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Cytotoxic Constituents of the Twigs and Leaves of *Aglaia rubiginosa*

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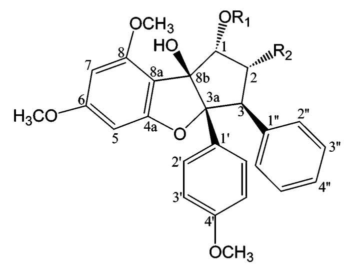
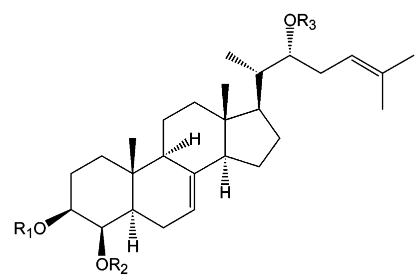
Activity-guided fractionation of a $CHCl_3$-soluble extract of the twigs of *Aglaia rubiginosa*, using human oral epidermoid carcinoma (KB) cells as a monitor, led to the isolation of a new naturally occurring cyclopenta[*b*]benzofuran, 1-*O*-acetylrocaglaol (**1**), along with seven known compounds, methyl rocaglate (**2**), rocagloic acid (**3**), 1-*O*-acetylmethyl rocaglate (**4**), desyclamide, eryodictiol, 5-hydroxy-3,7,4′-trimethoxyflavone, and naringenin. A $CHCl_3$ extract of the leaves of *A. rubiginosa* yielded the new compound (3*S*,4*R*,22*R*)-cholest-7,24-diene-3,4,22-triol (**5**), as well as 11 known compounds, including **2** and **4** and cabraleone, dammarelonic acid, (20*S*,23*E*)-20,25-dihydroxy-3,4-secodammara-4(28),23-dienoic acid, (20*S*,23*E*)-20,25-dihydroxy-3,4-secodammara-4(28),23-dienoic acid methyl ester, (3*β*,4*β*,22*R*)-ergosta-5,24(24′)-diene-3,4,22-triol, ocotillone, shoreic acid, *β*-sitosterol, and *β*-sitosterol glycoside. The structures of **1** and **5** were elucidated by spectral and chemical methods. Isolates were evaluated with a human cancer cell panel, and compounds **1**−**4** were found to exhibit potent cytotoxic activity.

The genus *Aglaia* of the family Meliaceae is represented by over 100 known species.[1,2] Previous phytochemical studies of *Aglaia* species have resulted in the isolation of bisamides, cyclopenta[*b*]benzofurans (flavaglines), cyclopenta[*b*]benzopyrans, lignans, limonoids, sterols, and triterpenoids (e.g., cycloartanes, dammaranes, and tirucallanes).[1−4] *Aglaia rubiginosa* (Hiern) Pannell (syn. *Aglaia ignea* Valeton ex K. Heyne, *Amoora rubiginosa* Heyne) is an emergent tree of freshwater peat swamp forests, lowland primary forests, and hill forests that grows in Indonesia, the Philippines, Malaysia, and Singapore.[5−9] It is locally known in Indonesia as “kaje laki”, “parak merah”, “parak api”, and “parak talang”, and the wood is used for beams in house and boat building.[8,9] Previous phytochemical studies on *A. rubiginosa* have resulted in the isolation of several androstane derivatives,[2] sterols,[2] triterpenoids,[2] and the putrescine alkaloid, aglairubine.[10,11]

As a part of our ongoing program for the discovery of new anticancer agents from plants,[12] separate chloroform-soluble extracts of the twigs and leaves of *A. rubiginosa* were found to exhibit significant cytotoxic activity when evaluated against a panel of human cancer cell lines.[13,14] Fractionation of the active extracts led to the isolation of one new cyclopenta[*b*]benzofuran (**1**) and seven known compounds from the twig extract, and the new sterol derivative **5** and 11 known compounds from the leaf extract. The structures of compounds **1** and **5** were determined on the basis of various 1D and 2D NMR experiments. The amide, cyclopenta[*b*]benzofuran, flavonoid, and triterpenoid constituents obtained were evaluated biologically against a human cancer cell panel.



