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Bioactive sucrose esters from *Bidens parviflora*

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Abstract

An investigation on *Bidens parviflora* led to the isolation of three sucrose esters and a substituted truxillate. Their structures were elucidated as (6-*O*-(*E*)-*p*-coumaroyl)-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside, (6-*O*-(*E*)-*p*-coumaroyl)-β-D-fructofuranosyl-(2→1)-(6-*O*-(*E*)-*p*-coumaroyl)-α-D-glucopyranoside II, 6,6′-sucrose ester of (1α,2α,3β,4β)-3,4-bis(4-hydroxyphenyl)-1,2-cyclobutanedicarboxylic acid, dimethyl ester of (1α,2α,3α,4α)-2,4-bis(3,4-dihydroxyphenyl)-1,3-cyclobutanedicarboxylic acid on the basis of spectral and chemical evidence. These compounds were subjected to the following bioassays: the histamine release inhibition of rat mast cells induced by antigen-antibody reaction and the inhibitory activity of PGE_2 production by macrophages.

Keywords: *Bidens parviflora* Willd.; Compositae; Sucrose ester; Truxillic and truxinic acids; Histamine release inhibitor; PGE_2 production inhibitor

1. Introduction

Bidens parviflora Willd. (Xiaohua-Guizhencao) has been used as a traditional antipyretic, anti-inflammatory and anti-rheumatic medicine in China (Jiangxi New Medical University, 1986; Xia et al., 1985). During our search for antiallergic compounds from this species, five polyacetylene glucosides have been reported (Wang et al., 2001). In the present paper, three sucrose esters and one known lignan were found to inhibit both the release of histamine by rat mast cells induced by antigen-antibody reaction and PGE_2 production by macrophages. The 60% aq. EtOH extract of *B. parviflora* Willd. was partitioned with *n*-hexane, ethyl acetate and *n*-butanol, successively. Evaporation of the corresponding solvents yielded the hexane, ethyl acetate, and *n*-butanol fractions, while the residual water phase similarly provided an aqueous fraction. The fractions were tested for histamine release inhibitory activity from rat mast cells induced by Compound 48/80 (*p*-methoxy-*N*-methyl-phenethylamine-*N*-methyl-homoanisylamine) using the standard bioassay procedure (Ishii et al., 1999), with activity being detected in the *n*-butanol fraction. The *n*-butanol fraction was thus subjected to silica gel column chromatography to obtain nine fractions, which were separated by chromatography on Sephadex LH-20 and ODS columns to yield four active compounds, (6-*O*-(*E*)-*p*-coumaroyl)-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside 1, (6-*O*-(*E*)-*p*-coumaroyl)-β-D-fructofuranosyl-(2→1)-(6-*O*-(*E*)-*p*-coumaroyl)-α-D-glucopy ranoside **2**, 6,6′-sucrose ester of (1α, 2α, 3β, 4β)-3,4-bis (4-hydroxyphenyl)-1,2-cyclodicarboxylic acid **3**, and dimethyl ester of (1α, 2α, 3α, 4α)-2,4-bis(3,4-dihydroxyphenyl)-1,3-cyclobutanedicarboxylic acid **4**, respectively.

2. Results and discussion

Compound **1** was obtained as an amorphous powder. The FAB-MS of **1** gave a pseudomolecular ion at *m*/*z* 489 $[M+H]^+$ consistent with a molecular formula of $C_{21}H_{28}O_{13}$. The IR spectrum showed hydroxyl and carbonyl absorptions, whereas the ^1H NMR spectrum of **1**

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suggested a 1,4-disubstituted benzene group as well as signals for two *trans* double bond protons suggesting a *trans*-cinnamoyl moiety (Jose et al., 1999; Shimazaki et al., 1991). This was supported further by analysis of the ^{13}C NMR spectrum, which in the low field displayed signals for a cinnamoyl moiety. The ^{13}C NMR spectrum also revealed 12 carbon signals arising from a disaccharide in which the chemical shift of the two anomeric carbon signals, and three CH_2 groups suggested that it was sucrose (Miyase and Ueno, 1993; Wang et al., 1999). This was further supported by analysis of the ^1H NMR spectrum, which contained a characteristic doublet signal with a small coupling constant at δ 5.39 (1H, $J=3.7$ Hz), assignable to the anomeric proton in the α-D-glucopyranosyl moiety. In the HMBC spectrum, the carbonyl group not only showed correlation with protons assigned to a double bond but also had correlation with H-6 methylene, suggesting that a cinnamoyl moiety was connected to the C-6 of fructose. Compound **1** was therefore elucidated as (6-*O*-(*E*)-*p*-coumaroyl)-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside. The chemical shift of carbons are listed in Table 1.

