Structure of the Antifungal Nucleotide Antibiotic Phosmidosine

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The structure of phosmidosine (1), a novel proline-containing nucleotide antibiotic from *Streptomyces durhameusis*, active against the pathogenic fungus *Botrytis cinerea*, was determined by mass spectrometry and NMR spectroscopy. Homologs 2, 3, and the isomer 4 were detected and characterized using approaches based principally on tandem mass spectrometry and combined liquid chromatography–mass spectrometry which permitted assignment of most structural features directly in the crude isolate without prior isolation of individual components. Conversion of 1–4 to the *O*-isopropylidene derivatives 1a–4a by a microscale procedure resulted in enhanced fast atom bombardment ionization (FAB) signal-to-background sensitivity. Collision-induced dissociation mass spectra were acquired from molecular ions and ion source-generated fragment ions and used in conjunction with FAB–deuterium exchange methods for the assignment of structural differences between 1a–4a.

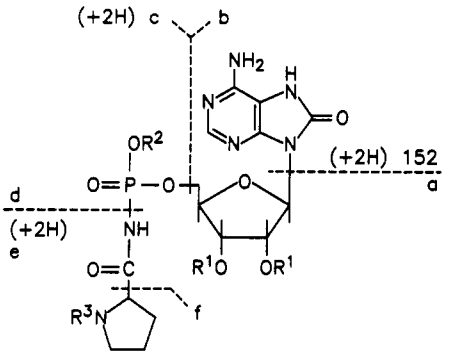
Phosmidosine is a new antifungal antibiotic recently isolated[1] from culture filtrates of *Streptomyces durhameusis*. It exhibits specific inhibitory activity against spore formation of *Botrytis cinerea*, a world-wide pathogenic fungus which causes gray mold disease in a variety of fruits and vegetables. We report the structure of phosmidosine (1), the compound on which biological testing was carried out, and on two homologs (2, 3) and an isomer (4). Compared with a large number of known nucleoside antibiotics,[3] four nucleotide antibiotics have been previously reported: agrocin-84 produced by *Agrobacterium radiobacter*,[2] thuringiensin from *Bacillus thuringiensis*,[3] fosfadecin from *Pseudomonas viridiflava*,[4] and fosfocytocin from *Pseudomonas fluorescens*.[4] The culture fermentation conditions, isolation, microbiological properties, and a brief description of chemical properties of phosmidosine were published with an initial communication of structure 1.[1]

The structure determination of phosmidosine was hampered due to low isolation yield from fermentation cultures and chemical instability over a period of several weeks. As a consequence, much of the principal structural data were acquired using tandem mass spectrometry, which permitted a number of structural features of 1 and related compounds 2–4 to be deduced without chromatographic separation or prior isolation of individual components of the culture isolate. HPLC of a crude phosmidosine isolate, on which most mass spectrometry experiments were carried out, shows four principal constituents,[5] corresponding to 1–4 as described in the following sections.

Results and Discussion

The exact molecular size and gross structural features of phosmidosine were established by high-resolution mass spectrometry, ^{31}P, ^{1}H, and ^{13}C NMR spectroscopy, and acid hydrolysis to yield the principal structural subunits. The molecular mass of phosmidosine determined from its fast atom bombardment mass spectrum was established as 473.1415 u, in support of elemental composition $C_{16}H_{24}N_7O_8P$. The presence of phosphorus was confirmed by the ^{31}P NMR spectrum (δ_P 10.5 ppm in D_2O; H_3PO_4 standard) and of a methyl phosphate group from a doublet of methoxyl methyl protons in the ^{1}H NMR spectrum (δ_H 3.48, d, J_{H-O-P} = 11.4 Hz). Phosmidosine was determined by mass spectrometry to contain seven exchangeable hydrogen atoms in the neutral molecule, based on an isotopic exchange method (MD^+, m/z 482) which employs deuterated matrix for FAB ionization.[6,7] Hydrolysis of a crude fermentation isolate by 6 N HCl yield L-proline and

Scheme I



1, $R^1 = R^3 = H$, $R^2 = CH_3$

2, $R^1 = R^2 = R^3 = H$

3, $R^1 = H$, $R^2 = R^3 = CH_3$

4, $R^1 = R^2 = H$, $R^3 = CH_3$

a series: $R^1 + R^1 = CH_3\overset{\diagdown\ \diagup}{C}CH_3$

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(1) Uramoto, M.; Kim, C. J.; Shin-ya, K.; Kusakabe, H.; Isono, K.; Phillips, D. R.; McCloskey, J. A. *J. Antibiot.* **1991**, *44*, 375–381.

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(3) Isono, K. *J. Antibiot.* **1988**, *41*, 1711–1739.

(4) Katayama, N.; Tsubotani, S.; Nozaki, Y.; Harada, S.; Ono, H. *J. Antibiot.* **1990**, *43*, 238–246.

(5) Data are shown in the supplementary material.

(6) Sethi, S. K.; Smith, D. L.; McCloskey, J. A. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 126–131.

