Artocarpol A, a Novel Constituent with Potent Anti-inflammatory Effect, Isolated from *Artocarpus rigida*

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A novel phenolic compound, artocarpol A (**1**), was isolated from the root bark of *Artocarpus rigida* and its structure determined by spectroscopic methods and by comparison with its diacetate derivative. Compound **1** strongly inhibited superoxide formation in phorbol 12-myristate 13-acetate (PMA) stimulated rat neutrophils in a concentration-dependent manner with an IC_{50} value of 13.7 ± 0.7 μM. Compound **1** also showed a significant inhibitory effect on tumor necrosis factor-α (TNF-α) formation in lipopolysaccharide(LPS)-stimulated RAW 264.7 cells.

1. Introduction. – Various constituents isolated from the bark of *Artocarpus rigida* (Moraceae) have been reported [1][2]. Within the scope of our search for biologically active compounds from Formosan *Artocarpus* plants, we investigated the constituents of the root bark of *A. rigida*. The root barks of *A. rigida* were collected at Ping-Tung Hsien, Taiwan, R.O.C., during July 1998. The $CHCl_3$ extract was chromatographed (silica gel) and yielded artocarpol A (**1**). In the present paper, the structural characterization, configuration, and anti-inflammatory activity of **1** are reported.

2. Results and Discussion. – Compound **1** was isolated as colorless needles. High-resolution MS revealed a M^+ at m/z 444.2394, which corresponds to the molecular formula $C_{29}H_{32}O_4$. The IR spectrum indicated the presence of OH groups (3399 cm^{-1}) and aromatic-ring moieties (1620 and 1593 cm^{-1}). The ^{1}H-NMR spectrum of **1** (*Table 1*) showed five aromatic proton signals (δ 6.51, 6.55, 6.80, 6.99, and 7.42 ppm) and proton signals of a γ,γ-dimethylallyl group (δ 1.64, 1.70, 3.18, and 5.20 ppm). Analysis of ^{1}H,^{1}H-COSY, HMQC, and HMBC data (*Fig. 1*) of **1** established the partial structures **a** and **b** (see *Fig. 1*). The ^{1}H-NMR spectrum of **1** also showed proton signals of a 'cyclol', *i.e.* partial structure **c** in *Fig. 1* (δ 0.60, 0.86, 1.27, 1.57, 1.62, 1.75, 2.02, 2.31, 2.45, and 3.01) [3], whose structure was confirmed by ^{1}H,^{1}H-COSY, HMQC, and HMBC (*Fig. 1*). The connectivities of partial structures **a**–**c** were deduced from HMBC data, the configurations from NOESY data (*Fig. 2*), and the structure of **1** was finally established by further ^{1}H- and ^{13}C-NMR and MS data of **1** and of its diacetate

derivative. Consequently, artocarpol A (**1**) was characterized as *rel*-(10a*R*,12a-*S*,12b*R*,13a*R*)-10a,11,12a,12b,13,13a-hexahydro-10a,13,13-trimethyl-2-(3-methylbut-2-enyl)-12*H*-5,10-dioxacyclobuta[*cd*]dibenzo[3,4 : 6,7]cyclohepta[1,2-*f*]indene-3,7-diol.

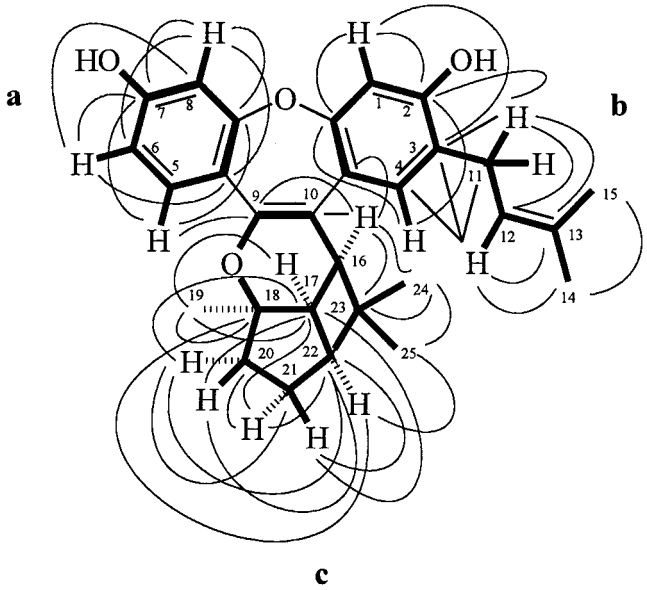

Fig. 1. *Structure of* **1** *and partial structures* **a**–**c** *of* **1**, *with some key HMBC correlations.* Bold lines represent ^{1}H,^{1}H and ^{1}H,^{13}C spin systems identified by ^{1}H,^{1}H-COSY, HMQC, and HMBC data. Arbitrary numbering.

