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ANTITUMOUR AND ANTI-AIDS TRITERPENES FROM *CELASTRUS HINDSII*

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Key Word Index—*Celastrus hindsii*; Celastraceae; cytotoxicity; anti-AIDS; anti-HIV; triterpenes; maytenfolone-A, celasdin-A; celasdin-B; celasdin-C.

Abstract—Four new triterpene compounds, celasdin-A, celasdin-C, anti-AIDS celasdin-B and cytotoxic maytenfolone-A, were isolated from *Celastrus hindsii*. The structural determination of maytenfolone-A, celasdin-A, celasdin-B and celasdin-C, as well as the structure–activity relationships of these new compounds and derivatives, are discussed. Maytenfolone-A was further confirmed by X-ray studies. Biological evaluation showed that Maytenfolone-A demonstrated cytotoxicity against hepatoma (HEPA-2B, ED_{50} = 2.3 μg ml^{-1}) and nasopharynx carcinoma (KB, ED_{50} = 3.8 μg ml^{-1}). Celasdin-B was found to exhibit anti-HIV replication activity in H9 lymphocyte cells with an EC_{50} of 0.8 μg ml^{-1}. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

In our search for potential antitumour agents from the plant family Celastraceae, we recently reported the isolation of new cytotoxic sesquiterpene pyridine alkaloids from the *Maytenus emarginata* (Gray) Hou [1–4], *M. diversifolia* [5] and *Celastrus hindsii* [6,7]. Now we report herein further investigations of an ethanol extract from *C. hindsii* Benth. It shows potent cytotoxicity against HEPA-2B (hepatoma), HELA (cervix carcinoma), COLO-205 (colon carcinoma) and KB (nasophyrnx carcinoma) cells, as well as against HIV replication activity in H9 lymphocytes *in vitro*. This led us to isolate and characterize four new triterpenes, maytenfolone-A (**1**), celasdin A (**2**), celasdin B (**3**) and celasdin C (**4**). In addition, the known friedelin (**6**) and canophyllol (**7**) were also isolated from the stems of *C. hindsii*. For the compounds **1–4**, the assignments of ^{1}H and ^{13}C NMR spectra were completed by employing 2D NMR techniques including ^{1}H–^{1}H COSY, NOESY and ^{1}H–^{13}C heteronuclear COSY as well as long-range COSY (HMBC and COLOC) experiments. The structure of **1** was also confirmed by X-ray studies and its spectra were compared with those of maytenfolone (**5**) which was isolated from the leaves of *M. diversifolia* [5]. The cytotoxicity and anti-HIV activity *in vitro* of these new compounds were tested.

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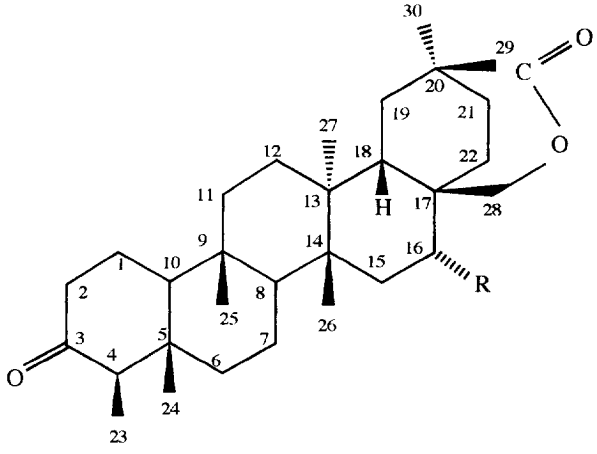
RESULTS AND DISCUSSION

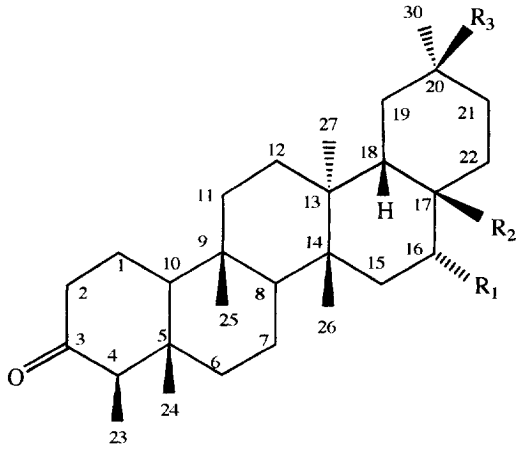
An ethanolic extract of the dried stems of *C. hindsii* was partitioned sucessively between *n*-hexane and chloroform. Repeated column chromatography of the chloroform extract yielded the novel triterpenes, **1–4**, **6** and **7**.

Compound **1**, white cubic crystals, had a molecular formula of $C_{30}H_{46}O_4$. Signals for one secondary, five tertiary methyl groups and 30 carbons were found in the ^{1}H NMR and ^{13}C NMR DEPT spectra, respectively. A characteristic peak at *m/z* 273 in the mass spectra revealed that **1** was the friedelin derivative [8–10]. The IR spectrum indicated a ketone (1715 cm^{-1}) and a hydroxyl group (3500 cm^{-1}). In addition, a carboxylic acid signal at δ_C 177.86, as well as typical two doublet protons at δ 3.95 and 4.02, and a carbon signal at δ_C 83.40, suggested that **1** is a lactone of friedelin triterpene and is an analogue of **5**) [5], except that it exhibits one hydroxyl group (δ_H 3.68; δ_C 70.24). Moreover, a detailed examination of the mass spectrum of **1** revealed a hydroxyl group on the D or E ring due to the obvious peak at *m/z* 221 [9–11].

