Potent Cytotoxic Rocaglamide Derivatives from the Fruits of *Amoora cucullata*

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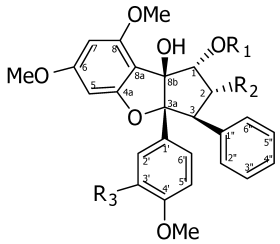
Two new rocaglamide derivatives, 1-*O*-formylrocagloic acid (1) and 3′-hydroxy rocagloic acid (2), together with five known compounds, rocaglaol (3), rocagloic acid (4), 3′-hydroxymethylrocaglate (5), 1-*O*-formylmethyl rocaglate (6), and methylrocaglate (7), were isolated from the fruits of *Amoora cucullata*. Their structures were elucidated by spectroscopic methods. Compounds 1—3, 6, and 7 exhibited potent cytotoxicity against KB, BC, and NCI-H187 cell lines, whereas 4 and 5 showed selective cytotoxicity against NCI-H187 cell line.

Key words *Amoora cucullata*; Meliaceae; rocaglamide derivative; cytotoxicity

Amoora cucullata (Meliaceae), is a mangrove plant, distributed in the coastal areas of Southeast Asia and the Indian Ocean. This plant has been by the local Thai people as a folk medicine for treatment of marrow, diarrhea, and inflammation.[1)] As part of our continuing search for bioactive constituents from Thai medicinal plants,[2—4)] separate hexane and dichloromethane soluble extracts of the fruits of *A. cucullata* were found to exhibit significant cytotoxic activity when evaluated against a panel of human cell lines. Fractionation of the hexane and dichloromethane extracts led to the isolation of two new naturally occurring cyclopenta[*b*]benzofuran, 1-*O*-formylrocagloic acid (**1**) and 3′-hydroxyrocagloic acid (**2**), along with five known compounds, rocaglaol (**3**),[5)] rocagloic acid (**4**),[6)] 3′-hydroxymethylrocaglate (**5**),[7)] 1-*O*-formylmethyl rocaglate (**6**),[5)] and methylrocaglate (**7**),[5)] from the fruits of *A. cucullata*. The structures of the known compounds were elucidated by comparison of their physical and spectral data with literature values. The obtained rocaglamide derivatives, **1**—**7**, were evaluated biologically against human cancer cell lines.

Compound (**1**) was obtained as a colorless gum. The molecular formula was determined as $C_{28}H_{26}O_9$ by HR-FAB-MS $[M]^+$ *m*/*z* 506.1580 (calcd for $C_{28}H_{26}O_9$: 506.1571). Its IR spectrum showed hydroxyl (3454 cm^{-1}) and carbonyl (1734, 1627 cm^{-1}) absorptions. The ^{1}H- and ^{13}C-NMR spectra of **1**



	R_1	R_2	R_3
1	CHO	COOH	H
2	H	COOH	OH
3	H	H	H
4	H	COOH	H
5	H	$COOCH_3$	OH
6	CHO	$COOCH_3$	H
7	H	$COOCH_3$	H

Fig. 1. Structures of Compounds Isolated from *Amoora cucullata*

(Table 1) exhibited signals similar to those of rocagloic acid,[6)] with the exception of an additional a signal as a singlet for a formyl group at δ_H 7.95 (δ_C 159.7), suggesting that the two compounds are based on the same carbon skeleton. Two *meta*-coupled aromatic protons were observed at δ_H 6.24 (1H, d, J=2.0 Hz, H-5) and 6.04 (1H, d, J=2.0 Hz, H-7) and also apparent were the characteristic AA′BB′ system of a *p*-disubstituted benzene ring at δ_H 7.12 (2H, d, J=9.0 Hz, H-2′, H-6′) and 6.62 (2H, d, J=9.0 Hz, H-3′, H-5′) and the signals of a monosubstituted benzene ring (5H, m, δ_H 7.09—7.01). The ^{1}H-NMR spectrum further exhibited signals at δ_H 6.20 (1H, d, J=5.5 Hz, H-1), 4.40 (1H, d, J=14.0 Hz, H-3), and 4.07 (1H, dd, J=5.5, 14.0 Hz, H-2) typical of H-1, H-2, and

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) Spectral Data for **1** and **2**

