# Cytotoxic and Anti-HIV Principles from the Rhizomes of *Begonia nantoensis*

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**Three new compounds: begonanline (1), nantoamide (2) and methyl (*S*)-glycerate (3) as well as forty-four known compounds have been isolated and characterized from the rhizomes of *Begonia nantoensis*. The structures of these compounds were determined by spectral analyses and/or X-ray crystallography. Among them, cucurbitacin B (4), dihydrocucurbitacin B (5), cucurbitacin E (6), dihydrocucurbitacin E (7), cucurbitacin I (8), and (−)-auranamide (9) showed cytotoxicity against four human cancer cell lines. 3$\beta$,22$\alpha$-Dihydroxyolean-12-en-29-oic acid (10), indole-3-carboxylic acid (11), 5,7-dihydroxychromone (12), and (−)-catechin (13) demonstrated significant activity against HIV replication in H9 lymphocyte cells.**

**Key words** *Begonia nantoensis*; Begoniaceae; cucurbitacin; dihydrocucurbitacin; cytotoxicity

*Begonia nantoensis* LAI & CHUNG (Begoniaceae), endemic to Taiwan, is a succulent and perennial herb.[1] It is widely distributed in woodland undergrowths in the mountains of central Taiwan. In our preliminary assay, the crude methanol extract of dry rhizomes of *B. nantoensis* exhibited cytotoxic activity against gastric carcinoma (NUGC-3) and nasopharyngeal carcinoma (HONE-1) cell lines. This ethnopharmacological property has inspired our attention to *B. nantoensis*. As a result, forty-seven compounds including three new begonanline (**1**), nantoamide (**2**) and methyl (*S*)-glycerate (**3**) and forty-four known compounds (see Experimental) were isolated and identified from the rhizomes of this herb. The isolation and structural elucidation of compounds **1**—**3** and five cucurbitacins **4**—**8** are discussed herein.

Begonanline (**1**) was obtained as yellow syrup. The high resolution (HR) EI-MS gave the molecular ion at *m/z* 242.0690 which was consistent with the molecular formula $C_{13}H_{10}N_2O_3$. The UV spectrum of **1** exhibited characteristic absorptions of a $\beta$-carboline chromophore at 206, 267, 316, 392 nm.[2] The IR absorption bands at 3400 and 1706 $cm^{-1}$ were indicative of hydroxyl, amino and carbonyl functionalities. Accordingly, the $^1$H-NMR spectrum displayed signals for NH and phenolic OH groups at $\delta$ 10.72 and 8.28, respectively. In the $^1$H-NMR spectrum, a set of ABX aromatic signals at $\delta$ 7.19 (1H, dd, $J$=8.8, 2.5 Hz, H-7), 7.64 (1H, d, $J$=8.8 Hz, H-8), and 7.66 (1H, d, $J$=2.5 Hz, H-5) indicated the presence of a monosubstituted aromatic ring in $\beta$-carboline. The *ortho* coupled doublets at $\delta$ 8.22 and 8.42 with smaller coupling constant of 4.8 Hz were typical of heteroaromatic protons, H-4 and H-3 of $\beta$-carboline skeleton. The location of a phenolic OH ($\delta$ 8.28) on C-6 was determined by nuclear Overhauser enhancement spectroscopy (NOESY) experiment in which the OH proton showed NOEs with H-7 ($\delta$ 7.19) and H-5 ($\delta$ 7.66). In turn, H-5 showed NOE with H-4 ($\delta$ 8.22) and an indolic NH ($\delta$ 10.72) showed NOE with H-8 ($\delta$ 7.64). The remaining proton signal at $\delta$ 4.00 (3H, s) and two carbon signals at $\delta$ 52.3 and 167.4 suggested the presence of a methoxycarbonyl group at C-1. The heteronuclear multiple bond connectivity (HMBC) correlations fully supported these assignments. Hence, **1** was 6-hydroxy-1-methoxycarbonyl-$\beta$-carboline and was called begonanline.

