Tetrahedron Letters,Vol.26,No.37,pp 4517-4520,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

PUREALIN, A NOVEL ENZYME ACTIVATOR FROM THE OKINAWAN MARINE SPONGE PSAMMAPLYSILLA PUREA[1]

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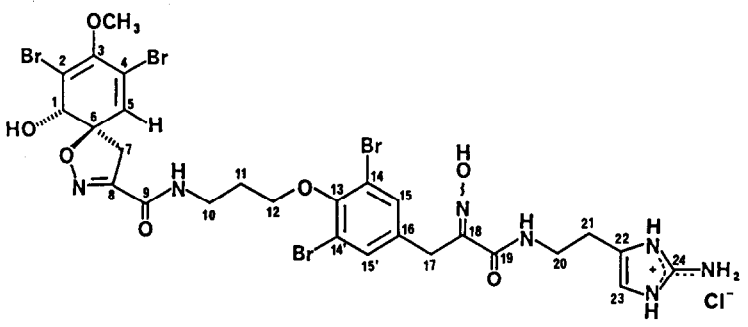
Summary: Purealin, a novel secondary metabolite, which modurates enzymic reactions of ATPases, has been isolated from the Okinawan marine sponge Psammaplysilla purea and the structure has been determined by the ^{1}H-^{1}H homonuclear and ^{1}H-^{13}C heteronuclear NMR chemical shift correlations and CD spectra.

In the course of our study on physiologically active substances from marine organisms,[3] we have examined extracts of various marine organisms for inhibitory activity against pharmacologically important enzymes.[4] As a result, we found that the extract of the Okinawan marine sponge Psammaplysilla purea showed a marked inhibitory effect on Na,K-ATPase. By bioassay-guided isolation, we obtained an active substance, purealin (1), which inhibited Na,K-ATPase and myosin Ca-ATPase, while it activated myosin K,EDTA-ATPase.

The marine sponge P. purea was collected at Ishigaki Island, Okinawa (-1 to -3m). The methanolic extract of the sponge (2.3 kg wet weight) was partitioned between ethyl acetate and water. The ethyl acetate soluble portion was chromatographed on a silica gel column (80:20:1:0.1, $CHCl_3$-MeOH-H_2O-AcOH), followed by an anion exchange column of Diaion WA 11 (Cl^- form, MeOH) and a Sephadex LH-20 column (MeOH) to give purealin (1) hydrochloride as colorless amorphous solids (380 mg, 0.017% of wet sponge, mp 142-145°)[5].

Purealin (1) showed the M+H ions at m/z 880, 882, 884, 886 and 888 (~



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1:4:6:4:1) in the FDMS and the signals due to 30 protons and 27 carbons were observed in the ^{1}H and ^{13}C NMR spectra, respectively, indicating the molecular formula of $C_{27}H_{29}Br_4N_7O_7 \cdot HCl$ for purealin hydrochloride. The ^{1}H-^{1}H homonuclear chemical shift correlations revealed the partial structures, $-O-CH_2-CH_2-CH_2-NH-C(=O)-$ and $Ar-CH_2-CH_2-NH-C(=O)-$, and the long range spin couplings were found between the isolated protons (Table 1). The H-C and C-C connectivities were elucidated by the direct and long range ^{1}H-^{13}C couplings (Table 2). These data suggested that 1 contained three isolated ring systems which were connected to each other via partial structures from C-9 to C-12 and from C-17 to C-21.

The chemical shifts of C-22, C-23 and C-24 and a large ^{1}H-^{13}C coupling constant of C-23 (198 Hz) indicated a 4-substituted-2-aminoimidazole functionality[6,7] for the terminal part, which must be connected to C-21. The 4-alkyl-2,5-dibromophenol constellation was determined on the basis of the ^{13}C chemical shifts.[8] The long range ^{1}H-^{13}C coupling between C-13 and H-12 and the long range ^{1}H-^{1}H coupling between H-17 and H-15 and H-15' strongly suggested that C-13 was linked to C-12 via an oxygen atom, whereas C-16 was linked to C-17. Furthermore, the partial structure from C-17 to C-19 was established by the long range ^{1}H-^{13}C couplings of C-18 and C-19 and H-17. The exchangeable proton signal at δ 12.10 as well as the chemical shift of C-18 suggested that C-18 was assignable to a carbon of an α-ketoxime.

