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

Pei-Lin WU,*[,a] Fu-Wen LIN,[a] Tian-Shung WU,[a] Chang-Sheng KUOH,[b] Kuo-Hsiung LEE,[c] and Shiow-Ju LEE[d]

[a] *Department of Chemistry, National Cheng Kung University;* [b] *Department of Biology, National Cheng Kung University; Tainan, 701, Taiwan:* [c] *Natural Products Laboratory, School of Pharmacy, University of North Carolina; Chapel Hill, North Carolina 27599, U.S.A.: and* [d] *Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes; Taipei, 221, Taiwan.* Received November 3, 2003; accepted December 4, 2003

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. 3β,22α-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

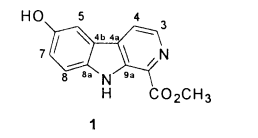
Begonia nantoensis LAI & CHUNG (Begoniaceae), endemic to Taiwan, is a succulent and perennial herb.[1] It is widely distributed in woodland undergrowths in the mountains of central Taiwan. In our preliminary assay, the crude methanol extract of dry rhizomes of *B. nantoensis* exhibited cytotoxic activity 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 β-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 δ 10.72 and 8.28, respectively. In the ^{1}H-NMR spectrum, a set of ABX aromatic signals at δ 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 β-carboline. The *ortho* coupled doublets at δ 8.22 and 8.42 with smaller coupling constant of 4.8 Hz were typical of heteroaromatic protons, H-4 and H-3 of β-carboline skeleton. The location of a phenolic OH (δ 8.28) on C-6 was determined by nuclear Overhauser enhancement spectroscopy (NOESY) experiment in which the OH proton showed NOEs with H-7 (δ 7.19) and H-5 (δ 7.66). In turn, H-5 showed NOE with H-4 (δ 8.22) and an indolic NH (δ 10.72) showed NOE with H-8 (δ 7.64). The remaining proton signal at δ 4.00 (3H, s) and two carbon signals at δ 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-β-carboline and was called begonanline.

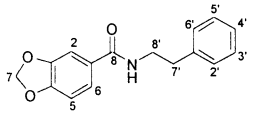
Nantoamide (**2**) was isolated as colorless syrup. Its molecular formula was determined to be $C_{16}H_{15}NO_3$ by high resolution EI-MS (*m/z* 269.1056, $[M]^+$). In the IR spectrum, a broad NH absorption at 3325 cm^{-1} and a strong C=O absorption at 1642 cm^{-1} indicated the presence of an amide 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 δ 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 δ 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 δ 6.05 (2H, s) constructed a 3,4-methylenedioxyphenyl moiety in **2**. The other set of five mutually coupled protons at δ 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′ (δ 2.89) with C-2′ and -6′ (δ 129.6); H-8′ (δ 3.58) with C-1′ (δ 130.1) as well as the NOE between NH (δ 7.63) and H-6 (δ 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 δ 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 δ 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 δ 173.5 and a methoxyl proton peak at δ 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-

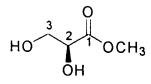
* To whom correspondence should be addressed. e-mail: wupl@mail.ncku.edu.tw



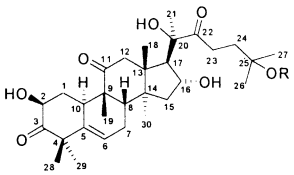
1



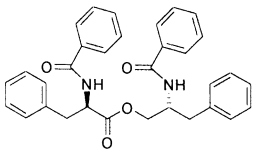
2



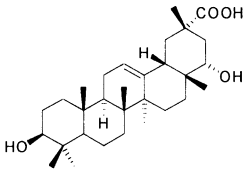
3



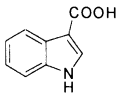
4: R = Ac, $\Delta^{23,24}$
5: R = Ac,
6: R = Ac, $\Delta^{1,2}$ and $\Delta^{23,24}$
7: R = Ac, $\Delta^{1,2}$
8: R = H, $\Delta^{1,2}$ and $\Delta^{23,24}$



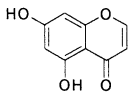
9



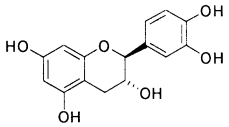
10



11



12



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 δ 1.01 exhibited key HMBC connectivities with carbons at δ 34.0 (C-10), 43.4 (C-8), and 212.7 (C-11) was assigned as H-19. A carbon signal at δ 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 δ 1.42 with carbons at δ 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 δ 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 μg/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, δ, Multiplicity, *J*, Hz in Parentheses)

