TECHNICAL NOTE

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^{1}H Nuclear Magnetic Resonance of Heroin's D Ring

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ABSTRACT: The ^{1}H nuclear magnetic resonance (NMR) chemical shifts and coupling constants of heroin's (1) D ring H-15$_\alpha$, H-15$_\beta$, and H-16$_\alpha$, and H-16$_\beta$ are presented. These assignments were accessible through the introduction of a double bond ($\Delta^{15,16}$) in heroin. The resulting compound, $\Delta^{15,16}$ didehydroheroin (2), was subjected to deuterium exchange or stereoselective reduction or both. Reduced products d$_1$-16$_\alpha$heroin (3), d$_2$-15$_\alpha$,15$_\beta$-heroin (4), and d$_3$-15$_\alpha$,15$_\beta$,16$_\alpha$-heroin (5) are presented. Heroin with deuterated acetyls is also presented for ^{1}H NMR spectral clarity in the D ring area.

KEYWORDS: toxicology, heroin, chemical analysis

There have been several articles treating the ^{1}H nuclear magnetic resonance (NMR) of morphine and its derivatives [*1-8*]. These investigations, in a first-order analysis, have treated assignments and coupling constants of Ring C's vicinal, allylic, homoallylic, and long-range interactions [*5*]. Treatment of Ring B's geminal and vicinal coupling have also been addressed [*2*]. However, Ring D's protons (H-15$_\alpha$, H-15$_\beta$, H-16$_\alpha$, and H-16$_\beta$) in the morphine series have remained unassigned. This situation was possibly a result of a lack of operative techniques to establish these chemical shift assignments.

An operative technique, although affordable through instrumental techniques [*9-11*], was provided in this study by the introduction of a double bond into the D ring of heroin [*1*]. This was accomplished via a modified Polonoski reaction [*12-18*], shown in Fig. 1. In this reaction, morphine *N*-oxide (6) [*19*] is reacted with acetyl chloride at 65°C. The product, the iminium acetate (7) form of $\Delta^{15,16}$ didehydroheroin (2) is a facile precursor to deuterium labeling at Carbon 15 through exchange and labeling at Carbon 16 through reduction.

Experimental Section

Proton magnetic resonance spectra were obtained on a Nicolet NT-200 WB Fourier transform spectrometer equipped with a NIC-1180 data system and a Model 293A programmable

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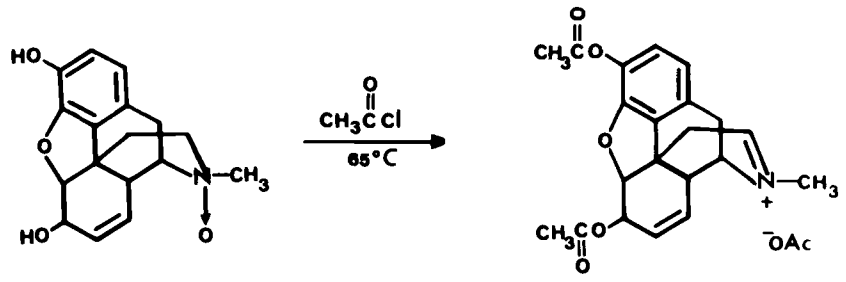


6. 7.

FIG. 1—*Morphine* N*-oxide* (6) *reacted with acetyl chloride at* 65°C yields $\Delta^{15,16}$ *didehydroheroinium acetate* (7). *The reaction has been termed a modified Polonoski reaction.*

pulser. Free induction decays (FIDs) were collected into 8K data points using a 90° pulse, with a spectral width of 1600 Hz. Sixty-four accumulations were taken on each sample with a repetition time of 20 s and an acquisition time of 5.1 s. A 10-mg sample per 1 mL of deuterated chloroform was analyzed using tetramethylsilane (TMS) and an internal standard.

$\Delta^{15,16}$ *Didehydroheroin (2) as the Iminium Acetate Form (7)*

Morphine *N*-oxide, 3 g, was placed into a 50-mL round bottom flask and 30 mL of acetyl chloride was added. The flask was lightly stoppered and immersed in an oil bath at 65°C. The reaction was maintained within an efficient hood, volatilizing acetyl chloride over 2 to 3 h to half volume. The homogeneous solution developed a light-orange color. The stopper was removed and the remainder of the acetyl chloride was allowed to volatilize. The dark resinous material was cooled, chloroform was added, swirling and decanting the solution removed any excess acetyl chloride, leaving a gummy residue. Adding chloroform again, agitating and suction filtering left the white crystaline product (7), 1.5 to 2.0 (50 to 70% yield).

