MAGNETIC RESONANCE IN CHEMISTRY
*Magn. Reson. Chem.* **37**, 60–64 (1999)

# Complete assignment of the $^{1}H$ and $^{13}C$ NMR spectra of the Coccinellidae-defensive alkaloids myrrhine, precoccinelline and hippodamine, their *N*-oxides and the corresponding hydrochlorides

**B. Lebrun, J. C. Braekman and D. Daloze***

Laboratory of Bio-Organic Chemistry, Department of Organic Chemistry, Faculty of Sciences, University of Brussels; CP 160/07, 50 Avenue F. D. Roosevelt, 1050 Brussels, Belgium

*Received 12 June 1998; accepted 29 August 1998*

ABSTRACT: The $^{1}H$ and $^{13}C$ NMR spectra of the 2-methylperhydro-9b-azaphenalene alkaloids characteristic of coccinellid beetles, myrrhine, precoccinelline, hippodamine, their *N*-oxides and the corresponding hydrochlorides were completely assigned for the first time by a one- and two-dimensional homo- and heteronuclear study ($^{1}H$, $^{13}C$, $^{1}H$–$^{1}H$ COSY, HMQC, HMBC) at 600 and 150.87 MHz. The influence of the ring junction stereochemistry and of the *N*-oxide function on the proton and carbon chemical shifts in this series of compounds is discussed. Protonation shifts are also considered 

KEYWORDS: NMR; $^{1}H$ NMR; $^{13}C$ NMR; alkaloids; 2-methylperhydro-9b-azaphenalene; *N*-oxides; hydrochlorides

## INTRODUCTION

Many coccinellid beetles are protected against vertebrate predators by the presence of repellent alkaloids in their hemolymph.[1,2] Different alkaloid types have been isolated, such as piperidines, pyrrolidines and homotropanes, but none is more characteristic of the coccinellids than the 2-methylperhydro-9b-azaphenalene skeleton[1,2] (Fig. 1). There are only three possible ring junction stereoisomers of this skeleton, and representatives of each of these isomers have been isolated from coccinellids. Myrrhine (**1**) is an achiral alkaloid based on the all-*trans* isomer, precoccinelline (**3**) and its *N*-oxide coccinelline (**4**) are representatives of another achiral isomer and hippodamine (**5**) and its *N*-oxide convergine (**6**) are representatives of the third stereoisomer, which is chiral. Myrrhine *N*-oxide (**2**), which is not a natural compound,[3] was included in this study for reasons of completeness. It should be pointed out that the three-dimensional shapes of these alkaloids differ from one stereoisomer to the other. Whereas **1** and **2** are flat molecules, with the three six-membered rings in one plane, **3** to **6** have two rings in one plane and the third one in a nearly perpendicular plane[1] (Fig. 2). Moreover, **3** and **4** differ from **5** and **6** only in the position of the $CH_3$ group on the perhydro-9b-azaphenalene skeleton (C-2 in **3** and **4**, and C-5 in **5** and **6**). To ensure that the chemical shifts of the carbon and hydrogen atoms of these isomers can be easily compared, we have changed the numbering used for **5** and **6** in previous publications.[4]

Although these alkaloids have been synthesized several times,[2] the $^{1}H$ and $^{13}C$ NMR data available until now are either incomplete or tentative, as the assignments were made before two-dimensional methods were available.[5,6] This is unfortunate since some of these compounds [e.g. precoccinelline (**3**) and hippodamine (**5**)] are not easily distinguished from each other. Thus, high-field $^{1}H$ and $^{13}C$ NMR spectra appear to provide the only reliable data to identify these

