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Cytotoxic and anti-HIV-1 constituents of *Gardenia obtusifolia* and their modified compounds

Patoomratana Tuchinda,[a,*] Wilart Pompimon,[a] Vichai Reutrakul,[a] Manat Pohmakotr,[a] Chalobon Yoosook,[b] Natedao Kongyai,[b] Samaisukh Sophasan,[c] Kulawee Sujarit,[c] Suchart E. Upathum[d] and Thawatchai Santisuk[e]

[a]*Department of Chemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand*
[b]*Department of Microbiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand*
[c]*Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand*
[d]*Department of Biology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand*
[e]*The Forest Herbarium, Royal Forest Department, Phaholyothin Road, Bangkok 10900, Thailand*

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Abstract—5α-Cycloart-24-ene-3,23-dione (**1**), 5α-cycloart-24-ene-3,16,23-trione (**2**) and methyl 3,4-*seco*-cycloart-4(28),24-diene-29-hydroxy-23-oxo-3-oate (**3**), together with five known flavones 5,7,4′-trihydroxy-3,8-dimethoxyflavone (**4**), 5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone (**5**), 5,7,4′-trihydroxy-3,6,8-trimethoxyflavone (**6**), 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone (**7**) and 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone (**8**) have been isolated from the leaves and twigs of *Gardenia obtusifolia*. The structures were assigned on the basis of spectroscopic methods. Compounds **3**–**8** and some of the modified compounds showed significant cytotoxic activities in several mammalian cell lines, especially **8** and its diacetate **21** which exhibited potent cytotoxicities (compound **8**: P-388 0.05 μg/mL, KB 0.09 μg/mL, BCA-1 0.63 μg/mL, Lu-1 0.09 μg/mL, ASK 0.70 μg/mL; its diacetate: P-388 0.27 μg/mL, KB 0.06 μg/mL, BCA-1 0.53 μg/mL, Lu-1 0.49 μg/mL). It was also found that **5**, **8** and **21** showed antimitotic acitivity in the ASK assay. Compounds **2**, **4**, **6**, **7** and some of the modified compounds displayed interesting anti-HIV activity in the syncytium assay, but were inactive or exhibited weak activity in the HIV-1 RT assay; while compound **3** was found to be active in the HIV-1 RT assay (99.9 % inhibition at 200 μg/mL), but cytotoxic in the syncytium assay.

1. Introduction

More than 80 species in the genus *Gardenia* (Rubiaceae) are widely distributed among the tropical forests in various parts of the world. Fifteen species of *Gardenia* were reported in Thailand.[1] Several species of *Gardenia* have been recorded to be used ethnomedically in various countries primarily for abortifacient[2] and contraceptive[2–4] purposes. Some species are used as a febrifuge,[5] for the treatment of headaches[6] and as a larvicides.[7] Extracts of various *Gardenia* species showing anti-implantation and abortifacient effects,[8] as well as antiulcer,[9] antibacterial,[10] analgesic,[11] diuretic,[11] hypertensive[11] and larvicidal activity[12] have been previously reported. As part of our ongoing project on the discovery of new anti-cancer and anti-HIV agents from plants, several species of *Gardenia* have been collected from various parts of Thailand. Our previous work on *Gardenia coronaria* and *G. sootepensis*[13] has resulted in the isolation of four ring-A *seco*-cycloartane triterpenes. In this work, the chloroform fraction of *Gardenia obtusifolia* Roxb. was studied and led to the isolation of 5α-cycloart-24-ene-3,23-dione (**1**), 5α-cycloart-24-ene-3,16,23-trione (**2**), methyl 3,4-*seco*-cycloart-4(28),24-diene-29-hydroxy-23-oxo-3-oate (**3**); along with five known flavones 5,7,4′-trihydroxy-3,8-dimethoxyflavone (**4**), 5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone (**5**), 5,7,4′-trihydroxy-3,6,8-trimethoxyflavone (**6**), 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone (**7**) and 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone (**8**) (Fig. 1). Compound **1** has been isolated previously from the stem bark of *Monocyclanthus vignei*,[14] the bud exudates of Fijian *Gardenia* species[15] and the leaves of *Guarea trichilioides*,[16] while **2** and **3** are new compounds. For further structure–activity studies, the new cycloartane derivatives **9**–**15** and the known flavone derivatives **16**–**22** (Fig. 1) were prepared from the isolated compounds. The structures of all compounds were elucidated on the basis of spectroscopic methods. We herein describe the isolation, the modification and the determination of the structures, including their cytotoxic and anti-HIV activities. There have been no reports either on phytochemistry or biological activity of *G. obtusifolia* prior to our work.

Keywords: cycloartane-3-one; ring-A *seco*-cycloartane; 3-methoxyflavone; cytotoxic activity; anti-HIV activity.

* Corresponding author. Tel.: +66-2-2015159; fax: +66-2-2477050; e-mail: scptc@mahidol.ac.th

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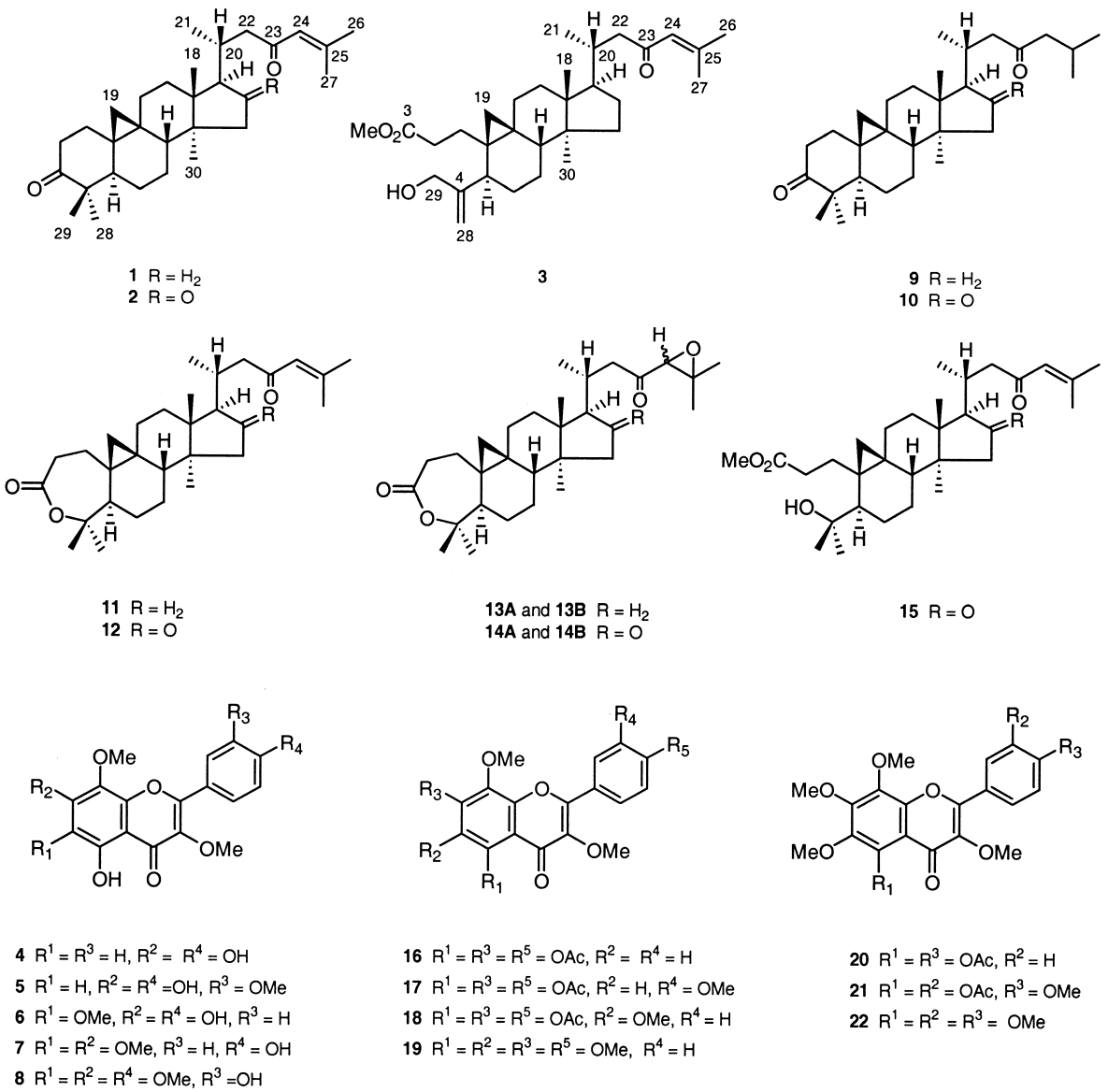


Figure 1.

2. Results and discussion

Compound **1** exhibited $[M]^+$ peak at *m/z* 438 in the EIMS corresponding to a molecular formula $C_{30}H_{46}O_2$. Its IR ($CHCl_3$) spectrum showed the absorption bands at ν_{max} 1698 cm^{-1} (for 6-membered ring ketone), 1683 cm^{-1} (for conjugated ketone), and 1616 cm^{-1} (for C=C). The ^1H NMR spectrum (Table 1) of **1** displayed a characteristic pair of doublets for cyclopropane methylene protons[17–24] at δ 0.58 and 0.79 (1H each, d, J=4.3 Hz), as well as the three singlet signals for the four methyl groups of the cycloartanone-fused moiety at δ 0.91 (3H), 1.05 (6H) and 1.10 (3H), which suggested that **1** was a normal cycloartanone-type triterpenoid. Additionally, the presence of a terminal dimethylvinyl group connected to a carbonyl group in the side chain was characterized by the low-field shifts of the signal of an olefinic proton at δ 6.06 (m), and also the signals of two geminal vinylmethyls at δ 2.15 and 1.87 (d each, J=1.1 Hz), respectively. Apart from the singlets of four tertiary methyls, a doublet of a secondary methyl group was observed at δ 0.89 (J=6.3 Hz), which was referred to the methyl at C-21. The fragmentation ions in the mass spectrum of **1** at *m/z* 438 $[M^+]$, 340, 125, 98, 83 (base peak) and 55 were useful in obtaining the structure of **1**.[15] The presence of the carbonyl group and the unsaturation in the side chain was confirmed as evidenced by the fragment ions at *m/z* 125 for $C_8H_{13}O^+$, 83 for $C_5H_7O^+$ and 55 for $C_4H_7^+$. The ions at *m/z* 340 and *m/z* 98 were the results of a McLafferty rearrangement involving the carbonyl group and the γ-hydrogen in the side chain. Compound **1** was finally proved to be 5α-cycloart-24-ene-3,23-dione by direct comparison of its ^1H and ^{13}C NMR (Table 1) spectral data with those reported in the literature.[14–16] The results from 2D NMR experiments supported the assignments of protons and carbons in the structure (see Section 5 and Table 2).

