Cytotoxic and Antiplatelet Aggregation Principles from *Aglaia elliptifolia*

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Two related 1*H*-2,3,3a,8b-tetrahydrocyclopenta[*b*]benzofurans, aglafolin (**1a**) and rocaglamide (**2**), isolated from the stems of *Aglaia elliptifolia*, showed significant cytotoxicity in six cancer cell lines. Aglafolin (**1a**) was also found to completely block platelet aggregation caused by arachidonic acid and platelet-activating factor at 100 μM and 2 ng/mL, respectively.

Previously, King *et al.* reported the isolation and structural characterization of rocaglamide (**2**), a novel 1*H*-2,3,3a,8b-tetrahydrocyclopenta[*b*]benzofuran, from an alcoholic extract of the dried roots and stems of *Aglaia elliptifolia* Merr. (Meliaceae).[1] This compound showed an optimal T/C value of ca. 156% at a dosage of 1.0 mg/kg against the P-388 murine lymphocytic leukemia *in vivo* test system.[1] In 1992, our investigation of a MeOH extract of the stems of this same plant resulted in the isolation of a related compound, aglafolin (**1a**), where the dimethylamino group of rocaglamide (**2**) is replaced by a methoxy group.[2] Aglafolin (**1a**) was described by us as a selective and effective inhibitor of platelet aggregation induced by platelet-activating factor (PAF) both *in vitro* and *in vivo*.[2] The isolation of this compound from a related species, *Aglaia odorata*, and its insecticidal activity was described by Ishibashi *et al.*;[3] however, the compound was named as methyl rocaglate in their paper. Herein, we describe the structural confirmation of aglafolin by NMR spectroscopy, including 2D methods, and by chemical transformation to rocaglamide. The antiplatelet aggregation activity and *in vitro* cytotoxicity of these two compounds are also reported.

The stems (2.7 kg) of *A. elliptifolia* were extracted four times with MeOH, and the MeOH extract (189.2 g) was partitioned between $CHCl_3$ and H_2O. Repeated column chromatography of the $CHCl_3$ extract (108.1 g) on Si gel gave a mixture (2.62 g) of the phytosterols β-sitosterol and stigmasterol, aglafolin (**1a**, 580 mg), scopoletin (25 mg), and rocaglamide (**2**, 135 mg). Like aglafolin (**1a**), scopoletin and rocaglamide (**2**) were both previously isolated from *A. odorata*; however, these latter two compounds were obtained as a mixture.[4]

The chemical conversion of **1a** to **2** was accomplished in two steps as shown in Scheme 1. First, saponification of **1a** provided the carboxylic acid **1b**; second, amidation with dimethylamine gave **2**.

The ^{1}H-NMR spectra of **1a** and **2** were similar to those reported in the literature[3,4] and to each other, except for the absence of the two *N*-Me singlets and presence of an OMe singlet in the former. The ^{13}C-NMR spectra (Table 1) of **1a**, **1b**, and **2** were also similar to each other, except for the absence and/or presence of the amide and ester methyl groups, and are reported here in $CDCl_3$ (the spectra of **1a** and **2** were previously reported in acetone-d_6).[3] The ^{1}H- and ^{13}C-NMR spectra of **1a**, **1b**, and **2** were completely assigned by 2D-NMR techniques including ^{1}H- and ^{13}C-NMR long-range correlations and NOESY correlations.

Because aglafolin (**1a**) has been previously identified as a PAF-antagonist, scopoletin and compounds **1a** and **2** were tested for inhibition of platelet aggregation induced by adenosine diphosphate (ADP), arachidonic acid (AA), PAF, and collagen. As shown in Table 2, at 100 μg/mL aglafolin (**1a**) completely blocked the platelet aggregation caused by PAF and AA but had no effect on that caused by ADP or collagen. Scopoletin and rocaglamide (**2**) were either completely inactive or showed only slight inhibition at the doses tested.

