Isolation of Hydromorphone and Dihydromorphine Glucuronides from Urine of the Rabbit After Hydromorphone Administration[1] (37291)

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Hydromorphone (dihydromorphinone, Dilaudid), Fig. 1, is one of the popularly prescribed potent narcotic analgesics, but little is known about its metabolism (1). Our interest in its metabolism derived from the fact that it possessed a ketone group in position 6. Because work with naloxone, a narcotic antagonist, had shown that the 6 position in naloxone is reduced to an alcoholic hydroxyl group (2, 3), by analogy, one would expect the same reduction to take place in the hydromorphone molecule to form dihydromorphine. Since dihydromorphine itself is a narcotic analgesic agent, its formation could have pharmacological importance. In the present study, we report on the isolation and characterization of hydromorphone-3-glucuronide and dihydromorphine-3-glucuronide as metabolic products of hydromorphone administered to the rabbit.

Materials and Methods. Two, white male rabbits, weighing 4.6 and 4.7 kg, respectively, were injected subcutaneously with 20 mg hydromorphone HCl/kg body weight. All the urine was collected during the next 5 days and pooled, yielding 900 ml. The urine was applied to 6 Amberlite XAD-2 resin (Rohm and Haas Co., Philadelphia, PA) columns (2.5 × 45 cm) using 150 ml urine/column. Then the columns were washed with 150 ml of distilled water followed by 300 ml methanol. Fifteen milliliter fractions were collected and monitored for the presence of metabolite. A sample of each fraction was spotted on silica gel impregnated thin layer sheets (Gelman Type SG ITLC) and chromatographed in solvent System I (2) composed of *n*-butanol, acetic acid and water (35:10:3, v/v). Alkaloids were detected as purple spots with iodoplatinate spray. Contaminating organic materials were determined by spraying with concentrated sulfuric acid followed by charring on a hot plate. The methanol fractions were found to contain hydromorphone ($R_f = 0.94$) and two metabolites with R_f values of about 0.4 and 0.3, the slower moving metabolite appearing to be in the greater amount. Methanol fractions containing the metabolites from the 6 columns were pooled and evaporated *in vacuo* to dryness on a rotary evaporator.

To reduce the amount of organic contamination an 8-transfer counter-current distribution was performed on the residue using 100 ml of each phase of a solvent system containing *n*-butanol, water and acetic acid (5:5:1, v/v). The lower aqueous phase was transferred with a syringe equipped with a long, 18 gauge needle. Thin layer chromatography in System I of the organic and aqueous phases show the two metabolites along with some organic contaminants in tubes 4–8 of the mobile aqueous phase. Attempts at crystallization using various mixtures of water, acetone and methanol were unsuccessful.

The solution was evaporated to dryness and the residue was dissolved in 8 ml water followed by addition of 10 ml methanol. This solution was applied to a basic aluminum oxide (Alupharm Chem., New Orleans, LA) column (2 × 12.5 cm) which had been previously saturated with methanol. The material was washed first with 5 ml methanol then with 400 ml of 70% methanol, 820 ml of 40% methanol, 750 ml of 10% methanol, and 400 ml water. Fractions were collected

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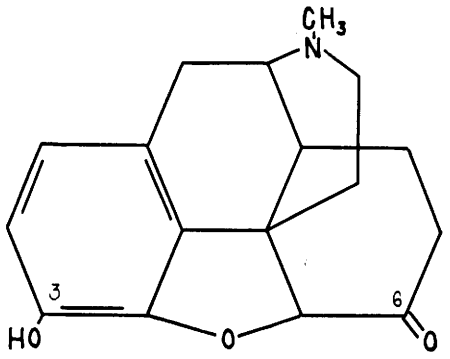

FIG. 1. Formula of hydromorphone.

and monitored for alkaloid content on thin layer chromatograms in System I. Metabolites appeared in the 40% methanol eluate. These fractions were pooled and evaporated to a small volume. Acetone, methanol and acetic acid were added and a white precipitate formed which contained the two metabolites. This precipitate (A) yielded 100 mg of the two metabolites.