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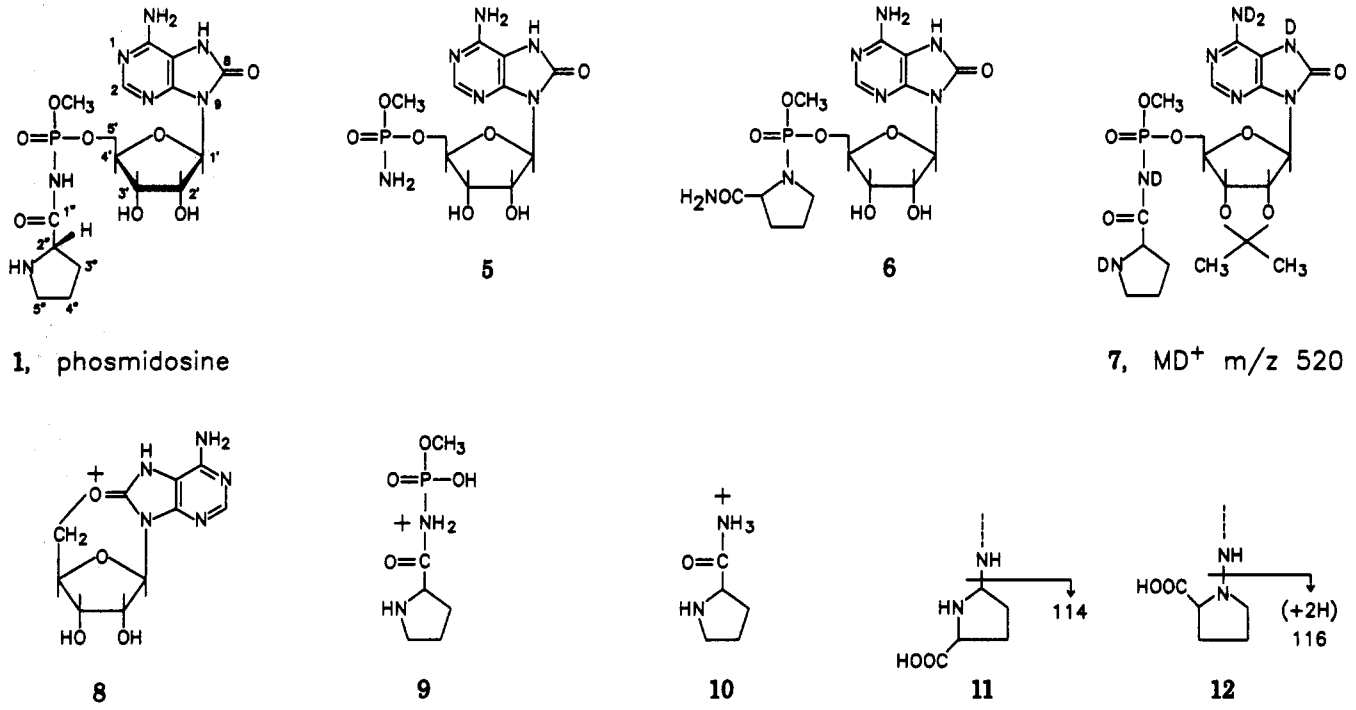
Chart I



8-oxoadenine,[8] as discussed below. Taken together with basic spectroscopic properties[1] these data indicate phosmidosine to be a nucleotide conjugate of proline. Relatively low ion yield in the FAB mass spectrum[5] of the isolate was attributed to the high polarity and hydrophilic character of the molecule, which is a surface activity-related effect commonly observed with peptides.[11,12] Microscale conversion to the more hydrophobic *O*-isopropylidene derivative was used to increase the surface concentration of the compound on the FAB matrix and to establish the presence and number of cis–diol groups in the molecule. The resulting mass spectrum, Figure 1, shows a 40-u molecular ion shift, from *m/z* 474 to 514, due to incorporation of one blocking group, as well as significantly improved signal-to-background ratio. The mass spectrum in Figure 1 also reveals molecular ions corresponding to **2a** (*m/z* 500) and **3a** (*m/z* 528) which were not evident (as the unblocked compounds **2** and **3**) in the mass spectrum[5] of the underivatized isolate. As a consequence, most further experiments on components of the phosmidosine isolate involving mass spectrometry were carried out using the isopropylidene derivatives.

