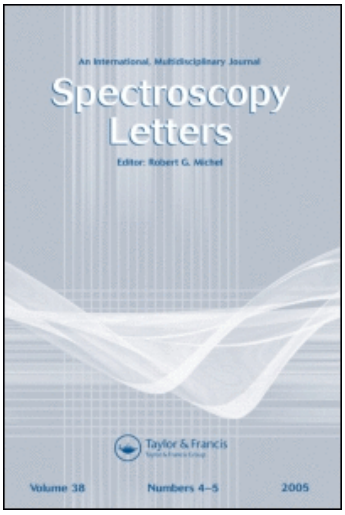


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# Assignments of $^{1}H$ and $^{13}C$ NMR Spectral Data for Benzoylecgonine, a Cocaine Metabolite

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**ABSTRACT** The complete assignment of the $^{1}H$ and $^{13}C$ NMR spectra of benzoylecgonine, a cocaine metabolite, was performed, with the aid of some 2D experiments such as gCOSY and gHSQC.

**KEYWORDS** benzoylecgonine, $^{13}C$ NMR, cocaine metabolite, $^{1}H$ NMR, NMR

## INTRODUCTION

Benzoylecgonine (BEG; Fig. 1) is a predominant cocaine metabolite. It is formed by hydrolysis of cocaine in the liver and is excreted mainly in the urine. Thus, it has been used as a valuable indicator of cocaine and is crack use.[1,2] BEG is routinely detected by a variety of techniques, as for instance by fluorescence polarized immunoassay (FPIA), for initial screening and is quantified by gas chromatography/mass spectrometry (GC/MS).[3]

Recently, high-resolution $^{1}H$ NMR spectroscopy has been employed to detect and quantify several metabolites in human urine and saliva.[4,5] A project of social significance has been launched in our laboratory, which involves the determination of BEG in the meconium (first bowel discharge) of newborn children whose mothers were suspected of drug abuse. This is very important for early medical treatment of these children. The proposed method of analysis involves the use of quantitative $^{1}H$ NMR spectroscopy, but the NMR data for BEG, in the literature, are either not complete or in disagreement among several authors.[5–7]

Therefore, BEG (**1**) (3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-4-carboxylic acid) was synthesized from cocaine, and its $^{1}H$ and $^{13}C$ NMR spectra were fully assigned. Careful analyses of the spectra were performed using the 2D NMR techniques Correlated Spectroscopy with field gradient (gCOSY), Heteronuclear Single Quantum Coherence with field gradient (gHSQC), and Heteronuclear Bond Correlation with field gradient (gHMBC).

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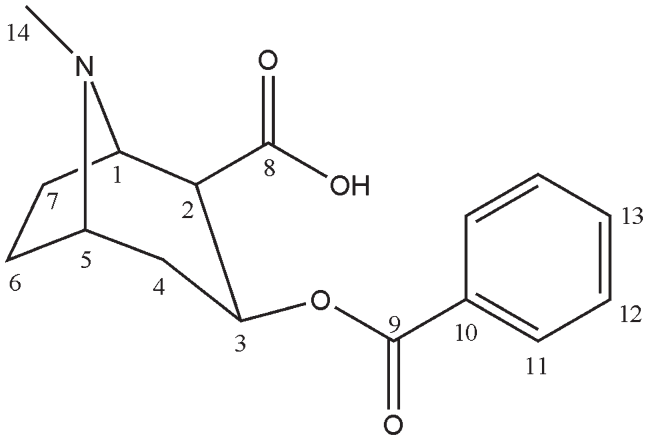


**FIGURE 1** **Structure and Atom Numbering for Benzoylecgonine (1).**

## MATERIALS AND METHODS

### Materials

Benzoylecgonine (**1**) was prepared through methods described in the literature.[8] Cocaine used in the synthesis was supplied by the Federal Police of Brazil.

