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Studies on the Constituents of the Seeds of *Hernandia ovigera* L. V.[1] Syntheses of Epipodophyllotoxin and Podophyllotoxin from Desoxypodophyllotoxin

HIDEO YAMAGUCHI,* MASAO ARIMOTO, SYUNJI NAKAJIMA, MARIKO TANOGUCHI and YOSHINARI FUKADA

Osaka College of Pharmacy, Kawai 2–10–65, Matsubara, Osaka 580, Japan

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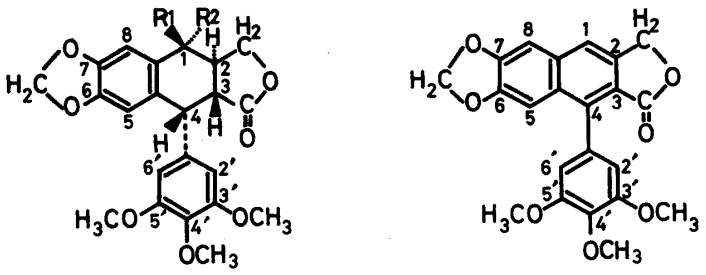
Epipodophyllotoxin (EPT) and podophyllotoxin (PT) were prepared from desoxypodophyllotoxin (DPT), obtained from the seeds of *Hernandia ovigera* L. (Hernandiaceae). EPT was obtained together with dehydrodesoxypodophyllotoxin (I) by a radical bromination of DPT with *N*-bromosuccinimide (NBS) followed by hydrolysis of the resultant 1-bromo-DPT, which was confirmed to be a mixture of 1α- and 1β-bromo compounds (7:3). EPT was oxidized with pyridinium chlorochromate to give podophyllotoxone (IV). The stereoselective reduction of IV to afford PT was examined with a variety of reagents, and borane–*tert*-butylamine complex was found to be effective.

Keywords——*Hernandia ovigera*; desoxypodophyllotoxin; dehydrodesoxypodophyllotoxin; podophyllotoxin; epipodophyllotoxin; 1-bromo-desoxypodophyllotoxin; pyridinium chlorochromate; borane–*tert*-butylamine complex

The preparation of 1-halo compounds of podophyllotoxin (PT) and analogous lignans has been reported by several workers.[2] They obtained predominantly 1β-bromo or -chloro compounds from 1α-hydroxy lignans such as PT[2a,b] and demethylene-PT dimethyl ether.[2c] These 1β-halo compounds are easily hydrolyzed to afford 1β-hydroxy compounds. On the other hand, no report has appeared on the preparation of 1-halo compounds from desoxypodophyllotoxin (DPT). In the present work, we aimed at developing syntheses of PT and epipodophyllotoxin (EPT) from DPT *via* its 1-bromo derivative, which could be obtained by a radical bromination. Treatment of DPT with *N*-bromosuccinimide (NBS) in the presence of benzoyl peroxide (BPO), followed by silica gel chromatography, gave two compounds which contained no bromine. These were identified as EPT and dehydrodesoxypodophyllotoxin (I)[3] by direct comparison with authentic samples in nuclear magnetic resonance (NMR) and infrared (IR) spectra and mixed melting point determination. The use of excess NBS did not improve the yield of EPT, but afforded 2′- and 8-bromo-I. We presumed that EPT was produced by the hydrolysis of the 1-bromo derivative of DPT during silica gel chromatography. The same result was reported in the reaction of the benzyl butyrolactone compound with NBS followed by hydrolysis on silica gel to give the hydroxy compound.[4] In order to isolate the bromo compound, silic AR (Mallinckrodt Works) was used in the chromatography of the reaction product to give a colorless solid containing bromine. The mass spectrum (MS) of this solid showed molecular ion peaks at *m/z* 476 and 478, corresponding to $C_{22}H_{21}BrO_7$. The NMR spectrum indicated that this substance was a 7:3 mixture of 1β-bromo-DPT (II) and 1α-bromo-DPT (III) based on the following findings. Signals were observed at 6.90, 7.18 (singlet) (C_8-H), 6.47, 6.49 (singlet) (C_5-H), 6.27, 6.40 (singlet) ($C_{2',6'}$-H), and 5.28 ($J=10.26$ Hz), 5.62 ($J=3.42$ Hz) (doublet) (C_1-H). The ratio of II and III was determined from the integrated intensity of C_1-proton in the NMR spectrum. The low yield of III can be



