Cytotoxic Terpenoids from the Formosan Soft Coral *Nephthea brassica*

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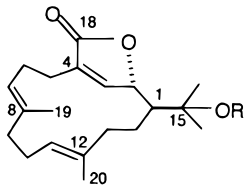
Two new cytotoxic cembranoid diterpenes, brassicolide (**1**) and brassicolide acetate (**2**); a new cytotoxic sesquiterpene, (−)-4α-*O*-acetyl-selin-11-en (**3**); and six cytotoxic terpenoids, (−)-selin-11-en-4α-ol (**4**), 2-hydroxynephthenol (**5**), nephthenol (**6**), cembrene A (**7**), epoxycembrene A (**8**), and (−)-*β*-elemene (**9**), have been isolated from the Formosan soft coral *Nephthea brassica*. The structures of compounds **1**−**9** were determined by spectral, chemical, and X-ray crystallographic analysis.

As part of our search for bioactive substances from marine organisms, the soft coral *Nephthea brassica* Kükenthal (Nephtheidae) was studied based on the CH_2-Cl_2 extracts showing significant cytotoxicity in A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), KB (human epidermoid carcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[1,2] Bioassay-guided fractionations resulted in the isolation of two new cytotoxic cembranoid diterpenes, brassicolide (**1**) and brassicolide acetate (**2**); a new cytotoxic sesquiterpene, (−)-4α-*O*-acetyl-selin-11-en (**3**); six cytotoxic terpenoids, (−)-selin-11-en-4α-ol (**4**), 2-hydroxynephthnol (**5**), nephthenol (**6**), cembrene A (**7**), epoxycembrene A (**8**), and (−)-*β*-elemene (**9**).



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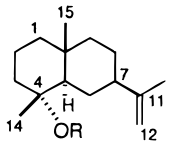

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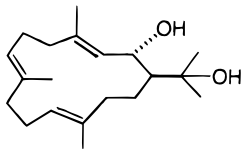

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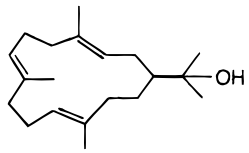

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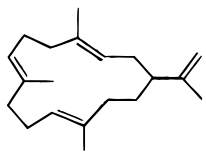

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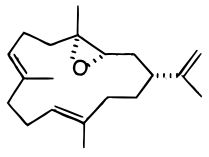

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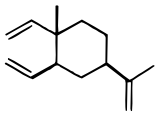

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Results and Discussion

Methylene chloride extraction of the freeze-dried animals followed by Si gel chromatography and recrystallization from hexane−acetone yielded **1** as a colorless crystalline solid, mp 115−116°. HRFABMS and the DEPT (135) established the molecular formula of **1** as $C_{20}H_{30}O_3$. Thus, 6 degrees of unsaturation were determined for **1**. The IR spectrum of **1** exhibited the presence of a carbonyl group of an α,*β*-unsaturated *γ*-lactone (ν_{max} 1750 cm^{-1}) and a hydroxyl group (ν_{max} 3502 cm^{-1}). A strong UV absorption at λ_{max} 236 nm suggested the presence of a α,*β*-unsaturated *γ*-lactone. ^1H and ^{13}C NMR spectral data (Table 1) showed the structure of **1** contained an isopropyl carbinol (δ_C 26.4, 28.5, q, C-16, 17; 73.0, s, C-15; δ_H 1.30, 1.32, 3H each, s each, H-16, 17), an α,*γ*-disubstituted α,*β*-unsaturated *γ*-lactone (δ_C 172.9, s, C-18; 133.1, s, C-4; 149.7, d, C-3; 83.1, d, C-2; δ_H 7.08, s, H-3; 4.89, d, J = 7.8 Hz, H-2), two isolated methyl-bearing trisubstituted double bonds (δ_H 4.96, 1H, m, H-7; 4.98, 1H, m, H-11; 1.54, 3H, br s, H-19; 1.61, 3H, br s, H-20; δ_C 123.3, d, C-7; 133.2, s, C-8; 126.9, d, C-11; 135.9, s, C-12), one methine carbon (δ_C 50.8, d, C-1), and six methylene carbons (δ_C 24.8, t, C-6; 25.0, t, C-5; 25.2, t, C-10; 25.5, t, C-14; 38.4, t, C-9; 39.4, t, C-13). These data suggested that **1** possessed a cembrane skeleton with functionalities of an isopropyl carbinol, an α,*γ*-disubstituted α,*β*-unsaturated *γ*-lactone, and two isolated methyl-bearing trisubstituted double bonds. In the HMBC experiment (Table 1) of **1**, the isopropyl carbinol group attached to C-1 was confirmed by long-range correlations between H-1 to C-2, C-3, C-13, C-14, C-15, C-16, and C-17; H-16 to C-1, C-15, and C-17; and H-17 to C-1, C-15, and C-16. The positioning of the α,*β*-unsaturated *γ*-lactone at C-4 (α), C-3 (*β*), C-2 (*γ*), and C-18 (carbonyl carbon) was deduced from HMBC correlations between H-2 and C-1, C-3, C-4, C-14, and C-15; H-3 and C-2, C-4, C-5, C-18; and H-5 and C-3, C-4, C-6, C-7, and C-18. The vinyl methyl group attached at C-8 was confirmed by HMBC correlations between H-19 and C-7, C-8, and C-9; H-7 and C-6 and C-19; and H-9 and C-7, C-8, C-10, and C-19. The other vinyl methyl group attached at C-12 was revealed by the HMBC correlations

