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Impact of the $^{1}H,^{15}N$-HMBC Experiment on the Constitutional Analysis of Alkaloids

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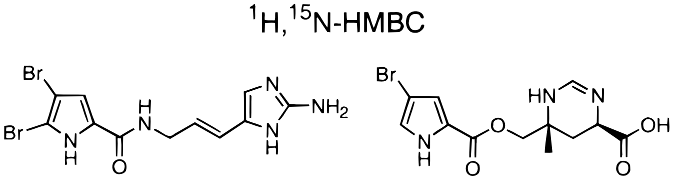
ABSTRACT



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The $^{1}H,^{15}N$-HMBC experiment is a very useful tool for the structure determination of alkaloids. In conjunction with the computer program COCON, we show how $^{1}H,^{15}N$-HMBC-derived connectivity information can assist in the assignment of constitution, by greatly reducing the number of possible structures compatible with a given data set. The method is illustrated for the marine natural products oroidin (1) and manzacidin A (2).

The HMBC (heteronuclear multiple bond correlation) experiment introduced in 1986 by Summers and Bax[1] is still the most important NMR experiment for the structure elucidation of natural products. The HMBC experiment is usually applied to proton−carbon correlations in natural products chemistry. In this Letter, the value of this experiment as applied to ^{15}N in natural products chemistry[2,3] is illustrated and quantified with the help of the computer program COCON.[4] It is shown how $^{1}H,^{15}N$-HMBC-derived connectivity information reduces the number of constitutions compatible with the data set. The $^{1}H,^{15}N$-HMBC experiment is only about three times less sensitive than the $^{1}H,^{13}C$-HMBC one as only the smaller natural abundance of ^{15}N to ^{13}C is of relevance.

The constitution of a new noncrystalline natural product is frequently considered elucidated as soon as an assigned structure is in accordance with the molecular formula and with connectivity information derived from 2D NMR experiments. But the most reliable approach is to examine all possible structures and find conclusive evidence for the elimination of some structures.[5] The computer program COCON (constitutions from connectivities), which was re-

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(1) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.

(2) For a review on the $^{1}H,^{15}N$-HMBC experiment, see: Koshino, H.; Uzawa, J. *Kagaku Seibutsu* **1995**, *33*, 252−258.

(3) (a) Crouch, R. C.; Martin, G. E. *J. Heterocycl. Chem.* **1995**, *32*, 1665−1669. (b) Martin, G. E.; Crouch, R. C.; Andrews, C. W. *J. Heterocycl. Chem.* **1995**, *32*, 1759−1766. (c) Martin, G. E.; Crouch, R. C. *J. Heterocycl. Chem.* **1995**, *32*, 1839−1842. (d) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1996**, *37*, 1447−1448. (e) Kolehmainen, E.; Puchala, A.; Suontamo, R.; Rasala, D.; Lysek, R. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2383−2387. (f) Martin, G. E.; Crouch, R. C.; Sharaf, M. H. M.; Schiff, J., Paul L. *J. Nat. Prod.* **1996**, *59*, 2−4. (g) Farley, K. A.; Walker, G. S.; Martin, G. E. *Magn. Reson. Chem.* **1997**, *35*, 671−679. (h) Lin, Q.; Ball, G. E.; Bishop, R. *Tetrahedron* **1997**, *53*, 10899−10910. (i) Hadden, C. E.; Martin, G. E. *J. Nat. Prod.* **1998**, *61*, 969−972.

(4) (a) Lindel, T.; Junker, J.; Köck, M. *J. Mol. Model.* **1997**, *3*, 364−368. (b) Lindel, T.; Junker, J.; Köck, M. *Eur. J. Org. Chem.* **1999**, 573−577. (c) Köck, M.; Junker, J.; Maier, W.; Will, M.; Lindel, T. *Eur. J. Org. Chem.* **1999**, 579−586. (d) Junker, J.; Maier, W.; Lindel, T.; Köck, M. *Org. Lett.* **1999**, *1*, 737−740. COCON calculations can be carried out on the Internet under http://cocon.org.chemie.uni-frankfurt.de.

(5) Faulkner, D. J. In *Marine Biotechnology*; Attaway, D. H., Zaborsky, O. R., Eds.; Plenum: New York, 1993; Vol. 1, pp 459−474.