Forty-five milligrams of A were redissolved in water and the metabolites were separated using 16 preparative thin layer chromatograms streaked with the material and developed in System I. The chromatograms were cut horizontally into strips corresponding to the location of the separated metabolites and the strips were eluted with water. Each metabolite was crystallized as glass-like needles from methanol, water and acetic acid. The crystals were water soluble and each showed 1 alkaloid spot on TLC in System I at $R_f = 0.36$ and $R_f = 0.27$, respectively.

Results and Discussion. Preliminary indication that the metabolites were glucuronides was obtained by working with precipitated material A (which contained both metabolites). Hydrolysis of A by β-glucuronidase liberated organic bases which corresponded in TLC characteristics to hydromorphone and dihydromorphine. Saccharo-1,4-lactone, an inhibitor of β-glucuronidase, completely prevented liberation of these bases. Sulfatase had no effect in either situation.

Infrared absorption spectra for the 2 crystalline metabolites are shown in Fig. 2. The metabolites with an $R_f = 0.27$ and 0.36 (System I) showed very similar general features in their spectra. Particularly noteworthy were the broad absorption bands in the 9 to 10 μm range and the strong absorption at about 6.3 μm. These are characteristic features seen previously for morphine-, nalorphine-, and naloxone-3-glucuronide, all of which exist as zwitterionic internal salts in the crystalline form (2, 4–6). This spectral evidence plus the β-glucuronidase hydrolysis experiments mentioned above indicate that

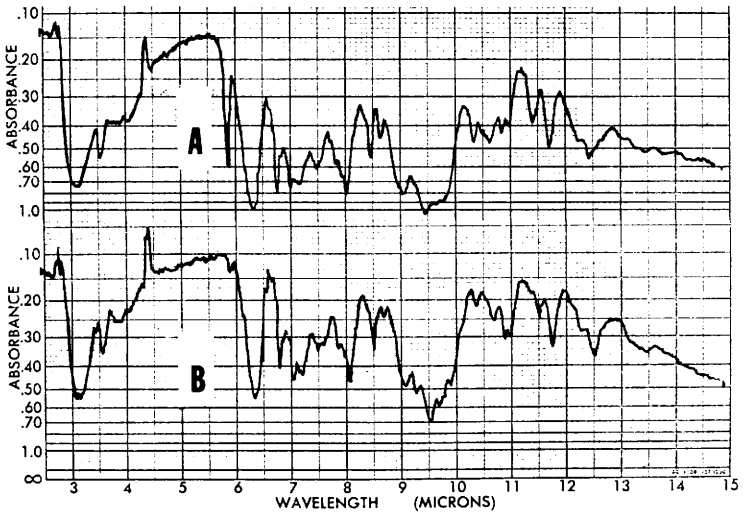

FIG. 2. Infrared spectra of crystalline metabolites in KBr pellets. Spectra for metabolites with $R_f = 0.27$ and 0.36 (TLC System I) are shown in (A and B), respectively.

both metabolites are glucuronides.

One important point of difference in the spectra seen between the two metabolites is that at 5:8 μm, a strong absorption band occurred for the R_f 0.27 compound which was absent in the other compound. By analogy to the naloxone metabolite study (2), the presence of the 5.8 μm band was attributed to presence of the keto group in position 6 of hydromorphone. The absence of this absorption band in the other metabolite was taken to indicate that the keto group of position 6 had been reduced. These results suggested that one metabolite (R_f 0.27) was hydromorphone glucuronide and the other (R_f 0.36) was dihydromorphine glucuronide.

To further establish the suggested structure of the alkaloid portion of the metabolite molecules, the metabolites were hydrolyzed for 1 hr in 6 *N* HCl under 15 lb pressure. The hydrolysates were adjusted to pH 8.5 with a few drops of concentrated NH_4OH and addition of solid $NaHCO_3$. The free bases were extracted into ethyl acetate and then returned from ethyl acetate into 0.1 *N* HCl. Small portions from the two samples of HCl phase were chromatographed in solvent System II, which consisted of benzene, hexane and diethylamine (25:10:2, v/v). One sample chromatographed identically with authentic hydromorphone (R_f 0.82) and the other with authentic dihydromorphine (R_f 0.48). The remaining portions of the HCl

