Eleven New 2-Pyrones from a Fungi Imperfecti, *Trichurus terrophilus*, Found in a Screening Study Guided by Immunomodulatory Activity

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In a screening study on immunomodulatory fungal constituents, eleven new 2-pyrones tentatively named TT-1 (1), and TT-2–11 (2–11) have been isolated from a Fungi Imperfecti, *Trichurus terrophilus*, and designated rasfonin (1), and trichurusin B–K (2–11), respectively. Compounds 1–4 exhibited considerably high immunosuppressive activities, and compounds 8–11 have shown moderate ones. The structure–activity relationships of these constituents have also been discussed.

Key words immunosuppressive fungal constituent; *Trichurus terrophilus*; Fungi Imperfecti; 2-pyrone; trichurusin; structure–activity relationship

In our screening program on immunomodulatory constituents from fungi, many immunosuppressive metabolites have already been isolated from various Ascomycetes belonging to *Gelasinospora*, *Diplogelasinospora*, *Microascus*, *Emericella*, *Eupenicillium*, *Chaetomium*, and *Zopfiella*.²⁾ The EtOAc extract of a Fungi Imperfecti, *Trichurus terrophilus* SWIFT & POVAH showed an appreciable suppressive effect on the proliferation (blastogenesis) of mouse splenic lymphocytes stimulated with mitogens, concanavalin A (Con A), and lipopolysaccharide (LPS). Solvent partitions followed by repeated chromatographic fractionations of the extract, guided by immunosuppressive activity, afforded eleven new compounds tentatively named TT-1–11 (1–11). This paper deals with the structures and immunosuppressive activities of these constituents.

The EtOAc extract of *T. terrophilus* IFM4606³⁾ cultivated on sterilized moistened-rice medium suppressed the Con A-induced proliferation of mouse splenic lymphocytes by 99.6% at 50 μ g/ml. The EtOAc extract was partitioned between *n*-hexane and water into an *n*-hexane layer and an aqueous suspension. The aqueous suspension was further partitioned between EtOAc and water into an EtOAc layer and an aqueous layer [yields (%) of the *n*-hexane, EtOAc, and aqueous layers after evaporation of the solvents from the EtOAc extract: 73.2, 17.4, and 2.3, respectively]. The IC₅₀ values of the *n*-hexane, EtOAc, and aqueous layers against the Con A-induced proliferation were 9.7, 2.5, and >50 μ g/ml, respectively. Repeated chromatographic fractionations monitored by the immunosuppressive activity of the EtOAc layer afforded four components tentatively named TT-1–4 (1–4) [yields (%) of 1, 2, 3, and 4 from the EtOAc extract: 0.26, 0.0078, 0.011, and 0.0089, respectively]. It was found out that the other strain of this fungus, *T. terrophilus* IMI46251³⁾ also produced 1 and its homologues in good yields. Repeated chromatographic fractionations of the EtOAc layer, which was obtained from the partition of the EtOAc extract of *T. terrophilus* IMI46251 in the same way as described for the partition of the EtOAc extract of *T. terrophilus* IFM4606, gave seven new constituents tentatively named TT-5–11 (5–11) in addition to 1–4 [yields (%) of 1, 2, 3, 4, 5, a mixture of 6 and 7 (6/7), 8, 9, 10, and 11 from the EtOAc extract: 1.7, 0.00088, 0.0091, 0.0044, 0.0025,

0.00094, 0.0019, 0.0036, 0.0011, and 0.00089, respectively].

TT-1 (1), obtained as an optically active white amorphous powder ($[\alpha]_D^{24}$ –223.6°), circular dichroism (CD) in MeOH (0.11 mM), $\Delta\epsilon$ (nm): –98 (266), –51 (239), –255 (212), gave C₂₅H₃₈O₆ as the molecular formula. The ¹H- and ¹³C-NMR spectral data of 1 (see Table 1) including the two dimensional ¹H–¹H shift correlation (COSY), ¹H-detected heteronuclear correlation through multiple quantum coherence (HMQC), and ¹H-detected heteronuclear multiple-bond correlation (HMBC) NMR data suggested a planar structure for TT-1. On acetylation with acetic anhydride in pyridine, 1 gave diacetate (12). Comparison of the ¹H- and ¹³C-NMR spectral data of 1 with those of 12 (Table 1) in reference to the acetylation shift rule,^{4,5)} confirmed the planar structure of TT-1 (1a) (see Fig. 1). TT-2 (2), obtained as an optically active colorless amorphous powder ($[\alpha]_D^{25}$ +18.4°), gave C₂₅H₄₀O₆ as the molecular formula. Comparison of the ¹H- and ¹³C-NMR spectral data of 2 (Table 1) with those of 1 revealed that the unsaturated C=C bond at position 3 in 1 was saturated in 2, indicating that the planar structure of TT-2 was expressed as 2 (Fig. 1). Then, in 2000 we reported the isolation and planar structures of TT-1 (1a) and 2 (2), two new immunosuppressive constituents from *T. terrophilus*.⁶⁾ Almost at the same time, Hayakawa *et al.* reported the isolation of rasfonin, a new apoptosis inducer from an Ascomycete, *Talaromyces* sp.⁷⁾ The planar structure and the physicochemical properties of rasfonin were the same as those of TT-1. In the absolute structure, the configurations of five chiral centers of TT-1 were determined to be 5*R*, 6*R*, 7*S*, 9*R*, and 6'*S* by the synthesis of diastereomers of two fragments of TT-1 (segments A, B) (Fig. 1) in optically active forms, and comparison of their spectral and optical data with those of natural specimens by us in 2003,⁸⁾ and finally by our total synthesis of TT-1 in 2005.⁹⁾ Thus, the structure of TT-1 was expressed as 1 including its absolute stereochemistry, as shown in Fig. 1.

Here, TT-1 (1), and TT-2–11 (2–11) from *Trichurus terrophilus* were designated as rasfonin, and trichurusin B–K, respectively. It was decided not to designate TT-1 (1) as trichurusin A because TT-1 was the same to rasfonin.