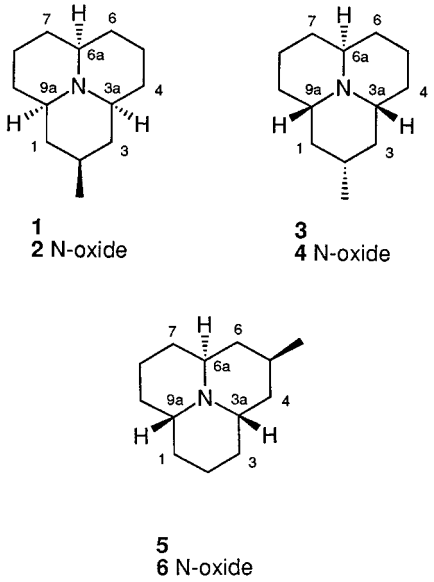


**Figure 1.** Compounds with the 2-methylperhydro-9b-azaphenalene skeleton.

* Correspondence to: D. Daloze, Laboratory of Bio-Organic Chemistry, Department of Organic Chemistry, Faculty of Sciences, University of Brussels; CP 160/07, 50 Avenue F. D. Roosevelt, 1050 Brussels, Belgium.
*Contract/grant sponsor*: Belgian Fund for Basic Research; *Contract/grant number*: 2.4513.90-96.
*Contract/grant sponsor*: French Community of Belgium; *Contract/grant number*: ARCC 93/98-137.
*Contract/grant sponsor*: FRIA.

 CCC 0749-1581/99/010060–05 $17.50

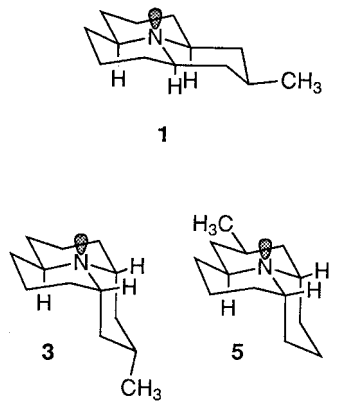


**Figure 2.** Three-dimensional shapes of **1**, **3** and **5**.

compounds unambiguously. This situation prompted us to make complete assignments of the signals of the $^{1}H$ and $^{13}C$ NMR spectra of **1–6**, which are reported in this paper. These assignments allowed us to analyze the influence of stereochemical parameters on the chemical shifts of carbon and hydrogen atoms in this series, and to discuss the differences observed between the spectra of free bases (**1**, **3** and **5**), the *N*-oxides derived therefrom (**2**, **4** and **6**) and the corresponding hydrochlorides.

## RESULTS AND DISCUSSION

### $^{1}H$ NMR spectra of 1–6 and their hydrochlorides

The $^{1}H$ NMR of the free bases **1**, **3** and **5** and of the hydrochlorides of **3** and **5** are reported in Table 1. The $^{1}H$ NMR spectra of *N*-oxides **2**, **4** and **6** and of the hydrochlorides of **4** and **6** are reported in Table 2. The spectra of the hydrochlorides of **1** and **2** could not be obtained owing to the small amount of material available.

**Influence of stereochemistry.** Comparison of the $^{1}H$ NMR of the free bases (Table 1) shows that the protons $\alpha$ to the nitrogen atom (H-3a, H-6a and H-9a) are more shielded in **1** than in **3** and **5**, the deshielding being about 1 ppm on average. This is also the case for the corresponding *N*-oxides **2**, **4** and **6** (Table 2). This effect is due to the different three-dimensional shapes of these molecules. Whereas H-3a and H-9a are in an equatorial position with respect to the *trans*-quinolizidine moiety of **3–6**, they are axial with respect to the three rings of **1** and **2**. Moreover, H-6a, which is axial in the three compounds, undergoes van der Waals interactions[7] with the axial protons at C-1 and C-3 in **3–6**, which explains its deshielding in these compounds in comparison with **1** and **2**. These van der Waals interactions also induce a strong deshielding of H-1ax and H-3ax in **3** and **5**, which become more deshielded than their equatorial counterparts. Furthermore, the axial carbon substituent at C-3a and C-9a in **3** also induces a strong deshielding of H-4ax and H-9ax in comparison with **1**.[8] In **5**, this effect is less marked for H-4ax, owing to the presence of the $CH_3$ group at C-5, whereas the uncertainty in the identification of H-9eq and H-9ax prevents any conclusion being drawn.

