$\Delta^{16,17}$-Dehydroheroinium Chloride: Synthesis and Characterization of a Novel Impurity Detected in Illicit Heroin

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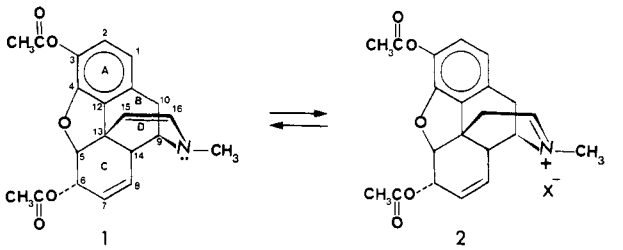
$\Delta^{15,16}$-Didehydroheroin (1) and $\Delta^{16,17}$-dehydroheroinium chloride (**2a**), an impurity detected in illicit heroin hydrochloride, have been synthesized in high yield by treatment of morphine *N*-oxide with acetyl chloride. The synthesis of 1 constitutes a route to $\Delta^{15,16}$-didehydro derivatives in the morphine series that are inaccessible via the classical mercuric acetate method. The synthesis and spectroscopic characterization of 1 and its iminium forms (**2a,b**) are described.

The characterization of manufacturing impurities in illicit drugs is important for forensic purposes. We have recently detected trace amounts of $\Delta^{15,16}$-didehydroheroin (1) and $\Delta^{16,17}$-dehydroheroinium chloride (**2a**) (see Scheme I) in samples of illicit heroin base and hydrochloride, respectively. Small quantities of 1 can also be formed as an injection-port artifact during the gas chromatographic analysis of heroin.

In addition to the rather interesting chemistry associated with 1 and 2, we believe that our synthesis of these compounds provides the most direct and high-yield procedure to date for the preparation of $\Delta^{15,16}$-didehydro compounds in the morphine series. The synthesis is simple and rapid and the product easily isolated with no significant byproduct contamination. Brine and Kepler[1] have previously described the use of the classical mercuric acetate dehydrogenation method for the preparation of $\Delta^{15,16}$-didehydro derivatives of naltrexone and naloxone. Haddlesey and co-workers[2] also used the mercuric acetate method for the preparation of $\Delta^{15,16}$-didehydro derivatives of 6,14-*endo*-etheno-6,7,8,14-tetrahydrothebaines and -oripavines. These dehydrogenation procedures, referred to as Leonard oxidations were unsuccessful on the morphine derivative containing the reactive olefinic bond at C7–C8, due apparently to the formation of intractable products as a result of attack at this site.[2] We were also unsuccessful in applying the Leonard oxidation in the preparation of the $\Delta^{15,16}$-didehydro derivative of codeine.

Our formation of **2b** by the action of acetyl chloride on morphine *N*-oxide is believed to proceed via a modified Polonovski reaction. The direct Polonovski product is realized when morphine *N*-oxide is reacted with acetic anhydride in lieu of acetyl chloride, the major product being O^3,O^6,*N*-triacetylnormorphine.[3] Since the early investigations by the Polonovskis,[4-6] there have been a significant number of modifications and variations of this reaction as well as postulated mechanisms. Sunjic and co-workers[7] have indicated that the reaction proceeds through a nonconcerted elimination–addition. Hayashi et al.[8] have implicated an ylide mechanism to account for the

Scheme I



1 2

a, X = Cl

b, X = OAc

course of the reaction. The preparation of the iminium salts via a modified Polonovski reaction was first reported by Ahond and co-workers.[9] They applied the reaction to steroidal compounds as well as vinblastine alkaloids.[10-13] These investigators stressed that the action of an acid anhydride on an *N*-oxide can give rise to either elimination or fragmentation. Preference for one pathway or the other is governed by electronic factors, such as nucleophilicity of the conjugate base and the nature of the leaving group, as well as steric factors such as the antiparallelism of the *N*-oxide bond with the hydrogen on the carbon α to the nitrogen.

