TECHNICAL NOTE

Harry D. Beckstead[1] *and George A. Neville,*[1] *Ph.D.*

Fourier Transform-Infrared (FT-IR) Characterization of the Ethyl Acetate Complex of O^6-Acetylmorphine

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ABSTRACT: The solid-state infrared spectral features of the (1 : 1) O^6-acetylmorphine-ethyl acetate complex are compared to those of its host, O^6-acetylmorphine base, and to its very similar analog, O^3,O^6-diacetylmorphine (heroin). The formation of a stable complex appears to be unique to O^6-acetylmorphine for neither morphine nor its closely related derivatives, codeine, thebaine, heroin, or O^3-acetylmorphine form isolable adducts during ethyl acetate extraction. Factors affecting formation of this complex during workup and extraction of forensic science exhibits are discussed.

KEYWORDS: toxicology, extraction, ethyl acetate complex, heroin, spectroscopic analysis

In the analysis of substances for illicit drugs generally, and particularly for the analysis of substances suspected to contain heroin (O^3,O^6-diacetylmorphine) or other morphine related drugs, it is common practice to employ aqueous or methanolic ammonia to liberate the desired free base(s). Extraction is most often performed with chloroform, but sometimes ethyl acetate (EtOAc) may be used to obtain the illicit material. Since recent work involving use of aqueous ammonia and EtOAc extraction resulted in discovery of formation of a 1:1 molar adduct of ethyl acetate by O^6-acetylmorphine [*1-3*], a Fourier transform-infrared (FT-IR) study of the nature of recovered product from closely related morphine derivatives, basified by either aqueous ammonia or sodium bicarbonate and extracted with either ethyl acetate or chloroform, was undertaken to see if such solvent complexation were an isolated phenomenon or a common occurrence.

Experimental Procedure

Equipment and Materials

FT-IR spectra were determined from 4000 to 400 cm^{-1} with resolution of 2 cm^{-1} using a Nicolet 60 SX instrument. Samples for IR determination were prepared as KBr disks (0.3% sample) using IR spectral grade potassium bromide.

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[1]Technologist and head of spectroscopy section, respectively. Drug Identification Division, Bureau of Drug Research, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada.

Crystalline O^6-acetylmorphine and the (1 : 1) ethyl acetate complex of O^6-acetylmorphine were prepared as previously reported [*1*]; O^3-acetylmorphine benzoate benzoic acid complex was obtained as previously reported [*4*]; morphine hydrochloride was used as supplied by Merck, Darmstadt, Germany; and codeine hydrochloride, thebaine hydrochloride, and diacetylmorphine (base) were used as supplied by T. & H. Smith Ltd., Edinburgh, Scotland.

Chloroform and ethyl acetate were high pressure liquid chromatographic (HPLC) grade solvents.

Sample Preparation

Each alkaloid (regardless of salt or base form) was dissolved in 0.1*N* sulfuric acid to obtain clear solutions; each solution was divided; one portion was made basic using concentrated aqueous ammonia, and the other portion was made basic with saturated aqueous sodium bicarbonate. Each solution was extracted with ethyl acetate; the extracts were filtered through a pledget of absorbent cotton, and evaporated to dryness in a conical centrifuge tube (2 mL) under a gentle stream of nitrogen at room temperature.

Results

FT-IR spectra of the crystalline EtOAc complex of O^6-acetylmorphine (6-O-AM), known to be a 1 : 1 molar adduct by both ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectroscopy and elemental microanalysis [*1*], are shown in Figs. 1 and 2 together with those of the crystalline parent substance (6-O-AM) and diacetylmorphine (heroin) from 4000 to 500 cm^{-1} (Fig. 1) and the expanded fingerprint region from 2000 to 500 cm^{-1} (Fig. 2). In Fig. 3, the spectral character of O^6-acetylmorphine (*a*) and its EtOAc complex (*b*) are compared in expanded format from 4000 to 2000 cm^{-1}. The spectral features of noncrystalline 6-O-AM · EtOAc complex, obtained under aqueous ammonia extraction with ethyl acetate followed by evaporation of the extract to dryness under a gentle stream of nitrogen, are shown in Fig. 4.