Nucleoside Moiety. Acid hydrolysis of **1** yielded a UV-absorbing product which was isolated by preparative TLC and shown to have composition $C_5H_5N_5O$ by EI mass spectrometry (M^+, 151.0495 found; 151.0494 calcd). Identity of this product as 8-oxoadenine was established by

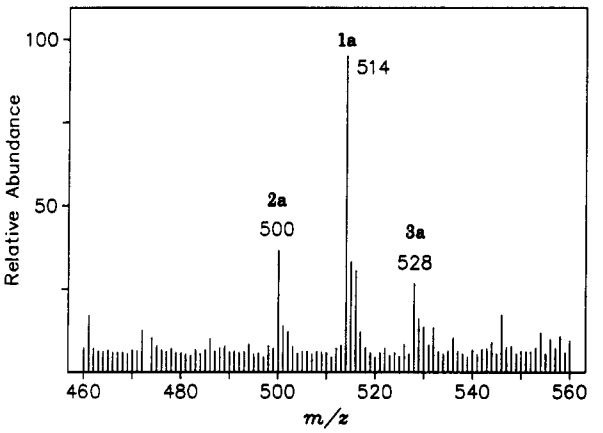


Figure 1. FAB mass spectrum of crude phosmidosine isolate following reaction with 2,2-dimethoxypropane to form isopropylidene derivatives **1a**, **2a**, and **3a**.

several independent methods including comparison of the collision-induced dissociation (CID) mass spectrum of the protonated base (*m/z* 152) generated by FAB of **1a** with those of the protonated bases generated by FAB of authentic 8-oxoadenosine.[13] The CID mass spectrum of the phosmidosine base closely matched that of the base from 8-oxoadenosine, but not those from the isomeric heterocycles guanosine and isoguanosine.[5,14] The CID mass spectrum from *m/z* 152 of 8-oxoadenosine is qualitatively similar to that reported by Alexander et al. who used a triple quadrupole instrument.[15]

(7) Verma, S.; Pomerantz, S. C.; Sethi, S. K.; McCloskey, J. A. *Anal. Chem.* **1986**, *58*, 2898–2902.

(8) Nomenclature and structural representations are made in terms of the 8-oxoadenine tautomer rather than the often-used 8-hydroxy form, in view of evidence from IR[9] and ^{15}N NMR spectroscopy.[10]

(9) Guy, A.; Duplaa, A. M.; Harel, P.; Teoule, R. *Helv. Chim. Acta* **1988**, *71*, 1566–1572.

(10) Cho, B. P.; Evans, F. E. *Nucleic Acids Res.* **1991**, *19*, 1041–1047.

(11) dePauw, E. D.; Pelzer, G.; Dung, D. V.; Marien, J. *Biochem. Biophys. Res. Commun.* **1984**, *123*, 27–32.

(12) Naylor, S.; Findeis, A. F.; Gibson, B. W.; Williams, D. H. *J. Am. Chem. Soc.* **1986**, *108*, 6359–6363.

(13) For discussion of the method of determining equivalence of gas-phase ion structures from collision-induced dissociation reactions, see: (a) Levsen, K.; Schwartz, H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 509–568. (b) Holmes, J. L. *Org. Mass Spectrom.* **1985**, *20*, 169–183.

(14) For leading references to the structural equivalence of base ions derived by dissociation of nucleosides and base molecular ions, see: Nelson, C. C.; McCloskey, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 3661–3668.

(15) Alexander, A. J.; Kebarle, P.; Fuciarelli, A. F.; Raleigh, J. A. *Anal. Chem.* **1987**, *59*, 2484–2491.

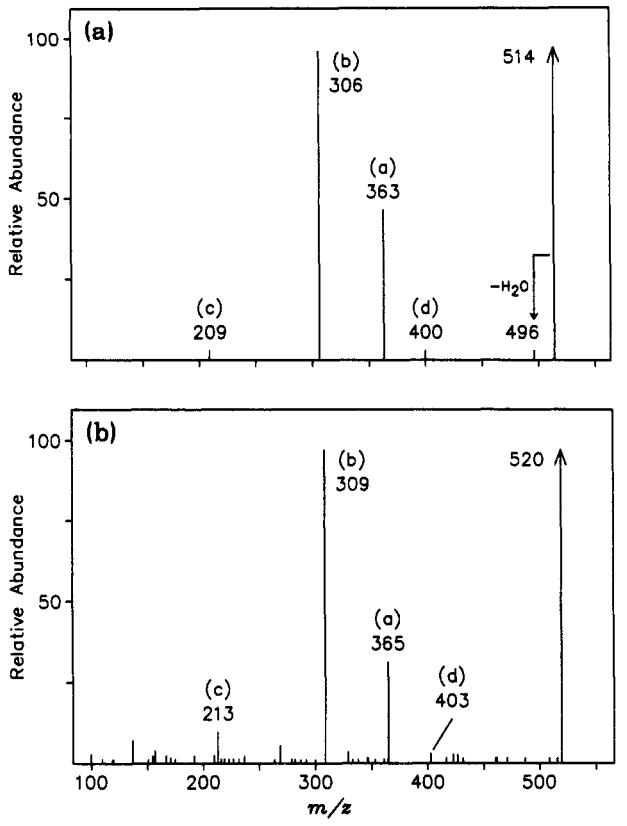


Figure 2. CID mass spectra of (a) **1a** and (b) **7**, resulting from deuterium exchange of **1a** in 3-nitrobenzyl alcohol-*O*-*d* FAB matrix. Both experiments were carried out using mass selection of precursor ions for the same sample mixture represented in Figure 1. Ion assignments are shown in Chart I.

