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Synthesis of Feruloyl-*myo*-insitol Derivatives and their Inhibitory Effects on Phorbol Ester-Induced Superoxide Generation and Esptein–Barr Virus Activation

Asao Hosoda,[a] Eisaku Nomura,[a] Akira Murakami,[b] Koichi Koshimizu,[b] Hajime Ohigashi,[c] Kazuhiko Mizuno[d] and Hisaji Taniguchi[a,*]

[a]*Industrial Technology Center of Wakayama Prefecture, 60 Ogura, Wakayama 649-6261, Japan*
[b]*Department of Biotechnological Science, Faculty of Biology-Oriented Science and Technology, Kinki University, Iwade-Uchita, Wakayama 649-6493, Japan*
[c]*Division of Applied Science, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*
[d]*Department of Applied Chemistry, College of Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan*

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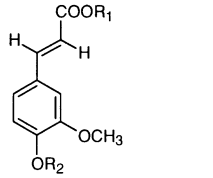
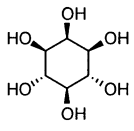
Abstract—We prepared 14 feruloyl-*myo*-inositol derivatives, and evaluated the relationships between their stereostructure and inhibitory activity toward the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced superoxide (O_2^-) generation. And further, their suppressive effect on the TPA-induced Epstein–Barr virus (EBV) activation was examined in order to estimate their anti-carcinogenic potentials. Among the derivatives tested, 1,6-*O*-bis[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**6b**) showed an excellent suppressive activity on the O_2^- generation at a concentration of 20 μM. For the suppressive effects on the EBV activation, 2,4,6-*O*-tris[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol 1,3,5-orthoformate (**9b**) showed the highest activity at a concentration of 100 μM among the derivatives tested. These results suggest that the inhibitory potencies of feruloyl-*myo*-inositol derivatives depend on the stereostructure of molecules rather than the hydrophobicity of molecules.

Introduction

Ferulic acid (**1a**) is a sort of polyphenolic compound occurring widely in the plant kingdom.[1,2] Some of the authors have recently developed a novel method for the convenient preparation of **1a** from the oily component of rice bran at kilogram or ton order.[3,4] Since then, the acid **1a** and the related compounds have attracted considerable attention in the field of cancer chemopreventive study. For example, a ferulic acid derivative, ethyl 3-(4′-geranyloxy-3′-methoxyphenyl)-2-propenoate (EGMP, **1b**),[4–6] in which geranyl group is bonded to the phenolic hydroxyl group of ethyl ferulate (**1d**), shows a suppressive effect on the formation of colonic tumor marker in rats.[7,8]

On the other hand, *myo*-inositol (**2**) is also obtained from rice bran, and it plays an important role in biological systems. It has become clear that D-*myo*-inositol-1,4,5-trisphosphate acts as an intracellular second messenger for calcium mobilization.[9,10] *myo*-Inositol hexaphosphate (IP_6) is shown to have an anticancer action in a variety of experimental tumor models.[11] Thus, we expected that feruloyl-*myo*-inositols, consisting of the acid **1a** and *myo*-inositol moieties, might have some biological activities, including anti-oxidation and anti-carcinogenesis.





2

1a :R_1=R_2=H
1b :R_1=ethyl, R_2=
1c :R_1=methyl, R_2=H
1d :R_1=ethyl, R_2=H
1e :R_1=H, R_2=Ac

*Corresponding author. Tel.: +81-73-477-1271; fax: +81-73-477-2880; e-mail: taniguti@wakayama-kg.go.jp

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In a preliminary paper, we reported the synthesis of seven ester compounds consisting of **1a** and **2** and their inhibitory effects on generation of the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced superoxide (O_2^-) in differentiated HL-60 cells, which contain the NADPH oxidase system generating O_2^- and have been used as a cellular system to search for anti-tumor promoters.[12] In this study, we found that only 3,4,5,6-tetra-*O*-acetyl-1,2-*O*-bis[3-(4′-acetoxy-3′-methoxyphenyl)-2-prop-enoyl]-*myo*-inositol (Fig. 1, **5a**), in which two feruloyl moieties are introduced to the hydroxyl groups bonded to the 1,2-vicinal carbons in *myo*-inositol, showed an excellent inhibitory activity on the assay system. This observation suggests that the inhibitory effect of bisferuloyl-*myo*-inositol derivatives depends on factors as follows: (1) The phenolic and inositol hydroxyl groups in the compounds are protected by acetyl group or not; (2) hydrophobicity of the molecules; (3) stereostructure of the molecules. Among these factors, we have been greatly interested in the relationships between the stereostructure of feruloyl-*myo*-inositols and their inhibitory effects toward the TPA-induced O_2^- generation, because 3,4,5,6-tetra-*O*-acetyl-1-*O*-[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (Fig. 1, **3**) and 1,2:4,5-di-*O*-cyclohexylidene-3,6-*O*-bis[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (Fig. 1, **6a**) showed no activity. Then, we designed to prepare a number of feruloyl-*myo*-inositol derivatives to discuss the relationships between their stereostructure and biological activity.

In the present study, we synthesized five newly novel feruloyl-*myo*-inositol derivatives and investigated their inhibitory activity and that of other nine feruloyl-*myo*-inositols toward the TPA-induced O_2^- generation.[13] The suppressive effect on the TPA-induced Epstein–Barr virus (EBV) activation was also examined in order to estimate their anti-carcinogenic potential.[14]

