Anti-inflammatory and Potential Cancer Chemopreventive Constituents of the Fruits of *Morinda citrifolia* (Noni)

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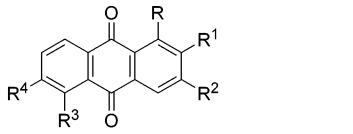
A new anthraquinone, 1,5,15-tri-*O*-methylmorindol (**1**), and two new saccharide fatty acid esters, 2-*O*-(*β*-D-glucopyranosyl)-1-*O*-hexanoyl-*β*-D-gluropyranose (**4**) and 2-*O*-(*β*-D-glucopyranosyl)-1-*O*-octanoyl-*β*-D-gluropyranose (**5**), have been isolated from a methanol extract of the fruits of *Morinda citrifolia* (noni) along with 10 known compounds, namely, two anthraquinones (**2**, **3**), six saccharide fatty acid esters (**6**−**11**), an iridoid glycoside (**12**), and a flavanol glycoside (**13**). Upon evaluation of six compounds (**5**−**7**, **9**, **10**, and **13**) for inhibitory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (1 *μ*g/ear) in mice, four saccharide fatty acid esters, **5**−**7** and **9**, exhibited potent anti-inflammatory activity, with ID_{50} values of 0.46−0.79 mg per ear. In addition, when compounds **1**−**13** were evaluated against the Epstein−Barr virus early antigen (EBV-EA) activation induced by TPA, all of the compounds exhibited moderate inhibitory effects (IC_{50} values of 386−578 mol ratio/32 pmol TPA).

Morinda citrifolia L. (Rubiaceae), known as "noni", is a small tree that grows widely across Polynesia.[1] The roots, barks, stems, leaves, and fruits have been used traditionally as a folk medicine for the treatment of many diseases[2,3] including diabetes, high blood pressure,[4] and cancer.[5] Furthermore, "noni juice", which is made from the fruits of this plant, is widely consumed today for the purported prevention of lifestyle-related diseases such as diabetes, high blood pressure, cardiopathy, and cerebral apoplexy caused by arteriosclerosis.[2] In this paper, we report the isolation and characterization of three new compounds, 1,5,15-tri-*O*-methylmorindol (**1**), 2-*O*-(*β*-D-glucopyranosyl)-1-*O*-hexanoyl-*β*-D-gluropyranose (**4**), and 2-*O*-(*β*-D-glucopyranosyl)-1-*O*-octanoyl-*β*-D-gluropyranose (**5**), and 10 known compounds, **2**, **3**, and **6**−**13**, from a methanol (MeOH) extract of the fruits of *M. citrifolia* L., as well as their inhibitory effects on TPA-induced inflammation in mice and on the EBV-EA activation induced by TPA. This is only the third report of anthraquinones in the fruits of *M. citrifolia*.[6,7]

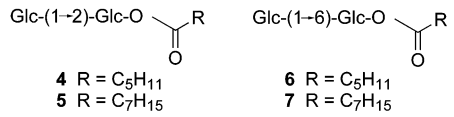
Results and Discussion

Three anthraquinones, **1**−**3**, eight saccharide fatty acid esters, **4**−**11**, an iridoid glycoside, **12**, and a flavonol glycoside, **13**, were isolated from the MeOH extract of *M. citrifolia* fruits as described in the Experimental Section.

The molecular formula of **1** was determined to be $C_{18}H_{16}O_6$ on the basis of the $[M - H]^-$ ion observed at *m*/*z* 327.0861 in the negative HRESIMS. The ^{13}C NMR spectrum indicated 18 carbon signals, including three methoxy carbons (δ_C 58.9, 62.2, 62.3), one methylene carbon (δ_C 69.0), and two carbonyl carbons (δ_C 181.5, 182.5). In the ^1H NMR spectrum, two pairs of *ortho*-coupled signals [one at δ_H 7.35 and 8.08 (each 1H, d, $J = 8.6$ Hz) and the other at δ_H 7.85 and 8.10 (each 1H, d, $J = 8.6$ Hz)] were observed. In addition, the presence of three methoxyl groups and one methylene group was suggested from the ^1H NMR resonances of δ_H 3.50, 3.94, and 4.02 (each 3H, s) and 4.64 (2H, s), respectively. The regiochemistry of each functional group was determined by a HMBC experiment (Figure 1). It was concluded that **1** is 6-hydroxy-1,5-dimethoxy-2-(methoxymethyl)anthraquinone, and this compound was named 1,5,15-tri-*O*-methylmorindol.

