Two Novel and Anti-Inflammatory Constituents of *Artocarpus rigida*

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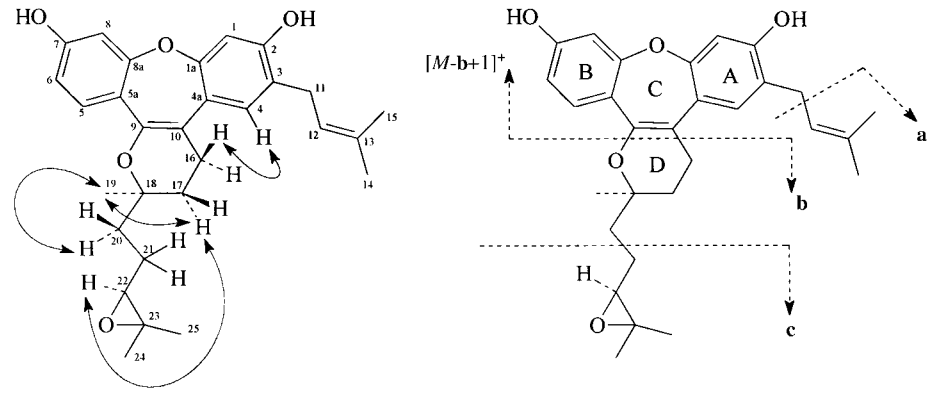
With the scope of our search for biologically active compounds, two new phenolic compounds, artocarpols G (**1**) and H (**2**), and two known compounds, rubraflavone C (**3**) and *trans*-stilbene-2,4,3′,5′-tetrol, were isolated from the root bark of *Artocarpus rigida*. Their structures were determined by spectroscopic methods and comparison with data reported in the literature. Compound **4**, previously isolated from this plant, strongly inhibited in a concentration-dependent manner the release of β-glucuronidase and histamine from mast cell degranulation caused by compound 48/80, with IC_{50} values of 10.9 ± 1.4 and 13.2 ± 0.6 μM, respectively. Compound **4** also showed a concentration-dependent inhibitory effect on the formyl-peptide-stimulated superoxide anion formation in neutrophils with an IC_{50} value of 26.0 ± 5.6 μM.

1. Introduction. – Previously, we reported the isolation of a series of novel structures containing an oxepane ring from the root bark of Formosan *Artocarpus rigida* [1–3]. These compounds were different from those of the same plant grown in Indonesia which were reported by *Hano et al.* [4][5]. Further examination of the $CHCl_3$ and acetone extracts of the root barks resulted in the isolation of two new compounds, named artocarpols G (**1**) and H (**2**), as well as of two known phenolic compounds, rubraflavone C (**3**) and *trans*-stilbene-2,4,3′,5′-tetrol [7] (see *Fig*). For rubraflavone C (**3**), a constituent of *Morus rubra*, no detailed spectral data were given in [6]. A compound, artocarpol A, strongly inhibited superoxide 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]. In the present paper, the structure characterization of **1** and **2**, the assignment of the ^{1}H- and ^{13}C-NMR spectra of **3**, and the anti-inflammatory activity of artocarpol C (**4**) [2] from this plant are reported.

2. Results and Discussion. – The optically active compound **1** obtained as colorless needles, gave a molecular-ion peak at *m*/*z* 462.2410 in the HR-EI-MS corresponding to



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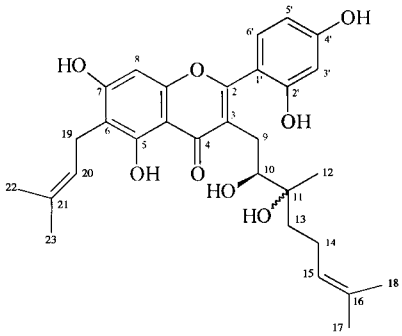

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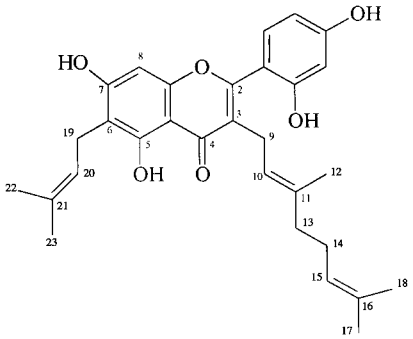
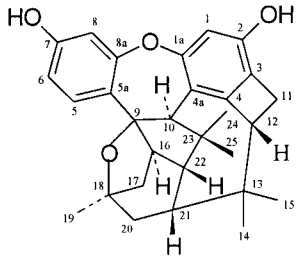


