Molecules **1996**, *1*, 41 – 45

Application of the 1,*n*-ADEQUATE Experiment in the Assignment of Highly Substituted Aromatic Compounds

Matthias Köck*, Bernd Reif, Matthias Gerlach and Michael Reggelin

Institut für Organische Chemie, Johann Wolfgang Goethe-Universität,
Marie-Curie-Str. 11, D-60439 Frankfurt/Main, Germany, Tel.: +49-69-798-29143, Fax: +49-69-798-29128
(km@krypton.org.chemie.uni-frankfurt.de)

Received: 30 May 1996 / Accepted: 11 July 1996 / Published: 19 August 1996

Abstract

This communication describes the application of the 1,*n*-ADEQUATE experiment to differentiate between the two possible products (**4** and **5**) of an acylation reaction of starting material **3**. The ADEQUATE experiments are new NMR based methods for assigning the constitution of natural products, combining a HSQC or HMBC step with a C,C correlation in natural abundance. The 1,*n*-ADEQUATE technique allows to observe *pseudo* $^4J_{CH}$ correlations which were necessary for the distinction of **4** and **5**. Different NMR methods for the constitutional assignment of natural products and other organic molecules are discussed in detail. This communication also demonstrates that the 1,*n*-ADEQUATE method is generally applicable for the constitutional assignment of highly substituted aromatic compounds as well as for the localization of O-acetyl groups bound to quarternary carbons.

Keywords: NMR, Constitutional assignment, HMBC, ADEQUATE, Euglobal

Introduction

The HMBC experiment [1] is widely accepted as the most important NMR experiment for the constitutional assignment of natural products and other organic molecules. Recently, we have emphasized that for certain classes of molecules the information obtained from HMBC spectra is not sufficient to elucidate the structure. Therefore, the ADEQUATE experiments [2] were introduced which are based on techniques described earlier. [3] The 1,*n*- and *n*,1-ADEQUATE experiments allow the observation of correlations with one bond more than in the HMBC experiment (pseudo $^4J_{CH}$ correlation, see Fig. 1). The utility of these experiments was demonstrated for the assignment of the constitution of the polyaromatic marine natural product 5,6-dihydro lamellarin H. [2]

In this communication the 1,*n*-ADEQUATE method was applied to distinguish between two possible intermediates in the total synthesis of euglobal-G1 (1) and euglobal-G2 (2, see Fig. 2) [4] via chiral, aracemic, allylic sulfoximines [5]. Furthermore the value of this experiment for the assignment of highly substituted aromatic ring systems is discussed in general and compared with other NMR methods.

Results and Discussion

The acylation reaction of the aldehyde **3** (see Fig. 3) led to only one of the two possible regioisomers **4** and **5**. [4] Both positions are sterically and electronically similar and therefore the regioselectivity cannot be predicted. The two regioisomers could not be distinguished by HMBC correlations ($^2J_{CH}$ and $^3J_{CH}$ correlations, for details see Table 1). For

* *To whom correspondence should be addressed*

Table 1: *Which correlations can be expected from an HMBC spectrum for **4** and **5**? [a]*

Position	from	to	Transfer	Comment
1	H-7	C-2, C-6	$^3J_{CH}$	same correlations for **4** and **5**
2	OH	C-1, C-3	$^3J_{CH}$	no correlations from the OH
3	H-9	C-3	$^3J_{CH}$	correlation to *ipso* position [b]
4	H-12	C-4	$^3J_{CH}$	correlation to *ipso* position [b]
5	H-5	C-4, C-6 [c]	$^2J_{CH}$	this correlation could not been observed [d]
	(H-3)	(C-2,C-4)		($^2J_{CH}$ in aromatic system)
6	H-13	C-6	$^3J_{CH}$	correlation to *ipso* position [b]

[a] Only $^2J_{CH}$ and $^3J_{CH}$ correlations are considered for this table. The delay for the evolution of the long range heteronuclear couplings in the HMBC experiment was set to 80 ms.