Discussion

If one were to come unsuspectingly upon an IR spectrum of the EtOAc adduct of 6-O-AM, one might easily be misled into thinking it represented a sample of heroin unless careful note was taken of the particular carbonyl frequencies (compare to Fig. 1). In Fig. 2, the higher carbonyl frequency (wave number) band (1737 cm^{-1}) arises from $V_{C=O}$ of the O^6-acetyl group whereas the lower band (1727 cm^{-1}) is due to $V_{C=O}$ of the EtOAc adduct. The additional intensity of the shoulder band at 1220 cm^{-1} in the complex would appear to arise from V_{C-O} of the EtOAc moiety. In heroin (Fig. 2), the higher frequency band (1762 cm^{-1}) is due to $V_{C=O}$ of the O^3-acetyl group whereas $V_{C=O}$ of the O^6-acetyl group arises at 1740 cm^{-1}. Because of the greater frequency difference between the two $V_{C=O}$ bands for heroin, the carbonyl bands are better resolved in heroin than in the EtOAc adduct of 6-O-AM. The somewhat lower $V_{C=O}$ of the O^6-acetyl group at 1734 cm^{-1} in the parent substance (6-O-AM) compared to that of the EtOAc complex and heroin serves to demonstrate the subtlety of closely related substances. In the V_{C-H} region of Fig. 3, one can see new bands (3034, 2975, and 2908 cm^{-1}) for the 6-O-AM · EtOAc complex compared to the spectrum of 6-O-AM itself which could be attributed to asymmetrical V_{C-H} and symmetrical V_{C-H} of the methyl and methylene groups of the EtOAc in the complex. When the 6-O-AM · EtOAc complex is obtained by extraction and evaporation under a gentle stream of nitrogen, the material obtained is amorphous but shows the essential IR features (Fig. 4) in somewhat broadened bands compared to those of the crystalline complex (Fig. 1).

