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# THE COMPLETE ASSIGNMENT OF THE $^1$H- AND $^{13}$C-NMR SPECTRA OF ISOCOCCULIDINE[1]

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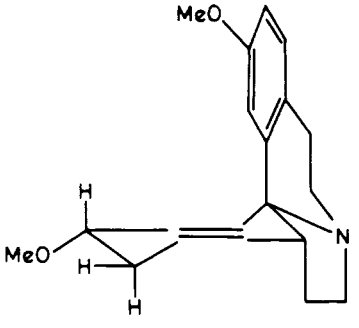
Isococculidine [**1**], a representative of abnormal *Erythrina* alkaloids isolated from the leaves of *Cocculus laurifolius* DC. (Menispermaceae), exhibited marked neuromuscular blocking and hypotensive activities (1). The stereostructure of **1** has been established by physicochemical methods (2,3). However, no detailed $^1$H- and $^{13}$C-nmr analysis of **1** and other abnormal *Erythrina* alkaloids has been previously made. It was thought desirable to complete its $^1$H- and $^{13}$C-nmr assignments, which could be very useful in studies of the biosynthetic pathways to these alkaloids using $^{13}$C-labeled precursors. The effect of the *N*-methiodide group on the chemical shifts of the methine and methylene carbons was also studied, because pachygonine, the *N*-methio salt of an abnormal *Erythrina* alkaloid, has been reported from *Pachygone ovata* (4).

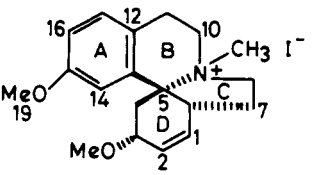



**1**

## RESULTS AND DISCUSSION

Preliminary assignments of the alicyclic protons of **1** (Table 1) were achieved from the homonuclear COSY spectrum. Inasmuch as the $^1$H-nmr assignments of the aromatic, olefinic, and H-3 protons of **1** have previously been established (2), they will not be discussed here. The starting point for $^1$H-nmr analysis was the olefinic methine signal at δ 6.03 which showed a cross peak at δ 2.76 with H-6 in the alicyclic region. Moreover, the signal at δ 2.76 was correlated to the signals at δ 2.17 and 1.85, which were, therefore, assigned to $H_2$-7. The latter resonance in turn showed cross peaks with $H_2$-8 at δ 3.20 and 2.76. It should be noted that the signal at δ 2.76 integrates for two protons, and one of the C-8 methylene protons (δ 2.76) was isochronous to H-6. The assignment of the C-4 methylene protons at δ 1.75 and 1.98 was straightforward as they exhibited cross peaks with the signal at δ 3.53 for H-3.

The distinction between $H_2$-10 and $H_2$-11 was carried out through identification of corresponding carbon chemical




**2**

shift and then correlating with the proton resonances in the 2D $^1$H-$^{13}$C COSY spectrum. These assignments were further confirmed by double resonance and nOe difference experiments. For example, irradiation of H-17 gave nOe's for H-16 and one of the H-11 protons.

The nOe difference studies (Table 1) gave a plethora of information regarding the stereostructure of **1** in solution. The most interesting result was obtained on

[1]CDRI Communication No. 4150.

TABLE 1. $^1$H Chemical Shifts (δH), Coupling Constants ($J_{H,H}$) and $^1$H, $^1$H nOe's for Isococculidine [**1**] and $^{13}$C Chemical Shifts (δC) for **1** and **2**.

