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Cyclooxygenase-Inhibitory and Antioxidant Constituents of the Aerial Parts of *Antirhea acutata*

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Abstract—Two new compounds, (6*S*)-hydroxy-29-*nor*-3,4-*seco*-cycloart-4(30),24-dien-3-oic acid (**1**) and 8-[1-(3,4-dihydroxyphenyl)-3-methoxy-3-oxopropyl]epicatechin (**3**), were isolated by bioassay-guided fractionation from the aerial parts of *Antirhea acutata* (DC.) Urb. (Rubiaceae). Compound **1** showed moderate inhibitory activities in cyclooxygenase-1 and -2 assays (IC_{50} 43.7 and 4.7 μM, respectively), while compound **3** was active in 1,1-diphenyl-2-picrylhydrazyl free-radical and cytochrome c reduction antioxidant assays (IC_{50} 29.1 and 16.3 μM, respectively). Additionally, one further new compound was isolated, (3*S*,24*S*)-25-trihydroxy-9,19-cycloartane-29-oic acid (**2**), but this was inactive in the bioassay systems used. Compound **1** is based on the unprecedented 29-*nor*-3,4-*seco*-cycloartane skeleton.

Cancer chemoprevention is an approach to reducing cancer risk in susceptible individuals by administering specific compounds to prevent, suppress, or reverse the process of carcinogenesis.[1,2] Chemopreventive agents can include substances that reduce the production of carcinogens, chemicals that inhibit the metabolic activation of carcinogens by Phase I enzymes or enhance their detoxification by Phase I or Phase II enzymes, antioxidants that scavenge free radicals, and chemicals that trap ultimate carcinogens, preventing their interaction with DNA. This broad category of compounds is referred to as 'carcinogen-blocking agents.' Other compounds, referred to as 'suppressing agents,' appear to inhibit the carcinogenic process subsequent to initiation. They include many antioxidants present in fruits and vegetables, estrogen analogues, and cyclooxygenase and lipoxygenase inhibitors.[3,4]

Antirhea acutata (DC.) Urb. (Rubiaceae), which was collected in Puerto Rico, is characterized by its sticky yellow-green leaves and small white tubular flowers.[5] No previous biological or phytochemical investigations on this plant have been reported. In our search for naturally occurring cancer chemopreventive agents, an EtOAc-soluble extract of *A. acutata* showed significant activities in a cyclooxygenase-1 (COX-1) inhibition assay (100% inhibition at 70 μg/mL) and in a 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical antioxidant assay (IC_{50} 34.7 μg/mL). Three new compounds (**1**–**3**) were isolated by bioassay-guided fractionation and their structures including absolute stereochemistry were elucidated by spectroscopic and chemical methods. This communication deals with the isolation, structure elucidation, and biological evaluation of these compounds.

The dried aerial parts of *A. acutata*[6] (1.5 kg) were ground and extracted with MeOH (3×5 L) by maceration. After filtration and concentration, the resultant extract was suspended in 90% MeOH and then partitioned with hexane to afford a hexane-soluble syrup (35.4 g). Then, the aqueous MeOH extract was concentrated and suspended in H_2O and partitioned with EtOAc to give an EtOAc-soluble residue (90.0 g). The EtOAc-soluble extract exhibited significant inhibitory activities when evaluated against COX-1 and DPPH free-radical antioxidant assays (100% inhibition at 70 μg/mL and IC_{50} 34.7 μg/mL, respectively). Bioassay-guided

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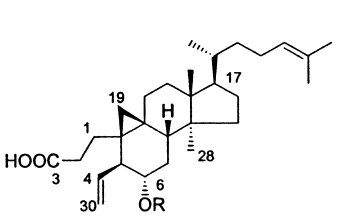

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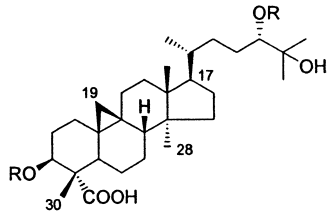
1s R = (*S*)-MTPA
1r R = (*R*)-MTPA



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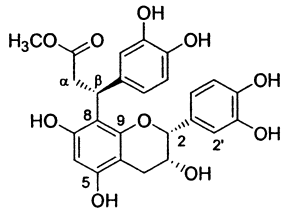
2r R = (*R*)-MTPA



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fractionation of the EtOAc-soluble extract using these in vitro assays, applying successive silica gel and reversed-phase (C_{18}) silica gel column chromatography and HPLC steps, resulted in the isolation of two new cycloartane type triterpenes (**1** and **2**) and one new phenylpropanoid-epicatechin (**3**).

