to ^{1}H NMR analysis consisted of **11** and **12** in a ratio of 4:1. The oil also contained a small amount (less than 6%) of dibenzylideneacetone as an impurity.

11: ^{1}H NMR (200 MHz) (assigned peaks in mixture with **12**) δ 5.9–5.6 (m, 4 H, olefinic), 3.75 (s, 3 H, one of CO_2Me), 3.74 (s, 3 H, one of CO_2Me), 3.51 (d, J = 8.8 Hz, 1 H, $CH(CO_2Me)_2$), 3.18 (m, 1 H, $CHCH(CO_2Me)_2$), 2.45–2.3 (m, 2 H, allylic CH_2), 1.95–1.75 (m, 2 H, CH_2).

12: ^{1}H NMR (200 MHz) (assigned peaks in mixture with **11**) δ 5.9–5.6 (m, 3 H, olefinic), 4.12 (s, 1 H, $CH(CO_2Me)_2$), 3.75 (s, 6 H, CO_2Me), 2.45–2.3 (m, 4 H, allylic CH_2), 1.95–1.75 (m, 2 H, CH_2).

Registry No. **1**, 96039-90-6; **2**, 96039-91-7; **3**, 82736-52-5; **4**, 110418-98-9; **5**, 39495-94-8; **7**, 110455-97-5; **8**, 110455-98-6; **9**, 15833-44-0; **10**, 110418-99-0; **11**, 91550-40-2; **12**, 110419-00-6; $Pd(dppe)_2$, 31277-98-2; $Pd(dba)_2$, 81141-80-2; dimethyl malonate, 108-59-8; (*E*)-(*R**,*R**)-2-acetoxy-5-chloro-3-hexene, 95177-49-4; (*E*)-(*R**,*S**)-2-acetoxy-5-chloro-3-hexene, 95177-50-7; sodium dimethyl malonate, 18424-76-5; *cis*-1-acetoxy-4-chloro-2-cyclohexene, 82736-39-8; *cis*-1-acetoxy-4-chloro-2-cycloheptene, 82736-40-1; dimethyl diazomalonate, 6773-29-1; (*E*,*E*)-2,4-hexadiene, 5194-51-4.

Stereochemistry of the Cyclic Tripeptide Antibiotic WS-43708A

Rajamoorthi Kannan and Dudley H. Williams*

University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.

Received April 27, 1987

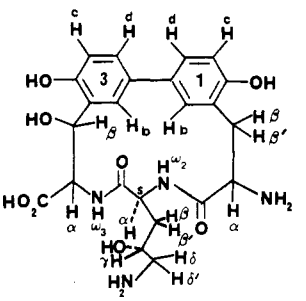
By the use of proton NMR spectroscopy, in particular of coupling constant and NOE data, it is shown that the cyclic tripeptide antibiotic WS-43708A has *S* stereochemistry at the α-carbon atom of all three amino acids and that the carbon atom at the benzylic position of residue 3 has *R* stereochemistry. The conformational preference of the biphenyl system has also been determined. Although WS-43708A appears to be the only reported antibiotic outside the vancomycin group to possess a biphenyl group, no evidence for binding of WS-43708A to the cell wall analogue N-Ac-D-Ala-D-Ala has been found. It is concluded therefore that WS-43708A exerts its antibacterial activity other than by binding to mucopeptide precursors terminating in D-Ala-D-Ala.

A covalent structure (**1**) for the cyclic tripeptide antibiotic WS-43708A has been reported by Hashimoto and co-workers.[1] The only stereochemical information which was available from their study was that residue 2 corresponds to *erythro*-γ-hydroxy-L-ornithine.[1] In view of a possible structural analogy between this structure and parts of the structures found for vancomycin group antibiotics,[2] we have fully determined and now report the stereochemistry of WS-43708A. Our interest in this stereochemistry was also initially aroused by the possibility that WS-43708A acts by inhibiting cell wall biosynthesis, as do also members of the vancomycin group.[2] We therefore wished to establish whether WS-43708A has a geometry suitable to bind mucopeptide precursors terminating in D-Ala-D-Ala.[2]

