before administration, but the ethanol solution was directly added to the culture. Less than 1 mL of medium and 0.1 mL of ethanol solution were used for administration into each flask at one time.

In feeding experiments with labeled acetate, a mixture of 50 mg of sodium [1-^{13}C]- or [2-^{13}C]acetate (99 atom % ^{13}C, Sigma) and 50 mg of unlabeled sodium acetate was added twice to each 500-mL flask containing 100 mL of the medium at the 24th and 48th hours of cultivation. After workup, this afforded 1.87 and 188 mg of **3a** from 15 × 100 mL broths in the experiments with sodium [1-^{13}C]- and [2-^{13}C]-acetate, respectively. For labeled isovalerate, 2.5 mg of sodium [1-^{13}C]isovalerate was fed to the culture four times (each 2-h interval from 24 to 30 h of cultivation) and again four times (from 48 to 54 h of cultivation); 0.33 mg of **3a** was obtained from 5 × 100 mL broths.

In feeding experiments with ^{13}C- and ^{2}H-labeled glycerol, the modified production medium was used for cultivation. Fifty milligrams of [1,3-$^{13}C_2$]glycerol was added twice to the culture at the 24th and 48th hours of cultivation, and 0.062 mg of **3a** was obtained from 5 × 100 mL broths. CI-MS of this labeled **3a**: *m/z* (rel intensity as that of *m/z* 439 $(M + H)^+$ is 100) 439 (100), 440 (38.7), 441 (14.6); that of natural **3a** 439 (100), 440 (30.2), 441 (5.9). From the MS data, the relative abundance of non-, mono-, and di-^{13}C-labeled molecules in the labeled **3a** was calibrated as 100:8.5:6.1,[19] indicating that the mono- and dilabeled forms increased by 7.4% and 5.3%, respectively. In the feeding experiment with [$^{2}H_5$]glycerol (98 atom % ^{2}H, CEA), 2.5 mg of the labeled glycerol was fed to the culture eight times in a manner similar to that of labeled isovalerate, and 15 mg of **3a** was obtained from 250 × 100 mL broths.

Doubly labeled β-keto acid or its derivative was fed to the culture eight times in the same manner as above. After workup, this afforded 0.18, 0.56, and 0.37 mg of **3a** from 5 × 100, 10 × 100, and 8 × 100 mL broths in feeding experiments in which each flask received at one time 1.25 mg of [2,3-$^{13}C_2$]-3-oxo-7-methyloctanoic acid, 1.1 mg of methyl [2,3-$^{13}C_2$]-3-oxo-7-methyloctanoate, and 1.2 mg of [2,3-$^{13}C_2$]-3-oxo-7-methyloctanoic acid *N*-acetylcysteamine thioester, respectively.

In feeding experiments with [4,5-^{2}H]-6-dehydro-VB A, 1.0 or 10 mg of the labeled compound was added to the culture eight times as above, and 0.48 and 2.84 mg of **3a** were obtained from each 2 × 100 mL broth, respectively.

Acknowledgment. We thank Professor A. L. Demain of the Massachusetts Institute of Technology for his critical reading of the manuscript. This work was supported by a Grant-in-Aid for scientific research from the Ministry of Education, Science and Culture and by a grant from the Naito Foundation.

Registry No. 3, 109215-47-6; **3a**, 135304-75-5; [4,5-^{2}H]-**3a**, 137570-41-3; **11**, 137570-40-2; **12**, 122922-47-8; glycerol, 56-81-5; isovaleric acid, 503-74-2; [1-^{13}C]isovaleric acid, 87994-84-1; [1,3-$^{13}C_2$]glycerol, 102088-01-7; [1-^{13}C]-5-methylhexanenitrile, 137570-37-7; methyl [2,3-$^{13}C_2$]-3-oxo-7-methyl loctanoate, 137570-38-8; [2,3-$^{13}C_2$]-3-oxo-7-methyloctanoic acid, 137570-39-9.

(19) Caprioli, R. M. *Biochemical Applications of Mass Spectrometry*; Wiley: New York, 1972; pp 735-776.