Compound **2** was obtained as a white powder. Its molecular formula $C_{30}H_{34}O_{15}$ was obtained from FAB–MS. Its IR spectrum showed a hydroxyl group, a conjugated ester carbonyl group, and an aromatic ring. In comparison of the ^1H NMR and ^{13}C NMR spectra with those of compound **1**, compound **2** also displayed the same signals for a sucrose moiety, but now with two sets of signals for cinnamoyl moieties (Sashida et al., 1991). From the HMBC spectrum, an ester group at 169.4 correlated with the protons at δ 4.58 and δ 4.26 assigned to the H-6 of the glucose moiety. The other ester group at δ 169.0 correlated with the protons at δ 4.50 and δ 4.42 assigned to the H-6 proton of the fructose moiety. Compound **2** was identified as (6-*O*-(*E*)-*p*-coumaroyl)-β-D-fructofuranosyl-(2→1)-(6-*O*-(*E*)-*p*-coumaroyl)-α-D-glucopyranoside.

Compound **3** was obtained as a white amorphous powder, $[\alpha]_D$ +37.1°(*c* 0.65, MeOH), which exhibited a phenol UV absorption. Compound **3** showed a pseudomolecular ion peak at *m*/*z* 635 $[M+H]^+$ in FAB–MS, and its molecular formula was determined to be $C_{30}H_{34}O_{15}$ by HR-FAB–MS. The presence of a lignan moiety, a bis(4-hydroxyphenyl) cyclobutanedicarboxyl group, as the acyl unit in the molecule was implied by two ester carbonyl carbon resonances, two A_2B_2-type aromatic proton signals, and an AA′BB′ system of methine proton signals [each *dd*, $^3J=12.8$ Hz (*cis*), $^3J=2.0$ Hz (*trans*)] which is characteristic of tetrasubstituted cyclobutane protons (Dimberg et al., 2001). From the HMQC and DEPT spectra, the signals at δ_C 45.2, 45.6, 44.3 and 44.7 were assigned to the cyclobutane methine carbons. The HMBC spectrum revealed that the former two signals are attributed to carboxyl-bearing carbons, while the other two have 4-hydroxyphenyl substituents. Based on these data, the acyl unit in **3** was assigned to the β-truxinyl type (7″,7‴, 8″,8‴) lignan (1α, 2α, 3β, 4β) (Jose et al., 1999). Comparison of the ^1H NMR and ^{13}C NMR spectroscopic data with those of compounds **1** and **2** suggested the same sucrose moiety was in compound **3**. Thus compound **3** was elucidated as 6′,6″-sucrose ester of (1α, 2α, 3β, 4β)-3,4-bis (4-hydroxyphenyl)-1,2-cyclobutanedicarboxylic acid. Compound **4** was obtained as colorless needles, $[\alpha]_D=0°$(*c* 0.47, MeOH), and exhibited a phenol UV absorption. Compound **4** showed a molecular ion $[M^+]$ peak at *m*/*z* 388 in EI-MS, and its molecular formula was determined to be $C_{20}H_{20}O_8$ by HR-EI–MS. From the ^1H NMR and ^{13}C NMR spectral data, compound **4** has a phenyl moiety, two saturated carbons, a carbonyl group, and a methoxyl moiety. From the HMBC spectral analysis, the methoxyl

Table 1
^{13}C NMR spectral data for compounds **1**–**3**[a,b,e]