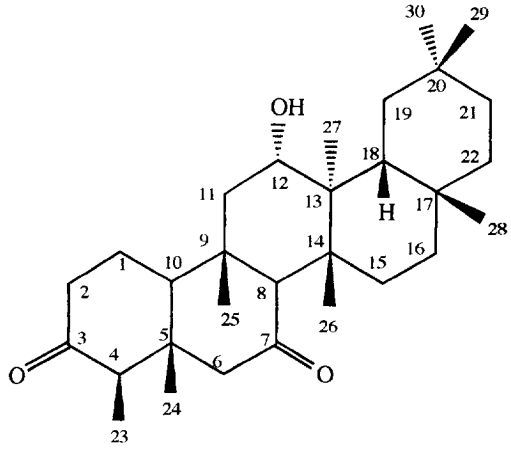
In a comparison of the ^{13}C NMR spectra of **1** and **5** (Table 1), the largest increase in the chemical shifts of C-15 (δ_C 38.04 in **1** to 28.98 in **5**), of C-16 (δ_C 70.24 in **1** to 29.54 in **5**) and of C-17 (δ_C 40.45–34.64) for **1** suggested the absence of one proton and the substitution of a hydroxyl at C-16. Moreover, inspection of the HMBC spectrum of **1** revealed that correlation between C-16 and H-15 also supported the location of a hydroxyl group at C-16. The stereochemical



	R
1	OH
1a	$O\overset{31}{C}\overset{32}{C}H_3$ (=O)
5	H



	R_1	R_2	R_3
2	OH	$\overset{28}{CH_3}$	$\overset{29}{CH_2OH}$
2a	$O\overset{31}{C}\overset{32}{CH_3}$ (=O)	CH_3	$\overset{29}{CH_2}O\overset{33}{C}\overset{34}{CH_3}$ (=O)
3	OH	$\overset{28}{CH_2OH}$	$\overset{29}{CH_3}$
3a	$O\overset{31}{C}\overset{32}{CH_3}$ (=O)	$\overset{28}{CH_2}O\overset{33}{C}\overset{34}{CH_3}$ (=O)	$\overset{29}{CH_3}$
6	H	CH_3	CH_3
7	H	CH_2OH	CH_3



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assignment of **1** was based on the NOESY (2D NOE) spectrum as well as on X-ray analysis (see Fig. 1 and Experimental). In the NOESY spectrum, the correlation between H-16 and Me-26, with no evidence of correlation between H-16 and Me-27, suggested the β-configuration for an H-16 and the α-configuration for a hydroxyl group. In addition, the α-configuration for a hydroxyl group and an H-22 that induced the obvious change of chemical shifts of H-22 between **1** and **5** (δ_C 23.43 in **1** to δ_C 41.38 in **5**), as well as between the acetylated compound **1** (**1a**) and **5** (δ_C 24.94 in **1a** to δ_C 41.38 in **5**), also agreed with this assignment. Notably, the quite different chemical shifts between H-28_a (δ 3.90) and H-28_b (δ 4.31), due to the existence of a hydroxyl at C-16, was relieved after acetylation. Thus, the acetate group at C-16 in **1a** had similar chemical shifts of H-28_a and of H-28_b as in **5**. Moreover, in view of the structure of **1** derived by X-ray analysis, the cyclohexane rings A, B, C and D in **1** all have a chair conformation with axial methyl groups and resemble an equatorially disposed hydroxyl. Ring E has a twisted envelope form due to the combination of a lactone between C-20 and C-28.

