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ScienceDirect
Phytochemistry 69 (2008) 206–211
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Flavaglines and triterpenoids from the leaves of *Aglaia forbesii*

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Received 21 February 2007; received in revised form 16 May 2007
Available online 20 August 2007

Abstract

Three structurally complex flavaglines of the cyclopenta[*bc*]benzopyran type, named desacetylpyramidaglains A, C, D (**1**–**3**), and the triterpene 23, 24, 25-trihydroxycycloartan-3-one (**4**) were isolated from the leaves of *Aglaia forbesii* together with the two rare pregnane steroids 2β,3β-dihydroxy-5α-pregn-17(*Z*)-en-16-one and 2β,3β-dihydroxy-5α-pregn-17(*E*)-en-16-one, as well as the bisamide pyramidatine, the sesquiterpene spathulenol, and the widespread triterpenoids lupeol, lupenone, and a mixture of β-sitosterol and stigmasterol. Their structures were elucidated by 1D and 2D NMR spectroscopy and mass spectrometry. Compounds **3**, **4**, **5**, and **6** were tested for antituberculosis and antiviral activity.

Keywords: *Aglaia forbesii*; Meliaceae; Flavaglines; Cyclopenta[*bc*]benzopyrans; Triterpenes; Cycloartane; Pregnane steroids; Bisamide; Sesquiterpene; Antituberculosis; Antiviral

1. Introduction

The genus *Aglaia* (Meliaceae) comprises around 100 species mainly distributed in the tropical rain forests of southeast Asia (Pannell, 1992). Previous phytochemical studies established the occurrence of characteristic flavaglines and bisamides, including cyclopenta[*b*]benzofurans, cyclopenta[*bc*]benzopyrans, and benzo[*b*]oxepines (Brader et al., 1998; Bacher et al., 1999; Saifah et al., 1999; Proksch et al., 2001). Some of these compounds were shown to exhibit interesting pharmacological properties including antiviral (Saifah et al., 1999), anticancer (Hayashi et al., 1982; Cui et al., 1997; Kim et al., 2006), antifungal (Engelmeier et al., 2000), anti-inflammatory (Baumann et al., 2002; Proksch et al., 2005) and insecticidal activities (Schneider et al., 2000; Greger et al., 2001). *Aglaia forbesii* King is a large tree widely distributed in southern Thailand. Previous studies on the bark extract of a Malaysian collection led to the isolation of the benzofuran flavaglines rocaglaol and ethylrocaglaol along with the benzopyran flavaglines aglain A, aglaforbesins A and B, and the benzoxepine flavaglines forbaglins A and B (Dumontet et al., 1996). In the present paper, we describe the structure elucidation of three new benzopyran flavaglines **1**–**3** from the leaf extract of a collection from southern Thailand, characterised by a cyclopenta[*bc*]benzopyran skeleton linked to a benzoyl-1,4-butanediamine, and a new cycloartane triterpene (**4**). Moreover, the two rare pregnane steroids 2β,3β-dihydroxy-5α-pregn-17(*Z*)-en-16-one (**5**) and 2β,3β-dihydroxy-5α-pregn-17(*E*)-en-16-one (**6**) were isolated together with the bisamide pyramidatine (**7**), the sesquiterpene spathulenol, and the widespread triterpenes lupeol, lupenone, and a mixture of β-sitosterol and stigmasterol. Compounds **3**, **4**, **5**, and **6** were tested for antituberculosis and antiviral activity.