Results and Discussion

In our study we were able to demonstrate the antiparallelism of H-16α and the *N*-oxide bond leading to elimination. This was easily accomplished by the stereoselective introduction of deuterium at the α position on C-16 by reduction of **2b** with sodium cyanoborodeuteride. This occurred by the approach of deuteride from the ring-A side, leading to [16α-^{2}H]heroin. This material was subsequently hydrolyzed to [16α-^{2}H]morphine and then oxidized with 30% hydrogen peroxide to yield [16α-^{2}H]morphine *N*-oxide. This product was reacted with acetyl chloride and after the usual workup yielded an enamine (1), which was shown mass spectrally to contain less than 12% deuterium. These results are consistent with a trans-coplanar elimination of H-16α and the acetoxyl substituent on nitrogen. The deuterium and tritium labeling at C-15, 16 for naltrexone and naloxone have been previously reported.[1]

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Table I. ^1H and ^{13}C NMR Chemical Shift Data for $\Delta^{15,16}$-Didehydroheroin (1), $\Delta^{16,17}$-Dehydroheroinoim Acetate (2b), Heroin, and Cyanoheroin[a]

				heroin		cyanoheroin[b]	
hydrogen no.	carbon no.	1[b]	2b[c]	$CDCl_3$	Me_2SO-d_6	16α	16β
1	[1]	6.617	6.71 [120.66]	6.584	6.58 [119.01]	6.65	6.64
2	[2]	6.692	6.90 [123.22]	6.765	6.73 [121.49]	6.81	6.86
	[3]		[131.94]		[132.51]		
	[4]		[148.77]		[148.80]		
5	[5]	5.314	5.73 [88.95]	5.108	5.50 [88.14]	5.15	5.09
6	[6]	5.185	5.73 [65.19]	5.164	5.50 [67.61]	5.15	5.14
7	[7]	5.677	5.39 [130.13]	5.625	5.30 [129.78]	5.68	5.68
8	[8]	5.513	5.20 [124.71]	5.434	5.13 [127.95]	5.43	5.44
9	[9]	3.714	4.82 [61.11]	3.367	3.31 [57.83]	3.48	3.45
10α	[10]	2.80	2.88 [26.64]	2.310	2.27 [20.29]	2.40	2.43
10β		3.103	3.33	3.062	2.96	3.07	3.34
	[11]		[126.46]		[131.40]		
	[12]		[130.38]		[131.00]		
	[13]		[36.90]		[42.53]		
14	[14]	2.746	3.25 [33.50]	2.743	2.73 [39.63]	2.85	2.71
15α	[15]	4.613	3.75 [41.07]	1.88	1.60 [34.41]	2.29	2.34
15β			3.75	2.04	2.01	2.29	2.29
16α	[16]	5.84	8.93 (177.93]	2.36	*d* [45.89]		3.93
16β				2.59	*d*	3.40	
N-CH_3	[N-CH_3]	2.86	3.84 [47.03]	2.44	2.31 [41.98]	2.64	2.64
O^3 $CH_3C{=}O$	[O^3 CH_3]	2.28	2.25 [20.20]	2.28	2.22 [20.20]	2.28	2.27
	[O^3 C=O]		[167.95]		[168.03]		
O^6 $CH_3C{=}O$	[O^6 CH_3]	2.15	2.03 [20.20]	2.14	2.05 [20.20]	2.15	2.13
	[O^6 C=O]		[169.44]		[169.61]		

[a] Chemical shifts are given in ppm and relative to tetramethylsilane. [b] In $CDCl_3$ at 30 mg/mL. [c] In Me_2SO-d_6 at 100 mg/mL. [d] Unable to make assignment due to resonance overlap.

