HYNAPENES A, B AND C, NEW ANTICOCCIDIAL AGENTS PRODUCED BY *Penicillium sp.*

II. STRUCTURE ELUCIDATION

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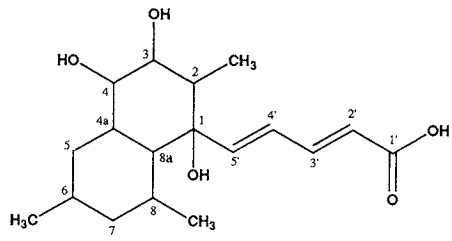
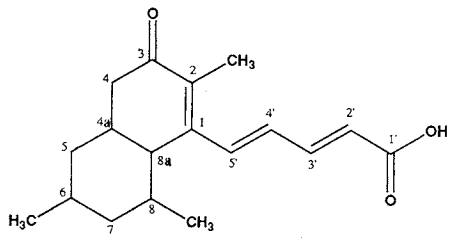
The structures of hynapenes A, B and C, novel anticoccidial agents, were determined by spectroscopic analyses. Hynapenes A, B and C were deduced to be (2*E*,4*E*)-5-(1,3,4-trihydroxy-2,6,8-trimethyldecalin)-2,4-pentadienoic acid, (2*E*,4*E*)-5-(1-ene-3-oxo-2,6,8-trimethyldecalin)-2,4-pentadienoic acid and (2*E*,4*E*)-5-(3-ene-1-oxo-2,6,8-trimethyldecalin)-2,2,4-pentadienoic acid, respectively.

In the course of our screening for anticoccidial agents of microbial origin, hynapenes A, B and C (Fig. 1) have been isolated from the fermentation broth of *Penicillium* sp. FO-1611. Taxonomy of the producing strain, fermentation, isolation and physico-chemical and biological characteristics of hynapenes were reported in the preceding paper[1]. In this report, we describe the structure elucidation of the hynapenes.

Fig. 1. Structures of hynapenes A, B and C.



Hynapene A



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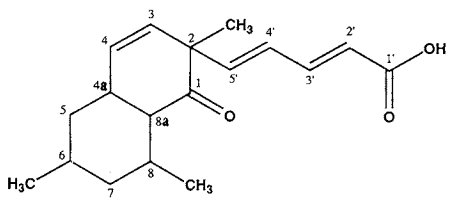


Hynapene C

Structure Elucidation of Hynapene A

The molecular formula of hynapene A was determined to be $C_{18}H_{28}O_5$ (*m/z* found 324.1926, calcd 342.1935) by HREI-MS analysis. The ^{13}C NMR spectrum (CD_3OD) showed 18 resolved peaks (Table 1), which were classified into three $-CH_3$, two $-CH_2-$, five $-CH-$, two $-O-CH-$, four $=CH-$ and two quaternary carbons by analysis of the DEPT spectrum. The ^1H NMR spectrum of hynapene A showed 24 proton signals (Table 1). To satisfy the molecular formula of hynapene A, the presence of three hydroxyl and one carboxylic acid groups is suggested, which is supported by the following evidence; 1) The fragment ion peaks of *m/z* 307 $[M+1-H_2O]^+$ and 289 $[M+1-2H_2O]^+$ in the FAB-MS spectrum indicate the presence of at least two hydroxyl groups, 2) the ^{13}C chemical shifts of C-1 (δ 79.82), C-3 (δ 77.80) and C-4 (δ 76.39) suggest the presence of adjacent oxygen atoms, 3) the chemical shift of C-1′ (δ 171.31) suggest a carbonyl carbon, and 4) the absorption at 1686 cm^{-1} in IR

Table 1. ^{1}H and ^{13}C NMR chemical shifts of hynapenes A, B and C.

