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Three plant growth inhibiting saponins from *Duranta repens*

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Abstract

Three new triterpenoid-type saponins were isolated from leaves of *Duranta repens* using plant growth inhibiting activity against seedlings of *Brassica juncea* var. *cernua* as a guide for fractionation. The structures of these compounds were elucidated as polygalacic acid-3-*O*-β-D-glucopyranosido-28-*O*-[α-L-rhamnopyranosyl-(1 → 3′)-β-D-apiofuranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-L-arabinopyranoside] (durantanin **I**), polygalacic acid-3-*O*-β-D-glucopyranosido-28-*O*-[α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-[β-D-apiofuranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-β-L-arabinopyranoside] (durantanin **II**), and polygalacic acid-3-*O*-β-D-glucopyranosido-28-*O*-[α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-L-arabinopyranoside] (durantanin **III**).

Keywords: *Duranta repens*; Verbenaceae; Golden dew drop; Allelopathy; Plant growth inhibitor; Polygalacic acid; Polygalacic acid glucoside; Polygalacic acid glycoside; Oleanane-type; Triterpenoid saponins; Durantanin **I**–**III**

1. Introduction

Duranta repens Linn. (Syn.: *Duranta plumieri* Jacq) is a tree-type ornamental plant grown in tropical countries. It is generally 2–4 m in height and its large number of leaves create shade year round. Weeds and shrubs growing around it are not healthy, suggesting the presence of water-soluble plant growth inhibitor(s) in the leaves (Zungsontiporn, 1995). This suggests a possible allelopathic effect. The presence of pectolinaringenin and scutellarein (Subramanian & Nair, 1972), lamiid and their esters with cinnamic acid (durantosid **I**), 4-methoxycinnamic acid (durantosid **II**), 3,4-dimethoxycinnamic acid (durantosid **III**), 4-hydroxycinnamic acid and 4-acetoxycinnamic acid have been reported in this plant (Kuo & Kubota, 1976; Rao, Rao & Vijayakumar, 1978; Rimpler & Timm, 1974), although their plant growth inhibitory activities and contributions to allelopathy are not clear. This report describes the isolation of three new candidate allelochemicals, named durantanin **I**–**III**, from the leaves of *D. repens* by focusing on the inhibitory effects on plant growth, and determining their structures from chemical and spectral evidences.

2. Results and discussion

Air-dried leaves of *D. repens* were extracted with 80% MeOH. Successive bioassay-directed liquid-liquid extraction, chromatography on activated charcoal and silica gel columns, solid phase extraction on Bondesil C18 columns, and prep. HPLC on reversed phase columns gave three plant growth inhibitors, compounds **1**–**3**, named durantanin **I**–**III**, respectively.

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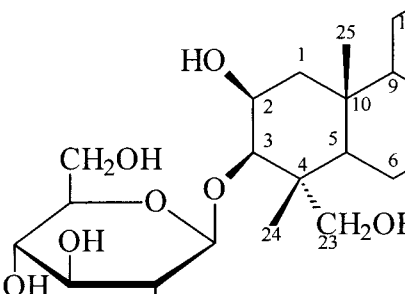

