Cytotoxic Constituents from Leaves of *Aglaia elliptifolia*

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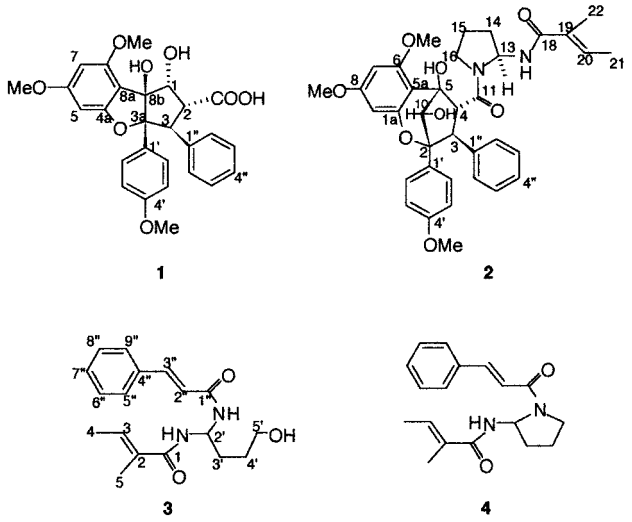
Three new cytotoxic compounds, rocagloic acid (**1**), elliptifoline (**2**), and elliptinol (**3**) were isolated from the leaves of *Aglaia elliptifolia*. The structures of compounds **1**−**3** were determined by spectral (NMR, MS) and chemical analysis.

In search for bioactive substances from natural sources, leaves of the Formasan plant *Aglaia elliptifolia* Merr. (Meliaceae) were studied. The methanol extract showed significant cytotoxicity in A549 (human lung adenocarcinoma), HT-29 (human colon aenocarcinoma), KB (human oral epidermoid carcinoma), HL-60 (human leukemia), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[1,2] Bioassay-guided fractionations resulted in the isolation of two new cytotoxic racaglamide derivatives, rocagloic acid (**1**) and elliptifoline (**2**); three diamides, elliptinol (**3**) (new compound), dehydroodorin (**4**),[3] and piriferine;[4] a damarane triterpene, foveolin B;[5] and a flavonoid, 3-hydroxy-5,7,4′-trimethoxyflavone.[6]



Rocagloic acid (**1**) was shown to possess a molecular formula of $C_{27}H_{26}O_8$ by HRFABMS. Its IR spectrum showed hydroxyl (3500 cm^{-1}) and carbonyl (1665 cm^{-1}) absorption. The ^1H NMR spectrum of **1** showed three methoxy groups at δ 3.65, 3.80, 3.81. In addition, signals of three aromatic rings similar to those of methyl rocaglate were observed,[7] that is, two meta-coupled aromatic protons at δ 6.09 and 6.25 (2d, J = 1.9 Hz), the characteristic AA′BB′ system of a *p*-disubstituted benzene ring at δ 6.61 and 7.09, and the signals of a monosubstituted benzene ring (5H, m, δ 7.01−7.08). The spectrum further exhibited signals at δ 4.89 (d, J = 5.6 Hz), 3.79 (dd, J = 14.1, 5.6 Hz) and 4.25 (d, J = 14.1 Hz) typical of H-1, H-2, and H-3 of methyl rocaglate.[7] The ^{13}C NMR spectrum of **1** was also similar to that of methyl rocaglate, showing the signals of a tetrasubstituted, a disubstituted, and a monosubstituted bezene ring; a carboxylic C=O at δ 172.8; and two characteristic quaternary carbons C−3a and C−8b at δ 101.9 and 93.6. Thus, owing to the elemental composition and the lack of a methyl group, the structure of **1** was assigned as shown. Saponification of methyl rocaglate[8] and conversion to **1** further confirmed the structure.

