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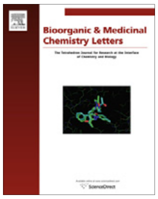

Cytotoxic cycloartane triterpene and rare isomeric bisclerodane diterpenes from the leaves of *Polyalthia longifolia* var. *pendula* ☆

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This Letter is dedicated to Koneni Joshitha Naidu (daughter of the senior author) on the occasion of her 8th birthday

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ABSTRACT

A 24-methylenecycloartane-3β, 16β, 23β-triol, Longitriol (**1**), rare bisclerodane imides, Longimide A (**2**) and previously known Longimide B (**3**) were isolated from ethanolic extract of the leaves of *Polyalthia longifolia* var. *pendula*. This is the first example of isolation of any cycloartane triterpene from this plant source. Structures were determined by extensive (1D and 2D NMR) spectroscopic data analysis combined with ESI MS/MS fragmentation and X-ray analysis. Furthermore, Compounds **1** and **2** were evaluated for their cytotoxic effects against four human cancer cell lines and found to be most active against cervical carcinoma cell lines with IC_{50} value of 10.03 and 4.12 μg/mL, respectively.

Genus '*Polyalthia*' has been an inexhaustible source of various novel bioactive natural products since three decades.[1] *Polyalthia longifolia* Benth. & Hook.f. var. *pendula* (Annonaceae) is cultivated widely throughout tropical and subtropical Asia as an evergreen ornamental tree and had been widely used in traditional medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis.[2] Aporphine alkaloids, clerodane and related diterpenes have been reported frequently, as the major secondary metabolites of this plant which showed varying degree of efficacy against different cancer cell lines and microbes.[3–6] As a part of our ongoing project on the discovery of new bioactive constituents from leaves of *P. longifolia* var. *pendula*,[7] we have isolated a 24-methylenecycloartane-3β, 16β, 23β-triol, a triterpene trivially named Longitriol (**1**), a bisclerodane imide named Longimide A (**2**) and a known isomeric (12*E*)-bisclerodane imide, which we named Longimide B (**3**).

Previously, a lanostane type triterpene as an anti-HIV principle from *Polyalthia suberosa* and two 24-methylene triterpenes from *Polyalthia lancilimba* were reported from this genus.[8,9] However, the isolation of **1** constitutes the first report of a cycloartane triterpene from this plant source. Isolation of bisclerodane imide is rare from any natural source. Although, few clerodane imides have been reported in recent years from different families,[10,11] this is the first report of the isolation of any dimeric clerodane **2** containing nitrogen atom in its core from this species and also this is the second report of isolation of any bisclerodane imide from entire plant kingdom. During our dereplication studies on **3** that was originally isolated from *Polyalthia viridis*, revealed minor discrepancies in the published structural assignment (^{13}C NMR data).[12] In this Letter, we describe the isolation and structure elucidation of two new compounds (**1** and **2**), together with revised complete structural assignment for the previously reported **3** and cytotoxicities of **1** and **2** against four human cancer cell lines is also provided.

Dried leaves of *P. longifolia* var. *pendula* were extracted with ethanol, extract thus obtained was partitioned with hexane, chloroform, butanol and water. A part of hexane extract was subjected

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☆ Part 10 in the series 'Search for new natural leads'

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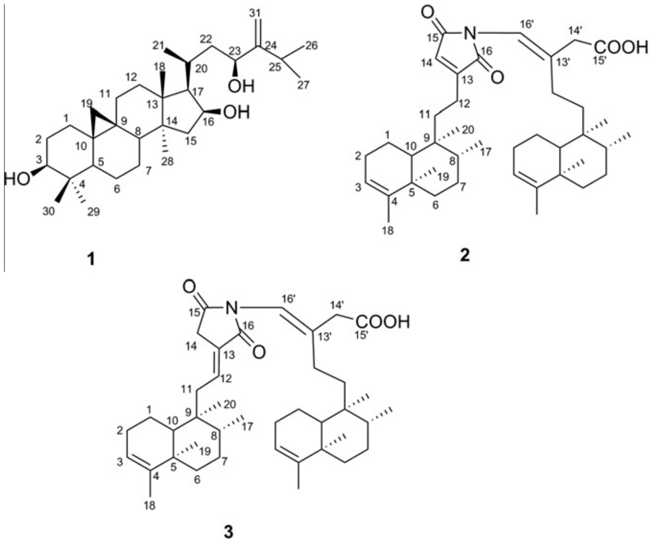

Figure 1. Chemical structure of the compounds (**1–3**) isolated from *P. longifolia* var. *pendula*.

to silica gel column chromatography with ethyl acetate–hexane (0–100%) followed by flash chromatography in ethyl acetate–hexane (5–30%). Repeated flash and thin layer chromatography of the subsequent fractions afforded Longitriol (**1**), Longimide A (**2**) and Longimide B (**3**) (Fig. 1).