Results and Discussion

Preparation of feruloyl-*myo*-inositols

We prepared 14 *myo*-inositol derivatives **3**, 1,3,4,5,6-penta-*O*-acetyl-2-*O*-[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**4**), **5a**, 1,2-*O*-bis[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**5b**), 3,4,5,6-tetra-*O*-acetyl-1,2-*O*-bis[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**5c**), **6a**, 1,2:4,5-di-*O*-cyclohexylidene-3,6-*O*-bis[3-(4′-hydoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**6b**), 1,2,4,5-tetra-*O*-acetyl-3,6-*O*-bis[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**7a**), 3,6-*O*-bis[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**7b**), 3,6-*O*-bis[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**7c**), 2,3,4,5-tetra-*O*-acetyl-1,6-*O*-bis[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**8a**), 1,6-*O*-bis[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**8b**), 2,4,6-*O*-tris[3-(4′-acetoxy-3′-methoxyphen-yl)-2-propenoyl]-*myo*-inositol 1,3,5-orthoformate (**9a**) and 2,4,6-*O*-tris[3-(4′-hydroxy-3′-methoxyphenyl)-2-propeno-yl]-*myo*-inositol 1,3,5-orthoformate (**9b**). And further, we classified these compounds into five types of molecular architectures in order to discuss the relationships between their stereostructure and biological activity. The structures of these compounds are shown in Figure 1 with the typical stereostructure of the molecules. 3,4,5,6-Tetra-*O*-acetyl-*myo*-inositol (**10**), 3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl chloride (**11**) and 1,6-*O*-bis[3-(4′-acetyloxy-3′-methoxyphenyl)-2-propenoyl]-3,4-*O*-(1,1,3,3-tetraisoprop-yldisiloxanedi-1,3-yl)-*myo*-inositol (**13**) were synthesized according to literatures.[15,16] Compounds **3**, **5a**, **5b**, **6a**, **6b**, **7b**, **7c**, **9a**, and **9b** were synthesized by the method described in previous papers.[12,17]

The treatment of two equivalent of acetyl chloride with **10** in the presence of a mixture of pyridine and 4-(dimethylamino)pyridine (DMAP) in dichloromethane at room temperature overnight afforded 1,3,4,5,6-penta-*O*-acetyl-*myo*-inositol **12** in a 79% yield which has a hydroxyl group on C-2. The reaction of **12** with two equivalent of **11** in the presence of a mixture of triethylamine (Et_3N) and DMAP in dichloromethane at room temperature overnight gave **4** in a 78% yield (Scheme 1). The treatment of hydrazine monohydrate with **5a** in a mixture of chloroform and methanol at room temperature for 5 h gave **5c** in an 80% yield (Scheme 2). Compound **7a** was obtained by a treatment of acetic anhydride with **7c** at 90 °C for 2 h in pyridine in an 82% yield (Scheme 2). Compounds **8a** and **8b** were synthesized as shown in Scheme 3. The desilylation of **13** was carried out by using a mixture of tetrabutylammonium fluoride and benzoic acid in tetrahydrofuran (THF) at −5 to 0 °C for 6 h to afford 1,6-*O*-bis[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**14**) in a quantitative yield. The reaction of **14** with acetic anhydride in the presence of DMAP in pyridine at room temperature for 2 h yielded **8a** in a 91% yield. The treatment of hydrazine monohydrate with **14** in methanol at room temperature for 2 h gave **8b** in an 80% yield.

Biological Evaluation

The suppressive effects on the NADPH oxidase system responsible for the TPA-induced O_2^- generation were examined using DMSO-differentiated HL-60 cells, a neutrophil model. The results, observed at a concentration of 100 μM, are shown in Figure 2.

Among the bisferuloyl-*myo*-inositols having the stereostructure of type A, compound **5a** exhibited a high suppressive activity toward the O_2^- generation (inhibitory rate of 100%) with moderate cytotoxicity, while **5b** showed a low activity (inhibitory rate of 12%).

The structural difference between **5a** and **5b** is whether the hydroxyl groups are protected or not. In addition, the compound **5c**, in which inositol hydroxyl groups are protected but phenolic ones are not, showed no activity.

These results show that it should be necessary that the phenolic hydroxyl groups are protected for activity-

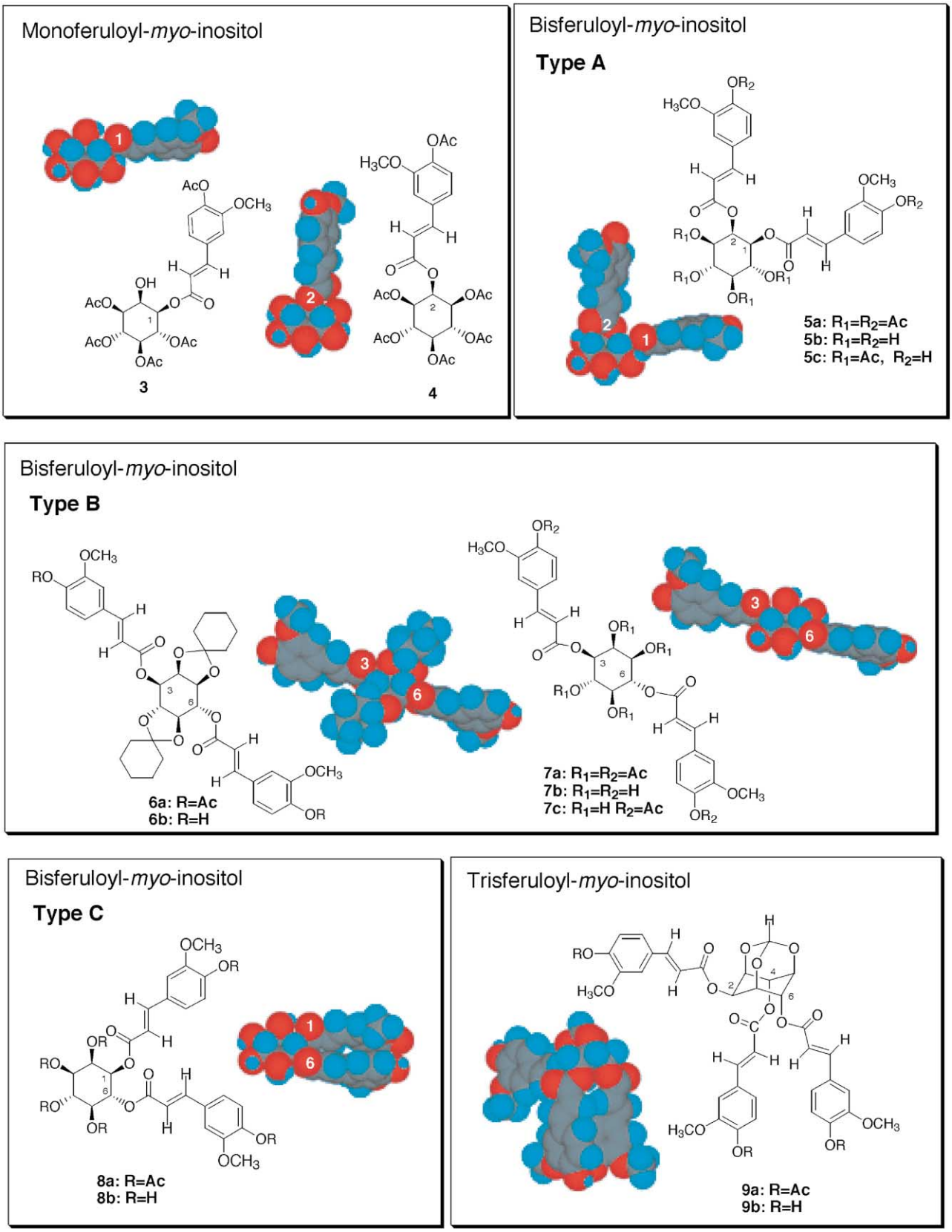
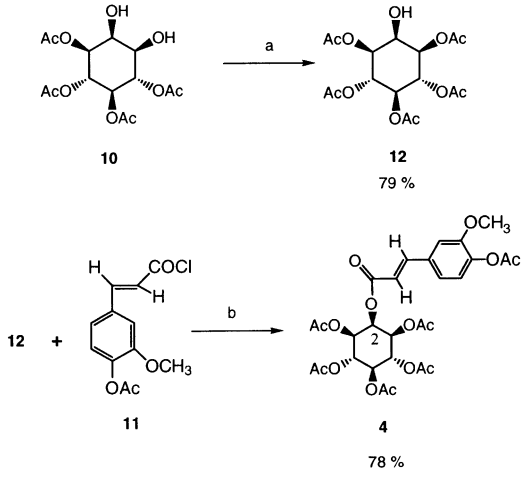


