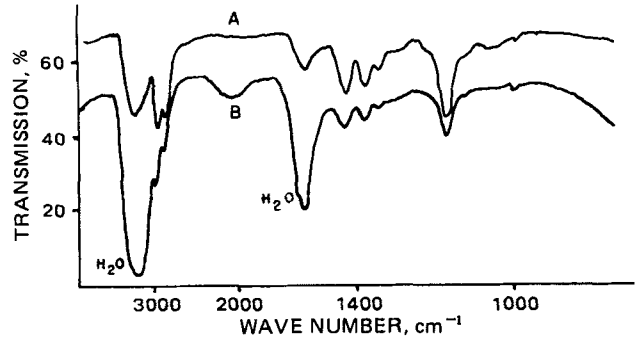

Figure 6—*IR spectra of (A) diethylamine reagent and (B) isolated decomposition product III.*

3000-cm^{-1} region indicated the absence of hydroxyl and primary or secondary amine groups.

The mass and IR spectra provided sufficient evidence for the elucidation of the structure of II. This compound was available commercially[13] and was purchased to serve as a reference material. The UV spectra of this reference material were nearly identical to those of the laboratory-produced decomposition product (Fig. 2). However, some background absorbance was apparent in the latter.

The IR and mass spectra of these two materials appeared identical (Figs. 3 and 4). The NMR spectra (Fig. 5) were also identical, with the exception of pentane peaks in the spectrum of the laboratory-produced material due to a residue remaining from the extraction step. A peak due to a chloroform impurity in the deuterated solvent was also visible in this spectrum because of the higher instrument gain used.

Gas and liquid chromatograms subsequently obtained for the reference material showed peaks identical to those of the previously unknown component. The chromatographic and spectral data combined with the identical physical characteristics of the cotton filler extract, the laboratory-produced material, and the reference material provided convincing evidence that II was formed as a major decomposition product of I.

Prior to the distillation of III from decomposed drug substance, the latter material was tested and found positive for secondary amines by the NF monograph test. The identity of III as a second decomposition product was then established by comparison of the gas chromatogram and the IR spectrum of the distillate with those of the diethylamine reagent[14]. Water was added to the latter prior to vaporization in the IR cell to simulate better its effect on the isolate spectrum (Fig. 6).

The distillate of an alkaline solution of undecomposed drug substance was found to contain no diethylamine, thus eliminating the suspicion that the latter may have been formed during the isolation procedure.

The route of decomposition from I to II and III has not been satisfactorily elucidated at this time and is apparently quite complex. However, it has been demonstrated that this type of decomposition has occurred in commercial tablet products and that it is accelerated by the presence of moisture.

The NF monograph assay procedure is specific for I in the presence of II, since it was found that II is retained in the sodium hydroxide solution while I is extracted into chloroform. However, the content uniformity procedure involves the direct UV determination of filtered hydrochloric acid solutions of the tablets. Since II was found to have considerable absorptivity and a maximum at 257 nm in this solvent, its presence in tablets could be expected to increase significantly the absorbance readings of their solutions, leading to inaccurately high results.

REFERENCES

(1) O. T. Bolding, *Curr. Ther. Res. Clin. Exp.*, **16**, 40 (1974).

(2) Y. K. Sedat and J. Reddy, *ibid.*, **16**, 398 (1974).

(3) D. E. Carney and E. D. Tweddell, *Med. J. Aust.*, **1**, 13 (1975).

(4) B. Testa and A. H. Beckett, *J. Chromatogr.*, **71**, 39 (1972).

(5) D. Mihailova, A. Rosen, B. Testa, and A. H. Beckett, *J. Pharm. Pharmacol.*, **26**, 711 (1974).

(6) E. C. Schreiber, B. H. Min, A. V. Zeiger, and J. F. Lang, *J. Pharmacol. Exp. Ther.*, **159**, 372 (1968).

(7) "The National Formulary," 14th ed., Mack Publishing Co., Easton, Pa., 1975, pp. 204, 205.

(8) K. G. Weidmann, A. Wolf, and J. Reisch, *Arch. Pharm.*, **306**, 954 (1973).

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Identification of Diacetylmorphine Metabolites in Humans

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Abstract □ With the techniques of column chromatography, TLC, and GLC, morphine, 6-acetylmorphine, normorphine, morphine 3-glucuronide, 6-acetylmorphine 3-glucuronide, and normorphine glucuronide were identified as metabolites of diacetylmorphine (heroin) in the urine of humans administered 10 mg iv/70 kg body weight.