Compound **1**, obtained as colorless crystals, $[\alpha]_D^{25}$ +43.5 (*c* 0.098, $CHCl_3$), showed pseudo molecular ion peak at *m*/*z* 454.3808 $[M{-}H_2O]^+$ in HREIMS (calcd 454.3811 for $C_{31}H_{50}O_2$), established the molecular formula as $C_{31}H_{52}O_3$ having six degrees of unsaturation.[13] This was supported by FABMS of **1** showing different fragment peaks at 495 $[M{+}Na]^+$, 472 $[M]^+$, 454 $[M{-}H_2O]^+$, 437 $[M{-}2H_2O{+}1]^+$, 419 $[M{-}3H_2O{+}1]^+$ and 313 $[M{-}C_9H_{17}O{-}H_2O]^+$ which indicated that the molecule has a side chain with nine carbons and three secondary hydroxyl groups. IR spectrum displayed bands at 3426 (OH group), 3021 (cyclopropane), 2939, 1629 (C=C), 1216 (C–O) and 767 cm^{-1}. The analysis of ^{13}C NMR data revealed the presence of seven methyls, ten methylenes, eight methines and six quaternary carbons, accounting for 31 carbon signals. Three signals at δ 72.5, δ 72.9 and δ 78.8 indicated oxygenated methines which together with mass fragmentation pattern confirmed them to be secondary hydroxyl groups (CH–OH). Carbon signal at δ 106.1 revealed that **1** contains exocyclic (=CH_2) methylene group (Table 1). The ^1H NMR spectrum of **1** displayed two highly shielded protons at δ 0.59 (1H, d, *J* = 4.0 Hz) and δ 0.34 (1H, d, *J* = 4.2 Hz) which are characteristic of two geminal protons of cyclopropane moiety. Further it showed the presence of four tertiary methyl singlets at δ 1.18, δ 0.88, δ 0.81 and δ 0.97, three secondary methyls

Table 1
1D and 2D NMR spectral data for Longitriol (**1**)

Position	δ_C, mult.[a,b]	δ_H[a] (*J* in Hz)	NOESY	HMBC[c]
1	31.9, CH_2	1.55, m	H-3, H-19b, H-2a	
		1.22, m	H-8	
2	30.3, CH_2	1.76, m	H-3, H-1a	
		1.55, m	H-7	
3	78.8, CH	3.28, dd (10.9, 4.3)	H-1a, H-29, H-5, H-2a	29, 30
4	40.5, qC			29, 30
5	47.1, CH	1.29, m	H-3, H-29	29, 30
6	21.0, CH_2	1.60, m	H-29, H-6b	
		0.81, m	H-6a	
7	26.2, CH_2	1.98, m	H-2b, H-11b	
8	48.0, CH	1.62, m	H-1b, H-18, H-19a	
9	19.8, qC			
10	26.1, qC			
11	26.1, CH_2	1.37, m	H-15a	
		1.10, m	H-7	
12	32.2, CH_2	1.60, m		18
13	45.2, qC			18
14	46.7, qC			28
15	47.0, CH_2	2.01, m	H-11a, H-28, H-16	
		1.40, m	H-18	
16	72.5, CH	4.46, m	H-15a, H-17, H-28	
17	56.7, CH	1.64, m	H-16, H-23, H-28	18, 21
18	18.8, CH_3	1.18, s	H-8, H-15b, H-21	
19	30.2, CH_2	0.59, d (4.0)	H-8, H-18, H-19b, H-30	
		0.34, d (4.2)	H-1a, H-19a	
20	28.0, CH	2.10, m	H-23	21
21	19.9, CH_3	1.02, d (6.5)	H-18	
22	44.8, CH_2	1.60, m	H-31a	21
		1.25, m		
23	72.9, CH	4.11, d (10.4)	H-17, H-20, H-25, H-31a	31a, b
24	159.8, qC			26, 27
25	30.4, CH	2.21, m	H-23, H-26	26, 27, 31a, b
26	23.4, CH_3	1.06, d (6.7)	H-25, H-31b	27
27	22.4, CH_3	1.08, d (6.7)		26
28	20.0, CH_3	0.88, s	H-15a, H-16, H-17	
29	25.4, CH_3	0.97, s	H-3, H-5, H-6a	30
30	14.0, CH_3	0.81, s		29
31	106.1, CH_2	5.09, s	H-22a, H-23, H-31b	
		4.87, s	H-26, H-31a	