3[l])



4[l])

Figure. *Structures of* **1**–**4**, *and the key NOESY interactions and MS fragmentation pattern of* **1**

the molecular formula $C_{29}H_{34}O_5$. The IR spectrum of **1** showed bands attributable to OH groups (3450 cm^{-1}) and aromatic moieties (1624 and 1600 cm^{-1}). The UV spectrum resembled that of artocarpols A–F [1–3]. The ^{1}H-NMR spectrum of **1** (*Table 1*) showed the presence of one set of *ABX*-type proton signals (σ 6.67,6.93, and 7.37), two *s* of aromatic protons signals (δ 6.47 and 6.71), and a 3,3-dimethylallyl group (δ 1.43, 1.59, 3.03, 3.18, and 5.10). By comparing the chemical shifts of **1** and those of artocarpols A and C–F [1–3], it was found that **1** also contained a 2,7-dihydroxy-3-isopentenyl dibenzo[*b*,*f*]oxepine skeleton (see *Fig.*)

In addition, the signals in the ^{13}C-NMR spectrum and DEPT experiment (δ 54.1 and 73.4) suggested an O-atom participating in an ether linkage, and also revealed the presence of one oxygenated quaternary C-atom (δ 74.3) and three Me, four sp^3 methylene, and one sp^3 methine graps. The proposed structure for artocarpol G (**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 connectivity of CH_2(16) to CH_2(17) and of CH_2(20) to H–C(22) in **1** were revealed by the COSY data[1]). The HMBC correlations Me(24)/C(23), Me(25)/C(23), CH_2(21)/C(23), and Me(24)/C(22) confirmed that the dimethyloxirane moiety was located at C(22). The HMBC correlations between CH_2(20)/C(18), CH_2(20)/C(19), Me(19)/C(18), and CH_2(20)/C(17) established that C(20) and C(17) were connected *via* C(18). H_β–C(16) showed HMBC correlations with C(4a), C(9), C(10), C(17), and C(18) supporting the connection of ring C and ring D by the bonds C(9)–O–C(18) and C(10)–C(16). The NOESY correlations H_α–C(17)/Me(19) and H_α–C(17)/H–C(22) suggested the α-configuration for Me(19) and H–C(22). The EI-MS (see *Fig.*) gave significant fragments at *m*/*z* 444 ($[M-H_2O]^+$), 389 ($[444-\mathbf{a}]^+$), and 361 ($[M-\mathbf{c}-2H]^+$), and 293 ($[M-\mathbf{b}+H]^+$), which also supported the characterization of **1**.

The optically active compound **2**, obtained as yellow needles, gave a molecular-ion peak at *m*/*z* 524.2305 in the HR-EI-MS corresponding to the molecular formula $C_{30}H_{36}O_8$. The IR spectrum of **2** showed the presence of OH groups (3446 cm^{-1}), a conjugated carbonyl group (1660 cm^{-1}), and aromatic moieties (1610, 1590 cm^{-1}). The UV spectrum of **2** resembled that of rubraflavone C (**3**) [6]. The ^{1}H-NMR spectrum of **2** revealed signals similar to those of **3**, except for the presence of a oxymethine signal and the absence of the olefinic-proton signal of the geranyl group (*Table 2*). The ^{13}C-NMR spectrum of **2** indicated also signals similar to those of **3**, except for the presence of an oxygenated tertiary and an oxygenated quaternary C-atom signal and the absence of an olefinic and a quarternary C-atom signal (*Table 2*). On the basis of the spectral evidence, artocarpol H was characterized as **2**. The ^{1}H- and ^{13}C-NMR assignments were confirmed by DEPT experiments and comparison with the corresponding data of **3** (*Table 2*) and reported data [4].