Keyphrases □ Diacetylmorphine—metabolites identified using TLC, GLC, and column chromatography, human urine □ Metabolites—diacetylmorphine, identified using TLC, GLC, and column chromatography, human urine □ TLC—identification, diacetylmorphine metabolites, human urine □ GLC—identification, diacetylmorphine metabolites, human urine □ Column chromatography—identification, diacetylmorphine metabolites, human urine □ Narcotics—diacetylmorphine, metabolites identified using TLC, GLC, and column chromatography, human urine

Studies in laboratory animals *in vitro* and *in vivo* indicated that diacetylmorphine (heroin) (I) was rapidly metabolized first to 6-acetylmorphine and then to morphine (1–5). In humans, diacetylmorphine was excreted in the urine mainly as morphine (6, 7). Approximately 50–60% of I administered to heroin addicts was excreted in the urine as conjugated morphine and 7% as free morphine (8, 9). In addition to free and conjugated morphine, small

amounts of I and free 6-acetylmorphine were observed in the urine of humans infused with a large dose of I intravenously (10). Morphine ethereal sulfate, free and conjugated normorphine, morphine 6-glucuronide, and dihydromorphinone could also be metabolites of I, since these substances have been identified as morphine metabolites in humans and laboratory animals (11–19). The present article reports the identification of I metabolites in humans following intravenous administration of a single 10-mg dose.

EXPERIMENTAL

Materials—Diacetylmorphine hydrochloride [free of contaminant when determined by TLC (20) but shown to contain about 5% 6-acetylmorphine as determined by GLC], 6-acetylmorphine hydrochloride, and normorphine hydrochloride were obtained[1]. Morphine sulfate and codeine phosphate were commercial products. Morphine 3-glucuronide and morphine 3-ethereal sulfate were isolated, respectively, from the urine of dogs and cats administered morphine (14, 21). Normorphine 3-glucuronide was isolated from the urine of dogs administered normorphine hydrochloride (22).

Methods—Twelve healthy, postaddict, male, federal prisoner volunteers, with an average age of 36 years and an average body weight of 77 kg, were administered 10 mg iv of I/70 kg body weight. Urine collections were made before drug administration and at intervals of 2, 4, 6, 8, 16, and 24 hr and *ad libitum* following drug administration. The urine was frozen after collection.

Extraction of I Metabolites—The method for extraction and determination of I metabolites was described previously (23).

Glucuronic Acid Determination—The content of glucuronic acid in the conjugated metabolites was determined according to the procedure of Dische (24).

Hydrolysis of Conjugated Metabolites: Acid Hydrolysis—Conjugated metabolites were hydrolyzed by autoclaving the sample in 2.2 *N* HCl [20% (v/v) concentrated hydrochloric acid] in a steam-jacketed autoclave at 6.8 kg of pressure and 115° for 30 min. After cooling, the sample was adjusted to about pH 10 and then extracted and silylated for GLC determination according to the procedure described (11, 23). Under these conditions, 6-acetylmorphine and its conjugates were hydrolyzed to morphine. Identical samples without hydrolysis were used as controls.

Hydrolysis of Conjugated Metabolites: Enzymatic Hydrolysis—Samples were adjusted to pH 7, buffered with 1.0 ml of 0.1 *M* phosphate buffer at pH 6.8, and incubated with 5 mg of β-glucuronidase[2] (type II bacterial powder, 170 Fishmen units/mg and no sulfatase activity) and 2 drops of chloroform at 37° for 4 hr (for hydrolysis of 6-acetylmorphine conjugate) or 18 hr (for hydrolysis of morphine and normorphine conjugates). Samples incubated without enzyme were used as the control. After incubation, the sample was adjusted to pH 8.5 (for extraction of 6-acetylmorphine and morphine) or pH ~10 (for extraction of normorphine and morphine) and then extracted and silylated for GLC determination according to the described procedure (11, 23).

TLC—TLC was performed with either instant TLC silica gel sheets[3] or linear preadsorbent TLC silica gel plates[4]. With the instant TLC silica gel sheets, samples were spotted 2.54 cm (1 in.) above the edge. The chromatogram was developed with 1-butanol–acetic acid–water (35:3:10). With the linear TLC silica gel plates, aliquots of unknown samples along with authentic samples were spotted on the preadsorbent section. The chromatogram was developed with ethyl acetate–methanol–ammonium hydroxide (17:2:1), and the R_f values were calculated using the beginning of the silica gel section as the origin. All chromatograms were visualized by spraying with iodoplatinate reagent[5].

