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Assignment of the Stereochemistry of Spiroxamine by Two-Dimensional NMR Spectroscopy and Stereoselective Chemical Synthesis†

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ABSTRACT: Spiroxamine is a new powdery mildew fungicide in cereals consisting of four biologically active isomers (two diastereomers, four enantiomers). The four isomers were separated by preparative high-performance liquid chromatography (HPLC) on a chiral stationary phase. At this stage it was not possible to assign their stereochemistry. Using stereoselective synthesis starting with the corresponding chirally pure glycerol derivates, the configuration at the asymmetric center, could be fixed. The resulting diastereomers were separated by preparative HPLC. Using COSY, HSQC and NOESY NMR spectroscopy it was possible to assign the configuration of the amino residue relative to the cyclohexyl ring. The 600 MHz ^{1}H NMR spectra permitted a complete assignment of all proton signals. The stereochemical assignment is based on NOEs observed in the NOESY spectrum. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: ^{1}H NMR; ^{13}C NMR; spiro-acetal amine; spiroxamine; fungicide; stereochemistry

INTRODUCTION

Spiroxamine is a new cereal fungicide, developed by Bayer.[1] It consists of four isomers (two diastereomers, four enantiomers) (see Scheme 1). All four isomers have partly different biological activity and interact with different steps in sterol biosynthesis.[2,3] Owing to the absence of a heavy atom and the physico-chemical and chemical properties of the spiro-acetal it was not possible to obtain x-ray-structures of the isomers.

Using preparative high-performance liquid chromatography (HPLC) on a chiral stationary phase, it was possible to separate all four isomers. For the assignment of the configurations of the isomers by NMR spectroscopy it was necessary to know the stereochemistry at C-2, which was achieved by stereoselective synthesis.

The assignment of the configurations of the isomers by NMR spectroscopy in common solvents such as $CDCl_3$, CD_3CN or methanol-d_4 was not possible because of the major signal overlap in the region of the cyclohexyl protons. Benzene was found to be the best solvent to reduce the signal overlap. In addition, 600 MHz measurements facilitated the assignments.

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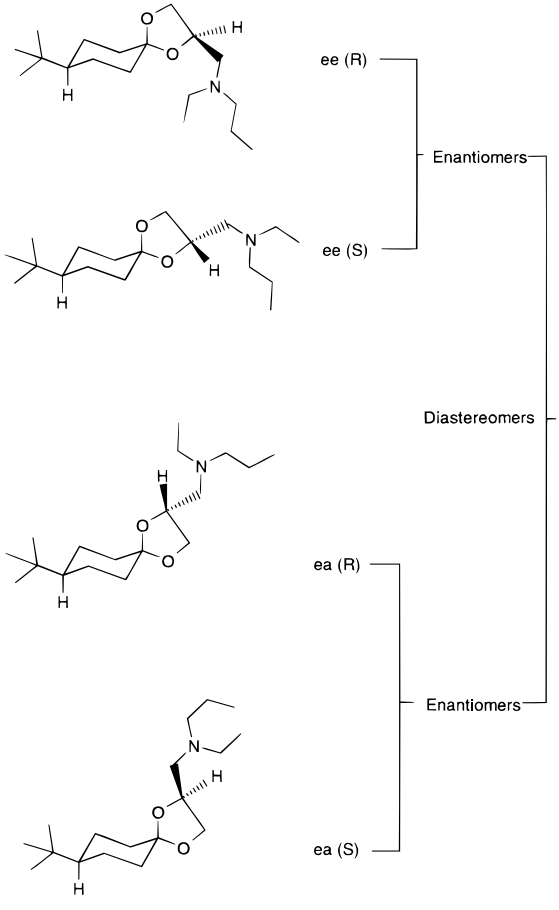
† Dedicated to Professor Buechel on the occasion of his 65th birthday.



Scheme 1. Isomers of spiroxamine.

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Results and Discussion

Enantioselective chemical synthesis

For the assignment of the configurations of the four isomers it was necessary to know the stereochemistry of C-2. Therefore, spiroxamine was enantioselectively synthesized using chiral glycerine derivatives [(*R*)- and (*S*)-2,2-dimethyl-1,3-dioxolane-4-methanol]. The synthesis of the *R*-isomer is shown in Scheme 2. The synthesis of the *S*-isomer starts with the corresponding *S*-dioxolane. The reactions are free of racemization, as shown by analytical HPLC on a chiral stationary phase. For each isomer the specific optical rotations were measured. The results are given in Table 1.

