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Configurational and conformational analysis of highly oxygenated pyrrolizidines: definitive identification of some naturally occurring 7a-epi-alexines

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Abstract

The NMR spectra of a number of naturally occurring alexines (tetrahydroxylated pyrrolizidine alkaloids) are analyzed and the consequences of changes in the configuration on the conformation of these bicyclic systems discussed. Unambiguous syntheses of australine (7-epi-alexine) and of 7,7a-epi-alexine have now unequivocally established the structures of two natural products isolated from *Castanospermum australe* which were insecure due to erroneous NMR data. Chemical shift parameters are unreliable as a method of comparing different samples of identical compounds; however, ^{1}H–^{1}H three bond coupling constants ($^{3}J_{HH}$) provide easy direct comparison between samples and allow assignments of both the relative configurations for the ring protons and the conformation of the pyrrolizidine framework.

1. Introduction

Pyrrolizidine alkaloids with a hydroxymethyl substituent at C3 (numbering scheme is given in Fig. 1) appear to be of very restricted natural occurrence.[1] The alexines and australines (7a-epi-alexines) with hydroxyl groups at positions C1, C2 and C7 have only been reported in two small genera of the *Leguminosae* (*Castanospermum* and *Alexa*), and casuarine with an extra hydroxyl at C6 occurs in a few related genera in the *Myrtaceae* and *Casuarinaceae*. There are many diastereoisomers in all the plants containing these highly oxygenated pyrrolizidines. These alkaloids selectively inhibit glycosidases and

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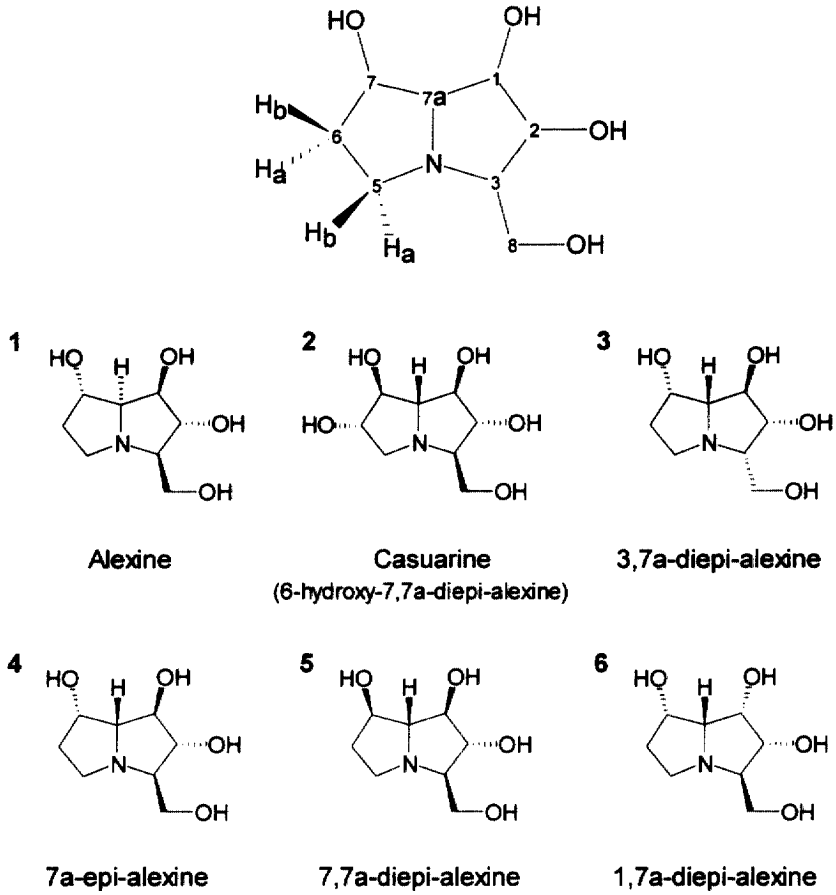

Fig. 1. Structures of alexines in this paper, together with labelling code for the compounds and the carbon numbering scheme used (top)

some show potent inhibition of glucosidase I, of interest as a possible approach to the treatment of cancer and AIDS. Supporting this are claims that some plants containing these alkaloids have such activity.[2] Unequivocal identification is important to explain or predict activity, and chemical synthesis can provide pure samples for unambiguous biological evaluation.

