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SYNTHESIS OF STEREOSPECIFICALLY DEUTERATED DESOXYPODOPHYLLOTOXINS AND ^{1}H-NMR ASSIGNMENT OF DESOXYPODOPHYLLOTOXIN

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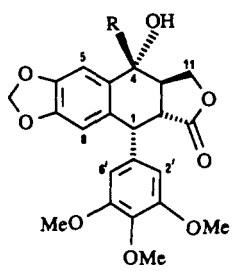
ABSTRACT.—[4β-^{2}H$_1$]Desoxypodophyllotoxin [**3**], [4α-^{2}H$_1$]desoxypodophyllotoxin [**4**], and [4,4-^{2}H$_2$]desoxypodophyllotoxin [**9**] were prepared from podophyllotoxin [**1**] via its chloride **5**. A complete assignment of the ^{1}H-nmr spectrum of desoxypodophyllotoxin [**2**] was made on the basis of the spectra of the deuterated compounds **3** and **4**.

The lignan podophyllotoxin [**1**] and its desoxy derivative desoxypodophyllotoxin [**2**] are potent cytotoxic compounds that occupy an important role in the development of natural products research, since work on these compounds at the National Cancer Institute gave some of the earliest indications of the potential of natural products as anticancer agents (1–3). Podophyllotoxin occurs in significant quantities in podophyllin, which is an extract of the dried roots and rhizomes of *Podophyllum peltatum* L. Desoxypodophyllotoxin occurs in small amounts in podophyllin (4), but it also occurs quite widely in other species and has been isolated, for example, as the major cytotoxic principle of *Austrocedrus chilensis* (D. Don) Florin and Boutelje (5), *Juniperus phoenicea* L. (6), and *Hyptis tomentosa* Poit. (7). The 4′-demethylepipodophyllotoxin derivatives,



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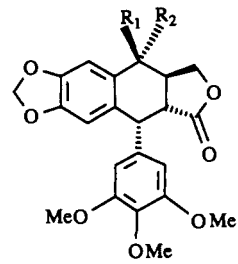
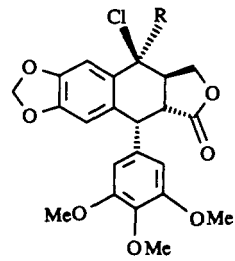
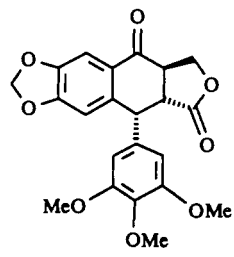
7 R=D



2 R_1=R_2=H
3 R_1=D, R_2=H
4 R_1=H, R_2=D
9 R_1=R_2=D



8 R=D
5 R=H



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etoposide and teniposide, have found application as clinically important anticancer drugs with activity against small cell lung cancer and other tumors (8).

The biosynthesis of podophyllotoxin has been investigated by Dewick and his collaborators (9–11), and they have reported that the formation of podophyllotoxin occurs through desoxypodophyllotoxin, with hydroxylation occurring stereospecifically at the 4 position (10). The stereochemistry of this hydroxylation process, in terms of whether it proceeded with retention or inversion of configuration at C-4, was, however, not determined.

The stereochemistry of enzymatic hydroxylation reactions is a subject of current interest to us, as exemplified by recent studies on the biosynthesis of virginiamycin S_1 (12) and virginiamycin M_1 (13), and we thus elected to begin a study of the stereochemistry of the hydroxylation of desoxypodophyllotoxin [**2**] to podophyllotoxin [**1**]. A necessary preliminary to such a study is the preparation of stereospecifically labeled precursors, and this paper reports the synthesis of stereospecifically deuterated desoxypodophyllotoxin and the complete assignment of the ^{1}H-nmr spectrum of desoxypodophyllotoxin.

RESULTS AND DISCUSSION

To investigate the stereospecificity of the conversion of **2** into **1** we require the labeled desoxypodophyllotoxins **3** and **4**; the stereochemistry of the conversion could then be monitored by mass spectrometric analysis of the resulting product.

This experiment requires that the reversible conversion of podophyllotoxin to its oxidation product podophyllotoxone be slow relative to the formation of podophyllotoxin. Published data (10) and the probability of a significant isotope effect suggest that this will be the case.

One attractive route to the preparation of labeled desoxypodophyllotoxin **3** was by catalytic hydrogenolysis of podophyllotoxin using deuterium in place of hydrogen, inasmuch as the conversion of **1** to **2** occurs readily by this route (2). However, catalytic deuteration of podophyllotoxin (D_2/MeCOOD/Pd/C) yielded a desoxypodophyllotoxin in which aromatic ring deuteration had occurred in addition to the anticipated deuteration at C-4, making this route unsuitable for our purposes.