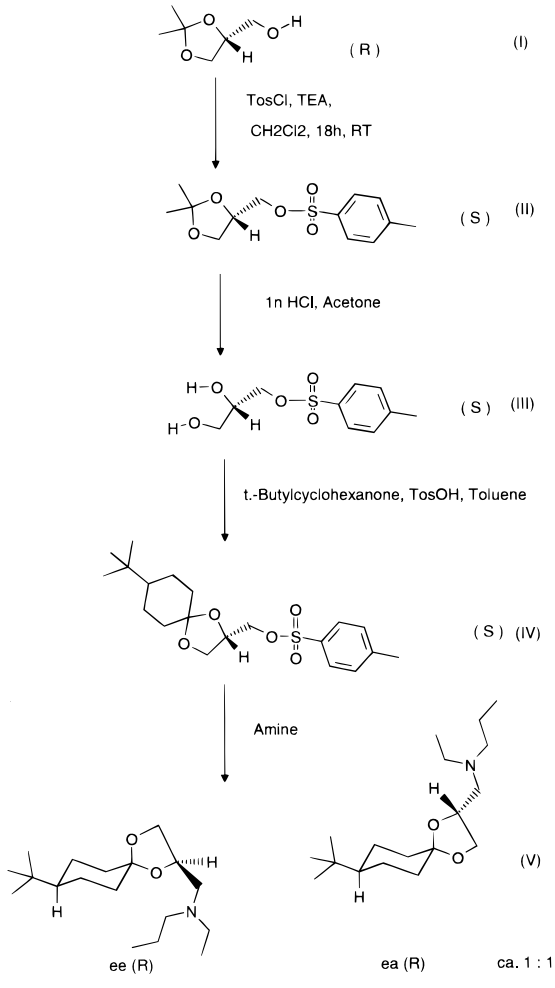
Table 1. Specific optical rotations of the four spiroxamine isomers

Peak	Configuration	$[\alpha]^{20}$ ($c = 1$)[a]
1	ea*S*	Hg_{546} + 43.5 Hg_{436} + 74.5 Hg_{365} + 117.8
2	ea*R*	Hg_{546} − 38.4 Hg_{436} − 65.9 Hg_{365} − 103.8
3	ee*S*	Hg_{546} + 36.8 Hg_{436} + 63.1 Hg_{365} + 99.5
4	ee*R*	Hg_{546} − 38.4 Hg_{436} − 66.3 Hg_{365} − 104.9

[a] Solvent, heptane; cell diameter, 1 cm.

HPLC

The assignment of each fraction was derived from a comparison of the retention times of the stereoselectively synthesized spiroxamine isomers. The first two peaks (4.97 and 5.57 min) and the last two peaks (6.14 and 6.69 min) contain isomers with the same position of the amino residue relative to the cyclohexyl ring. The absolute configuration of C-2 was determined by synthesis whereas the pseudo-axial or pseudo-equatorial position of the amino side-chain was elucidated from the NOESY spectra.



Scheme 2. Stereoselective synthesis of spiroxamine R-isomers.

NMR spectroscopy

The mixtures of the enantiomers of peaks 1 and 2 (ea*S*/ea*R*) and 3 and 4 (ee*S*/ee*R*) were used for the determination of the relative configuration of the amino residue [e and a denote the equatorial and axial orientation of the *tert*-butyl group (first letter) and the substituted carbon of the cyclic acetal (second letter)]. For the discussion of the NMR signals, the *S*- and *R*-enantiomeric

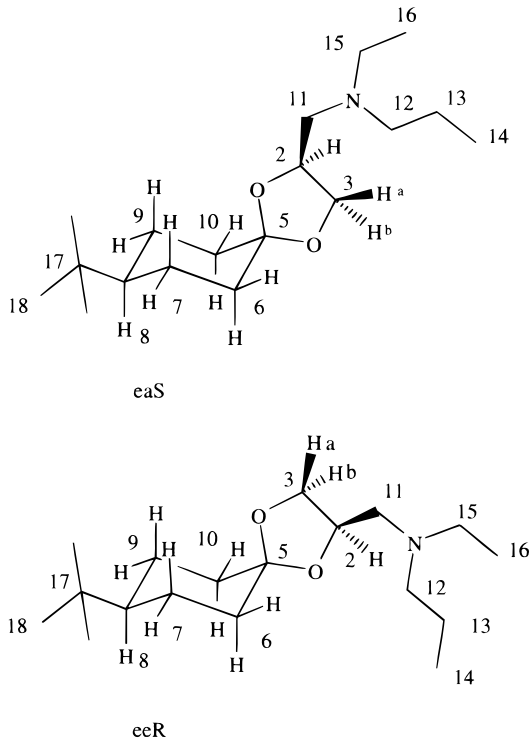


Figure 1. The eaS- and the eeR-isomers of spiroxamine.

structures are shown in Fig. 1. Of course, the *R*- and *S*-enantiomers cannot be distinguished by NMR.

