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F13459, a New Derivative of Mycophenolic Acid

II. Physico-chemical Properties and Structural Elucidation

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F13459 is a new inhibitor of synthesis and trafficking of virus glycoprotein isolated from the culture broth of a *Penicillium* sp. The molecular formula of F13459 was determined to be $C_{27}H_{28}O_{11}$ by HRFAB-MS and NMR spectral analyses. The structure of F13459 was elucidated to be 3,4-dihydro-3,4,6,8-tetrahydroxy-3-methyl-1*H*-2-benzopyran-1-one 4-*O*-mycophenolate, an ester derivative of mycophenolic acid. F13459 was isolated as the optically inactive form. F13459 exists in epimeric mixtures at C-3′ through relatively fast hemiacetal-ketone tautomerism and at C-4′ through slow keto-enol tautomerism. Those epimerizations were confirmed by NOE differential experiments for fast chemical exchange and equilibrium and by deuteration experiments in NMR for slow chemical exchange.

In the course of screening for inhibitors of intracellular trafficking of viral glycoproteins, we have reported brefeldin A,[1] concanamycin A,[2] leuconostatin A,[3] and effrapeptins[4] as the inhibitors. In the continued research, we found a new inhibitor produced by a *Penicillium* sp. This inhibitor, which we have named F13459, has structural features quite different from previously isolated inhibitors. The taxonomy of the producing strain, isolation, and biological properties are described in the preceding paper.[5] In this paper, the physico-chemical properties and structural elucidation are described.

Results and Discussion

Physico-chemical Properties

F13459 was obtained as a pale brown oil. The molecular formular was determined to be $C_{27}H_{28}O_{11}$ by HRFAB-MS spectral data as follows: found *m/z* 529.1748 $[M+H]^+$, calcd. 529.1710 for $C_{27}H_{29}O_{11}$. UV absorption peaks were observed at λ_{max} (ε) 213 (51,970), 247 (14,600), and 302 (7780) nm in MeOH. In the IR spectrum, characteristic absorption bands were observed at 3420, 2946, 1740, 1671, 1630 cm^{-1} indicating the presence of OH, COOR, and aromatic groups. Optical rotation value of F13459 was 0° and the lack of optical activity was confirmed by CD spectrum. In the ^1H spectrum of F13459 at room temperature, most signals were broad. These phenomena were caused by tautomerism, discussed later. The ^{13}C NMR spectrum and relatively sharp ^1H NMR signals were similar to those of authentic mycophenolic acid indicating that F13459 is a derivative of mycophenolic acid.

Planar Structure

To determine the structure of F13459 by NMR experimental data, we tried NMR measurements under several conditions of temperature using different solvent

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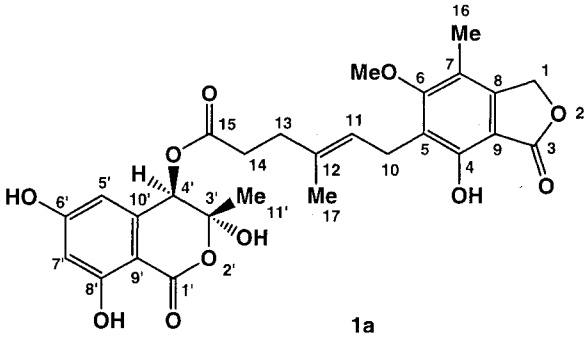
Table 1. Physico-chemical properties of F13459.

Characteristics	mixture of **1a** and **1b**
Appearance	pale brown oil
$[\alpha]_D^{24}$ (MeOH)	0° (c 1.0)
Molecular Formula	$C_{27}H_{28}O_{11}$
Molecular Weight	528
FAB-MS Pos. (*m/z*)	551$[M+Na]^+$, 529$[M+H]^+$, 303$[C_{17}H_{19}O_5]^+$, 207$[C_{10}H_9O_6-H_2O]^+$
FAB-MS Neg. (*m/z*)	527$[M-H]^-$
HRFAB-MS (*m/z*)	
found	529.1748 $[M+H]^+$
calcd.	529.1710 for $C_{27}H_{29}O_{11}$
UV λ_{max}^{MeOH} nm (ε)	213 (51,970), 247 (14,600), 302 (7780)
IR ν_{max}(KBr)(cm^{-1})	3420, 2946, 1740, 1671, 1630
TLC (Rf value) [a]	0.27

