Novel Anti-Inflammatory Constituents of *Artocarpus rigida*

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A novel phenolic compound containing an oxepine ring, artocarpol I (**1**), and a new biphenylpropanoid, artocarpol J (**2**), were isolated from the root bark of *Artocarpus rigida*. The structures, including relative configurations, were elucidated by spectroscopic data. Compound **1** and artocarpol J peracetate (**3**) strongly inhibited in a concentration-dependent manner the formyl-Met-Leu-Phe (fMLP)/cytochalasin B (CB)-stimulated superoxide anion formation in neutrophils with IC_{50} values of 17.1 ± 0.40 and 20.5 ± 2.60 μM, respectively. Biogenetically, all the novel phenolic compounds isolated from this plant seem to be derived from stilbene.

1. Introduction. – Previously, we reported the isolation of a series of phenolic compounds with a new skeleton from the root barks of Formosan *Artocarpus rigida* (Moraceae) [1–4]. These compounds were different from those of the same plant grown in Indonesia which were reported by *Hano et al.* [5][6]. Further examination of the $CHCl_3$ extract of the root barks resulted in the isolation of two new compounds, artocarpol I (**1**) and J (**2**). Artocarpol A strongly inhibited superoxide anion formation in phorbal 12-myristate 13-acetate (PMA) stimulated rat neutrophils and significantly inhibited tumor necrosis factor-α (TNF-α) formation in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells [1]. Artocarpol C strongly inhibited the release of β-glucuronidase and histamine from mast cell degranulation caused by compound 48/80 and also showed strongly inhibitory effect on the formyl-Met-Leu-Phe(fMLP)/cytochalasin B(CB)-stimulated superoxide anion formation in neutrophils [4]. In the present paper, the structure elucidation of **1** and **2** from this plant, and the anti-inflammatory activities of **1** and artocarpol J peracetate (**3**) are reported.

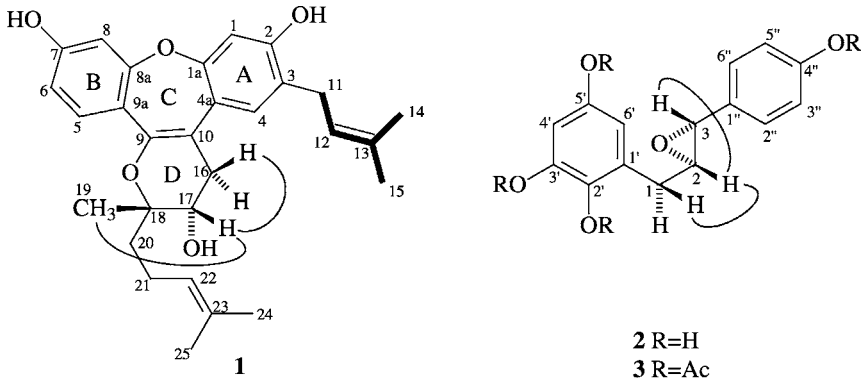

Figure. *Structures of* **1**–**3**, *and selected NOESY correlations and relative configurations for* **1** *and* **3**

2. Results and Discussion. – The molecular formula of artocarpol I (**1**) ($[\alpha]_D^{26} = 2$ ($c = 0.1$, $CHCl_3$)) was established as $C_{29}H_{34}O_5$ by HR-FAB-MS (*m/z* 463.2485, $[M+1]^+$].

The IR absorption of **1** implied the presence of OH (3417 cm^{-1}) and aromatic ring (1625 cm^{-1}) moieties. The UV spectrum was similar to that of artocarpol G [4], which indicated an unconjugated aromatic system [7].

^1H- and ^{13}C-NMR data (*Table 1*) revealed signals due to a trisubstituted and a tetrasubstituted benzene ring (B and A), two 3,3-dimethylallyl groups, two sp^3-CH_2 methylenes, a sp^3-oxymethine, a tertiary Me group, and an oxygenated C-atom. The proposed structure for **1** was deduced from extensive analysis of 1D- and 2D-NMR data, including those from COSY, HMQC, HMBC, and NOESY experiments in $CDCl_3$ (*Table 1*).

