cells. Differentiated HL-60 cells, suspended in 1 mL of Hank's buffer, were treated with 20 μM of each test compound (5 μL of stock solution), or the vehicle. After preincubation at 37 °C for 15 min, the suspension was centrifuged and the extracellular compounds were removed by washing with 1% bovine serum albumin (BSA) in Hank's buffer. The cells were then suspended in 1 mL of Hank's buffer, and incubated with 100 μM TPA or the vehicle and 1 mg/mL cytochrome *c* at 37 °C for 30 min. The reaction was terminated by adding a superoxide dismutase solution (10,000 U/mL) and being placed on ice. After centrifugation, the level of extracellular O_2^- was measured by the cytochrome *c* reduction method, in which reduced cytochrome *c* was quantified by measuring the visible absorption of the supernatant at 550 nm. Cells treated with a compound, cytochrome *c*, and the vehicle without TPA were used as a negative control, while cells with the vehicle, but without the compound, cytochrome *c*, or TPA were used as a positive control. Cells treated with the vehicle, but without the compound, cytochrome *c*, or the vehicle without TPA, were used as a blank. Inhibitory rates were calculated by the following formula: inhibitory rate (%) = {1 − [(test compound, Abs_{550}) − (negative, Abs_{550})/(positive, Abs_{550}) − (blank, Abs_{550})]} × 100(%). Cell viability was determined by a Trypan Blue dye exclusion test. Each experiment was done independently

in duplicate twice, and the data are shown as mean values.

General procedure of the reaction of alkyl ferulate with 3,4,5-triacetoxybenzoyl chloride

To a solution of 0.5 g of alkyl ferulate and triethylamine (two times molar amounts of alkyl ferulate) in anhydrous CH_2Cl_2 (20 mL) was added 3,4,5-triacetoxybenzoyl chloride (two times molar amounts of alkyl ferulate). The mixture was stirred for 4 h at rt under nitrogen. Ice-water was added to the reaction mixture, and the organic portion was extracted with CH_2Cl_2 (20 mL × 3) and dried over $MgSO_4$. After removal of the solvent, the product was purified through SiO_2 column ($CHCl_3$) and recrystallzed from $CHCl_3$/MeOH.

Ethyl 3-{4-(3,4,5-triactyloxybenzoayloxy)-3-methoxy phenyl}-2-propenoate (5a). 0.95 g (83%) of crystals; mp 148–149 °C; IR (KBr) ν 1782, 1747, 1713, 1641, 1599, 1508 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (t, 3H, J = 7.1 Hz, CH_3) 2.30 (s, 6H, Ac), 2.31 (s, 3H, Ac), 3.82 (s, 3H, OCH_3), 4.26 (q, 2H, J = 7.1 Hz, CH_2), 6.39 (d, 1H, J = 16.0 Hz, =CH), 7.11–7.13 (m, 3H, ArH), 7.64 (d, 1H,,J = 16.0 z, =CH), 7.94 (s, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 14.3, 20.2, 20.6, 55.9, 60.6, 111.2, 118.6, 121.2, 123, 123.2, 127.3, 133.7, 139.2, 141.2, 143.5, 143.8, 151.4, 162.3, 166.4, 166.8, 167.6. Anal. calcd for $C_{25}H_{24}O_{11}$: C, 60.00%; H, 4.83%; found: C, 59.96%; H, 4.72%.

Butyl 3-{4-(3,4,5-triacetyloxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (5b). 0.86 g (86%) of crystals; mp 143–144.5 °C; IR (KBr) ν 1784, 1753, 1701, 1638, 1601, 1508 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (t, 3H, J = 7.3 Hz, CH_3), 1.43 (m, 2H, CH_2), 1.68 (m, 2H, CH_2), 2.30 (s, 6H, Ac), 2.31 (s, 3H, Ac), 3.82 (s, 3H, OCH_3), 4.20 (t, 2H, J = 6.7 Hz, OCH_2), 6.39 (d, 1H, J = 15.9 Hz, =CH), 7.10–7.13 (m, 3H, ArH), 7.64 (d, 1H, J = 15.9 Hz, = CH), 7.94 (s, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 13.8, 19.2, 20.2, 20.6, 30.7, 55.9, 64.5, 111.2, 118.6, 121.2, 123.0, 123.2, 127.3, 133.7, 139.2, 141.2, 143.5, 143.8, 151.4, 162.3, 166.4, 167.0, 167.6. Anal. calcd for $C_{27}H_{28}O_{11}\cdot 0.5H_2O$: C, 60.33%; H, 5.44%; found: C, 60.00%; H, 5.10%.

