FR901459, A NOVEL IMMUNOSUPPRESSANT ISOLATED FROM *Stachybotrys chartarum* No. 19392

TAXONOMY OF THE PRODUCING ORGANISM, FERMENTATION, ISOLATION, PHYSICO-CHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITIES

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FR901459, a novel immunosuppressant, has been isolated from the fermentation broth of *Stachybotrys chartarum* No. 19392. The molecular formula of FR901459 was determined as $C_{62}H_{111}N_{11}O_{13}$. FR901459 was found to be a member of the cyclosporin family. However, it is structurally distinct from any other cyclosporins discovered so far, in that Leu is present at position 5 instead of Val. FR901459 was capable of prolonging the survival time of skin allografts in rats with one third the potency of cyclosporin A.

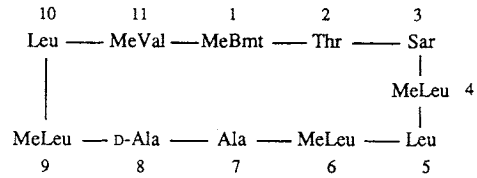
A considerable body of evidence has accumulated to support the idea that such immunosuppressants as cyclosporin A (CsA) and FK506 could be effective not only in preventing the rejection of organ transplants but also in the treatment of autoimmune diseases such as uveitis[1,2], type II diabetes[3,4] and rheumatoid arthritis[5,6] which do not respond to current drug therapy. Accordingly, we have established a screening system for microbial products with immunosuppressive activity.

FR901459, a novel immunosuppressant, was discovered in the fermentation broth of *Stachybotrys chartarum* No. 19392 with an assay for antifungal activity accompanied by striking morphologic changes. The details of the screening method will be published elsewhere (in preparation). FR901459 was found to be a member of the cyclosporin family. However, it has a few intriguing features in its chemical structure which make it different from the known cyclosporins. Fig. 1 shows the structure of FR901459 and CsA.

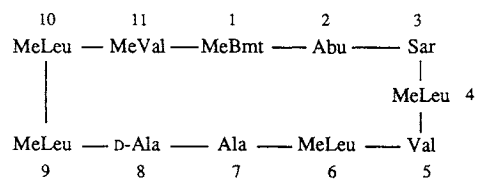
This paper describes the taxonomy of the

Fig. 1. Structure of FR901459 and cyclosporin A.

Bmt = (4*R*)-4-[(*E*)-2-butenyl]-4-methyl-L-threonine
Me = Methyl
Sar = Sarcosine
Abu = α-Aminobutyric acid



FR901459



Cyclosporin A

producing microorganism, fermentation, isolation and physico-chemical properties of FR901459, followed by a comparative study of its immunosuppressive activity with CsA.

Materials and Methods

Taxonomic Studies

The fungal strain No. 19392 was isolated from a soil sample collected on Hahajima Island, Tokyo Prefecture. Morphological observations were made after incubation at 25°C for 14 days on corn meal agar.

Fermentation

The strain No. 19392 on mature slant culture was inoculated into ten 500-ml Erlenmeyer flasks each containing 120 ml of sterilized seed medium. The seed medium consisted of sucrose 4%, Pharmamedia (Traders Protein Co., Ltd.) 2%, Molatein 1%, peptone 1%, KH_2PO_4 0.2%, $CaCO_3$ 0.2%, Tween 80 0.1% and Adekanol LG-109 0.025% (antifoam, Asahi Denka Co., Ltd.). The seed flasks were incubated for 3 days at 25°C on a rotary shaker (5.1 cm-throw) at 250 rpm.

All of the resultant seed cultures were transferred to a 200-liter jar fermentor containing 150 liters of production medium which had been sterilized at 125°C for 30 minutes in advance. The production medium for the strain No. 19392 consisted of modified starch 6%, wheat germ 2%, corn steap liquor 2%, soybean powder 2%, $(NH_4)_2SO_4$ 1%, $NaNO_3$ 0.2%, $CaCO_3$ 0.2%, Adekanol LG-109 0.025% and Silicone KM-70 (antifoam, Shin-Etsu Chemical Co., Ltd.) 0.025%. This medium was adjusted to pH 6.1 before sterilization. Fermentation was carried out at 25°C for 4 days with an air flow rate of 100 liters/minute and an agitation rate of 200 rpm.

FR901459 was quantified by high performance liquid chromatography (HPLC) using a YMC AM-303 column (4.6 mm inner diameter × 250 mm length, YMC Co. Ltd.) at 210 nm with a mobile phase of 90% MeOH in 2.5 mM KH_2PO_4-K_3HPO_4 buffer (pH 7.0) and a flow rate of 1 ml/minute. To prepare the test samples for HPLC, a broth was vigorously admixed with an equal volume of acetone and then centrifuged at 2,500 rpm for 10 minutes to give a debris-free supernatant. The supernatant was appropriately concentrated and 5 μl of concentrate was injected into the injector of a Hitachi Model L-6000 HPLC. The retention time of FR901459 was about 9.1 minutes.

