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Determination of Configuration at the Biaryl Axes of Naphthylisoquinoline Alkaloids by Long-Range NOE Effects†

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One- and two-dimensional NOE and ROE experiments were applied to investigate the relative configuration at the stereogenic biaryl axes of naphthylisoquinoline alkaloids, natural biaryl compounds mainly occurring as stable atropisomers. The influence of *ortho* substitution at the biaryl axes on the dynamic behavior of the atropodiastereomers and the determined NOE effects was investigated. As an example, the axial configuration of ancistrobrevine A was elucidated by the method. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

Naphthylisoquinoline alkaloids are a young and rapidly growing group of more than 50 related natural biaryl products.[2] Their occurrence is, as yet, restricted to the tropical plant families Ancistrocladaceae and Dioncophyllaceae. Alkaloid-rich species of these families have been used in folk medicine in West Africa and South-East Asia against several diseases.[2] More recently, naphthylisoquinoline alkaloids have been found to show fungicidal, insect antifeedant and antimalarial activity.[3–5] All of these compounds consist of a naphthalene and an isoquinoline part covalently joined together by a biaryl axis. For most of the alkaloids, *e.g.* for ancistrocladine (**1a**) and hamatine (**1b**) (Fig. 1),[6–8] this axis shows hindered rotation and thus gives rise to stable atropisomers.

The configuration at the biaryl axes was deduced by comparison of experimental circular dichroism (CD) spectra with calculated spectra[9,10] or with those of related, configuratively known compounds,[11] by total synthesis[12] or by special chemical procedures.[13] For as yet very few selected cases, namely for 5,8′-coupled naphthylisoquinolines[14–17] such as ancistrobrevine B (**2**), in which the naphthalene part is not too far away from the chiral part of the tetrahydroisoquinoline ring, it has been possible to elucidate the relative axial *vs.* central configuration by NOE experiments. In this paper, we report NOE experiments that allow one to determine the configuration at the biaryl linkage relative to the stereocenters also for 5,1′-coupled alkaloids such as **1a** and **1b** and even for compounds such as dioncophylline A (**3a**) and ancistrobrevine A (**4**, axial

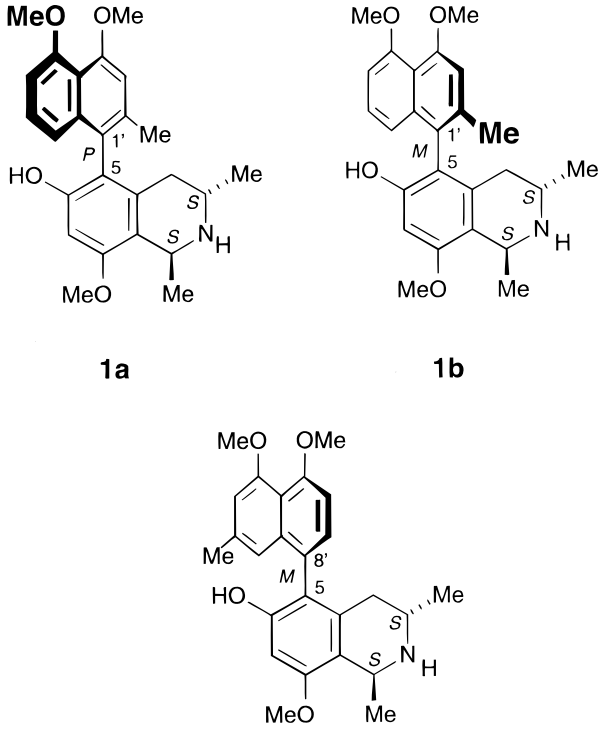

Figure 1. Ancistrocladine (**1a**), hamatine (**1b**) and ancistrobrevine B (**2**): naturally occurring biaryls from tropical lianas.