The molecular formula of compound **2** was determined as $C_{30}H_{44}O_3$ by its EIMS which showed the molecular ion peak at *m/z* 452 $[M]^+$. Compound **2** is clearly related to compound **1**, except that **2** has one extra carbonyl group in the structure. The IR ($CHCl_3$) spectrum of **2** exhibited three carbonyl stretching bands at 1727, 1700 and 1682 cm^{-1}

Table 1. 300 MHz ^{1}H- and 75 MHz ^{13}C NMR data of cycloartanes **1**–**3** ($CDCl_3$)

Position	δ_H[a]			δ_C[b]		
	1	**2**	**3**	**1**	**2**	**3**
1	(a) 1.85 (obsc.) (b) 1.53 (ddd, 14, 6.5, 2.6)	(a) 1.89 (obsc.) (b) 1.56 (obsc.)	(a) 2.16 (obsc.) (b) 1.37 (obsc.)	33.41	33.13	28.90
2	(a) 2.71 (ddd, 14, 14, 6.4) (b) 2.30 (ddd, 14, 4.3, 2.6)	(a) 2.72 (ddd, 13.9, 13.9, 6.3) (b) 2.32 (obsc.)	(a) 2.50 (obsc.) (b) 2.28 (ddd, 15.5, 11, 4.5)	37.44	37.30	31.55
3	–	–	–	216.50	215.99	174.50
4	–	–	–	50.21	50.15	152.38
5	1.71 (dd, 12.5, 4.6)	1.75 (dd, 12.2, 4.3)	2.53 (obsc)	48.42	48.30	42.07
6	(a) 1.56 (obsc.) (b) 0.95 (dddd, 12.5, 12.5, 12.5, 2.4)	(a) 1.62 (obsc.) (b) 0.97 (dddd, 12.6, 12.6, 12.6, 2.6)	(a) 1.67 (obsc) (b) 1.03 (obsc.)	21.47	21.27	28.85
7	(a) 1.39 (obsc.) (b) 1.14 (obsc.)	(a) 1.37 (obsc.) (b) 1.21 (obsc.)	(a) 1.30 (obsc.) (b) 1.06 (obsc.)	25.83	26.18	25.21
8	1.60 (obsc)	1.69 (dd, 12.3, 4.6)	1.56 (obsc.)	47.82	47.28	47.85
9	–	–	–	21.01	20.37	21.72
10	–	–	–	25.98	26.45	27.37
11	(a) 2.02 (obsc.) (b) 1.20 (obsc.)	(a) 2.16 (obsc.) (b) 1.28 (obsc.)	(a) 2.12 (obsc.) (b) 1.23 (obsc.)	26.66	26.20	26.85
12	1.64 (obsc)	1.85 (obsc.)	1.66 (obsc.)	32.69	31.25	32.84
13	–	–	–	45.45	42.07	45.15
14	–	–	–	48.87	45.29	48.96
15	1.33 (obsc.)	(a) 2.07 (d, 18.5) (b) 2.01 (d, 18.5)	1.32 (obsc.)	35.51	50.90	35.58
16	(a) 1.90 (obsc.) (b) 1.31 (obsc.)	–	(a) 1.87 (obsc.) (b) 1.30 (obsc.)	28.34	219.26	28.24
17	1.66 (obsc.)	2.30 (obsc.)	1.63 (obsc.)	52.53	60.87	52.44
18	1.05 (s)	1.20 (s)	1.01 (obsc.)	18.12	18.95	18.19
19	(a) 0.79 (br d, 4.3, *endo*) (b) 0.58 (d, 4.3, *exo*)	(a) 0.86 (br d, 4.4, *endo*) (b) 0.65 (d, 4.4, *exo*)	(a) 0.73 (br d, 4.4, *endo*) (b) 0.48 (d, 4.4, *exo*)	29.53	30.04	30.20
20	2.04 (obsc.)	2.33 (obsc.)	2.04 (obsc.)	33.39	27.40	33.39
21	0.89 (d, 6.3)	0.99 (d, 5.7)	0.88 (d, 6.1)	19.29	20.21	19.28
22	(a) 2.51 (dd, 14.4, 2.3) (b) 2.12 (obsc.)	(a) 3.20 (m) (b) 2.34 (obsc.)	(a) 2.51 (obsc.) (b) 2.13 (obsc.)	51.72	49.98	51.67
23	–	–	–	201.52	200.71	201.59
24	6.06 (m)	6.11 (m)	6.06 (m)	124.40	124.40	124.32
25	–	–	–	154.61	154.27	154.67
26	2.15 (d, 1.1)	2.14 (d, 1.0)	2.14 (d, 1.3)	20.65	20.72	20.62
27	1.87 (d, 1.1)	1.88 (d, 1.3)	1.89 (d, 1.1)	27.65	27.60	27.63
28	1.05 (s)	1.06 (s)	(a) 5.11 (br dd, 2.9, 1.5) (b) 5.08 (br s)	22.17	22.15	110.37
29	1.10 (s)	1.11 (s)	4.14 (br s)	20.75	20.64	64.62
30	0.91 (s)	1.10 (s)	0.94 (s)	19.37	19.70	19.31
3-OMe	–	–	3.64 (s)	–	–	51.50

[a] Chemical shift given in ppm using TMS as internal reference; multiplicities and coupling constants (Hz) are given in parentheses; obsc.=obscured signal.
[b] Chemical shift given in ppm; $CDCl_3$ signal at δ_C 77.00 as reference.

corresponding to the C=O of 5-membered ring ketone, C=O of 6-membered ring ketone and C=O of conjugated ketone, respectively. In its ^{1}H NMR spectrum, the characteristic pair of doublets at δ 0.65 and 0.86 (J=4.4 Hz) in combination with the four singlets at δ 1.06, 1.10, 1.11 and 1.20 are compatible with those obtained for a cycloartane triterpene bearing a carbonyl group at C-3. The low-field shifts of the signals of H-15a, H-15b, H-17, H-20, H-21, H-22a and H-22b (δ 2.07, 2.01, 2.30, 2.33, 0.99, 3.20 and 2.34, respectively), comparable with the data of **1**, supported the proximity of these protons to the C-16 carbonyl group (see Table 1). Compound **2** was suggested to have the same side chain as in **1**. The fragment ions in the mass spectrum at *m/z* 354, 125, 98, 83 (base peak) and 55 were in accordance with those occurred in compound **1**. The similarity of the side chain as in **1** was confirmed by the low-field signals of the vinyl proton at δ 6.11 (m) together with the two geminal vinylmethyls at δ 2.14 (d, J=1.0 Hz) and 1.88 (d, J=1.3 Hz). On the basis of 2D NMR spectral analyses (see Section 5 and Table 2), the chemical shifts in the ^{1}H and ^{13}C NMR spectra (Table 1) were assigned. Consequently, the structure of compound **2** was established as 5α-cycloart-24-ene-3,16,23-trione.

Compound **3** was obtained as a colorless oil. The IR ($CHCl_3$) spectrum of **3** exhibited the absorption bands at 1681 cm^{-1} (conjugated C=O stretching) and 1615 cm^{-1} (C=C stretching) which suggested that **3** had a conjugated ketone in the structure. The EIMS of **3** showed the $[M]^+$ at *m/z* 484, corresponding to a molecular formula of $C_{31}H_{48}O_4$. The presence of the ion peaks at *m/z* 386, 125, 98, 83 (base peak) and 55 supported the same side chain as in **1** and **2**. Compound **3** was characterized to contain an ester group by the observed C=O stretching band at 1729 cm^{-1}. The ^{13}C NMR spectral data at δ_C 201.59 and 174.50 confirmed the presence of the conjugated carbonyl and the ester groups, respectively. The ^{1}H NMR data (Table 1) of compound **3** showed signals of C-19 methylene in the cyclopropane ring of a cycloartane triterpene (δ 0.48 and 0.73, d, J=4.4 Hz), along with the signals of two tertiary methyls (δ 0.94 and 1.01, s), one secondary methyl (δ 0.88, d, J=6.1 Hz) and two geminal vinylmethyls connected to a carbonyl group (δ

Table 2. Observed HMBC correlations in compounds **1**–**3**

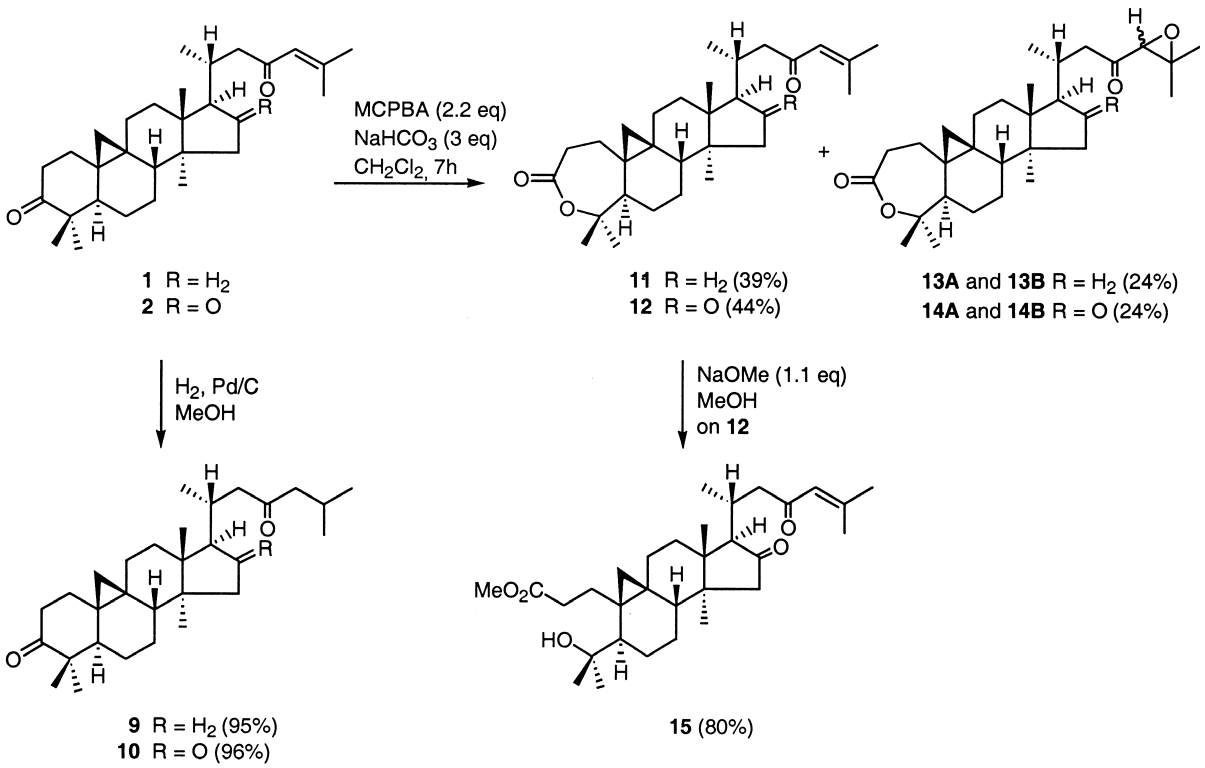
C	**1** correlated H	**2** correlated H	**3** correlated H
1	2a, 2b, 5, 19a, 19b	2b, 5, 19a, 19b	2a, 2b, 5, 19a, 19b
2	1a, 1b	1a, 1b	1a, 1b
3	1b, 2a, 2b, 28, 29	1a, 1b, 2a, 2b, 5, 28, 29	1a, 1b, 2a, 2b, 3-OMe
4	2a (w), 5, 28, 29	2a, 5, 28, 29	5, 28a, 28b, 29
5	1b, 6a, 6b, 7a, 19a, 19b, 28, 29	1a, 1b, 6a, 6b, 7a, 7b, 19b, 28, 29	1b, 6a, 7a, 7b, 19a, 19b, 28a, 28b, 29
6	5, 7a, 7b	5, 8	5, 7b, 8
7	5, 6a, 6b, 8	5, 8	6b, 8
8	1a (w), 6a, 7a, 11b, 15, 19a, 19b, 30	7b, 11b, 15a, 15b, 19a, 19b, 30	6a, 6b, 7a, 7b, 15, 19a, 19b, 30
9	1b, 5, 7a, 7b, 8, 11a, 11b, 19a, 19b	1a, 5, 7a, 7b, 8, 11a, 11b, 12, 19a, 19b	1a, 1b, 6a, 6b, 8, 12, 19a, 19b
10	1a, 1b, 2a, 2b, 5, 6a, 6b, 8, 19a, 19b	1a, 1b, 2a, 2b, 5, 6a, 8, 19a, 19b, 28 (w), 29 (w)	1a, 1b, 2a, 2b, 6a, 8, 19a, 19b
11	8, 12, 19a, 19b	8, 12	12, 19a, 19b
12	11a, 11b, 18	11a, 11b, 15, 17, 18	11a, 11b, 17, 18
13	11b, 12, 15, 16a, 17, 18, 30	8, 12, 15a, 15b, 18, 30	8, 11a, 12, 15, 16a, 16b, 17, 18, 30
14	8, 12, 16a, 16b, 17, 30	7a, 12, 15a, 15b, 17, 18, 30	8, 12, 15, 16b, 17, 18, 30
15	8, 16a, 16b, 17, 30	8, 17, 30	16b, 30
16	15	15a, 15b, 17	15, 17
17	12, 15, 18, 22a (w), 22b (w)	12, 18, 21, 22a, 22b	12, 15, 18, 22a, 22b
18	12, 17	12, 17	12, 17
19	1a, 1b, 5, 8, 11a, 11b	1a, 5, 8, 11a, 11b	1a, 5, 8, 11a, 11b
20	17, 21, 22a, 22b	17, 21, 22a, 22b	16b, 17, 21, 22a, 22b
21	17, 22a, 22b	17, 20, 22a, 22b	17, 22a, 22b
22	21	17, 20, 21	21
23	20 (w), 22a, 22b, 24	22a, 22b, 24, 26 (w), 27 (w)	22a, 22b, 24, 27 (w)
24	26, 27	26, 27	26, 27
25	26, 27	26, 27	24, 26, 27
26	24, 27	24, 27	24, 27
27	24, 26	24, 26	24, 26
28	5, 29	5, 29	5, 29
29	5, 28	5, 28	5, 28a, 28b
30	8, 15	8, 15a, 15b	8, 15, 16b
3-OMe	–	–	2b

w=weak correlation.

