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Studies on the Constituents of the Seeds of *Hernandia ovigera* L. III.[1] Structures of Two New Lignans

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Two kinds of lignans were isolated from the seeds of *Hernandia ovigera* L. (Hernandiaceae) besides the previously reported desoxypodophyllotoxin (DPT), desoxypicropodophyllin, bursehernin and podorhizol, and the structures of the lignans were clarified.

One, $C_{23}H_{24}O_8$, named hernandin, was identified as 5-methoxy-desoxypodophyllotoxin (I) by both chemical and X-ray crystallographic methods. The other, $C_{22}H_{18}O_7$, was obtained by purification of a previously isolated impure substance, mp 270—275°C. This compound was identified as 1,2,3,4-dehydrodesoxypodophyllotoxin (II). This is the first report of the natural occurrence of II in plants.

Keywords——lignans; *Hernandia ovigera* L.; hernandin; 1,2,3,4-dehydrodesoxypodophyllotoxin; picrohernandin; 1,2,3,4-dehydro-β-peltatin A methyl ether; 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ); optical rotatory dispersion (ORD) and circular dichroism (CD) of 4-aryltetralin lignans; mass spectra (MS) of 4-aryltetralin lignans; X-ray analysis of hernandin

In the first paper of this series,[2] the systematic extraction and isolation of the components of the seeds of *Hernandia ovigera* L. (Hernandiaceae) were carried out. Several lignans, such as desoxypodophyllotoxin (DPT), desoxypicropodophyllin, bursehernin and podorhizol were identified and an unknown substance, mp 270—275°C, was isolated as a minor constituent.

This paper describes the structure elucidation of a new lignan (I) as well as a lignan (II) which was obtained by purification of the above-mentioned unknown minor substance (mp 270—275°C).

Compound I, named hernandin, was obtained as floating feather crystals in the upper layer of the recrystallizing mother liquor of the DPT fraction, in which DPT crystallized as heavy prisms at the bottom of the flask. It gave mp 210—213°C and $[\alpha]_D$ −70° (in $CHCl_3$). A molecular formula of $C_{23}H_{24}O_8$ and molecular weight of 428 were deduced by means of mass spectrometry (MS). In the infrared (IR) and nuclear magnetic resonance (NMR) spectra, signals due to a lactone methylene, one methylenedioxy group, three aromatic protons and four methoxy groups were apparent. A known lignan with the same molecular formula and functional groups is β-peltatin A methyl ether (III),[3a-c] mp 162—163°C, $[\alpha]_D$ −118° (in $CHCl_3$).

In the NMR spectra, the greater part of the signals of I and III coincided almost exactly, with the exception that an aromatic proton of I in the tetralin ring appears at lower magnetic field than in III (I; δ 6.40, III; δ 6.24) and a methoxy group of I in the tetralin ring appears at higher magnetic field than in III (I; δ 3.60, III; δ 4.04).

In the MS of I and III, as shown in Fig. 1, not only the molecular peak at *m/e* 428 but also other prominent ion peaks at *m/e* 260, 215, 203, 181 and 168 are common to both compounds.[4] The above result shows that I has a trimethoxyphenyl group and a tetralin ring, and a methoxy and a methylenedioxy group are substituted on the benzene nucleus.

By aromatization using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), I was led to the naphthalene-type compound IV, mp 310—313°C which had no asymmetric carbons. This was compared with 1,2,3,4-dehydro-β-peltatin A methyl ether (V), mp 273.5—275.5°C, which