### NMR Spectra

All 1D ($^1$H and $^{13}$C) and 2D NMR experiments were performed on a Varian INOVA 500 spectrometer, Palo Alto, Ca, USA (499.88 MHz for $^1$H and 125.70 MHz for $^{13}$C). The $^1$H NMR spectra were acquired with a spectral width of 5.0 kHz, 32 K data points zero-filled to 128 K, acquisition time 3.277 s, 16 transients, providing a digital resolution of about 0.076 Hz. For the $^{13}$C NMR spectra, a spectral width of 27.5 kHz was used, 64 K (K = 1000) data points zero-filled to 256 K, acquisition time 1.19 s, 1024 transients, giving a digital resolution of about 0.21 Hz. The experiments were performed with standard pulse sequences, as suggested by the equipment manufacturer. All experiments were performed at 300 K, and all sample concentrations ranged from 20 to 30 mg mL$^{-1}$, with a total volume of 0.6 mL for each sample in $CD_3CN$ with TMS as internal reference.

For gCOSY spectra, a matrix of 2048 × 128 data points was collected with eight scans for each increment. For gHSQC spectra, a matrix of 2048 × 128 data points was collected with 128 scans for each increment. For gHMBC spectra, a matrix of 2048 × 256 data points was collected with 128 scans for each increment. The incremented spectra were obtained with delay values optimized for 8 Hz.

## RESULTS AND DISCUSSION

The $^1$H and $^{13}$C NMR data for benzoylecgonine (**1**) are presented in Table 1. The $^1$H NMR spectra contained no overlapped signals, thus the chemical shifts and the multiplicities could be easily determined.



**TABLE 1** **$^1$H and $^{13}$C NMR Chemical Shifts, Multiplicities, Coupling Constants, H-H and H-C Correlations in gHMBC, gCOSY, and gHSQC Experiments for Benzoylecgonine (1) in $CD_3CN$**

| C | δ C (ppm) | H | δ H (ppm) | Multiplicity | Coupling constants (Hz) | gHMBC | gCOSY | gHSQC |
|---|---|---|---|---|---|---|---|---|
| 1 | 65.3 | 1 | 3.71(1H) | dd | $J_{1,2} = 3.1$; $J_{1,7\alpha} = 6.1$ | C-2, 3, 5, 6, 14 | $H_2$, $H_{7\alpha}$, $H_{7\beta}$ | $H_1$ |
| 2 | 49.3 | 2 | 2.99(1H) | dd | $J_{2,3} = 6.5$; $J_{2,1} = 3.1$ | C-3, 4, 8 | $H_1$, $H_3$ | $H_2$ |
| 3 | 66.3 | 3 | 5.32(1H) | dt | $J_{3,4\alpha} = 11.3$; $J_{3,4\beta} = J_{3,2} = 6.5$ | C-2, 4, 8, 9 | $H_2$, $H_{4\alpha}$, $H_{4\beta}$ | $H_3$ |
| 4 | 34.6 | $4_\alpha$ | 2.33(1H) | ddd | $J_{4\alpha,4\beta} = 13.5$; $J_{4\alpha,3} = 11.3$; $J_{4\alpha,5} = 3.1$ | C-3, 5, 6 | $H_3$, $H_{4\beta}$, $H_5$ | $H_{4\alpha}$, $H_{4\beta}$ |
| 4 | | $4_\beta$ | 2.14(1H) | ddd | $J_{4\beta,4\alpha} = 13.5$; $J_{4\beta,3} = 6.5$; $J_{4\beta,5} = 3.1$ | C-2, 3 | $H_3$, $H_{4\alpha}$, $H_5$ | |
| 5 | 61.9 | 5 | 3.64(1H) | dt | $J_{5,6\alpha} = 6.4$; $J_{5,4\alpha} = J_{5,4\beta} = 3.1$ | C-3, 7 | $H_{6\alpha}$, $H_{4\alpha}$, $H_{4\beta}$ | $H_5$ |
| 6 | 25.7 | $6_\alpha$ | 2.26(1H) | dd | $J_{6\alpha,6\beta} \approx 9.8$; $J_{6\alpha,5} = 6.4$ | C-1, 4, 5, 7 | $H_5$, $H_{6\alpha}$, $H_{7\beta}$ | $H_{6\alpha}$, $H_{6\beta}$ |
| 6 | | $6_\beta$ | 1.96(1H) | t | $J_{6\beta,7\beta} = J_{6\beta,6\alpha} = 9.8$ | C-1, 4, 5, 7 | $H_5$, $H_{6\beta}$, $H_{7\alpha}$ | |
| 7 | 24.6 | $7_\alpha$ | 2.26(1H) | dd | $J_{7\alpha,7\beta} = 9.8$; $J_{7\alpha,1} = 6.1$ | C-1, 2, 5, 6 | $H_1$, $H_{6\beta}$, $H_{7\beta}$ | $H_{7\alpha}$, $H_{7\beta}$ |
| 7 | | $7_\beta$ | 1.89(1H) | t | $J_{7\beta,7\alpha} = J_{7\beta,6\beta} = 9.8$ | C-1, 2, 5, 6 | $H_1$, $H_{6\alpha}$, $H_{6\beta}$, $H_{7\alpha}$ | |
| 8 | 174.0 | 8 | — | — | — | — | — | — |
| 9 | 166.4 | 9 | — | — | — | — | — | — |
| 10 | 131.2 | 10 | — | — | — | — | — | — |
| 11 | 130.4 | 11 | 7.96(2H) | dd | $J_{11,12} = 8.5$; $J_{11,3} = 1.4$ | C-9, 10, 12, 13 | $H_{12}$, $H_{13}$ | $H_{11}$ |
| 12 | 129.6 | 12 | 7.48(2H) | dd | $J_{12,11} = 8.5$; $J_{12,13} = 7.3$ | C-9, 10, 11, 13 | $H_{11}$, $H_{13}$ | $H_{12}$ |
| 13 | 134.2 | 13 | 7.61(1H) | tt | $J_{13,12} = 7.3$; $J_{13,11} = 1.4$ | C-11 | $H_{11}$, $H_{12}$ | $H_{13}$ |
| 14 | 38.5 | 14 | 2.54(3H) | s | — | C-1, 5 | — | $H_{14}$ |

