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Jolkinolide D pharmacophore: synthesis and reaction with amino acids, nucleosides, and DNA

Akira Sakakura, Yui Takayanagi and Hideo Kigoshi*

Department of Chemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8571, Japan

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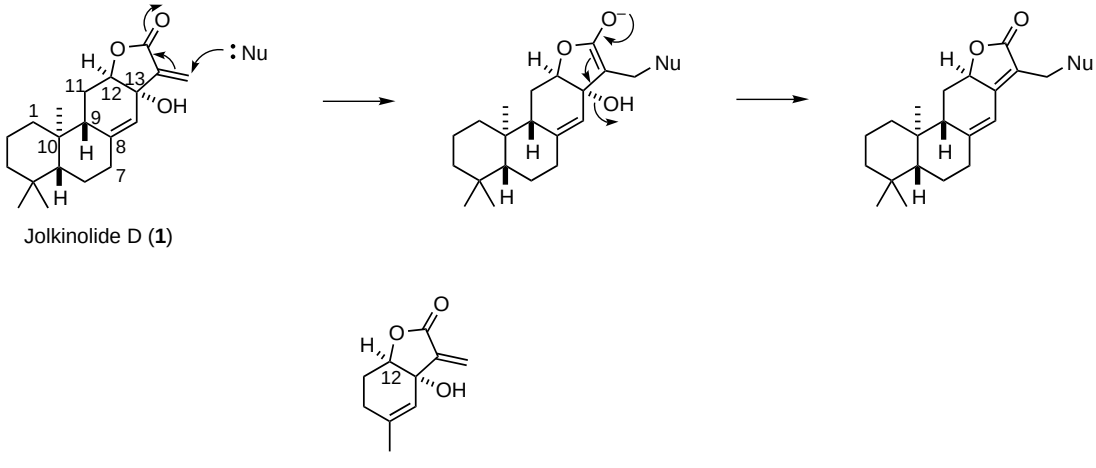
Abstract—For the purpose of obtaining preliminary information on the chemical modification of biomolecules with jolkinolide D, a bioactive diterpenoid of plant origin, jolkinolide D pharmacophore, was synthesized and its reactivity toward amino acids, nucleosides, and DNA was investigated. © 2002 Published by Elsevier Science Ltd.

Jolkinolide D (**1**) is a diterpenoid isolated from *Euphorbia jolkini* Boiss in 1974,[1] and its stereostructure was recently determined by X-ray crystallographic analysis.[2] It inhibited tumor invasion into the basement membrane and induced apoptosis in tumor cells.[2]

Jolkinolide D (**1**) has a γ,δ-unsaturated-β-hydroxy-α-methylene lactone unit as the pharmacophore structure, which suggests that jolkinolide D (**1**) might alkylate biomolecules such as proteins and DNA irreversibly in contrast to popular α-methylene lactones such as in germacranolides, eudesmanolides, guaianolides, and pseudoguaianolides[3] that would alkylate biomolecules reversibly (Scheme 1). Since there are no other compounds that have the pharmacophore, reactivities of the unit toward nucleophiles have not hitherto been investigated. We describe herein the synthesis of jolkinolide D pharmacophore (**2**) and its reactivities toward amino acids, nucleosides, and DNA.



Scheme 1. Jolkinolide D (**1**) and its pharmacophore (**2**).

Keywords: diterpenoid; jolkinolide D; pharmacophore; synthesis; reactivity.