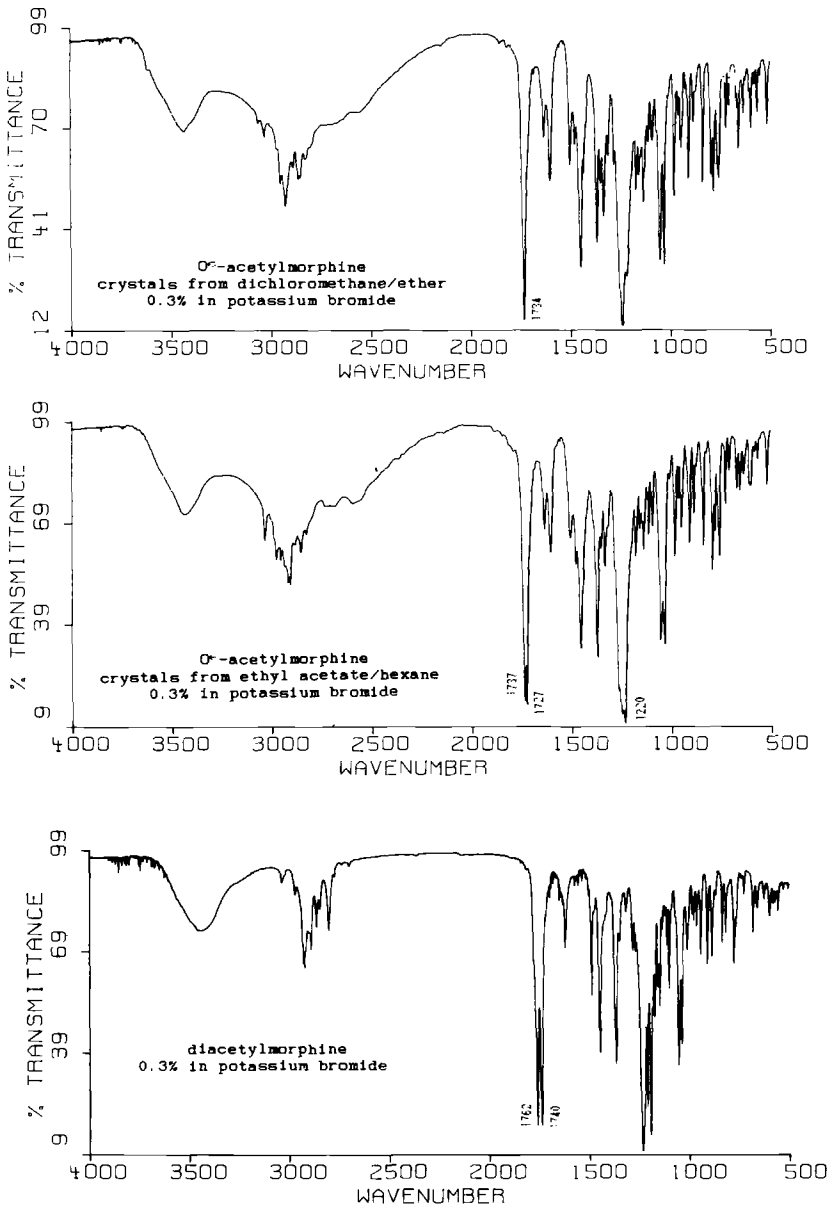


FIG. 1—*Comparison of FT-IR spectra of crystalline O⁶-acetylmorphine* (top), *the O⁶-acetylmorphine-ethyl acetate (1 : 1) complex* (middle), *and diacetylmorphine (heroin)* (bottom) *from 4000 to 500 cm^{-1} determined as KBr disks.*

When saturated aqueous sodium bicarbonate (pH ≃ 8) was used instead of aqueous ammonia (pH ≃ 12) to basify O⁶-acetylmorphine hydrochloride followed by extraction of the organic base with ethyl acetate, no solvent of complexation was found with the recovered 6-O-AM. Presumably the greater basicity of the 6-O-AM tertiary amine (pK_b of morphine = 6.1) over that of aqueous sodium bicarbonate (pK_h = 7.6) at room temperature results in intermolecular protonation in solution of the piperidine N by the phenolic OH group of