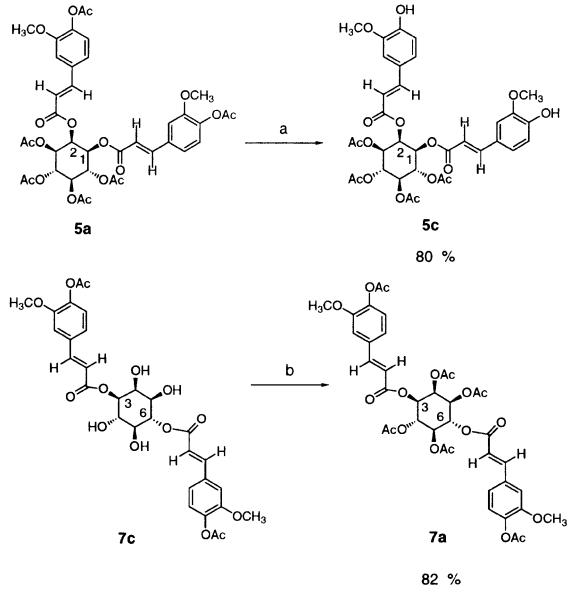
Figure 1. The structure of feruloyl-*myo*-inositols. Typical stereostructures of the molecules (space-filling model) are provided as the energy-minimized structures.

exhibition on this assay system. Moreover, mono-feruloyl-*myo*-inositol derivatives **3** and **4** also exhibited low suppressive activities toward the O_2^- generation. Therefore, the stereostructure of type A, in which the feruloyl group bonded to 2-position of *myo*-inositol is perpendicular to that bonded to 1-position, should be important for the suppression activity.

On the other hand, compounds **6a**, **6b**, **7a**, **7b** and **7c** exhibited low activities (inhibitory rates of 0–30%). From these observations, it became clear that the stereostructure of type B, in which two feruloyl moieties are introduced into the equatorial hydroxyl groups bonded to the 3 and 6 carbons in *myo*-inositol, was unimportant for this assay system. These results also suggest that the inhibitory effects on the O_2^- generation depend on the stereostructure of molecules rather than the hydrophobicity of molecules.



Scheme 1. (a) CH_3COCl, pyridine, DMAP, CH_2Cl_2, rt; (b) Et_3N, DMAP, CH_2Cl_2, rt.



Scheme 2. (a) $NH_2NH_2{\cdot}H_2O$, $CHCl_3$/MeOH (10:1), rt; (b) $(CH_3CO)_2O$, pyridine, 90 °C.

The stereostructure of type C has two feruloyl moieties which are introduced into the equatorial hydroxyl groups bonded to the vicinal 1 and 6 carbons in *myo*-inositol. Compound **8a** showed no activity, but compound **8b** exhibited a high suppressive activity on the O_2^- generation (inhibitory rate of 100%) without notable cytotoxicity. The structural difference between **8a** and **8b** is whether the hydroxyl groups are protected or not. This observation suggests that the stereostructure of type C should be important toward this assay system and the hydroxyl groups play important roles on the suppression of the O_2^- generation.