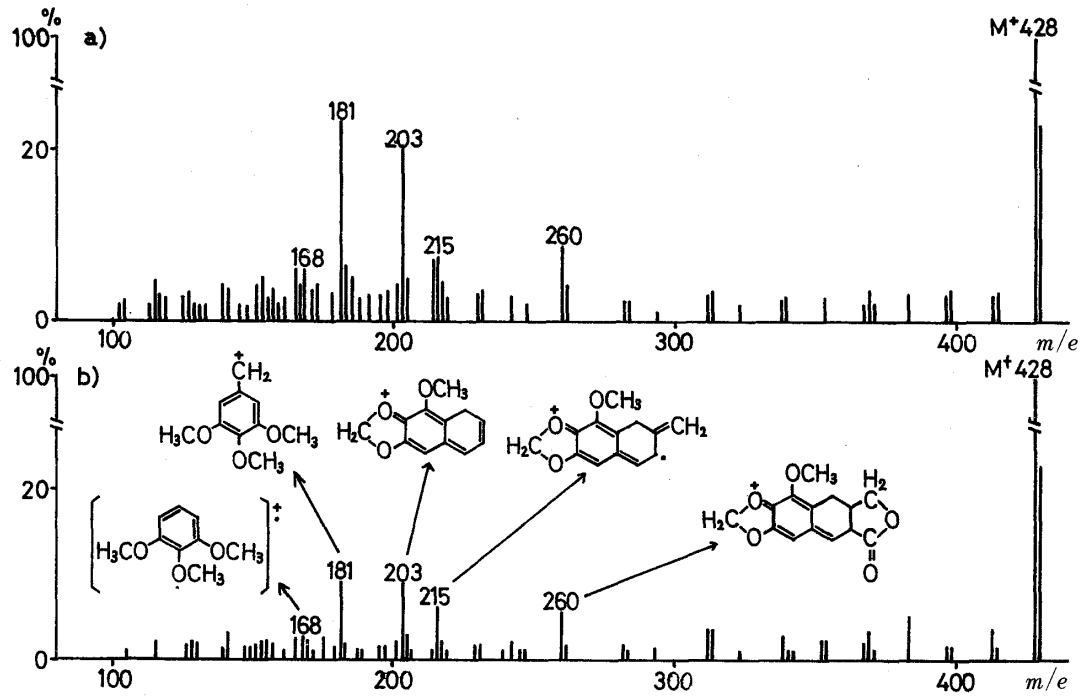


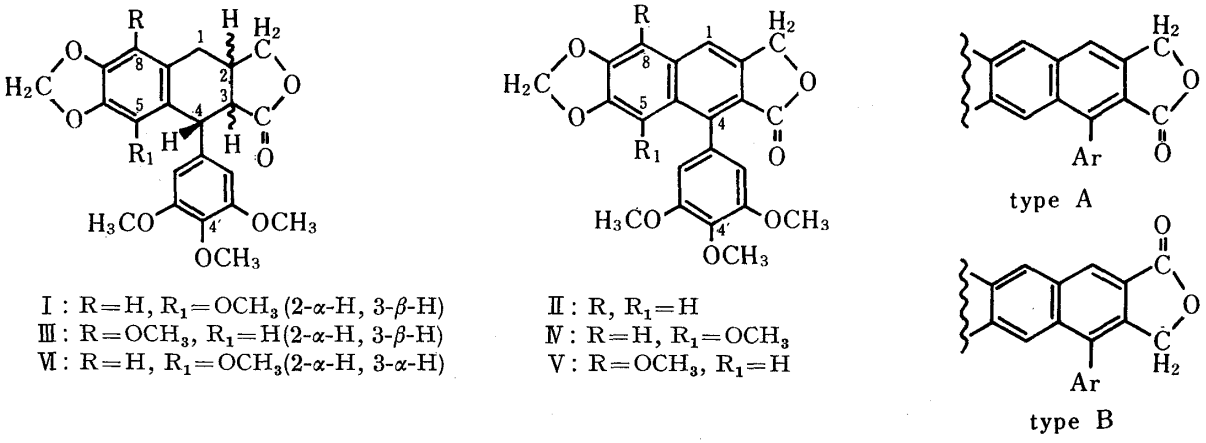
Fig. 1. Mass Spectra of Hernandin (I) (a) and β-Peltatin A Methyl Ether (b)

has been derived from III by the same reaction.[1] The fact that IV and V are different compounds offers evidence that the original compounds I and III are not stereoisomers.