Nantoamide (**2**) was isolated as colorless syrup. Its molecular formula was determined to be $C_{16}H_{15}NO_3$ by high resolution EI-MS (*m/z* 269.1056, $[M]^+$). In the IR spectrum, a broad NH absorption at 3325 $cm^{-1}$ and a strong C=O absorption at 1642 $cm^{-1}$ indicated the presence of an amide functional group in this compound. In the aliphatic region of the $^1$H-NMR spectrum, an ethylene group bearing a NH substituent at one end was deduced by the mutually coupled proton signals at $\delta$ 2.89 (2H, t, $J$=7.2 Hz, H-7′) and 3.58 (2H, td, $J$=7.2, 5.6 Hz, H-8′). In the aromatic region, a set of ABX proton signals at $\delta$ 6.86 (1H, d, $J$=8.0 Hz, H-5), 7.33 (1H, d, $J$=2.0 Hz, H-2) and 7.43 (1H, dd, $J$=8.0, 2.0 Hz, H-6) together with a signal at $\delta$ 6.05 (2H, s) constructed a 3,4-methylenedioxyphenyl moiety in **2**. The other set of five mutually coupled protons at $\delta$ 7.17 (1H, t, $J$=7.8 Hz, H-4′) and 7.26 (4H, m, H-2′, 3′, 5′, 6′) indicated a monosubstituted benzene unit. The HMBC correlations of H-7′ ($\delta$ 2.89) with C-2′ and -6′ ($\delta$ 129.6); H-8′ ($\delta$ 3.58) with C-1′ ($\delta$ 130.1) as well as the NOE between NH ($\delta$ 7.63) and H-6 ($\delta$ 7.43) defined the structure of **2** as *N*-(2-phenyl)ethyl-3,4-methylenedioxybenzamide and it was called as nantoamide.

Methyl (*S*)-glycerate (**3**), colorless oil, had the molecular formula $C_4H_8O_4$ from its high resolution EI-MS. In the $^1$H-NMR spectrum, two diastereotopic methylene protons at $\delta$ 3.84 (1H, dd, $J$=9.9, 3.4 Hz, H-3a) and 3.91 (1H, dd, $J$=9.9, 3.4 Hz, H-3b) coupled with a methine proton at $\delta$ 4.28 (1H, t, $J$=3.4 Hz, H-2) inferred the partial structure $HOCH_2CH(OH)$–. A methyl ester group was indicated by an IR absorption at 1740 $cm^{-1}$, a carbonyl carbon signal at $\delta$ 173.5 and a methoxyl proton peak at $\delta$ 3.83. A broad IR band at 3417 $cm^{-1}$ revealed the presence of hydroxyl functionality in the molecule. These foregoing spectral data confirmed the structure of **3** as methyl glycerate. This is the first reported isolation of **3** from a natural source, although it has been synthesized asymmetrically by Welzel and his colleagues.[3] The levorotatory optical rotation suggested the ab-

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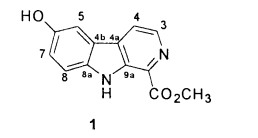

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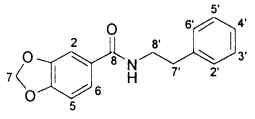

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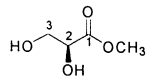

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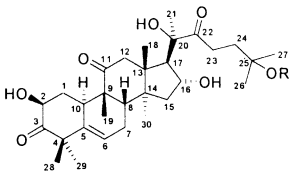
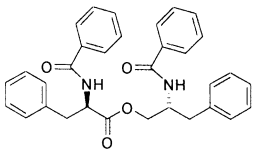


4: R = Ac, $\Delta^{23,24}$
5: R = Ac,
6: R = Ac, $\Delta^{1,2}$ and $\Delta^{23,24}$
7: R = Ac, $\Delta^{1,2}$
8: R = H, $\Delta^{1,2}$ and $\Delta^{23,24}$



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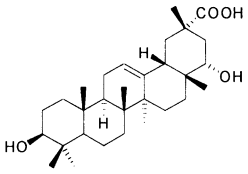

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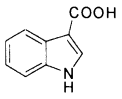

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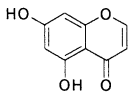

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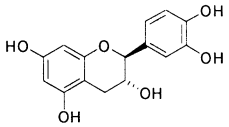

13

solute configuration of C-2 as *S*. Consequently, methyl (*S*)-glycerate was assigned for **3**.

