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Isolation of Nalorphine Ethereal Sulfate and Nalorphine Glucuronide from Urine of Cat and Rabbit* (35602)

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In a recent paper (1), we reported the isolation of morphine-3-ethereal sulfate, a major metabolite, from the urine of chicken and cats given morphine. The general value of the isolation procedure was demonstrated by crystallization of morphine-3-glucuronide from the urine of the rabbit.

In the present study, the above method of isolation is used to crystallize two metabolites of nalorphine, one from the urine of the cat and another from the urine of the rabbit. The material isolated from the cat is shown to be nalorphine-3-ethereal sulfate and that from the rabbit nalorphine-3-glucuronide. This latter compound also appears to be present in the urine of the cat. This study forms an integral part of our investigation of the renal tubular transport and metabolism of narcotic analgesics (2).

Methods. Two male cats (2.9 and 2.1 kg), fasted overnight, were each given 30 mg(0.9 ml) nalorphine hydrochloride, s.c. At 4.5 hr, doses of 200 and 150 mg, i.p., were given to the larger and smaller cat, respectively. On the second and third days, doses of 200 mg, i.p., were repeated. The urine was collected (1) for several days after the last dose and the samples were pooled.

The primary method used for the purification process was described for the isolation of the metabolites of morphine (1) and only details essential to the immediate procedure are given below. After filtration through diatomaceous earth, the volume of the urine was 590 ml. Portions of 100, 100, 140, and 150 ml were run through the Amberlite XAD-2 resin columns. The main fractions from the 4 runs were pooled (230 ml) and extracted with 100 ml of petroleum ether. The ether phase was discarded. The aqueous phase was adjusted to pH 8.5 by dropwise addition of concentrated ammonium hydroxide and the free nalorphine was removed by extracting twice with a mixture of 100 ml of chloroform and 5 ml of isoamyl alcohol. The aqueous phase was evaporated under vacuum to a tar-like residue. The residue was taken up in 5 ml of water and 50 ml of methanol; 0.5 ml of glacial acetic acid was added. Addition of acetone and methanol yielded a brown grey precipitate (I) that weighed 35 mg after filtration and drying. The filtrate was evaporated to dryness and the resulting tar-like

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residue was dissolved in 10 ml of warm methanol. After several hours in the cold room, a precipitate formed. The precipitate after filtration and washing with 3 ml of methanol weighed 55 mg. This precipitate was dissolved in 4 ml of 1 *N* sodium hydroxide and the solution was filtered; 0.7 ml of glacial acetic acid was added to the filtrate. Crystals appeared gradually and they were collected by filtration to yield 31 mg of golden needles (II).

A large male New Zealand rabbit, fasted overnight, was given a total of 1.6 g of nalorphine hydrochloride in equally divided doses administered s.c. at 0, 2, 6, and 12 hr. Urine was collected by catheterization (3) at convenient intervals during a 4-day period. The pooled urine sample after filtration through diatomaceous earth was 170 ml. This urine was processed in portions of 100 and 70 ml through the Amberlite XAD-2 columns. The eluate fractions were stored overnight in the cold room. Since crystals were forming in the peak tubes, the fractions were left in the cold room for 5 more days. The white crystals that formed were filtered into a sintered glass funnel and washed with 5 ml of acetone. The yield was 31 mg (III). The filtrate was combined with the remainder of the eluate fractions in which there were appreciable quantities of uncrystallized alkaloidal material. This combined mixture was reduced under vacuum to about ½ volume (100 ml). The precipitate which formed was filtered, washed with 5 ml each of ethanol and acetone. The yield of brown grey powdery precipitate was 36 mg (IV).

Thin-layer chromatography consisting of a solvent system of *n*-butanol, acetic acid and water (35:3:10) with Gelman type SG thin-layer sheets was used to follow the purification process. Alkaloids were detected with iodoplatinate reagent and organic compounds with sulfuric acid charring (2).

Chemical synthesis of nalorphine-3-ethereal sulfate was accomplished by using the same chemical reaction scheme as had been used for synthesis of morphine-3-ethereal sulfate (1). From a reaction mixture containing 3.38 g of nalorphine, 2.8 g of potassium pyrosulfate, water, and potassium hydroxide, 59 mg of nalorphine-3-ethereal sulfate was obtained following several steps of purification, crystallization, and recrystallization. Elemental analyses were : C 58.45, 58.19; H 5.51, 5.42; N 3.63, 3.54; S 7.94%. $C_{19}H_{21}NO_6S$ requires: C 58.30; H 5.41; N 3.58; S 8.19. The white needles decomposed at 235°.

Infrared spectra were obtained on a Beckman Microspec spectrophotometer using 1% KBr pellets of the material. Ultraviolet spectra were obtained with a Beckman DB spectrophotometer with a 10-in. recorder.