Position	^{1}H		^{13}C	
	1[a)]	**2**[a)]	**1**[a)]	**2**[a)]
1	6.20 (d, 5.5)	4.93 (d, 5.5)	79.1	78.8
2	4.07 (dd, 5.5, 14.0)	3.87 (dd, 5.5, 14.5)	50.6	51.4
3	4.40 (d, 14.0)	4.29 (d, 14.5)	55.9	55.9
3a	—	—	101.9	101.7
4a	—	—	160.8	161.1
5	6.24 (d, 2.0)	6.28 (d, 2.0)	88.5	88.9
6	—	—	164.1	163.9
7	6.04 (d, 2.0)	6.13 (d, 2.0)	92.2	92.4
8	—	—	158.7	157.4
8a	—	—	105.9	106.9
8b	—	—	92.5	93.7
1′	—	—	126.8	127.7
2′	7.12 (d, 9.0)	6.84 (d, 2.5)	128.8	114.6
3′	6.62 (d, 9.0)	5.54 (bs)	112.7	144.3
4′	—	—	157.7	145.8
5′	6.62 (d, 9.0)	6.60 (d, 8.5)	112.7	109.6
6′	7.12 (d, 9.0)	6.71 (dd, 2.5, 8.5)	128.8	119.5
1″	—	—	136.3	136.5
2″/6″	7.09—7.01 (m)	7.14—7.06 (m)	128	128.2
3″/5″	7.09—7.01 (m)	7.14—7.06 (m)	128	128.8
4″	7.09—7.01 (m)	7.14—7.06 (m)	126.6	126.7
COOH	—	—	170.3	173.2
OCHO	7.95 (bs)	—	159.7	—
Ar-OCH_3	3.83 (s)	3.86 (s)	55.6	55.6
	3.73 (s)	3.84 (s)	55.3	55.6
	3.66 (s)	3.77 (s)	55	55.6

a) TMS was used as an internal standard, and chemical shifts are presented in parts per million (δ). J values are given in Hz in parentheses.

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H-3 of rocagloic acid,[6] and three aromatic methoxy groups at δ_H 3.83, 3.73, and 3.66. The ^{13}C-NMR spectrum of **1** also showed the signals of a tetrasubstituted, a disubstituted, and a monosubstituted benzene ring; a carboxylic C=O at δ_C 170.3; and two characteristic quaternary carbons C-3a and C-8b at δ_C 101.9 and 92.5. In the HMBC spectrum of **1**, a correlation from δ_H 6.20 (H-1) to δ_C 159.7 suggested the presence of a formyl functional group in the molecule of **1**, and this could be located at C-1. The relative configuration of **1** was established primarily by analysis of the splitting patterns, and the coupling constant values between H-1, H-2, and H-3 indicating a 1α, 2α, 3β configuration, as well as a *cis*-BC ring junction.[8—10] These relative configurations were confirmed by NOESY experiments, wherein correlations were observed from H-1 to H-2 and H-6′ and from H-2 to H-3. Due to their structural similarity, all rocaglamide-related natural products that have so far been examined display CD spectra very similar to that of rocaglamide itself.[8,10,11] The CD curve of **1** was also very similar to that of rocaglamide, with a prominent negative cotton effect at 274 nm as the most characteristic feature, suggesting the presence of the usual rocaglamide-analogue absolute stereostructure, with a 1*R*, 2*R*, 3*S*, 3a*R*, 8b*S*-configuration. On the basis of the above evidence, the structure of **1** was characterized as 1-*O*-formylrocagloic acid.

Compound (**2**) was isolated as a colorless gum. The molecular formula $C_{27}H_{26}O_9$ was established by HR-FAB-MS *m*/*z* found 494.1605 (calcd for $C_{27}H_{26}O_9$: 494.1571). The IR spectrum showed hydroxyl absorption at 3460 cm^{-1} and carbonyl absorption at 1668 cm^{-1}. Inspection of ^{1}H- and ^{13}C-NMR spectra of **2** allowed unambiguous assignment of a hydroxyl substituent at C-3′ (Table 1). Analysis of the ^{1}H-NMR signal at δ_H 5.54 (1H, br s) and a corresponding ^{13}C-NMR signal at δ_C 144.3, indicated the appearance of the hydroxyl group, but no signal of an aromatic H-3′ position was observed in the molecule of **2**. Other ^{1}H-NMR signals at δ_H 6.84 (1H, d, J=2.0 Hz), 6.71 (1H, dd, J=2.0, 8.5 Hz), and 6.60 (1H, d, J=8.5 Hz) were attributable to H-2′, H-6′, and H-5′, respectively, and suggested the replacement of the aromatic proton at C-3′ in the molecule of **4** by 3′-hydroxyrocagloic acid in the molecule of **2**. The configuration around C-1, C-2, C-3, C-3a, and C-8b in the molecule of **2** was established on the basis of similar chemical shifts and vicinal coupling constant values of the methine protons at C-1, C-2, and C-3 ($J_{1,2}$=5.5 Hz, $J_{2,3}$=14.0 Hz) to those of **1** ($J_{1,2}$=5.5 Hz, $J_{2,3}$=14.5 Hz), which indicated the existence of the same 1α, 2α, 3β-configuration and ring B/C junction in the two compounds. The relative configurations were confirmed by a NOESY spectrum. A cross-peak was observed between proton H-1 and H-2, indicating that they have a *cis* configuration. In turn, the stereochemistry of protons H-2 and H-3 was identified from the coupling constant ($J_{2,3}$=14.5 Hz) typical of a *trans* configuration. The above evidence suggests that the structure of **2** can be assigned as 3′-hydroxyrocagloic acid.