There are currently reports in the literature of the isolation of compounds identified as alexine, 7a-epi-alexine (australine), 3,7a-diepi-alexine, 1,7a-diepi-alexine, 7,7a-diepi-alexine and casuarine (Fig. 1). The relative and absolute configurations of alexine **1**,[3] casuarine **2**[4] and 3,7a-diepi-alexine **3**[5] have been determined by X-ray crystallography. The relative and absolute configurations of 1,7a-diepi-alexine **6** have been determined by X-ray analysis of the acetonide.[6] The structure of 7a-epi-alexine **4** was established by X-ray crystallographic analysis and the NMR data reported.[7] A further alexine was also isolated and tentatively assigned as 7,7a-diepi-alexine '**5**'[6] based on the difference between its NMR parameters and those reported for 7a-epi-alexine **4**.

Very recently a sample of 7,7a-diepi-alexine **5** prepared by an unambiguous synthesis[8] showed that the natural product described as '**5**' was different from the synthesized compound. A further unambiguous synthesis of 7a-epi-alexine **4**[9] showed that the natural product described as '**5**' is **4**. The synthetic **4** is also identical to the natural product **4** as identified by X-ray crystallography.[7] However, the NMR data for the synthetic **4** is inconsistent with the reported NMR data for the natural product '**4**'.[7] The NMR analysis of

these sets of compounds shows that the published NMR data given for '**4**' are those for 1,7a-diepi-alexine **6**.

This paper reports the full NMR analysis of these compounds. The use of chemical shift parameters proved to be unreliable as a method of comparing different samples of identical compounds. However, use of ^1H–^1H three bond coupling constants ($^3J_{HH}$) allows easy direct comparison between samples and extraction of both the relative configurations for the ring protons and the conformation of the pyrrolizidine framework.

2. Experimental methods

The natural products **1**,[3] **2**,[4] **3**[5] and **6**[6] were the same materials as reported. The natural product **6** was also isolated from *Castanospermum australe* by ion exchange chromatography[6] and co-chromatographed by GC–MS as the pertrimethylsilylderivative with the natural product published as '**4**'.[7] The compound **4** was unambiguously synthesized[9] and was the same material as the natural product published as '**5**'.[6] The compound **5** was unambiguously synthesized.[8] The pertrimethylsilyl derivatives of all these isomers have characteristically different retention times by GC–MS. All samples were dissolved in ^2H_2O and the pH adjusted to that of the free base (**1** pH=11.3, **2** pH=8.3, **3** pH=12.3, **4** pH=8.6, **5** pH=8.9, **6** pH=10.5). ^1H NMR spectra were recorded on a Varian Unity 500, with a probe temperature of 30°C. Resonance assignments were obtained from the one-dimensional (1D) and phase-sensitive two-dimensional (2D) COSY spectra, referenced to trimethylsilylpropanesulphonic acid at 0.00 ppm. Phase-sensitive 2D NOESY spectra were recorded with mixing times of 100 to 400 ms without any random variation. Stereospecific assignments for ring CH_2 proton resonances were obtained from the pattern of NOEs, particularly from C3H to the two C5Hs, and coupling constants, particularly between the two C5Hs and two C6Hs (see Fig. 1 for numbering scheme). Molecular modelling was performed on a Silicon Graphics Indigo 2 workstation using InsightII and Discover software (MSI). Energy minimizations were performed by the steepest descent method, using the cvff forcefield *in vacuo* and with a dielectric constant of 80. X-Ray crystal structures for the natural products **1**, **2** and **3** were obtained from searching the Cambridge Crystallographic Database[10] at the Chemical Database Service at Daresbury.[11] Descriptions of the ring conformations as *endo*- or *exo*-buckled refer to whether the C6 or C2 carbon is *endo* or *exo* relative to the nitrogen lone pair.