| Atom | C[1] | C[2] | H[1] | $J_{H,H}$ (Hz) | $^1$H, $^1$H nOe's |
|---|---|---|---|---|---|
| 1 | 128.7 | 127.6 | 6.03 | $J_{1,2}=10.6, J_{1,3}=2.3, J_{1,6}=4.1$ | 2,6 |
| 2 | 131.2 | 130.5 | 5.91 | $J_{2,1}=10.6, J_{2,3}=1.8$ | 1,18-OMe |
| 3 | 75.1 | 74.0 | 3.53 | $J_{3,1}=2.3, J_{3,2}=1.8, J_{3,4a}=11.7, J_{3,4e}=5.3$ | 4e,14 |
| 4a | 37.5 | 32.9 | 1.75 | $J_{gem}=14.9, J_{4,3}=11.7$ | 4e,18-OMe |
| 4e | | | 1.98 | $J_{gem}=14.9, J_{4,3}=5.3$ | 3,4a,18-OMe |
| 5 | 62.9 | 76.2 | — | — | — |
| 6 | 45.1 | 36.4 | 2.76 | $J_{6,1}=4.1, J_{6,7\alpha}=1.8, J_{6,7\beta}=9.4$ | 1,7α,14 |
| 7β | 31.1 | 22.0 | 1.85 | $J_{7,6}=9.4, J_{7,8\beta}=5.8, J_{7,8\alpha}=10.0, J_{gem}=12.0$ | 7α,8β |
| 7α | | | 2.17 | $J_{7,6}=1.8, J_{7,8\beta}=2.9, J_{7,8\alpha}=4.1, J_{gem}=12.0$ | 7β,6 |
| 8α | 52.9 | 59.0 | 2.79 | $J_{8,7\alpha}=4.1, J_{8,7\beta}=10.0, J_{gem}=12.0$ | 8β,7α |
| 8β | | | 3.20 | $J_{8,7\alpha}=2.9, J_{8,7\beta}=5.8, J_{gem}=12.0$ | 8α |
| 10a | 47.1 | 51.4 | 2.64 | $J_{10,11e}=3.0, J_{10,11a}=5.8, J_{gem}=12.3$ | 10e |
| 10e | | | 3.38 | $J_{10,11e}=5.8, J_{10,11a}=7.0, J_{gem}=12.3$ | 10a |
| 11 | 27.5 | 27.0 | 2.87 | m | |
| 12 | 128.0 | 122.3 | — | — | |
| 13 | 144.0 | 134.6 | — | — | |
| 14 | 110.0 | 110.3 | 6.76 | $J_{14,16}=2.9$ | 3,6,19-OMe |
| 15 | 157.8 | 157.2 | — | — | |
| 16 | 111.0 | 113.9 | 6.73 | $J_{14,16}=2.9, J_{16,17}=9.0$ | |
| 17 | 128.8 | 128.5 | 7.09 | $J_{17,16}=9.0$ | 16 |
| 18-OMe | 55.5 | 55.5 | 3.26 | s | 2 |
| 19-OMe | 55.0 | 55.1 | 3.76 | s | 14,16 |
| MeN | — | 45.9 | — | | |

irradiating the signal at δ 3.53 (H-3), which resulted in the enhancement of the signal at δ 6.76 (H-14) indicating that these protons are close in space. The 1.8 Hz coupling between H-2 and H-3 indicated a β-axial conformation for the latter (5). This was confirmed by the 11.7 Hz coupling between H-3 and H-4a. It was interesting that H-6 showed a strong nOe with H-14 and a 4.1-Hz coupling with H-1 indicating that ring D has a half-chair conformation.

The $^{13}$C chemical shift data for **1** listed in Table 1 were achieved on the basis of their multiplicities in the single frequency off-resonance decoupled spectra as well as using the DEPT pulse sequence that provided the number of attached protons to individual carbons. Wherever it was difficult, the assignments were accomplished by combined application of 2D hetero COSY and specific proton decoupled $^{13}$C-nmr techniques.

The DEPT spectra of **1** indicated methylene carbons at δ 27.5, 31.1, 31.5, 47.1, and 52.9. The C-4, C-7, and C-8 methylenes were readily identified in the 2D $^1$H-$^{13}$C hetero COSY spectrum as their $^1$H chemical shift has been established. Of the remaining signals at δ 27.5 and 47.1, the latter was assigned to C-10 because of the attached hetero atom. The $^{13}$C chemical shifts of quaternary carbons C-5, C-12, C-13, and C-15 were assigned by comparison with the spectra of related *Erythrina* alkaloids (6).[2]

[2]Om Prakash, Raja Roy, S. Jain, and D.S. Bhakuni (unpublished results).

Conversion of **1** into the corresponding methiodide **2** gave an upfield shift of carbons which are two bonds away (β) from the nitrogen and a downfield shift for α carbons. For example, the signal at 31.1 (C-7) in **1** appeared at δ 22.0 in **2**. On the other hand, the signal at δ 47.1 (C-10) in **1** appeared at δ 51.4 in **2**.

## EXPERIMENTAL

All the spectra were recorded with a Bruker WM-400 multinuclear FT nmr spectrometer. Samples for $^{1}H$ measurements of **1** and **2** were carried out in a 5-mm $^{1}H/^{13}C$ dual probe containing 4 mg of sample in 0.5 ml of $CDCl_3$. The $^{13}C$-nmr samples were prepared in 5-mm tubes by mixing 50 mg of sample with 0.5 ml of $CDCl_3$; TMS was used as an internal standard in both measurements. The 2D COSY spectrum was obtained using a pulse sequence 90°-$t_1$-90°-acq (7). Proton coupling constants in **1** were extracted from the spin decoupling difference spectroscopy (SDDS) technique and from a resolution-enhanced $^{1}H$ spectrum using the Gaussian multiplication technique (8).

Resonance multiplicities for $^{13}C$ were established via the acquisition of SFORD and DEPT spectra (9). The 2D $^{1}H$-$^{13}C$ chemical shift correlation nmr spectrum was obtained using DEPT 2D pulse sequence (10).

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