The cytotoxic activities of compounds **1**—**7** were studied. The rocaglamide derivatives **1**—**3**, **6**, and **7** showed strong cytotoxicities against KB, BC, and NCI-H187 cell lines, whereas **4** and **5** were found to be selectively cytotoxicity against NCI-H187 cell lines as shown in Table 2. It is interesting to note that the absence of either 3′-OH or the substitution of methyl ester in the place of a carboxyl group resulted in reduced activity in **4** and **5**, respectively, as compared to the parent compound, rocaglamide **2**.

Table 2. Cytotoxic Activity of Compounds **1**—**7**

Sample	Cell lines[a]		
	KB	BC	NCI-H187
1	0.002	0.06	0.019
2	0.005	0.002	0.0000028
3	0.1	0.08	0.000031
4	>50	>50	0.000036
5	>50	>50	0.0019
6	0.3	0.07	0.00073
7	0.02	0.002	0.000037

a) Results are expressed as ED_{50} values (μg/ml); activity: <5 strong, 5—20 moderate, 20—50 weak, >50 inactive. Key to cell lines used: KB=oral human epidermoid carcinoma; BC=human breast cancer cell; NCI-H187=human small cell lung cancer.

Experimental

General Experimental Procedures Specific rotations were determined with an Autopol II automatic polarimeter. UV spectra were measured with a UV-160A spectrophotometer (Shimadzu), and IR spectra were recorded on a Perkin-Elmer 1750 FTIR spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ using a 500 MHz Varian Unity INOVA spectrometer. Chemical shifts are recorded in parts per million (δ) in $CDCl_3$. Mass spectra (EI or FAB) were recorded on a Finnigan-MAT 95 XL spectrometer. Column chromatography was carried out on silica gel 60 GF_{254} (Merck).

Plant Material The fruits of *Amoora cucullata* were collected in Khanom, Nakhon Si Thammarat, Thailand, in May 2004. A voucher specimen (number WU-0145) was deposited in the herbarium of the School of Science, Walailak University, Thasala, Nakhon Si Thammarat, Thailand.

Extraction and Isolation Air-dried fruits of *A. cucullata* (8.0 kg) was ground and extracted successively with hexane and dichloromethane at room temperature. The hexane extract (50.7 g) was subjected to quick column chromatography over silica gel and eluted with increasing concentrations of EtOAc in hexane followed by MeOH in EtOAc to yield six fractions. Fraction 5 (0.52 g) was further rechromatographed on a silica gel column, eluted with a gradient system of CH_2Cl_2–EtOAc, to afford **6** (21.0 mg, *Rf* 0.82). Fraction 6 (0.93 g) was purified by silica gel column chromatography to give **7** (40.0 mg, *Rf* 0.77). The dichloromethane extract (47.0 g) was initially separated by passage over silica gel rapid column chromatography eluted with a gradient system of CH_2Cl_2–hexane to yield seven fractions. Fractions 4, 5, and 7 were further separated on a silica gel column, eluted with 0—100% CH_2Cl_2–EtOAc mixture to give **3** (20.0 mg, *Rf* 0.72), **5** (43.3 mg, *Rf* 0.63), and **2** (19.5 mg, *Rf* 0.08), respectively. Purification of fraction 6 (0.42 g) was also performed by preparative TLC using 40% hexane–EtOAc to provide **1** (41.8 mg, *Rf* 0.88) and **4** (27.5 mg, *Rf* 0.12).

1-*O*-Formylrocagloic Acid (**1**): Colorless gum, $[\alpha]_D^{28}$ −23.2° (c=0.040, $CHCl_3$), UV λ_{max} ($CHCl_3$) nm (log ε): 232 (6.09), 275 (5.19), CD (c=0.25, $CHCl_3$) $\Delta\varepsilon$ (nm): −24.17 (274), +2.49 (334), IR ($CHCl_3$) cm^{-1}: 3454 (OH), 1734 (C=O), 1627 (C=O), ^{1}H-NMR, see Table 1, ^{13}C-NMR, see Table 1, HR-EI-MS *m*/*z* 506.1580 $[M]^+$ (calcd for $C_{28}H_{26}O_9$: 506.1571).

3′-Hydroxyrocagloic Acid (**2**): Colorless gum; $[\alpha]_D^{28}$ −42.6° (c=0.019, $CHCl_3$); UV λ_{max} ($CHCl_3$) nm (log ε): 228 (6.09), 279 (5.18), CD (c=0.04, $CHCl_3$) $\Delta\varepsilon$ (nm): −14.53 (274), +1.95 (332), IR ($CHCl_3$) cm^{-1}: 3460 (OH), 1668 (C=O), ^{1}H-NMR, see Table 1, ^{13}C-NMR, see Table 1, HR-EI-MS *m*/*z* 494.1605 $[M]^+$ (calcd for $C_{27}H_{26}O_9$: 494.1571).

Cytotoxicity Assay The cytotoxicity assay employed the colorimetric method.[12] Ellipticine, the reference substance, exhibited activity toward BC, KB, and NCI-H187 cell lines, with the IC_{50} range of 0.3—0.6 μg/ml (Table 2).

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