Isolation of 6,7-Demethylenedesoxypodophyllotoxin from *Hernandia ovigera*

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6,7-Demethylenedesoxypodophyllotoxin (1) was isolated from the seeds of *Hernandia ovigera* L. (Hernandiaceae) collected in Taiwan, together with several known lignans. This is the first example of the occurrence of 1 in a natural source. The assignments of the ^{13}C-nuclear magnetic resonance signals of several podophyllotoxin analogues isolated from this plant were also established by means of two-dimensional H–C correlation spectroscopy (COSY) and H–C long range COSY techniques.

Keywords *Hernandia ovigera*; Hernandiaceae; 6,7-demethylenedesoxypodophyllotoxin; podophyllotoxin; lignan; ^{13}C-NMR; ^{1}H-NMR

Several lignans,[1-3] aporphines,[4-6] and benzylisoquinoline alkaloids[6] have been isolated from *Hernandia ovigera* L. (Hernandiaceae). This time, the systematic extraction and isolation (Chart 1) of the constituents of the seeds of *Hernandia ovigera* L. collected in Taiwan gave 6,7-demethylenedesoxypodophyllotoxin (**1**) as a new natural lignan, along with several known lignans (**2**—**7**).

Results and Discussion

6,7-Demethylenedesoxypodophyllotoxin (**1**) was isolated as colorless needles, mp 238—241 °C, $[\alpha]_D$ −113° (chloroform), from the methanolic extract as shown in Chart 1. The molecular formula was determined as $C_{21}H_{22}O_7$ by high resolution mass spectrometry (HR-MS). The ultraviolet (UV) spectrum of **1** showed absorption bands at λ_{max} 208, 231 (sh), and 287 nm and infrared (IR) bands at ν_{max} 3550, 3350 (br), and 1780 cm^{-1} due to hydroxy and carbonyl groups, respectively. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum in dimethyl sulfoxide (DMSO) revealed signals due to three methoxyls [δ 3.63 (6H, s) and 3.61 (3H, s)] and four aromatic protons [δ 6.60 (1H, s), 6.35 (1H, s), and 6.31 (2H, s)] as well as seven aliphatic protons (see Table I). The relationships of aliphatic protons were established by H–H correlation spectroscopy (COSY). As shown in Table I, the signal pattern of the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** was similar to that of desoxypodophyllotoxin (**2**)[1,3,7] except for the lack of a methylenedioxy carbon signal at δ 100.93 and some differences of chemical shifts of signals due to carbons on ring A in the ^{13}C-NMR spectrum of **2**. Based on these spectral data, together with the results of H–C long range COSY as shown by arrows in Chart 2, we proposed the structure **1**, corresponding to a 6,7-demethylene derivative of desoxypodophyllotoxin (**2**), for this compound.

To confirm this structure, **2** was treated in acetic acid

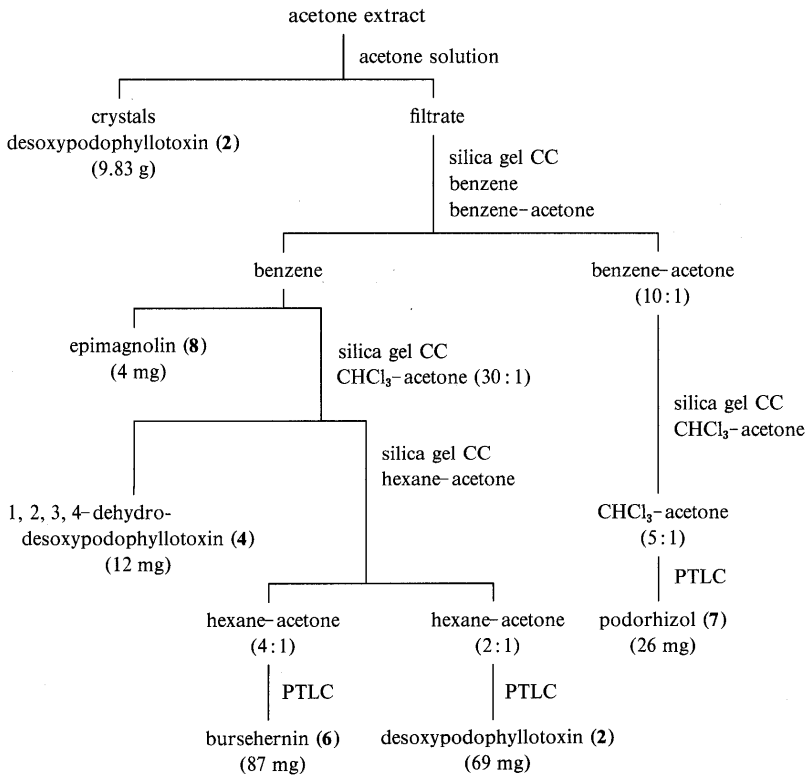


Chart 1 (continued)