In addition, the $^{1}$H- and $^{13}$C-NMR signals for cucurbitacins **4**—**8** in acetone-$d_6$ were re-assigned unambiguously by the aid of correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), HMBC, and NOESY spectra, as shown in Tables 1 and 2, and it was evident that the values for H-19 and H-30; C-19 and C-30 had been reversely assigned in the literature.[4] For example, in cucurbitacin B (**4**) a proton signal at $\delta$ 1.01 exhibited key HMBC connectivities with carbons at $\delta$ 34.0 (C-10), 43.4 (C-8), and 212.7 (C-11) was assigned as H-19. A carbon signal at $\delta$ 20.1 was attributed to C-19, as it had HMQC correlation with H-19. Similarly, on the basis of HMBC cross peaks of a proton at $\delta$ 1.42 with carbons at $\delta$ 43.4 (C-8), 46.4 (C-15), and 48.8 (C-13), it was ascribed to H-30 and it connected to a carbon at $\delta$ 19.1 in the HMQC spectrum, which therefore was assigned as C-30. The configuration of the cucurbitacin E (**6**) was further supported by single-crystal X-ray diffraction study as shown in Fig. 1.

Compounds **2**, **4**—**13**, and some others were subjected to anti-HIV evaluation. Among them, the four compounds **10**—**13** inhibited HIV replication in H9 lymphocyte cells with $EC_{50}$ values of 5.65, 2.41, 18.65, and 14.32 $\mu$g/ml and their therapeutic indexes ($IC_{50}/EC_{50}$) of 4.40, 6.79, 1.34, and 1.75, respectively (Table 3). Those compounds were also examined for their cytotoxicity and cucurbitacins **4**—**8** exhibited strong cytotoxic activity against two human cancer cell lines, NUGC-3 and HONE-1. Compound **9** showed moderate activity against NUGC-3 and HONE-1 cell lines (Table 4). Furthermore, compounds **2**, **4**, **6**, **8**—**13**, were assayed for an-

Table 1. The $^{1}$H-NMR Data of Cucurbitacins **4**—**8** (Acetone-$d_6$, $\delta$, Multiplicity, *J*, Hz in Parentheses)

| | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|
| H-1 | $\alpha$: 2.09 m | $\alpha$: 2.11 ddd (12.6, 6.2, 3.6) | 5.75 d (2.5) | 5.76 d (2.5) | 5.76 d (2.9) |
| | $\beta$: 1.13 q (12.6) | $\beta$: 1.11 q (12.6) | | | |
| H-2 | 4.52 m | 4.56 ddd (12.6, 6.2, 4.5) | — | — | — |
| H-6 | 5.81 m | 5.81 m | 5.80 m | 5.80 m | 5.79 m |
| H-7 | $\alpha$: 1.97 m | $\alpha$: 2.04 m | $\alpha$: 2.05 m | $\alpha$: 2.08 m | $\alpha$: 2.06 m |
| | $\beta$: 2.39 m | $\beta$: 2.40 m | $\beta$: 2.38 m | $\beta$: 2.38 m | $\beta$: 2.37 m |
| H-8 | 1.94 m | 1.96 m | 2.03 m | 2.07 m | 2.04 m |
| H-10 | 3.00 br d (12.6) | 3.02 dd (12.6, 3.6) | 3.65 m | 3.65 m | 3.67 d (2.9) |
| H-12 | $\alpha$: 3.38 d (14.6) | $\alpha$: 3.44 d (15.0) | $\alpha$: 3.38 d (14.7) | $\alpha$: 3.44 d (14.8) | $\alpha$: 3.43 d (14.6) |
| | $\beta$: 2.50 d (14.6) | $\beta$: 2.54 d (15.0) | $\beta$: 2.54 d (14.7) | $\beta$: 2.58 d (14.8) | $\beta$: 2.60 d (14.6) |
| H-15 | $\alpha$: 1.40 m | $\alpha$: 1.42 m | $\alpha$: 1.45 m | $\alpha$: 1.44 m | $\alpha$: 1.43 m |
| | $\beta$: 1.83 dd (12.5, 8.9) | $\beta$: 1.82 dd (13.0, 8.5) | $\beta$: 1.85 dd (12.4, 8.7) | $\beta$: 1.85 m | $\beta$: 1.84 dd (12.8, 9.0) |
| H-16 | 4.45 m | 4.35 m | 4.46 m | 4.38 m | 4.44 m |
| H-17 | 2.64 d (7.1) | 2.66 d (7.3) | 2.66 d (7.1) | 2.66 m | 2.66 d (7.1) |
| H-18 | 0.90 s | 0.93 s | 0.93 s | 0.95 s | 0.95 s |
| H-19 | 1.01 s | 1.02 s | 0.96 s | 0.96 s | 0.96 s |
| H-21 | 1.39 s | 1.39 s | 1.40 s | 1.40 s | 1.38 s |
| H-23 | 6.79 d (15.8) | 2.63 and 2.91 m | 6.81 d (15.7) | 2.68 and 2.94 m | 6.85 d (15.3) |
| H-24 | 6.97 d (15.8) | 1.99 t (7.8) | 6.98 d (15.7) | 2.03 m | 6.96 d (15.3) |
| H-26 | 1.50 s[a)] | 1.41 s | 1.51 s[a)] | 1.40 s | 1.29 s |
| H-27 | 1.54 s[a)] | 1.41 s | 1.54 s[a)] | 1.40 s | 1.29 s |
| H-28 | 1.31 s | 1.32 s | 1.24 s | 1.24 s | 1.24 s |
| H-29 | 1.27 s | 1.28 s | 1.29 s | 1.29 s | 1.30 s |
| H-30 | 1.42 s | 1.44 s | 1.46 s | 1.47 s | 1.46 s |
| 2-OH | 3.85 d (4.2) | 3.83 d (4.5) | 6.97 s | 6.96 s | 6.98 s |
| 16-OH | 3.65 d (5.0) | 3.89 d (4.2) | 3.66 d (4.5) | 3.88 d (4.5) | 3.68 d (4.8) |
| 20-OH | 4.51 s | 4.41 s | 4.50 s | 4.41 s | 4.48 s |
| 25-OH | — | — | — | — | 4.00 s |
| 25-OAc | 1.96 s | 1.89 s | 1.95 s | 1.88 s | — |