Position	1	2	3
1‴	127.7*s*	127.2*s*	131.0*s*
2‴	131.3*d*	131.4*d*	130.3*d*
3‴	116.8*d*	116.8*d*	115.8*d*
4‴	161.4*s*	161.3*s*	156.7*s*
5‴	116.8*d*	116.8*d*	115.8*d*
6‴	131.3*d*	131.4*d*	130.3*d*
7‴	146.9*d*	146.9*d*	45.2*d*
8‴	114.9*d*	115.1*d*	44.7*d*
9‴	169.1[c] *s*	169.4[d] *s*	174.3*s*
1″		127.1*s*	131.0*s*
2″		131.2*d*	129.9*d*
3″		116.8*d*	115.6*s*
4″		160.9*s*	156.7*s*
5″		116.8*d*	115.6*s*
6″		131.2*d*	129.9*d*
7″		146.8*d*	45.6*d*
8″		114.9*d*	44.3*d*
9″		169.0 [d] *s*	174.3*s*
OCH_3			
Glucose moiety			
1	94.3*d*	93.2*d*	92.7*d*
2	73.4*d*	73.3*d*	73.0*d*
3	74.8*d*	74.8*d*	74.5*d*
4	71.6*d*	72.2*d*	72.5*d*
5	74.3*d*	72.2*d*	72.4*d*
6	62.5*t*	65.5*t*	68.6*t*
Fructose moiety			
1′	63.8*t*	64.1*t*	63.8*t*
2′	105.5*s*	105.5*s*	105.6*s*
3′	78.9*d*	79.0*d*	79.2*d*
4′	76.8*d*	77.0*d*	77.9*d*
5′	80.8*d*	80.8*d*	79.1*d*
6′	66.8*t*	66.7*t*	67.5*t*

[a] Assigned by the ^1H–^1H COSY, and HMBC spectra.
[b] 125 MHz, TMS as int. standard (ppm, in MeOH-d_4).
[c] Carbonyl carbon attached to C-6 of fructose.
[d] Carbonyl carbon attached to C-6 of glucose.
[e] Carbonyl carbon attached to C-6 of fructose.

moiety was related to the carbonyl group. This generated a C_8O_2 fragment which we designated "A unit" for convenience (Fig. 1). The molecular formula $C_{20}H_{20}O_8$ suggested that two "A units" were present, but the degree of unsaturation requires an additional ring, or a double bond ($C_{20}H_{20}O_8$: 11, two "A units": 10). It is thus possible that the 7,8,7′,8′ carbons make up a cyclobutane moiety. From the MS spectral data, the fragment in *m/z* 194 $[(HO)_2ph–CH–CH–CO–OCH_3]^+$ was observed, but not *m/z* 144 $[CH_3O–CO–CH–CH–CO–OCH_3]^+$, nor *m/z* 244 $[(HO)_2ph–CH–CH\text{-}ph(OH)_2]^+$. Therefore, it should have the truxillic, and not the truxinic structure (Kulanthaivel and Benn, 1986) (Figs. 2 and 3).

From the ^1H NMR spectral data, H-7, H-8, H-7′, H-8′ are all triplets, suggesting that two *ortho* protons had both chemical environment and magnetic equivalency. So the pairs H-7/H-7′ and H-8/H-8′ should be at one side of the cyclobutane ring .The large coupling constant ($^3J_{7,8} = {}^3J_{7',8'} = 9.8$ Hz) (Dimberg et al., 2001) and the NOE data established a *cis* configuration for the pairs H-7/H-8 and H-7′/H-8′. Thus compound **4** was elucidated as the dimethyl ester of (1α, 2α, 3α, 4α)-2,4-bis (3,4-dihydroxyphenyl)- 1,3-cyclobutanedicarboxylic acid.

Compounds **1–4** were extracted and purified from *B. parviflora* Willd. through bioassay-guided isolation. Based on the bioassay activity data (Tables 3 and 4), compounds **1** and **4** show good PGE_2 inhibition property, and all four compounds **1–4** had better inhibitory effects on histamine release than indomethacin, a commonly used non-steroidal anti-inflammatory drug (Table 5).

Table 2
^1H NMR spectral data for compounds **1–3**[a,b]

