

Article

## Triterpenoids from the Roots of *Rhaphiolepis indica* var. *tashiroid* and Their Anti-Inflammatory Activity

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**Abstract:** Two new triterpenoids, 2 $\alpha$ ,3 $\beta$ -dihydroxyolean-11,13(18)-dien-19 $\beta$ ,28-olide (**1**) and 3 $\beta$ ,5 $\beta$ -dihydroxyglutanol (**2**), together with eight known compounds (**3–10**) were isolated from the roots of *Rhaphiolepis indica* var. *tashiroid* (Rosaceae). The structures of **1–10** were determined by spectroscopic techniques. Among these isolates, 2 $\alpha$ ,3 $\beta$ -dihydroxyolean-13(18)-en-28-oic acid (**9**) exhibited inhibitory effect on *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced superoxide production, with an IC<sub>50</sub> value of 16.50  $\mu$ M.

**Keywords:** *Rhaphiolepis indica* var. *tashiroid*; Rosaceae; roots; triterpenoids; anti-inflammatory activity; inhibition of superoxide production

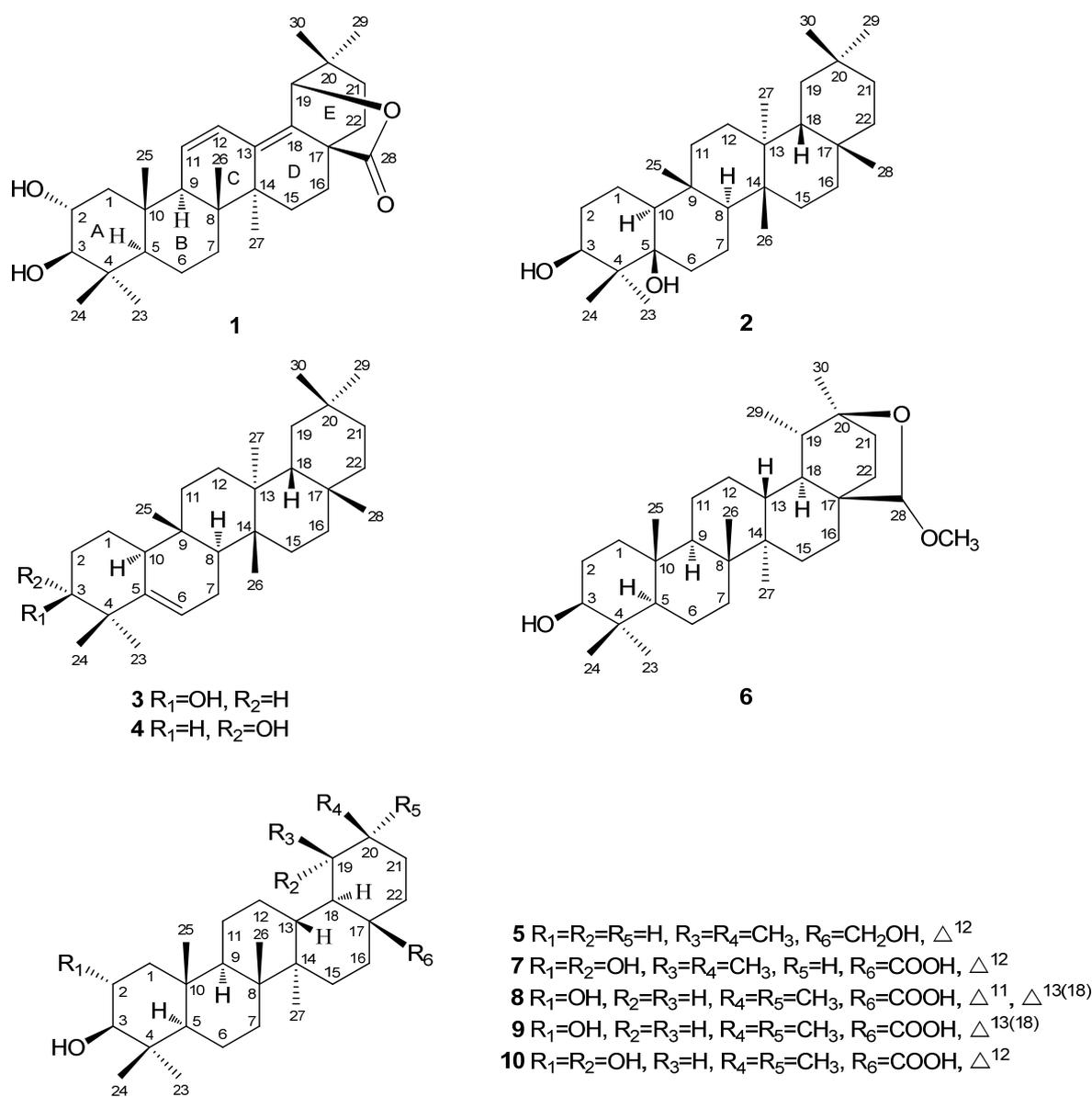
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### 1. Introduction