3. Results

The naturally occurring pyrrolizidines **1**,[3] **2**[4] and **3**[5] are well-characterized compounds and are the only underivatized 3-hydroxymethyl-pyrrolizidines with crystal structures available in the public domain. Full proton assignments (Table 1) were obtained for these three compounds using 1D and 2D COSY NMR spectra. The stereospecific assignments of the C5 and C6 protons (where appropriate) were obtained by analysis of the coupling constants and pattern of NOEs, obtained from 2D NOESY spectra. Table 2 gives the three-bond ($^3J_{HH}$) coupling constants obtained from the NMR spectra together with the torsion angles obtained from the crystal structures for all the ring proton pairs in these compounds.

These three compounds all have different ring conformations (Fig. 2), the rings in **2** being *exo*-/*endo*-buckled (C6 *exo* relative to the nitrogen lone pair and C2 *endo*), the rings in **3** being *endo*-/*exo*-buckled and the rings in **1** being nearly planar. The ring proton pair torsion angles for these three compounds cover the full range of values from 0° to 180°. The plot of coupling constant versus torsion angle (Fig. 3)

Table 1
Chemical shifts (ppm) for **1** (pH=11.3), **2** (pH=8.3), **3** (pH=12.3), **4** (pH=8.6), **5** (pH=8.9) and **6** (pH=10.5) in ^2H_2O and at a temperature of 30°C. See Fig. 1 for numbering scheme

Proton	Chemical Shift (ppm)					
	1 Alexine	**2** Casuarine	**3** 3,7a-diepi-alexine	**4** 7a-epi-alexine	**5** 7,7a-diepi-alexine	**6** 1,7a-epi-alexine
C5Ha	2.952	2.911	3.142	2.804	2.881	2.850
C5Hb	2.865	3.270	2.894	3.229	3.093	3.162
C6Ha	1.755	--	1.961	2.097	2.084	1.936
C6Hb	2.197	4.210	1.901	2.002	1.781	1.866
C7H	4.444	4.190	4.394	4.434	4.353	4.537
C7aH	3.297	3.071	3.395	3.272	3.029	3.295
C1H	4.202	4.162	4.297	4.291	3.723	4.409
C2H	3.798	3.796	4.140	3.959	3.770	3.794
C3H	2.921	3.036	3.297	2.803	2.682	2.914
C8H	3.84	3.771	4.023	3.850	3.776	3.788
	3.84	3.611	3.942	3.677	3.645	3.622

shows a Karplus type curve, confirming that these compounds adopt the same ring conformations in solution as in the crystal structures. The scatter of the points is fairly large, however this is to be expected in strained ring systems. More significantly, the data points fall into three distinct sets that correlate perfectly with the relative configurations of the protons (shown by the boxes in Fig. 3). The range of coupling constants found for these three sets is also given in Table 2. Although there is some overlap in these ranges, coupling constants above 7.5 Hz are only found for *trans* diaxial proton pairs, coupling constants below 3.8 Hz are only found for *trans* non-diaxial proton pairs and coupling constants between 4.0 Hz and 5.5 Hz are only found for *cis* proton pairs. This suggests that a full analysis of the coupling constants could provide considerable or complete information about the relative proton configurations within pyrrolizidines and their solution conformations.

The above approach has been used on three pyrrolizidines, **4**, **5** and **6**, which have previously been reported as natural products but where there is now considerable uncertainty about the original structures or data. Table 1 gives the full resonance assignments and Table 3 gives the $^3J_{HH}$ coupling constants for all the ring proton pairs in these three samples, together with the predicted relative configurations for the proton pairs based solely on the coupling data.

The pyrrolizidine **5** has recently been synthesized.[8] This compound is different from the natural product which has been isolated and identified as '**5**'.[6] The $^3J_{HH}$ coupling constants of greater than 7.5 Hz indicate that C3H, C2H, C1H and C7aH are all *trans* and axial. The $^3J_{HH}$ values of less than 3.8 Hz indicate that C7aH, C7H, C6Hb and C5Ha are also *trans* but not diaxial pairs. This gives C6Ha and C7H as *cis*, consistent with their coupling of 5.6 Hz. As C7aH is axial, C7H must be equatorial. The C6Ha to C5Hb coupling of greater than 7.5 Hz indicates that these protons are *trans* diaxial, leaving C6Hb and C5Ha as *trans* diequatorial. The observation of coupling constants outside the range 4–6 Hz (expected average J value) in both rings argues against any extensive ring-flipping in solution. This arrangement places the C3H, C1H, C7H, C6Ha and C5Ha protons on the same side of the ring system. This is confirmed by the observation of a strong NOE from C5Ha to C3H and NOEs from C6Ha to C3H and C1H. All these data are only consistent with **5** being 7,7a-diepi-alexine or its enantiomer and having very similar ring conformations to casuarine **2**.

