Structural Elucidation and Solution Conformation of the Novel Herbicide Hydantocidin

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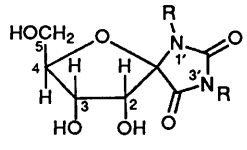
The structure of a novel herbicide, hydantocidin isolated from the fermentation broth of *Streptomyces hygroscopics* SANK 63584, was determined by the combined analysis of MS and ^{1}H NMR spectra. Hydantocidin is a novel spiro compound containing a subofuranoid ring, at the anomeric position of which a hydantoin ring is used such that the C(1)–N(1) linkage is β. The relative configuration and the conformation in solution was determined by quantitative analysis of the NOE spectra and T_1 values. In CD_3OD and [$^2H_6$]DMSO (dimethyl sulphoxide) solutions, the ribofuranose moiety of hydantocidin was found to be fixed in a C_2-*endo* conformation, probably due to the rigidity of the spiro structure and hydrogen bonding between 3-OH and the carbonyl group at C-4′.

In the course of our screening program for novel microbial metabolites, which exhibit herbicidal activity, hydantocidin **1** was isolated from the fermentation broth of the *Streptomyces hygroscopicus* SANK 63584 found in Annaka City, Japan.[1]

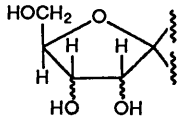
This paper describes the structural determination of hydantocidin by a combination of mass spectrometry (MS) and ^{1}H NMR spectroscopy. The solution conformation of **1** was also analysed by quantitative analysis of the NOEs and longitudinal relaxation times (T_1). Hydantocidin is a novel type of herbicide which has a spiro-hydantoin ring fused at the anomeric position of ribofuranose. The close structural similarity of hydantocidin with nucleosides made it an interesting task to determine its three-dimensional structure in solution in order to allow a comparison of its structure–activity relationship with those of biologically active nucleoside derivatives.[2]

Results and Discussion

Planar Structure.—The molecular formula $C_7H_{10}N_2O_6$ of **1** was established by high resolution mass measurement (Found:



1 R = H
2 R = Me



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219.061 75. Calc. for $C_7H_{11}N_2O_6$: 219.061 75) on the quasimolecular ion ($M + H^+$) at *m/z* 219 in the fast atom bombardment mass spectrum (FAB MS).

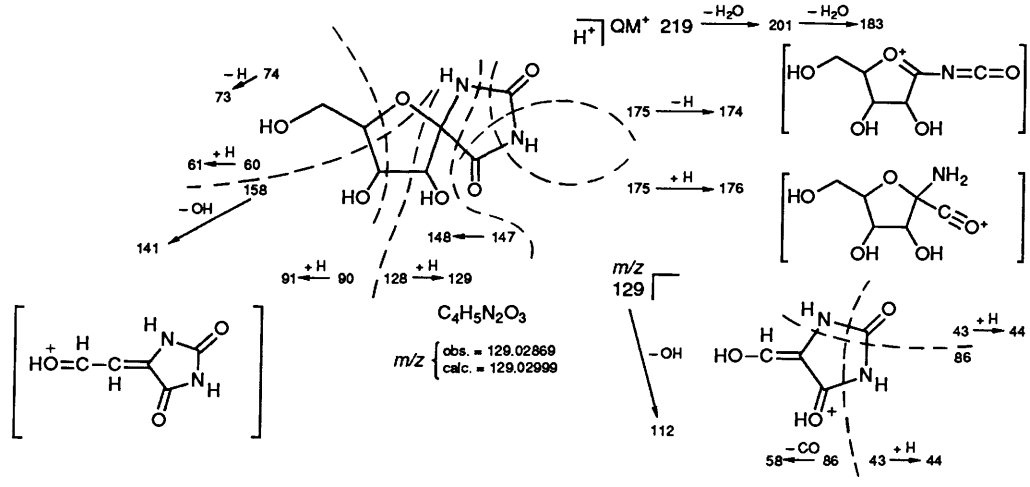
As reported in Table 1, the ^{1}H NMR spectrum of **1** in CD_3OD exhibited a relatively simple spectral pattern in the range 3.6–4.2 ppm. The decoupling experiments made clear the connectivity, OCH_2–CH(O)–CH(O)–CH(O)–. Three hydroxy protons were observed in [$^2H_6$]DMSO at 5.60 and 4.68 ppm as doublets, and at 4.78 ppm as a triplet. These hydroxy protons could be assigned to 2-OH, 3-OH and 5-OH, respectively. Thus the partial structure **3** could be derived. The rest of the molecule must, therefore, have the atomic composition $C_2H_2N_2O_2$, which involves two labile protons observed at 8.48 and 10.93 ppm in [$^2H_6$]DMSO.