Hydrothermolysis (Kim, Higuchi & Komori, 1992) of durantanin **I** in hot water gave a mixture of compound **4** (prosapogenin) and sugars. The FAB-mass spectrum of compound **4** showed a protonated molecular ion $[M+Na]^+$ at *m/z* 689, indicating a molecular formula $C_{36}H_{58}O_{11}$. The broad-band decoupled ^{13}C and DEPT NMR spectra of purified compound **4** showed 36 signals due to six methyl, 10 methylene, 12 methine and eight quaternary carbons. The presence of one carboxyl group (C-28, δ 181.3), six tertiary methyl proton groups (δ 0.81–1.39, *s*, 3H each), one olefinic proton (H-12, δ 5.31, *br* t, $J = 3.2$ Hz), and one anomeric carbon (δ 105.5) and proton (δ 4.43, *d*, $J = 7.8$ Hz) were indicated by their ^1H and ^{13}C NMR spectra. The olean-12-ene triterpene skeleton was inferred from the chemical shifts of C-12 (δ 123.5) and C-13 (δ 145.2) (Fullas et al., 1996). After determining the connectivity between each carbon and proton by ^{13}C-^1H COSY NMR spectrum, these C-H units were constructed according to the results of ^1H-^1H COSY, HOHAHA and HMBC NMR spectra, indicating the presence of substituted 2,3,16,23-tetrahydroxyolean-12-en-28-oic acid (polygalacic acid). Polygalacic acid had been isolated from *Polygala paenea* L. (Polonski, Pourrat & Seiligmann, 1960) and its chemical structure had been confirmed by spectral evidence (Kubota & Kitatani, 1968). The acid hydrolysate of compound **4** was converted to its TMS ether derivative and analyzed by GC, which showed the presence of a glucosyl residue. The HMBC spectrum showed correlation between the glucose-C-1 (δ 105.5) and polygalacic acid-H-3 (δ 3.61) and between glucose-H-1 (δ 4.43) and polygalacic acid-C-3 (δ 83.9). Based on this evidence, compound **4** was identified as polygalacic acid-3-*O*-glucoside.

Durantanin **I** has the molecular formula $C_{58}H_{94}O_{27}$ (HR FAB MS spectrometry). GC-MS analysis of the acid hydrolysate of durantanin **I** derivatized to the per-*O*-acetylated alditol showed the presence of one glucose, one arabinose, two rhamnoses and one apiose. A similar result was obtained from TMS ether derivative of methyl glycoside of acid hydrolysate of durantanin **I**. Glycosyl-linkage analysis (York, Darvill, McNeil, Stevenson & Albersheim, 1985) using the partially methylated alditol acetate derivative of acid hydrolysate of durantanin **I** showed the presence of terminal glucopyranose, terminal rhamnopyranose, 3-linked apiofuranose, 4-linked rhamnopyranose and 2-linked arabinopyranose. From the on-line optical rotation analysis of the hydrolysate of durantanin **I** by HPLC, the configurations of glucose, rhamnose, apiose and arabinose in durantanin **I** were determined to be D, L, D and L, respectively. The HMBC spectrum showed correlations between the D-glucopyranose-H-1 (δ 4.43) and polygalacic acid-C-3 (δ 84.0), terminal L-rhamnopyranose-C-1 (δ 102.0) and D-apiofuranose-H-3′ (δ 3.45, 3.77), D-apiofuranose-C-1 (δ 111.6) and inner L-rhamnopyranose-H-4 (δ 3.52), inner L-rhamnopyranose-H-1 (δ 4.94) and L-arabinopyranose-C-2 (δ 76.4), and L-arabinopyranose-H-1 (δ 5.72) and polygalacic acid-C-28 (δ 177.1). From the J_{H-H} values of the anomeric protons of D-glucopyranose (7.7 Hz) and L-arabinopyranose (2.8 Hz), both configurations were concluded to be β. The configurations of inner and terminal L-rhamnopyranoses were assigned as α from the chemical shifts at C-1 (δ 102.0, Rha-t; δ 101.7, Rha-i), C-3 (δ 72.4, Rha-t; δ 72.5, Rha-i) and C-5 (δ 70.0, Rha-t; δ 69.2, Rha-i), and the $^1J_{C-H}$ values (*d*, $J = 168.7$ Hz, Rha-t; *d*, $J = 169.3$ Hz, Rha-i) of its anomeric carbon signals in the ^1H non-decoupled ^{13}C-NMR spectrum (Asada, Ueoka & Furuya, 1989). The configuration of D-apiofuranose was determined to be β on the basis of comparison of chemical shifts at C-1 (δ 111.6) and C-2 (δ 78.3) with previous studies (Asada et al., 1989; Ishii & Yanagisawa, 1999; Snyder & Serianni, 1987). Therefore, the structure of compound **4** was determined to be polygalacic acid-3-*O*-β-D-glucopyranoside, and the structure of durantanin **I** was determined to be polygalacic acid-3-*O*-β-D-glucopyranoside-28-*O*-[α-L-rhamnopyranosyl-(1 → 3′)-β-D-apiofuranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-L-arabinopyranoside].

