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Novel 29-*nor*-3,4-*seco*-cycloartane triterpene methyl esters from the aerial parts of *Antirhea acutata*

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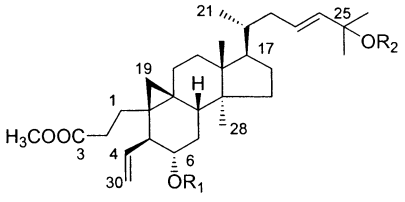
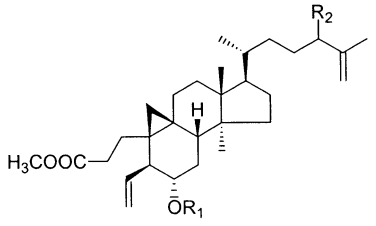
Abstract—Four novel 29-*nor*-3,4-*seco*-cycloartanes, (6*S*)-hydroxy-25-methoxy-29-*nor*-3,4-*seco*-cycloart-4(30),23-dien-3-oic acid methyl ester (**1**), (6*S*,25)-dihydroxy-29-*nor*-3,4-*seco*-cycloart-4(30),23-dien-3-oic acid methyl ester (**2**), (6*S*)-hydroxy-24-oxo-29-*nor*-3,4-*seco*-cycloart-4(30),25-dien-3-oic acid methyl ester (**3**), and (6*S*)-hydroxy-(24ξ)-hydroperoxy-29-*nor*-3,4-*seco*-cycloart-4(30),25-dien-3-oic acid methyl ester (**4**), along with a semi-synthetic acetylated derivative, (6*S*,24ξ)-diacetyloxy-29-*nor*-3,4-*seco*-cycloart-4(30),25-dien-3-oic acid methyl ester (**5**), were isolated and characterized from the aerial parts of *Antirhea acutata* (DC.) Urb. (Rubiaceae). Their structures and absolute stereochemistry were elucidated by spectroscopic and chemical methods. Compounds **1**–**5** are based on the unprecedented 29-*nor*-3,4-*seco*-cycloartane skeleton. Compound **4** showed moderate inhibitory activities in cyclooxygenase-1 and -2 assays (IC_{50} 45.7 and 18.4 μM, respectively), while the other four isolates were inactive.

1. Introduction

Members of the genus *Antirhea* (Rubiaceae) are shrubs or trees and represent about 40 species that grow in the West Indies, Central America, and East Asia.[1] The whole plant of *A. borbonica* is used in folk medicine for the treatment of cholera, diarrhea, hemorrhage, and stomach ulcers.[2] Several indole alkaloids have been reported from *A. lucida*,[3] *A. protoricensis*,[4] and *A. putaminosa*.[5] In our search for naturally occurring cancer chemopreventive agents, the aerial parts of *Antirhea acutata* (DC.) Urb.[6] were selected for detailed investigation, since its ethyl acetate-soluble extract 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 free-radical anti-oxidant assay (IC_{50} 34.7 μg/mL). Agents which inhibit the carcinogenic process subsequent to initiation, including estrogen analogues and COX inhibitors, and antioxidants that scavenge free radicals, are considered as carcinogen-suppressing and carcinogen-blocking agents, respectively.[7,8] In a preliminary communication, we have reported an initial phytochemical and biological investigation on this plant, with the isolation of a new COX-inhibitory 29-*nor*-3,4-*seco*-cycloartane (**6**), as well as a new antioxidant phenylpropanoid-epicatechin, and a new cycloartane derivative, which was not active in any of the bioassays used.[9] We currently report the isolation of five further novel 29-*nor*-3,4-*seco*-cycloartane derivatives (**1–5**) from *A. acutata*, in addition to their absolute stereochemistry and biological evaluation. Prior to our work with *A. acutata*, there have been no reports on either the biological activity or phytochemistry of this plant.



