Pyripyropenes, Novel ACAT Inhibitors Produced by *Aspergillus fumigatus*

IV. Structure Elucidation of Pyripyropenes M to R

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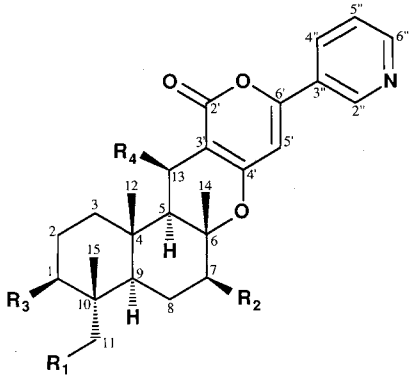
Six new pyripyropenes, M to R, were isolated from the ethyl acetate extracts of the jar fermentation broth of *Aspergillus fumigatus* FO-1289-2501. Structural elucidation indicated that all the pyripyropenes have the same pyridino-α-pyrone sesquiterpene core as pyripyropenes A to L. Among them pyripyropene M showed the most potent inhibition against acyl-CoA : cholesterol acyltransferase activity with an IC_{50} value of 3.80 μM in rat liver microsomes, but pyripyropenes N to R showed moderate inhibitory activity (IC_{50} 11.0~78.0 μM).

We have reported pyripyropenes A to L, a novel series of polyoxygenated metabolites produced by *Aspergillus fumigatus* FO-1289-2501, as inhibitors of acyl-CoA : cholesterol acyltransferase (ACAT)[1~6]. Among them pyripyropenes C, A, L and B showed very potent inhibitory activity in rat liver microsomes with IC_{50} values of 0.15, 0.16, 0.27 and 0.32 μM, respectively, indicating that they represent the most potent naturally occurring ACAT inhibitors reported to date. Further isolation study from the ethyl acetate extracts of the jar fermentation broth led to the discovery of six new components of pyripyropenes M to R (Fig. 1). In this paper, the isolation, structure elucidation and biological properties of these pyripyropenes are described.

Fig. 1. Structures of pyripyropenes A to R.



	R_1	R_2	R_3	R_4
Pyripyropene A	$-OCOCH_3$	$-OCOCH_3$	$-OCOCH_3$	-OH
Pyripyropene B	$-OCOCH_2CH_3$	$-OCOCH_3$	$-OCOCH_3$	-OH
Pyripyropene C	$-OCOCH_3$	$-OCOCH_2CH_3$	$-OCOCH_3$	-OH
Pyripyropene D	$-OCOCH_3$	$-OCOCH_3$	$-OCOCH_2CH_3$	-OH
Pyripyropene E	-H	-H	$-OCOCH_3$	-H
Pyripyropene F	-H	-H	$-OCOCH_2CH_3$	-H
Pyripyropene G	-H	-H	$-OCOCH_3$	-OH
Pyripyropene H	-H	-H	$-OCOCH_2CH_3$	-OH
Pyripyropene I	$-OCOCH_2CH_3$	$-OCOCH_2CH_3$	$-OCOCH_2CH_3$	-OH
Pyripyropene J	$-OCOCH_3$	$-OCOCH_2CH_3$	$-OCOCH_2CH_3$	-OH
Pyripyropene K	$-OCOCH_2CH_3$	$-OCOCH_3$	$-OCOCH_2CH_3$	-OH
Pyripyropene L	$-OCOCH_2CH_3$	$-OCOCH_2CH_3$	$-OCOCH_3$	-OH
Pyripyropene M	$-OCOCH_3$	$-OCOCH_2CH_3$	$-OCOCH_3$	-H
Pyripyropene N	$-OCOCH_2CH_3$	-H	$-OCOCH_2CH_3$	-OH
Pyripyropene O	$-OCOCH_3$	-H	$-OCOCH_3$	-H
Pyripyropene P	$-OCOCH_2CH_3$	-H	$-OCOCH_3$	-H
Pyripyropene Q	$-OCOCH_2CH_3$	-H	$-OCOCH_3$	-OH
Pyripyropene R	$-OCOCH_3$	-H	$-OCOCH_2CH_3$	-H

