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Studies on the Biological Activities of Islandic Acid and Related Compounds

YASUO FUJIMOTO,*,[a] TSUYOSHI KIHARA,[a] KIYOSHI ISONO,[a] HIROSHI TSUNODA,[a] TAKASHI TATSUNO,[a] KOUJI MATSUMOTO,[b] and HIDEO HIROKAWA[b]

The Institute of Physical and Chemical Research,[a] Wako-shi, Saitama 351, Japan and Sophia University,[b] 7–1 Kioi-cho, Chiyoda-ku, Tokyo 102, Japan

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The effects of islandic acid-I (**1**) and related compounds on the growth of Yosida sarcoma cells and on the transfection of *Bacillus* phage M2-DNA have been investigated. Compound **1** inhibited the growth of Yoshida sarcoma cells at 100 μg/ml, while its methyl ester (**2**) was 100 times more potent. Islandic acid-II methyl ester (**4**) and **2** produced 83 and 97% inhibition of the transfection of *Bacillus* phage M2-DNA at 33 and 66 μg/ml, respectively. Methyl (2*Z*,4*E*)-hexadienoate (**5**), having the same configuration as the side chain of **1**, also inhibited the transfection of M2-DNA over a wide concentration range, but did not show cytotoxicity against Yoshida sarcoma cells in tissue culture even at 100 μg/ml.

Keywords——*Penicillium islandicum*; islandic acid; Yoshida sarcoma; phage M2-DNA transfection; methylhexadienoate; antitumor activity

Penicillium islandicum Sopp. is a well known toxic fungus isolated from imported yellowed rice. It produces two major mycotoxins, luteoskyrin and cyclochlorotin, which cause serious liver damage.[1] In our previous paper,[2] we have reported the isolation and structural elucidation of islandic acid, which was produced by *P. islandicum* Sopp, under modified culture conditions.

In this paper, we describe the biological activities of islandic acid and related compounds on cytotoxicity against Yoshida sarcoma cells in tissue culture, and the effects on the transfection of *Bacillus* phage M2-DNA.

Materials and Methods

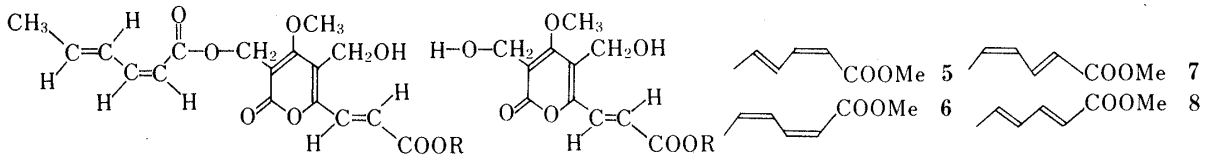
Isolation of Islandic Acid-I (1) and -II (3)——*P. islandicum* Sopp. was grown in yeast peptone-Czapek medium at 25 °C. After 2 weeks, the mycelia were resuspended in the medium, and cultivation was continued for a further 10 d. The culture filtrate was mixed with active charcoal and the mixture was allowed to stand for a few hours. The charcoal adsorbates were extracted with acetone and the extracts were concentrated under reduced pressure. The residual acetone–water solution was extracted with *n*-butanol. The extracts were concentrated *in vacuo* and then treated with a small amount of methanol to yield solid cyclochlorotine (*ca.* 200 mg). After removal of the cyclochlorotine by filtration, the filtrate was concentrated and chromatographed on silica gel ($CHCl_3$: MeOH = 4 : 1) to give islandic acid-I (*ca.* 200 mg): [mp 165—168 °C (from MeOH–H_2O), mass spectra (MS) *m/z*: 350 (M^+), $C_{17}H_{18}O_8$, infrared (IR) ν_{max}^{KBr} cm^{-1}: 3300 (OH), 2800—2300 (COOH), 1750, 1700 and 1650 (COO), ultraviolet (UV) λ_{max}^{MeOH} nm (ε): 234 (32000), 260 (23000), 335 (14500), proton nuclear magnetic resonance (^{1}H-NMR) (60 MHz, CD_3OD) δ: 1.88 (3H, d, J = 7.0 Hz, $C\underline{H}_3CH=C$), 4.13 (3H, s, OCH_3), 4.54 (2H, s, $C\underline{H}_2OH$), 5.12 (2H, s, $COOC\underline{H}_2$), 5.54 (1H, d, J = 11.5 Hz, $CH=C\underline{H}$–$COOCH_2$), 6.16 (1H, m, $CH_3C\underline{H}=CH$), 6.63 (1H, t, J = 11.5 Hz, $C\underline{H}=CH$–$COOCH_2$), 6.65, 7.68 (2H, ABq, J = 15.4 Hz, $C\underline{H}=C\underline{H}$–COOH), 7.35 (1H, br dd, J = 11.5, 15.4 Hz, CH_3–$CH=C\underline{H}$)] and islandic acid II (**3**) (*ca.* 70 mg) [mp 175—178 °C (from MeOH–H_2O), MS *m/z*: 256 (M^+), $C_{11}H_{12}O_7$, IR ν_{max}^{KBr} cm^{-1}: 3250 (OH), 1700 (sh), 1685 (COO), ^{1}H-NMR (60 MHz, CD_3OD) δ: 4.18 (3H, s, OCH_3), 4.54 (2H, s, $C\underline{H}_2OH$), 4.56 (2H, s, $C\underline{H}_2OH$), 6.62, 7.68 (2H, ABq, J = 15.4 Hz)].