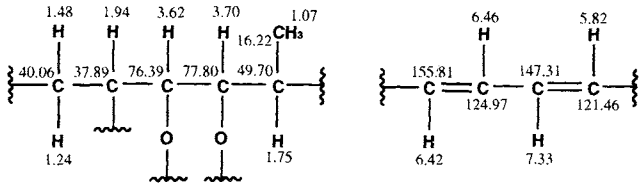
Carbon No.	Hynapene A		Hynapene B		Hynapene C	
	^{13}C chemical shifts ppm[a]	^{1}H chemical shifts ppm[b]	^{13}C chemical shifts ppm[a]	^{1}H chemical shifts ppm[b]	^{13}C chemical shifts ppm[a]	^{1}H chemical shifts ppm[b]
C-1	79.82		159.65		212.05	
C-2	49.70	1.75 (1H, dd, J=8.0, 3.0 Hz)	134.34		55.98	
C-2-CH_3	16.22	1.07 (3H, d, J=8.0 Hz)	14.21	1.77 (3H, s)	23.15	1.19 (3H, s)
C-3	77.80	3.70 (1H, br d, J=3.0 Hz)	202.52		133.78	5.53 (1H, dd, J=9.6, 2.1 Hz)
C-4	76.39	3.62 (1H, dd, J=3.0, 3.0 Hz)	47.75	2.42 (1H, dd, J=17.0, 3.7 Hz), 2.18 (1H, dd, J=17.0, 12.8 Hz)	134.40	5.71 (1H, d, J=9.6 Hz)
C-4a	37.89	1.94 (1H, dddd, J=11.5, 11.5, 3.0, 3.0 Hz)	42.98	1.84 (1H, m)	46.36	2.17 (1H, br d, J=4.3 Hz)
C-5	40.06	1.48 (1H, m), 1.24 (1H, br t, J=13.5, 11.5 Hz)	43.37	1.65 (1H, m), 0.87 (1H, m)	43.33	1.87 (1H, br d, J=12.9 Hz), 0.94 (1H, m)
C-6	33.98	1.49 (1H, m)	32.90	1.50 (1H, m)	33.63	1.45 (1H, m)
C-6-CH_3	23.66	0.89 (3H, d, J=6.0 Hz)	23.23	0.90 (3H, d, J=6.3 Hz)	23.20	0.91 (3H, br d, J=6.6 Hz)
C-7	48.46	1.58 (1H, ddd, J=12.0, 6.0, 3.0 Hz), 0.81 (1H, br d, J=12.0 Hz)	48.06	1.75 (1H, m), 0.85 (1H, m)	45.10	1.70 (1H, br d, J=12.5 Hz), 0.68 (1H, m)
C-8	34.72	1.64 (1H, m)	35.62	1.65 (1H, m)	31.72	1.76 (1H, m)
C-8-CH_3	24.76	0.91 (3H, d, J=6.0 Hz)	25.48	1.08 (3H, d, J=6.3 Hz)	20.96	0.84 (3H, d, J=6.3 Hz)
C-8a	46.79	1.51 (1H, m)	53.50	1.90 (1H, m)	58.33	2.19 (1H, m)
C-1′	171.31		170.74		170.08	
C-2′	121.46	5.82 (1H, d, J=15.8 Hz)	124.25	5.97 (1H, d, J=15.0 Hz)	123.67	5.90 (1H, d, J=15.3 Hz)
C-3′	147.31	7.33 (1H, dd, J=15.8, 8.6 Hz)	145.99	7.38 (1H, dd, J=15.0, 11.0 Hz)	145.98	7.24 (1H, dd, J=15.3, 10.9 Hz)
C-4′	124.97	6.46 (1H, dd, J=15.0, 8.6 Hz)	133.07	6.29 (1H, dd, J=16.0, 11.0 Hz)	131.12	6.34 (1H, dd, J=15.3, 10.9 Hz)
C-5′	155.81	6.42 (1H, d, J=15.0 Hz)	142.44	6.86 (1H, d, J=16.0 Hz)	147.17	6.06 (1H, d, J=15.3 Hz)

[a] Each sample were dissolved in CD_3OD. Chemical shifts are shown with reference to CD_3OD as 49.8 ppm.

[b] Chemical shifts are shown with reference to CD_3OD as 3.3 ppm.