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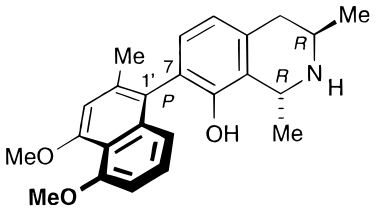

3a

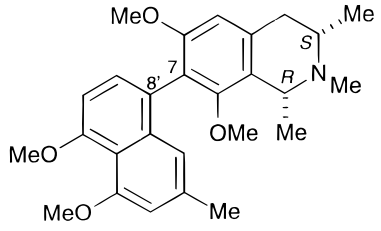

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Figure 2. Dioncophylline A (**3a**) and ancistrobrevine A (**4**), two naphthylisoquinoline alkaloids with the naphthyl substituent at the 7-position of the isoquinoline moiety.

configuration as yet unknown) (Fig. 2), in which, owing to a 7,1′- or 7,8′-coupling type, the relevant protons of the two ring systems are more distant from each other. The advantages of transient *vs.* steady-state experiments are discussed. Furthermore, the importance of flexibility around the biaryl axis on the NOE effect is reported.[18]

EXPERIMENTAL

All compounds of natural origin were isolated earlier.[2] The synthetic derivatives of dioncophylline A (**5a**, **6a** and **7a**) were prepared by standard methods applied previously;[19] details will be given elsewhere.[20] NMR spectra were recorded on Bruker AC 200 and WM 400, Varian UNITY 500 and Bruker DMX 600 spectrometers. Solutions of *ca.* 0.01 mmol l^{-1} in $CDCl_3$ were prepared in 5 mm tubes, thoroughly degassed and sealed. Typical acquisition parameters for the 1D experiments were appropriate for spectral widths corresponding to 9 ppm with 32K or 64K data points, using the chloroform signal (^{1}H: $\delta = 7.26$) for internal calibration. T_1 measurements were made using the inversion–recovery method, with delays from 0.015–5 s, and with a relaxation delay of 10 s. 1D NOE difference experiments were acquired non-spinning at 300K in blocks of 40 off- and 40 on-resonance scans in an interleaved manner. Subtraction of data was performed in the frequency domain. Line broadening of 0.5 Hz was used in order to minimize digitization artifacts. The build-up curves were measured over six presaturation times (0.1, 0.3, 0.5, 1, 3 and 5 s), thus allowing one to distinguish direct NOE effects from three-spin effects. The 1D ROE experiment was executed by employing the DANTE pulse train or a Gaussian soft pulse (30 ms) for selective irradiation. For the phase-sensitive NOESY and ROESY experiments,[21] the same ^{1}H spectral window as in the 1D experiments was used. In the ROESY experiments the strength of the spin-lock field was about 2 kHz. Interproton distances were derived from energy minimization using the SYBYL program.[22]

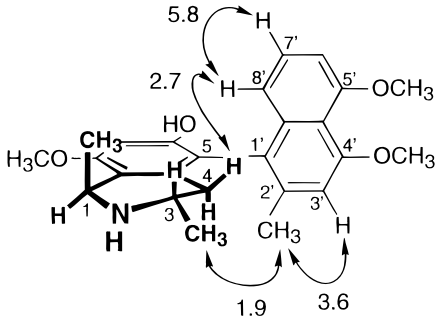
RESULTS AND DISCUSSION

^{1}H data for **1a**, **1b**, **3a**, **3b** and **4** (Table 1) were assigned using conventional one- and two-dimensional NMR techniques.

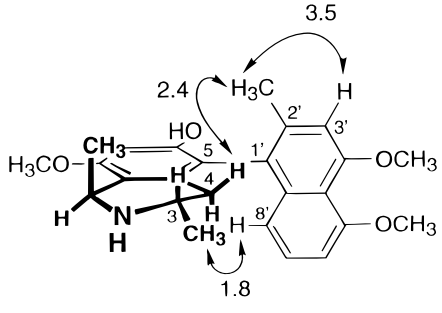
In the NOE difference spectra of the naturally occurring pair of atropisomers **1a** and **1b**, several through-space interactions between protons of the two ring systems were detected that allow the determination of the axial configuration (Fig. 3), which was known from independent investigations.[6,7] For naphthylisoquinoline alkaloids of the 5,1′-coupling type, the occurrence of strong NOE effects between the isoquinoline and naphthalene moieties was to be expected because of the spatial proximity ($\leqslant 3.5$ Å) of the relevant protons.

In the case of a 7,1′-coupling site, as is typical for dioncophylline A (**3a**) and its naturally occurring atropisomer 7-*epi*-dioncophylline A (**3b**), or a 7,8′-linkage as in ancistrobrevine A (**4**), minimal distances between relevant protons of the two parts of the molecule are much larger ($\geqslant 4$ Å). Nevertheless, in the steady-state NOE difference experiment small but significant interactions were found (Fig. 4) for presaturation times longer than 3 s. To ensure that the small effects observed were not a result of spin diffusion, NOE experiments with varying irradiation times were recorded in all critical cases.