**Influence of protonation state of 1, 3 and 5.** The acid-induced shifts in the $^{1}H$ NMR spectra of alkaloids are well documented,[9] and here the spectra of the

**Table 1.** $^{1}H$ NMR spectra of **1**, **3**, **3**·HCl, **5** and **5**·HCl (600 MHz, $CDCl_3$, $\delta$, *J* in Hz)

| Position | 1 | 3 | 3·HCl | 5 | 5·HCl |
|---|---|---|---|---|---|
| 1eq | 1.54, m | 1.02, bd, 13.0 | 1.48, bd, 13.6 | 0.95, bd, 11.0 | 1.60, m |
| 1ax | 1.04, q, 12.0 | 1.50, m | 1.58, m | 1.78, bt, 10.5 | 1.88, m |
| 2eq | — | — | — | 1.83, m | 1.93, m |
| 2ax | 1.47, m | 1.62, m | 1.76, m | 1.48, m | 1.45, m |
| 3eq | 1.54, m | 1.02, bd, 13.0 | 1.48, bd, 13.6 | 0.92, bd, 11.0 | 1.48, m |
| 3ax | 1.04, q, 12.0 | 1.50, m | 1.58, m | 1.82, bt, 10.5 | 1.88, m |
| 3a | 1.85, bt, 10.5 | 2.95, bd, 9.0 | 3.42, bd, 11.5 | 2.92, m | 3.51, bd, 11.4 |
| 4eq | 1.56, m | 1.50, m | 1.58, m | 1.40, m | 1.53, m |
| 4ax | 1.38, m | 1.88, m | 2.55, tt, 13.6, 4.2 | 1.40, m | 2.18, dt, 5.2, 13.6 |
| 5eq | 1.65, m | 1.50, m | 1.62, m | — | — |
| 5ax | 1.34, m | 1.50, m | 1.54, m | 1.60, m | 1.75, m |
| 6eq | 1.56, m | 1.56, m | 1.68, bd, 14.0 | 1.48, m | 1.70, m |
| 6ax | 1.38, m | 1.18, m | 1.94, dq, 13.5, 4.1 | 0.78, m | 1.60, m |
| 6a | 1.82, bt, 10.0 | 2.74, bt, 11.0 | 3.20, q, 9.3 | 2.76, bt, 10.0 | 3.28, bq, 10.5 |
| 7eq | 1.56, m | 1.56, m | 1.68, bd, 14.0 | 1.49, m | 1.70, m |
| 7ax | 1.38, m | 1.18, m | 1.94, dq, 13.5, 4.1 | 1.10, m | 1.90, m |
| 8eq | 1.65, m | 1.50, m | 1.62, m | 1.40, m | 1.60[a], m |
| 8ax | 1.34, m | 1.50, m | 1.54, m | 1.40, m | 1.50[a], m |
| 9eq | 1.56, m | 1.50, m | 1.58, m | 1.78[a], m | 1.55, m |
| 9ax | 1.38, m | 1.88, m | 2.55, tt, 13.6, 4.2 | 1.44[a], m | 2.54, tt, 14.8, 4.8 |
| 9a | 1.85, bt, 10.5 | 2.95, bd, 11.5 | 3.42, bd, 9.3 | 2.90, m | 3.53, bd, 12.4 |
| $CH_3$ | 0.86, d, 6.2 | 0.94, d, 6.5 | 0.98, d, 6.2 | 0.78, d, 6.5 | 0.89, d, 6.2 |

[a] ax and eq may be interchanged.