2. Results and discussion

Chromatographic separation of the *n*-hexane fraction of the methanolic leaf extract of *A. forbesii* led to isolation of

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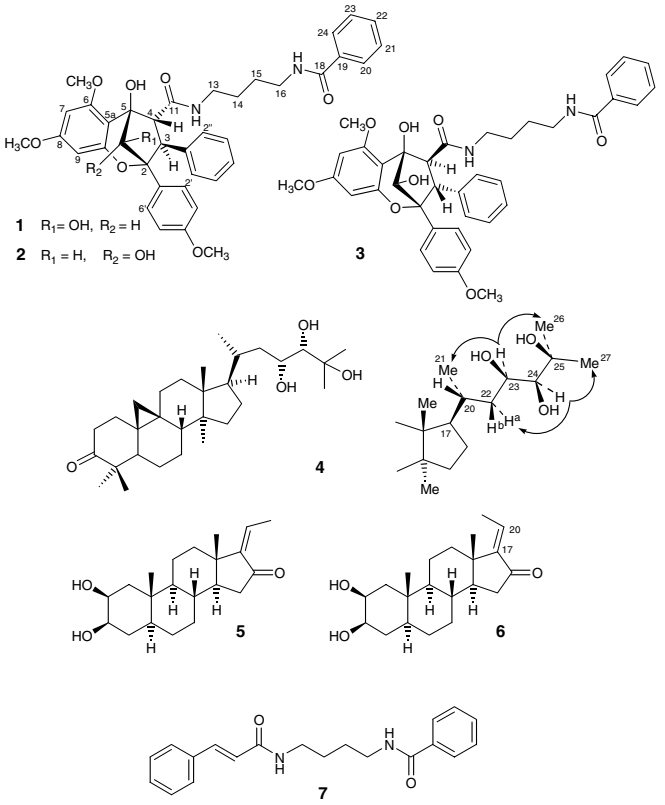
doi:10.1016/j.phytochem.2007.06.016

the sesquiterpene spathulenol and the common triterpenes lupeol, lupenone, and a mixture of β-sitosterol and stigmasterol. Their structures were elucidated by comparison with previously reported spectroscopic data (Rubinstein et al., 1976; Carpenter et al., 1980; Reynolds et al., 1986; Jahodar et al., 1988). The CH_2Cl_2 fraction yielded three new cyclopenta[*bc*]benzopyran flavaglines, named desacetylpyramidaglains A, C, and D (**1**–**3**), and a new triterpene, 23,24,25-trihydroxycycloartan-3-one (**4**), together with two rare pregnane steroids, 2β,3β-dihydroxy-5α-pregn-17 (*Z*)-en-16-one (**5**) and 2β,3β-dihydroxy-5α-pregn-17(*E*)-en-16-one (**6**) (Inada et al., 1997), and the bisamide pyramidatine (**7**) (Saifah et al., 1993).

The spectroscopic data of the newly isolated compounds **1**–**3** showed closely related ^1H and ^{13}C NMR characteristic signals for two monosubstituted aromatic rings, a *para*-substituted aromatic ring, and a fourfold substituted aromatic ring. The ^{13}C NMR spectrum showed 38 carbons signals and the 1D and 2D NMR data corresponded to cyclopenta[*bc*]benzopyran flavaglines with a benzoyl-1, 4-butaneamine moiety linked to the flavagline skeleton by an amide function. This benzoyl-1,4-butanebisamide structure was known from the pyramidaglains A and B (Puripattanavong et al., 2000) and the bisamide pyramidatine. MS data and IR spectra were also almost identical for the three compounds. The ESI-TOFMS showed $[M+Na]^+$ peaks at $m/z = 675$ matching a molecular formula of $C_{38}H_{40}N_2O_8$ and the IR absorptions indicated the presence of hydroxy at 3479 cm^{-1} and amide functions at 1620 and 1633 cm^{-1}.