Elliptifoline (**2**) was shown to possess a molecular formula of $C_{36}H_{40}N_2O_8$ by HRFABMS. Its IR spectrum showed hydroxyl absorption at 3650 cm^{-1} and carbonyl absorptions at 1635 and 1620 cm^{-1}, corresponding to amide functions. The ^1H and ^{13}C NMR spectra of **2** indicated three methoxyl groups and three benzene rings similar to **1**. In addition, signals of a tigloyl amide and a 2-aminopyrrolidine ring reminiscent of those reported for dehydroodorin were observed, suggesting that part of the dehydroodorin (**4**) structure could be linked through an amide function to an acid moiety of the rocagloic acid instead of the OH group. The ^1H NMR spectrum of **1** also showed three signals of methine protons at δ 4.45, 4.15, and 4.80, which could correspond to the 1, 2, 3 position in rocagloic acid. However, the coupling patterns of the three protons differed from those of rocagloic acid. The proton at δ 4.80 appeared as a sharp singlet, while the two others were coupled (9.2 Hz), which is compatible with 3α,4*β*-configuration.[9] Signals of quaternary carbons C-3a and C-8b with typical chemical shifts present at δ 101.9 and 93.6 were lacking and replaced by two quaternary carbon signals at δ 88.9 and 81.9. These ^1H and ^{13}C NMR data suggested that elliptifoline has the formula depicted in **2**,[9] with a pyran ring in place of the furan of rocagloic acid. In the HMBC experiment, the cross-peaks H-10/C-2, C-3, C-4, C-5a; H-4/C-3, C-5, C-5a, C-10, C-11, C-1″; and H-3/C-2, C-4, C-10 supported the relative positions 2, 3, 4, 5, and 10, whereas the correlations from H-3 to C-1″,-2″,-6″ and H-4 to C-11 indicated the connectivities of C-3 to the monosubstituted benzene ring and C-4 to C-11, respectively. NOESY relationships H-4/H-2″,-6″, H-3/H2″-6″, H-4/OH-10 further confirmed these assignments and established the relative configuration at C-2, C-3, C-4, C-5, and C-10 as depicted in formula **2**. The ^{13}C NMR chemical shift of C-10 (δ ca. 80)[9] also confirmed the relative configuration at this carbon. In addition, the NOESY cross-peak H-4/H-13 established that the relative configuration at C-13 is *S*, as depicted in **2**.

Compound **3** had molecular formula $C_{18}H_{24}O_3N_2$ as determined by HREIMS. Presence of a cinnamoyl moiety was indicated by the appearance of mass peaks at *m*/*z* 131

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(PhCH=CHCO) and 103 (PhCH=CH) and by IR bands at 1665, 1620, and 1550 cm^{-1}, and was substantiated by the presence in the ^1H NMR spectrum of characteristic low-field signals at δ 7.57 (1H, d, J = 15.6 Hz), 6.39 (1H, d, J = 15.6 Hz), and 7.35 (3H, m), 7.47 (2H, m). The presence of a tigloyl moiety was indicated by ^{13}C NMR signals at δ 169.9 s, 131.4 s, 132.2 d, 14.1 q, and 12.3 q. Comparing the rest of ^{13}C NMR signals (δ 62.4 d, 28.7 t, 30.6 t, and 58.8 t) and the molecular formula of **3** with those of **4**[3] suggested that elliptinol had the structure depicted in formula **3**, with a 1-hydroxy-4-aminobuta-4-yl moiety in place of the pyrrolidin-2-yl moiety in **4**. This structure was further confirmed by COSY and HMBC experiments.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micromelting point apparatus and are reported uncorrected. Optical rotations were determined on a JASCO DIP-181 polarimeter. IR spectra were recorded on a Hitachi 26-30 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a Bruker AMX 400 NMR spectrometer at 400 and 100.6 MHz, respectively, in $CDCl_3$ using TMS as internal standard. EIMS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Plant Material. The leaves of *A. elliptifolia* were collected in Pintong County, Taiwan, in February 1990. A voucher specimen (PT-012) is deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. Dry ground leaves (10.2 kg) of *A. elliptifolia* were extracted with MeOH. After removal of solvent in vacuo, the residue (760 g) was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble extract exhibited significant cytotoxicity against several cell lines tested. The $CHCl_3$-soluble extract (630 g) was chromatographed over Si gel 60 using $CHCl_3$ and MeOH−$CHCl_3$ mixtures of increasing polarity. The fraction (ED_{50} 0.06 μg/mL, 20 g) that was eluted with MeOH−$CHCl_3$ (1:49) was subjected to repeated Si gel column chromatography (CC) and Sephadex LH-20 CC yielding elliptifoline (**2**) (72 mg) (a) silica CC *n*-hexane−EtOAc (1:1) (b) silica CC *n*-hexane−EtOAc−Me_2CO−$CHCl_3$ (2:1:1:1), and (c) LH-20 CC *n*-hexane−CH_2Cl_2 (1:1). The fraction (ED_{50} 0.5 μg/mL, 12 g) that was eluted with MeOH−$CHCl_3$ (1:9) was subjected to repeated Si gel CC and Sephadex LH-20 CC yielding elliptinol (**3**) (5 mg) (a) silica CC *n*-hexane−EtOAc (1:4), (b) silica CC *n*-hexane−$CHCl_3$−Me_2CO−MeOH (8:4:3:1), and (c) LH-20 CC *n*-hexane−CH_2Cl_2 (1:1); rocagloic acid (**1**) (36 mg) (a) silica CC $CHCl_3$−Me_2CO (2:3) and (b) LH-20 CC Me_2CO−CH_2Cl_2 (2:3); fovelin B (20 mg) (a) silica CC *n*-hexane−Me_2CO (2:3) and (b) LH-20 CC *n*-hexane−CH_2Cl_2 (1:1). The fraction (ED_{50} 5.6 μg/mL, 15 g) that was eluted with $CHCl_3$ was subjected to repeated Si gel CC and Sephadex LH-20 CC yielding 3-hydroxy-5,7,4′-trimethoxyflavone (25 mg) (a) silica CC *n*-hexane−EtOAc (1:1) and (b) LH-20 CC *n*-hexane−CH_2Cl_2 (1:1); dehydroodorin (106 mg) (a) *n*-hexane−EtOAc (1:1). The fraction (ED_{50} 6.7 μg/mL, 10 g) that was eluted with MeOH−$CHCl_3$ (1:19) was subjected to Sephadex LH-20 CC [*n*-hexane−acetone (1:1)] yielding piriferine (15 mg).

