PURPACTINS, NEW INHIBITORS OF ACYL-CoA:CHOLESTEROL ACYLTRANSFERASE PRODUCED BY *Penicillium purpurogenum*

II. STRUCTURE ELUCIDATION OF PURPACTINS A, B AND C

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The structure of purpactins, novel acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors, was determined by spectroscopic analyses. Purpactin A was deduced to be 3-1′-acetoxy-11-hydroxy-4-methoxy-9-methyl-3′-methylbutyl-5*H*,7*H*-dibenzo[b,g]-1,5-dioxocin-5-one, purpactin B was 5-1″-acetoxy-6′-hydroxymethyl-4-methoxy-4′-methyl-3″-methylbutyl-spiro[benzofuran-2,1′-cyclohexa-3′,5′-diene]-2′,3(2*H*)-dione and purpactin C was 5-1″-acetoxy-6′-formyl-4-methoxy-4′-methyl-3″-methylbutyl-spiro[benzofuran-2,1′-cyclohexa-3′,5′-diene]-2′,3(2*H*)-dione. Purpactin A was attributed to 1′-*O*-acetylpenicillide.

In the course of our screening for acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor, purpactins A, B and C have been isolated from the fermentation broth of *Penicillium purpurogenum* FO-608. The taxonomy of the producing strain and fermentation, isolation and physico-chemical and biological characteristics of purpactins were reported in the preceding paper.[1] In this report, we describe the structure elucidation of purpactins.

Structure Elucidation of Purpactin A (1)

The MW and molecular formula of purpactin A (**1**) were determined to be 414.1678 and $C_{23}H_{26}O_7$ by analysis of HREI-MS spectrum, respectively. EI-MS spectrum suggested that **1** possesses acetoxy moiety because of the existance of a fragment ion peak at *m/z* 354 $(M-CH_3COOH)^+$ (Fig. 1). The ^1H-^{13}C COSY spectrum (Fig. 2 and Table 1) revealed the presence of 23 carbon signals, which were classified as four $-CH_3$, one aromatic $-OCH_3$, one $-CH_2-$, one $-O-CH_2-$, one $-O-CH-$, four $-CH=$, eight $-C=$ and two $-O-CO-$. The ^1H NMR spectrum of **1** (Fig. 3A) showed 25 non-exchangeable and one exchangeable proton signals. The exchangeable signal was attributed to be a phenol hydroxy proton because a bathochromic shift in the UV spectrum under the alkaline condition was observed. Analysis of ^1H-^1H COSY spectrum[2] revealed the presence of 1-oxy-isopentyl moiety and *ortho* and *meta* coupling protons (Fig. 4). The connection of unassigned carbons was confirmed by ^1H-^{13}C long range selective proton decoupling (LSPD) experiments. By irradiating the 1′-H (δ 6.11) and 1′-O-CO-CH_3 (δ 2.06), multiplicities at δ 170.2 and δ 134.3 were simplified, respectively, and the position of the 1-acetoxy-isopentyl moiety was determined (Fig. 5A). Also by irradiating the protons of 4-OCH_3 (δ 4.03), 1-H (δ 7.44) and 2-H (δ 6.87), the position of a methoxy moiety and the substitution pattern of four substituted benzene ring moiety were elucidated (Fig. 5A). In the NOE experiments, the irradiation of 4-OCH_3 (δ 4.03) enhanced

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Fig. 1. EI-MS spectrum of purpactin A.