Deuterium Exchange of $\Delta^{15,16}$ *Didehydroheroin (2)*

Of (7), 100 mg was placed into a test tube and 1 mL of deuterochloroform ($CDCl_3$) and 1 mL of deuterium oxide (D_2O) were added. The contents were shaken, and the $CDCl_3$ removed and discarded. Another 1.5 mL of $CDCl_3$ was added and the contents transferred to an NMR tube. Anhydrous sodium carbonate was added in small quantities. After each addition the tube was shaken and the ^{1}H NMR of the $CDCL_3$ layer examined to monitor the exchange of protons at Carbon 15. If an excess of sodium carbonate was added in one quantity, the rapid increase in pH converted (7) to (2) without exchange. Optimum conditions for exchange occurred at pH 4 to 6, at which sufficient small amounts of the exchanged enamine (2) was extracted into the organic layer to observe that complete exchange had occurred in the D_2O. The pH was then increased with excess sodium carbonate (Na_2CO_3) to extract the bulk of the exchanged enamine (2) into the $CDCl_3$.

d$_2$-15$_\alpha$, 15$_\beta$-*Heroin (4)*

The contents of the $CDCl_3$ solution described above were tranferred and diluted with 10 mL of deutero methanol (CD_3OD). Of sodium cyanoborahydride ($NaCNBH_3$) 50 mg were added and the solution stirred for ½ h. The solvent was removed via a rotary evaporator. Extraction of the residue from 2.8*N* hydrochloride (HCl) with chloroform ($CHCl_3$) afforded the ion-paired product in the $CHCl_3$. Evaporation of the solvent gave d_2-15$_\alpha$, 15$_\beta$-heroin hy-

drochloride (4). Elution of the hydrochloride salt through alumina (neutral) with $CHCl_3$ or extraction from saturated sodium bacarbonate ($NaHCO_3$) with $CHCl_3$ gave the base.

d_1-16$_\alpha$-Heroin (3)

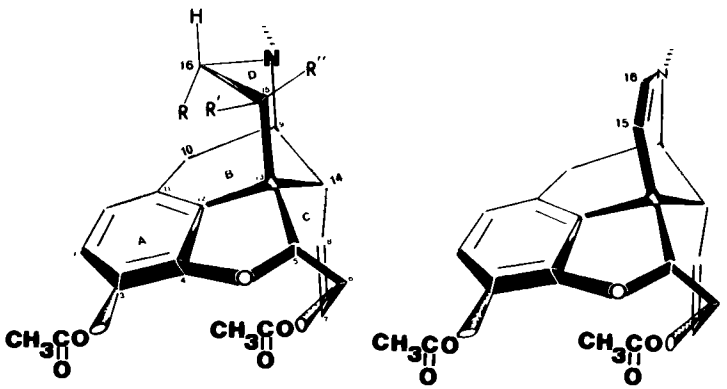
Of (7), 100 mg was dissolved in 10 mL of a water/methanol (H_2O/CH_3OH) mixture. Fifty milligrams of sodium cyanaboradeutiride ($NaCNBD_3$) was added and the contents stirred one-half hour. Isolation was effected as outlined above in (4).

d_3-15$_\alpha$, 15$_\beta$, 16$_\alpha$-Heroin (5) (Fig. 2)

Of (7), 100 mg worked up as outlined above in the deuterium exchange of $\Delta^{15,16}$ didehydroheroin was diluted with 10 mL of CD_3OD. Fifty milligrams of $NaCNBD_3$ was added and the solution stirred for one-half hour. Isolation was effected as outlined above to afford the hydrochloride salt of d_3-15$_\alpha$, 15$_\beta$, 16$_\alpha$-heroin (5).

Results and Discussion

The result of deuterium exchange through the iminium (7)-enamine (2) forms is spectrally presented in Fig. 3*b* and *c* (enamine forms). The iminium form (7) may exchange through a transient intermediate—the result of the attack at Carbon 16 by water, followed by decomposition with loss of a proton at Carbon 15—to the enamine (2) [*20*]. Exchange may also be effected on the iminium form (7) direct with buffer and solvent assistance. This is due to the increased acidity of the hydrogen at Carbon 15 in the iminium form (7). In this study we utilized the later approach in the interest of expediency. The exchange of the iminium form (7) in acid pH is virtually instantaneous. In contrast, at neutral pH the enamine-iminium equilibrium is sufficiently slow that exchange is not complete after three days. Implicit in the