In the NMR spectrum of IV, the methylenedioxy group and one of the methoxy groups appeared at δ 6.06 and δ 3.47 respectively, suggesting that the position of the methylenedioxy group was at C-6 and C-7,[5] while that of the methoxy group was at C-5[6] since the signal was shifted to much higher magnetic field than that of the C-8 methoxy group of V (δ 4.20) by the anisotropic effect of the 4-phenyl group.

As regards the direction of the lactone carbonyl group of 2,3-naphthalide lignans, it was reported that, in the NMR spectra, the lactone methylene protons and C-1 proton should appear at δ 5.32—5.52 and δ 7.6—7.7, respectively, in type A but at δ 5.08—5.23 and δ 8.25 in type B.[7a,b]

In the NMR spectrum of IV, they appeared at δ 5.30 and δ 7.64, respectively, showing that IV belongs to type A.



I : R=H, R_1=OCH_3 (2-α-H, 3-β-H)
III : R=OCH_3, R_1=H (2-α-H, 3-β-H)
VI : R=H, R_1=OCH_3 (2-α-H, 3-α-H)

II : R, R_1=H
IV : R=H, R_1=OCH_3
V : R=OCH_3, R_1=H

Chart 1

It was reported by Klyne and Sakakibara[8a,b] that optical rotatory dispersion (ORD) and circular dichroism (CD) were applicable to determine the configuration of the 4-aryl group in 4-aryltetralin-type lignans. They clarified that all 4-α-aryltetralin lignans gave a positive first Cotton effect at 290—280 nm in their ORD and CD, whereas 4-β-aryl compounds gave a negative effect. As shown in Fig. 2, the authors measured the ORD and CD spectra of I, DPT and III and compared them with the reported data for retroresinolide.[8] Even though it was not marked in the ORD, a clear positive effect was observed in the CD suggesting that I had 4-α-aryl configuration.

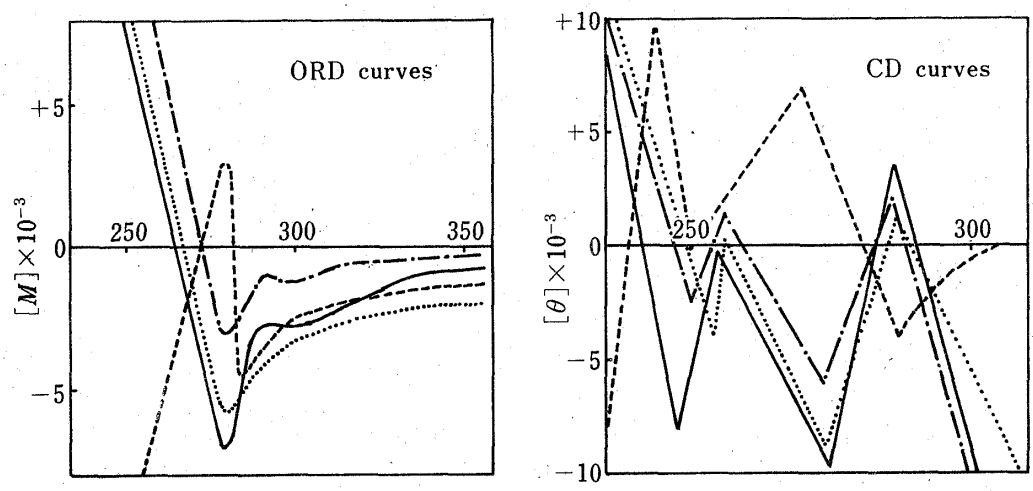


Fig. 2. ORD and CD Spectra of I, DPT, III and Retroresinolide
—·—·—: I, ——: DPT, ·····: III, ----: retroresinolide.