The connectivities of CH_2(11) to CH(12), CH_2(16) to H–C(17), and CH_2(21) to H–C(22) were revealed by the COSY data. In addition, the HMBC correlations between Me(14)/C(13), Me(15)/C(14) and C(12), CH_2(11)/C(2), C(3), C(12), and C(13) established the prenyl group at C(3), indicated with bold lines in **1** (see *Fig.*). The HMBC correlations between Me(24)/C(25), Me(25)/C(22) and C(23), CH_2(21)/C(23) and C(20), and CH_2(20)/C(21) confirmed the other prenyl group at C(20). The HMBC correlations between CH_2(16)/C(4a) and C(9), Me(19)/C(18) and C(17), the NOESY correlations between H_β–C(16)/H–C(17) and H–C(17)/Me(19), and the C(9) and C(18) indicated an oxygenated quarternary C-atom, established the connectivities between C(10) and C(16), C(17) and C(18), and C(18) and C(20), and supported the connection of the C- and D-ring by C(9)–O–C(18) and C(10)–C(16) bonds.

The NOESY experiment with **1** displayed cross-peaks as shown in the *Figure*. The relative configurations at C(17) and C(18) were based on NOESY correlations of H_β–C(16)/H–C(17) and H–C(17)/Me(19), while H–C(17) and Me(19), and OH–C(17) adopted the β-and α-configuration, respectively. Further experiments are required to elucidate the absolute configuration of **1**. From the above results,

Table 1. *1D- and 2D-NMR Data* (δ in ppm, J in Hz) *of* **1** *in* $CDCl_3$. Arbitrary numbering (see *Fig. 1*)[a]).

	δ(H)	δ(C)	HMBC (^{1}H)
H−C(1)	6.57 (*s*)	106.4	5.28 (HO−C(2))
C(1a)		151.9	6.57 (H−C(1)), 6.48 (H−C(4))
HO−C(2)	5.28 (*s*)	154.5	6.57 (H−C(1)), 3.23 (C−H_2(11))
C(3)		120.7	6.48 (H−C(4)), 3.23 (C−H_2(11))
H−C(4)	6.48 (*s*)	106.0	
C(4a)		132.0	2.52 (H_α−C(16))
H−C(5)	7.42 (*d*, *J* = 8.8)	121.1	
H−C(6)	6.80 (*dd*, *J* = 8.8, 2.0)	111.9	6.98 (H−C(8))
C(7)		153.4	7.42 (H−C(5))
H−C(8)	6.98 (*d*, *J* = 2.0)	98.3	6.80 (H−C(6))
C(8a)		155.6	7.42 (H−C(5)), 6.98 (H−C(8))
C(9)		152.6	2.52 (H_α−C(16))
C(9a)		122.1	6.80 (H−C(6))
C(10)		118.0	
C−H_2(11)	3.23 (*d*, *J* = 6.8)	27.2	
H−C(12)	5.20 (*t*, *J* = 6.8)	122.4	3.23 (C−H_2(11)), 1.67 (Me(15))
C(13)		131.6	3.23 (C−H_2(11)), 1.59 (Me(14))
Me(14)	1.59 (*s*)	17.7	1.67 (Me(15))
Me(15)	1.67 (*s*)	16.1	
H_α−C(16)	2.52 (*dd*, *J* = 17.2, 6.0)	30.0	
H_β−C(16)	2.81 (*dd*, *J* = 17.2, 4.8)		
H_β−C(17)	3.70 (*dd*, *J* = 6.0, 4.8)	69.8	1.32 (Me(19))
C(18)		76.5	1.32 (Me(19))
Me(19)	1.32 (*s*)	24.7	
C−H_2(20)	2.03 (*t*, *J* = 6.8)	39.6	2.08 (C−H_2(21))
C−H_2(21)	2.08 (*t*, *J* = 6.8)	26.5	2.03 (C−H_2(20))
H−C(22)	5.03 (*t*, *J* = 6.8)	123.7	1.64 (Me(25))
C(23)		138.2	2.08 (C−H_2(21)), 1.64 (Me(25))
Me(24)	1.35 (*s*)	22.0	
Me(25)	1.64 (*s*)	25.7	1.35 (Me(24))