The ^1H and ^{13}C NMR spectroscopic data for flavagline **1** (see Table 1) were almost identical with those of pyramidaglain A (Puripattanavong et al., 2000). The only difference was the lack of acetylation of the 10-OH function in **1**. The relative configurations in the hydroxylated methano bridge and the two substituents could be derived from the characteristic NOESY cross peaks 3-H → 4, NH-12, 2′/6′, 2″/6″; 4-H → 3, 10-OH, NH-12, 2″/6″; 10-H → 2′/6′; and 10-OH → 4. The most important cross peak was 10-OH ↔ 4-H because it proved, directly the relative configurations at C-3, C-4 and C-10. Since the resonances for 10-OH and 10-H were very close at room temperature (4.88 and 4.90 ppm), the NOESY spectrum was recorded also at lower temperatures. At 278 K, the broad doublet of 10-OH shifted to 5.25 ppm and the cross-peak between 10-OH and 4-H allowed a clear decision. Thus, as structure of **1** corresponded to the acetylated derivative pyramidaglain A (Puripattanavong et al., 2000) and was therefore designated as desacetylpyramidaglain A. However, in contrast to the usual presentation in the literature, the less likely enantiomer of pyramidaglain A was shown in that paper (Puripattanavong et al., 2000). The known absolute stereochemistry of cyclopenta[*b*]benzofurans (Trost et al., 1990) can be adopted to the closely related cyclopenta[*bc*]benzopyrans by means of biochemically reasonable considerations (Nugroho et al., 1999; Bacher et al., 1999). The stereo center at C-2 is not affected by bond forming and bond breaking steps and should possess the same absolute configuration 2*R* in both series (methano bridge up) and not 2*S* (Puripattanavong et al., 2000).



Compound **2** was a stereoisomer of **1**. NOESY cross peaks 3-H → 4, NH-12, 2′/6′, 2″/6″; 4-H → 3, 10-H, 2′/6″; and 10-H → 4, 2′/6′ proved the relative configurations shown in the formula. The most important cross-peak for the position of the methano bridge 10-OH relative to the substituents at C-3 and C-4 was the strong cross peak 4-H ↔ 10-H. The chemical shift of the OH group was at a remarkable high field which was probably due to the ring current effect of the close aromatic ring 5a–9a. A model showed that the proton of the 10-OH group was situated above this ring. The compound was named desacetylpyramidaglain C (**2**).

Compound **3** was characterised by 3-Hβ, 4-Hα, and 10-OH groups pointing towards positions 3 and 4. The typical NOESY cross peaks of the cyclopenta[*b*]benzofuran system were 3-H → 4, 10-OH, 2′/6′, 2″/6″; 4-H →3, NH-12, 2″/6″; 10-H → 10-OH, 2′/6′; and 10-OH → 3,5-OH. The stereochemistry followed immediately from the most informative NOESY cross peak 3-H ↔ 10-OH. The compound was designated as desacetylpyramidaglain D (**3**).

HREIMS of compound **4** showed a molecular mass of $m/z = 474.3715$ corresponding to the molecular formula $C_{30}H_{50}O_4$ and the ^1H and ^{13}C NMR spectra were typical for triterpenes of the cycloartane series. The high field pair of doublets at $\delta = 0.79$ and 0.58 ppm with a geminal coupling constant $J = 4.3$ Hz was characteristic for this class of triterpenes. The typical 3-keto carbonyl was found in

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data for desacetylpyramidaglains **A**, **C** and **D** (**1–3**) in $CDCl_3$