A Corrected Structure for Pyrrolosine

Brian A. Otter,*,† Shirish A. Patil,† Robert S. Klein,† and Steven E. Ealick‡

Contribution from the Medicinal Chemistry Laboratory, Department of Oncology, Montefiore Medical Center, 111 East 210th Street, Bronx, New York 10467, and Center for Macromolecular Crystallography, University of Alabama at Birmingham, Birmingham, Alabama 35294.
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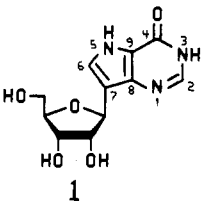
Abstract: Pyrrolosine, a C-nucleoside recently isolated from *Streptomyces albus*, was assigned structure **1** by Ikegami and co-workers largely on the basis of single-crystal X-ray diffraction. This is also the structure previously assigned to the synthetic C-nucleoside, 9-deazainosine. Because the physical and biological properties of the two compounds differ, Ikegami and co-workers suggested that the structure of 9-deazainosine should be reinvestigated. However, it is shown in this paper that the structure they proposed for pyrrolosine is incorrect. Furthermore, on the basis of the reported physical properties, it is shown that pyrrolosine is actually the known furo[3,2-*d*]pyrimidine C-nucleoside adenosine analogue **2**. This conclusion, which is more consistent with the types of biological activity reported for pyrrolosine, has now been confirmed by reanalysis of the X-ray data published by Ikegami and co-workers. All bond angles and distances in the reinterpreted model are consistent with structure **2** for pyrrolosine. The model also accounts more adequately for the hydrogen atoms of the base and the methanol of solvation and for all potential hydrogen bond donors. After the appropriate changes were made, the crystallographic *R* factor decreased from the reported value of 0.065 to 0.039. X-ray crystallographic data as well as additional UV and NMR studies have been used to reaffirm that 9-deazainosine is indeed the pyrrolo[3,2-*d*]pyrimidin-4-one **1**.

Pyrrolosine, a new inhibitor of starfish RNA synthesis, was isolated recently from the culture broth of *Streptomyces albus* by Ikegami and co-workers.[1] Based principally on their interpretation of single-crystal X-ray diffraction data, these investigators concluded that the structure of pyrrolosine is 1,5-dihydro-7-β-D-ribofuranosyl-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**1**). This is also the structure assigned to 9-deazainosine, a synthetic C-nucleoside that we originally reported in 1980.[2] Since a comparison of the physical and biological properties revealed that the two substances are different, Ikegami and co-workers suggested that the chemical structure of our "reputed" 9-deazainosine should be promptly reinvestigated. In fact the structure of 9-deazainosine already rests solidly on an unambiguous synthetic route,[2,3] on



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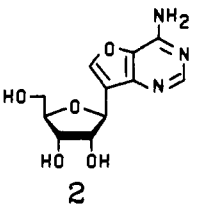

2

convincing spectroscopic evidence,[2,3] and on a substantial body of chemical transformations.[4] However, in view of the important

† Montefiore Medical Center.
‡ University of Alabama.

(1) Ikegami, S.; Isomura, H.; Tsuchimori, N.; Osano, Y. T.; Hayase, T.; Yugami, T.; Ohkishi, H.; Matsuzaki, T. *J. Am. Chem. Soc.* **1990**, *112*, 9668-9669.
(2) Lim, M.-I.; Klein, R. S.; Fox, J. J. *Tetrahedron Lett.* **1980**, *21*, 1013-1016.