Hexyl 3-{4-(3,4,5-triacetyloxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (5c). 0.84 g (84%) of crystals; mp 144–145 °C; IR (KBr) ν 1798, 1774, 1740, 1713, 1639, 1599, 1515 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 7.1 Hz, CH_3), 1.31–1.40 (m, 6H, CH_2), 1.67–1.71 (m, 2H, CH_2), 2.30 (s, 6H, Ac), 2.31 (s, 3H, Ac), 3.82 (s, 3H, OCH_3), 4.19 (t, 2H, J = 6.9 Hz, OCH_2), 6.39 (d, 1H, J = 15.8 Hz, =CH), 7.10–7.13 (m, 3H, ArH), 7.64 (d, 1H, J = 15.8 Hz, =CH), 7.94 (s, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 14.0, 20.2, 20.6, 22.5, 25.6, 28.7, 31.5, 55.9, 64.8, 111.2, 118.6, 121.2, 123.0, 123.2, 127.3, 133.7, 139.2, 141.1, 143.5, 143.8, 151.4, 162.3, 166.4, 166.9, 167.6. Anal. calcd for $C_{29}H_{32}O_{11}$: C, 62.58%; H, 5.80%; found: C, 62.71%; H, 5.72%.

Decyl 3-{4-(3,4,5-triacetyloxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (5d). 0.67 g (73%) of crystals; mp 118–120 °C; IR (KBr) ν 1778, 1740, 1717, 1639, 1601, 1508 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 6.9 Hz, CH_3), 1.2–1.5 (m, 14H, CH_2), 1.66–1.71 (m, 2H, CH_2), 2.30 (s, 6H, Ac), 2.31 (s, 3H, Ac), 3.82 (s, 3H, OCH_3), 4.19 (t, 2H, J = 6.8 Hz, OCH_2), 6.39 (d, 1H, J = 16.0 Hz, =CH), 7.10–7.13 (m, 3H, ArH), 7.64 (d, 1H, J = 16.0 Hz, =CH), 7.94 (s, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 14.1, 20.2, 20.6, 22.7, 26.0, 28.7, 29.3, 29.5, 32.0, 56.0, 64.8, 111.2, 118.6, 121.2, 123.0, 123.2, 127.3, 133.7, 139.2, 141.2, 143.5, 143.8, 151.4, 162.3, 166.4, 166.9, 167.6. Anal. calcd for $C_{33}H_{40}O_{11}$: C, 64.69%; H, 6.55%; found: C, 64.31%; H, 6.55%.

Undecyl 3-{4-(3,4,5-triacetyloxybenzoayloxy)-3-meth-oxyphenyl}-2-propenoate (5e). 0.59 g (67%); mp 97–99 °C; IR (KBr)? ν 1774, 1746, 1715, 1636, 1601, 1508 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 6.9 Hz, CH_3), 1.2–1.4 (m, 16H, CH_2), 1.67–1.71 (m, 2H, CH_2), 2.30 (s, 6H, Ac), 2.31 (s, 3H, Ac), 3.82 (s, 3H, OCH_3), 4.19 (t, 2H, J = 6.8 Hz, OCH_2), 6.39 (d, 1H, J = 15.9 Hz, =CH), 7.10–7.13 (m, 3H, ArH), 7.64 (d, 1H, J = 15.9 Hz, =CH), 7.94 (s, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 14.1, 20.2, 20.6, 22.7, 26.0, 28.7, 29.3, 29.3, 29.5, 29.6, 31.9, 55.9, 64.8, 111.2, 118.7, 121.2, 123.0, 123.2, 127.4, 133.7, 139.2, 141.2, 143.5, 143.8, 151.4, 162.3, 166.4, 166.9, 167.6. Anal. calcd for $C_{34}H_{42}O_{11}$: C, 65.16%; H, 6.76%; found: C, 64.71%; H, 6.68%.

2-Methyl-1-butyl 3-{4-(3,4,5-triacetyloxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (5f). 0.69 g (67%); mp 140–142 °C; IR (KBr) ν 1784, 1753, 1699, 1636, 1601, 1508 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91–0.97 (m, 6H, CH_3), 1.19–1.26 (m, 1H, CH_2), 1.26–1.54 (m, 1H, CH_2), 1.74–1.82 (m, 1H, CH), 2.30 (s, 6H, OAc), 2.31 (s, 3H, OAc), 3.83 (s, 3H, OCH_3), 3.98–4.11 (m, 2H, OCH_2), 6.40 (d, 1H, J = 15.9 Hz, =CH), 7.10–7.16 (m, 3H, ArH), 7.64 (d, 1H, J = 15.9 Hz, =CH), 7.94 (s, 2H, ArH); ^{13}C NMR ($CDCl_3$) δ 11.3, 16.5, 20.2, 20.6, 26.1, 34.2, 55.9, 69.3, 111.2, 118.7, 121.2, 123.0, 123.2, 127.3, 133.7, 139.2, 141.2, 143.5, 143.8, 151.4, 162.3, 166.4, 166.9, 167.6. Anal. calcd for $C_{28}H_{30}O_{11}$: C, 61.99%; H, 5.57%; found: C, 61.41%; H, 5.45%.

