Terpendoles, Novel ACAT Inhibitors Produced by *Albophoma yamanashiensis*

III. Production, Isolation and Structure Elucidation of New Components

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Eight new components of terpendoles E to L were isolated and characterized from the culture broth of *Albophoma yamanashiensis* using a different production medium. All the structures were elucidated by spectroscopic analyses including various NMR experiments, indicating that all the terpendoles have the same indoloditerpene core as terpendoles A to D. Terpendoles J, K and L showed the moderate inhibition against acyl-CoA : cholesterol acyltransferase (ACAT) activity with IC_{50} values of 38.8, 38.0 and 32.4 μM in rat liver microsomes, respectively. But terpendoles E~I showed weak activities (IC_{50} 145~388 μM).

We reported new fungal indoloditerpenes named terpendoles as inhibitors of acyl-CoA : cholesterol acyltransferase (ACAT)[1,2]. Four terpendoles A to D, emindole SB and paspaline were isolated from the culture broth when the producer of *Albophoma yamanashiensis*[5] was fermented in a soluble starch and soybean meal-based medium. Further isolation study from the culture broth of the strain fermented in a different fermentation medium led to the discovery of eight more components of terpendoles E~L (Fig. 1). In this paper, the fermentation, isolation and structure elucidation of the terpendoles are described.

Materials and Methods

General Experimental Procedures

Albophoma yamanashiensis[5] was used for production of terpendoles. Kieselgel 60 (E. Merck) 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. Melting points were measured with a Yanaco micro melting point apparatus. 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.

Single Crystal X-Ray Analysis

Terpendole E was recrystallized from MeOH to establish the relative stereochemistry. The colorless plate crystal having approximate dimensions of $0.4 \times 0.5 \times 0.1$ mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC-5R diffractometer with graphite monochromated CuK_α radiation. The data were collected at a temperature of 23 ± 1°C using the ω–2θ scan technique to a maximum 2θ value of 140.3°. Pertinent crystal, data collection, and refinement parameters are summarized in Table 3. Neutral atom scattering factors were taken from CROMER and WABER[6]. Anomalous dispersion effects were included in Fcalc[7]; the values for $\Delta f'$ and $\Delta f''$ were those of CROMER[6]. All calculations were performed using the TEXSAN[8] crystallographic software package of Molecular Structure Corporation. The structure was solved by direct methods[9,10].

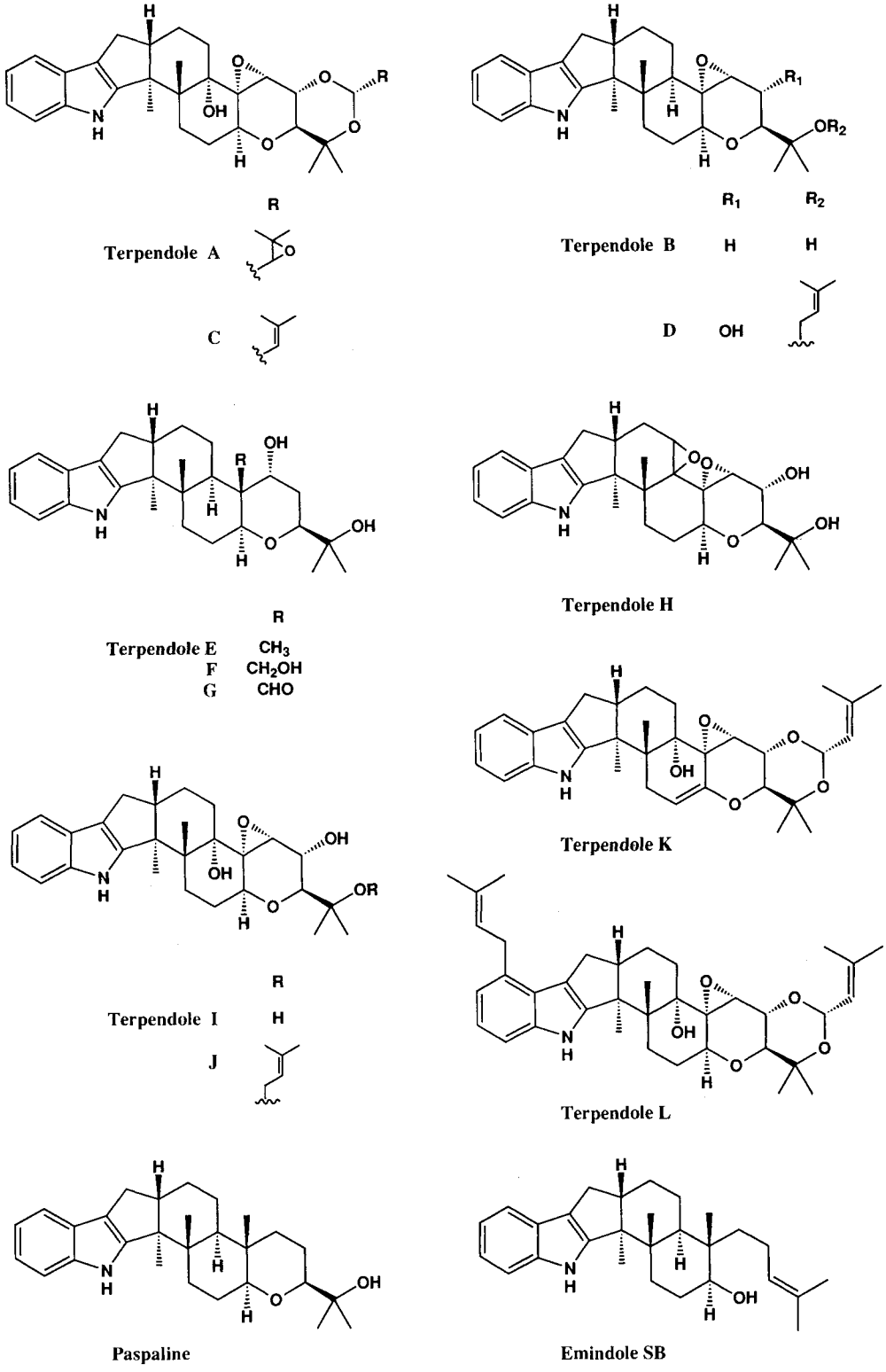
ACAT Activity

ACAT activity was measured in an enzyme assay using rat liver microsomes as reported previously[11].

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.

Fig. 1. Structures of terpendoles A to L, paspaline and emindole SB.



Results

Fermentation

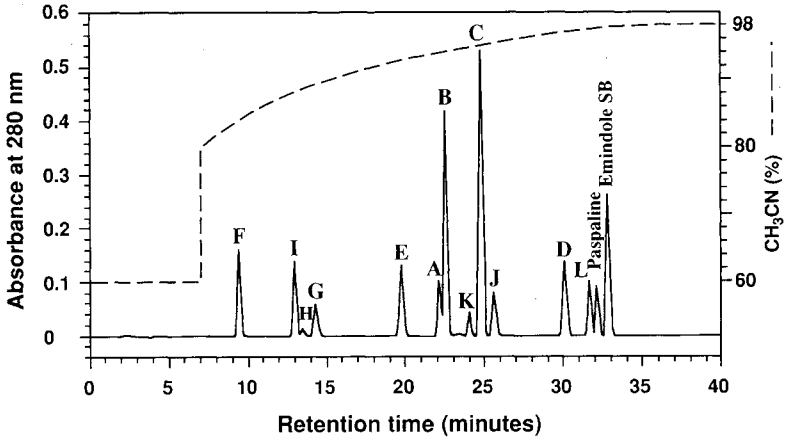
A slant culture of *A. yamanashiensis* grown on YpSs agar (soluble starch 1.5%, yeast extract 0.4%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05% and agar 2.0%, pH 6.0) was used to inoculate 500-ml Erlenmeyer flasks containing 100 ml of the seed medium (glucose 2.0%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, Polypepton 0.5%, KH_2PO_4 0.1% and agar 0.1%, pH 6.0). The flasks were

shaken on a rotary shaker for 2 days at 27°C. Two hundred milliliters of the seed culture were transferred into 20 liters of a production medium (maltose 5.0%, "Ebios" (Asahi Beer Co.) 2.5%, dry yeast 1.5%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, and KBr 1.0%, pH 7.0) in a 30-liter jar fermentor. The fermentation was carried out under the conditions as follows: aeration at 10 liters/minute, agitation at 250 rpm and temperature at 27°C. The production of terpendoles was measured by HPLC under the following conditions : Senshu pak ODS-H-1251, 4.6 × 250 mm; an isocratic 60% CH_3CN, at 0.7 ml/minute (0 ~ 7 minutes) and a convex gradient of program No. 5 (Waters 600E) from 80% CH_3CN to 98% CH_3CN, 1.0 ml/minute (7 ~ 40 minutes); UV detection at 280 nm. All terpendoles A to L, paspaline and emindole SB were separated (Fig. 2) to determine their titers in the culture broth. A typical time course of the fermentation is shown in Fig. 3. The production of terpendoles A and B was very low under this fermentation condition.