**Table 2.** $^1$H NMR spectra of **2**, **4**, **4** · HCl, **6** and **6** · HCl (600 MHz, $CDCl_3$, $\delta$, $J$ in Hz)

| Position | **2** | **4** | **4** · HCl | **6** | **6** · HCl |
|---|---|---|---|---|---|
| 1eq | 1.41, m | 1.65, bd, 13.0 | 1.72, bd, 14.0 | 1.66, m | 1.80, m |
| 1ax | 2.02, q, 12.5 | 1.82, q, 13.5 | 1.83, q, 13.0 | 2.12, m | 2.25, m |
| 2eq | — | — | — | 1.88, bd, 11.0 | 1.94, m |
| 2ax | 1.68, m | 1.96, m | 2.13, m | 1.65, m | 1.94, m |
| 3eq | 1.41, m | 1.65, bd, 13.0 | 1.72, bd, 14.0 | 1.68, m | 1.84, m |
| 3ax | 2.02, q, 12.5 | 1.82, q, 13.5 | 1.83, q, 13.0 | 2.10, m | 2.25, m |
| 3a | 3.0, bt, 11.0 | 3.87, bd, 10.8 | 4.67, bd, 12.7 | 3.39, m | 4.55[a], m |
| 4eq | 1.44, m | 1.38, bd, 13.8 | 1.50, bd, 14.0 | 1.31, m | 1.55, m |
| 4ax | 2.30, m | 2.74, tt, 13.5, 5.4 | 2.70, m | 2.54, td, 13.2, 4.8 | 2.36, dt, 4.9, 13.6 |
| 5eq | 1.76, bd, 13.2 | 1.58, m | 1.63, m | — | — |
| 5ax | 1.45, m | 1.58, m | 1.63, m | 1.78, m | 1.98, m |
| 6eq | 1.44, m | 1.40, bd, 14.0 | 1.52, bd, 12.8 | 1.34, m | 1.65, m |
| 6ax | 2.30, m | 2.05, qd, 12.5, 5.6 | 2.02, m | 1.82, m | 1.65, m |
| 6a | 2.98, bt, 11.0 | 3.55, bt, 11.7 | 3.67, bt, 11.7 | 3.53, bt, 11.4 | 3.92, tt, 11.8, 3.0 |
| 7eq | 1.44, m | 1.40, bd, 14.0 | 1.52, bd, 12.8 | 1.32, m | 1.60, m |
| 7ax | 2.30, m | 2.05, qd, 12.5, 5.6 | 2.02, m | 2.12, m | 2.0, dt, 4.0, 13.4 |
| 8eq | 1.76, bd, 13.2 | 1.58, m | 1.63, m | 1.56, m | 1.70, m |
| 8ax | 1.45, m | 1.58, m | 1.63, m | 1.56, m | 1.70, m |
| 9eq | 1.44, m | 1.38, bd, 13.8 | 1.50, bd, 14.0 | 1.33, m | 1.55, m |
| 9ax | 2.30, m | 2.74, tt, 13.5, 5.4 | 2.70, m | 2.78, m | 2.68, tt, 14.0, 4.8 |
| 9a | 3.0, bt, 11.0 | 3.87, bd, 10.8 | 4.67, bd, 12.7 | 3.42, m | 4.55[a], m |
| $CH_3$ | 0.94, d, 6.8 | 1.0, d, 6.5 | 1.0, d, 6.6 | 0.91, d, 5.7 | 0.96, d, 6.5 |

[a] Superimposed signals.

hydrochlorides of **3** and **5** differ substantially from those of the free bases. Most of the signals were better resolved in the hydrochlorides, thus allowing a more accurate determination of their coupling pattern. Nearly all of the proton signals underwent a downfield shift in comparison with the free bases (Table 1). However, contrary to predictions based on inductive effects, the downfield shifts of the protons $\alpha$ to nitrogen ($\Delta\delta$ between $+0.46$ and $+0.63$ ppm) are not the most pronounced. Indeed, in **3** · HCl, H-4ax/H-9ax and H-6ax/H-7ax experience a downfield shift of $+0.67$ and $+0.76$ ppm respectively. As a consequence, these hydrogens appear at a lower field than their equatorial counterparts, the $\Delta\delta$ difference sometimes being larger than 1 ppm (see Table 1). This effect may be ascribed to the positively charged nitrogen, which deshields all the hydrogen atoms experiencing a 1,3-diaxial relationship with it.[7] In contrast, the downfield shifts of equatorial protons are much smaller. This difference between axial and equatorial protons has been ascribed to a different angle between the C—H and $N^+$—H bond.[9] The effects are similar in **5** · HCl, but, again, the presence of the methyl group at C-5 reduces the deshielding of H-6ax.

