NMR Studies of Phakellins and Isophakellins

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Phakellins **3** and **4** and isophakellins **5** and **6** are tetracyclic pyrrole-imidazole alkaloids. Their structure elucidation is herein reviewed using state-of-the-art NMR methods. Special emphasis has been given to the application of ADEQUATE NMR pulse sequences. Furthermore, ^{15}N NMR chemical shifts, proton T_1 relaxation times, and $^1J_{CH}$ coupling constants were determined.

Sponges of the order Agelasida and Halichondrida are a rich source of brominated pyrrole-imidazole alkaloids (PIAs).[1] The structural diversity of this alkaloid family[2] results from a series of cyclization reactions of the biosynthetic precursors hymenidin (**1**)[3] and oroidin (**2**).[4] The group of tetracyclic pyrrole-imidazole alkaloids can be divided into phakellins **3** and **4** and isophakellins **5** and **6**. The iso-form of the phakellins is characterized by a different orientation of the pyrrole ring with respect to the central tricyclic core. So far, five compounds of this class have been published: monobromophakellin (**3**),[5] dibromophakellin (**4**),[5] monobromoisophakellin (**5**),[6] dibromoisophakellin (**6**),[7] and *N*-methyldibromoisophakellin.[8] While the monobrominated compounds **3** and **5** are intramolecularly cyclized hymenidin-derived compounds, the dibrominated alkaloids **4** and **6** are derived from oroidin (**2**).[4]

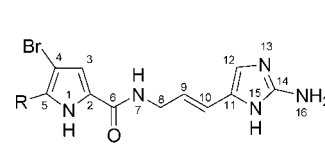
Several larger alkaloids (dimeric PIAs) include the phakellin/isophakellin moiety as a substructure. Examples are the palau'amines (**7**)[9] (**7b**, containing **3**, and **7c** containing **4**), the konbu'acidines (**8**)[10,11] (**8a**, containing **3**, and **8b** containing **4**), and the styloguanidines (**9**)[12,13] (**9b**, containing **5**, and **9c** and **9d** containing **6**). The structures of compounds **7a**, **7b**, **7c**, **8a**, **9a**, **9b**, and **9c** are drawn according to the reassignment of the relative configuration of the palau'amine congeners in 2007.[14] Our detailed analysis using a DG/DDD approach with interproton distances allowed an unambiguous reassignment of two stereogenic centers of the central cyclopentane ring. The most prominent change was the *trans* junction of the 3-azabicyclo[3.3.0]octane moiety, which was originally assigned to be *cis*. The debromo derivatives were not isolated for the monomeric PIAs but are seen in the phakellin/isophakellin substructure of the dimeric PIAs palau'amine (**7a**) and styloguanidine (**9a**).

Sponges are particularly well known as important sources of chemically interesting substances. Because they are sessile and benthic and lack motility, many sponges defend themselves against predators by producing deterrent metabolites, such as pyrrole-imidazole alkaloids. The PIAs display several biological activities including chitinase inhibiting,[12] feeding deterrent,[15] or antihistamine effects.[16] These activities may also play an important role in the ecological relevance of these metabolites.

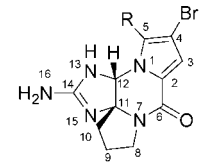
To our knowledge, a full description of the NMR properties of members of the phakellin/isophakellin family (monomeric PIAs) has been completed only for compounds **5** and **6**.[6,7] For compounds **3** and **4**, the ^1H and ^{13}C NMR chemical shifts are published, but no correlation data are available.[5] In order to fill this gap, we decided to reinvestigate this exciting class of alkaloids using state-of-the-art NMR methods. Furthermore, special emphasis was given to the general application of the ADEQUATE pulse sequence[17] in the structure elucidation of natural products. This will be demonstrated for compounds **3**−**6**. Finally, the NMR correlations obtained will be discussed in light of their importance for the constitutional assignment using the NMR-based structure generator COCON (Constitutions from Connectivities).[18]

COCON allows the comprehensive generation of the entirety of molecular constitutions compatible with a 2D NMR data set,

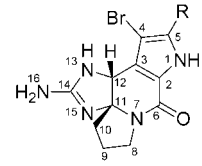
Chart 1



hymenidin (**1**, R = H)
oroidin (**2**, R = Br)