Also, because rocaglamide (**2**) has been previously reported to show antileukemic activity *in vitro*, compounds **1a** and **2** were tested against the KB (nasal pharyngeal carcinoma), A-549 (human lung carcinoma), HCT-8 (human colon carcinoma), P-388 (murine leukemia), RPMI-7951 (human melanoma), and TE-671 (human medulloblastoma) cancer cell lines, and scopoletin was tested against the KB cell line. As shown in Table 3, compounds **1a** and **2** were broadly cytotoxic in all cell lines, with IC_{50} values in the ng/mL range. These compounds deserve further investigation as potentially useful antitumor agents.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanagimoto MP-S3 apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 polarimeter in $CHCl_3$. IR spectra were recorded on a Shimadzu FTIR-8501 spectrophotometer as KBr disks. UV spectra were recorded on a Hitachi UV-3210 spectrophotometer in MeOH. Mass spectra were determined on a VG 70-250S spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AC-300 and AMNX-400 spectrometers with TMS as an internal standard.

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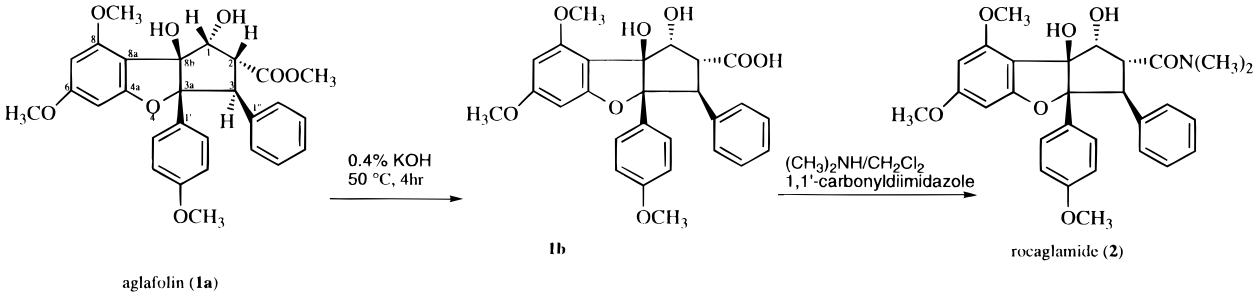
Scheme 1. Interconversion of **1a**, **1b**, and **2**



aglafolin (**1a**) **1b** rocaglamide (**2**)

Table 1. ^{13}C-NMR Assignments of Compounds **1a**, **1b**, and **2** in $CDCl_3$

position	1a	1b	2
1	79.6	79.2	78.5
2	50.5	51.2	47.5
3	55.0	55.1	55.8
3a	101.9	102.1	101.5
4a	160.9	161.0	161.0
5	89.5	89.0	89.2
6	164.1	163.7	163.8
7	92.6	92.2	92.4
8	157.0	157.3	157.1
8a	107.7	107.3	107.6
8b	93.7	93.6	93.9
1′	126.5	127.0	127.1
3′,5′	112.7	112.0	112.6
2′,6′	129.0	128.8	128.8
4′	158.7	158.4	158.5
1″	137.0	137.7	137.6
3″, 5″	127.7	127.6	127.6
2″, 6″	127.8	128.0	127.7
4″	126.5	126.1	126.2
MeO-6	55.7	55.4	55.6
MeO-8	55.8	55.5	55.6
MeO-4′	55.1	54.9	54.9
NMe			35.6 36.9
CO_2CH_3	52.0		
CO_2CH_3	170.5	175.8	169.5

Table 2. Antiplatelet Aggregation Activity (%) of Compounds **1a** and **2** and Scopoletin[a,b]

compd (100 μg/mL)	ADP (100 μM)	AA (100 μM)	PAF (2 ng/mL)	collagen (10 μg/mL)
aglafolin (**1a**)	85.2 ± 0.7	0.0 ± 0	0.0 ± 0	88.6 ± 8.6
rocaglamide (**2**)	77.6 ± 0.8	90.5 ± 0	85.5 ± 7.1	83.8 ± 7.9
scopoletin	65.3 ± 2.6	79.2 ± 8.0	90.5 ± 0.6	79.2 ± 7.4
control	88.6 ± 0.6	87.9 ± 7.7	95.2 ± 0.6	90.5 ± 1.3

[a] ADP = adenosine diphosphate, AA = arachinonic acid, PAF = platelet-activating factor. [b] Platelets were incubated with test sample or 0.5% DMSO at 37 °C for 1 min, then ADP (100 μm), AA (100 μm), collagen (10 μg/mL), or PAF (2 ng/mL) was added to trigger the aggregation. Values are presented as mean ± S.D.

