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COMMUNESINS, CYTOTOXIC METABOLITES OF A FUNGUS ISOLATED FROM A MARINE ALGA

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Summary: Communesins A and B, exhibiting cytotoxic activity against the cultured P-388 cells, were isolated from the mycelium of a strain of Penicillium sp. stuck on the marine alga Enteromorpha intestinalis. Their structures were elucidated by spectroscopic analyses.

Our continuing search for antineoplastic and/or cytotoxic metabolites from marine microorganism[1] has led us to examine the active materials from a strain of Penicillium sp. which was isolated from the marine alga Enteromorpha intestinalis. This investigation revealed that the present fungal strain produces the novel metabolites communesins A(1) and B(2) which exhibit cytotoxic activity against the cultured P-388 lymphocytic leukemia cells.

The fungal strain was cultivated at 27°C for three weeks in a medium (10 l) containing 2% glucose, 1% peptone and 0.5% yeast extract in artificial seawater, pH 7.5. The MeOH extract of the mycelium was purified by bioassay-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatographies and reverse phased HPLC to afford communesins A(1)(20 mg) and B(2)(13 mg).

Communesin A(1), mp 194-196°C(amorphous powder), $[\alpha]_D^{22}$ -58°(c=0.14, $CHCl_3$), analysed for $C_{28}H_{32}N_4O_2$ by HREIMS(m/z 456.2523[M]$^+$). Its IR spectrum exhibited bands at 3319, 1642, 1610 and 1596 cm^{-1}, characteristic of an amine, an amide and an aromatic ring. A close inspection of the ^1H and ^{13}C NMR spectra of 1 (Table 1) by DEPT and ^1H-^1H and ^1H-^{13}C COSY experiments revealed signals for two groups of methines (C6 and C9) linked to two nitrogens and a quaternary sp^3-hybridized carbon (partial structures A and B in Fig.1), two groups of ethylenes (C17 to C20) bearing a nitrogen and a quaternary sp^3-carbon in the both ends (partial structures C and D), N-acetyl (C1" and C2") and N-methyl (C1') groups, 1,2-disubstituted (C1 to C8a) and 1,2,3-trisubstituted (C7a to

Table 1. ^{1}H (300 MHz) and ^{13}C (75.4 MHz) NMR spectral data of communesins in $CDCl_3$

	1				2			
	H			C	H			C
position	(ppm)		J(Hz)	(ppm)	(ppm)		J(Hz)	(ppm)
1	6.70	d	2.8	123.21 (t)[a]	6.66	d	3.5	123.41 (t)
2	6.71	d	7.5	120.54 (t)	6.67	d	7.8	120.52 (t)
3	7.01	td	7.5	127.43 (t)	6.98	ddd	7.8	127.38 (t)
			2.8				5.2	
							3.5	
4	6.69	d	7.5	116.97 (t)	6.66	d	5.2	116.82 (t)
4a				142.69 (q)				142.65 (q)
6	4.70	s		82.44 (t)	4.70	s		82.39 (t)
7				51.42 (q)				51.40 (q)
7a				132.23 (q)				132.25 (q)
8				51.92 (q)				52.14 (q)
8a				132.38 (q)				132.32 (q)
9	5.03	s		79.65 (t)	5.11	s		79.00 (t)
11	4.08	d	9.0	65.38 (t)	4.18	d	9.0	65.55 (t)
11a				136.78 (q)				136.57 (q)
12	6.07	d	7.8	113.19 (t)	6.08	d	7.8	113.23 (t)
13	6.89	t	7.8	128.89 (t)	6.87	t	7.8	128.87 (t)
14	5.95	d	7.8	101.81 (t)	5.95	d	7.8	101.85 (t)
14a				150.55 (q)				150.53 (q)
17 A	3.01	td	12.0	44.08 (s)	3.07	td	12.5	44.21 (s)
			7.2				7.0	
B	3.89	dd	12.0		3.87	dd	12.5	
			8.8				8.4	
18 A	1.98	dd	12.0	30.81 (s)	2.00	dd	12.5	30.46 (s)
			7.2				7.0	
B	2.74	td	12.0		2.71	td	12.5	
			8.8				8.4	
19 A	2.26	dd	12.4	38.03 (s)	2.25	dd	12.8	37.82 (s)
			9.2				8.5	
B	2.35	m			2.34	ddd	12.8	
							9.2	
							8.5	
20 A	3.35	dt	16.0	36.03 (s)	3.40	dt	16.0	36.03 (s)
			9.2				8.5	
B	3.47	dd	16.0		3.48	dd	16.0	
			9.0				9.2	
21	2.87	d	9.0	64.01 (t)	2.90	d	9.0	63.95 (t)
22				59.79 (q)				59.75 (q)
23	1.39	s		24.80 (p)	1.42	s		24.89 (p)
24	1.54	s		20.50 (p)	1.65	s		20.54 (p)
1'	2.85	s		29.63 (p)	2.85	s		29.60 (p)
1"				170.02 (q)				168.43 (q)
2"	2.34	s		22.65 (p)	6.55	d	15.2	121.27 (t)
3"					7.32	dd	15.2	141.83 (t)
							10.1	
4"					6.18	dd	15.5	130.72 (t)
							10.1	
5"					6.12	dq	15.5	137.13 (t)
							5.8	
6"					1.85	d	5.8	18.71 (p)
NH	4.62	brs			4.60	brs		

[a] Letters, p, s, t and q, in parenthesis indicate respectively primary, secondary, tertiary and quaternary carbons, assigned by DEPT.