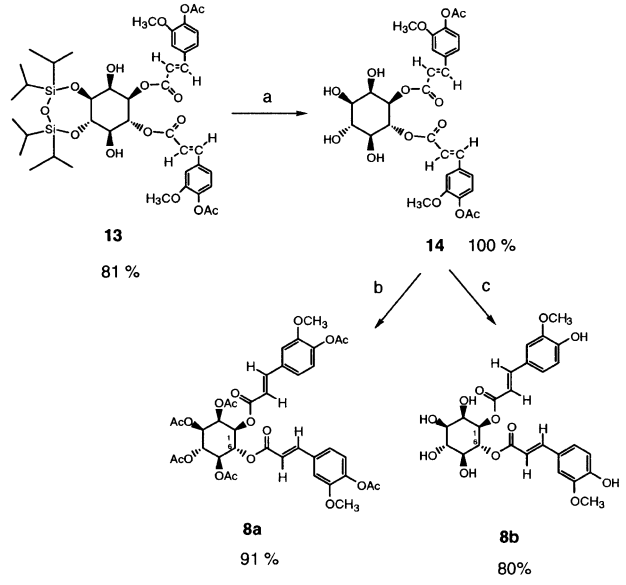
In addition, trisferuloyl-*myo*-inositol derivative **9b**, which is converted into an adamantane type, showed a moderate suppressive activity (inhibitory rate 62%), while **9a** showed no activity on the O_2^- generation. The structural difference between **9a** and **9b** is whether the hydroxyl groups are protected or not.

On the other hand, **1a**, EGMP (**1b**), methyl ferulate (**1c**), **1d**, *O*-acetyl ferulate (**1e**) and **2** showed low suppression activities (inhibitory rates of 0–42%). These findings show that it is important that both ferulic acid and *myo*-inositol are converted into feruloyl-*myo*-inositol in order to enhance their attributes.

Then, to clarify the suppressive potencies of **5a**, **8b**, and **9b**, which showed high activities at a concentration of 100 μM, we examined their suppressive effects at a concentration of 20 μM (Fig. 3).

Compound **8b** showed an excellent suppressive activity toward the O_2^- generation (inhibitory rate of 93%) without marked cytotoxicity. On the other hand, compound **5a** showed weak (inhibitory rate of 9%) and **9b** moderate (inhibitory rate of 62%) activities at 20 μM.

These results suggest that the inhibitory potencies of feruloyl-*myo*-inositol derivatives may depend on the



Scheme 3. (a) *n*-$Bu_4NF{\cdot}3H_2O$, $PhCO_2H$, THF, −5 to 0 °C; (b) $(CH_3CO)_2O$, DMAP, pyridine, rt; (c) $NH_2NH_2{\cdot}H_2O$, MeOH, rt.

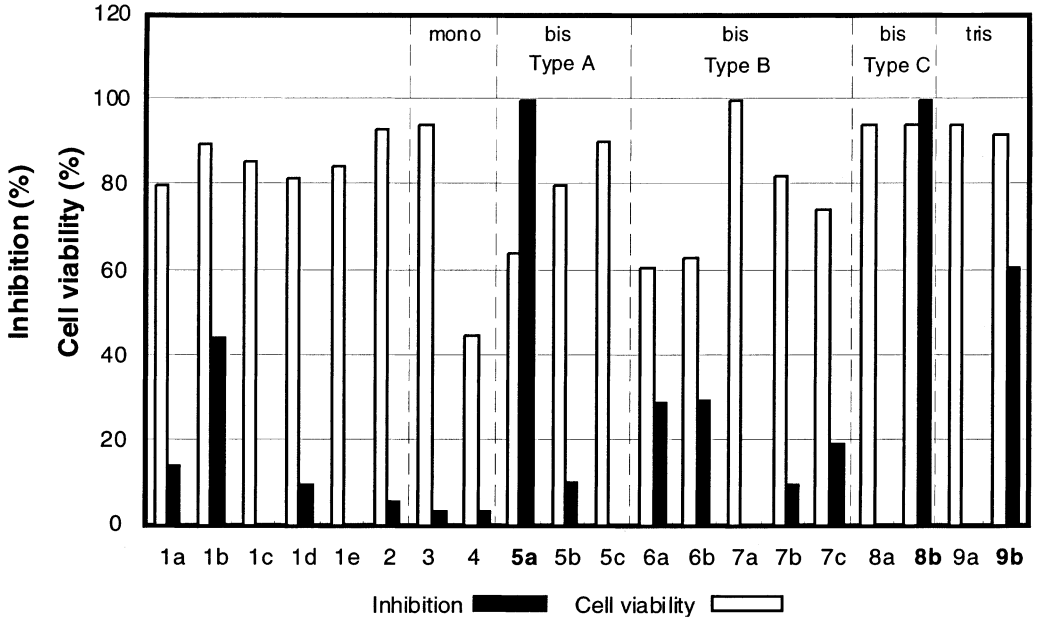


Figure 2. Suppressive effects of feruloyl-*myo*-inositol derivatives on TPA-induced O_2^- generation in differentiated HL-60 cells. O_2^- generation was induced by TPA (50 nM) and detected by cytochrome *c*. Sample concentration was 100 μM. Open and closed bars represent cell viability (%) and O_2^- generation suppression (%), respectively. Data are shown as mean values from duplicate experiments.

stereostructure of molecules rather than the hydrophorbicity of molecules.

The suppressive effects on the TPA-induced EBV activation in vitro were examined in order to estimate the anti-carcinogenic potential. We selected 16 compounds **1a**, **1b**, **1c**, **1d**, **1e**, **2**, **5a**, **5b**, **6a**, **6b**, **7a**, **7b**, **8a**, **8b**, **9a** and **9b**, and examined their suppressive effects at a concentration of 100 μM. The results were shown in Figure 4. The acid **1a** was inactive at a concentration of 100 μM and methyl ferulate (**1c**) and *myo*-inositol (**2**), which are control compounds, showed low suppression (inhibitory rates of 17–30%) on the EBV activation. On the other hand, the compound **1b** exhibited high suppression (inhibitory rate of 78%) without notable cytotoxicity.

As shown in Figure 4, most of bisferuloyl-*myo*-inositol derivatives showed moderate suppression (inhibitory rates of 30–58%), and it became clear that the protection of hydroxyl groups by acetyl group may increase the suppressive activity on this assay system.