Trichurusin C (TT-3) (3), obtained as an optically active colorless amorphous powder ($[\alpha]_D^{25}$ –166.6°), has C₂₅H₃₈O₇

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Table 1. ^1H - and ^{13}C -NMR Data for TT-1 (Rasfonin), TT-1 (Rasfonin) Diacetate, Trichurusin B, Trichurusin J, and Trichurusin K in CDCl_3 , δ (ppm) from TMS as an Internal Standard

Position	TT-1 (Rasfonin) (1)		TT-1 (Rasfonin) diacetate (12)		Trichurusin B (2)		Trichurusin J (10)		Trichurusin K (11)	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2		163.3 (s)		163.2 (s)		176.9 (s)		163.4 (s)		163.4 (s)
3	6.21 (d, 9.8)	124.8 (d)	6.22 (d, 9.6)	125.0 (d)	2.49 (2H, m)	28.3 (t)	6.21 (d, 9.6)	125.0 (d)	6.21 (d, 9.6)	125.0 (d)
4	7.06 (dd, 9.8, 6.1)	140.6 (d)	7.05 (dd, 9.6, 5.9)	140.5 (d)	1.94 (m), 2.29 (m)	24.6 (t)	7.04 (dd, 9.6, 5.9)	140.6 (d)	7.04 (dd, 9.6, 5.9)	140.6 (d)
5	5.35 (dd, 6.1, 2.5)	61.6 (d)	5.36 (dd, 5.9, 2.1)	61.8 (d)	4.75 (m)	79.3 (d)	5.35 (dd, 5.9, 2.7)	61.8 (d)	5.35 (dd, 5.9, 2.7)	61.8 (d)
6	4.14 (dd, 8.9, 2.5)	83.3 (d)	4.14 (dd, 8.9, 2.1)	83.2 (d)	4.97 (dd, 5.8, 4.1)	76.9 (d)	4.12 (br d, 8.8)	83.3 (d)	4.12 (br d, 8.8)	83.4 (d)
7	2.18 (m)	31.3 (d)	2.17 (m)	31.4 (d)	2.02 (m)	31.9 (d)	2.16 (m)	31.5 (d)	2.16 (m)	31.5 (d)
8	1.04 (ddd, 13.4, 9.7, 4.5)	39.9 (t)	1.04 (m)	39.9 (t)	1.00 (m)	40.9 (t)	1.03 (m)	40.1 (t)	1.03 (m)	40.0 (t)
	1.20 (ddd, 13.4, 9.2, 4.0)		1.23 (m)		1.36 (m)		1.23 (m)		1.23 (m)	
9	1.69 (m)	27.8 (d)	1.64 (m)	27.9 (d)	1.71 (m)	28.1 (d)	1.67 (m)	28.0 (d)	1.67 (m)	28.0 (d)
10	1.44 (dd, 13.2, 9.9), 2.06 (br d, 13.2)	46.2 (t)	1.44 (dd, 13.1, 9.8), 2.08 (br d, 13.1)	46.3 (t)	1.63 (m), 2.02 (m)	47.2 (t)	1.43 (m), 2.06 (m)	46.4 (t)	1.43 (m), 2.06 (m)	46.4 (t)
11		134.1 (s)		134.1 (s)		134.4 (s)		134.3 (s)		134.2 (s)
12	5.12 (q, 6.7)	120.0 (d)	5.12 (q, 6.6)	120.0 (d)	5.16 (q, 6.3)	120.1 (d)	5.11 (q, 6.0)	120.2 (d)	5.11 (q, 6.0)	120.1 (d)
13	1.55 (3H, d, 6.7)	13.3 (q)	1.54 (3H, d, 6.6)	13.3 (q)	1.55 (3H, d, 6.3)	13.4 (q)	1.54 (3H, d, 6.3)	13.4 (q)	1.54 (3H, d, 6.3)	13.4 (q)
14	1.15 (3H, d, 6.7)	15.8 (q)	1.15 (3H, d, 6.4)	15.8 (q)	0.95 (3H, d, 6.9)	15.7 (q)	1.14 (3H, d, 6.3)	15.9 (q)	1.14 (3H, d, 6.3)	15.9 (q)
15	0.78 (3H, d, 6.7)	20.5 (q)	0.79 (3H, d, 6.4)	20.6 (q)	0.81 (3H, d, 6.6)	20.4 (q)	0.77 (3H, d, 6.3)	20.7 (q)	0.77 (3H, d, 6.3)	20.7 (q)
16	1.53 (3H, s)	15.5 (q)	1.53 (3H, s)	15.5 (q)	1.55 (3H, s)	15.7 (q)	1.52 (3H, s)	15.6 (q)	1.52 (3H, s)	15.6 (q)
1'		166.1 (s)		165.9 (s)		166.8 (s)		166.1 (s)		166.1 (s)
2'	5.80 (d, 15.6)	114.8 (d)	5.82 (d, 15.8)	115.6 (d)	5.86 (d, 15.7)	116.0 (d)	5.81 (d, 15.7)	115.4 (d)	5.81 (d, 15.7)	115.3 (d)
3'	7.34 (d, 15.6)	151.0 (d)	7.33 (d, 15.8)	150.5 (d)	7.35 (d, 15.7)	150.2 (d)	7.34 (d, 15.7)	150.8 (d)	7.33 (d, 15.7)	150.9 (d)
4'		134.3 (s)		135.2 (s)		134.9 (s)		135.5 (s)		135.0 (s)
5'	5.78 (br d, 8.9)	143.4 (d)	5.69 (d, 10.1)	141.4 (d)	5.76 (br d, 9.9)	142.7 (d)	5.71 (d, 9.9)	142.7 (d)	5.71 (d, 9.9)	142.5 (d)
6'	2.87 (m)	39.2 (d)	2.98 (m)	35.3 (d)	2.89 (m)	39.3 (d)	2.84 (m)	38.9 (d)	3.05 (m)	35.1 (d)
7'	1.61 (m), 1.79 (m)	34.8 (t)	1.62 (m), 1.91 (m)	30.5 (t)	1.63 (m), 1.82 (m)	34.9 (t)	1.57 (m), 1.91 (m)	30.4 (t)	1.52 (m), 1.80 (m)	34.4 (t)
8'	3.59 (m), 3.72 (m)	60.4 (t)	3.94 (m), 4.10 (m)	61.9 (t)	3.61 (m), 3.75 (m)	60.8 (t)	3.95 (m), 4.10 (m)	62.3 (t)	3.56 (m), 3.67 (m)	60.3 (t)
9'	1.83 (3H, s)	12.5 (q)	1.80 (3H, s)	12.5 (q)	1.86 (3H, s)	12.8 (q)	1.81 (3H, s)	12.7 (q)	1.81 (3H, s)	12.6 (q)
10'	3.59 (2H, d, 6.1)	65.6 (t)	4.02 (2H, d, 6.4)	66.5 (t)	3.61 (2H, m)	66.0 (t)	3.56 (m), 3.62 (m)	65.8 (t)	4.03 (2H, m)	66.9 (t)
8'-OCOCH ₃			2.04 (3H, s)	20.8 (q)			2.02 (3H, s)	21.0 (q)		
8'-OCOCH ₃				170.8 (s)				171.0 (s)		
10'-OCOCH ₃			2.03 (3H, s)	20.9 (q)					2.02 (3H, s)	20.9 (q)
10'-OCOCH ₃				170.8 (s)						171.0 (s)