[a] The spectra were recorded in $CDCl_3$ at 300 MHz (^1H NMR) and 75 MHz (^{13}C NMR).
[b] Multiplicity was determined by DEPT experiments.
[c] HMBC correlations are from carbons to the indicated protons.

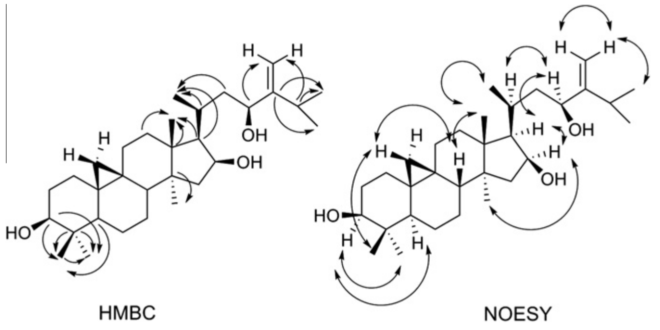

Figure 2. Key HMBC and NOESY correlations of **1**.

doublets at δ 1.02 (3H, d, $J = 6.5$ Hz) and the other two fused at δ 1.08 and δ 1.06 (6H, d, $J = 6.7$ Hz). Two singlet peaks at δ 5.09 (1H, s) and δ 4.87 (1H, s) confirmed the presence of olefinic methylene and other peaks at δ 4.46 (1H, m), δ 4.11 (1H, d, $J = 10.4$ Hz) and δ 3.28 (1H, dd, $J = 10.9$, 4.3 Hz) confirmed the presence of oxygenated carbons, that is, three hydroxyl groups. These data suggested **1** to be a lanostane type 24-methylenecycloartane triterpene. The multiplicities and connectivity of proton to carbon were confirmed by HSQC experiment. The COSY spectrum showed three spin systems (H_2–H_3, H_{15}–H_{16}–H_{17} and H_{22}–H_{23}–H_{31}) which coupled with the two and three bond cross correlations (HMBC spectrum) of C-3 with H-29 and H-30, C-17 with H-21 and H-18, C-23 with H-31a,b, C-24 with H-26 and H-27, C-25 with H-31a,b, H-26 and H-27, C-22 with H-21 established the position of hydroxyl groups at C-3, C-16 and C-23 and olefinic methylene at C-24 along with terminal isopropyl group and side chain at C-17. Besides this, the chemical shift value and splitting pattern of proton at H-3, confirmed C-3 hydroxyl group as β-oriented.[14] The NOESY data analysis helped in assigning the relative stereochemistry of **1** (Fig. 2). All these experimental data correlated well with the other published data of cycloartane tritepenes.[15–17] The relative stereochemistry and structure was finally confirmed by X-ray diffraction analysis[18] (ORTEP diagram shown in Fig. 3). Thus, the structure of **1** was identified as 24-methylenecycloartane-3β, 16β, 23β-triol, for which we proposed the name Longitriol.