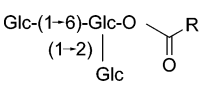


1 R = R^3 = OMe, R^1 = CH_2OMe, R^2 = H, R^4 = OH
2 R = R^4 = OH, R^1 = CH_2OMe, R^2 = H, R^3 = OMe
3 R = R^2 = OH, R^1 = OMe, R^3 = R^4 = H

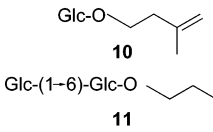


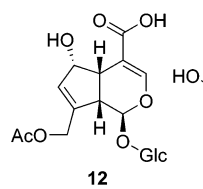
4 R = C_5H_{11}
5 R = C_7H_{15}

6 R = C_5H_{11}
7 R = C_7H_{15}

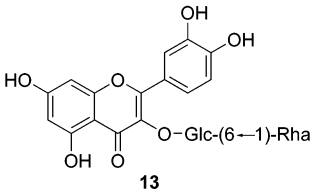


8 R = C_5H_{11}
9 R = C_7H_{15}





12



13

Compound **4** exhibited a sodiated molecular ion $[M + Na]^+$ in the positive HRESIMS at *m*/*z* 463.1788, compatible with the molecular formula $C_{18}H_{32}O_{12}$. In its ^1H and ^{13}C NMR spectra, compound **4** showed signals consistent with a hexanoyl partial structure. The ^1H NMR spectrum of **4** displayed two anomeric proton signals at δ_H 4.56 (1H, d, $J = 7.9$ Hz) and 5.61 (1H, d, $J = 7.9$ Hz). The ^{13}C NMR spectrum of **4** displayed signals at δ_C 63.1 (t), 72.3 (d), 76.8 (d), 79.0 (d), 79.5 (d), and 106.4 (d) attributable to a terminal *β*-D-glucose[8,9] and at δ_C 63.6 (t), 71.6 (d), 78.6 (d, 2 × C), 83.3 (d), and 94.8 (d) for the inner *β*-D-glucose. The

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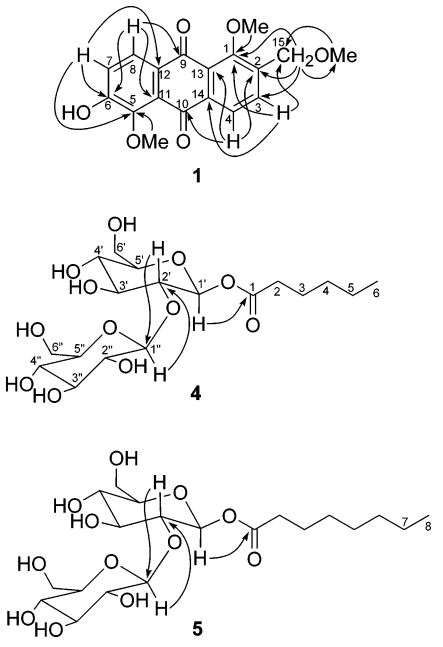

Figure 1. Major HMBC correlations for compounds **1**, **4**, and **5**.

glycosylation shifts of the C-2 signal, on comparison with the signal of methyl β-D-glucopyranoside,[10] suggested that the terminal glucose unit is connected to C-2 of the inner glucose. This evidence was used to propose the structure of **4** as 2-*O*-(β-D-glucopyranosyl)-1-*O*-hexanoyl-β-D-gluropyranose, and this was confirmed from the ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC spectra. HMBC experiments showed correlation contours between H-1 of the inner glucose (δ_H 5.61) and the carbonyl carbon of the hexanoyl moiety (δ_C 174.1) and between H-1 of the terminal glucose (δ_H 4.56) and C-2 of the inner glucose (δ_C 83.3).