No.	^{1}H			^{13}C		
	1	**2**	**3**	**1**	**2**	**3**
2				86.8 *s*	85.6 *s*	89.9 *s*
3	3.91 *d*, 8.6 Hz	3.22 *d*, 8.9 Hz	4.67 *d*, 6.0 Hz	61.7 *d*	59.0 *d*	55.9 *d*
4	4.12 *d*, 8.6 Hz	4.00 *d*, 8.9 Hz	3.44 *d*, 6.0 Hz	61.6 *d*	57.1 *d*	65.6 *d*
5				83.2 *s*	81.8 *s*	79.6 *s*
5a				106.0 *s*	104.2 *s*	110.5 *s*
6				158.8 *s*	160.3 *s*	156.3 *s*
7	5.77 *d*, 2.3 Hz	5.86 *d*, 2.3 Hz	6.12 *d*, 2.3 Hz	92.8 *d*	93.0 *d*	92.5 *d*
8				161.0 *s*	160.8 *s*	160.9 *s*
9	6.04 *d*, 2.3 Hz	6.25 *d*, $J = 2.3$ Hz	6.10 *d*, 2.3 Hz	93.7 *d*	93.9 *d*	93.9 *d*
9a				152.8 *s*	152.9 *s*	153.8 *s*
10	4.90 *d*, 4.8 Hz	4.90 *d*, 4.4 Hz	4.28 *d*, 9.5 Hz	78.7 *d*	73.5 *d*	82.6 *d*
11				173.3 *s*	170.0 *s*	173.8 *s*
NH-12	6.79 br *t*, 5.5 Hz	5.52 br *t*, 5.5 Hz	6.49[a] br *t*, 5.5 Hz			
13	2.98 *m*, 2.90 *m*	2.92 *m*, 2.62 *m*	3.48 *m*, 3.31 *m*	39.0 *t*	39.0 *t*	39.3 *t*
14	1.14 *m*, 2H	0.98 *m*, 2H	1.60–1.70 *m*, 2H	26.0 *t*	26.2 *t*	26.3 *t*
15	1.20 *m*, 2H	1.15 *m*, 2H	1.60–1.70 *m*, 2H	26.5 *t*	26.3 *t*	26.9 *t*
16	3.23 *m*, 2H	3.28 *m*, 3.15 *m*	3.44–3.50 *m*, 2H	39.4 *t*	39.4 *t*	39.7 *t*
NH-17	6.31 br *t*, 5.5 Hz	6.46 br *t*, 5.5 Hz	6.52[a], br *t*, 5.5 Hz			
18				167.6 *s*	167.5 *s*	167.7 *s*
19				134.5 *s*	134.5 *s*	134.4 *s*
20,24	7.78 ps *d*[b], 2H	7.81 ps *d*[b], 2H	7.77 ps *d*[b], 2H	126.9 *d*	126.9 *d*	126.9 *d*
21,23	7.45 ps *t*[c], 2H	7.46 ps *t*[c], 2H	7.42 ps *t*[c], 2H	128.6 *d*	128.6 *d*	128.6 *d*
22	7.51 ps *t*[c]	7.52 ps *t*[c]	7.49 ps *t*[c]	131.4 *d*	131.4 *d*	131.5 *d*
1′				130.3 *s*	129.2 *s*	129.8 *s*
2′,6′	7.74 ps *d*[b], 2H	7.61 ps *d*[b], 2H	7.64 ps *d*[b], 2H	127.7 *d*	128.0 *d*	128.0 *d*
3′,5′	6.89 ps *d*[b], 2H	6.89 ps *d*[b], 2H	6.86 ps *d*[b], 2H	113.7 *d*	113.6 *d*	113.1 *d*
4′				159.6 *s*	159.3 *s*	159.0 *s*
1″				136.7 *s*	136.8 *s*	137.5 *s*
2″,6″	6.98 *m*, 2H	6.92 *m*, 2H	6.89 *m*, 2H	129.3 *d*	128.6 *d*	129.2 *d*
3″,5″	7.15 *m*, 2H	7.15 *m*, 2H	7.10 *m*, 2H	128.6 *d*	127.7 *d*	128.6 d
4″	7.15 *m*	7.15 *m*	7.10 *m*	127.1 *d*	127.0 *d*	127.0 *d*
5-OH	5.42 *s*	5.43 *s*	5.79 *s*			
10-OH	4.88 br *d*, 4.8 Hz	2.32 br *d*, 4.4 Hz	5.96 *d*, 9.5 Hz			
6-OMe	3.08 *s*	3.11 *s*	3.86 *s*	55.7 *q*	55.5 *q*	56.2 *q*
8-OMe	3.71 *s*	3.78 *s*[a]	3.77 *s*	55.4 *q*	55.4 *q*	55.5 *q*
4′-OMe	3.77 *s*	3.77 *s*[a]	3.79 *s*	55.3 *q*	55.4 *q*	55.2 *q*

[a] Assignments are interchangeable in the same column.
[b] ps *d* = pseudo doublet.
[c] ps *t* = pseudo triplet.