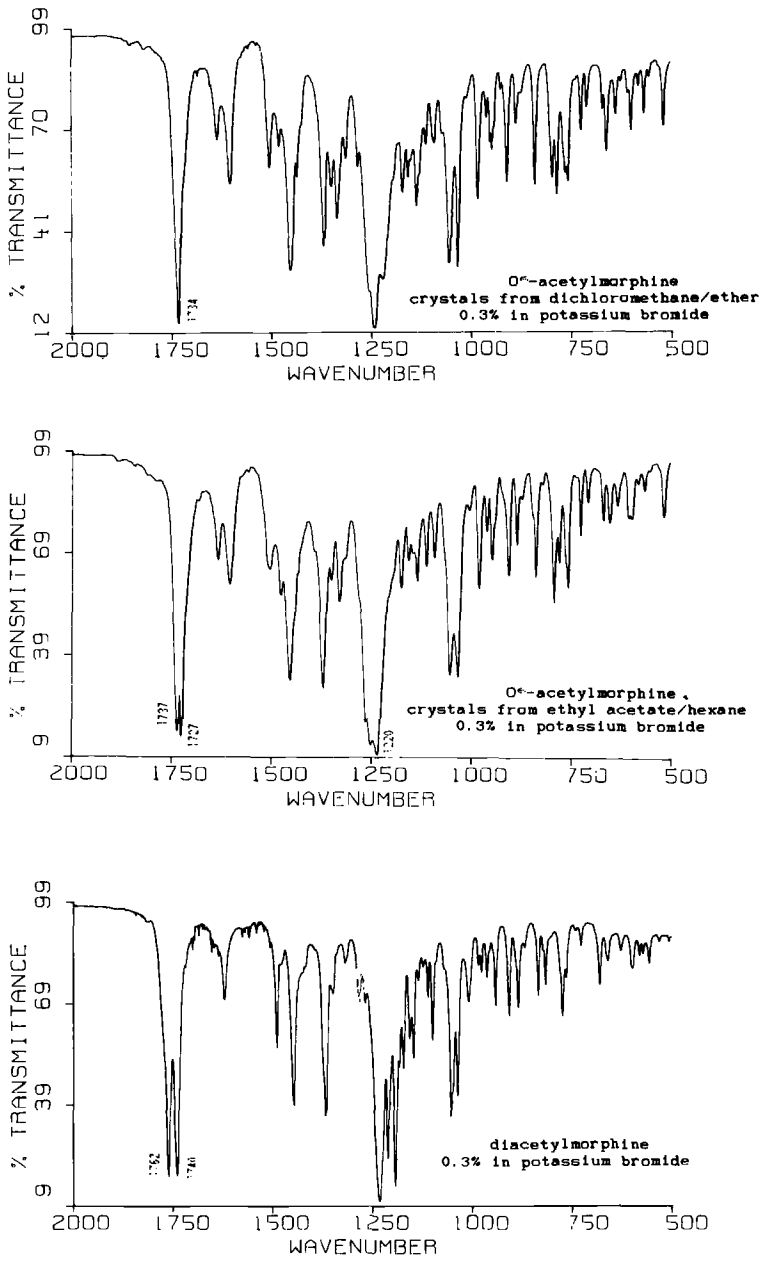


FIG. 2—*Comparison of the FT-IR expanded fingerprint regions (2000 to 500 cm^{-1}) of crystalline O^6-acetylmorphine* (top), *the O^6-acetylmorphine-ethyl acetate (1 : 1) complex* (middle), *and diacetylmorphine (heroin)* (bottom) *determined as KBr disks.*

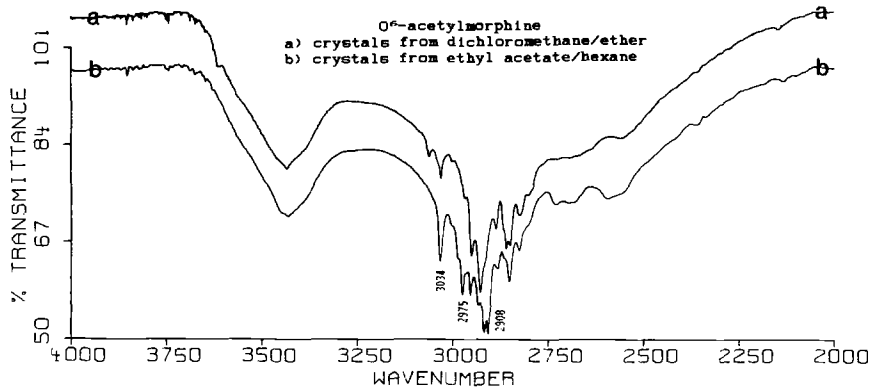


FIG. 3—*Comparison of the FT-IR expanded V_{C-OH} and V_{C-H} regions (4000 to 2000 cm^{-1}) of crystalline O⁶-acetylmorphine* (a), *and the O⁶-acetylmorphine-ethyl acetate (1 : 1) complex* (b) *determined as KBr disks.*

