introduced into the treated urine and carried out as mentioned above. Recovery was 83.2(±0.5)%. Total \( p \)-ethoxyphenylurea minus free \( p \)-ethoxyphenylurea equals \( p \)-ethoxyphenylurea N-glucuronide.

The authors are indebted to Chugai Pharmaceutical Co. Ltd., for their kind supply of \( \alpha \)-glucuronic acid for the present synthesis. This work was supported in part by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education, to which the authors are indebted.

**Summary**

\( N \)-Glucosiduronates of arylureas excreted in the rabbit urine dosed with arylureas were isolated and confirmed to be identical with ammonium 1-[3-(aryl)ureido]-1-deoxy-\( \alpha \)-glucopyranosiduronates which were synthesized from glucuronic acid and arylureas in pyridine.

It is suggested that \( N \)-glucosiduronate conjugation is a general pathway of arylurea metabolism in the rabbit.

(Received June 1, 1965)

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3. Masuo Akagi, Isamu Aoki, Masanobu Haga, Takayoshi Uematsu, and Masakatsu Sakata: Studies on Food Additives. XII.\(^1\)

Synthesis of \( p \)-Ethoxyphenylurea N-Glucuronide, a Metabolite in Rabbit.

(Faculty of Pharmaceutical Sciences, Hokkaido University\(^2\))

In the preceding papers,\(^1,13\) it was reported that oral administration of \( p \)-ethoxyphenylurea, \( o \)-tolylurea, \( p \)-chlorophenylurea and phenylurea to rabbits resulted in the excretion of a new type \( N \)-glucuronide and these were identical with the compounds prepared by the condensation of glucuronic acid and arylureas in pyridine.

In the present paper, the synthesis of some arylurea \( N \)-glucuronides and the determination of their structure are described.

The condensation of arylamine with glycosyl-isocyanate and isothiocyanate had been favorably used to obtain glycosylurea and thiourea.\(^2,4,6\) 2,3,4,6-Tetra-O-acetyl-\( \beta \)-d-glucopyranosylisocyanate\(^6\) was refluxed with \( p \)-phenetidine in chloroform-pyridine, and 1-[3-(\( p \)-ethoxyphenyl)ureido]-1-deoxy-2,3,4,6-tetra-O-acetyl-\( \beta \)-d-glucopyranose (VII) was obtained in a yield of 73%. The same compound was also prepared from 1-[3-(\( p \)-ethoxyphenyl)thiourea]-1-deoxy-2,3,4,6-tetra-O-acetyl-\( \beta \)-d-glucopyranose (K) in a yield of 20% when the aqueous methanolic solution of K was desulfurized with silver nitrate. K was synthesized from 2,3,4,6-tetra-O-acetyl-\( \beta \)-d-glucopyranosylisothiocyanate and \( p \)-phenetidine in the similar way as in the case of the isocyanate in a good yield. VII was converted to 1-[3-(\( p \)-ethoxyphenyl)ureido]-1-deoxy-\( \beta \)-d-glucopyranose

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in 90% yield, by treatment with ammonia-methanol. Ⅲ was catalytically oxidized by the method of Marsh\(^5\) to potassium 1-[3-(p-ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranuronate (I), in 30% yield, which was identified by mixed fusion with the metabolite obtained from the urine of rabbits dosed with p-ethoxyphenylurea.\(^5\)

Further, synthetic studies on starting material of methyl 1-[3-(p-ethoxyphenyl)-thioureido]-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate\(^6\) (Ⅲ) were established. Ⅲ was desulfured with silver nitrate to give methyl 1-[3-(p-ethoxyphenyl)ureido]-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (Ⅳ), in 60% yield. Ammonium 1-[3-(p-ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranuronate (Ⅱ), and I were prepared by hydrolysis of Ⅳ with barium methoxide and removal of barium with potassium sulfate, and ammonium sulfate respectively. The products, I and II were identical with the compounds prepared from glucuronic acid and p-ethoxyphenylurea,\(^6\) and the metabolite isolated from the urine dosed with p-ethoxyphenylurea.