Materials and Methods

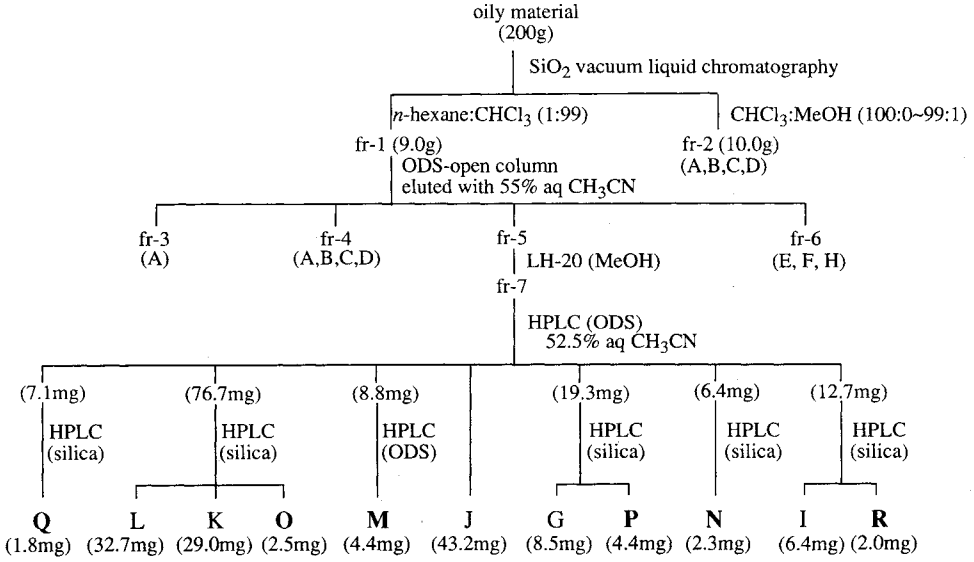
General Experimental Procedures

Aspergillus fumigatus FO-1289-2501 was used for production of pyripyropenes. Kieselgel 60 (E. Merck), SS 1020T (Senshu Sci. Co.) and Sephadex LH-20 (Pharmacia) were used for column chromatography. HPLC was carried out using JASCO (TRI ROTAR V) and Waters 600E systems.

Spectroscopic Studies

UV spectra were recorded on a Shimadzu UV-200S spectrophotometer. IR spectra were recorded on a Horiba FT-210 infrared spectrometer. Optical rotations were obtained with a JASCO DIP-370 digital polarime-

Fig. 2. Isolation procedures of pyripyropenes M to R.



ter. EI-MS spectra were recorded on a JEOL JMS-D 100 mass spectrometer at 20 eV. FAB-MS spectra were recorded on a JMS-DX300 mass spectrometer. The various NMR spectra were obtained on a Varian XL-400 spectrometer.

ACAT Activity

ACAT activity was carried out in an enzyme assay using rat liver microsomes as reported previously[7].

Antimicrobial Activity

Antimicrobial activity was tested using paper disks (i.d. 6 mm, ADVANTEC). Bacteria were grown on Müeller-Hinton agar medium (Difco), and fungi and yeasts were grown on potato broth agar medium. Antimicrobial activity was observed after 24-hour incubation at 37°C for bacteria and after 48-hour incubation at 27°C for fungi and yeasts.

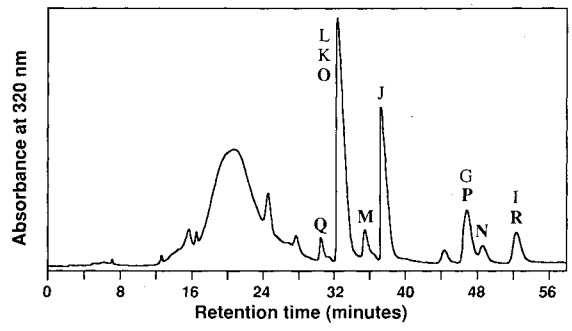
Results

Isolation

As described previously, about half of ethyl acetate extracts (410 g) obtained from 200 liters of 120-hour old whole broth was used for isolation of pyripyropenes E to L[4]. The other half of the oily extracts was used for isolation of pyripyropenes M to R as summarized in Fig. 2. The material (200 g) was purified by vacuum liquid chromatography using silica gel (Kieselgel 60, 400 g, 10.5 × 10 cm). The material was eluted stepwise with *n*-hexane - chloroform - methanol solutions (1200 ml each, 20 : 80 : 0, 1 : 99 : 0, 0 : 100 : 0, and 0 : 99 : 1, v/v), and each 200 ml of the elution was collected. The active 7th to 12th fractions, termed fr-1 were concentrated *in vacuo* to give a brown material (9.0 g). Then, the brown material was subjected to an octadecyl silyl (ODS) column (Senshu SS 1020T, 200 g). The materials were eluted with 55% aq acetonitrile and each 50 ml of the elution was collected. The 31st to 61st fractions termed fr-5 contained pyripyropenes M to R as well as pyripyropenes G, I, J, K and L. After concentration, the fr-5 was subjected to gel filtration using Sephadex LH-20 (35 × 415 mm; solvent, methanol; 5ml/fraction). The 39th to 58th fractions (fr-7) containing the pyripyropenes were concentrated *in vacuo* to a small volume, which were purified by preparative HPLC (Senshu pak ODS-H-6251, 30 × 250 mm; 52.5% aq CH_3CN; UV at 320 nm; 20.0 ml/minute). As shown in Fig. 3, pyripyropene Q was