It is well known that 2,3-*trans* configuration of these lignans is converted to *cis* type by base treatment.[2,3c,9] By heating with sodium methoxide, I was readily converted to an isomer named picrohernandin (VI), mp 90—92°C, $[\alpha]_D$ +100°. The fact that the optical rotation changed to dextrorotatory suggests that the configuration at the 2 and 3 positions is *trans*.

On the other hand, the crystal structures of 5′-demethoxy-β-peltatin A methyl ether and 2′-bromopodophyllotoxin were clarified by X-ray analyses[10,11] and it was established that the configurations of these lignans were 2-α-H, 3-β-H and 4-α-aryl. In view of the above experimental results and the conclusions obtained from X-ray analyses of analogous lignans, the structure of hernandin is presumed to be 5-methoxydesoxypodophyllotoxin (I). To confirm this, the absolute configuration of I was determined by X-ray analysis.

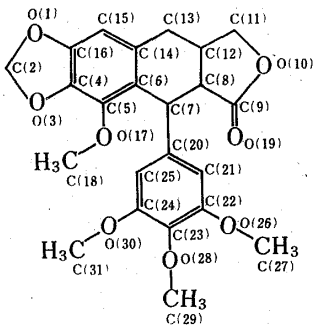


Fig. 3. Atomic Numbering of I

Colorless needle crystals of I were grown by slow evaporation of an ethanol solution at room temperature. Oscillation and Weissenberg photographs showed the space group to be $P2_12_12_1$. The density was measured by floatation in a mixture of water and saturated aqueous potassium iodide. A crystal, $0.4\times0.6\times0.2$ mm in size, was mounted on a Rigaku automated four-circle diffractometer. Graphite-monochromated CuK_α radiation was used. The unit-cell dimensions were determined by least-squares calculation with 2θ values of 25 high-angle reflections. The atomic numbering of I is shown in Fig. 3, and the crystallographic data are summarized in Table I.

Table I. Crystal Data

Chemical formula	$C_{23}H_{24}O_8$
Molecular weihgt	428.4
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Cell constant	
a/Å	8.519(2)
b/Å	13.125(3)
c/Å	18.497(4)
Volume/Å^3	2068.2 (9)
Z	4
D_m/gcm^{-3}	1.371(1)
D_x/gcm^{-3}	1.376
μ(Cu-K_α)/cm^{-1}	8.84
F(0 0 0)	904

Intensity data were collected on the diffractometer by using the $\omega-2\theta$ scanning mode and a scan rate of 4°/min. Stationary background counts of 5 s were taken at both limits of each scan. Four reference reflections were monitored periodically and showed no significant intensity deterioration. Corrections were made for Lorentz and polarization factors, but not for absorption effects. A total of 2024 unique reflections, of which 24 had no net intensities, were measured to the limit $2\theta=130°$.

The structure was solved by the direct method using the MULTAN program.[12] An E-map, calculated by using 310 reflections ($|E|\geqq1.39$) with the phase set of the highest combined figure of merit (2.788), revealed the locations of all the nonhydrogen atoms. The structure was refined by the block-diagonal least-squares method with anisotropic temperature factors. All the hydrogen atoms, found on a difference Fourier map, were included with anisotropic thermal factors. The quantity minimized was $\sum w(|F_o|-|F_c|)^2$. In the last refinements, the following weighting scheme was used: $w=0.47$ for $F_o=0.0$, $w=1.0$ for $0<F_o\leqq12.0$, and $w=1.0/[1.0+0.370\ (F_o-12.0)]$ for $F_o>12.0$. The final R value was 0.044. The final positional parameters with their estimated standard deviations are listed in Table II. The atomic scattering factors for all atoms were taken from the International Tables for X-ray Crystallography.[13] All numerical calculations were carried out at the Crystallographic Research Center, Institute for Protein Research, and the Computing Center of Osaka University using the UNICS programs.[14]