The remaining part of 1 consisted of $C_9H_8NO_3Br_2$, which was deduced to be a spiroisoxazole structure by comparing the ^{1}H and ^{13}C NMR data of 1 with those reported for aerothionin-related compounds.[7,8] The proposed structure was also supported by the ^{1}H-^{13}C long range couplings. The trans geometry of the vicinal oxygen atoms was established by the chemical shift of H-7[9] and the absolute configuration was decided as illustrated on the basis of the rotation and CD spectra of 1, $[\alpha]_D$ -85° (C 2.10, MeOH), and $[\theta]_{284}$ = -30200 and $[\theta]_{245}$ = -31400 (MeOH).[10]

Purealin appears to be closely related biogenetically to secondary metabolites derived from bromotyrosine which were isolated from marine sponges of the order Verongida.[12]

Table 1. 400 MHz ^{1}H NMR data for purealin (1)in DMSO-d_6.

Proton	δ (m,J in Hz)	C[a]	Proton	δ (m,J in Hz)	C[a]
H-1	3.98 (brs)		H-15,15'	7.46 (s)	
OH-1	6.48 (brs)		H-17	3.78 (s)	
OCH_3	3.67 (s)		NOH-18	12.10 (s)	
H-5	6.58 (brs)		NH-19	8.15 (t, 6)	
H-7	3.24 (d, 18)		H-20	3.42 (dt, 6, 7)	
	3.71 (d, 18)		H-21	2.66 (t, 7)	
NH-9	8.57 (t, 6)		NH-22	11.74 (brs)[b]	
H-10	3.43 (dt, 6, 7)		H-23	6.59 (s)	
H-11	2.02 (tt, 7)		NH-23	12.16 (brs)[b]	
H-12	3.97 (t, 7)		NH_2-24	7.38 (brs)	

a: Correlated by homonuclear spin decoupling and two dimensional shift correlation experiments.
b: Each assignment may be exchanged.

Table 2. 100 MHz ^{13}C NMR data for purealin (1) in DMSO-d_6

Carbon	δ	Direct H-C coupling m[a]	J(Hz)[b]	Long range H-C coupling m[b]	J(Hz)[b]	correlated H[c]
C-1	73.45	d	148	d	5	H-5
C-2	120.69	s		d	5	H-5
C-3	147.00	s		dq	5	H-5, OCH_3
C-4	113.01	s		brd	8	H-1, H-5
C-5	131.16	d	177	m		H-1, H-7
C-6	90.16	s		brs		H-1, H-5, H-7
C-7	39.37	t	143	brs		H-5
C-8	154.38	s		t	7	H-7
C-9	158.87	s		m		H-10, NH-9
C-10	36.14	t	135	brs		H-11
C-11	29.27	t	127	brs		H-10, H-12
C-12	71.18	t	148	brs		H-11
C-13	150.69	s		brt	7	H-12, H-15,15'
C-14,14'	117.14	s		dd	2, 3	H-12, H-15,15'
C-15,15'	132.77	d	163	dt	8, 5	H-17
C-16	136.08	s		t	7	H-15,15', H-17
C-17	27.80	t	130	brs		
C-18	150.71	s		t	7	H-17, H-15,15'
C-19	163.07	s		m		H-17, H-20, NH-19
C-20	37.26	t	143	brs		H-21
C-21	24.28	t	130	brs		H-20
C-22	124.11	s		brs		H-23
C-23	109.00	t	198	brs		H-21
C-24	146.74	s		d	9	H-23
OCH_3	59.50	q	142	s		

a: Determined by proton off-resonance decoupled ^{13}C NMR spectra and INEPT technique.
b: Defined by proton coupled with NOE spectra at 67.5 MHz.
c: Correlated by low power proton selective decoupling and heteronuclear two dimensional shift correlation experiments.

Purealin inhibited the activity of myosin Ca-ATPase and Na,K-ATPase. However, the activity of myosin K,EDTA-ATPase was enhanced by purealin. Purealin is the first natural product which activates myosin K,EDTA-ATPase. The unique activity of purealin will be reported in detail elsewhere.[13]

Acknowledgement: The authors acknowledge Dr. T. Hoshino (Mukaishima Marine Biological Station, Hiroshima University) for his kind identification of the marine sponge, Prof. S. Yamamura and Dr. S. Nishiyama (Department of Chemistry, Keio University) for their useful suggestion and Mrs. M. Imachi (Bruker Japan Co. Ltd.) for two dimensional shift correlation experiments. We also thank Dr. H. Ohtani and Mr. T. Hayase (Central Research Laboratories, Mitsubishi Chemical Industries Ltd.) for FDMS measurements, Mr. Z. Nagahama for his help to collect the marine sponge and Miss R. Abe for her technical assistance.

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(Received in Japan 4 July 1985)