Table 1 ^{1}H Chemical shifts (δ values from TMS) and *J* values (Hz in parentheses) of hydantocidin **1** and *N,N′*-dimethylhydantocidin **2**

Proton	**1** in CD_3OD	**1** in [$^2H_6$]DMSO	**2** in CD_3OD
2	4.24 (d, 6.0)	4.11 (dd, 5.9, 5.1)	4.46 (d, 5.9)
3	4.04 (dd, 6.0, 2.2)	3.87 (ddd, 5.9, 2.2, 10.2)	4.08 (dd, 5.9, 1.5)
4	4.22 (dt, 2.2, 4.2)	4.03 (dt, 2.2, 5.0)	4.27 (dt, 1.5, 3.6)
5, 5′	3.62 (m)	3.41 (m)	3.67 (m)
2-OH	—	5.60 (d, 5.1)	—
3-OH	—	4.68 (d, 10.2)	—
5-OH	—	4.78 (t, 5.1)	—
1′-NH	—	8.48 (s)	—
1′-NMe	—	—	2.93 (s)
3′-NH	—	10.93 (s)	—
3′-NMe	—	—	2.97 (s)

Taking into account the unsaturation number of 4, hydantocidin must have a spiro ring system fused at C-1. Among the several possible structures satisfying the formula $C_3H_2N_2O_2$, a hydantoin moiety was selected on the basis of the following mass spectral analysis. The $M^{+\cdot} = 578$ of the trimethylsilylated derivative indicated the presence of five labile protons, which correspond to the three hydroxy groups in the sugar moiety and two amide or phenolic protons in the rest of the molecule identified in the [$^2H_6$]DMSO ^{1}H NMR spectrum.

Among the labile protons, one labile hydrogen was suggested to be acidic, or sterically hindered, from the observation that this active hydrogen resisted acetylation under mild conditions.



Scheme 1 MS fragmentation pattern of **1**

The introduction of five bulky TMS groups into hydantocidin **1** ruled out the possibility of steric hindrance and the most probable structure was, therefore, considered to be **1**, where the amide proton at 3′-N would be both acidic and resistant to the acetylation. On treating this acetylated derivative with diazomethane, one methyl group was found to be introduced into the 3′-N position by the analysis of its MS spectrum.

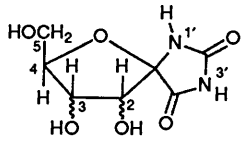
The structure of the major fragment ions were elucidated by analysis of tandem mass (MS/MS) spectra, and the fragmentation scheme depicted in Scheme 1 was obtained, which supported the proposed structure.

The Relative Configuration and Solution Conformation of Hydantocidin **1**.—The qualitative use of NOE spectroscopy is now a well established and commonly used technique for the solution of sterochemical problems in organic molecules. The NOEs were observed at 2-H, 3-H and 4-H on irradiating 5-H and 5′-H, while the enhancement of the signals 2-H, 5-H and 5′-H, were observed on the irradiation of 3-H. Thus the configuration of the five-membered ring of **1** could be assigned as that of ribofuranose. The relation of the hydantoin moiety with the sugar-like moiety was determined using the *N*-methylated derivative **2**, where the *N*-methyl proton signal at 2.93 ppm was enhanced on irradiating 2-H and *vice versa*.

On the other hand, the present authors and other groups have shown that quantitative analysis of the relaxation parameters, NOEs and longitudinal relaxation times (T_1), can give useful information about molecular geometry in solution.[3,4] This method was applied to hydantocidin not only because of interest in its solution conformation, which may be responsible for its herbicidal activity, but also in order to confirm the configuration derived from the qualitative interpretation of the NOE data.

The Quantitative Analysis of NOE *Intensities and* T_1 *Values*.—In solution, the major contribution to longitudinal relaxation may be considered to be intramolecular dipole–dipole interaction.[5] Thus, when molecular tumbling can be assumed to be isotropic and its internal motion negligible, the NOEs and T_1 values can be described as a function of the interatomic distances in a given molecular model. That is to say, a set of the observed NOE and/or T_1 data is equivalent to a set of inter-proton distances within a molecule. If reasonably good agreement between the observed and the calculated NOEs and/or T_1 values can be obtained in one of the assumed geometries, this geometry may be assigned to the actual geometry of the molecule under consideration.

With RSCA software, the expected NOEs and T_1 values for the given geometries are calculated by solving the Bloch equations numerically, where the molecular motion is represented by two effective correlation times, one applied to the inter-proton vectors involving methyl protons (τ_c^{eff}), and one applied to the rest of the inter-proton vectors (τ_c). The terms τ_c^{eff} and τ_c are treated as adjustable parameters and fixed in the course of the calculation so as to attain the best fitting to the observed parameters.[3] Details of the procedure for treating the internal motion of methyl groups are described in refs. 3*a* and 4.