* Corresponding author. Fax: +81-298-53-4313; e-mail: kigoshi@chem.tsukuba.ac.jp

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Synthesis of jolkinolide D pharmacophore (2)

Jolkinolide D pharmacophore (**2**) was synthesized from 6-(*tert*-butyldimethylsilyloxy)-3-methyl-2-cyclohexenone (**3**)[4] and 2-iodoallyl alcohol (**4**)[5] (Scheme 2). Addition of the vinyllithium reagent, generated from iodide **4** and *t*-BuLi, to ketone **3** afforded diastereomeric diols **5** (56%) and **6** (33%). Diol **5** could be transformed into jolkinolide D pharmacophore (**2**)[6] by removal of the TBS group and subsequent oxidation with MnO_2 in 43% yield whereas diol **6** was converted into aldehyde **7** by the same procedure (24%). The stereochemistry of the ring juncture in **2** was established by the small coupling constant of H-12 (ddd, $J=1.3$, 3.0, 4.0 Hz).[7]

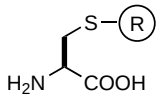
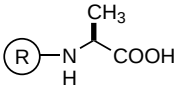
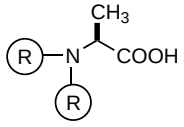
Reactions of jolkinolide D pharmacophore (2) with amino acids, nucleosides, and DNA

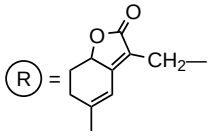
The reactivities of jolkinolide D pharmacophore (**2**) toward amino acids, nucleosides, and DNA have been investigated in order to obtain preliminary and basic information on the chemical modification of proteins and DNA with jolkinolide D (**1**).

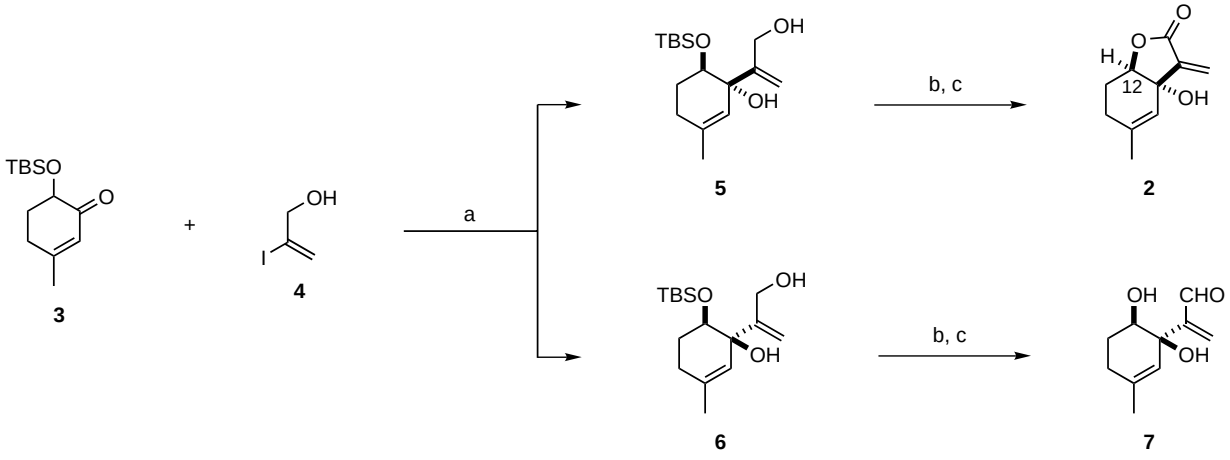
Two amino acids, a typical amino acid alanine and a sulfur-containing cysteine, were chosen as nucleophiles toward jolkinolide D pharmacophore (**2**). The alkylation conditions for these amino acids are as follows: 0.23 M jolkinolide D pharmacophore (**2**) and amino acid in aqueous THF, pH 7.5, ambient temperature, and 16 hours. The products were separated and purified by chromatography (ODS), and their structures were established by the spectroscopic method (Table 1). The thiol group in cysteine was alkylated rapidly with **2** in high yield (64%).[8,9] In the case of alanine, which has no nucleophilic functional group in the amino acid residue, alkylation occurred only at the amino group to afford the monoalkylated product **9**[8,10] (53%) and dialkylated product **10**[8,11] (4.3%). These results indicate that the thiol group is the most reactive toward **2** among the nucleophilic groups in cysteine and that the amino group is more reactive than the carboxyl group in alanine at pH 7.5.