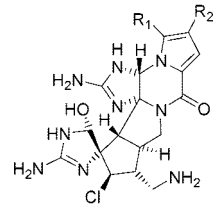


monobromophakellin (**3**, R = H)
dibromophakellin (**4**, R = Br)

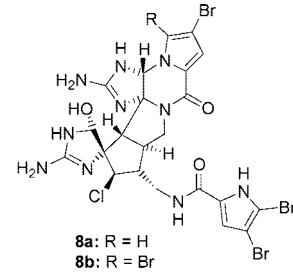


monobromoisophakellin (**5**, R = H)
dibromoisophakellin (**6**, R = Br)

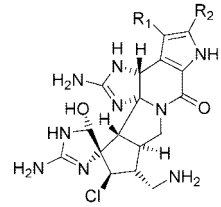
Chart 2



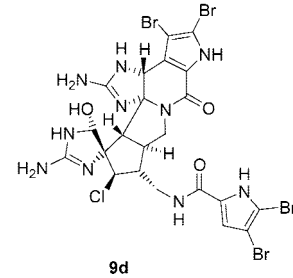
7a: R^1, R^2 = H
7b: R^1 = H, R^2 = Br
7c: R^1, R^2 = Br



8a: R = H
8b: R = Br



9a: R^1, R^2 = H
9b: R^1 = H, R^2 = Br
9c: R^1, R^2 = Br



9d

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Table 1. NMR Data for Monobromophakellin (**3**)[a]

no.	$\delta(^{13}C)$	$\delta(^{15}N)$[b]	$\delta(^{1}H)$	COSY[c]	$^{1}H,^{13}C$-HMBC[c]	1,1-ADEQUATE	$^{1}H,^{15}N$-HMBC
1		169					
2	123.9						
3	113.3		6.81	5	2, 4, 5, 6, 12	2, 4	1
4	98.1						
5	121.7		7.29	3	2, 3, 4, 6, 12	4	1
6	154.2						
7		121					
8	45.0		3.64/3.49	9	2, 6, 9, 10, 11	9	7
9	19.3		2.06	8, 10	8, 10, 11	8, 10	7
10	38.1		2.35/2.25	8, 9	6, 8, 9, 11, 12	9, 11	7, 15
11	82.1						
12	68.1		6.10		2, 5, 10, 11, 14	11	1, 13, 15
13		95	10.04		11, 12, 14		15
14	156.6						
15		109	10.21		11, 12, 14		13
16		77	8.63		11, 12, 14		13, 15

[a] ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 signal (2.50 and 39.5 ppm, respectively). [b] ^{15}N NMR spectra were not calibrated with an external standard. The δ value has an accuracy of about 1 ppm in reference to NH_3 (0 ppm). [c] The underlined numbers represent correlations over four bonds ($^{4}J_{HH}$ or $^{4}J_{CH}$).

Table 2. NMR Data for Dibromophakellin (**4**)[a]

no.	$\delta(^{13}C)$	$\delta(^{15}N)$[b]	$\delta(^{1}H)$	COSY[c]	$^{1}H,^{13}C$-HMBC[c]	1,1-ADEQUATE	$^{1}H,^{15}N$-HMBC
1		168					
2	125.0						
3	114.8		7.02		2, 4, 5, 6, 12	2, 4	1
4	102.0						
5	106.1						
6	153.7						
7		122					
8	44.6		3.66/3.51	9	2, 6, 9, 10, 11	9	7
9	19.0		2.07	8, 10	8, 10, 11	8, 10	7
10	38.5		2.41/2.29	9	6, 8, 9, 11, 12	9, 11	7, 15
11	84.4						
12	68.3		6.32	13	2, 3, 5, 6, 10, 11, 14	11	1, 13, 15
13		94	10.02	12, 15	11, 12, 14		15
14	156.7						
15		110	10.53	13	11, 12, 14		13
16		77	8.75/8.43				

[a] ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 signal (2.50 and 39.5 ppm, respectively). [b] ^{15}N NMR spectra were not calibrated with an external standard. The δ value has an accuracy of about 1 ppm in reference to NH_3 (0 ppm). [c] The underlined numbers represent correlations over four bonds ($^{4}J_{HH}$ or $^{4}J_{CH}$).