The positive HRESIMS of compound **5** exhibited a sodiated molecular ion $[M + Na]^+$ at *m*/*z* 491.2110, suggesting the molecular formula $C_{20}H_{36}O_{12}$. Compound **5** exhibited two anomeric signals at δ_H 4.56 and 5.60 in the ^{1}H NMR spectrum, and this spectrum was almost identical with that of **4**. Only slight differences were observed in the high-field region, where, instead of the signals for a hexanoyl moiety, signals for an octanoyl moiety were observed. This observation was supported by the ^{13}C NMR spectrum, which showed signals at δ_C 14.4 (q), 23.6 (t), 25.5 (t), 30.1 (t, 2 × C), 32.8 (t), 34.9 (t), and 174.0 (s), assignable to an octanoyl moiety.[8,9] The remaining ^{13}C NMR signals for the two glucose moieties were almost identical with those of **4**. Analysis of the ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC spectra led to the assignment of all of the ^{1}H and ^{13}C NMR signals for **5**. Thus, compound **5** was determined as 2-*O*-(β-D-glucopyranosyl)-1-*O*-octanoyl-β-D-glucopyranose.

Ten other compounds isolated from the MeOH extract of *M. citrifolia* fruits were identified as the known compounds 5,15-di-*O*-methylmorindol (**2**),[7] anthragallol 2-methyl ether (**3**),[11] 6-*O*-(β-D-glucopyranosyl)-1-*O*-hexanoyl-β-D-glucopyranose (**6**),[8] 6-*O*-(β-D-glucopyranosyl)-1-*O*-octanoyl-β-D-glucopyranose (**7**),[8] 2,6-di-*O*-(β-D-glucopyranosyl)-1-*O*-hexanoyl-β-D-glucopyranose (**8**),[9] 2,6-di-*O*-(β-D-glucopyranosyl)-1-*O*-octanoyl-β-D-glucopyranose (**9**),[9] 3-methylbut-3-enyl-β-D-glucopyranose (**10**),[12] 3-methylbut-3-enyl-6-*O*-β-D-glucopyranosyl-β-D-glucopyranose (**11**),[8] asperulosidic acid (**12**),[9] and rutin (**13**).[13]

Five saccharide fatty acid esters (**5**−**7**, **9**, **10**) and **13** were evaluated with respect to their anti-inflammatory activity against TPA-induced inflammation in mice, and the inhibitory effects were compared with those of quercetin (3,5,7,3′,4′-pentahydroxyflavone), a known inhibitor of TPA-induced inflammation in mice, and indomethacin, a commercially available anti-inflammatory drug, as shown in Table 1. Four saccharide fatty acid esters, **5**−**7** and **9**, exhibited potent inhibitory activity, with ID_{50} (50% inhibitory dose) values of 0.46−0.79 mg/ear, which were more highly inhibitory than quercetin (ID_{50} 1.6 mg/ear) while less inhibitory than indomethacin (ID_{50} 0.30 mg/ear).

The inhibitory effect on EBV-EA activation induced by TPA was further examined as a preliminary evaluation of the potential anti-tumor-promoting effects of the 13 compounds, **1**−**13**. The results are shown in Table 1, together with comparable data for quercetin as well as β-carotene, a vitamin A precursor that has been intensively studied in cancer chemoprevention by using in vitro, in vivo, and epidemiological test systems.[14] All of the compounds tested showed inhibitory effects, with IC_{50} values of 386−512 mol ratio/32 pmol TPA, which were almost comparable with or more inhibitory than quercetin (IC_{50} 560 mol ratio/32 pmol TPA) while, except for **1** (386 mol ratio/32 pmol TPA), less inhibitory than β-carotene (397 mol ratio/32 pmol TPA).

Anthraquinones appear to be rare in the fruits of *M. citrifolia*,[6,7] whereas the roots of noni are well known to contain these compounds.[3,15] Since the inhibitory effect against TPA-induced inflammation has been demonstrated to closely parallel that of the inhibition of tumor promotion in two-stage carcinogenesis initiated by 7,12-dimethylbenz[*a*]anthracene (DMBA) and promoted by TPA in a mouse skin model,[16] four saccharide fatty acid esters, **5**−**7** and **9**, which exhibited potent inhibitory activity in the mouse ear edema assay, in addition to an anthraquinone, **1**, which showed potent inhibitory effect against EBV-EA activation induced by TPA, may be potential inhibitors of tumor promotion (potential cancer chemopreventive agent). Potent cancer chemopreventive activity for an another anthraquinone, 2-methoxy-1,3,6-trihydroxyanthraquinone, from noni fruits has been suggested recently from the observation of the ability to induce quinone reductase (QR) activity with cultured murine hepatoma cells.[6] In view of the widespread use of noni fruits as a botanical dietary supplement and the few reports that have described the chemical constituents of the fruits,[3,6−9,17,18] it might be worthwhile to undertake further investigation of the bioactive constituents of noni fruits including those with potential anti-inflammatory and cancer chemopreventive activities.