Owing to the rigidity and the molecular weight of these types of molecules, their correlation times τ_c were



1a



1b

Figure 3. Selected NOE interactions (%) observed for the 5,1′-coupled atropo-diastereomers ancistrocladine (**1a**) and hamatine (**1b**).

Table 1. Proton assignments (δ, ppm) and T_1 data (s)[a]

	1a		1b		3a		3b		4	
Proton	δ	T_1	δ	T_1	δ	T_1	δ	T_1	δ	T_1
3-CH_3	0.89	0.3	0.99	0.4	1.41	0.35	1.44	0.4	1.34	0.3
1-CH_3	1.47	0.4	1.47	0.4	1.57	0.45	1.57	0.4	1.51	0.35
H-4_{ax}	1.75	0.25	1.74	0.3	2.74	0.3	2.82	0.3	2.88	0.4
H-4_{eq}	1.98	0.25	2.08	0.3	2.91	0.3	2.89	0.35	2.73	0.3
2′-CH_3	2.15	0.5	2.18	0.4	2.18	0.7	2.17	0.6	2.35	0.4
N-CH_3	—	—	—	—	—	—	—	—	2.54	0.3
H-3	3.12	0.4	3.19	0.45	3.49	0.4	3.51	0.5	3.00	0.5
6-OCH_3	—	—	—	—	—	—	—	—	3.04	
8-OCH_3	3.86	0.6	3.90	0.65	—	—	—	—	3.61	0.6
5′-OCH_3	3.99	0.6	4.00	0.6	3.96	0.6	3.95	0.7	3.97	0.7
4′-OCH_3	4.02	0.6	4.04	0.6	4.00	0.6	3.96	0.65	3.99	
H-1	4.16	0.5	4.40	0.55	4.57	0.6	4.56	0.7	3.78	0.5
H-7	6.50	1.1	6.51	1.0	—	—	—	—	—	
H-5	—	—	—	—	6.76	1.0	6.74	0.9	6.51	1.0
H-6′	6.83	0.8	6.86	0.7	6.80	0.85	6.78	0.9	7.26	0.8
H-3′	6.86	0.8	6.90	0.7	6.79	1.2	6.87	1.3	6.83	1.1
H-6	—	—	—	—	6.89	1.5	6.77	1.4	—	
H-8′	6.97	1.3	6.89	1.1	6.96	1.6	6.92	1.5	6.67	1.2
H-7′	7.26	1.2	7.22	1.3	7.24	1.2	7.23	1.4	6.87	1.0

[a] The spectra were taken at 300 K in $CDCl_3$ at 400 MHz for **1a**, **1b** and **4** and at 600 MHz for **3a** and **3b**.

expected to be outside the extreme narrowing limit ($\omega\tau_c \leqslant 1$), and the NOE effects thus might be very small or even not detectable. Therefore, 1D and 2D rotating frame experiments were performed on these molecules. For dioncophylline A (**3a**), a significant cross peak between 1-CH_3 and 2′-CH_3 was observed in the ROESY experiment at a mixing time of 300 ms (Fig. 5).

This interaction was observed in both directions, and also in the steady-state 1D NOE experiment, as a weak but significant enhancement for presaturation times longer than 3 s. NOE experiments with variation of the irradiation time from 0.1 to 5 s (Table 2) revealed that the effects observed are not a result of spin diffusion. A three-step contribution involving the hydroxyl at C-8 was excluded by further NOE experiments in MeOH-d_4, which gave a similar enhancement.

The correlation between 1-CH_3 and 2′-CH_3 established the *syn* array and thus spatial proximity of these groups and, with the known 1*R*-configuration, allowed

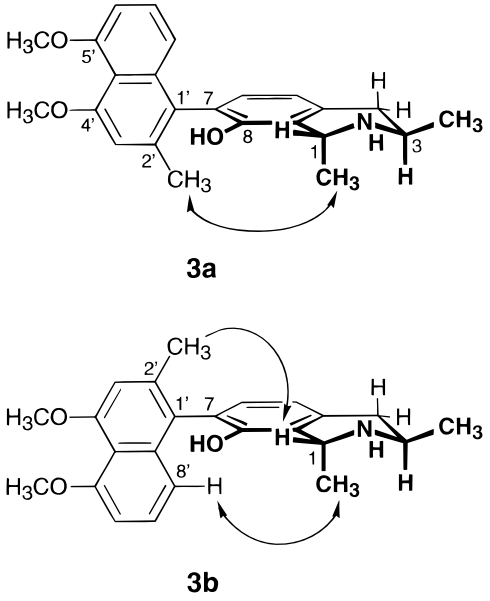


Figure 4. Specific NOE interactions that allow one to attribute the relative axial configuration of the 7,1′-coupled atropodiastereomers dioncophylline A (**3a**) and 7-*epi*-dioncophylline A (**3b**).