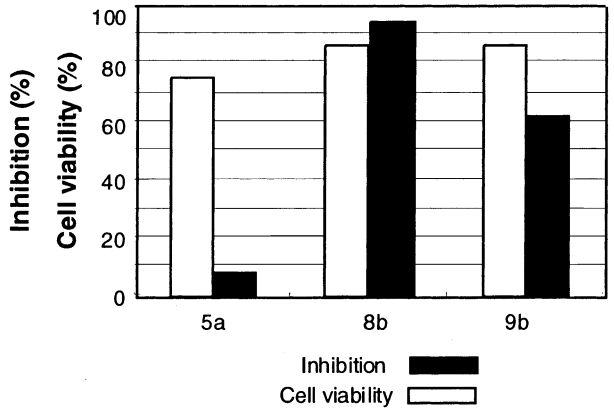


Figure 3. Suppressive effects of feruloyl-*myo*-inositol derivatives **5a**, **8b**, and **9b** at a concentration of 20 μM. Data are shown as mean values from duplicate experiments.

Among the compounds tested, the compound **9b** showed marked suppression on the EBV activation (inhibitory rate of 100%) without notable cytotoxicity. The compound **9b** has three feruloyl groups and has a unique structure in which two ferulic acids are facing each other by using a *myo*-inositol 1,3,5-orthoformate skeleton. In a previous paper, we have reported that compound **9b** shows high suppressive effect on the cyclooxygenase-2 promoter activity.[17] Therefore, the remarkable inhibitory effect on the EBV activation of **9b** should be attributed to that special structure.

Conclusion

We synthesized newly five feruloyl-*myo*-inositol derivatives **4**, **5c**, **7a**, **8a** and **8b**. We evaluated the relationships between their stereostructure and inhibitory activity of fourteen feruloyl-*myo*-inositol derivatives on the TPA-induced superoxide generation. Among these compounds, we found that a bisferuloyl-*myo*-inositol **8b** in which two feruloyl moieties are introduced into the hydroxyl groups bonded to 1,6-vicinal carbons in *myo*-inositol shows an excellent activity on the assay system. These results suggest that the inhibitory potencies of feruloyl-*myo*-inositol derivatives depend on the stereostructure of molecules rather than the hydrophorbicity of molecules. The derivative **8b** may warrant further evaluation in other biological assay systems with respect to its anti-oxidation and anti-carcinogenesis activities.

On the other hand, it should be noted that trisferuloyl-*myo*-inositol derivative **9b** showed a distinct inhibitory activity toward the TPA-induced EBV activation. It is

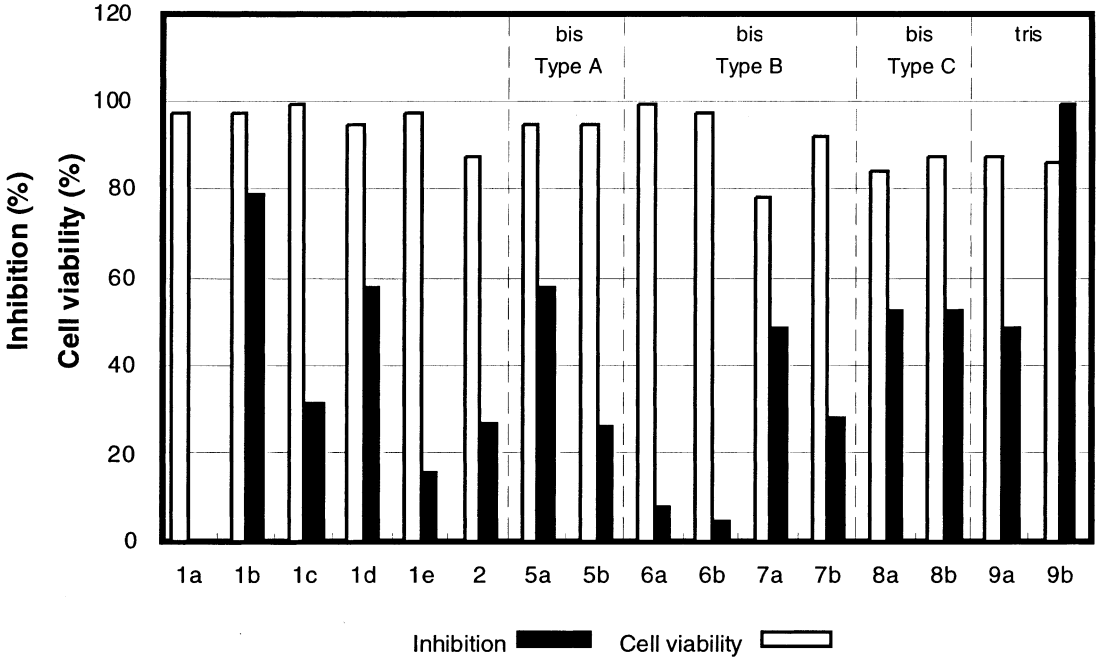


Figure 4. Suppressive effects of feruloyl-*myo*-inositol derivatives on TPA-linduced EBV activation in Raji cells. Cells were incubated with *n*-butyric acid (3 mM), TPA (50 nM), and test compound (100 μM). EBV activation was measured by detecting early antigen (EA)-induction. Open and closed bars represent cell viability (%) and EBV activation suppression (%), respectively. Data are shown as mean values from duplicate experiments.

suggested that the suppressive activity by compound **9b** also may be attributable to its special molecular structure in which two ferulic acids are facing each other by the use of a *myo*-inositol 1,3,5-orthoformate skeleton. These results also suggest that the inhibitory potencies of feruloyl-*myo*-inositol derivatives depend on the stereostructure of molecules rather than the hydrophorbicity of molecules.

The recent progress of molecular biology makes it possible to diagnose the susceptibility of cancer. Therefore, the cancer chemopreventive study and cancer chemopreventive agents will be increasingly attracted much attention. Many natural products are reported as cancer chemopreventive agents, however, it is difficult for these cancer chemopreventive agents to be used practically, because the amounts of these agents are very small in plants. Therefore, the synthetic compounds are in great demand for cancer chemopreventive agents.