Multiplicities and coupling constants (Hz) in parentheses.

Fig. 1

as the molecular formula. Comparison of the ^1H - and ^{13}C -NMR spectral data of **3** (Table 2) with those of **1** showed that instead of the signals of CH_3 -13 [δ_{H} 1.55 (3H, d), δ_{C} 13.3 (q)] and C-11 [δ_{C} 134.1 (s)], those of $\text{CH}_2=\text{CH}$ -

[δ_{H} 5.03, 5.19 (each 1H, dd), δ_{C} 111.7 (t)] and $>\text{C}(\text{O})$ - [δ_{C} 73.7 (s)] newly appeared, suggesting that the group $\text{CH}_3-\text{CH}=\text{C}(\text{CH}_3)-$ in segment A of **1** was replaced with the group $\text{CH}_2=\text{CH}-\text{C}(\text{CH}_3)(\text{OH})-$ in that of **3**. This was also

Fig. 2

Table 2. ^1H - and ^{13}C -NMR Data for Trichurusins C, D, and E in CDCl_3 , δ (ppm) from TMS as an Internal Standard

Position	Trichurusin C (3)		Trichurusin D (4)		Trichurusin E (5)	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2		163.4 (s)		163.3 (s)		163.3 (s)
3	6.21 (d, 9.6)	125.1 (d)	6.22 (d, 9.6)	125.1 (d)	6.21 (d, 9.6)	125.1 (d)
4	7.02 (dd, 9.6, 6.0)	140.5 (d)	7.02 (dd, 9.6, 6.1)	140.4 (d)	7.03 (dd, 9.6, 5.9)	140.5 (d)
5	5.39 (dd, 6.0, 2.5)	61.6 (d)	5.43 (dd, 6.1, 2.5)	61.4 (d)	5.42 (dd, 5.9, 2.6)	61.5 (d)
6	4.12 (dd, 9.1, 2.5)	83.2 (d)	4.13 (dd, 9.4, 2.5)	83.3 (d)	4.11 (dd, 9.3, 2.6)	83.4 (d)
7	2.08 (m)	31.5 (d)	2.14 (m)	31.7 (d)	2.11 (m)	31.6 (d)
8	0.94 (m), 1.25 (m)	41.3 (t)	1.05 (m), 1.25 (m)	40.1 (t)	0.94 (m), 1.23 (m)	41.3 (t)
9	1.62 (m)	26.5 (d)	1.76 (m)	28.5 (d)	1.63 (m)	26.4 (d)
10	1.19 (m), 1.46 (dd, 14.2, 2.6)	48.1 (t)	1.53 (dd, 14.9, 10.2), 2.11 (dd, 14.9, 3.5)	38.8 (t)	1.23 (m), 1.52 (m)	44.1 (t)
11		73.7 (s)		151.5 (s)		84.8 (s)
12	5.83 (dd, 17.3, 10.7)	145.2 (d)	4.12 (q, 6.5)	70.6 (d)	5.82 (dd, 12.4, 10.4)	141.8 (d)
13	5.03 (dd, 10.7, 1.2), 5.19 (dd, 17.3, 1.2)	111.7 (t)	1.22 (3H, d, 6.5)	22.4 (q)	5.17 (br d, 10.4), 5.18 (br d, 12.4)	115.3 (t)
14	1.12 (3H, d, 6.6)	16.2 (q)	1.16 (3H, d, 6.6)	16.3 (q)	1.15 (3H, d, 5.5)	16.5 (q)
15	0.96 (3H, d, 6.6)	23.3 (q)	0.87 (3H, d, 6.3)	20.9 (q)	0.96 (3H, d, 6.6)	23.2 (q)
16	1.20 (3H, s)	29.2 (q)	4.75 (s), 5.07 (s)	109.5 (t)	1.30 (3H, s)	21.6 (q)
1'		166.2 (s)		166.2 (s)		166.3 (s)
2'	5.82 (d, 15.5)	115.1 (d)	5.82 (d, 15.7)	115.0 (d)	5.83 (d, 15.6)	115.2 (d)
3'	7.35 (d, 15.5)	151.2 (d)	7.36 (d, 15.7)	151.3 (d)	7.37 (d, 15.6)	151.2 (d)
4'		134.6 (s)		134.8 (s)		135.0 (s)
5'	5.81 (d, 8.5)	143.8 (d)	5.80 (d, 11.3)	144.0 (d)	5.83 (d, 6.9)	143.6 (d)
6'	2.87 (m)	39.4 (d)	2.87 (m)	39.3 (d)	2.90 (m)	39.3 (d)
7'	1.58 (m), 1.80 (m)	34.8 (t)	1.59 (dd, 13.9, 8.2), 1.79 (dd, 13.9, 8.1)	34.7 (t)	1.60 (m), 1.81 (m)	34.6 (t)
8'	3.60 (m), 3.72 (m)	60.6 (t)	3.59 (m), 3.70 (m)	60.6 (t)	3.60 (m), 3.73 (m)	60.8 (t)
9'	1.83 (3H, s)	12.7 (q)	1.83 (3H, s)	12.7 (q)	1.86 (3H, s)	12.7 (q)
10'	3.59 (dd, 7.6, 6.3), 3.60 (dd, 7.6, 6.0)	65.8 (t)	3.57 (dd, 10.7, 6.5), 3.63 (dd, 10.7, 5.8)	65.9 (t)	3.60 (m), 3.65 (m)	65.9 (t)

Multiplicities and coupling constants (in Hz) in parentheses.

supported by the molecular formula and the HMBC NMR data of **3** (Fig. 2). Accordingly, the structure of trichurusin C was deduced to be **3**, as shown in Fig. 2.