Position	1	2	3
2‴	7.47 *d* (8.8)	7.46 *d* (8.5)	6.88 *d* (8.4)
3‴	6.81 *d* (8.8)	6.78 *d* (8.5)	6.57 *d* (8.4)
5‴	6.81 *d* (8.8)	6.78 *d* (8.5)	6.57 *d* (8.4)
6‴	7.47 *d* (8.8)	7.46 *d* (8.5)	6.88 *d* (8.4)
7‴	7.62 *d* (16.0)	7.64 *d* (15.8)	3.82 *dd* (9.3, 2.4)
8‴	6.63 *d* (16.0)	6.42 *d* (15.8)	4.21 *dd* (9.3, 2.2)
2″		7.38 *d* (8.5)	6.72 *d* (8.6)
3″		6.77 *d* (8.5)	6.51 *d* (8.6)
5″		6.77 *d* (8.5)	6.51 *d* (8.6)
6″		7.38 *d* (8.5)	6.72 *d* (8.6)
7″		7.62 *d* (15.8)	4.11 *dd* (12.8, 2.4)
8″		6.27 *d* (15.8)	4.24 *dd* (12.8, 2.2)
Glucose moiety			
1	5.39 *d*	5.43 *d*	5.32 *d*
2	3.42 *dd*	3.47 *dd*	3.47 *dd*
3	3.73 *dd*	3.76 *dd*	3.75 *dd*
4	3.34 *dd*	3.24 *dd*	3.09 *dd*
5	3.88 *dd*	4.18 *dd*	4.13 *dd*
6	3.84 *d* (10.1)	4.58 *dd* (11.9, 1.9)	4.55 *d* (10.1)
	3.69 *d* (10.1)	4.26 *dd* (11.9, 6.4)	4.00 *d* (10.1)
Fructose moiety			
1′	3.64, 2H, *s*	3.64, 2H,*s*	3.63, 2H,*s*
3′	4.12 *d*	4.13 *d*	4.09 *d*
4′	4.08 *dd*	4.15 *dd*	4.29 *dd*
5′	3.99 *m*	4.00 *m*	4.14 *m*
6′	4.49 *dd* (11.9, 7.6)	4.50 *dd* (11.9, 7.6)	4.55 *dd* (11.9, 7.6)
	4.42 *dd* (11.9, 3.4)	4.42 *dd* (11.9, 3.4)	4.33 *dd* (11.9, 3.4)

[a] Assigned by the ^1H–^1H COSY, HMQC and HMBC spectra.
[b] 500 MHz, TMS as int. standard (ppm, in MeOH-d_4).

Table 3
^{13}C NMR and ^1H NMR spectral data for compound **4**

Position	δC[b]	δH[a,c]
1	133.6 *s*	
2	114.7 *d*	6.74 *d* (1.9)
3	146.2 s	
4	145.3 s	
5	116.3 *d*	6.73 *d* (8.2)
6	118.9 *d*	6.62 *dd* (8.2, 1.9)
7	43.9 *d*	3.65 *t* (9.8)
8	50.3 *d*	3.11 *t* (9.8)
9	175.0 *s*	6.74 *d* (1.9)
1′	133.6 *s*	
2′	114.7 *d*	6.74 *d* (1.9)
3′	146.2 *s*	
4′	145.3 *s*	
5′	116.3 *d*	6.73 *d* (8.2)
6′	118.9 *d*	6.62 *dd* (8.2, 1.9)
7′	43.9 *d*	3.65 *t* (9.8)
8′	50.3 *d*	3.11 *t* (9.8)
9′	175.0 *s*	
	52.5 *q* (OCH_3)	3.72 *s* (–$COOCH_3$)

[a] Assigned by the ^1H–^1H COSY, HMQC and HMBC spectra.
[b] 125 MHz, TMS as int. standard (ppm, in MeOH-d_4).
[c] 500 MHz, TMS as int. standard (ppm, in MeOH-d_4).

3. Experimental

3.1. Plant materials

Whole plants of *B. parviflora* Willd. were collected at Da-Hei-Shan country of Liaoning province in China, in July 1999 and identified by Professor Weichun Wu (Department of Medical Plants, Shenyang Pharmaceutical University, China). A voucher specimen (99-DHS-953) is deposited at the College of Pharmacy, Nihon University and the Department of Natural Products Chemistry of Shenyang Pharmaceutical University.

3.2. General experimental procedures

All melting points were determined on a Yanagimoto micro-melting-point apparatus, and are uncorrected. Optical rotations $[\alpha]_D$, were taken on a Tri Rotar DIP-360 (JASCO). The UV spectrum was obtained in MeOH on a Hitachi 200–10 spectrophotometer, and the IR spectrum was recorded on a JASCO IR A-2 spectrophotometer. The NMR spectra were taken on JEOL GL-500 spectrometer, with TMS as an internal standard.

Table 4
Inhibition of PGE_2 production

Sample	Concentration (μg/ml)	PGE_2 concentration (pg/ml)	Inhibition ratio (%)
+NONE		16	
+LPS only		473	
Sample+LPS			
Compound			
1	30	214	58.1
2	30	536	−0.10
3	30	485	1
4	30	127	76.53
P-O[a]	30	465	−0.05
P-I[b]	30	308	38.23
P-II[c]	30	178	65.75
P-III[d]	30	502	−0.03
P-IV[e]	30	666	−37.4

[a] P-O: the alcohol extract.
[b] P-I: water fraction.
[c] P-II: *n*-butanol fraction.
[d] P-III: ethyl acetate fraction.
[e] P-IV: *n*-hexane fraction.