The pyrrolizidine **4** is the natural product initially identified as '**5**'.[6] The $^3J_{HH}$ coupling constants of less than 3.8 Hz indicate that C5Hb, C6Ha and C7H are all *trans* (but not in diaxial pairs). This is consistent with the C6Hb to C7H coupling of between 4.0 Hz and 5.5 Hz, indicating a *cis* arrangement of

Table 2
The relative configurations, modulus of the H–C–C–H torsion angle (degrees), measured from the crystal structures, and the $^3J_{HH}$ coupling constants (Hz), measured from the 1D NMR spectra, for all ring proton pairs in **1**, **2** and **3**. The numbering scheme is shown in Fig. 1. The plot of $^3J_{HH}$ versus torsion angle is shown in Fig. 3

Proton pair	Relative configuration	Modulus of H-C-C-H torsion angle (°)	$^3J_{HH}$ (Hz)
1 - Alexine			
C5Ha - C6Ha	*cis* (eq - ax)	31	6.9
C5Ha - C6Hb	*trans* (eq - eq)	94	3.2
C5Hb - C6Ha	*trans* (ax - ax)	151	10.0
C5Hb - C6Hb	*cis* (ax - eq)	26	5.8
C6Ha - C7H	*trans* (ax - ax)	145	7.5
C6Hb - C7H	*cis* (eq - ax)	21	6.6
C7H - C7aH	*trans* (ax - ax)	122	5.6
C7aH - C1H	*cis* (ax - ax)	8	7.4
C1H - C2H	*trans* (ax - ax)	134	6.6
C2H - C3H	*trans* (ax - ax)	163	8.9
2 - Casuarine			
C5Ha - C6Hb	*trans* (eq - eq)	92	4.0
C5Hb - C6Hb	*cis* (ax - eq)	33	4.7
C6Hb - C7H	*trans* (eq - eq)	87	3.1
C7H - C7aH	*trans* (eq - ax)	100	3.5
C7aH - C1H	*trans* (ax - ax)	151	8.0
C1H - C2H	*trans* (ax - ax)	170	8.0
C2H - C3H	*trans* (ax - ax)	175	8.0
3 - 3,7a-diepi-alexine			
C5Ha - C6Ha	*cis* (ax - eq)	57	5.9
C5Ha - C6Hb	*trans* (ax - ax)	176	12.2
C5Hb - C6Ha	*trans* (eq - eq)	81	0.9
C5Hb - C6Hb	*cis* (eq - ax)	38	7.0
C6Ha - C7H	*trans* (eq - eq)	74	1.0
C6Hb - C7H	*cis* (ax - eq)	49	3.9
C7H - C7aH	*cis* (eq - ax)	44	4.2
C7aH - C1H	*trans* (ax - eq)	105	3.0
C1H - C2H	*trans* (eq - eq)	81	3.0
C2H - C3H	*cis* (eq - ax)	47	4.1
Range of $^3J_{HH}$ observed for the different relative configurations			
	trans (ax - ax)		5.6 - 12.2
	cis		3.9 - 7.4
	trans (ax - eq) or (eq - eq)		0.9 - 4.0

these protons. The C5Ha to C6Hb coupling of greater than 7.5 Hz indicates that these protons are *trans* diaxial, thus C5Hb and C6Ha are *trans* diequatorial. The coupling of between 4.0 Hz and 5.5 Hz indicates a *cis* arrangement for C7H and C7aH. Interpretation of the C7aH to C1H coupling (7.4 Hz) is ambiguous and is consistent with either a *cis* or *trans* diaxial proton arrangement. The couplings of greater than 7.5 Hz for C1H to C2H and C2H to C3H indicate that these proton pairs are all *trans* diaxial. The only remaining ambiguity is removed by the observation of a strong NOE from C7aH to C2H indicating that these two protons are on the same side of the ring, giving a *trans* arrangement for C7aH and C1H. Again, observation of coupling constants outside the 4–6 Hz range in both rings suggest little or no ring-flipping. This places C5Hb, C6Hb, C7H, C7aH and C2H as all on the same side of the ring system and

