Pergamon

Tetrahedron Letters, Vol. 35, No. 31, pp. 5579-5582, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01146-X

Pokepola Ester: A Phosphate Diester from a Maui Sponge

Raju S. Kalidindi, Wesley Y. Yoshida, Jorge A. Palermo and Paul J. Scheuer*

Department of Chemistry, University of Hawaii at Manoa,
2545 The Mall, Honolulu, Hawaii 96822

Michelle Kelly - Borges[1]

Harbor Branch Oceanographic Institution,[2]
5600 North U. S.1, Fort Pierce, Florida 34936

Abstract: Pokepola ester (1), a phosphate diester of mixed biogenetic origin was isolated from a Maui sponge, *Spongia oceania*. It showed mild anti HIV activity.

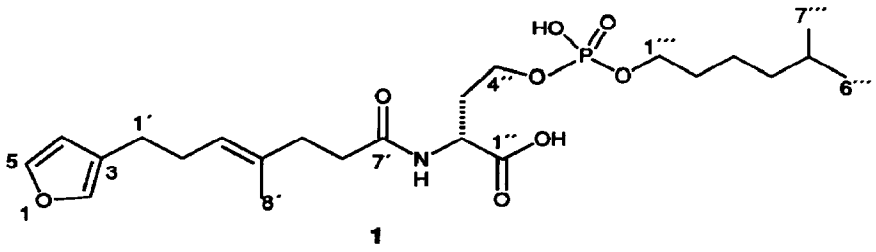
Phosphate esters are essential elements of nucleotides, which are primary metabolites. They have only rarely been encountered as building blocks of secondary metabolites. Recent examples of phosphate esters as moieties of marine natural products have been the sponge - derived calyculins[3] and some polyhydroxysterols from a sea star.[4]

We now report isolation and structural elucidation of another example of a phosphorus-containing marine natural product. Pokepola[5] ester (1) is a diester of phosphoric acid; the alcohols are 5-methylhexanol and homoserine. The amine of homoserine, in turn, forms an amide with a C_{12} carboxylic acid, which terminates in a β-substituted furan and appears to be a truncated sesquiterpene.

The sponge was collected at a depth of -12 to 14 m on the south shore of Maui, Hawaii in July, 1993. The sponge was identified as *Spongia oceania* (Spongiidae:Dictyoceratida).[6,7] The freeze-dried specimen (350 g) was extracted with ethanol (2 x 4L), and the solvent was removed *in vacuo*. The resulting residue (9 g) was partitioned between water and *n*-butanol to furnish 5.0 g of nonpolar extract (upper layer). This extract was again partitioned with hexane/methanol/water (5:4:1) which gave 3 g of polar residue (bottom layer). The polar residue was subjected to high-speed countercurrent chromatography with CH_2Cl_2/MeOH/H_2O (13:7:8) using the nonpolar layer as stationary phase and separated into 25 fractions. Two fractions (fractions 5 and 6) were combined (190 mg) and subjected to gel permeation chromatography with Sephadex LH-20 (methanol). Fractions 10 and 11 were combined and repeated RP C-18 HPLC (CH_3CN/H_2O, 35:65, YMC - Pack ODS-A , 250x10 mm column) was performed to give 10 mg of compound **1**.[8]

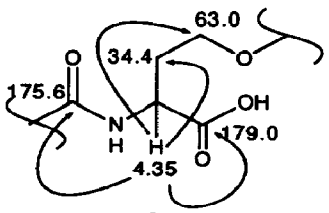
Compound **1** was obtained as an oil; its molecular formula, $C_{23}H_{38}NO_8P$, was deduced by a series of high mass ion peaks in the FAB mass spectrum at *m/z* 494 (M+Li), 510 (M+Na), 602 (M+Na+glycerol). It was also confirmed by HR FAB MS (M+Na 510.2120 Da, Δ 11.4 mmu). The proton NMR spectrum of the compound showed three aromatic signals at δ 7.35 (1H, bs), 7.24 (1H, bs), and 6.29 (1H, bs), characteristic of a β-

substituted furan. IR absorptions at 3600-3400 and 1640 cm^{-1} indicated the presence of an amide group. In the HMBC spectrum of the compound, a signal at δ 4.35 (1H, t, J=6.3 Hz) showed correlations to two carbonyl carbons at δ 175.5 and 179.0 and two methylene carbons at δ 34.5 and 63.0. A two-proton signal at δ 2.01 (m, H_2-3") exhibited correlations to carbons that resonated at δ 63.0, 53.5 and 179.0.