the ^{13}C NMR spectrum at 216.6 ppm and the pair 2-Hax and 2-Heq at 2.71 and 2.30 ppm in the ^{1}H NMR. Two low field protons at 4.13 and 3.18 ppm indicated two secondary alcohol functions. This was supported by the corresponding doublets in the ^{13}C NMR spectrum at 75.0 and 69.7 ppm. Additionally, a singlet at 74.3 ppm indicated a tertiary alcohol. The molecular formula $C_{30}H_{50}O_4$ (*m*/*z* = 474) is in agreement with one keto and three alcohol functions. The ^{13}C resonances of the basic four-ring system of cycloartanes were almost identical with the literature data, e.g. 21*S*,24*R*-dihydroxycycloart-25-en-3-one isolated from *A. rubiginosa* (Weber et al., 2000). The structure and shift assignments of the remaining 23,24,25-triol side-chain atoms were straightforward using 2D methods (HH-COSY, NOESY, HMBC, and HMQC). However, the stereochemistry of 23-OH and 24-OH represented a special problem.

Contrary to cycloartanes, this triol side-chain is rather common in the related tirucallanes. An X-ray analysis of piscidinol A and 24-*epi*-piscidinol A proved 23*R*,24*S* configuration for the former and 23*R*, 24*R* for the latter (McChesney et al., 1997). 24-*epi*-Piscidinol A was found also in *A. andamanica* (Puripattanavong et al., 2000); however, the chemical ^{13}C shifts of the side-chain did not agree with our data. The most striking difference in the ^{1}H NMR spectrum was the coupling constant *J*(23,24) which was 8.1 Hz, compared to almost zero in our case. The resonance for 24-H for compound **4** appeared as a slightly broadened singlet (3.18 ppm, $J < 0.5$ Hz) and as a consequence 23-H was a *dd* (4.13, $J = 9.4$, 4.8 Hz). A broad singlet for the 24-H was also observed for piscidinol A and B (Govindachari et al., 1995; McChesney et al., 1997) and the ^{13}C resonances of the side chain agreed also quite well. This implied that the configurations of the side chain in cycloartane **4** are 23*R*,24*S* or 24*S*,24*R*. These two possibilities would show the same NMR pattern and, additionally, one should have in mind that the configurations at carbon

atoms 13, 14, 17, and 20 are reversed in the tirucallanes and the same may be true for positions 23 and 24. The known absolute configuration 20*R*, common for all cycloartanes, could be used for the correlation of the side chain configurations. Characteristic NOEs (compare the formula scheme) prove that 23*R*, 24*S* is indeed correct. It is interesting that the absolute configurations of the two alcohol functions were identical in tirucallanes and cycloartanes, whereas all other comparable configurations were opposite in the two series. The enzymatic oxidation to the polyol chain seems to be similar and independent of the very different enzymatic formation of the basic terpenoid structures.

The two views of molecule **4** in the formula scheme needs some comments. The complete structure corresponds to the usual representation in literature. The partial structure shows a different conformation (rotation about the C20–C22 bond) and is based on the X-ray structure of piscidinol A. Note that a change in conformation by rotation about a single bond inverts the up and down positions within the chain attached to this bond, the absolute configurations remain of course unchanged. Only this more natural presentation is compatible with the NOEs and all observed couplings. Large coupling constants were observed for transoid arrangements like $J(22a,20) = 9.6$ Hz and $J(22b,23) = 9.4$ Hz and small values for cisoid relationships like $J(22a,23) = 4.8$ Hz, $J(22b,20) \sim 4$ Hz, and $J(23,24) < 0.5$ Hz (transoid means one H up and the other one down, cisoid means both protons on the same side, either both up or both down, see formula scheme). The two methyl resonances 26 and 27 can also be discriminated by means of different NOE effects. Both terminal methyl groups show NOE contacts to both protons 23-H and 24-H; however, in the case of 23-H the NOE to 26-H_3 is clearly stronger, in the case of 24-H the NOE to 27-H_3. This results in a geometry with all three OH groups pointing upwards in the correct stereochemical view of **4** (see formula scheme), an arrangement which is stabilised by hydrogen bonding. Optimised hydrogen bonds are also the reason that the vicinal torsional angle between 23-H and 24-H is widened up to 80° or 90° (therefore $J(23, 24) < 0.5$). This results in a smaller torsional angle of about 30° or 40° between 23-OH and 24-OH, allowing better formation of a hydrogen bond. Due to the many substituents and extensive hydrogen bridging of the three OH functions, the chain is relatively rigid and a detailed conformational analysis was possible. The derived structure **4** was (23*R*, 24*S*)-23, 24, 25-trihydroxycycloartan-3-one.