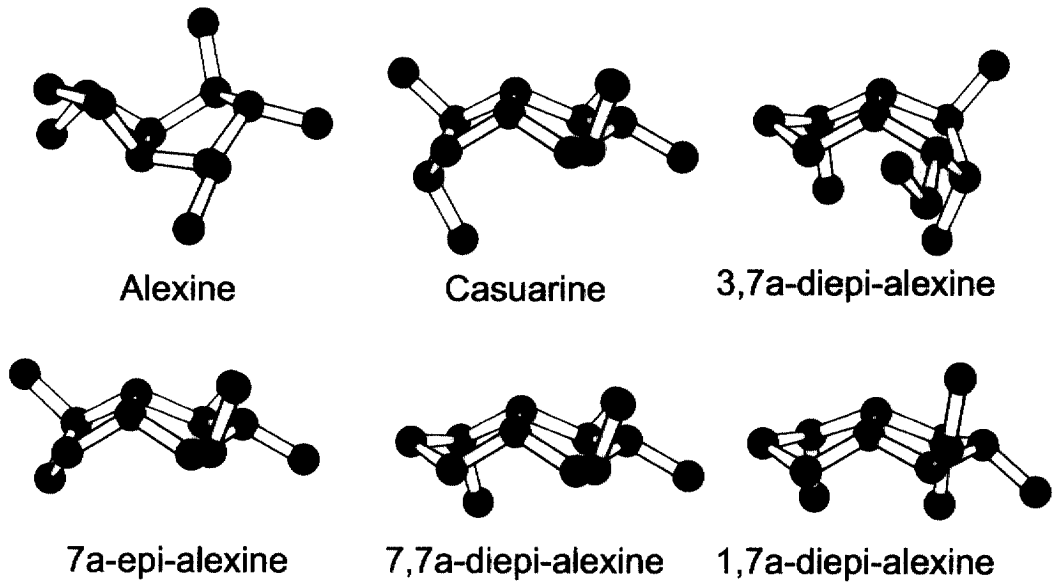

Fig. 2. X-Ray crystal structures of the natural products **1**, **2** and **3** and the molecular models generated for **4**, **5** and **6**. The nitrogen atoms are blue, oxygen red and carbon black

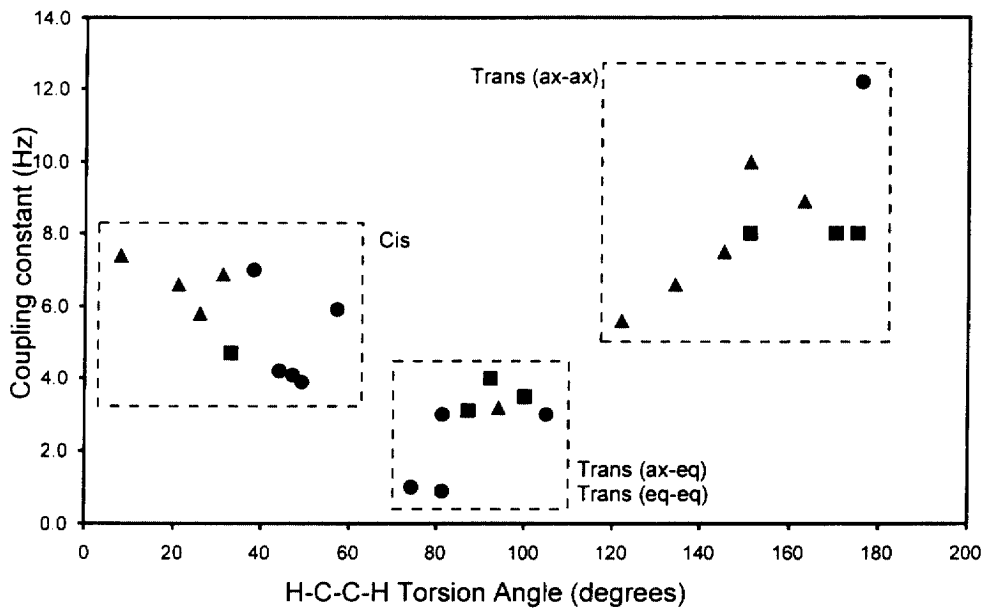

Fig. 3. Plot of $^{3}J_{HH}$ coupling constant (Hz), measured from the 1D NMR spectra, versus the modulus of the H–C–C–H torsion angle (degrees), measured from the crystal structures, for all the ring proton pairs of **1** (▲), **2** (■) and **3** (•)