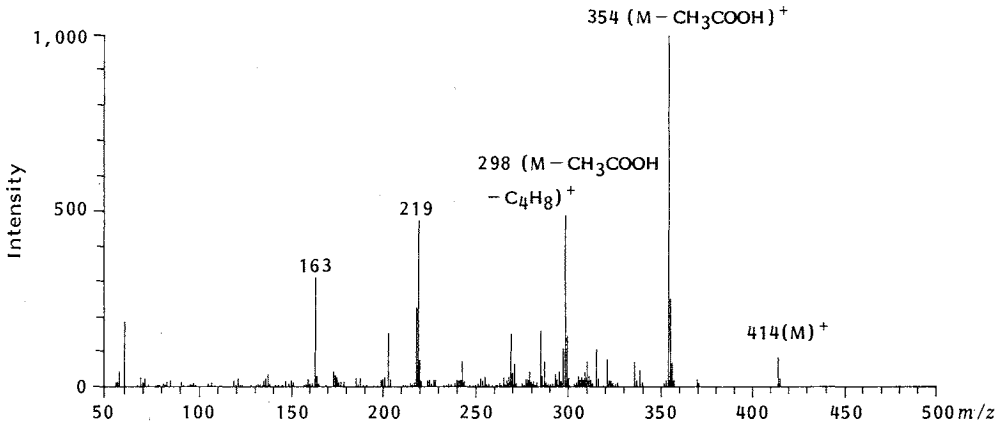
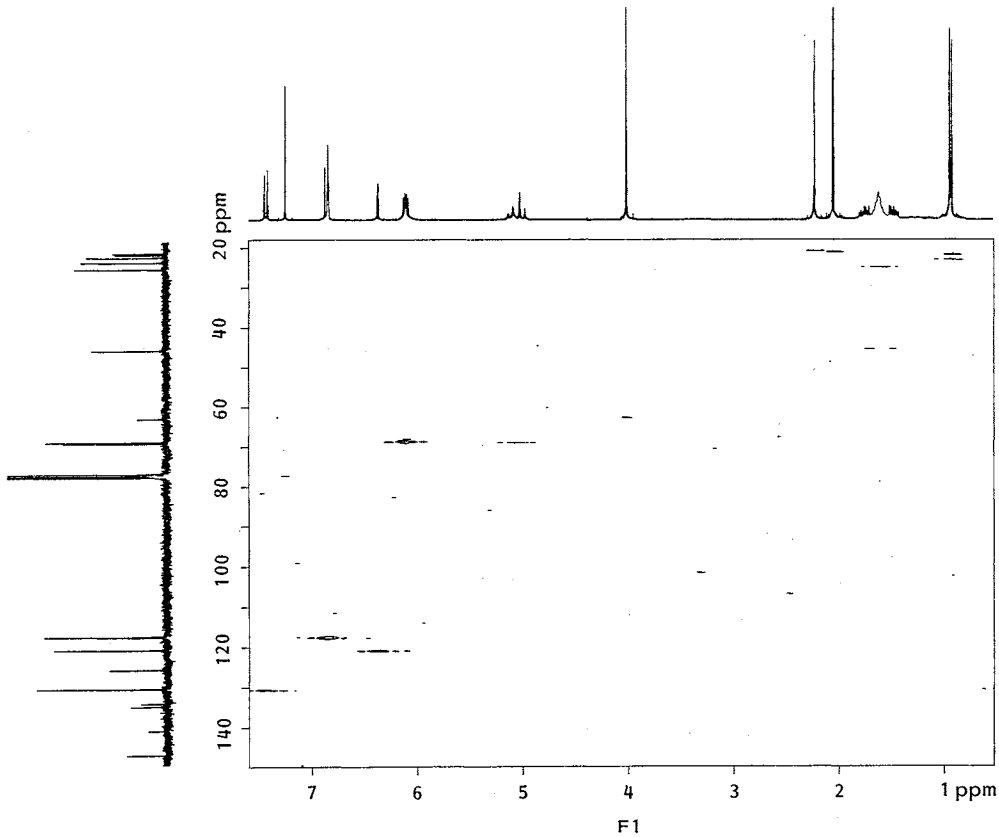


Fig. 2. ^{1}H-^{13}C COSY of purpactin A.



the intensity of the proton at δ 6.11 (1′-H), supporting the partial structure A as elucidated above. On the other hand, the irradiation of 7-H (δ 5.01 and δ 5.12) simplified the carbon multiplicities at δ 167.0, δ 125.8, δ 120.9 and δ 141.2 and the position of an oxymethylene moiety was clarified (Fig. 5B). Irradiating the protons of 9-CH_3 (δ 2.24), 8-H (δ 6.38) and 10-H (δ 6.85), the position of an aromatic methyl and

Table 1. ^1H and ^{13}C NMR shifts of **1** and its monomethylether (**1'**).