The ^{1}H-NMR spectrum of **2** indicated on oxymethine signal at δ 4.38 (*dd*, J = 9.6, 2.4 Hz, H–C(10)), and methylene signals at δ 2.62 (*dd*, J = 16.8, 9.6 Hz, H_α–C(9)) and 3.74 (*dd*, J = 16.8, 2.4 Hz, H_β–C(9)). The coupling constant of these protons suggested that the relative configuration of the OH group at C(10) adopted the β-configuration. The EI-MS of **2** gave significant fragments at *m*/*z* 522 ($[M-2]^+$), 506 ($[M-H_2O]^+$), 437 ($[506-C_5H_9]^+$), 383 ($[437-C_4H_7+2H]^+$), and 365 ($[383-H_2O]^+$). The assignments of the ^{1}H- and ^{13}C-NMR data of **3** (*Table 2*) were confirmed by DEPT experiments and comparison with reported data [4].

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[1]) Arbitrary numbering; for systematic names, see *Exper. Part*. In [2], the systematic numbering of artocarpol D was erroneous, see systematic name of **1**.

Table 1. *^{1}H- and ^{13}C-NMR Data* (δ in ppm, J in Hz) *of* **1** *in $CDCl_3$.* Arbitrary numbering (see *Fig.*)[a])

	δ(H)	δ(C)	HMBC (^{1}H)
H–C(1)	6.47 (*s*)	105.0	
C(1a)		152.6	
C(2)		154.4	6.47 (H–C(1)), 3.18 (H_β–C(11)), 5.10 (H–C(12)), 6.61 (H–C(4))
C(3)		120.9	6.47 (H–C(1)), 3.18 (H_β–C(11))
H–C(4)	6.61 (*s*)	107.9	
C(4a)		130.0	3.74 (H_β–C(16))
H–C(5)	7.37 (*d*, *J* = 8.4)	121.3	6.67 (H–C(6))
C(5a)		119.7	
H–C(6)	6.67 (*dd*, *J* = 8.4, 2.4)	112.6	6.93 (H–C(8))
C(7)		154.6	6.67 (H–C(6)), 6.93 (H–C(8)), 7.37 (H–C(5))
H–C(8)	6.93 (*d*, *J* = 2.4)	98.3	6.67 (H–C(6))
C(8a)		155.5	
C(9)		156.2	3.74 (H_β–C(16))
C(10)		116.9	3.74 (H_β–C(16))
H_α–C(11)	3.03 (*dd*, *J* = 16.0, 5.6)	27.2	
H_β–C(11)	3.18 (*dd*, *J* = 16.0, 5.6)		
H–C(12)	5.10 (*t*, *J* = 5.6)	122.8	3.18 (H_β–C(11))
C(13)		133.9	1.59 (Me(15))
Me(14)	1.43 (*s*)	26.9	
Me(15)	1.59 (*s*)	17.8	5.10 (H–C(12))
H_α–C(16)	1.23 (*dd*, *J* = 7.6, 3.2)	29.8	1.87 (H_α–C(17)), 1.93 (H_β–C(17))
H_β–C(16)	3.74 (br. *s*)		
H_α–C(17)	1.87 (*dd*, *J* = 12.8, 3.2)	38.9	2.02 (H_β–C(20)), 3.74 (H_β–C(16))
H_β–C(17)	1.93 (*dd*, *J* = 12.8, 2.8)		
C(18)		74.3	2.02 (H_β–C(20)), 3.74 (H_β–C(16))
Me(19)	1.36 (*s*)	28.4	1.51 (H_α–C(20))
H_α–C(20)	1.51 (*m*)	40.3	1.48 (H_α–C(21))
H_β–C(20)	2.02 (*m*)		
H_α–C(21)	1.48 (*m*)	20.9	
H_β–C(21)	1.56 (*dd*, *J* = 16.0, 5.6)		
H–C(22)	1.59 (*s*)	54.1	0.88 (Me(24)), 0.91 (Me(25)), 1.48 (H_α–C(21))
C(23)		73.4	0.88 (Me(24)), 0.91 (Me(25)), 1.56 (H_β–C(21))
Me(24)	0.88 (*s*)	25.7	0.91 (Me(25)),
Me(25)	0.91 (*s*)	29.2	

[a]) Signals obtained by ^{1}H,^{1}H COSY, HMBC, and NOESY techniques.