One-way Mixed Lymphocyte Reaction (MLR)

Female Balb/c (H-2^d) and C57BL/6 (H-2^b) mice 6 to 10 weeks old were purchased from Charles River Japan Inc. (Atsugi). Spleen cell suspensions of both strains were prepared as described previously[7]. The MLR test was performed in flat-bottomed microtiter plates, with each well containing 5×10^5 Balb/c responder cells and 2.5×10^5 X-irradiated C57BL/6 stimulator cells in 100 μl RPMI1640 medium supplemented with 10% fetal calf serum, 50 μM 2-mercaptoethanol, 100 units/ml penicillin and 100 μg/ml streptomycin (referred to as PRMI1640 complete medium). The cells were incubated at 37°C for 72 hours in humidified atmosphere of 5% CO_2 - 95% air. The cultures were pulsed with 18.5 kBq of tritiated thymidine (New England Nuclear, Boston, MA) during the final 4 hours and harvested onto glass fiber strips with a microharvester (Bellco Glass, Inc., Vineland, NJ). The test compound was dissolved in methanol and further diluted in RPMI1640 medium and added to the cultures in triplicate immediately after responder and stimulator cells were mixed.

Mitogen Response

Balb/c spleen cells (1.25×10^5 cells) suspended in 100 μl RPMI1640 complete medium containing 5 μg/ml concanavalin A (ConA, Sigma) were cultured in round-bottomed microtiter plates at 37°C for 72 hours in a CO_2 incubator with or without test compound. Incorporation of tritiated thymidine during the final 4 hours of culture was assessed as described above.

Delayed-type Hypersensitivity (DTH) Reaction

Female Balb/c mice (6~8 weeks old) were sensitized by subcutaneous injection of 1×10^8 sheep red

blood cells (SRBC) that had been washed three times with saline. Five days after the sensitization, 1.25×10^8 SRBC were injected subcutaneously into the left hind footpad to elicit the hypersensitivity reaction which was evaluated 24 hours later by measuring the increase in footpad thickness. The test compound was dissolved in olive oil and administered orally for 6 consecutive days, beginning at the day of sensitization.

Skin Grafting

Skin grafting was performed according to the method described previously[8], using fully allogeneic WKA (RT-1^k) and F344 (RT-1^{lvl}) rats as recipients and donors, respectively. Both strains of rats, 6 to 7 weeks old, were obtained from Charles River Japan Inc. and were kept under specific pathogen-free conditions until used. Full-thickness ear skin of a F344 rat was engrafted onto the left side of the lateral thorax of a WKA rat. The graft was covered with sterile bactericidal gauze (Sofratulle, Roussel Laboratories, England) and dressing which were removed 5 days after transplantation. Each graft was inspected daily until rejection (defined as >90% necrosis of the graft epithelium). The test compound was dissolved in olive oil and administered orally for 14 consecutive days, beginning at the day of transplantation.

Results

Taxonomy of the Producing Strain

This organism grew restrictedly, attaining 1.5~2.0 cm in diameter on corn meal agar (Difco 0386) after two weeks at 25°C, and formed yellowish-brown (5F4)[9] colonies.

The strain No. 19392 formed anamorph, consisting of macronematous conidiophores with apical cluster of several phialides bearing slimy heads of conidia (Fig. 2). The strips were hyaline at base, dark olivaceous toward the apex, sometimes minutely rough-walled at the upper parts, up to 70 μm in length, 2.5~3.5 μm thick and slightly enlarged at the apex which bore 5~9 terminal phialides. The phialides were obovate, aseptate, at first hyaline, later dark olivaceous, smooth walled, with conspicuous collarettes and 7.5~11.5 × 4.5~5.5 μm, and produced conidia. The conidia were acrogenous, aseptate, at first hyaline, when mature dark olive grey, smooth or ridged, ellipsoidal and 6.5~8.5 × 3.0~4.0 μm. They aggregated in slimy masses. The strain did not produce teleomorph structures. The strain No. 19392 grew from 8 to 35°C on potato dextrose agar (Nissui Pharmaceutical Co. Ltd.).

On the basis of its morphological characteristics, the strain No. 19392 resembled *Stachybotrys chartarum* Hughes 1958[10]. Therefore, we have designated FR901459 producers *Stachybotrys chartarum* No. 19392, and deposited it in the National Institute of Bioscience and Human-Technology (formerly the Fermentation

Fig. 2. Scanning electron micrograph of *Stachybotrys chartarum* No. 19392.
A: Conidial structures (scale: 50 μm), B: ridged conidia (scale: 10 μm).

