Lipopurealins, novel bromotyrosine derivatives with long chain acyl groups, from the marine sponge *Psammaplysilla purea*[1]

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Summary. Lipopurealin-A, -B and -C have been isolated from the Okinawan marine sponge *Psammaplysilla purea.* They are inhibitors of Na,K-ATPase, and the structures have been elucidated on the basis of spectral data. They are bromotyrosine derived structures with saturated long chain acyl parts.

Key words. Marine sponge; *Psammaplysilla purea;* enzyme inhibitor; Na,K-ATPase; purealin; lipopurealin.

Recently, we reported the isolation and the structure of purealin[1] (**1**) from the Okinawan marine sponge *Psammaplysilla purea*[1]. Purealin (**1**) is the first natural product which has been shown to activate myosin K,EDTA-ATPase[4]. Our continuing study on the physiologically active constituents in the sponge led to the isolation of novel compounds, lipopurealins, which have inhibitory effects on Na,K-ATPase. Lipopurealins contained long chain acyl moieties instead of the spiroisoxazole ring in purealin (**1**). We wish to report here the isolation and the structures of lipopurealin-A, -B and -C, which have saturated long chain acyl groups (fig. 1).

The less polar UV absorbing fraction obtained during the separation of purealin (**1**) was separated into 10 fractions by HPLC on a C_{18} column, using a methanol-water solvent system containing 0.2 M sodium chloride for elution. The fractions 1, 3 and 7 furnished hydrochlorides of lipopurealin-A (**2**), -B (**3**) and -C (**4**), respectively[5]. The isolation yields of **2**–**4** were 0.0001%, 0.0012% and 0.00013%, respectively, from the wet sponge.

Lipopurealin-A (**2**)[6], -B (**3**)[7] and -C (**4**)[8] showed intense M + H ions in the ratio of about 1:2:1 at m/z 727, 729 and 731 ($C_{31}H_{48}N_6O_4Br_2$), 741, 743 and 745 ($C_{32}H_{50}N_6O_4Br_2$), and 755, 757 and 759 ($C_{33}H_{52}N_6O_4Br_2$) in the SIMS spectra, respectively, thus indicating the presence of two bromine atoms in the compounds. Comparison of ^{1}H-NMR spectra of **2**–**4** with that of **1** (table 1) suggested that compounds **1**–**4** contained a common UV absorbing chromophore (λ_{max} 284 nm) composed of $C_{17}H_{21}N_6O_3Br_2$ which was connected to acyl groups (IR ν_{max} 1675 cm^{-1}). The molecular formulae of **2**–**4** indicated that the acyl groups of **2**–**4** were saturated long acyl chains of $C_{14}H_{27}O$, $C_{15}H_{29}O$ and $C_{16}H_{31}O$, respectively.

Lipopurealin-B (**3**) showed a triplet signal (2 H) at δ2.20 and a quartet signal (2 H) at δ1.16 due to the α- and β-protons of the acyl group, respectively, in the ^{1}H-NMR spectrum. The terminal iso-branched structure of this long chain was clearly indicated by a doublet signal (6 H) at δ0.87 assignable to two terminal methyls, and a nonalet signal (1 H) at δ1.51 due to a branched methine proton. In the ^{13}C-NMR spectrum of **3**, the signals assignable to the common UV chromophore were consistent with those of purealin (**1**)[1]. These data suggested that the acyl group of **3** was a 13-methyl-tetradecanoyl group; the calculated values of ^{13}C-chemical shifts for the long chain part also supported the structure[9].

In contrast to lipopurealin-B (**3**), lipopurealin-A (**2**) and -C (**4**) showed triplet methyl signals at δ0.90 and 0.88, respectively, due to one terminal methyl group, indicating that the acyl groups of **2** and **4** were straight chains. On the basis of the molecular formulae of **2** and **4**, n-tetradecanoyl and n-hexadecanoyl groups were assigned to the acyl groups of **2** and **4**, respectively.

Numerous bromotyrosine-derived metabolites have been isolated from sponges of the order Verongida and considered to be characteristic metabolites of this order[10]. From the biogenic point of view, these compounds can be interpreted as arising from bromotyrosines and amines, which may be derived from amino acids. However, lipopurealins belong to a new type of bromotyrosine-derived metabolite, which is coupled to long chain fatty acids. Lipopurealin-A, -B, and -C exhibited inhibitory activities on Na,K-ATPase purified from porcine brain and dog kidney, lipopurealin-B being the most potent inhibitor. In cardiac Na,K-ATPase all three compounds showed only weak activity (table 2). In addition, myosin K,EDTA-ATPase was markedly activated by purealin[4], whereas the enzyme was inhibited by lipopurealin-B.