Plant Material. *A. elliptifolia* was collected at Orchid Island, Taiwan, in September 1985. A voucher specimen is deposited at the Department of Biology, National Cheng-Kung University, Tainan, Taiwan.

Extraction and Isolation of Aglafolin (1a) and Rocaglamide (2). The stems of *A. elliptifolia* (2.7 kg) were extracted four times with MeOH. After concentration, the MeOH extract (189.2 g) was suspended in H_2O and extracted with $CHCl_3$. After concentration, the $CHCl_3$ extract (108.1 g) was chromatographed on Si gel using $CHCl_3$ as eluent to give four fractions, fractions A−D. Fraction B was chromatographed repeatedly on Si gel using isopropyl ether−C_6H_6 (1:3), $CHCl_3$−MeOH (200:1 to 70:1), and C_6H_6−Me_2CO (4:1) as eluents to yield 13 subfractions. A mixture (2.62 g) of the phytosterols β-sitosterol and stigmasterol was obtained from subfraction 6. Subfraction 7 afforded aglafolin (**1a**, 580 mg, 0.021%). Rocaglamide (**2**) was isolated from subfraction 12 (135 mg, 0.005%) and also from fraction C (1.21 g, 0.045%). The physical and spectral data of compounds **1a** and **2** were consistent with those in the literature; ^{13}C-NMR data in $CDCl_3$ are included in Table 1.

Saponification of 1a and Conversion to 1b. Compound **1a** (50 mg) was dissolved in 0.4% KOH/aqueous MeOH [MeOH−H_2O (5:10)] and heated at 50 °C for 4 h. After being cooled to room temperature, the reaction solution was neutralized with dilute HCl and then diluted with saturated NaCl solution (30 mL). The solution was extracted with Et_2O four times, and the ether layers were combined. After evaporation of solvent, the residue was crystallized from Me_2CO to give **1b** (32 mg, 66%): colorless needles from Me_2CO: mp 144−146 °C; $[\alpha]_D$ −70.0° (*c* 0.62, $CHCl_3$); IR υ_{max} 3450, 1620, 1605, 1515 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.77 (3H, s, MeO-4′), 3.83 (6H, overlapping s, MeO-6, MeO-8), 3.85 (1H, dd, *J* = 8 Hz, H-2), 4.28 (1H, d, *J* = 16 Hz, H-3), 4.86 (1H, d, *J* = 8 Hz, H-1), 6.01 (1H, d, *J* = 2 Hz, H-7), 6.22 (1H, d, *J* = 2 Hz, H-5), 6.59 (2H, dd, *J* = 2 and 8 Hz, H-3′, H-5′), 6.93 (2H, dd, *J* = 2, 8 Hz, H-2″, H-6″), 6.97 (3H, m, H-3″, H-4″, H-5″), 7.04 (2H, dd, *J* = 2, 8 Hz, H-2′, H-6′); ^{13}C-NMR data, see Table 1; HRFABMS *m/z* $[M + H]^+$ 497.172 ($C_{27}H_{27}O_8$ requires 479.170).

Amidation of 1b and Conversion to 2. Compound **1b** (20 mg) was dissolved in CH_2Cl_2 (2 mL), and dimethylamine (0.02 mL) was added at 0 °C under a N_2 current. The solution was stirred for 3 min, and then 1,1′-carbonyldiimidazole−CH_2Cl_2 solution (0.04 mL/1 mL) was added. After being stirred at 0 °C for 6 h, the solution was warmed to room temperature, and a few drops of dilute HCl were added. The reaction mixture was diluted with saturated NaCl solution and extracted three times with CH_2Cl_2. The CH_2Cl_2 layer was combined, dried, and evaporated to afford **2** (5 mg, 24%). The physical and spectral data of the synthetic compound agreed with those of the known compound.