2.14, d, J=1.3 Hz and 1.89, d, J=1.1 Hz). Furthermore, a broad doublet of doublets (δ 5.11, J=2.9, 1.5 Hz) and a broad singlet (δ 5.08) of an sp^2 methylene of H-28 signified a terminal double bond which exhibited allylic coupling with the two-proton broad singlet of H-29 at δ 4.14. Comparable with the analogous signals in compounds **1** and **2**, the pair of doublets corresponding to H-19a and H-19b of compound **3** appeared shifted downfield. All of these data suggested a change in ring-B substitution pattern, which led to the proposal for **3** of a 3,4-*seco*-cycloartane structure with a primary alcohol group at C-29. Information from the ^{13}C and DEPT-spectra indicated that one of the methylene carbons resonated at δ 64.62, providing a strong support for the primary alcohol unit. The presence of a carbomethoxy ester group in the structure was confirmed by the methyl ester signals at δ_H 3.64 (s, COOCH_3) and δ_C 51.50 (COOCH_3). In general, the ^{13}C NMR data correlated closely with our caronalolic acid previously isolated from *G. coronaria*,[13] which differed from compound **3** only in the side chain and the methyl ester group at C-3. By complete analyses of these 2D NMR spectra (see Section 5 and Table 2) in combination with the ^1H and ^{13}C NMR data (Table 1), compound **3** was identified as methyl 3,4-*seco*-cycloart-4(28),24-diene-29-hydroxy-23-oxo-3-oate (**3**).

In order to gain more information about the structure–activity relationship, the isolated cycloartanes **1** and **2** were modified as shown in Scheme 1. It was found that the side chain was easily hydrogenated (H_2, 10% Pd/C in MeOH) and gave the corresponding **9** and **10** in 95 and 96% yields, respectively. Compounds **9** and **10** were clearly proved to be the dihydro-derivatives of **1** and **2** as evidenced by their molecular ions at m/z 440 (for $C_{30}H_{48}O_2$) and 454 (for $C_{30}H_{46}O_3$), respectively, in the EIMS. The disappearance of the olefinic proton signal of H-24 in the ^1H NMR spectrum (Table 3) of each compound indicated that compounds **1** and **2** were completely hydrogenated. The correlations observed in the COSY (see Section 5) and HMQC spectra, in combination with the HMBC (Table 5) spectral data made the assignments of the chemical shifts in the ^{13}C NMR spectra of **9** and **10** (Table 4) possible.

Since there have been few natural *seco*-cycloartanes reported and these compounds exhibited interesting biological activities, hence the oxidative transformation of ring A of the isolated cycloartanes was undertaken. The Baeyer–Villiger oxidation of cycloartanone **1** with MCPBA (1.1 equiv.) in CH_2Cl_2 at room temperature for 7 h afforded the heptanolide **11** (36% yield) and the heptanolide–epoxides **13A**, **13B** (ratio 1:1.2) as an inseparable mixture of diastereomers (3% yield), along with the recovered starting material (24% yield). However, the oxidation reaction was completed by using 2.2 equiv. of MCPBA. Under these conditions, the heptanolide **11** and the inseparable diastereomers **13A**, **13B** (ratio 1:1.2) were isolated in 39 and 24% yields, respectively. Similarly, the Baeyer–Villiger oxidation of cycloartanone **2** with 1.1 equiv. of MCPBA gave the heptanolide **12**, the inseparable diastereomers of heptanolide–epoxides **14A**, **14B** (ratio 1:1.3) and the recovered starting material in 46, 8 and 25% yields, respectively. The complete oxidation of **2** could also be achieved by treatment with 2.2 equiv. of MCPBA under the same conditions, and led to the formation of the heptanolide **12** (44% yield) and the inseparable diastereomers of heptanolide–epoxides **14A**, **14B** (ratio 1:1.3, 24% yield). The heptanolides **11** and **12** were determined to have one oxygen more than **1** and **2**, respectively, by their EIMS at m/z 454 $[M]^+$ for **11** ($C_{30}H_{46}O_3$) and m/z 468 $[M]^+$ for **12** ($C_{30}H_{44}O_4$). The IR ($CHCl_3$) spectra of both compounds showed the absorption band corresponding to the C=O stretching of 7-membered ring lactone at 1702 cm^{-1}, while the C=O of 5-membered ring ketone in **12** absorbed at 1727 cm^{-1}. The ^1H and ^{13}C NMR spectral data of **11** and **12** were quite similar to those of **1** and **2** with some differences observed in ring-A. With respect to the analogous signals observed in **1** and **2**, the downfield shifts of the C-4 *gem*-dimethyl signals (H-28: δ_H 1.40 for **11** and 1.42 for **12**; H-29: δ_H 1.46 for **11** and 1.47



Scheme 1.

for **12**) and of the C-28 signals in the ^{13}C NMR spectra at δ_C 31.01 for **11** and δ_C 30.91 for **12** were due to the γ-effect of the C-5–C-6 bond and the C-3 carbonyl in the ring-A lactone. Other connectivities were confirmed by 2D NMR correlation experiments (see Section 5 and Table 5). Cycloartanes with 7-membered lactone in ring-A have been reported from natural sources, such as kadsudilactone isolated from *Kadsura coccinea*[22] and root of *Buxus papillosa*.[25] The diastereomers A and B of heptanolide–epoxides in **13** or **14** could not be separated from each other by chromatography; they were characterized as a mixture of diastereomers. Although the structure of each diastereomer of **13** possesses two carbonyl groups, the IR spectrum ($CHCl_3$) of the mixture shows only one C=O stretching band at 1703 cm^{-1}. In this case, the C=O absorptions of both 7-membered lactone and side-chain ketone appeared at the same position. The IR spectrum ($CHCl_3$) of **14A**, **14B** mixture exhibited one extra C=O absorption band at 1725 cm^{-1}, which was assigned to the absorption of 5-membered ring ketone, while the C=O absorptions of 7-membered lactone and the side-chain ketone were also observed as a strong band at 1706 cm^{-1}. The ratios of the diastereomers **13A/13B** and **14A/14B** were determined from the integration of the separated singlet signals of H-24 in the ^1H NMR spectra. The chemical shifts of other obscured signals in the ^1H NMR spectra of both mixtures were deduced from the correlation positions observed in the 2D-spectra (see Section 5 and Table 6).

The heptanolide **12** was used as an example for the synthesis of *seco*-cycloartane. The *seco*-cycloartane **15** was obtained in 80% yield by the cleavage of the heptanolide **12** with 1.1 equiv. of NaOMe in MeOH at room temperature for 6 h. The structure of **15** was confirmed by its spectral data. The singlet of three protons at δ 3.66 and the carbon signal at δ 51.58 were referred to the methyl signals of the ester group. Information from the mass spectrum, which showed the fragment ion at *m/z* 59 ($C_3H_7O^+$), confirmed the presence of the isopropanol group in the structure of **15**. All of the signals in the ^1H and ^{13}C NMR spectra were completely assigned by analyses of the 2D NMR spectral data (see Section 5 and Table 5).

The relative stereochemistry of the isolated compounds **1**–**3** and their modified products **9**–**15** were determined by NOESY NMR experiments. The results are in agreement with the ROE correlations of nigranoic acid observed in the ROESY spectrum.[18] The NOESY correlations observed are as shown in Fig. 2.

Compounds **4**–**8** are 3-methoxyflavone derivatives. The structures were identified by direct comparison of their melting points and spectral data to the values recorded in the literature.[26–33] The acetylated and methylated derivatives **16**–**22** were prepared by using Ac_2O/4-dimethylamino-pyridine (DMAP) at room temperature and Me_2SO_4/K_2CO_3 in acetone at reflux, respectively.

3. Bioassay evaluations

Pure compounds **1**–**22** were evaluated for cytotoxic effects against a panel of cultured mammalian cell lines.[34] The results including their antimitotic activities are as shown in Table 7. Moderate to high potency of cytotoxicities were found in compounds **3**–**8**, **12**, **20** and **21**, while compounds **5**, **8** and **21** exhibited antimitotic effects in the ASK assay. It should be noted that compound **8** and its diacetate **21**

Table 3. 300 MHz ^{1}H NMR data of compounds **9**–**12** and **15** ($CDCl_3$)

Position	δ_H[a]				
	9	**10**	**11**	**12**	**15**
1	(a) 1.86 (obsc.) (b) 1.55 (obsc.)	(a) 1.89 (obsc.) (b) 1.56 (obsc.)	(a) 1.80 (obsc.) (b) 1.52 (obsc.)	(a) 1.85 (obsc.) (b) 1.57 (obsc.)	(a) 2.71 (obsc.) (b) 1.40 (obsc.)
2	(a) 2.71 (ddd, 13.9, 13.9, 6.5) (b) 2.31 (obsc.)	(a) 2.72 (ddd, 13.8, 13.8, 6.5) (b) 2.34 (obsc.)	(a) 2.71 (obsc.) (b) 2.66 (obsc.)	(a) 2.73 (obsc.) (b) 2.69 (obsc.)	(a) 2.67 (obsc.) (b) 2.25 (obsc.)
3	–	–	–	–	–
4	–	–	–	–	–
5	1.72 (dd, 12.3, 4.4)	1.76 (obsc.)	2.04 (obsc.)	2.12 (obsc.)	1.92 (obsc.)
6	(a) 1.56 (obsc.) (b) 0.96 (obsc.)	(a) 1.62 (obsc.) (b) 0.98 (obsc.)	(a) 1.81 (obsc.) (b) 0.67 (obsc.)	(a) 1.86 (obsc.) (b) 0.71 (obsc.)	(a) 1.76 (m) (b) 0.72 (obsc.)
7	(a) 1.39 (obsc.) (b) 1.14 (obsc.)	(a) 1.35 (obsc.) (b) 1.20 (obsc.)	(a) 1.32 (obsc.) (b) 1.08 (obsc.)	(a) 1.32 (obsc.) (b) 1.18 (obsc.)	(a) 1.26 (obsc.) (b) 1.10 (obsc.)
8	1.61 (obsc.)	1.70 (obsc.)	1.43 (obsc.)	1.53 (obsc.)	1.43 (obsc.)
9	–	–	–	–	–
10	–	–	–	–	–
11	(a) 2.04 (obsc.) (b) 1.18 (obsc.)	(a) 2.17 (obsc.) (b) 1.26 (obsc.)	(a) 2.06 (obsc.) (b) 1.09 (obsc.)	(a) 2.21 (obsc.) (b) 1.22 (obsc.)	(a) 2.24 (obsc.) (b) 1.30 (obsc.)
12	1.65 (obsc.)	1.86 (obsc.)	1.69 (obsc.)	1.87 (obsc.)	1.85 (obsc.)
13	–	–	–	–	–
14	–	–	–	–	–
15	1.34 (obsc.)	(a) 2.07 (d, 18.4) (b) 2.00 (d, 18.4)	1.34 (obsc.)	2.04 (br.s)	2.03 (br.s)
16	(a) 1.88 (obsc.) (b) 1.31 (obsc.)	–	(a) 1.88 (obsc.) (b)1.31 (obsc.)	–	–
17	1.64 (obsc.)	2.27 (obsc.)	1.64 (obsc.)	2.32 (obsc.)	2.30 (obsc.)
18	1.03 (s)	1.18 (s)	1.03 (s)	1.17 (s)	1.16 (s)
19	(a) 0.80 (d, 4.1) (b) 0.59 (d, 4.1)	(a) 0.86 (d, 4.4) (b) 0.66 (d, 4.4)	(a) 0.66 (obsc.) (b) 0.61 (d, 4.9)	(a) 0.73 (d, 4.6) (b) 0.69 (d, 4.6)	(a) 0.75 (d, 4.8) (b) 0.61 (d, 4.8)
20	2.05 (obsc.)	2.35 (obsc.)	2.03 (obsc.)	2.34 (obsc.)	2.32 (obsc.)
21	0.88 (d, 6.2)	0.97 (d, 5.9)	0.89 (d, 6.2)	0.98 (d, 5.7)	0.97 (d, 5.8)
22	(a) 2.44 (dd, 15.7, 2.2) (b) 2.14 (obsc.)	(a) 3.15 (m) (b) 2.38 (obsc.)	(a) 2.51 (dd, 14.7, 2.3) (b) 2.12 (obsc.)	(a) 3.22 (m) (b) 2.35 (obsc.)	(a) 3.22 (m) (b) 2.31 (obsc.)
23	–	–	–	–	–
24	2.26 (obsc.)	2.30 (obsc.)	6.06 (m)	6.11 (m)	6.11 (m)
25	2.15 (obsc.)	2.15 (obsc.)	–	–	–
26	0.92 (d, 6.7)	0.92 (d, 6.5)	2.16 (d, 1.0)	2.14 (d, 1.1)	2.14 (d, 1.1)
27	0.91 (d, 6.5)	0.91 (d, 6.6)	1.89 (d, 1.1)	1.88 (d, 1.2)	1.88 (d, 1.1)
28	1.04 (s)	1.06 (s)	1.40 (s)	1.42 (s)	1.25 (s)
29	1.10 (s)	1.11 (s)	1.46 (s)	1.47 (s)	1.23 (s)
30	0.91 (s)	1.09 (s)	0.92 (s)	1.12 (s)	1.12 (s)
3-OMe	–	–	–	–	3.66 (s)

[a] Chemical shift given in ppm using TMS as internal reference; multiplicities and coupling constants (Hz) are given in parentheses.

showed high potent cytotoxicity comparable to other compounds in Table 7.