Fig. 3. A chromatographic profile of pyripyropenes M to R separated by preparative HPLC.

Column, Senshu pak ODS-H-6251 (30 × 250 mm); solvent, 52.5% aq CH_3CN; UV at 320 nm; 20.0 ml/minute.



eluted first as a peak with the retention time of 31.0 minutes, followed by a mixture of pyripyropenes L, K and O (32.5 minutes), M (35.5 minutes), J (37.5 minutes), a mixture of G and P (47.0 minutes), N (49.0 minutes) and a mixture of I and R (52.5 minutes). After concentration and ethyl acetate extraction of the fractions, new pyripyropenes were further isolated as follows. Therefore, pyripyropene O was further purified from the mixture (L, K and O) by preparative HPLC using a silica gel column (Senshu pak silica-5251-N, 20 × 250 mm; *n*-hexane - tetrahydrofuran - isopropyl alcohol (80 : 10 : 22, v/v); UV at 320 nm; 8 ml/minute). A peak with a retention time of 19.5 minutes was pooled and concentrated to give pure pyripyropene O (2.5 mg) as a white powder. Under the same HPLC conditions using the silica gel column, pyripyropenes Q (a retention time of 14.0 minutes), P (16.0 minutes), N (13.5 minutes) and R (17.0 minutes) were also purified to give pure white

Table 1. Physico-chemical properties of pyripyropenes M to R.

	Pyripyropene M	Pyripyropene N	Pyripyropene O
Appearence	White powder	White powder	White powder
Molecular weight	581	553	509
Molecular formula	$C_{32}H_{39}NO_9$	$C_{31}H_{39}NO_8$	$C_{29}H_{35}NO_7$
FAB-MS (*m/z*)			
Positive	582[M+H]$^+$	554[M+H]$^+$	510[M+H]$^+$
	604[M+Na]$^+$	576[M+Na]$^+$	532[M+Na]$^+$
EI-MS(*m/z*)	581[M]$^+$	553[M]$^+$	509[M]$^+$
	521[M-AcOH]$^+$	535[M-H_2O]$^+$	449[M-AcOH]$^+$
	432[M-AcOH-PrOH-CH_3]$^+$	520[M-H_2O-CH_3]$^+$	434[M-AcOH-CH_3]$^+$
	387[M-2AcOH-PrOH]$^+$	479[M-PrOH]$^+$	389[M-2AcOH]$^+$
	372[M-2AcOH-PrOH-CH_3]$^+$	405[M-2PrOH]$^+$	374[M-2AcOH-CH_3]$^+$
		372[M-2PrOH-H_2O-CH_3]$^+$	
HRFAB-MS(*m/z*)			
Found	582.2700	554.2751	510.2487
Calcd	582.2703 (for $C_{32}H_{40}NO_9$)	554.2754 (for $C_{31}H_{40}NO_8$)	510.2492 (for $C_{29}H_{36}NO_7$)
$[\alpha]_D^{28}$ (CH_3OH)	NT	+97 ° (*c* 0.05)	+103 ° (*c* 0.2)
UV $\lambda_{max}^{CH_3OH}$(nm)	231, 320	231, 320	231, 320
IR ν_{max}^{KBr} (cm^{-1})	3450, 2943, 1736, 1645, 1581, 1373, 1246, 1182, 1039	3429, 2943, 1716, 1652, 1635, 1384, 1306, 1122	3448, 2941, 1732, 1716, 1643, 1579, 1384, 1248, 1122