Four nucleosides, 2′-deoxyadenosine, 2′-deoxyguanosine, 2′-deoxycytidine, and thymidine were made to react with an equal amount of jolkinolide D pharmacophore (**2**) under the same conditions as those employed for amino acids except for concentration: 0.12 M for 2′-deoxyadenosine, 0.021 M for 2′-deoxyguanosine, 0.14 M for 2′-deoxycytidine, and 0.20 M for thymidine. The results are listed in Table 2. The sites of alkylation in the purine and pyrimidine bases of nucleosides were determined by comparison of the difference UV spectra between alkylated nucleosides and compound **9** with the UV spectra of the known ethylated nucleosides in acidic, neutral, and basic solutions (Table 3).[12,13] Only in the case of 2′-deoxyguanosine, alkylation reaction occurred to afford **11**,[8,14] the product alkylated at the N-1 position of guanine, in 21% yield. When alkylation of thymidine was executed under basic conditions (pH 8.7), the product **12**,[8,15] formed by alkylation at the N-3 position of thymine, was obtained in 22% yield. Both in the reactions,

Table 1. Alkylation of amino acids with jolkinolide D pharmacophore (**2**)

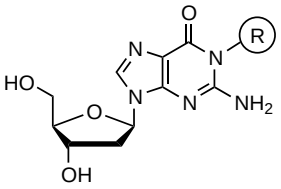
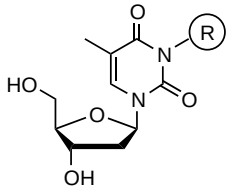
amino acid	products (yield)
L-cysteine	 **8** (64%)
L-alanine	 **9** (53%)  **10** (4.3%)

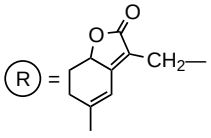




Scheme 2. *Reagents and conditions*: (a) *t*-BuLi, Et_2O, 0°C; (b) Bu_4NF, THF, 0°C; (c) MnO_2, $CHCl_3$, rt.

Table 2. Alkylation of nucleosides with jolkinolide D pharmacophore (**2**)

nucleoside	product (yield)
2'-deoxyguanosine	 **11** (21%)
thymidine	 **12** (22%, pH 8.7)
2'-deoxyadenosine	no reaction
2'-deoxycytidine	no reaction



a considerable amount (ca. 40%) of unreacted **2** was recovered after 16 h.

DNA from salmon spermary (0.060 M in nucleotide) was reacted with 0.060 M of jolkinolide D pharmacophore (**2**) in aqueous methanol (pH 7.5) at 37°C for a week. After the enzymatic hydrolysis,[16,17] the alkylated nucleosides were isolated and characterized as described above. There were obtained two products **11** (0.48%) and **12** (0.26%), the same products as in the reaction of nucleosides with **2**, but the yields were very low.[18]

In conclusion, we have investigated the reactivities of jolkinolide D pharmacophore (**2**) toward amino acids, nucleosides, and DNA. The order of reactivity of jolkinolide D pharmacophore (**2**) toward the nucleophiles in amino acids at pH 7.5 is the thiol group in cysteine>the α-amino groups in amino acids>the carboxyl groups in amino acids. Jolkinolide D pharmacophore (**2**) alkylated 2′-deoxyguanosine at the N-1 position (pH 7.5) and thymidine at the N-3 position (pH 8.7) and modified DNA at the same sites in lower yields. It is worth mentioning that the well-known alkylating agent, dimethyl sulfate, alkylates guanosine at the N-7 position[19] and thymidine at the N-3 position[20] under neutral and aqueous conditions. N-1 alkylation of 2′-deoxyguanosine was found on treatment with methyl iodide in dimethyl sulfoxide under basic conditions.[21]

Acknowledgements

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6. Jolkinolide D pharmacophore (**2**): UV (CH_3CN) λ_{max} 223 nm (ε 5500); IR (neat) 3400, 1760, 1670, 1270, 1150, 1010, 950 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 6.27 (s, 1H), 5.85 (s, 1H), 5.24 (m, 1H), 4.49 (ddd, $J=1.3$, 3.0, 4.0 Hz, 1H), 2.22–2.07 (m, 2H), 1.98–1.84 (m, 2H), 1.74 (s, 3H),