Compound **2**, $C_{30}H_{50}O_3$ ([M]$^+$ at m/z 458), which

Table 1. ^{1}H NMR data* for compounds **1–5**

H	1	2	3	4	5
1	1.91, 1.67 (*m*)	1.68, 1.67 (*m*)	1.91, † (*m*)	†, 2.02 (*m*)	1.93, 1.63 (*m*)
2	2.37, 2.19 (*m*)	2.35, † (*m*)	2.25, 2.37 (*m*)	2.46, † (*m*)	2.35, 2.22 (*m*)
4	2.22 (*m*)	2.22 (*m*)	2.25 (*m*)	2.50 (*m*)	2.23 (*m*)
6	1.26, 1.77 (*m*)	1.26, 1.75 (*m*)	1.26, 1.74 (*m*)	1.52, 1.72 (*m*)	1.22, 1.72 (*m*)
7	1.29, 1.47 (*m*)	1.32, 1.47 (*m*)	†, 1.55 (*m*)	—	1.27, 1.46 (*m*)
8	1.40 (*m*)	1.38 (*m*)	1.47 (*m*)	2.82 (*m*)	1.38 (*m*)
10	1.52 (*m*)	1.50 (*m*)	1.55 (*m*)	2.14 (*m*)	1.52 (*m*)
11	1.25, 1.42 (*m*)	1.21, 1.40 (*m*)	1.42, † (*m*)	2.21, 2.35 (*m*)	1.25, 1.44 (*m*)
12	1.19, 1.38 (*m*)	†, 1.32 (*m*)	1.22, † (*m*)	3.92 (*m*)	1.32, 1.45 (*m*)
15	1.29, 1.78 (*m*)	1.26, 1.78 (*m*)	1.20, 1.78 (*m*)	1.24, 1.91 (*m*)	1.25, 1.46 (*m*)
16	3.68 (*m*)	4.0 (*t*, J = 8.9)	4.24 (*dd*, J = 6.4, 11.6)	0.92†, 1.50 (*m*)	1.31, 1.43 (*m*)
18	1.93 (*m*)	1.52 (*m*)	1.34 (*m*)	1.94 (*m*)	1.96 (*m*)
19	1.58, 1.94 (*m*)	1.50, † (*m*)	1.50, † (*m*)	1.47, 1.75 (*m*)	147, 1.75 (*m*)
21	1.42, 1.90 (*m*)	1.17, 1.45 (*m*)	1.33, † (*m*)	†, 1.72 (*m*)	1.48, 1.93 (*m*)
22	1.82, 1.92 (*m*)	1.28, 1.33 (*m*)	1.83, 1.91 (*m*)	†, 1.37 (*m*)	1.42, 2.12 (*m*)
23	0.85 (*d*, J = 6.7)	0.85 (*d*, J = 6.8)	0.82 (*d*, J = 6.8)	0.86 (*d*, J = 6.8)	0.84 (*d*, J = 4.8)
24	0.69 (*s*)	0.69 (*s*)	0.68 (*s*)	0.69 (*s*)	0.69 (*s*)
25	0.88 (*s*)	0.83 (*s*)	0.86 (*s*)	1.42 (*s*)	0.86 (*s*)
26	0.89 (*s*)	1.03 (*s*)	0.85 (*s*)	0.96 (*s*)	0.83 (*s*)
27	1.10 (*s*)	0.98 (*s*)	1.10 (*s*)	1.05 (*s*)	1.08 (*s*)
28	3.90, 4.31 (ABq, J = 11.3)	1.12 (*s*)	3.31, 4.00 (ABq, J = 10.0)	1.19 (*s*)	3.89, 3.97 (ABq, J = 11.5)
29	—	3.46, 3.36 (ABq, J = 10.6)	1.01 (*s*)	0.99 (*s*)	—
30	1.19 (*s*)	0.97 (*s*)	0.92 (*s*)	0.94 (*s*)	1.18 (*s*)

* Measured in $CDCl_3$ (multiplicities, J in Hz).
† These assignments are uncertain and may be interchangeable.

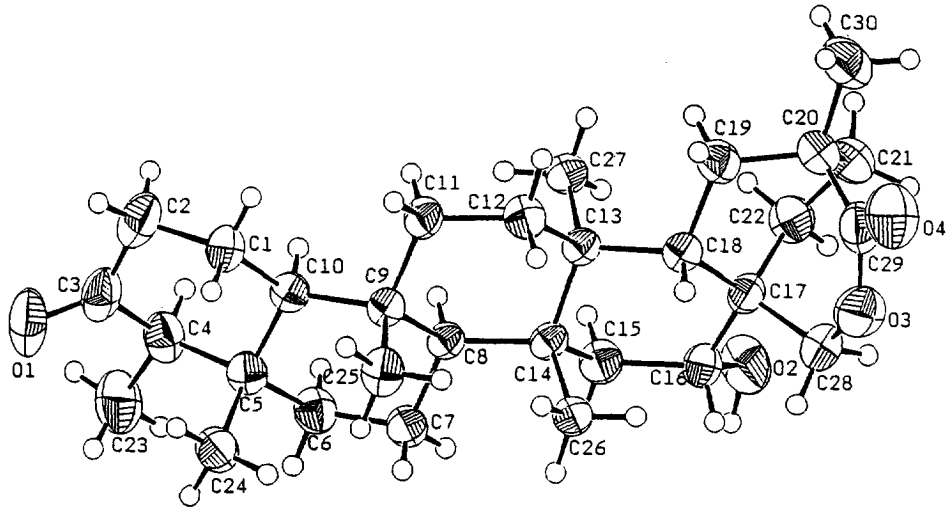

Fig. 1. Structure and solid-state conformation of maytenfolone-A (**1**).