GLC—A gas chromatograph[6] equipped with dual flame-ionization detectors and dual columns was used. A 0.9-m (3-ft) × 2-mm glass column (Column 1) was packed with 3% OV-17 coated on Gas Chrom Q (60–80 mesh). A 1.5-m (5-ft) × 2-mm stainless steel column (Column 2) was packed with 3% SE-30 coated on Varaport (100–200 mesh). The temperatures of the injector and detector were set at 255 and 295°, respectively; gas flow rates were 30 ml/min for nitrogen and hydrogen and 300–400 ml/min for compressed dried air.

Derivatization—An aliquot of the sample was placed in an acylation tube and evaporated to dryness under a stream of nitrogen in a water bath at about 60°. The residue was derivated to make silyl, trifluoroacetyl, acetyl, or propionyl derivatives. Silylated derivatives were prepared by heating the residue with 50 μl of 25% trimethylsilylimidazole in pyridine[7] at 90–95° for 1 hr. Trifluoroacetyl derivatives were prepared by heating the residue with 0.2 ml of trifluoroacetic anhydride at 60–70° for 0.5 hr. Acetyl derivatives were made by heating the residue with 0.2 ml of acetic anhydride and 0.1 ml of pyridine at 60–70° for 0.5 hr. Propionyl derivatives were prepared by heating the residue with 0.2 ml of propionic anhydride (glass redistilled) and 0.1 ml of pyridine at 60–70° for 0.5 hr. The excess anhydride and pyridine were removed by evaporation, and the residue was reconstituted with 50 μl of ethyl acetate. One microliter of the solution was injected into the gas chromatograph.

GLC–Mass Spectrometry— The chemical ionization mass spectral data were obtained[8] using a glass column [1.5 m (5 ft) × 2 mm] packed with 3% SE-30 coated on Gas Chrom Q. Methane was used as the carrier gas as well as the chemical ionization reagent gas in the ion source. The column, injector, and ion source temperatures were 240, 250, and 100°, respectively. The electron energy was 80 ev, and ion repeller voltage was 3 v.

Isolation with Column Chromatography—The urine samples collected during the 0–16-hr period from 10 subjects were pooled, centrifuged to remove insoluble substances, and chromatographed with a resin[9] column. Two hundred milliliters of the urine was passed, at a flow rate of about 2–4 ml/min, through the column (2.1 × 40 cm) previously washed with acetone, methanol, and water. The column then was washed with 150 ml of distilled water and eluted with 250 ml of methanol. The first 100 ml of washing was discarded, and the second 50 ml of washing was combined with the eluate. The eluate was evaporated to dryness under vacuum in a rotary evaporator in a water bath between 40 and 50° (all evaporation was done in the same manner). The residue was triturated with methanol and yielded methanol-soluble and insoluble (water-soluble) fractions.

Methanol-Soluble (Free Base Drug) Fraction—The methanol-soluble fraction was evaporated to dryness. The residue was dissolved with 15 ml of 10% acetic acid solution and extracted three times with 15 ml of *n*-butyl chloride to remove such substances as nicotine and caffeine. Morphine, I, and acetylmorphine were not extracted under these circumstances. The aqueous phase, after extraction with *n*-butyl chloride, was adjusted to pH 4.5 with 1 *N* NaOH and extracted with 15 ml of chloroform three times to attempt to extract I. After extraction with chloroform, the aqueous phase was adjusted to pH 8.5 with 1 *N* NaOH and extracted three times with 15 ml of 1,2-dichloroethane containing 30% (v/v) 2-propanol.

After extraction, the aqueous phase was again evaporated to dryness and triturated with methanol. The methanol-soluble portion was combined with the extract of 1,2-dichloroethane containing 30% 2-propanol. The methanol-insoluble portion was discarded. The extracts of *n*-butyl chloride, chloroform, and 1,2-dichloroethane containing 30% 2-propanol were separately pooled and evaporated to dryness, and the residues were dissolved with 1 ml of *n*-butyl chloride, chloroform, and ethyl acetate, respectively.