	R_1	R_2
1	$COCH_3$	H
2	H	$COOCH_3$
3	H	COOH
4	$COCH_3$	COOH



	R_1	R_2	R_3
5	H	H	H
5a	$C(CH_3)_2$		H
5b	*p*-BrBz	*p*-BrBz	*p*-BrBz
5ar	$C(CH_3)_2$		*R*-(MTPA)
5as	$C(CH_3)_2$		*S*-(MTPA)

Results and Discussion

Four cyclopenta[*b*]benzofuran lignan derivatives, constituted by methyl rocaglate (**2**),[15] rocagloic acid (**3**),[16] 1-*O*-acetylmethyl rocaglate (**4**),[16] and the new 1-*O*-acetylrocaglaol (**1**), three known flavonoids, eryodictiol,[18] 5-hydroxy-3,7,4′-trimethoxyflavone,[2] and naringenin,[19] and one amide, desyclamide,[16] were isolated from *A. rubiginosa* twigs by

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Table 1. ^{1}H (360 MHz), ^{13}C (90 MHz), HMBC, and NOESY NMR Data for 1-*O*-Acetylrocaglaol (**1**)[a]

position	^{13}C	^{1}H	HMBC	NOESY
1	79.5	5.84 dd (5.4, 2.0)	2α, 2β, 3,8b O*C*OCH$_3$-1	2α, 2β, 2′, 6′
2α	35.6	2.30 ddd (13.3, 6.2, 2.0)	1, 3a	3α
2β		2.82 dd (13.3, 5.4)		1, 2″, 6″
3	53.9	4.06 dd (13.3, 6.2)	3a, 8b	
3a	103.3		2″, 6″	
4a	161.0			
5	88.1	6.23 d (1.9)		OCH$_3$-6
6	158.2			
7	91.8	6.10 d (1.9)		OCH$_3$-6, OCH$_3$-8
8a	161.0			
8b	91.9			
1′	126.5			
2′, 6′	129.0	7.13 d (8.9)	1′, 3a	2β
3′, 5′	112.9	6.64 d (8.9)		
4′	158.8			
1″	138.8			
2″, 6″	127.4	7.05 m		2b
3″, 5″	127.4	7.11 m		OCH$_3$-4′
4″	126.5	7.11 m		
OCH$_3$-4′	55.2	3.75 s	4′	3″, 5″
OCH$_3$-6	55.5	3.84 s	6	5, 7
OCH$_3$-8	55.8	3.77 s	8	7
O*C*OCH$_3$	170.5			
OCO*C*H$_3$	21.1	1.87 s	1	

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

activity-guided fractionation using KB (human oral epidermoid carcinoma) cells. From the leaves of this same plant, activity-directed fractionation, using KB cells, afforded the new (3*S*,4*R*,24*R*)-cholest-7,24-diene-3,4,22-triol (**5**), as well as 11 known compounds, cabraleone,[20] dammarenolic acid,[21] (20*S*,23*E*)-20,25-dihydroxy-3,4-secodammara-4(28),23-dienoic acid,[21] (20*S*,23*E*)-20,25-dihydroxy-3,4-secodammara-4(28),23-dienoic acid methyl ester,[21] (3β,4β,22*R*)-ergosta-5,24(24′)-diene-3,4,22-triol,[22] methyl rocaglate (**2**),[15] 1-*O*-acetylmethyl rocaglate (**4**),[16] ocotillone,[20] shoreic acid,[23] β-sitosterol, and β-sitosterol glycoside. The known compounds were identified by physical and spectroscopic data measurement ([α]$_D$, ^{1}H NMR, ^{13}C NMR, DEPT, 2D NMR, and MS) and by comparing the data obtained with those of published values. This is the first report of cyclopenta[*b*]benzofurans as chemical constituents of *A. rubiginosa*.