Cyano Addition to 2b. Treatment of **2b** with aqueous sodium cyanide proceeded rapidly to completion to yield 16α-cyanoheroin. Nucleophilic attack of cyano at C-16 was stereoselective as the approach was limited to the ring-A side of **2b** to yield 16α-cyanoheroin. However, this kinetic product was unstable due to steric interaction between the aromatic π cloud and the α-cyano group. As a result, inversion of configuration resulted to yield the more stable thermodynamic product, 16β-cyanoheroin. This epimerization has also been reported for the 16-cyano derivative of 6,14-*endo*-ethenotetrahydrothebaine.[14] We were able to monitor both 16α- and 16β-cyanoheroin by thin-layer chromatography. The mass spectral analysis of cyanoheroin was not possible due presumably to the thermal elimination of HCN. The infrared spectrum was similar to heroin and indicated the absence of the double bond in ring D. A very weak doublet at about 2230 cm^{-1} and absent in heroin and **1** was attributed to ν_{CN}. Cyano addition was unequivocally established by ^1H NMR. The ^1H chemical shifts for 16α- and 16β-cyanoheroin are given in Table I.

Spectroscopic Characterization of 1 and 2a,b. As expected, the IR spectrum of **1** in KBr was similar to heroin base, but with the former exhibiting more diffuse absorption bands. These included 1767 and 1738 cm^{-1} due to $\nu_{C=O}$ at O^3 and O^6, respectively. The most intense band in the spectrum appeared at 1233 cm^{-1} and was attributed to ν_{C-O}. Support for C-15, 16 unsaturation in **1** was found upon comparing it with heroin. In heroin, bands due to the phenyl nucleus occurred at 1490 and 1622 cm^{-1}, with the former being more intense. In **1**, phenyl absorption appeared at 1490 cm^{-1}, but a significantly more intense band occurred at 1630 cm^{-1} and was attributed in part to $\nu_{C=C}$ at C-15, 16. The spectrum of **2b** exhibited the expected acetate anion and O^3 and O^6 ester carbonyl bands between 1680 and 1780 cm^{-1}. The 1630-cm^{-1} band observed in the spectrum of **1** was not present in that of **2b** and was apparently shifted to higher frequency as $C{=}N{<}^+$. This is in agreement with the behavior of enamine–iminium pairs studied by Leonard and Gach.[15] This shift upon iminium salt formation was not observed for **2a**, perhaps reflecting the counterion influence on $C{=}N{<}^+$. As expected, the broad amine band in heroin hydrochloride between 2400 and 2800 cm^{-1} was not observed in **2a,b**, again lending support to $C{=}N{<}^+$.

The electron-impact mass spectral (EIMS) analysis was provided for standard **1** as well as **1** isolated from illicit heroin.[16] The EIMS spectrum of **1** revealed extensive fragmentation, including ions at *m/z* (approximate relative intensity): 367 (100) ($M^{+\cdot}$), 325 (99), 282 (48), 266 (18), 226 (45), 176 (75), 144 (25), 115 (13), 99 (20), and 81 (37). The molecular ion at *m/z* 367 supported the structure of spectrum **1**. The losses of acetyl and acetoxyl from **1** also appear in the EIMS of heroin, though quantitatively different. These quantitative differences can be explained, in part, by the presence of an enamine moiety in ring D. Acid hydrolysis of **1** followed by reaction with *N,O*-bis(trimethylsilyl)acetamide (BSA) introduced Me_3Si groups at O^3 and O^6 and yielded the expected molecular ion at *m/z* 427. Conspicuously absent from this spectrum was any significant ion at *m/z* 146. This ion, present in the Me_3Si derivatives of morphine and related compounds having a hydrogen at C-14, is composed of elements of rings C and D.[17] Its absence in the spectrum of **1** suggested that modification of ring C or D had occurred as a result of the action of acetyl chloride on morphine *N*-oxide. The chemical ionization mass spectrum (CIMS) of **1** using methane confirmed the EIMS molecular ion by yielding a quasi-molecular ion at *m/z* 368 and the expected adduct ions at *m/z* 396 and 408. Of particular interest was the base peak ion at *m/z* 308, which was accounted for by