RESULTS

Aliquots of the extracts of *n*-butyl chloride, chloroform, and 1,2-dichloroethane containing 30% 2-propanol were chromatographed on linear TLC silica gel plates with ethyl acetate–methanol–ammonium hydroxide (17:2:1) as the developing solvent. The chromatogram of the extract of *n*-butyl chloride showed two spots with R_f 0.35 and 0.8. The retention time of the peaks of the extract did not correspond to any hypothesized possible I metabolite. The chromatogram of the chloroform extract showed three spots with R_f 0.43 (morphine), 0.8 (to be identified), and 0.9 (6-acetylmorphine and I). The chromatogram of the 1,2-dichloroethane containing 30% 2-propanol extract showed three spots with R_f 0.23 (normorphine), 0.43, and 0.9.

Preparative TLC of the chloroform extract was performed on two large

[1] Courtesy of Dr. E. L. May, National Institutes of Health.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Gelman Instrument Co., Ann Arbor, Mich.
[4] Quantum Industries, Fairfield, N.J.
[5] Consisting of 0.25 g of chloroplatinic acid and 5 g of potassium iodide/100 ml of water.
[6] Varian Aerograph, Series 2700.
[7] Tri-Sil-Z, Pierce Chemical Co., Rockford, Ill.
[8] Model 3300 GLC–mass spectrometer equipped with a model 6000 interactive data system, Finnigan, Sunnyvale, Calif.
[9] Amberlite XAD-2, a styrene–divinyl benzene copolymer, Rohm & Haas, Philadelphia, Pa.

Table I—GLC Retention Time (Minutes) of Diacetylmorphine Metabolites and Standards

Sample	Trifluoroacetyl Derivative	Trimethylsilyl Derivative	
	3% OV-17, 210°	3% OV-17, 220°	3% OV-17, 230°
6-Acetylmorphine hydrochloride	6.9	—	7.1
Codeine phosphate	5.8	—	—
Morphine sulfate	3.8	7.4	4.3
Norcodeine hydrochloride	9.1	—	—
Normorphine hydrochloride	6.2	8.8	—
Methanol-soluble fraction separated on TLC[a]			
R_f 0.23	3.8, 6.2	7.4, 8.8	—
R_f 0.40	3.8	7.4	—
R_f 0.9	6.9	—	7.1

[a] Linear TLC silica gel plate using ethyl acetate–methanol–ammonium hydroxide (17:2:1).

Table II—R_f Values of Diacetylmorphine Metabolites and Standards

Sample	Linear TLC Silica Gel Plate	Instant TLC Silica Gel Sheet
Authentic samples		
6-Acetylmorphine hydrochloride	0.90	0.9
Diacetylmorphine hydrochloride	0.90	0.9
Morphine sulfate	0.40	0.9
Normorphine hydrochloride	0.20	0.8
Morphine 3-glucuronide	—	0.40
Morphine 3-ethereal sulfate	—	0.65
Normorphine 3-glucuronide	—	0.66
Acetylcodeine	0.84	—
Codeine	0.67	—
Norcodeine	0.47	—
Codeine 6-glucuronide	—	0.60
Metabolites		
Methanol-soluble fraction		
n-Butyl chloride extract	0.35, 0.80	—
Chloroform extract	0.40, 0.80, 0.87	—
1,2-Dichloroethane–2-propanol extract	0.23, 0.40, 0.90	—
Methanol-insoluble fraction	—	0.03, 0.40, 0.76, 0.90

plates (20 × 20 cm), and preparative TLC of the 1,2-dichloroethane–2-propanol extract was performed on one plate. A section of the chromatogram was sprayed with iodoplatinate reagent. The zones corresponding to the iodoplatinate-positive regions and to R_f 0.7 (where codeine would be expected) were horizontally scraped off and eluted with methanol. The eluate was concentrated and aliquots were derivatized and determined by GLC. The identity of metabolites with R_f 0.23, 0.4, and 0.9 was further supported as normorphine, morphine, and 6-acetylmorphine, respectively, by cochromatography with authentic samples and by GLC (Table I).

Compound I was not detected in the eluate obtained from the region with R_f 0.9. The retention time of the peaks of the eluate with R_f 0.8 did not correspond to any hypothesized possible I metabolite. Norcodeine and codeine were not detected in the eluate obtained from the zone with R_f 0.4 and 0.7.