Isolation

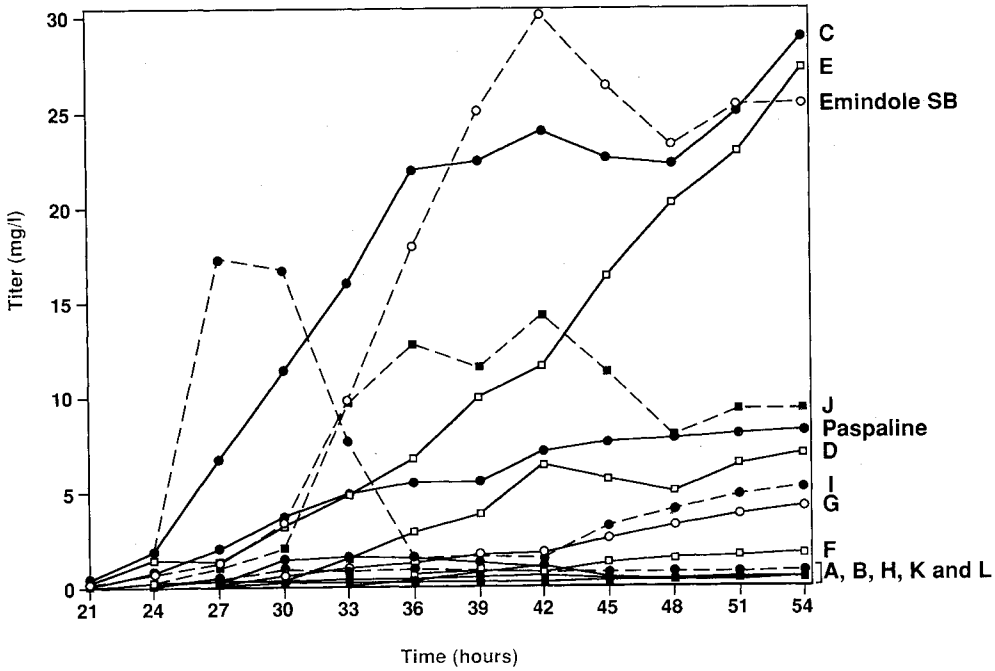
The isolation procedure for terpendoles E to L is summarized in Fig. 4. The 96-hour old whole broth (20 liters) was extracted with 20 liters of ethyl acetate. The extracts were dried over Na_2SO_4 and concentrated *in*

Fig. 2. Separation of all terpendoles by HPLC.



Terpendoles F 0.24 μg, I 0.26 μg, H 0.04 μg, G 0.14 μg, E 0.26 μg, A 0.20 μg, B 0.97 μg, K 0.11 μg, C 1.18 μg, J 0.19 μg, D 0.22 μg and L 0.14 μg, paspaline 0.16 μg and emindole SB 0.15 μg were injected.

Fig. 3. Time course of terpendoles production in a 30-liter jar fermentor.



vacuo to dryness to yield a brown oil (13.5 g). The material was distributed in a solution of *n*-hexane - methanol - H_2O (570 ml, 40 : 16 : 1, v/v). Then the lower layer was concentrated *in vacuo* to dryness to yield a brown oil (9.5 g). The oil was applied on a silica gel column (Kieselgel 60, 200 ml) previously equilibrated with chloroform. The materials were eluted with chloroform (1000 ml) and chloroform - methanol (98 : 2, 1000 ml). Each 100 ml of the elution was successively collected. The three active fractions were pooled; fr-1 (2nd ~ 10th

Fig. 4. Summary of isolation procedure of terpendoles E to L.

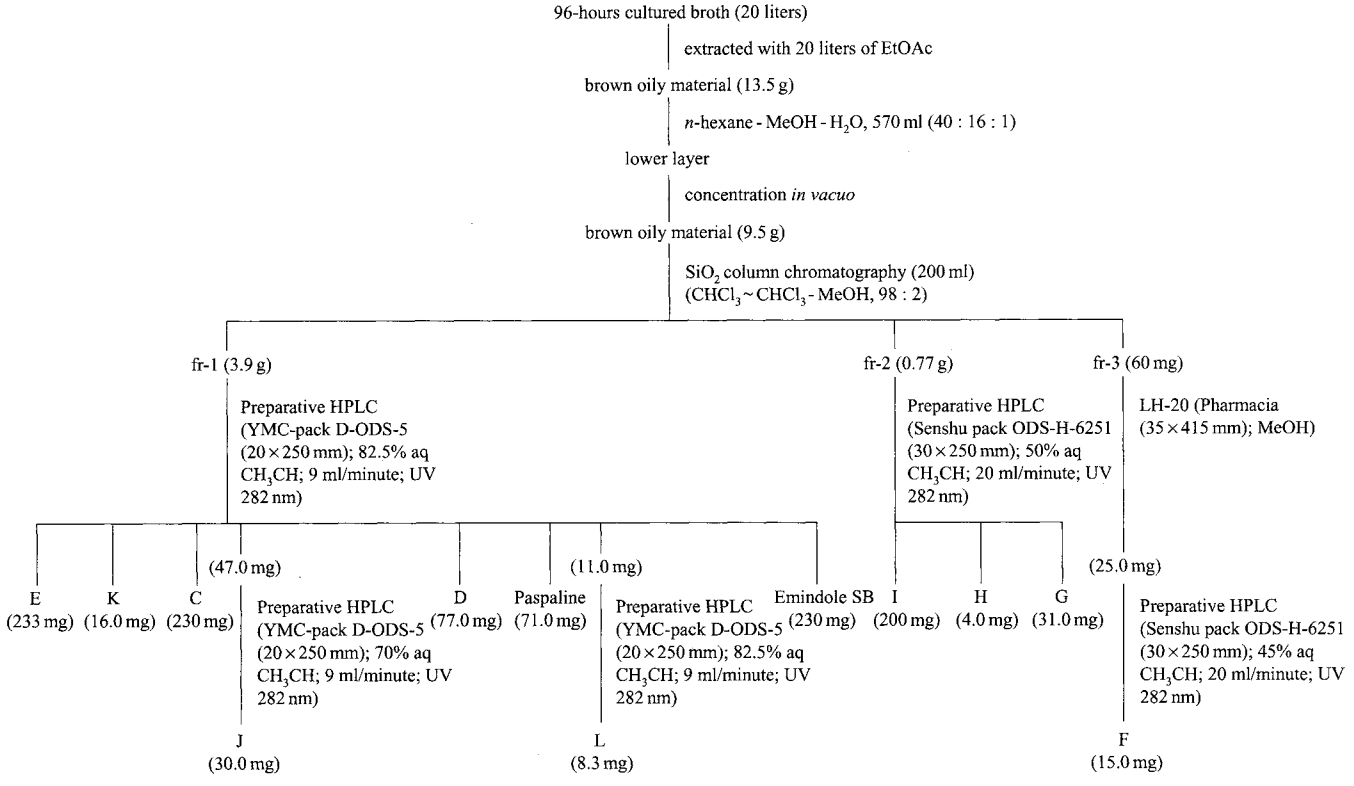


Table 1. Physico-chemical properties of terpendoles E, F, G, H, I, J, K and L.