Compound **2**, obtained as colorless gum, $[\alpha]^{25}_{D}$ −26.9 (*c* 0.078, CHCl$_3$) was characterized by analyzing various spectroscopic data including 1D and 2D NMR, Infrared absorption, ESIMS and HR-

Table 2
1D and 2D NMR spectral data for Longimide A (**2**) and Longimide B (**3**)[a]

Position	**2**			**3**	
	δ_C, mult.	δ_H (*J* in Hz)	HMBC	δ_C, mult.	δ_H (*J* in Hz)
1	18.4, CH$_2$	1.40, m		18.5, CH$_2$	1.42 m
2	27.1, CH$_2$	2.04, m		27.1, CH$_2$	1.99 m
3	120.7, CH	5.20, br s	18	120.6, CH	5.16 br s
4	144.4, qC		18, 19	144.1, qC	
5	38.4, qC		19	38.5, qC	
6	36.9, CH$_2$	1.69, m 1.13, m		36.8, CH$_2$	1.67 m 1.16 m
7	27.6, CH$_2$	1.44, m	17	27.6, CH$_2$	1.43 m
8	36.3, CH	1.38, m	17, 20	36.3, CH	1.36 m
9	39.1, qC		20	41.1, qC	
10	46.7, CH	1.38, m	19, 20	48.2, CH	1.34 m
11	35.8, CH$_2$	1.64, m 1.49, m	20	39.1, CH$_2$	2.31 m 2.18 m
12	19.7, CH$_2$	2.29, m	20	137.6, CH	6.90 br t (7.9)
13	151.8, qC		14	126.4, qC	
14	126.3, CH	6.32, s		32.5, CH$_2$	3.26 br s
15	170.4, qC		14	173.3, qC	
16	169.8, qC		14	168.7, qC	
17	16.2, CH$_3$	0.73, fused		16.1, CH$_3$	0.73 d (5.6)
18	18.2, CH$_3$	1.59, s		18.2, CH$_3$	1.56 s
19	20.2, CH$_3$	1.0, s		20.1, CH$_3$	1.0 s
20	18.3, CH$_3$	0.76, s		17.9, CH$_3$	0.82 s
1′	18.4, CH$_2$	1.48, m		19.2, CH$_2$	1.53 m
2′	27.0, CH$_2$	1.81, m 1.94, m		27.0, CH$_2$	1.94 m
3′	120.5, CH	5.11, br s	18′	120.5, CH	5.10 br s
4′	144.6, qC		18′, 19′	144.6, qC	
5′	38.3,qC		19′	38.3, qC	
6′	36.8, CH$_2$	1.69, m 1.13, m		36.4, CH$_2$	1.67 m 1.16 m
7′	27.7, CH$_2$	1.37, m	17′	27.7, CH$_2$	1.36 m
8′	36.6, CH	1.47, m	17′, 20′	37.9, CH	1.42 m
9′	38.9, qC		20′	38.9, qC	
10′	46.5, CH	1.22, m	19′, 20′	46.5, CH	1.22 m
11′	35.3, CH$_2$	1.38, m	20′	35.1, CH$_2$	1.36 m
12′	24.3, CH$_2$	1.94, m	14′, 20′	24.6, CH$_2$	1.91 m
13′	140.2, qC		14′, 16′	140.9, qC	
14′	39.4, CH$_2$	3.21, br s	16′	39.8, CH$_2$	3.23 br s
15′	175.4, qC		14′	174.6, qC	
16′	116.3, CH	5.83, s	14′	116.9, CH	5.89 s
17′	16.2, CH$_3$	0.82, d (5.6)		16.7, CH$_3$	0.86 d (5.8)
18′	18.2, CH$_3$	1.54, s		18.2, CH$_3$	1.54 s
19′	20.1, CH$_3$	0.95, s		20.1, CH$_3$	0.95 s
20′	18.5, CH$_3$	0.68, s		18.4, CH$_3$	0.68 s

[a] The spectra were recorded in CDCl$_3$ at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR).