3. Concluding remarks

The present data from the leaves of *A. forbesii* clearly deviated from the previously published chemical profile obtained from the stem bark of a collection from Malaysia (Dumontet et al., 1996). Apart from the expected variations between leaf and bark extracts (Brem, 2002), the different amide moieties incorporated in the characteristic benzopyran flavaglines suggested extracts originating from different species. The present derivatives resembled the flavagline and bisamide profile reported for *A. andamanica* (Puripattanavong et al., 2000). In a previous preliminary UV-HPLC comparison of small quantities of leaves, stem and root bark from another Thai provenance of *A. forbesii* no flavaglines could be detected in all three plant parts (Brem, 2002). However, it should be pointed out that, in spite of the large quantities of dried leaves (3.5 kg) extracted in the present analysis, only small amounts of flavaglines (10 mg and 6 mg) were isolated, which most likely would have not been detected in a UV-HPLC comparison with smaller quantities of plant material.

In the course of our current search for natural products with anti-TB and anti-HSV1 activity compounds **3**, **4**, **5**, and **6** were selected for bioassays against *Mycobacterium tuberculosis* H37Ra (Collins and Franzblau, 1997) and *Herpes simplex* virus type 1 (HSV-1) (Skehan et al., 1990). The benzopyran flavagline **3** exhibited the highest activity against *M. tuberculosis* with a MIC-value at 25 μg/ml which was compared with the two positive controls isoniazid with 0.25 μg/ml and kanamycin with 1.25 μg/ml. Flavagline **3** was moderately active against *H. simplex*, whereas the other compounds were either only weakly active (**4**, **6**) or inactive (**5**) at the non-cytotoxic concentration of 50 μg/ml.

4. Experimental

4.1. General

Mps: uncorr. NMR: Bruker, DRX 400 WB ($CDCl_3$, ^1H δ7.26, ^{13}C δ7.0). MS: Finnigan MAT 900 S. IR: Perkin–Elmer FT-IR 1760X Spectrometer. UV: Shimadzu UV-160A Spectrophotometer. Optical rotation: Perkin–Elmer Polarimeter 241. CC: silica gel (Merck 60, 230–400). PTLC: silica gel (Merck 60, 0.5 mm).

4.2. Plant material

Leaves of *A. forbesii* were collected in March 2004 near Ai-Kiew waterfall in Khao Luang National Park, Nakhon Sri Thammarat, S-Thailand and compared with authentic specimens of the Herbarium of the Institute of Botany, University of Vienna, Austria, WU, identified by Dr. Caroline Pannell, Herbarium University of Oxford, England. A voucher specimen (ES-0403) was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

4.3. Extraction and isolation

The dried, powdered leaves (3.5 kg) of *A. forbesii* were exhaustively extracted with MeOH and filtered. After