*a*) Assignments in each column may be interchangeable.

Table 2. The $^{13}$C-NMR Data of Cucurbitacins **4**—**8** (Acetone-$d_6$, $\delta$)

| | **4** | **5** | **6** | **7** | **8** |
|---|---|---|---|---|---|
| C-1 | 36.9 | 37.0 | 115.7 | 115.8 | 115.8 |
| C-2 | 72.2 | 72.2 | 146.0 | 146.1 | 146.0 |
| C-3 | 213.6 | 213.6 | 198.8 | 198.9 | 198.9 |
| C-4 | 51.0 | 51.0 | 48.5 | 48.6 | 48.5 |
| C-5 | 141.8 | 141.9 | 138.0 | 138.1 | 138.0 |
| C-6 | 120.7 | 120.7 | 121.3 | 121.4 | 121.3 |
| C-7 | 24.5 | 24.5 | 24.3 | 24.4 | 24.3 |
| C-8 | 43.4 | 43.4 | 42.6 | 42.6 | 42.6 |
| C-9 | 49.0 | 49.0 | 49.4 | 49.5 | 49.4 |
| C-10 | 34.0 | 34.1 | 35.3 | 35.3 | 35.2 |
| C-11 | 212.7 | 212.6 | 213.5 | 213.5 | 213.5 |
| C-12 | 49.3 | 49.6 | 49.5 | 49.9 | 49.7 |
| C-13 | 48.8 | 49.0 | 48.9 | 49.1 | 48.9 |
| C-14 | 51.3 | 51.4 | 51.3 | 51.4 | 51.4 |
| C-15 | 46.4 | 46.5 | 46.6 | 46.8 | 46.7 |
| C-16 | 71.2 | 71.0 | 71.3 | 71.1 | 71.1 |
| C-17 | 59.0 | 58.6 | 58.9 | 58.7 | 58.7 |
| C-18 | 20.5 | 20.4 | 20.5 | 20.5 | 20.5 |
| C-19 | 20.1 | 20.1 | 20.2 | 20.3 | 20.3 |
| C-20 | 79.5 | 80.0 | 79.5 | 80.1 | 79.1 |
| C-21 | 25.0 | 25.4 | 25.0 | 25.5 | 25.2 |
| C-22 | 203.4 | 214.5 | 203.3 | 214.5 | 203.6 |
| C-23 | 122.1 | 31.7 | 122.2 | 31.8 | 120.6 |
| C-24 | 150.9 | 35.4 | 150.9 | 35.5 | 155.3 |
| C-25 | 80.0 | 81.7 | 80.0 | 81.8 | 70.1 |
| C-26 | 26.3[a] | 26.1[a] | 26.4[a] | 26.1[a] | 29.8 |
| C-27 | 26.9[a] | 26.2[a] | 27.0[a] | 26.3[a] | 29.8 |
| C-28 | 29.7 | 29.5 | 28.2 | 28.2 | 28.1 |
| C-29 | 21.7 | 21.7 | 20.6 | 20.7 | 20.6 |
| C-30 | 19.1 | 19.1 | 18.6 | 18.7 | 18.6 |
| $OCOCH_3$ | 170.2, 21.8 | 170.3, 22.2 | 170.2, 21.8 | 170.4, 22.3 | — |