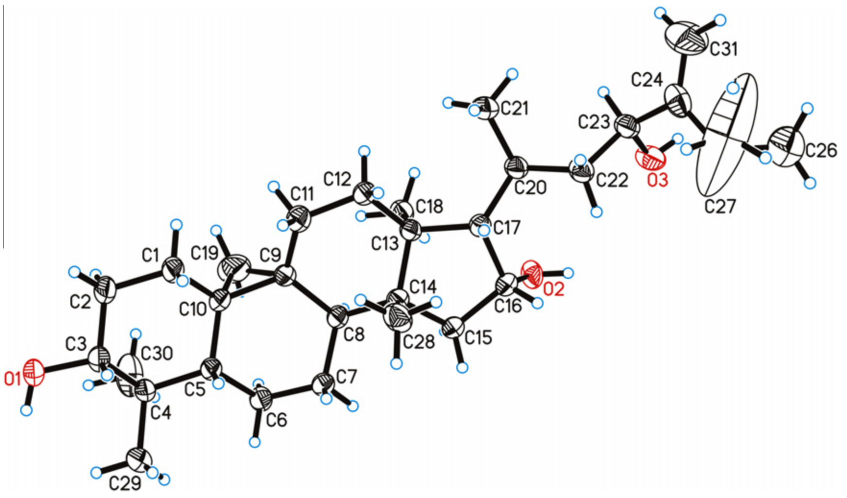

Figure 3. ORTEP diagram with atomic numbering scheme showing molecular structure of **1** at 30% probability.

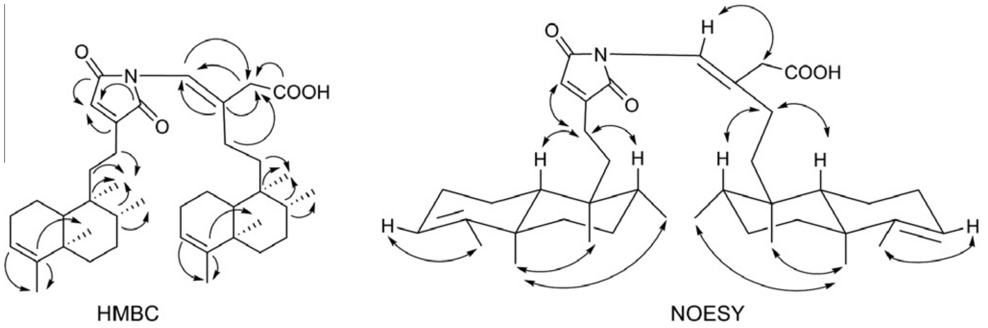

Figure 4. Key HMBC and NOESY correlations of **2**.

DART. The molecular formula was established as $C_{40}H_{59}NO_4$ on the basis of positive-ion HR-DART giving molecular ion peak at *m/z* 618.4516 (calcd for $C_{40}H_{60}NO_4$, 618.4522).[19] The IR spectrum showed absorption bands at 3405, 1712, 1633 and 1366 cm^{-1} denoting presence of a carboxylic acid and maleimide group. ^{13}C NMR spectra showed 40 carbon signals, most of the signals in aliphatic region were in pairs, indicating a dimer of diterpene. Thus, Compound **2** contained two clerodane moieties which was evident from mass spectrum and from the duplication of 15 signals (corresponding to C-1 to C-11 and C-17 to C-20, representing the trans-decalin system of clerodane diterpenes containing side chain at C-9) in the ^{13}C NMR spectrum (Table 2). The difference in chemical shifts of remaining ten carbons was apparent, of which one was a carboxylic acid (C-15′) that resonated at δ 175.4, two were α,β-unsaturated keto groups (C-15 and C-16) resonating at δ 170.4 and δ 169.8, two olefinic methine (C-14 and C-16′) resonating at δ 126.3 and δ 116.3, three methylenes (C-12, C-12′ and C-14′) resonating at δ 19.7, δ 24.3 and δ 39.4 and two quaternary carbons (C-13 and C-13′) at δ 151.8 and δ 140.2. Besides this, ^1H NMR spectrum displayed characteristic peaks at δ 6.32 (1H, s), δ 5.83 (1H, s) and δ 3.21 (2H, br s) that were assigned to H-14, H-16′ and H-14′, respectively. The COSY spectrum revealed two allylic spin systems, $H_{16'}$–H'_{14} and H_{14}–H_{12}. The proton to carbon connectivities were established with the help of HSQC experiment. Long range ^1H–^{13}C couplings observed in the HMBC spectrum supported the connectivity of important spin systems (Fig. 4). HMBC correlations were observed for the maleimide core, where H-14 correlated with C-15, C-16 and C-13 and furthermore, H-16′ correlated with C-13′ and C-14′ while, H-14′ correlated with C-15′, C-16′, C-13′ and C-12′confirming the presence of the β,γ-unsaturated carboxylic acid. NOESY correlations established the relative configurations of **2**. The observation of strong cross-peak in the NOESY between H-16′ and H-14′ permitted the assignment of orientation of the C-13′ olefinic bond as ‘*E*’. These correlations were in good agreement with that expected for α-methylene and β,γ-unsaturated carboxylic acid and were in good accord with published literature values of similar compound.[12]