[b] This correlation yields no constitutional information.

*[c] For compound **4** the transfer is from H-5 to C-2 and C-4.*

[d] In aromatic and olefinic systems the $^2J_{CH}$ couplings are usually very small.

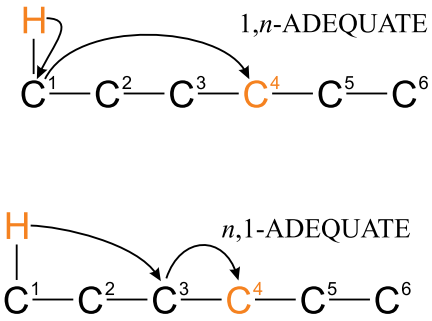
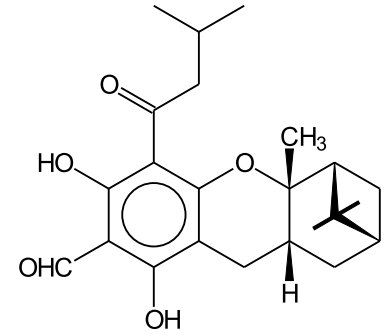
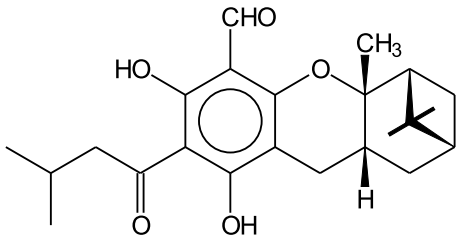


Fig. 1: *Schematic representation of the pseudo $^4J_{CH}$ correlations in the 1,n-ADEQUATE ($^1J_{CH}$ + $^3J_{CC}$) and n,1-ADEQUATE ($^3J_{CH}$ + $^1J_{CC}$) experiment.*



1



2

Fig. 2: *Structural formulae of euglobal-G1 (**1**) and euglobal-G2 (**2**).*

the positions 3, 4 and 6 of the aromatic ring in the compounds **4** and **5** there is no information available from the first proton of the corresponding side chain to the *ortho* position. The longest transfer ($^3J_{CH}$) is usually observed at the *ipso* position which yields no information about the spatial arrangement of the substituents. For the other three positions (1, 2 and 5), where in principle an HMBC correlation could be detected, these are either not observed or not useful for several reasons (see Table 1).

However, using *pseudo* $^4J_{CH}$ correlations (see Fig. 1), as observed in the 1,*n*-ADEQUATE [2a], between the two acylation products can be differentiated. In the case of compound **4** only one correlation is expected from the protons of the OMe groups (H-12 and H-13) to the unsubstituted carbon (C-5), whereas in compound **5** both OMe groups are expected to correlate with C-5.

The latter case was observed in the 1,*n*-ADEQUATE spectrum as shown in Fig. 4. The 1,*n*-ADEQUATE experiment was run in the ω_1-refocused version, allowing analysis of the spectrum in the same way as an HMBC. [2] The measurement time for the experiment was about four hours, which could have been further reduced because of the comparatively good resolution in ω_1.

The alternative analysis using correlations between H-5 and the carbons of the OMe groups (C12 and C13) was not possible because the chemical shifts of C-12 and C-13 are

Table 2. *NMR experiments used to solve the regioselectivity of the acylation reaction of **3**.*

Experiment	**Transfer**	**Can 4 and 5 be distinguished by the method? [a]** Case A H12, H13 anisochronous C12, C13 isochronous	Case B H12, H13 isochronous C12, C13 anisochronous
long range COSY	$^5J_{HH}$	? *(H12,H13 → H5)*	No
NOESY	NOE	? *(H12,H13 → H5)*	No
HMBC	$^3J_{CH}$	No	No
long range HMBC	$^4J_{CH}$	? *(H12,H13 → C5)*	? *(H5 → C12,C13)*
1,*n*-ADEQUATE	"$^4J_{CH}$" ($^1J_{CH}$ + $^3J_{CC}$)	**Yes *(H12,H13 → C5)***	**Yes *(H5 → C12,C13)***
n,1-ADEQUATE	"$^4J_{CH}$" ($^3J_{CH}$ + $^1J_{CC}$)	Yes *(H12,H13 → C5)*	No [b]