Experimental

Computational methods

Energy minimization was carried out by a MM2 force field in the program CAChe WorkSystem released 3.8 (CAChe Scientific, Inc.) on a Machintosh computer.

Chemistry

The ^{1}H NMR (400 MHz) spectra were recorded on a Varian unity-plus 400 spectrometer using tetramethylsilane as internal standard. Chemical shifts are reported in δ (ppm) and coupling constants (*J*) are given in Hertz. FT-IR spectra were recorded on a Shimadzu FTIR-8200D instrument using a diffuse reflectance cell. Melting points were measured in capillary tubes with a Yanagimoto micro melting point apparatus, and are uncorrected. Elemental analyses were performed on a Perkin–Elmer 2400II. Compound purity was checked by TLC on Silica Gel 60 F254 (E. Merck) with detection by charring with phosphomolybdic acid (10% in EtOH solution). Column chromatography was performed on Silica Gel, Wakogel C-200 (Wako Pure Chemical Industry). Ferulic acid and *myo*-inositol were provided by Tsuno Food Industrial Co., Ltd. Ferulic acid was recrystallized from ethanol. Other chemicals were commercial products and used without further purification. Solvents were reagent grade and in most cases dried prior to use.

1,3,4,5,6-Penta-*O*-acetyl-*myo*-inositol (12). To a solution of **10** (3.48 g, 10 mmol) in 50 mL CH_2Cl_2 were added successively pyridine (1.58 g, 20 mmol), a catalytic amount of DMAP and acetyl chloride (1.57 g, 20 mmol). The mixture was stirred at room temperature overnight, and the reaction was quenched by adding water. The organic layer was washed successively with saturated aqueous $NaHCO_3$, and brine. After the organic layer was dried over Na_2SO_4, the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, $CHCl_3$) to give **12** (3.08 g, 79%). Recrystallization of **12** from ethanol gave colorless prisms: mp 170–173 °C; IR (KBr) ν 3496, 2985, 2925, 1747, 1369, 1236, 1043 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 9H, Ac), 2.10 (s, 6H, Ac), 2.61 (d, 1H, J = 2.6 Hz, OH), 4.33 (dd, 1H, J = 2.4, J = 2.6 Hz, H-2), 5.02 (dd, 2H, J = 2.4, 10.0 Hz, H-1, and H-3), 5.19 (t, 1H, J = 10.0 Hz, H-5), 5.60 (t, 2H, J = 10.0 Hz, H-4 and

H-6); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 68.4, 69.4, 70.7, 70.9, 169.6, 169.7, 169.8. Anal. calcd for $C_{16}H_{22}O_{11}\cdot 0.5H_2O$: C, 48.12; H, 5.81. Found: C, 48.40; H, 5.77.

1,3,4,5,6-Penta-*O*-acetyl-2-*O*-[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (4). To a solution of **12** (1.56 g, 4.0 mmol) in 50 mL CH_2Cl_2 were added successively Et_3N (0.814 g, 8.0 mmol), a catalytic amount of DMAP and acid chloride **11** (2.04 g, 8.0 mmol). The mixture was stirred at room temperature overnight. The reaction was quenched by adding water, and the organic layer was washed successively with saturated aqueous $NaHCO_3$ and brine. After the organic layer was dried over Na_2SO_4, the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, 7:1 $CHCl_3$/*n*-hexane) to give **4** (1.91 g, 78%) as a white solid: mp 182–184 °C; IR (KBr) ν 2947, 2849, 1761, 1732, 1637, 1601, 1508, 1226, 1151, 1045, 984, 908 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H, Ac), 2.03 (s, 6H, Ac), 2.04 (s, 3H, Ac), 2.34 (s, 3H, Ac), 3.94 (s, 3H, OCH_3), 5.16 (dd, 2H, *J* = 2.8, 10.0 Hz, H-1 and H-3), 5.22 (t, 1H, *J* = 10.0 Hz, H-5), 5.59 (t, 2H, *J* = 10.0 Hz, H-4 and H-6), 5.75 (t, 1H, *J* = 2.8 Hz, H-2), 6.58 (d, 1H, *J* = 15.6 Hz, CH=), 7.08–7.23 (m, 3H, ArH), 7.72 (d, 1H, *J* = 15.6 Hz, CH=); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.6, 56.0, 68.2, 68.5, 69.5, 71.1, 110.9, 116.5, 122.1, 123.3, 132.8, 141.9, 146.1, 151.5, 165.6, 168.6, 169.5, 169.6, 169.7. Anal. calcd for $C_{28}H_{32}O_{15}$: C, 55.26; H, 5.30. Found: C, 54.98; H, 5.31.

3,4,5,6-Tetra-*O*-acetyl-1, 2-*O*-bis[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (5c). The compound **5a** (0.39 g, 0.5 mmol) was dissolved in a mixture of $CHCl_3$ (10 mL) and MeOH (1 mL) and treated with $NH_2NH_2\cdot H_2O$ (48.5 μL, 1.0 mmol). After being stirred at room temperature for 5 h, the reaction mixture was diluted with $CHCl_3$ and successively washed with saturated aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was recrystallized from EtOH/H_2O to provide **5c** as a white solid (0.28 g, 80%): mp 121–124 °C; IR (KBr) ν 3430, 2943, 2843, 1755, 1724, 1631, 1590, 1516, 1431, 1369, 1229, 1153, 1043, 982, 847, 820 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.92 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.99 (s, 3H, Ac), 3.72 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 5.40–5.69 (m, 6H, CH), 6.35 (d, 1H, *J* = 16.0 Hz), 6.70–7.45 (m, 8H, CH= and ArH), 7.61 (d, 1H, *J* = 16.0 Hz), 9.67 (s, 1H, ArOH), 9.72 (s, 1H, ArOH); ^{13}C NMR (DMSO-d_6) δ 20.3, 20.5, 55.8, 56.0, 67.7, 68.3, 68.7, 69.3, 69.6, 70.4, 111.1, 111.3, 112.9, 113.1, 115.6, 124.0, 124.5, 125.4, 125.5, 146.8, 147.4, 148.1, 148.2, 149.9, 150.0, 165.7, 166.2, 169.4, 169.6, 169.7. Anal. calcd for $C_{34}H_{36}O_{16}$: C, 58.28; H, 5.18. Found: C, 58.05; H, 5.19.