1 R_1 = H R_2 = CH_3
1s R_1 = (*S*)-MTPA R_2 = CH_3
1r R_1 = (*R*)-MTPA R_2 = CH_3
2 R_1 = H R_2 = H



3 R_1 = H R_2 = =O
4 R_1 = H R_2 = OOH
5 R_1 = $COCH_3$ R_2 = $OCOCH_3$

Keywords: *Antirhea acutata*; Rubiaceae; 29-*nor*-3,4-*seco*-cycloartane; cyclooxygenase-1 and -2.

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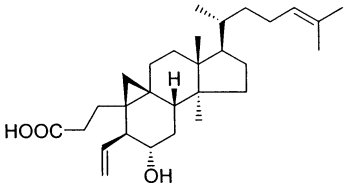
Table 1. ^{1}H NMR data for compounds **1**–**6** in $CDCl_3$

Position	1	2	3	4	5	6
1	1.20 obsc.[a]	1.35 obsc.	1.41 obsc.	1.25 obsc.	1.27 obsc.	1.37 obsc.
	1.98 obsc.	2.02 obsc.	2.06 obsc.	2.05 obsc.	2.05 obsc.	2.12 obsc.
2	2.07 obsc.	2.29 obsc.	2.30 obsc.	2.29 obsc.	2.28 obsc.	2.30 obsc.
	2.33 obsc.	2.46 obsc.	2.48 obsc.	2.45 obsc.	2.46 obsc.	2.46 obsc.
4	5.68 m	5.66 m	5.66 m	5.66 m	5.56 m	5.63 m
5	2.10 obsc.	2.09 obsc.	2.10 obsc.	2.09 obsc.	2.29 obsc.	2.01 obsc.
6	3.08 brt	3.09 brt	3.09 brt	3.09 brt	4.44 brt	3.07 brt
7	1.19 obsc.	1.17 obsc.	1.21 obsc.	1.19 obsc.	1.26 obsc.	1.18 obsc.
	1.52 obsc.	1.55 obsc.	1.55 obsc.	1.52 obsc.	1.43 obsc.	1.50 obsc.
8	1.56 obsc.	1.67 obsc.	1.65 obsc.	1.63 obsc.	1.72 obsc.	1.62 obsc.
11	1.09 obsc.	1.18 obsc.	1.19 obsc.	1.15 obsc.	1.42 obsc.	1.18 obsc.
	2.03 obsc.	2.13 obsc.	2.15 obsc.	2.13 obsc.	2.11 obsc.	2.17 obsc.
12	1.55 obsc.	1.66 obsc.	1.68 obsc.	1.65 obsc.	1.63 obsc.	1.65 obsc.
15	1.22 obsc.	1.29 obsc.	1.30 obsc.	1.31 obsc.	1.29 obsc.	1.28 obsc.
16	1.20 obsc.	1.30 obsc.	1.37 obsc.	1.31 obsc.	1.28 obsc.	1.19 obsc.
	1.84 obsc	1.93 obsc.	1.95 obsc.	1.91 obsc.	1.87 obsc.	1.90 obsc.
17	1.56 obsc.	1.60 obsc.	1.61 obsc.	1.59 obsc.	1.55 obsc.	1.59 obsc.
18	0.98 s	0.97 s	0.98 s	0.96 s	0.94 s	0.93 s
19	0.40 brs	0.40 brs	0.40 d (4.5)	0.39 d (4.3)	0.39 d (4.6)	0.36 brs
	0.42 brs	0.42 brs	0.43 d (4.5)	0.42 d (4.3)	0.49 d (4.6)	0.39 brs
20	1.39 obsc.	1.34 obsc.	1.38 obsc.	1.36 obsc.	1.40 obsc.	1.39 obsc.
21	0.88 d (6.4)	0.88 d (6.3)	0.89 d (6.4)	0.86 d (6.5)	0.86 d (6.4)	0.86 d (6.2)
22	1.68 obsc.	1.73 obsc.	2.63 obsc.	1.01 obsc.	1.15 obsc.	1.03 obsc.
	2.07 obsc.	2.17 obsc.	2.74 obsc.	1.46 obsc.	1.36 obsc.	1.48 obsc.
23	5.50 m	5.60 brs	1.25 obsc.	1.29 obsc.	1.50 obsc.	1.88 obsc.
			1.82 obsc.	1.51 obsc.	1.64 obsc.	2.00 obsc.
24	5.39 d (15.7)	5.60 brs	–	4.27 brt	5.10 (overlapped)	5.07 brt
26	1.26 s	1.31 s	5.77 brs	5.01 brs	4.86 brd	1.58 s
			5.97 brs	5.02 m	4.91 brd	
27	1.26 s	1.31 s	1.88 s	1.73 s	1.70 s	1.66 s
28	0.96 s	0.97 s	0.98 s	0.96 s	0.92 s	0.93 s
29	–	–	–	–	–	–
30	5.22 m	5.26 m	5.25 (overlapped)	5.26 m	5.08 (overlapped)	5.19 brd
	5.26 m		5.28 dd (1.8, 10.1)			5.24 brs
$COOCH_3$	3.64 s	3.65 s	3.66 s	3.65 s	3.64 s	–
OCH_3–C-25	3.02 s	–	–	–	–	–
$OCOCH_3$–C-6	–	–	–	–	1.97 s	–
$OCOCH_3$–C-24	–	–	–	–	2.03 s (2.04 s)	–