PT : R1=H, R2=OH
EPT : R1=OH, R2=H
I : R1=Br, R2=H
II : R1=H, R2=Br
IV : R1,R2=O

I

Fig. 1[13]

TABLE I. Stereoselective Reduction of Podophyllotoxone (IV) with Various Reducing Agents

Reducing agent[a]	Conditions			Ratio of products[b]	
	Time (h)	Solvent	Temp. (°C)	PT : EPT	(% yield)
$Zn(BH_4)_2$	3.5	Et_2O–benzene (4 : 1)	r.t.	7.5 : 1	(96)
$NH_3 \cdot BH_3$	1	Et_2O	r.t.	7 : 1	(95)
tert-$BuNH_2 \cdot BH_3$	3.5	Et_2O	r.t.	6 : 1	(91)
$[(CH_3)_2CH]_2NH \cdot BH_3$	2.5	Et_2O	r.t.	2.5 : 1	(10)
Borane-2,6-lutidine	45	Et_2O	r.t.	2.6 : 1	(3)
$BH_3 \cdot THF$	17	THF	−75—10	1 : 2	(16)
$NaBH_4$	17.5	Et_2O	r.t.	4 : 1	(19)
$NaAlH_2(OCH_2CH_2OCH_3)_2$	20	Et_2O	−75—10	32 : 1	(59)
$LiAlH_2$ (*tert*-BuO)$_3$	1.5	Et_2O	−75	20 : 1	(79)

a) Molar ratio of the reducing agent to IV was 10 : 1. *b*) Determined from the integrated intensity in the NMR (300 MHz) spectrum. r.t.: room temperature. THF: tetrahydrofuran.

ascribed to the steric hindrance of the C_4-α-phenyl group on the C_1-α-hydrogen.

Recrystallization of the above mixture from hexane–ether–benzene afforded a substance, $C_{22}H_{21}BrO_7$, mp 151—154 °C, $[\alpha]_D$ +11.4° ($CHCl_3$) which was identical with authentic II prepared from PT.[2a] However, an attempt to isolate III as crystals was unsuccessful. Treatment of the mixture with aqueous acetone or with silica gel in hexane–ethyl acetate (1 : 1) gave EPT as a sole product. When the mixture was refluxed in methanol, only EPT methyl ether was obtained. These results suggest that the substitution of secondary bromine at C_1 with a hydroxy or methoxy group proceeds by an S_N1 reaction mechanism, and the nucleophile attacks from the non-hindered β-side.[2c]

Since it was difficult to obtain PT from the 1-bromo compound, we examined another route to PT from EPT *via* podophyllotoxone (IV).[5] Although IV was obtained from PT in fairly good yield by oxidation with manganese dioxide,[5] this reagent gave unsatisfactory or negative results with isopodophyllotoxin[6] and epiisopodophyllotoxin.[7] We also had a negative result with EPT, recovering the starting material. Another reagent, pyridinium chlorochromate,[8] was found to be effective, affording IV in 76.2% yield from EPT.

As a reagent for the reduction of these keto-lignans with retention of their lactone functions, zinc borohydride was recommended by Gensler *et al.*[5] For example, PT and DL-epiisopodophyllotoxin were obtained in good yield from the corresponding keto-lignans.[5,6] However, good results were unobtainable with picropodophyllone and L-isopodophyllotoxone.[5,7] We tried to reduce IV with a variety of reagents and examined the ratio of

PT and EPT in the resulting products. The results are summarized in Table I.

Sodium bis-(2-methoxyethoxy)aluminum hydride[9] and lithium tri-*tert*-butoxyaluminum hydride[10] showed excellent stereoselectivity for PT compared with other reagents. However, these reagents have the disadvantage that low temperature (−75°C) is required to obtain satisfactory yield. Although the stereoselectivity was inferior to that of the above-mentioned aluminum hydride complexes, borane–*tert*-butylamine complex[11] and borane ammonia complex[12] gave the products in more than 90% yield. The reactions proceeded at room temperature, and borane–*tert*-butylamine complex did not require the use of a nitrogen stream.

In conclusion, two kinds of 1-hydroxyphenyltetralin-type lignans, EPT and PT, were derived from DPT, which is available in large quantities from the seeds of *Hernandia ovigera* L.