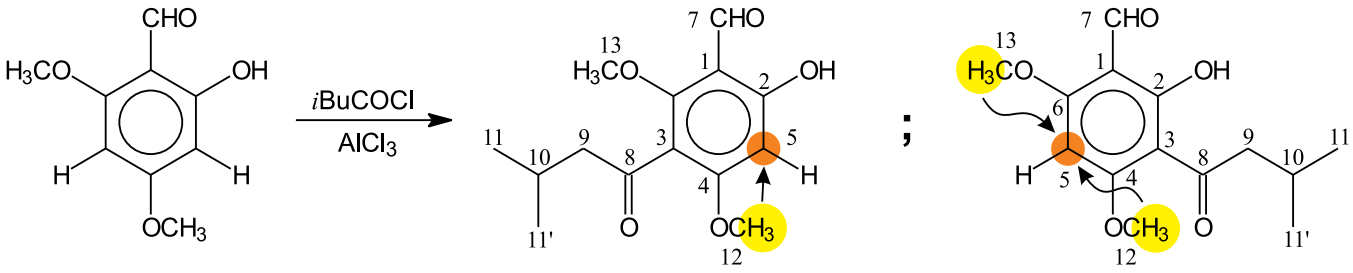
[a] The question mark (?) indicates that a result can not be predicted from these experiments. The corresponding correlations are shown in parenthesis.

[b] The HMBC transfer ($^3J_{CH}$) cannot be observed, because it would end at the oxygen atom of the methoxy group.

degenerate. But, in contrast to other methods it would have been theoretically possible to solve the constitutional problem even in this case with the 1,*n*-ADEQUATE experiment (see Table 2, case B). Further possibilities for solving the regioselectivity of this acylation reaction are homonuclear proton experiments, e.g. the long range COSY and the NOESY experiment. For the first experiment a $^5J_{HH}$ coupling from H12 and H13 to H5 is necessary to distinguish between the two possible products **4** and **5**. These couplings are usually very small and therefore difficult to observe. A NOESY experiment is also not the method of choice because small aromatic molecules show a high degree of external relaxation which makes the observation of NOE effects difficult. Furthermore, both experiments would, of course, not work in the theoretical case B with isochronous protons H12 and H13 (see Table 2). The HMBC experiment optimized to small couplings as $^4J_{CH}$ is also not a method which is generally applicable because these couplings are usually very small. The 1,*n*-ADEQUATE experiment is the only technique shown in Table 2 for which a positive result can be expected in both cases with a maximum probability. The *n*,1-ADEQUATE experiment would not work in case B because the first transfer of this experiment ($^3J_{CH}$, HMBC) ends at the oxygen atom of the methoxy group.

By application of the 1,*n*-ADEQUATE experiment a differentiation of the two possible reaction products **4** and **5** was possible. (see Fig. 3 and 4) The ADEQUATE data is in accordance to compound **5**, for which all proton and carbon resonances were assigned. The proton and carbon chemical shifts of compound **5** are given in Table 3.