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** and **2** in pyridine-d_5[a]

	δ_H		δ_C	
Position	**1**	**2**	**1**	**2**
1	1.57 m, 2.40 m		29.5	32.2
2	2.48 m, 2.74 m		32.2	30.9
3		4.79 dd (4.3, 11.7)	175.6	75.4
4	5.83 m		140.4	55.5
5	2.48 m	2.44 m	1.6	44.7
6	3.30 m		70.8	23.6
7	1.41 m, 1.64 m		35.1	28.4
8	1.58 m		47.7	47.9
9			23.7	20.6
10			30.4	26.0
11			26.2	26.7
12			32.8	33.2
13			44.9	45.6
14			48.5	49.0
15			35.7	35.6
16	1.28 m, 1.87 m		27.9	25.8
17	1.58 m		52.2	52.8
18	0.97 s	1.01 s	18.3	18.3
19	0.39 d (3.8), 0.44 d (3.8)	0.41 d (4.0), 0.68 d (3.9)	29.7	29.9
20			35.7	37.0
21	0.91 d (6.2)	1.02 d (6.4)	17.9	18.9
22	1.15 m, 1.49 m		36.2	34.5
23	0.93 m, 2.09 m		24.8	29.4
24	5.18 brt	3.75 brd (9.9)	125.3	80.0
25			130.3	72.8
26	1.60 s	1.55 s	17.3	26.0
27	1.67 s	1.58 s	25.4	26.1
28	0.94 s	0.89 s	19.3	19.5
29	—		—	180.0
30	5.21 brd, 5.35 brd	1.72 s	116.8	10.6

[a]TMS was used as the internal standard; chemical shifts are shown in the δ scale with *J* values (Hz) in parentheses.

Compound **1** was obtained as a gum and was shown to possess a molecular formula of $C_{29}H_{46}O_3$ by HREIMS. The IR spectrum of **1** indicated the presence of a hydroxyl group (3392 cm^{-1}) and a free carboxyl group (1714 cm^{-1}). The ^{1}H NMR spectrum of **1** (Table 1) exhibited a characteristic pair of doublets at δ_H 0.39 ($J = 3.8$ Hz) and δ_H 0.44 ($J = 3.8$ Hz), corresponding to the C-19 methylene protons of the cyclopropane ring of a cycloartane triterpene.[7] A complete analysis of a combination of the ^{1}H, APT, COSY, HMQC, HMBC, and TOCSY NMR spectra suggested that compound **1** is a 3,4-*seco*-cycloartane.[8,9] In particular, this was supported by salient HMBC correlations (H-1/C-3, H-2/C-3, H-4/C-6, H-30/C-4, and H-30/C-5) and TOCSY cross peaks (H-4/H-6 and H-6/H-30). However, the absence of any signal corresponding to a methyl group at C-29 and the presence of signals at δ_H 5.83 (1H, m, H-4) and δ_C 140.4 (C-4), and δ_H 5.21 (1H, brd, H-30), δ_H 5.35

Table 2. Partial ^{1}H NMR data of the (*S*)- and (*R*)-Mosher ester derivatives of compounds **1** and **2** in $CDCl_3$

	δ_H			δ_H		
Position	**1s**	**1r**	$\Delta\delta_{S-R}$	**2s**	**2r**	$\Delta\delta_{S-R}$
2				1.55	1.70	−0.15
3				5.35	5.38	*S*[a]
4	5.65	5.55	+0.10			
5	2.44	2.38	+0.05			
6	4.71	4.72	*S*[a]			
7	1.24	1.40	−0.16			
	1.48	1.54	−0.06			
23				1.38	1.44	−0.06
24				4.94	4.95	*S*[a]
26				1.16	1.14	+0.02
27				1.23	1.18	+0.05
28	0.92	0.96	−0.04			
30	5.13	4.88	+0.25	1.15	1.19	+0.04
	5.19	4.98	+0.21			

[a]Absolute configuration.

(1H, brd, H-30), and δ_C 116.8 (C-30), led to the inference that compound **1** is a 29-*nor*-3,4-*seco*-cycloartane. Unambiguous assignments of ^1H and ^{13}C signals were made using 1-D and 2-D NMR techniques. The relative stereochemistry of **1** was determined by a NOESY NMR experiment and the comparison of chemical shift data with literature values.[8,9] Important NOE correlation peaks were H-4/H-6, H-4/H-19, H-6/H-8, H-6/H-19, H-6/H-30, H-8/H-18, H-17/H-28, and H-19/H-30. The absolute configuration of the chiral centers in **1** was established using Mosher ester methodology.[10,11] Compound **1** was treated with (*R*)- and (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) to obtain the (*S*)- and (*R*)-ester C-6 analogues (**1s** and **1r**, respectively).[12] The positive values ($\Delta\delta_{S-R}$) obtained for H-4, H-5, and H-30 and the negative differences for H-7 and H-28 indicated the absolute stereochemistry of the chiral center at C-6 was *S* (Table 2). Hence, the absolute stereochemistry of all chiral centers of **1** was established as shown in the structure. Thus, compound **1** was elucidated as (6*S*)-hydroxy-29-*nor*-3,4-*seco*-cycloart-4(30),24-dien-3-oic acid.[13] There are several reports of ring-A 3,4-*seco*-cycloartanes from natural sources, even though such compounds are rare.[8,9] Compound **1** represents the first report of a 29-*nor*-3,4-*seco*-cycloartane derivative.