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



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2. Results and discussion

Compound **1** was obtained as a gum and shown to possess a molecular formula of $C_{31}H_{50}O_4$ by positive HRFABMS. The ^{1}H NMR spectrum of **1** (Table 1) exhibited a characteristic pair of broad singlets at δ_H 0.40 and 0.42, corresponding to the C-19 methylene protons of the cyclopropane ring of a cycloartane triterpene.[10,11] 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,[10,11] which 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 5.68 (1H, m, H-4) and δ_C 139.0 (C-4), and δ_H 5.22 (1H, m, H-30), δ_H 5.26 (1H, m, H-30), and δ_C 118.3 (C-30), led to the inference that compound **1** has the same 29-*nor*-3,4-*seco*-cycloartane skeleton as that of **6**, which was reported recently.[9] The ^{1}H- and ^{13}C NMR data of **1** (Tables 1 and 2) exhibited similar profiles to those of **6** except for the absence of signals for C-24 and C-25 and the presence of signals for a *trans* double bond at δ_H 5.50 (1H, m, H-23), δ_H 5.39 (1H, d, J=15.7 Hz, H-24), δ_C 128.2 (C-23), and δ_C 136.4 (C-24), a quaternary methoxyl signal at δ_H 3.02 (3H, s, OCH_3–C-25) and δ_C 49.9 (OCH_3–C-25), and an ester methyl signal at δ_H 3.64 (3H, s, $COOCH_3$) and δ_C 51.2 ($COOCH_3$).[10,12] The positions of each functional group were determined by the HMBC NMR experiment. Thus, the long-range correlations H-23/C-25, H-24/C-25, H-26 (H-27)/C-24, H-26 (H-27)/C-25, and OCH_3/C-25, suggested the location of a *trans* double bond between C-23 and C-24, and a methoxyl group at C-25. Additionally, the HMBC cross peak of the ester methyl signal (δ_H 3.64) with C-3 (δ_C 173.7) confirmed the presence of a methyl ester. The relative stereochemistry of **1** was determined by a NOESY NMR experiment (H-4/H-6, H-4/H-19, H-6/H-19, H-6/H-30, and H-19/H-30) and the comparison of chemical shift data with literature values.[9,11] The absolute

