The absolute configuration of a nitroxide spin labeled derivative of podophyllotoxin determined by the crystal structure

Baohan Zhou, Guodong Yin, Xianggao Meng, Yitao Li, and Anxin Wu

Abstract: The crystal structure of the nitroxide spin labeled derivative of podophyllotoxin was first reported. X-ray analysis demonstrated that four contiguous chiral centers in the molecule, C1, C2, C3, and C4, adopt *cis*- (1:2), *trans*- (2:3), and *cis*- (3:4) arrangement.

Key words: crystal structure, synthesis, nitroxyl radical, podophyllotoxin.

Résumé : On a déterminé pour la première fois la structure cristalline d'un dérivé de la podophyllotoxine portant un groupe nitroxyde comme marqueur de spin. Les analyses par diffraction des rayons X ont permis de démontrer que quatre centres chiraux contigus de la molécule, C1, C2, C3 et C4, adoptent des arrangements *cis*- (1,2), *trans*- (2,3) et *cis*- (3,4).

Mots clés : structure cristalline, synthèse, radical nitroxyle, podophyllotoxine.

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Introduction

Podophyllotoxin (**1**) and its many related derivatives are well known to possess pronounced antitumor and antiviral properties. However, the clinical application of podophyllotoxin and its analogues in the treatment of cancer has been limited by severe toxic side effects during the administration of the drugs (1–4). It was previously found that the introduction of a stable nitroxyl radical into the molecule of podophyllotoxin could result in novel compounds that have significant antitumor activity with marked decrease in toxicity compared with podophyllotoxin itself (5–7). Though the structure of spin-labeled podophyllotoxin derivatives can be indirectly characterized by mp, ESR, IR, MS, and HR-MS spectral analyses, its elaborate structure can't be confirmed by ^{1}H NMR because of its paramagnetic properties. Moreover, there are four contiguous chiral centers in the molecule, and its absolute configuration was not determined until now. In this paper, we synthesized one nitroxide spin labeled derivative of podophyllotoxin (**3**), first reported the crystal structure, and confirmed its absolute configuration.

Experimental section

4-[4″-(2″,2″,6″,6″-tetramethyl-1″-piperidinyloxy)amino]-4′-demethylepipodophyllotoxin (**3**) was prepared as shown in Scheme 1 (8). Podophyllotoxin (**1**), commercial agent (J & K Chemical Ltd., Shanghai, China), was used as a starting material.

The demethylation and bromination of **1** gave 4-bromo-4′-demethyl-epipodophyllotoxin (**2**). 33 g of **1** was suspended in 300 mL of 1,2-dichloroethane and 30 mL of ether and cooled to 0 °C. A current of dry hydrobromide was passed in until an increased mass of 82.5 g was obtained. After standing at 0–2 °C for 20 h, the solvent was removed under vacuum. The residue was recrystallized from acetone to give 12 g of **2**: 36% yield; mp 180–190 °C (dec.). A solution of **2** (2.77 g, 6 mmol) and 2,2,6,6-tetramethyl-1-piperidinyloxy-4-amine (2.57 g, 15 mmol) in 35 mL of dry THF and 2 drops of dry pyridine was refluxed under N_2 for 4 h. After filtering off the insoluble solids and removing the solvent under reduced pressure, the crude product was chromatographed twice through silica gel using CH_2Cl_2–acetone–Et_2NH (40:1:1, *v*/*v*) as an eluent to obtain 1.07 g (32% yield). Crystallization from acetone–hexane afforded the spin-labeled derivative of podophyllotoxin (**3**): red solid; mp 218–220 °C. UV (methanol) λ_{max} (nm): 283, 242, 220. IR (cm^{-1}): 3338 υ(NH), 1757 υ(C=O), 1609 υ(C=C), 1516 υ(C=C), 1479 $\delta_S(CH_3)$. ESR: 3 Lines, A_n = 16.2 G, ΔH_o = 2.66 G, g_o = 2.0061. MS–FAB *m*/*z* (%): 554 (13), 553 (16), 537 (7), 383 (100). Anal. calcd. (%): C 65.08, H 6.74, N 5.06; found: C 65.36, H 6.505, N 4.035.

A red crystal of the synthesized compound **3** was mounted on a glass fiber in a random orientation at 292(2) K. The determination of the unit cell and the data collection were performed with Mo Kα radiation (λ = 0.71073 Å) on a Bruker SMART APEX-CCD diffactometer with a ψ-ω scan mode. The structure was solved by direct methods with SHELXS-97 program and expanded by Fourier technique. The non-hydrogen atoms were refined anisotropically, and the hydrogen atoms were determined by theoretical calculations.