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cently described, is able to generate all possible structures for unknown compounds compatible with their 2D NMR data and molecular formula.[4] Besides ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HMBC, and 1,1-ADEQUATE correlations, Cocon is also able to use ^{1}H,^{15}N-HMBC correlations. The ^{1}H,^{15}N-HMBC correlations are implemented in the same way in Cocon as the ^{1}H,^{13}C-HMBC correlations. The only difference to the ^{1}H,^{13}C-HMBC correlations is that 1,1-ADEQUATE correlations cannot be used in order to distinguish between two- and three-bond correlations. Furthermore, the ^{15}N chemical shifts are not used in the Cocon program. The impact of the ^{1}H,^{15}N-HMBC correlations will be demonstrated for oroidin (**1**)[6] and manzacidin A (**2**).[7]

The first example to be discussed is oroidin (**1**), a marine alkaloid first isolated in 1971 from the sponge *Agelas oroides*.[6] The sample used in this investigation was isolated from *Agelas clathrodes* (0.4% of dry weight of the particular specimen). Several different 2D NMR experiments were acquired to examine the constitution of oroidin (**1**). Furthermore, data sets of different quality (for details, see footnote a of Table 1) were obtained for **1** in order to investigate the influence of correlation data sets on the number of possible structures. If the data set consisted of only a small number of ^{1}H,^{13}C-HMBC correlations (e.g., about 30% of the theoretical maximum), the number of possible constitutions was exorbitant. For this case, data set A considering ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HMBC correlations, Cocon generated 234336 possible solutions. With increasing numbers of correlations, the number of possibilities rapidly decreased: 27142 for data set B, 690 for C, and 60 for D (see Table 1). All theoretically possible $^2J_{NH}$ and $^3J_{NH}$ correlations for **1** were observed in the ^{1}H,^{15}N-HMBC spectrum. The traces of four nitrogens from the ^{1}H,^{15}N-HMBC spectrum are

Table 1. Cocon Results for Oroidin (**1**) and Manzacidin A (**2**)

		no. of structures generated from	
compd (data set[a])	no. of HMBC correlations[b]	COSY, HMBC	COSY, HMBC and ^{15}N-HMBC
1 (A)	9 (29%)	234336	10
1 (B)	18 (58%)	27142	10
1 (C)	23 (74%)	690	10
1 (D)	26 (84%)	60	6
2[c]	18 (51%)	190	8

[a] Data set A of **1** (^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HMBC) was obtained from measurements on a Varian VXR 500 spectrometer (DMSO-d_6). The ^{1}H,^{13}C-HMBC for data set A was the only one without gradients. The ^{1}H,^{15}N-HMBC data were taken from data set B of **1**. Data sets B, C, and D were obtained from measurements on a Bruker DRX600 spectrometer. Data set B was obtained from a sample of oroidin (**1**) in DMSO-d_6 (49.9 mg of **1** in 0.45 mL). For data set C, the ^{1}H,^{13}C-HMBC correlations obtained from a 100 mM sample of oroidin (**1**) in a 3:2 mixture of chloroform-d_1/DMSO-d_6 (49.6 mg of **1** in 0.50 mL) were combined with the correlation data of data set B. In data set D, the ^{1}H,^{13}C-HMBC results obtained for 4,5-dibromopyrrole-2-carboxylic acid (55.0 mg in 0.50 mL of DMSO-d_6, respectively, 63.9 mg in 0.60 mL of chloroform-d_1/DMSO-d_6 (1:1)) which represents the left part of oroidin and data set C are combined. The ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HMBC, and ^{1}H,^{15}N-HMBC correlations for all data sets (A−D) are given explicitly in the Supporting Information. [b] The percentage of the theoretical maximum is given in parentheses. The theoretical maximum includes all possible two- and three-bond correlation from protons (also protonated heteroatoms, see **1** and **2**) to carbons. [c] The Cocon calculation time was 2 min 9 s for COSY and HMBC with the version used in refs 4b and 4c; the time is reduced to 3.9 s with the new version (ref 4d). Including the ^{1}H,^{15}N-HMBC data, the calculation time is reduced to 7.3 s (0.1 s). All Cocon calculations were carried out on a SGI R10000 computer, 195 MHz processor, the source code was 64-bit optimized.