Table 1. *^{1}H- and ^{13}C-NMR Spectra* ($CDCl_3$) *of* **1**[a]. Arbitrary numbering according to *Fig. 1*

	δ(C)	δ(H)		δ(C)	δ(H)
H–C(1)	107.7	6.55 (*s*)	H–C(12)	122.6	5.20 (*t*, *J* = 6.8)
C(1a)	155.4		C(13)	134.1	
C(2)	153.7		Me(14)	17.8	1.64 (*s*)
C(3)	120.5		Me(15)	25.7	1.70 (*s*)
H–C(4)	106.5	6.51 (*s*)	H–C(16)	38.3	3.01 (*d*, *J* = 9.6)
C(4a)	130.7		H–C(17)	40.6	2.45 (*t*, *J* = 9.6)
H–C(5)	120.9	7.42 (*d*, *J* = 8.4)	C(18)	84.2	
C(5a)	122.3		Me(19)	25.3	1.27 (*s*)
H–C(6)	111.8	6.80 (*dd*, *J* = 8.4, 2.4)	CH_2(20)	25.1	1.62 (*m*), 1.75 (*m*)
C(7)	153.2[b])		CH_2(21)	41.0	1.57 (*m*), 2.02 (*m*)
H–C(8)	98.3	6.99 (*d*, *J* = 2.4)	H–C(22)	46.6	2.31 (*dt*, *J* = 8.8, 4.4)
C(8a)	153.1[b])		C(23)	40.1	
C(9)	153.3[b])		Me(24)	19.0	0.60 (*s*)
C(10)	119.7		Me(25)	33.3	0.86 (*s*)
CH_2(11)	27.4	3.18 (*d*, *J* = 6.8)			

[a]) All assignments were confirmed by HMQC, HMBC, and NOESY data. Chemical shifts δ in ppm and coupling constants *J* in Hz. [b]) Attributes may be reversed.

In structure **1** (*Fig. 1*), the HMBC of H–C(5) to C(9) confirmed the connectivity of partial structure **a** to partial structure **c**, and the HMBC of H–C(16) to C(4a) confirmed that the partial structure **c** was linked to partial structure **b** by C(10)–C(4a). In the NOESY experiment with **1**, the correlations between Me(19)/H–C(17)/H_α–C(20), H_α–C(20)/H_α–C(21)/H–C(22), H–C(22)/H–C(16)/H–C(17)/H_α–C(20), and H–C(16)/H–C(17) (*Fig. 2*) suggested the α-configuration for H–C(16), H–C(17), Me(19), and H–C(22). These results and the molecular model of **1** suggested a *trans* arrangement of Me(19) and the geminal Me groups at C(23), and the latter two (δ 0.60 and 0.86) experienced a positive aryl-shielding contribution [3]. The ^{1}H-NMR spectrum of the diacetate obtained from **1** showed two acetyl signals at δ 2.56 (*s*) and 2.34 (*s*) and five aromatic-proton signals at δ 6.61 (*s*), 6.75 (*s*), 7.01 (*dd*, $J = 8.4$, 2.4 Hz), 7.27 (*d*, $J = 2.4$ Hz) and 7.55 (*d*, $J = 8.4$ Hz). By comparing the aromatic-proton chemical shifts of **1** (*Table 1*) and of its diacetate, it was found that the signal at δ 6.55, 6.80, and 6.99 of **1** experienced a significant downfield shift of $\Delta\delta = +0.20$, $+0.21$, and $+0.27$ ppm, respectively, upon acetylation, whereas the signal at δ 6.51 and 7.42 of **1** showed only a slight downfield shift of $\Delta\delta = +0.10$ and 0.13 ppm, respectively. In addition to the acetylation-induced shifts [4], **1** showed a negative *Gibb's* test and significant NOESY interactions between a phenol signal at δ 4.88 (*s*, 1 H, exchange with D_2O) and H–C(12), Me(14), and Me(15), thus establishing an ether linkage between C(1a) and C(8a) of partial structures **a** and **b**, respectively (*Fig. 1*).