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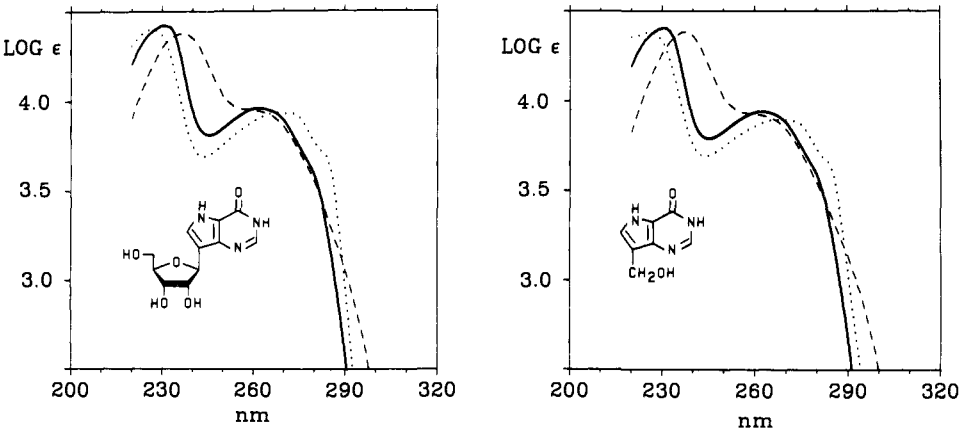

Figure 1. Comparison of UV spectra of 9-deazainosine and a model pyrrolo[3,2-*d*]pyrimidin-4-one (both 1.33×10^{-4} M) in water (solid lines), at pH 1 (dashed lines), and at pH 13 (dotted lines).

biological properties exhibited by 9-deazainosine,[5] we have carried out some additional studies, and we reaffirm in this paper that 9-deazainosine is indeed structure **1**. We also present evidence to show that pyrrolosine is the isomeric C-nucleoside adenosine analogue **2**.

The physical properties of the pyrrolo[3,2-*d*]pyrimidine ring system have been investigated rather extensively, and detailed UV spectral studies, for example, were reported by Imai[6a] as early as 1964. A comparison (Figure 1) of the UV spectra of 9-deazainosine and the model 7-(hydroxymethyl)pyrrolo[3,2-*d*]pyrimidin-4-one base[7] provides compelling evidence that these two compounds contain the same chromophore. Evidence for the identity of this chromophore is made even more conclusive by the fact that the two compounds were synthesized via fundamentally different routes. The spectra in Figure 1 are virtually superimposable on those of the parent base—9-deazahypoxanthine—published by Imai.[6a,b] In contrast, the UV spectral data reported for pyrrolosine[1] differ substantially from the spectra of known pyrrolo[3,2-*d*]pyrimidin-4-ones; we shall return to this point in later discussion.

Equally compelling evidence for the pyrrolo[3,2-*d*]pyrimidine structure of 9-deazainosine comes from NMR studies. Conventional 1D 200 MHz proton spectra of 9-deazainosine are first-order, and they are entirely in accord with structure **1**. For example, resonances from all of the exchangeable protons are seen in DMSO-d_6 (28 °C), where N(3)-H (δ 11.92) is coupled to H-2 (δ 7.81, J = 2.4 Hz), and N(5)-H (δ 12.00) is coupled to H-6 (δ 7.38, J = 2.8 Hz). In the ^{13}C NMR spectrum, the magnitudes of the one-bond coupling constants are also diagnostic for structure **1** because the values $^1J_{C6,H6}$ = 187 Hz and $^1J_{C2,H2}$ = 207 Hz are precisely those expected for sp^2 carbons flanked, respectively, by one and two nitrogen atoms.[8] No comparable information about exchangeable protons or the size of the one-bond coupling constants has been reported for pyrrolosine.[1]

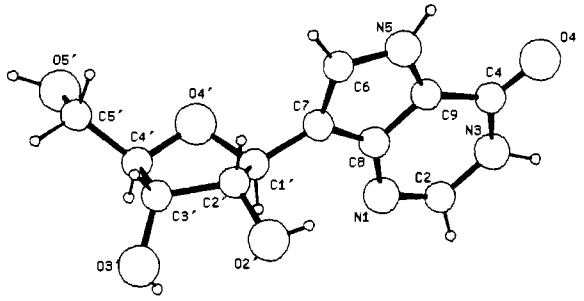

Figure 2. Computer-generated perspective drawing of 9-deazainosine.

Additional evidence for the structure of 9-deazainosine, albeit of an indirect nature, concerns its lack of toxicity toward mammalian cells. This is the pattern normally seen for inosine analogues.[9] Pyrrolosine, on the other hand, is *extremely* toxic to mammalian cells,[1] which is atypical of inosine analogues in general.