Under a screening program of Formosan plants on inhibition of *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) induced superoxide production assay, a methanolic extract of the roots of *Rhaphiolepis indica* var. *tashiroid* (Rosaceae) has shown suppression of *N*-formyl-methionyl-leucyl-phenylalanine

(fMLP)-induced superoxide production with no cytotoxicity against neutrophils. *R. indica* var. *tashiroi* is an evergreen shrub or small tree, which is distributed in countries throughout Asia, including India, southern China, the Ryukyus, Korea, and Taiwan [1]. We previously reported six new compounds, including four dibenzofurans, 2-hydroxy-3,4,6-trimethoxydibenzofuran, 2-hydroxy-3,4,9-trimethoxydibenzofuran, 2-hydroxy-3,4,6,9-tetramethoxydibenzofuran, and 1,2-methylenedioxy-3,4,6-trimethoxydibenzofuran, two new biphenyls, 3-hydroxy-2',5-dimethoxybiphenyl and 2',3-dihydroxy-5-methoxybiphenyl, and one known 3-hydroxy-5-methoxybiphenyl from the roots of *R. indica* var. *tashiroi* [2]. Continuous study from the roots of this plant led to the isolation of two new triterpenoids, one oleanane-type 2 $\alpha$ ,3 $\beta$ -dihydroxy-olean-11,13(18)-dien-19 $\beta$ ,28-olide (**1**), and one glutinane-type 3 $\beta$ ,5 $\beta$ -dihydroxyglutinol (**2**), together with eight known compounds (**3–10**) (Figure 1). This paper describes the structural elucidations and inhibition of superoxide production activity of these isolates.