	Pyripyropene P	Pyripyropene Q	Pyripyropene R
Appearence	White powder	White powder	White powder
Molecular weight	523	539	523
Molecular formula	$C_{30}H_{37}NO_7$	$C_{30}H_{37}NO_8$	$C_{30}H_{37}NO_7$
FAB-MS (*m/z*)			
Positive	524[M+H]$^+$	540[M+H]$^+$	524[M+H]$^+$
	546[M+Na]$^+$	562[M+Na]$^+$	546[M+Na]$^+$
EI-MS(*m/z*)	523[M]$^+$	539[M]$^+$	523[M]$^+$
	463[M-AcOH]$^+$	521[M-H_2O]$^+$	463[M-AcOH]$^+$
	448[M-AcOH-CH_3]$^+$	506[M-H_2O-CH_3]$^+$	449[M+1-AcOH-CH_3]$^+$
	387[M-2-AcOH-PrOH]$^+$	461[M-AcOH-H_2O]$^+$	434[M+1-AcOH-2CH_3]$^+$
	372[M-2-AcOH-PrOH-CH_3]$^+$	447[M-PrOH-H_2O]$^+$	389[M-AcOH-PrOH]$^+$
		387[M-AcOH-PrOH-H_2O]$^+$	374[M-AcOH-PrOH-CH_3]$^+$
HRFAB-MS(*m/z*)			
Found	524.2645	540.2596	524.2645
Calcd	524.2648 (for $C_{30}H_{38}NO_7$)	540.2597 (for $C_{30}H_{38}NO_8$)	524.2648 (for $C_{30}H_{38}NO_7$)
$[\alpha]_D^{28}$ (CH_3OH)	+95 ° (*c* 0.4)	NT	+146 ° (*c* 0.1)
UV $\lambda_{max}^{CH_3OH}$(nm)	231, 320	231, 320	231, 320
IR ν_{max}^{KBr} (cm^{-1})	3450, 2941, 1736, 1645, 1549, 1404, 1244, 1148, 1122	3442, 2943, 1733, 1639, 1578, 1435, 1385, 1246	3473, 2942, 1728, 1645, 1579, 1481, 1429, 1404, 1238, 1122

PrOH: propionic acid, AcOH: acetic acid, NT: not tested

powders (1.8, 4.4, 2.3 and 2.0 mg, respectively). Pyripyropene M was further purified by preparative HPLC using an ODS gel column (Senshu pak ODS-H-6251, 30 × 250 mm; 60% aq CH_3CN; UV at 320 nm; 20.0 ml/minute). A peak with a retention time of 25.5 minutes was pooled. After concentration and ethyl acetate extraction of the fraction, pure pyripyropene M was obtained as a white powder (4.4 mg).

Physico-chemical Properties of Pyripyropenes M to R

The physico-chemical properties of pyripyropenes M to R are summarized in Table 1. All the pyripyropenes showed the same UV absorption maxima at 231 and 320 nm as pyripyropenes A to L[2,4], suggesting the presence of a common pyridino-α-pyrone sesquiterpene skeleton.

Structure of Pyripyropene P

The molecular formula of pyripyropene P was determined to be $C_{30}H_{37}NO_7$ on the basis of HRFAB-MS measurements (*m/z* found 524.2645, calcd 524.2648 for $C_{30}H_{38}NO_7$ $(M+1)^+$). The ^1H NMR spectrum ($CDCl_3$) displayed 37 proton signals and the ^{13}C NMR spectrum showed 30 resolved peaks (Table 2), supporting the molecular formula. The carbons were classified into five methyl, six methylene, one oxy methylene, two methine, one oxy methine, three quaternary, five sp^2 methine, four sp^2 quarternary and three carbonyl carbons by analysis of the DEPT spectra. The NMR spectra are very similar to those of pyripyropene E[4,8] except that a propionyloxy unit is present. The connectivity of proton and carbon atoms was confirmed by the ^1H-detected multiple quantum coherence (HMQC) spectrum (Table 2). ^1H-^1H COSY spectrum revealed the five partial structures I to V, and ^{13}C-^1H long-range couplings of ^2J and ^3J in the ^1H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) spectrum confirmed the structure of pyripyropene P as shown in Fig. 4. Concerning the C-11 position, the cross peaks from H_2-11 (δ 3.77 and 3.87), 11-*O*-CO-C*H*$_2$-CH$_3$ (δ 2.36) and 11-*O*-CO-CH$_2$-C*H*$_3$ (δ 1.16) to 11-*O*-*C*O-CH$_2$-CH$_3$ (δ 174.2) indicated that a propionyloxy residue was attached at C-11 for pyripyropene P. Fragment ion peaks of *m/z* 448 (M−

Table 2-1. ^1H and ^{13}C NMR chemical shifts of pyripyropenes M and N.