Compound **2** demonstrated a molecular formula of $C_{30}H_{50}O_5$ by HREIMS. The ^1H NMR spectrum of **2** (Table 1) also exhibited two characteristic doublets at δ_H 0.41 ($J=4.0$ Hz) and δ_H 0.68 ($J=3.9$ Hz), corresponding to the C-19 methylene protons of the cyclopropane ring of a cycloartane triterpene.[7] The signals at δ_H 4.79 (1H, dd, $J=4.3$ and 11.7 Hz, H-3), δ_H 3.75 (1H, brd, $J=9.9$ Hz, H-24), and δ_C 180.0 (C-29) indicated the presence of two methine protons and a carboxyl carbon, which was confirmed using the APT and HMQC NMR procedures. The positions of each functional group were determined as C-3, C-24, and C-25 (three hydroxyls) and C-29 (carboxyl) with the COSY and HMBC NMR techniques. The relative stereochemistry of **2** was also determined by NOESY correlation peaks at H-3/H-5, H-17/H-28, and H-19/H-30. The di-Mosher esters of **2** indicated the *S* configurations at C-3 and C-24, because of the negative difference values for H-2 and H-23, and the positive differences for H-30 and the two methyls (H-26 and H-27) (Table 2). Therefore, the absolute configuration of all stereogenic centers of **2** could be deduced. Thus, the structure of compound **2** was assigned as (3*S*,24*S*),25-trihydroxy-9,19-cycloartane-29-oic acid.[13]

Compound **3** exhibited a molecular formula of $C_{25}H_{24}O_{10}$ from its positive HRFABMS data (m/z $[M+Na]^+$, 507.1246). The ^1H NMR spectrum of **3** showed characteristic epicatechin protons at δ_H 4.82 (1H, brs, H-2) and δ_H 4.18 (1H, brs, H-3).[14,15] Also, the signals at δ_H 3.13 (1H, dd, $J=7.2$ and 15.5 Hz, H-α), δ_H 3.29 (1H, dd, $J=8.5$ and 15.4 Hz, H-α), δ_H 5.04 (1H, t, $J=7.8$, H-β), and δ_C 173.3 (COO) indicated the presence of a β-substituted phenylpropanoate unit.[15,16] The positions of each functional group were determined using COSY and HMBC NMR techniques. In particular, the linkage between the epicatechin (C-8) and phenylpropanoate (C-β) unit was deduced by HMBC cross peaks (H-2/C-9, H-α/C-8, H-β/C-8, and H-β/C-9). The stereochemistry of the epicatechin moiety and C-β of **3** was determined by *J* value comparison and by comparing the circular dichroism curve of **3** with literature values of several cinchonains.[14,15] Therefore, the structure of this new phenylpropanoid-epicatechin, including the absolute stereochemistry, was elucidated as 8-[1-(3,4-dihydroxyphenyl)-3-methoxy-3-oxopropyl]-epicatechin (**3**).[13]

Compounds **1**–**3** were evaluated for their potential as cancer chemopreventive agents using cyclooxygenase-1 and -2 (COX-1 and COX-2) inhibition, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical and cytochrome c reduction antioxidant assays, performed according to established protocols.[17–19] Compound **2** was inactive (IC_{50} values >70 μg/mL) in both the COX-1 and COX-2 inhibition assays. Compound **1** showed IC_{50} values of 43.7 and 4.7 μM in the COX-1 and COX-2 inhibition assays, respectively. Compound **3** exhibited moderate DPPH free-radical and cytochrome c reduction antioxidant activity with IC_{50} values of 29.1 and 16.3 μM, respectively.