Treatment of **1** with 0.2 N NaOH by procedure A (see Experimental Section) produced two hydrolysis products, characterized as the nucleotide analog **5** as described in the following section, and the rearranged compound **6**.[16] Upon further hydrolysis of **5** by 1 N NH_4OH (110 °C, 3 h) a product was obtained which was indistinguishable from authentic 8-oxoadenosine by thermospray liquid chromatography–mass spectrometry (LC/MS) analysis and which produced no peak broadening when coinjected with 8-oxoadenosine. In addition, trimethylsilylation of the hydrolysis product followed by GC/MS analysis resulted in a product having a retention time 16:03 min and an EI mass spectrum indistinguishable[5] from the pentasilyl derivative of authentic 8-oxoadenosine (retention time 16:01 min). Following alkaline hydrolysis of **1** by 0.5 N NaOH (procedure B), a product was isolated having a ^1H NMR spectrum and optical rotation properties (see Experimental Section) identical to 8-oxoadenosine. The nucleoside moiety of phosmidosine is therefore rigorously characterized as 9-β-D-ribofuranosyl-8-oxoadenine.

Nucleotide Linkage and Side-Chain Substituents. Mass selection and collision-induced dissociation of the protonated isopropylidene derivative **1a** using tandem mass spectrometry (*m/z* 514 in Figure 1) provides a mass spectrum (Figure 2a) free of contributions from homologs **2a** and **3a**, which exhibits ions representing the principal structural subunits of phosmidosine. The analogous mass spectrum derived from the deuterated FAB matrix 3-nitrobenzyl alcohol-*O*-*d*, Figure 2b, shows ions in which all active hydrogen (protium) has been exchanged by deuterium (structure **7**) and which are used to corroborate the structural assignments to peaks in Figure 2a, summarized in Chart I. Elimination of a neutral molecule of prolylamide (ion d, Figure 2a) is supported by the presence of three exchangeable hydrogen atoms in the neutral species lost (derived from *m/z* 520–403 and 520–213 differences in Figure 2b). Loss of the neutral 8-oxoadenine base yields ion a, from a common but usually minor dissociation reaction.[17] The assignment for ion a is supported by a 40-u shift (to *m/z* 323) in the CID spectrum[5] from **1**, without the isopropylidene function. The active hydrogen content of ion a (two atoms) is as required for the total number in the precursor *m/z* 514 ion (six atoms), minus 8-oxoadenine (four atoms). Cleavage at C-5′ generates ion b, representing the nucleoside portion of the molecule in which stabilization is envisioned as shown in **8**.[18] Ions of this type are not usually observed in mass spectra of nucleosides and nucleotides[21] and so participation of the 8-oxo function as shown appears to be an essential structural requirement. The overall assignment for ion b as shown in Scheme I is supported by two observations: (i) a downward 40 u shift (to *m/z* 266) in the CID mass spectrum of **1**[5] and (ii) sufficient amounts of the *m/z* 306 ion are formed in the ion source following FAB ionization to permit further analysis by collision-induced dissociation. The resulting mass spectrum[5] after collisional activation shows *m/z* 152 as the principal product ion, demonstrating presence of the 8-oxoadenine group in ion b (Figure 2a).

The minor product ion c (Figure 2a) is of structural importance because it represents the intact C-5′ side-chain moiety. The assignment is supported by mass and active hydrogen interrelationships with **1a** and in particular by the CID mass spectrum of *m/z* 209 (Figure 3), which demonstrates presence of the prolyl group (ions e, f; see Scheme I). Evidence for the 5′-*O*-phosphate linkage was gained from the ^{13}C NMR spectrum of **1**, which showed four signals observed as doublets coupled with phosphorus: 84.4 (C-4′, J_{CP} = 7.3 Hz), 67.7 (C-5′, J_{CP} = 5.9 Hz), 64.9 (C-2″, J_{CP} = 24.9 Hz), and 56.4 (OCH_3, J_{CP} = 5.9 Hz). C–P coupling assignments were supported by the invariance of the *J* values measured at 25 and 100 MHz.

The structural assignments **9** (*m/z* 209, Figure 2a) and **10** (*m/z* 115, Figure 3), taken in concert with the elemental composition of **1** and presence of the C-5′–O bond (^{31}P NMR and recovery of 8-oxoadenosine from alkaline hydrolysis), require the sequence of atoms C-5′–O–P–N–proline as shown in structure **1**. This interpretation is verified by the molecular weight and structure of the alkaline hydrolysis product **5**, shown by NMR (see Experimental Section) to contain the P OCH_3 group. These data along with the even numerical value of molecular

(16) The structure of the second hydrolysis product **6** was established[5] by ^1H and ^{13}C NMR and by CID of protonated **6** and of three side-chain fragment ions, from which **6** is concluded to have arisen by side chain N–N′ migration.

(17) Crow, F. W.; Tomer, K. B.; Gross, M. L.; McCloskey, J. A.; Bergstrom, D. E. *Anal. Biochem.* **1984**, *139*, 243–262.