1,2,4,5-Tetra-*O*-acetyl-3, 6-*O*-bis[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (7a). To a solution of **7c** (0.123 g, 0.2 mmol) in 2 mL pyridine was added acetic anhydride (380 μL, 4.0 mmol). The mixture was stirred for 2 h at 90 °C. The cooled mixture was poured on ice, and resulting powder was collected. Recrystallization from EtOH gave **7a** as a white solid (0.128 g, 82%): mp 235–238 °C; IR (KBr) ν 3072, 2982, 2945, 2847, 1765, 1716, 1632, 1601, 1508, 1421, 1369, 1230, 1036, 984, 905, 837 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H, Ac), 2.00 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.24 (s, 3H, Ac), 2.33 (s, 6H, Ac), 3.88 (s, 6H, OCH_3), 5.20–5.35 (m, 3H, CH), 5.62–5.74 (m, 3H, CH), 6.28 (d, 1H, *J* = 16.0 Hz, CH=), 6.31 (d, 1H, *J* = 16.0 Hz, CH=), 7.00–7.12 (m, 6H, ArH), 7.63 (d, 1H, *J* = 16.0 Hz, CH=), 7.64 (d, 1H, *J* = 16.0 Hz, CH=); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.6, 20.8, 56.0, 68.3, 68.7, 69.5, 69.6, 70.8, 111.3, 116.3, 116.4, 121.8, 123.3, 132.7, 132.8, 141.9, 145.9, 146.1, 151.5, 165.2, 165.4, 168.7, 169.5, 169.6, 169.7, 169.9. Anal. calcd for $C_{38}H_{40}O_{18}$: C, 58.16; H, 5.14. Found: C, 57.98; H, 5.13.

1,6-*O*-Bis[3-(4′-acetyloxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (14). The compound **13** (0.859 g, 1.0 mmol) was dissolved in THF (20 mL) and treated with *n*-$Bu_4NF\cdot 3H_2O$ (0.80 mg, 2.54 mmol) in the presence of benzoic acid (0.366 g, 3.0 mmol). After being stirred at −5 to 0 °C for 6 h, the reaction mixture was diluted with ethyl acetate and successively washed with brine, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was chromatographed (silica gel, $CHCl_3$ then 1:1 ethyl acetate/EtOH,) to give **14** (0.62 g, 100%) as a white solid: mp 129–132 °C; IR (KBr) ν 3350, 2941, 2843, 1765, 1716, 1636, 1599, 1510, 1261, 1155, 1034, 1010, 904, 833 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.24 (s, 3H, Ac), 2.25 (s, 3H, Ac), 3.30–3.54 (m, 3H, CH), 3.79 (s, 6H, OCH_3), 3.97 (m, 1H, H-2), 4.48 (d, 1H, *J* = 5.6 Hz, OH), 4.87 (dd, 1H, *J* = 2.0, 10.0 Hz, H-1), 4.92 (d, 1H, *J* = 4.8 Hz, OH), 5.19 (d, 1H, *J* = 5.2 Hz, OH), 5.28 (d, 1H, *J* = 4.4 Hz, OH), 5.43 (t, 1H, *J* = 10.0 Hz, H-6), 6.61 (d, 1H, *J* = 16.0 Hz, CH=), 6.66 (d, 1H, *J* = 16.0 Hz, CH=), 7.07–7.47 (m, 6H, ArH), 7.56 (d, 1H, *J* = 16.0 Hz, CH=), 7.59 (d, 1H, *J* = 16.0 Hz, CH=2); ^{13}C NMR (DMSO-d_6) δ 20.3, 55.8, 55.9, 69.9, 71.0, 72.4, 72.5, 111.4, 111.5, 118.0, 118.5, 121.6, 121.8, 123.0, 123.1, 132.7, 132.9, 140.8, 141.0, 143.7, 144.3, 151.0, 151.1, 165.6, 168.3. Anal. calcd for $C_{30}H_{32}O_{14}\cdot H_2O$: C, 56.78; H, 5.40. Found: C, 56.98; H, 5.33.

2,3,4,5-Tetra-*O*-acetyl-1,6-*O*-bis[3-(4′-acetyloxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (8a). To a solution of **14** (0.312 g, 0.507 mmol) in 2 mL pyridine were added acetic anhydride (1 mL, 10.0 mmol) and catalytic amount of DMAP. The mixture was stirred for 2 h at room temperature. The mixture was poured on ice, and resulting powder was collected. Recrystallization from *n*-hexane/ethyl acetate gave **8a** as a white solid (0.361 g, 91%): mp 118–121 °C; IR (KBr) ν 3074, 2943, 2843, 1759, 1724, 1635, 1601, 1369, 1155, 1036, 903 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.24 (s, 3H, Ac), 2.31 (s, 6H, Ac), 3.84 (s, 6H, OCH_3), 5.18 (dd, 1H, *J* = 2.8, 10.0 Hz, H-3), 5.30–5.56 (m, 2H, H-1 and H-5), 5.57 (t, 1H, *J* = 10.0 Hz, H-4), 5.73–5.78 (m, 2H, H-2 and H-6), 6.25 (d, 1H, *J* = 16.0 Hz, CH=), 6.28 (d, 1H, *J* = 16.0 Hz, CH=), 7.00–7.08 (m, 6H, ArH), 7.58 (d, 1H, *J* = 16.0 Hz, CH=), 7.60 (d, 1H, *J* = 16.0 Hz, CH=); ^{13}C NMR

($CDCl_3$) δ 20.4, 20.5, 20.6, 20.8, 55.9, 68.3, 68.6, 68.8, 69.5, 70.8, 111.2, 116.3, 116.4, 121.8, 123.2, 123.3, 132.7, 132.8, 141.8, 145.9, 146.1, 149.5, 151.4, 165.2, 165.5, 168.6, 169.5, 169.7. Anal. calcd for $C_{38}H_{40}O_{18}$: C, 58.16; H, 5.14. Found: C, 58.03; H, 5.09.