As a result, the presumed structure of I based on the chemical studies was confirmed by

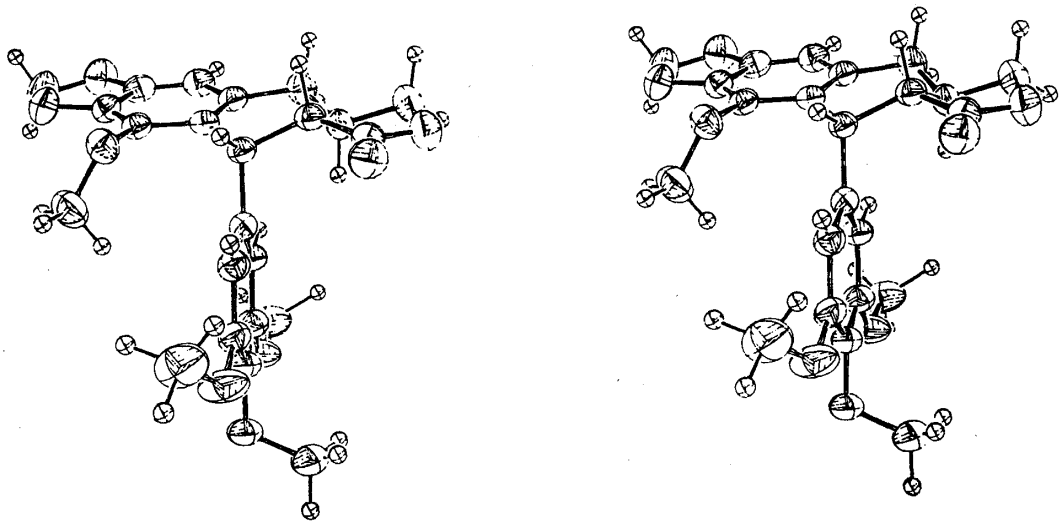


Fig. 4. ORTEP Drawing of I

TABLE II. The Final Atomic Coordinates ($\times 10^4$ for C, O Atoms, $\times 10^3$ for H Atoms) with Estimated Standard Deviations in Parentheses

Atom	x	y	z
O (1)	10046 (3)	4168 (2)	2690 (1)
C (2)	9496 (5)	3175 (3)	2474 (3)
O (3)	7818 (3)	3200 (2)	2478 (1)
C (4)	7425 (4)	4083 (2)	2836 (2)
C (5)	5981 (3)	4408 (2)	3055 (1)
C (6)	5861 (3)	5371 (2)	3397 (1)
C (7)	4253 (3)	5666 (2)	3687 (1)
C (8)	4251 (4)	6828 (2)	3756 (1)
C (9)	2852 (4)	7358 (2)	4087 (2)
O (10)	3361 (3)	8216 (2)	4415 (1)
C (11)	5058 (5)	8335 (2)	4311 (2)
C (12)	5607 (4)	7238 (2)	4185 (2)
C (13)	7122 (4)	7064 (2)	3777 (2)
C (14)	7200 (3)	5980 (2)	3474 (1)
C (15)	8676 (4)	5621 (2)	3251 (2)
C (16)	8744 (4)	4682 (3)	2951 (2)
O (17)	4636 (3)	3858 (2)	2923 (1)
C (18)	4474 (5)	2936 (3)	3328 (2)
O (19)	1487 (3)	7136 (2)	4071 (1)
C (20)	3853 (3)	5099 (2)	4388 (1)
C (21)	4969 (3)	4941 (2)	4927 (1)
C (22)	4531 (4)	4422 (2)	5554 (2)
C (23)	3015 (4)	4056 (2)	5644 (1)
C (24)	1940 (4)	4187 (2)	5091 (2)
C (25)	2347 (3)	4710 (2)	4466 (2)
O (26)	5523 (3)	4199 (2)	6116 (1)
C (27)	7032 (5)	4580 (4)	6117 (2)
O (28)	2629 (3)	3526 (2)	6257 (1)
C (29)	2243 (6)	4172 (3)	6847 (2)
O (30)	488 (3)	3772 (2)	5215 (1)
C (31)	−429 (5)	3506 (4)	4623 (2)
H (2a)	995 (6)	259 (3)	283 (2)
H (2b)	994 (6)	308 (4)	193 (3)
H (7)	339 (4)	548 (3)	333 (2)
H (8)	435 (5)	707 (3)	320 (2)
H (11a)	524 (4)	881 (3)	387 (2)
H (11b)	549 (5)	872 (3)	473 (2)
H (12)	568 (5)	683 (3)	472 (2)
H (13a)	803 (5)	723 (3)	413 (2)
H (13b)	723 (5)	757 (3)	333 (2)
H (15)	960 (4)	602 (3)	330 (2)
H (18a)	358 (6)	256 (3)	321 (2)
H (18b)	446 (6)	306 (3)	387 (2)
H (18c)	518 (7)	247 (4)	328 (3)
H (21)	603 (4)	526 (3)	486 (2)
H (25)	157 (4)	484 (3)	412 (2)
H (27a)	754 (5)	431 (4)	660 (2)
H (27b)	686 (7)	558 (4)	606 (3)
H (27c)	757 (5)	438 (4)	572 (2)
H (29a)	209 (5)	378 (3)	734 (2)
H (29b)	133 (5)	461 (4)	673 (2)
H (29c)	297 (6)	468 (4)	700 (3)
H (31a)	−136 (5)	306 (3)	476 (2)
H (31b)	16 (6)	297 (4)	426 (2)
H (31c)	−68 (7)	417 (4)	439 (3)