C5Ha, C6Ha, C1H and C3H on the other side. This is only consistent with **4** being 7a-epi-alexine or its enantiomer and having *endo-/endo*-buckled ring conformations (Fig. 2).

The pyrrolizidine **6** is the natural product identified as '**4**' on the basis of its GC retention time and NMR parameters.[7] The $^{3}J_{HH}$ coupling constants of less than 3.8 Hz indicates that C5Hb, C6Ha and C7H are all *trans* (but not diaxial). This is consistent with the C6Hb to C7H coupling of between 4.0 Hz and 5.5 Hz, indicating a *cis* arrangement of these protons. The C5Ha to C6Hb coupling of greater than 7.5

Table 3

The $^3J_{HH}$ coupling constants (Hz), measured from the 1D NMR spectra, and predicted relative configurations based on the coupling constants for all ring proton pairs in **4**, **5** and **6**. The modulus of the H–C–C–H torsion angles (degrees), determined by molecular modelling for the final predicted structures are also given. The plot of $^3J_{HH}$ versus torsion angle is shown in Fig. 4

Proton pair	$^3J_{HH}$ (Hz)	Predicted relative configuration	Torsion angle from modelling
4 - 7a-epi-alexine			
C5Ha - C6Ha	6.0	*cis* (ax - eq)	47
C5Ha - C6Hb	11.5	*trans* (ax - ax)	170
C5Hb - C6Ha	2.1	*trans* (eq - eq)	80
C5Hb - C6Hb	7.5	*cis* (eq - ax)	42
C6Ha - C7H	2.4	*trans*	83
C6Hb - C7H	4.2	*cis*	39
C7H - C7aH	4.4	*cis*	25
C7aH - C1H	7.4	*trans* (ax - ax) or *cis*	151
C1H - C2H	8.2	*trans* (ax - ax)	169
C2H - C3H	9.5	*trans* (ax - ax)	174
5 - 7,7a-diepi-alexine			
C5Ha - C6Ha	7.3	*cis* (eq - ax)	30
C5Ha - C6Hb	3.5	*trans* (eq - eq)	90
C5Hb - C6Ha	10.5	*trans* (ax - ax)	157
C5Hb - C6Hb	5.9	*cis* (ax - eq)	36
C6Ha - C7H	5.6	*cis* (ax - eq)	27
C6Hb - C7H	2.6	*trans* (eq - eq)	93
C7H - C7aH	1.8	*trans* (eq - ax)	103
C7aH - C1H	7.8	*trans* (ax - ax)	158
C1H - C2H	8.0	*trans* (ax - ax)	176
C2H - C3H	8.7	*trans* (ax - ax)	173
6 - 1,7a-epi-alexine			
C5Ha - C6Ha	6.1	*cis* (ax - eq)	47
C5Ha - C6Hb	10.2	*trans* (ax - ax)	170
C5Hb - C6Ha	2.2	*trans* (eq - eq)	80
C5Hb - C6Hb	7.5	*cis* (eq - ax)	42
C6Ha - C7H	2.3	*trans* (eq - eq) or *trans* (eq - ax)	83
C6Hb - C7H	4.2	*cis*	39
C7H - C7aH	4.2	*cis*	25
C7aH - C1H	4.9	*cis*	36
C1H - C2H	4.5	*cis*	54
C2H - C3H	8.4	*trans* (ax - ax)	174

Hz indicates that these are *trans* diaxial, thus C5Hb and C6Ha are *trans* diequatorial. The coupling of between 4.0 Hz and 5.5 Hz indicates *cis* arrangements for C7H to C7aH, C7aH to C1H and C1H to C2H. The coupling of greater than 7.5 Hz for C2H to C3H indicates that these protons are *trans* diaxial. Again, observation of coupling constants outside the 4–6 Hz range in both rings suggests little or no ring-flipping. This places C5Hb, C6Hb, C7H, C7aH, C1H and C2H as all on the same side of the ring system and C5Ha, C6Ha and C3H on the other side. This arrangement is confirmed by NOEs from C1H to C7H, from C2H to C7aH, from C3H to C5Ha and from C6Hb to C7aH. All these data are only consistent with **6** being 1,7a-diepi-alexine or its enantiomer and having ring conformations very similar to **4**.