[a]) Signals obtained by ^{1}H- and ^{13}C-NMR, DEPT, and HMBC experiments.

artocarpol I was characterized as 3,4-dihydro-2-methyl-6-(3-methylbut-2-enyl)-2-(4-methylpent-3-enyl)-2*H*-dibenzo[*b*,*f*]pyrano[2,3-*d*]oxepine-3α,7,11-triol (**1**).

We acylated compound **2**, since we were unable to isolate it in pure form. The peracetate **3** of **2** ($[\alpha]_D^{27} = +46$ ($c = 1.0$, $CHCl_3$)) displayed $[M+1]^+$ ion peak at m/z 443.1342 in the HR-FAB-MS, corresponding to the molecular formula $C_{23}H_{22}O_9$. The IR absorption of **3** showed the presence of AcO groups (1766 cm^{-1}) and an aromatic moiety (1622 cm^{-1}). The UV spectrum of **2** showed absorption maxima at 275 (3.73) and 210 (4.53) nm, indicating the presence of an unconjugated aromatic system [7].

^{1}H- and ^{13}C-NMR data (*Table 2*) revealed signals due to a tetrasubstituted benzene ring, a disubstituted benzene ring, four Ac groups, a sp^3-CH_2, and two sp^3-oxymethines. The proposed structure for **3** was deduced from extensive analysis of 1D- and 2D-NMR data, including those from COSY, HMQC, HMBC, and NOESY experiments in $CDCl_3$ (*Table 2*). The connectivities of H−C(3) to H−C(2) and H−C(2) to CH_2(1) were confirmed by the COSY data. The HMBC correlations between CH_2(1)/C(1′), H_α−C(1)/C(2′), and H−C(6′)/C(1) confirmed the connectivity between C(1′) to

Table 2. *1D- and 2D-NMR Data* (δ in ppm, J in Hz) *of* **3** *in* $CDCl_3$. Arbitrary numbering (see *Fig. 1*)[a]).

	δ(H)	δ(C)	HMBC (^{1}H)
H_α–C(1)	2.66 (*dd*, J = 16.8, 6.4)	23.7	
H_β–C(1)	2.83 (*dd*, J = 16.8, 4.8)		
H–C(2)	5.29 (*m*)	68.3	2.83 (H_β–C(1)), 5.17 (H–C(3))
H–C(3)	5.17 (*d*, J = 6.0)	78.0	2.83 (H_β–C(1))
C(1′)		110.2	2.66 (H_α–C(1)), 2.83 (H_β–C(1)), 6.66 (H–C(6′))
C(2′)		154.5	2.83 (H_β–C(1))
C(3′)		149.4	6.58 (H–C(4′))
H–C(4′)	6.58 (*d*, J = 2.0)	108.6	
C(5′)		149.8	6.58 (H–C(4′)), 6.66 (H–C(6′))
H–C(6′)	6.66 (*d*, J = 2.0)	107.6	
C(1″)		134.9	5.17 (H–C(3))
H–C(2″)	7.35 (*d*, J = 8.6)	127.4	5.17 (H–C(3)), 7.09 (H–C(3″))
H–C(3″)	7.09 (*d*, J = 8.6)	121.8	
C(4″)		150.6	7.35 (H–C(2″)), 7.09 (H–C(3″)), 7.09 (H–C(5″)), 7.35 (H–C(6″))
H–C(5″)	7.09 (*d*, J = 8.6)	121.8	
H–C(6″)	7.35 (*d*, J = 8.6)	127.4	5.17 (H–C(3)), 7.09 (H–C(5″))

[a]) Signals obtained by ^{1}H- and ^{13}C-NMR, DEPT, and HMBC experiments.