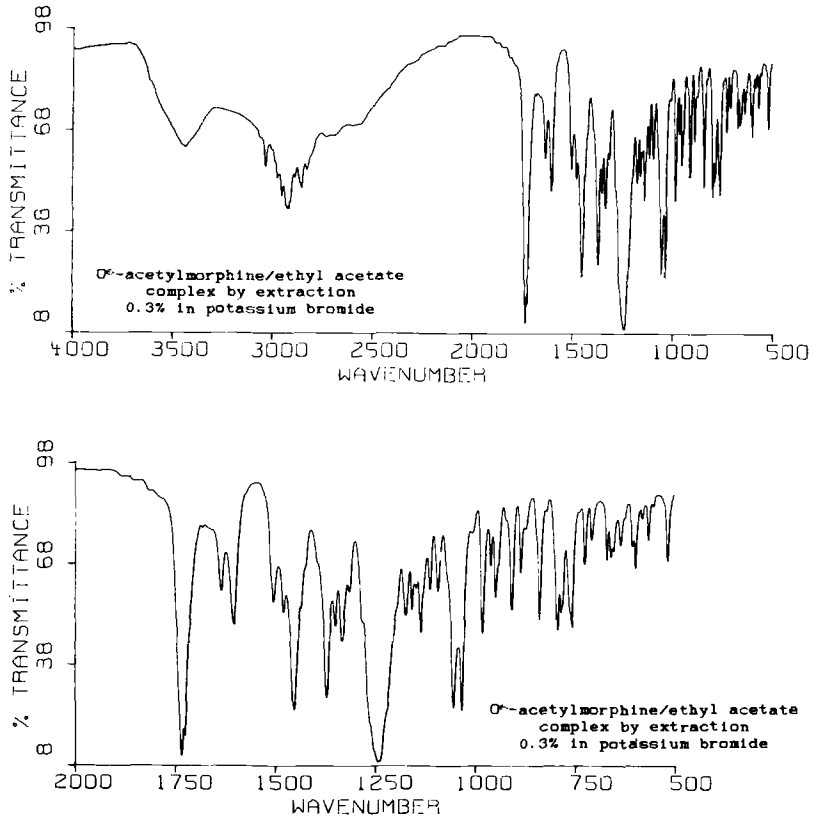


FIG. 4—*A typical FT-IR spectrum of amorphous O⁶-acetylmorphine-ethyl acetate complex obtained by extraction and determined as a KBr disk, 4000 to 500 cm^{-1}* (top) *and with expanded fingerprint region (2000 to 500 cm^{-1})* (bottom).

6-O-AM whereas in aqueous ammonia ($pK_b = 4.7$), the latter agent would be engaged in ammonium phenolate formation. In the solid-state, the phenolic OH of 6-O-AM is intermolecularly hydrogen bonded to the piperidinium N,[2] the O-H...N bond length being 0.2740 nm. When a salt of a strong acid is formed as for codeine hydrobromide (dihydrate) [5], morphine hydroiodide (dihydrate) [6], and morphine hydrochloride (trihydrate) [7], the piperidinium N is protonated, and the water molecules serve to interconnect chains of the host molecules by a well developed and similar N-H...O hydrogen bonding system [7]. No comparable system of H-bonding with the solvent exists in the solid state for the 6-O-AM · EtOAc complex,[2] the solvent molecules simply being retained in their host molecular cage by van der Waals forces.

In spite of the affinity of 6-O-AM for EtOAc under moderately basic conditions, it was surprising to find that no EtOAc adduct of O^6-acetylmorphine could be recovered when the (aq NH_3) basified mixture was extracted then left to evaporate slowly (for example, without the N_2 stream). While the EtOAc adduct can be obtained by recrystallization of 6-O-AM from excess EtOAc by treatment with hexane [1], or by extraction from an aqueous ammonia solution with ethyl acetate and by evaporation of the extract under a (cooling) stream of N_2, it would seem that an EtOAc extract of 6-O-AM is unable to form the adduct during slow evaporation because of loss of EtOAc at room temperature. Similar behavior is exhibited by the solid complex stored unsealed at room temperature—the transparent crystals become cloudy from formation of opaque pockets throughout the needle-like crystals, and lose their EtOAc over a period of a few weeks.

Heroin is particularly labile under aqueous ammonia treatment resulting in significant hydrolysis within minutes to O^6-acetylmorphine as found by IR analysis following extraction by either chloroform or ethyl acetate. If, however, heroin is treated with aqueous sodium bicarbonate before extraction with either chloroform or ethyl acetate, little or no hydrolysis occurs. In spite of the relative ease with which the O^3-acetyl group is cleaved from heroin in the presence of ammonia, O^3-acetylmorphine is apparently immune to such hydrolysis under similar conditions of sample basification and extraction into $CHCl_3$ or EtOAc by IR analysis. This observation is compatible with the demonstration by Bernhauer et al. [3] of the relative stability of O^3-acetylmorphine at pH 8.5 (50°C) in 0.01*M* phosphate buffer for approximately the first 10 h. These procedures, however, are inefficient for the extraction of morphine because of its relative insolubility in either $CHCl_3$ or EtOAc.