Experimental

All melting points are uncorrected. The instruments used in this study were as follows; IR spectra, Jasco IR-A-1; MS, Hitachi MU-6D; ^{1}H-NMR spectra, JEOL JNM-GX-400, Varian XL-300, Hitachi R-40 (90 MHz) with tetramethylsilane as an internal standard; optical rotation, Jasco DIP-181. Precoated silica gel plates used in preparative thin layer chromatography (PTLC) were Merck Kiesel gel 60-F_{254}, 0.5 mm thickness. Columns for ordinary column chromatography were prepared with silica gel (Merck Kiesel gel 60).

Bromination of DPT——A: A mixture of DPT (1.5 g), NBS (1 eq, 0.67 g) and BPO (0.01 g) in CCl_4 (100 ml) was refluxed for 2 h under a stream of dry N_2. After cooling, the reaction mixture was poured into water and extracted with $CHCl_3$. The extract was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the residue was stirred with silica gel (2 g) in hexane–AcOEt (1 : 1) (100 ml) for 3 h at room temperature. The silica gel was filtered off, and the solution was evaporated *in vacuo* to leave a colorless solid. This was fractionated by silica gel column chromatography with hexane–AcOEt (1 : 1). The first eluate, upon recrystallization from EtOH, gave I, 340 mg (23%), needles, mp 273—275°C, (lit.[3] mp 271—272°C). $[\alpha]_D \pm 0°$ ($c=0.5$, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{18}O_7$: C, 67.00; H, 4.60. Found: C, 66.76; H, 4.75. UV_{max}^{MeOH} nm (ε): 205 (49200), 256 (62500), 310 (10000), 350 (4900). IR cm^{-1} ($CHCl_3$): 1765 (C=O), 940 (–OCH_2O–). NMR ($CDCl_3$) δ ppm: 7.69 (1H, s, C_1-H), 7.19 (1H, s, C_8-H), 7.11 (1H, s, C_5-H), 6.54 (2H, s, $C_{2',6'}$-H), 6.07 (2H, s, –OCH_2O–), 5.37 (2H, s, lactone-CH_2), 3.96 (3H, s, $C_{4'}$-OCH_3), 3.83 (6H, s, $C_{3',5'}$-OCH_3). The second eluate, upon recrystallization from Et_2O–hexane, gave EPT, 827 mg (53%). mp 181—183 °C, mp 160—162 °C from EtOH–H_2O, (lit.[2a] 158.4—161.2 °C from EtOH–H_2O), $[\alpha]_D^{25} -69°$ ($c=0.5$, $CHCl_3$). MS *m/z*: 414(M^+). *Anal.* Calcd for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35. Found: C, 63.63; H, 5.35. IR cm^{-1} ($CHCl_3$): 3450 (OH), 1780 (C=O), 940 (–OCH_2O–). NMR ($CDCl_3$) δ ppm: 6.88 (1H, s, C_8-H), 6.55 (1H, s, C_5-H), 6.28 (2H, s, $C_{2',6'}$-H), 5.99 (2H, dd, $J=6$, 1 Hz, –OCH_2O–), 4.87 (1H, d, $J=3.5$ Hz, C_1-H), 4.62 (1H, d, $J=5.5$ Hz, C_4-H), 4.32—4.43 (2H, m, lactone-CH_2), 3.81 (3H, s, $C_{4'}$-OCH_3), 3.75 (6H, s, $C_{3',5'}$-OCH_3), 3.28 (1H, dd, $J=14$, 5 Hz, C_3-H), 2.76—2.92 (1H, m, C_2-H), 2.15 (1H, br s, C_1-OH, disappeared on addition of D_2O). Two by-products were obtained with excess NBS.

i) 2′-Bromo-I: Recrystallized from EtOH. mp 255—257 °C, $[\alpha]_D \pm 0°$ ($c=0.5$, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{17}BrO_7$: C, 55.83; H, 3.62. Found: C, 55.98; H, 3.48. UV_{max}^{MeOH} nm (ε): 207 (51261), 250 (59664), 257 (62185), 310 (10084), 330 (5462), 350 (5882). MS *m/z*: 472, 474 (M^+). IR cm^{-1} ($CHCl_3$): 1770 (C=O), 930 (–OCH_2O–). NMR ($CDCl_3$) δ ppm: 7.73 (1H, s, C_1-H), 7.21 (1H, s, C_8-H), 6.85 (1H, s, C_5-H), 6.59 (1H, s, $C_{6'}$-H), 6.08 (2H, s, –OCH_2O–), 5.40 (2H, s, lactone-CH_2), 4.00 (3H, s, $C_{3'}$-OCH_3), 3.97 (3H, s, $C_{5'}$-OCH_3), 3.80 (3H, s, $C_{4'}$-OCH_3).

