Erabulenols, Inhibitors of Cholesteryl Ester Transfer Protein Produced by *Penicillium* sp. FO-5637

II. Structure Elucidation of Erabulenols A and B

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Structures of erabulenols A and B, novel fungal inhibitors of cholesteryl ester transfer protein were elucidated by spectroscopic studies including various NMR measurements. Erabulenols consist of a phenalenone skeleton and a 1,2,2-trimethyltetrahydrofuran moiety in common. Erabulenol B possesses an additional 2,6-dihydroxy-5-methyl-3-methylketonyl benzyl moiety. The absolute stereochemistry at the C-2′ position of erabulenol A was deduced as *S* by comparison of the optical rotation with that of other related compounds.

Erabulenols A (**1**) and B (**2**) were isolated from the culture broth of *Penicillium* sp. FO-5637 as inhibitors of cholesteryl ester transfer protein (CETP). A known related compound, scleroderolide (**3**)[1], was also isolated from the culture broth of the producer. The fermentation, isolation, and biological properties of erabulenols have been described in the preceding paper[2]. We will report herein the structure elucidation of erabulenols A and B.

Materials and Methods

Materials

Erabulenols and scleroderolide were isolated from the culture broth of *Penicillium* sp. FO-5637 as described in the preceding paper[2].

General Experimental Procedures

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 polarimeter. 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.

Results

Physico-chemical Properties of Erabulenols

Physico-chemical properties of erabulenols A and B and scleroderolide are summarized in Table 1. They are soluble in ethanol, acetonitrile, methanol, chloroform and ethyl acetate and insoluble in water and *n*-hexane. The UV spectra (Fig. 1) show a maxima at 208 (ε 26,300), 226 sh (ε 17,400), 263 (ε 27,800), 297 sh (ε 6,400), 356 (ε 15,700), 372 (ε 17,400) and 435 nm (ε 1,100) for erabulenol A and at 216 (ε 75,900), 240 (ε 69,900), 246 sh (ε 67,700), 286 (ε 51,200), 322 sh (ε 18,700) and 407 (ε 25,300) for erabulenol B. The IR spectra 1595 cm^{-1} for erabulenol A and 1624 cm^{-1} for erabulenol B suggest the presence of a carbonyl group in their structures.

Structure of Erabulenol B

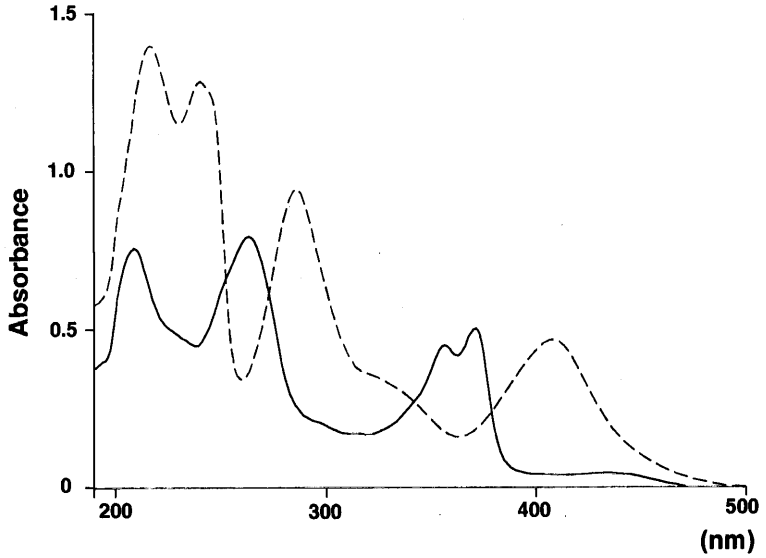
The molecular formula of erabulenol B was determined to be $C_{30}H_{30}O_{10}$ on the basis of HRFAB-MS measurement (*m/z*, found 551.1956, calcd 551.1970 for $C_{30}H_{31}O_{10}$ $[M+1]^+$). The ^{13}C NMR spectrum ($CDCl_3$) showed 30 resolved peaks (Table 2), which were classified into six $-CH_3$, one $-CH_2-$, one $-O-CH_3-$, one $-O-CH$, one $-CH=$, and 20 quaternary carbons by analysis of the DEPT spectra. The ^1H NMR spectrum displayed 29 proton signals (Table 2). Three downfield singlet protons (δ 14.8 and 13.2 (2H)) suggested the presence of hydrogen

Table 1. Physico-chemical properties of erabulenols A and B and sclerodeloride.