	Terpendole E	Terpendole F	Terpendole G	Terpendole H	Terpendole I	Terpendole J	Terpendole K	Terpendole L
Appearence	Colorless crystal	White powder	White powder	White powder	White powder	White powder	White powder	White powder
Molecular weight	437	453	451	451	453	521	517	587
Molecular formula	$C_{28}H_{39}NO_3$	$C_{28}H_{39}NO_4$	$C_{28}H_{37}NO_4$	$C_{27}H_{33}NO_5$	$C_{27}H_{35}NO_5$	$C_{32}H_{43}NO_5$	$C_{32}H_{39}NO_5$	$C_{37}H_{49}NO_5$
FAB-MS (*m/z*)								
Positive	437[M]$^+$ 460[M+Na]$^+$	453[M]$^+$ 476[M+Na]$^+$	451[M]$^+$ 474[M+Na]$^+$	451[M]$^+$	453[M]$^+$	521[M]$^+$ 544[M+Na]$^+$	518[M+H]$^+$	587[M]$^+$ 610[M+Na]$^+$
Negative	436[M-H]$^+$	452[M-H]$^+$	450[M-H]$^+$	450[M-H]$^+$	452[M-H]$^+$	NT	NT	NT
EI-MS(*m/z*)	437[M]$^+$	453[M]$^+$	451[M]$^+$	451[M]$^+$	453[M]$^+$	521[M]$^+$	517[M]$^+$	587[M]$^+$
HREI-MS(*m/z*)								
Calcd	437.2920 (for $C_{28}H_{39}NO_3$)	453.2879 (for $C_{28}H_{39}NO_4$)	451.2713 (for $C_{28}H_{37}NO_4$)	451.2350 (for $C_{27}H_{33}NO_5$)	453.2506 (for $C_{27}H_{35}NO_5$)	521.3130 (for $C_{32}H_{43}NO_5$)	517.2816 (for $C_{32}H_{39}NO_5$)	587.3598 (for $C_{37}H_{49}NO_5$)
Found	437.2924	453.2879	451.2703	451.2373	453.2496	521.3168	517.2828	587.3546
$[\alpha]_D^{28}$ (*c* 1.0, CH_3OH)	-36.4 °	-35.8 °	-28.0 °	-47.0 °	+7.0 °	-30.3 °[a]	+21.8 °	+16.5 °
UV $\lambda_{max}^{CH_3OH}$ nm (ε)	280 (8,700) 228 (38,600) 213sh (21,000)	280 (10,900) 228 (48,000) 207sh (25,400)	280 (11,800) 228 (49,400) 206sh (23,900)	279 (7,900) 227 (35,400) 203sh (19,100)	280 (11,200) 228 (47,400) 204sh (27,100)	282 (9,400)[a] 238 (13,100)	280 (11,200) 228 (38,600) 204sh (29,000)	283 (9,500) 230 (32,500) 203sh (29,400)
IR ν_{max}^{KBr} (cm^{-1})	2939, 1635, 1454 1373, 1302, 1228 1163	2939, 1454, 1376 1302, 1230	2935, 1706, 1666 1454, 1376, 1301 1230	2935, 1450, 1367 1230, 1176, 1105	2939, 1452, 1302 1259, 1213, 1174	2939, 1452, 1365 1302, 1255	2935, 1385, 1302 1232, 1169, 1090	2935, 1576, 1454 1382, 1305, 1209 1097
Melting point	174~176°C	NT	248~250°C (dec.)	NT	NT	248~250°C (dec.)	NT	148~150°C

a) measured in $CHCl_3$, NT: not tested

fractions) containing terpendoles C, D, E, J, K and L, paspaline and emindole SB, fr-2 (11th ~ 15th fractions) containing terpendoles G, H and I, and fr-3 (16th ~ 20th fractions) containing terpendole F. The fr-1 was concentrated *in vacuo* to give a brown material (3.9 g). The components were purified by preparative HPLC (YMC-pack D-ODS-5, 20 × 250 mm; solvent, 82.5% CH_3CN; UV at 282 nm; 9.0 ml/minute). Under the conditions, terpendoles C, D, E, J, K and L, emindole SB and paspaline were eluted with retention times of 19.5, 33.5, 13.0, 21.5, 17.0, 40.5, 42.0 and 38.5 minutes, respectively. All these fractions were concentrated and extracted with ethyl acetate to give pure terpendoles E (233 mg), K (16.0 mg), C (230 mg) and D (77.0 mg), paspaline (71.0 mg) and emindole SB (230 mg) as white powders, but fractions of terpendoles J (47.0 mg) and L (11.0 mg) still contained impurity. Therefore, terpendole L was re-purified by preparative HPLC under the same conditions to give pure terpendole L (8.3 mg) as white powder. Terpendole J was further purified by preparative HPLC using a different solvent of 70% CH_3CN. The fraction eluting with a retention time of 56.5 minutes was concentrated and extracted with ethyl acetate to give pure terpendole J (30.0 mg) as white powder. The fr-2 was concentrated *in vacuo* to give a pale yellow material (0.77 g). Terpendoles G, H and I were finally purified by

Table 2-1. ^1H and ^{13}C NMR chemical shifts of terpendoles E, F and G.

Carbon No.	Terpendole E		Terpendole F		Terpendole G	
	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]
NH-1		10.54 (1H, s)		10.53 (1H, s)		10.64 (1H, s)
C-2	151.3		151.5		150.8	
C-3	52.7		53.0		51.4	
C-4	40.1		39.2		40.1	
C-5	32.1	Ha 1.86 (1H, m)	29.5	Ha 1.88 (1H, m)	31.3	Ha 1.99 (1H, m)
		Hb 1.69 (1H, m)		Hb 1.68 (1H, m)		Hb 1.75 (1H, m)
C-6	25.0	1.67 (2H, m)	24.3	1.52 (1H, m)	24.3	Ha 1.85 (1H, m)
				1.58 (1H, m)		Hb 2.16 (1H, m)
C-7	76.9	3.48 (1H, dd, *J*=11.0, 4.0 Hz)	76.7	3.49 (1H, dd, *J*=11.0, 4.0 Hz)	75.1	3.64 (1H, t, *J*=9.0 Hz)
C-9	78.9	3.42 (1H, dd, *J*=12.0, 2.5 Hz)	78.8	3.45 (1H, dd, *J*=12.0, 2.5 Hz)	78.4	3.43 (1H, d, *J*=9.0 Hz)
C-10	29.3	1.45 (1H, dt, *J*=14.0, 2.5 Hz)	32.2	1.46 (1H, dt, *J*=14.0, 2.5 Hz)	31.2	1.21 (1H, d, *J*=9.0 Hz)
		1.78 (1H, dd, *J*=14.0, 6.0 Hz)		1.85 (1H, dd, *J*=14.0, 6.0 Hz)		1.59 (1H, d, *J*=9.0 Hz)
C-10-OH						
C-11	68.4	3.59 (1H, brd, *J*=3.0 Hz)	63.4	4.18 (1H, brd, *J*=3.0 Hz)	65.4	4.05 (1H, s)
C-11-OH		4.59 (1H, d, *J*=4.0 Hz)		4.48 (1H, d, *J*=4.0 Hz)		5.20 (1H, s)
C-12	39.2		43.4		53.9	
C-13	37.0	2.13 (1H, dd, *J*=12.5, 2.0 Hz)	37.8	2.10 (1H, dd, *J*=12.5, 2.0 Hz)	40.9	2.06 (1H, dd, *J*=12.5, 2.0 Hz)
C-13-OH						
C-14	21.0	Ha 1.62 (1H, m)	23.0	Ha 1.70 (1H, m)	21.5	Ha 1.79 (1H, m)
		Hb 1.23 (1H, m)		Hb 1.68 (1H, m)		Hb 1.32 (1H, m)
C-15	24.3	1.57 (2H, m)	25.5	1.46 (1H, m)	24.6	Ha 1.50 (1H, m)
				1.60 (1H, m)		Hb 1.62 (1H, m)
C-16	48.6	2.63 (1H, m)	48.9	2.60 (1H, m)	48.5	2.52 (1H, brs)
C-17	27.1	Ha 2.22 (1H, dd, *J*=13.0, 2.0 Hz)	27.2	Ha 2.21 (1H, dd, *J*=13.0, 2.0 Hz)	27.0	Ha 2.21 (1H, m)
		Hb 2.54 (1H, dd, *J*=13.0, 6.0 Hz)		Hb 2.54 (1H, dd, *J*=13.0, 6.0 Hz)		Hb 2.55 (1H, t, *J*=6.0Hz)
C-18	115.7		115.8		115.8	
C-19	124.4		124.3		124.3	
C-20	117.5	7.24 (1H, m)	117.5	7.23 (1H, m)	117.6	7.24 (1H, d, *J*=8.0 Hz)
C-21	118.3	6.87 (1H, td, *J*=7.0, 1.5 Hz)	118.3	6.87 (1H, td, *J*=7.0, 1.5 Hz)	118.4	6.88 (1H, d, *J*=7.0 Hz)
C-22	119.2	6.91 (1H, td, *J*=7.0, 1.5 Hz)	119.2	6.90 (1H, td, *J*=7.0, 1.5 Hz)	119.3	6.90 (1H, dd, *J*=8.0, 7.0 Hz)
C-23	111.8	7.25 (1H, m)	111.8	7.24 (1H, m)	111.8	7.27 (1H, d, *J*=8.0 Hz)
C-24	140.2		140.2		140.2	
C-25	14.5	0.95 (3H, s)	14.7	0.94 (3H, s)	14.8	0.91 (3H, s)
C-26	19.4	1.03 (3H, s)	19.2	1.14 (3H, s)	19.3	0.78 (3H, s)
C-27	70.3		70.3		70.0	
C-27-OH		4.00 (1H, s)		4.00 (1H, s)		
C-28	24.8	1.02 (3H, s)	24.9	1.02 (3H, s)	24.7	0.98 (3H, s)
C-29	26.7	1.06 (3H, s)	26.7	1.06 (3H, s)	26.7	1.04 (3H, s)
C-31						
C-33						
C-34						
C-35						
C-36						
C-37	13.1	0.77 (3H, s)	59.8	3.72 (1H, m)	208.0	10.1 (1H, d, *J*=6.5 Hz)
				3.90 (1H, m)		
C-37-OH				3.97 (1H, m)		
C-38						
C-39						
C-40						
C-41						
C-42						