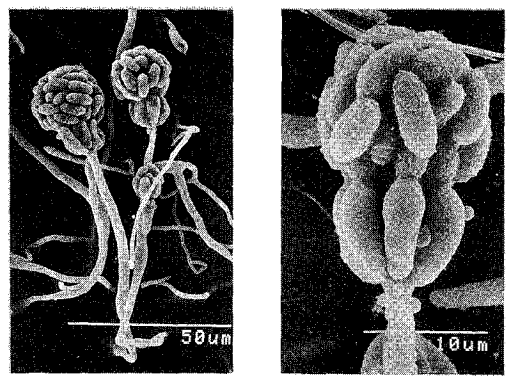


Fig. 3. Time course of FR901459 production in a 200-liter jar fermentor.

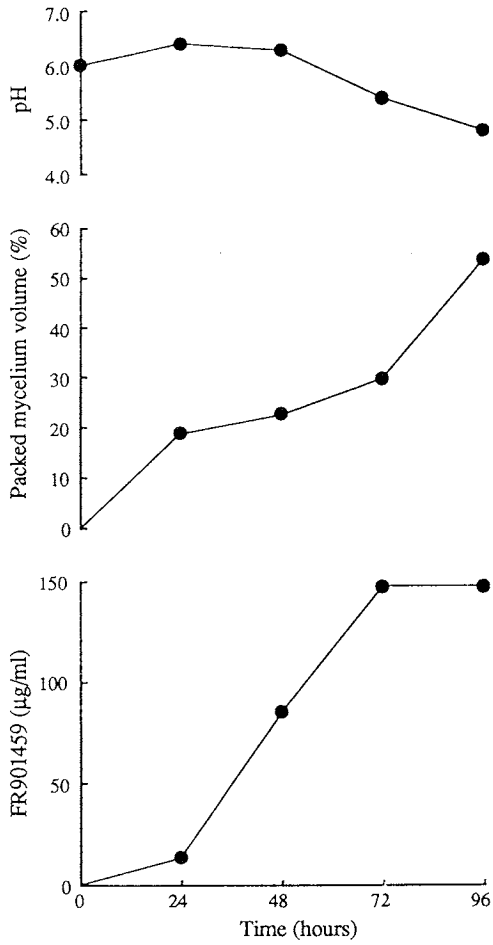


Fig. 4. Isolation procedure for FR901459.

Fermentation broth (225 liters)
↓ added acetone (225 liters)
Acetone extract (360 liters)
↓ adjusted to pH 8.9
Diaion HP-20 column (16 liters)
↓ eluted with 90% aq acetone
Activated carbon column (4 liters)
↓ eluted with acetone
Silica gel column (900 ml)
↓ eluted with CH_2Cl_2 - MeOH (50 : 1)
Silica gel column (1 liter)
↓ eluted with EtOAc
White powder (16 g)

Research Institute), Agency of Industrial Science and Technology, Japan, as FERM BP-3364.

Production of FR901459

Fig. 3 shows the time course of FR901459 production by *Stachybotrys chartarum* No. 19392 in a 200-liter fermentor, along with the pH of the medium and the packed mycelium volume. A maximal yield of 148 μg/ml was observed after 96 hours of cultivation.

Isolation and Purification

The purification scheme is shown in Fig. 4. The cultured broth (225 liters) was adjusted to pH 8.9 with 6 N NaOH, to which an equal volume of acetone was added and mixed extensively, and extraction was carried out overnight at room temperature. The extract was filtered with the aid of diatomaceous earth (5 kg) and the filtrate (360 liters) was loaded on a Diaion HP-20 column (16 liters). After washing with 50% aqueous acetone (72 liters), the active fractions containing FR901459 were eluted with 90% aqueous acetone (32 liters). These fractions were then applied to an activated carbon column (4 liters) and the activity was eluted with 40 liters of acetone.

The eluate was concentrated to a small volume and the resultant solution was mixed with 200 ml of silica gel (Kieselgel 60, 70~230 mesh, Merck Co. Ltd.). After the solvent was evaporated, the resultant dry powder was applied on a top of the silica gel column (700 ml) which had been prepacked with dichloromethane. The column was washed with a mixture of dichloromethane - methanol (100 : 1, 2.7 liters) and then the activity was eluted with a mixture of dichloromethane - methanol (50 : 1, 2.7 liters). The eluate was concentrated to dryness under reduced pressure. The residue (18 g) was redissolved with 600 ml of

n-hexane - ethyl acetate (5 : 1) and subjected again to a silica gel column chromatography (1 liter). After the column was developed with 7 liters of *n*-hexane - ethyl acetate (1 : 1), the activity was eluted with 4 liters of ethyl acetate. The eluate was concentrated under reduced pressure and *n*-hexane was added to the resultant solution with stirring. The mixture was allowed to stand for 1 hour at room temperature to give FR901459 (16 g) as a white powder.