the X-ray crystallographic method. An ORTEP drawing of the molecule of I is shown in Fig. 4.

Lignan (II) was obtained by purification of the previously isolated minor substance, mp 270—275°C.[2] It shows mp 276—278°C and has the molecular formula $C_{22}H_{18}O_7$. It has no optical rotation and its ultraviolet (UV) spectrum suggests that this compound is a naphthalene-type lignan. All spectral data coincide well with those of 1,2,3,4-dehydrodesoxypodophyllotoxin[1] which was derived from DPT by reaction with DDQ. By direct comparison of their IR and NMR spectra and by mixed melting point determination, it was confirmed that the compounds were identical. Although II is a known compound, this is the first report of its natural occurrence in plants.

Experimental

All melting points are uncorrected. The instruments used in this study were as follows; IR spectra, Jasco IR-A-1, UV spectra, Hitachi 200-10; MS, Hitachi MU-6D; NMR spectra, Hitachi R-40 (using tetramethylsilane as an internal standard); optical rotation, Jasco DIP-181; ORD and CD, Hitachi ORD-CD UV-5.

Hernandin (5-Methoxy-desoxypodophyllotoxin) (I)——The ethanolic mother liquor of fract. 2[2] from which DPT had been separated was allowed to stand for several days. Floating light feathers were deposited in the upper layer, while the remaining DPT crystallized as heavy prisms at the bottom of the flask. The upper layer including light feathers was decanted off and the product was recrystallized from ethanol. Fine needles, mp 210—213°C, $[\alpha]_D^{24}$ −70° (c=0.7 in $CHCl_3$). *Anal.* Calcd for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.20; H, 5.62. MS *m/e* 428 (M^+, 100%), 260 (8.7%), 215 (6.8%), 203 (20%), 181 (23.5%), 168 (6%). UV λ_{max}^{EtOH} nm (ε): 204 (58344), 262 (58921), 318 (9743), 354 (4544). IR cm^{-1} (KBr): 1770 (C=O), 940 (–O–CH_2–O–). NMR ($CDCl_3$) δ ppm: 6.40 (1H, s, C_8–H), 6.35 (2H, s, $C_{2',6'}$–H), 5.90 (2H, s, –O–CH_2–O–), 4.84 (1H, d, J=10 Hz, C_4–H), 3.95—4.50 (2H, m, lactone CH_2), 3.78 (3H, s, $C_{4'}$–OCH_3), 3.73 (6H, s, $C_{3',5'}$–OCH_3), 3.60 (3H, s, C_5–OCH_3), 2.50—3.30 (4H, m, $C_{1,2,3}$–H). ORD (c=0.07632, EtOH) $[M]^{29}$ (nm): −1050 (291) (trough); −2999 (278) (peak); +7900 (246) (trough). CD (c=0.16944, EtOH) $[\theta]^{27}$ (nm): +2083 (286); −6670 (274), +1250 (256); −2500 (250).