[a] Each sample was dissolved in DMSO-d_6. Chemical shifts are shown with reference to DMSO-d_6 as 39.5 ppm. [b] Chemical shifts are shown with reference to DMSO-d_6 as 2.50 ppm.

preparative HPLC (Senshu ODS-H-6251, 30×250 mm; solvent, 50% CH_3CN; UV at 282 nm; 20 ml/minute). Under the conditions, terpendoles G, H and I were eluted with retention times of 36.5, 33.0 and 31.0 minutes, respectively. The fractions were concentrated and extracted with ethyl acetate to give pure terpendoles G (31.0 mg), H (4.0 mg) and I (200 mg) as white powders. The fr-3 was concentrated *in vacuo* to give a pale yellow oily material (60 mg). Further purification of terpendole F was carried out by gel filtration using Sephadex LH-20 (35×415 mm; solvent, methanol). Each 5.0 ml was successively collected, and the 24th to 37th fractions were enriched with terpendole F. They were pooled and concentrated *in vacuo* to give a white powder (25.0 mg). Terpendole F was finally purified by preparative HPLC (Senshu ODS-H-6251, 30×250 mm; solvent, 45% CH_3CN; UV at 282 nm; 20 ml/minute). The peak eluting with a retention time of 28.0 minutes was concentrated and extracted with ethyl acetate to give pure terpendole F (15.0 mg) as white powder.

Table 2-2. ^1H and ^{13}C NMR chemical shifts of terpendoles H, I and J.

Carbon No.	Terpendole H		Terpendole I		Terpendole J	
	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]
NH-1		10.80 (1H, s)				7.76 (1H, bs)
C-2	150.8		154.0		151.9	
C-3	48.5		52.2		50.7	
C-4	38.5		43.8		42.3	
C-5	28.0	2.08 (2H, m)	27.5 Ha	1.62 (1H, dd, *J*=13.0, 5.5 Hz)	27.4 Ha	1.33 (1H, m)
			Hb	2.57 (1H, dd, *J*=13.0, 5.5 Hz)	Hb	2.70 (1H, m)
C-6	28.4 Ha	1.78 (1H, m)	29.6	1.78 (1H, m)	27.8	1.76 (1H, m)
	Hb	2.30 (1H, m)		2.24 (1H, m)		2.28 (1H, m)
C-7	70.7	3.90 (1H, t, *J*=9.0 Hz)	73.1	4.15 (1H, t, *J*=9.0 Hz)	71.4	4.20 (1H, t, *J*=9.0 Hz)
C-9	76.3	3.18 (1H, d, *J*=9.0 Hz)	77.9	3.33 (1H, d, *J*=9.0 Hz)	74.7	3.43 (1H, d, *J*=9.0 Hz)
C-10	66.2	3.93 (1H, dd, *J*=9.0, 1.5 Hz)	68.8	3.91 (1H, dd, *J*=9.0, 1.5 Hz)	67.2	3.99 (1H, dd, *J*=9.0, 1.5 Hz)
C-10-OH		5.24 (1H, m)				4.65 (1H, s)
C-11	63.0	3.32 (1H, m)	65.2	3.43 (1H, bs)	64.3	3.61 (1H, s)
C-11-OH						
C-12	62.8		70.8		68.8	
C-13	67.1		78.7		77.9	
C-13-OH						
C-14	54.9	3.22 (1H, m)	30.8 Ha	1.28 (1H, m)	30.4 Ha	1.48 (1H, dt, *J*=13.2, 4.8 Hz)
			Hb	1.57 (1H, m)	Hb	1.58 (1H, m)
C-15	24.7 Ha	1.88 (1H, m)	22.1	1.51 (1H, m)	20.6	1.63 (1H, m)
	Hb	2.07 (1H, dd, *J*=12.5, 3.5 Hz)		1.88 (1H, m)		1.93 (1H, dd, *J*=12.5, 3.5 Hz)
C-16	45.1	2.65 (1H, m)	51.7	2.69 (1H, m)	50.1	2.80 (1H, m)
C-17	26.8 Ha	2.20 (1H, dd, *J*=13.0, 11.0 Hz)	28.4 Ha	2.32 (1H, dd, *J*=12.0, 10.5 Hz)	27.2	2.42 (1H, dd, *J*=13.0, 11.0 Hz)
	Hb	2.64 (1H, dd, *J*=13.0, 6.0 Hz)	Hb	2.63 (1H, m, *J*=12.0 Hz)		2.71 (1H, dd, *J*=13.0, 6.0 Hz)
C-18	114.8		117.4		117.6	
C-19	124.3		126.7		125.2	
C-20	117.9	7.27 (1H, d, *J*=8.0 Hz)	119.0	7.29 (1H, dd, *J*=7.0, 1.5 Hz)	118.5	7.44 (1H, d, *J*=8.0 Hz)
C-21	118.5	6.90 (1H, t, *J*=8.0 Hz)	120.0	6.91 (1H, dt, *J*=7.0, 1.5 Hz)	119.6	7.08 (1H, t, *J*=8.0 Hz)
C-22	119.6	6.95 (1H, t, *J*=8.0 Hz)	120.9	6.96 (1H, dt, *J*=7.0, 1.5 Hz)	120.4	7.08 (1H, t, *J*=8.0 Hz)
C-23	111.8	7.26 (1H, d, *J*=8.0 Hz)	112.9	7.27 (1H, dd, *J*=7.0, 1.5 Hz)	111.4	7.31 (1H, d, *J*=8.0 Hz)
C-24	140.2		142.1		139.7	
C-25	16.4	1.02 (3H, s)	16.8	1.24 (3H, s)	16.0	1.27 (3H, s)
C-26	18.2	1.14 (3H, s)	19.2	1.01 (3H, s)	18.8	1.12 (3H, s)
C-27	71.7		73.9		79.1	
C-27-OH		4.76 (1H, s)				
C-28	27.8	1.13 (3H, s)	27.8	1.212 (3H, s)	19.2	1.23 (3H, s)
C-29	24.7	1.12 (3H, s)	25.3	1.208 (3H, s)	23.6	1.29 (3H, s)
C-31					58.1	3.96 (2H, d, *J*=7.0 Hz)
C-33					120.6	5.26 (1H, tt, *J*=7.0, 0.5 Hz)
C-34					137.2	
C-35					17.9	1.71 (3H, d, *J*=0.5 Hz)
C-36					25.7	1.65 (3H, d, *J*= 0.5 Hz)
C-37						
C-37-OH						
C-38						
C-39						
C-40						
C-41						
C-42						