Preparation of Islandic Acid-I Methyl Ester (2) and -II Methyl Ester (4)——A methanol solution of islandic acid-I (**1**) was treated with an equimolar amount of diazomethane in ether under ice cooling.[3] After a few minutes, the

reaction mixture was concentrated *in vacuo*, and then the residual oily material was chromatographed on silica gel (hexane : ethyl acetate = 1 : 2) to give islandic acid-I methyl ester (**2**). The physical data for islandic acid-I methyl ester: see ref. 2.

Islandic acid-II was methylated as above to give islandic acid-II methyl ester (**4**) [mp 116—117 °C (from methanol–ethyl acetate), IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1720 (sh), 1700 (sh) and 1680 (COO), 1635 (C=C), ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 3.82 (3H, s, $COOCH_3$), 4.17 (3H, s, OCH_3), 4.59 (2H, s, CH_2OH), 6.78, 7.59 (2H, ABq, J = 15.4 Hz, CH=CH)].

Synthesis of Methyl Hexadienoates——A solution of sorbic acid (300 mg) in benzene (50 ml) was irradiated with a low pressure mercury lamp (10 W) for 18 h and then the solution was concentrated under reduced pressure to leave an oil. The residual oily material was dissolved in methanol and then treated with diazomethane. Evaporation of the solvent gave a mixture of methyl hexadienoates and an unidentified compound. A part of this mixture was separated by high pressure liquid chromatography (hexane : ethyl acetate = 100 : 1) to give (2*Z*,4*E*)-(**5**: 41.9%), (2*Z*,4*Z*)-(**6**: 14.4%), (2*E*,4*Z*)-(**7**: 11.2%), (2*E*,4*E*)-hexadienoic acid methyl ester[4] (**8**: 24.4%) and an unidentified compound (8.1%).



1 : R=H
2 : R=CH_3

3 : R=H
4 : R=CH_3

Chart 2

Growth Inhibition against Yoshida Sarcoma Cells——The effects of samples on the growth of Yoshida sarcoma cells were investigated as follows. Cells were suspended in DM-160 culture medium containing 20% fetal bovine serum. Two ml of cell suspension (approximately 20×10^4 cells/ml) was transferred into each vial. After addition of the sample to be tested at the concentration indicated, the vials were incubated at 37 °C with rubber stoppers. Viable cell number was counted after incubation for 4 d.

Transfection Assay——*Bacillus subtilis* strain SR22 (*trp* C2, *spo* 0A12) was used for multiplication of phage M2 in TY medium.[5] Procedures of propagation and purification of phage M2 particles and extraction of phage DNA were as described in the previous paper.[6] *Bacillus subtilis* strain 222 (*arg* A15, *trp* B160) was used as competent cells in transfection. Competent cells of strain 222 were prepared as described by Spatz and Trautner.[7] Samples were dissolved in dimethyl sulfoxide and diluted with 1/10 SSG (15 mM NaCl, 1.5 mM Na_3 citrate) to appropriate concentrations. M2-DNA (0.1 ml of 1 μg/ml) was mixed with 0.1 ml of a diluted sample solution. The mixture was incubated for 10—30 min at 30 °C and then 0.2 ml of competent cells was added. After a 40 min incubation at 30 °C, transfection was terminated by adding DNase I (50 μg/ml, 5 min, 30 °C).

Indicator cells (0.1 ml of noncompetent cells of strain 222) were mixed with transfection culture and 0.2 ml of the culture was withdrawn and spread on an LTT plate[6] in duplicate. After a 16—18 h incubation at 30 °C, the number of infective centers on the plate was counted. The effect of a sample on transfection was expressed as the number of infective centers per plate as a percentage of the number in a control transfection without the sample.

In order to determine the direct effect of a sample on competent cells, the same concentration of sample was added immediately before plating.

Results and Discussion

As shown in Table I, islandic acid-I (**1**) completely inhibited the growth of Yoshida sarcoma cells at 100 μg/ml, while its methyl ester (**2**) was 100 times more potent. In order to investigate the active site of islandic acid-I methyl ester (**2**), the cytotoxicity of side chain analogues of **2** and the α-pyrone moiety (**4**) against Yoshida sarcoma cells was examined.

However, neither methyl hexadienoates (**5**, **6**, **7**, **8**) nor the α-pyrone moiety (**4**) showed growth inhibition even at 100 μg/ml. Therefore, it may be considered that the ester linkage of hexadienoic acid with the α-pyrone moiety is necessary for the appearance of cytotoxicity against Yoshida sarcoma cells.