The molecular formula of durantanins **II** and **III** were $C_{63}H_{102}O_{31}$ and $C_{58}H_{94}O_{27}$ (HR FAB MS spectrometry), respectively. The hydrothermolysis of durantanins **II** and **III** each gave compound **4**. The sugar analyses using per-*O*-acetylated alditol and methyl TMS derivatives showed the presence of one glucose, two rhamnoses, one xylose, one apiose and one arabinose in durantanin **II**, and one glucose, two rhamnoses, one xylose and one arabinose in durantanin **III**. The on-line optical rotation analysis showed the configurations of glucose, rhamnose, xylose, apiose and arabinose in durantanins **II** and **III** are D, L, D, D and L, respectively.

Glycosyl-linkage analysis of durantanin **II** indicated

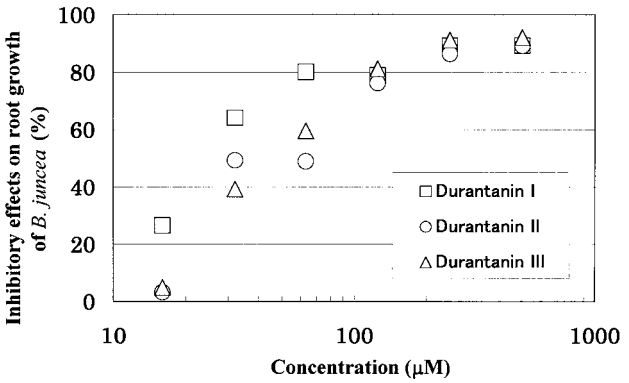

Fig. 1. Inhibitory effects of durantanin **I**–**III** on root growth of *B. juncea*.

the presence of terminal D-glucopyranose, terminal L-rhamnopyranose, terminal D-apiofuranose, 3-linked D-xylopyranose, 3,4-di-linked L-rhamnopyranose and 2-linked L-arabinopyranose. The HMBC spectrum of durantanin **II** showed correlations between D-glucopyranose-H-1 (δ 4.43) and polygalacic acid-C-3 (δ 84.0), terminal L-rhamnopyranose-H-1 (δ 5.12) and D-xylopyranose-C-3 (δ 84.0), D-xylopyranose-H-1 (δ 4.65) and inner L-rhamnopyranose-C-4 (δ 78.3), terminal D-apiofuranose-H-1 (δ 5.24) and inner L-rhamnopyranose-C-3 (δ 81.9), inner L-rhamnopyranose-H-1 (δ 4.94) and L-arabinopyranose-C-2 (δ 76.1), and L-arabinopyranose-H-1 (δ 5.68) and polygalacic acid-C-28 (δ 177.1). The configuration of D-xylopyranose was assigned as β because of the J_{H-H} value (7.7 Hz) between H-1 and H-2. The other anomeric configurations of sugar moieties in durantanin **II** were the same as those of durantanin **I**. Therefore, the structure of durantanin **II** was determined to be polygalacic acid-3-*O*-β-D-glucopyranosido-28-*O*-[α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-[β-D-apiofuranosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-β-L-arabinopyranoside].