The anti-inflammatory activity of artocarpol C (**4**) was studied *in vitro* by measuring the inhibitory effect on the chemical-mediator release from mast cells, neutrophils, macrophages, and microglial cells. Compound **4** did not cause a significant inhibition of neutrophil degranulation stimulated with formyl-Met-Leu-Phe (fMLP)(1 μM)/cytochalasin B (CB) (5 μg/ml) (data not shown) [8–10]. However, it strongly inhibited in a concentration-dependent manner the β-glucuronidase and histamine release in compound-48/80-stimulated rat mast cells, with IC_{50} values of 10.9 ± 1.4 and 13.2 ± 0.6 μM, respectively (*Table 3*). Both fMLP (0.3 μM)/CB (5 μg/ml) and phorbol myristate acetate (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 neutrophil [11]. As shown in

Table 2. *^{1}H- and ^{13}C-NMR Data* (δ in ppm, J in Hz) *of* **2** *and* **3**. Arbitrary numbering (see *Fig.*)[a]).

	2		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)
C(2)	160.7		160.7	
C(3)	123.9		122.2	
C(4)	182.5		183.7	
C(4a)	104.7		105.7	
C(5)	157.1		157.7	
C(6)	115.7		111.9	
C(7)	163.2		163.0	
H–C(8)	94.2	6.56 (*s*)	94.2	6.37 (*s*)
C(8a)	161.8		162.0	
CH_2(9)	26.2,	2.62 (*dd*, *J* = 16.8, 9.6)	25.3	3.08 (br. *d*, *J* = 7.2)
		3.47 (*dd*, *J* = 16.8, 2.4)		
H–C(10)	87.5	4.38 (*dd*, *J* = 9.6, 2.4)	123.9	5.09 (*m*)
C(11)	84.1		132.6	
Me(12)	20.9	1.30 (*s*)	16.3	1.40 (*s*)
CH_2(13)	41.2	1.97 (*m*)	41.2	1.93 (*m*)
CH_2(14)	28.1	2.05 (*m*)	28.1	2.03 (*m*)
H–C(15)	125.8	5.05 (*t*, *J* = 7.0)	125.8	5.05 (*m*)
C(16)	136.0		135.9	
Me(17)	18.4	1.45 (*s*)	26.5	1.58 (*s*)
Me(18)	23.1	1.55 (*s*)	18.3	1.76 (*s*)
CH_2(19)	22.7	3.35 (*d*, *J* = 7.0)	22.6	3.35 (br. *d*, *J* = 7.2)
H–C(20)	123.9	5.30 (*t*, *J* = 7.0)	123.4	5.27 (br. *d*, *J* = 7.2)
C(21)	132.3		132.2	
Me(22)	17.0	1.60 (*s*)	26.5	1.52 (*s*)
Me(23)	26.5	1.79 (*s*)	18.3	1.52 (*s*)
C(1′)	117.9		113.7	
C(2′)	159.2		157.8	
H–C(3′)	109.3	6.62 (*d*, *J* = 2.4)	104.5	6.53 (*d*, *J* = 2.4)
C(4′)	163.0		162.7	
H–C(5′)	112.7	6.75 (*dd*, *J* = 8.8, 2.4)	108.7	6.48 (*dd*, *J* = 8.4, 2.4)
H–C(6′)	131.8	7.96 (*d*, *J* = 8.8)	132.9	7.15 (*d*, *J* = 8.4)

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

Table 4, compound **4** inhibited in a concentration-dependent manner the superoxide-anion formation in fMLP/CB- and PMA-stimulated rat neutrophils ($IC_{50} = 26.0 \pm 5.6$ μM for the fMLP-induced response). In the determination of nitrogen monoxide (NO) and tumor-necrosis factor-α (TNF-α) formation, the macrophage-like cell line RAW 264.7 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 **4** had no significant effect on NO and TNF-α production from both cell lines (data not shown). The present study shows that **4** attenuates the respiratory burst in neutrophils and suppresses the release of β-glucuronidase and histamine from mast cells. These results imply that **4** has anti-inflammatory activity.