Experimental Section

General Experimental Procedures. Crystallizations were performed in EtOAc−MeOH, and melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 polarimeter in MeOH at 25 °C. IR spectra were recorded in KBr disks. NMR spectra were recorded with a JEOL ECA-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) or with a JEOL LA-500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer in CD_3OD or in $CDCl_3$ with tetramethylsilane as an internal standard. ESIMS and HRESIMS were recorded on an Agilent 1100 LC/MSD TOF (time-of-flight) system [ionization mode: positive; nebulizing gas (N_2) pressure: 35 psig; drying-gas (N_2): flow, 12 L/min, temp, 325 °C; capillary voltage: 3000 V; fragmentor voltage: 225 V]. Silica gel (silica gel 60, 220−400 mesh, Merck), Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), and C_{18} silica (Chromatorex-ODS, 100−200 mesh; Fuji Silysia Chemical, Ltd., Aichi, Japan) were used for open column chromatography. Preparative TLC on silica gel (silica gel 60G, Merck; 0.5 mm thick; 20 × 20 cm) was developed using *n*-hexane−EtOAc−AcOH (60:40:1). Reversed-phase preparative HPLC (with a refractive index detector) was carried out on C_{18} silica columns (25 cm × 10 cm i.d.) at 25 °C at a flow rate of 2.0 mL/min of the eluent, on a TSK ODS-120A 5 μm column (Toso Co., Tokyo, Japan) [eluents: MeOH−H_2O−AcOH (50:50:1) (HPLC system I), MeOH−H_2O−AcOH (40:60:1) (HPLC system II), or MeOH−H_2O−AcOH (35:65:1) (HPLC system III)] and on a Pegasil ODS II 5 μm column (Senshu Scientific Co., Ltd., Tokyo, Japan) [eluent: MeOH−H_2O−AcOH (70:30:1) (system IV)].

Plant Material. *Morinda citrifolia* L. (Rubiaceae) was cultivated on a farm at Nakajo (Okinawa prefecture, Japan), and the fruit was harvested from a 2-year-old tree in April 2004. The plant was

Table 1. Inhibitory Effects of Compounds from *Morinda citrifolia* Fruits and Reference Compounds on TPA-Induced Inflammation in Mice and on the Induction of Epstein−Barr Virus Early Antigen

	compound	inhibition of inflammation ID_{50}[a] (mg/ear)	percentage of EBV-EA induction[b] concentration (mol ratio/ 32 pmol TPA) 1000	500	100	10	IC_{50}[c] (mol ratio/32 pmol TPA)
1	1,5,15-tri-*O*-methyl morindol		10.1 (60)	29.4	75.6	100	386
2	5,15-di-*O*-methyl morindol		13.7 (60)	45.1	69.3	95.1	475
3	anthragallol 2-methyl ether		14.3 (60)	46.7	71.2	96.0	483
4	2-*O*-(*β*-D-glucopyranosyl)-1-*O*-hexanoyl-*β*-D-glucopyranose		16.0 (70)	61.5	86.2	100	512
5	2-*O*-(*β*-D-glucopyranosyl)-1-*O*-octanoyl-*β*-D-glucopyranose	0.79	15.3 (60)	56.4	85.7	100	570
6	6-*O*-(*β*-D-glucopyranosyl)-1-*O*-hexanoyl-*β*-D-glucopyranose	0.64	15.6 (60)	59.2	84.0	100	571
7	6-*O*-(*β*-D-glucopyranosyl)-1-*O*-octanoyl-*β*-D-glucopyranose	0.46	15.3 (60)	59.7	84.3	100	499
8	2,6-di-*O*-(*β*-D-glucopyranosyl)-1-*O*-hexanoyl-*β*-D-glucopyranose		14.1 (60)	58.6	83.1	100	495
9	2,6-di-*O*-(*β*-D-glucopyranosyl)-1-*O*-octanoyl-*β*-D-glucopyranose	0.79	16.1 (70)	61.9	86.3	100	507
10	3-methylbut-3-enyl-*β*-D-glucopyranose	>1.0	10.5 (60)	55.5	80.1	100	494
11	3-methylbut-3-enyl-6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranose		14.2 (60)	58.3	85.4	100	504
12	asperulosidic acid		13.5 (60)	47.2	82.5	100	485
13	rutin	>1.0	16.2 (70)	60.1	81.1	100	578
	reference compounds						
	quercetin	1.6	21.6 (60)	55.7	82.7	100	560
	indomethacin	0.30					
	β-carotene		8.6 (70)	34.2	82.1	100	397