We thus turned to reduction of a suitable podophyllotoxin derivative with a complex hydride. Our initial venture was to make the 4-tosylate (or 4-mesylate), because both podophyllotoxin and 4-*epi*-podophyllotoxin are available and thus both stereoisomers could in principle be made in a stereochemically unambiguous manner. However, the 4-sulfonates proved to be too labile for our purposes and could not be prepared and characterized. We thus selected the previously prepared 4-chloroderivative.

4-Chlorodesoxypodophyllotoxin [**5**] has been prepared by chlorination of podophyllotoxin with various chlorinating reagents (1), and the corresponding 4-bromo analogue has been prepared from both podophyllotoxin and 4-*epi*-podophyllotoxin (1). The stereochemistry of **5** has been established as 4β by ^{1}H-nmr studies on **5** itself (14) and its 4′-demethyl derivative (15). These studies suggest that the pathway to the 4-halodesoxypodophyllotoxins proceeds via an ionic route with preferential attack of halide ion from the less hindered β face of the molecule (14) (Figure 1). It was thus not entirely surprising to find that reduction of 4-chlorodesoxypodophyllotoxin [**5**] with sodium cyanoborodeuteride (16) in THF yielded [4β-^{2}H$_1$]desoxypodophyllotoxin [**3**] in 70% yield from podophyllotoxin and 97% isotopic enrichment. Isotopic enrichment was determined by ms (Table 1). Analysis of **3** by ^{1}H nmr, discussed below, established its stereochemistry and showed that the 4β isomer predominated over the 4α isomer by a factor of greater than 20:1. A similar nucleophilic substitution of an analogue of **5** with retention of configuration at the 4 position is found in the

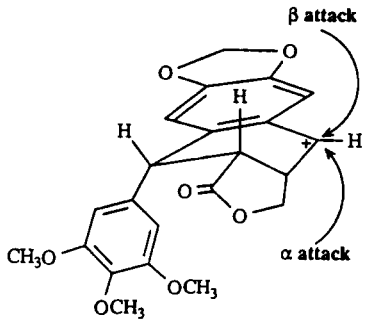

FIGURE 1. Stereoview of the carbocation intermediate in reactions of podophyllotoxin showing preferential attack from the β-face.

methanolysis of a 4-chlorodesoxypodophyllotoxin derivative to the corresponding methyl ether (14).

Synthesis of the 4α isomer **4** was achieved by reduction of [4α-$^2H_1$]4β-chlorodesoxypodophyllotoxin with sodium cyanoborohydride. To do this, podophyllotoxin [**1**] was first oxidized with MnO_2 to podophyllotoxone [**6**] and then reduced with zinc borodeuteride (17) to afford [4β-$^2H_1$]podophyllotoxin [**7**]. Subsequent chlorination with phosphorous trichloride gave the chloroderivative **8**, and reductive displacement with sodium cyanoborohydride gave [4α-$^2H_1$]desoxypodophyllotoxin [**4**] in 37% yield from podophyllotoxin and in 95% isotopic enrichment (Table 1). Again, ^{1}H-nmr analysis indicated the product to be the 4α isomer with no more than a few percent of the 4β isomer present.

A final preparation of [4,4-$^2H_2$]desoxypodophyllotoxin [**9**] was accomplished by reduction of the chloro compound **8** with sodium cyanoborodeuteride; the product had an isotopic enrichment of 94.5% $^2H_2$ and 5.5% $^2H_1$ (Table 1).

The ^{1}H-nmr spectra of podophyllotoxin and desoxypodophyllotoxin have been published on several occasions (14,15, 18–21), but a complete assignment of the spec-

TABLE 1. Isotopic Enrichment of Desoxypodophyllotoxins.

Compound	*m/z*	Observed Intensity	Corrected Intensity[a]	Corresponding Species
2	398	100.0	100.0	$^2H_0$
	399	24.8	0.0	
	400	4.3	0.0	
3	398	3.0	2.9	$^2H_0$
	399	100.0	97.1	$^2H_1$
	400	24.1	0.0	
	401	4.6	0.0	
4	398	4.5	4.3	$^2H_0$
	399	100.0	95.4	$^2H_1$
	400	25.2	0.0	
	401	4.3	0.0	
8	399	5.7	5.5	$^2H_1$
	400	100.0	94.5	$^2H_2$
	401	24.5	0.0	
	402	4.2	0.0	

[a]Corrected for the contributions to peak intensities from natural abundance ^{13}C and ^{18}O isotopes.

trum of desoxypodophyllotoxin has not previously been made because of the problems of overlapping resonances. This problem is particularly acute in $CDCl_3$ as solvent, because the H-2, H-3, and one of the H-4 protons of desoxypodophyllotoxin all resonate at about 2.75 ppm in this solvent (20). C_6H_6 forms stable inclusion compounds with the aryltetrahydronaphthalene lignans, however (2), and the use of C_6D_6 as a solvent provided a very much improved spectrum in which resonances of all the protons of desoxypodophyllotoxin could be observed.