**Figure 1.** Structures of compounds 1–10.



## 2. Results and Discussion

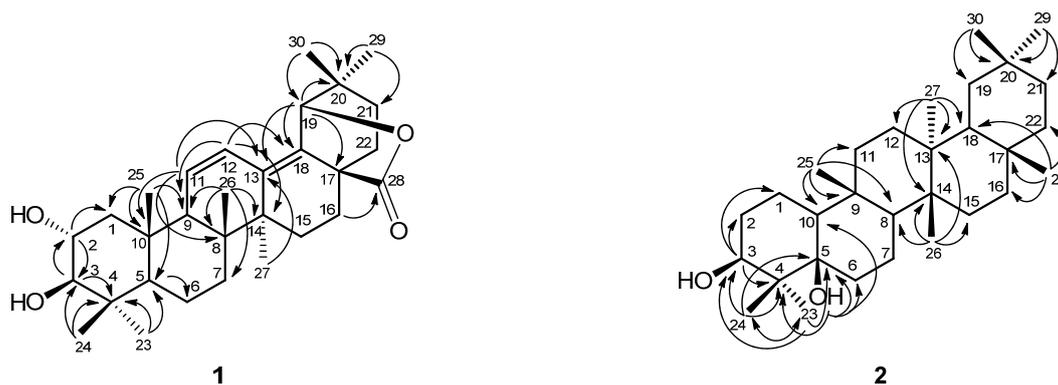
### 2.1. Structure Elucidation

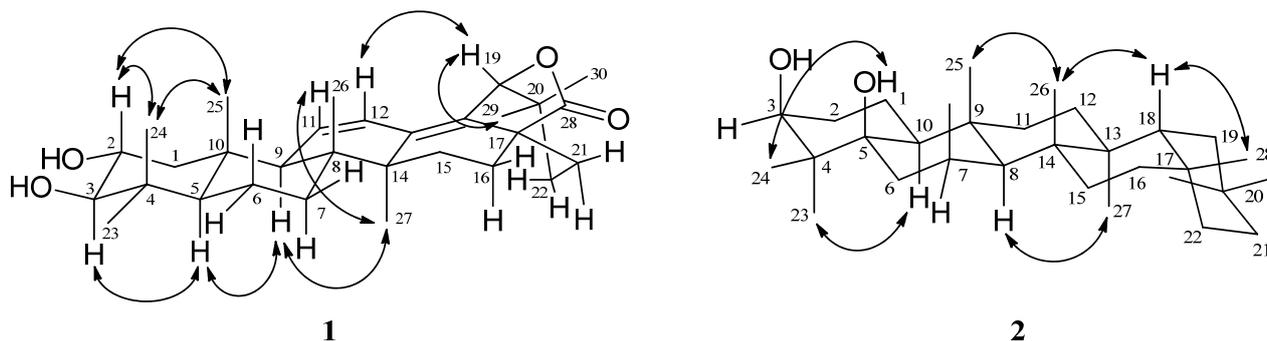
Compound **1** was obtained as amorphous powder. ESIMS ( $m/z$  491  $[M + Na]^+$ ) and HRESIMS ( $m/z$  491.3136  $[M + Na]^+$ ) analysis established the molecular formula of **1** as  $C_{30}H_{44}O_4$ . The IR absorption bands suggested the presence of hydroxy ( $3440\text{ cm}^{-1}$ ),  $\gamma$ -lactonic carbonyl ( $1767\text{ cm}^{-1}$ ), and olefinic ( $1630, 1600\text{ cm}^{-1}$ ) groups. The  $^1\text{H}$  NMR spectrum (Table 1) of **1** indicated seven methyl singlets ( $\delta_{\text{H}}$  0.76, 0.82, 0.95, 1.01, 1.02, 1.04, and 1.10), and a pair of *cis* olefinic protons at  $\delta$  5.77 (1H, dd,  $J = 10.2, 2.0$  Hz, H-11) and 6.15 (1H, dd,  $J = 10.2, 2.8$  Hz, H-12). The  $^{13}\text{C}$  NMR and DEPT spectra revealed that **1** had 30 carbons. Two tertiary olefinic carbons at  $\delta$  123.3 (C-12) and 129.1 (C-11), and two quaternary olefinic carbons at  $\delta$  132.9 (C-18) and 134.7 (C-13) indicated the presence of two double bonds. Two oxymethine carbons at  $\delta$  68.8 and 83.9, and a  $\gamma$ -lactonic carbonyl carbon at  $\delta$  178.1 supported the proposed structure as **1**, which was similar to that of camaldulenic acid (**8**) with oleanane-type skeleton [3]. The major difference was a  $\gamma$ -lactonic carbonyl group in ring E in **1** instead of the COOH group in **8**. The observed oxygen-bearing tertiary carbon signal at C-19 ( $\delta_{\text{C}}$  85.0) as well as the HMBC correlations from H-19 ( $\delta$  4.72, s) to C-13, C-17, C-18, C-20, and C-21 indicated a five-membered lactone ring between C-17 and C-19 which are also supported by IR absorption and nine degrees of unsaturation. The locations of two double bonds at  $\Delta^{11}$  and  $\Delta^{13(18)}$  were determined by the HMBC correlations from H-11 to C-8, C-10, and C-13, from H-12 to C-9, C-14, and C-18, from H-27 to C-13, and from H-19 to C-13 and C-18 (Figure 2). The relative configurations of **1** were determined through inspection of the NOESY spectrum (Figure 3). The NOESY cross-peaks of H-2/H-25 and H-3/H-5 established the  $\beta$ -orientation of H-2 and  $\alpha$ -orientation of H-3 in *trans* A/B ring junction [4]. Thus, **1** was elucidated as  $2\alpha,3\beta$ -dihydroxyolean-11,13(18)-dien-19 $\beta$ ,28-olide.