removing the solvent in vacuo, a residue (450 g) was obtained, which was mixed with silica, packed in a column and eluted with *n*-hexane, CH_2Cl_2, EtOAc and MeOH, successively. The *n*-hexane fraction (15 g) was subjected to silica gel CC eluting with hexane:CH_2Cl_2 = 1:1 to efford (15 mg) lupeol, (31 mg) a mixture of β-sitosterol and stigmasterol, and a mixture (58 mg) which was further purified by CC eluting with 4% acetone in hexane, followed by Sephadex LH 20 CC eluted with CH_2Cl_2–MeOH (1:1) to yield spathulenol (5 mg) and lupenone (10 mg). The CH_2Cl_2 fraction (20 g) was subjected to repeated CC (i) CH_2Cl_2:EtOAc = 1:1, (ii) gradient system of CH_2Cl_2:MeOH (9:1 to 0:1) and (iii) CH_2Cl_2:EtOAc:MeOH = 80:15:5 to give a fraction (32 mg) which led to 12 mg pyramidatine (**7**) by recrystallization in MeOH , a fraction containing **1**, **2** and **3** (68 mg), and a fraction containing **4**, **5** and **6** (48 mg) which were further purified by prep. TLC using hexane:acetone:MeOH = 60:34:6 as eluent for the former and hexane:acetone = 72:28 for the latter fraction to yield desacetylpyramidaglain A (**1**) (10 mg), desacetylpyramidaglain C (**2**) (6 mg), desacetylpyramidaglain D (**3**) (10 mg), 23, 24, 25-trihydroxycycloartan-3-one (**4**) (3 mg), 2β,3β-dihydroxy-5α-pregn-17 (*Z*)-en-16-one (**5**) (3 mg), and 2β,3β-dihydroxy-5α-pregn-17(*E*)-en-16-one (**6**) (3 mg), respectively.

4.4. Desacetylpyramidaglain A (*1*)

Amorphous powder; m.p. 129–131 °C. $[\alpha]_D^{20} = +75$ (c = 0.5, $CHCl_3$). UV λ^{MeOH} nm 208. IR ν^{CHCl_3} cm^{-1} 3479 *w*, 3300 *m*, 2927 *s*, 1633 *s*, 1620 *s*, 1588 *s*, 1519 *m*, 1455 *m*. ^1H and ^{13}C NMR spectra, see Table 1. EIMS (70 eV, 200 °C): m/z = 502 (3%), 416 (11), 330 (26), 322 (44), 313 (60), 281 (15), 181 (21), 135 (44), 105 (74), 55 (100); ESIMS: m/z = 675 $[M+Na]^+$, M = 652 ($C_{38}H_{40}N_2O_8$).

4.5. Desacetylpyramidaglain C (*2*)

Amorphous powder; m.p. 144–146 °C. $[\alpha]_D^{20} = -36$ (c = 0.4, $CHCl_3$). UV λ^{MeOH} nm 208. IR ν^{CHCl_3} cm^{-1} 3480 *w*, 3315 *m*, 2937 *s*,1640 *s*, 1619 *s*, 1589 *s*, 1517 *m*,1455 *m*. For ^1H and ^{13}C NMR spectra, see Table 1. EIMS (70 eV, 210 °C): m/z = 416 (22%), 254 (11), 218 (18), 162 (52), 134 (25), 105 (100), 77 (95), 55 (44); ESIMS: m/z = 675 $[M+Na]^+$, M = 652 ($C_{38}H_{40}N_2O_8$).

4.6. Desacetylpyramidaglain D (*3*)

Amorphous powder; m.p. 120–122 °C. $[\alpha]_D^20 = +20$ (c = 0.5, $CHCl_3$). UV λ^{MeOH} nm 208. IR ν^{CHCl_3} cm^{-1} 3478 *w*, 3287 *m*, 2931 *s*, 1633 *s*, 1620 *s*, 1589 *s*, 1515 *m*, 1455 *m*. For ^1H and ^{13}C NMR spectroscopy, see Table 1. EIMS (70 eV, 200 °C): m/z = 416 (7%), 330 (15), 322 (42), 313 (100), 181 (35), 135 (35), 131 (54), 105 (93), 55 (40); ESIMS: m/z = 675 $[M+Na]^+$, M = 652 ($C_{38}H_{40}N_2O_8$).