The procedure described for solving the regioselectivity of this acylation reaction using the 1,*n*-ADEQUATE experiment can be seen as a general approach for the assignment of highly substituted aromatic rings. The method makes it possible to correlate OMe groups attached to aromatic rings with



3 **4** **5**

Fig. 3: *The starting material (**3**) and the two possible products (**4** and **5**) of the acylation reaction are shown. The arrows in the structural formulae of **4** and **5** indicate the correlations from H-12 or/and H-13 (yellow circles) to the only non-substituted aromatic position (orange circle)..*

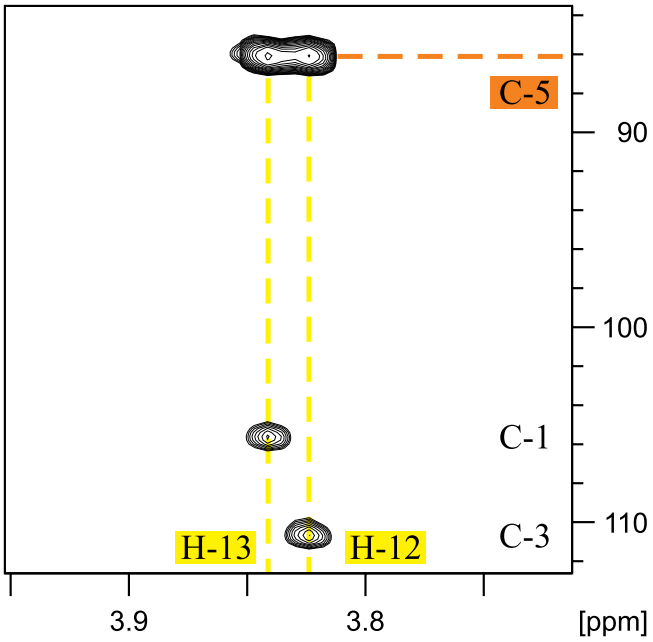


Fig. 4: *The expansion of the 1,n-ADEQUATE spectrum[5] shows correlations from both OMe groups to C-5. Also the correlations from H-13 to C-1 and from H-12 to C-3 represent pseudo* $^4J_{CH}$ *correlations.*

the corresponding *ortho* positions. The same is true for other side chains which have a heteroatom or a non-protonated carbon in the position which is attached to the aromatic ring. Furthermore, the 1,*n*-ADEQUATE experiment can also be of great help for the assignment of acetoxy groups which are bound to quarternary carbons, because there is no HMBC correlation available from the OAc to the position where it is bound. In this special case, only the 1,*n*-ADEQUATE experiment is useful and not the *n*,1-ADEQUATE because the first transfer of the latter experiment would end at an oxygen (see Fig. 5).

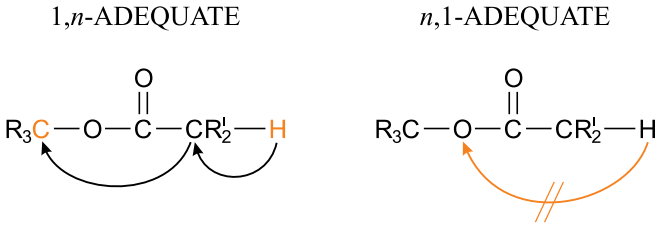


Fig. 5: *Schematic correlations in an O-acetyl group (R' = H) attached to a quarternary carbon atom (R ≠ H) for an 1,n- and n,1-ADEQUATE experiment. The 1,n-ADEQUATE experiment allows to observe a correlation from the protons of the methyl group to the quarternary carbon atom. For the n,1-ADEQUATE experiment no correlation is expected in this case because the first transfer (*$^3J_{CH}$*, HMBC) would end at an oxygen atom.*

Table 3. *Proton and carbon chemical shifts [ppm] of compound* ***5*** *in* $CDCl_3$ *at 300 K.[8]*

No.	δ (^{1}H)	δ (^{13}C)	No.	δ (^{1}H)	δ (^{13}C)
1	—-	105.9	8	—-	202.6
2	—-	163.3	9	2.60	53.8
3	—-	111.4	10	2.11	25.0
4	—-	165.3	11,11'	0.87	22.7
5	5.88	86.0	12	3.82	55.9
6	—-	165.3	13	3.84	55.9
7	10.03	191.5	OH	12.95	—-

Conclusion

In summary, the 1,*n*-ADEQUATE experiment has proved to be a very useful NMR method for solving constitutional problems in synthetic organic chemistry. Synthetic intermediates are often obtained in such amounts that the ADEQUATE experiments can be run in reasonable measuring times of 3 to 6 h using ordinary 5 mm NMR tubes and probes.