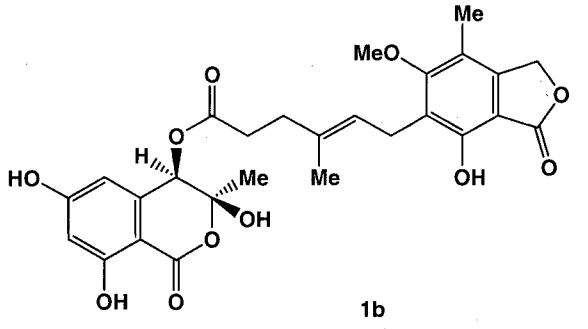
[a] Silica gel TLC : $CHCl_3$-MeOH (10:1)

including the presence of an acid. We found that acetone-d_6 solution at −30°C or lower temperature is suitable for analyses by NMR spectra (Fig. 2). At −40°C in CD_3OD solution, some signals were still broadened and this condition was not suitable for 2D experiments. In the ^1H NMR spectra at −40°C in acetone-d_6, most proton signals were observed as sharp signals, so most of NMR experiments were performed under this condition except as noted. Some separated signals with different intensities in the ^1H NMR spectrum suggested that F13459 is a mixture of two components in the ratio of 7 : 2. The two components were exchangeable at −40°C in acetone-d_6, which was confirmed by NOE differential experiments. Observed chemical exchange of ^1H NMR signals were useful to assign minor components of F13459. To elucidate the planar structure of F13459, PFG-DQFCOSY, PFG-HMQC and PFG-HMBC experiments were performed at −40°C. Partial structures of the mycophenolic acid portion was easily assigned by 2D NMR data. Especially useful were heteronuclear long-range correlations of PFG-HMBC data, summarized in Fig. 3. In the ^{13}C NMR spectrum, chemical shift differences between major (**1a**) and minor (**1b**) forms for the mycophenolate portion were very small, except for the carbonyl carbon, C-15, the carboxylic group of mycophenolic acid, with chemical shift values of 172.18 and 172.79 ppm for **1a** and **1b**, respectively. The chemical shift of the lactone carbonyl carbon (C-3) of the phthalide part was 172.18 ppm and overlaped the signal from C-15 of

Fig. 1. Structures of major (**1a**) and minor (**1b**) form of F13459.



1a



1b

1a is a racemic mixture of 3′*R*,4′*R*- and 3′*S*,4′*S* enantiomers, only the *RR* isomer is shown, similarly **1b** is also racemic mixture and only the 3′*S*,4′*R* enantiomer is depicted.

1a. This was easily confirmed by HMBC data. In the ^{1}H NMR spectrum, proton signals for the α, β, and δ positions from the carbonyl carbon of the sidechain have large differences between the major and minor forms. In the PFG-HMBC spectrum, H-4′ at 5.66 (s) and 6.09 (s) ppm for **1a** and **1b**, respectively, had long-range correlations to the carbonyl carbon C-15. These data indicated that F13459 is an ester derivative of mycophenolic acid. From the ester linked methine proton H-4′, networks of H-C long-range coupling were analyzed (Fig. 3) and the remaining part of F13459 was assigned as 3,4-dihydro-3,4,6,8-tetrahydroxy-3-methyl-1*H*-2-benzopyran-1-one.[6,7] The network of long-range coupling connectivities and the similarity of chemical shift values for major and minor components (Table 2) suggested that the planar structure of the major and minor forms of F13459 are the same. Relatively large chemical shift differences in ^{1}H NMR data were observed at the 3,4-dihydro-3,4,6,8-tetrahydroxy-3-methyl-1*H*-2-benzopyran-1-one portion. In the case of ^{13}C NMR spectral data, chemical shifts of carbons in the vicinity of the two chiral centers, *e.g.* C-3′ and C-4′, showed some characteristic differences. These data suggested that structural differences between the major and minor forms are derived from epimerism of the C-3′ hemiacetal carbon. In the positive mode FAB-MS spectral data, characteristic fragment ions, which arose by ester cleavage as follows, *m/z* 303 for mycophenolate portion and *m/z* 207 for 3,4-dihydro-3,4,6,8-tetrahydroxy-3-methyl-1*H*-2-benzopyran-1-one portion with dehydration were observed. This FAB-MS fragmentations and the result of alkaline hydrolysis[5] also supported the proposed planar structure for F13459.