Compound **2** was isolated as amorphous powder. The HRESIMS analysis of **2** revealed an  $[M + Na]^+$  ion peak at  $m/z$  467.3862 (calcd. 467.3865), which corresponds to the molecular formula  $C_{30}H_{52}O_2$ , accounting for five degrees of unsaturation. The IR absorption bands showed a hydroxy group at  $3400\text{ cm}^{-1}$ . The  $^1\text{H}$  NMR spectrum (Table 1) revealed eight methyl groups ( $\delta_{\text{H}}$  0.89, 0.94, 0.95, 0.97, 0.99, 1.01, 1.01, and 1.17). The NMR data of **2** resembles to those of glutinol (**3**) with glutinane-type skeleton. The major differences between **2** and **3** were having a quaternary 5-OH group ( $\delta_{\text{H}}$  1.10;  $\delta_{\text{C}}$  77.4) and a C-6 methylene ( $\delta_{\text{H}}$  1.55, 1.77;  $\delta_{\text{C}}$  34.4) in **2** instead of trisubstituted olefinic signals at C-5 and C-6 in **3**. The  $^{13}\text{C}$  NMR signal at  $\delta_{\text{C}}$  73.5 and its corresponding proton signal at  $\delta_{\text{H}}$  3.76 (1H, dd,  $J = 11.2, 4.0$  Hz, H-3), were assigned as C-3 and H-3 $\alpha$ , respectively [5]. Moreover, the HMBC correlations (Figure 2) from H-3 to C-1, C-2, and C-4, from H-23 to C-3, C-4 C-5 and C-24, from H-24 to C-3, C-4, C-5, and C-23, and from OH-5 to C-4, C-5, C-6, and C-10, suggested that two hydroxy groups were attached to C-3 and C-5, respectively. The relative configurations of **2** were determined through inspection of the NOESY spectrum (Figure 3). The several key NOESY correlations (H-24/OH-5; H-23/H-10; H-8/H-27; H-25/H-26; H-18/H-26; H-18/H-28) suggested that the  $\beta$ -axial orientation of OH-3 and  $\alpha$ -orientation of H-10 in *trans* A/B ring junction (Figure 3). On the basis of these data, **2** was established as  $3\beta,5\beta$ -dihydroxyglutinol.

**Table 1.**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  (100 MHz) NMR data of **1** and **2** ( $\text{CDCl}_3$ ).