The information presented above, together with that published previously,[2-4] would ordinarily be considered sufficient to establish that 9-deazainosine is structure **1**. However, that conclusion implies that the X-ray-based structure reported[1] for pyrrolosine must be incorrect, which goes against the commonly held view that X-ray crystallographic data are the definitive authority in matters of structural assignments. Consequently, we have subjected 9-deazainosine itself to single-crystal X-ray diffraction, using material obtained by crystallization from 90% ethanol. The resulting needles belong to the $P2_1$ space group, with a = 10.801 (2) Å, b = 4.997 (1) Å, c = 11.474 (2) Å, β = 96.73 (2)°, and Z = 2. Intensities were measured using ω–2θ scans with an Enraf-Nonius CAD-4 diffractometer using Cu Kα radiation and a graphite monochromator (λ = 1.5418 Å). Corrections were made for Lorentz and polarization factors and for X-ray absorption (μ = 9.422 cm^{-1}). A total of 1424 independent intensities were measured with $2\theta < 150$; 41 reflections had net intensities of <0 and were omitted from all further calculation.

The structure was determined by direct methods using the program MULTAN80.[10] All hydrogen atom positions and a water molecule of solvation were located using difference Fourier maps. The structure was refined using full-matrix least-squares techniques in which non-hydrogen atoms were given anisotropic temperature factors, and hydrogen atoms were assigned a fixed isotropic temperature factor equal to 1.5 times the equivalent isotropic temperature factor of the atom to which it was attached.

(3) Lim, M.-I.; Ren, W.-Y.; Otter, B. A.; Klein, R. S. *J. Org. Chem.* **1983**, *48*, 780–788. 9-Deazainosine was reported in refs 2 and 3 as the hydrochloride salt. The crystalline free base (monohydrate from 90% ethanol; mp 238–244 °C dec, with prior softening) was used for the present study.

(4) Published examples include the synthesis of 5′-tritiated 9-deazainosine via an oxidation–reduction process (Singh, A. K.; Klein, R. S. *J. Labelled Compd. Radiopharm.* **1988**, *25*, 1220–1228) and the synthesis of the 5′-iodo derivative as an inhibitor of purine nucleoside phosphorylase (Stoeckler, J. D.; Ryden, J. B.; Parks, R. E.; Chu, M.-Y.; Lim, M.-I.; Ren, W.-Y.; Klein, R. S. *Cancer Res.* **1986**, *46*, 1774–1778). In all cases, the spectral properties of the intermediates and final products, as well as an X-ray determination of the 5′-iodo compound (S. E. Ealick, unpublished results), are entirely consistent with the pyrrolo[3,2-*d*]pyrimidin-4-one structure.

(5) 9-Deazainosine inhibits the growth of *Trypanosoma* sp., *Leishmania* sp., and *Pneumocystis carinii* (see ref 13c and references therein). More recently, in vitro activity against *Cryptosporidium* has been observed (Soave, R.; Tong, W. P.; Harrison, E. H.; Bernard, E.; Armstrong, D. *Clinical Res.* **1990**, *38*(2), 269A).

(6) (a) Imai, K. *Chem. Pharm. Bull.* **1964**, *12*, 1030–1042. (b) This traditional methodology of comparing UVs was in fact used in our original proof of structure of 9-deazainosine, as we stated in ref 3.

(7) Ektova, L. V.; Tolkachev, V. N.; Sizova, O. S.; Nikolaeva, T. G.; Pilipenko, T. V.; Dobrynin, Ya. V.; Preobrazhenskaya, M. N. *Bioorg. Khim.* **1985**, *11*, 1105–1109.

(8) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH Publishers: Weinheim, Germany, 1987; p 288.

(9) See ref 13c and references therein.

(10) Main, P.; Fiske, S. J.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woodfson, M. M. *MULTAN80, A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data*; Universities of York, England, and Louvain, Belgium, 1980.

