^{1}H NMR study of the hydrolysis of *N*-acylhydroxy[^{2}H]methylpyrroles

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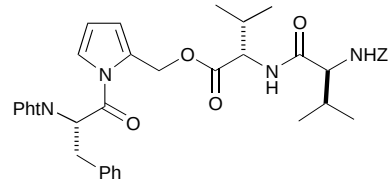
KOH catalysed hydrolysis of the (*S*)- and (*R*)-*N*-(*N*-phthalylleucinyl)hydroxy[^{2}H]methylpyrroles 6b and 6c, in CD_3CN containing 1 equiv. of (+)-*sec*-butylamine, proceeds by an initial *N*- to *O*-acyl transfer with retention of configuration at the labelled centre, followed by trapping of an azafulvene to give (*S*)- and (*R*)-*sec*-butylamino[^{2}H]methylpyrroles 9b.

An electron-withdrawing group (EWG) on the nitrogen of a pyrrole of the type **5** is thought to suppress the formation of a highly electrophilic azafulvenium species **4** in nucleophilic substitution reactions (Scheme 1). In the absence of such deactivation, the analogous pyrroles **1** readily react with a nucleophile, *via* the postulated azafulvene intermediate **2**, to give products of the type **3a**.[1,2] Such a sequence is thought to be involved in the biosynthesis of uroporphyrinogen III, an important intermediate in the biosynthesis of vitamin B_{12} and related pigments.[3]

Pyrroles substituted with an EWG are found in natural products,[4] and they are also useful intermediates in organic synthesis[2,5] (*e.g.* in the synthesis deuterium-labelled porphobilinogen[2,6]—an important probe for studying the biosynthesis of vitamin B_{12}). The idea of suppressing azafulvene formation, with the introduction of an EWG on nitrogen, has also been used to develop latent reactive inhibitors of serine proteases.[7] Here, a hydroxymethylpyrrole derivative such as **6a** (a peptidic example of the general compound **5**) is stabilised by *N*-acylation with an amino acid—chosen to be recognised by the target enzyme. Enzyme catalysed deacylation yields a reactive azafulvene **2** which is then thought to lead to covalent inactivation of the enzyme by alkylation (see Scheme 1 where Nu^- is an amino acid in the enzyme's active site).

Removal of an EWG from a pyrrole nitrogen, by chemical or enzymatic hydrolysis, represents a key step in all the above applications of compounds of the type **5**. Here we report the stereochemical fate of the deuterium label of (*S*)- and (*R*)-*N*-(*N*-phthalylleucinyl)hydroxy[^{2}H]methylpyrroles **6b** and **6c** upon KOH catalysed hydrolysis in the presence of an external nucleophile [(+)-*sec*-butylamine]. This study provides evidence for (i) an intramolecular acyl transfer to give an *O*-acylpyrrole **8** [pathway (*a*), Scheme 2] as an intermediate in the deacylation sequence, and (ii) the facile formation of an azafulvene **2** on deacylation as evidenced by its trapping with an external nucleophile to give equal amounts of the (*S*)- and (*R*)-amino[^{2}H]methylpyrroles **9**. This sequence also serves to model the proposed mechanism of action of **6a** as a latent reactive inhibitor of serine proteases—the KOH mimics the protease catalysed deacylation and the external nucleophile mimics the final inactivation step. It should also be noted that the *O*-acyl intermediate **8** bears a strong resemblence to **10** which is also an inhibitor of serine proteases.[7]