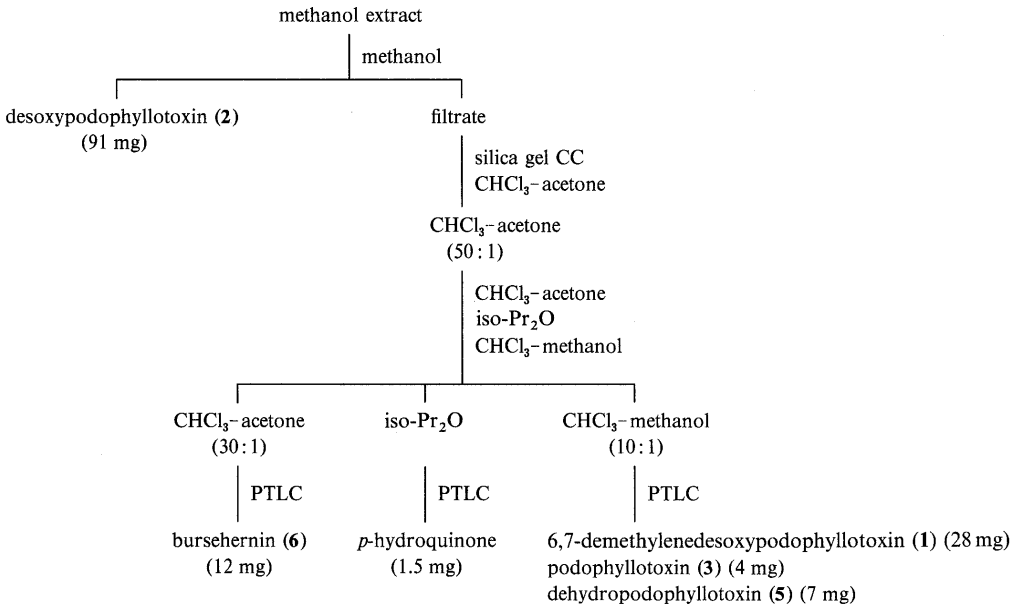
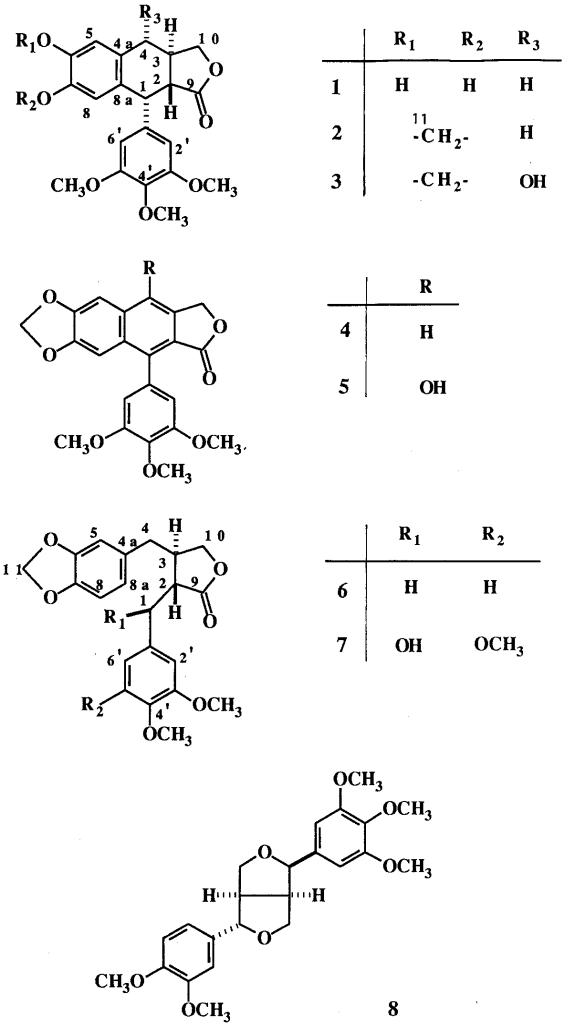