	Erabulenol A	Erabulenol B	Sclerodeloride
Appearance	Orange powder	Orange powder	Yellow powder
Molecular formula	$C_{20}H_{20}O_6$	$C_{30}H_{30}O_{10}$	$C_{18}H_{16}O_6$
Molecular weight	356	550	328
FAB-MS (*m/z*)			
Positive	357 $[M+H]^+$	551 $[M+H]^+$	329 $[M+H]^+$
	379 $[M+Na]^+$	573 $[M+Na]^+$	351 $[M+Na]^+$
Negative		549 $[M-H]^-$	
HRFAB-MS (*m/z*) (positive)			
MF+H	$C_{20}H_{21}O_6$	$C_{30}H_{31}O_{10}$	$C_{18}H_{17}O_6$
Calcd:	357.1338	551.1970	329.1025
Found:	357.1338	551.1956	329.1025
$[\alpha]_D^{23}$ (*c* 1.0, MeOH)	−220°	−223°	−117°
UV λ_{max}^{MeOH} nm (ε)	208 (26,300), 226 sh (17,400), 263 (27,800), 297 sh (6,400), 356 (15,700), 372 (17,400), 435 (1,100)	216 (75,900), 240 (69,900), 246 sh (67,700), 286 (51,200), 322 sh (18,700), 407 (25,300)	204 (31,500), 246 (47,600), 257 (47,200), 283 (18,400), 293 (17,400), 404 (12,800), 425 (12,800)
IR ν_{max}^{KBr} (cm^{-1})	3400, 1595, 1458, 1419, 1383, 1294, 1215, 1165, 1128, 1036	3400, 1624, 1504, 1446, 1388, 1369, 1180, 1025	3400, 1624, 1603, 1460, 1381, 1155, 1130, 1107, 1065, 1034
Solubility			
Soluble	EtOH, CH_3CN, MeOH, $CHCl_3$, EtOAc	EtOH, CH_3CN, MeOH, $CHCl_3$, EtOAc	EtOH, CH_3CN, MeOH, $CHCl_3$, EtOAc
Insoluble	H_2O, *n*-hexane	H_2O, *n*-hexane	H_2O, *n*-hexane

Fig. 1. UV spectra of erabulenols A and B (10 μg/ml in MeOH).

A: ——, B: – – –



bonded OH protons. The connectivity of proton and carbon atoms was confirmed by the HMQC spectrum.

Analyses of ^1H-^1H coupling observed in COSY spectrum and ^{13}C-^1H long-range couplings of ^2J and ^3J in the HMBC spectrum revealed the three partial structures as shown in Fig. 2. The partial structure I was elucidated because the long-range couplings of ^2J or ^3J were observed from C-7-OH (δ 13.2) to C-6 (δ 112.48), C-7 (δ 161.6) and C-8 (δ 130.61), from C-9-OH (δ 14.8) to C-8, C-9 (δ 162.7) and C-10 (δ 109.0), and from H_3-15

Table 2. ^{1}H and ^{13}C NMR chemical shifts of erabulenols A and B and scleroderolide.