position	<b>1</b>		<b>2</b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)
1	41.6 ( $\text{CH}_2$ )	0.97, m; 2.24, dd (12.4, 4.4)	19.1 ( $\text{CH}_2$ )	1.52, m; 1.56, m
2	68.8 (CH)	3.77, ddd (9.6, 4.4, 2.0)	30.6 ( $\text{CH}_2$ )	1.39, m; 1.78, m
3	83.9 (CH)	3.03, d (9.6)	73.5 (CH)	3.76, dd (11.2, 4.0)
4	39.2 (C)	–	43.9 (C)	–
5	54.9 (CH)	0.91, m	77.4 (C)	–
6	18.1 ( $\text{CH}_2$ )	1.44, m	34.4 ( $\text{CH}_2$ )	1.55, m; 1.77, m
7	33.0 ( $\text{CH}_2$ )	1.39, m; 1.46, m	17.2 ( $\text{CH}_2$ )	1.45, m; 1.50, m
8	41.0 (C)	–	52.5 (CH)	1.24, m
9	52.8 (CH)	2.14, br s	36.8 (C)	–
10	37.9 (C)	2.40, m	50.9 (CH)	1.31, m
11	129.1 (CH)	5.77, dd (10.2, 2.0)	34.9 ( $\text{CH}_2$ )	1.18, m; 1.54, m
12	123.3 (CH)	6.15, dd (10.2, 2.8)	30.3 ( $\text{CH}_2$ )	1.31, m; 1.39, m
13	134.7 (C)	–	38.2 (C)	–
14	40.6 (C)	–	39.5 (C)	–
15	25.5 ( $\text{CH}_2$ )	1.27, m; 1.39, m	32.5 ( $\text{CH}_2$ )	1.28, m; 1.51, m
16	24.2 ( $\text{CH}_2$ )	2.33, ddd (14.4, 5.2, 2.6)	39.2 ( $\text{CH}_2$ )	0.92, m; 1.48, m
17	44.0 (C)	–	29.8 (C)	–
18	132.9 (C)	–	42.7 (CH)	1.54, m
19	85.0 (CH)	4.72, s	32.8 ( $\text{CH}_2$ )	1.28, m; 1.51, m
20	35.7 (C)	–	28.2 (C)	–
21	32.6 ( $\text{CH}_2$ )	1.45, m; 1.62, m	35.3 ( $\text{CH}_2$ )	1.18, m; 1.38, m
22	34.5 ( $\text{CH}_2$ )	1.63, m; 1.83, m	35.9 ( $\text{CH}_2$ )	1.37, m; 1.56, m
23	28.3 ( $\text{CH}_3$ )	1.04, s	19.2 ( $\text{CH}_3$ )	0.97, s
24	16.3 ( $\text{CH}_3$ )	0.82, s	16.5 ( $\text{CH}_3$ )	0.89, s
25	19.1 ( $\text{CH}_3$ )	1.02, s	17.1 ( $\text{CH}_3$ )	0.95, s
26	16.9 ( $\text{CH}_3$ )	0.76, s	20.4 ( $\text{CH}_3$ )	1.01, s
27	19.4 ( $\text{CH}_3$ )	1.01, s	18.7 ( $\text{CH}_3$ )	1.01, s
28	178.1 (C)	–	32.1 ( $\text{CH}_3$ )	1.17, s
29	27.8 ( $\text{CH}_3$ )	1.10, s	35.0 ( $\text{CH}_3$ )	0.94, s
30	23.3 ( $\text{CH}_3$ )	0.95, s	31.8 ( $\text{CH}_3$ )	0.99, s
OH-5	–	–	–	1.10, br s check

**Figure 2.** Key HMBC ( $\curvearrowright$ ) connectivities for compounds **1** and **2**.

**Figure 3.** Key NOESY ( $\curvearrowright$ ) connectivities for compounds **1** and **2**.

The known compounds, glutinol (**3**) [6], 5(6)-gluten-3 $\alpha$ -ol (**4**) [7], uvaol (**5**) [8], 20 $\beta$ ,28-epoxy-28 $\alpha$ -methoxytaraxasteran-3 $\beta$ -ol (**6**) [9], tormentic acid (**7**) [10], camaldulenic acid (**8**) [3], 2 $\alpha$ ,3 $\beta$ -dihydroxyolean-13(18)-en-28-oic acid (**9**) [4], arjunic acid (**10**) [8] were identified by comparison of their physical and spectroscopic data with values reported in the literatures.

## 2.2. Inhibition of Superoxide Production Activities

The inhibition of superoxide production of the compounds *in vitro* was estimated by an assay on inhibitory activity of induction of the *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced generation of the superoxide anion, an inflammatory mediator produced by neutrophils. The clinical anti-inflammatory agent ibuprofen was used as the positive control. The results are shown in Table 2. The effects of compound **9** ( $IC_{50}$  16.50  $\pm$  0.56  $\mu$ M) on fMLP-induced superoxide generation was more potent than that of ibuprofen ( $IC_{50}$  27.53  $\pm$  3.58  $\mu$ M). However, compounds **1**, **2**, and **8** showed weaker activities than that of ibuprofen with  $IC_{50}$  values of 98.37, 56.08 and 89.42  $\mu$ M.

**Table 2.**  $IC_{50}$  Values for the isolates of the roots of *R. indica* var. *tashiroi* in the inhibition on *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced superoxide generation in human neutrophils.