The ^{13}C-NMR spectrum of **1** (*Table 1*) supported the structure assignment. The base peak at *m*/*z* 361 in the MS of **1** was attributed to the fragments $[429 - b - H]^+$ (*Fig. 2*). This and characteristic peaks at *m*/*z* 429 ($[M - a]^+$), 305 ($[429 - CO - 3\,H]^+$), 235 ($[305 - b - H]^+$), and 223 ($[361 - c - H]^+$) (*Fig. 2*) also confirmed the structure assignment of **1**.

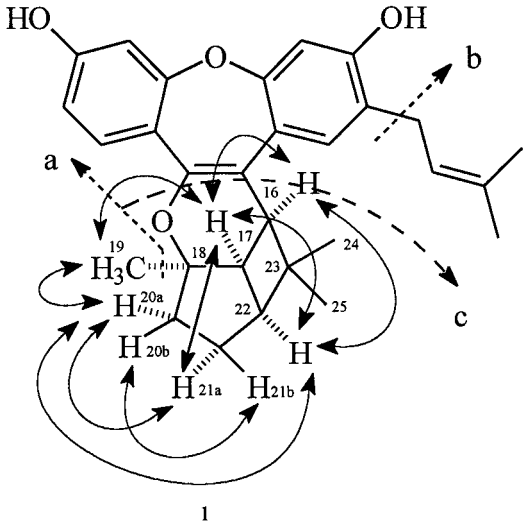

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Fig. 2. *Some key NOESY interactions and EI-MS fragmentation patterns of* **1**

The anti-inflammatory activities of **1** were studied *in vitro* by measuring the inhibitory effects on the chemical mediators released from mast cells, neutrophils, macrophages, and microglial cells. Compound **1** did not cause significant inhibition of mast-cell degranulation stimulated with compound 48/80 and neutrophil degranulation stimulated with formyl-Met-Leu-Phe (fMLP) (1 μM)/cytochalasin B (CB) (5 μg/ml) (data not shown) [5–7]. fMLP (0.3 μM)/CB (5 μg/ml) or PMA (3 nM) also induced the superoxide-anion formation from rat neutrophils. As shown in *Table 2*, **1** strongly inhibited superoxide anion formation in phorbol 12-myristate 13-acetate (PMA) stimulated rat neutrophils in a concentration-dependent manner with an IC_{50} value of 13.7 ± 0.7 μM, while it did not significantly inhibit the superoxide-anion formation from

rat neutrophils stimulated with fMLP/CB (data not shown). The data indicate that fMLP/CB and PMA induce the superoxide-anion formation from rat neutrophils, but that they utilize different transduction mechanisms and are regulated differently [8][9].

Table 2. *The Inhibitory Effect of* **1** *on Superoxide-Anion Formation from Rat Neutrophils Stimulated with PMA* [a])

Compound	Superoxide formation	
	x mol/10^6 cells/30 min	% inhibition
Control	3.14 ± 0.12	
1 1 μM	4.38 ± 0.74	-37.3 ± 3.0
3 μM	3.55 ± 0.68	-10.6 ± 7.8
10 μM	0.57 ± 0.03 [b])	80.7 ± 3.9
30 μM	0.35 ± 0.09 [b])	88.6 ± 3.2
Trifluoperazine 1 μM	2.55 ± 0.23	16.6 ± 0.1
3 μM	1.59 ± 0.43 [b])	49.5 ± 7.4
10 μM	0.39 ± 0.19 [b])	87.1 ± 4.9

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

Following the activation of mouse macrophage-like cell line RAW 264.7 and murine microglial cell line N9, NO and TNF-α were generated in response to LPS and LPS/interferon-γ (IFN-γ), respectively [10–12]. Compound **1** did not cause significant inhibitory effects on NO accumulation from RAW 264.7 and N9 cells in response to LPS (1 μg/ml) and LPS (10 ng/ml)/IFN-γ (10 unit/ml) (data not shown), respectively [13]. Compound **1** (3 μM) caused potent inhibitory effects on the product of TNF-α from RAW 264.7 cells induced by LPS (1 μg/ml) with a % inhibition of 40.1 ± 2.0, while it (1 μM) showed a slight inhibitory effect on the production of TNF-α from N9 cells induced by LPS (10 ng/ml)/IFN-γ (10 unit/ml) with a % inhibition of 15.7 ± 1.5. These results indicate that **1** may attenuate the respiratory burst in neutrophils and suppress the TNF-α formation from macrophages.