Results and Discussion. Figure 1 shows

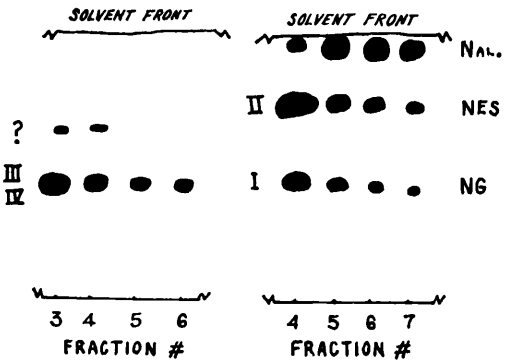

FIG. 1. Thin-layer chromatogram of eluate fractions (indicated by Arabic numerals from Amberlite XAD-2 resin columns of urine of the cat (right panel) and rabbit (left panel). The TLC solvent system was *n*-butanol, acetic acid, and water (35:3:10) used with Gelman type SG sheets; spray reagent was iodoplatinate; Roman numerals refer to products designated in "Methods;" Nal. is nalorphine; NES is nalorphine etheral sulfate; NG is nalorphine glucuronide; ? is unidentified product.

thin-layer chromatograms of cat and rabbit urine that has been processed through the Amberlite XAD-2 resin columns. In the sample from the cat, nalorphine and products designated I and II are present. In the rabbit, products III, IV and an unidentified alkaloid are seen; no free nalorphine was present. Product II is nalorphine-3-ethereal sulfate; III and IV and probably I are nalorphine-3-glucuronide. The evidence to support the identification of these products will be presented next.

Figure 2 (top) shows the IR spectrum of

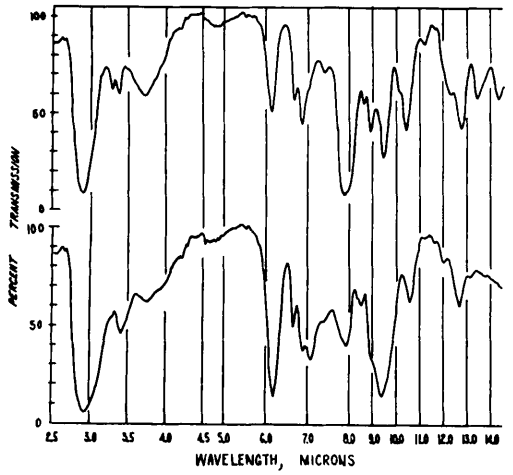

FIG. 2. Infrared spectra of chemically synthesized nalorphine ethereal sulfate (top) and biologically derived nalorphine glucuronide from the urine of the rabbit (bottom).

chemically synthesized nalorphine-3-ethereal sulfate. Product II, isolated in crystalline form from the urine of the cat, had an IR spectrum that was identical to the chemically synthesized compound. The ultraviolet absorption spectra are shown in Fig. 3 (right) for II. It had the same spectra as the chemically synthesized product. The conjugation of the sulfuric acid occurs at the 3-OH position of nalorphine since no bathochromic shift occurs in the ultraviolet spectra with a change from acidic to basic conditions (1, 4–6).

It appears that the nalorphine-3-etheral sulfate occurs as an internal salt as indicated by the elemental analyses and the characteristic broad IR absorption peaks in the 3–4 μ range. An internal salt is the expected form based on the examples given by Kumler (7). Morphine-3-ethereal sulfate also occurs in this form (1).

The IR spectrum for the crystalline compound III, obtained from the urine of the rabbit, is shown in Fig. 2 (bottom). An identical spectrum was obtained for IV Comparison of the IR spectra of these compounds with that of morphine-3-glucuronide (6) leads to our conclusion that III and IV are nalorphine glucuronide. Acid hydrolysis yields free nalorphine (thin-layer chromatography). Also, enzymatic hydrolysis by bovine liver β-glucuronidase yields free nalorphine. The estimated content of nalorphine, quantitated in the acid hydrolysate by using the bathochromic shift at 300 mμ (4), agreed with the expected content of 49.5% for nalorphine monoglucuronide·H_2O. The ultraviolet spectrum of the unhydrolyzed compound given in Fig. 3 (left) indicates conjugation of the glucuronic acid with the 3-OH group of nalorphine. The arguments here are the same as for nalorphine-3-ethereal sulfate given above. The purity of III and IV is open to some slight question. Elemental analyses for III and IV gave, respectively: C 56.28; H 6.07; N 2.94 and C 54.95; H 5.89; N 2.78. Calculation for nalorphine glucuronide, $C_{25}H_{29}O_9N \cdot 2\frac{1}{2}H_2O$ requires: C 56.4; H 6.45; N 2.63. However, the body of evidence supports the definitive identification of the products as nalorphine-3-glucuronide.

In analogy to morphine-3-ethereal sulfate, morphine-3-glucuronide (1, 6) and nalorphine-3-ethereal sulfate it would be expected that nalorphine-3-glucuronide would be an internal salt. The IR spectrum is in accord with this expectation.