Trichurusin D (TT-4) (**4**), obtained as an optically active white amorphous powder ($[\alpha]_{\text{D}}^{25} -78.1^\circ$), has $\text{C}_{25}\text{H}_{38}\text{O}_7$ as the molecular formula. Comparison of the ^1H - and ^{13}C -NMR

spectral data of **4** (Table 2) with those of **1** showed that instead of the signals of CH_3 -16 [δ_{H} 1.53 (3H, s), δ_{C} 15.5 (q)] and CH -12 [δ_{H} 5.12 (1H, q), δ_{C} 120.0 (d)], those of $\text{CH}_2=\text{C}<$ [δ_{H} 4.75, 5.07 (each 1H, s), δ_{C} 109.5 (t)] and $-\text{CH}(\text{O})-$ [δ_{H} 4.12 (q), δ_{C} 70.6 (d)] newly appeared, suggesting that the group $\text{CH}_3-\text{CH}=\text{C}(\text{CH}_3)-$ in segment A of **1**

Table 3. ^1H - and ^{13}C -NMR Data for Trichurusins F, G, H, and I in CDCl_3 , δ (ppm) from TMS as an Internal Standard

Position	Trichurusin F (6)		Trichurusin G (7)		Trichurusin H (8)		Trichurusin I (9)	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2		163.0 (s)		163.5 (s)		163.2 (s)		163.2 (s)
3	6.23 (d, 9.3)	125.0 (d)	6.25 (d, 9.3)	125.0 (d)	6.22 (d, 9.6)	125.1 (d)	6.22 (d, 9.6)	125.1 (d)
4	7.02 (m)	140.3 (d)	6.99 (m)	140.3 (d)	7.03 (dd, 9.6, 6.0)	140.5 (d)	7.02 (dd, 9.6, 6.0)	140.4 (d)
5	5.41 (br d, 6.0)	61.5 (d)	5.48 (br d, 5.5)	61.5 (d)	5.37 (dd, 6.0, 2.7)	61.7 (d)	5.38 (dd, 6.0, 2.5)	61.3 (d)
6	4.16 (br d, 6.9)	83.0 (d)	4.17 (br d, 9.1)	83.0 (d)	4.15 (dd, 8.7, 2.7)	83.0 (d)	4.11 (dd, 9.3, 2.5)	83.3 (d)
7	2.17 (m)	31.7 (d)	2.17 (m)	31.7 (d)	2.09 (m)	31.6 (d)	2.14 (m)	31.3 (d)
8	1.11 (m), 1.26 (m)	39.8 (t)	1.11 (m), 1.26 (m)	39.8 (t)	1.03 (m), 1.25 (m)	40.4 (t)	1.06 (m), 1.20 (m)	40.2 (t)
9	1.85 (m)	28.4 (d)	1.85 (m)	28.4 (d)	1.77 (m)	27.4 (d)	1.71 (m)	27.4 (d)
10	1.66 (m), 2.33 (dd, 13.1, 4.3)	47.4 (t)	1.70 (m), 2.36 (br d, 9.9)	38.4 (t)	1.00 (br d, 12.0), 1.66 (dd, 12.0, 4.4)	44.3 (t)	0.54 (t, 12.7), 1.97 (dd, 12.7, 3.6)	45.0 (t)
11		163.0 (s)		164.0 (s)		60.1 (s)		60.1 (s)
12	5.78 (d, 8.0)	129.0 (d)	5.93 (d, 8.4)	130.5 (d)	2.69 (q, 5.5)	58.2 (d)	2.76 (q, 5.6)	60.5 (d)
13	9.94 (d, 8.0)	191.2 (d)	9.75 (d, 8.4)	190.8 (d)	1.26 (3H, d, 5.5)	14.0 (q)	1.25 (3H, d, 5.6)	14.0 (q)
14	1.18 (3H, d, 6.9)	16.0 (q)	1.19 (3H, d, 6.9)	16.0 (q)	1.14 (3H, d, 6.6)	16.0 (q)	1.15 (3H, d, 6.6)	15.7 (q)
15	0.86 (3H, d, 6.6)	20.6 (q)	0.87 (3H, d, 6.6)	20.6 (q)	0.97 (3H, d, 6.6)	21.5 (q)	0.87 (3H, d, 6.6)	21.0 (q)
16	2.12 (3H, s)	17.3 (q)	1.93 (3H, s)	24.7 (q)	1.22 (3H, s)	16.6 (q)	1.19 (3H, s)	16.2 (q)
1'		166.2 (s)		166.2 (s)		166.1 (s)		166.2 (s)
2'	5.81 (d, 15.4)	114.8 (d)	5.81 (d, 15.6)	114.8 (d)	5.80 (d, 15.3)	115.0 (d)	5.79 (d, 15.7)	114.8 (d)
3'	7.38 (d, 15.4)	151.5 (d)	7.41 (d, 15.6)	151.7 (d)	7.34 (d, 15.3)	151.2 (d)	7.32 (d, 15.7)	151.5 (d)
4'		134.6 (s)		134.6 (s)		134.7 (s)		134.9 (s)
5'	5.84 (d, 11.5)	144.3 (d)	5.84 (d, 11.5)	144.3 (d)	5.80 (d, 10.7)	143.7 (d)	5.79 (d, 12.1)	144.0 (d)
6'	2.89 (m)	39.2 (d)	2.89 (m)	39.2 (d)	2.89 (m)	39.3 (d)	2.89 (m)	39.4 (d)
7'	1.61 (m), 1.81 (m)	34.7 (t)	1.61 (m), 1.81 (m)	34.7 (t)	1.61 (m), 1.83 (m)	34.8 (t)	1.60 (m), 1.80 (m)	34.6 (t)
8'	3.59 (m), 3.71 (m)	60.7 (t)	3.59 (m), 3.71 (m)	60.7 (t)	3.61 (m), 3.73 (m)	60.7 (t)	3.61 (m), 3.70 (m)	60.6 (t)
9'	1.84 (3H, s)	12.7 (q)	1.81 (3H, s)	12.7 (q)	1.85 (3H, s)	12.7 (q)	1.85 (3H, s)	12.8 (q)
10'	3.61 (m), 3.64 (m)	65.9 (t)	3.61 (m), 3.64 (m)	65.9 (t)	3.60 (dd, 10.4, 6.2), 3.63 (dd, 10.4, 6.9)	65.9 (t)	3.57 (dd, 11.0, 6.7), 3.63 (dd, 11.0, 5.8)	66.1 (t)