Chemical shift assignments of all the protons except the H-2, H-3, and H-4 protons were made on the basis of normal chemical shift theory and by comparison with published spectra, and they agree with those previously published (18–21). The assignments for the 4α and 4β protons were made from the spectra of the deuterium-labeled compounds **3**, **4**, and **9**, and the assignments of H-2 and H-3 followed directly from analysis of coupling constants and specific proton decoupling measurements.

In the [4β-$^2H_1$] derivative **3**, the signal for H-4β at 1.88 ppm was missing, and the signal for H-4α appeared as a doublet at 2.18 ppm with $J = 5.3$ Hz. In the [4α-$^2H_1$] derivative **4**, the signal of H-4α was missing, and H-4β appeared as a doublet at 1.89 ppm with $J = 11.4$ Hz. The assignments of H-4α and H-4β were made on the basis of these coupling constants. Inspection of a Dreiding model indicates that H-4β is almost trans-diaxial to H-3 and thus should have a large coupling constant (in the range 8.5–16 Hz) according to the Karplus equation (22). H-4α has a dihedral angle with H-3 of approximately 45° and would thus have a coupling constant approximately half that of H-4β, consistent with the observed value of 5.3 Hz.

The remaining assignments were made on the basis of chemical shift and coupling constant data; specific proton-proton decouplings of the H-1/H-2 and the H-3/H-11α, H-11β pairs were carried out. Unfortunately the H-3/H-11α and H-3/H-11β dihedral angles are very similar, and this is reflected in the similarity of the coupling constants of 7.7 and 8.5 Hz. It was thus not possible to assign the individual H-11 protons, but we have arbitrarily assigned the upfield signal to H-11β to ensure consistency with the previous arbitrary assignment by Ayres *et al.* (19). The complete chemical shift and coupling constant data for desoxypodophyllotoxin [**2**] are given in Table 2.

TABLE 2. ^{1}H-nmr Spectral Assignments for Desoxypodophyllotoxin.[a]

Proton	Chemical shift (ppm)	Multiplicity	Coupling Constant (Hz)
H-1	4.41	d	$J_{1,2} = 4.8$
H-2	1.96	dd	$J_{2,1} = 4.8$; $J_{2,3} = 13.6$
H-3	2.31	m	$J_{3,2} = 13.6$; $J_{3,4\alpha} = 5.3$; $J_{3,4\beta} = 11.4$; $J_{3,11\alpha} = 7.7$; $J_{3,11\beta} = 8.5$
H-4α	2.20	dd	$J_{4\alpha,3} = 5.3$; $J_{4\alpha,4\beta} = 15.9$
H-4β	1.88	dd	$J_{4\beta,3} = 11.4$; $J_{4\alpha,4\beta} = 15.9$
H-5	6.47	s	
OCH_2O	5.33	dd	
H-8	6.42	s	
H-11α	3.65[b]	dd	$J_{11\alpha,3} = 7.7$; $J_{11\alpha,11\beta} = 10.6$
H-11β	3.06[b]	dd	$J_{11\beta,3} = 8.5$; $J_{11\alpha,11\beta} = 10.6$
H-2′, -6′	6.57	s	
4′-OMe	3.78	s	
3′-, 5′-OMe	3.51	s	

[a]Spectrum determined in C_6D_6 at 270 MHz.
[b]Assignments may be reversed: see text.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr spectra were obtained on an IBM WP-270 spectrometer in C_6D_6 as solvent; chemical shifts are reported in ppm downfield from internal TMS, and coupling constants are in Hz. Mass spectra were recorded on a VG 7070E-HF instrument in the electron ionization mode. Melting points are uncorrected. Flash chromatography was carried out on E. Merck Si gel, 230–400 mesh, and tlc was carried out on E. Merck precoated Si gel plates, 0.2 mm, with $CHCl_3$-iPrOH (10.7:0.3) as solvent. All solvents were distilled prior to use. Podophyllin was obtained from Sigma Chemical Company.