Compound **1**, [α]$^{23}_{D}$ −115.2° (*c* 0.2, MeOH), was obtained as a colorless gum. The molecular formula was determined as $C_{28}H_{28}O_7$ by HRFABMS (obsd *m*/*z* 499.1734). The ^{1}H NMR and ^{13}C NMR spectra of **1** (Table 1) exhibited signals similar to those of rocaglaol,[24] with the exception of additional signals for an acetyl group [δ_H 1.87 (3H, s)/δ_C 170.5, 21.1] in **1**, suggesting that the two compounds are based on the same carbon skeleton. Two *meta*-coupled aromatic protons were observed at δ_H 6.23 (1H, d, $J = 1.9$ Hz, H-5) and 6.10 (1H, d, $J = 1.9$ Hz, H-7), and also apparent were the characteristic AA′BB′ signals of a *p*-disubstituted benzene ring at δ_H 7.13 (2H, d, $J = 8.9$ Hz, H-2′ and H-6′) and 6.64 (2H, d, $J = 8.9$ Hz, H-3′ and H-5′) and the signals of a monosubstituted benzene ring at δ_H 7.11 (3H, m, H-3″, H-4″, and H-5″) and 7.05 (2H, m, H-2″ and H-6″). The ^{1}H NMR spectrum further exhibited signals at δ_H 5.84 (1H, dd, $J = 5.4$, 2.0 Hz, H-1), 4.06 (1H, dd, $J = 13.3$, 6.2 Hz, H-3), 2.82 (1H, dd, $J = 13.3$, 5.4 Hz, H-2β), and 2.30 (1H, ddd, $J = 13.3$, 6.2, 2.0 Hz, H-2α), typical of H-1, H-2α, H-2β, and H-3 of rocaglaol,[24] and two aromatic methoxyl groups at δ_H 3.84 and 3.77. In the HMBC spectrum of **1**, a correlation from δ_H 5.84 (H-1) to δ_C 170.5 indicated the presence of an *O*-acetyl 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-2α, H-2β, and H-3 indicated a 1α,3β configuration, as well as a *cis*-BC ring junction.[13,23,24] These relative configurations were confirmed by 2D NOESY experiments, wherein correlations were observed from H-1 to H-2α, H-2β, H-2′, and H-6′ and from H-2α 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.[15,24,25] Since the absolute stereostructure of rocaglamide has been elucidated by enantioselective total synthesis,[25−29] the absolute configuration of new rocaglamide-related compounds has been assigned by chiroptical comparison with rocaglamide as the “parent compound”.[24−29] The CD curve of **1** was very similar to that of rocaglamide, with a prominent negative Cotton effect at 218 nm as the most characteristic feature, suggesting the presence of the usual rocaglamide-analogue absolute stereostructure, with a 1*R*,3*S*,3a*R*,8b*S*-configuration. On the basis of the above evidence, the structure of **1** was characterized as 1-*O*-acetylrocaglaol.

Compound **5** was obtained as colorless needles ($CHCl_3$−MeOH, 9:1), mp 235−237 °C, [α]$^{23}_{D}$ +29.3° (*c* 1.0, MeOH). The molecular formula, $C_{27}H_{44}O_3$, was determined for this compound from the molecular ion peak at *m*/*z* 416.3207 [M]$^+$ (calcd for $C_{27}H_{44}O_3$, 416.3209) obtained by HREIMS, consistent with six degrees of unsaturation. The ^{1}H NMR spectrum in $CDCl_3$ displayed characteristic signals for five methyl groups (four singlets at δ_H 1.74, 1.64, 1.23, and 0.57, and one doublet at δ_H 0.96) and two olefinic proton signals (δ_H 5.26, br s, H-7 and 5.17, t, $J = 6.8$ Hz, H-24). Consistent with the determined molecular formula and the above ^{1}H NMR spectral data analysis, the ^{13}C and DEPT NMR spectra of **5** showed 27 carbon signals, including five methyl groups (δ_C 25.9, 18.0, 15.2, 12.7, and 11.8), three oxymethines (δ_C 73.2, 73.1, and 72.6), and four olefinic carbons (δ_C 138.9, 135.3, 121.3, and 118.0). Accordingly, compound **5** could be assigned a cholesterol-type structure.[4,20] In the COSY spectrum of **5**, a cross-peak between H-3 (δ_H 3.57) and a second oxymethine hydrogen at δ_H 3.90 (H-4) indicated that two secondary alcohol groups occupied positions 3 and 4.[20] The positions of the other functional groups were determined by a HMBC NMR experiment (Table S1; Supporting Information). Thus, long-range correlations between C-24/H-23, H-26, H-27, C-23/H-24, and C-22/H-21, H-23 suggested the location of a double bond between C-24 and C-25 and a hydroxyl group at C-22. The relative stereochemistry of **5** was determined by a NOESY NMR experiment and the comparison of chemical shift data with literature values.[4,22] Recently, the absolute stereochemistry of certain natural products was determined by a convenient Mosher ester procedure, in which the samples were treated with (*R*)- and (*S*)-MTPA-Cl in deuterated pyridine directly in NMR tubes to afford the (*S*)- and (*R*)-MTPA esters, respectively.[30] Accordingly, the reactions can be monitored by running ^{1}H NMR spectra at intervals, and the NMR data of the esters can be acquired without purification.[30] To determine the absolute stereochemistry of compound **5** by this method, the 3,4-isopropylidene derivative of **5** was prepared using dry acetone, dimethoxypropane (DMP), and camphorsulfonic acid (CSA). The 3,4-isopropylidene derivative of **5** (**5a**) was used to determine the absolute stereochemistry using the above-mentioned