Chart 1. Isolation Procedure of the Constituents



with $Pb(OAc)_4$ at 78 °C for 3 h to give **1**, mp 238—241 °C, $[\alpha]_D$ −124° (chloroform), which was found to be identical with the natural specimen and an authentic sample[8] of **1**

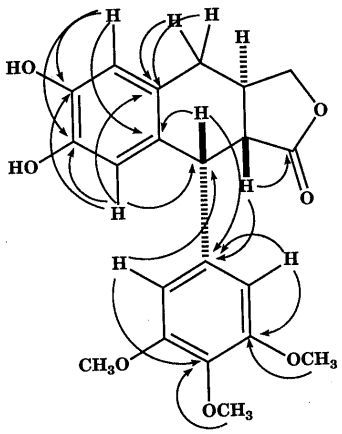


Chart 2. H–C Long Range Correlations in the H–C Long Range COSY of **1**

by IR, ^{1}H-NMR, and $[\alpha]_D$ comparisons. 6,7-Demethylenedesoxypodophyllotoxin (**1**) has been prepared from **2** by Yamaguchi and collaborators.[8] However, this is the first isolation of **1** from a natural source.

Known lignans, desoxypodophyllotoxin (**2**),[1,7] podophyllotoxin (**3**), 1,2,3,4-dehydrodesoxypodophyllotoxin (**4**),[3] dehydropodophyllotoxin (**5**),[2,9] bursehernin (**6**),[1,10] and podorhizol (**7**),[1,11] were also isolated and characterized by comparisons of physical and spectroscopic data (UV, IR, and ^{1}H-NMR) with the literature values, and full assignments of the signals of their ^{13}C-NMR spectra using H–C COSY and H–C long range COSY were also carried out for their structural identifications (see Table I).

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a GX-270 (JEOL) or GX-400 (JEOL) spectrometer in $CDCl_3$, unless otherwise stated. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. H–C long range COSY spectra were recorded at J_{HC} 5 Hz in DMSO on a GX-400 (JEOL) spectrometer. All mass spectra (MS) and HR-MS were taken under electron impact (EI) conditions using a Hitachi M-80 or JEOL JMS-HX-110 mass spectrometer having a direct

TABLE I. ^{1}H- and ^{13}C-NMR Data for the Constituents of Seeds of *Hernandia ovigera*