First, a full assignment of all proton signals was necessary. The signal assignment was complicated owing to severe overlap of the cyclohexyl proton signals. Nevertheless, we succeeded by using COSY-DQF[4] (not shown) and GRASP-HSQC[5–7] NMR spectra (see Figs 2 and 3). In the HSQC spectrum the protons attached to a particular carbon are assigned and with the help of the phase-sensitive DQF-COSY spectrum the connection of the protons of one carbon with the protons of neighbouring carbons could be established. The signals of carbons 3,5 and 2,6 are clearly separated by their chemical shifts, which is in agreement with literature data.[8] The proton and carbon chemical shifts of the two diastereomers are summarized in Table 2.

The assignment of the pseudo-axial and pseudo-equatorial position of the amino residue was achieved by the observation of NOE peaks in the NOESY[9] spectrum between proton 2 and proton 9ax in the ea*S*-isomer and between protons 3a and 3b and protons 7ax and 9ax, respectively, in the ee*R*-isomer (Figs 2 and 3). Comparing the corresponding sections of the NOESY spectra of the ea- and ee-isomers, they can clearly be distinguished owing to an additional NOE signal for the ee-isomer. Assuming a minimum distance of 2.6 Å between the protons 2,3 and 6eq,10eq, the distance between protons 2 and 3 and protons 7ax and 9ax can

Table 2. ^{1}H and ^{13}C NMR chemical shifts of the ea- and the ee-isomers of spiroxamine

	δ_H (ppm)			δ_C (ppm)	
H	ea-Isomer	ee-Isomer	C	ea-Isomer	ee-Isomer
2	4.17	4.21	2	75.2	75.0
3a	3.70	3.69	3	68.7	68.7
3b	3.99	3.96	5	109.6	109.5
6ax	1.68	1.69	6	37.5	37.1
6eq	1.93	1.92	7	25.1	25.3
7ax	1.49	1.53	8	47.5	47.4
7e	1.67	1.67	9	25.1	25.0
8ax	0.94	0.94	10	35.6	35.8
9ax	1.48	1.47	11	57.2	57.6
9eq	1.67	1.65	12	56.7	56.7
10ax	1.49	1.52	13	20.9	21.0
10eq	1.93	1.96	14	12.0	11.9
11a	2.60	2.63	15	48.8	48.7
11b	2.46	2.47	16	12.3	12.3
12a	2.28	2.26	17	32.3	32.3
12b	2.34	2.32	18	27.8	27.8
13	1.35	1.34			
14	0.83	0.82			
15a	2.44	2.45			
15b	2.36	2.36			
16	0.90	0.90			
18	0.85	0.85			

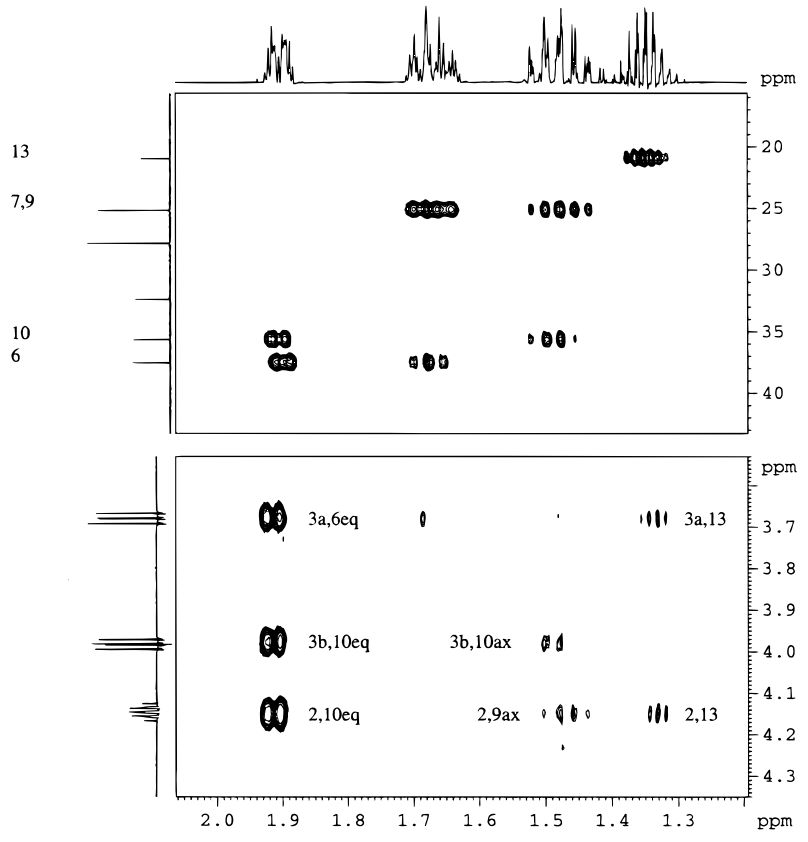


Figure 2. Expansions of the HSQC and NOESY spectra of the eaS -isomer.