Multiplicities and coupling constants (in Hz) in parentheses.

was replaced with $\text{CH}_3\text{--CH(OH)--C(=CH}_2\text{)--}$ in that of **4**. This was also supported by the molecular formula and the HMBC NMR data of **4** (Fig. 2). Accordingly, the structure of trichurusin D was deduced to be **4**, as shown in Fig. 2.

Trichurusin E (TT-5) (**5**) was obtained as a white amorphous powder. Comparison of the ^{13}C -NMR spectral data of **5** (Table 2) with those of **3** showed that all of the signals of **5** were quite similar to those of **3** except that the signals of C-10, -11, -12, -13, and -16 were shifted to δ_{C} 44.1 (−4.0), 84.8 (+11.1), 141.8 (−3.4), 115.3 (+3.6), and 21.6 (−7.6), respectively, suggesting that **5** might be the epimer of **3** at position 11, as shown in Fig. 2. This was also supported by the fact that the signals of $\text{H}_2\text{--10}$, $\text{H}_2\text{--13}$, and $\text{H}_3\text{--16}$ were only shifted in comparison to the ^1H -NMR spectral data of **5** (Table 2) with those of **3**. Though other physicochemical properties of **5** remained unclear because this compound was gradually decomposed while standing at room temperature, we proposed the tentative structure as shown in Fig. 2 to **5**.

A mixture of trichurusins F (TT-6) (**6**) and G (TT-7) (**7**) was obtained as a uniformly white amorphous powder, which was estimated to be a mixture of **6** and its stereoisomer (**7**) in the ratio of *ca.* 3 to 2 by the ^1H - and ^{13}C -NMR spectral data (Table 3). The mixture of **6** and **7** (**6/7**) gave $\text{C}_{25}\text{H}_{36}\text{O}_7$ as their common molecular formulae. In the ^1H - and ^{13}C -NMR spectra, each signal due to **6** was independently observed from each corresponding one due to **7**. Comparison of the ^1H - and ^{13}C -NMR spectral data of both **6** and **7** with those of **1** showed that instead of the signal of $\text{CH}_3\text{--13}$, those of CHO--13 of **6** [δ_{H} 9.94 (d), δ_{C} 191.2 (d)] and **7** [δ_{H} 9.75 (d), δ_{C} 190.8 (d)] newly appeared, respectively, suggesting that the group $\text{CH}_3\text{--CH=C(CH}_3\text{)--}$ in segment A of **1** was replaced

with $\text{CHO--CH=C(CH}_3\text{)--}$ in that of both **6** and **7**. This was also supported by the common molecular formulae and the HMBC NMR data (Fig. 3). In the differential nuclear Overhauser effect (DifNOE) experiment, 2.3% of NOE was observed between CHO--13 (δ_{H} 9.94) and $\text{CH}_3\text{--16}$ [δ_{H} 2.12 (3H, s)] of **6**, meanwhile, 4.8% of NOE was observed between CHO--13 (δ_{H} 9.75) and one H of $\text{CH}_2\text{--10}$ [δ_{H} 2.36 (1H, br d)] of **7**, indicating that the configuration at position 11 might be *E* in **6**, but *Z* in **7**. Accordingly, the structures of trichurusins F and G were deduced to be **6** and **7**, respectively, as shown in Fig. 3.

Trichurusin H (TT-8) (**8**), obtained as an optically active white amorphous powder ($[\alpha]_{\text{D}}^{25} -124.9^\circ$), has $\text{C}_{25}\text{H}_{38}\text{O}_7$ as its molecular formula. Comparison of the ^1H - and ^{13}C -NMR spectral data of **8** (Table 3) with those of **1** revealed that large shifts were observed on the signals of C-11 [δ_{C} 60.1 (−74.0)] and CH-12 [δ_{H} 2.69 (−2.43), δ_{C} 58.2 (−61.8)], and on the signals of $\text{CH}_2\text{--10}$ [δ_{H} 1.00 (br d), 1.66 (dd)], $\text{CH}_3\text{--13}$ [δ_{H} 1.26 (3H, d)], and $\text{CH}_3\text{--16}$ [δ_{H} 1.22 (3H, s)], suggesting that instead of the C=C bond at position 11(12), an epoxy ring might be present between C-11 and C-12 of **8**. This was also supported by the molecular formula and the HMBC NMR data (Fig. 3). Accordingly, the structure of trichurusin H was deduced to be **8**, as shown in Fig. 3.

Trichurusin I (TT-9) (**9**), obtained as an optically active white amorphous powder ($[\alpha]_{\text{D}}^{25} -167.3^\circ$), gave $\text{C}_{25}\text{H}_{38}\text{O}_7$ as the molecular formula, indicating that **9** was an isomer of **8**. Comparison of the ^1H - and ^{13}C -NMR spectral data of **9** (Table 3) with those of **8** showed that all of the signals of **9** were quite similar to those of **8** except for the presence of considerably large shifts in the signals of $\text{CH}_2\text{--10}$ [δ_{H} 0.54

Fig. 3

(-0.46), 1.97 ($+0.31$), δ_C 45.0 ($+0.7$)] and CH-12 [δ_H 2.76 ($+0.07$), δ_C 60.5 ($+2.3$)], suggesting that **9** might be a diastereomer of **8** concerning the configuration of the epoxy ring between C-11 and C-12. This was also supported by the HMBC NMR data (Fig. 3). Accordingly, the structure of trichurusin I was deduced to be **9**, as shown in Fig. 3.