Carbon No.	**1**		**1'**	
	^{13}C Shift	^1H Shift	^{13}C Shift	^1H Shift
C-1	117.7	6.87 (1H, d, J=8.5)	118.2	6.93 (1H, d, J=8.5)
C-2	130.7	7.44 (1H, d, J=8.5)	130.6	7.41 (1H, d, J=8.5)
C-3	134.3		134.6	
C-4	154.7		154.7	
C-4-OCH_3	62.7	4.03 (3H, s)	62.7	4.00 (3H, s)
C-4a	119.8		119.8	
C-5	167.0		167.1	
C-7	68.9	5.01 (1H, d, J=14.0), 5.12 (1H, d, J=14.0)	69.0	5.00 (1H, d, J=14.0), 5.12 (1H, d, J=14.0)
C-7a	125.8		127.3	
C-8	120.9	6.38 (1H, d, J=1.7)	121.2	6.42 (1H, d, J=1.2)
C-9	135.1		133.4	
C-9-CH_3	20.9	2.24 (3H, s)	21.1	2.27 (3H, s)
C-10	117.5	6.85 (1H, d, J=1.7)	114.4	6.78 (1H, d, J=1.2)
C-11	147.2		152.5	
C-11-OH		6.12 (1H, br s)		
C-11-OCH_3			56.4	3.92 (3H, s)
C-11a	141.2		143.4	
C-12a	151.5		151.2	
C-1'	68.6	6.11 (1H, dd, J=4.0, 9.0)	68.7	6.12 (1H, dd, J=4.0, 9.0)
C-1'-CO	170.2		170.2	
C-1'-$COCH_3$	21.2	2.06 (3H, s)	21.2	2.04 (3H, s)
C-2'	45.3	1.48 (1H, ddd), 1.77 (1H, ddd)	45.3	1.49 (1H, ddd), 1.76 (1H, ddd)
C-3'	24.9	1.63 (1H, m)	24.9	1.61 (1H, m)
C-3'-CH_3	21.9	0.95 (3H, d, J=6.0)	21.9	0.93 (3H, d, J=6.0)
C-4'	23.1	0.95 (3H, d, J=6.0)	23.1	0.93 (3H, d, J=6.0)

J=Hz.