The earlier structural work on WS-43708A had been carried out in D_2O/DCl and CD_3OD/D_2O. Since we wished to utilize NOE and coupling data for the amide protons, it was desirable to work in DMSO-d_6 solution. As the dihydrochloride of the antibiotic (kindly provided by Dr. Hashimoto) was not soluble in DMSO-d_6, it was converted to the free base by dissolving in an aqueous solution of ammonium carbonate. The solution was then lyophilized several times to remove excess ammonium carbonate.

Proton NMR spectra of the above free base were obtained in DMSO-d_6 solution on a Bruker AM-400 spectrometer. Two and three bond proton–proton couplings were determined from a double quantum filtered phase-sensitive COSY spectrum. This spectrum also exposed the four bond couplings between benzylic protons and protons ortho to the benzylic position. Chemical shifts and coupling constants are summarized in Table I, by using the proton code given in **1**, and are in accord with the reported structure. Phase-sensitive NOESY and CAMELSPIN[3]

Table I. ^{1}H NMR Chemical Shifts and Coupling Constants for WS-437083A[a]



1

proton	chem shift, δ	J, Hz
ω_2	8.94	9.2
ω_3	8.40	9.7
3b	7.44	2.6
1d, 3d	7.18	m
1b	6.94	2.2
1c, 3c	6.9	7.5
β_3	5.72	s
α_2	5.04	m
α_3	4.52	9.7
α_1	4.14	m
γ_2	3.92	m
β_1	3.24	15, 6
β_1'	3.02	15, 3
δ_2	2.98	13, 3
δ_2'	2.78	13, 8
β_2, β_2'	1.84	m

[a] Spectrum of the free base in DMSO-d_6 at 330 K: m = multiplet: s = singlet. The amino acid residues are coded 1, 2, and 3 from the N- to the C-terminus.

(1) Uchida, I.; Ezaki, M.; Shigematsu, N.; Hashimoto, M. *J. Org. Chem.* **1985**, *50*, 1341–1342.
(2) Barna, J. C. J.; Williams, D. H. *Annu. Rev. Microbiol.* **1984**, *38*, 339–357.

0022-3263/87/1952-5435$01.50/0

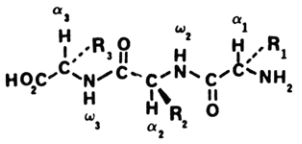
Table II. NOEs Observed from Phase Sensitive NOESY[a] and CAMELSPIN[b] Spectra of WS-437083A[c]

$\omega_2 \leftrightarrow \alpha_1$	$1b \leftrightarrow \beta_1'$	$\gamma_2 \leftrightarrow (\beta_2, \beta_2')$
$\omega_2 \leftrightarrow (\beta_2, \beta_2')$	$\beta_3 \leftrightarrow \alpha_3$	$\gamma_2 \leftrightarrow \delta_2$
$\omega_3 \leftrightarrow \alpha_2$	$\alpha_2 \leftrightarrow (\beta_2, \beta_2')$	$\beta_1 \leftrightarrow \beta_1'$
$\omega_3 \leftrightarrow 3b$	$\alpha_1 \leftrightarrow \beta_1$	$\delta_2 \leftrightarrow \alpha_2$
$3b \leftrightarrow \alpha_3$	$\alpha_1 \leftrightarrow \beta_1'$	$\delta_2 \leftrightarrow \delta_2'$
$3b \leftrightarrow 1b$		

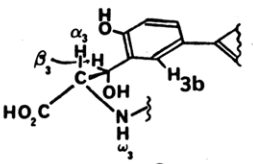
[a] Obtained in DMSO-d_6 at normal probe temperature (290–293 K). [b] Obtained in DMSO-d_6 containing 2% TFA (v/v) at 320 K, with a spin-locking duration of 200 ms; the experiment was repeated with a different transmitter offset to check for any false enhancement.[4] [c] All NOEs were negative in the NOESY spectrum; their magnitudes lay in the range 2–15%. The CAMELSPIN NOEs are positive.