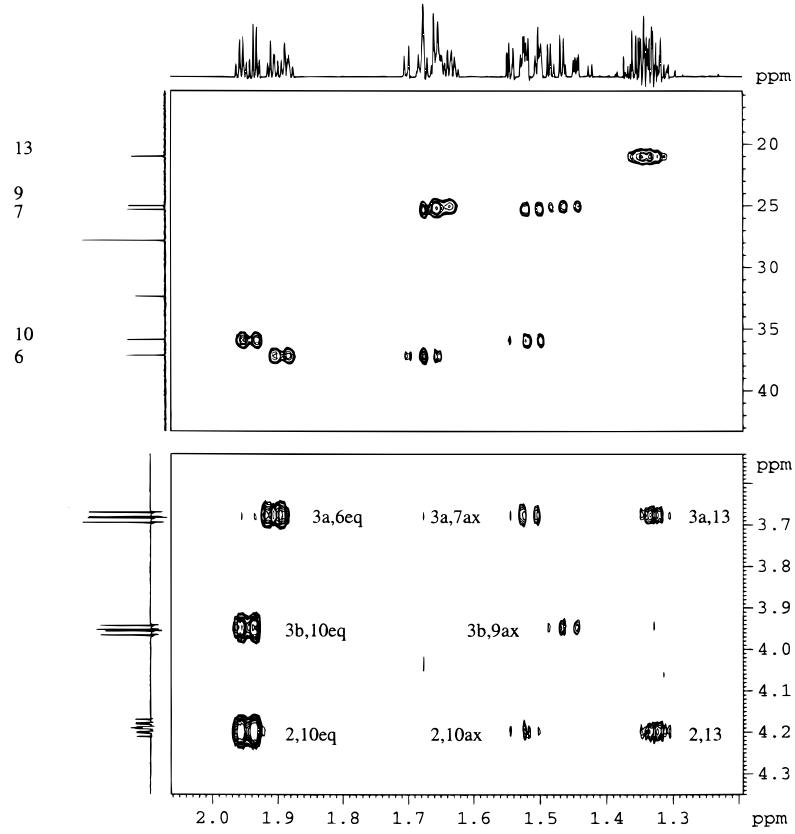


Figure 3. Expansions of the HSQC and NOESY spectra of the eeR-isomer.

be determined to be *ca.* 3.7 Å from the integrals of the corresponding NOE cross peaks in the NOESY spectrum.[10] The NOESY spectra showed different phases for the diagonal signals and the NOE cross peaks according to a positive NOE for molecules in the extreme narrowing limit. Comparison of the spectra of the ee- and ea-isomers shows a high field shift of the 6eq proton in the ee-isomer. This may be due to the shielding by methylene group 11. Because of the low intensity, the full multiplicity of the proton signals in the NOE cross peaks between the 7,9 and 2,3 protons could be observed.

Experimental

Compounds

To 5.3 g of (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol (**I**) in 40 ml of CH_2Cl_2, 6.95 ml of triethylamine and 8 g of toluenesulfonyl chloride at 0 °C were added and the solution was stirred for 16 h at room temperature. After hydrolysis and extraction, 10.1 g (88.2% yield) of (*S*)-2,2-dimethyl-1,3-dioxolane-4-methanoltoluene-4-sulfonate (**II**) were obtained. A 6 g amount of **II** in 5 ml of acetone and 15 ml of 1 M HCl were heated at 90–95 °C for 30 min. The solvent was evaporated under vacuum and, after aceotropic distillation with acetonitrile, the residue was dissolved in 50 ml of CH_2Cl_2. The solution was dried and after distillation of the solvent 7.6 g (yield 95.4%) of the diol (**III**) were obtained. A 4.8 g amount of **III** with 3 g of 4-*tert*-butylcyclohexanone, 10 ml of *n*-butanol and 0.5 g of *p*-toluenesufonic acid in 120 ml of toluene were heated for 4 h in a water separator. The organic phase was washed with NH_4CO_3 solution and water, dried and evaporated under vacuum. A 5 g amount (yield 86.4%) of the *S*-configured cyclohexanonacetal (**V**) in a diastereomeric ratio of 42.4:47.1 (as determined by GC–MS) were obtained. The *R*-configured isomers were obtained by an analogous procedure by starting with (*S*)-**I**.