When codeine hydrochloride, thebaine hydrochloride, and O^3-acetylmorphine benzoate benzoic acid complex, analogs of O^6-acetylmorphine, were each subjected to the conditions of concentrated aqueous ammonia/EtOAc extraction, each of these substances was recovered in its respective free base form without any evidence of EtOAc complexation (Table 1). Similarly, attempts to obtain EtOAc complexation by recrystallizing these free bases from EtOAc/hexane at room temperature, as achieved with O^6-acetylmorphine [1], failed.

Conclusion

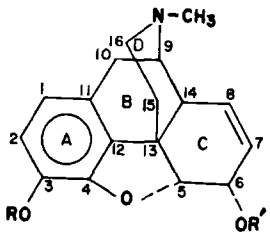
Formation of the EtOAc complex of O^6-acetylmorphine appears to be specific and unique to O^6-acetylmorphine for neither morphine nor its closely related derivatives, codeine, thebaine, heroin, or O^3-acetylmorphine form EtOAc adducts under the described extraction conditions.

Acknowledgment

We are grateful to Dr. F. R. Ahmed of the Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, for having determined the X-ray crystal structures of both O^6-acetylmorphine and its ethyl acetate solvated complex.

[2]F. R. Ahmed, Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, personal communication, 1986.

TABLE 1—*Formation of an ethyl acetate complex by morphine analogs at room temperature.*



R	R'	Name	EtOAc Complex*
H	Ac	O^6-Acetylmorphine	Yes
Ac	H	O^3-Acetylmorphine	No
Ac	Ac	Heroin	No
H	H	Morphine	No
CH_3	H	Codeine	No
CH_3	CH_3	Thebaine	No

with $\Delta^{6,8}$ in ring C

* Upon extraction of free base with EtOAc.

References

[1] Sy, W.-W., By, A. W., Neville, G. A., and Wilson, W. L., "A Direct Synthesis of O^6-Monoacetylmorphine from Morphine," *Canadian Society of Forensic Science Journal*, Vol. 18, No. 2, 1985, pp. 86-91.

[2] Neville, G. A., Ekiel, I., and Smith, I. C. P., "500 MHz PMR Characterization of the Ethyl Acetate Adduct of 6-O-Acetylmorphine," *Canadian Journal of Spectroscopy*, Vol. 30, No. 4, 1985, pp. 86-91.

[3] Bernhauer, D., Fuchs, E.-F., and Neumann, H., "Nachweis von 3-O-Acetylmorphin als Zersetzungsprodukt des Diacetylmorphins (Heroin) mit HPLC und Capillar-GC, Reaktionsablant der Heroinzersetzung und Bedeutung der Zersetzungsprodukte für die Charakterisierung von illegalen Heroinproben," *Fresenius' Zeitschrift für Analytische Chemie*, Vol. 316, 1983, pp. 501-504.

[4] Sy, W.-W., By, A. W., Avdovich, H. W., and Neville, G. A., "Spectral Characterization of 3-O-Acetylmorphine and its Benzoate Salt Complex," *Canadian Journal of Spectroscopy*, Vol. 30, No. 3, 1985, pp. 56-63.

[5] Kartha, G., Ahmed, F. R., and Barnes, W. H., "Refinement of the Crystal Structure of Codeine Hydrobromide Dihydrate and Establishment of the Absolute Configuration of the Codeine Molecule," *Acta Crystallographica*, Vol. 15, No. 4, 1962, pp. 326-333.

[6] MacKay, M. and Hodgkin, D. C., "A Crystallographic Examination of the Structure of Morphine," *Journal of the Chemical Society*, 1955, p. 3261.

[7] Gylbert, L., "The Crystal and Molecular Structure of Morphine Hydrochloride Trihydrate," *Acta Crystallographica*, Vol. B29, 1973, pp. 1630-1635.

Address requests for reprints or additional information to
George A. Neville
Health and Welfare Canada
Banting Bldg., Tunney's Pasture
Ottawa, Ontario, K1A 0L2 Canada