was more polar than **1**, had IR absorption bands at 3450 (OH) and 1715 (CO) cm^{-1}. The mass spectrum of **2**, showing a characteristic peak at m/z 273, as well as one secondary, five tertiary methyl groups and 30 carbons in the ^{1}H NMR and ^{13}C NMR DEPT spectra, revealed that **2** belonged to the friedelin type of triterpene [8–10]. Thus, **2** was similar to **1** except that we found one more methyl group in **2** and that the hydroxyl and oxidized methylene chemical shifts had changed. In the ^{13}C NMR spectrum, a signal at δ 70.58 was found. Its appearance is common in this type of CH_2OH group in the friedelin compounds [9, 12] and quite different from an oxidized methylene (δ_C 82.45 ppm) in a lactone compound such as **1**. After a detailed inspection of the HMBC spectrum (Fig. 1), the long-range correlation between C-21 (δ_C 29.63) and the proton of CH_2OH at δ_H 3.46 and 3.36, as well as between the carbon of CH_2OH (δ_C 70.60) and the

methyl group (H-30), led to the assignment of the CH_2OH group (H-29) being linked to C-20. In addition, the long-range correlations between the hydroxyl carbon at δ 75.30 and H-15, and H-28, suggested that the position of the hydroxyl group would be at C-16. Furthermore, from the 2D NOE spectrum, the correlations between β-methyl at C-17 and β-CH_2OH at C-20, and the obvious interactions among H-16, H-26 and H-18 for the β-feature, were consistent with the β-CH_2OH and α-OH for the stereochemistry of **2** at C-20 and C-16, respectively. Moreover, in the HMBC spectrum of **2a** (acetate of **2**), not only did the two acetyl methyl groups correlate with carbonyl carbons at δ_C 171.44 (C-31) and 171.11 (C-33), respectively, but the long-range correlations between C-16 (δ_C 78.16), H-28 (δ_H 1.22), and H-15 (δ_H 1.31), respectively, and between C-29 (δ_C 71.63) and H-30 (δ_H 0.98), also supported the assignments of hydroxyl and CH_2OH at C-16 and C-20, respectively. From the above evidence, together with the molecular ion at m/z 458 $[M]^+$, the structure of celasdin A (**2**) was unambiguously confirmed.

Compound **3**, $C_{30}H_{50}O_3$ ($[M]^+$ at m/z 458), was obtained as a white amorphous powder. An inspection of the mass spectrum of **3** showed the characteristic peak m/z 273; there were IR absorptions at 3450 (OH) and 1715 (CO) cm^{-1} and NMR spectra chemical shifts at δ_H 4.24 and δ_C 74.30 (C-OH). The oxidized methylene signals at δ_H 4.00, 3.31 (ABq, J = 10 Hz) and δ_C 71.28 revealed that **3** belonged to the friedelin type of triterpene and was similar to **2**, except for the change of position of one oxidized methylene group in **3**.

In an investigation of the HMBC spectrum of **3**, the carbon signal (δ_C 74.30) of C-16 correlated with an oxidized methylene signal at δ_H 4.00, 3.31 (H-28) and its adjacent methylene at $\delta_{H\text{-}a}$ 1.74 (H-15). This result clearly indicated that a hydroxyl group and a oxidized methylene are positioned at C-16 and C-17, respectively. Furthermore, the extremely close chemical shifts of C-13 and C-14 were distinguished at δ_C 39.88 and 39.19, respectively. These assignments were based on the correlation between C-13 and H-12 (δ_H 1.22), whereas there was no obvious correlation with C-14. From the NOESY studies, the correlations between H-16, H-18 and H-26 were consistent with an α-hydroxyl at C-16, and completed the structure of **3**. In addition, the acetylation of **3** resulted in **3a**, which indicated the downshift chemical shifts of one hydroxyl group (H-16) at δ_H 5.40, an oxidized methylene (H-28) at δ_H 4.03 (q, J = 13 Hz) and two methyl groups at δ_H 2.01 ($s \times 2$, H-32, 34) all due to the long-range correlations between carbonyl carbons at C-31 and C-33 (δ_C 170.11 and 171.22). This observation was also consistent with the unambiguous assignment of structure **3** and its chemical shifts. Moreover, the difference between H-28_a and H-28_b, due to the hydroxyl at C-16 of **3** seemed to be insignificant after the acetylation of **3**. Similarly, in **1** and **1a**, the chemical shifts of H-$28_{a,b}$ were influenced by the hydroxyl at C-16 more than by acetate.

Compound **4** ($[M]^+$ at m/z 456, $C_{30}H_{48}O_3$), was obtained as an amorphous powder. Its ^1H NMR, ^{13}C NMR, ^1H–^{13}C COSY and DEPT spectra suggested a triterpene skeleton containing two carbonyl carbons (δ_C 210.15 and 210.56), and one hydroxyl group (δ_C 72.79 and δ_H 3.92). From the mass spectrum, the molecular ion at m/z 456 and characteristic peaks at m/z 288 and 273 units, suggested a friedelin type triterpene and two carbonyl oxygens at the A and B ring, respectively [13]. The other diagnostically important peak at m/z 205, derived from the D and E rings, therefore provided evidence for the lack of a hydroxyl group in these rings in **4** [10,11,13]. Furthermore, in the HMBC spectrum, the carbonyl carbon at δ_C 210.15 correlated with singlet proton signals at H-8 (δ_H 2.82) and methyl groups of C-25 and C-26. There was also correlation between the other carbonyl carbon at δ_C 210.56 and the doublet methyl group (δ_H 0.86) of C-23. These correlations suggested the ketones should be assigned at C-7 and C-3, respectively, the same diketone positions as in putranjivadione (**6**) [13], which was also isolated from the title plant. The position of the hydroxyl group could be assigned at C-12, due to the long-range correlation between the carbon signal at δ_C 72.79 (C-12) and the proton signals at δ_H 1.05 (H-27). In addition, the correlations between C-15, H-26 and H-16, as well as the correlations between C-19, H-29 and H-30, respectively, excluded the probable hydroxyl position at C-15 or C-19 and was therefore consistent with the arrangement of an hydroxyl group at C-12. Furthermore, examination of a 2D NOE (NOESY) spectrum for the stereochemistry of structure **4** showed that a methyl group at δ 1.05 (H-27) correlated with a methine at δ 2.82 (H-8), and the respective methyl groups at δ 1.42 (H-25) and 0.96 (H-26) correlated with δ 3.92 (H-12). These observations reveal that H-8, OH-12 and C-27 have α-configurations, and therefore completely confirm the structure of celasdin C (**4**).