Table 2. Cytotoxic Activity of Compounds Isolated from *A. rubiginosa*[a]

compound[b]	Col2	HUVEC	KB	LCNaP	Lu1	hTERT-RPE1
1-*O*-acetylrocaglaol (**1**)	189	231	147	273	210	210
methyl rocaglate (**2**)	243	203	142	325	163	183
rocagloic acid (**3**)	209	209	204	104	63	209
1-*O*-acetylmethylrocaglate (**4**)	149	205	168	224	112	206
paclitaxel[c]	46	105	0.4	5	2	23
camptothecin[c]	57	258	22	28	29	230

[a] Results are expressed as ED_{50} values (nM). Key to cell lines used: Col2 = human colon cancer; HUVEC = human umbilical vein endothelial; KB = human oral epidermoid carcinoma; LNCaP = human hormone-dependent prostate cancer; Lu1 = human lung cancer; hTERT-RPE1 = human telomerase reverse transcriptase-retinal pigment epithelial. [b] Compound **5** and the known compounds cabraleone, dammarelonic acid, desyclamide (20*S*,23*E*)-20,25-dihydroxy-3,4-secodammara-4(28),23-dienoic acid, (20*S*,23*E*)-20,25-dihydroxy-3,4-secodammara-4(28),23-dienoic acid methyl ester, (3*β*,4*β*,22*R*)-ergosta-5,24(24′)-diene-3,4,22-triol, eryodictiol, 5-hydroxy-3,7,4′-trimethoxyflavone, naringenin, ocotillone, and shoreic acid were inactive (ED_{50} values > 1 × 10^3 nM). [c] Positive control substance.

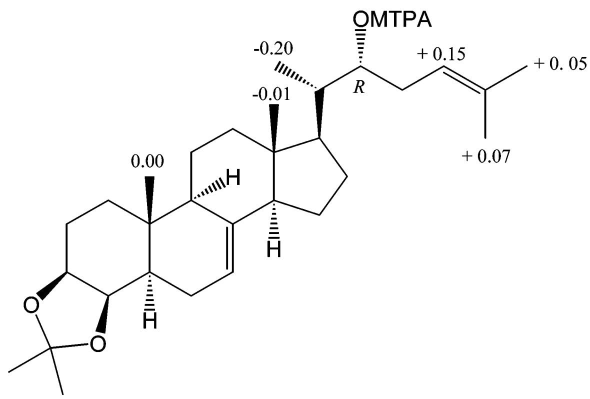

Figure 1. Distribution of Δδ (=δ*S* − δ*R*) values for compounds **5ar** and **5as** (ppm, 300 MHz).

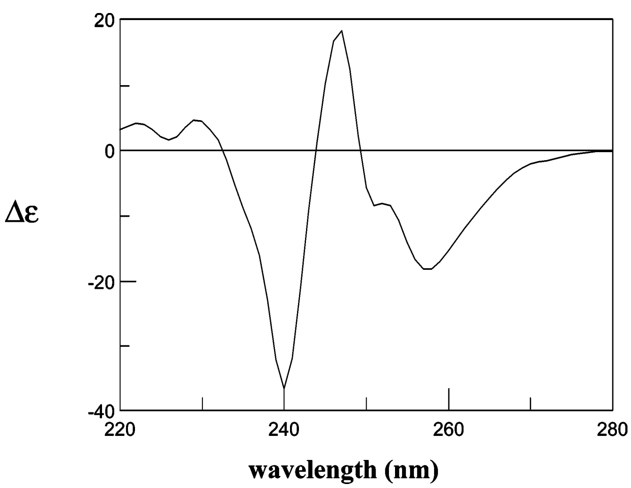

Figure 2. CD spectrum of **5b**.