spectra were obtained in the same solvent (but the latter after addition of 2% v/v TFA). The latter spectrum is useful in obtaining specific NOEs even in cases where spin diffusion is normally a problem, and in cases where the NOE would be near zero because $\omega_o\tau_c \simeq 1$. The NOE data obtained from these spectra are summarized in Table II. Since the pattern of NOEs was essentially the same from both types of experiment, it is clear that significant spin diffusion had been avoided at the mixing time (0.3 s) used in the NOESY experiment. It is also clear from these data that the conformation of the antibiotic, in the regions in which NOEs are observed, is essentially the same for the protonated form (after addition of TFA) and for the free base.

The vicinal coupling constants between the amide N*H* protons of amino acid residues **2** and **3** (ω_2 and ω_3) and their respective α-C*H*s both lie in the range 9–10 Hz.



2



3

These facts, coupled with the observation that neither of the α-CH protons shows an NOE to the amide NH of its own residue, indicate that the α-C*H*N*H* protons are approximately trans-orientated (see **2**). Additionally, the strong NOEs $\alpha_1 \leftrightarrow \omega_2$ and $\alpha_2 \leftrightarrow \omega_3$ indicate that these two pairs of protons are on the same side of the molecule, as also indicated in **2**.

Since the side chains of the oxidized phenylalanine units (R_1 and R_3 in **2**) are connected (presumably via phenol oxidative coupling), then they must be orientated in the same direction from the peptide backbone unit **2**. CPK models show that this direction must be downwards from the plane of the paper (see **2**); if they orientated upwards from the plane of the paper, then the side chain of the γ-hydroxyornithine residue (R_2 in **2**) cannot be accommodated within the 15-membered ring. Thus, both residues **1** and **3** have the *S* absolute configuration of their α-carbon atoms (see **2**).

There is no discernible coupling between α_3 and β_3, and therefore the dihedral angle between these vicinal protons must be near to 90° (see also ref 1). CPK models show that this geometry can only be accommodated if the benzylic position of residue **3** has the *R* configuration, i.e., with the benzylic hydroxyl group approximately trans-orientated with respect to the C–$H_{\alpha 3}$ bond. Additionally, the fact that β_3 shows an NOE to α_3 but to no other proton (Table II) demands, when taken in conjunction with the other constraints, the *R* configuration at the benzylic center. This geometry is reproduced in **3** and is in accord with the observation that **3b** shows NOEs to both ω_3 and α_3 but not to β_3.

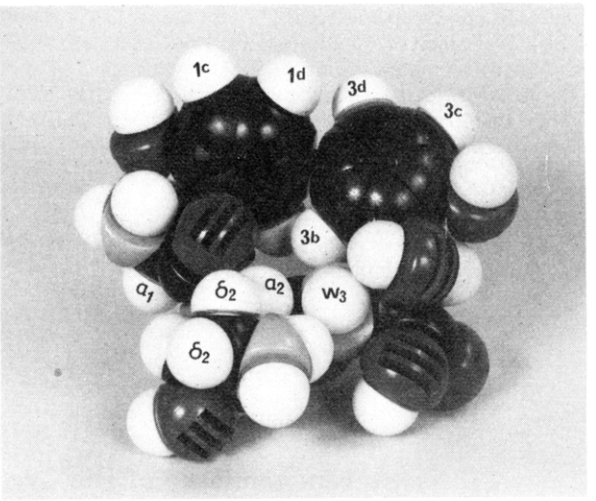
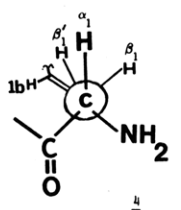
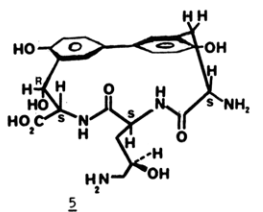


Figure 1. CPK model of WS-43708A.