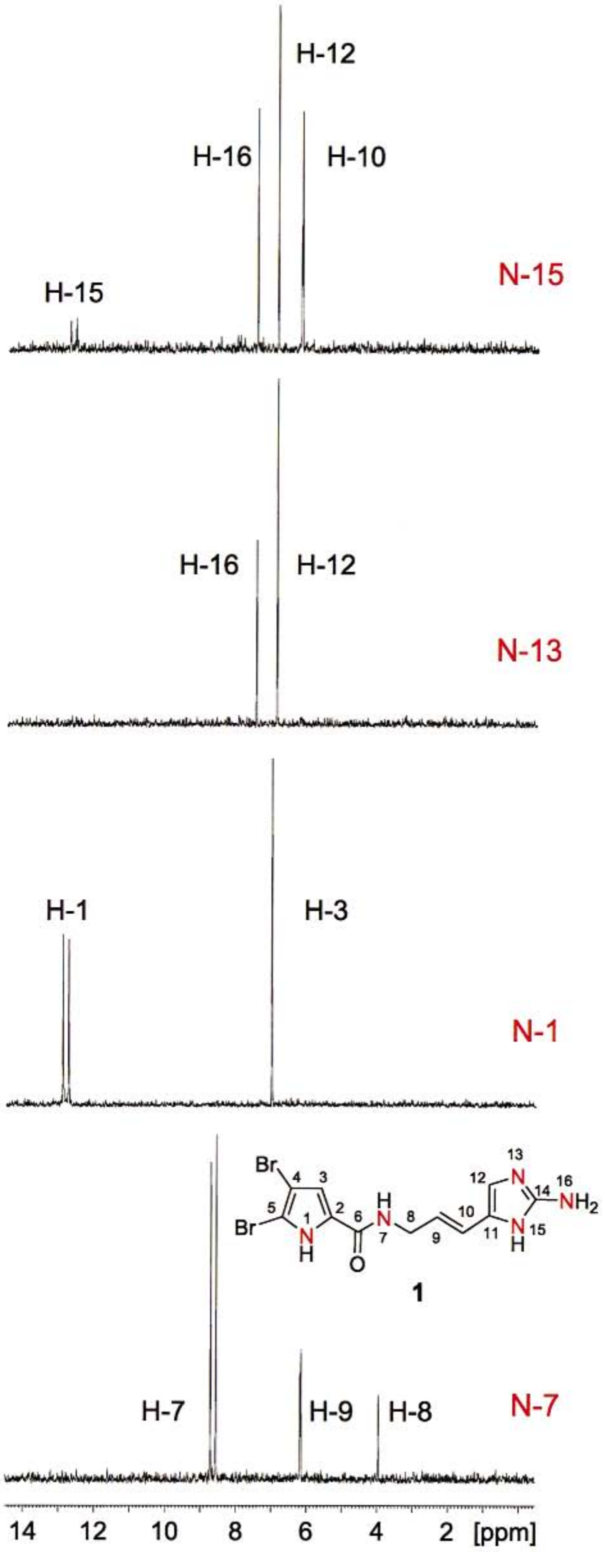

Figure 1. Four rows of the 600 MHz ^{1}H,^{15}N-HMBC spectrum of oroidin (**1**). The delay for the evolution of the long-range couplings was set to 100 ms.[8]

shown in Figure 1. The reduction of the number of possible structures by the introduction of the ^{1}H,^{15}N-HMBC correlations is dramatic, especially for the poorly defined data sets A and B (see Table 1). The 10 solutions derived from data set C consist of two groups of assignment isomers (i.e., only five different constitutions were obtained, Figure 2). The 2-aminoimidazol substructure is present in all proposed constitutions. This part of the molecule is especially poorly defined when omitting the ^{1}H,^{15}N-HMBC-derived connectivity information. For all structures a ^{13}C chemical shift calculation using the computer program SpecEdit[9] was performed (see Table 2). The deviations between the calculated and experimental ^{13}C chemical shifts were calculated for each carbon and averaged over all carbons of the molecule (⟨Δδ(^{13}C)⟩). The structures **1**-3 and **1**-6 are favored on the basis of these calculated deviations of δ(^{13}C). The results are even more clearly defined if the carbons C-9 to C-14 from the 2-aminoimidazol part (which are identical for all five structures) are not considered. The final decision between the 3,5-dibromo (**1**-6)[10] and the 4,5-dibromo substitution (**1**-3) is carried out using the ^{13}C chemical shift of C-4 (98 ppm) which is only fulfilled for structure **1**-3.