Compounds **1**, **1a**, **2**, **2a**, **3**, **3a** and **4**–**7** were tested for cytotoxicity in four cancer cell lines (KB, COLO-205, HEPA-2B and HELA). Compound **1** showed cytotoxicity against hepatoma (HEPA-2B, ED_{50} = 2.3 μg ml^{-1}) and nasopharynx carcinoma (KB, ED_{50} = 3.8 μg ml^{-1}) cells, whereas the other compounds were inactive due to the $ED_{50} > 10$ μg ml^{-1}, respectively, under the reference compound, mitomycin C against above four cell lines ($ED_{50} < 0.04$ μg ml^{-1}). These results indicate that the friedelin derived seven-membered ring lactone between C-17 and C-20 may be important if the hydroxyl is located at C-16. Furthermore, in the anti-HIV bioassay of compounds **1**–**7**, the anti-HIV potential was poor, except for **1** and **3**, because the EC_{50} values exceeded 10 μg ml^{-1}. Compound **3** inhibited HIV replication in H9 lymphocyte cells with an EC_{50} of 0.8 μg ml^{-1} and exhibited toxicity at 5.5 μg ml^{-1}. This therapeuric index of **3** is better than that of **1** with an EC_{50} of 1.8 μg ml^{-1} and a lower toxicity at

7.0 μg ml^{-1}. The standard agent, AZT, showed an EC_{50} of 0.01 μg ml^{-1} and toxicity at 500 μg ml^{-1}. The detailed structure–cytotoxicity relationship remains to be explored.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were recorded at 300.13 and 75.46 MHz on a Bruber 300 AC spectrometer, respectively. The spectra of heteronuclear correlation, HMBC and COLOC, were established by coupling constants of 8 Hz. EIMS were performed on a JEOL SX-102A instrument. X-ray analysis was performed with a Nonius (CAD4) instrument. Silica gel (Merck, 70–230 mesh) was used for CC, and precoated silica gel (Merck 60F_{254}) plates were used for TLC. HPLC was accomplished on a SPD-6AV liquid chromatograph employing a semi-prep. silica gel column. Mps were determined on a Fisher–Johns apparatus and are uncorr.

Plant material. Stems of *C. hindsii* were collected in September 1992 in Taichung County, Taiwan. A voucher specimen is deposited at the National Research Institute of Chinese Medicine, Taipei Hsien, Taiwan.

Isolation of compounds **1–4**. Dried stems of *C. hindsii* (5.2 kg) were extracted exhaustively with EtOH. The crude EtOH extract (200 g) was subjected to CC on silica gel. Fr. 6 was further sepd repeatedly by HPLC (silica gel, CH_2Cl_2–EtOAc, 1 : 3) to afford **1** (20 mg), **2** (13 mg), and **3** (9 mg). Fr. 2 was rechromatographed on silica gel with hexane–EtOAc (3 : 1) as eluent to yield 12 mg **4**.

Maytenfolone-A (**1**). Cubic crystalline solid from EtOAc, mp 166–168°. $[\alpha]_D$ −85° ($CHCl_3$; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3500, 1715; EIMS *m/z* (rel. int.): 470 [M]$^+$ (86), 452 (20), 410 (47), 385 (100); HRMS: ($C_{30}H_{46}O_4$) found 470.3387, required 470.3397; ^{1}H and ^{13}C NMR,: Tables 1 and 2, respectively. X-ray crystal data: $C_{30}H_{46}O_4$, M_r 470.69, monoclinic, $P2_1$, a = 6.643 Å, b = 13.767 Å, c = 14.203 Å, β = 101.99°, V = 1270.6 Å^3, D_c = 1.230 g cm^{-3} for Z = 2, F11(000) = 517, μ = 5.866 cm^{-1}.

Celasdin-A (**2**). Amorphous powder, mp 251–253°. $[\alpha]_D$-55° ($CHCl_3$; *c* 0.1) IR ν_{max}^{KBr} cm^{-1}: 3450, 1715; EIMS *m/z* (rel. int.) 458 [M]$^+$ (13), 440 (16), 410 (34), 385 (31), 317 (16), 273 (50), 247 (35), 231 (34), 193 (55), 109 (100); HRMS: ($C_{30}H_{50}H_3$) found 458.3732, required 458.3762; ^{1}H and ^{13}C NMR,: Tables 1 and 2, respectively.