required *respectively* forbidden substructures, and the molecular formula. The high calculation speed of the COCON program is based on the integrated evaluation of HMBC information simultaneously with the construction of constitutions and on the detection of constitutionally equivalent but combinatorial different isomers at the earliest possible stage of their generation. For compounds **3**−**6** two different COCON calculations were performed. In the first analysis (A) the hybridization states of every atom are considered as they are given by the constitutions **3**−**6**. In practice, however, the hybridization states especially of heteroatoms are usually unknown. In the more in-depth second analysis (B), the program starts with only the true molecular formula and the degree of protonation of each atom (available, for example, from DEPT for carbon atoms). Restricted by only a very limited number of ^{13}C NMR chemical shift rules, every possible combination of hybridization states is generated and afterward analyzed independently. Of course, the solutions generated in the first analysis A are a subgroup of those of the second analysis B. In both calculation modes, identical 2D NMR-derived connectivity information was regarded.

Results and Discussion

A complete NMR data set was acquired for compounds **1**−**6** using a 600 MHz NMR spectrometer with a cryo probe. This included the following 2D experiments: $^{1}H,^{1}H$-COSY, $^{1}H,^{13}C$-HSQC, $^{1}H,^{13}C$-HMBC, 1,1-ADEQUATE, 1,*n*-ADEQUATE, INADEQUATE, $^{1}H,^{15}N$-HSQC, and $^{1}H,^{15}N$-HMBC. All assignments for compounds **3**−**6** as well as all correlations gained from $^{1}H,^{1}H$-COSY, $^{1}H,^{13}C$-HMBC, 1,1-ADEQUATE, and $^{1}H,^{15}N$-HMBC experiments are displayed in Tables 1−4. Comparison of the carbon chemical shifts of phakellins **3** and **4** and isophakellins **5** and **6** with their probable respective biosynthetic precursors hymenidin (**1**) and oroidin (**2**) is carried out in Figures S1 and S2 (see Supporting Information). All $^{1}J_{CH}$ coupling constants for **1**−**6** were extracted from the $^{1}H,^{13}C$-HMBC spectra (Table 5). The values for methylene protons are inaccurate, as their signals were broad or overlaid by the water signal. The olefinic/aromatc $^{1}J_{CH}$ coupling constants range from 175 to 194 Hz, while the aliphatic ones vary from 130 to 150 Hz. An exception is proton H-12, with coupling constants of ∼200 Hz (in **1** and **2**), ∼175 Hz (in **3** and **4**), and ∼155 Hz (in **5** and **6**). Where possible, the $^{1}J_{CC}$ coupling constants were extracted from the INADEQUATE spectra (Table 6). For the sample of dibromophakellin (**4**, 44 mg) all coupling constant values could be extracted, while in the spectrum of the lower concentrated sample of monobromophakellin (**3**, 21 mg), three correlations were missing (C-2/C-6, C-8/C-9, and C-9/C-10). The *S*/*N* ratio of the INADEQUATE spectrum of dibromoisophakellin (**6**) was too low to assign any $^{1}J_{CC}$ coupling constants. The assignments of the respective spin systems of monobromoisophakellin (**5**) within the INADEQUATE spectrum are shown in Figure S3 (Supporting Information). Also in the INADEQUTE spectrum of **5** two correlations were not observed (Table 6). The olefinic/aromatic $^{1}J_{CC}$ constants vary in the range 60 to 86 Hz and the aliphatic values in the range 29 to 41 Hz. The proton T_1 relaxation times of the phakellins and isophakellins also exhibit a wide range. In particular,

Table 3. NMR Data for Monobromoisophakellin (**5**)[a]

no.	$\delta(^{13}C)$	$\delta(^{15}N)$[b]	$\delta(^{1}H)$	COSY[c]	$^{1}H,^{13}C$-HMBC[c]	1,1-ADEQUATE	$^{1}H,^{15}N$-HMBC
1		156	12.40	5	2, 3, 4, 5, 6, 12		
2	121.5						
3	121.7						
4	93.5						
5	124.5		7.18	1	2, 3, 4, 6, 12	4	1
6	155.7						
7		124					
8	44.1		3.56/3.46	9	6, 9, 10, 11	9	7
9	19.4		2.03	8, 10	8, 10, 11	8, 10	7
10	39.0		2.21	9	8, 9, 11, 12	9, 11	7, 15
11	84.4						
12	54.3		5.21		2, 3, 4, 6, 10, 11, 14	3, 11	13, 15
13		109	9.87	15	11, 12, 14		15
14	157.1						
15		89	8.83	13	11, 12, 14		13
16			8.02				