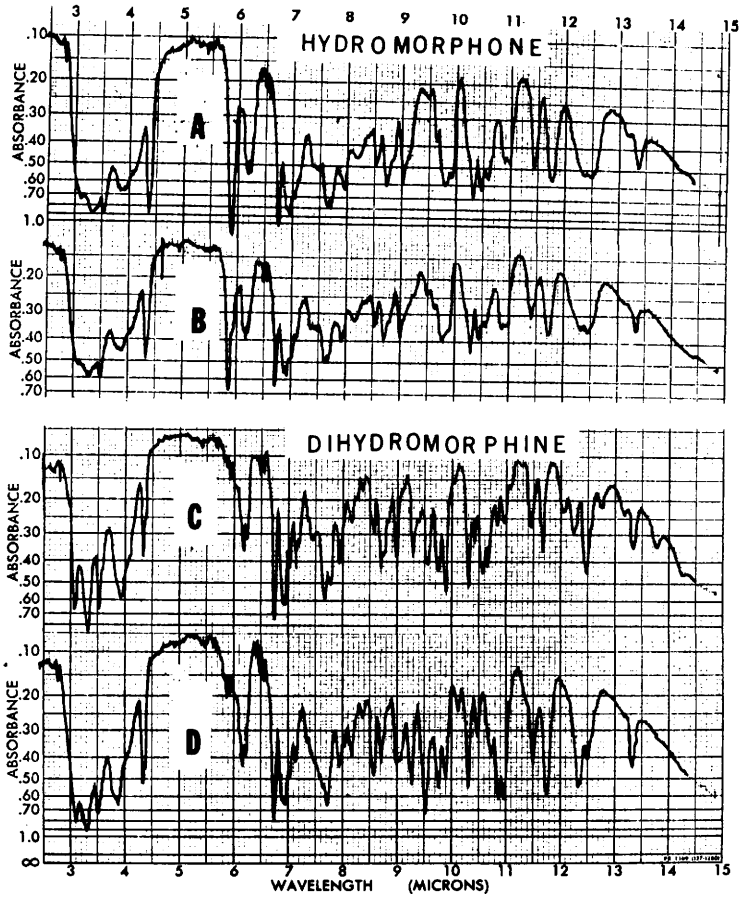

FIG. 3. Infrared spectra of bases recovered from the two hydrolyzed metabolites. (A) Authentic hydromorphone, (B) compound from R_f 0.27 (TLC System I) metabolite, (C) authentic dihydromorphone, (D) compound from R_f 0.36 (TLC System I) metabolite.

phases were evaporated to dryness. The solid residues were taken for ir analysis and compared to authentic hydromorphone and dihydromorphine treated in the same way as the unknowns. These ir curves are given in Fig. 3. It was evident that one isolated alkaloid was hydromorphone and the other was dihydromorphine.

Even though at this point, the glucuronic acid and base composition were known for the metabolites, a further experiment was necessary to establish the site on the base molecules at which the glucuronic acid was conjugated. Determination of ultraviolet absorption spectra of both metabolites showed no bathochromic shift in going from acid to alkaline pH. Since a bathochromic shift should occur if the phenol group of the base were free (7), it was concluded that the glucuronic acid was conjugated at the 3 position of the base in each case. Because of the drastic conditions necessary to hydrolyze off the glucuronic acid chemically, it is likely that this linkage occurs through an ether type bond. Thus, we concluded that the two metabolites after hydromorphone administration to the rabbit are hydromorphone-3-glucuronide and dihydromorphine-3-glucuronide.

Furthermore on a semiquantitative basis, the two metabolites isolated were the major metabolites present in the urine. This impression was based on the relative intensity of the spots after thin layer chromatography by the urine analysis method of Fujimoto and Wang (8).

Because there are species differences in the extent to which naloxone is reduced (2, 3, 6), it will be of interest in the future to see whether species differences will occur for reduction of hydromorphone.

Summary. Two metabolites of hydromorphone were isolated from the urine of the rabbit. By infrared analyses, thin layer chromatography, β-glucuronidase hydrolysis and ultraviolet spectroscopy, the crystalline metabolites were found to be hydromorphone-3-glucuronide and dihydromorphine-3-glucuronide.

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