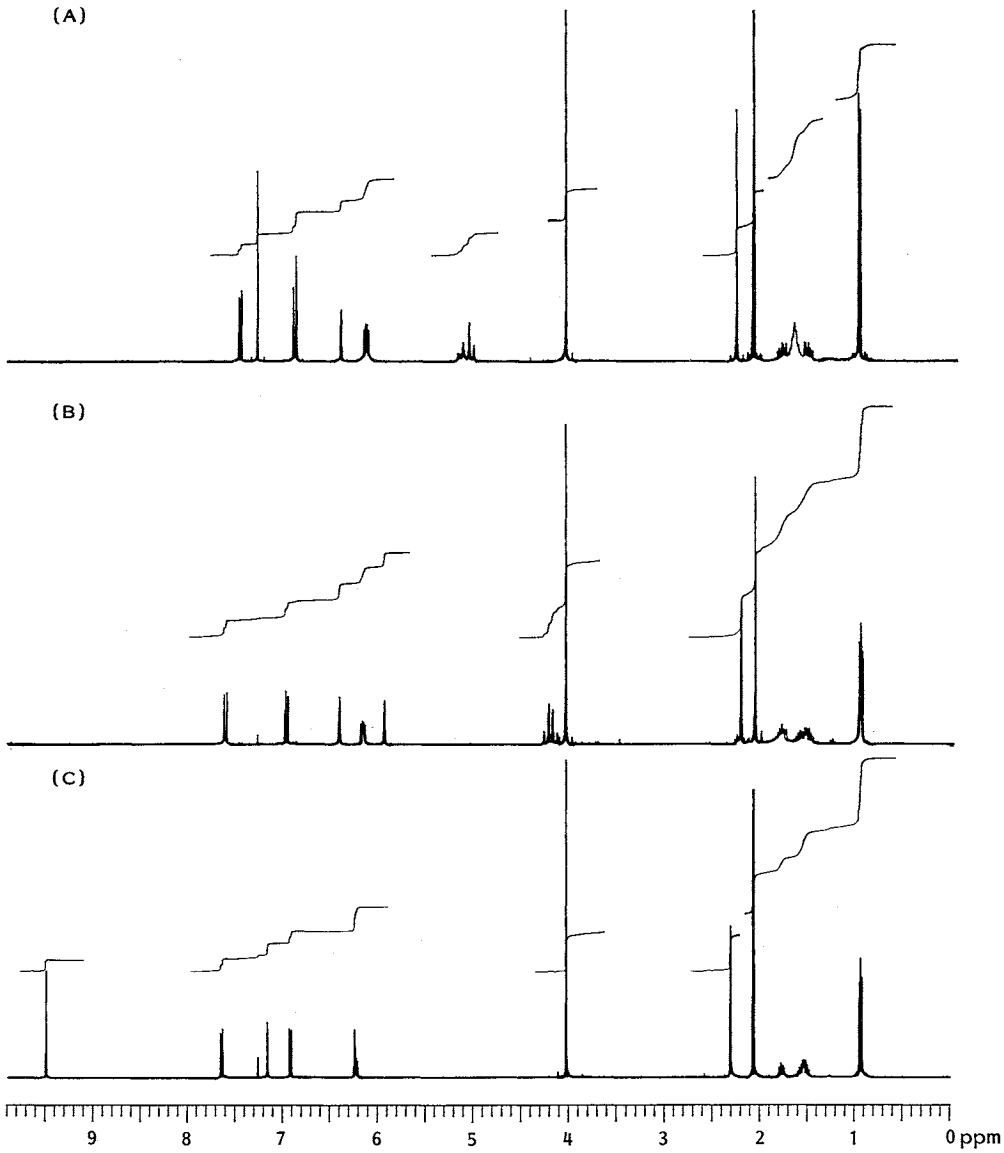
the substitution pattern of four substituted benzene ring were concluded as shown in Fig. 5B. The NOE study showed that the irradiations of 7-H (δ 5.01 and δ 5.12) enhanced the intensity of the proton at δ 6.38 (8-H), supporting the partial structure obtained through ^1H-^{13}C LSPD experiments as described above. To determine the phenol hydroxy position, monomethyl purpactin A (**1'**) was prepared by treating with diazomethane. Comparing with both ^{13}C NMR spectra of **1** and **1'**, the phenol position was deduced to be C-11 because of the observation of downfield shift of the olefinic quaternary carbon at δ 147.2 to δ 151.2 ppm (Table 1). From all of the observations described above, the partial structures of compound **1** are concluded as shown in Fig. 5A and 5B. Considering the carbon chemical shifts at C-4a (δ 119.8), C-5 (δ 167.0), C-11a (δ 141.2) and C-12a (δ 151.5), it is concluded that **1** has 5*H*,7*H*-dibenzo[b,g]-1,5-dioxocin 5-one skeltone. Thus, the structure of **1** was determined to be 3-1'-acetoxy-11-hydroxy-4-methoxy-9-methyl-3'-methylbutyl-5*H*,7*H*-dibenzo[b,g]-1,5-dioxocin-5-one (Fig. 6).

Structure Elucidation of Purpactin B (2)

Purpactin B (**2**) possesses the same molecular formula ($C_{23}H_{26}O_7$) and a similar EI-MS fragmentation pattern (m/z 354 $(M-CH_3COOH)^+$ and m/z 298 $(M-CH_3COOH-C_4H_8)^+$) with purpactin A (**1**). In the ^1H NMR spectrum of **2** (Fig. 3B and Table 2), it was concluded that **2** also possesses the partial structure A as shown in the structure of **1**. And the highfield shift was observed on the signals of the

Fig. 3. ^{1}H NMR spectra of purpactins A, B and C (300 and 400 MHz, $CDCl_3$).

(A) Purpactin A, (B) purpactin B, (C) purpactin C.

