Stereostructure and Bioactivities of Jolkinolide D

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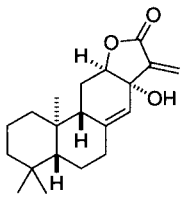
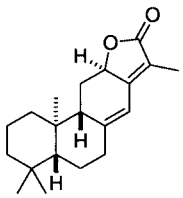
The stereostructure and bioactivities of jolkinolide D, a diterpenoid from *Euphorbia jolkini*, were investigated. Jolkinolide D inhibited tumor invasion into the basement membrane and induced apoptosis in tumor cells.

Chemotherapy is one of the medical treatments for cancer. Drugs for chemotherapy are being investigated on the basis of many kinds of modes of action against tumor cells. The inhibition of invasion[1] and induction of apoptosis[2] of tumor cells are promising ways to develop new types of anticancer drugs.

Jolkinolide D is a diterpenoid isolated from *Euphorbia jolkini* Boiss in 1974, and the structure with partial stereochemistry was determined by spectroscopic analysis and chemical derivation.[3] It has a pharmacophore structure, an α-methylene lactone unit, but its bioactivities have not yet been investigated. We describe herein the stereostructure and bioactivities of jolkinolide D.



Jolkinolide D (**1**)



Jolkinolide E (**2**)

The stereostructure was determined by X-ray crystallographic analysis. Although the crystallization of jolkinolide D from methanol gave well-formed and colorless crystals, its analysis was difficult because the α and β values were close to each other and 90°. After several trials, we determined the crystal system to be triclinic.[4] Four independent molecules of jolkinolide D were in the unit cell ($Z = 4$). The X-ray crystal structure of jolkinolide D is shown in Figure 1. Considering the absolute stereochemistry of jolkinolide E (**2**),[5] which was chemically correlated with jolkinolide D,[3] the absolute stereochemistry of jolkinolide D was established as structural formula **1**.

The bioactivities of jolkinolide D were next investigated.

The apoptosis-inducing activity of jolkinolide D was determined by measuring the DNA fragmentation induced in human leukemia HL-60 cells (Table 1). The ratio of the fragmented DNA was determined by a method established by Burton. [6] Jolkinolide D induced apoptosis as strongly as etoposide, which is known as an anti-cancer drug that induces apoptosis.

The inhibiting activity of jolkinolide D against tumor invasion into basement membranes was evaluated by using the reconstituted basement membrane Matrigel, prepared as previously described.[7] The tumor cells invading through a Matrigel-coated filter of the transwell chamber were visually counted, and the results are summarized in Table 2. Jolkinolide D was proved to inhibit 92% of the tumor invasion at the concentration of 1 μg/mL. Even 0.1 μg/mL of jolkinolide D inhibited 57% of the invasion similarly to 1 μg/mL of doxorubicin.

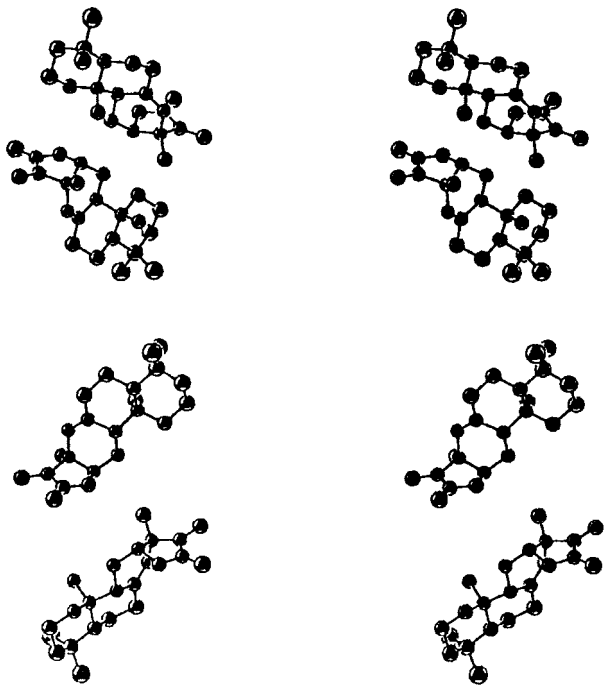


Figure 1. Stereoscopic view of the crystal structure of jolkinolide D

Table 1. Apoptosis-inducing activity of jolkinolide D

	Concentration / $\mu g \cdot mL^{-1}$	DNA fragmentation[a] / % 20 h	43 h
Control	0	11	16
Jolkinolide D	10	40	46
Etoposide	10	56	72

[a]Human leukemia HL-60 cells were treated with the test drug in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum in a humidified atmosphere of 5% CO_2 and 95% air. The DNA fragmentation was analyzed by the Burton method.

Table 2. Invasion-inhibiting activity of jolkinolide D

	Concentration / μg·mL^{-1}	Invasion cell number[a]	rel. value
Control	0	158 ± 12	100
Jolkinolide D	0.001	131 ± 10	83
	0.01	124 ± 7	78
	0.1	68 ± 9	43
	1	13 ± 2	8
Doxorubicin	1	68 ± 11	43

[a]Human fibrosarcoma HT-1080 was incubated in the upper compartment of the transwell chamber assembly fitted with a Matrigel-coated filter for 4 h, and the invasion cells that passed through the filter were visually counted under a microscope.

Furthermore, jolkinolide D exhibited cytotoxicities against P388 murine leukemia and DLD-1 human colon cancer with IC_{50} values of 0.16 and 3.0 μg/mL, respectively.

The mechanism of action and the in vivo behavior of jolkinolide D are currently under investigation.

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References and Notes

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4 The crystallographic data for jolkinolide D are as follows: $C_{20}H_{28}O_3$, M_r = 316.00; triclinic; space group $P1$ with a = 6.977(2) Å, b = 7.012(2) Å, c = 37.568(5) Å, α = 89.93(2)°, β = 89.91(2)°, γ = 75.01(2)°, V = 1775.3(7) Å^3, Z = 4; D_x = 1.182 Mg m^{-3}, D_m = 1.100 Mg m^{-3}, μ(Cu Kα) = 5.813 cm^{-1}. A Mac Science MXC18 diffractometer was used throughout this study. The final R and R_w values for 5628 reflections ($I > 2\sigma(I)$) were 0.039 and 0.041, respectively.
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