All of the isolated and modified compounds were also tested employing HIV-1 reverse transcriptase (RT),[35] and a syncytium assay[36] using $^{\Delta Tat/Rev}$MC99 virus and 1A2 cell line system[37] (see Table 8). It was found that only **3** showed potent inhibitory activity (99.9% inhibition at 200 μg/mL) against HIV-1 RT (fagaronine chloride was used as a positive control[35]). Some of the compounds were active in the $^{\Delta Tat/Rev}$MC99 syncytium assay, using AZT as a reference.[38] However, compounds **3**, **5**, **8**, **12**, **17**, **21** and **22** were found to be very toxic to the cell lines used in this assay. It is noteworthy that compound **10**, which was modified from the isolated **2**, gave the best therapeutic index (TI>32.1; EC_{50}<3.9 μg/mL; IC_{50}>125 μg/mL) comparable with other compounds.

4. Conclusion

In conclusion, it is interesting to note that although the occurrence of cycloartane triterpenes and highly oxygenated flavonol methyl ethers have been reported in several species of plants, compounds possessing significant cytotoxic and anti-HIV activities are particularly rare. To the best of our knowledge, the structure modification of the isolated cycloartanes to obtain better biological activities has not been attempted. Our work represents an example of an investigation of both chemical and biological aspects.

5. Experimental

5.1. General procedures

Mps: uncorr.; UV: EtOH or MeOH; IR: $CHCl_3$ or KBr. NMR spectra were recorded on a Bruker DPX 300 in $CDCl_3$ using TMS as an internal standard, otherwise stated; CC was carried out on silica 60, 70–230 mesh.

5.2. Plant material

The leaves and twigs of *G. obtusifolia* Roxb. (Rubiaceae) were collected from Chiang Mai Province of Thailand in March, 1996, and identified by one of us (T. S.). A voucher

Table 4. 75 MHz ^{13}C NMR data of compounds **9**–**12** and **15** ($CDCl_3$)

Position	δ_C[a]				
	9	**10**	**11**	**12**	**15**
1	33.36	33.11	30.17	29.79	29.90
2	37.41	37.27	35.09	34.87	32.05
3	216.58	215.98	175.46	175.20	174.91
4	50.17	50.12	87.23	86.94	76.04
5	48.36	48.25	49.83	49.61	45.10
6	21.42	21.23	26.05	25.58	25.06
7	25.78	26.14	25.38	25.64	26.00
8	47.76	47.24	48.74	47.92	47.91
9	20.94	20.31	23.01	22.19	21.87
10	25.91	26.39	27.26	27.68	27.18
11	26.59	26.14	27.27	26.77	26.20
12	32.63	31.25	32.77	31.30	31.66
13	45.37	42.03	45.03	41.97	42.21
14	48.82	45.19	48.88	44.74	44.86
15	35.42	50.87	35.75	51.00	51.39
16	28.26	219.35	28.38	219.08	219.53
17	52.25	60.65	52.56	60.73	60.93
18	18.08	18.89	18.39	19.05	19.22
19	29.48	30.00	29.61	29.86	31.38
20	32.72	26.81	33.31	27.32	27.48
21	19.36	20.39	19.35	20.02	20.03
22	50.67	49.03	51.65	49.88	50.05
23	211.30	210.51	201.46	200.63	200.78
24	52.57	52.24	124.41	124.28	124.38
25	24.49	24.60	154.66	154.32	154.26
26	22.51	22.52	20.65	20.57	20.63
27	22.63	22.62	27.64	27.55	27.60
28	22.12	22.11	31.01	30.91	31.75
29	20.71	20.70	23.11	22.98	26.16
30	19.23	19.64	19.42	19.75	19.92
3-OMe	–	–	–	–	51.58

[a] Chemical shift given in ppm; $CDCl_3$ signal at δ_C 77.00 as reference.

specimen (BKF no. 092419) of *G. obtusifolia* has been deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

5.3. Extraction and isolation

The air-dried and finely powdered leaves and twigs of *G. obtusifolia* (7.9 kg) were successively extracted with methanol (75 L×21 days×5 times) at room temperature, followed by filtration. The filtrates were combined and evaporated to dryness under vacuum to afford a crude MeOH extract (1093 g). The methanol extract was suspended in water (1 L) and partitioned with $CHCl_3$ (5×2.5 L), EtOAc (3×2.5 L) and *n*-BuOH (2×2 L), respectively. Removal of solvents from each fraction yielded the $CHCl_3$ fraction (496 g), EtOAc fraction (31 g), *n*-BuOH fraction (196 g) and H_2O fraction (360 g), respectively.

The $CHCl_3$ extract (496 g) was subjected to a coarse separation on a silica gel column (1.8 kg), eluting with various proportions of CH_3COCH_3-*n*-hexane, followed by increasing amount of MeOH in CH_3COCH_3 and finally with MeOH. Fractions (500 mL each) were collected and combined on the basis of TLC behavior. Elution with 15% CH_3COCH_3-*n*-hexane yielded a fraction (115 g), which consisted of three compounds. Repeated column chromatography of this fraction (silica gel, CH_3COCH_3-*n*-hexane gradient) resulted in the isolation of three compounds. Further purification of the two isolated compounds by recrystallization gave colorless needles of 5α-cycloart-24-ene-3,23-dione (**1**) (180.1 mg) and 5α-cycloart-24-ene-3,16,23-trione (**2**) (4.11 g). Another compound was isolated as a white semi-solid and identified as methyl 3,4-*seco*-cycloart-4(28),24-diene-29-hydroxy-23-oxo-3-oate (**3**) (53.8 mg). Elution with 15–20% CH_3COCH_3-*n*-hexane led to the separation of a fraction (36.3 g). Repeated CC (silica gel, CH_3COCH_3-*n*-hexane gradient) afforded two flavones which were recrystallized with EtOH and identified as 5,7,4′-trihydroxy-3,8-dimethoxyflavone (**4**) (2.52 g) and 5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone (**5**) (1.22 g). Elution with 20–30% CH_3COCH_3-*n*-hexane yielded a semi-solid mixture (29.3 g) of three compounds. Further separation by CC (silica gel, CH_3COCH_3/*n*-hexane gradient) followed by recrystallization from EtOH resulted in the isolation of 5,7,4′-trihydroxy-3,6,8-trimethoxyflavone (**6**) (1.30 g), 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone (**7**) (1.02 g) and 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone (**8**) (59.4 mg), respectively. Elution with 30–50% CH_3COCH_3-*n*-hexane yielded a gum (95.5 g). This fraction was further purified by CC (silica gel, CH_3COCH_3-*n*-hexane gradient) twice, followed by recrystallization from EtOH to give **7** (2.98 g) and **8** (572.4 mg).

5.3.1. 5α-Cycloart-24-ene-3,23-dione (1). Colorless needles from MeOH–$CHCl_3$, mp 135.8–136.0°C (Lit.[14] 137–138°C). $[\alpha]_{589}^{26}$=+7.29 (*c* 0.85, $CHCl_3$) [Lit.[14] $[\alpha]_D^{21}$=+7 (*c* 1.14)]. UV (EtOH) λ_{max} nm (log ε): 235 (4.76). IR ($CHCl_3$) ν_{max}: 1698 (C=O stretching of 6-membered ring ketone), 1683 (C=O stretching of conjugated ketone), 1616 (C=C), 1447, 1382, 1235, 1113, 1038, 973 cm^{-1}. ^1H and ^{13}C NMR data: see Table 1. COSY correlations H/H: 1a/1b, 2a, 2b, 19a; 1b/1a, 2a, 2b; 2a/1a, 1b, 2b; 2b/1a, 1b, 2a; 5/6a, 6b, 19a; 6a/5, 7a, 7b; 6b/5, 7a; 7a/6a, 6b, 8; 7b/6a; 8/7a, 19b, 30; 11a/11b, 12; 11b/11a, 12; 12/11a, 11b, 18; 15/16a, 16b, 30; 16a/15, 16b; 16b/15, 16a; 17/16b, 18, 20; 18/12, 17, 30; 19a/1a, 5, 19b; 19b/5, 8, 19a; 20/17, 21, 22a, 22b; 21/17, 20, 22a, 22b; 22a/20, 21, 22b; 22b/20, 21, 22a; 24/26, 27; 26/24, 27; 27/24, 26. HMBC correlations: see Table 2. EIMS (70 eV) *m/z* (%): 438 $[M]^+$ (3), 423 (1), 355 (1), 340 (13), 325 (3), 313 (3), 298 (1), 285 (1), 271 (1), 255 (1), 243 (1), 231 (1), 217 (2), 202 (5), 187 (5), 175 (5), 161 (5), 147 (21), 125 (56), 98 (42), 91 (13), 83 (100), 67 (12), 55 (42). Anal. calcd for $C_{30}H_{46}O_2$: C, 82.14; H, 10.57. Found: C, 81.93; H, 10.41.

5.3.2. 5α-Cycloart-24-ene-3,16,23-trione (2). Colorless needles from EtOAc, mp 173.0–173.8°C. $[\alpha]_{589}^{26}$=−92.7 (*c* 0.85; $CHCl_3$). UV (EtOH) λ_{max} nm (log ε): 236 (4.19). IR ($CHCl_3$) ν_{max}: 1727 (C=O of 5-membered ring ketone), 1700 (C=O stretching of 6-membered ring ketone), 1682 (C=O stretching of conjugated ketone), 1618 (C=C), 1448, 1385, 1243, 1114, 1037, 974 cm^{-1}. ^1H and ^{13}C NMR data: see Table 1. COSY correlations H/H: 1a/1b, 2a, 2b, 19a; 1b/1a, 2a, 2b; 2a/1a, 1b, 2b, 29; 2b/1a, 1b, 2a, 28; 5/6a, 6b, 19a, 19b; 6a/5, 6b, 7a, 7b; 6b/5, 6a, 7a, 7b; 7a/6a, 6b, 7b; 7b/6a, 6b, 7a, 8; 8/7a, 7b, 15, 19a, 19b, 30; 11a/11b, 12, 19a, 19b; 11b/11a, 12, 19b; 12/11a, 11b, 18; 15a/15b, 17, 30; 15b/15a, 17, 30; 17/15a, 15b, 18; 18/12, 17; 19a/1a, 5, 8, 11a, 19b; 19b/1a, 5, 8, 11a, 11b, 19a; 20/21, 22a, 22b; 21/20, 22a, 22b; 22a/20, 21, 22b; 22b/20, 21, 22a; 24/26, 27; 26/24, 27; 27/24, 26; 28/2b, 5, 29; 29/2a, 28; 30/8, 15a, 15b. HMBC correlations: see Table 2. EIMS (70 eV) *m/z* (%): 452 $[M]^+$ (1), 437 (16), 381 (8), 355 (2), 354 (<1), 339 (1),

Table 5. Observed HMBC correlations in compounds **9**–**12** and **15** (in $CDCl_3$)