	4	5	6	7	8
H-1	α: 2.09 m	α: 2.11 ddd (12.6, 6.2, 3.6)	5.75 d (2.5)	5.76 d (2.5)	5.76 d (2.9)
	β: 1.13 q (12.6)	β: 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	α: 1.97 m	α: 2.04 m	α: 2.05 m	α: 2.08 m	α: 2.06 m
	β: 2.39 m	β: 2.40 m	β: 2.38 m	β: 2.38 m	β: 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	α: 3.38 d (14.6)	α: 3.44 d (15.0)	α: 3.38 d (14.7)	α: 3.44 d (14.8)	α: 3.43 d (14.6)
	β: 2.50 d (14.6)	β: 2.54 d (15.0)	β: 2.54 d (14.7)	β: 2.58 d (14.8)	β: 2.60 d (14.6)
H-15	α: 1.40 m	α: 1.42 m	α: 1.45 m	α: 1.44 m	α: 1.43 m
	β: 1.83 dd (12.5, 8.9)	β: 1.82 dd (13.0, 8.5)	β: 1.85 dd (12.4, 8.7)	β: 1.85 m	β: 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, δ)

	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.

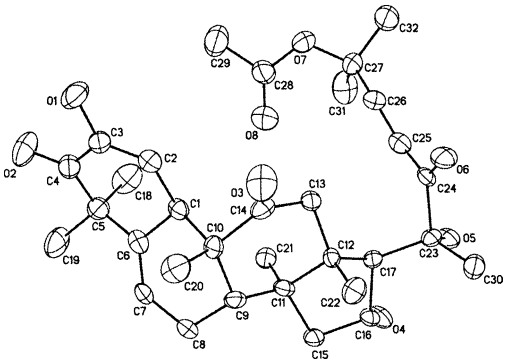


Fig. 1. The X-Ray Structure of Cucurbitacin E (**6**)

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} (μg/ml)[a]	EC_{50} (μg/ml)[b]	TI[c]
Nantoamide (**2**)	>25	NS[d]	NS[d]
3β,22α-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} (μg/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 μg/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} (μg/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 μg/ml or inhibition <50% at 20 μg/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 β-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 β-sitosteryl-β-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 6β-hydroxysitost-4-en-3-one and 6β-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] 3β,22α-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 λ_{max} (MeOH) nm (log ε): 206 (3.82), 267 (3.04), 316 (2.56), 392 (2.04). IR (film) ν_{max} cm^{-1}: 3400, 1706, 1602, 1492. ^{1}H-NMR (acetone-d_6) δ: 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) δ: 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 λ_{max} (MeOH) nm (log ε): 293 (2.88). IR (film) ν_{max} cm^{-1}: 3325, 1642, 1604, 1544, 1503. ^{1}H-NMR (acetone-d_6) δ: 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) δ: 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 λ_{max} (MeOH) nm (log ε): 204 (3.11), 222 (2.97). IR (film) ν_{max} cm^{-1}: 3417, 1740, 1441. ^{1}H-NMR ($CDCl_3$) δ: 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 ($CDCl_3$) δ: 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-Kα radiation (λ=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, ρ_{calcd}=1.266 mg·m^{-3}, μ=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).