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Table 1. NMR Data of **1**[a]

position	δ_H, mult. (Hz)	δ_C; mult.	HMBC	NOESY
1	1.62−1.63 m	50.8; d[c]	2, 3, 13, 14, 15, 16, 17	13
2	4.89 d (7.8)[b]	83.1; d	1, 3, 4, 14, 15	1, 3, 14β
3	7.08 s	149.7; d	2, 4, 5, 18	2, 14α, 14β
4		133.1; s		
5α	2.31−2.33 m	25.0; t	3, 4, 6, 7, 18	6α
β	2.51−2.53 m		3, 4, 6, 7, 18	6β
6α	2.07−2.09 m	24.8; t	4, 5, 7	5α
β	2.46−2.49 m		4, 5, 7	5β, 7, 19
7	4.96 m	123.3; d	6, 19	6α, 9
8		133.2; s		
9	2.10−2.13 m	38.4; t	7, 8, 10, 19	7
10α	2.18−2.20 m	25.2; t	9, 11	20
β	2.42−2.44 m		9, 11	
11	4.98 m	126.9; d	9, 10, 13, 20	
12		135.9; s		
13	2.03−2.06 m	39.4; t	11, 14, 20	1, 20
14α	1.11−1.15 m	25.5; t	1, 2, 13	1, 3
β	1.61−1.63 m		1, 2, 13	2, 16, 17
15		73.0; s		3
16	1.32 s	28.5; q	1, 15, 17	14β
17	1.30 s	26.4; q	1, 15, 16	14β
18		172.9; s		
19	1.54 s	16.1; q	7, 8, 9	6β
20	1.61 s	16.0; q	11, 12, 13	10α, 13

[a] Spectra recorded in $CDCl_3$. [b] *J* values (in Hz) in parentheses. [c] Multiplicity deduced by DEPT and indicated by usual symbols.

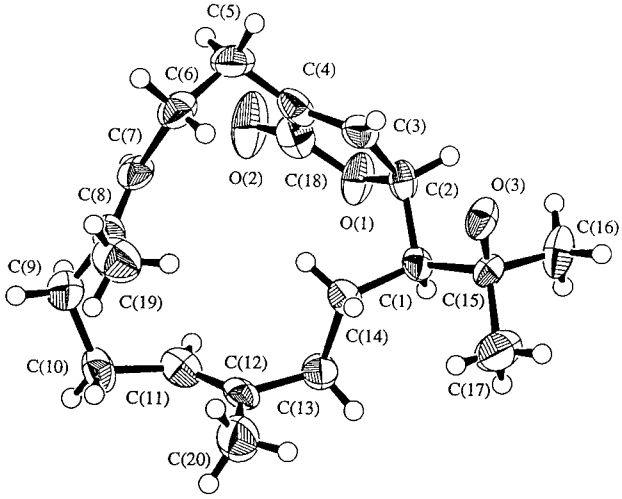

Figure 1. Molecular structure (relative configuration) of compound **1**.

between H-20 and C-11, C-12, and C-13; H-11 and C-9, C-10, C-13, and C-20; and H-13 and C-11, C-14, and C-20. The relative configuration of **1** was finally established by its X-ray diffraction analysis (Figure 1).[3]