Carbon No.	Pyripyropene M		Pyripyropene N	
	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]
C-1	73.4	4.79 (1H, dd, *J*=12.0, 5.0 Hz)	73.6	4.82 (1H, dd, *J*=12.0, 5.0 Hz)
C-2	22.9	1.69 (1H, m)	22.8	1.69 (1H, m)
		1.89 (1H, m)		1.86 (1H, m)
C-3	36.6[c]	1.21 (1H, m)	36.5	1.21 (1H, m)
		1.81 (1H, m)		1.81 (1H, m)
C-4	36.6[c]		38.0	
C-5	50.3	1.65 (1H, dd, *J*=13.0, 4.5 Hz)	56.2	1.65 (1H, d, *J*=4.0 Hz)
C-6	82.0		82.1	
C-7	77.2	5.04 (1H, m)	41.1	1.62 (1H, m)
				2.13 (1H, m)
C-8	25.0	1.49 (1H, dd, *J*=24.0, 11.5 Hz)	19.2	1.63 (1H, dd, *J*=24.0, 11.5 Hz)
		1.80 (1H, m)		1.79 (1H, m)
C-9	45.1	1.68 (1H, m)	48.2	1.41 (1H, m)
C-10	40.3		40.7	
C-11	64.8	3.70 (1H, d, *J*=12.0 Hz)	65.0	3.72 (1H, d, *J*=12.0 Hz)
		3.81 (1H, d, *J*=12.0 Hz)		3.85 (1H, d, *J*=12.0 Hz)
C-12	15.6	1.05 (3H, s)	17.5	1.41 (3H, s)
C-13	16.9	2.33 (1H, dd, *J*=17.5, 4.5 Hz)	60.4	4.98 (1H, d, *J*=4.0 Hz)
		2.57 (1H, dd, *J*=17.5, 13.0 Hz)		
C-13-OH				2.83 (1H, brs)
C-14	15.3	1.32 (3H, s)	22.1	1.63 (3H, s)
C-15	13.3	0.74 (3H, s)	13.1	0.90 (3H, s)
1-O-CO-(CH2)-CH3	21.1	2.03 (3H, s)	9.29	1.130 (3H, t, *J*=7.5 Hz)
1-O-CO-CH2-CH3			27.6	2.32 (2H, dq, *J*=1.5, 7.5 Hz)
1-O-CO-(CH2)-CH3	170.4		173.8	
7-O-CO-(CH2)-CH3	9.18	1.25 (3H, t, *J*=7.5 Hz)		
7-O-CO-CH2-CH3	27.9	2.43 (2H, dq, *J*=4.0, 7.5 Hz)		
7-O-CO-(CH2)-CH3	173.3			
11-O-CO-(CH2)-CH3	20.8	2.11 (3H, s)	9.21	1.135 (3H, t, *J*=7.5 Hz)
11-O-CO-CH2-CH3			27.6	2.33 (2H, dq, *J*=1.5, 7.5 Hz)
11-O-CO-(CH2)-CH3	171.0		173.9	
C-2'	163.7		164.1	
C-3'	99.8		103.0	
C-4'	162.2		162.3	
C-5'	99.2	6.40 (1H, s)	99.5	6.45 (1H, s)
C-6'	155.9		157.2	
C-2"	146.7	8.99 (1H, brs)	146.8	9.00 (1H, d, *J*=2.0 Hz)
C-3"	127.4		127.0	
C-4"	132.8	8.09 (1H, ddd, *J*=8.0, 1.5, 0.5 Hz)	133.0	8.10 (1H, ddd, *J*=8.0, 2.0, 1.0 Hz)
C-5"	123.6	7.38 (1H, dd, *J*=8.0, 4.5 Hz)	123.6	7.41 (1H, dd, *J*=8.0, 5.0 Hz)
C-6"	151.2	8.66 (1H, brd, *J*=4.5 Hz)	151.5	8.68 (1H, dd, *J*=5.0, 1.0 Hz)

[a] Each sample was dissolved in $CDCl_3$. Chemical shifts are shown with reference to $CDCl_3$ as 77.7 ppm. [b] Chemical shifts are shown with reference to $CDCl_3$ as 7.26 ppm. [c] The signals were observed as the same chemical shifts.

Table 2-2. ^{1}H and ^{13}C NMR chemical shifts of pyripyropenes O and P.