1

Based on these data, the presence of a homoserine moiety was suggested and partial structure (a) represents this portion of the molecule.



a

HMBC and COSY correlations of proton and carbon resonances of the single olefin (δ 5.23 and 125.7, 135.6) and the vinyl methyl (δ 1.61 and 16.1) together with the adjacent methylene protons and carbons revealed a C_{12} carboxylic acid terminating in a furan ring at one end and a carboxyl function at the other, which formed an amide with the amino group of homoserine .

The ^1H NMR spectrum of **1** showed the presence of a methyl doublet at δ 0.87 (6H, d, J = 6.8 Hz) and a one proton multiplet at δ 1.52 (2H, m) characteristic of an isopropyl group. HMBC and COSY correlations starting from the isopropyl group revealed the presence of an esterified 2-methylhexanol.

The ^{13}C NMR spectrum (DMSO-d_6) at 50°C showed signals at δ 66.7, 63.0, 34.5 and 32.0 which appeared as doublets due to ^{13}C-^{31}P coupling through two and three bonds, thus indicating the presence of a phosphate group. This was confirmed by a ^{31}P NMR spectrum in which phosphorus resonated at -1.13 ppm, when the spectrum was recorded in CD_3OD with phoshoric acid as the external standard.

The ^1H and ^{13}C NMR spectral data of pokepola ester (**1**) are presented in Table I. The three exchangeable protons present in the molecule were not observed in ^1H NMR spectrum, because the spectrum was recorded in CD_3OD. The geometry of the double bond was ascertained by NOE experiment. An NOE was shown between H-3' and H_2-5' and also between H_2-2' and Me-8'. No NOE was observed for H-3' and Me-8', which establishes *E* geometry for the double bond.

Table I. NMR Data for **1**(500 MHz, CD_3OD)

#[a]	^{13}C	^{1}H (J in Hz)	HMBC	COSY
2	140.1	7.24 (1H, bs)	H-4, H-5, H-1'	
3	126.1	–	H-2, H-4, H-5, H_2-1', H_2-2"	
4	112.0	6.29 (1H, bs)	H-2, H-5, H_2-1'	H-5
5	143.8	7.35 (1H, bs)	H-2, H-4ß	H-4
1'	25.8	2.43 (2H, bt, 7.0)	H-4, H_2-2', H-3'	H_2-2'
2'	29.6	2.23 (2H, bt, 7.4)	H_2-1', H-3'	H_2-1',H-3'
3'	125.7	5.23 (1H, td, 6.8, 1.1)	H_2-1', H_2-2', H_2-5', H_3-8'	H_2-2',H_3-8'
4'	135.6	–	H_2-2', H_2-5', H_2-6', H_3-8'	
5'	36.6	2.29 (2H, m)	H-3', H_2-6', H_3-8'	H_2-6'
6'	36.0	2.33 (2H, m)	H_2-5'	H_2-5'
7'	175.6	–	H_2-5', H_2-6', H-2"	
8'	16.1	1.61 (3H, bs)	H-3', H_2-5'	H-3'
1"	179.0	–	H-2", H_2-3"	
2"	53.5	4.35 (1H, t, 6.2)	H_2-3", H_2-4"	H_2-3"
3"	34.5[b]	2.01 (2H, m)	H-2", H_2-4"	H-2",H_2-4"
4"	63.0[b]	4.02 (1H, m)		H_2-3"
		3.90 (1H, m)	H-2", H_2-3"	
1"'	66.7[b]	3.85 (2H, m)	H_2-2"', H_2-3"'	H_2-2"'
2"'	32.0[b]	1.55 (2H, m)	H_2-1"', H_2-3"', H_2-4"'	H_2-1"', H_2-3"'
3"'	24.7	1.38 (2H, m)	H_2-1"', H_2-2"' H_2-4"', H_2-5"'	H_2-2"', H_2-4"'
4"'	39.8	1.19 (2H, m)	H_2-2"', H_2-3"', H_2-5"' H_3-6"' and H_3-7"'	H_2-3"', H-5"'
5"'	29.1	1.52 (1H, m)	H_2-4"', H_3-6"' and H_3-7"'	H_2-4"', H_3-6"', H_3-7"'
6"'	23.0	0.87 (3H, d, 6.8)	H_2-4"', H_2-5"'	H-5"'
7"'	23.0	0.87 (3H, d, 6.8)	H_2-4"', H_2-5"'	H-5"'

[a] Assignments were made by COSY, HMQC and HMBC correlations
[b] These signals appeared as doublets when the spectrum was run at 50°C due to ^{13}C-^{31}P coupling through two and three bonds.