Mosher ester procedure.[30] The ^{1}H NMR spectra of the diasteromeric MTPA esters (**5ar** and **5as**) of **5a** were obtained by monitoring the reaction NMR tubes directly. Although strong proton signals of the excess MTPA chlorides, MTPA acids, and DMAP were present in the spectra, most of the signals of **5as** and **5ar** were undisturbed. The absolute stereochemistry of C-22 was determined as *R* on the basis of the observed chemical shift differences (Figure 1) of **5ar** and **5as**.[31] The absolute configurations at C-3 and C-4 were determined for **5** using the CD exciton coupling method of Harada and Nakanishi.[32−34] Treatment of **5** with *p*-bromobenzoyl chloride gave a tris-*p*-bromobenzoyl derivative, **5b**. The CD spectrum of **5b** showed a typical split (Figure 2), and this established the chirality of OBz-3/OBz-4 as positive.[30−32] Thus, the stereogenic centers at C-3 and C-4 were determined as 3*S*,4*R*, respectively. On the basis of the above evidence, the structure of the new sterol **5** was established as (3*S*,4*R*,22*R*)-cholest-7,24-diene-3,4,-22-triol.

The cytotoxic activities of the compounds isolated from the twigs and the leaves of *A. rubiginosa* were evaluated against a panel of human cancer cell lines (Table 2), according to established protocols.[13,14] Compounds **1**−**4** exhibited broad cytotoxic activity, with ED_{50} values in the range 63−325 nM (Table 2). Compound **5** and the other nine known compounds tested were noncytotoxic (ED_{50} values ≥1 × 10^3 nM for all cell lines).

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. IR spectra were run on a ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectral data were recorded on Bruker Advance DPX-300 and Varian 360 MHz spectrometers with tetramethylsilane (TMS) as internal standard. EIMS and HREIMS were recorded on a Finnigan MAT-90 instrument operating at 70 eV. A YMC-pack ODC-AQ column (5 mm, 15 × 2 cm i.d., YMC Co., Wilmington, NC) and a YMC-guardpack ODC-AQ guard column (5 mm, 5 × 2 cm i.d.) were used for preparative HPLC, along with two Waters 515 HPLC pumps and a Waters 2487 dual *λ* absorbance detector (Waters, Millford, MA). Column chromatography was carried out with Si gel G (Merck 230−400 mesh). Analytical thin-layer chromatography (TLC) was performed on precoated 250 *μ*m thickness Merck Si gel 60 F_{254} aluminum plates, while preparative TLC was carried out on precoated 20 × 20 cm, 250 or 1000 *μ*m thickness Merck silica gel 60 F_{254} glass plates.

Plant Material. The twigs and leaves of *Aglaia rubiginosa* (Hiern) Pannell were collected in a secondary forest at Timpah District, Kapuas Regent, Central Kalimantan, Indonesia, in August 2000. The plant was identified by J.J.A. and S.R., and a voucher specimen (KP-05) was deposited at the Herbarium Bogoriense, Bogor, Indonesia.

Extraction and Isolation. The dried and milled twigs of *A. rubiginosa* (825 g) were extracted by maceration with MeOH three times at room temperature, for up to 3 days each. The resultant extracts were combined, concentrated under a vacuum, dissolved in MeOH (500 mL), and washed with hexane (3 × 500 mL). The lower layer was dried under reduced pressure to produce a residue (45.3 g), which was partitioned between 5% aqueous H_2O (500 mL) and $CHCl_3$ (3 × 500 mL). The $CHCl_3$-soluble extract [68.4 g; ED_{50} 2.7 *μ*g/mL against the KB (human oral epidermoid carcinoma) cell line] was subjected to silica gel (375 g) column chromatography and eluted with a gradient mixture of $CHCl_3$−MeOH (1:0 → 0:1, 250 mL per fraction) to give 10 pooled fractions. Fractions 5−7 were active when tested against the KB cell line (ED_{50} 0.1, 0.3, and 0.8 *μ*g/mL, respectively). Fraction 5, eluted with $CHCl_3$−MeOH (10:1), was purified by semipreparative HPLC eluting with CH_3CN−H_2O (75:25; 8.2 mL/min), to afford compound **1** (t_R =

23.2 min, 6 mg), methyl rocaglate (**2**; t_R = 25.7, 3 mg), and 1-*O*-acetylmethyl rocaglate (**4**; t_R = 28.9 min, 2 mg). Fraction 6, eluted with $CHCl_3$−MeOH (9:1), was chromatographed over a silica gel column (2.8 × 35 cm), using hexane−EtOAc−MeOH (1:1:0.1) as solvent system, to give additional amounts of methyl rocaglate (**2**, 2.2 mg) and rocagloic acid (**3**, 3.1 mg). Fraction 7, eluted with $CHCl_3$−MeOH (8:2), was chromatographed over a silica gel column (2.8 × 35 cm), eluted with $CHCl_3$−MeOH (30:1, 20:1, 15:1, 10:1, 5:1, 2:1), and afforded, in turn, desyclamide (7.6 mg), 5-hydroxy-3,7,4′-trimethoxyflavone (5.6 mg), eryodictiol (20 mg), and naringenin (12.0 mg).