[a] ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 signal (2.50 and 39.5 ppm, respectively). [b] ^{15}N NMR spectra were not calibrated with an external standard. The δ value has an accuracy of about 1 ppm in reference to NH_3 (0 ppm). [c] The underlined numbers represent correlations over four bonds ($^{4}J_{HH}$ or $^{4}J_{CH}$).

Table 4. NMR Data for Dibromoisophakellin (**6**)[a]

no.	$\delta(^{13}C)$	$\delta(^{15}N)$[b]	$\delta(^{1}H)$	COSY[c]	$^{1}H,^{13}C$-HMBC[c]	1,1-ADEQUATE	$^{1}H,^{15}N$-HMBC
1		161	13.35		2, 3, 4, 5, 6, 12		
2	122.5						
3	122.8						
4	96.3						
5	108.4						
6	154.7						
7		123					
8	44.1		3.56/3.47	9	6, 9, 10, 11	9	7
9	19.1		2.03	8, 10	8, 10, 11	8, 10	
10	39.0		2.22	9	8, 9, 11, 12	9, 11	7, 15
11	84.1						
12	54.0		5.23	13	2, 3, 4, 5, 6, 10, 11, 14	3, 11	7, 13, 15
13		88	8.92	12, 15	11, 12, 14		15
14	156.9						
15		109	9.89	13	11, 12, 14		13
16		72					

[a] ^{1}H and ^{13}C chemical shifts are referenced to the DMSO-d_6 signal (2.50 and 39.5 ppm, respectively). [b] ^{15}N NMR spectra were not calibrated with an external standard. The δ value has an accuracy of about 1 ppm in reference to NH_3 (0 ppm). [c] The underlined numbers represent correlations over four bonds ($^{4}J_{HH}$ or $^{4}J_{CH}$).

Table 5. $^{1}J_{CH}$ Coupling Constants [Hz] of Compounds **1**−**6**[a]

atom no.	**1**	**2**	**3**	**4**	**5**	**6**
C-3	176	177	180	183	-	-
C-5	191	-	193	-	193	-
C-8	137	137	145/142	147/144	144/144	148/144
C-9	150	142	133	134	144	136
C-10	145	164	132/133	133/137	133	127
C-12	200	201	177	173	153	157

[a] The underlined values are inaccurate due to broad and/or overlaid signals. The hyphens indicate quaternary carbon atoms.

Table 6. $^{1}J_{CC}$ Coupling Constants [Hz] of Compounds **3**−**5**[a]

CC correlation	**3**	**4**	**5**
C-2/C-3	69	68	-
C-2/C-6	-	~77	76
C-3/C-4	61	60	64
C-4/C-5	77	~86	76
C-8/C-9	-	32	-
C-9/C-10	-	30	29
C-10/C-11	38	38	38
C-11/C-12	41	41	38

[a] The *S*/*N* ratio of the INADEQUATE spectrum of **6** was too low to assign any $^{1}J_{CC}$ coupling constants. The hyphens indicate CC correlations that did not show up in the INADEQUATE experiment.

Table 7. Proton T_1 Relaxation Times [ms] of Compounds **1**−**6**[a]

atom no.	**1**	**2**	**3**	**4**	**5**	**6**
H-3	2847	2451	7561	7070		
H-5	4003		2512		2891	
H-8	569	559	544/754	567/[b]	[b]	557/601
H-9	1515	1406	439	461	519	464
H-10	2137	1795	424/461	459/489	526	503
H-12	2847	2199	1332	1433	1552	1515

[a] The underlined values are inaccurate due to broad and/or overlaid signals. [b] Not analyzed.

the protons of the pyrrole ring show long T_1 times with up to 7.5 s for H-3 and up to 2.9 s for H-5 (Table 7).