Trichurusin J (TT-10) (**10**), obtained as an optically active colorless amorphous powder ($[\alpha]_D^{24} -135.1^\circ$), gave $C_{27}H_{40}O_7$ as the molecular formula. Comparison of the 1H - and ^{13}C -NMR spectral data of **10** (Table 1) with those of **1** showed that the signal of CH_3CO newly appeared at δ_H 2.02 (3H, s), δ_C 21.0 (q), and 171.0 (s), and the signals of C-8' and -7' were shifted to δ_C 62.3 ($+1.9$) and 30.4 (-4.4), respectively, indicating that, considering the acetylation shift rule,^{4,5} **10** might be an 8'-O-acetyl ester of **1**. This was also supported by the molecular formula. Accordingly, the structure of trichurusin J was deduced to be **10**, as shown in Fig. 1.

Trichurusin K (TT-11) (**11**), obtained as an optically active colorless amorphous powder ($[\alpha]_D^{25} -122.3^\circ$), gave $C_{27}H_{40}O_7$ as the molecular formula. Comparison of the 1H - and ^{13}C -NMR spectral data of **11** (Table 1) with those of **1** showed that the signal of CH_3CO newly appeared at δ_H 2.02 (3H, s), δ_C 20.9 (q), and 171.0 (s), and the signals of C-10' and -6' were shifted to δ_C 66.9 ($+1.3$) and 35.1 (-4.1), respectively, indicating that, **11** might be a 10'-O-acetyl ester of **1**. This was also supported by the molecular formula. Accordingly, the structure of trichurusin K was deduced to be **11**, as shown in Fig. 1.

The fact that the specific rotations and the CD curves of trichurusins C (**3**), D (**4**), the mixture of trichurusins F and G (**6/7**), H (**8**), I (**9**), J (**10**), and K (**11**) (see Experimental) were similar to those of **1** suggested that the absolute configurations at positions 5, 6, 7, 9, and 6' in **3**, **4**, **6**, **7**, **8**, **9**, **10**, and **11** might be the same as those in **1** (5*R*, 6*R*, 7*S*, 9*R*, and 6'*S*), as shown in Figs. 1, 2, and 3. Though the optical data of trichurusin E (**5**) were not obtained from its decomposition, **5** was considered to be the epimer of **3** at position 11 from the 1H - and ^{13}C -NMR spectral data as mentioned above. Mean-

Table 4. Immunosuppressive Effects of TT-1 (Rasfonin), Trichurusins B—D, H—K, TT-1 (Rasfonin) Diacetate, and Azathioprine, Cyclosporin A, and Tacrolimus (FK506) on the Con A-Induced and LPS-Induced Proliferations of Mouse Splenic Lymphocytes

Compound	IC ₅₀ (μ g/ml)	
	Con A-induced	LPS-induced
TT-1 (Rasfonin) (1)	0.7	0.5
Trichurusin B (2)	2.0	0.8
Trichurusin C (3)	1.2	0.4
Trichurusin D (4)	0.9	0.4
Trichurusin H (8)	6.2	6.0
Trichurusin I (9)	4.7	4.5
Trichurusin J (10)	n.t.	6.4
Trichurusin K (11)	6.9	6.9
TT-1 (Rasfonin) diacetate (12)	6.0	6.9
Azathioprine	2.7	2.7
Cyclosporin A	0.04	0.07
Tacrolimus (FK506)	1.5×10^{-5}	1.6×10^{-3}

The IC₅₀ value of each sample was calculated from the correlation curve between the sample concentration (horizontal axis) and the cell proliferation (vertical axis). The curve of each sample was drawn with 7 points, each of which represented the mean of three experiments on each correlation between 7 different concentrations and cell proliferations. n.t.: not tested.

while, the absolute stereochemistry of trichurusin B (**2**) remained pending, because the specific rotation and the CD curve of **2** (see Experimental) were different from those of **1**.

The immunosuppressive activities (IC₅₀ values) of **1—4**, **8—11**, and diacetate of **1** (**12**) were calculated against Con A-induced (T cell) and LPS-induced (B cell) proliferations of mouse splenic lymphocytes, as shown in Table 4. Comparison of the IC₅₀ values of these new compounds with those of some known immunosuppressants in Table 4 showed that **1—4** possessed considerably high immunosuppressive activities, while **8—11** possessed moderate ones. The fact that the immunosuppressive activities of **10**, **11** and **12** were lower than that of **1** suggests that the presence of both free OH groups at positions 8' and 10' might be important for the appearance of the immunosuppressive activity of **1**.

Experimental

The general procedures for chemical experiments and other experimental conditions, including those for the evaluation of suppressive activity (IC_{50} values) of samples against the proliferation of mouse splenic lymphocytes stimulated with Con A and LPS, were the same as those described in our previous reports [this method is based on the formation ratio of MTT-formazan from exogenous 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) in lymphocytes].²⁾ Chemical shifts are expressed in δ (ppm) values from tetramethylsilane (TMS) as an internal standard.