C(1). The HMBC correlation between H–C(3)/C(1″), C(2″), and C(6″) established the connectivity between C(3) to C(1″).

The NOESY experiment with **3** showed cross-peaks as shown in the *Figure*. The relative configurations at C(2) and C(3) were based on NOESY correlations of H_β–C(1)/H–C(2) and H–C(2)/H–C(3), while H–C(2) and H–C(3) adopted the β-configuration. Further experiments are required to elucidate the absolute configuration of **3**. From the above results, artocarpol J (**2**) was characterized as 6-{[3-(4-acetoxyphenyl)oxiran-2-yl]methyl}benzene-1,2,4-triyl triacetate (**2**).

The anti-inflammatory activities of artocarpol I (**1**) and artocarpol J peracetate (**3**) were studied *in vitro* by measuring the inhibitory effect on the chemical-mediator release from mast cells, neutrophils, macrophages, and microglial cells. Compounds **1** and **3** did not cause a significant inhibition of mast cell and neutrophil degranulation stimulated with compound 48/80 (10 μg/ml) and fMLP (1 μM)/CB (5 μg/ml) (data not shown) [8–10]. Both fMLP (0.3 μM)/CB (5 μg/ml) and PMA (3 nM) stimulate the superoxide anion formation from rat neutrophils. These two stimulants activate the same oxidase, but utilize different transduction mechanisms and are regulated differently in neutrophils [11]. As shown in *Table 3*, compounds **1** and **3** inhibited in a concentration-dependent manner the superoxide anion formation in fMLP/CB- and PMA-stimulated rat neutrophils (IC_{50} = 17.1 ± 0.40 and 20.5 ± 2.60 μM, resp., for the fMLP/CB-induced response) except for **3** induced the superoxide anion formation in PMA-stimulated rat neutrophils. In the determination of nitrogen monoxide (NO) and tumor-necrosis factor-α (TNF-α) formation, the macrophage-like cell line RAW264.7 and the microglia-like cell line N9 were stimulated with lipopolysaccharide (LPS) (1 μg/ml) and LPS (10 ng/ml)/IFN-γ (interferon-γ)(10 units/ml), respectively [12–14]. Compound **1** had no significant effect on NO and TNF-α production from both cell

lines (data not shown). Compound **3** (10 μM) showed slight inhibitory effect on NO production from N9 cells induced by LPS/IFN-γ with a % inhibition of 20.2 ± 0.50, while it had no significant effect on NO production from RAW 264.7 cells and TNF-α production from both cell lines (data not shown). These results indicate that **1** and **3** may attenuate the respiratory burst in neutrophils, and **3** may also suppress the NO production from microglial cells.

Table 3. *Inhibitory Effect of* **1** *and* **3** *on Superoxide-Anion Formation from Rat Neutrophils Stimulated with fMLP* (0.3 μM)/*CB* (5 μg/ml) *and PMA* (3 nM)[a])

Compound ([μM])	Superoxide formation [mmol/10^6 cells/30 min]	
	fMLP/CB	PMA
Control	2.49 ± 0.24	2.75 ± 0.10
1 (3)	2.52 ± 0.33	n.d.[b])
(10)	2.06 ± 0.30	1.63 ± 0.13[c])
(30)	0.74 ± 0.10[d])	1.32 ± 0.19[c])
IC_{50}	17.1 ± 0.40 μM	
3 (3)	1.90 ± 0.21[c])	n.d.[b])
(10)	1.50 ± 0.14[d])	3.20 ± 0.21
(30)	1.09 ± 0.10[d])	3.23 ± 0.15
IC_{50}	20.5 ± 2.60 μM	
Triphcoperazine (1)	n.d.[b])	1.98 ± 0.13
(3)	2.41 ± 0.27	1.21 ± 0.27[d])
(5)	1.85 ± 0.09[c])	n.d.[b])
(10)	0.47 ± 0.22[d])	0.45 ± 0.16[d])
IC_{50}	6.60 ± 0.20 μM	2.70 ± 0.60 μM

[a]) Values are expressed as the means ± s.e.m. ($n = 3$). [b]) n.d.: Not determined. [c]) $P < 0.05$ compared with the corresponding control values. [d]) $P < 0.01$.

