Potential Anti-Inflammatory Activities of Bractelactone and other Compounds Isolated from *Fissistigma bracteolatum*

by **Yu-Hsuan Lan**[a]), **Yi-Chen Chia**[b]), **Fang-Rong Chang**[a]), **Tsong-Long Hwang**[c]), **Chih-Chaung Liaw**[a]), and **Yang-Chang Wu***[a])

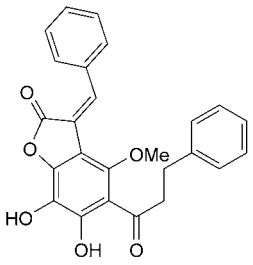
[a]) Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan (phone: +886-7-3121101 ext. 2197; fax: +886-7-3114773; e-mail: yachwu@kmu.edu.tw)
[b]) Department of Food Science & Technology, Tajen Institute of Technology, Ping Tung Hsien 907, Taiwan
[c]) Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan

From the stems of *Fissistigma bracteolatum*, a novel natural product with an unprecedented skeleton, bractelactone (**1**), was isolated, together with four known compounds: piperolactam A (**2**), aristololactam BIII (**3**), aristololactam BII (**4**), and fissilandione (**5**). The structure of **1** was established on the basis of spectroscopic data as (3*Z*)-6,7-dihydroxy-4-methoxy-3-(phenylmethylidene)-5-(3-phenylpropanoyl)-1-benzofuran-2(3*H*)-one. This compound may be derived from a hybrid of a chalcone and a cinnamic acid, or from a degradation product of a dichalcone. Compounds **1**, **2**, and **5** showed inhibitory effects on NO generation by RAW264.7 macrophages in response to lipopolysaccharide. Compounds **2** and **5** showed inhibitory effects on formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)-induced superoxide anion ($O_2^{\cdot-}$) generation in human neutrophils.

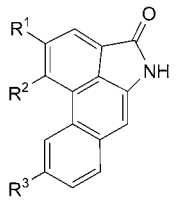
Introduction. – In our previous studies, alkaloids, furano-fissohamione, cyclopentenones, and flavonoids have been reported from *Fissistigma* plants [1–8]. *Fissistigma bracteolatum* CHATT. (Annonaceae), a climbing shrub, grows mainly in the southern part of China and Vietnam. In traditional medicine, this plant is used for curing broken bones and to stop bleeding [9]. This paper describes the structural elucidation and biological activity of a novel compound named bractelactone (**1**), isolated from the stems of *F. bracteolatum*, together with four known compounds: piperolactam A (**2**) [10], aristololactam BIII (**3**) [11], aristololactam BII (**4**) [11], and fissilandione (**5**) [6]. Compounds **1**–**5** were investigated for their effects of formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)-induced superoxide anion ($O_2^{\cdot-}$) generation in human neutrophils, as well as the nitric oxide (NO) generation by RAW264.7 macrophages in response to lipopolysaccharide (LPS).

Results and Discussion. – Compound **1**, obtained as yellow needles, displayed UV absorptions at 294 and 228 nm. The molecular formula, $C_{25}H_{20}O_6$, was confirmed by HR-FAB-MS (*m*/*z* 416.1252; calc. 416.1260). The presence of OH and C=O groups was indicated from IR absorptions at 3492, and 1776 and 1640 cm^{-1}, respectively. From the ^{1}H-NMR spectrum of **1** (*Table 1*), a dihydrochalcone moiety comprising a 2′-OH group[1]) on ring *A* was identified, with signals for a mono-substituted aromatic ring at δ(H) 7.30–7.43 (3 H) and 7.22 (2 H), α- and β-CH_2 signals at δ(H) 3.41 and 3.05 (2*t*,

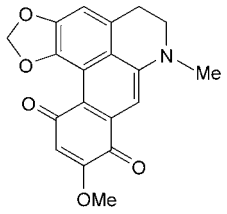
[1]) Arbitrary atom numbering, see *Fig. 1*.



1



2 R^1 = MeO, R^2 = OH, R^3 = H
3 R^1 = R^2 = R^3 = MeO
4 R^1 = R^2 = MeO, R^3 = H



5

$J = 7.6$ Hz each), and a H-bonded phenolic signal at δ(H) 13.50, which was oriented towards a C=O group. As shown in *Fig. 1,a*, these assignments were confirmed by HMBC correlations from δ(H) 13.50 to δ(C) 152.3 (C(2′)), 101.8 (C(1′)), and 132.1 (C(3′)); from δ(H) 3.05 to δ(C) 203.6 (C(β')), 140.6 (C(1)), and 128.5 (C(2)); and from δ(H) 3.41 to δ(C) 203.6 (C(β')) and 140.6 (C(1)). The NOESY correlation between δ(H) 5.50 (OH) and 13.50 (2′-OH) indicated an additional 3′-OH group. A MeO signal at δ(H) 4.26 showed correlations with the aromatic signals of ring *B* and the β-CH_2 moiety, but no correlation with the 3′-OH group. Thus, the MeO group had to be attached at C(6′).