Durantanin **III** was shown to have terminal D-glucopyranose, terminal L-rhamnopyranose, 3-linked D-xylopyranose, 4-linked L-rhamnopyranose and 2-linked L-arabinopyranose in glycosyl-linkage analyses. The connectivities between D-glucopyranose-H-1 (δ 4.43) and polygalacic acid-C-3 (δ 84.0), terminal L-rhamnopyranose-H-1 (δ 5.14) and D-xylopyranose-C-3 (δ 84.2), D-xylopyranose-H-1 (δ 4.53) and L-rhamnopyranose-C-4 (δ 78.5), L-rhamnopyranose-H-1 (δ 5.03) and L-arabinopyranose-C-2 (δ 75.8), and L-arabinopyranose-H-1 (δ 5.62) and polygalacic acid-C-28 (δ 177.1) were observed in HMBC. The configurations of sugar residues in durantanin **III** were same as those of durantanin **II**. Therefore, the structure of durantanin **III** is polygalacic acid-3-*O*-β-D-glucopyranosido-28-*O*-[α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-L-arabinopyranoside].

Table 1
^{13}C NMR spectral data for durantanin **I**–**III** (compounds **1**–**3**) and compound **4** (150.8 MHz, CD_3OD)

C	Durantanin **I**	Durantanin **II**	Durantanin **III**	Compound **4**[a]
1	44.5	44.5	44.5	44.4
2	71.2	71.2	71.2	71.2
3	84.0	84.0	84.0	83.9
4	43.2	43.2	43.2	43.1
5	48.3	48.3	48.3	48.2
6	18.7	18.7	18.8	18.6
7	33.8	33.8	33.7	33.7
8	41.0	40.9	40.9	40.7
9	48.5	48.5	48.5	48.4
10	37.6	37.6	37.6	37.5
11	24.7	24.7	24.7	24.6
12	124.0	123.9	123.9	123.5
13	144.8	144.8	144.8	145.2
14	43.0	42.9	43.0	42.9
15	36.4	36.3	36.4	36.1
16	74.7	74.8	74.6	75.3
17	50.4	50.4	50.4	49.6
18	42.1	42.2	42.2	42.1
19	47.6	47.6	47.7	47.7
20	31.4	31.4	31.4	31.4
21	36.4	36.4	36.5	36.6
22	31.9	31.8	32.0	32.7
23	65.7	65.8	65.9	65.6
24	14.8	14.9	14.9	14.7
25	18.1	18.1	18.0	17.8
26	17.7	17.7	17.7	17.5
27	27.5	27.4	27.4	27.3
28	177.1	177.1	177.1	181.2
29	33.4	33.4	33.4	33.4
30	25.3	25.3	25.2	24.9

[a] Compound **4**: prosapogenin of durantanin **I**–**III**.

Inhibitory effects of durantanin **I**–**III** on root growth of seedlings of *B. juncea* were found to have the same order (Fig. 1), and 50% of the root growth was inhibited around 50 μM of durantanin **I**–**III**.

3. Experimental

3.1. General

Mps: uncorr; NMR: JEOL JNM α-600 spectrometer, CD_3OD with TMS as int. ref; MS: JEOL SX102A spectrometer, direct inlet system, glycerol was used as a matrix; IR: KBr disc; CC: activated charcoal (Charcoal, Activated, 031-02135, Wako Pure Chem. Ind., Ltd), silica gel (Wakogel C-200, 74–149 μm, Wako Pure Chem. Ind., Ltd); solid phase extraction: chemically modified silica gel (Analytichem Bondesil C18, 40 μm, preparative grade, varian); prep. HPLC:

Table 2
^{13}C NMR spectral data for the sugar moieties of durantanin **I**–**III** (compounds **1**–**3**) and compound **4** (150.8 MHz, CD_3OD)