2-Ethyl-1-hexyl 3-{4-(3,4,5-triacetyloxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (5g). 0.76 g (81%); mp 116–118 °C; IR (KBr) ν 1784, 1726, 1593, 1491 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.87–0.93 (m, 6H, CH_3), 1.31–1.43 (m, 8H, CH_2), 1.61–1.65 (m, 1H, CH), 2.30 (s, 6H, OAc), 2.31 (s, 3H, OAc), 3.83 (s, 3H, OCH_3), 4.10–4.13 (m, 2H, OCH_2), 6.39 (d, 1H, J = 15.9 Hz, =CH), 7.10–7.14 (m, 3H, ArH), 7.63 (d, 1H, J = 15.9 Hz, =CH), 7.94 (s, 2H, ArH). ^{13}C NMR ($CDCl_3$) δ 11.0, 14.1, 20.2, 20.6, 23.0, 23.8, 29.0, 30.4, 38.9, 55.9, 67.1, 111.2, 118.7, 121.2, 123.0, 123.2, 127.3, 133.7, 139.2, 141.2, 143.5, 143.7, 151.4, 162.3, 166.4, 167.0, 167.6. Anal. calcd for $C_{31}H_{36}O_{11}$: C, 63.69%; H, 6.21%; found: C, 63.48%; H, 6.12%.

General procedure of the reaction of deacetylation

To a solution of 0.5 g of the esters **5** in 20 mL acetonitrile was added hydrazine monohydrate (three times molar amounts of alkyl ferulate). The mixture was stirred for 15 min at rt. Acetic acid was added to the mix-

ture. The organic portion was extracted with ethyl acetate (50 mL), washed with water (30 mL × 2) and satd aqueous NaCl (20 mL), and dried over $MgSO_4$. The solvent was evaporated to give a sold.

Ethyl 3-{4-(3,4,5-trihydroxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (6a). Recrystallized from MeOH to afford 0.224 g (60%) of crystals: mp 241–244 °C; IR (KBr) ν 3477, 1736, 1666, 1612, 1537, 1508 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.26 (t, 3H, J = 7.1 Hz, CH_3), 3.80 (s, 3H, OCH_3), 4.19 (q, 2H, J = 7.1 Hz, CH_2), 6.72 (d, 1H, J = 16.0 Hz, =CH), 7.06 (s, 2H, ArH), 7.20 (d, 1H, J = 8.2 Hz, ArH), 7.32 (d, 1H, J = 8.2 Hz, ArH), 7.54 (s, 1H, ArH), 7.65 (d, 1H, J = 16.0 Hz, =CH), 9.3 (brs, 3H, OH); ^{13}C NMR (DMSO-d_6) δ 14.4, 56.2, 60.3, 109.4, 112.1, 118.0, 118.6, 121.9, 123.7, 133.1, 139.5, 141.6, 144.1, 146.0, 151.6, 164.1, 166.5. Anal. calcd for $C_{19}H_{18}O_8$: C, 60.96%; H, 4.85%; found: C, 60.74%; H, 4.80%.

Butyl 3-{4-(3,4,5-trihydroxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (6b). Recrystallized from MeOH to afford 0.28 g (70%) of crystals: mp 168–169 °C; IR (KBr) ν 3476, 3310, 1724, 1684, 1635, 1610, 1601, 1541, 1514 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.91 (t, 3H, J = 7.3 Hz, CH_3), 1.33–1.43 (m, 2H, CH_2), 1.59–1.66 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.15 (t, 2H, J = 6.7 Hz, OCH_2), 6.71 (d, 1H, J = 16.0 Hz, =CH), 7.06 (s, 2H, ArH), 7.20 (d, 1H, J = 8.4 Hz, ArH), 7.32 (dd, 1H, J = 8.4, 1.6 Hz, ArH), 7.54 (d, 1H, J = 1.6 Hz, ArH), 7.65 (d, 1H, J = 16.0 Hz, =CH), 9.3 (brs, 3H, OH); ^{13}C NMR (DMSO-d_6) δ 13.81, 18.9, 30.5, 56.2, 64.0, 109.4, 112.1, 118.0, 118.5, 121.9, 123.7, 133.1, 139.5, 141.6, 144.1, 146.0, 151.6, 164.1, 165.6. Anal. calcd for $C_{21}H_{22}O_8 \cdot H_2O$: C, 59.99%; H, 5.75%; found: C, 60.02%; H, 5.31%.