Molecular models of **4**, **5** and **6** were generated and energy minimized. The torsion angles obtained for all the ring proton pairs from these structures are also given in Table 3. Fig. 4 shows the plot of experimental coupling constants versus modeled torsion angle for **4**, **5** and **6**, superimposed on the data for **1**, **2** and **3**. As can be seen, the data for **4**, **5** and **6** fit the same Karplus type curve as the first three

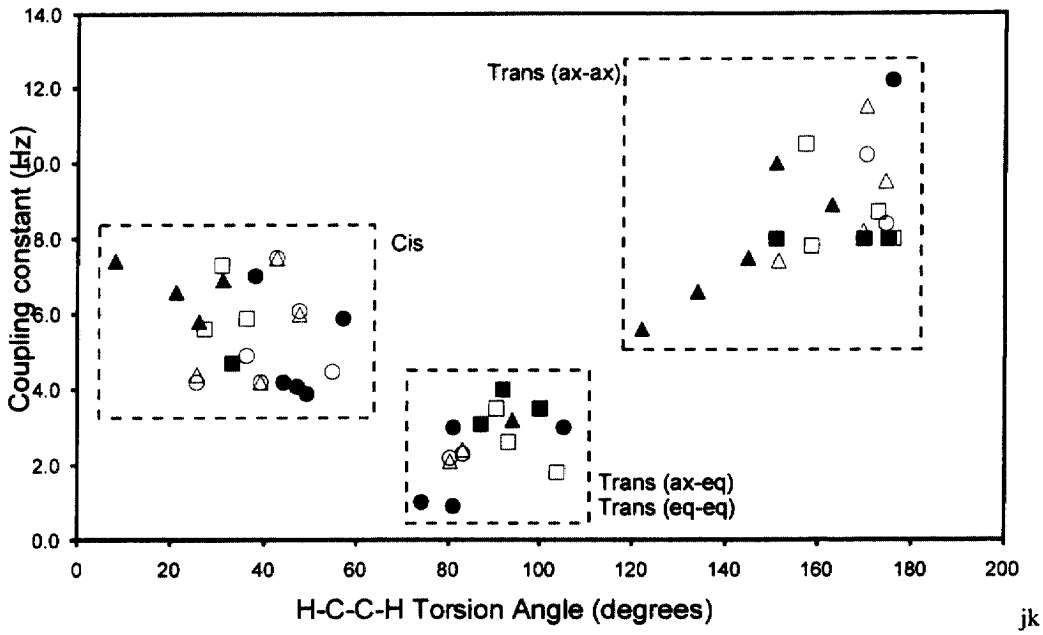

Fig. 4. Plot of $^3J_{HH}$ coupling constant (Hz), measured from the 1D NMR spectra, versus the modulus of the H–C–C–H torsion angle (degrees), determined by molecular modelling of the proposed structure, for all the ring proton pairs of **4** (△), **5** (□) and **6** (○), together with the data for **1**, **2** and **3** from Fig. 3

compounds exceedingly well, showing that their proposed structures fit all the experimental coupling data. These structures can also be used to predict the NOE patterns for these compounds and again this gives a very good fit to the experimental data (not shown).

4. Discussion

On the basis of the three reference compounds used, **1**, **2** and **3**, proton–proton three-bond coupling constants provide an accurate method of determining the relative configuration of ring protons in pyrrolizidines and the ring conformations, as long as the ring system does not undergo ring-flipping. This criterion can be established by the observation of either large or small coupling constants within each ring. In the majority of cases, a full analysis of the ring coupling constants could provide unambiguous relative configurations. Where ambiguities still remain, these can be resolved by use of NOEs. We have applied this technique to the synthetic compound **5** that has already been fully characterized by X-ray crystallography.[8] Analysis of the coupling constant data alone allowed this compound to be identified as 7,7a-diepi-alexine or its enantiomer, identical to the structure to be published and thus verifying the approach used.