From the foregoing experimental results, it was concluded that I and II had a β-configuration at anomic center and the position of the bonding of glucuronic acid was in N\(^\circ\) of ureido group.

**Chart 1.**

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### Table I. Stability of \(\beta\)-Ethoxyphenylurea N-Glucuronide

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stability Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benedict's reagent</td>
<td>((-)) () () constant (48 hr.) ()</td>
</tr>
<tr>
<td>Fehling's reagent</td>
<td>((+)) (0%) (room temp.), 72 hr.</td>
</tr>
<tr>
<td>Mutarotation</td>
<td>(10% \text{ K}_2\text{CO}_3) (5%) (room temp.), 24 hr.</td>
</tr>
<tr>
<td></td>
<td>(1/10\text{N HCl}) (100%) (90-100\degree C), 30 min.</td>
</tr>
<tr>
<td></td>
<td>(1/2\text{N HCl}) (40%) (room temp.), 24 hr.</td>
</tr>
<tr>
<td></td>
<td>Neutral (0%) (room temp.), 72 hr.</td>
</tr>
<tr>
<td></td>
<td>(4%) (90-100\degree C), 30 min.</td>
</tr>
</tbody>
</table>

The same conclusion might apply to other arylurea homologs obtained from the rabbit urine.

\(\beta\)-Ethoxyphenylurea N-glucuronide, as shown in Table I, did not show any mutarotation during 48 hours, and was stable in neutral or alkaline media, unstable in acidic media, but seemed to be more stable than other amine N-glucuronide, e.g., a labile N-glucuronide.\(^7\)\(^8\) It was reported that meprombamate,\(^9\) sulfisoxazole and sulfathiazole\(^10\)\(^11\) N-glucuronides were very stable. These properties of \(\beta\)-ethoxyphenylurea N-glucuronide appeared to be similar to those of 1-\(\beta\)-glucosylurea.\(^12\)

Besides, 1-[3-(\(\beta\)-ethoxyphenyl)ureido]-1-deoxy-\(\beta\)-d-glucopyranuramide (V) was prepared by treatment of N with ammonia-methanol, and was acetylated to give 1-[3-(\(\beta\)-ethoxyphenyl)ureido]-1-deoxy-2,3,4-tri-O-acetyl-\(\beta\)-d-glucopyranuramide (VI). Methyl 1-[3-(\(\beta\)-tolyl)ureido]-1-deoxy-2,3,4-tri-O-acetyl-\(\beta\)-d-glucopyranuronate (X) was synthesized in the same manner as mentioned for V.

### Experimental\(^{12}\)

**Ammonium 1-[3-(\(\beta\)-Ethoxyphenyl)ureido]-1-deoxy-\(\beta\)-d-glucopyranuronate (II)——One gram of N was dissolved in 50 ml of MeOH. After the addition of 0.47 mol of 0.3N Ba(OH)\(_2\), the mixture was kept in a refrigerator for 48 hr. To the reaction mixture were added 0.4 ml of conc. NH\(_2\)OH and 0.6 ml of 0.3N (NH\(_4\))\(_2\)SO\(_4\) and filtered to remove BaSO\(_4\). The filtrate was treated with activated charcoal and was evaporated to dryness in vacuo. The residue was recrystallized from MeOH to 200 mg (27%) of colorless needles, m.p. 158\degree C (decomp.), \(\alpha\)\(^D\) = -45.0\degree C (c=1.00, H\(_2\)O), (Anal. Calcd. for \(\text{C}_{18}\text{H}_{16}\text{O}_{6}\text{N}_{4}\): C, 48.26; H, 6.00; N, 11.26. Found: C, 48.62; H, 5.77; N, 11.38).