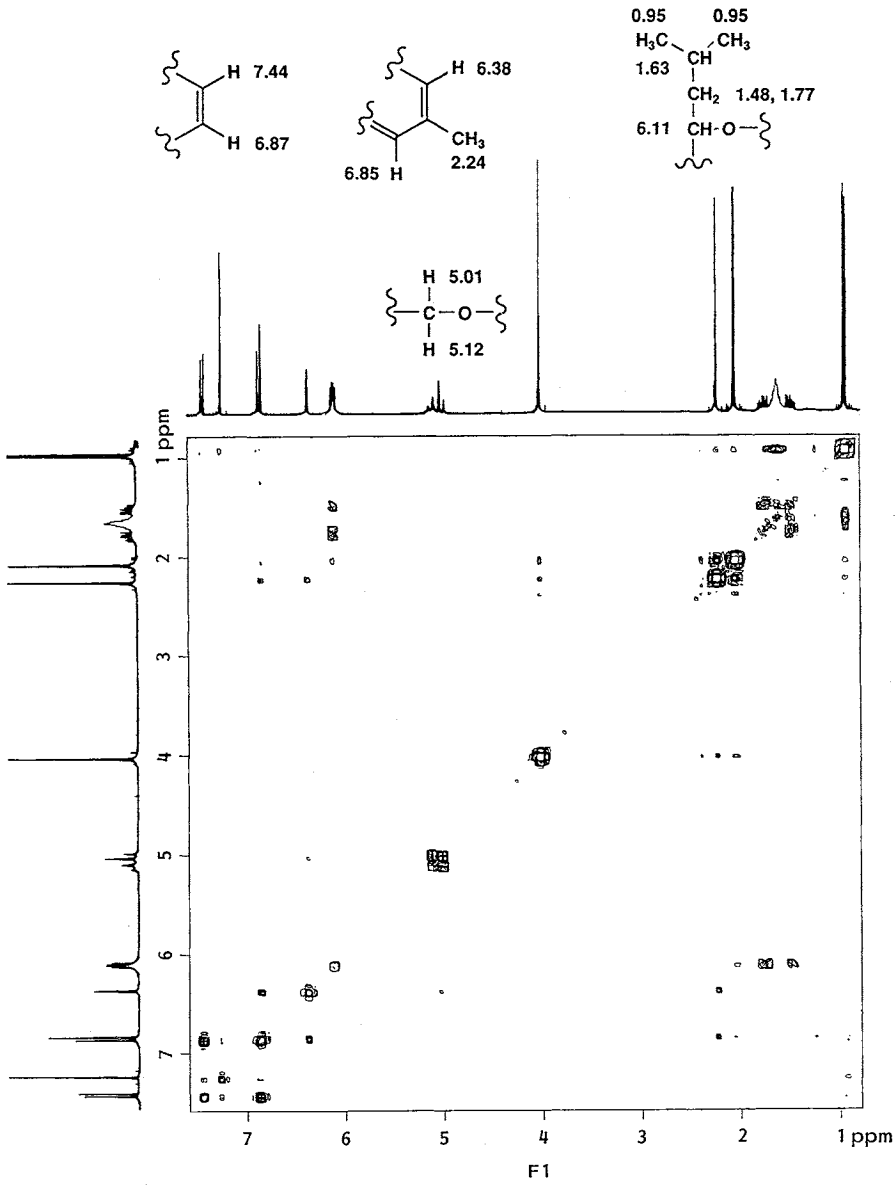


oxymethylene protons (δ 4.14 and δ 4.23) and hydroxy proton (δ 1.78) compared with those of **1**. The NOE study on **2** showed that the irradiations of 4-OCH_3 (δ 4.02), 6′-O–CH_2– (δ 4.14 and δ 4.23), 4′-CH_3 (δ 2.18) and 3′-H enhanced the intensities of the protons at δ 6.15 (1″-H), δ 6.39 (5′-H) and δ 5.93 (3′-H) and of the carbon at δ 191.2 (C-2′), respectively. The connection between unassigned carbons was also determined by LSPD experiments. From all of the observation described above, the structure of **2** was concluded to be 5-1″-acetoxy-6′-hydroxymethyl-4-methoxy-4′-methyl-3″-methylbutyl-spiro[benzofuran-2,1′-cyclohexa-3′,5′-diene]-2′,3(2*H*)-dione (Fig. 6).

Structure Elucidation of Purpactin C (3)

The ^{1}H NMR spectrum of purpactin C (**3**) (Fig. 3C and Table 2) was quite similar to that of purpactin

Fig. 4. ^{1}H-^{1}H COSY of purpactin A.



B (**2**) except for the presence of aldehyde signal (δ 9.49) and the absence of hydroxymethyl (δ 4.14 and δ 4.23) and δ 1.78 (–OH). The molecular formula of **3** ($C_{23}H_{24}O_7$) suggested that **3** was oxidative compound of **2**. The assignments of each signal were achieved by comparison with those of **2** and confirmed by LSPD and NOE experiments. Consequently, the structure of **3** was determined to be 5-1″-acetoxy-6′-formyl-4-methoxy-4-methyl-3″-methylbutylspiro[benzofuran-2,1′-cyclohexa-3′,5′-diene]-2′,3(2*H*)-dione (Fig. 6).