ii) 8-Bromo-I: Recrystallized from EtOH. mp 296—297 °C, $[\alpha]_D \pm 0°$ ($c=0.5$, $CHCl_3$). *Anal.* Calcd for $C_{22}H_{17}BrO_7$: C, 55.83; H, 3.62. Found: C, 55.70; H, 3.66. UV_{max}^{MeOH} nm (ε): 205 (40976), 260 (59024), 315 (11707), 352 (5854). MS *m/z*: 472, 474 (M^+). IR cm^{-1} ($CHCl_3$): 1770 (C=O), 940 (–OCH_2O–). NMR ($CDCl_3$) δ ppm: 7.63 (1H, s, C_1-H), 7.10 (1H, s, C_5-H), 6.50 (2H, s, $C_{2',6'}$-H), 6.10 (2H, s, –OCH_2O–), 5.26 (2H, s, lactone-CH_2), 3.94 (3H, s, $C_{4'}$-OCH_3), 3.82 (6H, s, $C_{3',5'}$-OCH_3).

B: Bromination was carried out as described above using 1 eq of NBS. The crude bromination product was immediately applied to a column of silic AR (100—200 Mesh Type 60 Å Special, Mallinckrodt, Inc.) and eluted with hexane–AcOEt (1 : 1). The first eluate was found by NMR spectroscopy to be a mixture of 1α- and 1β-bromo compounds which was recrystallized from hexane–ether–benzene to afford II as colorless needles, mp 151—154 °C, $[\alpha]_D +11.4°$ ($c=0.5$, $CHCl_3$) (lit.[2a] mp 154 °C, $[\alpha]_D +15.8°$ ($c=1$, ethanol-free $CHCl_3$)). *Anal.* Calcd for $C_{22}H_{21}BrO_7$: C, 55.36; H, 4.44. Found: C, 55.74, H, 4.30. MS *m/z*: 476, 478 (M^+). IR cm^{-1} ($CHCl_3$): 1780 (C=O), 940 (–OCH_2O–). NMR ($CDCl_3$) δ ppm: 6.90 (1H, s, C_8-H), 6.47 (1H, s, C_5-H), 6.27 (2H, s, $C_{2',6'}$-H), 5.98 (2H, d, $J=6$ Hz, –OCH_2O–), 5.62 (1H, d, $J=3.5$ Hz, C_1-H), 4.69 (1H, d, $J=5$ Hz, C_4-H), 4.32—4.48 (2H, m, lactone-CH_2), 3.81 (3H, s, $C_{4'}$-OCH_3), 3.75 (6H, s, $C_{3',5'}$-OCH_3), 3.39 (1H, dd, $J=15$, 6 Hz, C_3-H), 2.80—2.92 (1H, m, C_2-H). On

stirring II with silica gel in aqueous acetone or hexane–AcOEt for 2 h at room temperature, EPT was obtained in quantitative yield.

EPT *O*-Methyl Ether——A 7 : 3 mixture of II and III obtained by chromatography of the bromination products was refluxed in MeOH for 2 h. The MeOH was evaporated off, and the residue was recrystallized from EtOH to afford EPT *O*-methyl ether in quantitative yield. Needles, mp 205—207 °C. $[\alpha]_D^{25}$ −97.8 ° (c=0.5, $CHCl_3$). (lit.[2c] mp 203—204 °C, $[\alpha]_D$ −96 ° ($CHCl_3$)). *Anal.* Calcd for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.50; H, 5.37. MS m/z: 428 (M^+). IR cm^{-1} ($CHCl_3$): 1780 (C=O), 940 (–OCH_2O–). NMR ($CDCl_3$) δ ppm: 6.82 (1H, s, C_8-H), 6.55 (1H, s, C_5-H), 6.25 (2H, s, $C_{2',6'}$-H), 5.97 (2H, m, –OCH_2O–), 4.60 (1H, d, J=6.7 Hz, C_1-H), 4.00—4.50 (3H, m, C_4-H, lactone-CH_2), 3.80 (3H, s, $C_{4'}$-OCH_3), 3.73 (6H, s, $C_{3',5'}$-OCH_3), 3.46 (3H, s, C_1-OCH_3), 2.60—3.40 (2H, m, $C_{2,3}$-H).