**Influence of the *N*-oxide function.** In the three *N*-oxides (**2**, **4** and **6**) (Table 2), the most characteristic effect of the N—O dipole is to induce a strong downfield shift of all axial protons which are in a 1,3-diaxial interaction with it, a situation well known for other polar substituents.[7] Thus, except for H-5ax and H-8ax in **2** and **4** and H-2ax and H-8ax in **6**, all axial hydrogens are more deshielded than the corresponding equatorial hydrogen. In **4** and **6**, the deshielding of H-1ax and H-3ax is due to a combination of van der Waals and *N*-oxide effects.

**Influence of protonation of the *N*-oxide.** In **4** · HCl and **6** · HCl, the protonation of the *N*-oxide function leads to a deshielding of the $\alpha$-hydrogens (Table 2). This effect is larger for H-3a and H-9a ($\Delta\delta + 0.80$ in **4** · HCl; $\Delta\delta + 1.16$ and $+1.13$, respectively, in **6** · HCl) than for H-6a ($\Delta\delta + 0.12$ in **4** · HCl; $\Delta\delta + 0.39$ in **6** · HCl). It should be noted that the protons which are in a 1,3-diaxal relationship with the *N*-oxide function are not affected or even slightly shielded upon protonation (e.g. H-4ax, H-6ax, H-7ax, H-9ax).

## $^{13}$C NMR spectra of 1–6 and their hydrochlorides

The $^{13}$C NMR of the free bases **1**, **3** and **5** and of the hydrochlorides of **3** and **5** are reported in Table 3. The $^{13}$C NMR spectra of the *N*-oxides **2**, **4** and **6** and of the hydrochlorides of **4** and **6** are reported in Table 4.

Owing to the presence of a symmetry plane, the spectra of **1** and **3** exhibit only eight signals, whereas the spectrum of **5** displays the expected 13 signals. Moreover, the chemical shifts of the carbon atoms of **1** and **3** are very different (see the next section). The same conclusions also apply to the $^{13}$C NMR spectra of the *N*-oxides **2** and **4** (Table 4). $^{13}$C NMR thus seems to be the method of choice to identify unambiguously the stereoisomeric 2-methylperhydro-9b-azaphenalene alkaloids and their *N*-oxides.

It should be mentioned that there is an error in the data reported for myrrhine *N*-oxide (**2**) by Ayer *et al.*[10]

**Table 3.** $^{13}C$ NMR spectra of **1**, **3**, **3**·HCl, **5** and **5**·HCl (150.87 MHz, $CDCl_3$, $\delta$)

| Position | 1 | 3 | 3·HCl | 5 | 5·HCl |
|---|---|---|---|---|---|
| 1 | 42.6 | 31.2[a] | 31.4 | 23.0 | 23.1 |
| 2 | 30.3 | 32.6 | 30.3 | 26.0 | 22.9 |
| 3 | 42.6 | 31.2[a] | 31.4 | 22.3 | 23.6 |
| 3a | 62.2 | 58.1 | 59.2 | 58.0 | 59.8 |
| 4 | 34.0 | 31.1[a] | 28.1 | 39.9 | 36.6 |
| 5 | 24.3 | 19.8 | 17.8 | 25.6 | 24.4 |
| 6 | 34.0 | 34.6 | 30.8 | 43.3 | 39.2 |
| 6a | 62.2 | 48.3 | 51.6 | 47.9 | 51.5 |
| 7 | 34.0 | 34.6 | 30.8 | 34.5 | 30.8 |
| 8 | 24.3 | 19.8 | 17.8 | 19.7 | 17.8 |
| 9 | 34.0 | 31.1[a] | 28.1 | 31.3 | 28.3 |
| 9a | 62.2 | 58.1 | 59.2 | 58.8 | 59.3 |
| $CH_3$ | 22.0 | 22.7 | 21.6 | 22.4 | 21.2 |

[a] These assignments may be interchanged.