Discussion

Chemical structures of purpactins A (**1**), B (**2**) and C (**3**) were elucidated mainly by analyzing NMR

Fig. 5. ^{1}H-^{13}C LSPD and NOE experiments of purpactin A.

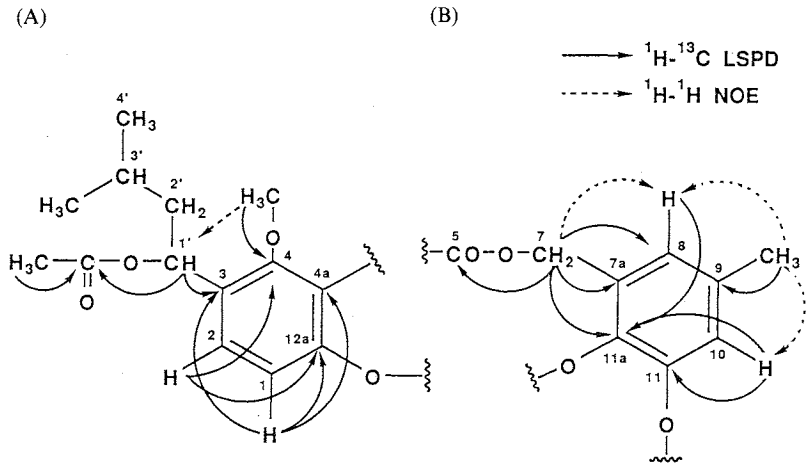
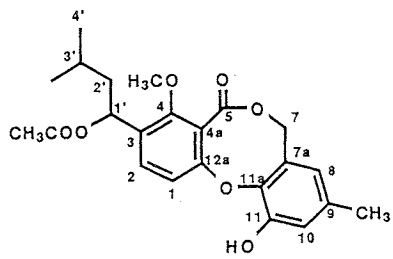
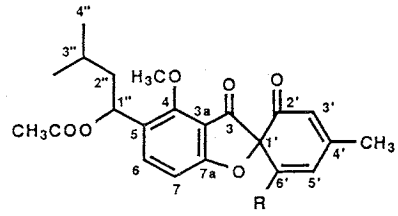


Fig. 6. Structures of purpactins A, B and C.



Purpactin A (**1**)



