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Isolation of Dephosphonocalyculin A from the Marine Sponge, *Discodermia calyx*[1]

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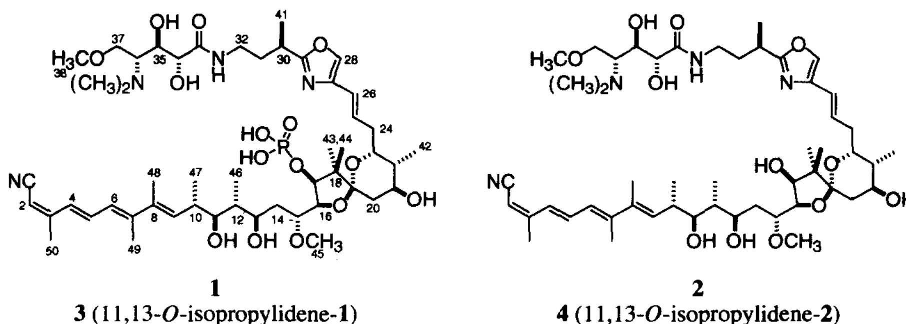
Abstract : Fractionation of the EtOH extract of the marine sponge *Discodermia calyx* led to the isolation of dephosphonocalyculin A (**2**). The gross structure **2** was deduced on the basis of spectral data. The structure including the absolute stereochemistry has been confirmed by chemical transformation of **2** and calyculin A to a common compound. Dephosphonocalyculin A inhibits protein phosphatases 1 and 2A with IC_{50} values of 3.0 and 8.2 nM, respectively. © 1997 Elsevier Science Ltd.

The calyculins [calyculin A (**1**)], unique metabolites of the marine sponge *Discodermia calyx*, have become increasingly important due to their potent inhibitory activity against protein phophatases 1 and 2A,[2-6] which illuminates the interactions between the calyculins and the phosphatases. In the course of our search for new calyculin derivatives,[7] we have found that a polar fraction of the EtOH extract of *D. calyx* contained a considerable amount of a related compound, which we have isolated and found to be dephosphonocalyculin A.

The EtOH extract of *D. calyx* (4.0 kg, wet weight) collected by SCUBA off the Izu Peninsula was partitioned between H_2O and CH_2Cl_2; the organic layer was repeatedly fractionated by silica gel chromatography, Sephadex LH-20 chromatography, and ODS HPLC to yield dephosphonocalyculin A (**2**; 25.5 mg) as a colorless solid. The FAB mass spectrum exhibited an $(M+H)^+$ ion at *m/z* 929, 80 units smaller than that of calyculin A, whereas the ^1H NMR spectrum contained signals characteristic for the calyculins, e.g. an oxazole singlet, two *O*-methyls, *N*, *N*-dimethyl, three olefinic methyls, four doublet *C*-methyls, and two singlet *C*-methyls.[8] However, some differences were observed between the spectra of **1** and **2**; two *O*-methyl and *N*, *N*-dimethyl signals were



1
3 (11,13-*O*-isopropylidene-**1**)

2
4 (11,13-*O*-isopropylidene-**2**)

observed at 3.50, 3.40, and 2.87 ppm in **1**, but at 3.60, 3.24, and 2.27 ppm in **2**. The gross structure of **2**, which was identical with that of **1** except for the phosphate ester at the C17 hydroxyl, was easily assigned on the basis of COSY, HMQC, and HMBC spectra. These features would indicate that compound **2** was simply a dephosphorylated analog of **1**, but perhaps the stereochemistry of the two compounds might be different at several chiral centers, because chemical shifts differed at atoms distant from C17. In order to solve this problem dephosphorylation of calyculin A was attempted; it was not successful due to the strong intra-molecular hydrogen bonding network present in **1**.[2] This feature prevents the approach of the hydrolytic or solvolytic agents to the phosphate group. Then we changed our strategy; calyculin A (**1**) was first converted to the 11,13-acetonide **3** which was solvolyzed with a mixture of dioxane and pyridine to furnish **4**[9, 10] which could be also derived from **2** by treatment with 2,2-dimethoxypropane in the presence of PPTS. Thus the structure of **2** was unambiguously assigned as the dephosphorylated derivative of calyculin A.