*a*) Assignments in each column may be interchangeable.

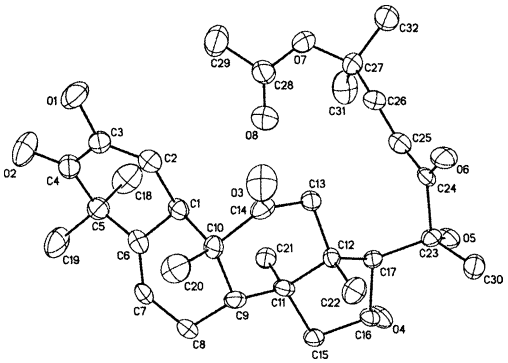


Fig. 1. The X-Ray Structure of Cucurbitacin E (**6**)

other two human cancer cells, breast carcinoma (MCF-7) and lung carcinoma (A549). Compounds **4**, **6**, **8**, and **9** showed strong cytotoxic activity. **11** exhibited marginal cytotoxicity against A549 and MCF-7 (Table 5). This is an interesting result about cucurbitacins not only due to their potency but also to the consistency with recently reported anti-cancer activity.[5,6]

## Experimental

**General Procedures** UV spectra were recorded on an Agilent 8453 spectrophotometer. IR spectra were measured on a Nicolet Magna FT-IR spectrophotometer. NMR spectra were recorded on Bruker AMX-300 and AMX-400 FT-NMR spectrometers; all chemical shifts were given in ppm from tetramethylsilane as an internal standard. Mass spectra were obtained on Finnigan Trace and VG 70-250S spectrometer by a direct inlet system.

Table 3. Inhibition of HIV Replication in H9 Lymphocytic Cells for Compounds from the Rhizomes of *Begonia nantoensis*

| Compound | $IC_{50}$ ($\mu$g/ml)[a] | $EC_{50}$ ($\mu$g/ml)[b] | TI[c] |
|---|---|---|---|
| Nantoamide (**2**) | >25 | NS[d] | NS[d] |
| 3$\beta$,22$\alpha$-Dihydroxyolean-12-en-29-oic acid (**10**) | >25 | 5.65 | 4.40 |
| Indole-3-carboxylic acid (**11**) | 16.40 | 2.41 | 6.79 |
| 5,7-Dihydroxychromone (**12**) | >25.00 | 18.65 | 1.34 |
| (−)-Catechin (**13**) | >25 | 14.32 | 1.75 |
| AZT | 500 | 0.0007 | 737207 |

*a*) Concentration that inhibits uninfected H9 cell growth by 50%. *b*) Concentration that inhibits viral replication by 50%. *c*) Therapeutic index=$IC_{50}/EC_{50}$. *d*) No suppression.