Podophyllotoxone (IV)——A solution of EPT (1.227 g, 2.96 mmol) in dry CH_2Cl_2 (5 ml) was added to a suspension of pyridinium chlorochromate (989.8 mg, 4.59 mmol) in dry CH_2Cl_2 (5 ml) under N_2. The reaction mixture was stirred at room temperature for 6 h, anhydrous Et_2O was added, and the solvent layer was separated from the gummy residue by decantation. The precipitate was extracted several times with Et_2O. The combined solvent layer was concentrated and purified by passing it through a short column (13 g) of Florisil (Floridin Co.). After the removal of the solvent, the residue was recrystallized from EtOH to give IV as colorless needles, yield 929.4 mg (76.2%). mp 187—189 °C, $[\alpha]_D^{25}$ −123 ° (c=0.56, $CHCl_3$). (lit.[5] mp 190—191.5 °C, $[\alpha]_D$ −125 °). MS m/z 412 (M^+). *Anal.* Calcd for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found: C, 64.12; H, 5.16. IR cm^{-1} ($CHCl_3$): 1790 (C=O), 1700 (C=O), 940 (–OCH_2O–). NMR ($CDCl_3$) δ ppm: 7.55 (1H, s, C_8-H), 6.69 (1H, s, C_5-H), 6.38 (2H, s, $C_{2',6'}$-H), 6.08 (2H, dd, J=6, 1 Hz, –OCH_2O–), 4.84 (1H, d, J=4 Hz, C_4-H), 4.57, 4.35 (each 1H, dd, J=9, 7 Hz and J=10, 9 Hz, lactone-CH_2), 3.82 (3H, s, $C_{4'}$-OCH_3), 3.76 (6H, s, $C_{3',5'}$-OCH_3), 3.43—3.57 (1H, m, C_2-H), 3.27 (1H, dd, J=16, 4 Hz, C_3-H).

Reduction of IV——The results with a variety of reagents are summarized in Table I. In every case the product was examined by NMR spectroscopy to determine the ratio of EPT and PT prior to the isolation of each component by column chromatography or PTLC. Reduction with $Zn(BH_4)_2$ was done by the method of Gensler *et al.*[5] The general procedure for reduction is as follows: reagent was added to a stirred solution of IV in the organic solvent and under the reaction conditions described in Table I. When the reaction was completed, the mixture was treated with dil. HCl. The solvent layer was separated and the aqueous layer was extracted with $CHCl_3$. The combined extract was washed with brine and dried over anhydrous $MgSO_4$. After the removal of the solvent *in vacuo*, the residue was purified on a silica gel column or by PTLC with $CHCl_3$–acetone (10 : 1). EPT was obtained from the first eluate and PT was isolated from the second eluate.

EPT: mp 158—161 °C (from EtOH–H_2O), $[\alpha]_D$ −72 ° ($CHCl_3$). Identified by direct comparison with an authentic sample.

PT: mp 183—184 °C (from EtOH), $[\alpha]_D$ −127 ° (c=0.43, $CHCl_3$). (lit.[2a] mp 183—184 °C, $[\alpha]_D$ −132 ° (c=1, $CHCl_3$)). MS m/z: 414 (M^+). *Anal.* Calcd for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35. Found: C, 63.76; H, 5.11. IR cm^{-1} ($CHCl_3$): 3450 (OH), 1785 (C=O), 940 (–OCH_2O–). NMR ($CDCl_3$) δ ppm: 7.12 (1H, s, C_8-H), 6.51 (1H, s, C_5-H), 6.37 (2H, s, $C_{2',6'}$-H), 5.97 (2H, dd, J=6, 1 Hz, –OCH_2O–), 4.77 (1H, m, C_1-H), 4.59 (2H, m, C_4-H and lactone 1H), 4.07 (1H, dd, J=10, 1 Hz, another lactone 1H), 3.82 (3H, s, $C_{4'}$-OCH_3), 3.76 (6H, s, $C_{3',5'}$-OCH_3), 2.70—2.88 (2H, m, $C_{2,3}$-H), 2.24 (1H, d, J=6 Hz, C_1-OH, disappeared with D_2O).

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