Table 1. *^{1}H- and ^{13}C-NMR Data of Compound* **1**. At 400/100 MHz, in $CDCl_3$; δ in ppm, *J* in Hz. Assignments based on ^{1}H,^{1}H-COSY, NOESY, DEPT, HMQC, and HMBC experiments.

Position[1])	^{1}H	^{13}C	Position[1])	^{1}H	^{13}C
C(1)	–	140.6	6′-MeO	4.27 (*s*)	60.6
H–C(2,6)	7.30–7.43 (*m*)	128.5	2′-OH	13.51 (*s*)	–
H–C(3,5)	7.30–7.43 (*m*)	128.5	3′-OH	5.50 (*s*)	–
H–C(4)	7.22 (*m*)	126.2	C(1″)	–	164.8
$CH_2(\alpha)$	3.41 (*t*, *J* = 7.2)	44.4	C(2″)	–	119.1
$CH_2(\beta)$	3.05 (*t*, *J* = 7.2)	30.0	H–C(3″)	8.13 (*s*)	142.7
C(β')	–	203.6	C(4″)	–	133.7
C(1′)	–	101.8	H–C(5″,9″)	8.00–8.02 (*m*)	131.5
C(2′)	–	152.3	H–C(6″,8″)	7.43–7.45 (*m*)	130.7
C(3′)	–	132.1	H–C(7″)	7.43–7.45 (*m*)	128.3
C(4′)	–	146.5			
C(5′)	–	107.6			
C(6′)	–	147.3			

The remaining ^{1}H- and ^{13}C-NMR signals of **1** (δ(H) 8.00–8.02 (2 H), 7.43–7.45 (3 H), and 8.13 (1 H); δ(C) 119.1, 128.3, 130.7 (2C), 131.5 (2C), 133.7, 142.7, and 164) indicated a mono-substituted aromatic ring, a trisubstituted C=C bond, and a conjugated C=O system. HMBC correlations from δ(H) 8.13 (H–C(3″)) to δ(C) 133.7 (C(4″)), 164.8 (C(1″)), and 107.6 (C(5′)) confirmed the linkage between C(2″) and C(5′). The resonance at δ(C) 164.8 (C(1″)) indicated a lactone ring with an

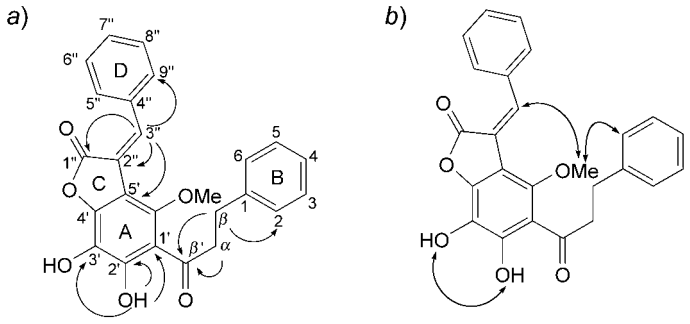


Fig. 1. *Key HMBC* (a) *and NOESY* (b) *correlations for* **1**

O–C(4′) linkage. These assignments were further confirmed by analysis of the MS fragmentation pattern (*Fig. 2*).

NOESY Correlations observed between the 6′-MeO group and H–C(3″) indicated the C=C bond in **1** to be (*Z*)-configured (*Fig. 1,b*). The same conclusion was drawn from the ^{13}C-NMR chemical shift of C(3″). On the basis of the previous reports [12][13], the chemical shift of the exocyclic methine C-atom of (*E*)-isoaurone is *ca.* 152 ppm, whereas that of (*Z*)-isoaurone is *ca.* 141 ppm. Hence, the observed δ(C) value of 142.7 for C(3″) of **1** was consistent with a (*Z*)-configured olefin. From these data, the structure of compound **1** was determined as (3*Z*)-6,7-dihydroxy-4-methoxy-3-(phenylmethylidene)-5-(3-phenylpropanoyl)-1-benzofuran-2(3*H*)-one, and named *bractelactone.*

Bractelactone (**1**) represents an unprecedented natural product, with a hexasubstituted aromatic ring. Probably, **1** is formed as a hybrid of a chalcone and a cinnamic acid, or by degradation of a dichalcone. In general, 3,8-linked diflavonoids represent a common skeleton in the classes of diflavonoids [14]. It suggests that a 5,α-linked dichalcone may also be a reasonable precursor of **1**.