Product I from the urine of the cat has the same chromatographic characteristics as III and IV from the rabbit. This result would suggest that I is also nalorphine-3-glucuronide. Further attempts at crystallization of I were unsuccessful.

Quantitatively, it would appear from Fig. 1 that the nalorphine-3-glucuronide is a major metabolite in the urine of the rabbit. Nalorphine-3-ethereal sulfate may predominate in the urine of the cat but appreciable quantities of the glucuronide may also be present. These findings correlate with the differences found between the cat and rabbit in the metabolism of morphine (1). The ethereal sulfate and glucuronide appear to be major metabolites of morphine in the cat and rabbit, respectively.

Summary. Isolation of a major metabolite of nalorphine from the urine of the cat was accomplished. This metabolite was nalorphine-3-ethereal sulfate by chemical synthesis

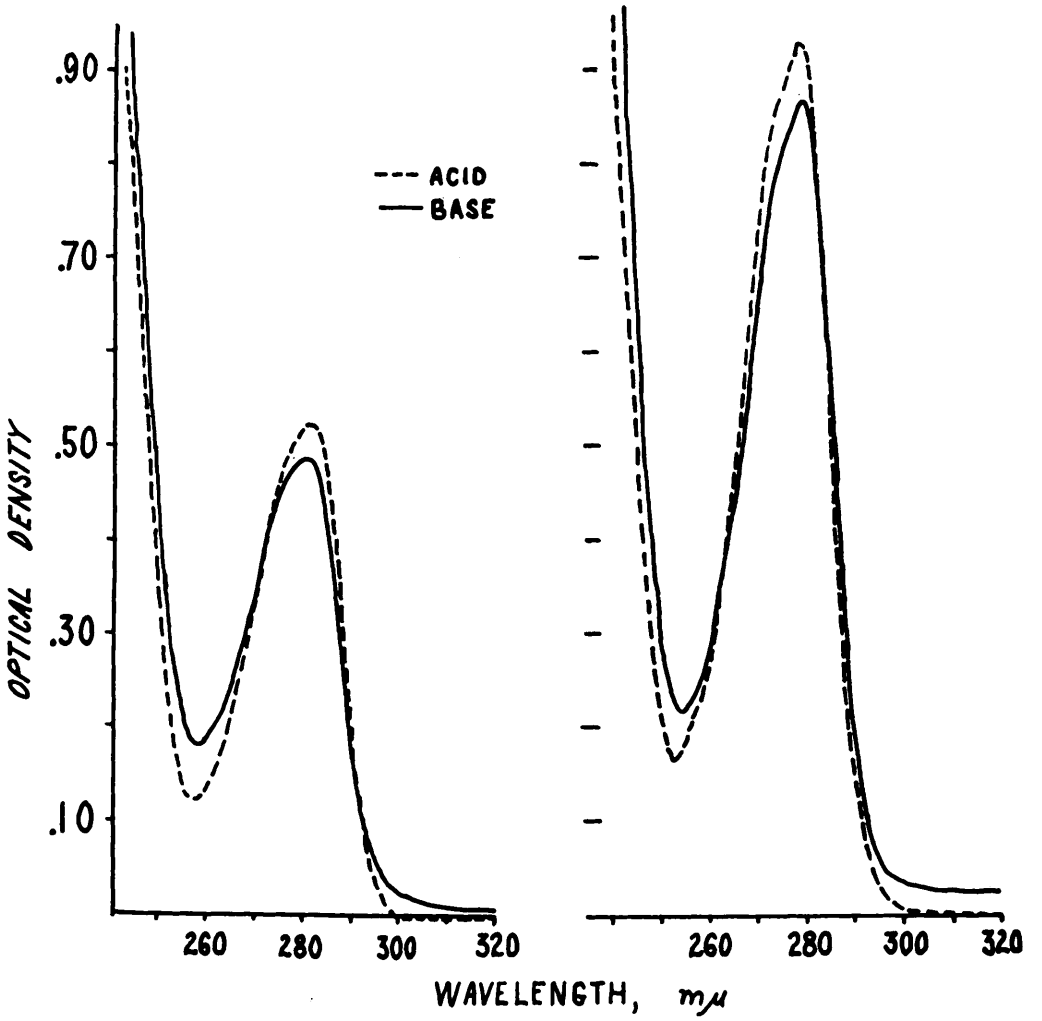

FIG. 3. Ultraviolet spectra of biologically derived nalorphine ethereal sulfate from the cat (right) and nalorphine glucuronide from the rabbit (left). The concentrations were 160 μg of compound/ml in hydrochloric acid and sodium hydroxide.

of the compound. Evidence is presented to characterize the crystalline metabolite isolated from the urine of the rabbit as nalorphine-3-glucuronide.

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