[a] ID_{50}: 50% inhibitory dose. [b]Values represent percentages relative to the positive control value. TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cells. [c]IC_{50} represents the molar ratio to TPA that inhibits 50% of positive control (100%) activated with 32 pmol of TPA.

authenticated by one (H.K.) of the authors, and a voucher specimen (No. 024040) has been deposited in the Research Laboratory, Nakazen Co. Ltd.

Chemicals and Reagents. Chemicals were purchased as follows: TPA from ChemSyn Laboratories (Lenexa, KS), quercetin, indomethacin, hydrocortisone, and *β*-carotene from Sigma Chemical Co. (St. Louis, MO), and the EBV cell culture reagents and *n*-butanoic acid from Nacalai Tesque, Inc. (Kyoto, Japan).

Extraction and Isolation. Air-dried and powdered fruits (1.31 kg) from the fresh fruits (24.6 kg) of *M. citrifolia* were extracted three times with MeOH (reflux, 3 h) to yield a MeOH extract (228 g). This extract was suspended in water and partitioned successively with $CHCl_3$ and *n*-butanol (*n*-BuOH) to yield $CHCl_3$ (13.1 g), *n*-BuOH (61.0 g), and H_2O (120.8 g) extracts sequentially. The $CHCl_3$ extract was partitioned with *n*-hexane−MeOH−H_2O (19:19:2), giving *n*-hexane- (8.2 g) and MeOH−H_2O (3.3 g)-soluble fractions.

The MeOH−H_2O-soluble fraction was chromatographed on a silica gel (150 g) column, which was eluted successively with solvents of increasing polarity [*n*-hexane−EtOAc (4:1→0:1) and EtOAc−MeOH (19:1 → 0:1)] to afford 13 fractions, A−M. Fraction B (44 mg) from the eluate of *n*-hexane−EtOAc (4:1) was subjected to TLC, which gave two fractions, B1 (15 mg; R_f 0.5) and B2 (19 mg; R_f 0.50). Fraction B1 was subjected to chromatography on an ODS (13 g) column using MeOH−H_2O (4:0) to give compound **3** (1.3 mg). Fraction B2, upon chromatography on a silica gel column (13 g) [eluent: EtOAc−MeOH (1:0 → 19:1)], yielded compound **2** (2.7 mg). Fraction C (303 mg) from the eluate of *n*-hexane−EtOAc (3:2) was subjected to TLC to give a fraction (33 mg; R_f 0.50), which upon separation using HPLC system IV yielded compound **1** [1.4 mg, retention time (t_R) 7.0 min].