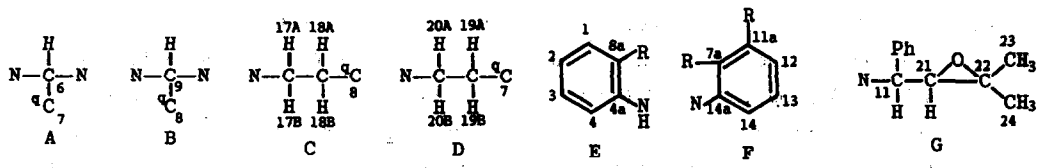

Fig. 1 Partial structures of communesin A (^{q}C indicates a quaternary carbon)

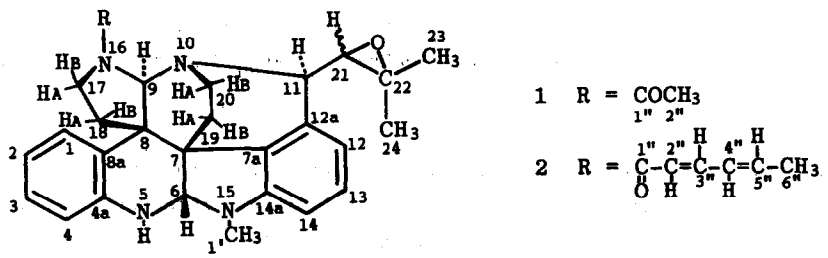

Fig. 2 Structures of communesins A (1) and B (2) (relative stereochemistry)

C14a) benzenes, an epoxide (C21 and C22), gem-dimethyls (C23 and C24) and a methine (C11) bonded to a nitrogen and a phenyl group. The signals for one quaternary carbon each (C4a and C14a) of the two aromatic rings appeared lowfield and the H13 aromatic proton signal had a cross peak with the C14a carbon signal in the HMBC spectrum. This indicated partial structures E and F (Fig. 1).

The coupling relationship between the H11 and H21 protons showed the epoxide to be linked to the methine (C11) bearing a nitrogen function and a phenyl group. In addition, HMBC cross peaks were observed from the H23 and H24 protons of the gem-dimethyl group to the C21 carbon of the epoxide. These data are consistent with partial structure G in Fig. 1.

The connection of the partial structures (A to G) thus established was determined on the basis of three-bond HMBC correlations as follows; H1 to C8, H6 to C4a, C7a, C14a and C19, H9 to C8a, C11, C17, C18, and C20, H11 to C7a, C9 and C20, H18 to C8a and C9, and H19 to C7a and C8. The positions of N-acetyl and N-methyl groups were confirmed by NOEs between H1'-H6 and H14, and H2"-H9 and H11 which were observed in the NOESY spectrum. This evidence led to planar structure 1 for communesin A (Fig.2).

The relative stereochemistry of 1 except for the C21 configuration was deduced from NOEs between the protons as follows; H6-H19A, H19B and H1', H9-H1, H11 and H2", H18B-H19A and H20A, together with the above-mentioned NOEs of H1' and H2". The

configuration at C21 could not be deduced from NOEs between H21-H20B, H23 and H24, and H24-H11 and H12 because these NOEs can be observed in both the C21R and S configurations.

Communesin B (2), mp 152-154°C (amorphous powder), $[\alpha]_D^{22}$ +8.7 (c=0.23, $CHCl_3$), was assigned a molecular formula of $C_{32}H_{36}N_4O_2$ by HREIMS (m/z 508.2834 $[M]^+$). The general features of the UV, IR, NMR and CD spectra of 2 closely resembled those of 1 except that the acetyl signals in the NMR spectrum of 1 were replaced by 2,4-hexadienoyl ones (Table 1). The geometry of the double bonds in the hexadienoyl group was deduced as 2"-trans, 3"-s-trans and 4"-trans from the coupling constants ($J_{2",3"}$=15.2 Hz, $J_{3",4"}$=10.1 Hz and $J_{4",5"}$=16.2 Hz) of the olefinic protons.[2] The above evidence allowed assignment of structure 2 to communesin B (Fig. 2).

Communesins A (1) and B (2) exhibited moderate to potent cytotoxic activity in the P-388 lymphocytic leukemia test system in cell culture[3] (ED_{50} 3.5 and 0.45 μg/ml, respectively).

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