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Use of ^{15}N-HMBC NMR Techniques to Determine the Orientation of the Steroidal Units in Ritterazine A[1]

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Abstract: Orientation of the two steroidal units about the pyrazine ring in ritterazine A, a cytotoxic dimeric steroid from the tunicate *Ritterella tokioka*, was determined by ^{15}N-HMBC spectroscopy.

The ritterazines, highly cytotoxic metabolites of the Japanese tunicate *Ritterella tokioka*,[2] encompass two polyoxygenated hexacyclic steroidal units joined through a pyrazine ring. These structural features are reminiscent of the cephalostatins isolated from the Indian Ocean hemichordate *Cephalodiscus gilchristi*.[3] The most difficult aspect of the structural elucidation of this class of compounds lies in determining the orientation of the steroidal units with respect to the pyrazine ring, as no ^{1}H-^{1}H or ^{1}H-^{13}C connectivities were observed between the atoms of the two steroidal units. Pettit and coworkers determined the orientation in cephalostatin 1[4] by X-ray crystallography; they proposed the same orientation for the other congeners by analogy with cephalostatin 1. We prepared *N*-methyl derivatives of ritterazine B (**1**) in order to use *N*-methyl groups for NOESY experiments, which was successful, and revealed that the orientation of the steroidal units in **1** is identical with that in cephalostatin 1.[5] Since ritterazine C is derived from ritterazine B (**1**) by mild acid treatment, identical steroidal orientation is implied, but orientation of the other ritterazines had to be established.[6]

Since ^{15}N chemical shifts are more widely dispersed than those of ^{13}C or ^{1}H as evidenced in protein NMR studies,[7] the two nitrogens in the pyrazine ring were expected to give rise to different chemical shift values, thus allowing long-range ^{1}H-^{15}N coupling data to be used to solve our problem. Thanks to the recently developed pulsed field gradient technique which significantly decreases the *t1*-noise,[8,9] we have succeeded in applying ^{15}N-HMBC spectroscopy to determine the orientation of the two steroidal units in ritterazine A (**2**), which is the subject in this paper.

We first ran a ^{15}N-HMBC experiment for ritterazine B, to validate this methodology. The τ delay was set to 60 ms to observe ^{1}H, ^{15}N long-range coupling.[10] Cross peaks were observed between H_2-1 and H_2-1' and nitrogens having different ^{15}N chemical shifts, which was consistent with the previously determined orientation.[11] The ^{15}N-HMBC spectrum of ritterazine A (**2**) provided virtually identical results (Fig. 1), allowing us to conclude that the orientation of the steroidal units in **2** was the same as that in **1**.[12]

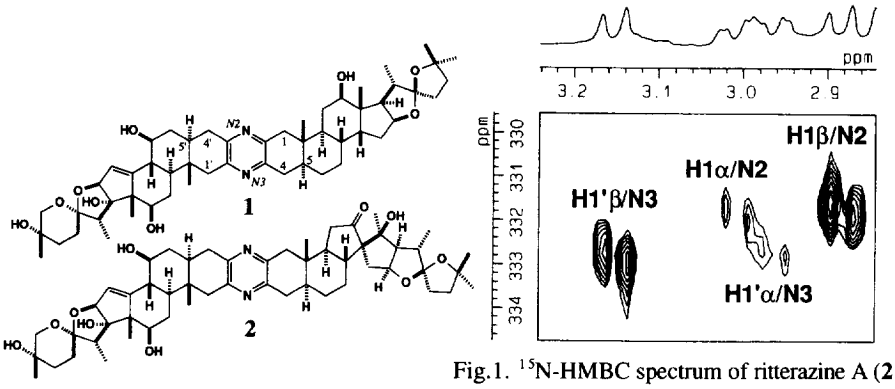

Fig.1. ^{15}N-HMBC spectrum of ritterazine A (**2**).

Thus, ^{15}N-HMBC experiments aided by the pulsed field gradient technique made it possible to determine the orientation of the steroidal units in ritterazine A. Without this technique we would have had to run a cumbersome sequence of experiments, i.e., *N*-methylation of the pyrazine nitrogens, causing isomerization of the terminal 5,6-spiroketal, HPLC separation of the resulting four isomers, measurement of COSY and HMQC[13] spectra to assign NMR signals, and finally a NOESY spectrum to observe the cross peaks from the *N*-methyl groups.[5] With the recent improved stability of NMR spectrometers, the time required to obtain an ^{15}N-HMBC spectrum has been shortened considerably. Without doubt, ^{15}N-HMBC experiments will become popular in structure determination of nitrogenous natural products.

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11. Probably due to further coupling to H5 and H5', H4 and H4' gave no HMBC correlations with nitrogens.
12. Ritterazines A (ca. 20 mg) and B (ca. 29 mg) were dissolved in pyridine-d_5 (0.2 mL). The ^{15}N-HMBC spectrum of ritterazine A was recorded on a 600 MHz NMR spectrometer at 300K. Over a period of 16 h 512 increments with 64 scans per increment were acquired. The gradient strength values are G1:G2 = 4.94:1.0 G cm^{-1}. Data sets consisted of 512 FIDs (*t1*) and 1024 data points in *t2*. A relaxation delay interval of 1.6 s was set for each pulse sequence. The spectrum was zero-filled to 1024 points in *t1* prior to Fourier transformation. ^{15}N chemical shifts were referenced to 0 ppm for NH_3.
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