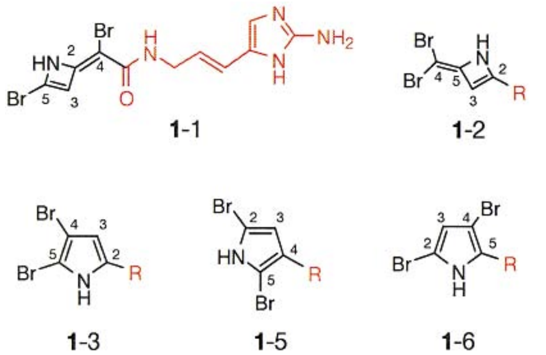
1-1
1-2
1-3
1-5
1-6

Figure 2. Final five structural proposals of oroidin (**1**) generated by COCON.

Table 2. SpecEdit Results for the Five Structural Proposals of Oroidin (**1**) Given in Figure 2

no. of atom	δ(^{13}C) [ppm]	**1**-3	**1**-6	**1**-1	**1**-2	**1**-5
⟨Δδ(^{13}C)⟩		2.7	3.5	6.1	6.7	6.8
⟨Δδ(^{13}C)⟩^{a}		2.0	3.3	7.7	8.7	8.8
C-2	128.0	1.0	0.0	22.0	12.0	−23.0
C-3	113.0	2.0	2.0	3.0	1.0	0.0
C-4	98.0	2.0	10.0	19.0	−7.0	24.0
C-5	105.0	1.0	−4.0	1.0	22.0	−1.0
C-6	159.0	3.0	−1.0	−1.0	7.0	5.0
C-8	40.0	−3.0	−3.0	−3.0	−3.0	0.0
C-9	127.0	−4.0	−4.0	−4.0	−4.0	−4.0
C-10	116.0	5.0	5.0	5.0	5.0	5.0
C-11	125.0^b	–	–	–	–	–
C-12	111.0	4.0	4.0	4.0	4.0	4.0
C-14	148.0	2.0	2.0	2.0	2.0	2.0

[a] Average over C-2 to C-8, the carbon atoms C-9 to C-14 are not considered. [b] Each carbon in SpecEdit is defined by a six-sphere HOSE code which defines the neighbors in the six spheres around the carbon. If no HOSE code larger or equal than two spheres was found, SpecEdit calculates δ(^{13}C) with an increment system which was not considered here. Therefore, no chemical shift is given.

The second example is manzacidin A (**2**), an alkaloid isolated from the Okinawan sponge *Hymeniacidon* sp.[7] In addition to its 4-bromopyrrole-2-carbonyl unit, it possesses a tetrahydropyrimidine moiety. With the experimental ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HMBC data as well as all hybridization states of the atoms as described by Kobayashi et al., COCON generated 190 possible constitutions. Since no ^{15}N data were published and we had no sample of **2**, a theoretical ^{1}H,^{15}N-HMBC data set was used. Given that all theoretically possible ^{1}H,^{15}N-HMBC correlations were observed for oroidin (**1**), the same was assumed for manzacidin A. Using these data, the number of proposed constitutions by COCON is reduced from 190 to 8 (see Figure 3). For all structures, a SpecEdit calculation was performed, resulting in the subsequent ranking: **2**-5 ⟨Δδ(^{13}C)⟩ = 3.0 ppm, **2**-6 (6.3 ppm), **2**-7 (6.4 ppm), **2**-2 (10.0 ppm), **2**-1 (11.4 ppm), **2**-4 (12.8 ppm), **2**-3