Table 4. Cytotoxicity of the Compounds from the Rhizomes of *Begonia nantoensis* toward Two Human Cancer Lines NUGC-3 and HONE-1[a]

| Compound | $IC_{50}$ ($\mu$g/ml)[b] | |
|---|---|---|
| | NUGC-3 | HONE-1 |
| Nantoamide (**2**) | >20 (2) | >20 (3) |
| Cucurbitacin B (**4**) | 0.22 | 0.05 |
| Dihydroucurbitacin B (**5**) | 3.26 | 1.55 |
| Cucurbitacin E (**6**) | 0.34 | 0.08 |
| Dihydroucurbitacin E (**7**) | 8.60 | 2.68 |
| Cucurbitacin I (**8**) | 2.14 | 0.89 |
| (−)-Auranamide (**9**) | 17.12 | 8.68 |

*a*) NUGC-3=human gastric carcinoma; HONE-1=human nasopharyngeal carcinoma. *b*) If inhibition <50% at 20 $\mu$g/ml, percent observed is the value in brackets.

Table 5. Cytotoxicity of the Compounds from the Rhizomes of *Begonia nantoensis* toward Two Human Cancer Lines A549 and MCF-7[a]

| Compound | $EC_{50}$ ($\mu$g/ml)[b] | |
|---|---|---|
| | A549 | MCF-7 |
| Nantoamide (**2**) | >20 (17) | >20 (47) |
| Cucurbitacin B (**4**) | <2.5 (87) | <2.5 (91) |
| Cucurbitacin E (**6**) | <2.5 (81) | <2.5 (82) |
| Cucurbitacin I (**8**) | <2.5 (82) | <2.5 (78) |
| (−)-Auranamide (**9**) | <2.5 (81) | <2.5 (78) |
| Indole-3-carboxylic acid (**11**) | 4.6 | 12.9 |

*a*) A549=human lung carcinoma; MCF-7=human breast carcinoma. *b*) If inhibition >50% at 2.5 $\mu$g/ml or inhibition <50% at 20 $\mu$g/ml, percent observed is the value in brackets.

**Plant Material** The rhizomes of *Begonia nantoensis* were collected from Nanto Hsien, Taiwan, Republic of China, in February 2002; the plant was authenticated by Professor C. S. Kuoh. A voucher specimen (No: PLW-020001) was deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

**Extraction and Isolation** The air-dried rhizomes of *Begonia nantoensis* (5.5 kg) were powdered and extracted under reflux with MeOH 6 times. The combined extracts were concentrated under reduced pressure to give dark brown syrup. The syrup was then suspended in $H_2O$ and partitioned with hexane, $CHCl_3$ and EtOAc, successively. The concentrated hexane layer (64 g) was fractionated on a silica gel column with a gradient of hexane and $Me_2CO$ (5 : 1 to pure $Me_2CO$) into nine fractions. Fraction 2 was chromatographed on silica gel eluting with hexane–EtOAc (99 : 1 to 9 : 1) to obtain 2,4-diphenylbut-1-ene (2 mg)[7] and 2,4,6-triphenylhex-1-ene (4 mg).[8] Fractions 3 and 4 were chromatographed on silica gel column with hexane–EtOAc (5 : 1) eluent to yield a mixture of $\beta$-sitosterol and stigmasterol (850 mg)[9] and stigmast-4-en-3-one (38 mg),[10] respectively. Fraction 5 was further purified by silica gel column chromatography using a hexane–EtOAc