On the basis of the spectral evidence, it was concluded that **2** is a dimer of clerodane imide that is linked through N-atom of maleimide core to C-16′ atom of the second clerodane moiety. The

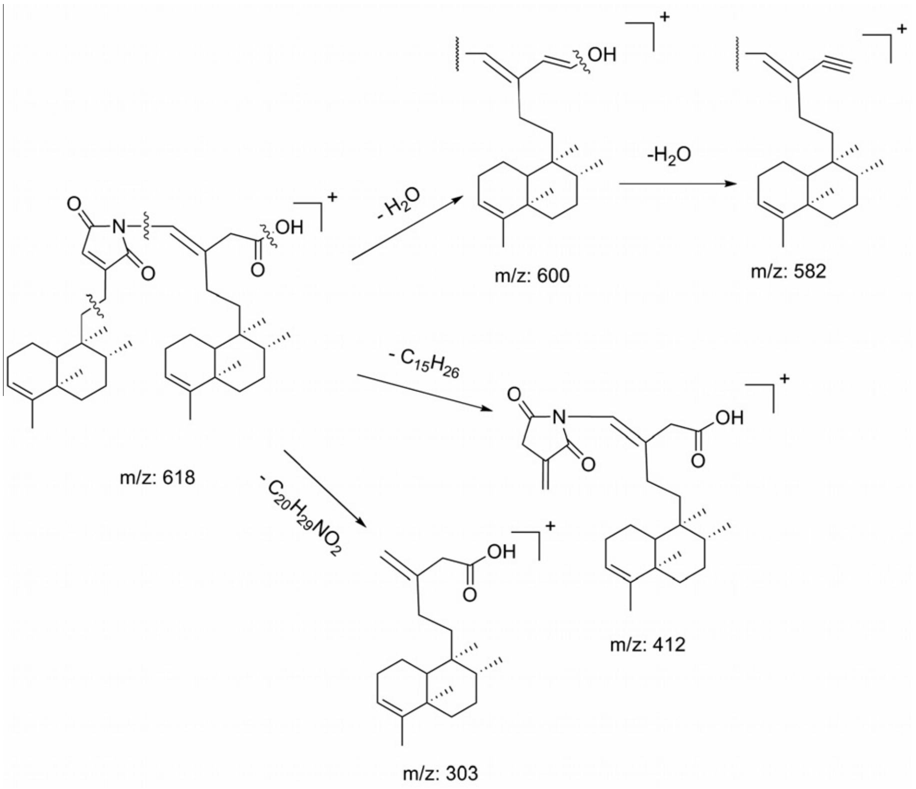

Figure 5. Proposed fragmentation of **2** by Tandem Mass (MS/MS).

Table 3
In vitro cytotoxic activity of **1** and **2** on different cell lines

Cell line	IC_{50}[a] (in µg/mL)		
	Doxorubicin	Longitriol (**1**)	Longimide A (**2**)
KB	0.12	24.11	8.85
MCF-7	0.45	30.88	9.23
A549	0.33	13.19	10.13
C33A	0.28	10.03	4.12
NIH3T3	nd[b]	>50	44.7

[a] Values shown are obtained from single independent experiments done in triplicate.
[b] nd—not done.

proposed structure was confirmed by Tandem mass (MS/MS) spectrometry. The proposed key fragmentations that corroborate the proposed structure are shown in Figure 5. Consequently, structure of **2** was established as shown and has been named Longimide A.

