THE STRUCTURE OF VACIDIN A, AN AROMATIC HEPTAENE MACROLIDE ANTIBIOTIC

II. STEREOCHEMISTRY OF THE ANTIBIOTIC

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On the basis of coupling constants and rotating frame nuclear Overhauser effect spectroscopy of vacidin A methoxycarbonylmethylamide, the stereochemistry of the antibiotic was established. The configuration of the aglycone was determined as (3*R*,7*R*,9*R*,11*S*,13*S*,15*R*,17*S*,18*R*,19*S*,21*R*, 36*S*,37*R*,38*S*). The aminosugar constituent of the antibiotic was identified as β-(D)-mycosamine. The chiral center at C-41 remains to be assigned.

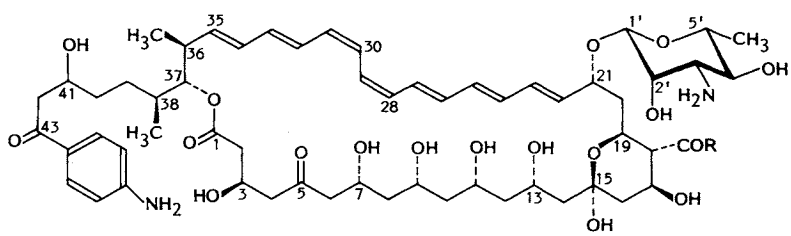
A gross structure of vacidin A along with the geometry of the heptaene chromophore have been presented in Part I of this report[1]. In this paper the ^{1}H NMR spectral data are discussed from the point of view of vacidin stereochemistry. The investigation of vacidin A resulted in the assignment of all but one chiral center of the antibiotic (Fig. 1).

The stereochemical studies of vacidin A were based upon analysis of coupling constants and nuclear Overhauser enhancement (NOE) which are listed in Table 2 of Part I[1].

The ^{1}H NMR spectrum of **2** was of unprecedented quality as compared to the other reported spectra of polyene macrolides. Therefore, it was possible to assign the majority of coupling constants; only a few of them could not be determined directly.

It should also be mentioned that a full set of NOE's was not observed in rotating frame nuclear Overhauser effect spectroscopy (ROESY) of **2**, but this concerned only those cases in which expected NOE's were masked by overlapping protons of interest or by other strong effects such as NOE's between geminal protons.

Fig. 1. Structure of vacidin A.



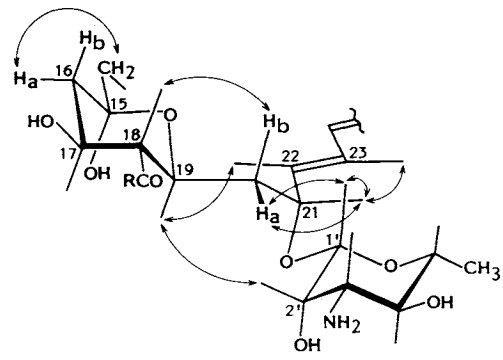
Vacidin A (**1**) R=OH
Vacidin A methoxycarbonylmethylamide (**2**) R=NHCH$_2$COOCH$_3$

Results and Discussion

The values of vicinal coupling constants between the protons 16-H_a, 16-H_b, 17-H, 18-H and 19-H as well as the 14-H–16-H_a NOE, shown in Fig. 2, pointed out a chair conformation of the hemiketal ring and the relative configuration of the C-15, C-17, C-18 and C-19 chiral centers. It should be mentioned that 14-H–17-H and 14-H–19-H NOE's were not observed.

Analysis of vicinal coupling constants and NOE's within the mycosamine moiety (Table 2 of Part I), supplemented by the specific rotation of mycosamine obtained by acidic hydrolysis of vacidin A, clearly indicated the absolute configuration of this sugar substituent. Thus, the value of $J_{19,20b}$= 10.1 Hz and 20-H_b–18-H, 20-H_a–21-H, 21-H–1′-H, 20-H_a–1′-H and 2′-H–19-H NOE's depicted in Fig. 2 pointed out a spatial relation between mycosamine moiety and the C-15–C-21 fragment, thereby proving its absolute configuration.

Fig. 2. Conformation of the hemiketal region of vacidin A.



Observed NOE's are depicted as bidirectional arrows.

	J (Hz)
16-H_a, 17-H	4.7
16-H_b, 17-H	10.3
17-H, 18-H	10.3
18-H, 19-H	10.1
19-H, 20-H_b	10.1
21-H, 22-H	9.0

The antiperiplanar position of 21-H and 22-H, derived from $J_{21,22}$=9 Hz and the 21-H–23-H NOE combined with the 19-H–22-H NOE (Fig. 2) unambiguously situated the C-14–C-21 fragment in relation to the plane of the heptaene chromophore.