(and cited in the review of Tourwé and Van Binst[5]): for the methylene carbons, they reported[10] two C at $\delta$ 35.8, two C at $\delta$ 35.6, two C at $\delta$ 27.4, and two C at $\delta$ 23.4, whereas we found by two-dimensional methods two C at $\delta$ 35.0, four C at $\delta$ 27.0 and two C at $\delta$ 22.5. These latter values are in good agreement with the upfield shifts expected on going from the free base to the *N*-oxide (see below).

**Influence of stereochemistry.** Except for C-2, C-6 and C-7, all ring carbon atoms of **3** are more shielded than those of **1**. This is easily explained by a *γ-gauche* effect[11] between C-1, C-6a and C-8 and between C-3, C-5 and C-6a. Thus, C-1, C-3 and C-6a, which experience two *γ-gauche* effects in **3**, are the most shielded (−11.4, −11.4 and −13.9 ppm, respectively), whereas C-5 and C-8, which suffer only one *γ-gauche* interaction, are shielded by −4.5 ppm. C-3a, C-4, C-9 and C-9a undergo smaller upfield shifts, which can be explained by the loss of one *anti* vicinal hydrogen–hydrogen interaction[12] for each of these carbons on going from **1** to **3**. Each of these carbons has two such interactions in **1** and only one in **3** (Fig. 2). In simple hydrocarbons, the loss of one *anti* vicinal hydrogen–hydrogen interaction has been estimated to shield the corresponding carbon by about −3 ppm.[12] The values we observed in our systems are slightly larger ($\Delta\delta$, C-3a and C-9a: −4.1 ppm; C-4 and C-9: −2.9 ppm), but are still of the same order of magnitude. The comparison between **1** and **5** is less straightforward, as the $CH_3$ group occupies a different position in these two compounds. However, when the comparison is limited to the carbon atoms which are sufficiently remote from the $CH_3$ group (C-6a, C-7, C-8, C-9 and C-9a), we can see that the chemical shifts are nearly the same as those of the corresponding carbons of **3**.

**Influence of protonation of the free bases.** On protonation of the nitrogen atom of the free bases **3** and **5**, the expected[5] deshielding of the α-carbons is observed (from +1.1 to +3.3 ppm for **3** and from +0.5 to +3.6 ppm for **5**). The magnitude of this effect is clearly correlated with the stereochemical relationship between the hydrogen atom of the α-carbon and the nitrogen lone pair, the two carbons bearing an equatorial hydrogen being more deshielded (*ca.* +3.5 ppm). As already reported for other alkaloid skeletons,[5] the *β*- and *γ*-carbons are slightly shielded in the hydrochlorides ($\Delta\delta$ − 2.0 to −4.1 ppm), except for C-1 and C-3, which have the same $\delta$ in the hydrochlorides as in the free bases. This shows that the shielding effect depends on the stereochemical relationship between the $N^+$—H bond and the carbon atom.

**Influence of the *N*-oxide function.** The influence of an *N*-oxide function on the chemical shift of the carbon atoms of alkaloids has already been studied.[5] The $\Delta\delta$ measured for our compounds are reported in Table 5. The values confirm those reported in the literature[5] except, as already mentioned, that the values reported by Ayer *et al.*[10] for **2** should be changed to those in Table 4. The expected pattern of deshielding of the α-carbons (+10.8 to +16.3 ppm) and shielding of *β*- and