Physico-chemical Properties

The physico-chemical properties of FR901459 are summarized in Table 1. FR901459 is soluble in methanol, acetone, ethyl acetate and diethyl ether, and it is insoluble in *n*-hexane and water. Color reactions of FR901459 are as follows: Positive in iodine vapor ceric sulfate, potassium permanganate and Dragendorff tests. Negative in ferric chloride, Molish and Ehlrich tests. The Rf values of FR901459 on silica gel TLC (Silica gel 60 F_{254}, E. Merck) developed with ethyl acetate - acetone (4 : 1) and chloroform - methanol (10 : 1) were 0.28 and 0.54, respectively. The molecular formula was determined to be $C_{62}H_{111}N_{11}O_{13}$ (molecular weight: 1,217) by FAB-MS and elementary analysis.

The ^1H NMR spectrum, ^{13}C NMR spectrum and IR spectrum of FR901459 are shown in Figs. 5, 6 and 7, respectively.

Table 1. Physico-chemical properties of FR901459.

Appearance	White powder
mp	156～158°C
$[\alpha]_D^{23}$	−230° (*c* 1.0, $CHCl_3$)
UV λ_{max}^{MeOH} nm (ε)	End absorption
Molecular formula	$C_{62}H_{111}N_{11}O_{13}$
Mass spectrum (FAB-MS)	1,218 $(M+H)^+$
Elementary analysis	
Calcd for $C_{62}H_{111}N_{11}O_{13}\cdot H_2O$:	C 60.22, H 9.21, N 12.46
Found:	C 60.09, H 9.31, N 12.34

Structure Determination

The molecular formula of FR901459 was established to be $C_{62}H_{111}N_{11}O_{13}$ by FAB-MS and elementary analysis. Compound **I** in Fig. 8 was negative to Ninhydrin reagent. Acetylation of **I** with

Fig. 5. ^1H NMR spectrum of FR901459 in pyridine-d_5 (400 MHz).

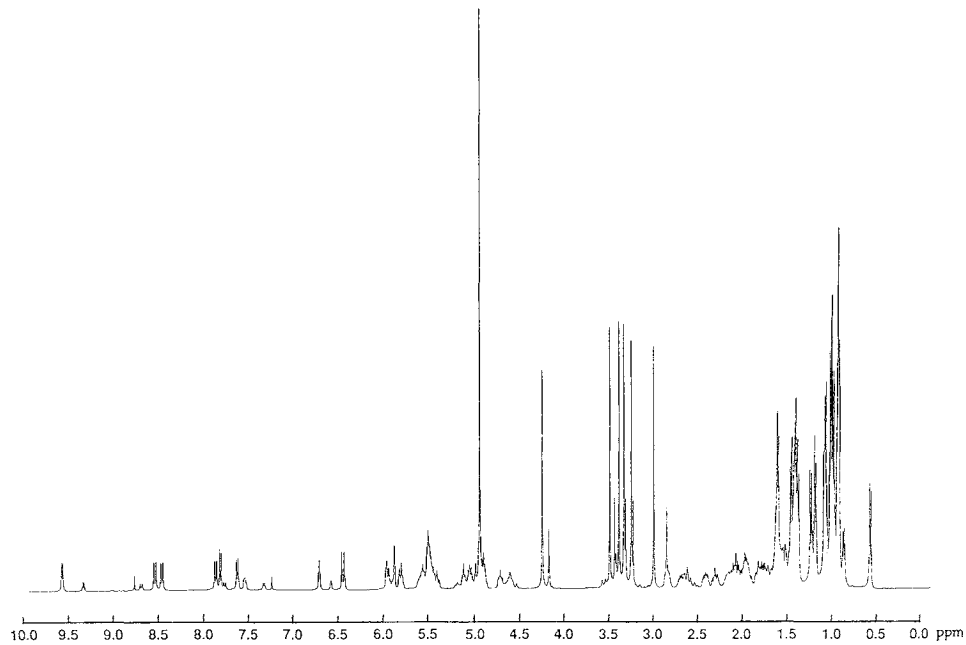


Fig. 6. ^{13}C NMR spectrum of FR901459 in pyridine-d_5 (100 MHz).