Table 2. ^{13}C NMR data for compounds **1–6** in $CDCl_3$

Position	1	2	3	4	5	6
1	28.8	29.1	29.1	29.1	28.8	29.0
2	31.2	31.5	31.5	31.5	31.5	31.6
3	173.7	174.1	174.1	174.1	173.8	178.1
4	139.0	139.2	139.2	139.2	138.3	139.1
5	51.7	52.3	52.4	52.3	48.4	52.0
6	70.6	70.8	70.8	70.8	73.8	71.0
7	33.2	33.1	33.1	33.1	31.4	33.2
8	47.3	47.5	47.5	47.5	47.2	47.5
9	23.5	23.8	23.8	23.8	23.5	22.7
10	30.0	30.3	30.3	30.3	30.2	30.2
11	26.4	26.8	26.8	26.8	26.6	26.7
12	32.4	32.8	32.8	32.8	32.6	32.7
13	44.8	45.1	45.1	45.1	45.0	45.0
14	48.4	48.6	48.7	48.7	48.6	48.5
15	35.5	35.8	35.8	35.8	35.6	35.6
16	27.8	28.0	28.0	27.9	27.8	28.0
17	51.8	52.0	52.3	52.1	52.1	52.2
18	18.2	18.4	18.4	18.4	18.3	18.3
19	29.4	29.6	29.7	29.6	29.4	29.6
20	35.9	36.3	35.8	35.9	35.7	35.7
21	18.0	18.2	18.1	18.1 (18.2)[a]	18.1	18.1
22	38.9	38.9	34.7	31.8 (31.9)[a]	31.3	36.1
23	128.2	125.4	30.9	27.2 (27.5)[a]	29.1 (29.3)[a]	24.8
24	136.4	139.4	202.8	90.1 (90.3)[a]	77.5 (77.9)[a]	125.1
25	74.6	70.9	144.2	143.6 (143.9)[a]	142.9 (143.2)[a]	130.8
26	25.4	29.8	124.3	114.2 (114.7)[a]	112.4 (113.1)[a]	17.6
27	25.9	29.9	17.7	16.8 (17.1)[a]	18.0	25.6
28	19.2	19.5	19.5	19.5	19.4	19.4
29	–	–	–	–	–	–
30	118.3	118.9	118.9	118.9	117.5	118.7
$COOCH_3$	51.2	51.6	51.6	51.6	51.5	–
OCH_3–C-25	49.9	–	–	–	–	–
$OCOCH_3$–C-6	–	–	–	–	170.5, 21.2	–
$OCOCH_3$–C-24	–	–	–	–	170.2, 21.2	–

TMS was used as the internal standard; chemical shifts are shown in the δ scale.
[a] Assignment between paired signals interchangeable.

configuration of the chiral centers in **1** was established using Mosher ester methodology.[13,14] The Mosher esters of **1** indicated the *S* configuration at C-6, because of the negative difference values for H-7, and the positive differences for H-4, H-5, and H-30 (Table 3). Accordingly, the absolute configuration of all stereogenic centers of **1** could be deduced. Therefore, the structure of compound **1** was assigned as (6*S*)-hydroxy-25-methoxy-29-*nor*-3,4-*seco*-cycloart-4(30),23-dien-3-oic acid methyl ester.

The ^1H- and ^{13}C NMR spectra of compound **2** (Tables 1 and 2) were almost superimposable with those of compound **1**, except for the absence of a C-25 methoxyl signal. This was consistent with the molecular formula ($C_{30}H_{48}O_4Na$; HRFABMS, *m/z* 495.3415) obtained. Also, the chemical shift differences for the *trans* double bond and C-25 at δ_C 125.4 (C-23), δ_C 139.4 (C-24), and δ_C 70.9 (C-25) for **2** and at δ_C 128.2 (C-23), δ_C 136.4 (C-24), and δ_C 74.6 (C-25) for **1** supported this inference.[10,12] The HMBC correlations H-1/C-3, H-4/C-6, H-23/C-25, H-24/C-25, H-26/C-24, H-26/C-25, and H-30/C-5 confirmed the position of each functional group. The relative stereochemistry was inferred by NOE cross peaks (H-4/H-6, H-4/H-19, H-6/H-19, and H-19/H-30). The absolute stereochemistry of **2** was determined by comparing its CD spectrum with those of compounds **1** and **6**. Thus, the structure of compound **2** was determined as (6*S*,25)-dihydroxy-29-*nor*-3,4-*seco*-cycloart-4(30),23-dien-3-oic acid methyl ester.