(3 : 1) mixture as eluent to give nantoamide (**2**, 9 mg), oleanoic acid (1 mg),[11] (−)-auranamide (**9**, 7 mg),[12] glyceryl-1-tetracosanoate (6 mg),[13] lutein (3 mg),[14] methyl vanillate (2 mg),[9] vanillin (4 mg),[15] and eudesmic acid (3 mg).[16] Fractions 6 and 7 were chromatographed on silica gel column with $CHCl_3$–MeOH (19 : 1) eluent to give 9-hydroxylinoleic acid (8 mg)[17] and a mixture of $\beta$-sitosteryl-$\beta$-D-glucoside (2.5 g).[9] The $CHCl_3$ layer (41 g) was fractionated on a silica gel column by eluting with a gradient of hexane and $Me_2CO$ (3 : 1 to pure $Me_2CO$) to obtain seven fractions. Fraction 3 was chromatographed on silica gel column by eluting with hexane–EtOAc (5 : 1) to give a mixture of 6$\beta$-hydroxysitost-4-en-3-one and 6$\beta$-hydroxystigmasta-4,22-dien-3-one (46 mg).[18,19] Fraction 4 was chromatographed on silica gel column with a gradient of *i*-$Pr_2O$–MeOH (49 : 1 to pure MeOH) to give 2-(2-hydroxytricosanoylamino)-1,3,4-hexadecanetriol (28 mg),[20] methylparaben (1 mg),[21] *p*-hydroxybenzaldehyde (4 mg),[21] and *trans*-docosanylferulate (15 mg).[22] Fraction 5, on repeated chromatography on silica gel column with a gradient of *i*-$Pr_2O$–MeOH (49 : 1 to pure MeOH) afforded cucurbitacin B (**4**, 60 mg),[23] dihydrocucurbitacin B (**5**, 5 mg),[24] cucurbitacin E (**6**, 18 mg),[4] dihydrocucurbitacin E (**7**, 4 mg),[23] cucurbitacin I (**8**, 3 mg),[4] dihydrocucurbitacin I (1 mg),[4] 3$\beta$,22$\alpha$-dihydroxyolean-12-en-29-oic acid (**10**, 5 mg),[25] indole-3-carboxaldehyde (15 mg),[26] indole-3-carboxylic acid (**11**, 4 mg),[27] 5,7-dihydroxychromone (**12**, 4 mg),[28] (*S*)-*N*-(1-hydroxymethyl-2-phenylethyl)-benzamide (5 mg),[29] vanillic acid (4 mg),[9] piperonylic acid (1 mg),[30] benzoic acid (20 mg),[31] and caffeic acid (2 mg).[15] The concentrated EtOAc layer (16 g) was subjected to column chromatography over silica gel and eluted with a gradient of *i*-$Pr_2O$–MeOH (5 : 1 to pure MeOH) to give nine fractions. Purification of fraction 2 by silica gel column with $CHCl_3$–MeOH (9 : 1) furnished daidzein (2 mg),[32] protocatechuic acid (6 mg),[33] protocatechuic acid methyl ester (11 mg),[34] 4-hydroxybenzoic acid (5 mg),[9] and *p*-coumaric acid (1 mg).[9] Further separation of fraction 3 on silica gel column with a gradient of $CHCl_3$–MeOH (9 : 1 to pure MeOH) yielded begonanline (**1**, 5 mg), methyl (*S*)-glycerate (**3**, 9 mg), and (−)-catechin (**13**, 998 mg).[35] Fraction 6 was chromatographed on silica gel column with $CHCl_3$–MeOH (5 : 1) eluent to yield vitexin (50 mg)[36] and fraction 9 give nicotinic acid (5 mg),[37] uracil (3 mg),[9] 1,2,4-trihydroxybenzene (2 mg),[38] and cucurbitacin F (0.6 mg).[39]

Begonanline (**1**): Yellow syrup. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 206 (3.82), 267 (3.04), 316 (2.56), 392 (2.04). IR (film) $\nu_{max}$ $cm^{-1}$: 3400, 1706, 1602, 1492. $^1$H-NMR (acetone-$d_6$) $\delta$: 4.00 (3H, s, $OCH_3$), 7.19 (1H, dd, $J$=8.8, 2.5 Hz, H-7), 7.64 (1H, d, $J$=8.8 Hz, H-8), 7.66 (1H, d, $J$=2.5 Hz, H-5), 8.22 (1H, d, $J$=4.8 Hz, H-4), 8.28 (1H, s, 6-OH), 8.42 (1H, d, $J$=4.8 Hz, H-3), 10.72 (1H, br s, NH). $^{13}$C-NMR (acetone-$d_6$) $\delta$: 52.3 ($OCH_3$), 106.8 (C-5), 113.9 (C-8), 119.3 (C-4), 119.8 (C-7), 122.3 (C-4b), 130.8 (C-4a), 131.8 (C-1), 136.5 (C-8a), 138.2 (C-9a), 138.4 (C-3), 152.7 (C-6), 167.4 (C=O). EI-MS *m*/*z*: 242 ($M^+$, 67), 210 (20), 182 (100); HR-EI-MS *m*/*z*: 242.0690 $[M]^+$ (Calcd for $C_{13}H_{10}N_2O_3$: 242.0691).

