Pergamon

Tetrahedron Letters, Vol. 38, No. 7, pp. 1201-1202, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00016-6

Akaterpin, a Novel Bioactive Triterpene from the Marine Sponge *Callyspongia* sp.

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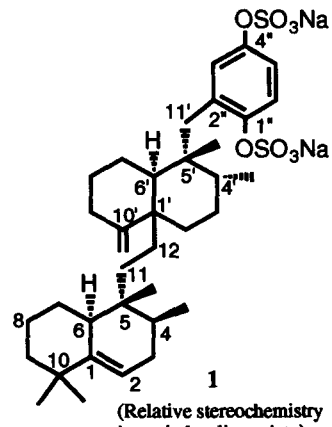
Abstract: Akaterpin (1), an inhibitor of phosphatidylinositol-specific phospholipase C, was isolated from an acetone extract of the marine sponge, *Callyspongia* sp. Structural determination by 2D-NMR spectroscopy revealed that it was a novel triterpene with the hydroquinone disulfate.

Many growth factors and hormones induce cellular phosphatidylinositol turnover[1], in which phosphatidylinositol-specific phospholipase C (PI-PLC) is the rate-limiting enzyme. For the isolation of akaterpin, an inhibitor of PI-PLC, the inhibitor was extracted from the marine sponge *Callyspongia* sp. (27.9 g) with 30 ml of butanol after an initial extraction with the same volume of acetone. The butanol extract was concentrated *in vacuo* and the residue was applied to a silica gel column. The column was eluted with chloroform-methanol (10:0-10:5). The active fraction (eluted with $CHCl_3$:MeOH=10:3-10:4) was subjected to preparative HPLC (Senshu Pak, Pegasil-ODS, 20 ϕ x 250 mm, flow rate 5 ml/min) with elution with a linear gradient of 35-50% aqueous acetonitrile. Akaterpin (12.0 mg) was eluted with a peak at 118 min.

Akaterpin (1) was obtained as a colorless solid, mp >240°C; $[\alpha]_D^{24}$ +15° (*c* 0.59, MeOH); UV (MeOH): λ_{max} (ε) 207 (16800), 275 nm (1070); IR (KBr): ν_{max} 3504, 3392, 2962, 2929, 2887, 1630, 1485, 1460, 1381, 1265 (SO_2), 1232 (SO_2), 1182, 1061, 1045, 964, 889, 854 cm^{-1}; FAB-MS (pos.): *m/z* 745 $(M+Na)^+$, 723 $(M+H)^+$, 643 $(M+H-SO_3)^+$; FAB-MS (neg.): *m/z* 699 $(M-Na)^-$; FAB-HRMS (neg.): *m/z* 677.3184 $(M+H-Na_2)^-$, calcd for $C_{36}H_{53}O_8S_2$, 677.3182. The 1D-NMR (^1H, ^{13}C and differential NOE) and 2D-NMR (^1H-^1H COSY, HMQC, HMBC and NOESY) spectra in CD_3OD were obtained on a 400 MHz or 500 MHz spectrometer (Table 1).



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(Relative stereochemistry in each decalin moiety)

The formula $C_{36}H_{52}O_8S_2Na_2$, having the sodium salt of the hydroquinone disulfate, was supported by the spectral analyses. The hexaisoprenoid structure consisting of two decalin moieties was determined by extensive NMR measurements (^{1}H-^{1}H COSY, HMQC and HMBC). The ^{1}H and ^{13}C chemical shifts were assigned as shown in Table 1. The relative stereochemistry of akaterpin was elucidated from NOESY and differential NOE experiments. Among six methyl groups on the decalin moieties, a *cis* relation of 4-CH_3 (δ 0.86) and 5-CH_3 (δ 0.59) on the opposite side of 6-H (δ 2.23) was suggested by NOE. In addition, two NOE's between 4'-CH_3 (δ 1.15) and 6'-H (δ 1.68) and between 4'-CH_3 and 11'-H (δ 2.40) suggested that 6'-H and 11'-H are on the same side as 4'-CH_3. An NOE between 5'-CH_3 (δ 1.03) and 12-Ha (δ 2.13) could not be identified, because the 5'-CH_3 signal overlapped with 12-Hb (δ 1.04). No cross peak between 5'-CH_3 and 12-Ha, or between 6'-H and 12-Ha was observed in NOESY. Therefore, the stereochemistry at C-1' remained undefined, and the relative stereochemistry in each decaline moiety was determined, as shown in structure **1**. The triterpenoid structure of **1** is clearly different from that of toxiusol[2], which has the same molecular formula in the hexaisoprenoid moiety. The exo-ene structure at C-10' is also found in sponge-derived siphonodictyoic acid[3].

In vitro PI-PLC activity was measured as previously described[4], and the IC_{50} value of **1** was 0.5 μg/ml. Akaterpin also inhibited neutral sphingomyelinase weakly with an IC_{50} of 30 μg/ml. Other PI-PLC inhibitors include fluvirucin B_2[4] and pholipeptin[5]. Akaterpin was the most potent in inhibiting the enzyme.

Table 1. ^{1}H and ^{13}C NMR data (δ) in CD_3OD for akaterpin

Position	^{13}C	^{1}H	Position	^{13}C	^{1}H	Position	^{13}C	^{1}H
1	147.4		10-$(CH_3)_2$	30.3	1.06s	7'	22.4	2.20m
2	117.4	5.47ddd		29.5	1.04s			1.99m
3	32.7	1.84ddd	11	30.2	1.15m	8'	25.7	1.65m
		1.77ddd	12	36.4	2.13ddd	9'	34.0	2.32ddd
4	34.6	1.47m			1.04m			2.18m
4-CH_3	15.6	0.86d	1'	44.1		10'	153.4	
5	37.6		2'	28.3	1.98m	10'-CH_2	109.4	4.76brs
5-CH_3	16.8	0.59s			1.46m			4.73brs
6	41.0	2.23m	3'	26.2	1.96m	11'	37.3	3.32d
7	28.9	1.68m			1.20m			2.40d
		0.98m	4'	38.6	1.65m	1"	150.1	
8	23.3	1.57m	4'-CH_3	16.5	1.15d	2"	135.2	
9	42.2	1.42m	5'	43.1		3"	125.1	7.30d[a]
		1.20m	5'-CH_3	25.2	1.03s	4"	149.9	
10	37.1		6'	47.0	1.68m	5"	120.4	7.09dd[b]
						6"	123.2	7.38d[c]

[a] J=2.4 Hz [b] J=2.4, 8.5 Hz [c] J=8.5 Hz

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(*Received in Japan* 21 *October* 1996; *revised* 19 *December* 1996; *accepted* 25 *December* 1996)