Compound **2** was isolated as a colorless oil, whose molecular formula, $C_{22}H_{32}O_4$, was revealed by HRFABMS. The IR spectrum of **2** exhibited the presence of a carbonyl group of an α,β-unsaturated γ-lactone (ν_{max} 1755 cm^{-1}) and an ester carbonyl (ν_{max} 1725 cm^{-1}). A strong UV absorption at λ_{max} 234 nm suggested the presence of a α,β-unsaturated γ-lactone. ^1H and ^{13}C NMR spectral data (Table 2) showed the structure of **2** contained an acetoxyisopropyl side chain (δ_C 24.7, 24.6, q, C-16, 17; 83.5, s, C-15, 22.5, q; 170.0, s; δ_H 1.59, 1.56, 3H each, s each, H-16, 17; 2.06, 3H, s), an α,γ-disubstituted α,β-unsaturated γ-lactone (δ_C 173.3, s, C-18; 134.6, s, C-4; 148.6, d, C-3; 81.9, d, C-2; δ_H 7.05, s, H-3; 5.01, d, H-2), two isolated methyl-bearing trisubstituted double bonds (δ_H 4.98, 1H, m, H-7; 4.95, 1H, m, H-11; 1.50, 3H, br s, H-19; 1.55, 3H, br s, H-20; δ_C 124.0, d, C-7; 134.6, s, C-8; 125.8, d, C-11; 133.9, s, C-12), one methine carbon (δ_C 49.4, d, C-1), and six methylene carbons (δ_C 25.6, t, C-6; 25.0, t, C-5; 24.1, t, C-10; 25.7, t, C-14; 38.8, t, C-9; 39.4, t, C-13). These data suggested that **2** possessed a cembrane skeleton with functionalities of an acetoxyisopropyl, an α,γ-disubstituted α,β-unsaturated γ-lactone, and two isolated methyl-bearing trisubstituted double bonds. In the HMBC spectrum (Table 2) of **2**, the acetoxyisopropyl attached to C-1 was confirmed by correlations between H-1 to C-13, C-14, C-16, and C-17; H-16 to C-1, C-15, and C-17; H-17 to C-1, C-15, and C-16. HMBC correlations between H-2 and C-1, C-3, C-4, and C-14; H-3 and C-2, C-4, C-5, and C-18; H-5 and C-6, C-7, and C-18 revealed the position of α,β-unsaturated γ-lactone. The vinyl methyl group attached at C-8 was confirmed by the HMBC correlations between H-19 and C-7, C-8, and C-9; H-7 and C-9 and C-19; H-9 and C-7, C-10, and C-19. The other vinyl methyl group attached at C-12 was confirmed by the HMBC correlations between H-20 and C-11, C-12, and C-13; H-13 and C-11, C-12, C-14, and C-20. NMR data of **2** looked similar to those of **1** except for an additional methyl and a carbonyl at δ_H 2.06, 3H, s; δ_C 22.5, q and δ_C 170.0, s, respectively. In addition, downfield shift of H-16, 17 from δ 1.30, 1.32 in **1** to δ 1.56, 1.58 in **2**, as well as 10.5-ppm downfield shift of C-15 from δ 73.0 in **1** to δ 83.5 in **2** indicated that **2** may be a 15-acetate of **1**. The relative configuration of **2** was deduced from similarity of NOESY spectra and $^1H-^1H$ coupling patterns between **2** and **1**. Thus, **2** was confirmed as 15-acetate of **1**.

Table 2. NMR Data of **2**[a]

position	δ_H, mult. (Hz)	δ_C; mult.	HMBC	NOESY
1	2.18−2.21 m	49.4; d[c]	13, 14, 16, 17	2, 13
2	5.01 d (6.3)[b]	81.9; d	1, 3, 4, 14	1, 3, 14β
3	7.05 s	148.6; d	2, 4, 5, 18	2, 14α, 14β
4		134.6; s		
5α	2.39−2.41 m	25.0; t	6, 7, 18	6α
β	2.49−2.52 m		6, 7, 18	6β
6α	2.17−2.19 m	25.6; t	7, 8	5α, 7
β	2.52−2.55 m		7, 8	5β, 19
7	4.98 m	124.0; d	9, 19	6α
8		134.6; s		
9	2.06−2.09 m	38.8; t	7, 10, 19	7
10α	2.06−2.09 m	24.1; t	9, 11	20
β	2.15−2.18 m		9, 11	
11	4.95 m	125.8; d		
12		133.9; s		
13	2.03−2.06 m	39.4; t	11, 12, 14, 20	1, 20
14α	1.12−1.16 m	25.7; t	2	1, 3, 11
β	2.16−2.20 m		1, 13	2, 16, 17
15		83.5; s		3
16	1.59 s	24.7; q	1, 15, 17	14β
17	1.56 s	24.6; q	1, 15, 16	14β
18		173.3; s		
19	1.50 s	15.5; q	7, 8, 9	6β, 10, 14
20	1.55 s	15.9; q	11, 12, 13	10α, 13
OAc	2.06 s	22.5; q		
		170.0; s		