Acknowledgment: This work was supported by the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft (Gr 1211/6-1 and Re 1007/1-4/5). We acknowledge the support of Prof. Dr. C. Griesinger. M. K. thanks the Fonds der Chemischen Industrie for a fellowship (Li 149/4). We thank Dr. John P. Marino for careful reading of the manuscript.

References

1. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093-2094.
2. a) Reif, B.; Köck, M.; Kerssebaum, R.; Kang, H.; Fenical, W.; Griesinger, C. *J. Magn. Reson.* **1996**, *A118*, 282-285; b) Köck, M.; Reif, B.; Fenical, W.; Griesinger, C. *Tetrahedron Lett.* **1996**, *37*, 363-366.
3. a) Gosser, Y. Q.; Howard, K. P.; Prestegard, J. H. *J. Magn. Reson.* **1993,** *B101*, 126-133; b) Chung, J.; Tolman, J. R.; Howard, K. P.; Prestegard, J. H. *J. Magn. Reson.* **1993,** *B102*, 137-147; c) Pratum, T. K.; Moore, B. S. *J. Magn. Reson.* **1993,** *B102*, 91-97; d) Weigelt, J.; Otting, G. *J. Magn. Reson.* **1995**, *A113*, 128-130.
4. Takasaki, M.; Konoshima, T.; Kozuka, M.; Haruna, M.; Ito, k.; Shingu, T. *Chem. Pharm. Bull.* **1994**, *42*, 2591-2597.
5. a) Reggelin, M.; Weinberger, H.; Gerlach, M.; Welcker, R. *J. Am. Chem. Soc.* **1996**, *118*, 4765 - 4777; b) Reggelin, M.; Weinberger, H. *Angew. Chem.* **1994**, *106*, 489-491; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 444-446.

6. Preparation of compound **5** by Friedel-Crafts acylation: To an ice-cold suspension of 5.5 g (41.2 mmol) of dry aluminium chloride in 40 ml abs. dichloromethane was added a solution of 5.0 g (5.1 ml, 41.2 mmol) 3-methyl-butyric acid chloride in 20 ml abs. dichloromethane. After having been stirred for another thirty minutes at 0 °C 5.0 g (27.4 mmol) 4,6-dimethoxysalicylaldehyde (**3**) dissolved in 30 ml of dichloromethane was added slowly at this temperature. The mixture was allowed to reach room temperature and stirred for another 20 h. Aqueous workup (50 ml cold water) and threefold extraction of the aqueous phase with dichloromethane yields, after evaporation of the volatile components, a yellow oil which was purified by column filtration (36 g silica gel, ethyl acetate/hexane = 1:3). The final yield of the pure acylation product was 5.8 g (80%) as a colorless, crystalline solid (mp. 91 °C).
7. The experiment was run with 100 mg of compound **5** in 0.5 ml $CDCl_3$ at 300 K using a 5 mm NMR tube and 5 mm NMR probe on a Bruker DRX600 spectrometer. The pulse sequence for the refocused 1,1-ADEQUATE as described in detail in Ref. 2a was used. Only the delay for the evolution of the C,C-couplings was changed to long range couplings for the refocused 1,*n*-ADEQUATE experiment. Acquisition parameters: 2048 data points in F_2, 192 data points in F_1, 48 scans, acquisition time of 142.6 ms, relaxation delay of 1.5 s and delay for evolution of $^nJ_{CC}$ couplings 31.8 ms. Processing parameters: the FIDs were multiplied with a squared cosine function prior to Fourier transformation, 2048 data points in F_2, 512 data points in F_1.
8. We have used two different samples of compound **5** for this project (50 and 100 mg) which slightly differ in their chemical shifts.