The vicinal coupling constants of α_1 to β_1 and β_1' (6 and 3 Hz, respectively) establish that neither of these benzylic protons is held in a specific trans conformation relative to α_1. This conclusion, in conjunction with the observation of NOEs from α_1 to both β_1 and β_1', shows that these benzylic protons are on the same side of the structure as α_1. In the NOESY spectrum the cross peak between α_1 and β_1' is larger than that between α_1 and β_1; moreover, in the CAMELSPIN spectrum, only the $\alpha_1 \leftrightarrow \beta_1'$ NOE is observed. Hence α_1 is closer to β_1' than to β_1, which requires a smaller dihedral angle on average between α_1 and β_1' than between α_1 and β_1. Additionally, **1b** shows an NOE to β_1' but not to β_1. These data demand a geometry of this part of the molecule which is close to that shown in **4**. The original form of the Karplus equation would



4



5

of course require $J_{\alpha_1,\beta_1'} > J_{\alpha_1,\beta_1}$, but account must also be taken of the fact that vicinal coupling is reduced when one of the protons involved is approximately trans-coplanar to an electronegative substituents.[5] To take account of this type of effect (which operates here, since β_1' is almost trans-coplanar to the NH_2 substituent—see **4**), we have used the equation of Gandour and co-workers.[6] The observed values of $J_{\alpha_1,\beta_1'} = 3$ Hz and $J_{\alpha_1,\beta_1} = 6$ Hz (Table I) in this equation give dihedral angles of 42° and 60°, respectively. Although the sum of these is somewhat less than the required 120°, their relative magnitudes are

(3) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloy, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811–813.
(4) Neuhaus, D.; Keeler, J. *J. Magn. Reson.* **1986**, *68*, 568–574.
(5) Williams, D. H.; Bhacca, N. S. *J. Am. Chem. Soc.* **1964**, *86*, 2742–2743.
(6) Colucci, W. J.; Jungk, S. J.; Gandour, R. D. *Magn. Reson. Chem.* **1985**, *23*, 335–343.

consistent with the conclusions summarized in 4.

Other NOEs (Table II) give information about possible conformational states of the peptide. Proton **3b** gives NOEs to both α_3 and ω_3 with the former being more intense. Protons α_3 and ω_3 are on opposite sides of the molecule—see **2**); thus there is probably some conformational flexibility of the aromatic ring **3**, with **3b** on average being somewhat nearer to α_3 than to ω_3. Also noteworthy is the NOE $\alpha_2 \leftrightarrow \delta_2$ (Table II). This NOE establishes that the side chain of residue **2** spends some of its time coiled up such that the terminal carbon atom of the sidechain is proximate to its α-CH proton. The structure of WS-43708A is reproduced with stereochemical detail in **5**; and, as a CPK model, in a conformation which is in accord with the above findings, in Figure 1. In this latter representation, we have rotated the covalent structure **1** through 180° about a vertical axis bisecting the C–C bond connecting the aromatic residues **1** and **3**. This has been done because what transpires to be a concave face of the molecule is now presented upwards.

The site to which WS-43708A binds in exerting its antibacterial activity is unknown. However, addition of the cell wall analogue N-Ac-D-Ala-D-Ala[7] to a DMSO-d_6 solution of the antibiotic free base affected neither the chemical shifts nor the line shapes of the proton resonances. This situation still pertained as trifluoroacetic acid was then added stepwise to the solution to reduce the "pH". Additionally, the addition of N-Ac-D-Ala-D-Ala, or di-N-Ac-Lys-D-Ala-D-Ala[7] to a solution containing WS-43708A (0.1–0.2 mg/mL in 0.02 M sodium citrate buffer, pH 5.1) did not affect its original UV spectrum. We conclude that WS-43708A exerts its physiological action on cell wall biosynthesis other than by binding to mucopeptide precursors terminating in D-Ala-D-Ala.

(7) Nieto, M.; Perkins, H. R. *Biochem. J.* **1971**, *123*, 789–803.