Rocagloic acid (1): white powder; mp 145−146°; $[\alpha]^{22}_D$ −47.6° (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 273 (3.36) nm; IR (KBr) ν_{max} 3500, 1665, 1600, 1510 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.65 (3H, s, OMe-4′), 3.79 (1H, dd, J = 14.1, 5.6 Hz, H-2), 3.80 (3H, s, OMe-8), 3.81 (3H, s, OMe-6), 4.25 (1H, d, J = 14.1 Hz, H-3), 4.89 (1H, d, J = 5.6 Hz, H-1), 6.09 (1H, d, J = 1.9 Hz, H-7), 6.25 (1H, d, J = 1.9 Hz, H-5), 6.61 (2H, d, J = 9.0 Hz, H-3′,-5′), 7.01 (2H, m, H-2″,-6″), 7.05 (2H, m, H-3″,-5″), 7.08 (1H, m, H-4″), 7.09 (2H, d, J = 9 Hz, H-2′,-6′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 51.7 (d, C-2), 55.2 (s, OMe-4′), 55.7 (s, OMe-6), 55.8 (s, OMe-8), 56.3 (d, C-3), 79.0 (d, C-1), 89.3 (d, C-5), 92.5 (d, C-7), 93.6 (s, C-8b), 101.9 (s, C-3a), 107.2 (s, C-8a), 112.7 (d, C-3′,-5′), 126.6 (s, C-1′), 127.0 (d, C-4″), 128.0 (d, C-3″,-5″), 128.3 (d, C-2″,-6″), 129.1 (d, C-2′,-6′), 136.4 (s, C-1″), 157.4 (s, C-8), 158.8 (s, C-4′), 161.1 (s, C-4a), 164.1 (s, C-6), 172.8 (s); FABMS m/z $[MH]^+$ 479 (1), 461 (15), 443 (13), 415 (20), 390 (20), 313 (92), 300 (100); HRFABMS m/z 479.1703 (calcd for $C_{27}H_{27}O_8$ 479.1698).