1,6-*O*-Bis[3-(4′-hydroxy-3′-methoxyphenyl)-2-propeno-yl]-*myo*-inositol (8b). The compound **14** (0.247 g, 0.4 mmol) was dissolved in methanol (5 mL) and treated with $NH_2NH_2 \cdot H_2O$ (43 μL, 0.88 mmol). After being stirred at room temperature for 2 h, the reaction mixture was diluted with ethyl acetate and successively washed with saturated aqueous $KHSO_4$ and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was recrystallized from $EtOH/H_2O$ to provide **8b** as white a solid (0.17 g, 80%): mp 183–186 C; IR (KBr) ν 3320, 2947, 2844, 1695, 1633, 1593, 1512, 1275, 1180, 1033, 1010, 845, 820 cm^{-1}; ^1H NMR (DMSO-d_6) 3.30–3.60 (m, 3H, CH), 3.77 (s, 6H, OCH_3), 3.94 (m, 1H, H-2), 4.75 (d, 1H, $J = 4.8$ Hz, OH), 4.80 (dd, 1H, $J = 2.4$, 10.0 Hz, H-1), 4.86 (d, 1H, $J = 4.4$ Hz, OH), 5.11 (d, 1H, $J = 5.2$ Hz, OH), 5.20 (d, 1H, $J = 4.4$ Hz, OH), 5.38 (t, 1H, $J = 10.0$ Hz, H-6), 6.34 (d, 1H, $J = 16.0$ Hz, CH=), 6.39 (d, 1H, $J = 16.0$ Hz, CH=), 6.70–7.24 (m, 6H, ArH), 7.46 (d, 1H, $J = 16.0$ Hz, CH=), 7.48 (d, 1H, $J = 16.0$ Hz, CH=), 9.59 (m, 2H, ArOH); ^{13}C NMR ($CDCl_3$) δ 55.8, 55.9, 70.1, 71.2, 72.3, 72.7, 111.1, 111.3, 114.5, 115.0, 115.6, 123.2, 123.5, 125.7, 125.8, 145.0, 145.6, 148.0, 148.1, 149.3, 149.5, 166.3. Anal. calcd for $C_{26}H_{28}O_{12} \cdot 3H_2O$: C, 53.24; H, 5.84. Found: C, 53.10; H, 5.71.

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).

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 1.25% dimethylsulfoxide in RPMI medium (supplemented with 10% FBS) for 6 days to induce differentiation into granulocyte-like cells. Differentiated HL-60 cells, suspended in 1 mL of Hank's buffer, were treated with 100 μM (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.

EBV activation

An EBV activation inhibitory test was performed as previously reported.[14] 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 (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.

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References and Notes

1. Clifford, M. N. *J. Sci. Food Agric.* **1999**, *79*, 362.
2. Kroon, P. A.; Garcia-Conesa, M. T.; Fillingham, I. J.; Hazlewood, G. P.; Williamson, G. *J. Sci. Food Agric.* **1999**, *79*, 428.
3. Taniguchi, H.; Nomura, E.; Tsuno, T.; Minami, S.; Kato, K.; Hayashi, C. JP-2095088.

4. Taniguchi, H.; Hosoda, A.; Tsuno, T.; Maruta, Y.; Nomura, E. *Anticancer Res*. **1999**, *19*, 3757.
5. Miyake, Y.; Hosoda, A.; Takagaki, M.; Nomura, E.; Taniguchi, H. *Acta Crystallogr*. *E* **2001**, *57*, 1096.
6. Hosoda, A.; Miyake, Y.; Nomura, E.; Mizuno, K.; Taniguch, H. *ITE Lett*. *Batt*. *New Tech*. *Med*. **2001**, *2*, 659.
7. Tsuda, H.; Park, C. M.; Takasuka, N.; Baba-Toriyama, H.; Sekine, K.; Moore, M. A.; Nomura, E.; Taniguchi, H. *Anticancer Res*. **1999**, *19*, 3779.
8. Han, B. S.; Park, C. B.; Takasuka, N.; Naito, A.; Sekine, K.; Nomura, E.; Taniguchi, H.; Tsuno, T.; Tsuda, H. *Jpn*. *J*. *Cancer Res*. **2001**, *92*, 404.
9. Berridge, M. J.; Irvine, R. F. *Nature* **1984**, *312*, 315.
10. Berridge, M. J. *Nature* **1993**, *361*, 315.
11. Shamsuddin, A. M. *Anticancer Res*. **1999**, *19*, 3733.
12. Hosoda, A.; Nomura, E.; Murakami, A.; Koshimizu, K.; Ohigashi, H.; Mizuno, K.; Taniguchi, H. *Bioorg*. *Med*. *Chem*. *Lett*. **2000**, *10*, 1439.
13. Murakami, A.; Kuki, W.; Takahashi, Y.; Yonei, H.; Nakamura, Y.; Ohto, Y.; Ohigashi, H.; Koshimizu, K. *Jpn*. *J*. *Cancer Res*. **1997**, *88*, 443.
14. Kim, O.-K.; Murakami, A.; Nakamura, Y.; Ohigashi, H. *Can*. *Lett*. **1998**, *125*, 199.
15. Angyal, S. J.; Tate, M. E.; Gero, S. D. *J*. *Chem*. *Soc* **1961**, 4116.
16. Hosoda, A.; Nomura, E.; Mizuno, K.; Taniguchi, H. *J*. *Org*. *Chem*. **2001**, *66*, 7199.
17. Hosoda A.; Ozaki, Y.; Kashiwada, A.; Mutoh, M.; Wakabayashi, K.; Mizuno, K.; Nomura, E.; Taniguchi, H. *Bioorg*. *Med*. *Chem*. In press.