Experimental Section

^{1}H NMR spectra were obtained on a Brucker AM-400 spectrometer using approximately 20 mg/mL solutions in DMSO-d_6. One-dimensional spectra were usually recorded with a spectral width of 3500–4500 Hz and 16K data points. Two-dimensional double quantum filtered COSY (DQFCOSY), NOESY, and CAMELSPIN experiments were all run in the phase-sensitive mode. NOESY spectra were measured with various mixing times (0.3–0.5 s) to obtain the optimum NOES without spin diffusion. Two CAMELSPIN experiments were recorded with different transmitter offsets to check for false enhancements;[4] the same spin-locking durations of 200 ms was used in both cases. All the 2D data matrices consisted of 512 2K spectra, which yielded, after zero-filling in F1 and Fourier transformation, a 1-MW square matrix. A maximum of 32 scans were accumulated in each t_1 fid with proper phase cycling. The data were subjected to Lorentzian–Gaussian multiplications in t_2 and sine bell multiplication (SSB1 valve of 3) in t_1 prior to transformation. UV studies were carried out with a Pye Unicam PU 8800 UV/Vis spectrometer and followed a previously published procedure.[7]

Acknowledgment. We thank SERC for financial support and Dr. M. Hashimoto (Fujisawa Pharmaceutical Co. Ltd.) for a sample of WS-43708A.

Registry No. Antibiotic WS-43708A, 100296-21-7.

Formal Transfers of Hydride from Carbon–Hydrogen Bonds. Attempted Generation of Molecular Hydrogen by Intramolecular Reduction of Protons Bound by 2,3-Dihydro-1,3-dimethyl-2-(2-pyridinyl)-1*H*-benzimidazole

Socorro M. Ramos, Micheline Tarazi, and James D. Wuest*

Département de Chimie, Université de Montréal, Montréal, Québec H3C 3J7, Canada

Received May 4, 1987

2,3-Dihydro-1,3-dimethyl-2-(2-pyridinyl)-1*H*-benzimidazole (**5**) is designed to bind electrophilic substrates and reduce them by an internal transfer of hydride. The intended binding site is the nitrogen atom of the pyridine ring, and the source of hydride is the carbon–hydrogen bond at C_2 of the dihydrobenzimidazole ring. In the preferred conformation of compound **5**, the carbon–hydrogen bond is activated as a donor of hydride by two antiperiplanar lone pairs, and the intended binding site is nearby. Protons bind primarily to the nitrogen atom of the pyridine ring as expected, but they are not reduced to molecular hydrogen. Instead, two other reactions are triggered by protonation. One is heterolysis of the carbon–carbon bond between the dihydrobenzimidazole and pyridinium rings, and the other is a 1,2-shift of hydride from the activated carbon–hydrogen bond to the pyridinium ring. The failure of compound **5** to reduce protons to molecular hydrogen can be attributed primarily to unfavorable thermodynamics; the hydridic carbon–hydrogen and acidic nitrogen–hydrogen bonds of salt **5**-H$^+$ are inadequately activated, so alternative reactions are more rapid. Formation of hydrogen may also be disfavored because salt **5**-H$^+$ adopts an unsuitable conformation or because an appropriate trajectory for intramolecular protonation of the activated carbon–hydrogen bond is unattainable.

We are interested in the design and synthesis of reducing agents that have some of the features of redox enzymes, including a receptor site that recognizes and binds reducible substrates, and an adjacent site that acts as a donor of hydride. In enzymatic reductions involving the coenzyme NADH, Cannizzaro reactions, Meerwein–Ponndorf–Verley reductions, and other important redox reactions, carbon–hydrogen bonds serve as the donors of hydride. The subdued reactivity of these bonds makes them particularly suitable when the substrate must be recognized, oriented, and activated by a receptor before the transfer of hydride takes place. We have therefore begun to study compounds in which a reducible substrate is held close to a suitably activated carbon–hydrogen bond.[1]

0022-3263/87/1952-5437$01.50/0