Isolation of Rasfonin (1) and Trichurusins B–D (2–4) from *T. terrophilus* IFM4606 *T. terrophilus* IFM4606³⁾ was cultivated on sterilized moistened-rice in Roux flasks (200 g/flask $\times$ 85) at 25 °C for 20 d. The whitish gray moldy-rice was extracted with EtOAc (20 l) with shaking at room temperature for 6 h twice to give an EtOAc solution (ca. 40 l), which gave, after evaporation *in vacuo*, an EtOAc extract (32.44 g). A concentrated solution of the EtOAc extract in MeOH (30 ml) was suspended in H₂O (600 ml). The suspension was partitioned with *n*-hexane (600 ml) twice into an *n*-hexane layer (after evaporation *in vacuo*, 23.75 g) and an aqueous suspension. The aqueous suspension was further partitioned with EtOAc (600 ml) twice into an EtOAc layer (5.63 g) and an aqueous layer (0.75 g). The IC_{50} values of the *n*-hexane, EtOAc, and aqueous layers against the Con A-induced proliferation of mouse splenic lymphocytes were 9.7, 2.5, and >50 μ g/ml, respectively. The EtOAc layer was subjected to chromatography on a silica gel (Wakogel C-200, Wako) column with *n*-hexane–EtOAc (15 : 1, v/v), (7.5 : 1), (5 : 1), (2 : 1), (1 : 3), EtOAc, and MeOH to give seven fractions 1a–g (73, 99, 758, 714, 431, 479, 987 mg), whose IC_{50} values against the Con A-induced proliferation were 39.3, 19.3, 22.1, 1.6, 1.8, 1.2, and 18.7 μ g/ml, respectively. Fraction 1f was further chromatographed on a silica gel column with CHCl₃–MeOH (50 : 1), (37.5 : 1), (17.5 : 1), (10 : 1), (7.5 : 1), and (2.5 : 1) to give six fractions 2a–f (19, 230, 105, 70, 22, 13 mg), respectively. Fraction 2b was separated by HPLC on an octadecyl silica gel (ODS) column (Pegasil ODS, Senshu) with MeOH–H₂O (3 : 1) at a flow rate of 8.0 ml/min to afford seven fractions 3a–g (25.0, 5.9, 83.6, 13.3, 4.1, 2.5, 19.8 mg), respectively. Fractions 3c and f directly afforded **1** (83.6 mg) and **2** (2.5 mg), respectively. Fraction 3a was further separated by HPLC on a silica gel column (Aqasil SS-652, Senshu) with CHCl₃–MeOH (30 : 1) at a flow rate of 2.0 ml/min to afford **3** (0.9 mg) and **4** (0.7 mg). Fraction 2c was treated with MeOH to give a MeOH-soluble portion (72 mg), which was separated by HPLC on an ODS column with CH₃CN–H₂O (3 : 7) at a flow rate of 8.0 ml/min to give six fractions 4a–f (7.2, 1.8, 3.2, 9.7, 1.8, 24.5 mg), respectively. Fraction 4c was further separated by HPLC on a silica gel column with CHCl₃–MeOH (20 : 1) at a flow rate of 2.0 ml/min to afford **3** (1.3 mg) and **4** (1.1 mg). Fraction 4d was also separated by HPLC on an ODS column with CH₃CN–H₂O (1 : 3) to afford **3** (1.3 mg) and **4** (1.1 mg).

Isolation of Rasfonin (1) and Trichurusins B–K (2–11) from *T. terrophilus* IFM46251 *T. terrophilus* IFM46251³⁾ was cultivated on sterilized moistened-rice in Roux flasks (200 g/flask $\times$ 150) at 25 °C for 20 d. The greenish gray moldy-rice was extracted with EtOAc (40 l) with shaking at room temperature for 6 h twice to give an EtOAc extract (159.8 g), which was dissolved in MeOH (100 ml) and then suspended in H₂O (1000 ml). The suspension was partitioned with *n*-hexane twice (750, 375 ml) into an *n*-hexane layer (70.2 g) and an aqueous suspension. The aqueous suspension was further partitioned with EtOAc twice (750, 375 ml) into an EtOAc layer (34.9 g) and an aqueous layer (40.1 g). The EtOAc layer was subjected to chromatography on a silica gel (PSQ100B, Fuji Silysia) column with *n*-hexane–*n*-hexane–EtOAc (3 : 1), (1 : 1), (1 : 3), EtOAc, and MeOH to give five fractions 1a–e (19.78, 3.00, 1.37, 4.41, 5.03 g), respectively. Fraction 1d was further chromatographed on a silica gel column with CHCl₃–MeOH (50 : 1), (50 : 1), (25 : 1), (10 : 1), (10 : 1), and MeOH to give six fractions 2a–f (176, 2593, 403, 934, 83, 211 mg), respectively. Fraction 2b was separated by chromatography on an ODS (Chromatorex, Fuji Silysia) column with CH₃CN–H₂O (2 : 3), (1 : 1), (3 : 2), and CH₃CN to give four fractions 3a–d (285, 1675, 62, 230 mg), respectively. Fraction 3b was separated by HPLC on an ODS column (ODS-5251S, Senshu) with CH₃CN–H₂O (1 : 1), (1 : 1), (1 : 1), (3 : 2), (3 : 2), (3 : 2), and (3 : 2) at a flow rate of 7.0 ml/min to afford eight fractions 4a–h (20, 10, 19, 15, 11, 1328, 47, 36 mg), respectively. Fraction 4f directly afforded **1** (1328 mg). Fractions 4c, d, and g were purified by chromatography on silica gel columns with CHCl₃–MeOH (30 : 1) to afford **8** (3.0 mg), a fraction 4d' (8.9 mg), and a fraction 4g' (13 mg), respectively. Fractions 4d' and g' were further purified by HPLC on ODS columns with CH₃CN–H₂O (1 : 1) to afford **9** (5.8 mg) and **2** (1.4 mg), respectively. Fraction 2d was separated by chromatography

on an ODS column with CH₃CN–H₂O (2 : 3), (2 : 3), (2 : 3), (2 : 3), (1 : 1), (1 : 1), and CHCl₃ to give seven fractions 5a–g (69, 159, 343, 39, 26, 26, 158 mg), respectively. Fraction 5c was further chromatographed by HPLC on an ODS column with CH₃CN–H₂O (2 : 3) to give six fractions 6a–f (5, 15, 16, 23, 38, 118 mg), respectively. Fraction 6a directly afforded **4** (5.0 mg). Fractions 6b, c, and d were purified by HPLC on ODS columns with CH₃CN–H₂O (2 : 3) to afford **3** (10 mg), both **3** (4.6 mg) and **4** (2.0 mg), and both **5** (4.0 mg) and **6/7** (1.5 mg), respectively. The *n*-hexane layer was subjected to chromatography on a silica gel column with *n*-hexane–*n*-hexane–EtOAc (1 : 1), (1 : 3), (1 : 6), EtOAc–EtOAc–MeOH (15 : 1), (7.5 : 1), (3.5 : 1)–(1 : 1), and MeOH to give seven fractions 7a–g (4.80, 6.98, 3.51, 3.11, 2.20, 1.86, 1.13 g), respectively. Fraction 7c was further chromatographed on an ODS column with CH₃CN–H₂O (2 : 3), (2 : 3), (2 : 3), (1 : 1), (3 : 2), (3 : 2), CH₃CN–CHCl₃ to give seven fractions 8a–g (171, 52, 1405, 31, 58, 284, 552 mg), respectively. Fraction 8c directly afforded **1** (1405 mg). Fraction 8e was separated by HPLC on an ODS column (Develosil ODS-SR-5, Nomura) with CH₃CN–H₂O (7 : 3) at a flow rate of 1.8 ml/min to give four fractions 9a–d (1.1, 3.2, 24.9, 5.2 mg), respectively. Fractions 9b and c were separated by preparative TLC on silica gel plates (Kieselgel 60, Merck) with CHCl₃–MeOH (20 : 1) to afford **10** (1.8 mg) and **11** (1.4 mg).