**Table 4.** $^{13}C$ NMR spectra of **2**, **4**, **4**·HCl, **6** and **6**·HCl (150.87 MHz, $CDCl_3$, $\delta$)

| Position | 2 | 4 | 4·HCl | 6 | 6·HCl |
|---|---|---|---|---|---|
| 1 | 35.0 | 35.5 | 35.5 | 27.29 | 27.1 |
| 2 | 29.6 | 29.8 | 29.3 | 23.5 | 22.3 |
| 3 | 35.0 | 35.5 | 35.5 | 28.0 | 27.8 |
| 3a | 74.2 | 72.7 | 71.5 | 74.3 | 72.5 |
| 4 | 27.0 | 25.2 | 25.0 | 33.9 | 33.3 |
| 5 | 22.5 | 17.5 | 17.2 | 24.5 | 23.5 |
| 6 | 27.0 | 27.0 | 27.2 | 35.8 | 35.3 |
| 6a | 74.2 | 59.5 | 60.2 | 58.7 | 60.1 |
| 7 | 27.0 | 27.0 | 27.2 | 27.33 | 27.0 |
| 8 | 22.5 | 17.5 | 17.2 | 18.0 | 17.0 |
| 9 | 27.0 | 25.2 | 25.0 | 25.6 | 25.1 |
| 9a | 74.2 | 72.7 | 71.5 | 73.7 | 72.1 |
| $CH_3$ | 21.0 | 21.0 | 20.9 | 21..5 | 21.1 |

**Table 5.** $\Delta\delta$ of the carbon chemical shifts between the free bases **1**, **3** and **5** and the corresponding *N*-oxides **2**, **4** and **6**

| Position | $\Delta\delta$ (**1** → **2**) | $\Delta\delta$ (**3** → **4**) | $\Delta\delta$ (**5** → **6**) |
|---|---|---|---|
| 1 | −7.6 | +4.3 | +4.3 |
| 2 | −0.7 | −2.8 | −2.5 |
| 3 | −7.6 | +4.3 | +5.7 |
| 3a | +12.0 | +14.6 | +16.3 |
| 4 | −7.0 | −5.9 | −6.0 |
| 5 | −1.8 | −2.3 | −1.1 |
| 6 | −7.0 | −7.6 | −7.5 |
| 6a | +12.0 | +11.2 | +10.8 |
| 7 | −7.0 | −7.6 | −7.1 |
| 8 | −1.8 | −2.3 | −1.7 |
| 9 | −7.0 | −5.9 | −5.7 |
| 9a | +12.0 | +14.6 | +14.9 |

$\gamma$-carbons is observed. In the case of $\beta$-carbons, the strongest shielding ($-5.7$ to $-7.6$ ppm) is experienced by those which have one hydrogen atom in a 1,3-diaxial relationship with the *N*-oxide function (van der Waals effect), such as C-4, C-6, C-7 and C-9 in the three *N*-oxides, plus C-1 and C-3 in **2** (Table 5). The $\beta$-carbons which are not in this situation, such as C-1 and C-3 in **4** and **6**, are deshielded ($+4.3$ and $+5.7$ ppm) in comparison with the free base. In the case of $\gamma$-carbons (C-2, C-5 and C-8), the trend is towards shielding but less so than for the $\beta$-carbons ($-0.7$ to $-2.8$ ppm).

**Influence of protonation of the *N*-oxide.** The protonation shifts on going from an *N*-oxide to the corresponding protonated species are much less pronounced than for the protonation of the free bases. In **4** and **6**, C-3a and C-9a are slightly shielded in the protonated compound ($-1.2$ to $-1.8$ ppm), C-6a is slightly deshielded ($+0.7$ and $+1.4$ ppm) and there is nearly no effect on the other carbons.

## CONCLUSIONS

This paper reports the first complete assignment of the $^1$H and $^{13}$C NMR spectra of the three stereoisomeric 2-methylperhydro-9b-azaphenalene alkaloids from coccinellid beetles and of the corresponding *N*-oxides. These data show that the $^{13}$C NMR spectra are much less influenced than the $^1$H NMR spectra by the extent of protonation, a situation which, together with the clear differences between the $^{13}$C NMR spectra of these compounds, makes a strong case for using this method for identification purposes in this series. The differences in chemical shifts observed for the carbon and hydrogen atoms of compounds **1**–**6** and their hydrochlorides have been rationalized by taking into account a combination of *γ-gauche*, van der Waals, protonation and *anti* vicinal hydrogen–hydrogen effects.