	1		2		3		4		5		6		7	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.40 (m)	42.43	4.51 (d, 5.1)	43.03	4.49 (m)	43.26	—	139.03	—	130.22	2.86 (dd) (5.5, 13.6)	33.76	5.10 (br s)	70.53
											2.78 (dd) (7.0, 13.6)		5.85 (d, OH) (4.4)	
2	2.93 (m)	46.24	2.96 (dd) (5.1, 13.9)	46.10	2.60 (m)	43.99	—	140.19	—	122.22	2.48 (m)	45.66	2.64 (m)	52.23
3	2.63 (m)	32.80	2.62 (m)	32.57	3.14 (dd) (4.7, 14.7)	39.00	—	118.30	—	119.00	2.48 (m)	40.80	2.75 (m)	35.68
4	2.68 (d, 11.7)	31.28	2.74 (dd) (15.8, 11.7)	32.00	4.63 (d, 7.3)	70.95	7.96 (s)	119.56	—	145.33	2.48 (2H, m)	37.09	2.40 (dd) (8.1, 13.6)	38.62
	2.92 (m)		3.02 (dd) (4.8, 15.8)		5.78 (d, OH) (7.3)				5.76 (s, OH)				2.02 (dd) (7.3, 13.6)	
4a	—	126.07	—	129.07	—	134.92	—	134.22	—	124.51	—	132.55	—	132.34
5	6.60 (s)	115.49	6.80 (s)	108.52	7.10 (s)	106.24	7.53 (s)	103.65	7.62 (s)	97.95	6.65 (d, 1.5)	108.86	6.37 (s)	108.62
6	—	144.33	—	146.35	—	146.51	—	148.58	—	148.67	—	145.68	—	145.51
7	—	143.79	—	145.93	—	146.36	—	148.37	—	148.27	—	147.33	—	147.15
8	6.35 (s)	117.01	6.50 (s)	109.92	6.84 (s)	109.00	6.92 (s)	102.26	6.86 (s)	102.47	6.80 (d, 8.1)	113.03	6.64 (d, 8.1)	107.82
8a	—	127.88	—	130.56	—	130.64	—	129.19	—	130.80[a]	6.56 (dd) (8.1, 1.5)	121.47	6.33 (d, 8.1)	121.35
9	—	175.07	—	174.96	—	174.62	—	169.05	—	169.26	—	178.37	—	177.72
10	4.40 (m)	71.52	4.41 (t, 7.3)	71.55	4.49 (m)	70.48	5.44 (2H, s)	67.93	5.36 (2H, s)	66.50	4.10 (dd) (7.3, 8.8)	70.65	4.23 (dd) (8.1, 8.4)	72.02
	3.93 (dd) (9.5, 8.8)		3.95 (dd) (10.3, 8.4)		4.09 (dd) (8,8, 10.3)						3.88 (t, 8.8)		3.92 (dd) (8.4, 5.9)	
11	—		5.96 (s)	100.93	5.99 (s)	100.90	6.19 (2H, s)	102.10	6.17 (2H, s)	101.90	5.98 (s)	100.79	5.96 (s)	100.74
			5.95 (s)		5.97 (s)						5.97 (s)		5.92 (s)	
1′	—	137.58	—	137.01	—	136.47	—	130.38	—	130.76[a]	—	130.52	—	138.51
2′	6.31 (s)	108.18	6.30 (s)	108.19	6.33 (s)	108.31	6.59 (s)	107.37	6.53 (s)	107.67	6.78 (d, 1.8)	108.10	6.61 (s)	102.55
3′	—	151.82	—	151.97	—	151.83	—	152.50	—	152.32	—	148.59[a]	—	152.67
3′-OMe	3.63 (3H, s)	55.73	3.64 (3H, s)	55.76	3.64 (3H, s)	55.77	3.74 (3H, s)	55.95	3.73 (3H, s)	55.80	3.73 (3H, s)	55.39[a]	3.75 (3H, s)	55.79
4′	—	136.20	—	136.33	—	136.32	—	137.00	—	136.70	—	147.49[a]	—	136.22
4′-OMe	3.61 (3H, s)	59.84	3.62 (3H, s)	59.90	3.62 (3H, s)	59.81	3.78 (3H, s)	60.07	3.77 (3H, s)	59.97	3.73 (3H, s)	55.34[a]	3.64 (3H, s)	60.02
5′	—	151.82	—	151.97	—	151.83	—	152.50	—	152.32	6.86 (d, 8.1)	111.67	—	152.67
5′-OMe	3.63 (3H, s)	55.73	3.64 (3H, s)	55.76	3.64 (3H, s)	55.77	3.74 (3H, s)	55.95	3.73 (3H, s)	55.80	—		3.75 (3H, s)	55.79
6′	6.31 (s)	108.18	6.30 (s)	108.19	6.33 (s)	108.31	6.59 (s)	107.37	6.53 (s)	107.67	6.73 (dd) (8.1, 1.8)	121.25	6.61 (s)	102.55

Vatues are in ppm (δ_H and δ_C). ^{1}H- and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, in $CDCl_3$. Each signals corresponds to 1H, unless otherwise stated. Figures in parentheses are coupling constants (J) in Hz. *a*) Assignments may be interchanged.

inlet system. IR spectra were recorded on a JASCO IR-810 IR spectrophotometer in $CHCl_3$, unless otherwise stated, UV spectra on a JASCO UVIDEC-610C double beam spectrophotometer in MeOH, and $[\alpha]_D$ on a DIP-181 (JASCO) in $CHCl_3$ at 20 °C.

Extraction and Isolation Dried and powdered seeds (1900 g) of *Hernandia ovigera* L. (Hernandiaceae) collected in Taiwan were defatted with hexane, and extracted with acetone and then methanol at room temperature. The acetone and methanol extracts were treated according to the procedures shown in Chart 1, to give the indicated components.

6,7-Demethylenedesoxypodophyllotoxin (1) Colorless needles from ethanol, mp 238—241 °C, $[\alpha]_D$ −113° (c=0.086, $CHCl_3$). IR ν_{max} cm^{-1}: 3550, 3350 (br), 1780. UV λ_{max} nm: 208, 231 sh, 287. MS m/z (%): 386 (M^+, 93), 358 (19), 262 (14), 182 (13), 181 (44), 174 (12), 173 (15), 168 (18), 167 (12), 161 (19), 153 (12), 152 (14), 151 (57), 137 (14), 135 (10), 123 (27), 115 (21), 114 (68). HR-MS: Calcd for $C_{21}H_{22}O_7$: 386.1364. Found: 386.1361.