The dried and milled leaves of *A. rubiginosa* (1046 g) were extracted and partitioned in the same manner as described above for the twigs. The resultant $CHCl_3$-soluble extract (88.27 g; ED_{50} 2.7 μg/mL against the KB cell line) was fractionated by silica gel (525 g) column chromatography and eluted with a gradient mixture of $CHCl_3$−MeOH (1:0 → 0:1, 250 mL per fraction). Eight fractions were collected and evaluated against the KB cell line. The active fractions 3 and 4 (ED_{50} 1.7 and 2.1 μg/mL, respectively) were combined and chomatographed over a Sephadex LH-20 (75 g, 2.5 × 40 cm) column eluted with MeOH. Subfractions 5−10 were combined (869 mg) and purified by silica gel column chromatography eluted with hexane−EtOAc−MeOH (10:10:0.1 → 1:1:0.1) to afford 40 fractions, of which combined fractions 12−20 and 25−36 were further purified over a silica gel column using the same solvent systems to yield, in turn, β-sitosterol (40 mg), (20*S*,23*E*)-20,-25-dihydroxy-3,4-secodammara-4(28),23-dienoic acid methyl ester (8.0 mg), methyl rocaglate (**2**, 4.0 mg), and 1-*O*-acetylmethyl rocaglate (**4**, 2.3 mg). A sample of the active fraction 5 (200 mg, ED_{50} 1.9 μg/mL) was purified by semipreparative HPLC, by eluting with MeOH−H_2O−CH_3COOH (75:25:0.01; 8.2 mL/min), to afford pure shoreic acid (t_R = 16.3 min, 9.0 mg), dammarenolic acid (t_R = 17.5 min, 12 mg), (3*S*,4*S*,24*R*)-cholest-7,24-diene-3,4,22-triol (**5**) (t_R = 20.4 min, 12 mg), (22*R*,3β,4β)-ergosta-5,24(24′)-diene-3,4,22-triol (t_R = 22.3 min, 8.7 mg), (20*S*,23*E*)-20,25-dihydroxy-3,4-secodammara-4(28),-23-dienoic acid (t_R = 26.9 min, 6.1 mg), cabraleone (t_R = 32.2 min, 4.3 mg), and ocotillone (t_R = 33.1 min, 5.8 mg). From the inactive fraction 7, β-sitosterol glycoside (40 mg) was obtained.

1-*O*-Acetylrocaglaol (1): colorless gum; $[\alpha]^{23}_D$ −115.2° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.12), 271 (3.20) nm; CD (*c* 0.45, MeOH) 218 (Δε −4.77), 291 (Δε +0.43) nm; IR (film) ν_{max} 2950, 2839, 1745, 1613, 1453, 1251, 1217, 1063, 755 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (90 MHz, $CDCl_3$), see Table 1; FABMS *m/z* 499 [M + Na]$^+$; HRFABMS *m/z* 499.1734 (calcd for $C_{28}H_{28}O_7Na$ [M + Na]$^+$, 499.1732).