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Scheme 1.

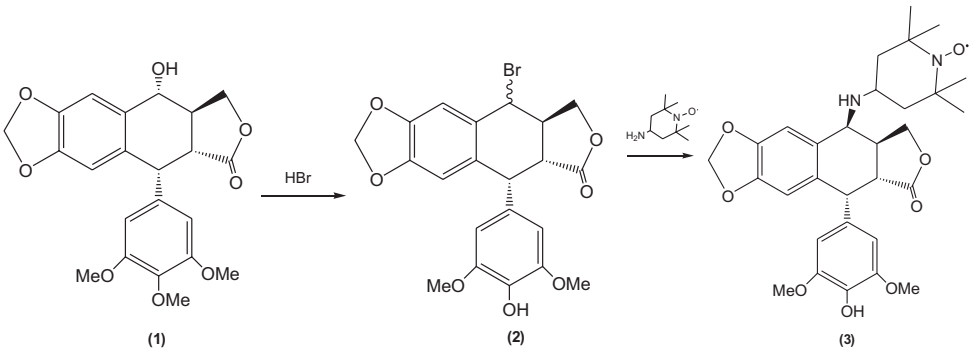


Table 1. Crystal data and structure refinement for **3**.

Empirical formula	$C_{30}H_{37}N_2O_8$
Formula weight	553.62
Temperature	292(2) K
Wavelength (Å)	0.71073
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	8.1082(6)
b (Å)	19.3396(13)
c (Å)	42.130(3)
$\alpha = \beta = \gamma$	90°
Volume (Å^3)	6606.4(8)
Z	4
D_{calcd} (mg/m^3)	1.222
Absorption coefficient (μ) (mm^{-1})	0.087
F(000)	2576
Crystal size (mm^3)	0.30 × 0.20 × 0.20
θ range for data collection (°)	2.16–25.00
Limiting indices	$-9 \le h \le 9$, $-19 \le k \le 23$, $-50 \le l \le 48$
Reflections collected	33402
Independent reflections	6467 (R_{int} = 0.0851)
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	6467/0/853
Goodness-of-fit on F^2	1.023
Final R indices ($I > 2\sigma(I)$)	R_1 = 0.0614, wR_2 = 0.1453
R indices (all data)	R_1 = 0.1039, wR_2 = 0.1692
Extinction coefficient	0.0002(4)
Largest diff. Peak and hole (e Å^{-3})	0.189 and –0.188

Crystal data and structure refinement for compound **3** are listed in Table 1. The selected bond lengths and bond angles are given in Table 2 and 3, respectively. The other tables of CIF have been deposited as supplementary material.[2]

Results and discussion

Figure 1 shows the molecular structure of compound **3**, and the atomic numbering of the same compound is shown in Fig. 2.

Because the tumor-damaging potency of podophyllotoxin and related compounds is closely associated with their stereochemistry (9, 10), the determination of their absolute configuration might shed further light on the mechanism of their action, a question of importance in the search for effective chemotherapeutic agents. Synthetic organic chemists have been attracted to the stereochemical challenge represented by the four contiguous chiral centers of podophyllotoxin, the rigid *trans*-lactone, and the axially locked 1-aryl substituent. The early syntheses (11–14) generated

[2] Supplementary data for this article are available on the journal Web site or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada. DUD 5111. For more information on obtaining material, refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml. CCDC 298236 contains the crystallographic data for this manuscript. These data can be obtained, free of charge, via ww.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033; or deposit@ccdc.cam.ac.uk).

Table 2. Selected Bond lengths (Å).

Bond	Distance
C1—N1	1.472(6)
C1—C9	1.525(7)
C1—C2	1.541(7)
C2—C3	1.513(7)
C2—C12	1.522(7)
C3—C13	1.497(8)
C3—C4	1.524(7)
C4—C10	1.516(8)
C4—C1′	1.523(6)
C5—C6	1.349(9)
C5—C10	1.406(7)
C6—O2	1.388(7)
C7—C8	1.359(8)
C7—O1	1.374(7)
C8—C9	1.424(8)
C9—C10	1.398(7)
C11—O1	1.409(11)
C11—O2	1.434(11)
C12—O3	1.444(8)
C13—O4	1.205(7)
C13—O3	1.362(8)
C6—C7	1.381(9)

Table 3. Selected bond angles (°).