Demethylenation of 2 A mixture of **2** (100 mg) and $Pb(OAc)_4$ (200 mg) in AcOH (4 ml) was heated at 78 °C for 3 h. The solvent was evaporated off and the residue was subjected to preparative TLC ($CHCl_3$–acetone, 5:1) to give **1** (15 mg) as colorless needles, mp 238—241 °C, $[\alpha]_D$ −124° (c=0.054, $CHCl_3$). This compound was found to be identical with the natural specimen and an authentic sample of **1** provided by Professor Yamaguchi, by comparisons of IR and ^{1}H-NMR spectra and $[\alpha]_D$.

Desoxypodophyllotoxin (2) Colorless needles, mp 167—168 °C, $[\alpha]_D$ −110° (c=0.159, $CHCl_3$). IR ν_{max} cm^{-1}: 1780, 1595. UV λ_{max} nm: 208, 242 sh, 289. MS m/z (%): 398 (M^+, 100), 370 (9), 283 (5), 230 (9), 199 (5).

Podophyllotoxin (3) Colorless oil, $[\alpha]_D$ −122° (c=0.125, $CHCl_3$). IR ν_{max} cm^{-1}:3400 br, 1780, 1595. UV λ_{max} nm: 215, 243 sh, 292. MS m/z (%): 414 (M^+, 100), 202 (16), 201 (22), 200 (16), 189 (22), 185 (16), 181 (30), 173 (11), 169 (41), 168 (55), 165 (14), 163 (12).

1,2,3,4-Dehydrodesoxypodophyllotoxin (4) Colorless needles, mp 269—270 °C. IR ν_{max} cm^{-1}: 1770, 1585. UV λ_{max} nm: 206, 223 sh, 256, 296, 308, 347. MS m/z(%): 394 (M^+, 100), 380 (8), 379 (31), 351 (9), 328 (5), 321 (7), 293 (6), 277 (6), 270 (5), 265 (8), 197 (5), 182 (6), 175 (6).

Dehydropodophyllotoxin (5) Colorless plates, mp 280—282 °C. IR ν_{max} cm^{-1}: 3400, 1770, 1595. UV λ_{max} nm: 206, 223 sh, 263, 309, 323, 357. MS m/z: (%): 410 (M^+, 100), 395 (37), 367 (13), 135 (26), 108 (10).

Bursehernin (6) Colorless oil, $[\alpha]_D$ −29° (c=0.28, $CHCl_3$). IR ν_{max} cm^{-1}: 1770, 1600. UV λ_{max} nm: 206, 232, 283. MS m/z (%): 370 (M^+, 55), 234 (6), 208 (8), 162 (7), 161 (7), 152 (25), 151 (100).

Podorhizol (7) Amorphous powder, $[\alpha]_D$ −43° (c=0.159, $CHCl_3$). IR ν_{max} cm^{-1}: 3625, 1765, 1600. UV λ_{max} nm: 206, 237 sh, 286. MS m/z (%): 416 (M^+, 13), 221 (28), 220 (99), 198 (12), 197 (81), 196 (99), 195 (21), 182 (17), 181 (98), 169 (26), 167 (17), 166 (34), 165 (27).

Epimagnolin (8) Amorphous powder, $[\alpha]_D$ +108° (c=0.103, $CHCl_3$). IR ν_{max} cm^{-1}: 1600, 1520. UV λ_{max} nm: 209, 232, 278. MS m/z (%): 416 (M^+, 81), 224 (15), 220 (15), 219 (14), 208 (11), 207 (26), 197 (34), 196 (18), 195 (22), 194 (21), 189 (13), 182 (31), 181 (53). ^{1}H-NMR δ: 6.92 (1H, br s), 6.89 (2H, m), 6.59 (2H, s), 4.86 (1H, d, J=5.4 Hz), 4.46 (1H, d, J=7.1 Hz), 4.14 (1H, d, J=9.4 Hz), 3.91 (3H, s), 3.88 (9H, s), 3.86 (3H, s), 3.80—4.10 (2H, overlapped m), 3.35 (2H, overlapped m), 2.93 (1H, m).

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