Celasdin-B (**3**). Amorphous powder, mp 171–173°. $[\alpha]_D$ −35° ($CHCl_3$; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3450, 1715; EIMS *m/z* (rel. int.): 458 [M]$^+$ (8), 440 (16), 410 (24), 409 (42), 303 (40), 273 (45), 234 (100), 109 (50); ^{1}H and ^{13}C NMR,: Tables 1 and 2, respectively.

Celasdin-C (**4**). Amorphous powder, mp 256–258°. $[\alpha]_D$ −60° ($CHCl_3$; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3450, 1715; EIMS *m/z* (rel. int.): 456 [M]$^+$ (5), 455 (11), 439 (11), 409 (8), 287 (10), 273 (23), 221 (16); ^{1}H and ^{13}C NMR,: Tables 1 and 2, respectively.

Acetylation of **1**, **2** *and* **3**. A soln of **1** (5 mg), **2** (5 mg) and **3** (5 mg) in pyridine (1 ml), respectively, was added to Ac_2O (1 ml) at room temp. and stood for 20 hr. The reaction products, following general procedures for purification, yielded **1a**, **2a** and **3a**, respectively.

Compound **1a**. ^{1}H NMR ($CDCl_3$): δ 4.04, 3.98 (2H, AB*q*, J = 11.6 Hz, H-28), 4.94 (1H, *dd*, J = 6.8, 11.0 Hz, H-16), 0.70 (3H, *s*, H-24), 0.86 (3H, *d*, J = 6.6 Hz, H-23), 0.89 (3H, *s*, H-26), 0.95 (3H, *s*, H-25), 1.14 (3H, *s*, H-27), 1.21 (3H, *s*, H-30), 2.03 (3H, *s*, H-31); ^{13}C NMR ($CDCl_3$): δ 22.18 (*t*, C-1), 41.40 (*t*, C-2), 213.00 (*s*, C-3), 58.15 (*d*, C-4), 41.96 (*s*, C-5), 40.98 (*t*, C-6), 18.28 (*t*, C-7), 50.12 (*d*, C-8), 37.65 (*s*, C-9), 59.52 (*d*, C-10), 34.67 (*t*, C-11), 27.94 (*t*, C-12), 39.73 (*s*, C-13), 39.63 (*s*, C-14), 34.67 (*t*, C-15), 72.67 (*d*, C-16), 39.29 (*s*, C-17), 41.61 (*d*, C-18), 29.68 (*t*, C-19), 37.65 (*s*, C-20), 32.95 (*t*, C-21), 24.94 (*t*, C-22), 6.80 (*q*, C-23), 14.64 (*q*, C-24), 19.02 (*q*, C-25), 16.76 (*q*, C-26), 15.08 (*q*, C-27), 82.45 (*q*, C-28), 177.34 (*q*, C-29), 28.09 (*q*, C-30), 170.48 (*s*, C-31), 21.11 (*q*, C-32).

Compound **2a**. ^{1}H NMR ($CDCl_3$): δ 5.19 (1H, *t*, J = 6.2 Hz, H-16), 3.89 (2H, *s*, H-29), 0.70 (3H, *s*, H-24), 0.85 (3H, *d* = 6.8 Hz, H-23), 0.85 (3H, *s*, H-25), 0.98 (3H, *s*, H-30), 1.06 (3H, *s*, H-27), 1.07 (3H, *s*, H-26), 1.22 (3H, *s*, H-28), 1.98 (3H, *s*, H-34), 2.06 (3H, *s*, H-32); ^{13}C NMR ($CDCl_3$): δ 22.21 (*t*, C-1), 41.42 (*t*, C-2), 213.00 (*s*, C-3), 58.18 (*d*, C-4), 42.04 (*s*, C-5), 41.06 (*t*, C-6), 18.42 (*t*, C-7), 53.29 (*d*, C-8), 37.48 (*s*, C-9), 59.42 (*d*, C-10), 35.52 (*t*, C-11), 30.48 (*t*, C-12), 39.97 (*s*, C-13), 39.19 (*s*, C-14), 40.10 (*t*, C-15), 78.16 (*t*, C-16), 34.46 (*s*, C-17), 44.63 (*d*, C-18), 34.83 (*t*, C-19), 31.76 (*s*, C-20), 29.69 (*t*, C-21), 27.39 (*t*, C-22), 6.81 (*q*, C-23), 14.66 (*q*, C-24), 18.17 (*q*, C-25), 19.88 (*q*, C-26), 21.27 (*q*, C-27), 29.78 (*q*, C-28), 71.67 (*q*, C-29), 26.37 (*q*, C-30), 171.44 (*s*, C-31), 20.99 (*q*, C-32), 171.11 (*s*, C-33), 21.27 (*q*, C-34).