Carbon No.	Pyripyropene O		Pyripyropene P	
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]
C-1	73.7	4.80 (1H, dd, *J*=11.5, 4.5 Hz)	73.7	4.81 (1H, dd, *J*=11.5, 4.5 Hz)
C-2	22.9	1.69 (1H, m)	22.9	1.77 (1H, m)
		1.85 (1H, m)		1.86 (1H, m)
C-3	36.8	1.21 (1H, m)	36.9	1.22 (1H, m)
		1.82 (1H, m)		1.83 (1H, m)
C-4	36.7		36.7	
C-5	51.5	1.57 (1H, m)	51.6	1.58 (1H, m)
C-6	80.7		80.7	
C-7	39.9	1.66 (1H, m)	40.4	1.67 (1H, m)
		2.12 (1H, m)		2.15 (1H, m)
C-8	19.0	1.47 (1H, m)	19.0	1.49 (1H, m)
		1.66 (1H, m)		1.67 (1H, m)
C-9	47.7	1.46 (1H, m)	47.7	1.49 (1H, m)
C-10	40.5		40.6	
C-11	65.1	3.77 (1H, d, *J*=12.0 Hz)	64.8	3.77 (1H, d, *J*=12.0 Hz)
		3.84 (1H, d, *J*=12.0 Hz)		3.87 (1H, d, *J*=12.0 Hz)
C-12	15.6	0.98 (3H, s)	15.6	0.99 (3H, s)
C-13	17.3	2.26 (1H, dd, *J*=17.0, 12.5 Hz)	17.3	2.27 (1H, dd, *J*=17.0, 12.5 Hz)
		2.55 (1H, dd, *J*=17.0, 5.0 Hz)		2.56 (1H, dd, *J*=17.0, 5.0 Hz)
C-13-OH				
C-14	20.7	1.28 (3H, s)	20.7	1.29 (3H, s)
C-15	13.1	0.87 (3H, s)	13.1	0.89 (3H, s)
1-O-CO-(CH2)-CH3	21.2	2.04 (3H, s)	21.2	2.05 (3H, s)
1-O-CO-CH2-CH3				
1-O-CO-(CH2)-CH3	170.4		170.5	
7-O-CO-(CH2)-CH3				
7-O-CO-CH2-CH3				
7-O-CO-(CH2)-CH3				
11-O-CO-(CH2)-CH3	20.9	2.07 (3H, s)	9.26	1.16 (3H, t, *J*=7.5 Hz)
11-O-CO-CH2-CH3			27.6	2.36 (2H, dq, *J*=1.5, 7.5 Hz)
11-O-CO-(CH2)-CH3	170.9		174.2	
C-2'	164.0		163.9	
C-3'	100.2		100.2	
C-4'	162.7		162.7	
C-5'	99.3	6.42 (1H, s)	99.3	6.43 (1H, s)
C-6'	155.8		155.7	
C-2"	146.7	8.99 (1H, d, *J*=2.0 Hz)	146.6	9.00 (1H, dd, *J*=2.0, 1.0 Hz)
C-3"	127.5		127.6	
C-4"	132.8	8.10 (1H, ddd, *J*=8.0, 3.0, 1.0 Hz)	132.8	8.12 (1H, ddd, *J*=8.0, 3.0, 1.5 Hz)
C-5"	123.6	7.39 (1H, ddd, *J*=8.0, 5.0, 1.0 Hz)	123.6	7.40 (1H, ddd, *J*=8.0, 3.5, 1.0 Hz)
C-6"	151.1	8.66 (1H, dd, *J*=5.0, 2.0 Hz)	151.1	8.67 (1H, dd, *J*=5.0, 2.0 Hz)

[a] Each sample was dissolved in CDCl3. Chemical shifts are shown with reference to CDCl3 as 77.7 ppm. [b] Chemical shifts are shown with reference to CDCl3 as 7.26 ppm.

$PrOH)^+$ and 389 $(M-PrOH-AcOH)^+$ of EI-MS supported the presence of one acetyl and one propionyl residues. Taken together, the structure of pyripyropene P was elucidated as shown in Fig. 1.

Structures of Pyripyropenes M, N, O, Q and R

The molecular formula of pyripyropene R is the same as that of pyripyropene P (Table 1), suggesting that they are the positional stereoisomers of acyloxy groups. In fact, all the spectral data including ^{1}H and ^{13}C NMR (Table 2~3), HMBC and EI-MS indicated that pyripyropene R has a propionyloxy residue at C-1 and an acetoxy residue at C-11. The positions of the two acyloxy residues were confirmed by the HMBC experiments.