Compounds	$IC_{50}$ ( $\mu$ M) <sup>a</sup>
2 $\alpha$ ,3 $\beta$ -dihydroxyolean-11,13(18)-dien-19 $\beta$ ,28-olide ( <b>1</b> )	98.37 $\pm$ 6.84
3 $\beta$ ,5 $\beta$ -dihydroxyglutinol ( <b>2</b> )	56.08 $\pm$ 0.57
glutinol ( <b>3</b> )	>100
5(6)-gluten-3 $\alpha$ -ol ( <b>4</b> )	>100
uvaol ( <b>5</b> )	>100
20 $\beta$ ,28-epoxy-28 $\alpha$ -methoxytaraxasteran-3 $\beta$ -ol ( <b>6</b> )	>100
tormentic acid ( <b>7</b> )	>100
camaldulenic acid ( <b>8</b> )	89.42 $\pm$ 4.26
2 $\alpha$ ,3 $\beta$ -dihydroxyolean-13(18)-en-28-oic acid ( <b>9</b> )	16.50 $\pm$ 0.56
Ibuprofen <sup>b</sup>	27.53 $\pm$ 3.58

<sup>a</sup> The  $IC_{50}$  values were calculated from the slopes of the dose–response curves. The values are expressed as the means  $\pm$  standard errors of the means (SEM) of three independent experiments. <sup>b</sup> Positive control.

### 3. Experimental Section

#### 3.1. General Experimental Procedures

All melting points were measured on a Yanaco micro-melting apparatus and were uncorrected. Optical rotations were measured on a Jasco P-1020 digital polarimeter. The UV spectra were obtained with a Jasco V-530 UV/VIS spectrophotometer, and IR spectra (KBr or neat) were taken on a Perkin-Elmer System 2000 FT-IR spectrometer. 1D ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT) and 2D (COSY, NOESY, HSQC, HMBC) NMR spectra using  $\text{CDCl}_3$  or  $\text{CD}_3\text{OD}$  as solvent were recorded on Varian Gemini 2000-200 (200 MHz for  $^1\text{H}$  NMR, 50 MHz for  $^{13}\text{C}$  NMR), Varian Unity Plus 400 (400 MHz for  $^1\text{H}$  NMR, 100 MHz for  $^{13}\text{C}$  NMR) and Varian VNMRS-600 (600 MHz for  $^1\text{H}$  NMR, 150 MHz for  $^{13}\text{C}$  NMR) spectrometers. Chemical shifts were internally referenced to the solvent signals in  $\text{CDCl}_3$  ( $^1\text{H}$ ,  $\delta$  7.26;  $^{13}\text{C}$ ,  $\delta$  77.0) or  $\text{CD}_3\text{OD}$  ( $^1\text{H}$ ,  $\delta$  3.31;  $^{13}\text{C}$ ,  $\delta$  49.0), with TMS as the internal standard. ESIMS were obtained on an API 3000 mass spectrometer (Applied Biosystems) and HRESIMS on a Bruker Daltonics APEX II 30e mass spectrometer. Silica gels (70–230, 230–400 mesh) (Merck) were used for column chromatography (CC), and silica gel 60 F-254 (Merck) was used for analytical and preparative TLC. A spherical C18 100 Å column (20–40  $\mu\text{M}$ ) (Silicycle) was used for medium-pressure liquid chromatography.

#### 3.2. Plant Material

The dried roots of *R. indica* var. *tashiroi* were collected at Wutai, Pingtung County, Taiwan, in September 2007, and identified by one of the authors (I.-S.C.). A voucher specimen (Chen 6060) was deposited in the Herbarium of the School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, China.