The absolute configuration of homoserine was determined to be D ([*R*]-2-amino-4-hydroxybutyric acid) by Marfey's method.[9] To a 1 mL vial containing 25 µg of pure amino acid standard (D and L homoserine) in 50 µL H_2O was added 1 mg of FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) in 100 µL of acetone, followed by 20 µL of 1N $NaHCO_3$. The mixture was heated for 1 h at 40°C. After cooling to room temperature, 10 µL of

2N HCl was added. The resulting solution was concentrated under reduced pressure. The residue was dissolved in 500 μL of DMSO and stored in the dark until HPLC analysis. To prepare the FDAA derivative of the amino acid of **1**, it was dissolved in 150 μL of methanol-water (1:1) and 150 μL of 6N constant boiling HCl was added. The mixture was heated for 12 h at 100°C and cooled to room temperature; the solvent was removed *in vacuo*. The hydrolyzate was dissolved in 50 μL of water and added 1 mg of FDAA in 100 μL of acetone, followed by 20 μL of 1N $NaHCO_3$. The mixture was heated for 1 h at 40°C and after cooling to room temperature, 10 μL of 2N HCl was added.The residue was dissolved in 500 μL DMSO. A 20 μL aliquot of the resulting mixture of FDAA derivatives was analyzed by HPLC by eluting with 30% CH_3CN in 50 mM - TEAP (triethylammine phosphate buffer), pH 3.0. A YMC pack RP C-18A column was used with a flow rate of 2 mL/min, UV detector at λ_{max} 340 nm. From the HPLC trace of the product obtained after Marfey's derivatization of **1**, it was observed that both D- and L- isomers are present in a 4:1 ratio. Apparently partial epimerization had occurred. Hence **1** and authentic samples of D- and L- homoserine were independently subjected to the same hydrolysis conditions and then FDAA derivatization was performed. In all cases the epimerized product was obtained as a minor component (< 20% of the original).

Pokepola ester (**1**) showed mild anti HIV activity at a concentration of 0.2 μg/mL without showing any cytotoxicity. The C_{12} carboxylic acid appears to be a trisnorsesquiterpene. The presence of a D amino acid in the molecule suggests a microbial origin.[10]

Acknowledgment. We thank Mike Severns for specimen collection, Professor K.L. Rinehart, Jr. (University of Illinois) for mass spectral support, Toshio Ichiba and John Carney for valuable discussions. Bioassay was performed by Pharma Mar, S. A. Financial support by NCI through the National Cooperative Drug Discovery Group Program, the National Science Foundation, the Sea Grant College Program, and Pharma Mar, S. A. is gratefully acknowledged.

References and Notes

1. Present address: Department of Zoology, The Natural History Museum, London SW7 5BD, U. K.
2. HBOI Contribution No. 0000.
3. Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K. *J. Org. Chem.* **1988**, *53*, 3930-3932.
4. De Riccardis, F.; Iorizzi, M.;Minale, L.; Riccio, R.; Debitus, C. *Tetrahedron Lett.***1992**, *33*, 1097-1100.
5. The Hawaiian transliteration of phosphorus is pokepola.
6. de Laubenfels, M. W. *The Sponges of Kaneohe Bay*, Oahu. **1950** *Pac. Sci. 4*:3-36.
7. A Voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (Catalogue No. 003:898).
8. **1**: oil;$[\alpha]_D$ -4.5° (*c* 0.5, MeOH); HR FABMS m/z 510.2120 (M+Na calcd for $C_{23}H_{38}NO_8PNa$ 510. 2223 ; IR (AgCl plates) 3400-3200, 1710, 1640, 1220 cm^{-1}; NMR data in Table I.
9. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591-596.
10. Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793-1806.

(*Received in USA* 24 *May* 1994; *accepted* 9 *June* 1994)