C	Durantanin **I**	Durantanin **II**	Durantanin **III**	Compound **4**[a]
C-3-*O*-β-D-Glc				
1	105.5	105.5	105.5	105.5
2	75.4	75.4	75.4	75.4
3	78.3	78.3	78.3	78.2
4	71.2	71.2	71.2	71.1
5	77.8	77.8	77.8	77.7
6	62.4	62.4	62.4	62.3
α-L-Rha-t[a]				
1	102.0	102.7	102.6	
2	72.1	72.4	72.3	
3	72.4	72.3	72.3	
4	74.0	74.0	74.1	
5	70.0	70.1	70.1	
6	18.3	17.9	17.9	
β-D-Xyl				
1		105.0	106.6	
2		76.0	76.3	
3		84.0	84.2	
4		70.2	69.9	
5		67.0	67.2	
β-D-Api				
1	111.6	112.1		
2	78.3	78.2		
3	79.5	80.1		
3′	70.6	64.9		
4	75.1	74.9		
α-L-Rha-i[b]				
1	101.7	101.2	101.4	
2	72.7	72.0	72.2	
3	72.5	81.9	72.4	
4	80.3	78.3	83.5	
5	69.2	69.2	69.1	
6	18.5	18.3	18.2	
C-28-*O*-β-L-Ara				
1	93.7	93.8	94.1	
2	76.4	76.1	75.8	
3	70.2	70.2	71.2	
4	66.3	66.3	67.1	
5	62.8	62.9	63.8	

[a] α-L-Rha-t: terminal α-L-rhamnopyranose.
[b] α-L-Rha-i: inner α-L-rhamnopyranose.

Shim-pack PREP-ODS(H) (20 mm × 25 cm, Shimadzu).

3.2. Extraction and separation

Air-dried leaves of *D. repens* (from Bangkok, Thailand, 200 g) were extracted with 80% MeOH. The resulting water suspension of the MeOH extract was divided into frs soluble in hexane (7 g), acidic EtOAc (21 g), acidic *n*-BuOH (44 g), and water (23 g). The *n*-BuOH fr. was chromatographed on an activated charcoal column and eluted with H_2O, MeOH, Me_2CO and EtOAc, successively. The MeOH eluate (11 g) was sepd into 11 frs on a column of silica gel ($CHCl_3$, successively and finally MeOH). The 40% MeOH/$CHCl_3$ eluate (3 g) was sepd into eight frs by solid phase extraction (successively from 40% aq. MeOH to pure MeOH). The 50 and 55% aq. MeOH frs were chromatographed by prep. HPLC [Shim-pack PREP-ODS(H), 25 cm × 20 mm, eluted with 50% aq. MeOH] to obtain active durantanin **I** (0.42 g), **II** (2.01 g) and **III** (0.37 g).

3.2.1. Durantanin *I*

White powder, mp 216–220°, $[\alpha]_D^{22}$ −57.4° (MeOH; *c* 1.21). HR FAB MS: *m/z* 1223.6053, $C_{58}H_{94}O_{27}+H$

requires 1223.6061. IRν_{max} (KBr) cm^{-1}: 3402, 2936, 1736, 1720, 1638, 1052. ^{1}H NMR (600 MHz, CD_3OD) δ: 0.80, 0.88, 0.94, 0.99, 1.30, 1.38 (all 3H, *s*), 1.25 (3H, *d*, J = 6.2 Hz, H-6 of Rha-i), 1.27 (3H, *d*, J = 6.2 Hz, H-6 of Rha-t), 4.43 (1H, *d*, J = 7.7 Hz, H-1 of Glc), 4.71 (1H, *d*, J = 1.5 Hz, H-1 of Rha-t), 4.94 (1H, *d*, J = 1.5 Hz, H-1 of Rha-i), 5.34 (1H, *d*, J = 2.4 Hz, H-1 of Api), 5.72 (1H, *d*, J = 2.8 Hz, H-1 of Ara). ^{13}C NMR: Tables 1 and 2.