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Table II. ^{1}H NMR Coupling Constants for $\Delta^{15,16}$-Didehydroheroin (1) and Heroin[a]

	coupling constants, Hz			coupling constants, Hz	
$J_{^1H-^1H}$	heroin	1	$J_{^1H-^1H}$	heroin	1
1-2	8.2	8.1	7-8	10.0	9.9
1-10α	0.8	0.8	7-14	2.9	3.1
1-10β	0.8	0.8	8-14	1.9	2.1
5-6	6.6	6.2	9-10α	6.0	5.4
5-7	1.1	1.1	9-10β	0.8	0.5
6-7	1.9	2.0	9-14	3.3	3.2
6-8	2.7	3.0	9-14	3.3	3.2
6-14	3.7	3.0	10α-10β	18.8	18.2

[a] Spectra recorded in $CDCl_3$; tetramethylsilane used as internal standard.

the loss of acetic acid at C-6 from the quasi-molecular ion. The analogous ion also appeared in the CIMS spectrum of heroin, its intensity being accounted for by the stability of the resultant allyl carbonium ion at C-6. This suggested that the assignment of an additional double bond in 1 be in ring D, for if it were in ring C to give $\Delta^{8,14}$–$\Delta^{6,7}$ or $\Delta^{5,6}$–$\Delta^{7,8}$ conjugation, a carbonium ion at C-6 would be less effectively stabilized.

The ^{1}H and ^{13}C NMR spectra of 1 and **2b** were recorded and compared to those of heroin and O^6-acetylcodeine. A study of these spectra allowed for the unequivocal assignment of an additional olefinic bond in 1 at C-15,16 in ring D. Our initial attempts at producing an interpretable ^{1}H of 1 proved unsuccessful due to interconversion of enamine and iminium forms.[18] This interconversion was also noted during the TLC analysis of 1.[19] Acceptable spectra for 1 were obtained by using base-treated $CDCl_3$.[18] Good spectra were obtained for **2b** in dimethyl-d_6 sulfoxide while not experiencing the difficulties associated with 1.

The ^{1}H and ^{13}C chemical shift data for 1, **2b**, and heroin base are given in Table I. When compared to heroin, virtually every proton in 1 and **2b** experienced downfield chemical shifts. Furthermore, the data revealed that ring C had not undergone chemical modification. The striking similarity of coupling patterns between O^6-acetylcodeine, heroin, and 1 allowed for the facile assignment of protons. The obvious chemical shift of H-5 in 1 can be attributed to anisotropic deshielding. Proton H-5 is close to end-on alignment with the $\Delta^{15,16}$ double bond, and the McConnell approximation of this deshielding agrees moderately with that observed (0.3 ppm).

Whereas well-defined differences existed in the chemical shifts of heroin and 1, there were no significant variations in their coupling constants as illustrated in Table II. Small differences in the coupling constants of H-5 to H-6 and H-9 to H-10 can be interpreted as the result of shortening the ring-D bridge across C-13 to C-9 in going from sp^3 to sp^2 at C-15 and C-16. Inspection of Drieding models reveals that the shortening of the bridge rotates H-9 away from its near-elliptical relationship with H-10α, thus lowering their coupling value, while concomitantly H-9 moves closer to an orthogonal relationship to H-10β. Thus, $J_{9-10\beta}$ decreased in accordance with the Karplus equation. The shortening of the ring-D bridge in 1 is similar to Sinnema's observations concerning the piperidine ring in cocaine.[20] The shortened ring-D bridge in 1 moves C-14 away from the plane of C-9, 10, 12, and 13. Because of the entire ring system of morphine is so rigid, the movement of C-14 is translated to the point of bond rotation C-6 to C-5. The net result is that H-6 moves away from H-5, increasing the dihedral angle, thus lowering the coupling constant from 6.6 to 6.2 Hz.

The ^{13}C NMR spectrum of **2b** was acquired in Me_2SO-d_6, and the chemical shifts compared to heroin and given in Table I. The large increase in chemical shift for C-16 in **2b** allowed for its assignment as the iminium carbon. All assignments were based on ^{13}C NMR chemical shift theory,[21-23] single-frequency off-resonance decoupling experiments, and comparison to structurally related compounds, including heroin.[24-27]

Experimental Section

All ^{1}H and ^{13}C NMR spectra were obtained on a Nicolet 200-MHz spectrometer interfaced with an 1180 data system and 293A pulser. Tetramethylsilane was used as an internal standard. Infrared spectra were recorded in KBr on a Beckman 4240 spectrophotometer. Electron-impact mass spectra were acquired on a Finnigan 4000 quadrupole mass spectrometer at an ionizing potential of 70 eV and an ionizing current of 30 μA. Chemical ionization mass spectra were obtained with methane as the reagent gas. The mass spectrometer was equipped with a 6 ft. × 2 mm i.d. glass column packed with 3% OV-1 on Gas Chrom Q (100/120M).