Special emphasis was given on the application of the ADEQUATE pulse sequences in the structure elucidation of these compounds. A 1,1-ADEQUATE spectrum allows for selective observation of two-bond correlations and thereby differentiation between two- and three-bond correlations when used in combination with a $^{1}H,^{13}C$-HMBC spectrum (Figure 1).[17b] Usually, the one-bond correlations ($^{1}J_{CH}$) are also observed in the 1,1-ADEQUATE spectrum, which can be easily identified by comparison with the $^{1}H,^{13}C$-HSQC spectrum (or $^{1}H,^{13}C$-HMBC spectrum as shown in

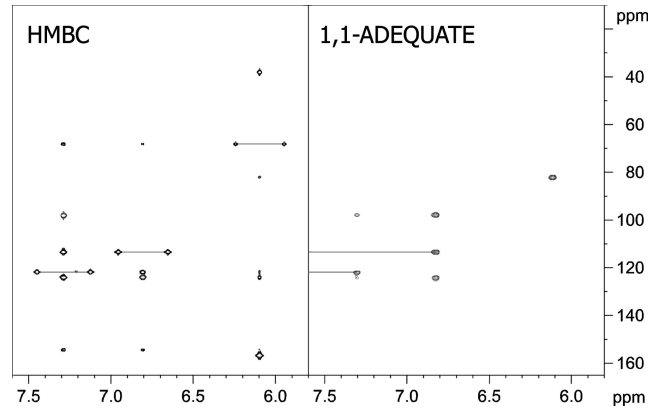

Figure 1. 1,1-ADEQUATE allows for differentiation between two- and three bond correlations. For monobromoisophakellin (**3**) the HMBC (left) and 1,1-ADEQUATE (right) spectra are shown.

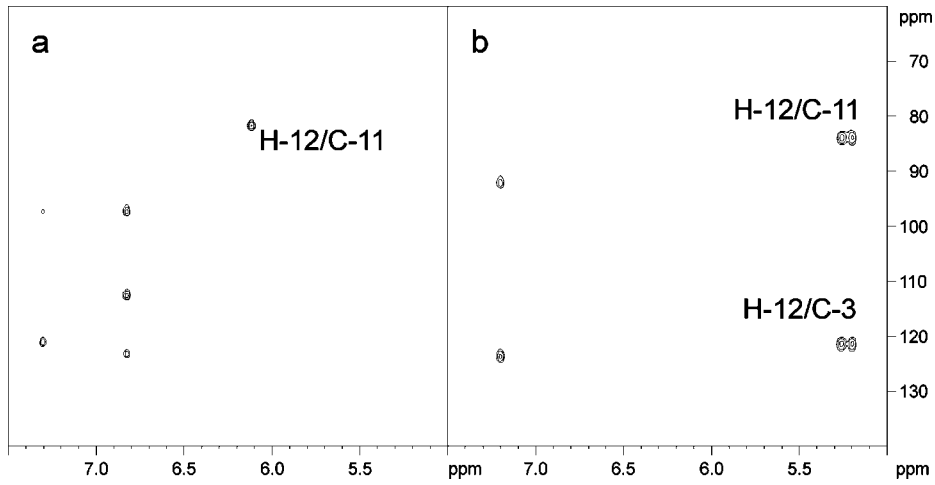

Figure 2. 1,1-ADEQUATE allows one to selectively observe two-bond correlations. For monobromophakellin (**3**) one $^2J_{CH}$ connection for H-12 can be observed (a), while monobromoisophakellin (**5**) yields two (b).