a) Each sample was dissolved in DMSO-d_6. Chemical shifts are shown with reference to DMSO-d_6 as 39.5 ppm. b) Chemical shifts are shown with reference to DMSO-d_6 as 2.50 ppm.
c) Each sample was dissolved in CD_3OD. Chemical shifts are shown with reference to CD_3OD as 49.8 ppm. d) Chemical shifts are shown with reference to CD_3OD as 3.30 ppm.
e) Each sample was dissolved in $CDCl_3$. Chemical shifts are shown with reference to $CDCl_3$ as 77.7 ppm. f) Chemical shifts are shown with reference to $CDCl_3$ as 7.26 ppm.

Physico-chemical Properties of Terpendoles E to L

Physico-chemical properties of terpendoles E to L are summarized in Table 1. All the terpendoles showed the same UV absorption maxima at 279~283 and 228~238 nm and the same fragment ion peak of m/z 130 in the EI-MS spectra, suggesting the presence of a common indole moiety in their structures.

Structure of Terpendole L

The molecular formula of terpendole L was determined to be $C_{37}H_{49}NO_5$ on the basis of HREI-MS measurement (m/z, found 587.3546, calcd 587.3598). The ^{13}C NMR spectrum (DMSO-d_6) showed 37 resolved peaks (Table 2), which were classified into eight $-CH_3$, six $-CH_2$, one $-CH-$, five $-O-CH-$, five $-CH=$, and 12 quaternary carbons by analysis of the DEPT spectra. The ^1H NMR spectrum displayed 49 proton signals (Table 2-3). The results supported the molecular formula. The connectivity of proton and carbon atoms was confirmed by the HMQC spectrum (Table 2-3). Two singlet protons (δ 10.64 and 4.52) suggested the presence of NH and OH protons from the molecular formula and HMQC spectrum. Analyses of ^1H-^1H COSY spectrum revealed the six partial structures I to VI (Fig. 5). ^{13}C-^1H long-range couplings of ^2J and ^3J observed in the HMBC

Table 2-3. ^1H and ^{13}C NMR chemical shifts of terpendoles K and L.

Carbon No.	Terpendole K		Terpendole L	
	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^1H chemical shifts (ppm)[b]
NH-1		10.72(1H, bs)		10.64 (1H, s)
C-2	152.6		152.0	
C-3	50.2		49.9	
C-4	43.3		42.2	
C-5	28.8	Ha 2.20 (1H, dd, J=16.0, 7.0 Hz)	25.5	2.42 (2H, m)
		Hb 2.90 (1H, d, J=16.0 Hz)		
C-6	105.4	5.25 (1H, dd, J=6.5, 2.0 Hz)	28.4	Ha 1.63 (1H, m)
				Hb 2.14 (1H, m)
C-7	145.0		70.7	4.26 (1H, t, J=9.0 Hz)
C-9	74.0	3.836 (1H, s)	71.1	3.40 (1H, d, J=9.0 Hz)
C-10	70.3	4.16 (1H, d, J=10.0 Hz)	70.1	4.06 (1H, d, J=9.0 Hz)
C-10-OH				
C-11	58.5	3.84 (1H, d, J=10.0 Hz)	58.9	3.51 (1H, s)
C-11-OH				
C-12	64.5		67.0	
C-13	74.9		76.5	
C-13-OH				4.52 (1H, s)
C-14	28.8	1.53~1.58 (2H, m)	28.6	1.46~1.50 (2H, m)
C-15	20.6	Ha 1.53 (1H, m)	20.4	Ha 1.48 (1H, m)
		Hb 1.83 (1H, m)		Hb 1.78 (1H, m)
C-16	49.7	2.65 (1H, m)	49.7	2.69 (1H, brs)
C-17	27.0	Hb 2.30 (1H, dd, J=12.5, 10.5 Hz)	28.5	Ha 2.42 (1H, m)
		Ha 2.60 (1H, m)		Hb 2.70 (1H, t, J=6.0Hz)
C-18	115.0		114.4	
C-19	124.6		123.9	
C-20	117.7	7.26 (1H, d, J=7.0 Hz)	131.4	
C-21	118.5	6.90 (1H, t, J=7.0 Hz)	117.6	6.64 (1H, d, J=7.0 Hz)
C-22	119.4	6.94 (1H, t, J=7.0 Hz)	119.5	6.81 (1H, dd, J=8.0, 7.0 Hz)
C-23	111.9	7.28 (1H, d, J=7.0 Hz)	109.6	7.06 (1H, d, J=8.0 Hz)
C-24	140.0		139.8	
C-25	16.4	1.25 (3H, s)	16.0	1.16 (3H, s)
C-26	19.3	1.04 (3H, s)	18.0	1.03 (3H, s)
C-27	74.5		74.1	
C-27-OH				
C-28	16.7	1.28 (3H, s)	16.7	1.22 (3H, s)
C-29	27.9	1.19 (3H, s)	28.3	1.13 (3H, s)
C-31	92.3	5.57 (1H, d, J=6.5 Hz)	92.0	5.51 (1H, d, J=6.5 Hz)
C-33	122.2	5.12 (1H, brd, J=6.5 Hz)	122.5	5.10 (1H, dt, J=6.5, 1.0 Hz)
C-34	138.0		137.4	
C-35	18.5	1.66 (3H, s)	25.0	1.65 (3H, d, J=1.0 Hz)
C-36	25.2	1.67 (3H, s)	18.3	1.64 (3H, d, J=1.0 Hz)
C-37				
C-37-OH				
C-38			31.6	3.46 (2H, d, J=7.0 Hz)
C-39			124.0	5.30 (1H, tt, J=7.0, 1.5 Hz)
C-40			130.6	
C-41			17.7	1.69 (3H, d, J=0.5 Hz)
C-42			25.5	1.66 (3H, d, J=0.5 Hz)

[a] Each sample was dissolved in DMSO-d_6. Chemical shifts are shown with reference to DMSO-d_6 as 39.5 ppm. [b] Chemical shifts are shown with reference to DMSO-d_6 as 2.50 ppm.