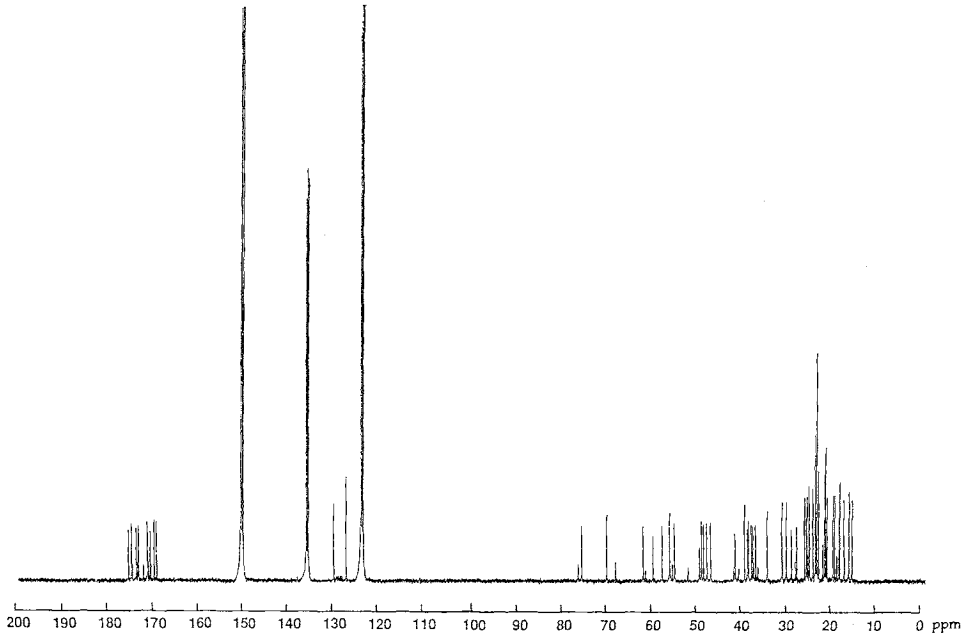
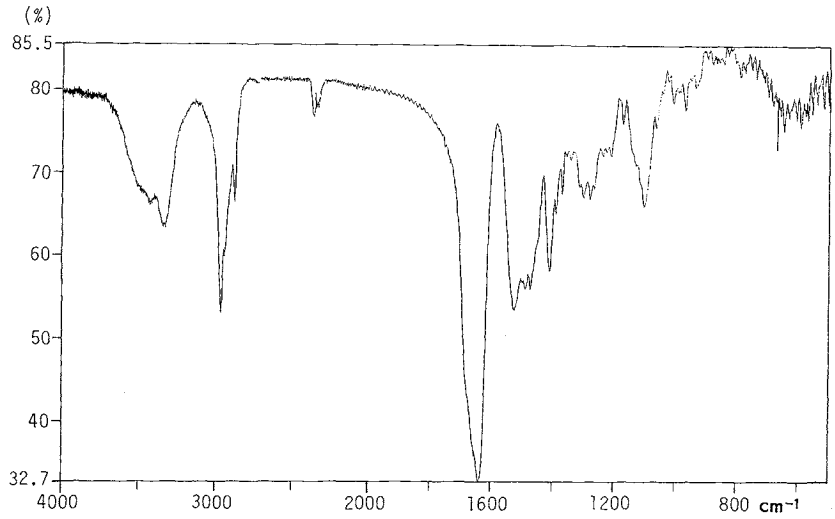
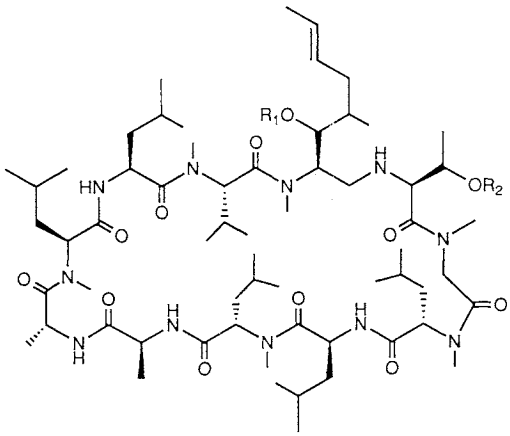


Fig. 7. IR spectrum of FR901459 in $CHCl_3$.



Ac_2O-pyridine gave a mixture of monoacetyl (**II**) and diacetyl (**III**) derivatives (FAB-MS *m/z* 1,259 (**II**), *m/z* 1,301 (**III**)). A strong absorption at 1635 cm^{-1} in IR spectrum suggested the presence of a peptide linkage. Acid hydrolysis of **I** (6 N HCl, 110°C, 18 hours) gave products which were positive to Ninhydrin on a TLC plate. This further substantiated the peptide-like nature of the molecule. Amino acid analysis of the hydrolysate revealed the presence of one residue each of Thr and Sar, two residues of Ala and Leu, and two unknown peaks were also observed. To determine the stereochemistry of the standard amino

Fig. 8. Structures of FR901459 (**I**), monoacetyl-FR901459 (**II**) and diacetyl-FR901459 (**III**).