Purpactin B (**2**) R=CH_2OH
Purpactin C (**3**) R=CHO

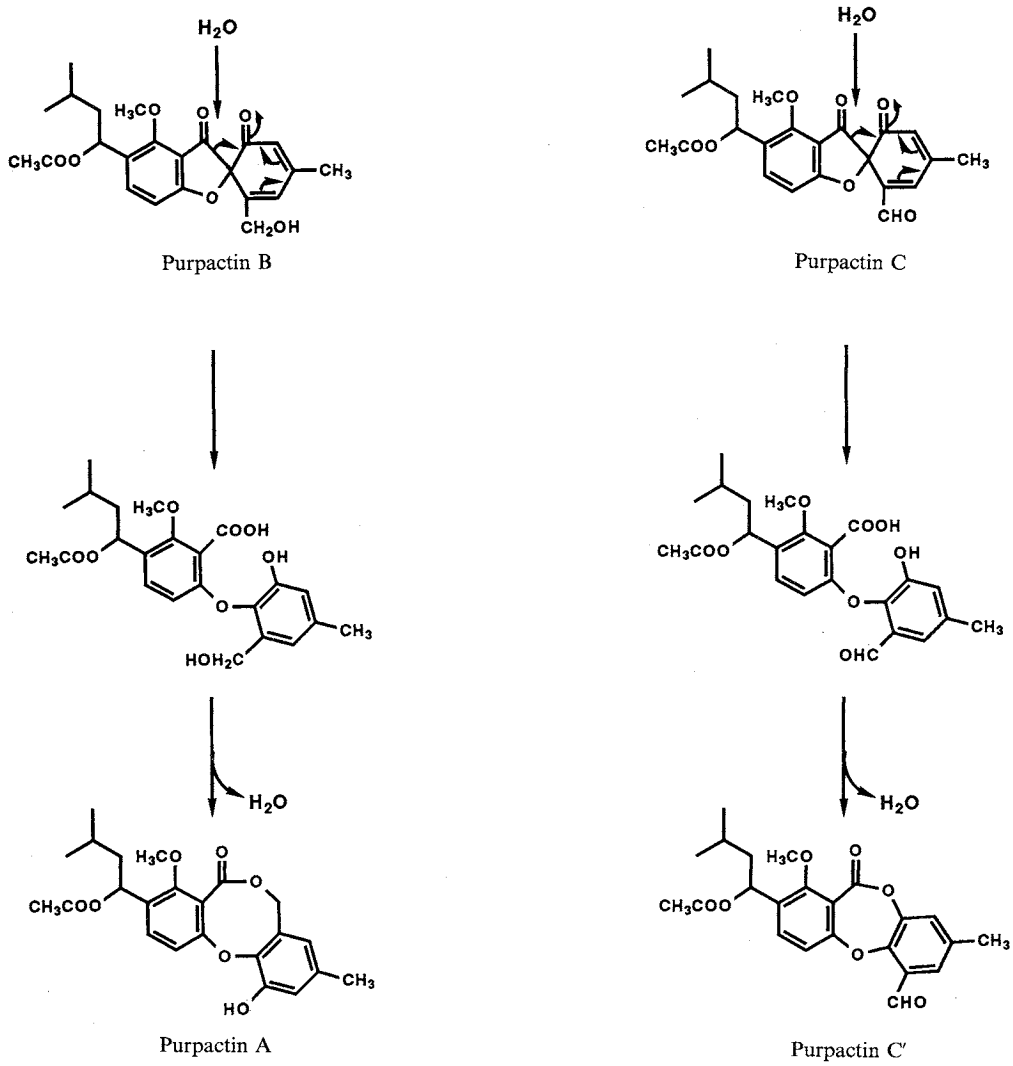
spectral data. All of these compounds possess a 1-acetoxy-3-methylbutyl phenyl moiety in common. Structurally related penicillide (**1**″)[3] was also obtained from the culture broth of *P. purpurogenum* FO-608 as a minor component. Purpactin A (**1**) attributes 1′-*O*-acetylpenicillide. On the other hand, purpactins B (**2**) and C (**3**) possess a similar skeltone with that of isogriseofulvin and showed the good coincidence of the chemical shifts at C-1′ and C-2′ position in ^{13}C NMR spectra.[4] Purpactin B (**2**) was converted to purpactin A (**1**) in an aqueous alcohol solution and it is likely that such cunversion occurs during fermentation as described in the preceding paper.[1] On the other hand, purpactin C (**3**) was also converted to purpactin C′ in pyridine at room temperature. The possible mechanism of this conversion was shown in scheme, but the postulated intermediates (**2a** and **3a**) have not been detected until now.[5] In addition, there might be the same kind of precursor of penicillide in the cultured broth.

Studies on the absolute configurations of purpactins (C-1′ of **1**, and C-1′ and C-1″ of both **2** and **3**) are in progress.

Experimental

UV and IR spectra were recorded on a Shimadzu model UV-200S spectrophotometer and a Jasco model A-102 interferometer, respectively. ^{1}H NMR (300 and 400 MHz) and ^{13}C NMR (75 and 100 MHz) spectra were obtained on a Varian XVK-300 and XL-400 spectrometer. MS was obtained with a Jeol model DX-300 mass spectrometer.

Scheme 1. Conversion of purpactin B to purpactin A and purpactin C to purpactin C'.



Preparation of Monomethyl-purpactin A (**1'**)

A solution of 10 mg of **1** in 3 ml of diethylether containing 5 mg of silica gel (Kieselgel 60, Merck) was kept under diazomethane gas at 0°C for 4 hours. The solution was applied to preparative TLC (Kieselgel 60 F_{254}, $CHCl_3$) and monomethylpurpactin A was obtained as a colorless powder (yield 10 mg, 97%); UV λ_{max}^{EtOH} nm (ε) 280 (2,000); EI-MS (*m/z*) 428 ($C_{24}H_{28}O_7$, M^+), 386 $(M-C_2H_2O)^+$, 368 $(M-C_2H_4O_2)^+$, 329 $(M-C_2H_2O-C_4H_9)^+$, 311 $(M-C_2H_4O_2-C_4H_9)^+$, 219 $(C_{13}H_{15}O_3)^+$, 163 $(C_{13}H_{15}O_3-C_4H_8)^+$; Rf value ($CHCl_3$-MeOH, 98:2) 0.60.