A portion (50.8 g) of the *n*-BuOH fraction was subjected to chromatography on a Diaion HP-20 (420 g) column. A step gradient elution was conducted with H_2O−MeOH (1:0 → 0:1) to give fractions Ba (18.0 g; from the eluate of 100% H_2O), Bb (8.7 g; 30% MeOH), Bc (6.8 g; 50% MeOH), Bd (3.5 g; 80% MeOH), and Be (0.7 g; 100% MeOH). A portion (1.00 g) of fraction Bb was separated by ODS (26 g) column chromatography [eluent: H_2O−MeOH (1:1 → 4:1)] to give six fractions, Bb1−Bb6, listed in decreasing order of polarity. Application of HPLC (system II) to fraction Bb3 (187 mg) gave compound **10** (19.2 mg, t_R 9.6 min). Fraction Bb4 (349 mg), using HPLC system III, yielded compounds **6** (14.1 mg, t_R 11.3 min), **11** (8.2 mg, t_R 5.2 min), and **12** (2.8 mg, t_R 3.3 min). Fraction Bb5 (176 mg) was subjected to separation with HPLC system II to afford compounds **4** (20.5 mg, t_R 19.2 min), **6** (5.8 mg, t_R 18.0 min), and **8** (5.3 mg, t_R 9.6 min). A portion (0.94 g) of fraction Bc was subjected to ODS (26 g) column chromatography [eluent: H_2O−MeOH (1:0 → 1:1)] to give five fractions, Bc1−Bc5, which are numbered in decreasing order of polarity. Preparative HPLC (HPLC system I) of fractions Bc3 (198 mg) and Bc4 (238 mg) gave compound **9** (46.1 mg, t_R 13.2 min) and compound **7** (13.6 mg, t_R 26.0 min) and further compound **9** (30.3 mg), respectively. Fraction Bc5 (205 mg), upon HPLC system I, yielded compounds **5** (9.6 mg, t_R 22.4 min), **7** (14.2 mg), and **13** (3.3 mg, t_R 8.8 min).

1,5,15-Tri-*O*-methylmorindol [6-hydroxy-1,5-dimethoxy-2-(methoxymethyl)anthraquinone] (1): yellow-brown, fine needles; mp 186−190 °C; UV (MeOH) λ_{max} (log ϵ) 218 (4.18), 248 (4.15), 270 (4.12), 355 (3.57) nm; IR (KBr) ν_{max} 3388 (OH), 2927, 1666 (C=O), 1576 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.10 (1H, d, J = 8.6 Hz, H-4), 8.08 (1H, d, J = 8.6 Hz, H-8), 7.85 (1H, d, J = 8.6 Hz, H-3), 7.35 (1H, d, J = 8.6 Hz, H-7), 4.64 (2H, s, H-15), 4.02 (3H, s, OMe-5), 3.94 (3H, s, OMe-1), 3.50 (3H, s, OMe-15); ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.5 (s, C-10), 181.5 (s, C-9), 158.0 (s, C-1), 154.9 (s, C-6), 146.0 (s, C-5), 140.3 (s, C-2), 135.8 (s, C-14), 133.6 (d, C-3), 128.8 (s, C-12), 125.6 (d, C-8), 125.0 (s, C-13), 124.9 (s, C-11), 123.4 (d, C-4), 120.4 (d, C-7), 69.0 (t, C-15), 62.3 (q, OMe-5), 62.2 (q, OMe-1), 58.9 (q, OMe-15); HMBC data, see Table S1; negative HRESIMS *m/z* 327.0861 [M − H]$^-$ (calcd for $C_{18}H_{15}O_6$, 327.0868).

2-*O*-(*β*-D-Glucopyranosyl)-1-*O*-hexanoyl-*β*-D-glucopyranose (4): colorless gum; $[\alpha]^{25}_D$ +13.9 (*c* 1.02, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (2.67) nm; IR (KBr) ν_{max} 3378 (OH), 2929, 1754 (C=O), 1641 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 5.61 (1H, d, J = 7.9 Hz, H-1′), 4.56 (1H, d, J = 7.9, H-1″), 3.83 (2H, dt, J = 12.1, 2.1 Hz, Hb-6′ and Hb-6″), 3.68 (2H, dd, J = 4.8, 12.1 Hz, Ha-6′ and Ha-6″), 3.63 (1H, dd, J = 8.2, 9.3 Hz, H-3′), 3.59 (1H, dd, J = 7.9, 9.3 Hz, H-2′), 3.40 (1H, H-5′), 3.38 (1H, dd, J = 7.6, 9.3 Hz, H-4′), 3.36 (1H, dd, J = 8.6, 9.3 Hz, H-3″), 3.30 (1H, dd, J = 8.2, 9.3 Hz, H-4″), 3.28 (1H, H-5″), 3.19 (1H, dd, J = 7.9, 9.3 Hz, H-2″), 3.19 (1H, dd, J = 7.9, 9.3 Hz, H-2″), 2.47 (1H, dt, J = 16.5, 7.6 Hz, H-2b), 2.38 (1H, dt, J = 16.5, 7.6 Hz, H-2a), 1.63 (2H, quint., J = 7.3 Hz, H-3), 1.34 (4H, H-4 and H-5), 0.92 (3H, t, J = 7.0 Hz, H-6); ^{13}C NMR (150 MHz, CD_3OD) δ 174.1 (s, C-1), 106.4 (d, C-1″), 94.8 (d, C-1′), 83.3 (d, C-2′), 79.5 (d, C-5′), 79.0 (d, C-5″), 78.6 (2 × C, d, C-3′ and C-3″), 76.8 (d, C-2″), 72.3 (d, C-4″), 71.6 (d, C-4′), 63.6 (t, C-6″), 63.1 (d, C-6′),