[a] Spectra recorded in $CDCl_3$. [b] *J* values (in Hz) in parentheses. [c] Multiplicity deduced by DEPT and indicated by usual symbols.

Compound **4** was isolated as colorless needles, mp 92−94°, $[\alpha]^{20}_D$ −16° (*c* 0.5, $CHCl_3$). IR absortion at 3305 and 1641 cm^{-1} indicated the presence of a hydroxyl group and a terminal double bond. In the ^1H and ^{13}C NMR spectra, the presence of the following moieties were deduced: an isopropenyl group [δ_H 4.65, 2H, d, J = 6 Hz; 1.68, 3H, s; δ_C 108.1, t; 150.5, s; 22.5, q], two aliphatic tertiary methyl groups [δ_H 0.83, 3H, s; 1.06, 3H, s; δ_C 18.6, q; 21.0, q], an aliphatic quaternary carbon [$\delta_{C\ 34.5}$], an oxygenated quaternary carbon [$\delta_{C\ 72.1}$], two sp^3 methine carbons [δ_C 46.2, d; 54.8, d], and six sp^3 methylene carbons [δ_C 20.0, 25.9, 26.8, 41.0, 43.3, 44.6]. The identity of **4** as (−)-selin-11-en-4α-ol was established by direct comparison of the $[\alpha]^{20}_D$, IR, EIMS, ^1H NMR, and ^{13}C NMR data with those reported in the literature.[4−6]

Table 3. NMR Data of **3**[a]

position	δ_H, mult. (Hz)	δ_C; mult.	HMBC	NOESY
1	1.14 m	40.6; t[c]	2	
	1.37 m			
2	1.53 m	19.6; t	1	
3α	2.63 m	37.8; t	1, 4, 5	5
β	1.50 m			
4		85.8; s		
5	1.62 dd (10.1, 3.1)[b]	51.9; d	3, 4, 6	3α
6	1.76 m	26.3; t	5	
7	1.98 m	46.2; d		
8	1.52 m	26.9; t	6	
9	1.27 m	44.9; t	5, 8, 15	
	1.41 m			
10		34.7; s		
11		150.8; s		
12	4.72 d (6.0)	108.2; t	7, 13	
13	1.76 s	20.9; q	7, 11, 12	
14	1.41 s	19.1; q	4, 5	
15	0.92 s	18.8; q	1, 5, 9, 10	
OAc	1.95 s	22.8; q		
		170.5; q		

[a] Spectra recorded in $CDCl_3$. [b] *J* values (in Hz) in parentheses. [c] Multiplicity deduced by DEPT and indicated by usual symbols.

Table 4. Cytotoxicitiy[a] of **1**−**9** ($n = 8$)

	ED_{50} (μg /mL) in indicated cell line			
compound	A549	HT-29	KB	P-388
1	>50	5.81	2.40	2.44
2	3.03	0.81	0.72	1.20
3	0.12	>50	>50	2.44
4	>50	>50	>50	3.86
5	0.71	1.02	0.23	1.80
6	2.72	3.01	1.81	0.42
7	1.11	3.42	3.73	1.10
8	3.61	2.72	3.41	0.40
9	>50	>50	>50	0.92

[a] For significant activity of pure compounds, an ED_{50} of ≤ 4.0 μg/mL is required.[1]