Elliptifoline (2): white powder; mp 184−185°; $[\alpha]^{22}_D$ −88.9° (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 273 (3.36) nm; IR (KBr) ν_{max} 3650, 3440, 1635, 1620, 1598 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.47 (3H, s, H-22), 1.63 (3H, d, J = 7.0 Hz, H-21), 1.84 (2H, m, H-15), 2.02 (2H, m, H-14), 3.21, 3.58 (1H each, m, H-16), 3.66 (3H, s, OMe-4′), 3.71 (3H, m, OMe-8), 3.80 (3H, m, OMe-6), 4.15 (1H, d, J = 9.2 Hz, H-4), 4.45 (1H, d, J = 9.2 Hz, H-3), 4.62 (1H, s, OH-10), 4.80 (1H, s, H-10), 5.57 (1H, d, J = 7.9 Hz, NH-17), 5.88 (1H, s, OH-5), 6.02 (1H, m, H-20), 6.06 (1H, m, H-7), 6.09 (1H, m, H-9), 6.60 (2H, m, H-3′,-5′), 6.63 (1H, m, H-13), 6.89 (2H, m, H-3″,-5″), 6.91 (1H, m, H-4″), 7.11 (2H, m, H-2″,-6″), 7.41 (2H, m, H-2′,-6′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.0 (q, C-22), 13.9 (q, C-21), 21.4 (t, C-15), 34.5 (t, C-14), 46.1 (t, C-16), 55.0 (s, OMe-4′), 55.2 (s, OMe-8), 56.0 (s, OMe-6), 57.1 (d, C-3), 62.3 (d, C-4), 63.7 (d, C-13), 79.7 (d, C-10), 81.9 (s, C-5), 88.9 (s, C-2), 91.9 (d, C-7), 94.2 (d, C-9), 105.4 (s, C-5a), 112.6 (d, C-3′,-5′), 125.6 (d, C-4″), 127.7 (d, C-3″,-5″), 130.2 (d, C-2″,-6″), 130.4 (d, C-2′,-6′), 130.7 (s, C-19), 131.8 (d, C-20), 141.8 (s, C-1″), 153.7 (s, C-1a), 158.1 (s, C-6), 158.4 (s, C-4′), 161.0 (s, C-8), 167.9 (s, C-18), 169.9 (s, C-11); FABMS m/z $[MH]^+$ 629 (6), 530 (1), 461 (1), 376 (17), 313 (84), 181 (53), 70 (100); HRFABMS m/z 629.2863 (calcd for $C_{36}H_{41}N_2O_8$ 629.2863).

Elliptinol (3): white powder; mp 162−163°; $[\alpha]^{22}_D$ +38.6° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 283 (4.66) nm; IR (KBr) ν_{max} 3650, 1665, 1620, 1550 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.61 (2H, m, H-3′), 1.75 (3H, d, J = 6.9 Hz, H-4), 1.83 (3H, s, H-5), 2.15 (2H, m, H-4′), 3.71 (1H, t, J = 6.0 Hz, H-2′), 5.41 (1H, m, H-5′), 6.39 (1H, d, J = 15.6 Hz, H-2″), 6.49 (1H, q, J = 6.9 Hz, H-3), 7.35 (3H, m, H-5″,-6″,-7″); 7.47 (2H, m, H-5″,-9″), 7.57 (1H, H-3″); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.3 (q, C-5), 14.1 (q, C-4), 28.7 (t, C-3′), 30.6 (t, C-4′), 58.8 (t, C-5′), 62.4 (d, C-2′), 120.5 (d, C-2″), 127.9 (d, C-5″,-9″), 128.9 (d, C-6″,-8″), 129.9 (d, C-7″), 131.4 (s, C-2), 132.2 (d, C-3), 134.7 (s, C-4″), 141.7 (d, C-3″), 166.3 (s, C-1″), 169.6 (s, C-1); EIMS m/z $[M]^+$ 316 (0.5), 258 (8), 257 (47), 217 (5), 189 (5), 146 (17), 131 (100), 103 (39); HREIMS m/z 316.1787 (calcd for $C_{18}H_{24}N_2O_3$ 316.1788).

Saponification of Methyl Rocaglate and Conversion to 1. Methyl rocaglate (30 mg) was dissolved in 0.5% KOH−aqueous MeOH [MeOH−H_2O (1:2)] and refluxed at 65 °C for 5 h. The reaction mixture was neutralized with diluted HCl and diluted with saturated NaCl. The solution was then extracted with Et_2O to get 20 mg of **1** [crystallized from Me_2CO−MeOH (1:1)].

Cytotoxicity Testing. KB and P-388 cells were supplied by Prof. J. M. Pezzuto, University of Illinois at Chicago. The A549, HL-60, and HT-29 cells were purchased from the American Type Culture Collection. Cytotoxicity asssays were carried out according to the procedure described previously.[10] Cytotoxicity of **1**−**3** is shown in Table 1.

Table 1. Cytotoxicitiy[a] of **1**−**3** (n = 8)

compound	ED_{50} (μg/mL) in indicated cell line				
	A549	HL-60	HT-29	KB	P-388
1	0.00074	0.00084	0.00084	0.0023	0.0012
2	18.9	>50	>50	>50	3.41
3	>50	32.1	>50	>50	3.62

[a] For significant activity of pure compounds, an ED_{50} of ≤ 4.0 μg/mL is required.[1]

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