Compound	R_1	R_2
I	H	H
II	$COCH_3$	H
III	$COCH_3$	$COCH_3$

Fig. 9. X-ray structure of monoacetyl-FR901459.

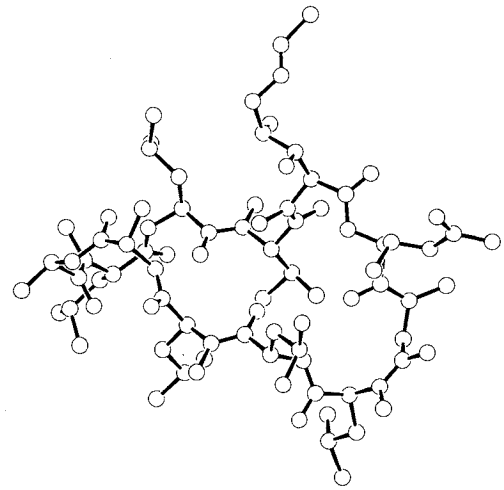
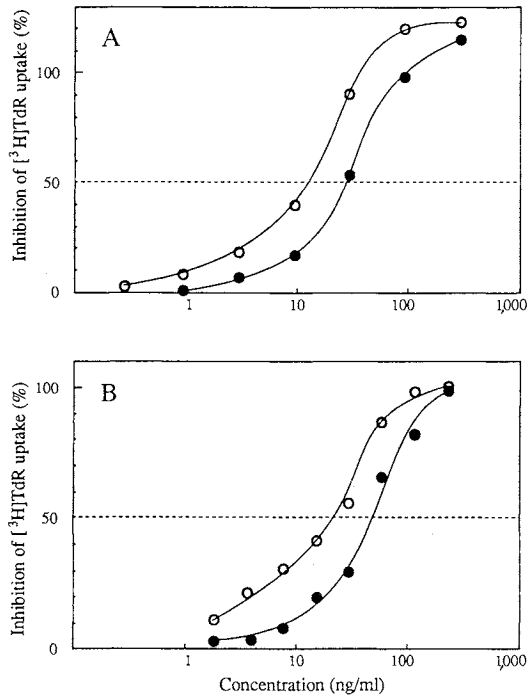


Fig. 10. Effect of FR901459 and cyclosporin A (CsA) on the proliferative responses in mixed lymphocyte reaction (A) and concanavalin A-induced mitogenesis (B).

The control responses (cpm) in experiments A and B were 73,496±1,343 and 148,100±6,271, respectively. ● FR901459, ○ CsA.



acids, the acid hydrolysates described above were derivatized with 15% HCl-*n*-BuOH followed by trifluoroacetic anhydride to *N,O*-trifluoroacetyl *n*-butyl ester derivatives. Chiral column GC-MS analysis of the derivative allowed us to assign the L configuration for the Leu, Thr and Ala and the D configuration for the another Ala, and the existence of MeLeu and MeVal.

Careful examination of the ^{13}C NMR spectrum (100 MHz, pyridine-d_5) indicated that **I** exists as a mixture of conformers in solution (4:1). This resulted in complicated ^1H and ^{13}C NMR spectra and also made it difficult to connect the fragments by application of modern 2D NMR techniques. Since these chemical and spectroscopic methods were found to be impractical for structural determination of this peptide, we decided to submit crystals of **II** to X-ray crystal analysis.

The crystals of **II** were found to be optimum, which formed in the orthohombic space group $P2_12_12_1$ with a=24.773(2) Å, b=23.709(3) Å, c=14.658(2) Å; V=8609(1) Å; Z=4; D 1.04 g/cm^3. The structure was determined by direct methods (DIRDIF and RANTAN). Parameters were refined by using anisotropic temperature factors to R=0.131 for 4382

independent reflections. The absolute structure of monoacetyl-FR901459 was established after confirming that the Thr was in the L configuration. A perspective drawing of the final X-ray model of **II** is given in Fig. 9.

Biological Activities

Fig. 10A shows representative data demonstrating the suppressive effect of FR901459 and CsA on the proliferative response in murine MLR. FR901459 was capable of inhibiting lymphocyte proliferation in a dose-dependent fashion, approximately one third the potency of CsA. The IC_{50} values of FR901459 and CsA were 26.8 and 9.9 ng/ml, respectively. The immunosuppressive effect of CsA is primarily mediated by the transcriptinal inhibition of lymphokine mRNA including interleukin 2 (IL-2) and interferon γ (IFN-γ)[11,12] which are considered to play a critical role in T cell activation. Accordingly, the effect of FR901459 on IL-2 production was examined in MLR and it was found that FR901459 efficiently suppressed IL-2 production as well as proliferation (data not shown). Similarly, Fig. 10B shows that FR901459 was also capable of suppressing the mitogenic responses induced by plant lectin, ConA. The IC_{50} values of FR901459 and CsA in this system were 50.1 and 21.9 ng/ml, respectively.