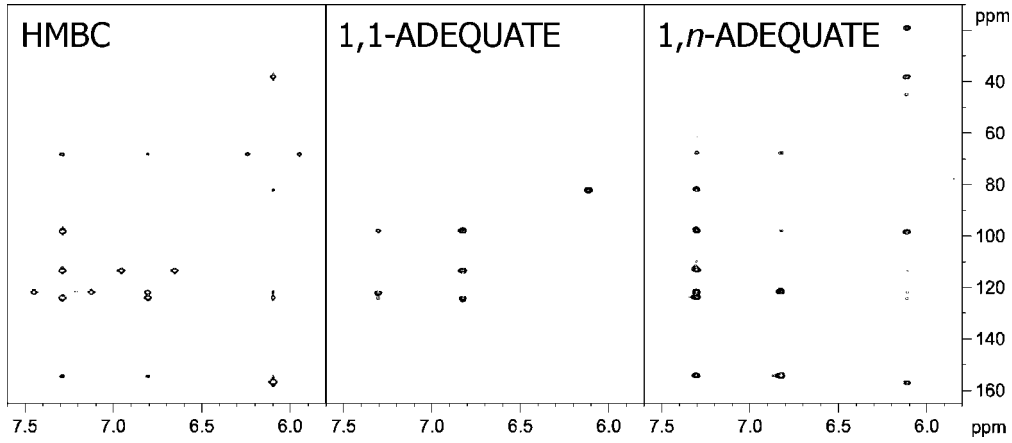

Figure 3. Comparison of 1,1-ADEQUATE and 1,*n*-ADEQUATE to an HMBC spectrum of monobromophakellin (**3**).

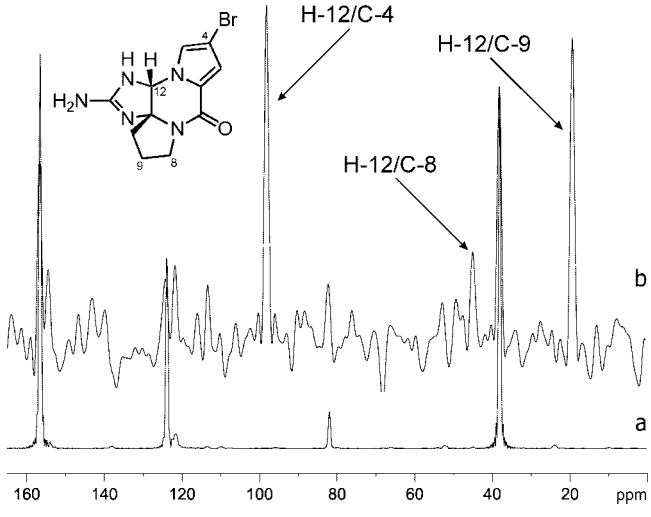

Figure 4. Comparison of single columns taken out of a 1,*n*-ADEQUATE (a) and a HMBC spectrum (b). For H-12 in monobromophakellin (**3**) 1,*n*-ADEQUATE reveals three additional correlations over four bonds, as indicated by arrows.

Figure 1). As an example, the 1,1-ADEQUATE spectra of monobromophakellin (**3**) and monobromoisophakellin (**5**) are shown in Figure 2. One $^2J_{CH}$ correlation can be observed for H-12 of monobromophakellin (**3**; H-12/C-11), while two such correlations are seen in monobromoisophakellin (**5**; H-12/C-11 and H-12/C-3).

Table 8. Additional $^4J_{CH}$ Correlations of Compounds **3**−**6** from the 1,*n*-ADEQUATE Experiment

	3	**4**	**5**	**6**
H-3				
H-5	11			
H-8				
H-9		6, 12	6, 12	6
H-10			3, 6	3, 6
H-12	3, 4, 9	4, 9	8, 9	9

Figure 3 compares the 1,1- and 1,*n*-ADEQUATE spectra of monobromophakellin (**3**) with the ^{1}H,^{13}C-HMBC spectrum. The 1,*n*-ADEQUATE enables the observation of additional pseudo $^4J_{CH}$ couplings. In Figure 4 this is demonstrated for H-12 in monobromophakellin (**3**). Three correlations revealed in the 1,*n*-ADEQUATE were not observed in the standard HMBC spectrum: H-12/C-4, H-12/C-8, and H-12/C-9. These supplementary correlations may further simplify structure elucidation. Table 8 shows the additional $^4J_{CH}$ linkages that were not observed in the respective HMBC experiments of compounds **3**−**6**.