Carbon No.	Erabulenol A				Erabulenol B		Scleroderolide	
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[c]	^{1}H chemical shifts (ppm)[d]	^{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	126.8		126.8		138.7		122.1	
C-2	111.6		113.5		105.5		108.7	
C-3	160.6		163.0		179.8		169.6	
C-4	118.4		118.4		79.6		119.3	
C-5	157.4		156.9		196.1		167.4	
C-5-OH				9.6 (1H, bs)				13.7 (1H, s)
C-6	98.02		97.72		112.48		107.3	
C-7	155.1		159.0		161.6		170.1	
C-7-OH				9.6 (1H, bs)		13.2 (1H, s)		
C-8	133.6		132.8		130.61		155.8	
C-9	177.3		175.9		162.7			
C-9-OH		8.3 (1H, bs)		15.5 (1H, s)		14.8 (1H, s)		
C-10	109.3		108.4		109.0		129.8	
C-11	171.7		170.0		185.8		144.6	
C-11-OH								3.49 (1H, s)
C-12	120.5	6.78 (1H, d, J=1.0 Hz)	121.9	6.80 (1H, d, J=1.0 Hz)	151.6		117.2	6.90 (1H, s)
C-13	146.3		147.9		131.3		137.1	
C-14	25.33	2.91 (3H, d, J=1.0 Hz)	25.66	2.90 (3H, d, J=1.0 Hz)	21.2	2.86 (3H, s)	22.4	2.75 (3H, s)
C-15	60.51	3.95 (3H, s)	59.72	3.80 (3H, s)	60.7	3.81 (3H, s)		
C-16					19.6	3.77 (1H, d, J=14.0 Hz)		
						3.97 (1H, d, J=14.0 Hz)		
C-17					112.46			
C-18					162.3			
C-18-OH						13.2 (1H, s)		
C-19					102.2			
C-20					130.60	7.32 (1H, s)		
C-21					117.9			
C-22					161.9			
C-22-OH						10.1 (1H, s)		
C-23					202.5			
C-24					26.1	2.49 (3H, s)		
C-25					16.2	2.15 (3H, s)		
C-1′	14.07	1.55 (3H, d, J=6.5 Hz)	13.98	1.40 (3H, d, J=6.5 Hz)	19.0	1.70 (3H, d, J=7.0 Hz)	14.7	1.53 (3H, d, J=7.0 Hz)
C-2′	92.39	4.75 (1H, q, J=6.5 Hz)	90.41	4.75 (1H, q, J=6.5 Hz)	96.5	4.70 (1H, q, J=7.0 Hz)	92.8	4.75 (1H, q, J=7.0 Hz)
C-3′	43.14		42.68		43.7		43.1	
C-4′	21.06	1.33 (3H, s)	20.36	1.30 (3H, s)	16.0	1.50 (3H, s)	20.6	1.34 (3H, s)
C-5′	25.09	1.60 (3H, s)	24.21	1.55 (3H, s)	24.3	0.99 (3H, s)	25.6	1.56 (3H, s)

[a] 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] Chemical shifts are shown with reference to DMSO-d_6 as 39.5 ppm.
[d] Chemical shifts are shown with reference to DMSO-d_6 as 2.48 ppm.

(δ 3.81) to C-8. Additionally, the long-range coupling of ^{4}J from C-7-OH to C-5 (δ 196.1) supported the structure I. The partial structure II elucidated from the following results; The long-range couplings of ^{2}J or ^{3}J were observed from H_3-14 (δ 2.86) to C-2 (δ 105.5), C-12 (δ 151.6) and C-13 (δ 131.1), from H_2-16 (δ 3.77 and 3.97) to C-11 (δ 185.8), C-12, C-13, C-17 (δ 112.46), C-18 (δ 162.3) and C-22 (δ 161.9), from C-18-OH (δ 13.2) to C-17, C-18 and C-19 (δ 102.2), from H-20 (δ 7.32) to C-18, C-21 (δ 117.9), C-22, C-23 (δ 202.5) and C-25 (δ 16.2), from C-22-OH (δ 10.1) to C-17, C-21 and C-22, from H_3-24 (δ 2.49) to C-23, and from H_3-25 (δ 2.15) to C-20 (δ 130.60), C-21 and C-22. Furthermore, the long-range coupling of ^{4}J were observed from C-18-OH to C-23, supporting the structure II with the 2,6-dihydroxy-5-methyl-3-methylketonyl benzyl moiety. The partial structure III was also elucidated from the following observation; ^{1}H-^{1}H COSY couplings between H_3-1′ (δ 1.70) and H-2′ (δ 4.70) showed the sequence of $-O-CH-CH_3$. Furthermore, the long-range couplings from H_3-1′ to C-2′ (δ 96.5) and C-3′ (δ 43.7), from H-2′ to C-3 (δ 179.8), C-4 (δ 79.6), C-1′ (δ 19.0), C-3′ and

Fig. 2. Partial structures I, II and III of erabulenol B.