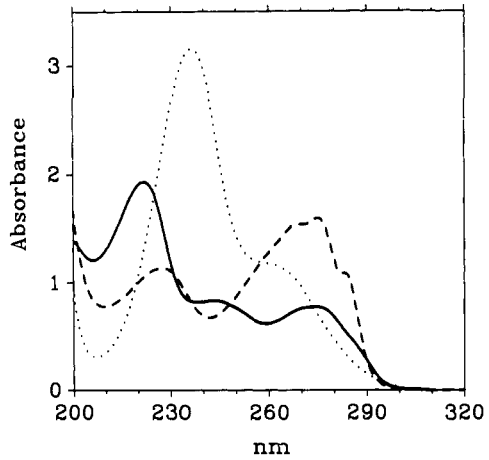

Figure 3. UV spectrum of synthetic **2** (1.33×10^{-4} M) in water (solid line) and at pH 1 (dashed line). The spectrum of an equimolar solution of 9-deazainosine at pH 1 (dotted line) is included for comparison.

The final R factor for 1383 observations and 226 variables was 0.042, and the goodness-of-fit was 1.35. All crystallographic calculations were carried out using the Enraf-Nonius Structure Determination Package.[11] The computer-generated model of 9-deazainosine shown in Figure 2 clearly confirms the structural conclusions already reached on the basis of UV and NMR spectroscopy. The model further indicates that the glycosyl torsion angle χ (O4′–C1′–C7–C8) is in the anti range[12] with a value of −116.7° and that the sugar puckering is the nonstandard O4′-endo–C1′-exo.

With the structure of 9-deazainosine established beyond question, we can now consider possible structures for pyrrolosine. The compound is evidently a ribosyl-C-nucleoside of formula $C_{11}H_{13}N_3O_5$ from the MS and ^{13}C NMR data obtained by Ikegami and co-workers,[1] and it seems reasonable to suppose that it is a positional isomer of 9-deazainosine, perhaps involving an interchange of some of the oxygen and nitrogen atoms. In fact, the large differences in the reported UV spectra of the neutral and protonated forms strongly suggest the presence of a readily protonated amidine structure rather than an amide. Of several possibilities, it appeared that the furo[3,2-*d*]pyrimidine C-nucleoside **2** could be a likely candidate. Ironically, we ourselves reported[13] the synthesis of the monohydrochloride salt of this compound in 1986. Using a newly prepared sample of the free base in order to facilitate comparison with the data obtained by Ikegami and co-workers, we found the UV spectra (Figure 3) of **2** at pH 7 and pH 1 to be strikingly similar to those reported for pyrrolosine, even down to the fine structure described in the spectrum of the protonated species.[1] Figure 3 also serves to illustrate the considerable differences between the UV spectra of pyrrolosine and a known pyrrolo[3,2-*d*]pyrimidin-4-one, namely 9-deazainosine.

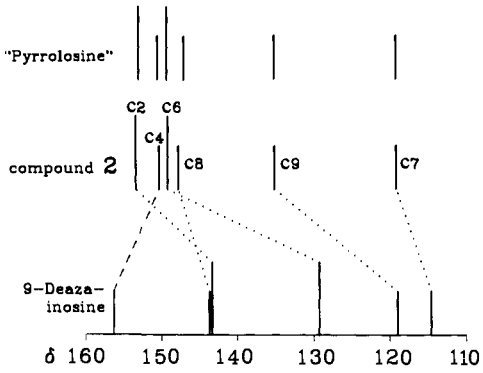

Figure 4. Comparison of ^{13}C chemical shifts in D_2O. Because of its limited solubility, the spectrum of 9-deazainosine was obtained at 45 °C. See footnote 15 for additional details.

The conclusion from the UV data that pyrrolosine is probably structure **2** receives strong support from the ^1H and ^{13}C NMR[14,15] spectra. In each case, the values found for synthetic **2** are in satisfactory agreement with those reported for pyrrolosine.[1] The close similarity of the chemical shifts of the heterocyclic base carbons of **2** and pyrrolosine is illustrated in Figure 4, along with comparable data for 9-deazainosine. The large downfield shifts of C6 and C9 noted for **2** (and pyrrolosine) relative to 9-deazainosine are comparable to the chemical shift differences seen between 2,3-benzofuran and indole,[8] and they add further weight to the structural assignments.