Stereochemistry and Epimerism

To determine the relative stereochemistry of the major and minor forms of F13459, NOE differential experiments were performed at several temperatures. At −40°C chemical exchanges between the major and minor forms were fast, and this condition was not suitable for study of stereochemistry. Chemical exchanges were observed between each protons attached to the same carbon atoms of major and minor form with different chemical shifts *e.g.* H-10, 11, 13, 14, 17, 4′, 5′, 7′ and 11′. Even at −60°C, those chemical exchanges caused by epimerization at the hemiacetal carbon, C-3′ were observed, but suppressed to *ca* 10% intensity of negative enhancements in NOE differential spectra except for the four exchangeable hydroxyl protons of OH-4, 3′, 6′ and 8′. Those hydroxyl protons were exchanged faster by chemical exchange through residual H_2O. However low temperature experiments were effective in suppressing chemical exchange effects on NOE differential spectra and the relative stereochemistry of C-3′ and C-4′ was determined by careful interpretation of NOE data at −60°C. For the major form **1a**, important NOEs were observed from the ester attached oxygenated methine proton, H-4′ to H-5′, H-11′ and OH-3′ indicating that H-4′ is oriented in the equatorial direction and the ester group of mycophenolate is oriented axially. On the contrary, for the minor form **1b**, an NOE was observed only to the methyl protons of C-11′ from H-4′ indicating that H-4′ and Me-11′ were oriented axially and equatorially, respectively. Those NOE data and the proposed relative stereochemitry are shown in Fig. 4. In addition to those NOE data, ^{13}C NMR chemical shift values supported the relative stereochemistry. In comparing ^{13}C NMR data of **1a** and **1b**, a large highfield shift of C-5′ (Δ 3.71 ppm) for **1b** can be considered to the γ-effect of the ester group in the peri position in the same plane as the aromatic ring system. In the ^{1}H NMR spectra, H-14 proton signals of **1a** were observed at 2.43 (m) and 2.50 (m) ppm

Fig. 2. ^{1}H NMR spectra (600 MHz) of F13459 in acetone-d_6 at 25°C (A) and −40°C (B).