(18) Stable cyclic nucleosides similar to **8** are known.[19] Alternatively, stabilization of a 5′-carbonium ion by electrons of N-3 of the adenine ring has precedent in the solution chemistry of 2′,3′-*O*-isopropylideneadenosine *p*-toluenesulfonate.[20]

(19) Ikehara, M.; Kaneko, M.; Okano, R. *Tetrahedron* **1970**, *26*, 5675–5682.

(20) Townsend, L. B. In *Synthetic Procedures in Nucleic Acid Chemistry*: Zorbach; W. W.; Tipson R. S., Eds.; Interscience Publishers: New York, 1973; Vol. 2, pp 328–330.

(21) Schram, K. H. In *Mass Spectrometry*; Lawson, A. M., Ed.; Walter deGruyter: New York, 1989; pp 507–570.

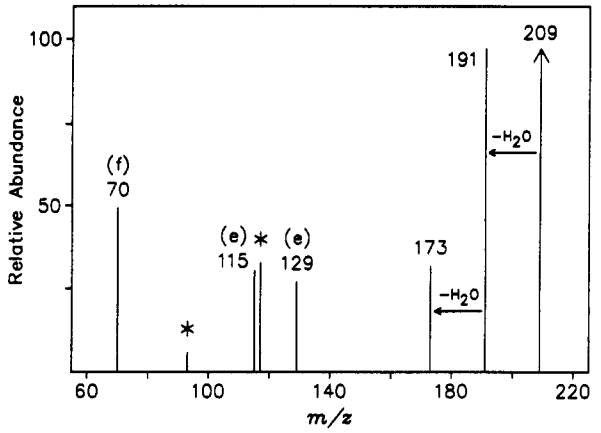


Figure 3. Mass spectrum resulting from CID of the m/z 209 ion produced by FAB ionization of **1a**. Asterisks denote background ions from FAB matrix.

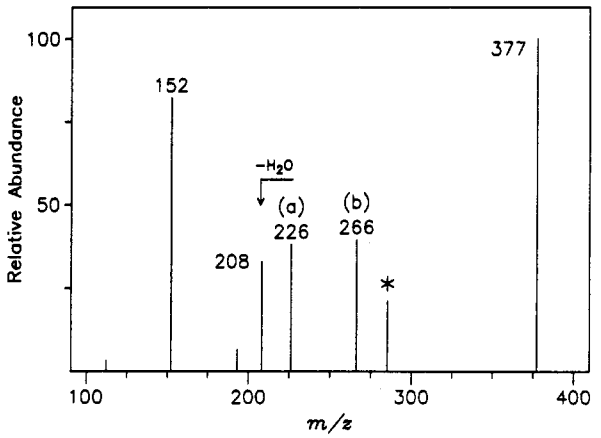


Figure 4. CID mass spectrum of **5** resulting from alkaline hydrolysis of **1**. The assignment for ion (a) is analogous to that shown in Scheme I.

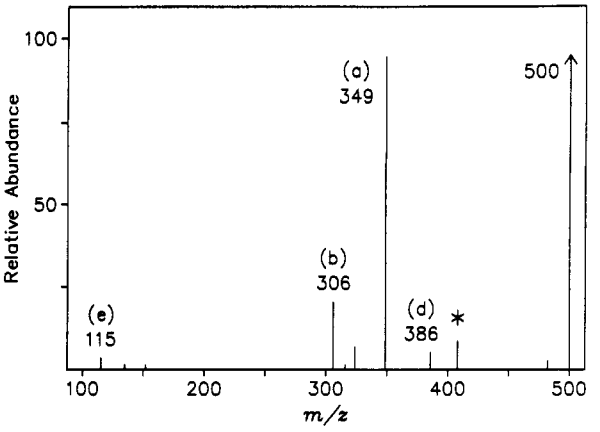


Figure 5. Mass spectrum of **2a**, produced by mass selection and collision-induced dissociation of m/z 500 ions from the mixture represented in Figure 1.

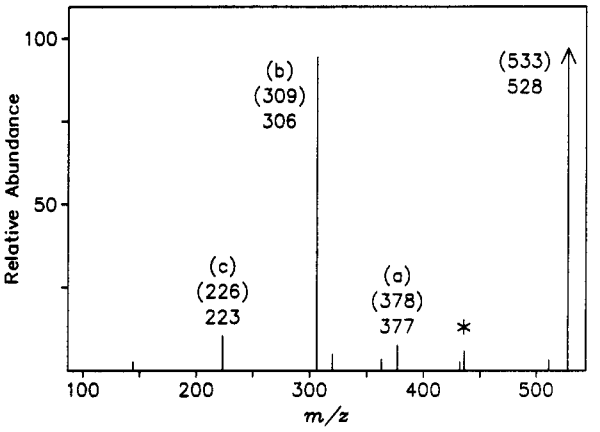


Figure 6. Mass spectrum of **3a**, produced by mass selection and collision-induced dissociation of m/z 528 ions shown in Figure 1. Values in parentheses indicate corresponding mass values from a separate experiment in which deuterium change of **3a** was carried out in 3-nitrobenzyl alcohol-*O*-*d* FAB matrix.

weight (requiring an even number of nitrogens) implies that **5** contains a P–N bond, including only one of the side-chain nitrogen atoms but none of the carbons of proline. This interpretation is supported by the CID mass spectrum of **5** (Figure 4) which shows the presence of 8-oxoadenosine (ions b and m/z 152) and by difference the CH_5NO_3P moiety substituted at C-5′.