Compound **3**, obtained as colorless gum, $[\alpha]_D^{25}$ −32.8 (*c* 0.06, $CHCl_3$), was found to be similar to **2** on the basis of 1D and 2D NMR spectra. ESIMS showed *m/z* at 618 $[M+H]^+$ and 635 $[M+NH_4]^+$ in positive-ion mode.[20] In the ^1H NMR, appearance of a peak at δ 6.90 (1H, br t, *J* = 7.9 Hz) and δ 3.26 (2H, br s) together with another peak at δ 5.89 (1H, s) and δ 3.23 (2H, br s) clearly indicated that the olefinic bond at C-13 now migrated to C-12 giving rise to methylene group at δ 32.5 (C-14) in the succinimide core. So, it was concluded that compound **3** is an olefinic '12*E*' isomer of compound **2** on the basis of complete spectral analysis. During our dereplication studies it was found that **3** is identical to the compound reported from *P. viridis*,[12] however minor discrepancies in the published structural assignment (^{13}C NMR data) were noticed. The chemical shift values assigned for four carbon signals were interchanged. Specifically, the chemical shift for C-11 at δ 39.1 (reported as δ 34.8) was interchanged with C-11′ at δ 35.1 (reported as δ 38.8). Similarly, C-13 at δ 126.4 (reported as δ 140.2) was interchanged with C-13′ at δ 140.9 (reported as δ 126.2). The chemical shift value assignments of these carbon atoms were confirmed unambiguously by detailed analysis of the HMBC data. Thus, complete and corrected structural assignment of compound **3** named Longimide B is also now reported (Table 2).

Compounds **1** and **2** were tested for their in vitro cytotoxic activity against four different cancer cell lines and one normal cell line using SRB assays.[21] Compound **3** could not be tested due to the paucity of the compound. IC_{50} values were based on dose–response curves (Table 3). Both compounds showed cytotoxicity against cancer cell lines, but **2** was more active comparatively. Longimide A (**2**) showed significant activity against cervical carcinoma cell lines (IC_{50} 4.12 µg/mL). Furthermore, it was found to be apparently non-toxic against normal cell lines (NIH3T3).

In conclusion, we have isolated and elucidated the structure of two new compounds, Longitriol (**1**) and Longimide A (**2**) along with a known compound Longimide B (**3**) from *P. longifolia* var. *pendula*. Both of them displayed noteworthy activity against cervical carcinoma cell lines in vitro, which is amongst the four most common form of cancer in females globally. Biogenetically, the isolation of cycloartane triterpene from this plant could be attributed to the fact that one of the simplest representative-cycloartenol is the key intermediate in the biosynthesis of phytosterols. Previous literature survey reveals that this class of triterpenoids displayed interesting biological activities including cytotoxic and anti-HIV effects.[22] The isolation of rare bisclerodane imides is interesting as maleimides are known to show cytotoxicity towards tumor cell lines through the inhibition of human topoisomerase II.[23] The fact could be utilized for further target based studies on these rare molecules. This study underscores the importance of this genus '*Polyalthia*' as a steady source of structurally diverse novel bioactive compounds that clearly merits further study.

Acknowledgments

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Supplementary data

Supplementary data (spectral data (1D and 2D) of isolated compounds **1**–**3** along with the general experimental details, extraction and isolation, crystallographic data and in vitro cytotoxicity assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.141.

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13. Longitriol (**1**): colorless crystals (MeOH); mp 230–231 °C; $[\alpha]_D^{25}$ +43.5 (*c* 0.098, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (3.04) nm; IR (KBr) ν_{max} 3426, 3021, 2939, 1629, 1216, 767 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; FABMS *m/z* 495 $[M+Na]^+$ (17), 472 $[M]^+$ (16), 454 (48), 437 (100), 419 (28), 313 (83); HREIMS *m/z* 454.3808 (calcd for $C_{31}H_{52}O_3$—H_2O, 454.3811).
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18. Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 752041). These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; email: deposit@ccdc.cam.ac.uk).
19. Longimide A (**2**): colorless gum; $[\alpha]_D^{25}$ −26.9 (*c* 0.078, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (4.32), 280(2.24) nm; IR (neat) ν_{max} 3405, 2930, 1712, 1633, 1366, 1218, 769 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; ESIMS *m/z* 618 $[M+H]^+$ (100), 640 $[M+Na]^+$ (43), 600 (25), 412 (6), 303 (6); HR-DART *m/z* 618.4516 (calcd for $C_{40}H_{60}NO_4$, 618.4522).
20. Longimide B (**3**): colorless gum; $[\alpha]_D^{25}$ −32.8 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (3.42) nm; IR (neat) ν_{max} 3447, 2928, 1708, 1594, 1458, 1384, 1352, 1193, 758 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; ESIMS *m/z* 618 $[M+H]^+$ (100), 635 $[M+NH_4]^+$ (50).
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