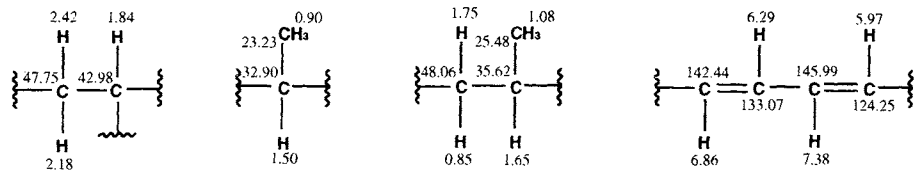
Fig. 2. Partial structures of hynapenes A (a), B (b) and C (c).

a)



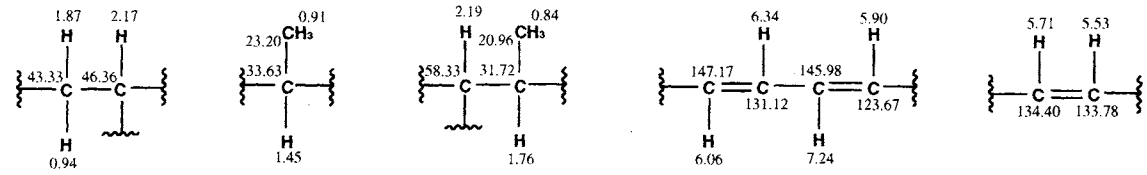
I II

b)



I II III IV

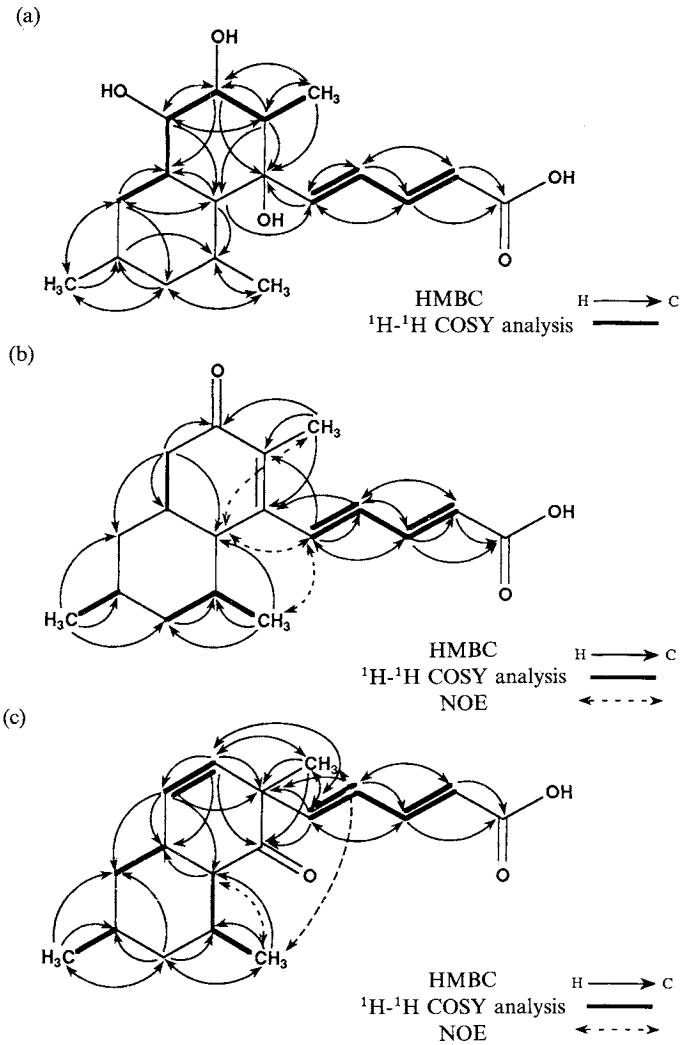
c)