Hexyl 3-{4-(3,4,5-trihydroxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (6c). Recrystallized from MeOH to afford 0.30 g (78%) of crystals: mp 138–140 °C; IR (KBr) ν 3580, 3477, 3327, 1774, 1724, 1690, 1639, 1599, 1537, 1514 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.87 (t, 3H, J = 6.9 Hz, CH_3), 1.28–1.36 (m, 6H, CH_2), 1.60–1.65 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.14 (t, 2H, J = 6.7 Hz, OCH_2), 6.72 (d, 1H, J = 16.0 Hz, =CH), 7.06 (s, 2H, ArH), 7.20 (d, 1H, J = 8.1 Hz, ArH), 7.32 (dd, 1H, J = 8.1, 1.6 Hz, ArH), 7.54 (d, 1H, J = 1.6 Hz, ArH), 7.65 (d, 1H, J = 16.0 Hz, =CH), 9.15 (bs, 1H, OH), 9.41 (bs, 2H, OH); ^{13}C NMR (DMSO-d_6) δ 14.1, 22.2, 25.3, 28.4, 31.1, 56.2, 64.2, 109.4, 112.0, 118.0, 118.5, 121.9, 123.7, 133.1, 139.5, 141.6, 144.1, 145.9, 151.6, 164.1, 166.6. Anal. calcd for $C_{23}H_{26}O_8 0.5H_2O$: C, 62.86%; H, 6.19%; found: C, 63.17%; H, 5.95%.

Decyl 3-{4-(3,4,5-trihydroxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (6d). Recrystallized from MeOH/$CHCl_3$ to afford 0.33 g (83%) of crystals: mp 114–115 °C; IR (KBr) ν 3490, 3305, 1726, 1690, 1634, 1601, 1551, 1514 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.84 (t, 3H, J = 6.7 Hz, CH_3), 1.2–1.4 (m, 14H, CH_2), 1.61–1.65 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.14 (t, 2H, J = 6.6 Hz, OCH_2), 6.72 (d, 1H, J = 15.9 Hz, =CH), 7.06 (s, 2H, ArH), 7.20 (d, 1H, J = 8.2 Hz, ArH), 7.32 (d, 1H, J = 8.2 Hz, ArH), 7.54 (d, 1H, J = 1.6 Hz, ArH), 7.64 (d, 1H, J = 15.9 Hz, =CH), 9.3 (brs, 3H, OH); ^{13}C NMR (DMSO-d_6) δ 14.2, 22.3, 25.6, 28.4, 28.9, 29.2, 31.5, 56.2, 64.2, 109.4, 112.0, 118.0, 118.5, 121.9, 123.7, 133.1, 139.5, 141.6, 144.1, 145.9, 151.6, 164.1, 166.5. Anal. calcd for $C_{27}H_{34}O_8 \cdot H_2O$: C, 64.27%; H, 7.19%; found: C, 63.98%; H, 7.12%.

Undecyl 3-{4-(3,4,5-trihydroxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (6e). Recrystallized from MeOH/$CHCl_3$ to afford 0.29 g (73%) of crystals: mp 92–94.5 °C; IR (KBr) ν 3659, 3558, 3518, 3269, 1728, 1684, 1634, 1601, 1512 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 6.9 Hz, CH_3), 1.1–1.4 (m, 16H, CH_2), 1.67–1.71 (m, 2H, CH_2), 3.76 (s, 3H, OCH_3), 4.20 (t, 2H, J = 6.8 Hz, OCH_2), 6.39 (d, 1H, J = 15.9 Hz, =CH), 7.06–7.13 (m, 3H, ArH), 7.31 (s, 2H, ArH), 7.65 (d, 1H, J = 15.9 Hz, =CH); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 26.0, 28.7, 29.27, 29.32, 29.5, 29.6, 31.9, 55.9, 65.1, 110.6, 111.4, 118.2, 120.4, 121.2 123.3, 133.3, 137.1, 141.7, 143.6, 144.3, 151.5, 164.5, 167.4. Anal. calcd for $C_{28}H_{36}O_8 \cdot H_2O$: C, 64.85%; H, 7.39%; found: C, 64.82%; H, 7.33%.