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12. Preparation of (*S*)- and (*R*)-MTPA ester derivatives of compounds **1** and **2**. To solutions of compounds **1** and **2** (2 mg in a 0.5 mL of $CHCl_3$) were added sequentially pyridine (100 μL), 4-dimethylaminopyridine (0.5 mg), and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 mg). Each mixture was heated at 50 °C for 4 h under N_2 and then passed through a disposable pipet (0.6×5 cm) packed with silica gel and eluted with 5 mL of $CHCl_3$. The solvent was removed in vacuo to obtain the respective *S*-Mosher esters **1s** (1.9 mg) and **2s** (1.7 mg). Individual treatment of compounds **1** and **2** [2 mg with (*S*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride], as described above, yielded the *R*-Mosher esters **1r** (1.8 mg) and **2r** (1.6 mg), respectively (^{1}H NMR data, Table 2).
13. Physical and spectroscopic data: **(6*S*)-Hydroxy-29-*nor*-3,4-*seco*-cycloart-4(30),24-diene-3-oic acid** (**1**): gum; $[\alpha]_D^{20}$ +53.9° (*c* 0.59, MeOH); UV (EtOH) λ_{max} (log ε) 219 (2.97), 258 (2.91) nm; IR (NaCl) ν_{max} 3392, 2940, 2891, 1714, 1454, 1376, 1291 cm^{-1}; ^{1}H and ^{13}C NMR data of **1**, see Table 1; EIMS *m*/*z* 442 (16), 424 (18), 409 (22), 313 (19), 259 (18), 219 (24), 205 (27), 147 (48), 109 (55), 95 (81), 69 (100); HREIMS *m*/*z* calcd for $C_{29}H_{46}O_3$ 442.3435, found 442.3420. **(3*S*,24*S*),25-Trihydroxy-9,19-cycloartane-29-oic acid** (**2**): white powder: $[\alpha]_D^{20}$ +24.0° (*c* 0.05, MeOH); UV (MeCN) λ_{max} (log ε) 210 (3.71), 258 (3.13), 332 (2.30) nm; IR (NaCl) ν_{max} 3395, 2925, 2859, 1635, 1558, 1456, 1375 cm^{-1}; ^{1}H and ^{13}C NMR data of **2**, see Table 1; EIMS *m*/*z* 490 (6), 472 (12), 457 (16), 439 (17), 411 (20), 345 (30), 327 (33), 320 (35), 175 (55), 147 (50), 133 (64), 121 (84), 107 (100); HREIMS *m*/*z* calcd for $C_{30}H_{50}O_5$ 490.3645, found 490.3654. **8-[1-(3,4-Dihydroxyphenyl)-3-methoxy-3-oxopropyl]-epicatechin** (**3**): brown powder; $[\alpha]_D^{20}$ −17.6° (*c* 0.25, acetone); UV (MeCN) λ_{max} (log ε) 220 (4.34), 281 (3.76) nm; CD (MeOH) nm $\Delta\varepsilon_{228}$ +43.5, $\Delta\varepsilon_{252}$ −43.1, $\Delta\varepsilon_{291}$ +21.3; IR (NaCl) ν_{max} 3335, 1733, 1617, 1522, 1455, 1362, 1284, 1113 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 500 MHz) δ 2.75 (1H, dd, *J* = 2.4 and 16.8 Hz, H-4), 2.86 (1H, dd, *J* = 4.7 and 16.7 Hz, H-4), 3.13 (1H, dd, *J* = 7.2 and 15.5 Hz, H-α), 3.29 (1H, dd, *J* = 8.5 and 15.4 Hz, H-α), 3.47 (3H, s, OCH_3), 4.18 (1H, brs, H-3), 4.82 (1H, brs, H-2), 5.04 (1H, t, *J* = 7.8, H-β), 6.07 (1H, s, H-6), 6.57 (1H, d, *J* = 8.2 Hz, H-5″), 6.71 (1H, dd, *J* = 1.9 and 8.2 Hz, H-6″), 6.80 (1H, d, *J* = 8.1 Hz, H-5′), 6.87 (overlap, H-6′), 6.89 (1H, brs, H-2″), 7.11 (1H, d, *J* = 1.8 Hz, H-2′); ^{13}C NMR (Me_2CO-d_6, 125 MHz) δ 28.7 (C-4), 35.5 (C-β), 38.1 (C-α), 50.5 (OCH_3), 65.7 (C-3), 78.8 (C-2), 95.7 (C-6), 99.2 (C-10), 109.8 (C-8), 114.3 (C-5″), 114.4 (C-2′), 114.7 (C-5′), 115.4 (C-2″), 118.6 (C-6′), 119.0 (C-6″), 131.4 (C-1′), 136.6 (C-1″), 142.7 (C-4″), 144.1 (C-3″), 144.4 (C-4′), 144.5 (C-3′), 154.0 (C-7), 154.1 (C-9), 154.5 (C-7), 173.3 (COO); FABMS *m*/*z* 485 $[M+1]^+$, 453 (5), 391 (6), 333 (7), 307 (23), 289 (15), 154 (100), 136 (87); HRFABMS *m*/*z* calcd for $C_{25}H_{24}O_{10}Na$ 507.1260, found 507.1246.
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