#### 3.3. Extraction and Isolation

Dried roots (32.8 kg) of *R. indica* var. *tashiroi* were extracted three times with cold MeOH (40 L) to yield a MeOH extract (1.9 kg), which was partitioned using EtOAc– $\text{H}_2\text{O}$  (1:1; 2 L  $\times$  3) to produce an EtOAc-soluble fraction (600 g) and an  $\text{H}_2\text{O}$ -soluble fraction. The  $\text{H}_2\text{O}$ -soluble fraction was partitioned in *n*-BuOH: $\text{H}_2\text{O}$  (1:1; 3L  $\times$  3) to obtain an *n*-BuOH-soluble fraction (700 g) and an  $\text{H}_2\text{O}$ -soluble fraction (400 g). The EtOAc-soluble fraction (100 g) was subjected to silica gel column chromatography (CC) using *n*-hexane as the primary eluting solvent and gradually increasing the eluent polarity with EtOAc and MeOH to produce 12 fractions (A-1–A-12). Fractions A-5 and A-7 showed anti-inflammatory activity. Fraction A-4 (590 mg) was subjected to MPLC using a mixture of *n*-hexane:EtOAc (15:1) to obtain seven fractions (A-4-1–A-4-7). Fraction A-4-3 (126 mg) was separated by MPLC using a mixture of *n*-hexane: $\text{CH}_2\text{Cl}_2$  (1:1) to obtain four fractions (A-4-3-1–A-4-3-4). Fraction A-4-3-1 (25.9 mg) was separated by MPLC using a mixture of *n*-hexane: $\text{CH}_2\text{Cl}_2$  (2:1) to afford **3** (21.9 mg). Fraction A-4-4 (94.5 mg) was separated by MPLC using a mixture of *n*-hexane: $\text{CH}_2\text{Cl}_2$  (1:1) to obtain nine fractions (A-4-4-1–A-4-4-9). Fraction A-4-4-7 (19.7 mg) was separated by MPLC using a mixture of *n*-hexane: $\text{CH}_2\text{Cl}_2$  (1:2) to afford **4** (9.5 mg). Fraction A-6 (1.46 g) was subjected to silica gel CC using a mixture of *n*-hexane:acetone (7:1) as the eluent to yield

eight fractions (A-6-1–A-6-8). Fraction A-6-4 (388 mg) was applied to MPLC using a mixture of *n*-hexane:acetone (10:1) to yield four fractions (A-6-4-1–A-6-4-4). Fraction A-6-4-1 (199 mg) was separated by MPLC (RP-18) using a mixture of acetone:H<sub>2</sub>O (3:1) to produce **5** (10.3 mg). Fraction A-7 (685 mg) was subjected to MPLC using a mixture of *n*-hexane:acetone (8:1) to obtain seven fractions (A-7-1–A-7-7). Fraction A-7-6 (77.4 mg) was separated by MPLC using a mixture of CH<sub>2</sub>Cl<sub>2</sub>:EtOAc (20:1) to obtain **2** (5 mg), Fraction A-7-7 (54.8 mg) was subjected to MPLC using a mixture of *n*-hexane:acetone (3:1) to obtain seven fractions (A-7-7-1–A-7-7-7). Fraction A-7-7-3 (13.9 mg) was purified by preparative TLC (RP-18) developed with acetone:H<sub>2</sub>O (1:6) to yield **6** (2.9 mg). Fraction A-9 (697 mg) was applied to MPLC using a mixture of CH<sub>2</sub>Cl<sub>2</sub>:MeOH (50:1) to provide six fractions (A-9-1–A-9-6). Fraction A-9-2 (98.2 mg) was subjected to MPLC (RP-18) using a mixture of acetone:H<sub>2</sub>O (2:1) to obtain **1** (4.6 mg). Fraction A-9-4 (139.9 mg) was applied to MPLC (RP-18) using a mixture of MeOH:H<sub>2</sub>O (1:1) to provide **7** (68.2 mg), **8** (9.3 mg), and **9** (74.7 mg). Fraction A-10 (811 mg) was separated by MPLC (RP-18) using a mixture of MeOH:H<sub>2</sub>O (3:1) to produce 12 fractions (A-10-1–A-10-12). Fraction A-10-12 (23.1 mg) was subjected to MPLC using a mixture of CH<sub>2</sub>Cl<sub>2</sub>:MeOH (20:1) to obtain **10** (7.6 mg).