1. R, R′, R″ = H
2.
3. R = D, R′, R″ = H
4. R′, R″ = D, R = H
5. R, R′, R″ = D

FIG. 2—*Three-dimensional structures of compounds used in this study.*

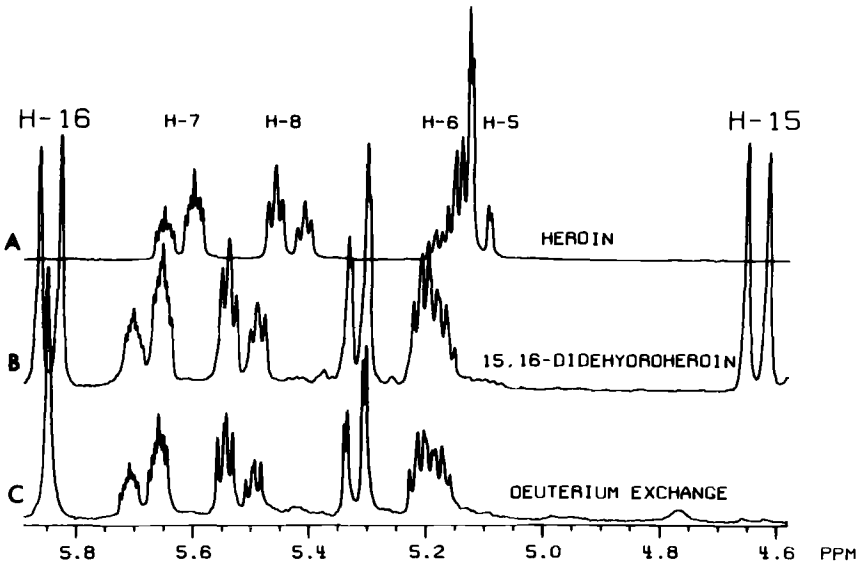


FIG. 3—*^{1}H NMR spectra in deutrochloroform (4.6 − 6.0 region): (a) heroin's (1) C ring (Hydrogen 7, Hydrogen 8, Hydrogen 6, and Hydrogen 5), (b) $\Delta^{15,16}$ didehydroheroin's (2) C ring and D ring (Hydrogen 16 and Hydrogen 15), and (c) $\Delta^{15,16}$ didehydroheroin (2) illustrating deuterium exchange at Carbon 15 with collapse of Hydrogen 16 to a singlet.*

foregoing is that Carbon 15 is the site of electrophilic attack, that is, deuterium exchange, or reaction with heptaflurobutyric anhydride [*21*]. Conversely, Carbon 16 is the site of nucleophilic attack, that is, hydride reduction, cyano, or Grignard addition [*22*].

Exchange with D_2O on (7) replaces both protons at 15 with deuterium. This was followed by reduction with $NaCNBH_3/CD_3OD$, resulting in the production of d_2-15_α,15_β-heroin (4). The proton spectra of (4) is illustrated in Fig. 4*a*, overlayed above heroin (1) in Fig. 4*d*. Assignment of absent (deuterium replaced protons) resonances, 2.04 and 1.88 ppm, to protons at Carbon 15 is decisive. Conversely, the remaining resonances, 2.59 and 2.35 ppm, are the result of protons at Carbon 16. Verification of the correctness of the Carbon 16 assignments is reflected in Fig. 4*b*. This was obtained by deuteride reduction of the iminium form (7) with $NaCNBD_3/CH_3OH$ without exchange at Carbon 15, resulting in d_1-16_α-heroin (3). This places one deuterium at Carbon 16, removing the resonance at 2.35 ppm. The reduction of the iminium (7) via $NaCNBD_3$ is stereoselective at Carbon 16. Were it not the case, the outcome would have been a diminution of resonances at both 2.35 and 2.59 ppm. Careful integration of the area under the resonances at 2.59 ppm (Hydrogen 16) relative to 1.88 ppm (Hydrogen 15) revealed a good 1:1 ratio. Furthermore, this steroselective reduction at Carbon 16 through the iminium form (7) is visually magnified in the tri-deuterated compound d_3-15_α,15_β,16_α-heroine (5) (Fig. 4*c*). These chemical shift assignments, Hydrogen 15's higher field and Hydrogen 16's lower field, are not unexpected considering the proximity of the protons at Carbon 15 to the aromatic ring, subject to diamagnetic anisotropic shielding from the aromatic ring [*23*, p. 95] and the deshielding of the protons at Carbon 16 because of the nitrogen's electron withdrawing ability [*23*, p. 64].