Rasfonin (1): White amorphous powder (lit.⁷⁾ colorless oil), $[\alpha]_D^{25}$ –223.6° (c =6.00, MeOH) (lit.⁷⁾ –170° (MeOH). HR-FAB-MS m/z : 435.2736 (Calcd for C₂₅H₃₉O₆ [(M+H)⁺]: 435.2747) (lit.⁷⁾ 435.2754). UV λ_{max}^{MeOH} nm (log ϵ): 202 (4.36), 270 (4.49) (lit.⁷⁾ 202 (4.21), 270 (4.36)). IR ν_{max}^{KBr} cm^{–1}: 3437, 2926, 1715, 1621, 1258, 1158 (lit.⁷⁾ 3450, 1730). CD (0.11 mm, MeOH) $\Delta\epsilon$ (nm): –98 (266), –51 (239), –255 (212).

Trichurusin B (2): Colorless amorphous powder, $[\alpha]_D^{25}$ +18.4° (c =0.71, MeOH). HR-FAB-MS m/z : 437.2878 (Calcd for C₂₅H₄₁O₆ [(M+H)⁺]: 437.2904). UV λ_{max}^{MeOH} nm (log ϵ): 203 (4.19), 268 (4.35). CD (0.062 mm, MeOH) $\Delta\epsilon$ (nm): +0.34 (285), –0.40 (253), +4.9 (211).

Trichurusin C (3): Colorless amorphous powder, $[\alpha]_D^{25}$ –166.6° (c =1.08, MeOH). HR-FAB-MS m/z : 473.2541 (Calcd for C₂₅H₃₈O₇Na [(M+Na)⁺]: 473.2516). UV λ_{max}^{MeOH} nm (log ϵ): 206 (4.32), 271 (4.37). CD (0.094 mm, MeOH) $\Delta\epsilon$ (nm): –64 (269), –40 (238), –214 (212).

Trichurusin D (4): White amorphous powder, $[\alpha]_D^{25}$ –78.1° (c =1.44, MeOH). HR-FAB-MS m/z : 473.2539 (Calcd for C₂₅H₃₈O₇Na [(M+Na)⁺]: 473.2515). UV λ_{max}^{MeOH} nm (log ϵ): 203 (4.18), 270 (4.29). CD (0.12 mm, MeOH) $\Delta\epsilon$ (nm): –32 (264), –17 (241), –100 (212).

Trichurusin E (5): White amorphous powder.

Mixture of Trichurusins F and G (6/7) (in the Ratio of ca. 3 to 2): Uniformly white amorphous powder, $[\alpha]_D^{25}$ –73.9° (c =0.45, MeOH). HR-FAB-MS m/z : 472.2421 (Calcd for C₂₅H₃₇O₇Na [(M+H+Na)⁺]: 472.2437). UV λ_{max}^{MeOH} nm (log ϵ): 206 (4.17), 241 (sh, 4.02), 269 (4.10). CD (0.17 mm, MeOH) $\Delta\epsilon$ (nm): –56 (266), –2.4 (241), –133 (211).

Trichurusin H (8): White amorphous powder, $[\alpha]_D^{25}$ –124.9° (c =0.80, MeOH). HR-FAB-MS m/z : 451.2677 (Calcd for C₂₅H₃₉O₇ [(M+H)⁺]: 451.2696). UV λ_{max}^{MeOH} nm (log ϵ): 205 (4.38), 270 (4.31). CD (0.092 mm, MeOH) $\Delta\epsilon$ (nm): –66 (271), –39 (241), –259 (214).

Trichurusin I (9): White amorphous powder, $[\alpha]_D^{25}$ –167.3° (c =2.9, MeOH). HR-FAB-MS m/z : 451.2691 (Calcd for C₂₅H₃₉O₇ [(M+H)⁺]: 451.2695). UV λ_{max}^{MeOH} nm (log ϵ): 204 (4.33), 272 (4.37). CD (0.13 mm, MeOH) $\Delta\epsilon$ (nm): –69 (273), –40 (239), –232 (212).

Trichurusin J (10): Colorless amorphous powder, $[\alpha]_D^{24}$ –135.1° (c =0.17, MeOH). HR-FAB-MS m/z : 477.2839 (Calcd for C₂₇H₄₁O₇ [(M+H)⁺]: 477.2852). CD (0.11 mm, MeOH) $\Delta\epsilon$ (nm): –9.2 (265), –5.4 (240), –31.1 (212).

Trichurusin K (11): Colorless amorphous powder, $[\alpha]_D^{25}$ –122.3° (c =0.23, MeOH). HR-FAB-MS m/z : 477.2822 (Calcd for C₂₇H₄₁O₇ [(M+H)⁺]: 477.2852). CD (0.090 mm, MeOH) $\Delta\epsilon$ (nm): +0.24 (325), –8.2 (264), –4.3 (240), –21.0 (212).

Acetylation of Rasfonin (1) A solution of **1** (10.6 mg) in pyridine (1.0 ml) and acetic anhydride (0.5 ml) was allowed to stand at room temperature for 2.5 h. A small volume of ice-water was added to the reaction mixture, which was then extracted with EtOAc. The EtOAc solution was treated as usual to afford **12** (10.9 mg).

Rasfonin Diacetate (12): Colorless amorphous powder, $[\alpha]_D^{23}$ –95.7° (c =0.72, MeOH), CD (0.12 mm, MeOH) $\Delta\epsilon$ (nm): +0.089 (319), –1.65 (264), –1.16 (248), –19.8 (214).

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References and Notes

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