3.2.2. Durantanin **II**

White powder, mp 225–227°, $[\alpha]_D^{22}$ −62.8° (MeOH; *c* 1.22). HR FAB MS: *m/z* 1355.6487, $C_{63}H_{102}O_{31}+H$ requires 1355.6484. IRν_{max} (KBr) cm^{-1}: 3437, 2935, 1736, 1720, 1638, 1048. ^{1}H NMR (600 MHz, CD_3OD) δ: 0.80, 0.88, 0.95, 0.98, 1.30, 1.37 (all 3H, *s*), 1.24 (3H, *d*, J = 5.6 Hz, H-6 of Rha-t), 1.26 (3H, *d*, J = 5.9 Hz, H-6 of Rha-i), 4.43 (1H, *d*, J = 7.7 Hz, H-1 of Glc), 4.65 (1H, *d*, J = 7.7 Hz, H-1 of Xyl), 4.95 (1H, *d*, J = 1.8 Hz, H-1 of Rha-i), 5.12 (1H, *d*, J = 1.5 Hz, H-1 of Rha-t), 5.24 (1H, *d*, J = 3.7 Hz, H-1 of Api), 5.68 (1H, *d*, J = 2.9 Hz, H-1 of Ara). ^{13}C NMR: Tables 1 and 2.

3.2.3. Durantanin **III**

White powder, mp 223–225°, $[\alpha]_D^{22}$ −45.6° (MeOH; *c* 1.37). HR FAB MS: *m/z* 1223.6104, $C_{58}H_{94}O_{27}+H$ requires 1223.6061. IRν_{max} (KBr) cm^{-1}: 3437, 2933, 1736, 1720, 1638, 1047. ^{1}H NMR (600 MHz, CD_3OD) δ: 0.79, 0.88, 0.96, 0.97, 1.30, 1.38 (all 3H, *s*), 1.25 (3H, *d*, J = 6.2 Hz, H-6 of Rha-t), 1.28 (3H, *d*, J = 6.2 Hz, H-6 of Rha-i), 4.44 (1H, *d*, J = 7.7 Hz, H-1 of Glc), 4.53 (1H, *d*, J = 7.7 Hz, H-1 of Xyl), 5.03 (1H, *d*, J = 1.5 Hz, H-1 of Rha-i), 5.14 (1H, *d*, J = 1.5 Hz, H-1 of Rha-t), 5.62 (1H, *d*, J = 3.7 Hz, H-1 of Ara). ^{13}C NMR: Tables 1 and 2.

3.2.4. Compound **4**

White powder, mp 226–230°, $[\alpha]_D^{22}$ +17.1° (MeOH; *c* 0.053). HR FAB MS: *m/z* 689.3868, $C_{36}H_{58}O_{11}+Na$ requires 689.3877. IRν_{max} (KBr) cm^{-1}: 3432, 2927, 1701, 1686, 1042. ^{1}H NMR (600 MHz, CD_3OD) δ: 0.81, 0.88, 0.94, 0.97, 1.29, 1.39 (all 3H, *s*), 4.45 (1H, *d*, J = 7.7 Hz, H-1 of Glc). ^{13}C NMR: Tables 1 and 2.

3.3. Hydrothermolysis

A solution of durantanin **I**, **II** or **III** (10 mg) in 1 ml of H_2O was allowed to stand for 2 days at 105°. The soln was subjected to sept on solid phase extraction (H_2O and 40, 55, 80 and 100% aq. MeOH). The 80% aq. MeOH eluate gave compound **4**.

3.4. Acid hydrolysis

Dry powder of durantanin **I**–**III** (1 mg) was mixed with 0.25 ml of 2 M TFA and allowed to stand at 121° for 1 h.

3.5. On-line optical rotation analysis by HPLC

Acid hydrolysate of durantanin **I**–**III** and standard sugar samples were analyzed using an HPLC system (Waters 626 LC System) equipped with a Shodex Asahipack NH2P-50 4E column (4.6 mm i.d. × 250 mm, H_3PO_4:water:acetonitrile = 1:19:80, 1 ml/min, 40°) and optical rotational detector (Shodex OR2).

3.6. Measurement of activity

Test solutions were placed into test tubes and dried completely under vacuum at 40°. One milliliter of hot agar aq. solution (40–50°, 0.5%) was added to the test tube, and the sample was dissolved or suspended. After solidification of the agar solution, seeds of *Brassica juncea* var. *cernua* were placed on it. After incubation at 25° in light conditions for 5 days, the inhibitory effect on elongation growth was observed by measuring the length of hypocotyls and roots.

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