The UV and NMR evidence presented above establishes the identity of pyrrolosine as 7-β-D-ribofuranosyl-4-aminofuro[3,2-*d*]pyrimidine (**2**). It should be noted that its structural similarity to adenosine (as opposed to inosine) is consistent with the pronounced in vitro cytotoxicity observed by Ikegami and co-workers[1] against human fibroblast KMST-6 and mouse mammary carcinoma FM3A cells, where the IC_{50} values are in the ng/mL range. We had earlier reported similarly low IC_{50} values for synthetic **2** against mouse P815 and L1210 leukemia cells in vitro.[13]

It remains to consider whether the X-ray diffraction data reported for pyrrolosine can be reinterpreted in light of the corrected structure. Certainly, the final atomic coordinates and bond distances published as supplementary material to the paper of Ikegami and co-workers[1] contain some curious features that should have argued against a pyrrolo[3,2-*d*]pyrimidin-4-one structure for pyrrolosine. These features include the apparently single-

(11) Frenz, B. S.; Okaya, Y. *Enraf-Nonius Structure Determination Package*; Enraf-Nonius, Delft, Holland, 1980.

(12) Although 9-deazainosine adopts the anti conformation in the crystalline state, the compound exists in solution in DMSO-d_6 predominantly in the syn form, which is apparently stabilized by an intramolecular 5′-OH···N(1) hydrogen bond. Such a hydrogen bond affects rotation about the C5′–O5′ bond, and the 5′-OH resonance of **1** consequently appears (28 °C) as a sharp doublet of doublets (δ 5.25) with $J_{5'S,OH}$ = 4.2 and $J_{5'R,OH}$ = 8.3 Hz. The 5′-OH resonance would have appeared as a triplet if the couplings were averaged by free rotation, which is the situation usually seen for nucleosides. Additional evidence that the 5′-hydroxyl proton is close to the pyrimidine ring comes from NOE difference spectroscopy, where preirradiation (5 s) of 5′-OH produces a 5% enhancement at H-2 (allowing for an additional small NOE to H-2 that results from partial transfer of saturation to N(3)-H). Also, the 8% enhancement seen at H-1′ on preirradiation of H-6 indicates a substantial population of syn conformers for **1** in DMSO-d_6. In the same experiment, H-2′ is enhanced by only 1%. An analogous intramolecular hydrogen bond between the hydroxymethyl group and the base has been observed previously with purine nucleosides in solution (where it involves the purine N-3), but only in highly substituted derivatives and in much less polar solvents. For an example, see: Plochocka, D.; Rabczenko, A.; Davies, D. B. *Biochim. Biophys. Acta* **1977**, *476*, 1–15.

(13) (a) Bhattacharya, B. K.; Lim, M.-I.; Otter, B. A.; Klein, R. S. *Tetrahedron Lett.* **1986**, *27*, 815–818. (b) Bhattacharya, B. K.; Lim, M.-I.; Otter, B. A.; Klein, R. S. 191st National Meeting of the American Chemical Society, New York, NY, April 1986; American Chemical Society: Washington, DC, 1986; CARB 005. (c) Bhattacharya, B. K.; Otter, B. A.; Berens, R. L.; Klein, R. S. *Nucleosides Nucleotides* **1990**, *9*, 1021–1043.