Analytical HPLC was carried out on an HP 1090 system with a Chiracel OD-H 5 μm column (250 × 4.6 mm i.d.) with *n*-heptane–propan-2-ol (99.9:0.1) as eluent at a flow rate of 1 ml min^{-1}. The injection volume was 5 μl of a 0.1% solution. UV detection at 210 nm was used. Preparative HPLC was run under the same conditions except for the column size (250 × 20 mm i.d.) and the injection volume (250 μl).

Spectra

All NMR experiments were performed at 298 K for solutions of 20 mg (^{1}H-detected experiments) or 100 mg (^{13}C spectra) of compound dissolved in 0.7 ml of benzene-d_6 on a Bruker DMX-600 instrument with a 5 mm dual probe head or a 5 mm inverse-triple resonance (^{1}H, ^{13}C, ^{15}N) probe head, both equipped with a shielded *Z*-gradient coil. ^{1}H NMR spectra were measured with a spectral width of 14.3 kHz on a data size of 128K. The acquisition time was 3.56 s and relaxation delay 0.4 s. The number of scans was 16. A Gaussian function was applied prior to Fourier transformation, which was done on a data size of 64K. The ^{13}C spectra were recorded with composite pulse decoupling with a spectral width of 40 kHz on a data size of 256 K. The

acquisition time was 3.3 s and relaxation delay 1.5 s. The number of scans was 128. Exponential multiplication was applied before Fourier transformation, which was done on a data size of 128K.

The phase-sensitive double quantum filtered COSY spectra resulted from a 256 × 4096 data matrix size with 16 scans per t_1 increment. The recycle time was 2.98 s and the total experiment time was 3.5 h. A Gaussian multiplication was done in F_2 and a non-shifted qsine function was applied in F_1. Zero-filling in F_1 resulted in a 2K × 2K matrix after Fourier transformation.

The gradient enhanced HSQC spectra resulted from a 256 × 4096 data matrix size with four scans per t_1 increment. A spectral width of 3 kHz in F_2 and 12.2 kHz in F_1 was recorded. The delays were set to 1.7 (1/4J) and 1.1 ms (1/6J) and the recycle time was 1.8 s, which resulted in a total experiment time of 0.5 h. The gradient pulses (80:20:80:−20) were sine-shaped pulses with a duration of 1 ms. A shifted qsine multiplication was used prior to Fourier transformation in F_2 and an unshifted qsine was used in F_1. Fourier transformation was done on a 2K × 2K data matrix.

The phase-sensitive NOESY spectra resulted from a 256 × 4096 data matrix with 16 scans per t_1 increment. The recycle time was 5.2 s and the total measurement time was 5.9 h. The mixing time was set to 0.7 s. A shifted qsine multiplication in both dimensions was applied before Fourier transformation, which resulted in a 1K × 2K data matrix.

REFERENCES

1. W. Krämer, J. Weissmüller, D. Berg and St. Dutzmann (to Bayer), *Ger. Pat.* DE 3735555 (1988); *Chem. Abstr.* **110**, 23868t (1989).
2. D. Berg, G. Holmwood, R. Pontzen, J. Weissmüller and R. Tiemann, *J. Prakt. Chem.* **335**, 569 (1993).
3. W. Krämer, J. Weissmüller, D. Berg, St. Dutzmann and P. Reinecke (to Bayer), *Ger. Pat.* DE 3730499 (1988); *Chem. Abstr.* **109**, 2300792h (1988).
4. A. E. Derome and M. P. Williamson, *J. Magn. Reson.* **88**, 177 (1990).
5. A. G. Palmer, J. Cavanagh, P. E. Wright and M. Rance. *J. Magn. Reson.* **93**, 151 (1991).
6. L. E. Kay, P. Keifer and T. Saarinen, *J. Am. Chem. Soc.* **114**, 10663 (1992).
7. J. Schleucher, M. Sattler and C. Griesinger, *Angew. Chem.* **105**, 1518 (1993).
8. *C-Search Carbon-13 Data Base.* Sadtler Software, Bio-Rad Laboratories, Richmond, CA 1991–94.
9. G. Bodenhausen, H. Kogler and R. R. Ernst, *J. Magn. Reson.* **58**, 370 (1984).
10. N. H. Andersen, H. L. Eaton and X. Lai, *Magn. Reson. Chem.* **27**, 515 (1989).