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Table 3. *Inhibitory Effect of* **4** *on the Release of β-Glucuronidase and Histamine from Rat Peritoneal Mast Cells Stimulated with Compound 48/80* (10 μg/ml)[a])

Compound ([μM])		Release (%)	
		β-glucuronidase	histamine
Control		43.2 ± 1.6	56.7 ± 2.0
4	(3)	28.2 ± 1.6[b])	41.6 ± 2.5[b])
	(10)	21.4 ± 2.1[b])	32.1 ± 0.8[b])
	(30)	7.4 ± 1.6[b])	7.2 ± 0.2[b])
	IC_{50}	10.9 ± 1.4 μM	13.2 ± 0.6 μM
Mepacrine	(10)	29.9 ± 0.6[b])	42.6 ± 2.7[b])
	(30)	18.2 ± 1.1[b])	31.6 ± 3.4[b])
	(100)	5.9 ± 0.8[b])	12.1 ± 1.2[b])
	IC_{50}	32.2 ± 3.6 μM	48.3 ± 3.8 μM

[a]) Values are expressed as the means ± s.e.m. ($n = 3$). [b]) $P < 0.01$ compared with the corresponding control values.

Table 4. *Inhibitory Effect of* **4** *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 [nmol/10^6 cells/30 min]	
		fMPLP/CB	PMA
Control		1.83 ± 0.05	2.81 ± 0.17
4	(10)	1.29 ± 0.04[b])	2.61 ± 0.09
	(20)	1.03 ± 0.08[b])	n.d.[c])
	(30)	0.72 ± 0.14[b])	2.00 ± 0.14[d])
	IC_{50}	26.0 ± 5.6 μM	
Trifluoperazine	(3)	2.50 ± 0.42	2.13 ± 0.04
	(10)	1.09 ± 0.28[b])	0.56 ± 0.08[b])
	(30)	0.11 ± 0.06[b])	0.22 ± 0.09[b])
	IC_{50}	12.9 ± 1.0 μM	9.7 ± 1.1 μM

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

Experimental Part

General. M.p.: uncorrected. UV Spectra: *Jasco-UV-VIS* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi-260-30* spectrometer; 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 (8.5 kg) 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 (0.79 kg) of *A. rigida* were chipped and extracted with $CHCl_3$ and acetone at r.t. The extract (57 g) was subjected to column chromatography (silica gel) to give **1** (10 mg) with C_6H_{12}/Me_2CO 1:1, **2** (100 mg) and **3** (300 mg) with $CHCl_3/Me_2CO$ 7:1 and *trans*-stilbene-2,4,3′,5′-tetrol (70 mg) with $CHCl_3/Me_2CO$ 1:1.

Artocarpol G (= *2-[2-(3,3-Dimethyloxiran-2-yl)ethyl]-3,4-dihydro-6-(3-methylbut-2-enyl)-2*H-*dibenzo-[*b,f*]pyrano[2,3-*d*]oxepin-7,11-diol*, **1**): Colorless needles ($CHCl_3$/MeOH). M.p. 189–190°, $[\alpha]_D^{27} = -41.8$ ($c = 0.1$, $CHCl_3$), UV (MeOH): 215 (3.53), 291 (3.16). IR (KBr): 3450, 1624, 1600. ^1H-NMR ($CDCl_3$, 400 MHz; for numbering, see *Fig.*): *Table 1*. ^{13}C-NMR: *Table 1*. EI-MS (70 eV): 462 (16, M^+), 444 (33), 377 (5), 361 (100), 305 (14), 293 (5), 277 (9). HR-EI-MS: 462.2410 ($C_{29}H_{34}O_5^+$; calc. 462.2406).

Artocarpol H (= *3-(2,3-Dihydroxy-3,7-dimethyloct-6-enyl)-2-(2,4-dihydroxyphenyl)-6-(3-methylbut-2-enyl)-4*H-*1-benzopyran-4-one*; **2**): Yellow needles (acetone). M.p. 199–200°. $[\alpha]_D^{27} = -13.53$ ($c = 0.1$, acetone).

UV (MeOH): 215 (3.53), 291 (3.16). IR (KBr): 3020, 1660, 1615. ^{1}H-NMR ((D$_6$)acetone, 400 MHz; for numbering, see *Fig.*): *Table 1*. ^{13}C-NMR: *Table 1*. EI-MS (70 eV): 524 (1, M^+), 523 (5, $[M-1]^+$), 522 (12, $[M-2]^+$), 506 (28), 463 (14), 419 (23), 407 (1), 383 (52), 323 (19), 203 (6). HR-EI-MS: 524.2385 ($C_{30}H_{36}O_8^+$; calc. 524.2410).

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