A Revised Structure for Sibiromycin

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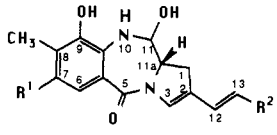
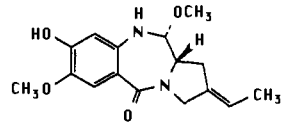
Received December 18, 1987

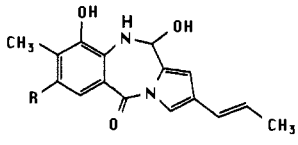
The pyrrolo[1,4]benzodiazepine antibiotics are believed to mediate their antitumor activity at the level of DNA binding.[1] These compounds have been shown to bind to DNA in the minor groove by an unusual mechanism; a key feature includes the formation of a covalent bond with N^2 of guanosine.[2] Also of apparent importance is the asymmetric center at C-11a which confers a right-handed twist to the pyrrolo[1,4]benzodiazepines that matches the curvature of the DNA helix and should thus facilitate binding in the minor groove of DNA. In this context, it is of interest that individual pyrrolo[1,4]benzodiazepines such as anthramycin (**3**) and tomaymycin (**4**) exhibit somewhat different DNA binding patterns,[2d] which may well derive from differences in the magnitude of the right hand twist for each.[3]



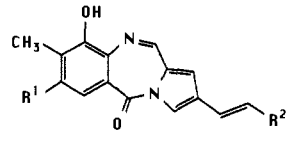
1. R^1=1'-O-sibirosamine, R^2=CH_3
2. R^1=OH, R^2=CH_3
3. R^1=H, R^2=$CONH_2$



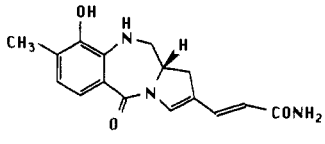
4



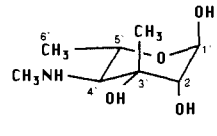
5. R=1'-O-sibirosamine



6. R^1=1'-O-sibiromycin, R^2=CH_3
7. R^1=OH, R^2=CH_3
8. R^1=H, R^2=$CONH_2$



9



sibirosamine

The pyrrolo[1,4]benzodiazepine sibiromycin has an assigned structure (**5**) that differs significantly from those of the other members of this series. In addition to the presence of a carbohydrate moiety at C-7 of the tricyclic nucleus, sibiromycin (**5**) has been reported to contain a pyrrole moiety.[4] Both structural features might be anticipated to alter the nature of the interaction of this pyrrolo[1,4]benzodiazepine with DNA; diminution of minor groove binding can be predicted both from the elimination of the right hand twist of sibiromycin via introduction of the pyrrole and from the known low reactivity of the imine form of **5** (**6**; anhydrosibiromycin[4]) which would presumably be responsible for reaction of sibiromycin with N^2 of guanosine.[1,2] That imine **6** exhibits poor reactivity and a lack of biological activity[1a,4,5] further complicates the analysis, as do the observations that sibiromycin is actually among the most potent members of the pyrrolo[1,4]-benzodiazepines and binds to DNA rapidly and with high affinity.[1a,5]

The anomalous behavior of sibiromycin could be due to an altered mode of DNA binding for this structurally unique pyrrolo[1,4]benzodiazepine, to the failure of the foregoing description to properly characterize the nature of pyrrolo[1,4]benzodiazepine–DNA interaction, or conceivably to the misassignment of structure to one or more members of this series. While the structures of anthramycin (**3**) and tomaymycin (**4**) have been established through X-ray crystallographic analysis and total synthesis,[6] the structure suggested for sibiromycin is based largely on spectroscopic data and a study of its chemical degradation products.[4] Although the stereochemistry of the carbohydrate moiety has been revised,[7] the aglycone structure has not been questioned. Correct structural assignments for sibiromycin degradation products **6** (anhydrosibiromycin) and **7** (anhydrosibiromycinone) are substantiated by the original characterization studies[4] and through the synthesis of **7**.[8] However, on the basis of the foregoing analysis of DNA binding by the pyrrolo[1,4]-benzodiazepines and additional studies of model compounds related to **7**, we believe that the structure assigned to the aglycone moiety of sibiromycin is incorrect. Reported herein are the data that support this assertion and a revised structure for sibiromycin consistent with all available data.