Table 5
Inhibitory effects on histamine release

Compound	IC_{50} (μg/ml)
1	21.7
2	23.5
3	41.2
4	16.9
Indomethacin[a]	89.5

[a] A medicine to diminish inflammation.

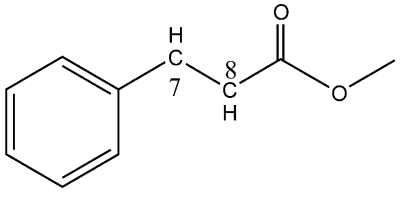

Fig. 1. Structure of "A unit".

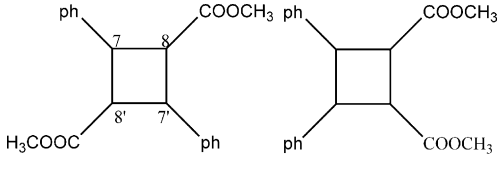

Fig. 2. Structure of truxillic and truxinic moiety.

The mass spectra (MS) were obtained on a Hitachi M-80B spectrometer. CC was carried out over silica gel (Wako gel C-300, Wako pure Chemical Industry Ltd.), and Sephadex LH-20 (20–100 μm, Pharmacia Fine Chemical Co., Ltd.). TLC was performed on Merck TLC plates (0.25 mm thickness), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in EtOH solution followed by heating.

3.3. Extraction and isolation

The air-dried whole plant (5.5 kg) was extracted twice with 60% aq. EtOH under reflux for 1 h 40 min. Evaporation of the solvent under reduced pressure from the combined extract gave the 60% aq. EtOH extract [674.2 g, inhibitory effect, 98.4% (100 μg/ml)]. The extract was dissolved and suspended in water (2.0 l) and partitioned with hexane (3×2 l), EtOAc (3×2 l) and *n*-butanol (3×2 l), successively, whose extract and inhibitory effect is 136 g, 76.5%, 126 g, 79.8%, 176 g, 96.2%, respectively, and yielded the aqueous extract [376 g, inhibitory effect, 58.4% (100 μg/ml)].

The m-butanol fraction was subjected to silica gel CC (SiO_2, 400 g, eluted with $CHCl_3$ and MeOH in increasing polarity) to obtain 9 frs. Frs. 4 was subjected to a Sephadex LH-20 CC and the fractions were further purified by prep. HPLC with 18% CH_3CN as eluant to provide compounds **1** (80 mg), **2** (140 mg), **3** (17 mg) and **4** (36 mg). The compounds were tested for inhibitory effect on histamine release from rat mast cells induced by antigen-antibody reaction.

Compound **1** white powder, mp 129–130 °C; $[\alpha]_D^{25}$ 56° (*c* 0.48, MeOH); FAB–MS *m/z*: 489 $(M+H)^+$; HR-FAB–MS, found 489.16082, calc. 489.16072; UV λ_{max}^{MeOH} nm (log ϵ) 312 (4.645), 229 (4.336); IR ν_{max}^{KBr} cm^{-1} 3380, 1693, 1598, 1513, 1448, 1267, 1174, 1060; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

Compound **2** white powder, mp 138–140 °C; $[\alpha]_D^{25}$ 61.4°(*c* 0.64, MeOH); FAB–MS *m/z*: 635 $(M+H)^+$; HR-FAB–MS, found 635.19619, calc. 635.19757; UV λ_{max}^{MeOH} nm (log ϵ) 312 (3.287), 229 (3.102); IR ν_{max}^{KBr} cm^{-1} 3399, 1691, 1598, 1513, 1446, 1267, 1174, 1060; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

Compound **3** white powder, mp 198–200 °C; $[\alpha]_D^{25}$ 37.1°(*c* 0.65, MeOH); FAB–MS *m/z*: 635 $(M+H)^+$; HR-FAB–MS, found 635.19761, calc. 635.19757; UV λ_{max}^{MeOH} nm (log ϵ) 326 (4.63), 229 (4.34); IR ν_{max}^{KBr} cm^{-1} 3380, 1729, 1610, 1515, 1452, 1365, 1135, 1062; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

Compound **4** colorless needles, mp 220 °C; $[\alpha]_D^{25}$ 0.00°(*c* 0.47, MeOH); EI-MS *m/z*: 388.1 $[M]^+$; HR-EI–MS, found 388.11550, calcd. 388.11580; UV λ_{max}^{MeOH} nm (log ϵ) 284 (4.36), 236 (4.50); IR ν_{max}^{KBr} cm^{-1} 3401, 3324, 1712, 1616, 1535, 1448, 1361, 1302; for ^1H and ^{13}C NMR spectra, see Table 3.