Preparation of Purpactin C' (**3'**)

A solution of 50 mg of **3** in 1 ml of pyridine was kept at room temperature for 3 hours. The solution was diluted with 10 ml of water and extracted with twice of 10 ml of EtOAc. The organic layer was concentrated and applied to preparative TLC (Kieselgel 60 F_{254}, $CHCl_3$-MeOH, 98:2) and **3'** was obtained as a colorless powder (yield 40 mg, 80%); HREI-MS (*m/z*) 412.1517 (calcd 412.1522) $(C_{23}H_{22}O_7)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (3H, d, 7.5), 0.94 (3H, d, 7.4), 1.48 (1H, m), 1.63 (1H, m), 1.75 (1H, m), 2.04 (3H, s), 2.37 (3H, s), 3.96 (3H, s), 6.10 (1H, dd, J=4.5 and 9.5 Hz), 7.03 (1H, d, J=8.5 Hz), 7.31

Table 2. ^{1}H and ^{13}C NMR shifts of **2** and **3**.

Carbon No.	**2**		**3**	
	^{13}C Shift	^{1}H Shift	^{13}C Shift	^{1}H Shift
C-3	187.8		188.4	
C-3a	109.8		110.5	
C-4	156.6		156.5	
C-4-OCH_3	62.4	4.02 (3H, s)	62.4	4.03 (3H, s)
C-5	127.6		127.4	
C-6	137.1	7.60 (1H, d, J=8.5)	137.5	7.64 (1H, d, J=8.5)
C-7	107.4	6.96 (1H, d, J=8.5)	107.6	6.92 (1H, d, J=8.5)
C-7a	173.9		174.5	
C-1′	95.2		89.9	
C-2′	191.2		189.6	
C-3′	121.2	5.93 (1H, dd, J=1.0, 0.8)	126.9	6.24 (1H, dd, J=1.1, 1.5)
C-4′	156.3		152.0	
C-4′-CH_3	23.4	2.18 (3H, s)	22.8	2.31 (3H, s)
C-5′	126.0	6.39 (1H, d, J=0.8)	143.6	7.16 (1H, d, J=1.1)
C-6′	146.5		142.2	
C-6′-CH_2OH	61.5	4.14 (1H, d, J=14.8), 4.23 (1H, d, J=14.8), 1.78 (1H, br s, OH)		
C-6′-CHO			187.5	9.49 (1H, s)
C-1″	68.4	6.15 (1H, dd, J=5.0, 8.5)	68.2	6.22 (1H, dd, J=5.0, 8.5)
C-1″-CO	170.2		170.0	
C-1″-$COCH_3$	21.2	2.04 (3H, s)	21.2	2.07 (3H, s)
C-2″	45.1	1.49 (1H, ddd), 1.76 (1H, ddd)	45.1	1.51 (1H, ddd), 1.78 (1H, ddd)
C-3″	24.8	1.57 (1H, m)	24.7	1.57 (1H, m)
C-3″-CH_3	22.8	0.94 (3H, d, J=4.2)	22.6	0.95 (3H, d, J=4.2)
C-4″	22.2	0.92 (3H, d, J=4.2)	22.2	0.93 (3H, d, J=4.3)

J=Hz.

(1H, d, J=2.0 Hz), 7.47 (1H, d, J=2.0 Hz), 7.48 (1H, d, J=8.5 Hz), 10.59 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7 (q), 21.1 (q), 21.8 (q), 23.1 (q), 24.9 (q), 45.1 (t), 63.1 (q), 68.6 (d), 114.7 (s), 114.8 (d), 125.3 (d), 127.3 (d), 128.5 (s), 132.0 (d), 133.8 (s), 136.9 (s), 144.3 (s), 150.6 (s), 159.1 (s), 160.4 (s), 160.6 (s), 170.2 (s), 187.6 (d).

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