Biogenetically, seven novel phenolic compounds containing an oxepin ring, artocarpols A (**5**), C (**6**), D (**9**), E (**4**), F (**7**), G (**8**), and I (**1**) (*Scheme*) seem to be derived from appropriate stilbenes. As shown in the *Scheme*, the cleavage of the pyran ring of **9** yields **10**. Compound **10** is prenylated at C(20) to yield **4**, and it undergoes cyclization between C(9)–OH and C(18), and is oxidized at C(17) to give **1**, and after cyclization and oxidation (between C(9)–OH and C(18), and C(22) and C(23), respectively, to give **8**. The cyclization and oxidation reactions between C(17)–OH and C(22), and C(21) and C(23) of **1**, respectively, give **7**. The cyclization reactions between C(9)–OH and C(18), C(17) and C(22), and C(16) and C(23) of **4** give **5**. The rearrangement between C(9) and C(16), and cyclization between C(9)–OH and C(18), C(21) and C(13), C(12) and C(4), C(22) and C(16), and C(23) and C(10) of **4**, lead to **7**.

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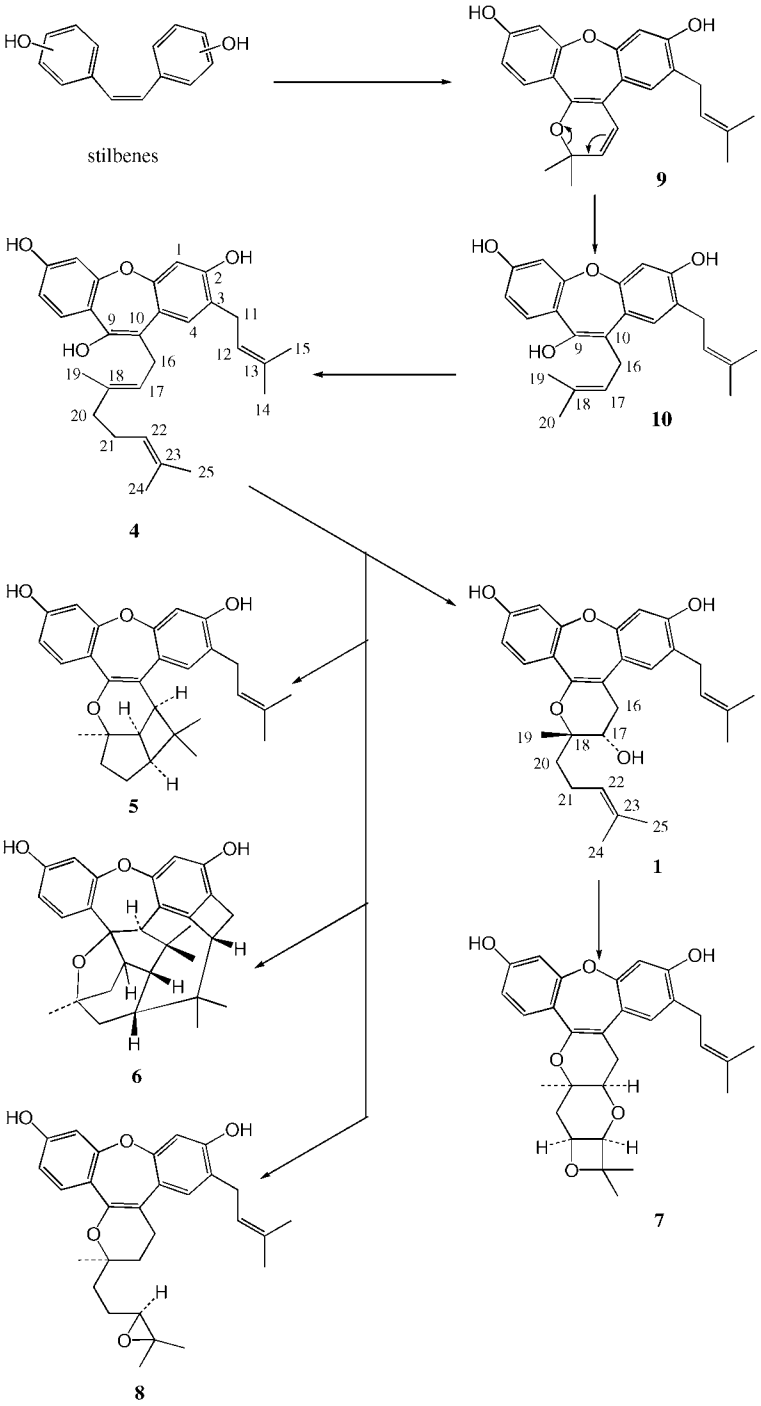
Scheme. *Hypothetical Biogenetic Route to Artocarpols A* (**5**), *C* (**6**), *D* (**9**), *E* (**4**), *F* (**7**), *G* (**8**), *and I* (**1**) *from Stilbenes*