On the other hand, the inhibitions by islandic acid-I methyl ester (**2**) and islandic acid-II methyl ester (**4**) of the transfection of *Bacillus* phage M2-DNA were similar in order of magnitude (Table II). It was interesting that among methyl hexadienoates, the 2*Z*,4*E*-isomer

TABLE I. Growth Inhibition of Yoshida Sarcoma Cells in Tissue Culture

Sample	Concentration (μg/ml)	Inhibition (%)
Islandic acid-I (**1**)	100	100
	50	
Islandic acid-I methyl ester (**2**)	10	100
	1	100
	0.5	43
	0.1	—
Islandic acid-II (**3**)	100	—
Islandic acid-II methyl ester (**4**)	100	—
Methyl (2*Z*, 4*E*)-hexadienoate (**5**)	100	—
Methyl (2*Z*, 4*Z*)-hexadienoate (**6**)	100	—
Methyl (2*E*, 4*Z*)-hexadienoate (**7**)	100	—
Methyl (2*E*, 4*E*)-hexadienoate (**8**)	100	—

TABLE II. Transfection Assay

Sample	Concentration (μg/ml)	Inhibition (%)
Islandic acid-I methyl ester (**2**)	66.0	97
	6.6	17
	0.6	0
Islandic acid-II methyl ester (**4**)	33.0	83
	3.3	24
	0.3	19
Methyl (2*Z*, 4*E*)-hexadienoate (**5**)	100.0	95
	50.0	87
	25.0	71
	10.0	43
Methyl (2*Z*, 4*Z*)-hexadienoate (**6**)	100.0	39
	50.0	29
	25.0	0
	10.0	0
Methyl (2*E*, 4*Z*)-hexadienoate (**7**)	100.0	99
	50.0	68
	25.0	51
	10.0	0
Methyl (2*E*, 4*E*)-hexadienoate (**8**)	100.0	99
	50.0	75
	25.0	37
	10.0	0

(**5**) having the same configuration as the side chain of islandic acid inhibited the transfection of *Bacillus* phage M2-DNA over a wide concentration range (Table II).

Thus, we showed that islandic acid-I methyl ester (**2**) inhibited the growth of Yoshida sarcoma cells in tissue culture and the transfection of *Bacillus* phage M2-DNA. However, the

structure–activity relationship is not yet clear, and requires further study. Studies on the mode of action and the cytotoxicity of **2** and related compounds against other tumor cells are in progress.

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References and Notes

1) M. Saito, E. Enomoto, and T. Tatsuno, *Microbial Toxins*, **1971**, **6**, 299.
2) Y. Fujimoto, H. Tsunoda, J. Uzawa, and T. Tatsuno, *J. Chem. Soc., Chem. Commum.*, **1982**, 83.
3) Esterification should be carried out with an equimolar amount of diazomethane under ice cooling, because treatment of islandic acid-I or -II with excess diazomethane gave a yellow adduct.
4) The structures of methyl hexadienoates (**5**, **6**, **7**, **8**) were determined from the ^{1}H-NMR (400 MHz, $CDCl_3$, δ) data. **5**: 1.88 (3H, dd, J=1.0, 7.0 Hz, CH_3), 3.73 (3H, s, OCH_3), 5.56 (1H, dd, J=1.0, 11.5 Hz, H-2), 6.10 (1H, dq, J=7.0, 15.4 Hz, H-5), 6.55 (1H, t, J=11.5 Hz, H-3), 7.37 (1H, br dd, J=11.5, 15.4 Hz, H-4). **6**: 1.85 (3H, dd, J=1.7, 7.3 Hz, CH_3), 3.73 (3H, s, OCH_3), 5.69 (1H, d, J=11.5 Hz, H-2), 6.00 (1H, m, H-5), 6.97 (1H, ddd, J=1.0, 11.5, 11.5 Hz, H-3), 7.29 (1H, br t, J=11.5 Hz, H-4), **7**: 1.89 (3H, dd, J=1.7, 7.1 Hz, CH_3), 3.75 (3H, s, OCH_3), 5.86 (1H, d, J=15.4 Hz, H-2), 5.95 (1H, m, H-5), 6.16 (1H, ddq, J=1.7, 11.5, 11.7 Hz, H-4), 7.65 (1H, ddd, J=1.0, 11.7, 15.4 Hz, H-3), **8**: 1.86 (3H, br d, J=6.9 Hz, CH_3), 3.74 (3H, s, OCH_3), 5.78 (1H, br d, J=15.4 Hz, H-2), 6.10—6.23 (2H, m, H-4 and H-5), 7.26 (1H, dd, J=10.0, 15.4 Hz, H-3).
5) N. Biswal, A. K. Kleinschmidt, H. CH. Spatz, and T. A. Trautner, *Mol. Gen. Genet.*, **100**, 39 (1967).
6) H. Hirokawa, *Proc. Natl. Acad. Sci., U.S.A.*, **69**, 1555 (1972).
7) H. CH. Spatz and T. A. Trautner, *Mol. Gen. Genet.*, **109**, 84 (1970).