All ADEQUATE spectra were acquired in ∼20 h. A 21 mg sample of monobromophakellin (**3**) allowed an acquisition time of 6 h and showed all possible $^2J_{CH}$ correlations. Six of the nine correlations were still observed with a 3 h acquisition (Figure 5) and maintained a sufficient resolution for the unambiguous assignment of carbon atoms. To demonstrate the application and general feasibility of the ADEQUATE pulse sequence for metabolites with a wide range of coupling constants, two samples of **3** with lower concentrations were prepared. The 5.5 mg sample of monobro-

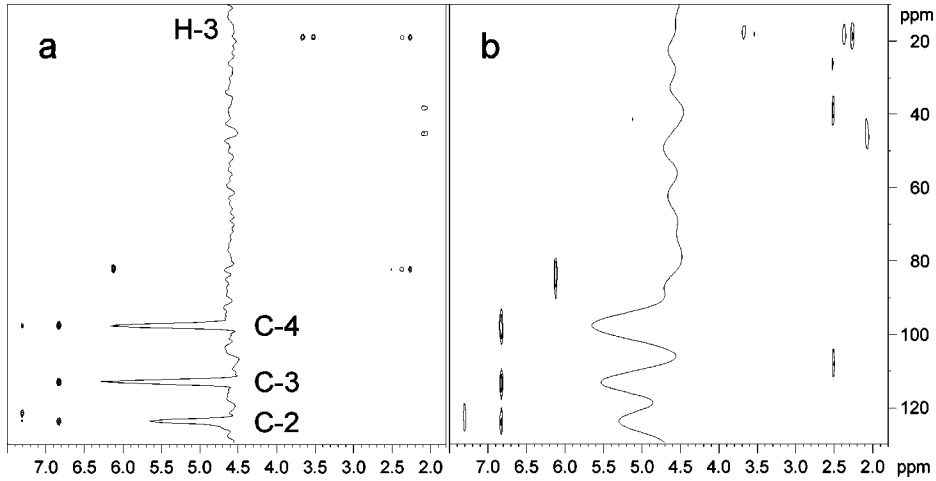

Figure 5. Comparison of two 1,1-ADEQUATE spectra of a 21 mg sample of monobromophakellin (**3**). Spectrum a was acquired in 19.3 h (128 scans, 256 increments) and b in 3 h (80 scans, 64 increments). The projections shown are columns extracted at 6.81 ppm (H-3).

Table 9. Numbers of Constitutional Proposals for Compounds **3**−**6** (COCON calculations; calculation time is given in parentheses [min:s])[a,b]

	data set A	data set B	data set C	data set D
3	1310 (2:06)	27 (0:03)	16 (0:02)	4 (<0:01)
	26 (<0:01)	6 (<0:01)	5 (<0:01)	2 (<0:01)
4	236161 (15:39)	16578 (0:39)	1105 (0:22)	176 (0:02)
	264 (0:02)	62 (<0:01)	20 (<0:01)	4 (<0:01)
5	212 (0:28)	55 (0:01)	21 (0:01)	7 (0:01)
	8 (<0:01)	4 (<0:01)	4 (<0:01)	2 (<0:01)
6	31286 (9:29)	10225 (0:46)	311 (0:10)	218 (0:02)
	67 (0:01)	36 (<0:01)	14 (<0:01)	5 (<0:01)

[a] COCON calculations are performed for four data sets of compounds **3**−**6**. Data set A (1100) consists of ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HMBC correlation data, data set B (1110) of ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HMBC, and 1,1-ADEQUATE correlation data, data set C (1101) of ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HMBC, and ^{1}H,^{15}N-HMBC, and data set D (1111) of ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HMBC, 1,1-ADEQUATE, and ^{1}H,^{15}N-HMBC. The number in parentheses is the nomenclature (correlation flag) used by COCON. [b] First line gives the results for the COCON calculations without atom types (without hybridization states), the second line for the COCON calculations with atom types (with predefined hybridization states).

mophakellin (**3**, 35 mM) allowed for the acquisition of a spectrum with a sufficient *S*/*N* ratio to show seven of nine possible correlations in ∼38 h. The correlations H-5/C-4 and H-10/C-11 were not visible or barely larger than the noise level since the $^1J_{CH}$ and $^1J_{CC}$ coupling constants are the largest (194 and 77 Hz) and smallest (135 and 38 Hz) values, respectively. This observation is not remarkable since the spectrum was acquired with delays optimized for coupling constants of 155 Hz ($^1J_{CH}$) and 55 Hz ($^1J_{CC}$). A 2.5 mg sample of **3** (16 mM) proved to be at the low end of a reasonable sample concentration for ADEQUATE experiments. Only three correlations (H-3/C-4, H-9/C-10, and H-12/C-11) were observed in a 77 h acquisition.