C	δ_H				
	9	**10**	**11**	**12**	**15**
1	2a, 2b, 5, 19a, 19b	2a, 2b, 5, 19a, 19b	2a, 2b, 5, 19a, 19b	2a, 2b, 5, 19a, 19b	2a, 2b, 5, 19a, 19b
2	1a, 1b	1a, 1b	1a, 1b	1a, 1b	1a, 1b, 19a
3	1a, 1b, 2a, 2b, 5, 28, 29	1a, 1b, 2a, 2b, 5, 28, 29	1a, 1b, 2a, 2b, 28, 29	1a, 1b, 2a, 2b, 28 (w)	1a, 1b, 2a, 2b, 3-OMe
4	2b, 5, 28, 29	2b, 5, 28, 29	5, 28, 29	5, 6b (w), 28, 29	5, 6b, 28, 29
5	1a, 1b, 6a, 6b, 7a, 7b, 19a, 19b, 28, 29	1a, 1b, 6a, 6b, 7a, 7b, 19a, 19b, 28, 29	1a, 1b, 6a, 7b, 19a, 19b, 28, 29	1a, 1b, 6a, 7a, 7b, 19a, 19b, 28, 29	19a, 19b
6	5, 7a, 7b, 8	5, 7a, 7b, 8	5, 8	5, 7a, 7b, 8	5, 8
7	5, 6a, 6b, 8	5, 6a, 6b, 8	5, 8	5, 6a, 6b, 8	5, 8
8	6a, 6b, 7a, 7b, 11a, 11b, 15, 19a, 19b, 30	6a, 6b, 7a, 7b, 11a, 11b, 15a, 15b, 19a, 19b, 30	6a (w), 7a, 7b, 11b, 19a, 19b, 30	6a (w), 7a, 7b, 15, 19a, 19b, 30	6a, 7a, 11b, 15, 19a, 19b, 30
9	5, 7a, 7b, 8, 11a, 11b, 19a, 19b	1b (w), 5, 7a, 7b, 8, 11a, 11b, 12, 19a, 19b	7b, 8, 11a, 11b, 12, 19a, 19b	1a, 1b, 7a, 7b, 8, 11a, 11b, 12, 19a, 19b	1a, 1b, 7a, 7b, 8, 11a, 11b, 12, 19a, 19b
10	1a, 1b, 2a, 2b, 5, 6a, 6b, 8, 11a, 11b, 19a, 19b	1a, 1b, 2a, 2b, 5, 6a, 6b, 8, 11a, 11b, 19a, 19b	1a, 1b, 2a, 2b, 8, 11a, 19a, 19b	1a, 1b, 2a, 2b, 5, 6a, 6b, 8, 11a	1a, 1b, 2a, 2b, 5, 6a, 19a, 19b
11	8, 12, 19a, 19b	8, 12, 19a, 19b	8, 11b, 12, 19a, 19b	8, 12, 19a, 19b	8, 12, 19a, 19b
12	11a, 11b, 17, 18	11a, 11b, 15a, 15b, 17, 18	11a, 11b, 18	11a, 11b, 18	11a, 11b, 18
13	8, 11a, 11b, 12, 15, 16a, 16b, 18, 30	8, 12, 15a, 15b, 18, 30	11b, 12, 16a, 16b, 17, 18, 30	8, 12, 15, 18, 30	8, 12, 15, 18, 30
14	8, 12, 16a, 16b, 17, 30	8, 11a, 11b, 12, 15a, 15b, 17, 18, 30	7a, 7b, 8, 12, 17, 18, 30	7b, 8 (w), 12, 15, 17, 18, 30	8, 12, 15, 17, 18, 30
15	8, 16a, 16b, 30	8, 17 (w)	16b, 30	8, 30	8, 30
16	15, 17, 21	15a, 15b, 17, 20	15	15, 17	15, 17, 20
17	12, 15, 16a, 16b, 18, 21, 30 (w)	12, 15a, 15b, 18, 20, 21, 22a, 22b	12, 18, 21	12, 15, 18, 20, 21, 22a, 22b	12, 15, 18, 21, 22a, 22b
18	12, 17	12, 17	12, 17	12, 17	12, 17
19	1a, 1b, 5, 8, 11a, 11b	1a, 1b, 5, 8, 11a, 11b	1a, 5, 8, 11a, 11b	1a, 1b, 5, 8, 11a, 11b	1b, 5, 11a, 11b
20	16a, 16b, 17, 21, 22a, 22b	17, 21, 22a, 22b	21, 22a, 22b	17, 21, 22a, 22b	17, 21, 22a
21	20, 22a, 22b	17, 20, 22a, 22b	20, 22a, 22b	17, 20, 22a, 22b	20, 22a
22	21	20, 21, 24	21	17, 20, 21	20, 21, 22
23	22a, 22b, 24, 25	21, 22a, 22b, 24, 25	22a, 22b, 24	22a, 22b, 24, 26 (w), 27 (w)	22a, 22b, 26 (w), 27 (w)
24	22a, 22b, 26, 27	25, 26, 27	22b, 26, 27	26, 27	22b (w), 26, 27
25	24, 26, 27	24, 26, 27	26, 27	26, 27	26, 27
26	24, 25, 27	24, 25, 27	24, 27	24, 27	27
27	24, 25, 26	24, 25, 26	24, 26	24, 26	26
28	5, 29	5, 29	29	5, 29	29
29	5, 28	2a, 2b, 5, 28	5, 28	5, 28	5, 28
30	15	8, 15a, 15b	8, 15	8, 15	8, 15
3-OMe	–	–	–	–	–

w=weak correlation.

313 (1), 299 (1), 269 (1), 233 (1), 203 (1), 185 (2), 177 (2), 161 (2), 147 (3), 135 (5), 125 (15), 98 (24), 91 (15), 83 (100), 69 (21), 55 (62). Anal. calcd for $C_{30}H_{44}O_3$: C, 79.60; H, 9.80. Found: C, 79.49; H, 9.61.

5.3.3. Methyl 3,4-*seco*-cycloart-4(28),24-diene-29-hydroxy-23-oxo-3-oate (3). Colorless semi-solid. $[\alpha]_{589}^{31}$= +58.7 (*c* 0.8; $CHCl_3$). UV (EtOH) λ_{max} nm (log ε): 237 (4.16). IR ($CHCl_3$) ν_{max}: 3483 (O–H stretching of alcohol), 1729 (C=O stretching of ester), 1681 (C=O stretching of conjugated ketone), 1615 (C=C), 1438, 1379, 1358, 1282, 1228, 1170, 1041, 904 cm^{-1}. ^1H and ^{13}C NMR: see Table 1. COSY correlations H/H: 1a/1b, 2a, 2b; 1b/1a, 2a, 2b; 2a/2b, 1a, 1b; 2b/1a, 1b, 2a; 5/6a, 6b, 19a, 19b; 6a/5, 6b, 7a, 7b; 6b/5, 6a, 7a, 7b; 7a/6a, 6b, 7b, 8; 7b/6a, 6b, 7a, 8; 8/7a, 7b, 19a, 19b, 30; 11a/11b, 12, 19a, 19b; 11b/11a, 12, 19b; 12/11a, 11b, 18; 15/16a, 16b; 16a/15, 16b, 17; 16b/15, 16a; 17/16a, 16b; 18/12, 17; 19a/1a, 5, 8, 11a, 19b; 19b/5, 8, 11a, 11b, 19a; 20/21, 22a, 22b; 21/20, 22a, 22b; 22a/20, 21, 22b; 22b/20, 21, 22a; 24/26, 27; 26/24, 27; 27/24, 26; 28a/28b, 29; 28b/28a, 29; 29/28a, 28b; 30/8, 15. HMBC correlations: see Table 2. EIMS (70 eV) *m/z* (%): 484 $[M]^+$ (1), 469 (9), 453 (8), 438 (5), 411 (1), 397 (3), 386 (7), 371 (12), 353 (7), 339 (3), 313 (2), 299 (3), 287 (4), 271 (2), 260 (4), 247 (5), 231 (5), 219 (5), 187 (10), 173 (12), 159 (12), 147 (38), 125 (34), 121 (38), 105 (28), 98 (13), 91 (32), 83 (100), 67 (20), 55 (55), 41 (28). HRFABMS calcd for $C_{31}H_{49}O_4$ $[M+H]^+$ 485.3618, found 485.3621.

5.3.4. 5,7,4′-Trihydroxy-3,8-dimethoxyflavone (4). Yellow needles from EtOH, mp 256.9–257.4°C after darkening at 245.1–245.6°C (Lit.[26] 259°C after darkening at 248°C, Lit.[27] 242–244°C and 254–256°C). IR (KBr) ν_{max}: 3424 (O–H stretching), 3227 (O–H stretching), 1656 (C=O stretching of conjugated ketone), 1610, 1555, 1504, 1436, 1365, 1287, 1230, 1172, 1013, 930 cm^{-1}. Anal. calcd for $C_{17}H_{14}O_7$: C, 61.82; H, 4.27. Found: C, 62.04; H, 4.27. The triacetate **16** crystallized from EtOH as colorless needles, mp 164.9–165.6°C. (Lit.[27] 167–168°C).

5.3.5. 5,7,4′-Trihydroxy-3,8,3′-trimethoxyflavone (5). Yellow needles from EtOH; mp 215.4–216.9°C (Lit.[26] 217–218°C, Lit.[27] 215–217°C, Lit.[28] 216–218°C). IR (KBr) ν_{max}: 3367 (O–H stretching), 1651 (C=O stretching of conjugated ketone), 1595, 1505, 1450, 1380, 1320, 1300, 1209, 1163, 1021, 983, 936 cm^{-1}. The triacetate **17**

Table 6. Observed HMBC correlations in compounds **13A**, **13B** and **14A**, **14B**

13A, **13B**		**14A**, **14B**	
C	Correlated H	C	Correlated H
1	2, 5, 19a, 19b	1	2, 5, 19a, 19b
2	1a, 1b	2	1a, 1b
3	1a, 1b, 2, 28	3	1a, 1b, 2, 28, 29
4	5, 28, 29	4	5, 28, 29
5 (**A**)	1a, 6a, 28, 29	5	1a, 1b, 6a, 6b, 7a, 7b, 19a, 19b, 28, 29
5 (**B**)	1a, 6a, 28, 29		
6	5, 7b, 8 (**A**), 8 (**B**)	6	5, 7a, 7b, 8
7	5, 6a, 6b	7	5, 6a, 6b, 8
8 (**A**)	6a, 7b, 19a, 19b	8 (**A**)	6a, 6b, 7a, 7b, 11b, 15, 19a, 19b, 30 (**A**)
8 (**B**)	6a, 7b, 19a, 19b	8 (**B**)	6a, 6b, 7a, 7b, 11b, 15, 19a, 19b, 30 (**B**)
9	5, 7b, 11b, 19a, 19b	9	5, 7a, 7b, 8, 11a, 11b, 12, 19a, 19b
10	2, 5, 6a, 11a, 19a, 19b	10	1a, 1b, 2, 5, 6a, 6b, 8, 11a, 19a, 19b
11	12, 19a, 19b	11	8, 12, 19a, 19b
12 (**A**)	11a, 11b, 18 (**A**)	12	11a, 11b, 17 (**A**), 17 (**B**), 18
12 (**B**)	11a, 11b, 18 (**B**)		
13 (**A**)	11b, 16a, 17 (**A**), 18 (**A**), 30	13	8, 12, 15, 18, 30 (**A**), 30 (**B**)
13 (**B**)	11b, 16a, 17 (**B**), 18 (**B**), 30		
14 (**A**)	17 (**A**), 18 (**A**)	14	7a, 12, 15, 17 (**A**), 17 (**B**), 18, 30 (**A**), 30 (**B**)
14 (**B**)	17 (**B**), 18 (**B**)		
15 (**A**)	16a, 16b, 30	15 (**A**)	8, 12, 17 (**A**), 30 (**A**)
15 (**B**)	16a, 16b, 30	15 (**B**)	8, 12, 17 (**B**), 30 (**B**)
16 (**A**)	15, 17 (**A**)	16 (**A**)	15, 17 (**A**)
16 (**B**)	15, 17 (**B**)	16 (**B**)	15, 17 (**B**)
17 (**A**)	18 (**A**), 21 (**A**), 22a, 22b, 30	17 (**A**)	12, 15, 18, 20, 21 (**A**), 22a (**A**), 22b (**A**)
17 (**B**)	18 (**B**), 21 (**B**), 22a, 22b, 30	17 (**B**)	12, 15, 18, 20, 21 (**B**), 22a (**B**), 22b (**B**)
18 (**A**)	12	18 (**A**)	12, 17 (**A**)
18 (**B**)	12	18 (**B**)	12, 17 (**B**)
19	1a, 1b, 5, 8 (**A**), 8 (**B**), 11a, 11b	19	1a, 1b, 5, 8, 11a, 11b
20 (**A**)	21 (**A**), 22a, 22b	20	17 (**A**), 17 (**B**), 21 (**A**), 21 (**B**), 22a (**A**), 22a (**B**), 22b (**A**), 22b (**B**)
20 (**B**)	21 (**B**), 22a, 22b		
21 (**A**)	22a, 22b	21	17 (**A**), 17 (**B**), 22a (**A**), 22a (**B**), 22b (**A**), 22 (**B**)
21 (**B**)	22a, 22b		
22 (**A**)	21 (**A**)	22 (**A**)	17 (**A**), 20, 21 (**A**)
22 (**B**)	21 (**B**)	22 (**B**)	17 (**B**), 20, 21 (**B**)
23 (**A**)	22a, 22b, 24 (**A**)	23 (**A**)	22a (**A**), 22b (**A**), 24 (**A**)
23 (**B**)	22a, 22b, 24 (**B**)	23 (**B**)	22a (**B**), 22b (**B**), 24 (**B**)
24 (**A**)	22b, 26 (**A**), 27	24	26 (**A**), 26 (**B**), 27
24 (**B**)	22b, 26 (**B**), 27		
25 (**A**)	24 (**A**), 26 (**A**), 27	25 (**A**)	24 (**A**), 26 (**A**), 27
25 (**B**)	24 (**B**), 26 (**B**), 27	25 (**B**)	24 (**B**), 26 (**B**), 27
26 (**A**)	24 (**A**), 27	26	24 (**A**), 24 (**B**), 27
26 (**B**)	24 (**B**), 27		
27	26 (**A**), 26 (**B**)	27 (**A**)	24 (**A**), 26 (**A**)
		27 (**B**)	24 (**B**), 26 (**B**)
28	5, 29	28	5, 29
29 (**A**)	5, 28	29	5, 28
29 (**B**)	5, 28		
30	8 (**A**), 8 (**B**), 15	30 (**A**)	8, 15
		30 (**B**)	8, 15

crystallized from EtOH as colorless plates, mp 138.6–139.4°C (Lit.[39] mp 138–139°C). HRFABMS calcd for $C_{24}H_{23}O_{11}$ $[M+H]^+$ 487.1233, found 487.1244.