Methanol-Insoluble Fraction—The chromatogram of the methanol-insoluble (water-soluble) fraction on an instant TLC silica gel sheet developed with 1-butanol–acetic acid–water (35:3:10) showed four iodoplatinate-positive spots with R_f 0.03, 0.4, 0.76, and 0.9 (Table II). Preparative TLC was performed on 10 instant TLC silica gel sheets (20 × 20 cm), and a section of the chromatogram was sprayed with iodoplatinate reagent. The zones corresponding to the iodoplatinate-positive spots were horizontally cut off and eluted with warm water. The material located at R_f 0.9 was eluted first with methanol and then with water. In addition, the iodoplatinate-negative regions with R_f 0.5 and 0.6 (corresponding to R_f values of morphine 6-glucuronide and morphine 3-ethereal sulfate) were isolated and eluted with water. It was thought that these metabolites could be present in small amounts, too low to show positive with iodoplatinate.

The metabolite with R_f 0.03 was hydrolyzed to yield morphine. Since this metabolite could have been due to morphine 3-glucuronide, held back by an impurity, it was rechromatographed. The chromatogram again showed one spot with R_f 0.03. The metabolite with R_f 0.03 was negative with the phenolic test and hydrolyzed with β-glucuronidase or with acid to yield morphine. This metabolite appears to be a polar morphine metabolite, but its identity has not yet been determined.

The metabolite with R_f 0.4 was negative with the phenolic test, positive with the glucuronic acid test, and hydrolyzed with β-glucuronidase or with acid to give morphine. Cochromatography of this metabolite with authentic morphine 3-glucuronide showed a single spot. Therefore, this metabolite was identified as morphine 3-glucuronide.

The metabolite with R_f 0.76 was positive with the glucuronic acid test and hydrolyzed with β-glucuronidase to yield 6-acetylmorphine and normorphine. These metabolites were tentatively identified as 6-acetylmorphine 3-glucuronide and normorphine glucuronide, respectively.

The material with R_f 0.9, corresponding to the R_f of free morphine and 6-acetylmorphine, showed peaks of morphine and 6-acetylmorphine on GLC. The concentration of morphine and 6-acetylmorphine did not significantly increase after enzymatic hydrolysis, suggesting that the compound was in the free form. Other peaks were also observed but did not correspond to those of any known I metabolites.

The eluate obtained from the iodoplatinate-negative region with R_f 0.5–0.6 was concentrated and passed through a neutral alumina column (1.0 × 2.5 cm). The column was eluted with 100 ml of water. The eluate was concentrated to about 1.5 ml and rechromatographed on one instant TLC silica gel sheet (20 × 20 cm), using 1-butanol–acetic acid–water (35:3:10) as the developing solvent. After a section of the chromatogram was sprayed with iodoplatinate reagent, a faint spot with R_f 0.5 was clearly distinguished from the spot of morphine 3-glucuronide (0.4) and codeine 6-glucuronide (0.6). The zone corresponding to R_f 0.5 was cut off and eluted with water, and the eluate was concentrated. The eluate was positive with the Folin–Ciocalteu phenolic test and with a glucuronic test. After hydrolysis with β-glucuronidase, the metabolite yielded morphine. The content of glucuronic acid in the eluate was determined according to the described procedure (24), and the content of morphine was determined by GLC. The ratio of morphine to glucuronic acid was about 1:1. These data suggest this metabolite to be morphine 6-glucuronide.

Identification of Normorphine by GLC–Mass Spectrometry—The chemical ionization mass spectral data of a sample (as the acetyl derivative) showed a peak with a retention time and mass fragmentation spectrum [with mass at *m/e* 398 (M − 1) and 338 (M − acetic acid + 1)] identical with those of authentic triacetylnormorphine (Fig. 1).

Search for Dihydromorphinone as Possible Metabolite of I—Aliquots (200 ml) of the first 8-hr urine from two subjects were acid hydrolyzed, buffered at pH ~10, and extracted with chloroform instead of 1,2-dichloroethane containing 30% 2-propanol according to a described triple-extraction procedure (11). The organic phase was concentrated and applied along with authentic samples to an aluminum oxide 1B plate[10]. The chromatogram was developed using the lower phase of the solvent mixture of chloroform–methanol–water–acetic acid (20:10:20:2) (19). A section of the plate was sprayed with iodoplatinate, and a spot corresponding to morphine was observed; no dihydromorphinone spot was seen. The presence of normorphine was inconclusive because of an impurity masking the spot.