The molecular formula of pyripyropene O is a CH_2 unit lower than that of pyripyropene P (Table 1), suggesting the presence of acetoxy residues at both C-1 and -11 positions. The molecular formula of pyripyropene Q is an oxygen atom larger than that of pyripyropene P (Table 1), suggesting the presence of a hydroxy group attached to the C-13 position. The molecular formula of pyripyropene N is a CH_2 unit larger than that of pyripyropene Q (Table 1), suggesting the presence of propionyloxy residues at both C-1 and C-11 positions. All the spectral data (Table 2) supported the structures of pyripyropenes O, Q and N. The molecular formula of pyripyropene M is an acetoxy unit larger than that of pyripyropene P (Table 1), suggesting the presence of acyloxy residues at all the C-1, -7 and -11 positions. The HMBC experiment indicated that pyripyropene M has acetoxy residues at C-1 and C-11 and a propionyloxy

Table 2-3. ^{1}H and ^{13}C NMR chemical shifts of pyripyropenes Q and R.

Carbon No.	Pyripyropene Q		Pyripyropene R	
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]
C-1	73.9	4.81 (1H, dd, *J*=11.5, 5.0 Hz)	73.4	4.81 (1H, dd, *J*=11.5, 4.5 Hz)
C-2	22.8	1.80 (1H, m)	22.9	1.69 (1H, m)
		1.86 (1H, m)		1.86 (1H, m)
C-3	36.5	1.38 (1H, m)	36.8	1.22 (1H, m)
		2.14 (1H, m)		1.82 (1H, m)
C-4	38.0		36.7	
C-5	56.2	1.53 (1H, m)	51.5	1.58 (1H, m)
C-6	82.1		80.7	
C-7	41.1	1.62 (1H, m)	39.9	1.67 (1H, m)
		2.13 (1H, m)		2.14 (1H, m)
C-8	19.2	1.63 (2H, m)	19.0	1.47 (1H, m)
				1.68 (1H, m)
C-9	48.2	1.41 (1H, m)	47.7	1.48 (1H, m)
C-10	40.6		40.6	
C-11	65.0	3.73 (1H, d, *J*=12.0 Hz)	65.1	3.75 (1H, d, *J*=12.0 Hz)
		3.84 (1H, d, *J*=12.0 Hz)		3.84 (1H, d, *J*=12.0 Hz)
C-12	17.5	1.42 (3H, s)	15.6	0.99 (3H, s)
C-13	60.4	4.98 (1H, d, *J*=4.0 Hz)	17.3	2.26 (1H, dd, *J*=17.0, 12.5 Hz)
				2.55 (1H, dd, *J*=17.0, 5.0 Hz)
C-13-OH		2.83 (1H, brs)		
C-14	22.1	1.63 (3H, s)	20.7	1.28 (3H, s)
C-15	13.1	0.89 (3H, s)	13.1	0.88 (3H, s)
1-O-CO-(CH2)-CH3	21.2	2.04 (3H, s)	9.19	1.13 (3H, t, *J*=7.5 Hz)
1-O-CO-CH2-CH3			27.9	2.32 (2H, dq, *J*=1.5, 7.5 Hz)
1-O-CO-(CH2)-CH3	170.5		173.8	
7-O-CO-(CH2)-CH3				
7-O-CO-CH2-CH3				
7-O-CO-(CH2)-CH3				
11-O-CO-(CH2)-CH3	9.27	1.13 (3H, t, *J*=7.5 Hz)	20.9	2.07 (3H, s)
11-O-CO-CH2-CH3	27.6	2.33 (2H, dq, *J*=1.5, 7.5 Hz)		
11-O-CO-(CH2)-CH3	174.2		170.9	
C-2'	164.2		164.0	
C-3'	103.3		100.2	
C-4'	162.7		162.7	
C-5'	99.5	6.46 (1H, s)	99.3	6.42 (1H, s)
C-6'	157.2		155.7	
C-2"	146.8	9.00 (1H, d, *J*=2.0 Hz)	146.6	8.99 (1H, d, *J*=2.0 Hz)
C-3"	127.3		127.5	
C-4"	132.9	8.10 (1H, ddd, *J*=8.0, 2.0, 1.0 Hz)	132.8	8.10 (1H, ddd, *J*=8.0, 2.0, 1.0 Hz)
C-5"	123.6	7.41 (1H, dd, *J*=8.0, 5.0 Hz)	123.6	7.39 (1H, ddd, *J*=8.0, 5.0 Hz)
C-6"	151.5	8.68 (1H, dd, *J*=5.0, 1.0 Hz)	151.1	8.66 (1H, dd, *J*=5.0, 1.0 Hz)

[a] Each sample was dissolved in CDCl3. Chemical shifts are shown with reference to CDCl3 as 77.7 ppm. [b] Chemical shifts are shown with reference to CDCl3 as 7.26 ppm.

residue at C-7.