I II III IV V

spectrum support the presence of a carboxylic acid group[2]. The connectivity of proton and carbon atoms was confirmed by the ^{13}C-^{1}H COSY spectrum as shown in Table 1. Analysis of ^{1}H-^{1}H COSY spectrum revealed the two partial structures I and II (Fig. 2a). Furthermore, the proton sequences were determined by differential selective proton decoupling spectra. Irradiation at H-6 (δ 1.49) and H-8 (δ 1.64) showed the positive signals at H-6-CH_3 (δ 0.89) and H-8-CH_3 (δ 0.91), respectively, suggesting the presence of two –CH–CH_3 sequences. ^{13}C-^{1}H long range couplings of ^{2}J and ^{3}J observed in the HMBC spectrum are shown in Fig. 3a; 1) Cross peaks from H-8a (δ 1.51) to C-4a (δ 37.89), C-5 (δ 40.04) and C-8 (δ 34.72), from H-2 (δ 1.75) to C-8a (δ 46.79) and C-1 (δ 79.82), from H-5 (δ 1.48) to C-7 (δ 48.46) and from H-6 (δ 1.49) to C-8 (δ 34.72) revealed the decalin skeleton, 2) cross peaks from H-5′ (δ 6.42) to C-1 (δ 79.82) and from H-8a (δ 1.51) to C-5′ (δ 155.81) indicated that the olefin moiety is attached at the C-1 position of decalin core, and 3) cross peaks from H-2′ (δ 5.82) and H-3′ (δ 7.33) to C-1′ (δ 171.31) indicated that the carbonyl carbon is attached to the terminal olefin moiety. The respective coupling constants between

Fig. 3. HBMC analysis of hynapene A (a) and HMBC analyses and NOE experiments of hynapenes B (b) and C (c).



H-2′ and H-3′ and between H-4′ and H-5′ were 15.8 and 15.0 Hz, suggesting that the both olefins in the side chain have *E* configurations[2]. On the basis of these results described above, the structure of hynapene A was deduced as shown in Fig. 1a.

Structure Elucidation of Hynapenes B and C

The same molecular formula of hynapenes B and C was determined to be $C_{18}H_{24}O_3$ (*m/z* calcd 288.1724, found 288.1725 for hynapene B and 288.1724 for hynapene C) by HREI-MS analyses. Both ^{13}C NMR and ^{1}H NMR spectra showed 18 carbons signals and 23 proton signals, respectively (Table 1). The DEPT spectra indicated the presence of three $-CH_3$, three $-CH_2-$, four $-CH-$, four $=CH-$ and four quaternary carbons for hynapene B and the presence of three $-CH_3$, two $-CH_2-$, four $-CH-$, six $=CH-$ and three quaternary carbons for hynapene C. The absorption at 1660 cm^{-1} for hynapene B and at 1691 cm^{-1} for hynapene C in IR spectra suggested the presence of a carboxylic acid residue, which was supported by the ^{13}C carbon chemical shifts of C-1′ (δ 170.74 for hynapene B and δ 170.08 for hynapene C). The connectivity of proton and carbon atoms was assigned by the ^{13}C-^{1}H COSY spectra as shown in Table 1. The ^{1}H-^{1}H COSY spectra suggested the presence of four partial structures I, II, III and IV for hynapene B (Fig. 2b) and five partial structures I, II, III, IV and V for hynapene C (Fig. 2c). The partial structures were connected by the HMBC spectra and NOE experiments as shown in Figs. 3b and 3c. Furthermore, the stereochemistries of the diene moiety in the side chains of both compounds are also determined to be all *E* by $J_{H2'-H3'}$ and $J_{H4'-H5'}$ (Table 1). Consequently, the structures of hynapenes B and C are shown in Figs. 1b and 1c.

Discussion

Chemical structures of hynapenes A, B and C were elucidated mainly by analyzing NMR spectral data. All of these compounds possess a 2,6,8-trimethyl decalin core having a side chain of (2*E*,4*E*)-2,4-pentadienoic acid in common. However, the side chain is bonded to the C-1 position of the decalin core for hynapenes A and B but to C-2 for hynapene C. It might be that hynapene C is biosynthesized from hynapenes A and/or B after rearrangement of the side chain.

Experimental

Various NMR spectra were recorded on a Varian XL-400 (400 MHz) NMR spectrometer. Mass spectra were obtained on a JEOL model JMS-D 100 mass spectrometer. UV-visible spectra were measured on Shimadzu UV-200S spectrometer. IR spectra recorded on a Horiba FT-210 diffraction infrared spectrometer.

Acknowledgments

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References

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