References

1) Liao J. C., "Flora of Taiwan," 2nd ed., Vol. 1, Editorial Committee of the Flora of Taiwan, Taipei, 1993, pp. 851—852.
2) Hashimoto Y., Kawanishi K., *Phytochemistry*, **14**, 1633—1635 (1975).
3) Eichelberger U., Mansourova M., Henning L., Findeisen M., Giesa S., Muller D., Welzel P., *Tetrahedron*, **57**, 9737—9742 (2001).
4) Velde V. V., Lavie D., *Tetrahedron*, **39**, 317—321 (1983).
5) Blaskovich M. A., Sun J. Z., Cantor A., Turkson J., Jove R., Sebti S. M., *Cancer Res.*, **63**, 1270—1279 (2003).
6) Frei B., Heinrich W., Herrmann D., Orjala J. E., Schmitt J., Sticher O., *Planta Med.*, **64**, 385—386 (1998).
7) Yayli N., *Indian J. Chem., Sec. B*, **33**, 556—561 (1994).
8) Ayer W. A., Muir D. J., Chakravarty P., *Phytochemistry*, **42**, 1321—1324 (1996).
9) Wu P. L., Su G. C., Wu T. S., *J. Nat. Prod.*, **66**, 996—998 (2003).
10) Wu T. S., Yang C. C., Wu P. L., Liu L. K., *Phytochemistry*, **40**, 1247—1250 (1995).
11) Zhang Z., Koike K., Jia Z., Nikaido T., Guo D., Zheng J., *Chem. Pharm. Bull.*, **47**, 388—393 (1999).
12) Jakupovic J., Chen Z. L., Bohlmann F., *Phytochemistry*, **26**, 2777—2780 (1987).
13) Sultana N., Armstrong J. A., Waterman P. G., *Phytochemistry*, **52**, 895—900 (1999).
14) Khachik F., Steck A., Pfander H., *J. Agri. Food Chem.*, **47**, 455—461 (1999).
15) Wu T. S., Tsang Z. J., Wu P. L., Lin F. W., Li C. Y., Teng C. M., Lee K. H., *Bioorg. Med. Chem.*, **9**, 77—84 (2001).
16) Chang Y. C., Chang F. R., Wu Y. C., *J. Chin. Chem. Soc.*, **47**, 373—380 (2000).
17) Kato T., Nakai T., Ishikawa R., Karasawa A., Namai T., *Tetrahedron: Asymmetry*, **12**, 2695—2701 (2001).
18) Arai Y., Nakagawa T., Hitosugi M., Shiojima K., Ageta H., Abdel-Halim O. B., *Phytochemistry*, **48**, 471—474 (1998).
19) Anjaneyulu V., Satyanarayana P., Viswanadham K. N., Jyothi V. G., Rao K. N., Radhika P., *Phytochemistry*, **50**, 1229—1236 (1999).
20) Inagaki M., Isobe R., Kawano Y., Miyamoto T., Komori T., Higuchi R., *Eur. J. Org. Chem.*, **1**, 129—132 (1998).
21) Wu T. S., Yeh J. H., Wu P. L., *Phytochemistry*, **40**, 121—124 (1995).
22) Kuo Y. H., Chen W. C., *J. Chin. Chem. Soc.*, **46**, 819—824 (1999).
23) Afifi M. S., Ross S. A., el Sohly M. A., Naeem Z. E., Halaweish F. T., *J. Chem. Ecol.*, **25**, 847—860 (1999).
24) Nishida R., Fukami H., Tanaka Y., Magalhaes B. P., Yokoyama M., Blumenschein A., *Agric. Biol. Chem.*, **50**, 2831—2836 (1986).
25) Kutney J. P., Hewitt G. M., Lee G., Piotrowska K., Roberts M., Rettig S. J., *Can. J. Chem.*, **70**, 1455—1480 (1992).
26) Yue Q., Miller C. J., White J. F. Jr., Richardson M. D., *J. Agric. Food Chem.*, **48**, 4687—4692 (2000).
27) Chan Y. Y., Leu Y. L., Wu T. S., *Chem. Pharm. Bull.*, **47**, 887—889 (1999).
28) Kuo Y. H., Yeh M. H., *J. Chin. Chem. Soc.*, **44**, 379—383 (1997).
29) Lee K. Y., Kim Y. H., Park M. S., Oh C. Y., Ham W. H., *J. Org. Chem.*, **64**, 9450—9458 (1999).
30) Koike K., Ohmoto T., *Phytochemistry*, **29**, 2617—2621 (1990).
31) Leu Y. L., Chan Y. Y., Hsu M. Y., Chen I. S., Wu T. S., *J. Chin. Chem. Soc.*, **45**, 539—542 (1998).
32) Kanakubo A., Koga K., Isobe M., Fushimi T., Saitoh T., Ohshima Y., Tsukamoto Y., *Tetrahedron*, **57**, 8801—8806 (2001).
33) Wu T. S., Chang F. C., Wu P. L., Kuoh C. S., Chen I. S., *J. Chin. Chem. Soc.*, **42**, 929—934 (1995).
34) Kita Y., Arisawa M., Gyoten M., Nakajima M., Hamada R., Tohma H., Takada T., *J. Org. Chem.*, **63**, 6625—6633 (1998).
35) Martin T. S., Kikuzaki H., Hisamoto M., Nakatani N., *J. Am. Oil*

Chem. Soc., **77**, 667—673 (2000).
36) Lin Y. L., Kuo Y. H., Shiao M. S., Chen C. C., Ou J. C., *J. Chin. Chem. Soc.*, **47**, 253—256 (2000).
37) Chen C. Y., Chang F. R., Teng C. M., Wu Y. C., *J. Chin. Chem. Soc.*, **46**, 77—86 (1999).
38) Hiramoto K., Li X. H., Makimoto M., Kato T., Kikugawa K., *Mutat. Res.*, **419**, 43—52 (1998).
39) Sarker S. D., Whiting P., Sik V., Dinan L., *Phytochemistry*, **50**, 1123—1128 (1999).
40) Kupchan S. M., Sigel C. W., Guttman L. J., Restivo R. J., Bryan R. F., *J. Am. Chem. Soc.*, **94**, 1353—1354 (1972).