The zones corresponding to normorphine (R_f 0.03), morphine (0.23), and dihydromorphinone (0.38) were separately scraped off and eluted with methanol. Aliquots of the eluate were evaporated to dryness, and the residues were made into propionyl derivatives. One microliter of the reconstituted solution was injected into Column 2 with an oven temperature at 240°. Peaks corresponding to normorphine (R_t 22) and morphine (7.2) were observed in the eluate. The dihydromorphinone peak (R_t 4.9) was not observed. A detailed procedure for the determination of dihydromorphinone as a morphine metabolite will be published later.

DISCUSSION

Free morphine, 6-acetylmorphine, free normorphine, morphine 3-glucuronide, morphine 6-glucoronide, 6-acetylmorphine 3-glucuronide,

[10] J. T. Baker, Phillipsburg, N.J.

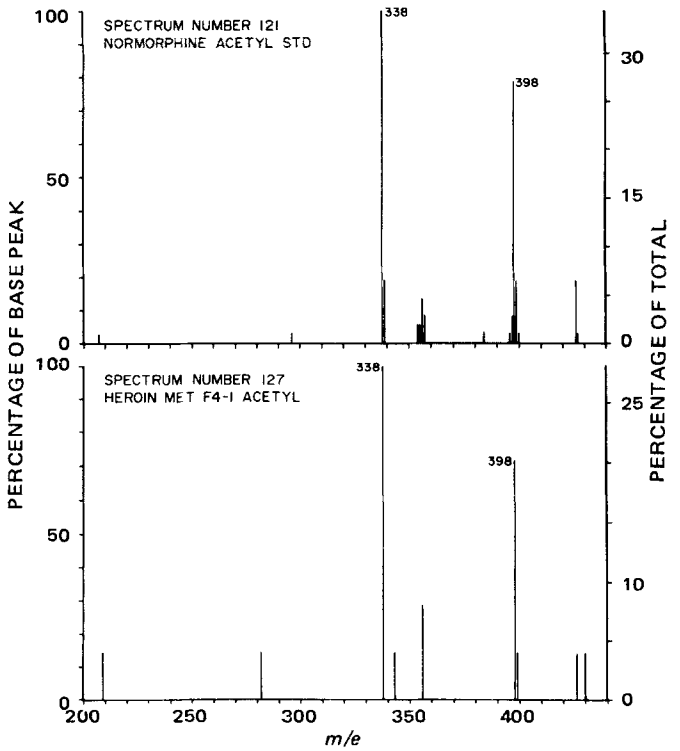

Figure 1—*GLC–mass spectrometric analysis of acetylated normorphine and the peak corresponding to acetylated normorphine of the extract of 5 ml of urine collected 0–8 hr from Subject F administered 10 mg of diacetylmorphine hydrochloride.*

and normorphine glucuronide were found as metabolites of I in human urine. Morphine 3-glucuronide was the major metabolite, accounting for approximately 50% of the administered dose. Free morphine accounted for about 7%, and the remaining metabolites were present in a magnitude of less than 5%. The quantitative studies on metabolism of I in humans will be published elsewhere (25).

Since heroin is deacetylated to 6-acetylmorphine and then to morphine (2, 3, 5), it seems likely that the 6-acetylmorphine present in the administered dose was deacetylated to morphine *in vivo* and the 6-acetylmorphine found in the urine was a minor I metabolite. However, the possibility could not be ruled out that it was an autohydrolysis product of I excreted in the urine or that it occurred during the process of extraction.

An attempt to isolate 6-acetylmorphine 3-glucuronide was unsuccessful because of the small amount of material and the labile acetyl group. The existence of 6-acetylmorphine 3-glucuronide could be further illustrated by incubation of the eluate obtained from the resin[9]. The eluate was evaporated to dryness and reconstituted with water prior to incubation with β-glucuronidase. The amount of 6-acetylmorphine was increased about twofold after incubation, while morphine increased five- to sixfold as compared to that without incubation.

In the present study, normorphine glucuronide together with 6-acetylmorphine 3-glucuronide was located at R_f 0.76. An attempt to isolate normorphine glucuronide was unsuccessful because of the small amount of material.