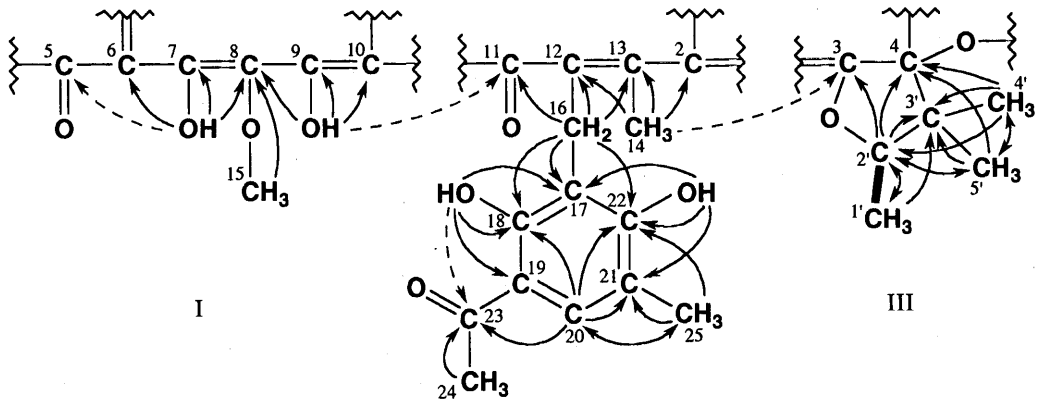
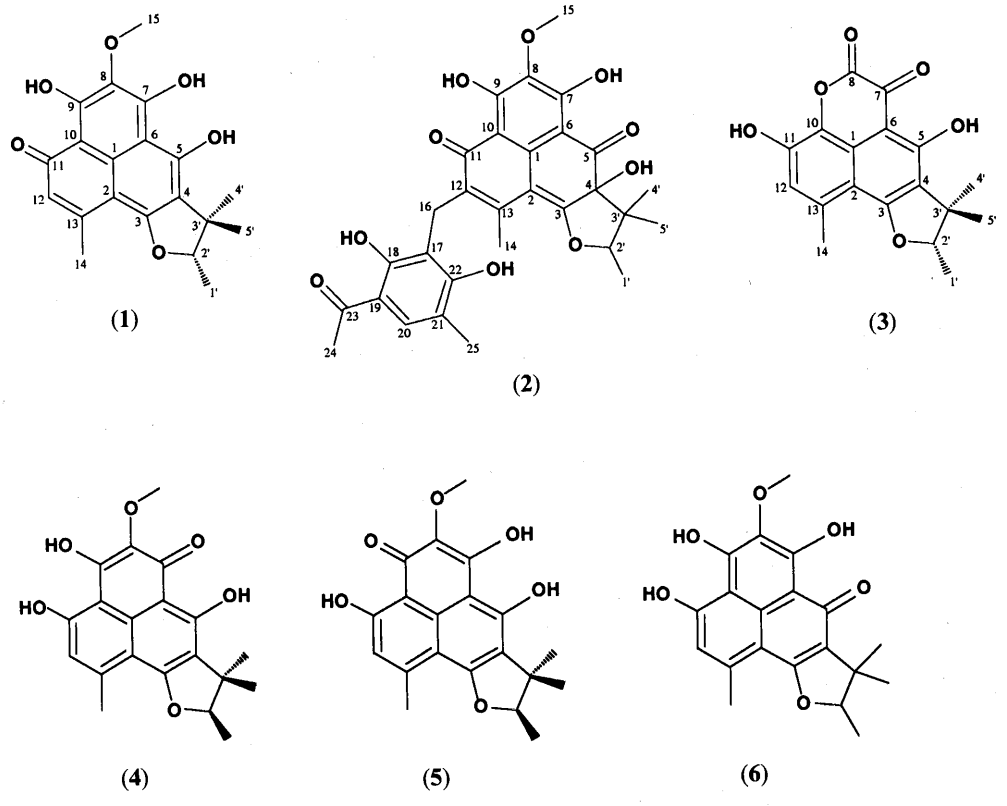


Fig. 3. Structures of erabulenols A (**1**) and B (**2**), scleroderolide (**3**) and deoxyherquinones (**4**~**6**).