As mentioned above, the obtained correlation data were used to evaluate the effect of different NMR experiments on the structure elucidation. This was carried out with the NMR-based structure generator COCON.[18] This program considers the correlation data information provided and generates the set of all structures in accordance with the input data and with certain shift rules. In this way, various calculations with input files including the information of different combinations of NMR experiments were performed for each compound (data sets A−D). In case of monobromophakellin (**3**) two constitutions were possible if all input data were used (data set D, for details see Table 9). It turned out that for the calculation of the structure of **3** the information gained from the ^{1}H,^{15}N-HMBC spectrum (data set C) was more important for structure elucidation than the 1,1-ADEQUATE correlations (data set B). COCON generated 16 structural proposals for data set C versus 27 for data set B. In comparison, 1310 solutions resulted if the input file only considered ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HMBC correlation data (data set A), which is supposed to be the standard correlation data set for the constitutional assignment of natural products. The 1,1-ADEQUATE correlations reduced the number of possible constitutions for monobromophakellin (**3**) to 2.1% and the ^{1}H,^{15}N-HMBC to 1.2% compared to the COCON input file, which initially included only ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HMBC data. Using all correlation data for monobromophakellin (**3**), the possible constitutions were reduced to 0.3%. The additional data should help to reduce the number of wrong structure elucidations in the future. To confirm this, a citation from the review by Nicolaou and Snyder covering the misassignment in the time period between 1990 and 2004 is given: "Although the past century has witnessed a remarkable improvement in our ability to isolate and characterize complex natural products, mistakes are still a relatively common occurence. However, stories relating to our own experiences hopefully indicate, this state of affairs is far from catastrophic."[19]

In conclusion, the phakellins and isophakellins were investigated by state-of-the-art NMR methods in order to contribute to the ongoing research on this important group of pyrrole-imidazole alkaloids. This work may ease the future structure elucidation of new members of this class of compounds. The investigation of the influence of different NMR experiments on the total number of possible constitutions further supports the benefit of conducting more sophisticated NMR experiments. The 1,1-ADEQUATE correlations reduced the number of possible constitutions for the phakellin/isophakellins (**3**−**6**) in the range 2−33% and the ^{1}H,^{15}N-HMBC to 1−10% compared to the COCON input file, which initially included only ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HMBC data. Using all correlation data for compounds **3**−**6** the possible constitutions were reduced to 0.1−3%. When using the hybridization states (atom types) of the atoms, this results in two constitutional proposals for monobromophakellin (**3**) and monobromoisophakellin (**3**), four for dibromophakellin (**4**), and five for dibromoisophakellin (**6**).

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer with a cryo system (5 mm TCI cryo probe). All spectra were measured at 303 K. Sample concentrations were 21 mg/500 μL (**3**), 44 mg/500 μL (**4**), 30 mg/500 μL (**5**), and 21 mg/500 μL (**6**) in DMSO-d_6. The lower concentration samples of **3** contained 5.5 mg/500 μL and 2.5 mg/500 μL, respectively. The ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC, ^{1}H,^{15}N-HSQC, ^{1}H,^{15}N-HMBC, 1,1-ADEQUATE, 1,*n*-ADEQUATE, and INADEQUATE experiments were carried out using standard param-

eters. The relaxation delay chosen for the acquisition of proton T_1 relaxation times was set to 70 s with an array of 23 delays (0.02, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.5, 10, 15, 20, 25, 30, 35 s). The acquisition parameters of ADEQUATE experiments were optimized (in particular the CH and CC coupling constants) in order to address the protons of either the olefinic/aromatic or the aliphatic region. As default parameters, 155 Hz ($^1J_{CH}$) and 55 Hz ($^1J_{CC}$) were chosen.

COCON. Although, the structures **3**−**6** are shown in their neutral form, the imidazole part of the molecules is protonated in solution with a positive charge delocalized over the guanidine system. In order to incorporate all obtained correlations into COCON calculations, the input file had to be adjusted, as COCON cannot calculate charged molecules. The COCON program was run on a SUNFire V20z with 2 AMD opteron CPUs of 2.2 GHz. Ubuntu 5.04 was used as operating system. The kernel was 2.6.10-5-amd64-generic, meaning that only one CPU was used for calculations.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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