Angle	(°)
N1–C1–C9	110.1(4)
N1–C1–C2	110.6(4)
C9–C1–C2	107.8(4)
C3–C2–C12	101.2(4)
C3–C2–C1	108.6(4)
C12–C2–C1	119.7(4)
C13–C3–C2	103.6(4)
C13–C3–C4	120.0(5)
C2–C3–C4	113.3(4)
C10–C4–C1′	113.2(4)
C10–C4–C3	108.2(4)
C1′–C4–C3	114.0(4)

much new and interesting chemistry but led to the inactive *cis*-lactone.

X-ray analysis demonstrated that four contiguous chiral centers in the molecule, C1, C2, C3, and C4, adopt *cis*-(1:2), *trans*- (2:3), and *cis*- (3:4) arrangement (Fig. 1). The angles of C3–C2–C12, C3–C2–C1, and C13–C3–C2 are 101.2(4)°,108.6(4)°, and 103.6(4)°, respectively, which are less than the normal 109.5° angle. The angles of C12–C2–C1, C13–C3–C4, and C2–C3–C4 are 119.7(4)°, 120.0(5)°, and 113.3(4)°, respectively, which are much larger than the normal 109.5° angle. These results show that C2 and C3 are distorted atoms. Perhaps because the *trans*-lactone is a rigid ring and can not rotate freely. The six-membered ring containing N–O radical adopts a chair conformation, and the bond length of N—O is 1.270(5) Å, which is in the normal range of nitroxyl radicals (1.25–1.31 Å) (15, 16).

Fig. 1. Molecular structure of compound **3**.

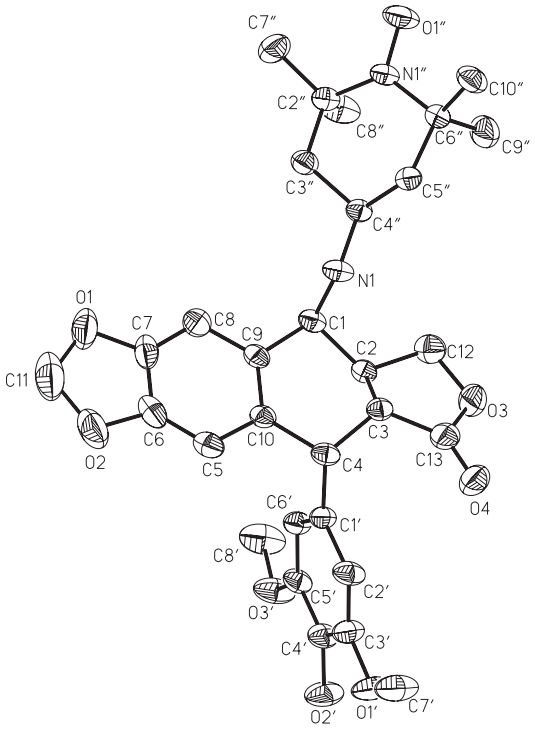
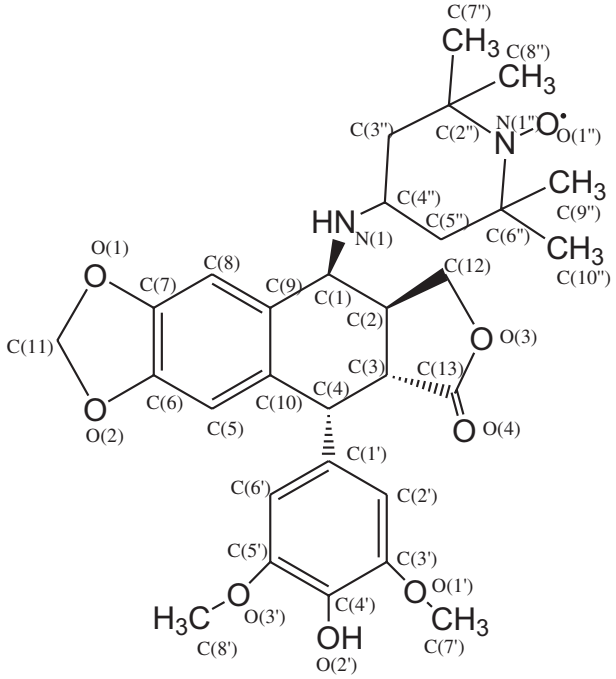


Fig. 2. Atomic numbering of compound **3**.



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