Compound **3a**. ^{1}H NMR ($CDCl_3$): δ 0.66 (3H, *s*, H-24), 0.86 (3H, *d*, J = 7.5 Hz, H-23), 0.94 (6H, *s*, H-26,27), 1.02 (3H, *s*, H-25), 1.23 (6H, *s*, H-29,30), 2.01 (6H, *s*, H-32,34), 3.99, 4.07 (2H, AB*q*, J = 11.2 Hz, H-28), 5.40 (1H, *m*, H-16); ^{13}C NMR ($CDCl_3$): δ 22.18 (*t*, C-1), 41.38 (*t*, C-2), 213.01 (*s*, C-3), 58.18 (*d*, C-4), 41.99 (*s*, C-5), 41.13 (*t*, C-6), 18.36 (*t*, C-7), 50.19 (*d*, C-8), 37.49 (*s*, C-9), 59.36 (*s*, C-10), 34.96 (*t*, C-11), 29.55 (*t*, C-12), 39.37 (*s*, C-13), 38.92 (*s*, C-14), 35.51 (*t*, C-15), 72.95 (*d*, C-16), 37.49 (*s*, C-17), 40.98 (*d*, C-18), 33.29 (*t*, C-19), 27.65 (*s*, C-20), 33.29 (*t*, C-21), 23.02 (*t*, C-22), 6.08 (*q*, C-23), 14.67 (*q*, C-24), 19.52 (*q*, C-25), 16.38 (*q*, C-26), 20.92 (*q*, C-27), 69.46 (*q*, C-28), 32.09 (*q*, C-29), 36.03 (*q*, C-30), 170.11 (*s*, C-31), 18.80 (*q*, C-32), 171.22 (*s*, C-33), 21.26 (*q*, C-34).

Friedelin (**6**). Mp 258–260°; $[\alpha]_D$ −13.5° ($CHCl_3$; *c* 0.1); EIMS *m/z* [M]$^+$ at 440. The identity of **6** was established by a spectroscopic comparison with an authentic sample [5].

Canophyllol (**7**). Mp 283–284°; $[\alpha]_D$ −30.5° ($CHCl_3$; *c* 0.1); EIMS *m/z* [M]$^+$ at 440. Compound **7** was confirmed by direct comparison with an authentic sample.

Table 2. ^{13}C NMR data* for compound **1**–**5**

C	1	^1H–^{13}C correlations†	2	^1H–^{13}C correlations†	3	^1H–^{13}C correlations†	4	^1H–^{13}C correlations†	5
1	22.16 (*t*)	H-2	22.20 (*t*)	—	22.18 (*t*)	H-2	21.80 (*t*)	H-25,2	22.22 (*t*)
2	41.23 (*t*)	H-10	41.40 (*t*)	—	41.42 (*t*)	—	40.87 (*t*)	H-23	41.38 (*t*)
3	212.9 (*s*)	H-2,4,23	213.0 (*s*)	H-2,23	213.0 (*s*)	H-2,23	210.6 (*s*)	H-4,H-2	212.8 (*s*)
4	58.07 (*d*)	H-23,24	58.10 (*d*)	H-23,24	58.11 (*d*)	H-23,24	57.95 (*d*)	H-23,24	58.12 (*d*)
5	41.98 (*s*)	H-23,24,4	42.20 (*s*)	H-23,24	42.04 (*s*)	H-24	43.14 (*s*)	H-8	41.91 (*s*)
6	40.98 (*t*)	H-24	41.10 (*t*)	H-24	41.15 (*t*)	H-24	47.23 (*t*)	H-4,6 H-23,24	41.01 (*t*)
7	18.20 (*t*)	H-8	18.40 (*t*)	H-8	18.31 (*t*)	H-8	210.2 (*s*)	H-8,24	18.08 (*t*)
8	50.04 (*d*)	H-25	53.30 (*d*)	H-26,15	49.86 (*d*)	H-26,15	63.37 (*d*)	H-6	50.70 (*d*)
9	37.55 (*s*)	H-25	37.40 (*s*)	H-25	37.39 (*s*)	H-25	39.77 (*s*)	H-8,25	37.71 (*s*)
10	59.46 (*d*)	H-25	59.40 (*d*)	H-24,25	59.53 (*d*)	H-25	59.24 (*d*)	H-25	59.36 (*d*)
11	34.68 (*t*)	H-25	35.60 (*t*)	H-25	34.89 (*t*)	H-25	57.04 (*t*)‡	H-25,12	34.72 (*t*)
12	27.84 (*t*)	H-27	30.70 (*t*)	H-27	29.35 (*t*)	H-27	72.79 (*d*)	H-26,27	28.28 (*t*)
13	40.45 (*s*)	H-27	40.10 (*s*)	H-15,26, H-27	39.88 (*s*)	H-27,15,H-12	44.84 (*s*)	H-18,25,H-27	38.97 (*s*)
14	39.88 (*s*)	H-26,27	39.10 (*s*)	H-12,26,H-27	39.19 (*s*)	H-27,15	47.60 (*s*)	H-8,26	38.47 (*s*)
15	38.40 (*t*)	H-26	44.10 (*t*)	H-26	36.80 (*t*)	H-26	32.60 (*t*)	H-16,26	28.98 (*t*)
16	70.24 (*d*)	H-15	75.30 (*d*)	H-15,28	74.30 (*d*)	H-28,15	39.06 (*t*)	H-18,15, H-28	29.54 (*d*)
17	40.45 (*s*)	H-15	35.60 (*s*)	H-18,22,H-28	41.02 (*s*)	H-18	31.07 (*s*)	H-18,28	34.64 (*s*)
18	41.81 (*d*)	H-27,28	44.50 (*s*)	H-22,27	41.75 (*d*)	H-27	43.27 (*d*)	H-27,19, H-28,16	39.47 (*d*)
19	29.96 (*t*)	Me-30	27.54 (*t*)	H-18,30	33.66 (*t*)	H-29,30, H-21	32.75 (*t*)	H-29,30	33.20 (*t*)
20	33.05 (*s*)	Me-30	33.30 (*s*)	H-21,30	28.02 (*s*)	H-28,29, H-21,19	28.46 (*s*)	H-29,30	33.32 (*s*)
21	33.05 (*t*)	H-30	29.63 (*t*)	H-19,30	33.78 (*t*)	H-30,29,H-19	38.30 (*t*)	H-29,30	30.03 (*t*)
22	23.43 (*t*)	H-28	35.00 (*t*)	H-28	19.85 (*t*)	H-21	35.64 (*t*)	H-28	41.38 (*t*)
23	6.77 (*q*)	H-4	6.80 (*q*)	H-4	6.75 (*q*)	H-4	6.97 (*q*)	H-4	6.77 (*q*)
24	14.62 (*q*)	H-4	14.60 (*q*)	H-4	14.66 (*q*)	H-4	15.27 (*q*)	H-4	14.59 (*q*)
25	19.30 (*q*)	H-11	18.10 (*q*)	—	19.25 (*q*)	H-10	19.76 (*q*)	H-8	18.09 (*q*)
26	16.72 (*q*)	H-8	19.90 (*q*)	H-8	16.47 (*q*)	H-8,27	20.63 (*q*)	H-18	16.63 (*q*)
27	14.87 (*q*)	H-12	21.30 (*q*)	H-18	19.70 (*q*)	H-18	11.67 (*q*)	H-18	15.23 (*q*)
28	83.40 (*t*)	H-18	25.09 (*q*)	H-16	71.28 (*t*)	H-16,18	31.38 (*q*)	H-18	83.80 (*t*)
29	177.9 (*s*)	H-28,30	70.60 (*t*)	H-30	36.96 (*q*)	H-30	34.56 (*q*)	H-30	180.0 (*s*)
30	28.20 (*q*)	—	29.30 (*q*)	H-29	31.76 (*q*)	H-29,19	32.24 (*q*)	H-29	27.85 (*q*)