The ^1H- and ^{13}C NMR spectra of compound **3** (Tables 1 and 2) also showed nearly the same profiles as those of **1** and **2** indicating that **3** has the same 29-*nor*-3,4-*seco*-cycloartane methyl ester backbone. Additionally, signals for an additional olefinic methylene at δ_H 5.77 (1H, brs, H-26), δ_H 5.97 (1H, brs, H-26), and δ_C 124.3 (C-26), and a carbonyl signal at δ_C 202.8 (C-24), were observed. The downfield shifts of the C-26 signals supported the presence of carbonyl group at the C-24 position,[10,15] consistent with the molecular formula ($C_{30}H_{46}O_4Na$; HRFABMS, *m/z* 493.3315) obtained. The positions of the functional groups including the carbonyl group were confirmed by the HMBC NMR experiment. The cross peaks H-22/C-24, H-23/C-24, H-26/C-24, H-27/C-24, and H-27/C-25 confirmed the position of the carbonyl group as C-24. Also, NOE correlations between H-4/H-6, H-4/H-19, and H-6/H-19 suggested the relative stereochemistry of **3**. The CD spectrum displayed the same profile as those of compounds **1** and **6**. Therefore, the structure of compound **3** was assigned as (6*S*)-hydroxy-24-oxo-29-*nor*-3,4-*seco*-cycloart-4(30),25-dien-3-oic acid methyl ester.

In the ^1H- and ^{13}C NMR spectra of compound **4** (Tables 1 and 2), characteristic signals for a hydroperoxyl group at δ_H 4.27 (1H, brt, H-24) and δ_C 90.1 (90.3, C-24) were observed.[10,15,16] The molecular formula was determined by positive HRFABMS ($C_{30}H_{48}O_5Na$, *m/z* 511.3431). Further evidence for the presence of a hydroperoxyl group was

Table 3. Partial ^{1}H NMR data of the (*S*)- and (*R*)-Mosher ester derivatives of compound **1** in $CDCl_3$

	δ_H		
Position	**1s**	**1r**	$\Delta\delta_{S-R}$
4	5.95	5.76	+0.19
5	2.67	2.62	+0.05
6	5.02	5.02	*S*[a]
7	1.40	1.59	−0.19
	1.75	1.80	−0.05
30	5.29	5.05	+0.24

[a] Absolute configuration.

proved by a color test with acidified ferrous thiocyanate reagent.[15] The position of this hydroperoxyl group was determined by a HMBC NMR experiment, in which HMBC cross peaks between H-26/C-24, H-26/C-27, H-27/C-24, H-27/C-25, and H-27/C-26 clearly indicated the position of this substituent as C-24. The relative stereochemistry of **4** was decided by a NOESY NMR experiment (H-4/H-6, H-4/H-19, H-6/H-19, H-6/H-30), with the absolute stereochemistry determined by comparison of its CD spectrum with those of compounds **1** and **6**. A careful analysis of its ^{13}C NMR spectrum showed that the C-21–C-27 resonances appeared as pairs of closely spaced signals, indicating that compound **4** was an inseparable mixture of 24*R* and 24*S* epimers.[10,15] Thus, the structure of compound **4** was elucidated as (6*S*)-hydroxy-(24ξ)-hydroperoxy-29-*nor*-3,4-*seco*-cycloart-4(30),25-dien-3-oic acid methyl ester.