Only H-2, H-3, and the aromatic hydrogens (H-11, 12, and 13) could be assigned through chemical shifts and coupling constants information. The pseudosymmetry of the molecule brought some difficulties in the assignment of the remaining hydrogens. However, from the unambiguous assignment of H-2, C-2, H-3, C-3, C-8, and C-9, through a combination of gHSQC and gHMBC data it was possible to complete the assignment of all signals in both spectra.

The coupling constants between H-3 and the two methylene hydrogens H-4 were different, being $J_{3,4\alpha} = 11.3$ Hz and $J_{3,4\beta} = 6.5$ Hz. However, the coupling constants between H-5 and H-4$\alpha$/H-4$\beta$ were equal ($J_{5,4\alpha} = J_{3,4\beta} = 3.1$ Hz). Analyzing the structure of **1**, optimized by molecular mechanics calculation,[9] the following dihedral angles were found: H-3 and H-4$\alpha$/H-4$\beta$: H-3/H-4$\alpha \approx 165^\circ$ and H-3/H-4$\beta \approx 48^\circ$. This does not occur with H-5, as H-5/H-4$\alpha \cong$ H-5/H-4$\beta \cong 60^\circ$, leading to the same coupling values according to the Karplus equations.[10,11]

The optimized structure also showed that a hydrogen bond between the nitrogen atom and the hydrogen of the hydroxyl group is formed, which contributes to the stability and rigidity of the molecule.

The assignment of *endo-exo* relative stereochemistry in this kind of structure could be achieved by examining the coupling constants of H-1 with H-7$\alpha$ and H-7$\beta$. For an *endo*-hydrogen, a $J_{1,7\alpha}$ value of 3–6 Hz is usually observed whereas for an *exo*-hydrogen the dihedral angle between H-1 and H-7$\beta$ is near $90^\circ$ and thus $J_{1,7\beta} \cong 0$ Hz.[10,11] For BEG, a $J_{1,7\alpha}$ value of 6.1 Hz and $J_{1,7\beta} = 0$ Hz were observed as expected. Similarly, a $J_{5,6\alpha}$ value of 6.4 Hz and $J_{5,6\beta} = 0$ Hz were found. The chemical shifts of H-6$\alpha$/H-7$\alpha$ are equal and those of H-6$\beta$/H-7$\beta$ very similar due to the pseudosymmetry of the molecule.

The chemical shift of the methyl group was easily assigned, a singlet at 2.54 ppm, which is expected to be used in the detection of BEG in crude urine samples, which are complex mixtures of several compounds.



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