Dehydrohernandin (IV)——DDQ (450 mg, 2 mmol) was added to a dry benzene (40 ml) solution of 428 mg (1 mmol) of I, and the mixture was refluxed for 18 h, then cooled. The precipitate was filtered off, and the benzene solution was concentrated *in vacuo*. The residue was chromatographed on a silica gel column with $CHCl_3$–Me_2CO (10: 1). The eluant was recrystallized from 35% EtOH. mp 310—313°C. $[\alpha]_D^{24}$ ±0° (c=0.26 in $CHCl_3$). *Anal.* Calcd for $C_{23}H_{20}O_8$: C, 65.09; H, 4.75. Found: C, 64.82; H, 4.79. IR cm^{-1} (KBr): 1770 (C=O), 940 (–O–CH_2–O–). UV λ_{max}^{EtOH} nm (ε): 204 (58343), 223 (30113), 262 (58921), 317 (9743), 354 (4544). NMR ($CDCl_3$) δ ppm: 7.64 (1H, s, C_1–H), 6.98 (1H, s, C_8–H), 6.47 (2H, s, $C_{2',6'}$–H), 6.06 (2H, s, –O–CH_2–O–), 5.30 (2H, s, lactone CH_2), 3.92 (3H, s, $C_{4'}$–OCH_3), 3.80 (6H, s, $C_{3',5'}$–OCH_3), 3.47 (3H, s, C_5–OCH_3).

Picrohernandin (VI)——Sodium methoxide (3 ml of MeOH and 100 mg of Na) was added to a solution of 30 mg of I and the mixture was refluxed for 45 min. After the reaction water was added and acidified with conc. HCl. The resulting crystals (24 mg, 80%) were filtered off and recrystallized from MeOH. mp 90—92°C. $[\alpha]_D^{20}$ +100° (c=0.45 in $CHCl_3$). *Anal.* Calcd for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.20; H, 5.62. IR cm^{-1} (KBr): 1770 (C=O), 935 (–O–CH_2–O–). NMR ($CDCl_3$) δ ppm: 6.40 (1H, s, C_8–H), 6.34 (2H, s, $C_{2',6'}$–H), 5.92 (2H, s, –O–CH_2–O–), 5.02 (1H, m, C_4–H), 3.90—4.60 (2H, m, lactone CH_2), 3.90 (3H, s, C_5–OCH_3), 3.80 (3H, s, $C_{4'}$–OCH_3), 3.76 (6H, s, $C_{3',5'}$–OCH_3), 3.46 (1H, m, C_3–H), 2.9—3.30 (1H, m, C_2–H), 2.25—3.00 (2H, m, C_1–H).

1,2,3,4-Dehydrodesoxypodophyllotoxin (II)——The crude substance, mp 270—275°C, obtained from fraction-5[2] was recrystallized from EtOH. mp 276—278°C. $[\alpha]_D^{24}$ ±0° (c=0.5 in $CHCl_3$). *Anal.* Calcd for $C_{22}H_{18}O_7$: C, 67.00; H, 4.60. Found: C, 67.12; H, 4.45. It was identified with an authentic sample[1] on the basis of mixed melting point determination and comparisons of UV, IR and NMR spectra.

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