The ^{1}H- and ^{13}C NMR spectra of chromatographic fraction F017 indicated that it contained a 29-*nor*-3,4-*seco*-cycloartane derivative with some impurities. Since all attempts to purify the natural product utilizing standard methodology were unsuccessful, F017 was acetylated under the usual reaction conditions. The HRFABMS of the peracetate **5** showed a molecular ion peak at *m/z* 579.3646, which corresponded to a molecular formula of $C_{34}H_{52}O_6Na$. The ^{1}H- and ^{13}C NMR spectra of **5** (Tables 1 and 2) displayed the same profiles as those of **4** except for the additional two acetyl signals of **5**. The signals at δ_H 4.27 (1H, brt, H-24) and δ_C 90.1 (90.3, C-24) were shifted to δ_H 5.10 (overlapped, H-24) and δ_C 77.5 (77.9, C-24) in **5**, suggesting the presence of an acetyloxy substituent at the C-24 position.[17] The full structure of **5** was determined by the HMBC NMR experiment. Thus, the cross peaks H-4/C-6, H-6/C-4, and H-6/O*C*OCH$_3$–C-6 and H-24/C-25, H-24/C-26, H-24/C-27, H-24/O*C*OCH$_3$–C-24, and H-27/C-24, enabled the assignment of acetoxy groups at C-6 and C-24, respectively. The relative stereochemistry was assumed to be the same as those of the 29-*nor*-3,4-*seco*-cycloartane derivatives previously mentioned because of the similar chemical shifts and coupling constants. The absolute stereochemistry of **5** was also determined by comparing its CD spectrum with those of compounds **1** and **6**. In its ^{13}C NMR spectrum, two closely spaced signals for C-23 through C-26 were also observed, indicating the presence of an inseparable mixture of 24*R* and 24*S* epimers.[10,15] Therefore, the structure of diacetate (**5**) was elucidated as (6*S*,24ξ)-diacetyloxy-29-*nor*-3,4-*seco*-cycloart-4(30),25-dien-3-oic acid methyl ester. Acetyl signals were absent in the ^{1}H- and ^{13}C NMR spectra of fraction F017. Thus, the natural form of **5** can be surmised as being the corresponding (6*S*,24ξ)-dihydroxy derivative.

All of the isolates obtained were evaluated with inhibition assays for cyclooxygenases-1 and -2 (COX-1 and COX-2).[18,19] Compound **4** inhibited COX-1 and COX-2 with IC_{50} values of 45.7 and 18.4 μM, respectively. Compounds **1**, **2**, **3**, and **5** were inactive (IC_{50} values >100 μg/mL) in both the COX-1 and COX-2 inhibition assays. The hydroperoxyl moiety of **1** and the free carboxyl group of **6**[9] might mediate in part the inhibitory activities of **1** and **6**, respectively, against COX-1 and COX-2.

Ring-A 3,4-*seco*-cycloartanes have been reported from natural sources, such as certain species of the Bromeliaceae,[10,20] Illiciaceae,[21,22] Meliaceae,[23] Pinaceae,[24] Rubiaceae,[11] and Schisandraceae,[16,25,26] even though they are rare. Such compounds include the coronalolides from *Gardenia coronaria*, which exhibited cytotoxicity against several human cancer cell lines,[11] lancilactone C from *Kadsura lancilimba*, with HIV replication inhibitory activity,[25] and nigranoic acid from *Schisandra sphaerandra*, with HIV reverse transcriptase inhibitory activity.[26] Compounds **1**–**6** are based on the unprecedented 29-*nor*-3,4-*seco*-cycloartane skeleton.

3. Experimental

3.1. General procedures

Optical rotations were determined on a Perkin–Elmer 241 polarimeter. UV spectra were recorded on a Beckman DU-7 spectrometer. CD measurements were performed using a JASCO 600 CD spectrometer. IR spectra were taken on a JASCO 410 FT-IR spectrometer. NMR spectra were measured on a Bruker DRX-500 MHz spectrometer using a 2.5 mm or a 5 mm sample tube. FABMS and HRFABMS were obtained on a VG 7070-HF instrument.

3.2. Plant material

Aerial parts of *A. acutata* were collected in Puerto Rico in July, 1999 by F. Axelrod and J. Turnquist. Voucher specimens have been deposited at the Herbarium of the Department of Biology, University of Puerto Rico-Río Piedras (accession number 10945) and at the Field Museum of Natural History, Chicago, IL (accession number 2218930).