(3*S*,4*R*,22*R*)-Cholest-7,24-diene-3,4,22-triol (5): colorless needles; 235−237 °C; $[\alpha]^{24}_D$ −29.3° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.17) nm; IR (film) ν_{max} 3429, 2960, 1450, 1363 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H 5.26 (1H, br s, H-7), 5.17 (1H, t, *J* = 6.8 Hz, H-24), 3.90 (1H, m, H-4), 3.59 (1H, m, H-22), 3.57 (1H, m, H-3), 2.36 (1H, m, H-6b), 2.03 (1H, m, H-12b), 1.90 (1H, m, H-2b), 1.80 (3H, m, H-1b, H-6a, H-20), 1.78 (2H, m, H-14, H-16b), 1.74 (3H, s, H_3-27), 1.65 (2H, m, H-9, H-23), 1.64 (3H, s, H_3-26), 1.60 (2H, m, H-2a, H-15b), 1.49 (1H, m, H-16a), 1.48 (1H, m, H-11), 1.40 (1H, m, H-15a), 1.36 (1H, m, H-5), 1.23 (3H, s, H_3-19), 1.20 (1H, m, H-12a), 1.18 (1H, m, H-17), 1.08 (1H, m, H-1a), 0.96 (3H, d, *J* = 6.6 Hz, H_3-21), 0.57 (3H, s, H_3-18); ^1H NMR (pyridine-d_5) δ 5.66 (1H, t, *J* = 7.0 Hz, H-24), 5.36 (1H, br s, H-7), 4.16 (1H, br s, H-3), 4.04 (1H, m, H-22), 3.85 (1H, m, H-4), 1.74 (3H, s, H_3-27), 1.71 (3H, s, H_3-26), 1.39 (3H, s, H_3-19), 1.28 (3H, d, *J* = 7.0 Hz, H-21), 0.62 (3H, s, H_3-18); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.9 (C, C-8), 135.3 (C, C-25), 121.3 (CH, C-7), 118.0 (CH, C-24), 73.2 (CH, C-3), 73.1 (CH, C-22), 72.6 (CH, C-4), 54.6 (CH, C-14), 53.2 (CH, C-17), 50.6 (CH, C-9), 44.3 (CH, C-5), 43.3 (CH, C-13), 41.5 (CH, C-20), 37.3 (CH_2, C-1), 37.2 (CH_2, C-12), 34.2 (C, C-10), 28.9 (CH_2, C-16), 27.2 (CH_2, C-6), 27.1 (CH_2, C-23), 25.9 (CH_2, C-2), 25.4 (CH_2, C-15), 21.0 (CH, C-11), 25.9 (CH_3, C-26), 18.0 (CH_3, C-27), 15.2 (CH_3, C-19), 12.7 (CH_3, C-21), 11.8 (CH_3, C-18); FABMS *m/z* 439 [M + Na]$^+$; HRFABMS *m/z* 439.3187 (calcd for $C_{27}H_{44}O_3Na$ [M + Na]$^+$, 439.3189); HREIMS *m/z* 416.3207 [M]$^+$ (calcd for $C_{27}H_{44}O_3$, 416.3209).

Preparation of (22*R*)-3,4-*O*,*O*′-Isopropylidenecholest-7,24-dien-22-ol (5a). To a solution in acetone (2 mL) of cholest-7,24-diene-3,4,22-triol (**5**, 5 mg) were added dimethoxypropane (DMP, 2 mL) and camphorsulfonic acid (CSA, 0.1 mg). The reaction mixture was stirred at room temperature for 12 h before being quenched with $NaHCO_3$ (5 mg), filtered, and concentrated in vacuo to give an oily residue. The residue was purified on a silica gel column using hexane−EtOAc (9:1) as eluent to yield **5a** (4.2 mg): ^1H NMR (pyridine-d_5) δ 5.67 (1H, t, *J* = 8.0 Hz, H-24), 5.33 (1H, br s, H-7), 4.16 (1H, t, *J* = 4.6 Hz, H-4), 4.10 (1H, m, H-3), 4.04 (1H, m, H-22), 1.74 (3H, s, H_3-27), 1.72 (3H, s, H_3-26), 1.58 (3H, s, O−C(C*H*$_3$)$_2$−O), 1.38 (3H, s, O−C(C*H*$_3$)$_2$−O), 1.28 (3H, d, *J* = 5.6 Hz, H_3-21), 1.19 (3H, s, H_3-19), 0.59 (3H, s, H_3-18); FABMS *m/z* 555 [M + Na]$^+$; HRFABMS *m/z* 555.1996 (calcd for $C_{31}H_{32}O_8Na$ [M + Na]$^+$, 555.1994).