spectrum (Fig. 6) gave the following evidences; 1) The cross peaks from H_2-38 (δ 3.46) to C-39 (δ 124.0) and C-40 (δ 130.6), from H-39 (δ 5.30) to C-38 (δ 31.6), C-41 (δ 17.7) and C-42 (δ 25.5), from H_3-41 (δ 1.69) to C-39, C-40, and C-42, and from H_3-42 (δ 1.66) to C-39, C-40 and C-41 showed the presence of an isoprenyl moiety containing the partial structure I. A fragment ion peak (*m/z* 69) of EI-MS also supported the presence of an isoprenyl moiety. 2) The cross peaks from H-21 (δ 6.64) to C-19 (δ 123.9) and C-23 (δ 109.6), from H-22 (δ 6.81) to C-20 (δ 131.4) and C-24 (δ 139.8), and from H-23 (δ 7.06) to C-19 and C-21 (δ 117.6) indicated a 1,2,3-trisubstituted benzene containing the partial structure II. 3) The long-range couplings from H_2-38 to C-19, C-20 and C-21, and from H-21 to C-38 showed that the isoprenyl group was attached to C-20 of the benzene. 4) The long-range couplings from NH-1 (δ 10.64) to C-2 (δ 152.0), C-18 (δ 114.4), C-19 and C-24 showed that a pyrrole ring was attached to benzene, leading to the constructions of 2,3,4- the trisubstituted indole moiety as expected from UV and EI-MS. 5) The long-range couplings from H-16 (δ 2.69) to C-2 and C-18, from H_2-17 (δ 2.42 and 2.70) to C-2 and C-18, and from H_3-25 (δ 1.16) to C-2, C-3 (δ 49.9) and C-16 (δ 49.7) suggested that a cyclopentane ring containing a part of the partial structure III was attached to the indole moiety. 6) The long-range couplings from H_2-14 (δ 1.46~1.50) to C-13 (δ 76.5) and C-4 (δ 4.22), from H_3-26 (δ 1.03) to C-3, C-4 and C-13, and from H_3-25 to C-4 suggested that a cyclohexane ring was attached to the cyclopentane ring. 7) The long-range couplings from H_2-6 (δ 1.63 and 2.14) to C-4, C-5 (δ 25.5), C-7 (δ 70.7) and C-12 (δ 67.0), from H-7 (δ 4.26) to C-6 (δ 28.4) and C-12, from 13-OH (δ 4.52) to C-4, C-12 and C-13, and from H_3-26 to C-5 suggested that another cyclohexane ring, which contained the partial structure IV, was attached to the cyclohexane

Fig. 5. Partial structures of terpendole L.

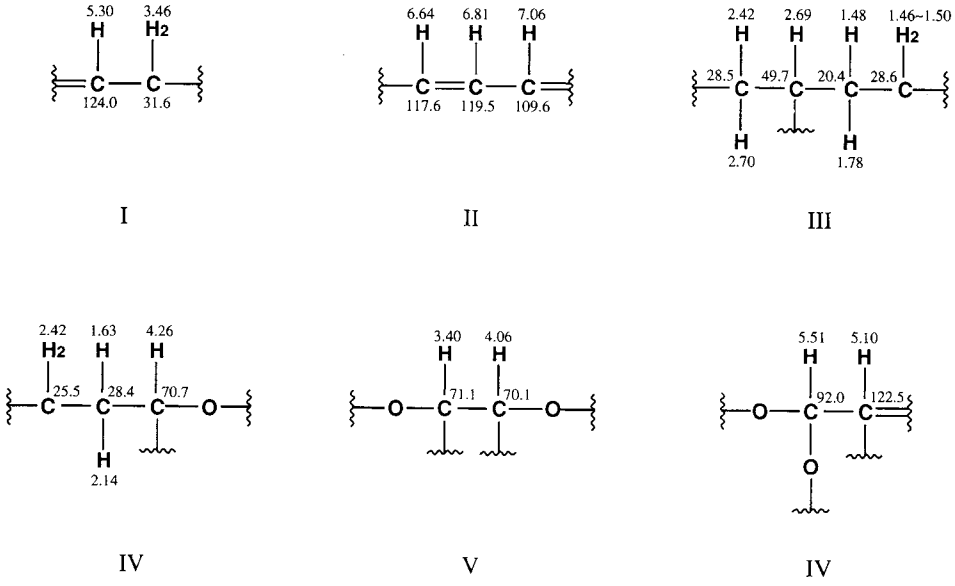
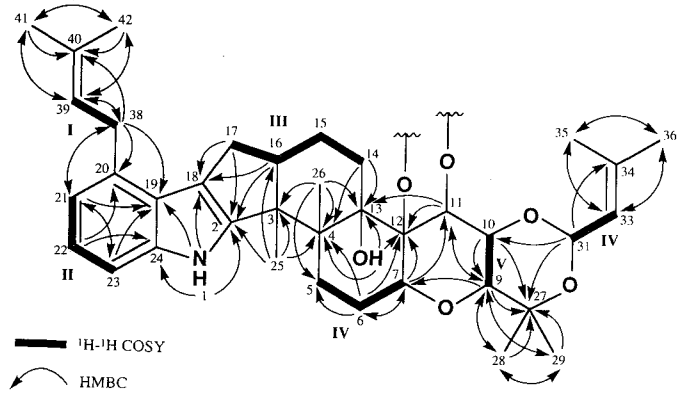


Fig. 6. ^1H-^1H COSY and HMBC experiments of terpendole L.



ring. 8) The long-range couplings from H-7 to C-11 (δ 58.9), from H-9 (δ 3.40) to C-7 and C-11, from H-10 (δ 4.06) to C-9 (δ 71.1), and from H-11 (δ 3.51) to C-9, C-12 and C-13 suggested that a tetrahydropyrane, which contained the partial structure V, was attached to the second cyclohexane moiety. 9) The long-range couplings were observed from H-9 to C-27 (δ 74.1), C-28 (δ 16.7) and C-29 (δ 28.3), from H-10 to C-27 from H_3-28 (δ 1.22) to C-9, C-27 and C-29, from H_3-29 (δ 1.13) to C-9, C-27 and C-28, and from H-31 (δ 5.51) to C-10 (δ 70.1) and C-27, and the chemical shift of C-31 (δ 92.0) was comparable with that of an acetal carbon. Therefore, 1,3-dioxane ring was suggested. 10) The long-range couplings were observed from H-31 to C-34 (δ 137.4), from H-33 (δ 5.10) to C-35 (δ 25.0) and C-36 (δ 18.3), from H_3-35 (δ 1.65) to C-33 (δ 122.5) and C-36, and from H_3-36 (δ 1.64) to C-33 and C-35. Therefore, an isobutenyl residue containing the partial structure VI was attached to C-31 of the 1,3-dioxane ring. Finally the presence of an 11,12-epoxide was suggested because of the degree of unsaturation and the molecular formula. Taken together, the structure of terpendole L was elucidated as shown in Fig. 1.

Structures of Terpendole K

Comparison of the NMR spectral data of terpendole K (Table 2-3 and Fig. 7) with those of terpendole L revealed that 1) one isoprenyl unit (C-38 ~ C-42) was lacking and correspondingly the sp^2 methine carbon of C-20 (δ 117.7) was observed in terpendole K instead of the quarternary one (δ 131.4) in terpendole L, and 2) the sp^2 methine carbon of C-6 (δ 105.4) and the quarternary carbon of C-7 (δ 145.0) were observed in terpendole K instead of the methylene carbon (δ 28.4) and the oxymethine carbon (δ 70.7) in terpendole L. The structure of terpendole K was confirmed by the HMBC experiments as shown in Fig. 7. Consequently, the structure of terpendole K was determined as shown in Fig. 1.

Structures of Terpendole J

The ^{13}C NMR spectrum (DMSO-d_6) of terpendole J (Table 2-2) was similar to those of terpendole K, but the three carbon signals of C-6, C-7 and C-31 were different, that is, the methylene C-6 (δ 27.8), the oxymethine C-7 (δ 71.4) and the oxymethylene C-31 (δ 58.1) carbons in terpendole J. The structural analyses were done by ^1H-^1H COSY and HMBC experiments as shown in Fig. 8. The sequence of C-6 and C-7 was confirmed, which was the same as that of terpendole L. Regarding the C-31 carbon, it was concluded that the ether bond between O-10 and C-31 in terpendole K was opened with hydration because of 1) the presence of the oxymethylene proton of H_2-31 (δ 3.96), which showed ^1H-^1H couplings with the sp^2 methine proton of H-33 (δ 5.26), 2) the fragment ion peak of m/z 69 corresponding to an isoprenyl residue in the EI-MS which was not observed in that for terpendole K, and 3) the long-range couplings from 10-OH (δ 4.65) to C-9 (δ 74.7), C-10 (δ 67.2) and C-11 (δ 64.3), from H-10 (δ 3.99) to C-9, C-11 and C-27 (δ 79.1) and from H_2-31 (δ 3.96) to C-27, C-33 (δ 120.6) and C-34 (δ 137.2) in the HMBC experiments. Thus, the structure of terpendole J was determined as shown in Fig. 1.