The immunosuppressive activity of FR901459 was further evaluated *in vivo* using two animal models.

Table 2. Effect of FR901459 and cyclosporin A (CsA) on delayed-type hypersensitivity reaction to sheep red blood cells in Balb/c mice.

Drug	Dose[a] (mg/kg)	n[b]	Increase in footpad thickness ($\times 10^{-1}$ mm)[c]	Inhibition (%)
Vehicle		3	8.7±0.6	0
FR901459	10	3	7.5±1.0	14.9
	32	3	9.0±0.5	−3.1
	100	3	5.3±2.5	40.8
CsA	10	3	9.0±0.3	−3.1
	32	3	7.7±0.8	12.2
	100	3	1.6±0.1[d]	83.5[d]

[a] The test compounds were dissolved in olive oil and administered orally for 6 days.
[b] Number of mice per group.
[c] Mean±S.E.
[d] $P<0.001$ as compared to vehicle control.

Table 3. Effect of FR901459 and cyclosporin A (CsA) on skin allograft survival in rats.

Drug	Dose[a] (mg/kg)	n[b]	Graft survival time (days)	Median survival time (days)
Vehicle		6	7, 7, 7, 7, 7, 7	7.0
FR901459	10	5	7, 7, 8, 8, 8	7.5
	32	5	8, 8, 8, 9, 9	8.0[d]
	100	5	9, 10, 10, 11, 11	10.0[d]
	320	5	27, 28, 28, 28, 30	28.0[d]
CsA	3.2	5	7, 7, 7, 8, 8	7.0
	10	5	7, 8, 8, 9, 9	8.0[c]
	32	5	19, 19, 19, 19, 21	19.0[d]
	100	5	19, 19, 19, 20, 22	19.0[d]

[a] The test compounds were dissolved in olive oil and administered po for 14 consecutive days, beginning at the day of transplantation.
[b] Number of rats per group.
[c] $P<0.05$ as compared with vehicle-treated group (MANN-WHITNEY's U-test).
[d] $P<0.01$ as compared with vehicle-treated group (MANN-WHITNEY's U-test).

We first tested the effect of FR901459 on DTH reaction in Balb/c mice in response to T cell-dependent particulate antigen, SRBC. Table 2 shows results demonstrating that oral administration of 100 mg/kg FR901459, although less potent than CsA, exerted suppression of DTH responses by 40.8%.

The ability of FR901459 to prevent rejection of skin allografts in rats was then determined. As shown in Table 3, the graft survival time in the groups of rats treated orally with 100 and 320 mg/kg FR901459 were significantly longer than those in the vehicle-treated group. On the other hand, significant prolongation of the skin graft survival was observed in rats treated with 32 and 100 mg/kg CsA. Thus FR901459 has approximately one third the potency of CsA in both *in vitro* and *in vivo* immunosuppression.

Discussion

We demonstrated in this paper that FR901459, a new cyclosporin, was isolated from the fermentation broth of *Stachybotrys chartarum* No. 19392. A total of 25 cyclosporins (CsA to I and CsK to Z) have been isolated so far from the fermentation broth of *Tolypocladium inflatum*[13]. However, the majority of natural cyclosporins other than CsA were isolated as the minor metabolites of the same structural type. On the other hand, the immunosuppressive activity produced by *Stachybotrys chartarum* No. 19392 is exclusively due to FR901459, and even trace amounts of CsA and CsC were not detected at all in the fermentation broth. Moreover, various fungal species have been reported to produce cyclosporins. Among them are moniliaceous hyphomycetes and ascomycetes, the former of which, include *Tolypocladium inflatum*[14], other *Tolypocladium* species[15], *Cylindrocarpon lucidum*[16], *Fusarium solani*[17] and *Beauveria bassina*[18] and the latter include *Neocosmospora vasinfecta*[19]. Here we report for the first time that the dematiaceous hyphomycete species, *Stachybotrys chartarum*, is also a cyclosporin producer.