Assessment of the entering deuteride's stereo location was based on coupling constant analysis. Had the borodeuteride approached the iminium derivative (7) from the C ring side, a deuterium would have occupied 16β (see Fig. 5.) In this situation, the Karplus equation [*23*, p. 281] would predict one small coupling, $J_{16\alpha-15\alpha} \cong 3.0$ Hz and one large coupling, $J_{16\alpha-15\beta} \cong 10.0$ Hz, for the resonance at 2.59 ppm (see Fig. 4*b*, d_1-16_α-heroin (3). Clearly,

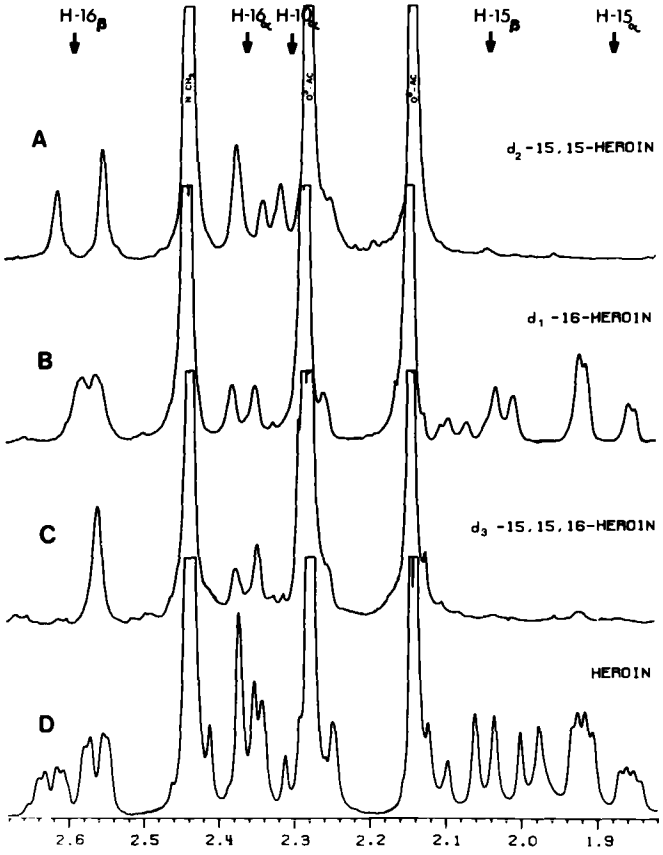


FIG. 4—*^{1}H NMR spectra in deutrochloroform (1.82 to 2.68 ppm region):* (a) *d_2-15$_{\alpha}$*, 15$_{\beta}$-heroin (4), (b) *d_1-16$_{\alpha}$*-heroin (3), (c) *d_3-15$_{\alpha}$*, 15$_{\beta}$, 16$_{\alpha}$-heroin (5), *and* (d) heroin (1).

this is not the case. The analysis of the coupling constants involved at 2.59 ppm (Fig. 4*b*) indicates two relatively small constants. This is consistent with the approach of the borodeuteride from the A ring side to yield d_1-16$_{\alpha}$-heroin (3). The coupling of Hydrogen 16β, at 2.59 ppm, is thus $J_{16\beta-15\alpha} = 1.8$ Hz and $J_{16\beta-15\beta} = 5.1$ Hz. Again, these assignments are consistent with the consideration of the closer proximity of the alpha hydrogens (higher field) relative to the beta pair (lower field) to the shielding region of the aromatic ring.

In consideration of the borodeuteride approach to the iminium (7) structure [*24*], Dreiding models reflect that the C ring is more sterically hindered, by virtue of the Hydrogen 14, than the A ring side. Hydrogen 10β, on the A ring side, is tilted away from the D ring bridge (Carbon 13 to Carbon 9). Conversely, Hydrogen 14, on the C ring side, is tilted towards Carbons 15 to 16, shielding the bridge in (7).

The chemical shift assignment of hydrogen 15α (1.88 ppm) and hydrogen 15β (2.04 ppm) was established by coupling constants. Referring to d_1-16$_{\alpha}$-heroin (3) (Fig. 4*b*) the resonance at 2.04 ppm has a large coupling constant—11.7 Hz—removed relative to its parent compound, heroin (1) (Fig. 4*d*). In heroin (1), this resonance, 2.04 ppm, contains three couplings: 12.5, 11.7 and 5.1 Hz. Decoupling experiments established the geminal coupling of hydrogen 15α to hydrogen 15β as 12.5 Hz. The assignment of hydrogen 15β to 2.04 ppm is then consistent with the Karplus equation, given the vicinal interaction with hydrogen 16β, 5.1 Hz, and hydrogen 16α, 11.7 Hz.