3.3. Extraction and isolation

The dried aerial parts of *A. acutata* (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. Then, the aqueous MeOH extract was concentrated and suspended in H_2O (2 L) and partitioned with EtOAc (3×2 L) to give an EtOAc-soluble residue [92.0 g, 100% inhibition at 70 μg/mL in the cyclooxygenase-1 (COX-1) inhibition assay].[18,19] The EtOAc-soluble extract was subjected to Si gel column chromatography using $CHCl_3$–MeOH mixtures (50:1→10:1) to give fractions F001–F010. Fractions F004 and F005 were

active in the COX-1 inhibition assay (96 and 91% inhibition at 70 μg/mL, respectively). Additional Si gel chromatography of fraction F004 with hexane–EtOAc (3:1), resulted in the isolation of **6** (150 mg). Fraction F005 was chromatographed over *Si* gel eluted with petroleum ether–EtOAc (4:1), and afforded the additional fractions F011–F019. Compound **1** (80 mg) was obtained in a pure form from F012. Fraction F014 was subjected to passage over C_{18} reversed-phase Si gel using 70% MeOH in H_2O, resulting in the purification of **2** (55 mg). Compounds **3** (4 mg) and **4** (3 mg) were purified by passage of F016 and F018, respectively, over Sephadex LH-20, using MeOH for elution. Fraction F017 (10 mg) was dissolved in pyridine (5 mL), with acetic anhydride (2 mL) added, and then the mixture was stirred at the room temperature overnight. After removing reagents, the product was chromatographed over *Si* gel eluted with $CHCl_3$, resulting in the purification of **5** (4 mg).

3.3.1. (6*S*)-Hydroxy-25-methoxy-29-*nor*-3,4-*seco*-cycloart-4(30),23-dien-3-oic acid methyl ester (1). Gum; $[\alpha]^{20}_D$=+68.9° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (3.05), 253 (1.96) nm; CD (MeOH) nm $\Delta\epsilon_{203}$+20.0, $\Delta\epsilon_{384}$+1.2; IR (NaCl) ν_{max} 3449, 2941, 1738, 1437, 1169 cm^{-1}; ^{1}H- and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations: H-1/C-3, H-2/C-3, H-4/C-5, C-6, C-10; H-5/C-4, C-6, C-30; H-6/C-4; H-19/C-5, C-8, C-9, C-10; H-23/C-22, C-24, C-25; H-24/C-22, C-23, C-25; H-26 (H-27)/C-24, C-25; H-30/C-4, C-5; OCH_3/C-25; $COOCH_3$/C-3; NOESY correlations: H-4/H-6, H-19; H-6/H-19, H-30; H-19/H-30; FABMS *m/z* 509 $[M+Na]^+$ (100), 493 (35), 477 (10), 437 (10), 199 (20), 176 (45), 109 (95); HRFABMS *m/z* 509.3577 (calcd for $C_{31}H_{50}O_4Na$, 509.3606).

3.3.2. (6*S*,25)-Dihydroxy-29-*nor*-3,4-*seco*-cycloart-4(30),23-dien-3-oic acid methyl ester (2). Gum; $[\alpha]^{20}_D$=+41.7° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.99), 214 (3.92), 279 (3.48), 325 (3.45) nm; CD (MeOH) nm $\Delta\epsilon_{204}$ +17.7, $\Delta\epsilon_{382}$ +0.6; ^{1}H- and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations: H-1/C-3; H-2/C-3; H-4/C-6; H-6/C-4; H-23/C-20, C-25; H-24/C-25; H-26 (H-27)/C-25; H-30/C-5; $COOCH_3$/C-3; NOESY correlations: H-4/H-19; H-6/H-19; H-19/H-30; FABMS *m/z* 495 $[M+Na]^+$ (40), 437 (25), 393 (45), 359 (30), 329 (30), 176 (100); HRFABMS *m/z* 495.3415 (calcd for $C_{30}H_{48}O_4Na$, 495.3450).