(14) ^1H NMR data for **2** (free base) in D_2O (23 °C, 200 MHz, reference standard = $(CH_3)_3SiCD_2CD_2COONa$ (TSP), all resonances integrate for 1 H): δ 8.19 (s, H-2), 8.07 (s, H-6), 5.02 (d, H-1′), 4.49 (dd, H-2′), 4.33 (dd, H-3′), 4.21 (m, H-4′), 3.91 (dd, H-5′), 3.81 (dd, H-5″), mean δ for 5′ and 5″ is 3.86 ppm; $J_{1',2'}$ = 7.3, $J_{2',3'}$ = 5.2, $J_{3',4'}$ = 3.1, $J_{4',5'}$ = 2.7, $J_{4',5''}$ = 3.4, $J_{5',5''}$ = 12.8 Hz. For comparison, the proton chemical shifts reported[1] for pyrrolosine in D_2O (temperature and reference standard unstated) are δ 8.15, 8.02, 5.04, 4.52, 4.32, 4.22 and 3.84 ppm. Ikegami et al.[1] reported only a few of the coupling constants for pyrrolosine. We assume that the value of $J_{2',3'}$ = 7.2 Hz implied in their report is an error since it exceeds the range of values generally seen for ribosyl nucleosides. In DMSO-d_6 solution, the NH_2 resonance of **2** appears as a two-proton singlet (D_2O-exchangeable) at δ 7.46.

(15) ^{13}C NMR data for **2** (free base) in D_2O (24 °C, 125 MHz, values in parentheses following the peak assignments are the differences between the observed shifts and those reported[1] for pyrrolosine): δ 153.4 (C2, 0.3), 150.3 (C4, −0.3), 149.2 (C6, −0.2) 147.8 (C8, 0.6), 135.2 (C9, −0.1), 119.2 (C7, −0.1), 86.2 (C4′, 0.0), 76.4 (C1′, 0.0), 75.5 (C2′, −0.1), 72.7 (C3′, 0.0), 62.9 (C5′, 0.0). The small differences in the chemical shifts presumably reflect the fact that the pyrrolosine obtained by Ikegami and co-workers was solvated with methanol, whereas our sample of **2** was not. Since the reference standard used by Ikegami et al. was not specified, C-5′ in **2** was set to 62.9 ppm (the value they found for C-5′ of pyrrolosine), which gives a shift for internal TSP of −2.08 ppm. In the spectrum of 9-deazainosine shown in Figure 4, TSP was set to −2.08 ppm in order to allow direct comparison with **2**. It should be noted that the $^1J_{C6,H6}$ value of 207 Hz found for **2** is consistent[8] with C6 being adjacent to an oxygen atom (cf. 9-deazainosine).

bonded nature of the 4-substituent—which means that if pyrrolosine was really **1** it would have to crystallize in the lactim form, which is itself improbable. The same data also indicate that the atom at position 5 does not serve as a hydrogen bond donor since the closest acceptor is more than 3.5 Å away. Such a feature is much more consistent with the existence of an oxygen atom at that position. In our crystal structure of 9-deazainosine, all hydrogen bond donors are accounted for, and a strong hydrogen bond is formed between N(5) and O(5′) of an associated molecule.

Because of the inconsistencies noted above between the crystallographic data and the reported structure of pyrrolosine, we have reanalyzed the X-ray intensity data obtained by Ikegami and co-workers. When the atom at position 5 was changed from N to O and the substituent atom at position 4 was changed from O to N, the crystallographic *R* factor dropped from 0.065 to 0.053. A second hydrogen atom attached to N(4) was located as the largest feature in a difference Fourier map and included in the model. All four hydrogen atoms associated with the solvent methanol were also located in the difference Fourier map and added. Finally, the disordered oxygen atom of the methanol molecule was replaced by a single anisotropic atom. After least-squares refinement, the *R* factor converged at 0.039.

All bond distances and angles in the reinterpreted model are consistent with the structure of the furo[3,2-*d*]pyrimidine C-nucleoside **2**. Furthermore, the hydrogen bonding scheme, which consists of five intermolecular and one intramolecular contact, accounts for all potential hydrogen bond donors.[16] The misinterpretation of their own X-ray data by Ikegami and co-workers in the structural determination of pyrrolosine illustrates the pitfall of relying too heavily on X-ray diffraction techniques without the corroboration of other synthetic and spectroscopic evidence.

The finding that the furo[3,2-*d*]pyrimidine C-nucleoside **2** is a natural product is of considerable interest given the fact that the compound has been obtained by synthesis in only modest overall yield via a demanding eight-step route starting with D-ribose.[13] Its natural occurrence also poses some interesting questions about its benefit to the organism that produces it and about its biosynthetic pathway. However, we suggest that future studies to investigate these questions should use a name other than "pyrrolosine" in order to more accurately reflect the actual structure of the compound.