Compound **3** was isolated as a colorless oil, whose molecular formula, $C_{17}H_{28}O_2$, was established by HREIMS. IR absortion at 1730 and 1643 cm^{-1} indicated the presence of an ester carbonyl group and a terminal double bond. The ^1H and ^{13}C NMR data (Table 3) showed the structure of **3** contained an isopropenyl group [δ_H 4.73, 1H, s; 4.71, 1H, s; 1.76, 3H, s; δ_C 108.2, t; 150.8, s; 20.9, q], two aliphatic tertiary methyl groups [δ_H 0.92, 3H, s; 1.41, 3H, s; δ_C 18.8, q; 29.1, q], an aliphatic quaternary carbon [δ_C 34.7], an oxygenated quaternary carbon [δ_C 85.8], two sp^3 methine carbons [δ_C 46.2, d; 51.9, d], and six sp^3 methylene carbons [δ_C 19.6, 26.3, 26.9, 37.8, 40.6, 44.9]. NMR data of **3** were similar to those of **4** except for an additional methyl and a carbonyl at δ_H 1.95, 3H, s; δ_C 22.8, q and δ_C 170.5, s, respectively. The 0.29 ppm downfield shift of H-14 from δ 1.12 in **4** to δ 1.41 in **3** and 14.0 ppm downfield shift of C-4 from δ 71.8 in **4** to δ 85.8 in **3** indicated **3** to be a 4-acetyl derivative of **4**. The C-4-acetoxy substitution in **3** was further confirmed by HMBC correlations (Table 3) between H-14 and C-3, C-4, and C-5; H-5 and C-3, C-4, C-6, and C-14. Hydrolysis of **3** with methanolic K_2CO_3 at 40 °C afforded **3**. The structure of **4** was thus established as (−)-4α-*O*-acetyl-selin-11-en. The identity of compounds **5**−**9** was established by direct comparison of the $[\alpha]^{20}_D$, IR, EIMS, ^1H NMR, and ^{13}C NMR data with literature data.[7−11]

Cytotoxicity of compounds **1**−**9** was shown in Table 4. Compounds **2** and **5**−**8** exhibited cytotoxicity against A549, HT-29, KB, and P-388 cell lines. Compounds **1** and **4** were not active against A549 cells; however, their acetates (**2** and **3**) exhibited cytotoxicity toward A549 cells. Compound **3** was not cytotoxic against HT-29 cells.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micromelting point apparatus and are reported uncorrected. Optical rotations were determined on a JASCO DIP-181 polarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra were recorded on a Hitachi 26−30 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a Brucker AMX 400 NMR spectrometer at 400 and 100.6 MHz, respectively, in $CDCl_3$ using TMS as internal standard. EIMS were obtained with a JEOL JMS−SX/SX 102A mass spectrometer at 70 ev. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *N. brassica* (Nephtheidae) was collected at Liouchou Island of Taiwan in September 1996, at a depth of 10 m and was stored for 2 days in a freezer until extraction. A voucher specimen, NSUSL-1001, was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. The bodies of the soft coral *N. brassica* were freeze-dried to give 610 g of a solid, which was extracted with CH_2Cl_2 (1.5 L × 3). After removal of solvent in vacuo, the residue (52 g) was chromatographed over Si gel 60 using $CHCl_3$ and $CHCl_3$−MeOH mixtures of increasing polarity. Elution by $CHCl_3$ afforded fractions containing compounds **3**, **4**, **6**, and **7**. Elution by $CHCl_3$−MeOH (99:1) afforded fractions containing cembranoids **8** and **9.** Elution by $CHCl_3$−MeOH (19:1) afforded fractions containing compounds **1**, **2**, and **5**. Compounds **7**, **3**, **4**, and **6** were obtained by Si gel column chromatography, by eluting with *n*-hexanes−EtOAc (100:1), *n*-hexanes−EtOAc (50:1), *n*-hexanes−EtOAc (30:1), and *n*-hexanes−EtOAc (10:1), respectively. Compounds **9** and **8** were obtained by Si gel column chromatography, by eluting with *n*-hexanes−EtOAc (100:1) and *n*-hexanes−EtOAc (30:1), respectively. Compounds **2**, **1**, and **5** were obtained by Si gel column chromatography, by eluting with *n*-hexane−acetone (6:1), *n*-hexane−acetone (3:1), and *n*-hexane−acetone (2:1), respectively.

Brassicolide (1): colorless prism (193 mg); mp 115−116 °C; $[\alpha]^{25}_D$ +50.3° (*c* 3.7, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 236 (4.1) nm; IR (KBr) ν_{max} 3502, 2972, 1750 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; FABMS *m/z* 319 $[M]^+$ (4), 301 (33), 255 (12), 215 (8), 165 (16), 133 (100), 121 (27), 91 (60, 77 (44); HR-FABMS *m/z* 319.2253 (calcd for $C_{20}H_{31}O_3$ 319.2265).

