A SIMPLE METHOD FOR THE ISOLATION OF MORPHINE GLUCURONIDE FROM URINE

TETSUO OKA

Department of Pharmacology, School of Medicine, Keio University,
Tokyo, Japan

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INTRODUCTION

It is often needed for researchers on morphine to isolate morphine glucuronide, a chief morphine metabolite in animal bodies. However, it is difficult to isolate morphine glucuronide from urine by simple method as it is not extracted by any volatile organic solvent. Woods (1954), and Fujimoto and Way (1957) described their excellent methods independently to get pure morphine glucuronide from the urine and bile of dogs administered morphine and from the urine of human addicts but they were really tedious works. Lately, the author succeeded to established a simple method to isolate morphine glucuronide from urine by utilizing the adsorptive natures of charcoal and some ion exchange resins.

METHODS

I. Materials and Tools.

Urine: The urine of non-tolerant dogs injected 100 mg/kg of morphine hydrochloride subcutaneously were collected by insertion of a Nelaton's catheter into urinary bladder.

Charcoal: Charcoal was treated as described in the foregoing paper (Oka and Hosoya, 1967).

Resin: 3 g of Amberlite CG-50 were stirred with 100 ml of 1 N HCl for 15 minutes three times and bufferized at pH 4.7 with 100 ml of acetate buffer. Three gram of Amberlite CG-400 were stirred with 100 ml or 1 N HCl for 15 minutes three times and then stirred with 100 ml of 1 N NaOH for 15 minutes three times. Three gram of resin were used for 50 ml of urines.
Column: The glass column of 30 cm high and 1 cm I. D. was used for 1 g of charcoal or 3 g of resin.

Reagents: All reagents used were reagent grades.

II. Procedures.

1. Pour the urine into a charcoal column: 1 g of charcoal was enough for 50 ml of urine. It must be noticed that air gases adsorbed on charcoal had to be driven in vacuum desiccator before charcoal is poured into the column. The control of pH of the urine was found unnecessary. Free morphine and morphine glucuronide were adsorbed on charcoal.

2. Elute adsorbed materials with 20 ml of glacial acetic acid after washing the charcoal column with 50 ml of water: Free morphine and morphine glucuronide were eluted by this procedure.

3. Evaporate the acetic acid eluate and dissolve the residue with 100 ml of distilled water: The acetic acid eluate was evaporated to dryness at room temperature in a ventilating hood. One hundred milliliter of the solution were adjusted to pH 4.7.

4. Pour the pH adjusted solution into Amberlite CG-50 column: 3 g of the resin were used for 50 ml of the original urine. Free morphine was adsorbed on the resin, whereas morphine glucuronide passed through the resin.

5. Pour the solution passed through the Amberlite CG-50 column, into Amberlite CG-400 column: 3 g of the resin were used. Morphine glucuronide was adsorbed on the resin.

6. Elute with 20 ml of 1 N HCl: Morphine glucuronide was eluted into HCl solution.

7. Pour the 1 N HCl eluate into charcoal column and eluate with acetic acid: 1 g of charcoal and 10 ml of acetic acid was used. Most parts of contaminating substances in urine was excluded until this stage.

8. Evaporate the acetic acid eluate.

9. Crystallize morphine glucuronide: The acetic acid residue was dissolved in 1 ml of water and filtered with glass filter. Methanol was added slowly to this filtrate, so colorless crystals appeared. The mixture was filtered with glass filter and the crystals were washed with 10 ml of the mixture of water (1 vol.) and methanol (9 vol.) and washed with 10 ml of ether. The washed crystals were desiccated in a vacuum over P₂O₅ for 48 hours at room temperature.
Fig. 1 Infrared spectrum for the crystal got at the final stage.
RESULTS AND DISCUSSION

I. Identifications of the crystal got at the final stage are done by the following tests:

1. **Melting point**: 229–230°C with decomposition. This value is almost the same as reported by Woods (1954).