C-5′ (δ 24.3), from H_3-4′ (δ 1.50) to C-4, C-2′, C-3′ and C-5′, and from H_3-5′ (δ 0.99) to C-4, C-2′, C-3′ and C-4′ (δ 16.0) gave the structure III with the 1,2,2-trimethyltetrahydrofuran moiety.

Next, regarding the connection of the three partial structures the cross peaks from C-9-OH to C-11 and from H_3-14 to C-3 were observed in the long-range couplings of ^4J, suggesting the I-II-III sequence as shown in Fig. 2.

In addition to the degree of unsaturation, the presence of the other three rings was suggested due to the following reasons; First, C-1 (δ 138.7) that showed no cross peak

in the HMBC experiments should be attached to the three sp^2 carbons, C-2 (δ 105.5), C-6 (δ 112.48) and C-10 (δ 109.0), which was supported by their ^{13}C chemical shifts and molecular modeling (actual models). Second, the ^{13}C chemical shift of C-5 (δ 196.1) indicated that the carbonyl carbon is linked directly to carbons. Finally, the remaining hydroxy group should be attached to C-4 (δ 79.6) due to the comparable ^{13}C chemical shift. Taken together, the general structure of erabulenol B (**2**) was elucidated as shown in Fig. 3. The preliminary data of X-ray crystallography (data not shown) supported the structure although the R-index was not good (R = 0.16).

Structure of Erabulenol A

The molecular formula of erabulenol A was determined to be $C_{20}H_{20}O_6$ on the basis of HRFAB-MS measurement (*m/z*, found 357.1338, calcd 357.1338 for $C_{20}H_{21}O_6$ $[M+1]^+$), which is the same as that of deoxyherqueinones[3~5] (**4**~**6**, Fig. 3). The NMR spectra in the two slovents (Table 2) showed structural similarity between erabulenol A and deoxyherqueinone[3] in that they possess the same phenalenone skeleton and 1,2,2-trimethyltetrahydrofuran ring (Fig. 3).

Comparison of the ^{13}C chemical shifts of erabulenol A with those of deoxyherqueinone suggested that they are the positional stereoisomers of the carbonyl group. The long-range couplings from C-9-OH (δ 15.5) to C-8 (δ 132.8), C-9 (δ 175.9) and C-10 (δ 108.4) and from H-12 (δ 6.80) to C-11 (δ 170.0). And the long range coupling from H_3-15 (δ 3.80) to C-8 suggested that a methoxy group is attached to C-8. Furthermore, the chemical shift of the quaternary C-8 (δ 133.6) was shifted to a higher field than that of deoxyherqueinone (δ 145.1), indicating that a hydroxy group should be attached to both C-7 and C-9. In fact, the C-8 chemical shift is in good agreement with the additivity rule of substituent.[6] Accordingly, the carbonyl carbon should be at the C-11 for erabulenol A.

Taken together, the structure of erabulenol A (**1**) was elucidated as shown in Fig. 3.

Stereochemistry of Erabulenol A

Erabulenol A, scleroderolide and deoxyherqueinone have the only stereogenic carbon at the same position in their 1,2,2-trimethylfuran moiety. The stereochemistry of scleroderolide with a minus $[\alpha]_D$ value ($-117°$, Table 1) was demonstrated to be *S*[1], whereas that of deoxyherqueinone (A) with plus $[\alpha]_D$ value ($+55.9°$) was reported to be *R*[3]. Therefore, erabulenol A with a minus $[\alpha]_D$ value ($-220°$, Table 1) was deduced to have the *S* configuration as shown in Fig. 3.

Discussion

Erabulenol B possesses the two chiral carbons at C-4 and C-2′ (Fig. 3). As described in the preceding paper[2], erabulenols and scleroderolide were produced by the same fungal strain (*Penicillium* sp. FO-5637) and are expected to share the same stereochemistry at the 1,2,2-trimethylfuran moiety. Therefore, it is plausible that erabulenol B has the *S* configuration at C-2′. The stereochemistry at C-4 of erabulenol B still remains to be investigated.

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