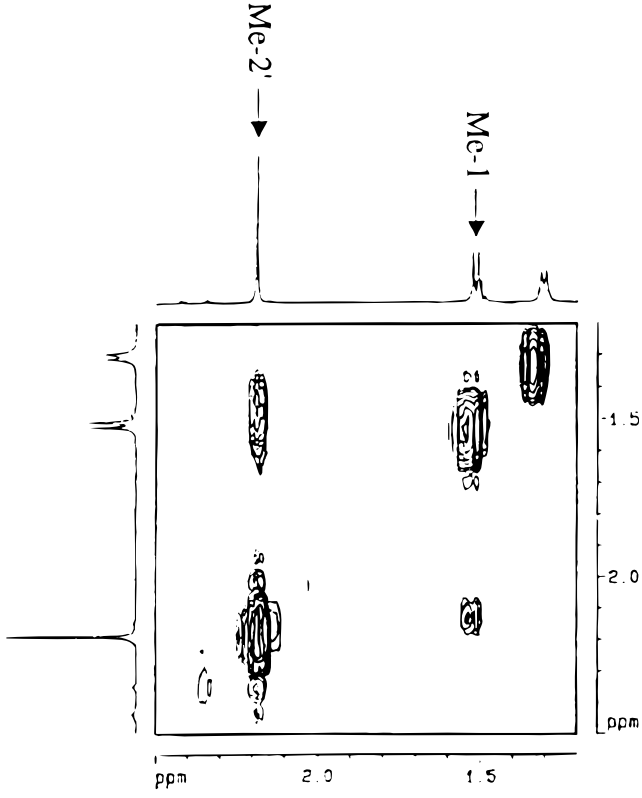
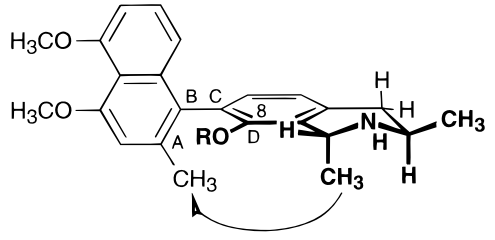


Figure 5. Expansion of the ROESY spectra of dioncophylline A (**3a**) (400 MHz, 2 kHz cw spin-lock, 300 ms mixing time, 512 t_1 increments, 1K data points) showing the spatial proximity between 1-CH_3 and 2′-CH_3.

Table 2. Dependence of the NOE effect (%) on the irradiation time for the relevant interaction between 1-CH_3 and 2′-CH_3 of dioncophylline A (3a)

Irradiated	Enhanced	0.1 s	0.3 s	0.6 s	1.5 s	2 s	3 s	5 s
1-CH_3	H-1	0.8	3.0	5.5	7.0	7.7	8.7	8.9
	H-3	0.6	1.8	3.0	5.0	5.8	6.3	6.1
	2′-CH_3				0.3	0.6	0.8	0.9
2′-CH_3	H-3′	0.3	1.8	3.6	7.3	9.1	10.0	10.4
	H-6			0.3	1.4	1.9	2.0	2.2
	H-8′				0.5	1.0	1.5	1.5
	1-CH_3					0.4	0.4	0.5

Table 3. Dependence of the NOE effect (%) from 1-CH_3 to 2′-CH_3 on the size of the *ortho*-substituent at C-8

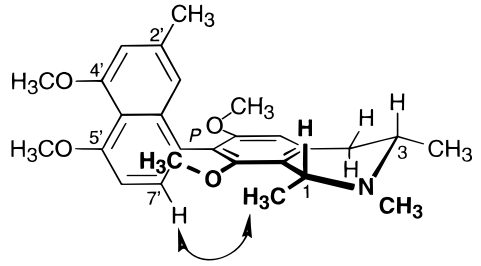


3a, 5a, 6a, 7a

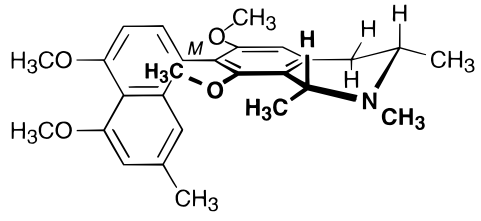
Compound	OR	NOE
3a	OH	0.8
5a	OCH_3	0.5
6a	OCH_2CH_3	0.5
7a	$OCH(CH_3)_2$	(≤0.3)

one to attribute (and thus confirm) the axial *P*-chirality for **3a**.[8]