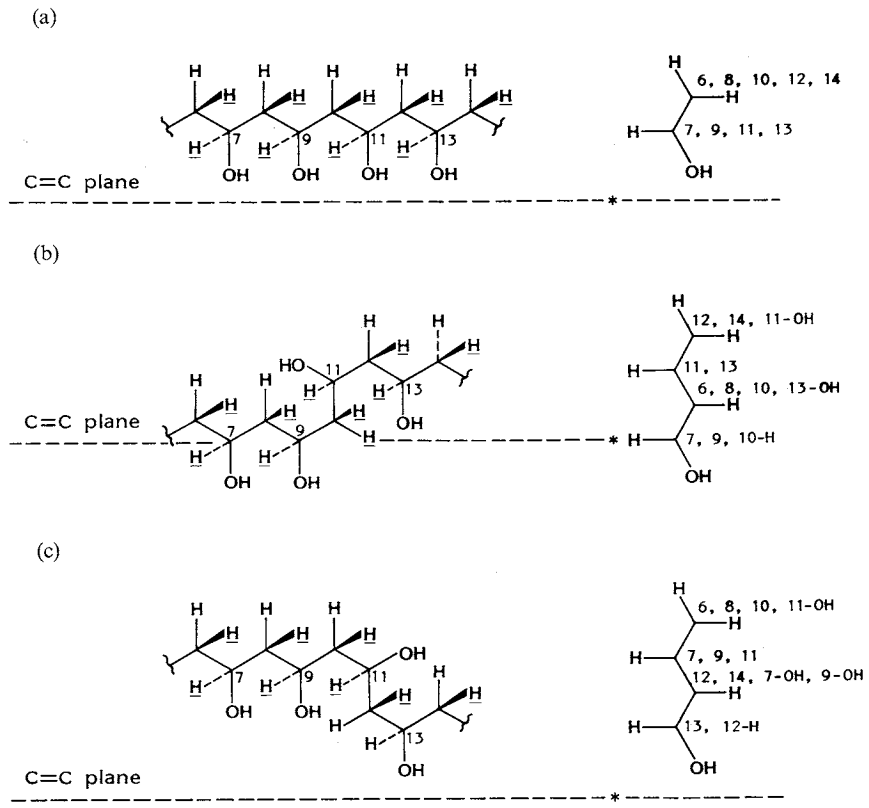
Subsequently, the relative configuration of the C-13–C-7 fragment was determined on the basis of coupling patterns of hydroxymethine protons and their NOE's to olefinic protons, which are discussed below.

The vicinal coupling constants between methylene protons (12-H_a, 10-H_a, 8-H_a) and hydroxymethine protons (13-H, 11-H, 9-H, 7-H), observed as $J\sim$ 10 Hz (Table 2 of Part I), could be matched with three types of conformation of the C-13–C-7 fragment, which are shown in Fig. 3 as (a), (b) and (c). However, the presence of 13-H, 11-H, 9-H and 7-H NOE's to the appropriate olefinic protons (Table 2 of Part I) allowed exclusion of (b) and (c) from our considerations. Namely, in the exemplary case of (b) the distances of olefinic protons to 7-H and 9-H were unacceptably short. In the case of (c) 7-H, 9-H and 11-H NOE's to olefinic protons were missing. Thus (a) remained the only solution for the conformation of the C-13–C-7 fragment.

It was possible to assign the absolute configuration of this fragment by finding the relation between 13-H and 16-H *via* C-14. An 13-H–14-H_a coupling constant of 10.5 Hz combined with an 14-H_b–13-H NOE and the absence of an NOE between 14-H_a–13-H clearly indicated staggered positions for the 14-H_a and 13-H protons. Thus, the 16-H_a–14-H_a NOE along with the steric relation of the heptaene chromophore and the C-13–C-7 fragment (Fig. 4) determined the absolute configuration of the C-13–C-7 fragment.

Relative configurations at C-36/C-37 and C-37/C-38 of **2** were derived from the $J_{36,37}$ and $J_{37,38}$ values as well as 36-CH_3–38-H and 36-H–38-CH_3 NOE's which are depicted in Fig. 5.

Fig. 3. Three conformational variants resulting from the coupling pattern of the C-6–C-14 polyhydroxylic fragment of vacidin A and their interference with the polyene chromophore.



The protons with large coupling constants are underlined. The end views of a, b and c are shown on the right-hand side.

* Olefinic proton.