Structures of Terpendoles H and I

Comparison of the molecular formulas between terpendoles I and J revealed that C_5H_8 corresponding to an isoprenyl unit was lacking in terpendole I. Furthermore, comparison of various NMR data (Table 1) confirmed that terpendole I is 30-deisoprenyl-terpendole J as shown in Fig. 1.

Comparison of the spectral data between terpendoles H and I indicated that two hydrogen atoms are lacking in terpendole H from the molecular formula (Table 1), and that the C-14 oxymethine (δ 54.9) carbon was observed for terpendole H in place of the corresponding methylene carbon for terpendole I from the ^{13}C NMR spectrum (Table 2-2). Furthermore, the quaternary carbon of C-13

Fig. 7. ^1H-^1H COSY and HMBC experiments of terpendole K.

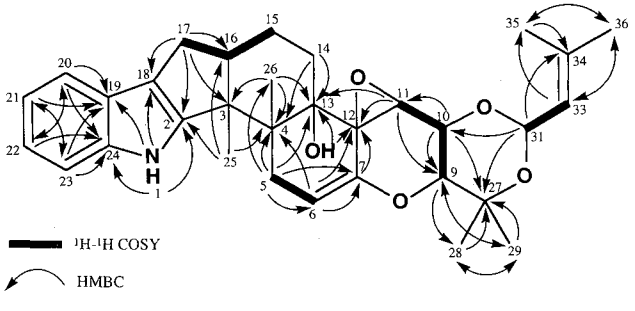
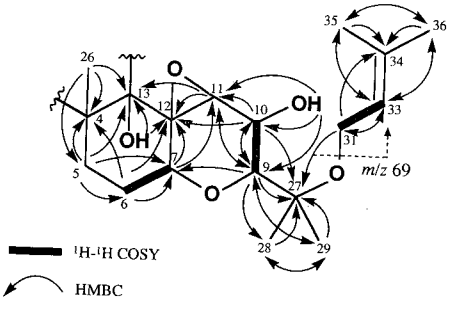


Fig. 8. ^1H-^1H COSY and HMBC experiments of terpendole J.



(δ 67.1) was shifted to a higher field than that of terpendole I. In the HMBC experiments the long-range couplings were observed from H_2-15 (δ 1.88, 2.07) to C-13 and C-14, and from H-14 (δ 3.22) to C-13 and C-16 (δ 45.1) (Fig. 9). These data revealed that terpendole H is 13,14-epoxy derivative of terpendole I as shown in Fig. 1.

Structures of Terpendole F

The structure of terpendole F was determined by comparison of various spectral data with those of terpendole I. The result are illustrated in Fig. 10. First, terpendole F lacks the 13-OH of terpendole I. In fact, the proton sequence of $-C^{13}H-C^{14}H_2-C^{15}H_2-$ was confirmed from the ^1H-^1H COSY spectrum. In the HMBC experiments the long-range couplings were observed from H_2-14 to C-13 (δ 37.8), from H-13 (δ 2.10) to C-4 (δ 39.2), C-12 (δ 43.4), C-14 (δ 23.0), and C-26 (δ 19.2), from H_2-5 (δ 1.68, 1.88) to C-13, and from H-7 (δ 3.49) to C-12. Second, terpendole I has a hydroxy group at the C-11 position. Because the ^1H-^1H COSY data revealed the proton sequence $-O-C^9H-C^{10}H_2-C^{11}H-OH$, and the long-range couplings were observed from H-11 (δ 4.18) to C-7 (δ 76.7), C-9 (δ 78.8) and C-12 from H_2-10 (δ 1.46, 1.85) to C-11 (δ 63.4), C-9 and C-12, and from 11-OH (δ 4.48) to C-11 and C-12 in the HMBC experiments. Finally, the long-range couplings were observed from H_2-37 (δ 3.72, 3.90) to C-11 and C-12, and the NOE was observed between the hydroxy proton of 37-OH (δ 3.97) and H-37 (δ 3.72). Therefore, the hydroxymethyl group is attached to the C-12 bridge head carbon in terpendole I.

Taken together, the structure of terpendole F was determined as shown in Fig. 1.

Structures of Terpendoles E and G

By comparison of various spectral data of terpendoles E and G with those of terpendole F, the structural differences were found to lie only in the C-37 position. Terpendoles E and G showed methyl (δ 13.1) and

Fig. 9. ^1H-^1H COSY and HMBC experiments of terpendole H.

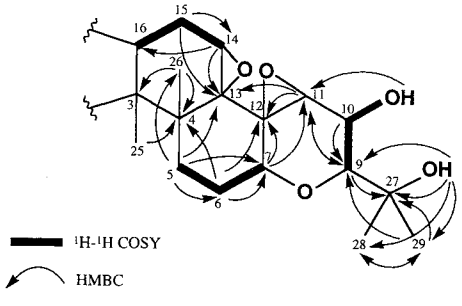


Fig. 10. ^1H-^1H COSY and HMBC experiments of terpendoles E, F and G.

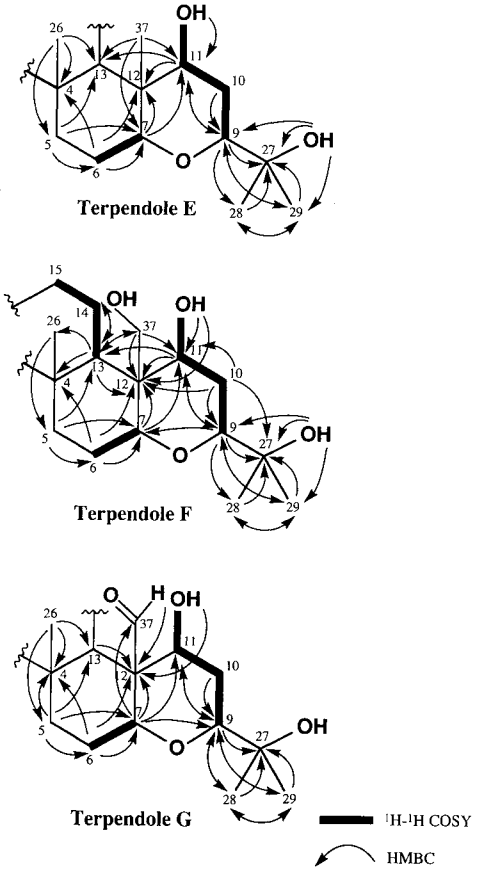


Table 3. Single crystal X-ray crystallographic analysis.

Crystal parameters	
Empirical fromula	$C_{28}H_{37}NO_3$
Formula weight	435.61
Crystal dimensions (mm)	0.4 x 0.5 x 0.1
Crystal system	Orthorhombic
Lattice Parameters:	a = 7.989 (9) Å
	b = 46.22 (2) Å
	c = 6.43 (1) Å
	V = 2375 (1) Å^3
Space group	$P2_12_12_1$ with Z=4
Density calc (g/cm^3)	1.218
Linear absorption factor (cm^{-1})	5.77
Refinement parameters	
No. of reflections measured	2,612
Nonzero reflections (I> 3.00σ)	1,363
R-index Residuals: R[a]	0.098
Residuals: R_w[b]	0.083
Goodness of fit indicator[c]	4.99

[a] Σ ||Fo|-|Fc||/Σ|Fo|

[b] $[(\Sigma w(|Fo|-|Fc|)^2/\Sigma wFo)]^{1/2}$

[c] $[\Sigma w(|Fo|^2-|Fc|)^2/(No-Nv)]^{1/2}$

No=number of observations

Nv=number of variables

Fig. 11. Relative molecular structure of terpendole E determined by X-ray crystallography.