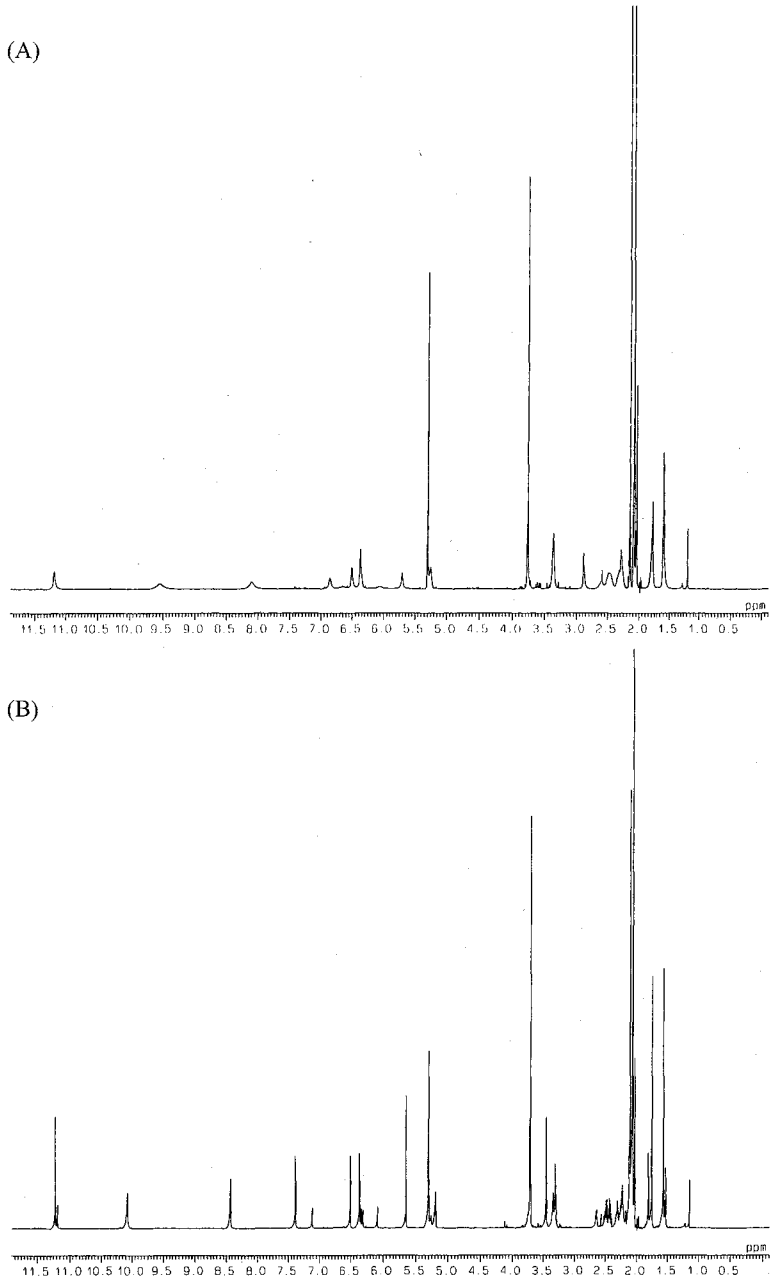


Fig. 3. Observed H-C long-range correlations in PFG-HMBC spectra for **1a** and **1b**.