Codeine and norcodeine were not observed as metabolites of I in the present studies, although they have been reported as metabolites of morphine in humans (26, 27). The existence of codeine and norcodeine as metabolites of morphine in humans has been questioned (28, 29). The sensitivity of the present GLC method could detect 0.5 μg of codeine, 1.0 μg of norcodeine, and 5 μg of dihydromorphinone. Dihydromorphinone has been reported as a morphine metabolite in the rat chronically administered morphine but was not observed in the present study. This result could be due to a species difference.

The chromatograms of the extract of *n*-butyl chloride on a linear TLC silica gel plate, developed with ethyl acetate–methanol–ammonium hydroxide (17:2:1), showed spots with R_f 0.35 and 0.8. These spots were probably due to nicotine and caffeine and their metabolites. A chromatographic study on the urine from cigarette smokers and coffee drinkers was described previously (30).

REFERENCES

(1) G. Rizzott, *Bull. Soc. Ital. Biol. Spec.*, **9**, 507 (1934); through *Chem. Abstr.*, **29**, 225 (1934).

(2) C. I. Wright, *J. Pharmacol. Exp. Ther.*, **71**, 164 (1941).

(3) E. L. Way, J. W. Kemp, J. M. Young, and D. R. Grassetti, *ibid.*, **129**, 144 (1960).

(4) E. L. Way, J. M. Young, and J. W. Kemp, *Bull. Narc.*, **17**(1), 15 (1965).

(5) G. L. Cohn, J. A. Cramer, and H. D. Kleber, *Proc. Soc. Exp. Biol. Med.*, **144**, 351 (1973).

(6) T. M. Shen, *J. Chin. Pharm. Assoc.*, **1**, 124 (1936); through *Chem. Abstr.*, **31**, 730 (1937).

(7) F. W. Oberst, *J. Pharmacol. Exp. Ther.*, **73**, 401 (1941).

(8) *Ibid.*, **79**, 266 (1943).

(9) P. N. Mo and E. L. Way, *J. Pharmacol. Exp. Ther.*, **154**, 142 (1966).

(10) H. W. Elliott, K. D. Parker, J. A. Wright, and N. Nomof, *Clin. Pharmacol. Ther.*, **12**, 806 (1971).

(11) S. Y. Yeh, *J. Pharmacol. Exp. Ther.*, **192**, 201 (1975).

(12) J. M. Fujimoto and V. B. Haarstad, *ibid.*, **165**, 45 (1969).

(13) D. G. May, J. M. Fujimoto, and C. E. Inturrisi, *ibid.*, **157**, 626 (1967).

(14) S. Y. Yeh, H. I. Chevnov, and L. A. Woods, *J. Pharm. Sci.*, **60**, 469 (1971).

(15) H. Yoshimura, K. Oguri, and H. Tsukamoto, *Biochem. Pharmacol.*, **18**, 279 (1968).

(16) A. L. Misra, S. Y. Yeh, and L. A. Woods, *ibid.*, **19**, 1536 (1970).

(17) H. Yoshimura, M. A. Mori, K. Oguri, and H. Tsukamoto, *ibid.*, **19**, 2353 (1970).

(18) K. Oguri, S. Ida, H. Yoshimura, and H. Tsukamoto, *Chem. Pharm. Bull.*, **18**, 2414 (1970).

(19) A. Klutch, *Drug Metab. Disp.*, **2**, 23 (1974).

(20) G. R. Nakamura, *J. Assoc. Offic. Anal. Chem.*, **49**, 1086 (1966).

(21) S. Y. Yeh and L. A. Woods, *J. Pharmacol. Exp. Ther.*, **173**, 21 (1970).

(22) S. Y. Yeh, *Fed. Proc.*, **34**, 814 (1975).

(23) S. Y. Yeh and R. L. McQuinn, *J. Pharm. Sci.*, **64**, 1237 (1975).

(24) Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947).

(25) S. Y. Yeh, C. W. Gorodetzky, and R. L. McQuinn, *J. Pharmacol. Exp. Ther.*, **196**, 249 (1976).

(26) U. Boerner and S. Abbott, *Experientia*, **29**, 180 (1973).

(27) U. Boerner, R. L. Roe, and C. E. Becker, *J. Pharm. Pharmacol.*, **26**, 393 (1974).

(28) S. Y. Yeh, *Experientia*, **30**, 265 (1974).

(29) S. Y. Yeh, *J. Pharm. Pharmacol.*, **27**, 214 (1975).

(30) S. Y. Yeh, *J. Pharm. Sci.*, **62**, 1827 (1973).

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[x] To whom inquiries should be directed.