Table 3. Difference UV spectra of jolkinolide D pharmacophore–nucleoside adducts and UV spectra of ethylated nucleosides

Alkylated nucleoside	pH 1[a]		pH 7[b]		pH 13[c]	
	λ_{max} (nm)	λ_{min} (nm)	λ_{max} (nm)	λ_{min} (nm)	λ_{max} (nm)	λ_{min} (nm)
11	262, 277	229	257, 272	227	258, 272 (sh)	226
N-1-Ethyl-2′-deoxyguanosine[d]	261, 272 (sh)	240	257, 270 (sh)	237	258, 270 (sh)	239
12	271	239	272	237	272	238
N-3-Ethylthymidine[d]	269		269		270	

[a] 0.1 M HCl/H_2O–MeOH (9:1).
[b] H_2O/MeOH (9:1).
[c] 0.1 M KOH/H_2O–MeOH (9:1).
[d] Ref. 13.

a signal of a hydroxyl proton was not detected; ^{13}C NMR ($CDCl_3$) δ 168.8, 142.1, 140.0, 121.9, 121.1, 81.1, 72.8, 23.8, 23.7, 22.4; MS (FAB) m/z 203 $(M+Na)^+$, 181 $(M+H)^+$; HRMS (FAB) calcd for $C_{10}H_{12}NaO_2$ $[(M+Na)^+]$ 203.0684, found 203.0685.