Crucial to the structure assignment are the ^1H signals corresponding to the hydrogens at the 1- and 11a-positions and the coupling mode of the H at the 11-position. As indicated in Table I, Parker and Babine[7b] observed six signals for aromatic and olefinic H's in anhydrosibiromycin (**6**): these appeared at 6.21, 6.37, 7.14, 7.94, 8.18, and 8.28 ppm and were assigned to positions 13, 12, 1, 6, 3, and 11, respectively. In comparison, we found that sibiromycin had only four such signals at 5.41, 6.31, 6.76, and 6.90 ppm, which were assigned to positions 13, 12, 6, and 3, respectively. The spectrum of sibiromycin also contained signals having chemical shifts and multiplicities consistent with a dihydropyrrole structure, including δ 2.85–3.05 (m) and 4.11 (dt) which were attributed to H's at positions 1 and 11a, respectively.[9] The multiplicity (doublet) of the signal for C-11 H in the D_2O-exchanged spectrum supported the assignment of the dihydropyrrole structure. The chemical shift of this signal (δ 3.91) was farther upfield than would be expected for a normal carbinolamine[11] but corresponded closely to those of the 11-bisulfite adducts of anthramycin (δ 4.00) and a synthetic sample of sibiromycin aglycone (δ 3.87) (**2**, vide infra). The above assignments

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(1) For reviews see: (a) Hurley, L. H. *J. Antibiot.* **1977**, *30*, 349. (b) Hurley, L. H.; Thurston, D. E. *Pharm. Res.* **1984**, 52.

(2) (a) Petrusek, R. L.; Anderson, G. L.; Garner, T. F.; Fannin, L. F.; Kaplan, D. J.; Zimmer, S. G.; Hurley, L. H. *Biochemistry* **1981**, *20*, 1111. (b) Graves, D. E.; Pattaroni, C.; Krishnan, B. S.; Ostrander, J. M.; Hurley, L. H.; Krugh, T. R. *J. Biol. Chem.* **1984**, *259*, 8202. (c) Barkley, M. D.; Cheatham, S.; Thurston, D. E.; Hurley, L. H. *Biochemistry* **1986**, *25*, 3021. (d) Hertzberg, R. P.; Hecht, S. M.; Reynolds, V. L.; Molineux, I. J.; Hurley, L. H. *Biochemistry* **1986**, *25*, 1249.

(3) X-ray crystallographic analysis and molecular modeling studies, for example, suggest that the torsion angle between the aromatic and diazepine rings is 35° and 9°, respectively, for anthramycin and tomaymycin.[2d]

(4) (a) Brazhnikova, M. G.; Konstantinova, N. V.; Mesentsev, A. S. *J. Antibiot.* **1972**, *25*, 668. (b) Mesentsev, A. S.; Kulgaeva, V. V.; Rubasheva, L. M. *J. Antibiot.* **1974**, *27*, 866.

(5) Hurley, L. H.; Gairola, C.; Zmijewski, M. *Biochim. Biophys. Acta* **1977**, *475*, 521.

(6) (a) Leimgruber, W.; Batcho, A. D.; Czajkowski, R. C. *J. Am. Chem. Soc.* **1968**, *90*, 5641. (b) Arora, S. K. *J. Antibiot.* **1981**, *34*, 462 and references therein. (c) Tozuka, Z.; Takasugi, H.; Takaya, T. *J. Antibiot.* **1983**, *36*, 276.

(7) (a) Parker, K. A.; Babine, R. E. *Tetrahedron Lett.* **1982**, 1763. (b) Parker, K. A.; Babine, R. E. *J. Am. Chem. Soc.* **1982**, *104*, 7330.

(8) Parker, K. A.; Fedynyshyn, T. H. *Tetrahedron Lett.* **1979**, 1657.

(9) Although unresolved at 360 MHz, the hydrogens at the 1-position were partially resolved at 500 MHz, permitting tentative assignment of H-1α (δ 2.89, dd) and H-1β (δ 2.99, dd).[10] The H-11a signal was resolved into a doublet of triplets with J = 10.1 Hz (H-11, H-1α) and 3.8 Hz (H-1β).

(10) The comparable values for anthramycin were δ 2.66 (dd) and δ 3.12 (dd). See: Kunimoto, S.; Masuda, T.; Kanbayashi, N.; Hamada, M.; Naganawa, H.; Miyamoto, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1980**, *33*, 665.