The compound **4** had been isolated and identified as '**5**'.[6] The original identification was based on an NMR analysis of the peracetylated compound using NOEs. This identified the relative configurations at C7a, C1, C2 and C3 as identical to australine **4**. The configuration at C7 was assigned based on the fact that the NMR spectrum of this compound was different from the previously reported spectrum of '**4**'.[7] However, the NMR data for this natural compound is significantly different from **5** as synthesized, particularly the chemical shift of C1H and, more importantly, the C7H–C7aH coupling constant. Our analysis, based on the coupling constants and NOEs, defines the structure of the natural product identified as '**5**' unambiguously as 7a-epi-alexine **4** or its enantiomer. This is consistent with the original

experimental data and the NMR analysis of the synthetic **5**, however is inconsistent with the reported NMR data for '**4**'. A synthesis for **4** has been designed very recently and the NMR spectrum of the synthetic compound produced is identical to that for the natural product.[9]

The natural product **6** had originally been identified as '**4**' based on comparison with the reported NMR and GC data for '**4**'.[7] The NMR coupling constants given in this original paper do not fit with the observed X-ray structure and this was rationalized by ring-flipping leading to the observation of average values.[7] However, observation of selected coupling constants outside the expected average range (C2H–C3H of 8.4 Hz, C6Ha–C7H of 2.3 Hz, C5Ha to C6Hb of 10.2 Hz) in both rings argues against such ring-flipping. Our analysis of coupling constants and NOEs identifies **6** unambiguously as 1,7a-diepi-alexine. This is fully consistent with all the NMR data and with the NMR analysis of **5** and **4** reported above. A natural compound identified as **6** by X-ray analysis of its 1,7-isopropylidene derivative has been previously reported.[6] The NMR spectrum of this compound gives significantly different chemical shifts to the original **6** but a very similar pattern of coupling constants. On combining the two samples in a two to one ratio, only one set of peaks is seen in the NMR spectrum with chemical shifts intermediate between the two starting samples. Thus, these two sample of **6** are identical. The difference in chemical shifts between the two samples is almost certainly due to slight differences in solvent (ionic strength, presence of metal ions, pH, etc.). Thus, coupling constant analysis is a more reliable method of identification than chemical shift comparison in these compounds.

Whilst there is no doubt about the structure of australine **4** as determined by X-ray diffraction,[7] the NMR data originally presented for '**4**' correspond to **6** and not to the compound obtained as crystals. A retrospective examination of the original data has revealed that the published spectrum was not that of the crystalline sample of australine **4** but of another (non-crystalline) sample isolated in the same manner which it is now apparent must have been the 1-epimer. The 200 MHz NMR spectrum recorded for the crystalline material corresponds to that of synthetic **4** published herein. The author (RJM) of the original publication[7] apologizes for any problems caused by this error. The original identification of the natural product '**5**' (and, presumably, any other compounds that relied upon the original NMR data given for '**4**') is thus also incorrect. This means that 7,7a-diepi-alexine **5** has not been found as a natural compound so far.

It is interesting to note the extreme sensitivity of the pyrrolizidine ring conformations to epimerization at selected sites. In the 7a-epi-alexines, the N–C5–C6–C7–C7a ring conformation appears to be governed by the configuration at C7 and the N–C7a–C1–C2–C3 ring conformation appears to be governed by the configuration at C3.

In summary, this paper reports the full NMR analysis of some naturally occurring highly oxygenated pyrrolizidines and corrects the errors in the original published spectrum of australine **4** and the consequent misassignment of the structure of the original natural product described as '**5**'. The NMR analysis of this ring system reported in this paper has provided evidence that the conformation of the alkaloids in solution is very similar to that of the crystal structures, and has also shown that the change of configuration of single positions in the rings can have profound effects on the conformations of apparently very similar structures; the dramatic changes in biological activities of such materials may be rationalized and modelling studies are in progress.

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