2. **Elementary analysis** is presented in Table 1.

<table>
<thead>
<tr>
<th>Elementary analysis</th>
<th>Theoretical per cent*</th>
<th>Observed per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>6.28%</td>
<td>6.07%</td>
</tr>
<tr>
<td>C</td>
<td>55.53%</td>
<td>55.49%</td>
</tr>
<tr>
<td>N</td>
<td>2.82%</td>
<td>2.61%</td>
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<tr>
<td>Ash</td>
<td>(−)</td>
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<table>
<thead>
<tr>
<th>Alkaline ferricyanide oxidation</th>
<th>Morphine</th>
<th>Codeine</th>
<th>the crystal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>blue</td>
<td>no blue</td>
<td>no blue</td>
</tr>
</tbody>
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* Theoretical per cent of H, C and N was calculated as morphine monoglucuronide dihydrate.

3. **Infrared spectrum**: The infrared spectrum of the final crystal was taken (Figure 1). Comparing the spectrum of the final crystal to those of Fujimoto and Way, it corresponds well to that crystal got at the final stage of their procedure.

4. **Alkaline ferricyanide oxidation** (Table 1): This result shows that the crystal has no phenolic hydroxyl group.

5. **Paper chromatography**:

   a) Two clear spots (Rf 0.68 and 0.22) were recognized on paper chromatogram developed descendingly with the solvent mixture, n-butanol, glacial acetic acid and water (4, 1, 2) and sprayed iodoplatinate reagent (Munier and Machéboeuf, 1949) by the concentrated urine of the dog administered 100 mg/kg of morphine hydrochloride subcutaneously (Figure 2). One spot (Rf 0.68) is due to free morphine and the other (Rf 0.22) to morphine glucuronide according to the study of Woods (1954).

   b) No spot was found on chromatogram by the same procedure in the urine of dog who had not been given morphine at all (Figure 2).
Fig. 2 Paper chromatogram developed with the solvent mixture, n-butanol, glacial acetic acid and water (4, 1, 2) and sprayed by iodoplatinate or aniline hydrogen phthalate reagent. a-d were sprayed by iodoplatinate reagent. e-h were sprayed by aniline hydrogen phthalate reagent. a: The urine of dog administered morphine. b: The urine of dog who had not been given morphine at all. c: The crystal got at the final stage. d: The hydrolysate of the solution of final crystal. e: The crystal got at the final stage. f: The hydrolysate of the solution of final crystal. g: The hydrolysate of glucuronolactone. h: Glucuronolactone.

c) By the same procedure, water solution of the crystal of the final stage described in the foregoing chapter showed only one spot at Rf 0.22 on paper chromatography (Figure 2).

d) After hydrolysis of the solution of the final crystal by boiling 15 minutes with 5 N HCl, one spot was found at Rf 0.68 instead of Rf 0.22 (Figure 2) by spraying iodoplatinate reagent. And by spraying aniline hydrogen phthalate reagent (Partridge, 1949) three spots (Rf 0.50, 0.31 and 0.25) were found (Figure 2). According to the study of Seibert et al. (1954), the spots of Rf 0.50 and 0.25 were due to glucuronolactone and glucuronic acid respectively, and the spot of Rf 0.31 was unknown.

The results a) b) c) and d) tell us the final crystal at 9th stage may be nothing but morphine glucuronide.

From these results as mentioned 1-5, the crystal got at the final stage is an almost completely pure morphine 3-monoglucuronide dihydrate.
II. The yield ranged 68–75% of the total amounts of morphine glucuronide in the urine calculated from the results by fluorophotometric method described by Akera and Hosoya (1965).

III. Pharmacological studies on morphine glucuronide is under investigation, which will be reported in the near future.

CONCLUSIONS

It is proved that by the simple procedure, utilizing the adsorptive natures of charcoal and ion exchange resins, morphine glucuronide in urine can be isolated in high purified state with good recovery rate.

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REFERENCES