7. The numbering adopted in this paper corresponds to that of jolkinolide D (**1**).
8. Because jolkinolide D pharmacophore (**2**) was synthesized in a racemic form, alkylated products were obtained as a 1:1 diastereomeric mixture, except for **10**. The diastereomeric ratio of **10** could not be obtained.
9. Compound **8**: UV (H_2O/MeOH 9:1) λ_{max} 283 nm (ε 11 000); IR (neat) 3450, 1740, 1650, 1495, 1390, 1340, 1040 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 6.56 (s, 0.5H), 6.54 (s, 0.5H), 4.94 (dd, J=5.1, 13.2 Hz, 1H), 3.74 (m, 1H), 3.54 (d, J=13.5 Hz, 0.5H), 3.53 (d, J=13.5 Hz, 0.5H), 3.46 (d, J=13.5 Hz, 1H), 3.18 (dd, J=6.2, 14.7 Hz, 0.5H), 3.17 (dd, J=6.3, 14.4 Hz, 0.5H), 2.90 (dd, J=8.2, 14.7 Hz, 0.5H), 2.89 (dd, J=9.4, 14.4 Hz, 0.5H), 2.55–2.36 (m, 3H), 1.96 (s, 3H), 1.67–1.53 (m, 1H); MS (FAB) m/z 284 $(M+H)^+$; HRMS (FAB) calcd for $C_{13}H_{17}NNaO_4S$ $[(M+Na)^+]$ 306.0776, found 306.0784.
10. Compound **9**: UV (H_2O/MeOH 9:1) λ_{max} 279 nm (ε 16 000); IR (KBr) 3510, 3360, 1760, 1665, 1590, 1400, 1365, 1335 cm^{-1}; ^{1}H NMR (270 MHz, CD_3OD) δ 6.61 (s, 0.5H), 6.57 (s, 0.5H), 5.05 (dd, J=5.3, 13.9 Hz, 1H), 4.03–3.73 (m, 2H), 3.54 (m, 1H), 2.55–2.43 (m, 3H), 2.00 (s, 3H), 1.95–1.65 (m, 1H), 1.48 (d, J=6.6 Hz, 3H); MS (FAB) m/z 252 $(M+H)^+$; HRMS (FAB) calcd for $C_{13}H_{18}NO_4$ $[(M+H)^+]$ 252.1236, found 252.1240.
11. Compound **10**: UV (H_2O/MeOH 9:1) λ_{max} 281 nm (ε 12 000); IR (neat) 1745, 1650, 1460, 1395, 1340, 1035 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.37 (s, 2H), 4.80–4.76 (m, 2H), 3.59–3.51 (m, 1H), 3.44–3.26 (m, 4H), 2.50–2.38 (m, 6H), 1.96 (s, 6H), 1.75–1.53 (m, 2H), 1.38 (d, J=7.0 Hz, 1.5H), 1.36 (d, J=7.0 Hz, 1.5H); MS (FAB) m/z 414 $(M+H)^+$; HRMS (FAB) calcd for $C_{23}H_{28}NO_6$ $[(M+H)^+]$ 414.1917, found 414.1889.
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14. Compound **11**: UV (H_2O/MeOH 9:1) λ_{max} 257 (ε 10 000), 272 nm (ε 12 000); IR (neat) 3330, 1730, 1640, 1580, 1540, 1440, 1100 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (s, 1H), 6.77 (s, 1H), 6.25 (dd, J=6.2, 7.3 Hz, 1H), 5.12 (d, J=15.1 Hz, 1H), 4.99 (dd, J=5.1, 14.0 Hz, 1H), 4.69 (d, J=15.1 Hz, 1H), 4.51 (ddd, J=3.1, 3.5, 6.2 Hz, 1H), 3.97 (ddd, J=3.5, 3.5, 4.1 Hz, 1H), 3.76 (dd, J=3.5, 12.2 Hz, 1H), 3.72 (dd, J=4.1, 12.2 Hz, 1H), 2.68 (ddd, J=6.2, 7.3, 13.5 Hz, 1H), 2.47–2.39 (m, 3H), 2.33 (ddd, J=3.1, 6.2, 13.5 Hz, 1H), 1.94 (s, 3H), 1.71–1.60 (m, 1H); MS (FAB) m/z 430 $(M+H)^+$; HRMS (FAB) calcd for $C_{20}H_{24}N_5O_6$ $[(M+H)^+]$ 430.1727, found 430.1708.
15. Compound **12**: UV (H_2O/MeOH 9:1) λ_{max} 274 nm (ε 18 000); IR (neat) 3430, 1740, 1700, 1650, 1340, 1050 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (q, J=1.2 Hz, 1H), 6.39 (s, 0.5H), 6.38 (s, 0.5H), 6.19 (t, J=6.5 Hz, 0.5H), 6.18 (t, J=6.5 Hz, 0.5H), 4.94 (d, J=14.7 Hz, 0.5H), 4.92 (d, J=14.7 Hz, 0.5H), 4.80 (d, J=14.7 Hz, 0.5H), 4.78 (d, J=14.7 Hz, 0.5H), 4.75 (dd, J=4.9, 13.5 Hz, 1H), 4.61 (m, 1H), 4.02 (ddd, J=3.3, 3.3, 3.3 Hz, 1H), 3.95 (br d, J=12.4 Hz, 1H), 3.87 (br d, J=12.4 Hz, 1H), 2.57 (br s, 1H), 2.45–2.34 (m, 5H), 1.94 (s, 3H), 1.92 (s, 3H), 1.64–1.59 (m, 1H), 1.58 (br s, 1H); MS (FAB) m/z 405 $(M+H)^+$; HRMS (FAB) calcd for $C_{20}H_{25}N_2O_7$ $[(M+H)^+]$ 405.1662, found 405.1667.
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17. The crude modified DNA (17 mM in nucleotide) was hydrolyzed with deoxyribonuclease I (Takara, 316 unit/mL) in aqueous buffer [50 mM Tris–HCl (pH 9.0), 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol] at 37°C overnight. To the reaction mixture were added snake venom phosphodiesterase (Worthington Biochemical Corporation, 10.9 unit/mL) and bacterial alkaline phosphatase (Takara, 5.26 unit/mL), and the mixture was incubated at 37°C for 24 h. The hydrolysate was separated by reversed-phase HPLC to afford **11** and **12** along with 2′-deoxyadenosine, 2′-deoxyguanosine, 2′-deoxycytidine, and thymidine.
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