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Experimental Part

General. M.p.: uncorrected. UV Spectra: *Jasco-UV-VIS* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi 260-30* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Varian-Unity-400* spectrometer; 400 and 100 MHz, resp.; δ in ppm, J in Hz. 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 of *A. rigida* were chipped and extracted with $CHCl_3$ at r.t. The extract was subjected to column chromatography (silica gel, cyclohexane/CH_2Cl_2/AcOEt 6:3:1): **1** (150 mg). Colorless needles. M.p. 181–182°. $[\alpha]_D^{25} = -8$ ($c = 0.1$, $CHCl_3$). UV (MeOH): 213 (4.45), 297 (4.15), 403 (2.87). IR (KBr): 3399, 1620, 1593. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS (70 eV; see *Fig. 2*): 444 (20, M^+), 429 (6), 361 (100), 317 (5), 305 (15), 277 (9).

Diacetate of **1**. Amorphous powder. $[\alpha]_D^{25} = -6$ ($c = 0.1$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz; for numbering, see *Fig. 1*): 0.62 (*s*, Me(24)); 0.88 (*s*, Me(25)); 1.28 (*s*, Me(19)); 1.42 (*s*, Me(14)); 1.57 (*s*, Me(15)); 1.60 (*m*, 1 H–C(21)); 1.66 (*m*, 1 H–C(20)); 1.76 (*m*, 1 H–C(20)); 2.03 (*m*, 1 H–C(21)); 2.35 (*m*, H–C(22)); 2.46 (*t*, $J = 9.2$, H–C(17)); 2.26, 2.34 (2 *s*, 2 Ac); 3.01 (*d*, $J = 9.2$, H–C(16)); 3.08 (*d*, $J = 4.8$, 1 H–C(11)); 4.97

(*t*, *J* = 4.8, H–C(12)); 6.61 (*s*, H–C(4)); 6.75 (*s*, H–C(1)); 7.01 (*dd*, *J* = 8.4, 2.4, H–C(6)); 7.27 (*d*, *J* = 2.4, H–C(8)); 7.55 (*d*, *J* = 8.4, H–C(5)).

Superoxide-Anion Formation. Superoxide-anion formation was measured in terms of superoxide dismutase inhibitable cytochrome *c* reduction [14]. Neutrophil suspension was preincubated at 37° with 0.5% DMSO or drugs for 3 min, and then superoxide dismutase of HBSS was added to the blank and test tubes, respectively. The reaction was initiated by challenge with fMLP (0.3 μM)/CB (5 μg/ml) of PMA (3 nM). After 30 min, the reaction was terminated by centrifugation and the absorbance change of supernatants was monitored at 550 nm in a microplate reader. The final concentration of DMSO was fixed at 0.5%.

Macrophage Cultures and Drugs Treatment. RAW 264.7 mouse macrophage-like cell line (American Type Culture Collection) was plated in 96-well tissue-culture plates in *Dulbecco*'s modified eagle medium supplemented with 5% fetal calf serum (FCS), 100 unit/ml of penicillin and streptomycin at $2 \cdot 10^5$ cells/200 μl per well. Cells were allowed to adhere overnight. Pretreatment of cells with test drugs was done at 37° for 1 h before stimulation with 1 μg/ml of LPS (*Escherichia coli*, serotype 0111 : B4) for 24 h, and then the medium was collected and stored at −70 °C until used. The final concentration of DMSO was fixed at 0.5%.

Microglial Cell Cultures and Drug Treatment. Murine microglial cell lines N9 [15] (kindly provided by Dr. *P. Ricciardi-Castagnoli*, CNR, Cellular and Molecular Pharmacology Center, Italy) was plated in 96-well tissue-culture plates in *Iscove*'s modified *Dulbecco*'s medium containing 2% heat-inactivated FCS and antibiotics at $8 \cdot 10^4$ cells/200 μl per well. Pretreatment of cells with test drugs was done at 37° for 1 h before stimulation with LPS (10 ng/ml/IFN-γ (10 unit/ml)) for 24 h, and then the medium was collected and stored at −70° until used. The final concentration of DMSO was fixed at 0.5%.

TNF-α Determination. TNF-α in medium was measured by an *EIA* kit according to the procedure described by the manufacturers.

Statistical Analysis. Data are presented as the means ± s.e.m. Statistical analyses were performed with the least-significant-difference test method after analysis of variance. *P* Values < 0.05 were considered to be significant. Analysis of the regression line was used to calculate IC_{50} values.

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