Brassicolide acetate (2): colorless oil (60 mg); $[\alpha]^{25}_D$ +101.0° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (4.4) nm; IR (KBr) ν_{max} 2924, 1755, 1725 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; FABMS *m/z* 361 $[MH]^+$ (1), 301 (27), 255 (7), 137 (45), 81 (86), 69 (100); HRFABMS *m/z* 361.2380 (calcd for $C_{22}H_{33}O_4$ 361.2370).

(−)-4α-*O*-Acetyl-selin-11-en (3): colorless oil (56 mg); $[\alpha]^{25}_D$ −41.6° (*c* 0.45, $CHCl_3$); IR (KBr) ν_{max} 1730, 1643 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; EIMS *m/z* 264 $[M]^+$ (1), 204 (59), 189 (100), 161 (37), 147 (46), 133 (67), 108 (52), 43 (60); HREIMS $[M]^+$ *m/z* 264.2162 (calcd for $C_{17}H_{28}O_2$ 264.2171).

Hydrolysis of (−)-4α-*O*-Acetyl-selin-11-en (3). Compound **3** (7 mg) was treated with 0.5% K_2CO_3−MeOH at 40 °C for 48 h. The resulting mixture was purified by column chromatography over Si gel using *n*-hexanes−EtOAc (10:1) as eluting solvent to afford **4** (4 mg).

Single-crystal X-ray Analysis of Brassicolide (1). Crystal data: $C_{20}H_{30}O_3$, space group $P2_1$, $a = 8.95$ (6) Å, $b = 24.3$ (2) Å, $c = 8.79$ (1) Å, $V = 1913$ (19) Å^3, $Z = 4$, Dcalc = 1.106 g/cm^3, λ (Mo Kα) = 0.71069 Å. Intensity data were measured on a Rigaku AFC6S diffractometer up to 2θ of 50.1°. A total of 1966 reflections were observed [$I > 3\sigma$(I)]. The structure was solved by the direct methods (SIR92),[12] and the final structure parameters were obtained by a full-matrix least-squares process. In view of the absence of heavy atom in the structure, Friedel pairs were not collected, and the absolute configuration of brassicolide (**1**) was not determined via diffraction method. Calculated hydrogen positions were put in the final cycle of

Table 5. Crystal Data and Intensity Collection Data for Brassicolide (**1**)

empirical formula	$C_{20}H_{30}O_3$
fw	318.46
color, habit	colorless, prism
diffractormeter used	Rigaku AFC6S
space group	$P2_12_12_1$(#19)
a, Å	8.95 (6)
b, Å	24.3 (2)
c, Å	8.79 (1)
V, Å^3	1913 (19)
Z	4
D_{calcd}, g cm^{-3}	1.106 g/cm^3
λ(Mo Kα), Å	0.71069
F(000)	696.00
unit cell detn (no. 2θ range, deg)	21, 2θ (17.1−25.3°)
scan type	ω−2θ
μ(Mo Kα), cm^{-1}	0.72 cm^{-1}
crystal size, mm	0.33 × 0.66 × 0.72 mm
transm factor	0.6273−1.000
temp, °C	23.0
no. of measd reflns	1966
no. of obsd reflns (N_o)	1119
R,[a] R_w[a]	0.079, 0.050
GOF[a]	4.53
no. of ref params (N_p)	208
max peak in final diff. map	0.33 e$^-$/Å^3
min peak in final diff. map	−0.31 e$^-$/Å^3

[a] $R = [\sum||F_o| - |Fc||/\sum|F_o|$. $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$. GOF $= [\sum w(|F_o| - |F_c|)^2/(N_o - N_p)]^{1/2}$.

structure factor calculation but not refined. The agreement indices were $R(F) = 0.079$, $Rw(F) = 0.050$ with anisotropic refinement done on all nonhydrogen atoms. Experimental details are shown in Table 5. Final atomic coordinates are listed in Table 6.

Cytotoxicity Testing. KB and P-388 cells were supplied by Prof. J. M. Pezzuto, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection.