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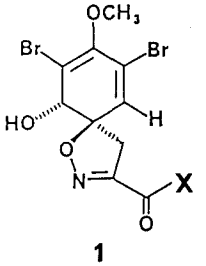

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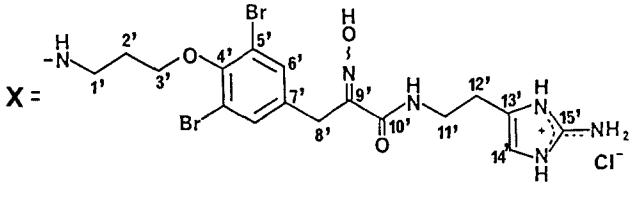

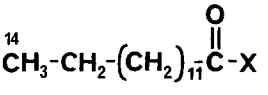

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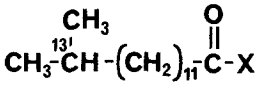

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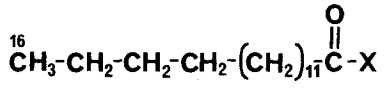

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Table 1. ^{1}H NMR data for **1**, **2**, **3** and **4** in CD_3OD[a]

Proton	1	2	3	4	
H-1′	3.58	3.44	3.44	3.44	(2H, t, J=7Hz)
H-2′	2.10	2.04	2.04	2.04	(2H, quin, J=7Hz)
H-3′	4.04	4.01	4.01	4.02	(2H, t, J=7Hz)
H-6′	7.47	7.48	7.48	7.48	(2H, s)
H-8′	3.82	3.83	3.82	3.83	(2H, brs)
H-11′	3.47	3.47	3.48	3.47	(2H, t, J=7Hz)
H-12′	2.70	2.70	2.71	2.71	(2H, t, J=7Hz)
H-14′	6.50	6.51	6.51	6.51	(1H, s)
H-1		2.19	2.20	2.19	(2H, t, J=7Hz)
H-2		1.61	1.61	1.61	(2H, quin, J=7Hz)
CH_3		0.90(t)	0.87(d)	0.88(t)	(J=7Hz)
CH		–	1.51	–	(1H, nona., J=7Hz)
CH_2		1.28[b]	1.28[c]	1.28[d]	(brs)
			1.16		(2H, q, J=7Hz)

[a] δ in ppm; [b] 20H; [c] 16H; [d] 24H.

Table 2. Inhibitory effects of lipopurealin-A, -B and -C on Na,K-ATPase

Compounds	Na,K-ATPase inhibition[a] IC_{50}[b] (M)		
	Brain[c]	Kidney[c]	Heart[d]
Lipopurealin-A	30	20	100
Lipopurealin-B	6	10	> 100
Lipopurealin-C	60	20	> 100

[a] The enzyme reaction was carried out at 37 °C for 15 min; [b] 50% inhibitory concentration; [c] Na,K-ATPase isolated from porcine brain or dog kidney was purchased from Sigma Chemical Company; [d] Cardiac Na, K-ATPase was prepared from guinea pig heart by the method of Pitts and Schwartz[11].

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5 Lipopurealins were obtained as hydrochlorides by the described isolation procedure and the counter ion was analyzed by ion chromatography.

6 **2**: colorless amorphous solids, m.p. 94–95 °C; UV (MeOH) λ_{max} 284 nm (ε 970); IR ($CHCl_3$) ν_{max} 2930, 2850, 1675, 1520, 1450 and 1250 cm^{-1}.

7 **3**: colorless amorphous solids, m.p. 93–95 °C; UV (MeOH) λ_{max} 284 nm (ε 930); IR (KBr) ν_{max} 2930, 2850, 1675, 1540, 1455 and 1260 cm^{-1}; ^{13}C-NMR (CD_3OD) δ37.7 (t, C-1′), 30.9 (t, C-2′), 72.3 (t, C-3′), 152.8 (s, C-4′), 118.7 (s, C-5′), 134.4 (d, C-6′), 137.2 (s, C-7′), 28.8 (t, C-8′), 151.9 (s, C-9′), 165.4 (s, C-10′), 38.9 (t, C-11′), 25.8 (t, C-12′), 126.0 (s, C-13′), 110.7 (d, C-14′), 148.5 (s, C-15′), 176.3 (s, C-1), 37.2 (t, C-2), 27.1 (t, C-3), 30.2 (t, C-4), 30.4 (t, C-5), 30.7 (t, C-6 ~ C-9), 31.0 (t, C-10), 28.5 (t, C-11), 40.2 (t, C-12), 29.0 (d, C-13), 23.1 (q, C-14 and C-15).

8 **4**: colorless amorphous solids, m.p. 108–110 °C; UVB (MeOH) λ_{max} 284 nm (ε 910); IR ($CHCl_3$) ν_{max} 2930, 2860, 1675, 1520 and 1450 cm^{-1}.

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