Acknowledgment. Support of this work by funds from the American Cancer Society (Grants CH-305 and CH-213) and the U.S. Department of Health and Human Services (Grants CA-13330, CA-24634, and GM-38823) is gratefully acknowledged.

Registry No. 1, 89458-19-5; **1**·H_2O, 137541-62-9; **2**, 86132-93-6; **2**·MeOH, 137623-78-0; 7-(hydroxymethyl)pyrrolo[3,2-*d*]pyrimidin-4-one, 104303-72-2.

Supplementary Material Available: Comparative perspective views of 9-deazainosine and "pyrrolosine" (Figure 5), tables of final atomic coordinates, thermal parameters, bond distances, bond angles, torsion angles, and hydrogen bonds for 9-deazainosine (**1**) (Tables 1–6), and corrected atomic coordinates, thermal factors, bond distances, bond angles, torsion angles, and hydrogen bonds for "pyrrolosine" (**2**) (Tables 8–13) (15 pages); listing of observed and calculated structure factors for **1** (Table 7) (7 pages). Ordering information is given on any current masthead page.

(16) The intramolecular contact is a 5′-OH···N(1) hydrogen bond. Coincidentally, the syn conformation of pyrrolosine (**2**) in the crystalline state is similar to that found for 9-deazainosine in solution in DMSO-d_6 (see footnote 12).

Stereochemistry of the Macrolactins

Scott D. Rychnovsky,*,†,1 Donald J. Skalitzky,† Charles Pathirana,‡ Paul R. Jensen,‡ and William Fenical*,‡

Contribution from the Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, and Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093-0236. *Received July 11, 1991*

Abstract: The macrolactins are a group of six 24-membered ring lactones isolated from a taxonomically-undefinable deep sea bacterium. Macrolactin A, the parent aglycone, shows a number of interesting biological activities, including the protection of T-lymphoblast cells against human HIV viral replication. Herein we report the stereochemistries of macrolactin B and macrolactin F, which were determined by a combination of ^{13}C-acetonide analysis using isotopically enriched acetone, oxidative degradation, and chemical correlation. Macrolactins B and F were found to have the same stereochemistry at each of the common stereogenic centers, and so we expect that macrolactin A, the aglycone of macrolactin B, has the stereochemistry 7*S*,13*S*,15*R*,23*R*.

The macrolactins are a group of six 24-membered ring lactones isolated from a taxonomically-undefinable deep sea bacterium.[2] Macrolactin A, the parent aglycone, shows selective antibacterial activity and inhibits B16–F10 murine melanoma cancer cells in in vitro assays.[2] Macrolactin A also shows significant inhibition of mammalian *Herpes simplex* viruses (types I and II) and protects T-lymphoblast cells against human HIV viral replication.[2] The structures of macrolactins A–F were determined by a combination of spectroscopic techniques that included extensive use of proton NMR spectroscopy. The macrolactins are not crystalline, and their derivatives are not suitable for X-ray analysis, so the absolute and relative stereochemistry of the macrolactins have remained undefined.[2] Unfortunately, fermentation of this deep sea bacterium has been unreliable, and macrolactin A is no longer available in significant yield. We turned to macrolactins B and F and report herein their complete stereochemistry, which was determined by a combination of ^{13}C-acetonide analysis, degradation, and chemical correlation (Figure 1).

^{13}C-Acetonide Analysis

The relative stereochemistry of 1,3-diols can be determined by preparing the acetonide derivative and inspecting the ^{13}C chemical shifts of the acetonide methyl groups.[3,4] As shown in Figure 2,

†University of Minnesota.
‡University of California at San Diego.

(1) Camille and Henry Dreyfus Teacher-Scholar, 1990–1995.
(2) Gustafson, K.; Roman, R.; Fenical, W. *J. Am. Chem. Soc.* **1989**, *111*, 7519–7524.

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