Calyculin A is believed to inhibit protein phosphatases due to the presence of the phosphate group, mimicking the substrate, phosphorylated proteins.[11] Thus it is unexpected that **2** also strongly inhibited protein phosphatase[5] 1 and 2A with IC_{50} values of 3.0 and 8.2 nM, respectively. It was also highly cytotoxic against P388 with an IC_{50} value of 18 ng/mL. Purity of **2** is unambiguous, because **2** is far more polar than other calyculins and elutes in separate fractions in both normal phase and reversed phase chromatographies. Hence the mode of interaction of **2** with the protein phosphatases becomes an important research objective.[11]

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8. NMR Data for **2**: ^{1}H NMR (600 MHz, CD_3OD); δ 5.20 (s, H-2), 6.69 (d, *J*=15.0 Hz, H-3), 7.06 (dd, *J*=15.0, 11.2 Hz, H-5), 6.32 (d, *J*=11.2 Hz, H-6), 5.92 (d, *J*=9.6 Hz, H-9), 2.66 (m, H-10), 3.31 (dd, *J*=2.7, 9.6 Hz, H-11), 1.55 (m, H-12), 3.80 (m, H-13), 1.85 (m, H-14a), 1.55 (m, H-14b), 3.58 (m, H-15), 4.06 (dd, *J*=9.0, 4.3 Hz, H-16), 3.48 (d, *J*=4.3 Hz, H-17), 1.44 (m, H-20a), 1.58 (m, H-20b), 4.01 (dt, *J*=1.9, 5.4 Hz, H-21), 1.65 (m, H-22), 4.51 (m, H-23), 2.24 (m, H-24a), 2.36 (m, H-24b), 6.24 (m, H-25), 6.26 (m, H-26), 7.76 (s, H-28), 2.98 (m, H-30), 1.74 (m, H-31a), 1.96 (m, H-31b), 3.14 (2H, t, *J*=7.2 Hz, H-32), 3.92 (d, *J*=6.1 Hz, H-34), 3.57 (m, H-35), 2.86 (ddd, *J*=9.6, 6.9, 3.4 Hz, H-36), 3.55 (m, H-37a), 3.64 (dd, *J*=10.8, 3.4 Hz, H-37b), 3.24 (3H, s, H-38), 2.26 (3H, s, H-39, 40), 1.25 (3H, d, *J*=6.9 Hz, H-41), 0.84 (3H, d, *J*=6.9 Hz, H-42), 0.86 (3H, s, H-43), 1.03 (3H, s, H-44), 3.60 (3H, s, H-45), 0.66 (3H, d, *J*=6.9 Hz, H-46), 0.98 (3H, d, *J*=6.9 Hz, H-47), 1.82 (3H, s,H-48), 1.97 (3H, s, H-49), 2.02 (3H, s, H-50); ^{13}C NMR (150 MHz, CD_3OD); δ 95.5 (C-2), 159.0 (C-3), 129.2 (C-4), 135.1 (C-5), 125.7 (C-6), 146.0 (C-7), 137.5 (C-8), 132.4 (C-9), 37.5 (C-10), 78.9 (C-11), 44.2 (C-12), 70.9 (C-13), 30.0 (C-14), 81.2 (C-15), 87.1 (C-16), 80.5 (C-17), 48.5 (C-18), 109.5 (C-19), 34.1 (C-20), 71.8 (C-21), 38.5 (C-22), 68.2 (C-23), 37.5 (C-24), 130.0 (C-25), 122.5 (C-26), 139.5 (C-27), 136.5 (C-28), 169.7 (C-29), 32.9 (C-30), 35.4 (C-31), 38.0 (C-32), 175.0 (C-33), 76.1 (C-34), 71.2 (C-35), 65.0 (C-36), 70.1 (C-37), 58.4 (C-38), 42.5 (C-39,40) 18.6 (C-41), 11.6 (C-42), 22.0 (C-43), 16.6 (C-44), 60.1 (C-45), 11.4 (C-46), 18.4 (C-47), 14.2 (C-48), 14.6 (C-49), 19.2 (C-50).
9. Calyculin A (**1**, 27 mg) was treated with PPTS (3 mg) in 2,2-dimethoxypropane/acetone (1:1, 2 mL) at rt for 3 days to furnish **3** (10 mg): FABMS [$(M+H)^+$ *m/z* 1049]. Subsequently, **3** was heated with a mixture of pyridine/dioxane (1:1, 0.3 mL) at 120 °C for 4 days. The reaction mixture was separated by HPLC on ODS to afforded **4** (1 mg).
10. **4** from calyculin A: colorless solid; $[\alpha]^{20}_D$ -117° (*c* 0.025, MeOH); FABMS [$(M+H)^+$ *m/z* 969]; **4** from **2**: colorless solid; $[\alpha]^{20}_D$ -131° (*c* 0.05, MeOH); FABMS [$(M+H)^+$ *m/z* 969].
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