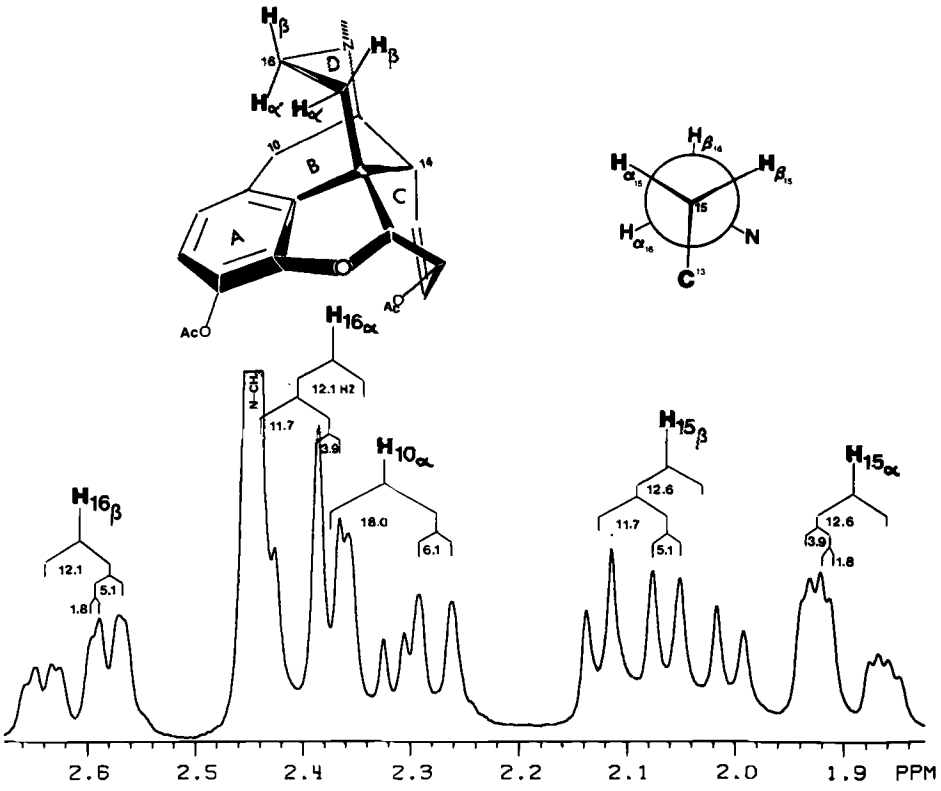


FIG. 5—*^{1}H NMR spectra in deutrochloroform (1.82 to 2.68 ppm region): heroin* (1) *with deuterated acetyls (o^3-acetyl 2.28 ppm and o^6-acetyl 2.14 ppm.) Illustration indicates nomenclature (α and β) used in this work. Assignments and coupling constants (Hz) are also given in Table 1.*

The results of the experiments reflected in Table 1 would seem to indicate that Hydrogen 16β is not symmetrically disposed in its dihedral relationship with Hydrogens 15α and 15β, $J_{16\beta-15_\alpha} = 1.8$ Hz and $J_{16\beta-15\beta} = 5.1$ Hz (see Fig. 5.) However, limitations must be exercised in converting coupling constants to dihedral angles. Electronegative substitutuents, like nitrogen, have been shown to impose deviation on its fundamental concept. It has been shown that these groups, like nitrogen, have their greatest effect, diminished coupling, when they are situated antiperiplanar in the bond path of the coupling hydrogens [*23*, p. 283, *25*].

TABLE 1—*First-order analysis of coupling constants for heroin's D ring.*

Chemical Shifts Relative to TMS	Coupling Constants
16α = 2.36 ppm	$J_{16\alpha-16\beta} = 12.1$ Hz
16β = 2.59 ppm	$J_{16\beta-15\beta} = 5.1$ Hz
15α = 1.88 ppm	$J_{16\beta-15\alpha} = 1.8$ Hz
15β = 2.04 ppm	$J_{16\alpha-15\beta} = 11.7$ Hz
10α = 2.31 ppm	$J_{16\alpha-15\alpha} = 3.9$ Hz
	$J_{15\beta-15\alpha} = 12.6$ Hz
	$J_{10\alpha-10\beta} = 18.0$ Hz
	$J_{10\alpha-9} = 6.1$ Hz

Since this situation exists (antiperiplanar to the coupling bond path) between the nitrogen and Hydrogen 15α, the diminished coupling between Hydrogen 16β and Hydrogen 15α relative to Hydrogen 15β further substantiates the foregoing assignment.

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