5.3.6. 5,7,4′-Trihydroxy-3,6,8-trimethoxyflavone (6). Yellow needles from EtOH, mp 244.1–245.6°C (Lit.[26] 244–245°C). UV (MeOH) λ_{max} nm (log ε): 280 (3.20), 332 (3.17). IR (KBr) ν_{max}: 3424 (O–H stretching), 3227 (O–H stretching), 1656 (C=O stretching of conjugated ketone), 1609, 1555, 1504, 1436, 1365, 1287, 1230, 1172, 1012, 930 cm^{-1}. The triacetate **18** crystallized from EtOH as pale yellow needles, mp 158.1–158.9°C (Lit.[40] 159–160°C). HRFABMS calcd for $C_{24}H_{23}O_{11}$ $[M+H]^+$ 487.1233, found 487.1258. The methylated product **19** crystallized from EtOH as pale yellow needles, mp 130.1–131.5°C (Lit.[40,41] 130–131°C, Lit.[42] 131–132°C).

5.3.7. 5,4′-Dihydroxy-3,6,7,8-tetramethoxyflavone (7). Yellow needles from EtOH, mp 226.2–226.6°C (Lit.[29] mp 225–226°C). UV (MeOH) λ_{max} nm (log ε): 283 (4.70), 343 (4.73). IR (KBr) ν_{max}: 3222 (O–H stretching), 1648 (C=O stretching of conjugated ketone), 1612, 1575, 1547, 1509, 1481, 1371, 1288, 1207, 1178, 1046, 1004, 920 cm^{-1}. Anal. calcd for $C_{19}H_{18}O_8$: C, 60.96; H, 4.85. Found: C, 61.21; H, 4.99. The diacetate **20** crystallized from EtOH as pale yellow needles, mp 128.0–129.5°C. The methylated product **19** crystallized from EtOH as pale yellow needles, mp 130.1–131.5°C (Lit.[40,41] 130–131°C, Lit.[42] 131–132°C).

5.3.8. 5,3′-Dihydroxy-3,6,7,8,4′-pentamethoxyflavone (8). Yellow needles from EtOH, mp 171.6–172.2°C (Lit.[31] 170°C, Lit.[32] 169–170°C, Lit.[33] 176–177°C). IR ($CHCl_3$)

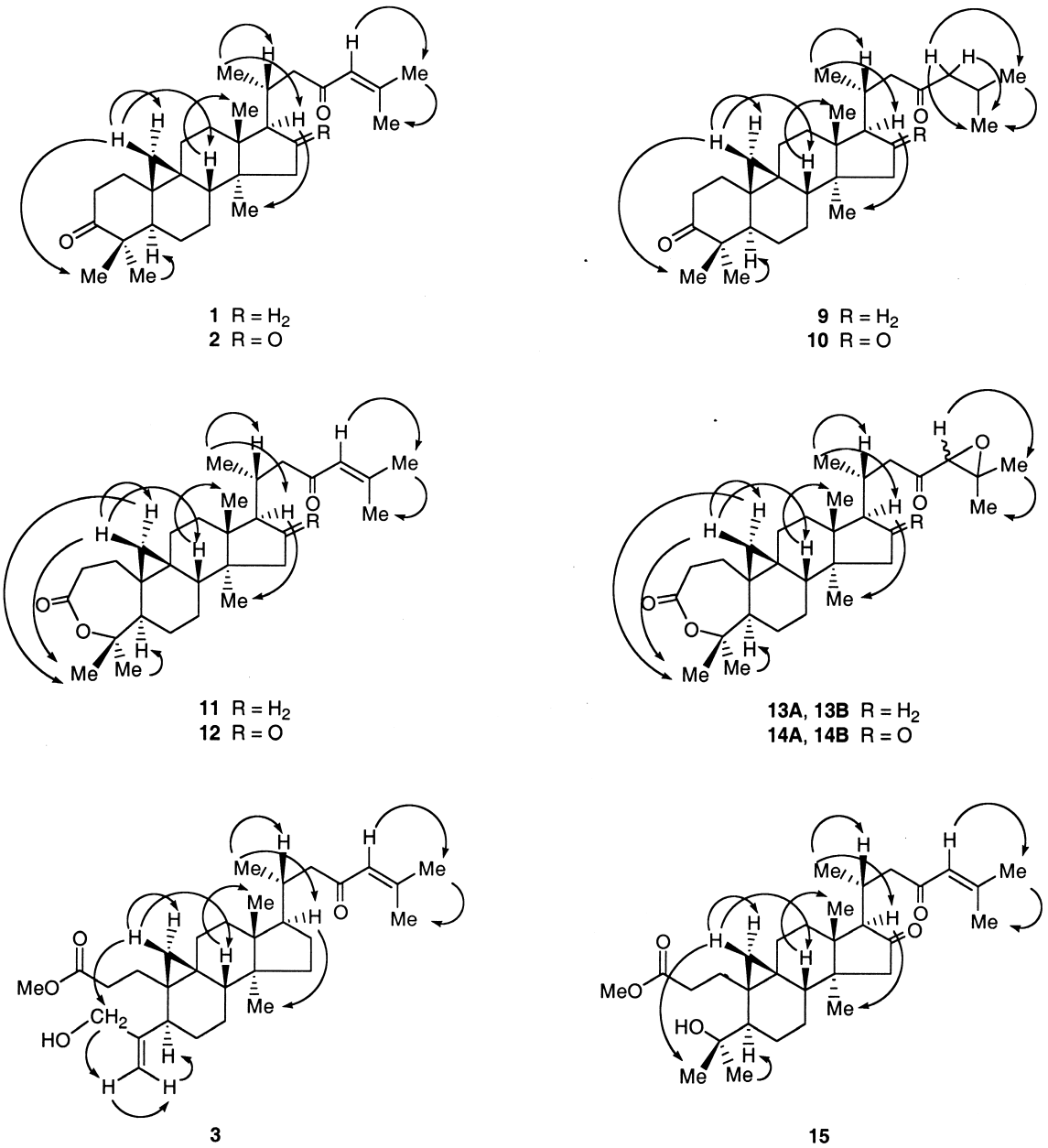


Figure 2. NOSEY correlations of the isolated **1**–**3** and the modified **9**–**15**.

ν_{max}: 3546 (O–H stretching), 1647 (C=O stretching of conjugated ketone), 1596, 1561, 1512, 1481, 1463, 1376, 1275, 1249, 1135, 1052, 1006, 981 cm^{-1}. Anal. calcd for $C_{20}H_{20}O_9$: C, 59.41; H, 4.99. Found: C, 59.61; H, 5.12. The diacetate **21** crystallized from EtOH as pale yellow needles, mp 165.8–166.3°C. The methylated product **22** crystallized from EtOH as pale yellow needles, mp 130.5–131.8°C (Lit.[43] 131–132°C).

5.4. Structure modification

5.4.1. Preparation of 5α-cycloart-24,25-dihydro-3,23-dione (9). Compound **1** (30.5 mg, 0.097 mmol) in MeOH (4 mL) was hydrogenated in the presence of 10% palladium on carbon catalyst (0.7 mg). The catalyst was filtered, and the filtrate after evaporation and recrystallization from EtOH gave **9** (29.1 mg, 95%) as colorless plates, mp 149.5–151.1°C. $[\alpha]_{589}^{30}$=+64.0 (*c* 0.05, $CHCl_3$). IR ($CHCl_3$) ν_{max}: 1702 (C=O of 6-membered ring ketone and C=O of the side-chain ketone), 1467, 1383, 1366, 1213, 1113, 1008, 916 cm^{-1}. ^1H and ^{13}C NMR data see Tables 3 and 4. COSY correlations H/H: 1a/1b, 2a, 2b, 19a; 1b/1a, 2a, 2b, 19b; 2a/1a, 1b, 2a, 29; 2b/1a, 1b, 2a, 28; 5/6a, 6b, 19a, 19b; 6a/5, 6b, 7a, 7b; 6b/5, 6a, 7a, 7b; 7a/6a, 6b, 7b; 7b/6a, 6b, 7a; 8/6b, 7a, 7b; 11a/11b, 12, 19a, 19b; 11b/11a, 12, 19b; 12/11a, 11b; 15/16a, 16b; 16a/15, 16b, 17; 16b/15, 16a, 17; 17/16a, 16b, 18, 20; 18/12, 17; 19a/1a, 5, 11a, 19b; 19b/1b, 5, 11a, 11b, 19a; 20/17, 21, 22a, 22b; 21/20, 22a; 22a/20, 21, 22b, 24; 22b/20, 22a, 24; 24/22a, 25, 26, 27; 25/24, 26, 27; 26/24, 25; 27/24, 25; 28/29; 29/28; 30/8, 15. HMBC correlations: see Table 5. EIMS (70 eV) *m/z* (%): 440 $[M]^+$ (11), 425 (6), 355 (8), 340 (66), 325 (21), 313 (8), 302 (20), 271 (6), 255 (6), 243 (6), 221 (9), 217 (12), 202 (27), 187 (23), 175 (27), 161 (28), 147 (99), 135 (40), 121 (99), 107

Table 7. Cytotoxic and antimitotic activities (ASK assay) of the isolated **1**–**8** and the modified compounds **9**–**22**

Compound	Cytotoxicity						ASK assay
	Cell line						
	P-388	KB	Col-2	BCA-1	Lu-1	ASK	
1	>20	>20	>20	>20	>20	>20	−
2	>20	>20	>20	>20	>20	>20	−
3	3.3	16.4	9.1	10.9	5.8	10.9	−
4	2.4	>20	>20	9.4	>20	16.3	−
5	3.1	14.8	15.7	2.7	13.1	5.9	+
6	13.8	>20	>20	1.2	>20	17.1	−
7	2.7	19.9	>20	4.8	13.0	3.9	−
8	0.05	0.09	8.8	0.63	0.09	0.70	+
9	13.8	>20	>20	>20	>20	>20	−
10	8.8	>20	>20	>20	>20	>20	−
11	>20	12.6	>20	>20	16.6	>20	−
12	1.8	8.7	4.2	13.3	5.9	11.2	−
13	7.3	>20	10.8	15.5	19.3	>20	−
14	>20	>20	>20	>20	>20	>20	−
15	14.0	11.6	9.4	>20	9.1	>20	−
16	5.3	>20	>20	11.7	16.9	13.1	−
17	18.3	>20	>20	>20	>20	>20	−
18	17.5	14.1	14.1	15.7	>20	14.7	−
19	>20	16.8	>20	>20	>20	>20	−
20	10.4	14.8	>20	2.7	11.4	11.1	−
21	0.27	0.06	13.0	0.53	0.49	2.36	+
22	18.1	7.00	>20	>20	>20	>20	−

Cytotoxic assay: $ED_{50} \leq 5$ μg/mL is considered active; P-388: murine lymphocytic leukemia, KB: human nasopharyngeal carcinoma, Col-2: human colon cancer, BCA-1: human breast cancer, Lu-1: human lung cancer, ASK: rat glioma; +=active; −=inactive.