Bromobenzoylation of 5. To a stirred solution of **5** (2.0 mg) and *p*-bromobenzoyl chloride (5.0 mg), in pyridine (1.5 mL), was added *N*,*N*-dimethyl-4-aminopyridine (0.5 mg). The reaction mixture was stirred at room temperature for 48 h, diluted with CH_2Cl_2 (10 mL), washed with 1 N HCl (3 × 10 mL), saturated $NaHCO_3$ solution (3 × 10 mL), and water (3 × 10 mL), and then dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The resulting mixture was purified by preparative TLC on silica gel, eluting with hexane−ethyl acetate (7:3), to yield a tris-*p*-bromobenzoyl derivative (**5b**, 1.9 mg, R_f 0.75): colorless needles; UV (MeOH) λ_{max} (log ε) 205 (4.08), 243 (3.47) nm; CD (*c* 1.2, MeOH) 240 (Δε −42.73), 247 (Δε +23.17), 257 (Δε −19.56) nm; ^1H NMR (300 MHz, $CHCl_3$) δ 7.86 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 7.73 (2H, d, *J* = 8.5 Hz, H-3″, H-5″), 7.60 (2H, d, *J* = 8.5 Hz, H-3‴, H-5‴), 7.56 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 7.53 (2H, dd, *J* = 8.5 Hz, H-2″, H-6″), 7.42 (2H, d, *J* = 8.5 Hz, H-2‴, H-6‴), 5.78 (1H, dd, *J* = 10.2, 3.5 Hz, H-4), 5.52 (1H, dd, *J* = 10.2, 3.5 Hz, H-3), 5.48 (1H, m, H-22), 5.33 (1H, br s, H-7), 5.27 (1H, t, *J* = 8.0, H-24), 1.71 (3H, s, H_3-27), 1.62 (3H, s, H_3-26), 1.17 (3H, s, H_3-19), 0.98 (3H, d, *J* = 5.6, H_3-21), 0.58 (3H, s, H_3-18); FABMS *m/z* 985 [M + Na]$^+$; HRFABMS *m/z* 985.1292 (calcd for $C_{48}H_{53}Br_3O_6Na$ [M + Na]$^+$, 985.1289).

Preparation of the (*R*)- and (*S*)-Mosher Ester Derivatives of 5a. Two portions of compound **5a** (each 1.1 mg) were treated separately with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (2 μL) in deuterated pyridine (0.5 mL), directly in NMR tubes, as described previously,[30] and afforded the (*R*)- and (*S*)-MTPA ester, respectively. It was necessary to add a small amount of the catalyst *N*,*N*-dimethyl-4-aminopyridine to both tubes to accelerate the reactions. ^1H NMR data of the (*R*)-MTPA ester derivative **5ar** of **5a** (300 MHz, pyridine-d_5; data were obtained from the reaction NMR tube directly and assigned on the basis of correlations in the ^1H−^1H COSY spectrum): δ 5.41 (1H, m, H-22), 5.34 (1H, br s, H-7), 5.13 (1H, t, *J* = 8.0 Hz, H-24), 4.16 (1H, t, *J* = 4.6 Hz, H-4), 4.10 (1H, m, H-3), 1.64 (3H, s, H_3-27), 1.58 (3H, s, O−C(C*H*$_3$)$_2$−O), 1.57 (3H, s, H_3-26), 1.38 (3H, s, O−C(C*H*$_3$)$_2$−O), 1.16 (3H, s, H_3-19), 1.15 (3H, d, *J* = 5.6 Hz, H_3-21), 0.59 (3H, s, H_3-18). ^1H NMR data of the (*R*)-MTPA ester derivative **5as** of **5a** (300 MHz, pyridine-d_5): δ 5.41 (1H, m, H-22), 5.35 (1H, br s, H-7), 5.28 (1H, t, *J* = 8.0, H-24), 4.16 (1H, t, *J* = 4.6, H-4), 4.10 (1H, m, H-3), 1.69 (3H, s, H_3-27), 1.62 (3H, s, H_3-26), 1.57 (3H, s, O−C(C*H*$_3$)$_2$−O), 1.38 (3H, s, O−C(C*H*$_3$)$_2$−O), 1.17 (3H, s, H_3-19), 0.95 (3H, d, *J* = 5.6, H_3-21), 0.59 (3H, s, H_3-18).

Cell Culture Panel Bioassay. Extracts, fractions, and isolates obtained from the twigs and leaves of *A. rubiginosa* (with the exception of β-sitosterol and β-sitosterol glycoside) were evaluated for cytotoxicity against a panel of human cancer cell lines, according to established protocols.[13,14] ED_{50} values of ≥1 × 10^3 nM are regarded as inactive.

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Supporting Information Available: The ^{1}H NMR spectra of compounds **1** and **5**; CD spectrum of compound **1**; NMR spectrum of compound **5a**; ^{1}H NMR spectra of (*R*)- and (*S*)-MTPA esters (**5ar** and **5as**) of compound **5a**; table of HMBC, COSY, and NOESY data for **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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