Taken together, the structures of pyripyropenes M, N, O, Q and R were elucidated as shown in Fig. 1.

Biological Properties

Effect of Pyripyropenes on ACAT Activity in Microsomes

As shown in Fig. 5, pyripyropenes inhibited ACAT activity dose-dependently in the enzyme assay. Among the new pyripyropenes, pyripyropene M showed the most potent inhibitory activity with an IC_{50} value of 3.80 μM. However, pyripyropenes N (IC_{50}: 48.0 μM), O (11.0 μM), P (44.0 μM), Q (40.0 μM) and R (78.0 μM) were less potent than pyripyropene M.

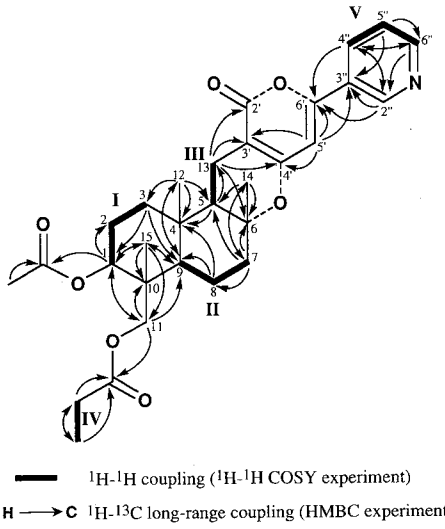
Other Biological Activities

No antimicrobial activity was observed at a concentration of 1 mg/ml (10 μg/disk) for pyripyropenes M to R against the following microorganisms; *Bacillus subtilis*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, *Candida albicans*, *Saccharomyces sake*, *Pyricularia oryzae*, *Mucor racemosus* and *Aspergillus niger*.

Discussion

From this series of our study[1~4], a total of 18 different pyripyropenes were isolated from *Aspergillus fumigatus* FO-1289-2501. All the pyripyropenes isolated have the common structure of pyridino-α-pyrone sesquiterpene (Fig. 1). Four possible oxygenated positions R_1-R_4 exist in the sesquiterpene moiety. Once the positions R_1

Fig. 4. ^1H-^1H COSY, HMQC and HMBC experiments of pyripyropene P.



to R_3 are oxygenated, the resulting hydroxy groups are exclusively acylated by acetyl or propionyl residues. However, R_4 position is not acylated even if it is oxygenated. The order of oxygenation at R_1-R_3 positions is deduced from the structures of all pyripyropenes. All the components of pyripyropenes have an acyloxy moiety at the R_3 position, indicating that this position is oxygenated first. Our recent study on biosynthetic origin of pyripyropene A[6] also suggested that R_3 oxygenated sesquiterpene moiety might be produced *via* cyclization of epoxidated farnesyl intermediate. Furthermore, we could isolate R_1, R_2-dideoxy (E to H) and R_2-monodeoxy (N to R) pyripyropenes (Fig. 1) but R_1-monodeoxy ones were not obtained, indicating that the R_1 position is oxygenated second, followed by the R_2 position.

Pyripyropenes are classified into three groups depending upon their potency of *in vitro* ACAT inhibitory activity; triacyloxy pyripyropenes (A ~ D and I ~ M) are the most potent group, diacyloxy ones (N ~ R) are the second potent group and monoacyloxy ones (E ~ H) are very weak inhibitors. Presence of R_4 hydroxy moiety in pyripyropenes is favorable for ACAT inhibition. Studies on structure-activity relationships of pyripyropenes are in progress[9] and will be reported in near future.

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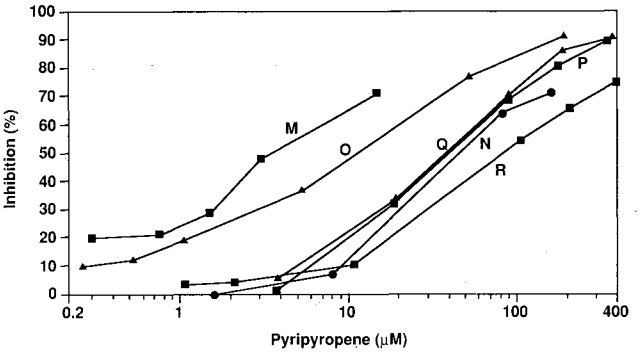
Fig. 5. ACAT inhibition by pyripyropenes M to R in the enzyme assay using rat liver microsomes.



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