In the case of **3b**, selective irradiation of the protons of 2′-Me gave a distinct NOE enhancement for H-1 in the steady-state NOE measurement, thus confirming the axial configuration of 7-*epi*-dioncophylline A (**3b**). The significant interaction observed in the NOE difference experiment in 7-*epi*-dioncophylline A (**3b**) between H-1 and 2′-CH_3 and *vice versa* was hardly detectable in the transient 1D ROE or 2D ROESY experiments. The combination of short relaxation times on the one hand and slow build-up rates for the long-range NOE effects on the other is an inherent problem with all transient experiments for this kind of molecule.



4a



4b

Figure 6. Determination of the axial configuration of ancistrobrevine A (**4a**) using NOE experiments.

Since both the naphthalene and the isoquinoline ring systems are fairly rigid, possible internal motions, apart from methyl group rotation, are mainly restricted to the torsion at the biaryl axis, so that these molecules serve as a good example for the dependence of the NOE effect on molecular motion.[23–25] The distance between protons of the two parts of the molecule is strongly dependent on the relative orientation of the two ring systems, and thus on the restricted torsion at the axis. The averaged dihedral torsion angle ABCD (see formula in Table 3) should be about 90° for all the molecules investigated here. Diagnostically useful minimum distances between the relevant protons will result from a maximum deviation from that median angle and thus from the size of the *ortho*-substituents, which, with increasing steric demand, may disfavor such analytically valuable tight conformations. Thus, because of the r^{-6} dependence of the NOE effect, the detectable enhancement depends on the mobility around the biaryl axis. We have examined the effect of steric hindrance of different *ortho*-substituents on the NOE. For this purpose, a whole series of derivatives of dioncophylline A, with greatly varying steric demand of the 8-*O*-substituent, were synthesized and NOE enhancements under identical experimental conditions were determined. The data in Table 3 reveal that the hindrance at the biaryl axis by sterically demanding substituents is of critical influence on the NOE measured.

As a first extension of the procedure to the stereoanalysis of 7,8′-instead of 7,1′-coupled alkaloids, we next investigated ancistrobrevine A (**4**), a naphthylisoquinoline alkaloid isolated from *Ancistrocladus abbreviatus*.[26] Its axial configuration was as yet unknown, so that observation of long-range interactions would allow one to distinguish between the two possible atropisomeric forms, **4a** and **4b**, and thus lead to a completion of the structure elucidation of this moderately antimalarial[27] compound.

A small but significant NOE between 1-Me and H-7′ was detected. (Fig. 6). Given the known absolute con-

figuration at the stereocenters[26] and thus the orientation of 1-Me to be α, *i.e.* below the tetrahydroisoquinoline 'plane,' H-7′ must likewise be below that plane, as in **4a**, thus clearly excluding the structure **4b** for the natural product. In this case the NOE methodology described here was indispensible for attribution of the structure of ancistrobrevine A as **4a**. A clear interpretation of the CD spectra was not possible owing to the similar substitution pattern around the axis (*i.e.* two identical *ortho*-OMe groups). This underlines the value of the NMR method presented in this paper.

CONCLUSION

With the help of NOE experiments, the axial configuration of naphthylisoquinoline alkaloids relative to the stereocenters can be determined. This was illustrated in several examples including a novel naphthylisoquinoline alkaloid with an unknown stereoorientation at the axis. Because of the long relevant interproton distances, a thorough optimization of the experimental conditions is crucial. For these naphthylisoquinoline alkaloids the conventional steady-state NOE difference experiment gives much better results than ROE measurements, which can be explained by the counterbalance of the slow build-up rates of long-range NOEs and the fast relaxation processes.

As discussed, the dynamic behavior of this kind of molecule has an important influence on the size of the observed NOE enhancement. Therefore, detailed NOE investigations allow statements about the rigidity at the biaryl axis. This is an important prerequisite for further structure–activity investigations on naphthylisoquinoline alkaloids.

Acknowledgements

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