2 $\alpha$ ,3 $\beta$ -Dihydroxyolean-11,13(18)-dien-19 $\beta$ ,28-olide (**1**): amorphous powder;  $[\alpha]_D^{24} +49.5$  (*c* 0.09, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3440 (OH), 1767 ( $\gamma$ -lactonic C=O), 1630, 1600; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; ESIMS: *m/z* 491 [M + Na]<sup>+</sup>; HRESIMS: *m/z* 491.3136 [M + Na]<sup>+</sup> (calcd. for C<sub>30</sub>H<sub>44</sub>O<sub>4</sub>Na, 491.3137).

3 $\beta$ ,5 $\beta$ -Dihydroxyglutininol (**2**): amorphous powder;  $[\alpha]_D^{24} +7.6$  (*c* 0.11, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3400 (OH); for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; ESIMS: *m/z* 467 [M + Na]<sup>+</sup>; HRESIMS: *m/z* 467.3862 [M + Na]<sup>+</sup> (calcd. for C<sub>30</sub>H<sub>52</sub>O<sub>2</sub>Na, 467.3865).

### 3.4. Inhibition of Superoxide Production Assay

#### 3.4.1. Evaluation of O<sub>2</sub><sup>-</sup> Release by Human Neutrophils

The anti-inflammatory effects of the compounds isolated from the roots of *R. indica var. tashiroi* were evaluated by measuring the inhibition of superoxide anion production. Superoxide anion production was tested using continuous spectrophotometric analysis of ferricytochrome *c* reduction by an isolated preparation of human neutrophils.

#### 3.4.2. Preparation of Human Neutrophils

Human neutrophils from the venous blood of healthy [11], adult volunteers (20–28 years old) were isolated using a standard method of dextran sedimentation, before centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of the erythrocytes [12]. The purified neutrophils containing >98% viable cells, as determined by the Trypan blue exclusion method, were resuspended in a Ca<sup>2+</sup> (1 mM) Hank's balanced salt solution (pH 7.4) and maintained at 4 °C until use.

#### 3.4.3. Measurement of O<sub>2</sub><sup>-</sup> Generation

The assay for measuring O<sub>2</sub><sup>-</sup> generation was based on the superoxide dismutase (SOD)-inhibitable reduction of ferricytochrome *c* [13]. Briefly, neutrophils (1 × 10<sup>6</sup> cells/mL), pretreated with various

concentrations of the test compounds for 5 min at 37 °C, were stimulated with fMLP (1 µmol/L) in the presence of ferricytochrome *c* (0.5 mg/mL). Extracellular O<sub>2</sub><sup>•-</sup> production was assessed using a UV spectrophotometer at 550 nm (Hitachi, UV-3010). The percentage of superoxide inhibition by the test compound was calculated as  $\{[(\text{control} - \text{resting}) - (\text{compound} - \text{resting})]/(\text{control} - \text{resting})\} \times 100$ . SigmaPlot software was used to determine the IC<sub>50</sub> value.

#### 3.4.4. Statistical Analysis

The results are expressed as the means ± SEM, and comparisons were made with the Student's *t* test. A probability of 0.05 or less was considered significant.

### 4. Conclusions

There are about five *Rhaphiolepis* species in India, southeastern and eastern Asia and one species with three varieties in Taiwan [1]. The leaves of *R. umbellata* were previously reported to have dibenzofurans [14], biphenyls [14–16] as phytoalexin with antifungal activity, and its bark contained flavanol glycosides [17] and procyanidins [18]. Two new triterpenoids were isolated from the roots of *R. indica* var. *tashiroi*. To our knowledge, this is the first report of triterpenoids from the *Rhaphiolepis* plants. The inhibition of superoxide production of the *Rhaphiolepis* genus has never been examined by other research groups. However, we previously reported anti-inflammatory biphenyls and dibenzofurans from the roots of this Formosan plant [2]. Compound **9** was previously reported to show the anti-inflammatory activity [4]. In this study, **9** showed stronger inhibition of superoxide production (IC<sub>50</sub> 16.50 ± 0.56 µM) than ibuprofen (IC<sub>50</sub> 27.53 ± 3.58 µM) suggesting the possibility of developing a new anti-inflammatory agent. This result implied that the anti-inflammatory mechanism of action is worth studying further.

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### Conflict of Interest

The authors declare no conflict of interest.

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