2,3-OH	1′β	1′α
Ribose	Cr	Cr′
Arabinose	Ca	Ca′
Xylose	Cx	Cx′
Lyxose	Cl	Cl′

Fig. 1 The possible combination of the configurations at C-2′, C-3 and the spiro juncture, and their notation referred in the text. The suffix *r*, *a*, *x*, *l* were added by the analogy with the pentose having the same configurations. The spiro juncture is differentiated by the presence or the absence of a prime.

Definition of the Geometry.—To determine the configuration and the conformation of hydantocidin, the simulation of NOEs and T_1 values should cover the model geometries for all possible combinations of chirality at the asymmetric carbons from C-1 to C-3, in each of which the conformational space must be searched for the furanose ring and the rotamer about C(4)–C(5).

To define the eight possible relative configurations, the notation depicted in Fig. 1 was used. The configuration of the sugar moiety was named by analogy with the configuration of the corresponding pentose, where a D-sugar was tentatively assumed. For example, the notation Cr means that the sugar moiety has the same configuration as that of ribose and the spiro junction at 1′-N is in the β-orientation, and Cr′ indicates that the configuration at 1′-N is in the α-orientation.

The pseudo-rotation of the five-membered ring is described by the formula,[6] where the *j*-th torsion angle is determined by

$$\varphi_j = \varphi_m \cdot \mathrm{COS}(p + \tfrac{4}{5}\pi \cdot j) \quad j = 0\text{–}4$$

the maximum torsion angle φ_m and phase angle P. Referring to the statistical distribution of φ_m and P seen in the X-ray derived conformation of nucleotides, the following ranges were selected; φ_m was fixed at 38.6°, the average of the X-ray derived conformation of the nucleotides,[7] and P was varied in the ranges 0–60° and 120–180° in steps of 20°. These P ranges corresponded to the C-2-*endo*, and C-3-*endo* conformations, respectively. The rotamer around C-4–C-5 was fixed to gg conformation, which was consistent with the observed vicinal coupling constants $J_{4,5} = J_{4,5'} = 4.2$ Hz.

Comparison of the Observed and Calculated Relaxation Parameters for N,N'-*Dimethylated Hydantocidin* **2**.—The T_1 measurement for **2** was carried out by the conventional inversion recovery method. The observed T_1 values are summarized in Table 2 with the calculated T_1 values at the phase angle which gave the best fit in each configurational model. The effective correlation times of $\tau_c = 2.2$ ns and $\tau_c^{eff} = 0.6$ ns were assumed in the present calculation.

As shown in Table 2, the best fit between observed and calculated T_1 values could be achieved, when the stereochemistry denoted by Cr (see Fig. 1) with the phase angle $P = 140°$ as assumed. The observed NOEs could also be reproduced by this model geometry (see Table 3). Thus, the configurational assignments discussed above could be confirmed quantitatively. The ribofuranose ring was concluded to be in C_2 *endo* conformation as depicted in Scheme 2.

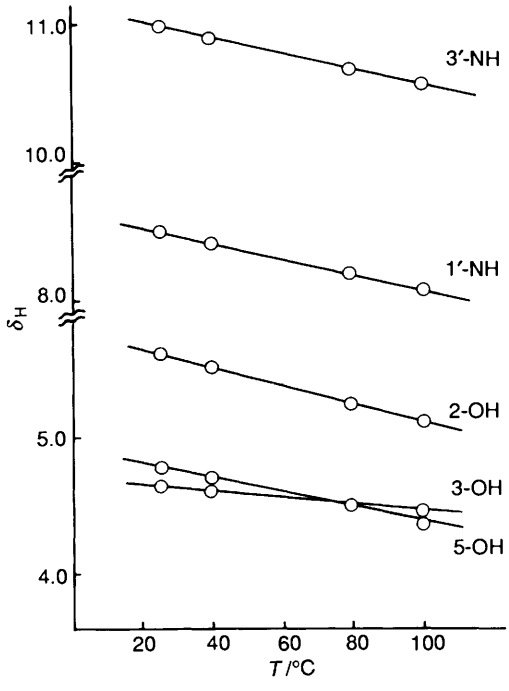


Fig. 2 The temperature dependence of the chemical shifts of the labile protons for **1** in [$^2H_6$]DMSO

Table 2 The observed and calculated T_1 (s) values for **2**

Proton	2	3	4	5,5'	1'-*N*Me	P^a	Q^b
$T_1^{obs.}$	3.7	4.6	5.5	1.3	4.2		
$T_1^{calc.}$							
Cr	4.5	4.7	5.7	1.4	3.2	140	0.15
Ca	16.1	6.1	5.5	1.4	3.4	160	0.36
Cx	7.5	5.5	3.8	1.5	3.3	60	0.34
Cl	9.8	4.7	3.8	1.5	3.4	60	0.36
Cr'	9.2	6.5	5.3	1.5	3.4	180	0.32
Ca'	2.5	13.4	4.6	1.5	2.9	180	0.43
Cx'	15.7	5.3	3.4	1.5	3.4	0	0.46
Cl'	2.1	4.5	3.1	1.5	2.9	0	0.53

[a] The phase angle P, at which the best fit between the observed and calculated T_1 values were obtained. [b] The Q value indicates the extent of the goodness of fit. The lower Q means the better agreement could be attained. For the definition of Q, see ref. 3.