Nantoamide (**2**): Colorless syrup. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 293 (2.88). IR (film) $\nu_{max}$ $cm^{-1}$: 3325, 1642, 1604, 1544, 1503. $^1$H-NMR (acetone-$d_6$) $\delta$: 2.89 (2H, t, $J$=7.2 Hz, H-7′), 3.58 (2H, td, $J$=7.2, 5.6 Hz, H-8′), 6.05 (2H, s, H-7), 6.86 (1H, d, $J$=8.0 Hz, H-5), 7.17 (1H, t, $J$=7.8 Hz, H-4′), 7.26 (4H, m, H-2′, 3′, 5′, 6′), 7.33 (1H, d, $J$=2.0 Hz, H-2), 7.43 (1H, dd, $J$=8.0, 2.0 Hz, H-6), 7.63 (1H, br s, NH). $^{13}$C-NMR (acetone-$d_6$) $\delta$: 36.5 (C-7′), 42.1 (C-8′), 102.6 (C-7), 108.1 (C-2), 108.4 (C-5), 122.6 (C-6), 126.9 (C-4′), 129.2 (C-3′ and -5′), 129.6 (C-2′ and -6′), 130.1 (C-1), 140.6 (C-1′), 148.7 (C-3), 151.6 (C-4), 166.3 (C-8). EI-MS *m*/*z*: 269 ($M^+$, 18), 165 (30), 149 (100), 121 (24), 91 (19), 65 (23). HR-EI-MS *m*/*z*: 269.1056 $[M]^+$ (Calcd for $C_{16}H_{15}NO_3$: 269.1052).

Methyl (*S*)-Glycerate (**3**): Colorless oil; $[\alpha]_D$ −8.4° ($c$=0.44, $CH_3OH$, lit.[37] −10.71°). UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 204 (3.11), 222 (2.97). IR (film) $\nu_{max}$ $cm^{-1}$: 3417, 1740, 1441. $^1$H-NMR ($CDCl_3$) $\delta$: 3.72 (1H, br s, OH), 3.83 (3H, s, $OCH_3$), 3.84 (1H, dd, $J$=9.9, 3.4 Hz, H-3a), 3.91 (1H, dd, $J$=9.9, 3.4 Hz, H-3b), 4.28 (1H, t, $J$=3.4 Hz, H-2). $^{13}$C-NMR ($CDCl_3$) $\delta$: 52.9 ($OCH_3$), 64.0 (C-3), 71.5 (C-2), 173.5 (C-1). EI-MS *m*/*z*: 121 ($[M+H]^+$, 20), 120 (4), 105 (45), 91 (49), 88 (40), 78 (89), 57 (80), 55 (100). HR-EI-MS *m*/*z*: 121.0500 $[M+H]^+$ (Calcd for $C_4H_9O_4$: 121.0501).

**X-Ray Crystal Data for Cucurbitacin E (6)** Data were acquired on a Simens Smart CCD 1000 diffractometer. All intensity measurements were performed using graphite monochromated Mo-K$\alpha$ radiation ($\lambda$=0.71073 Å). Cucurbitacin E (**6**), $C_{32}H_{44}O_8$ 556.69, was obtained as orthorhombic crystals, space group $P2_12_12_1$ with cell dimensions $a$=8.0423 (5), $b$=16.5503 (10), $c$=22.0277 (13) Å, $\alpha=\beta=\gamma$=90°, $V$=2931.9 (4) Å$^3$, $Z$=4, $F$(000)=1208, $\rho_{calcd}$=1.266 mg·m$^{-3}$, $\mu$=0.090 mm$^{-1}$, $2\theta_{max}$=56.66°, crystal dimensions 0.30×0.20×0.10 mm$^3$. The crystal structure was solved by a direct method. Full-matrix least-squares refinement of atomic parameters (anisotropic C, O; isotropic H) converged at $R_1$=0.0798, $wR_2$=0.16988 over 7039 reflections with $I \geq 2\sigma(I)$. The absolute stereochemistry cannot be directly determined from X-ray data, but it is correct as shown based on transformation of **4** to the known di-*p*-iodobenzoate ester of cucurbitacin D.[40]

**Anti-HIV Assay** The anti-HIV assay was carried out according to the procedure described in the literature.[9]

**Cytotoxicity Assay** The cytotoxicity assay was carried out according to the procedure described in the literature.[9]

**Acknowledgments** The authors would like to thank the National Science Council of the Republic of China for financial support (NSC 91-2323-B-006-003).

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