Morphine *N*-Oxide. To 5 g of morphine base in a round-bottomed flask was added 10 mL of ice-cooled 30% hydrogen peroxide in one quantity. The slurry was allowed to come to room temperature over a 15-min period. The flask was then placed in a container of hot water to effect complete solution. The contents were then transferred to a beaker, and acetone was added to effect precipitation of 3 g (60%) of morphine *N*-oxide. The product was filtered, washed with acetone, and dried. Alternately, morphine *N*-oxide was prepared by the *m*-chloroperbenzoic acid oxidation procedure of Craig and Purushothaman.[28] The IR of both standards were similar to that of a standard of morphine *N*-oxide obtained commercially.

$\Delta^{16,17}$-Dehydroheroinium Acetate (2b), $\Delta^{15,16}$-Didehydroheroin (1), and $\Delta^{16,17}$-Dehydroheronium Chloride (2a). To 3.0 g of morphine *N*-oxide was added 30 mL of acetyl chloride. The mixture was heated to 65 °C to effect dissolution, and heating was continued for an additional 2–3 h. The solvent was evaporated and the residue washed with chloroform, filtered, and dried to afford 1.5–2.0 g (50–70%) of **2b**. To an aqueous solution of **2b** was added dropwise stronger ammonia water until precipitation of 1 was complete. The precipitated 1 was filtered, washed with additional dilute ammonia water, and carefully dried. Dry HCl gas was slowly bubbled into an ethereal solution of 1 until precipitation of **2a** was complete. The precipitated **2a** was isolated, washed with acetone, and dried. One- and two-dimensional TLC, GC-MS, IR, and NMR analyses of the prepared standards revealed no significant byproducts. Complete spectroscopic characterization of 1, **2a**, and **2b** is given in the text.

(18) Dissolution of 1 in untreated $CDCl_3$ yielded a ^{1}H NMR spectrum that was rather diffuse in nature and as a result not easily interpreted. This is believed due to a composite of enamine and iminium forms and transient intermediates associated with the enamine–iminium interconversion (Scheme I). A well-defined spectrum of 1 was obtained by first extracting **2b** from aqueous sodium carbonate into $CDCl_3$ and then subjecting this solution to NMR analysis without an evaporation step.

(19) TLC was done on 100 μm silica gel GF with 20:3 acetone:methanol; approximate R_f values for enamine and iminium forms are 0.74 and 0.45, respectively.

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16-Cyanoheroin. To 500 mg of **2b** dissolved in 4 mL of water was added a small excess of an aqueous solution of potassium cyanide. The product, which precipitated immediately, was filtered, washed with water, and air-dried to afford 450 mg (90%) of 16α- and 16β-cyanoheroin (note: this product eventually epimerized to 16β-cyanoheroin). ^{1}H NMR chemical shift data for both 16α- and 16β-cyanoheroin are given in Table I.

Registry No. 1, 86993-77-3; **2a**, 86993-78-4; **2b**, 86993-80-8; morphine *N*-oxide, 639-46-3; 16(*S*)-cyanocheroin, 87011-55-0; 16(*R*)-cyanoheroin, 87011-56-1; morphine, 57-27-2; heroin, 561-27-3.

Synthetic Studies on Sequences of Vitamin K Dependent Proteins. Gla-Arg-Gla Sequences

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The synthesis of peptides containing the Gla-Arg-Gla sequence is reported. Nuclear magnetic resonance studies suggest that intramolecular salt bridge interactions occur between the guanidino group of arginine and the γ-carboxyglutamic acid.