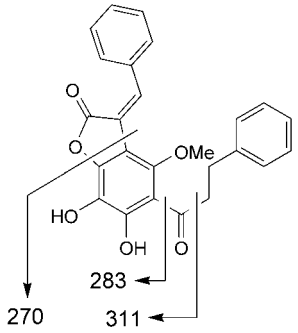


Fig. 2. *Assignment of EI-MS fragments for* **1**. In *m/z*.

According to our previous studies, piperolactam A (**2**) shows significant inhibitory effects against arachidonic acid and collagen [10]. To investigate the biological activities of compounds **1**–**5** from *F. bracteolatum*, and to rationalize the folk-medicinal

use of this plant, all compounds were screened for inhibition of $O_2^{\cdot-}$ release from human neutrophils. The effects on NO generation by RAW264.7 macrophages in response to LPS were also examined.

Our screening showed that compounds **2** and **5** significantly inhibit the fMLP-induced $O_2^{\cdot-}$ generation (*Table 2*). Compounds **1**, **2**, and **5** showed inhibitory effects on NO generation by RAW264.7 macrophages in response to LPS. However, compound **2** showed a cytotoxic effect towards RAW264.7 macrophages. These results, thus, support the beneficial folk-therapeutic use of this interesting plant.

Table 2. *Inhibitory Effects of Compounds* **1**–**5** *on* a) *Superoxide Anion* ($O_2^{\cdot-}$) *Generation by Human Neutrophils in Response to formyl-MetLeuPhe* (fMLP) *and* b) *Nitric Oxide Generation by RAW264.7 Macrophages in Response to Lipopolysaccharide* (LPS). For details, see *Exper. Part*. 'Diphenylene-iodonium' (DPI), an NADPH oxidase inhibitor, and N^{ω}-nitro-L-arginine methyl ester (L-NAME) were used as positive controls, resp. All values were averaged ($n = 3-4$).

Compound	IC_{50} [μg/ml][a])		Cytotoxic[b])
	fMLP/CB	LPS	
1	n.t.[c])	1.55 ± 0.42	no
2	4.68 ± 0.75	2.47 ± 0.76	yes
3	> 10	> 10	no
4	> 10	> 10	no
5	2.34 ± 0.09	0.85 ± 0.30	no
DPI	0.13 ± 0.06	–	–
L-NAME	–	47.31 ± 0.99	no

[a]) Concentration required for 50% inhibition. [b]) Examined by LDH release at a level of 10 μg/ml. [c]) Not tested. Compound **1** caused reduction of cytochrome *c* in a cell-free system.

Experimental Part

General. Column chromatography (CC): silica gel *60* (230–400 mesh; *Merck*). Thin-layer chromatography (TLC): silica gel GF_{254} precoated plates; detection by spraying with 50% H_2SO_4, followed by heating on a hot plate. Melting points (m.p.): *Yanagimoto* micro-melting-point apparatus; uncorrected. IR Spectra: *Mattson Genesis II* spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra (including HMQC, HMBC, ^1H,^1H-COSY, DEPT, and NOESY): *Varian Unity Plus* spectrometer; at 400/100 MHz, resp., in $CDCl_3$, δ in ppm, J in Hz. EI-MS: *JEOL JMS-SX/SX 102A* mass spectrometer; in m/z. High-resolution (HR) FAB-MS: *MAT-95XL* mass spectrometer; in m/z.

Plant Material. Fresh stems of *F. bracteolatum* were collected in Kunming, Yunnan, P. R. China. A voucher specimen (FB 1) was deposited at the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. The MeOH extract (48 g) of *F. bracteolatum* stem was subjected to CC (SiO_2; hexane/$CHCl_3$/AcOEt/MeOH gradient of increasing polarity): 15 fractions (*Fr.*) on the basis of TLC. *Fr. 6*, eluted with hexane/AcOEt 5:1, afforded aristololactam BII (**4**; 1.8 mg) and fissilandione (**5**; 3.5 mg). *Fr. 7*, eluted with $CHCl_3$, gave bractelactone (**1**; 42 mg). *Fr. 8*, eluted with hexane/AcOEt 3:1, provided aristololactam BIII (**3**; 4.2 mg); and *Fr. 10*, eluted with $CHCl_3$, gave piperlactam A (**2**; 2.4 mg).

Neutrophil Superoxide Anion Generation. Human neutrophils from venous blood of healthy, adult volunteers (18–32 years old) were isolated by means of standard dextran sedimentation prior to centrifugation in *Ficoll–Hypaque* gradient and hypotonic lysis of erythrocytes [15]. The generation of $O_2^{\cdot-}$ was determined with superoxide dismutase (SOD), which inhibits cytochrome *c* reduction. Cells were activated by formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) for 10 min. When fMLP was used as stimulant, cytochalasin B (CB; 1 μg/ml) was incubated for 3 min before peptide activation.