* Multiplicities were obtained ($CDCl_3$) from DEPT spectra.

† ^1H–^{13}C long-range correlation (HMBC) corresponding to 2- or 3-bond connectivities.

‡ These assignments could be interchangeable.

Cytotoxic assay. The *in vitro* cytotoxicity assay was investigated according to procedures previously described by [14,15]. The assay against KB (nasopharyngal carcinoma), HEPA-2B (hepatoma), HELA (cervix carcinoma) and COLO-205 (colon carcinoma) tumour cells was mainly based on a method reported in ref. [16]. These cells, except for KB, were provided by the Cell Bank of the Veterans General Hospital, Taipei. KB cells were purchased from the American Type Culture Collection. Cells for bioassay were cultured in RPMI-1640 medium supplemented with a 5% CO_2 incubator at 37°. The cytotoxicity assay by the methylene blue dying method was performed, employing a modification of published procedures [17,18]. The assay depends on binding the methylene blue to the fixed monolayer at pH 8.5 and after washing the monolayer, releasing dye by lowering the pH. In summary, test samples and control standard agents (anti-drug) were prepd at a concns of 1, 5, 10, 40 and 100 μg ml^{-1}, After seeding 2880 cells well^{-1} in a 96-well microplate for 3 h, 20 μl of sample or standard agents were placed in each well, and incubated at 37° for 3 days. After removing the medium from the microplates, the cells were fixed by 10% formal saline for 30 min, then dyed by 1% (w/v) methylene blue in 0.01 M borate buffer 100 μl well^{-1} for 30 min. The 96-well tray was dipped into a 0.01 M borate buffer soln 4× in order to remove the dye. Then, 100 μl well^{-1} EtOH–0.1 M HCl (1 : 1): was added as a dye eluting solvent and the absorbance was measured on a microtitre plate reader (Dynatech, MR 7000) at a wavelength of 650 nm. The ED_{50} was defined during a comparison with the untreated cells as the concn of test sample resulting in 50% reduction of absorbance.

HIV inhibition assay. HIV inhibition was measured as described previously [19]. Briefly, this assay was performed by incubation of H9 lymphocytes (1×10^7 cells ml^{-1}) in the presence or absence of HIV-1 (HTLV-IIIB) for 1 hr at 37°. Cells were washed thoroughly to remove unadsorbed virions and resuspended at 4×10^5 cells ml^{-1} in culture medium. Aliquots (1ml) were placed in the wells of 24-well culture

plates containing an equal vol. of test compound (diluted in test medium), after incubation for 3 days at 37°. Cell densities of uninfected cultures were determined to assess toxicity of the test compound. A p24 antigen capture assay was used to determine the level of HIV infection in HIV-treated cultures. The ability of test compounds to inhibit HIV replication was measured at 4 different concns of test compound and compared with infected, untreated cultures. Test compounds were considered to be active if the p24 levels were less than 70% of infected, untreated cultures.

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