## EXPERIMENTAL

Coccinelline (**4**) and convergine (**6**) were isolated from *Coccinella 7-punctata* and *Hippodamia convergens*, respectively. Owing to their relative instability, the corresponding free bases, precoccinelline (**3**) and hippodamine (**5**), were prepared by Zn–$H_2SO_4$ reduction[13] of **4** and **6**, just before obtaining the NMR spectra. Myrrhine (**1**) was provided by Dr M. Birkett (IACR, Rothamsted, UK) and its *N*-oxide (**2**) was prepared by *m*-chloroperoxybenzoic acid oxidation.[3] The hydrochlorides were prepared by bubbling gaseous HCl into an ethereal solution of the compound.

All NMR spectra ($^1$H, $^{13}$C, $^1$H–$^1$H COSY, HMQC[14] and HMBC[15]) were obtained on a Varian Unity 600 MHz spectrometer in $CDCl_3$ solutions (about 6–8 mg ml$^{-1}$) at 30 °C. The residual solvent peak was referenced to 7.3 ppm in $^1$H and 77.10 ppm in $^{13}$C NMR. 1D $^1$H NMR spectra were recorded with a spectral width of 5900 Hz, 2048 points. 1D $^{13}$C NMR spectra were recorded with a spectral width of 13 000 Hz, 32K points. 2D gradient DQF COSY were recorded with a spectral width of 5900 Hz, with 2048 points, 512 increments (zero-filling to 1K); four transients were recorded for each increment with a relaxation delay of 1 s. Two-dimensional gradient HMQC and HMBC (5 and 10 Hz) were recorded with a spectral width of 5900 Hz, 2048 points in the $^1$H dimension and 13 000 Hz in the $^{13}$C dimension, 400 increments (zero-filling to 1K); 32 transients were recorded for each increment, with a relaxation delay of 1 s.

## Acknowledgements

This work was supported by grants from the Belgian Fund for Basic Research (Grant 2.4513.90-96) and the French Community of Belgium (ARC 93/98-137). We thank Dr K. Bartik and Mr C. Maerschalk for the NMR spectra and Dr Mike Birkett (IACR, Rothamsted, UK) for a generous gift of myrrhine. One of us (B.L.) thanks the FRIA for financial support.

## REFERENCES

1. D. Daloze, J. C. Braekman and J. M. Pasteels, *Chemoecology* **5/6**, 173 (1995).
2. A. G. King and J. Meinwald, *Chem. Rev.* **96**, 1105 (1996).
3. B. Tursch, D. Daloze, J. C. Braekman, C. Hootelé and J. M. Pasteels, *Tetrahedron* **31**, 1541 (1975).
4. B. Tursch, D. Daloze, J. C. Braekman, C. Hootelé, A. Cravador, D. Losman and R. Karlsson, *Tetrahedron Lett.* 409 (1974).
5. D. Tourwé and G. Van Binst, *Heterocycles*, **9**, 507 (1978).
6. R. H. Mueller, M. E. Thompson and R. M. DiPardo, *J. Org. Chem.* **49**, 2217 (1984).
7. N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy to Organic Chemistry*, pp. 183–190. Holden-Day, San Francisco (1964).
8. L. M. Jackman and S. Sternhell, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, p. 78. Pergamon Press, Oxford (1969).
9. J. Schripsema, R. Verpoorte and A. Baerheim Svendsen, *Tetrahedron Lett.* **27**, 2523 (1986).
10. W. A. Ayer, R. Dawe, R. A. Eisner and K. Furuichi, *Can. J. Chem.* **54**, 473 (1976).
11. F. W. Wehrli and T. Wirthlin, *Interpretation of Carbon-13 NMR Spectra*. Heyden, London (1978).
12. J. K. Whitesell and M. A. Minton, *J. Am. Chem. Soc.* **109**, 225 (1987).
13. T. Hartmann and G. Toppel, *Phytochemistry* **26**, 1639 (1987).
14. M. F. Summers, L. G. Marzilli and A. Bax, *J. Am. Chem. Soc.* **108**, 4285 (1986).
15. A. Bax and M. F. J. Summers, *J. Am. Chem. Soc.* **108**, 2093 (1986).

 