Macrophage Nitric Oxide Generation. RAW264.7 Cells were obtained from *American Type Culture Collection* (Manassas, VA). Cells were seeded at a density of 10^6 cells/ml in 24-well *Costar* plates, and cultured in *Dulbecco*'s modified *Eagle* medium containing 2% heat-inactivated fetal calf serum (FCS) [16]. The cells were stimulated with lipopolysaccharide (LPS; 1 μg/ml) for 24 h. Measurement of NO was performed *via* an assay based on the accumulation of nitrite in the medium, as determined by colorimetry with *Griess* reagent [17], N^{ω}-nitro-L-arginine methyl ester (L-NAME) being used as positive control.

Lactate Dehydrogenase (LDH) *Release.* Cytotoxicity was expressed as the percent LDH activity obtained in cell-free medium compared to the total LDH activity. The latter was determined by lysing cells with 0.1% *Triton X-100* for 30 min at 37°.

Bractelactone (*=(3Z)-6,7-Dihydroxy-4-methoxy-3-(phenylmethylidene)-5-(3-phenylpropanoyl)-1-benzofuran-2(3*H*)-one*; **1**). Yellow needles. M.p. 132–134°. IR (KBr): 3492, 3026, 2938, 2217, 1776, 1640, 1600, 1424, 1348, 1268, 1174, 1008, 957. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS (70 eV): 416 (97, M^+), 398 (10, [$M - H_2O$]$^+$), 325 (5), 311 (10), 283 (23), 105 (37), 91 (100). HR-FAB-MS: 416.1252 (M^+, $C_{25}H_{20}O_6^+$; calc. 416.1260).

This work was supported by a grant from the *National Science Council of the Republic of China*.

REFERENCES

[1] S.-T. Lu, Y.-C. Wu, *Heterocycles* **1983**, *20*, 813.
[2] S.-T. Lu, Y.-C. Wu, S.-P. Leou, *Phytochemistry* **1985**, *24*, 1829.
[3] Y.-C. Wu, S.-T. Lu, T.-S. Wu, K.-H. Lee, *Heterocycles* **1987**, *26*, 9.
[4] Y.-C. Wu, S.-C. Kao, J.-F. Huang, C.-Y. Duh, S.-T. Lu, *Phytochemistry* **1990**, *29*, 2387.
[5] Y.-C. Chia, F.-R. Chang, C.-M. Li, Y.-C. Wu, *Phytochemistry* **1998**, *48*, 367.
[6] Y.-C. Chia, F.-R. Chang, Y.-C. Wu, *J. Nat. Prod.* **1998**, *61*, 1430.
[7] Y.-C. Chia, F.-R. Chang, Y.-C. Wu, *Tetrahedron Lett.* **1999**, *40*, 7513.
[8] Y.-C. Chia, J.-B. Wu, Y.-C. Wu, *Tetrahedron Lett.* **2000**, *41*, 2199.
[9] T.-P. Lien, A. Porzel, J. Schmidt, T.-V. Sung, G. Adam, *Phytochemistry* **2000**, *53*, 991.
[10] Y.-C. Chia, F.-R. Chang, C.-M. Teng, Y.-C. Wu, *J. Nat. Prod.* **2000**, *63*, 1160.
[11] S. J. Desai, B. R. Prabhu, N. B. Mulchandani, *Phytochemistry* **1988**, *27*, 1511.
[12] K. Suzuki, S. Yahara, K. Maehata, M. Uyeda, *J. Nat. Prod.* **2001**, *64*, 204.
[13] P. K. Agrawal, 'Carbon-13 NMR of Flavonoids', Elsevier, New York, 1989, p. 243–250.
[14] Y.-M. Lin, M. T. Flavin, C. S. Cassidy, A. Mar, F.-C. Chen, *BioMed. Chem. Lett.* **2001**, *11*, 2101.
[15] T.-L. Hwang, H.-W. Hung, S.-H. Kao, C.-M. Teng, C.-C. Wu, S. J.-S. Cheng, *Mol. Pharmacol.* **2003**, *64*, 1419.
[16] T.-L. Hwang, C.-C. Wu, J.-H. Guh, C.-M. Teng, *Biochem. Pharmacol.* **2003**, *66*, 149.
[17] L. C. Green, D. A. Wagner, J. Glogowski, P. L. Skipper, J. S. Wishnok, S. R. Tannenbaum, *Anal. Biochem.* **1982**, *126*, 131.

Received January 18, 2005