It is well known that CsA binds with high affinity to ubiquitous, cytoplasmic proteins, cyclophilins, that catalyze the *cis-trans* isomeration of X-Pro peptide bond (where X is any amino acid)[20,21] and a good correlation is observed between the binding affinity and immunosuppressive activities of CsA analogs[22]. Since our preliminary experiments have demonstrated that FR901459 competed with [^{3}H]CsA for binding to partially-purified bovine cyclophilin to a similar extent as that of CsA (data not shown), it appears that FR901459 interacts with the CsA binding site on cyclophilin, which probably exerts the immunosuppressive action.

FR901459 was found to be structurally different from the known cyclosporins in the following points. One is that the amino acid in position 5 of this compound is Leu, whereas the known cyclosporins have Val or MeVal in the same position. The second is that FR901459 exists in several different conformations in $CDCl_3$. On the other hand, CsA has been reported to exhibit two conformations in $CDCl_3$, the ratio of which is approximately 95 : 5[23,24].

Since FR901459 is structurally distinct from CsA, it would possibly be expected that FR901459 was less nephrotoxic than CsA. To clarify this point, the subacute toxicity of FR901459 was examined in rats at oral doses of 32, 100 and 320 mg/kg given for 14 consecutive days, with 32 mg/kg CsA as nephrotoxic control that is the minimal dose eliciting nephrotoxicity. Both FR901459 and CsA were that is the minimal dose eliciting nephrotoxicity. Both FR901459 and CsA were found to cause evident renal calcification at doses 100 and 32 mg/kg, respectively (data not shown).

Great efforts have been made to develop a CsA analog that might be improved in regard to nephrotoxicity. The fact that we could discover a new cyclosporin, FR901459, in microbial cultures with high productivity demonstrates the possibility of finding more effective and safer cyclosporins.

Experimental

Acid Hydrolysis of FR901459 (I)

For amino acid analysis, 2 mg of FR901459 (**I**) were dissolved in 0.8 ml of 6 N HCl in an evacuated glass tube and heated at 110°C for 16 hours. After evaporation, the residue was dissolved in 0.1 N HCl and subjected to amino acid analysis on a Hitachi 835 amino acid analyzer under conditions for standard

amino acids. Retention times in the amino acid analysis (minutes): Thr (19.58), Sar (23.05), Ala (38.94), Unknown-1 (42.54), Leu (53.69), NH_3 (75.78), Unknown-2 (63.01).

Chiral Column GS-MS Analysis

Chiral column GC-MS was carried out using a Chirasil-L-Val capillary column (0.25 mm × 25 mm) programmed from 80°C to 240°C to 3°C/minute and ZAB-SE mass spectrometer operating in the positive EI mode (scan range between *m/z* 35 and 600 with repetition time of 1.5 seconds). Derivatization of amino acid residues was done as follows. Acid hydrolysate of FR901459 (**I**) was heated in 10% HCl in *n*-BuOH (0.2 ml) at 110°C for 30 minutes in a screw-capped test tube. After removal of the *n*-butanolic HCl *in vacuo*, CH_2Cl_2 (0.2 ml) and trifluoroacetic anhydride (0.2 ml) were added, and the mixture was kept at 110°C for 5 minutes. The product was evaporated, dissolved in EtOAc, and subjected to the analysis. Retention time (minutes): Sar (6.18), D-Ala (6.54), L-Ala (7.54), MeVal (8.32), L-Thr (10.28), MeLeu (11.26), L-Leu (15.06).

Acetylation of FR901459

To a solution of **I** (80 mg) in pyridine (0.8 ml) was added Ac_2O (0.4 ml) and 4-dimethylaminopyridine. After stirring the solution overnight at room temperature, the solvent was evaporated *in vacuo*. The residue was purified by preparative TLC (5% MeOH-$CHCl_3$) to give monoacetyl-FR901459 (**II**, 20 mg) and diacetyl-FR901459 (**III**, 50 mg).

Monoacetyl-FR901459

Colorless prisms; mp 148~150°C; $[\alpha]_D^{19}$ −199° (*c* 0.5, $CHCl_3$); FAB-MS *m/z* 1,261 $(M+H)^+$; IR (KBr) 3330, 2960, 1745, 1635, 1525, 1240 cm^{-1}.

Anal Calcd for $C_{64}H_{113}N_{11}O_{14}\cdot 5H_2O$: C 56.91, H 9.16, N 11.41.
Found: C 56.64, H 8.82, N 11.27.

Diacetyl-FR901459

White powder; mp 138~140°C; $[\alpha]_D^{20}$ −199° (*c* 0.5, $CHCl_3$); FAB-MS *m/z* 1,303 $(M+H)^+$; IR (KBr) 3335, 2960, 1755, 1635, 1520, 1235 cm^{-1}.

Anal Calcd for $C_{66}H_{115}N_{11}O_{15}\cdot 4H_2O$: C 57.66, H 9.02, N 11.21.
Found: C 57.95, H 8.89, N 11.12.

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