This product was undepressed on admixture with the substance synthesized earlier and obtained from rabbit urine.\(^1\)

**Potassium 1-[3-(\(\beta\)-Ethoxyphenyl)ureido]-1-deoxy-\(\beta\)-d-glucopyranuronate (I)——a) One gram of N was dissolved in 50 ml of MeOH and 0.7 ml of 0.3N Ba(OH)\(_2\) was added. After kept in a refrigerator for 48 hr., the solution was treated with 0.7 ml of 0.3N K\(_2\)SO\(_4\) solution to remove barium ion and then was brought to pH 9 with aq. K\(_2\)CO\(_3\) solution to neutralize any excess carboxylic acid present. The resulting precipitates were discarded, the filtrate was concentrated in vacuo nearly to dryness and the yellowish residue triturated with MeOH. The product was recrystallized from H\(_2\)O-MeOH to give 250 mg of (33%) of colorless needles, m.p. 168\degree C (decomp.), \(\alpha\)\(^D\) = -46.7\degree C (c=1.00, H\(_2\)O), (Anal. Calcd. for \(\text{C}_{18}\text{H}_{16}\text{O}_{6}\text{N}_{4}\text{K}: C, 45.67; H, 4.86; N, 7.10. Found: C, 45.60; H, 5.08; N, 6.90). This compound was not depresssed by the authentic sample isolated from rabbit urine.

b) Two grams of (II) and 0.4 g. of platinum black were suspended in 150 ml of H\(_2\)O and vigorously stirred. Compressed oxygen was introduced into the mixture while it was maintained at 90\degree C on the hot magnetic stirrer and kept to pH 8 by addition of dilute potassium bicarbonate solution. After 2 hr.,

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\(^{12}\) All melting points were uncorrected.

4/5 equivalent of alkaline was consumed and no further change in pH occurred. The catalyst was removed by filtration treating with activated charcoal and the solution evaporated to about 1 ml. in vacuo and diluted with 20 ml. of MeOH. Recrystallization from 95% MeOH afforded 600 mg. (30%) of colorless needles, m.p. 185°C (decomp.), [α]D2 +46.0° (c=1.00, H2O). (Found : C, 45.42; H, 5.05; N, 6.71). This compound showed no depression of mixed melting point with the substance synthesized earlier and isolated from the rabbit urine.

**Methyl 1-[3-(p-Ethoxyphenyl)ureido]-1-deoxy-2,3,4,tri-O-acetyl-β-D-glucopyranuronate (IV)**—A solution of 7 g. of III in 140 ml. of EtOH and a solution of 7 g. of AgNO3 in 70 ml. of H2O were mixed at 50~60°C. After kept at this temperature for 5 min., the mixture was neutralized with 1/10N NaOH and further warmed at 50~60°C for 5 min. to promote the coagulation of silver sulfate. Then the solution was cooled, filtered, and the residue was washed with 50 ml. of hot EtOH. The combined filtrate and washing were concentrated in vacuo to about 100 ml. and extracted with 2×100 ml. of CHCl3. The CHCl3 layer was dried over anhyd. Na2SO4 and the solvent was evaporated in vacuo. Ten ml. of EtOH was added to the residue. The crystals that separated out were recrystallized from 50% EtOH to 2.7 g. (41%) of pinkish needles, m.p. 166°C, [α]D2 +15.4° (c=1.00, CHCl3). (Anal. Calcd. for C23H25O11N2·H2O: C, 51.16; H, 6.00; N, 5.42. Found : C, 51.03; H, 5.89; N, 5.73).

