Tetrahedron Letters,Vol.24,No.22,pp 2283-2286,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

THE STRUCTURES OF FOSFAZINOMYCINS A AND B

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Summary: The structures of fosfazinomycins A and B, phosphorus containing antibiotics, have been established by spectral evidence and degradation studies.

During the course of our new screening program[1] for anti-fungal antibiotics, fosfazinomycins B(1) and A(2), which are active against some filamentous fungi, were isolated from culture filtrate of Streptomyces lavendofoliae No.630. We will describe the structural elucidation of fosfazinomycins in this paper[2].

Fosfazinomycin B(1), $C_{10}H_{23}N_6O_6P$, [FDMS $M+1^+$ m/z 355, micro analyses; C: 30.80, H: 6.48, N: 20.33, P: 7.34 (found), C: 29.09, H: 6.01, N: 20.19, P: 7.54(calced. for $C_{10}H_{23}N_6O_6P \cdot H_2CO_3$)], m.p.148-150°C(dec.), $[\alpha]_D^{25}$ 17.2°(c 1, H_2O), 3300(NH and OH), 1740(ester) and 1680 cm^{-1}(amide), positive to ninhydrin and Sakaguchi reactions, is hygroscopic white powder and is very labile in acidic and alkaline media. L-Arginine was identified in the acid hydrolysate of 1(a.a. analysis and optical rotation) and isolation of DNP-arginine by acid hydrolysis of DNP-1 indicated that the N-terminus of the arginine must be free.

^{1}H-nmr of 1(D_2O) showed the signals assigned to a methoxy group(δ 3.77, s) and an N-methyl group(δ 2.87, d, $^3J_{H-P}$=6Hz). The partial structure, P-N-CH_3, was concluded by the coupling of the N-methyl proton with a phosphorous atom which was confirmed by ^{1}H-{^{31}P} nmr experiment.

^{13}C-nmr spectral data of 1, 2 and related compounds are summarized in Table 1. Since the twelve resonances were observed for the ten carbon atoms in the spectrum of 1, two carbons must be coupled with phosphorus. One of them was readily assigned to the N-methyl signal at 38.2 ppm($^2J_{C-P}$=7.8Hz)

Table 1. ^{13}C-nmr spectral data of 1, 2 and related compounds*

Carbon No.	1[a]	2[a]	4[a]	Arginine[4]	Valine[4]
1	176.8	176.4	168.7	174.9	
2	53.4	52.8	53.0	54.8	
3	31.6	28.9	29.1	28.2	
4	25.0	25.1	24.9	24.6	
5	41.6	41.4	41.6	41.2	
6	157.4	157.7	157.7	157.2	
1'	173.8	173.8[b]			
2'	71.5(J_{C-P}=125Hz)	71.5(J_{C-P}=125Hz)			
NCH_3	38.2(J_{C-P}=7.8Hz)	38.1(J_{C-P}=7.3Hz)	38.7		
OCH_3	53.6	53.5			
1"		174.1[b]			175.0
2"		60.7			61.3
3"		32.5			29.9
4"		17.8			17.6
5"		19.3			18.8

a) The spectra were recorded at 25MHz in D_2O solution.
b) These signals may be interchanged.
* in ppm relative to internal dioxane(67.4 ppm)

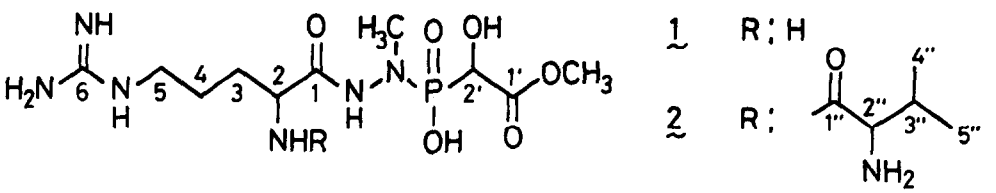
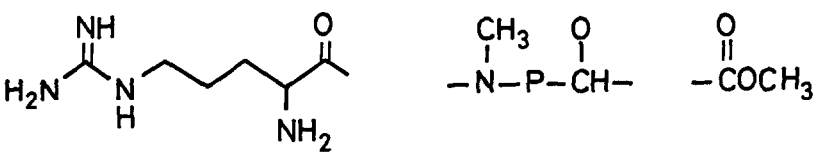


Fig.1 The structures of 1 and 2

based on the result of ^{1}H-nmr. The other resonance coupled with phosphorus was ascribed to the oxymethine at 71.5 ppm($^1J_{C-P}$=125Hz) because the J value of this oxymethine signal remained constant on the measurement at different magnetic fields(22.5 and 25MHz). This large coupling constant[3] due to one-bond C-P coupling indicated the partial structure O-CH-P.

The results described above suggested the following partial structures



Acid hydrolysis of 1(50% AcOH, r.t., 16hrs) gave an acidic substance 3, $C_3H_7O_6P$, and a basic compound 4, $C_7H_{18}N_6O$(FDMS M+1$^+$ m/z 203).

Spectral evidence of 3 revealed the presence of an oxymethine adjacent to phosphorus(δ_H 4.61, d, $^2J_{H-P}$=14Hz, and δ_C 65.7, $^1J_{C-P}$=143Hz) and a carbomethoxy group(δ_H 3.83, s, OCH_3, and δ_C 173.6, C=O).