3.4. Acid hydrolysis

Compounds **1**, **2** and **3** were subjected to acid hydrolysis, and showed the presence of D-fructose (R_f=0.45) and D-glucose (R_f=0.25) on TLC (solvent: $CHCl_3$–MeOH–H_2O, 65:30:5).

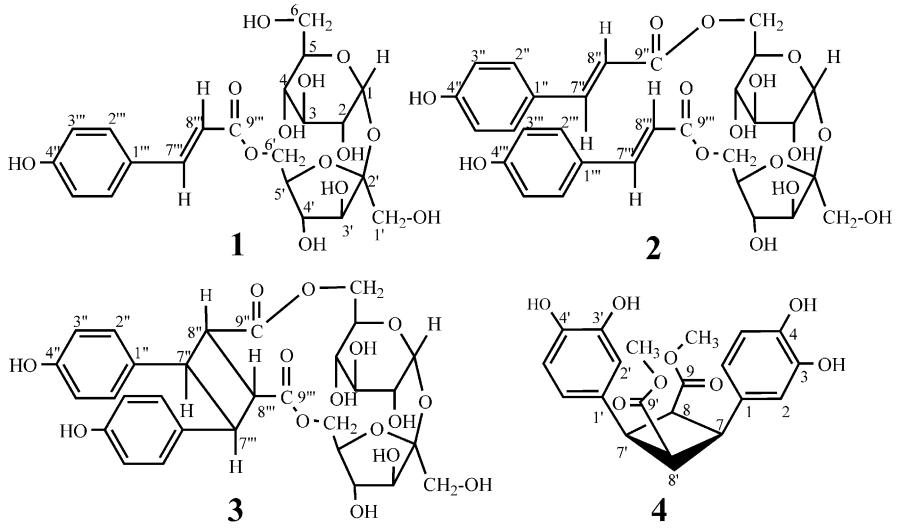

Fig. 3. Structures of compounds 1–4.

3.5. Assay of inhibitory activity on histamine release

Compounds 1–4 were assayed using the HPLC-fluorometry method previously described (Wang et al., 2001) (REF) with modifications. Male Wistar rats (Japan SLC, Shizuoka) weighing 180–200 g were exsanguinated and injected intraperitoneally with 10 ml of Tyrode solution. The abdominal region was gently massaged for 3 min and then the peritoneal exudates were collected. The peritoneal cavity fluid containing mast cells was suspended in PBS, and then layered on BSA ($d = 1.068$) in a test tube at room temp for 20 min. After centrifugation at 300 g and 4 °C for 10 min, the layer containing mast cells was pipetted out. The cells were washed with PBS (pH 7.0) (3 ml×3) and suspended in the same medium. Cell viability was determined using trypan blue. Mast cells (1.0×10^6 cells/ml) were pre-incubated with test samples (10 μl) at 37 °C for 10 min, followed by addition of histamine releasers, either compound 48/80 (5 μg/ml) or anti-DNP-IgE (5 μg/ml) and phosphatidyl serine (100 μg/ml). The mixtures were incubated again for 10 min. The quantity of histamine released was expressed in peak height, from which % inhibition was calculated.

3.6. PGE_2 production assay

RAW 264.7 cell, a mouse macrophage-like cell line transformed with the Abelson leukemia virus, were obtained from the American Type Culture Collection (Rockville, MD). The cells were suspended in the medium at a density of 2.0×10^5 cells/ml. A 400μl of the suspension was poured into each cell well of a 24-well microplate (Sumitomo Bakelite, #8024 R; Tokyo, Japan), and then incubated at 37 °C for 2 h. *Mallotus japonicus* or a test compound and 100 μl/ml lipopolysaccharide were added to the culture medium, and the cells were incubated at 37 °C for 16 h. The medium was collected in a microfuge tube to determine the amount of PGE_2 Enzyme-Immune-Assay kit (Cayman Chemical Company, MI, USA).

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