PODOPHYLLOTOXIN.—Podophyllin (5 g) was dissolved in hot EtOH (50 ml), the solution filtered, and the solvent evaporated in vacuo. The residue was dissolved in EtOAc, H_2O was added (20 ml), and the mixture was separated in a separatory funnel. The EtOAc layer was dried ($MgSO_4$) and evaporated, and the residue was subjected to flash chromatography with $CHCl_3$-MeOH (25:1) as eluent. Recrystallization of the appropriate fractions from EtOH yielded podophyllotoxin (1.56 g, 31%), mp 182° [lit. (1) 183–184°]. The sample was identical (tlc, co-tlc) with an authentic sample (Aldrich).

DESOXYPODOPHYLLOTOXIN [**2**].—Podophyllotoxin (12.4 mg, 0.0293 mmol) in HOAc (1 ml) was hydrogenated over 10% Pd-C (18 mg) at 95° for 3 h. The mixture was filtered, the catalyst washed with EtOH-H_2O (1:1) (30 ml), and the solution evaporated to dryness. The crude product was purified by preparative tlc to give pure material in 72% yield, mp 167.5°, identical with an authentic sample (7). For ^{1}H-nmr data see Table 2.

[4β-^{2}H$_1$]DESOXYPODOPHYLLOTOXIN [**3**].—Podophyllotoxin (250 mg, 0.604 mmol), previously dried in vacuo for 24 h at 100°, in dry C_6H_6 (5 ml) was heated under reflux with PCl_3 (0.31 ml of 2 M solution in CH_2Cl_2) for 45 min. The clear solution was decanted from the yellowish residue, which was washed with hot C_6H_6. The combined C_6H_6 solutions were evaporated and the residue dried in vacuo to give crude white amorphous podophyllotoxin chloride [**5**]. The crude chloride was dissolved in dry C_6H_6 (10 ml) and added dropwise via a cannula to a stirred suspension of excess $NaBD_3CN$ (98 atoms %, Aldrich) in freshly dried THF (15 ml) at 25°. The reaction mixture was stirred under N_2 for 15 h, and EtOAc was then added to decompose unreacted $NaBD_3CN$. The mixture was heated with H_2O, and the organic layer was separated, washed, dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography, with $CHCl_3$-iPrOH (10.7:0.3) as mobile phase, to give **3** (169 mg, 70%), identical with authentic material (tlc, co-tlc). ^{1}H nmr (C_6D_6) δ 6.57 (s, 2H, H-2′, -6′), 6.47 (s, 1H, H-5), 6.42 (s, 1H, H-8), 5.33 (overlapping d, 2H, OCH_2O), 4.41 (d, 1H, H-1, J = 4.8 Hz), 3.78 (s, 3H, 4′-OMe), 3.65 (dd, 1H, H-11α, J = 7.7, 10.6 Hz), 3.51 (s, 6H, 3′-, 5′-OMe), 3.06 (dd, 1H, H-11β, J = 8.5, 10.6 Hz), 2.31 (dddd, 1H, H-3, J = 13.8, 5.3, 8.5, 7.7 Hz), 2.18 (d, 1H, H-4α, J = 5.3 Hz), 1.96 (d, 1H, H-2, J = 4.8, 13.8 Hz); a small peak at 1.88 ppm (H-4β) was detected as a shoulder on the peak at 1.96 ppm, and was estimated at less than 0.05 of a proton.

PODOPHYLLOTOXONE [**6**].—Podophyllotoxin (0.5 g, 1.207 mmol) and MnO_2 (3.1 g) in $CHCl_3$ were heated under reflux for 1.5 h, until reaction was complete. The mixture was filtered, the solvent removed in vacuo, and the crude product purified by flash chromatography [$CHCl_3$-iPrOH (10.7:0.3)]. A total of 0.363 g (72%) of faintly yellow podophyllotoxone was obtained, mp 180°. ^{1}H nmr ($CDCl_3$) δ 7.55 (s, 1H, H-5), 6.71 (s, 1H, H-8), 6.39 (s, 2H, H-2′, H-6′), 6.1 (overlapping d, 2H, OCH_2O), 4.85 (d, 1H, H-1, J = 1.2 Hz), 4.56 (dd, 1H, H-11α, J = 9.1, 10.4 Hz), 3.82 (s, 3H, 4′-OMe), 3.75 (s, 6H, 3′-, 5′-OMe), 3.53 (m, 1H, H-3, J = 10.6, 15.5, 7.7 Hz), 3.29 (dd, 1H, H-2, J = 15.5, 4.3 Hz); eims *m/z* (rel. int.) [M]$^+$ 412 (100), 367 (20), 337 (10), 336 (8).