(56), 105 (51), 95 (45), 93 (41), 85 (57), 79 (29), 67 (35), 57 (100). HRFABMS calcd for $C_{30}H_{49}O_2$ $[M+H]^+$ 441.3720, found 441.3716.

5.4.2. Preparation of 5α-cycloart-24,25-dihydro-3,16,23-trione (10). Compound **2** (252.5 mg, 0.56 mmol) in MeOH (6 mL) was hydrogenated in the presence of 10% palladium on carbon catalyst (5.9 mg). After work-up and recrystallization from EtOH, **10** (243.8 mg, 96%) was obtained as colorless needles, mp 128.1–129.1°C. $[\alpha]_{589}^{30} = -58.18$ (*c* 0.06, $CHCl_3$). IR ($CHCl_3$) ν_{max}: 1727 (C=O of 5-membered ring ketone), 1703 (C=O of 6-membered ring ketone and C=O of saturated ketone in the side chain), 1466, 1417, 1386, 1367, 1245, 1167, 1145, 1114, 976 cm^{-1}. ^1H and ^{13}C NMR data: see Tables 3 and 4. COSY correlations H/H: 1a/1b, 2a, 2b, 19a; 1b/1a, 2a, 2b; 2a/1a, 1b, 2b; 2b/1a, 1b, 2a; 5/6a, 6b; 6a/5, 6b, 7a, 7b; 6b/5, 6a, 7a, 7b; 7a/6a, 6b, 7b; 7b/6a, 6b, 7a, 8; 8/7a, 7b; 11a/11b, 12; 11b/11a, 12; 12/11a, 11b; 15a/15b, 30; 15b/15a, 30; 17/18, 20, 21; 18/12, 17; 19a/1a, 19b; 19b/19a; 20/17, 21, 22a, 22b; 21/17, 20, 22a, 22b; 22a/21, 22b; 22b/20, 22a; 24/25, 26, 27; 25/24, 26, 27; 26/24, 25, 27; 27/24, 25, 26; 28/5, 29; 29/28; 30/15a, 15b, 18. HMBC correlations: see Table 5. EIMS (70 eV) *m/z* (%): 454 $[M]^+$ (2), 439 (100), 421 (4), 355 (3), 339 (4), 313 (4), 301 (8), 185 (3), 147 (3), 135 (4), 119 (3), 109 (3), 105 (5), 95 (3), 93 (4), 91 (5), 85 (8), 79 (4), 67 (4), 57 (12), 41 (8). HRFABMS calcd for $C_{30}H_{47}O_3$ $[M+H]^+$ 455.3513, found 455.3515.

5.4.3. Reaction of 5α-cycloart-24-ene-3,23-dione (1) with 2.2 equiv. of MCPBA. To a stirred solution of **1** (100 mg, 0.2283 mmol) in anhydrous CH_2Cl_2 (6 mL) was sequentially added MCPBA (85%, 102 mg, 0.5023 mmol, 2.2 equiv.) and anhydrous $NaHCO_3$ (57.5 mg, 0.6844 mmol, 3 equiv.). The reaction mixture was left stirring at room temperature for 7 h, then diluted with CH_2Cl_2 (15 mL), washed with a 1:1 mixture of 5% $Na_2S_2O_3$ and 5% $NaHCO_3$ (3×20 mL) to remove excess MCPBA, and finally with H_2O (3×20 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated to dryness to afford a white solid (115.2 mg). Purification by preparative thin-layer chromatography on silica plates (EtOAc/hexane, 1:4)

Table 8. Anti-HIV activities of the isolated **1**–**8** and the modified **9**–**22** by syncytium and HIV-1 RT assays

Compound	Anti-syncytium (MC99+1A2)				Anti-HIV-1 RT	
	IC_{50} (μg/mL)	EC_{50} (μg/mL)	TI (IC_{50}/EC_{50})	Activity	% Inhibition	Activity
1	215	–	–	Ia	12.0	I
2	>250	146.2	>1.7	Aa	7.0	I
3	<3.9	–	–	T	99.9	A
4	14.0	<3.9	>3.6	A	35.2	W
5	<3.9	–	–	T	19.1	I
6	41.8	<3.9	>10.7	A	25.2	I
7	77.0	<3.9	>19.5	Aa	9.4	I
8	<7.8	–	–	T	15.0	I
9	64.1	17.6	3.6	A	19.3	W
10	>125	<3.9	>32.1	Aa	22.0	I
11	18.0	6.2	2.9	A	35.8	W
12	<7.8	–	–	T	12.9	I
13	20.5	5.1	4.0	Aa	2.6	I
14	>125	–	–	Ia	7.8	I
15	12.3	<3.9	>3.1	Aa	13.9	I
16	16.4	<3.9	>4.2	Aa	8.3	I
17	<3.9	–	–	T	0	I
18	16.8	<3.9	>4.3	Aa	6.0	I
19	>125	–	–	Ia	0	I
20	6.7	<3.9	>1.7	Aa	0	I
21	<3.9	–	–	T	0	I
22	<3.9	–	–	T	0.8	I

Syncytium assay: A=active in the assay for inhibition of syncytium formation by MC99-infected cells, Aa=active in the assay for reduction of syncytium formation by MC99 virus, I=inactive in the syncytium inhibition assay, Ia=inactive in the reduction assay, T=toxic; Radioisotopic RT assay: A=very active (>70% inhibition), M=moderately active (>50–70% inhibition), W=weakly active (30–50% inhibition), I=inactive (<30% inhibition).

afforded heptanolide **11** (40.4 mg, 39%) and a 1:1.2 diastereomeric mixture of **13A** and **13B** (25.5 mg, 24%).

The heptanolide **11** was recrystallized from EtOH to give colorless needles, mp 134.8–135.4°C. $[\alpha]_{589}^{30}$=+18.3 (*c* 0.07, $CHCl_3$). UV (EtOH) λ_{max} nm (log ε): 234 (4.19). IR ($CHCl_3$) ν_{max}: 1702 (C=O of 7-membered lactone), 1685 (C=O of conjugated ketone), 1616 (C=C), 1446, 1389, 1294, 1225, 1193, 1113, 1103, 1035, 1012, 980 cm^{-1}. ^1H and ^{13}C NMR data see Tables 3 and 4. COSY correlations [δ_H/δ_H (H/H)]: 1a/1b, 2a, 2b, 19a, 19b; 1b/1a, 2a, 2b; 2a/1a, 1b, 2b; 2b/1a, 1b, 2a; 5/6a, 6b, 19a, 19b, 28, 29; 6a/5, 6b, 7a, 7b; 6b/5, 6a, 7a, 7b; 7a/6a, 6b, 7b, 8; 7b/6a, 6b, 7a, 8; 8/7a, 7b, 30, 19b; 11a/11b, 12, 19a, 19b; 11b/11a, 12, 19a, 19b; 12/11a, 11b, 18; 15/16a, 16b, 30; 16a/16b, 17; 16b/15, 16a, 17; 17/16a, 16b, 21; 18/12, 17; 19a/1a, 5, 11a, 11b, 19b; 19b/1a, 5, 8, 11a, 11b, 19a; 20/17, 21, 22a, 22b; 21/20, 22a, 22b; 22a/20, 21, 22b; 22b/20, 21, 22a; 24/26, 27; 26/24, 27; 27/24, 26; 28/29; 29/5, 28; 30/8. HMBC correlations: see Table 5. EIMS (70 eV) *m/z* (%): 454 $[M]^+$ (2), 439 (4), 421 (1), 371 (1), 356 (9), 341 (11), 327 (2), 313 (3), 298 (2), 283 (3), 257 (1), 233 (3), 219 (2), 201 (3), 187 (4), 175 (5), 161 (6), 147 (19), 133 (10), 125 (16), 121 (22), 105 (15), 91 (20), 83 (100), 79 (19), 67 (14), 55 (47), 41 (22). HRFABMS calcd for $C_{30}H_{47}O_3$ $[M+H]^+$ 455.3513, found 455.3527.

The mixture of **13A** and **13B** was obtained as a colorless solid, mp 129.1–130.7°C. $[\alpha]_{589}^{30}$=+25.5 (*c* 0.06, $CHCl_3$). IR ($CHCl_3$) ν_{max}: 1703 (C=O of 7-membered lactone and C=O of the side-chain ketone), 1447, 1459, 1390, 1294, 1193, 1114, 1030, 1012, 979 cm^{-1}. ^1H NMR (300 MHz) δ_H: 3.41 (s, H-24 of **A**), 3.34 (s, H-24 of **B**), 2.69 (obsc., H-2), 2.55 (obsc., H-22a) 2.34 (obsc., H-22b), 2.10 (obsc., H-11a), 2.09 (obsc., H-5), 2.08 (obsc., H-20), 1.93 (m, H-16a), 1.81 (obsc., H-1a), 1.80 (obsc., H-6a), 1.69 (obsc., H-17 of **A**), 1.67 (obsc., H-12), 1.65 (obsc., H-17 of **B**), 1.52 (m., H-1b), 1.46 (s, H-29), 1.45 (obsc., H-8 of **A**), 1.44 (s, H-26 of **A**), 1.43 (obsc., H-8 of **B**), 1.43 (s, H-26 of **B**), 1.40 (s, H-28), 1.36 (obsc., H-15), 1.35 (obsc., H-16b), 1.30 (obsc., H-7a), 1.27 (s, H-27), 1.10 (obsc., H-11b), 1.09 (obsc., H-7b), 1.02 (s, H-18 of **B**), 1.01 (s, H-18 of **A**), 0.93 (s, H-30), 0.91 (d, *J*=6.5 Hz, H-21 of **B**), 0.90 (d, *J*=6.5 Hz, H-21 of **A**), 0.69 (obsc., H-6b), 0.68 (d, *J*=5.0 Hz, H-19a), 0.62 (d, *J*=5.0 Hz, H-19b). ^{13}C NMR (75 MHz) δ_C: 206.89 (C-23 of **B**), 206.70 (C-23 of **A**), 175.43 (C-3), 87.19 (C-4), 65.73 (C-24 of **A**), 65.61 (C-24 of **B**), 61.03 (C-25 of **A**), 60.96 (C-25 of **B**), 52.32 (C-17 of **B**), 52.21 (C-17 of **A**), 49.76 (C-5 of **A**), 49.75 (C-5 of **B**), 48.89 (C-14 of **B**), 48.88 (C-14 of **A**), 48.67 (C-8 of **B**), 48.65 (C-8 of **A**), 48.14 (C-22 of **B**), 48.09 (C-22 of **A**), 45.00 (C-13 of **A**), 44.99 (C-13 of **B**), 35.65 (C-15 of **A**), 35.64 (C-15 of **B**), 35.05 (C-2), 32.85 (C-20 of **A**), 32.72 (C-12 of **A**), 32.70 (C-12 of **B**), 32.44 (C-20 of **B**), 30.97 (C-28), 30.13 (C-1), 29.56 (C-19), 28.41 (C-16 of **A**), 28.34 (C-16 of **B**), 27.24 (C-10), 27.16 (C-11), 26.00 (C-6), 25.32 (C-7), 24.76 (C-26 of **B**), 24.74 (C-26 of **A**), 23.08 (C-29 of **B**), 23.07 (C-29 of **A**), 22.91 (C-9), 19.47 (C-30), 19.39 (C-21 of **A**), 19.35 (C-21 of **B**), 18.51 (C-27), 18.36 (C-18 of **A**), 18.34 (C-18 of **B**). COSY correlations H/H: 1a/1b, 2, 19a; 1b/1a, 2; 2/1a, 1b; 5/6a, 6b, 19a, 19b, 29; 6a/5, 7b; 6b/5, 7a; 7a/6b, 8 of **A** and **B**; 7b/6a, 8 of **A** and **B**; 8 of **A** and **B**/7a, 7b, 19b; 11a/11b, 12; 11b/11a, 12; 12/11a, 11b; 15/16a, 16b, 30; 16a/15, 16b, 17 of **A** and **B**; 16b/15, 16a, 17 of **A** and **B**; 17 of **A** and **B**/16a, 16b, 20; **18** of **A**/12, **17** of **A**, 30; **18** of **B**/12, 17 of **B**, 30; 19a/1a, 5, 19b; 19b/5, 8 of **A** and **B**, 19a; 20/17 of **A** and **B**, 21 of **A** and **B**, 22a, 22b; 21 of **A**/20, 22a of **A**; 21 of **B**/20, 22a of **B**, 22a/20, 21 of **A** and **B**; 24 of **A**/26 of **A**, 27; 24 of **B**/26 of **B**, 27; 26 of **A**/24 of **A**, 27; 26 of **B**/24 of **B**, 27; 27/24 of **A** and **B**, 26 of **A** and **B**; 28/29; 29/5, 28; 30/8 of **A** and **B**, 15. HMBC correlations see Table 6. EIMS (70 eV) *m/z* (%): 470 $[M]^+$ (2), 455 (5), 430 (4), 397 (4), 383 (8), 365 (15), 356 (39), 341 (41), 329 (11), 327 (18), 313 (19), 283 (16), 269 (15), 253 (12), 241 (18), 233 (26), 213 (27), 201 (31), 189 (32), 187 (42), 175 (58), 173 (60), 161 (72), 147 (100), 141 (9), 133 (75), 121 (80), 107 (70), 99 (8), 93 (76), 91 (75), 79 (59), 67 (29), 55 (20). HRFABMS calcd for $C_{30}H_{46}O_4Na$ $[M+Na]^+$ 493.3282, found 493.3259.