3.3.3. (6*S*)-Hydroxy-24-oxo-29-*nor*-3,4-*seco*-cycloart-4(30),25-dien-3-oic acid methyl ester (3). Gum; $[\alpha]^{20}_D$=+45.0° (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.62), 218 (3.65) nm; CD (MeOH) nm $\Delta\epsilon_{202}$ +17.2, $\Delta\epsilon_{387}$ +2.0; ^{1}H- and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations: H-1/C-3; H-2/C-3; H-5/C-4, C-30; H-19/C-5, C-8, C-9, C-10; H-22/C-20, C-23, C-24; H-23/C-24; H-26/C-24, C-27; H-27/C-24, C-25, C-26; $COOCH_3$/C-3; NOESY correlations: H-4/H-19; H-6/H-8, H-19, H-30; FABMS *m/z* 493 $[M+Na]^+$ (35), 413 (10), 329 (60), 307 (15), 176 (100); HRFABMS *m/z* 493.3315 (calcd for $C_{30}H_{46}O_4Na$, 493.3293).

3.3.4. (6*S*)-Hydroxy-(24ξ)-hydroperoxy-29-*nor*-3,4-*seco*-cycloart-4(30),25-dien-3-oic acid methyl ester (4). Gum; $[\alpha]^{20}_D$=+40.6° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (3.74), 223 (3.60), 247 (3.22) nm; CD (MeOH) nm $\Delta\epsilon_{205}$ +9.1, $\Delta\epsilon_{386}$ +0.8; ^{1}H- and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations: H-1/C-3; H-2/C-3; H-4/C-6; H-6/C-4; H-19/C-5, C-8, C-9, C-10; H-26/C-24, C-27; H-27/C-24, C-25, C-26; H-30/C-5; $COOCH_3$/C-3; NOESY correlations: H-4/H-6, H-19; H-6/H-19, H-30; FABMS *m/z* 511 $[M+Na]^+$ (55), 495 (35), 481 (20), 413 (35), 365 (30), 329 (60), 301 (50), 199 (60), 176 (100); HRFABMS *m/z* 511.3431 (calcd for $C_{30}H_{48}O_5Na$, 511.3399).

3.3.5. (6*S*,24ξ)-Diacetyloxy-29-*nor*-3,4-*seco*-cycloart-4(30),25-dien-3-oic acid methyl ester (5). Gum; $[\alpha]^{20}_D$=+64.4° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.32), 227 (2.59) nm; CD (MeOH) nm $\Delta\epsilon_{205}$ +11.6, $\Delta\epsilon_{388}$ +0.5; ^{1}H- and ^{13}C NMR data, see Tables 1 and 2; HMBC correlations: H-2/C-3; H-4/C-6; H-5/C-4, C-6, C-30; H-6/C-4, C-5, O*C*OCH$_3$–C-6; H-24/C-24, C-25, C-26, O*C*OCH$_3$–C-24; H-27/C-24, C-25, C-26; $COOCH_3$/C-3; OCOCH$_3$–C-6/C-6, O*C*OCH$_3$–C-6; OCOCH$_3$–C-24/C-24, O*C*OCH$_3$–C-24; FABMS *m/z* 579 $[M+Na]^+$; HRFABMS *m/z* 579.3646 (calcd for $C_{34}H_{52}O_6Na$, 579.3662).

3.3.6. (6*S*)-Hydroxy-29-*nor*-3,4-*seco*-cycloart-4(30),24-dien-3-oic acid (6).[9] CD (MeOH) nm $\Delta\epsilon_{205}$ +32.1, $\Delta\epsilon_{383}$ +8.2.

3.4. Preparation of (*S*)- and (*R*)-MTPA ester derivatives of 1

To a solution of compound **1** (5 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). The 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 Si gel and eluted with 5 mL of $CHCl_3$. The solvent was removed in vacuo, to obtain the *S*-Mosher ester **1s** (4.5 mg). Treatment of **1** [5 mg with (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride], in a similar manner, yielded the *R*-Mosher ester **1r** (4.8 mg) (^{1}H NMR data of **1s** and **1r**, Table 3).

3.5. Inhibition assay for cyclooxygenase-1 and -2

The effect of test compounds on cyclooxygenase-1 and -2 activities were determined according to established protocols.[18,19] IC_{50} values of >100 μg/mL are regarded as inactive in either assay.

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