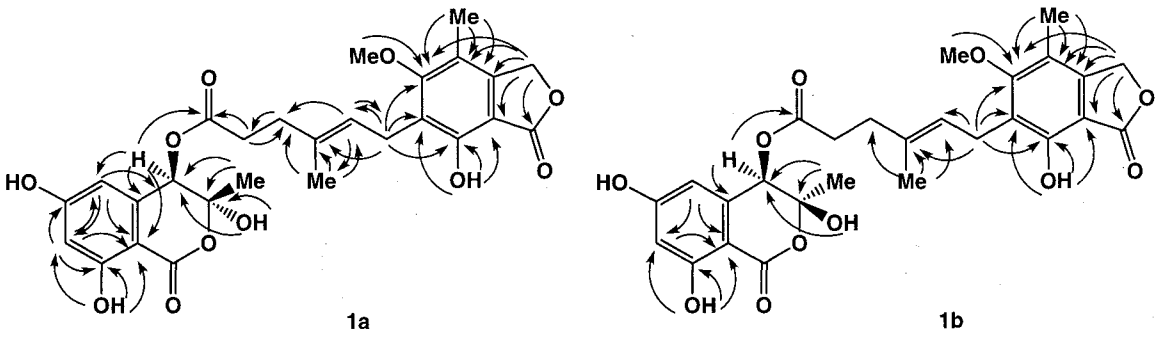


Table 2. NMR data for F13459 in acetone-d_6 at −40°C. *

	1a	**1b**	**1a**	**1b**
No.	C		H	
1	70.10	70.10	5.30 s	5.30 s
3	172.18	172.18	----	----
4	153.44	153.44	----	----
5	122.24	122.24	----	----
6	163.59	163.59	----	----
7	117.34	117.34	----	----
8	145.98	145.98	----	----
9	106.95	106.95	----	----
10	22.85	22.89	3.33 dd(14.3,6.8)	3.36 br.d (6.8)
			3.30 dd(14.3,6.8)	
11	123.52	123.47	5.20 t (6.8)	5.27 t (6.8)
12	134.02	134.12	----	----
13	34.59	34.59	2.24 m	2.32 br.t (7.3)
14	32.55	32.55	2.43 m, 2.50 m	2.64 t (7.3)
15	172.18	172.79	----	----
16	11.21	11.21	2.13 s	2.13 s
17	15.99	16.12	1.75 s	1.81 s
6-OMe	61.01	61.01	3.71 s	3.73 s
1'	168.81	168.69	----	----
3'	103.04	103.41	----	----
4'	69.56	70.66	5.66 s	6.09 s
5'	110.41	106.70	6.51 d (1.8)	6.35 br
6'	164.88	165.10	----	----
7'	103.56	102.49	6.37 d (1.8)	6.32 br
8'	164.43	164.43	----	----
9'	100.52	100.18	----	----
10'	139.11	140.26	----	----
11'	23.73	24.43	1.57 s	1.54 s
4-OH	----	----	8.44 s	8.44 s
3'-OH	----	----	7.39 s	7.12 s
6'-OH	----	----	10.08 s	10.08 s
8'-OH ----		----	11.23 s	11.19 s

* **1a** and **1b** are unseparable epimers and the ratio of **1a** : **1b** = 7 : 2.

with non equivalent chemical shift values, but for **1b** H-14 proton signals were observed at 2.64 (t) ppm, which could be explained by restricted rotation of the ester sidechain portion in axial orientation of **1a**, in support of the proposed relative stereochemistry.

Epimerization at C-4′ by keto-enol tautomerisation would explain the isolation of F13459 as a racemate. To confirm the C-4′ epimerisation, deuterium exchange experiments were performed. F13459 was dissolved in CD_3OD - acetone-d_6 (1 : 10) and ^1H NMR spectra were

Fig. 4. Relative stereochemistry of lactone ring portion of 1*H*-2-benzopyran-1-one unit and key NOE data from H-4′ proton for **1a** and **1b**.

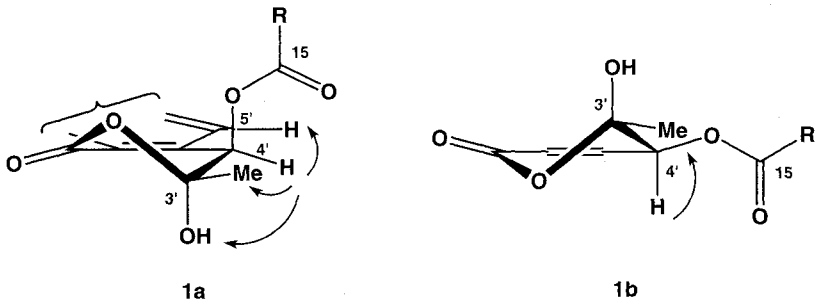
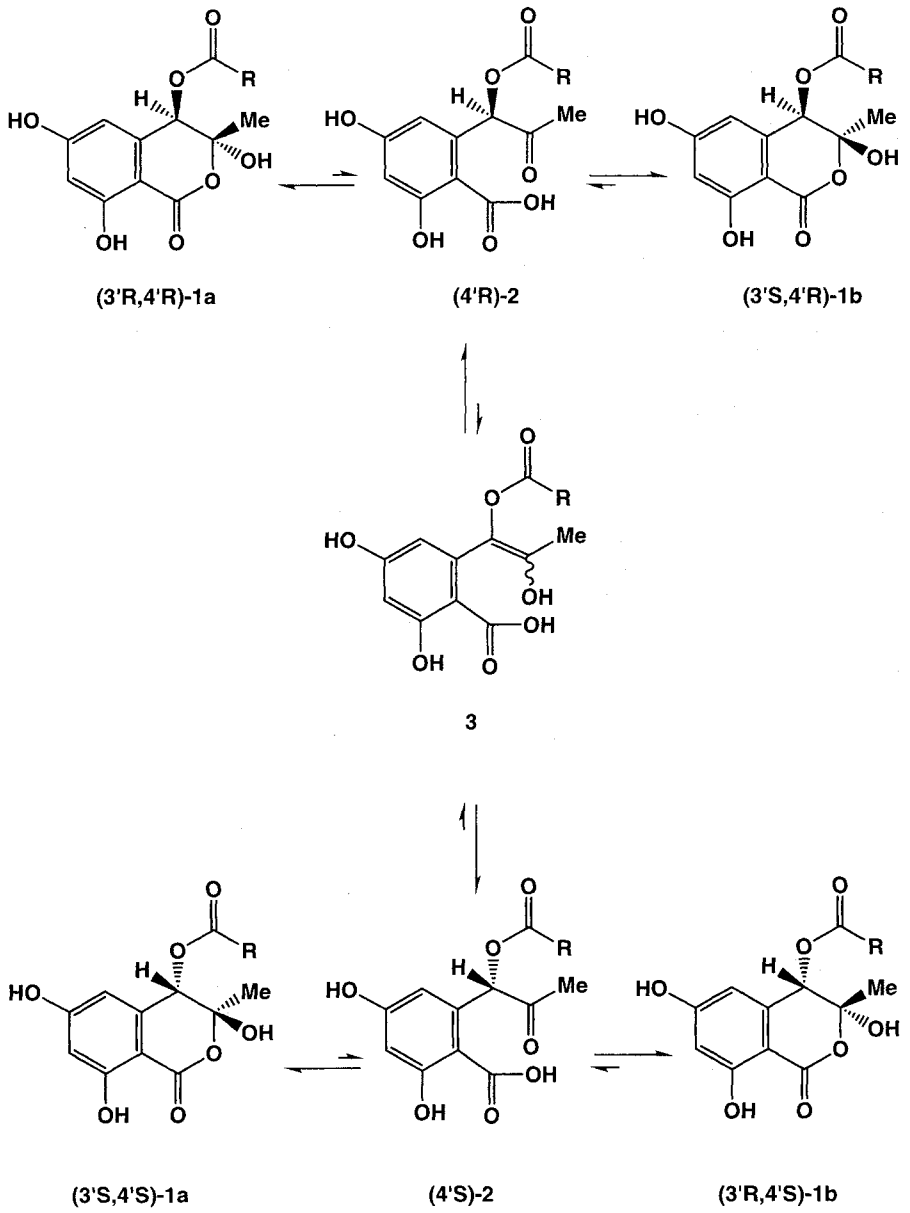