5.4.4. Reaction of 5α-cycloart-24-ene-3,16,23-trione (2) with 2.2 equiv. of MCPBA. According to the procedure described in Section 5.4.3, compound **2** (100 mg, 0.2212 mmol) in anhydrous CH_2Cl_2 (6 mL) was reacted with MCPBA (85%, 98.8 mg, 0.4867 mmol, 2.2 equiv.) and anhydrous $NaHCO_3$ (55.8 mg, 0.6637 mmol, 3 equiv.). After left stirring at room temperature for 7 h and usual work-up, a white solid (108.9 mg) was obtained. Purification by preparative thin-layer chromatography on silica plates (EtOAc/CH_2Cl_2/hexane, 3:1:6 as eluent) afforded heptanolide **12** (45.9 mg, 44%) and a 1:1.3 diastereomeric mixture of heptanolide–epoxides **14A/14B** (25.7 mg, 24%).

The heptanolide **12** was recrystallized from EtOH to give colorless needles, mp 117.8–119.0°C. $[\alpha]_{589}^{30}$=−48.6 (*c* 0.07, $CHCl_3$). UV (EtOH) λ_{max} nm (log ε): 231 (4.19). IR ($CHCl_3$) ν_{max} 1727 (C=O of 5-membered ring ketone), 1702 (C=O of 7-membered lactone), 1687 (C=O of conjugated ketone), 1618 (C=C), 1542, 1447, 1389, 1293, 1225, 1116, 1101, 980 cm^{-1}. ^1H and ^{13}C NMR data: see Tables 3 and 4. COSY correlations H/H: 1a/1b, 2a, 2b, 19a; 1b/1a, 2a, 2b, 19b; 2a/1a, 1b, 2b; 2b/1a, 1b, 2a; 5/6a, 6b, 29; 6a/5, 6b, 7a, 7b; 6b/5, 6a, 7a, 7b; 7a/6a, 6b, 7b, 8; 7b/6a, 6b, 7a, 8; 8/7a, 7b; 11a/11b, 12, 19a; 11b/11a, 12; 12/11a, 11b; 15/17, 30; 17/15, 18, 21; 18/12, 17, 30; 19a/1a, 5, 11a, 11b, 19b; 19b/1a, 5, 8, 11b, 19a; 20/17, 21, 22a, 22b; 21/17, 20, 22a, 22b; 22a/20, 21, 22b; 22b/20, 21, 22a; 24/26, 27; 26/24, 27; 27/24, 26; 28/29; 29/5, 28; 30/8, 15. HMBC correlations: see Table 5. EIMS (70 eV) *m/z* (%): 468 $[M]^+$ (8), 453 (63), 397 (19), 371 (8), 355 (8), 339 (10), 234 (6), 135 (10), 125 (23), 105 (13), 98 (16), 91 (17), 83 (100), 69 (17), 55 (41), 41 (17). HRFABMS calcd for $C_{30}H_{45}O_4$ $[M+H]^+$ 469.3306, found 469.3313.

The mixture of **14A** and **14B** was obtained as a colorless solid, mp 200.0–201.5°C. $[\alpha]_{589}^{31}$=+10 (*c* 0.06, $CHCl_3$). IR ($CHCl_3$) ν_{max} 1725 (C=O of 5-membered ring ketone), 1706 (C=O of 7-membered lactone and C=O of the side-chain ketone), 1459, 1447, 1389, 1293, 1245, 1187, 1115, 1101, 1033, 980 cm^{-1}. ^1H NMR (300 MHz) δ_H: 3.49 (s, H-24 of **A**), 3.44 (s, H-24 of **B**), 3.34 (dd, *J*=17.3, 2.5 Hz, H-22a of **A**), 3.29 (dd, *J*=17.3, 2.9 Hz, H-22a of **B**), 2.72 (m, H-2), 2.52 (dd, *J*=17.3, 11.2 Hz, H-22b of **A**), 2.50 (dd, *J*=17.3, 11.6 Hz, H-22b of **B**), 2.38 (m, H-20), 2.29 (d, *J*=9.1 Hz, H-17 of **B**), 2.27 (d, *J*=9.1 Hz, H-17 of **A**), 2.23 (m, H-11a), 2.12 (dd, *J*=12.7, 5.3 Hz, H-5), 2.04 (br s, H-15), 1.88 (obsc., H-12), 1.85 (obsc., H-6a), 1.84 (obsc., H-1a), 1.56 (obsc., H-1b), 1.54 (obsc., H-8), 1.47 (s,

H-29), 1.45 (s, H-26 of **A**), 1.44 (s, H-26 of **B**), 1.42 (s, H-28), 1.30 (obsc., H-7a), 1.29 (s, H-27), 1.24 (obsc., H-11b), 1.20 (obsc., H-7b), 1.16 (s, H-18), 1.12 (s, H-30 of **B**), 1.11 (s, H-30 of **A**), 1.00 (d, J=6.2 Hz, H-21 of **B**), 0.99 (d, J=6.2 Hz, H-21 of **A**), 0.73 (d, J=4.9 Hz, H-19a), 0.71 (obsc., H-6b), 0.69 (d, J=4.9 Hz, H-19b). ^{13}C NMR (75 MHz) δ_C: 219.10 (C-16 of **A**), 219.04 (C-16 of **B**), 205.78 (C-23 of **B**), 205.58 (C-23 of **A**), 175.15 (C-3), 86.92 (C-4), 65.57 (C-24), 60.96 (C-25 of **B**), 60.89 (C-25 of **A**), 60.54 (C-17 of **B**), 60.49 (C-17 of **A**), 50.98 (C-15 of **A**), 50.95 (C-15 of **B**), 49.62 (C-5), 47.96 (C-8 of **B**), 47.94 (C-8 of **A**), 46.83 (C-22 of **B**), 46.80 (C-22 of **A**), 44.72 (C-14), 42.02 (C-13), 34.92 (C-2), 31.41 (C-12), 30.94 (C-28), 29.84 (C-1 and C-19), 27.70 (C-10), 26.76 (C-11), 26.67 (C-20), 25.67 (C-6), 25.60 (C-7), 24.71 (C-26), 23.03 (C-29), 22.19 (C-9), 20.33 (C-21), 19.77 (C-30 of **B**), 19.75 (C-30 of **A**), 19.02 (C-18 of **A**), 18.97 (C-18 of **B**), 18.40 (C-27 of **B**), 18.16 (C-27 of **A**). COSY correlations H/H: 1a/1b, 2, 5, 19a; 1b/1a, 2, 19a, 19b; 2/1a, 1b; 5/1a, 6a, 6b, 19b, 29; 6a/5, 6b, 7a, 7b; 6b/5, 6a, 7a, 7b; 7a/6a, 6b, 7b, 8; 7b/6a, 6b, 7a, 8; 8/7a, 7b, 15; 11a/11b, 12, 19a; 11b/11a, 12; 12/11a, 11b, 18; 15/8, 17 of **A** and **B**, 30; 17 of **A**/15, 18, 20, 21 of **A**; 17 of **B**/15, 18, 20, 21 of **B**; 18/12, 17 of **A** and **B**; 19a/1a, 1b, 5, 8, 11a, 11b, 19b; 19b/1a, 1b, 5, 8, 11b, 19a; 20/17 of **A** and **B**, 21 of **A** and **B**, 22a of **A** and **B**, 22b of **A** and **B**; 21 of **A**/20, 22a of **A**, 22b of **A**; 21 of **B**/20, 22a of **B**, 22b of **B**; 22a of **A**/20, 21 of **A**, 22b of **A**; 22a of **B**/20, 21 of **B**, 22b of **B**; 22b of **A**/20, 21 of **A**, 22a of **A**; 22b of **B**/20, 21 of **B**, 22a of **B**; 24 of **A**/26 of **A**, 27; 24 of **B**/26 of **B**, 27; 26 of **A**/24 of **A**, 27; 26 of **B**/24 of **B**, 27; 27/24 of **A** and **B**, 26 of **A** and **B**; 28/5, 29; 29/5, 28; 30 of **A** and **B**/8, 15. HMBC correlations: see Table 6. EIMS (70 eV) m/z (%): 484 $[M]^+$ (8), 469 (9), 451 (6), 426 (2), 413 (11), 397 (100), 355 (6), 339 (25), 311 (3), 287 (7), 259 (5), 243 (4), 229 (8), 203 (5), 191 (11), 178 (24), 161 (10), 139 (15), 119 (21), 105 (21), 91 (26), 83 (33), 69 (55), 55 (37), 43 (63). HRFABMS calcd for $C_{30}H_{44}O_5Na$ $[M+Na]^+$ 507.3075, found 507.3072.

5.4.6. Methanolysis of heptanolide 12. A solution of heptanolide **12** (50 mg, 0.1068 mmol) in dry methanol (2 mL) was added dropwise to a stirred mixture of NaOMe (6.34 mg, 0.1177 mmol, 1.1 equiv.) in dry MeOH (6 mL) at 0°C. The reaction mixture was left stirring at room temperature for 6 h and then acidified with 2N HCl until the pH~6.5–7.0. After dilution with water (50 mL) and extraction with CH_2Cl_2 (3×20 mL), the combined organic layer was washed with water (3×50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to give a white semi-solid (48.2 mg). Purification by preparative thin-layer chromatography on silica plates, eluting with EtOAc–hexane (1:4) gave the hydroxy-ester **15** (42.6 mg, 80% yield) as a semi-solid. $[\alpha]_{589}^{30}=-59.5$ (c 0.39, $CHCl_3$). UV (EtOH) λ_{max} nm (log ε): 235 (5.57). IR ($CHCl_3$) ν_{max}: 3492 (O–H stretching), 1727 (C=O of ester and 5-membered ring ketone), 1684 (C=O of conjugated ketone), 1618 (C=C), 1447, 1387, 1292, 1244, 1187, 1173, 1116, 1101, 1053, 1035, 979 cm^{-1}. ^{1}H and ^{13}C NMR see Tables 3 and 4. COSY correlations H/H: 1a/1b, 2a, 2b; 1b/1a, 2a, 2b; 2a/1a, 1b, 2b; 2b/1a, 1b, 2a; 5/6a, 6b, 19a, 19b, 28; 6a/5, 6b, 7a, 7b; 6b/5, 6a, 7a, 7b; 7a/6a, 6b, 7b, 8; 7b/6a, 6b, 7a, 8; 8/7a, 7b, 15; 11a/11b, 12, 19a, 19b; 11b/11a, 12, 19b; 12/11a, 11b, 18; 15/8, 17, 30; 17/15, 18, 21; 18/12, 17; 19a/1b, 5, 11a, 19b; 19b/1b, 5, 11a, 19a; 20/21, 22a, 22b; 21/17, 22a, 22b; 22a/20, 21, 22b; 22b/20, 21, 22a; 24/26, 27; 26/24, 27; 27/24, 26; 28/29; 29/5, 28; 30/12, 15. HMBC correlations: see Table 5. EIMS (70 eV) m/z (%): 501 $[M+H]^+$ (3), 482 (5), 467 (16), 453 (45), 427 (51), 422 (8), 397 (19), 371 (27), 355 (8), 339 (14), 299 (6), 271 (3), 233 (8), 177 (11), 147 (8), 125 (26), 91 (17), 83 (100), 73 (24), 59 (42), 43 (16). HRFABMS calcd for $C_{31}H_{49}O_5$ $[M+H]^+$ 501.3567, found 501.3603.

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