The sequenced proteins shown to contain γ-carboxyglutamic acid residues (Gla) contain a high degree of sequence homology within the Gla-rich region. In addition to containing 10–14 Gla residues and a small cystine loop, the sequences Gla-Arg-gla and/or Arg-Gla-Gla invariably occur. Direct spectroscopic studies of Eu^{3+}–Gla peptide and protein interactions[1,2] and theoretical calculations involving malonate/metal ion interactions[3] suggest that a single Gla residue will contribute one ligand to a divalent ion even though two side-chain carboxyl groups exist per Gla residue. We suspected that the Arg-Gla sequences might contribute to the overall stability of the secondary structure via salt bridges formed by side-chain interactions between the guanidino and carboxyl side chains in the presence or absence of divalent metal ions.

Lancelot et al.[4] have shown that carboxylate–guanidinium ion interactions occur in peptides containing Arg-Glu sequences. Salt bridge formation leads to a downfield shift of the N$^\epsilon$-proton resonance of arginine in the NMR spectrum of these peptides. Protonation of the carboxylate anion with strong acids such as trifluoroacetic acid or perchloric acid disrupted the interaction and resulted in an upfield shift of the same proton resonance. Hence, we suspected that the chemical shift of the N$^\epsilon$-proton of Arg should indicate hydrogen bonding between Arg and Gla side chains. In order to evaluate possible hydrogen bonding interactions of this sort and to develop synthetic methods that would yield Arg-Gla and Gla-Arg peptides, we undertook the synthesis of the peptides **16** and **17** representing the sequence 15–17 and 11–17 of bovine prothrombin.

Solutions of L-arginine can be prepared in DMF containing 1 equiv of either trifluoroacetic acid or methanesulfonic acid. Addition of 1 equiv of triethylamine liberates free arginine, which precipitates; however, precipitation can be prevented by using a weaker base such as *N*-methylmorpholine. Acylation of L-arginine with the *N*-succinimidoyl ester of *N*-(benzoyloxycarbonyl)-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic acid (**2**) provided *N*-(benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamyl-L-arginine (**3**) in 85% yield. The reaction was slow, requiring up to 48 h for completion (Scheme I).

L-Arginine solutions could also be prepared in DMF containing 1 equiv of *N*-hydroxybenzotriazole. Acylation of L-arginine under these conditions using the pentachlorophenyl ester of *N*-(benzyloxycarbonyl)-γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic acid (**4**) provided 81% of **3** in 24 h.

DiBello et al.[5] have shown that Z-AA-Arg-OH (AA = Phe, glu-γ-Bzl) dipeptides can be coupled to other amino acids or peptides by the method of Konig and Geiger[6] without racemization. Acylation of γ,γ-di-*tert*-butyl-L-γ-carboxyglutamic acid α-methyl ester *p*-toluenesulfonate salt (**6**), generated from **5**, with the protected dipeptide acid **3** using DCC/HOBt provided the tripeptide **7** in 94% yield. When **7** was precipitated from the reaction by addition of ether, the peptide was obtained as the *N*-hydroxybenzotriazole salt. Alternatively, an aqueous sodium acetate wash of the reaction mixture in ethyl acetate

Table I. Chemical Shift Data (ppm) for 16 in the Presence and Absence of Cesium Carbonate/Me_2SO-d_6

resonance	no Cs_2CO_3	1 equiv of Cs_2CO_3
Gla γ-H	3.35	3.18
Arg δ-H	3.10	3.10
Arg N$^\epsilon$-H	7.57	7.83

Table II. Concentration Dependence of Chemical Shift Data (ppm) for 16 in the Presence of Cesium Carbonate/Me_2SO-d_6

	various concentrations of 16, M				
resonance	5×10^{-2}	2.5×10^{-2}	1×10^{-2}	5×10^{-3}	1×10^{-3}
Gla γ-H	3.18	3.12	3.10	3.10	3.10
Arg δ-H	3.10	3.10	3.08	3.10	3.10
Arg N$^\epsilon$-H	7.83	7.83	7.85	7.88	7.83

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