Attachment of the proline residue through an amide linkage is shown by the formation of ion f in Figure 3. Alternative structures such as **11** or **12** require a 16 u higher molecular weight than is observed and would be expected to produce ions analogous to f also at higher mass values. In addition, for **11** a larger number of exchangeable hydrogen atoms in the side chain of **1a** would be required (three) than is determined (two).

Homologs and Isomer of Phosmidosine. The initial assumption that ions of m/z 500 and 528 in Figure 1 represent homologs of phosmidosine, each differing by one methyl group, was conclusively established by the following experiments. (i) Mass shifts from the deuterium exchange FAB method showed the presence of six exchangeable hydrogen atoms in **2a** (MD^+, m/z 507) and four in **3a** (MD^+, m/z 533) as required for CH_3 versus H substitution on oxygen or nitrogen, relative to **1a** which contains five active hydrogens. (ii) CID mass spectra of m/z 500 (Figure 5) and 528 ions (Figure 6), acquired directly from the mixture represented in Figure 1, showed clear assignments analogous to the dissociation products of **1a**, bearing one fewer and one more methyl group, respectively, in the C-5′ side chain. In both mass spectra, ion b shows the nucleoside moiety to be unchanged from **1a**. In Figure 5, ions a, d and e all occur at mass values required for absence of side-chain methylation. In Figure 6, ions a and c indicate the presence of two methyl groups, but ions of type e and f which could be used to assign specific sites of methylation are not formed. Deuterium shift data indicated in Figure 6 confirm the methylation assignments, and the 3-u shift of m/z 223 requires that both methyls reside on N or O rather than on carbon. Determination of the sites of methylation in **3a** was made by directly-combined thermospray HPLC-mass spectrometry (LC/MS), discussed below.

While the dissociation products of the m/z 514 ion population show only the intact C-5′ side chain as m/z 209 (Figure 2a), CID of the 209-u side-chain ion carried out in a separate experiment (Figure 3) suggests the additional presence of an isomer of **1** in which the methyl group is located in the prolylamide moiety. Thus, the mass spectrum in Figure 3 exhibits a peak corresponding to cleavage e at m/z 129, which would be a homolog of the m/z 115 species derived from **1a**. Additional evidence from the corresponding ion of type f which would occur at m/z 84 is not observed. Because both e-type ions are products of monomethyl side chains of the same mass (209 u) they

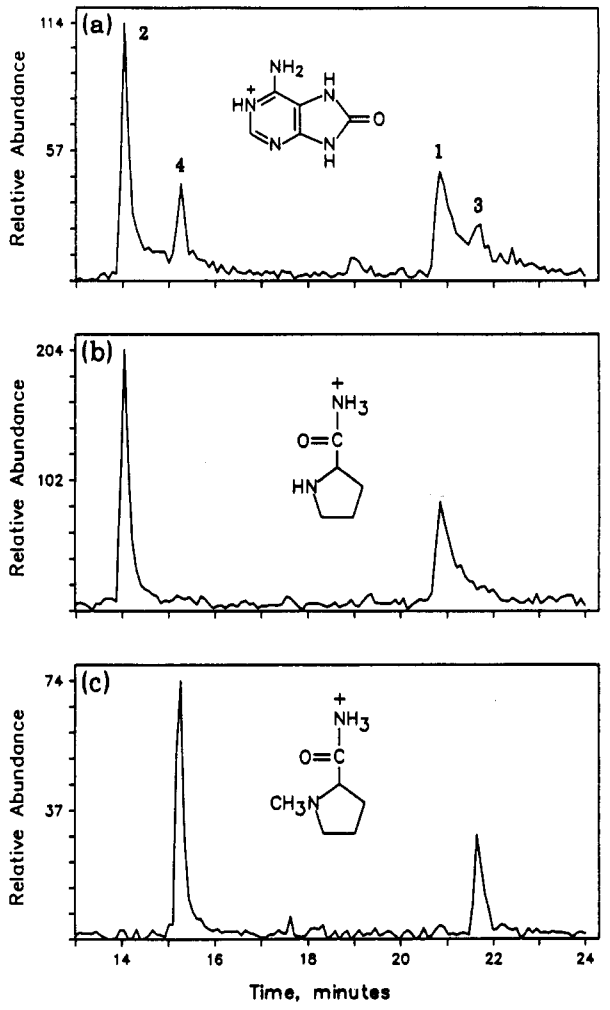


Figure 7. Reconstructed ion chromatograms from thermospray LC/MS of crude phosmidosine isolate: (a) *m/z* 152, (b) *m/z* 115, (c) *m/z* 129.

cannot be determined from the MS/MS experiment to originate from structurally different precursors (**1a** and **4a**).