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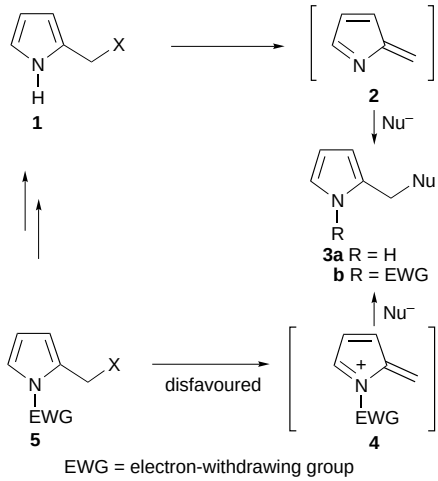
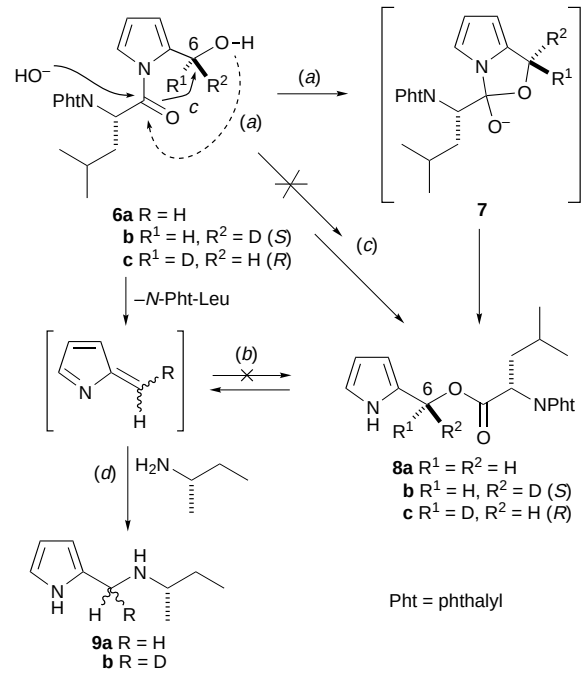
The key deuterium labelled hydroxymethylpyroles **6b** and **6c** were prepared from pyrrole-2-[^{2}H]carbaldehyde[2] by acylation with *N*-phthalylleucine acid fluoride according to the literature method,[8] followed by reduction with *R*- and *S*- Alpine-borane®, respectively.[2] The unlabelled analogue **6a** was similarly prepared by acylation of pyrrole-2-carbaldehyde followed by $Zn(BH_4)_2$ reduction of the formyl group. The epimeric purity at the deuterated centres of **6b** and **6c** was determined to be $> 9:1$ (see first column, Table 1) by integration of the pyrrole–CHD



Scheme 1



Scheme 2

Table 1 ^{1}H NMR resonances§ [CD_3CN, $CHCl_3$ internal standard (δ 7.25)] for the pyrrolemethylene group of **6** and the hydrolysis products **8** and **9** [KOH and (*S*)-(+)-*sec*-butylamine added]. In the spectra of **6a**–**c**, coupling of the signals with the OH proton were removed by homonuclear decoupling

	6a	**8a**	**9a**
	6c : **6b**	**8b** : **8c**	**9b**

singlet resonances at δ 4.21 and 4.24 respectively. The assigned configurations were consistent with related examples in which the absolute configuration has been determined by chemical conversion to the *O*-camphanate of [2-^{2}H] glycolic acid, a known literature reference compound.[2]

The mechanism of hydrolysis of compounds **6** was then studied.[7] In a typical experiment, an equivalent of KOH in D_2O (approx 10 μl) was added to a solution of either **6a**,**b** or **c** (3 mg) in CD_3CN (150 μl) containing $CHCl_3$ as an internal standard, and 1 equiv. of (+)-*sec*-butylamine (external nucleophile). The ratio of **6**, **8** and **9** was then monitored by ^{1}H NMR spectroscopy (the *exo*-methylene proton resonances of these compounds are shown in Table 1). The spectum taken immediately after mixing contained starting material **6**, *O*-acyl intermediate **8** and pyrrole amine **9** in a typical ratio of 1 : 1 : 1. ^{1}H NMR spectra of the mixtures after 18 h revealed that **6** and **8** had been completely converted to the pyrrole amine **9**.