2-Methyl-1-butyl 3-{4-(3,4,5-trihydroxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (6f). Recrystallized from MeOH/$CHCl_3$ to afford 0.27 g (71%) of crystals: mp 150–153 °C; IR (KBr) ν 3310, 1724, 1682, 1636, 1601, 1514 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.87–0.94 (m, 6H, CH_3), 1.16–1.21 (m, 1H, CH_2), 1.42–1.48 (m, 1H, CH_2), 1.71–1.76 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.95–4.06 (m, 2H, OCH_2), 6.73 (d, 1H, J = 15.9 Hz, =CH), 7.06 (s, 2H, ArH), 7.20 (d, 1H, J = 8.2 Hz, ArH), 7.32 (dd, 1H, J = 8.2, 1.8 Hz, ArH), 7.55 (d, 1H, J = 1.8 Hz, ArH), 7.65 (d, 1H, J = 15.9 Hz, =CH), 9.15 (brs, 1H, OH), 9.41 (brs, 2H, OH); ^{13}C NMR (DMSO-d_6) δ 11.3, 16.4, 25.7, 33.9, 56.2, 68.6, 109.4, 112.0, 118.0, 118.5, 122.0, 123.7, 133.1, 139.5, 141.6, 144.1, 145.9, 151.6, 164.1, 166.6. Anal. calcd for $C_{22}H_{24}O_8 \cdot 1.5H_2O$: C, 59.59%; H, 6.14%; found: C, 59.83%; H, 5.87%.

2-Ethyl-1-hexyl 3-{4-(3,4,5-trihydroxybenzoayloxy)-3-methoxyphenyl}-2-propenoate (6g). Recrystallized from MeOH/H_2O to afford 0.16 g (42%) of crystals: mp 130.5–132 °C; IR (KBr) ν 3320, 1726, 1688, 1600–1670, 1514 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.86–0.90 (m, 6H, CH_3), 1.29–1.39 (m, 8H, CH_2), 1.58–1.63 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 4.06–4.10 (m, 2H, OCH_2), 6.72 (d, 1H, J = 15.9 Hz, =CH), 7.06 (s, 2H, ArH), 7.20 (d, 1H, J = 8.2 Hz, ArH), 7.32 (d, 1H, J = 8.2 Hz, ArH), 7.55 (s, 1H, ArH), 7.64 (d, 1H, J = 15.9 Hz, =CH), 9.15 (brs, 1H, OH), 9.41 (brs, 2H, OH); ^{13}C NMR (DMSO-d_6) δ 11.0, 14.1, 22.6, 23.4, 28.6, 30.0, 38.5, 56.2, 66.3, 109.4, 112.0, 118.0, 118.5, 122.0, 123.7, 133.1, 139.5, 141.6, 144.1, 145.9, 151.6, 164.1, 166.6. Anal. calcd for $C_{25}H_{30}O_8 \cdot H_2O$: C, 63.01%; H, 6.77%; found: C, 63.09%; H, 6.69%.

Single crystal X-ray diffraction for 6f

The crystal mounted on a glass fiber. The single crystal X-ray data was collected on a Rigaku R-AXIS RAPID Imaging plate diffractometer. All calculations were performed with the crystallographic software package teX-

san (Molecular Structure Corporation, 1985 and 1999). The structure was solved by direct method (SHELXS-97)[17] and expanded using Fourier techniques (DIRDIF94).[18] Non-hydrogen atoms were refined anisotropically. Hydrogen atoms except OH groups were included at calculated positions: Crystal data for $C_{22}H_{24}O_8 \cdot 2(CH_4O)$ **6f**: M = 480.51, monoclinic, $a = 7.257(1)$, $b = 8.996(2)$, $c = 38.848(9)$ Å, $b = 92.341(9)^\circ$, $V = 2534.2(9)$ Å^3, $T = 23\,^\circ$C, space group $P2_1/c$ (no. 14), $Z = 4$, $\mu(\mathrm{Cu}K_\alpha) = 8.26\,\mathrm{cm}^{-1}$, $Dc = 1.259\,\mathrm{g\ cm}^{-3}$, 14,346 reflections measured, 4548 unique ($R_{\mathrm{int}} = 0.029$), Full-matrix least-squares refinement was based on 2915 observed reflections [$I > 2.00\sigma(I)$] and 307 variable parameters. $R = 0.074$, $Rw = 0.124$, GOF = 1.08. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 167664. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: http:www.deposit@ccdc.cam.ac.uk).

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