To address this ambiguity, the crude phosmidosine isolate was examined by LC/MS, permitting side-chain ion signals to be distinguished on the basis of differing chromatographic elution times. Chromatograms in Figure 7 were derived from full-scan mass spectral data and those in Figure 8 from selected ion recordings. In both figures, chromatographic alignment is made relative to *m/z* 152, the protonated 8-oxoadenine ion, which represents a structural feature common to all components. The assignment of elution order (as shown in Figure 7a) was made on the basis of MH^+ responses (data not shown); **1** and **4** which are isomers having the same MH^+ values were distinguished on the basis of absence of side-chain methylation in **1** (Figure 7b, 20.8 min.; Figure 8b, 21.6 min.). Figure 7 shows ion profile chromatograms for ions of type e (*m/z* 115 and 129) representing the prolylamide moiety. The results in panels b and c clearly indicate that the *m/z* 115 and 129 signals are associated with different components: nonmethylated prolylamide with **1** (20.9 min) and monomethylprolylamide with **4** (15.2 min). Further resolution as to the site of methylation in the prolylamide moiety of **4** was obtained by selected ion monitoring of the pyrrolidine ions of *m/z* 70 and 84 (f, Scheme I). The resulting ion chromatograms, Figure 8, show ring meth-

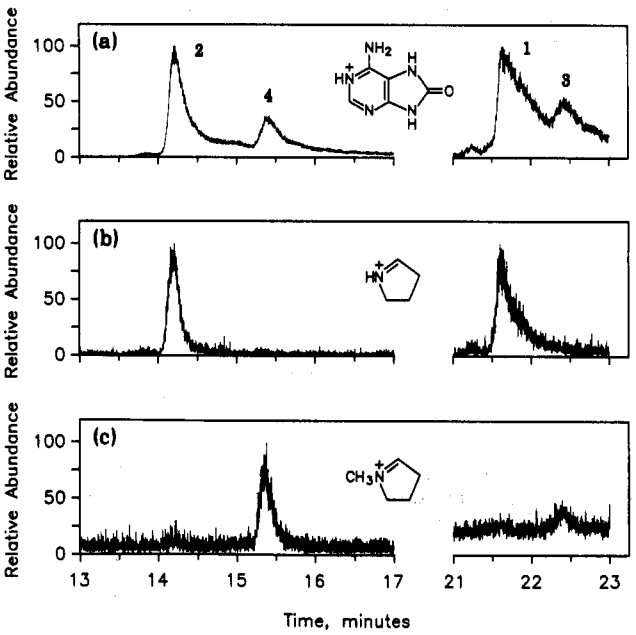


Figure 8. Selected ion recordings from thermospray LC/MS of crude phosmidosine isolate, showing pyrrolidinyl moiety signals associated with **1**–**4**. Left- and right-side panels were derived from separate experiments: (a) *m/z* 152, (b) *m/z* 70, (c) *m/z* 84.

ylation in **4** (panel c, 15.4 min), but not in **1** (panel b, 21.6 min), the latter in accord with mass spectral and NMR data concerning structure **1** as previously discussed. The structure of the phosmidosine isomer is therefore formulated as **4**.

LC/MS data in Figures 7 and 8 further confirm the conclusion that the prolylamide moiety in **2** is unmethylated and in addition defines the location of methyl groups in the molecular weight 527 homolog as shown in structure **3a**. Both methyl groups in **3** are confined to the C-5′ side chain and are on N or O as shown by the data in Figure 6. The *N*-methylpyrrolidinyl signal at 22.4 min in Figure 8c, and corresponding absence of a dimethylprolylamide signal (m/z 143) at the elution point of **3** in Figure 7 (data not shown), requires that the second methyl occurs as a phosphate methoxyl, as in phosmidosine **1**.

Experimental Section

Isolation of phosmidosine (**1**) from *S. durhameusis* was described earlier.[1] FAB-MS: MH^+ 474.1493 found, 474.1502 calcd for $C_{16}H_{25}N_7O_8P$. UV: (H_2O) nm 255 sh (12 300) and 269 (14 200); (0.1 N HCl) 264 (12 300) and 280 (11 350); (0.1 N NaOH) 280 (15 600). Chemical shifts from NMR spectra are reported in ppm and are referenced to TSP in D_2O for ^1H and to H_3PO_4 for ^{31}P.

Conventional FAB and collision-induced mass spectra were acquired using a VG 70-SEQ instrument (data in Figures 2–6), consisting of a double-focusing magnetic sector mass analyzer (MS-1), rf-only quadrupole gas collision cell, and quadrupole mass analyzer (MS-2), controlled by a VG-11/250J data system. The spectrum shown in Figure 1 was obtained using a MAT 731 mass spectrometer. Samples were dissolved in glycerol (data in Figures 1, 2a, 3–6) or 3-nitrobenzyl alcohol-*O*-*d* (Figures 2b, 6) and ionized by FAB using 7.2 kV Xe atoms. CID mass spectra were acquired at 1–2 unit mass resolution produced using 13–40 eV translational energy (lab frame) at Kr collision gas pressure corresponding to 25–40% beam transmission.