The key points to note from the results shown in Table 1 are that the configurational purity (> 9 : 1) and absolute configurations§ of the starting materials **6b** and **c** are retained in the corresponding *O*- acyl intermediates **8b** and **c** (rows 2 and 3, Table 1). In theory, the formation of the *O*-acylpyrrole **8** could occur by either an intramolecular acyl transfer *via* the tetrahedral intermediate **7** [Scheme 2, pathway (*a*), retention of configuration], trapping of the azafulvene **2** with released *N*-phthalylleucine [Scheme 2, pathway (*b*), epimerisation at the methylene] or by an S_N2 like displacement [Scheme 2, pathway (*c*), inversion of configuration). The fact that the configurational purity at the [^{2}H]methylene centre of **8b** and **c** is intact, and also did not change with time, implies that these species are not in equlibrium with the azafulvene **2b**, hence pathway (*b*) is not operating. In addition, an S_N2 displacement at the methylene position of a hydroxymethylpyrrole has only been reported in extreme examples using a combination of Mitsunobu reaction conditions and an *N*-triflyl substituent to strongly deactivate the pyrrole ring and hence suppress azafulvene formation.[2] An *N*-acyl group is not sufficiently deactivating to promote S_N2 like displacement,[2] and hence a reaction of the type shown in pathway (*c*) is chemically unlikely under the conditions of the hydrolysis experiment. Pathway (*a*) is, however, consistent with both the literature and the observed results, *i.e*. the formation of **8** occurs with retention of configuration at the deuterated methylene centre. A second point to note is that equal mixtures of the (*S*) and (*R*) deuterium-labelled amines **9b** were produced as the end product in the reactions of **6b** and **c**, a result clearly consistent with the intermediacy of the azafulvene **2** [Scheme 2, pathway (*d*)]. Finally, as expected, the two oppositely labelled series **6b** and **c** gave complimentary results (rows 2 and 3, Table 1).

In summary, the above observations suggest that the *O*-acylpyrrole **8** is most likely formed *via* an intramolecular acyl transfer on hydrolysis of the *N*-(*N*-phthalylleucinyl)hydroxymethylpyrroles **6**. Evidence for the subsequent release of an azafulvene **2** is gained from the observed scrambling of the deuterium label at the [^{2}H]methylene position of **9** on trapping with (+)-*sec*-butylamine. Ongoing work is centred on the further development of these compounds as useful synthetic intermediates and inhibitors of serine proteases.

Notes and References

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§ In the case of *N*-acylhydroxy[^{2}H]methylpyrroles, the methylene resonance is upfield for the (*S*)-isomer (*e.g.* **6b**) relative to the (*R*)-isomer (*e.g.* **6c**). This observation is reversed for *O*-acylpyrroles (*e.g.* **8b** and **8c**) (see ref. 2).

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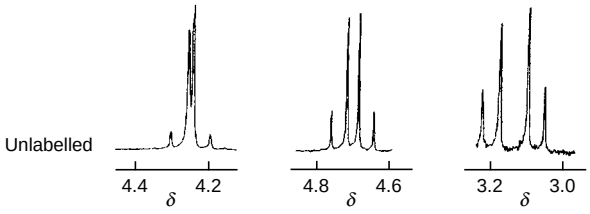

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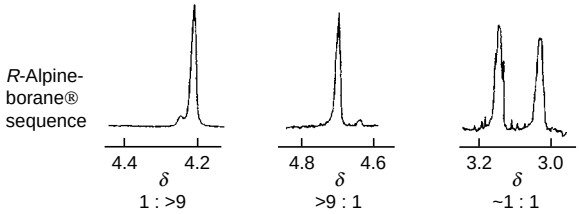

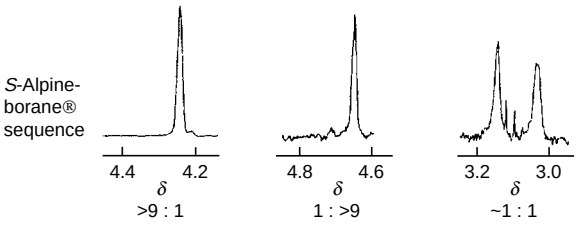

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