4.7. 23, 24, 25-Trihydroxycycloartan-3-one (*4*)

Amorphous powder; m.p. 77–79 °C. $[\alpha]_D^{20} = +17$ (c = 0.3, $CHCl_3$). IR ν^{CHCl_3} cm^{-1} 3419 br, 1706 *s*, 2928 *s*. ^1H NMR ($CDCl_3$, δ/ppm) 4.13 (*dd*, 1H, J = 9.4 and 4.8 Hz, 23-H), 3.18 (br *s*, 1H, 24-H), 2.71 (*ddd*, 1H, J = 13.6, 13.4 and 6.4 Hz, 2-H_{ax}), 2.56 (vbr *s*, 1 OH), 2.51 (vbr *s*, 2 OH), 2.30 (*ddd*, 1H, J = 13.6, 4.2 and 2.5 Hz, 2-H_{eq}), 1.89 (*ddd*, 1H, J = 13.2, 9.6 and 4.8 Hz, 22-H_a), 1.86 (*ddd*, J = 13.4, 13.4 and 4.2 Hz, 1H, 1-H_{ax}), 1.53 (*m*, 1H, 1-H_{eq}), 1.33 (*s*, 3H, Me-27), 1.32 (*s*, 3H, Me-26), 1.15 (*ddd*, 1H, J = 13.2, 9.4, and 4 Hz, 22-H_b), 1.10 (*s*, 3H, Me-29), 1.05 (*s*, 3H, Me-28), 1.01 (*s*, 3H, Me-18), 0.94 (*d*, 1H, J = 4.3 Hz, 21-H_a), 0.91 (*s*, 3H, Me-30), 0.79 (*d*, 1H, J = 4.3 Hz, 21-H_b); the coupling constants of 22-H_a, 22-H_b, and 1-H_{ax} were confirmed by analysis of the shape of the well isolated NOESY cross peaks. For ^{13}C NMR spectra, see Table 2. EIMS (70 eV, 200 °C): m/z = 474 (12%), 384 (7), 340 (11), 313 (8), 147 (13), 121 (14), 91 (23), 59 (100); HREIMS: m/z = 474.3715 (calcd. 474.3709 for $C_{30}H_{50}O_4$).

4.8. Anti-tuberculosis assay

The antituberculosis assay of the pure compounds against *Mycobacterium tuberculosis* H37Ra was performed in duplicate using the microplate Alamar blue assay (Collins and Franzblau, 1997). The reference compounds were isoniazid and kanamycin sulfate. The minimum inhibitory concentrations (MICs) of less than 200 μg/ml were considered as active.

4.9. Anti-herpes simplex activity

Anti-herpes simplex virus type 1 (HSV-1) activity of pure compounds was tested against HSV-1 strain ATCC VR 260, using a colorimetric microplate assay (Skehan et al., 1990). The growth of host cells (Vero cell line ATCC

Table 2
^{13}C (100 MHz) NMR spectroscopic data for 23,24,25-trihydroxycycloartan-3-one (**4**) in $CDCl_3$

No.	δC	No.	δC
1	33.4 *t*	16	28.4 *t*
2	37.5 *t*	17	53.1 *d*
3	216.6 *s*	18	18.2 *q*
4	50.2 *s*	19	29.5 *t*
5	48.4 *d*	20	33.6 *d*
6	21.5 *t*	21	18.8 *q*
7	25.8 *t*	22	40.7 *t*
8	47.8 *d*	23	69.7 *d*
9	21.0 *s*	24	75.0 *d*
10	26.0 *s*	25	74.3 *s*
11	26.7 *t*	26	26.2 *q*
12	32.9 *t*	27	27.5 *q*
13	45.4 *s*	28	22.2 *q*
14	48.8 *s*	29	20.8 *q*
15	35.5 *t*	30	19.3 *q*

CCL-81) infected with the virus and treated with the analyzed compounds was compared with control cells, which were infected with virus only. Acyclovir and DMSO were used as positive and negative control, respectively. The analyzed compounds were tested at non-cytotoxic concentrations (>50% host cell growth), of which the 50% inhibitory concentration (IC_{50}) represents the concentration of the compound that caused more than 50% viral inhibition.

Acknowledgement

This investigation was financially supported by Thailand Research Fund.

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