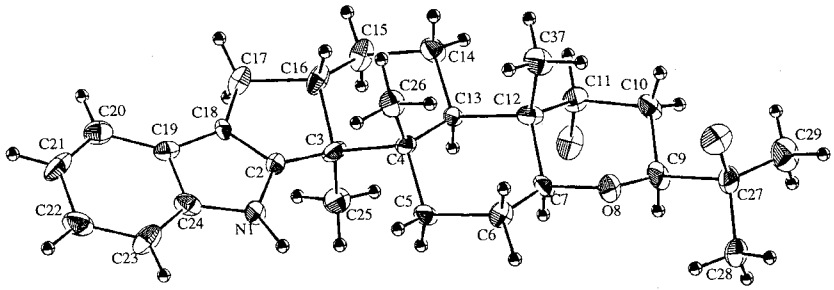
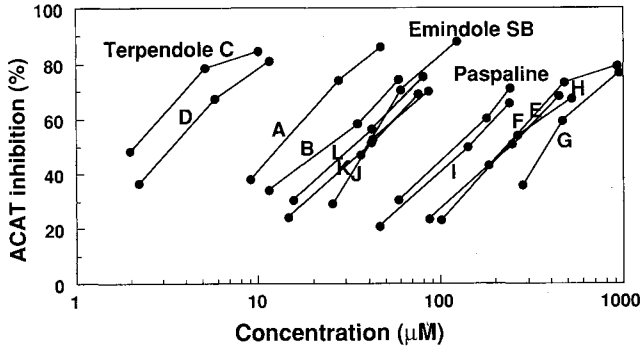


Fig. 12. ACAT inhibition by terpendoles A to L, paspaline and emindole SB in the enzyme assay using rat liver microsomes.



aldehyde (δ 208.0) carbon signals for the C-37 position, respectively, in place of C-37 oxymethylene carbon signal in terpendole F (Table 2-1). The structural analyses were done by the ^{1}H-^{1}H COSY and HMBC experiments (Fig. 10). As a result, the structures of terpendoles E and G were determined as shown in Fig. 1.

X-ray Crystallographic Analysis of Terpendole E

The data from a single crystal X-ray crystallographic analysis of terpendole E are summarized in Table 3. The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 1363 observed reflections and 289 variable parameters and converged with unweighted and weighted agreement factors of $R=0.098$, $R_w=0.083$. Thus, the structure of terpendole E described above was secured, and the relative configuration was also determined as shown in Fig. 11.

Biological Properties

Effect of Terpendoles on ACAT Activity in Microsomes

All the terpendoles inhibited ACAT activity in a dose-dependent fashion as shown in Fig. 12. Terpendoles J, K and L, having (an) additional isoprenyl unit(s) to the indoloditerpene core, exhibited moderate inhibitory activity with IC_{50} values of 38.8 μM, 38.0 μM and 32.4 μM, respectively, but the other terpendoles showed weak ACAT inhibition (IC_{50}: E 228 μM, F 221 μM, G 388 μM, H 230 μM and I 145 μM).

Other Biological Activities

No antimicrobial activity was observed at a concentration of 1 mg/ml (10 μg/paper disk) for terpendoles E to L 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

In the previous report, we used the fermentation medium consisting of soluble starch 3.0%, glycerol 1.0%, soybean meal 2.0%, dry yeast 0.3%, KH_2PO_4 0.05%, $CaCO_3$ 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05% and KCl 0.3% (pH 6.5) for production of terpendoles A to D[1]. From the culture broth, terpendoles K, H and I were also isolated in addition to terpendoles A to D, emindole SB and paspaline. Furthermore, the presence of novel other terpendoles was suggested from their specific UV spectra by HPLC analyses but their production was very low. By using the different fermentation medium as described in this paper, the production of terpendoles increased and enough amounts of novel other terpendoles were obtained to enable us to elucidate their structures. However, the production of terpendoles A and B was still low.

The planar structures of terpendoles were elucidated by spectral data mainly including various NMR experiments, revealing that they have a common indoloditerpene core. On the basis of the structural carbon skeleton, they are classified into 4 groups; 1) the fundamental indoloditerpene group such as terpendoles E, F and G, 2) the 12-demethylated indoloditerpene

group including terpendoles B, H and I, 3) the 12-demethylated and 27-*O*-prenylated indoloditerpene group such as terpendoles A, C, D, J and K, and 4) the 12-demethylated, 20-*C*-prenylated and 27-*O*-prenylated indoloditerpene of terpendole L. Emindole SB and paspaline belong to the first group. During the processing of the carbon skeleton, oxidation and/or epoxidation reactions occur at the certain positions of the terpene core to produce variety kinds of terpendoles.

The relative stereostructure of terpendole E was determined by X-ray crystallographic analysis (Fig. 11) although the R-indexes were not so good due to the very thin crystal (Table 3), showing good coincidence with those of terpendole D[2], paspaline[4] and emindole SB[3]. The other terpendoles F to L were expected to be biosynthetically related, and their stereostructures were deduced as shown in Fig. 1. However, the stereochemistry of 13,14-epoxy residue in terpendole H still remains to be defined.

As reported previously, an additional prenyl residue at the 27-*O*-position is important for potent ACAT inhibition[1]. Among the terpendoles appeared in this paper, terpendoles J, K and L have such a prenyl residue, and showed more potent ACAT inhibition (IC_{50} 32.4 ~ 38.8 μM) than the other terpendoles (145 ~ 388 μM). Unexpectedly, however, their inhibitory activity is one magnitude less potent than that of terpendoles C and D (IC_{50} 2.1 and 3.2 μM, respectively). One possible reason might be the poor solubility of terpendole L to both water and organic solvents. The hydroxy residue at the C-13 position of the diterpene is believed to be responsible for exhibiting tremorgenic activity[12]. Terpendoles A, C, I, J, K and L have such a hydroxy group. However, it is still unclear whether they exhibit tremorgenic activity or not.

Acknowledgment

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References

1) HUANG, X.-H.; H. TOMODA, H. NISHIDA, R. MASUMA & S. ŌMURA: Terpendoles, novel ACAT inhibitors produced by *Albophoma yamanashiensis*. I. Production, isolation and biological properties. J. Antibiotics 48: 1 ~ 4, 1995
2) HUANG, X.-H.; H. NISHIDA, H. TOMODA, N. TABATA, K. SHIOMI, D.-J. YANG, H. TAKAYANAGI & S. ŌMURA: Terpendoles, novel ACAT inhibitors produced by *Albophoma yamanashiensis*. II. Structure elucidation of terpendoles A, B, C and D. J. Antibiotics 48: 5 ~ 11, 1995
3) NOZAWA, K.; S. NAKAJIMA, K. KAWAI, S. UDAGAWA, Y. HORIE & N. YAMAZAKI: Novel indoloditerpenes, emindoles and related compounds from *Emericelia* spp. The 29th Symposium on Chemistry of Natural Products (Sapporo) pp. 637 ~ 643, 1987
4) SPRINGER, J. P. & J. CLARDY: Paspaline and paspalicine, two indole-mevalonate metabolites from *Claviceps paspali*. Tetrahedron Lett. 21: 231, 1980
5) KOBAYASHI, T.; R. MASUMA, S. ŌMURA & K. WATANABE: Materials for fungus flora of Japan (47). Mycoscience 35: 399 ~ 401, 1994
6) CROMER D. T. & J. T. WABER: International Tables for X-ray crystallography, Vol. IV, The Kynoch Press, Birmingham, England, 1974
7) IBERS, J. A. & W. C. HAMILTON: Acta Crystallogr. 17: 781 1964
8) TEXSAN-TEXRAY structure analysis package, Molecular Structure Corporation, 1985
9) GILMORE, C. J.: Mithril—an intergrated direct methods computer program. J. Appl. Cryst. 17: 42 ~ 46, Univ. of Glasgow, Scotland, 1984
10) BEURSKENS, P. & T. DIRDIF: Direct methods for difference structures—an automatic procedure for phase extension and refinement of difference structure factors. Technical report 1984/1 crystallography laboratory, Toernooiveld, 6525 *Ed.* NIJEMGEN, Netherlands
11) TOMODA, H.; H. NISHIDA, R. MASUMA, J. CAO, S. OKUDA & S. ŌMURA: Purpactins, new inhibitors of acyl-CoA : cholesterol acyltransferase produced by *Penicillium purpurogenum*. I. Production, isolation and physicochemical and biological properties. J. Antibiotics 44: 136 ~ 143, 1991
12) BERTINA, V.: *In* Mycotoxins. Chemical, Biological and Environmental Aspects. pp. 353 ~ 387. Elsevier, Amsterdam, 1989