[4β-^{2}H$_1$]PODOPHYLLOTOXIN [**7**].—A mixture of anhydrous reagent grade $ZnCl_2$ (4 g, 0.029 mol) was dissolved in dry Et_2O (50 ml) with gentle warming, and the solution was added to a stirred suspension of $NaBD_4$ (3.068, 0.073 mol) in 150 ml Et_2O and the resulting solution stirred for 12 h under N_2. Podophyllotoxone (0.363 g, 0.881 mmol) in dry C_6H_6 (5 ml) was added through a cannula at 25° to 5 ml of the $Zn(BD_4)_2$ solution prepared above, and the resulting mixture stirred for 24 h at 25°. H_2O (15 ml) was then added, followed by HOAc (5 ml) and H_2O (15 ml), and the organic layer was separated, washed, dried (Na_2SO_4), and evaporated. Purification by flash chromatography gave [4β-^{2}H$_1$]podophyllotoxin (0.236 g, 65%) as a white solid: mp 183°; ^{1}H-nmr ($CDCl_3$) δ 7.12 (s, 1H, H-5), 6.51 (s, 1H, H-8), 6.37 (s, 2H, H-2′, -6′), 5.99 (overlapping d, 2H, OCH_2O), 4.59 (m, 1H, H-11α), 4.08 (m, 1H, H-11β), 3.81 (s, 3H, 4′-OMe), 3.76 (s, 6H, 3′-, 5′-OMe), 2.9–2.7 (m, 2H, H-2, H-3); eims *m/z* (rel. int.) [M]$^+$ 415 (100), 369 (10).

[4α-^{2}H$_1$]DESOXYPODOPHYLLOTOXIN [**4**].—[4β-^{2}H$_1$]Podophyllotoxin (250 mg, 0.602 mmol) [**7**] was converted to the corresponding chloride by treatment with PCl_3 (0.05 ml of a 1 M solution in CH_2Cl_2) as previously described. The chloride was added to a suspension of excess $NaCNBH_4$ in freshly

dried THF at 25°, and the mixture was stirred for 12 h. Workup as previously described and purification by flash chromatography gave 178 mg of **4** (74%), mp 168°, identical with **2** by tlc and co-tlc: ^{1}H-nmr (C_6D_6) δ 6.57 (s, 2H, H-2′, -6′), 6.47 (s, 1H, H-5), 6.42 (s, 1H, H-8), 5.33 (overlapping d, 2H, OCH_2O), 4.41 (d, 1H, H-1, J = 4.8 Hz), 3.78 (s, 3H, 4′-OMe), 3.65 (dd, 1H, H-11α, J = 7.7, 10.6 Hz), 3.51 (s, 6H, 3′-, 5′-OMe), 3.06 (dd, 1H, H-11β, J = 8.5, 10.6 Hz), 2.31 (dddd, 1H, H-3, J = 13.8, 11.4, 8.5, 7.7 Hz), 1.96 (dd, 1H, H-2, J = 4.8, 13.8 Hz), 1.89 (d, 1H, H-4β, J = 11.4 Hz); a small peak at 2.20 ppm (H-4α) was detectable, integrating for less than 0.05 proton; eims *m/z* (rel. int.) $[M]^+$ 399 (100), 398 (5).

[4,4-$^2H_2$]DESOXYPODOPHYLLOTOXIN [**9**].—Reaction of [4β-$^2H_1$]podophyllotoxin (250 mg, 0.602 mmol) [**7**] with PCl_3 followed by $NaCNBD_4$ as described above yielded the dideuterated product **9** (169 mg, 70%): mp 168°; ^{1}H-nmr (C_6D_6) δ 6.57 (s, 2H, H-2′, -6′), 6.47 (s, 1H, H-5), 6.42 (s, 1H, H-8), 5.33 (overlapping d, 2H, OCH_2O), 4.41 (d, 1H, H-1, J = 4.8 Hz), 3.78 (s, 3H, 4′-OMe), 3.65 (dd, 1H, H-11α, J = 7.7, 10.6 Hz), 3.51 (s, 6H, 3′-, 5′-OMe), 3.06 (dd, 1H, H-11β, J = 8.5, 10.6 Hz), 2.31 (dd, 1H, H-3, J = 13.8, 11.4 Hz), 1.96 (dd, 1H, H-2, J = 4.8, 13.8 Hz); eims *m/z* (rel. int.) $[M]^+$ 400 (100), 399 (6).

ACKNOWLEDGMENTS

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