Directly combined HPLC mass spectrometry (LC/MS) experiments (data in Figures 7, 8) were carried out using a Vestec 201 instrument (Houston, TX) controlled by a Teknivent Corp. (St. Louis, MO) Vector/One data system. The mass spectrometer was interfaced through a Vestec thermospray ion source to a

Beckman 126 liquid chromatograph, with Supelcosil LC-18S column (4.6 × 250 mm) and Brownlee Spheri-5 C_{18} precolumn (3 cm). A HPLC gradient elution system similar to that of Buck et al.[22] was used; further details of the LC/MS experimental procedures have been described.[23]

GC/MS measurements (table in supplementary material) were made using a VG 70-SEQ instrument operated at 70 eV ionizing energy (EI), with Hewlett-Packard 5890 gas chromatograph and Pierce (Rockford, IL) SE-30 capillary column (15 m × 0.32 mm) temperature programmed at 10 °C/min from 120 to 300 °C.

Sample Preparation for Mass Spectrometry. Samples for FAB ionization were dissolved in glycerol (data in Figures 1, 2a, 3–6), 3-nitrobenzyl alcohol-*O*-*d* (NBAD), or glycerol-*O*-d_3 (Cambridge Isotope Laboratories, Woburn, MA) (Figures 2b, 6). NBAD was prepared by a D_2O-exchange method similar to that previously reported.[24] Details of the procedure for deuterium exchange in labeled FAB matrix solvents for the determination of exchangeable hydrogen atoms were earlier described.[25,26]

O-Isopropylidine derivatives were prepared by reaction of 1–5 μg of phosmidosine isolate with 30 μL of 2,2-dimethoxypropane and 1 μL of concd HCl for 5 min with continuous stirring and then dried thoroughly in a stream of N_2, a step found to be necessary for high reaction yield (estimated as 95%). Following additional overnight drying under vacuum, the sample was dissolved in H_2O for transfer to the FAB probe tip.

Trimethylsilyl derivatives of the product of alkaline hydrolysis (procedure B) of 1, and of 8-oxoadenosine, were prepared by dissolving of 5 μg of vacuum-dried material in 10 μL of pyridine, followed by addition of 20 μL of premixed solution (Pierce, Rockford, IL) consisting of *N*,*O*-bis(trimethylsilyl)acetamide with 1% trimethylchlorosilane. The mixture was sealed in a glass tube, heated at 100 °C for 1 h, and opened immediately before GC/MS analysis.[5]

Acid and Base Hydrolysis of 1. Hydrolysis experiments were carried out on 1 isolated from a crude culture filtrate as previously described.[1] Acidic hydrolysis (6 N HCl, 110 °C 16 h) produced 8-oxoadenine (EI-MS, M^+ 159.0495 found, 151.0493 calcd for $C_5H_5N_5O$. UV: $\lambda_{max}^{H^+}$ 265, 280 nm, $\lambda_{max}^{OH^-}$ 279 nm. ^1H NMR: δ_H (8.03) and L-proline (ORD $[\phi]_{203}$ −3849, H_2O).

Alkaline hydrolysis (procedure A: 0.2 N NaOH, 100 °C 1 h) produced **5** and rearranged isomer **6**.[5] The ^1H NMR spectrum of **5** (FAB-MS, MH^+ 377) in D_2O showed a methyl doublet at δ_H 3.59 (J = 11.3 Hz) coupled with phosphorus, but no signals from the prolyl moiety were observed. In the ^{13}C NMR spectrum, three doublets of ^{13}C signals coupled with phosphorus were observed: δ_C 55.3 ($POCH_3$, J_{CP} = 6.1 Hz), 67.5 (C-5, J_{CP} = 5.9 Hz), 84.9 (C-4′ J_{CP} = 8.8 Hz).

Treatment of 1 by 0.5 N NaOH, 100 °C, 2 h (procedure B) produced one principal product, isolated by HPLC and characterized as 8-oxoadenosine. FAB-MS: MH^+ 284. ^1H NMR: δ_H 8.04 (H-2, s), 5.86 (H-1′, d, J = 6.8 Hz), 4.95 (H-2′ dd, J = 5.7, 6.8 Hz), 4.39 (H-3′, dd, J = 2.8, 5.7 Hz), 4.18 (H-4′ m), 3.84 (H-5′a, dd, J = 2.8, 12.6 Hz), 3.75 (H-5′b, dd, J = 3.6, 12.6 Hz). CD: $[\theta]_{284}$ = +339, $[\theta]_{275}$ = +962, $[\theta]_{263}$ = −735 in H_2O.

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Supplementary Material Available: Chromatogram and FAB mass spectrum of culture isolate, characterization data for **6**, CID mass spectra of phosmidosine base, guanine, isoguanine, **1**, **6**, and *m/z* 306 ion from **1a**, and EI mass spectra of 8-oxoadenosine and the alkaline hydrolysis product of 1 (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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