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Table I. Partial ^1H NMR Spectra of Sibiromycin and Related Compounds[a]

assignment	sibiromycin (**1**·$NaHSO_3$)		anhydrosibiromycin[b] (**6**)	anthramycin[c] (**3**·$NaHSO_3$)	sibiromycin aglycone	
					(**2**·$NaHSO_3$)[c]	2-imine
	δ	2D-connectivity	δ	δ	δ	δ
1α	2.85–3.05 (m)	3, 11a	7.14 (d)	3.0 (m)	d	3.31 (dd)
1β				3.2 (m)	d	3.18 (dd)
3	6.90 (s)	1, 14	8.18 (d)	7.38 (s)	6.90 (s)	6.90 (s)
6	6.76 (s)		7.94 (s)	6.97 (d)	6.53	6.85 (s)
11	3.91 (d)	11a	8.28 (s)	4.00 (d)	3.87 (d)	7.82 (d)
11a	4.11 (dt)	1, 11		4.28 (dt)	4.3 (m)	4.3–4.4 (m)
12	6.31 (d)	13, 14	6.37 (d)	7.31 (d)	6.30 (d)	6.33 (d)
13	5.41 (dq)	12, 14	6.21 (dq)	5.79 (d)	5.6 (dq)	5.67 (dq)

[a] Chemical shifts (δ) are reported in ppm, relative to $(CH_3)_4Si$. Two-dimensional connectivities were determined with a COSY program. The spectrum of **1** was obtained in DMSO-d_6. [b] *Reference* 7b. [c] The anthramycin and sibiromycin aglycone bisulfite adducts were obtained by adding 4 equiv of $Na_2S_2O_5$ in D_2O to DMSO-d_6 solutions of these compounds. [d] Signal obscured.

were shown to be consistent with the connectivities determined by 2D homonuclear correlation NMR spectroscopy (Table I), providing further support for the proposed dihydropyrrole structure.

The revised structure **1** for sibiromycin has been confirmed by total synthesis of sibiromycin aglycone (**2**).[12] Comparison of the ^1H and ^{13}C NMR spectra of **1** and **2** indicated a correspondence in the chemical shifts and multiplicities for the atoms shared in common.[13] We suggest that the error in the original structure assignment of sibiromycin[14] can be attributed to a facile oxidative aromatization of the dihydropyrrole moiety under acidic conditions, such as those used to dehydrate the carbinolamine during the original structure determination.[4]

Acknowledgment. We thank Dr. Charles W. DeBrosse for the ^{13}C and COSY spectra and Dr. D. E. Thurston, University of Texas at Austin, for the 500 MHz spectrum of sibiromycin.

Supplementary Material Available: Table of ^1H and ^{13}C NMR spectral data of sibiromycin and related compounds (2 pages). Ordering information is given on any current masthead page.

(11) The chemical shift values for C-11 H of the carbinolamine form of anthramycin methyl ether[10] and our synthetic sibiromycin aglycone[12] were δ 4.78 and 4.71, respectively. In DMSO-d_6 the NMR spectrum of the sibiromycin sample (NSC 291320 ND) used in this study consisted only of signals corresponding to a derivative hydrated at C-11. Under the same conditions anthramycin and sibiromycin aglycone exhibited spectra for the imine form only, with carbinolamine signals appearing on addition of D_2O. These data, along with the differences in chemical shift of the C-11 H signals, indicated that the sibiromycin sample had been converted to the more stable bisulfite adduct, probably during isolation (see ref 4a).

(12) Hoover, J. R. E.; Leber, J. D.; Holden, K. G.; Hecht, S. M., in preparation.

(13) Complete ^1H and ^{13}C NMR spectra for **1**, **2**, **3**, and **6** are included as Supplementary Material.

(14) In trifluoroacetic acid (25 °C) anthramycin was dehydrated and aromatized to afford **8** in approximately 50% yield (Malhotra, R. K.; Ostrander, J. M.; Hurley, L. H.; McInnes, A. G.; Smith, D. G.; Walter, J. A.; Wright, J. L. C. *J. Nat. Prod.* **1981**, *44*, 38). The aromatization was assumed to occur through disproportionation, although coformation of a reduced product **9** was not explored. Upon reinvestigation of this reaction, we obtained both **8** and **9** (*m/z* 309.1352); however, the latter was present to a lesser extent than **8**. In addition, we have observed that sibiromycin and anthramycin were dehydrated and aromatized, without disproportionation, when dilute solutions of the antibiotics in DMSO-d_6 (NMR sample) were acidified without prior removal of dissolved air. Aromatization did not occur when the solutions were degassed and purged with argon.

Additions and Corrections

Rates and Mechanisms of Hydrolysis of Esters of Phosphorous Acid [*J. Am. Chem. Soc.* **1988**, *110*, 181–185]. F. H. WESTHEIMER,* SHAW HUANG, and FRANK COVITZ

Page 182: The names appearing in lines 9 and 12 of Table I should read "dimethyl hydrogenophosphonate" (instead of "dimethyl hydrogen phosphate"), and "diethyl hydrogenphosphonate" (instead of "diethyl hydrogen phosphate"), respectively. The name appearing on line 3 of Table II should read "trimethyl phosphite" (instead of "trimethyl phosphate").