The P-388 cells were cultured in Fisher's medium supplemented with 10% heat-inactivated fetal calf serum (FCS). The KB cells were maintained in Basal Medium Eagle (BME) containing 10% heat-inactivated FCS. The A549 cell line was cultured in Eagle Minimum Essential Medium (EMEM) containing Earle's salts and supplemented with 0.1 mM of nonessential amino acids and 10% heat-inactivated FCS. The HT-29 cell lines were maintained in Rosewell Park Memorial Institute (RPMI) 1640 medium containing 10% heat-inactivated FCS. All the cell lines were maintained in an incubator at 37 °C in humidified air containing 5% CO_2. For routine cytotoxicity assay, all four cell lines were adapted to one single medium, RPMI 1640 supplemented with 10% FCS and 1 mM glutamate.

The cytotoxic activities of tested compounds or fractions against P-388, KB, A549, and HT-29 were assayed with modification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. For P-388 cells, 200 μL of culture was established at 1500 cells/well in 96-well tissue culture plates (Falcon). Tested compounds were subsequently dispensed to the established culture plate at eight concentrations, each with three replicates. After 3 days of incubation, P-388 cells were enumerated with MTT.

To measure the cytotoxic activities of pure compounds or crude fractions against A549, HT-29, KB, and P-388, each cell line was initiated at 750, 750, 2000, and 1500 cells/well, respectively, in 96-well microtiter plates. Three to eight concentrations encompassing an 8- to 128-fold range were evaluated on each cell line. A549, HT-29, KB, and P-388 cells were enumerated using MTT after the exposure to test samples for 6, 6, 3, and 3 days, respectively. Of 1 mg/mL MTT, 50 mL was added to each well, and plates were incubated at 37 °C for a further 4 h. The supernatant was aspirated with a Dynatech automatic washer. Formazan crystals were redissolved in DMSO (Merck) for 10 min with shaking, and the plate was read immediately on a microtiter plate reader (Dynatech) at a wavelength of 540 nm. The ED_{50} value was defined as the concentration of test compound resulting in a 50% reduction of absorbance compared to untreated cell in the MTT assay.[13]

Table 6. Atomic Parameters for Brassicolide (**1**)

atom	*x*	*y*	*z*	*B*eq
O(1)	0.7896(8)	0.3674(3)	0.5101(6)	4.3(2)
O(2)	0.8212(9)	0.4021(3)	0.7445(7)	6.7(2)
O(3)	0.9032(7)	0.4048(2)	0.0538(6)	3.7(2)
C(1)	0.908(1)	0.3424(3)	0.2717(10)	2.9(2)
C(2)	0.812(1)	0.3869(4)	0.3541(9)	3.2(2)
C(3)	0.872(1)	0.4446(4)	0.3755(9)	3.6(3)
C(4)	0.888(1)	0.4568(4)	0.524(1)	3.7(3)
C(5)	0.952(1)	0.5084(4)	0.599(1)	4.5(3)
C(6)	1.123(1)	0.5080(4)	0.604(1)	3.9(3)
C(7)	1.189(1)	0.4593(3)	0.697(1)	3.3(2)
C(8)	1.321(1)	0.4378(4)	0.675(1)	4.2(3)
C(9)	1.378(1)	0.3913(4)	0.781(1)	4.8(3)
C(10)	1.418(1)	0.3368(4)	0.702(1)	4.3(3)
C(11)	1.293(1)	0.3158(3)	0.604(1)	3.8(3)
C(12)	1.289(1)	0.3050(3)	0.457(1)	3.2(2)
C(13)	1.141(1)	0.2939(3)	0.380(1)	3.6(3)
C(14)	1.0725(10)	0.3491(4)	0.326(1)	3.5(2)
C(15)	0.884(1)	0.3474(4)	0.0955(10)	3.4(2)
C(16)	0.726(1)	0.3281(3)	0.053(1)	4.7(3)
C(17)	0.993(1)	0.3105(3)	0.010(1)	5.6(3)
C(18)	0.833(1)	0.4080(4)	0.609(1)	4.4(3)
C(19)	1.428(1)	0.4547(4)	0.554(1)	5.9(3)
C(20)	1.421(1)	0.3089(4)	0.353(1)	5.6(3)

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Supporting Information Available: X-ray crystallograpphic data for brassicolide (**1**), including an ORTEP drawing, experimental and calculation details, and tables of atomic coordinates, anisotropic displacement parameters, bond lengths and angles, and nonbonded contacts. This material is available free of charge via the Internet at http://pubs.acs.org.

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(3) Crystallographic data for **1** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-(0)1223-336033 or E-mail: deposit@ccdc.cam.ac.uk).
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