Fig. 5. Tautomerism and epimerization of F13459.



measured at room temperature focussing on H-4′ in a time course experiment. The intensity of H-4′ decreased slowly by deuteration and almost in half after two days. This result indicated that there is tautomerism of keto-enol as shown in Fig. 5. Based on the evidence above, the structure of F13459 was determined to be 3,4-dihydro-3,4,6,8-tetrahydroxy-3-methyl-1*H*-2-benzopyran-1-one 4-*O*-mycophenolate in a tautomeric and racemic mixture of (3′*RS*,4′*RS*)-**1a** and (3′*SR*,4′*RS*)-**1b** forms in a ratio of 7 : 2 in acetone solution.

Mycophenolic acid has been well known as a classical antibiotic[8] and several structurally related metabolites have been reported.[9~12] 3,4-Dihydro-3,4,6,8-tetrahydroxy-3-methyl-1*H*-2-benzopyran-1-one has also been known as a fungal metabolite[6] and recently reported as a proto-inhibitor of the mammalian cell cycle and precursor of acetophthalidine.[7] Although two units of F13459 are known metabolites, interestingly an ester derivative such as F13459 is a very rare metabolite of fungi to the best of our knowledge.[13]

Experimental

General

NMR spectra were measured on a JEOL JNM A600 spectrometer for ^{1}H NMR at 600 MHz and ^{13}C NMR at 150 MHz and a JEOL JNM A400 spectrometer for ^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz in acetone-d_6 or other solvent. Acetone-d_6 at 29.8 ppm and acetone-d_5 at 2.04 ppm are used as internal reference for ^{13}C and ^{1}H NMR, respectively. FAB-MS spectra were measured on a JEOL JMS HX-110 mass spectrometer with a matrix of 3-nitrobenzyl alcohol. IR and UV spectra were recorded on a JASCO FT-IR 700 and Beckman DU-65 spectrophotometer, respectively. Optical rotation was measured on a JASCO DIP-370 digital polarimeter. CD spectra were recorded on a JASCO J-720 spectropolarimeter.

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