Acetylation(Ac_2O/Py) of 3 followed by methylation(CH_2N_2/MeOH) afforded 3a, $C_7H_{13}O_7P$ (EIMS M^+ m/z 240), a monoacetyl dimethyl ester of 3. The structural components of 3a were deduced by ^1H and ^{13}C-nmr analysis as follows; two methoxy groups adjacent to phosphorus (δ_H 3.87, d, $^3J_{H-P}$=11Hz and δ_H 3.88, d, $^3J_{H-P}$=11Hz), an acetyl methyl(δ_H 2.33, s), an acetylated oxymethine bonded directly to phosphorus(δ_H 5.48, d, $^2J_{H-P}$=17Hz, and δ_C 68.2, J_{C-P}=161Hz) and a carbomethoxy group(δ_H 3.84, d, $^5J_{H-P}$=0.5Hz, OCH_3 and δ_C 165.0, C=O).

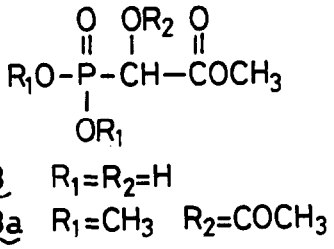


Fig.2

These results as well as the molecular formula determined the structure of 3a as depicted in Fig 2, thus, establishing 3 as methyl 2-phosphono-2-hydroxy-acetate.

Basic compound 4, $C_7H_{18}N_6O$(FDMS $M+1^+$ m/z 203), was assumed to be an N-methyl-hydrazide of arginine based on the findings that the ^{13}C-nmr of 4 revealed the resonances ascribed to the arginine[4] and N-methyl moieties (Table 1), and that the mass spectrum of the hexa-TMS derivative 4a, $C_{25}H_{66}N_6OSi_6$(EIMS M^+ m/z 634), showed characteristic peaks at m/z 259, 316 and 417(Fig.3). The N-methyl moiety was located at the terminal of the molecule by the diagnostic fragment ion at m/z 532.3160(found)(532.3172 calcd. for $C_{21}H_{54}N_5OSi_5$) in the mass spectrum of 4a. The foregoing evidence established 4 as N^1-methyl-N^2-arginyl hydrazine.

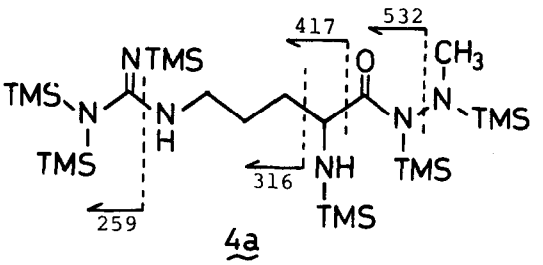


Fig. 3

Taking into consideration of the partial structures described above and isolation of 3 and 4, the structure of 1 was established as depicted in Fig. 1.

Fosfazinomycin A(2), $C_{15}H_{32}N_7O_7P$ [FDMS $M+1^+$ m/z 454, micro analyses; C: 37.88, H: 7.34, N: 21.88, P: 6.29(found), C: 39.73, H: 7.06, N: 21.63, P: 6.84(calced. for $C_{15}H_{32}N_7O_7P$)], m.p. 157-161°C(dec.), $[\alpha]_D^{25}$ 14.7°(c 1, H_2O), is also white hygroscopic powder and labile in acidic and alkaline media. The spectral evidence revealed 2 as an analogue of 1 and L-valine and L-arginine were detected in acid hydrolysate of 2.

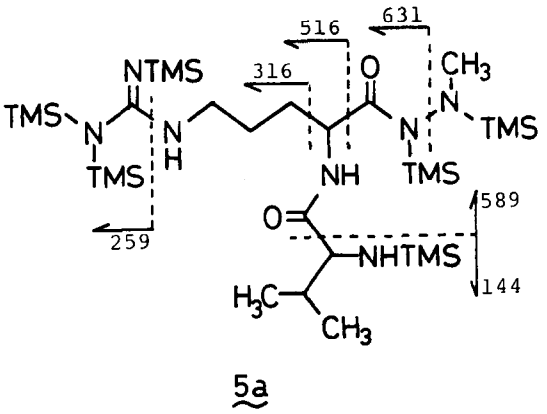
As shown in Table 1, ^{13}C-nmr of 2 was almost superimposable with that

of 1 except for the signals attributed to the valine moiety. The signal assigned to the β-carbon of the arginine was observed with the upfield shift in the spectrum of 2 compared with that of 1, indicating the substitution of the valine moiety on the α-amino group of the arginine[5].

Acid hydrolysis(2N-H_2SO_4, r.t., 16hrs) of 2 gave 3 and 5, $C_{12}H_{27}N_7O_2$ (FDMS M+1$^+$ m/z 302). The structure of 5 was established as N^1-methyl-N^2-valyl-arginyl-hydrazine based on the mass spectral analysis of hexa-TMS derivative 5a, $C_{30}H_{75}N_7O_4Si_6$(EIMS M$^+$ m/z 733)(Fig. 4).

The results obtained above confirmed the structure of 2 as depicted in Fig. 1.

The structures of fosfazinomycins revealed a unique feature possessing a phosphorous atom and an N-methyl hydrazine moiety and these were found to be related to that of FR-900137[6], the antibiotic with anti-bacterial activity.



5a

Fig. 4

Acknowledgement The authors are grateful to Dr. Jun Uzawa of Institute of Physical and Chemical Research for the ^1H-{^{31}P} nmr experiment.

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(Received in Japan 28 February 1983)