35.7 (t, C-2), 33.1 (t, C-4), 26.0 (t, C-3), 24.2 (t, C-5), 15.0 (q, C-6); HMBC data, see Table S2; positive HRESIMS *m*/*z* 463.1788 [M + Na]$^+$ (calcd for $C_{18}H_{32}O_{12}Na$, 463.1791).

2-*O*-(*β*-D-Glucopyranosyl)-1-*O*-octanoyl-*β*-D-glucopyranose (5): colorless gum; $[\alpha]^{25}_D$ −2.4 (*c* 1.95, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (2.83) nm; IR (KBr) ν_{max} 3402 (OH), 2927, 1745 (C═O), 1641 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 5.60 (1H, d, J = 7.7 Hz, H-1′), 4.56 (1H, d, J = 7.5 Hz, H-1″), 3.83 (2H, dt, J = 2.1, 2.0 Hz, Hb-6′ and Hb-6″), 3.68 (2H, dd, J = 4.5, 12.1 Hz, Ha-6′ and Ha-6″), 3.68 (2H, dd, J = 4.5, 12.1 Hz, Ha-6′ and Ha-6″), 3.62 (1H, dd, J = 7.7, 9.2 Hz, H-2′), 3.59 (1H, dd, J = 7.5, 9.2 Hz, H-3′), 3.40 (1H, dd, J = 7.5, 9.0 Hz, H-4′), 3.38 (1H, H-5′), 3.36 (1H, dd, J = 7.9, 9.2 Hz, H-3″), 3.29 (1H, dd, J = 7.9, 9.0 Hz, H-4″), 3.27 (1H, H-5″), 3.19 (1H, dd, J = 7.5, 9.2 Hz, H-2″), 2.47 (1H, dt, J = 16.3, 7.4 Hz, Hb-2), 2.38 (1H, dt, J = 16.3, 7.4 Hz, Ha-2), 1.63 (2H, quint., J = 7.2 Hz, H-3), 1.33 (8H, H-4, H-5, H-6, and H-7), 0.90 (t, J = 6.9 Hz, H-8); ^{13}C NMR (150 MHz, CD_3OD) δ 174.0 (s, C-1), 105.6 (d, C-1″), 94.0 (d, C-1′), 82.5 (d, C-2′), 78.7 (d, C-5′), 78.2 (d, C-5″), 77.8 (2 × C, d, C-3′ and C-3″), 76.0 (d, C-2″), 71.4 (d, C-4″), 70.8 (d, C-4′), 62.7 (t, C-6″), 62.2 (d, C-6′), 34.9 (t, C-2), 32.8 (t, C-6), 30.1 (2 × C, t, C-4 and C-5), 25.5 (t, C-3), 23.6 (t, C-7), 14.4 (t, C-8); HMBC data, see Table S2; positive HRESIMS *m*/*z* 491.2110 [M + Na]$^+$ (calcd for $C_{20}H_{36}O_{12}Na$, 491.2104).

Assay of TPA-Induced Inflammation Ear Edema in Mice. For the protocol for this in vivo assay, refer to a previous article.[19]

In Vitro EBV-EA Activation Experiment. For the protocol for this in vitro assay, refer to a previous article.[19]

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Supporting Information Available: Tables of HMBC NMR data for compounds **1**, **4**, and **5**. This information is available free of charge via the Internet at http://pubs.acs.org.

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