

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

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Summary. Lipopurealin-A, -B and -C have been isolated from the Okinawan marine sponge *Psammaphysilla 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; *Psammaphysilla purea*; enzyme inhibitor; Na,K-ATPase; purealin; lipopurealin.

Recently, we reported the isolation and the structure of purealin¹ (1) from the Okinawan marine sponge *Psammaphysilla purea*¹. Purealin (1) is the first natural product which has been shown to activate myosin K₂EDTA-ATPase⁴. 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 C₁₈ 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), respectively⁵. 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 (C₃₁H₄₈N₆O₄Br₂), 741, 743 and 745 (C₃₂H₅₀N₆O₄Br₂), and 755, 757 and 759 (C₃₃H₅₂N₆O₄Br₂) in the SIMS spectra, respectively, thus indicating the presence of two bromine atoms in the compounds. Comparison of ¹H-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 C₁₇H₂₁N₆O₃Br₂ 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 C₁₄H₂₇O, C₁₅H₂₉O and C₁₆H₃₁O, respectively.

Lipopurealin-B (3) showed a triplet signal (2 H) at δ 2.20 and a quartet signal (2 H) at δ 1.16 due to the α- and β-protons of the acyl group, respectively, in the ¹H-NMR spectrum. The terminal iso-branched structure of this long chain was clearly indicated by a doublet signal (6 H) at δ 0.87 assignable to two terminal methyls, and a nonalet signal (1 H) at δ 1.51 due to a branched methine proton. In the ¹³C-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 ¹³C-chemical shifts for the long chain part also supported the structure⁹.

In contrast to lipopurealin-B (3), lipopurealin-A (2) and -C (4) showed triplet methyl signals at δ 0.90 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 order¹⁰. 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 K₂EDTA-ATPase was markedly activated by purealin⁴, whereas the enzyme was inhibited by lipopurealin-B.

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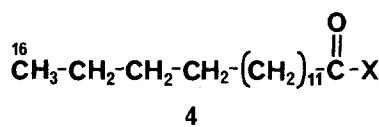
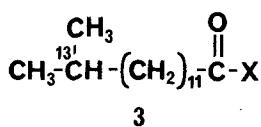
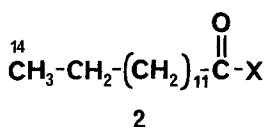
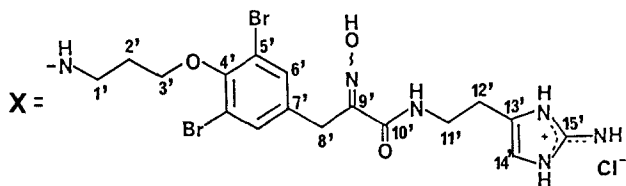
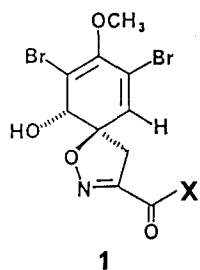


Table 1. ^1H NMR data for **1**, **2**, **3** and **4** in CD_3OD^a

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)	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 Schwartz¹¹.

- 1 Physiologically active marine natural products from Porifera X. Preceding paper: Nakamura, H., Wu, H., Kobayashi, J., Nakamura, Y., Ohizumi, Y., and Hirata, Y., *Tetrahedron Lett.* 26 (1985) 4517.
- 2 Present address: Shanghai Institute of Organic Chemistry, Academia Sinica, 345 Linglin Lu, Shanghai, China.
- 3 To whom reprint requests should be addressed at Mitsubishi-Kasai Institute of Life Sciences.
- 4 Nakamura, Y., Nakamura, H., Wu, H., Kobayashi, J., and Ohizumi, Y., submitted (1985).
- 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) δ 37.7 (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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- 11 Pitts, B.J.R., and Schwartz, A., *Biochim. biophys. Acta* 184 (1975) 401.

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