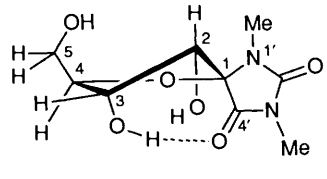
Table 3 The observed and calculated NOEs for **2**

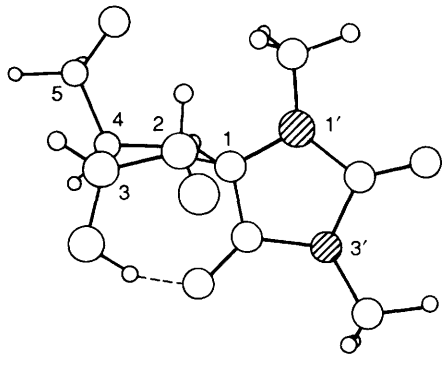
f_d (s)[a]	f_2 (3)	f_2 (1'-Me)	f_3 (4)	$f_{5,5'}$ (3)
Obs.	0.16	0.04	0.02	0.10
Calc.[b]	0.20	0.05	0.07	0.10

[a] The notation of f_d (s) means the fractional enhancement of H_s irradiating H_d. [b] The calculation was carried out based upon the geometry Cr with $P = 140$ and the same correlation times as used for the calculation of T_1 values.

Identification of the Hydrogen Bonding.—When the ribofuranose ring of hydantocidin is in C_2-*endo* conformation, the hydroxy group at C-3 is expected to be close enough to form a hydrogen bond with the carbonyl group at C-4'. The presence of this hydrogen bond was confirmed by the temperature dependence of the chemical shifts for the labile protons.

The chemical shifts of the labile protons involved in hydantocidin as a function of temperature in [$^2H_6$]DMSO are plotted in Fig. 2. The chemical shifts for the hydroxy and amide proton signals showed highfield shifts on elevating the temperature. The slopes of these lines were more than 0.004 ppm/deg for the protons other than that of 3-OH which was 0.0027 ppm/deg, a value which falls in the range expected for protons which are involved in hydrogen bonding.[8] This observation gave another line of the evidence supporting the configuration and the solution conformation of hydantocidin derived from the ^1H NMR relaxation data.





Scheme 2 A schematic representation of the conformation of hydantocidin **1** in solution (left), and the computer drawing of the geometry, in which the best reproducibility of the observed relaxation parameters could be obtained (right)

Experimental

Material.—Hydantocidin **1** was isolated from the fermentation broth of *Streptomyces hygroscopics* as reported previously.[1] The *N,N'*-dimethyl derivative was prepared by treating **1** with diazomethane in methanol for 2.5 h at ambient temperature.

MS.—Electron ionization mass spectra were acquired on a JEOL JMS-D300 mass spectrometer. FAB MS and FAB MS/MS spectra were obtained with a JEOL JMS-HX100 triple analyser tandem mass spectrometer (E/B/E configuration; *E*, electrostatic field; *B*, magnetic field).[9] The samples were dissolved in a glycerol matrix on the FAB probe tip and bombarded with 6 keV xenon atoms. FAB MS/MS spectra were obtained by collisional activation. The parent ions were collided with argon gas (75% beam reduction of parent ions) in the collison cell.

NMR Spectra.—The ^{1}H NMR spectra were recorded on a JEOL GX-400 NMR spectrometer operating at 399.6 MHz. *N,N'*-Dimethyl hydantocidin used for the NOE and the spin-lattice relaxation time measurements was degassed by repeating the freeze–pump–thaw cycles. The measurements of T_1 values were made by the inversion recovery method (180–*t*–90 pulse sequence) at ambient temperature (24.5 ± 0.5 °C) allowing *t* to vary in the range 0.1–5.0 s. 64 Free induction decays (FIDs) were accumulated for each experiment with 16 K data points; T_1 values were obtained by linear least-squares fitting to the initial part of the recovery curves (up to 50%). The NOE was measured in the conventional difference spectral mode.

NOE *and* T_1 *Simulation.*—The calculations of the NOE and T_1 were carried out using the RSCA program as described previously.[3] The coordinates of the geometries of hydantocidin used for the calculation were generated using the molecular modelling utility involved in RSCA.

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