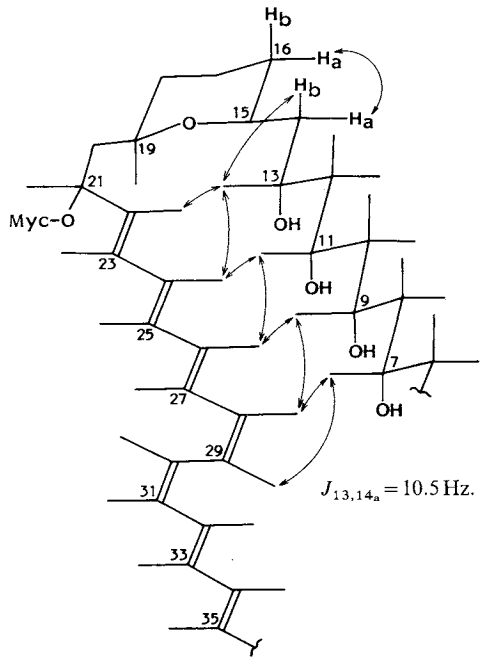
The configuration of C-3 results from placement of the C-3–3-H and C-3–C-4 bonds. The latter is determined by the requirement of closing the macrolide ring, while the former derives from 38-CH_3–3-H, 3-H–34-H, 34-H–36-H and 35-H–37-H NOE's (Fig. 5).

It was a very fortunate observation that it was not possible to construct the macrolactone ring from the enantiomer of the C-34–C-3 fragment presented in Fig. 5. Namely, its conformation drives C-3 out of the polyene chromophore plane. In order to construct the macrolactone ring, C-3 needed to be linked through C-4–C-6 with the C-7–C-13 fragment which was also located out of plane. This could be achieved only in the case where C-3 and C-7 were located on the same side of the chromophore plane. Thus, only one enantiomer of C-34–C-3 fulfilled this requirement. Therefore, the relative configuration of the C-36–C-3 fragment became absolute.

In conclusion, chiral centers of vacidin A were assigned as (3*R*,7*R*,9*R*,11*S*,13*S*,15*R*,17*S*,18*R*,19*S*, 21*R*,36*S*,37*R*,38*S*,1′*R*,2′*S*,3′*S*,4′*S*,5′*R*) (Fig. 1). Only the configuration at C-41 remains unknown, and work on this problem is under way.

Among the many polyene macrolide antibiotics reported, the complete absolute configuration has been assigned only for amphotericin B using X-ray analysis[2]. It is of interest that the comparison of steric relations of amphotericin B and vacidin A fragments with the same constitution, namely C-3 and C-11–C-21

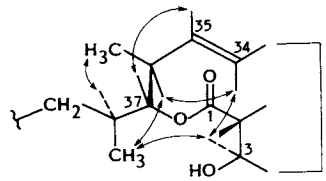
Fig. 4. Mutual alignment of polyhydroxylic and olefinic parts of vacidin A based on observed NOE's (bidirectional arrows).



The value of $J_{13,14_a}$ combined with the 14-H_a–16-H_a NOE correlate configurations of polyhydroxylic and hemiketal fragments.

Myc: Mycosaminyl.

Fig. 5. Configuration and conformation of the lactone part of vacidin A.



Observed NOE's are depicted as bidirectional arrows.

	J (Hz)
35-H, 36-H	9.0
36-H, 37-H	9.8
37-H, 38-H	2.2

(carbon atoms numbering for vacidin A), revealed their identity. The term configuration is not used in this case since it depends on the type of substituents.

Experimental

Mycosamine

100 mg of vacidin A was dissolved in 30 ml of methanol and 1 M hydrochloric acid (1 : 1) and kept for 1 hour at ambient temperature. Next, 1-butanol and water were added and methanol was evaporated. The aqueous solution was extracted three times with butanol and evaporated to dryness. The residue was purified on a Dowex 50WX8 (H^+) column (1 × 15 cm), employing 1 M hydrochloric acid as the eluent. The collected fractions were evaporated and dissolved in water, and then 30 μl of triethylamine and 20 μl of acetic anhydride were added. After 1 hour the solution was evaporated to dryness, dissolved in water and passed through a Dowex 50WX8 (H^+) column (1 × 15 cm), using water as eluent. Evaporation of collected fractions gave 12 mg of pure *N*-acetylmycosamine. The specific rotation, $[\alpha]_D^{20} -43°$ (*c* 1.0, EtOH) was comparable with that of *N*-acetylmycosamine prepared from amphotericin B: $[\alpha]_D^{20} -42°$ (*c* 1.0, EtOH).

Acknowledgments

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References

1) Sowiński, P.; P. Gariboldi, A. Czerwiński & E. Borowski: The structure of vacidin A, an aromatic heptaene macrolide antibiotic. I. Complete assignment of the ^{1}H NMR spectrum and geometry of the polyene chromophore. J. Antibiotics 42: 1631 ~ 1638, 1989

2) Mechlinski, W.; C. P. Schaffner, P. Gains & G. Avitabile: Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B. Tetrahedron Lett. 1970: 3873 ~ 3876, 1970