Cytotoxicity Assays. The *in vitro* KB cytotoxicity assay was carried out according to procedures described by Geran *et al.*[5] and Ferguson *et al.*[6] The asssays against A-549, HCT-8, P-388, RPMI-7951, and TE-671 tumor cells were based on a method reported by Lee *et al.*[7]

Platelet Aggregation Assays. Collagen (Type 1, bovine achilles tendon) obtained from Sigma Chemical Co. was homogenized in 25 mL of HOAc and then stored at −70 °C. Adachidonic acid, bovine serum albumen (BSA), EDTA (disodium salt), sodium citrate, dimethyl

Table 3. Cytotoxic Activity of **1a** and **2** and Scopoletin

	cell line (IC_{50} μg/mL)[b]					
compd[a]	KB	A-549	HCT-8	P-388	RPMI-7951	TE-671
aglafolin (**1a**)	<0.001	<0.001	0.005	0.002	<0.001	<0.001
rocaglamide (**2**)	0.006	0.006	0.007	0.005	0.002	0.006
scopoletin	4.0	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]

[a] Values (μg/mL) for standard antitumor drugs were:[7] etoposide 0.12 (KB) and 2.62 (P-388); vinblastine sulfate 0.002 (A-549), 0.005 (HLT-8); doxorubicin HCl 0.15 (A-549) and 0.3 (HCT-8). [b] Values were obtained as described in references 5−7; KB (nasal pharyngeal carcinoma), A-549 (human lung carcinoma), HCT-8 (human colon carcinoma), P-388 (murine leukemia), RPMI-7951 (human melanoma), TE-671 (human medullablastoma). [c] NT = not tested.

sulfoxide (DMSO), and platelet-activating factor (PAF) were purchased from Sigma Chemical Co.

Platelet Suspension Preparation. Blood was collected from the rabbit marginal ear vein and was mixed with EDTA to a final concentration of 6 mM. It was centrifuged at 90*g* for 10 min at room temperature, and the supernatant was obtained as platelet-rich plasma. The latter was further centrifuged at 500*g* for 10 min. The platelet pellets were washed with Tyrode's solution without EDTA. After centrifugation at the same conditions, the platelet pellets were finally suspended in Tyrode's solution of the following composition (mM): NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (1.1), NaH_2PO_4 (0.33), $CaCl_2$ (1.0), and glucose (11.2). Platelet numbers were counted by Coulter Counter (Model ZM) and adjusted to 4.5×10^8 platelets/mL.

Platelet Aggregation. Aggregation was measured by the turbidimetric method[8] with a dual-channel Lumi-aggregometer (Model 1020, Payton, Canada). All glassware was siliconized. One minute before the addition of the aggregation inducer, the platelet suspension was stirred at 900 rpm. The percentage of aggregation was calculated as described previously.[9]

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References and Notes

(1) King, M. L.; Chiang, C. C.; Ling, H. C.; Fujita, E.; Ochiai, M.; McPhail, A. T. *J. Chem. Soc., Chem. Commun.* **1982**, 1150−1151.
(2) Ko, F. N.; Wu, T. S.; Liou, M. J.; Huang, T. F.; Teng, C. M. *Eur. J. Pharmcol.* **1992**, *218*, 129−135.
(3) Ishibashi, F.; Satasook, C.; Isman, M. B. *Phytochemistry* **1993**, *32*, 307−310.
(4) Janprasert, J.; Satasook, C.; Sukumanlanand, P.; Champagne, D. E.; Isman, M. B.; Wiriyachitra, P. *Phytochemistry* **1993**, *32*, 67−69.
(5) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3 (2)*, 1−103.
(6) Ferguson, P. J.; Fisher, M. H.; Stephenson, J.; Li, D. H.; Zhou, B. S.; Cheng, Y. C. *Cancer Res.* **1988**, *48*, 5956−5964.
(7) Lee, K. H.; Lin, Y. M.; Wu, T. S.; Zhang, D. C.; Yamagishi, T.; Hayashi, T.; Hall, I. H.; Chang, J. J.; Wu, R. Y.; Yang, T. H. *Planta Med.* **1988**, *54*, 308−311.
(8) O'Brien, J. R. *J. Clin. Path.* **1962**, *15*, 446−455.
(9) Teng, C. M.; Chen, W. Y.; Ko, W. C.; Ouyang, C. *Biochem. Biophys. Acta* **1987**, *924*, 375−382.

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