Experimental Part

General. M.p.: uncorrected. UV Spectra: *Jasco* UV-VIS spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi 260-30* spectrometer; ν in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: at 400 and 100 MHz, resp.; *Varian Unity-400* spectrometer. MS: *JMS HX-100* mass spectrometer; *m/z* (rel. %).

Plant Material. Root barks (790 g) of *A. rigida* were collected at Ping-Tung Hsien, Taiwan, in July 1998. A voucher specimen is deposited in the laboratory of medicinal chemistry.

Extraction and Isolation. The root barks (790 g) were chipped and extracted with $CHCl_3$ at r. t. The extract (57 g) was eluted through column chromatography (CC; silica gel) to give **1** (10 mg) with hexane/AcOEt 4:1. Compound **2**, unstable in org. solvents at r.t., was acetylated with excess Ac_2O in anh. pyridine for 8 h. The acetylated product was worked up as usual. The residue was purified by CC (silica gel; cyclohexane/AcOEt 7:1) to give **3** (21 mg).

Artocarpol I (= *3,4-Dihydro-2-methyl-6-(3-methylbut-2-enyl)-2-(4-methylpent-3-enyl)-2*H-*dibenzo[*b,f*]pyrano[2,3-*d*]oxepine-3α,7,11-triol*; **1**). Colorless powder. $[\alpha]_D^{26} = +2$ ($c = 0.1$, $CHCl_3$). UV (MeOH): 295 (4.36). IR (film): 3417, 1625, 1600. ^1H-NMR ($CDCl_3$, 400 MHz; for numbering, see the *Figure*): see *Table 1*. ^{13}C-NMR: see *Table 1*. FAB-MS: 463 (3, $[M+1]^+$), 462 (6) 307 (11), 154 (100), 136 (87), 69 (40). HR-FAB-MS: 463.2485 ($C_{29}H_{34}O_5^+$; calc. 463.2484).

Artocarpol J Tetraacetate (= *6-[[3-(4-Acetoxyphenyl)oxiran-2-yl]methyl]benzene-1,2,4-triyl Triacetate*; **3**). Colorless oil. $[\alpha]_D^{27} = +46$ ($c = 1.0$, $CHCl_3$). UV (MeOH) 275 (3.73), 210 (4.53). IR (film): 1766, 1622, 1600. ^1H-NMR ($CDCl_3$, 400 MHz; for numbering, see *Figure*): see *Table 2*. ^{13}C-NMR: see *Table 2*. FAB-MS: 443 (3, $[M+1]^+$), 442 (3) , 382 (31), 340 (47), 323 (100), 136 (56), 107 (77). HR-FAB-MS: 443.1342 ($C_{23}H_{22}O_9^+$; calc. 443.1341).

Inhibitory assays for chemical mediator induced by various stimulants in mast cells, neutrophils, RAW 364.7 cells, and N9 cells were performed by the methods described in [15].

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