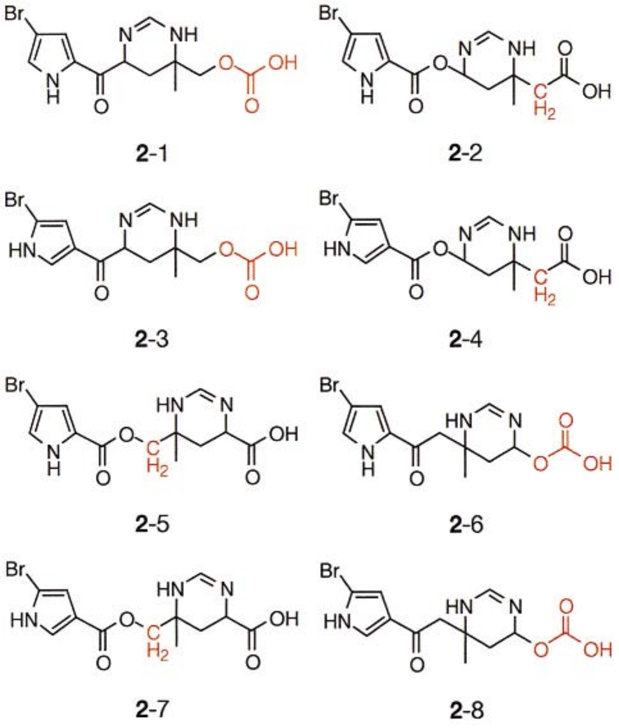
2-1
2-2
2-3
2-4
2-5
2-6
2-7
2-8

Figure 3. Final eight structural proposals of manzacidin A (**2**) generated by COCON.

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(6) (a) Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc., Chem. Commun.* **1971**, 1129−1130. (b) Garcia, E. E.; Benjamin, L. E.; Fryer, R. I. *J. Chem. Soc., Chem. Commun.* **1973**, 78−79. (c) Walker, R. P.; Faulkner, D. J.; van Engen, D.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 6772−6773.

(7) Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. *J. Org. Chem.* **1991**, *56*, 4574−4576.

(14.8 ppm), and **2**-8 (19.8 ppm). The structural proposals **2**-1, **2**-3, **2**-6, and **2**-8 can be excluded because carbonic acid hemiesters are not stable under laboratory conditions (see Figure 3). Structures **2**-5 and **2**-7 can be distinguished from **2**-2 and **2**-4 by considering the proton chemical shift of the exocyclic methylene group (see Figure 3): a proton chemical shift greater than 4.0 ppm is expected only for structures **2**-5 and **2**-7 (the experimental values are 4.15 and 4.26 ppm). The constitution of **2**-5 and **2**-7 differ only in the substitution pattern of the pyrrole ring. The results from SpecEdit clearly favor **2**-5, which represents the correct constitution.

The two bromopyrrole alkaloids examined here clearly show the importance of the ^{1}H,^{15}N-HMBC experiment for a comprehensive constitutional analysis. The combination of COCON and SpecEdit allows a systematic investigation of structural proposals based on ^{13}C chemical shift calculations. On the basis of our findings, the ^{1}H,^{15}N-HMBC experiment should be included in the routine structure elucidation of alkaloids. This is especially true with the recent introduction of cryogenic probes which overcome the reduced sensitivity of this experiment.

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Supporting Information Available: (1) Some comments about ^{15}N NMR spectroscopy, history of the ^{1}H,^{15}N-HMBC experiment, and some practical comments on the ^{1}H,^{15}N, HMBC experiment; (2) COCON input files for oroidin (**1**) and manzacidin A (**2**) including all correlation data used in the COCON analysis and numbered structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The ^{1}H,^{15}N-HMBC spectra were recorded with the standard Bruker pulse program (inv4gslrnd). The delay for the heteronuclear long-range couplings was set to 100, 150, and 200 ms (16 acquisitions and 512 increments with a spectral width of 100 ppm in F_1). The acquisition time was 227.8 ms, and the relaxation delay was set to 2.5 s.

(9) Maier, W. In *Computer-Enhanced Analitical Spectroscopy*; Wilkins, C. L., Ed.; Plenum: New York, 1993; Vol. 4, pp 37−55.

(10) This is the correct nomenclature, although the carbon numbering in Figure 2 is different.