1-[3-(p-Ethoxyphenyl)ureido]-1-deoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (VII)  a) Three grams of X and 1.2 g. of p-phenetidine were dissolved in 23 ml. of CHCl3 and 1.8 ml. of pyridine. The mixture was refluxed at 70°C for 5 hr. on a water bath and evaporated to dryness in vacuo. EtOH was added to the residue and the crystals that formed were recrystallized from EtOH to 2.9 g. (73%) of colorless prisms, m.p. 159°C, [α]D2 -8.0° (c=2.00, CHCl3). (Anal. Calcd. for C25H29O11N2 : C, 54.11; H, 5.92. Found : C, 54.20; H, 6.05).

b) To a solution of 6.5 g. of K in 150 ml. of MeOH, a solution of 4.5 g. of AgNO3 in 20 ml. of H2O was added and allowed to stand for 5 min. at 40~50°C. The reaction mixture was made pH 7 with 1/10N NaOH and maintained again for 5 min. at 50°C. After cooling, the solution was filtered and the filtrate was evaporated to dryness in vacuo. The yellow powder was recrystallized from EtOH to 1.3 g. (20%) of yellowish needles, m.p. 159°C and undepressed on admixture with authentic sample obtained by the method of a. [α]D2 -8.0° (c=2.00, CHCl3).

1-[3-(p-Ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranose (VIII)—Five grams of XI were dissolved in 50 ml. of NH4·MeOH in an ice bath. After the solution was allowed to stand overnight in a refrigerator, the crystals separated from it were filtered and recrystallized from EtOH to afford 3.9 g. (90%) of colorless needles, m.p. 211°C (decomp.), [α]D2 -7.0° (c=1.00, pyridine). (Anal. Calcd. for C15H23O11N2 : C, 52.62; H, 6.48; N, 8.18. Found : C, 52.54; H, 6.58; N, 8.23).

1-[3-(p-Ethoxyphenyl)thiourea]-1-deoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (IX)—Six grams of XII and 2.4 g. of p-phenetidine were dissolved in 40 ml. of CHCl3 and 2.5 ml. of pyridine, and refluxed at 70°C for 5 hr. The reaction mixture was evaporated to dryness in vacuo, and EtOH was added to the residue. The crystals that separated out were collected and recrystallized from EtOH to afford 5.6 g. (67%) of colorless needles, m.p. 150~151°C, [α]D2 -8.0° (c=2.00, CHCl3). (Anal. Calcd. for C23H25O12N2S: C, 52.43; H, 5.75; N, 5.32. Found : C, 52.24; H, 6.02; N, 5.34).

1-[3-(p-Ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranuronanamide (V)—A solution of 500 mg. of N in 100 ml. of MeOH was saturated with NH3 gas with cooling. The solution was kept overnight in a refrigerator. The solvent was removed in vacuo, and the residue was recrystallized from dil. EtOH to afford 100 mg. (29%) of colorless needles, m.p. 212~213°C, [α]D2 -21.7° (c=1.01, dimethylformamide). (Anal. Calcd. for C15H23O11N2·H2O·N2: C, 50.71; H, 5.90; N, 11.93. Found : C, 50.98; H, 6.01; N, 12.00).

1-[3-(p-Ethoxyphenyl)ureido]-1-deoxy-3,4,4-tri-O-acetyl-β-D-glucopyranuronanamide (VI)—Seven grams of V was acetylated as usual by 40 ml. of pyridine and 40 ml of Ac2O. After standing for 2 days at room temperature, the solution was poured into 200 ml. of ice-water. The precipitates were filtered and washed with H2O. Recrystallization from EtOH gave 3.5 g. (36%) of colorless needles, m.p. 225~227°C (decomp.), [α]D2 -13.8° (c=0.79, dioxane). (Anal. Calcd. for C25H29O11N2: C, 52.39; H, 5.65; N, 8.73. Found : C, 52.16; H, 5.71; N, 8.51).

**Methyl 1-[3-(p-Tolyl)ureido]-1-deoxy-β-D-glucopyranuronate (X)”—To a solution of 10 g. of methyl 1-[3-(p-tolyl)thiourea]-1-deoxy-2,3,4,tri-O-acetyl-β-D-glucopyranuronate in 200 ml. of EtOH, a solution of 10 g. of AgNO3 in 50 ml. of H2O was mixed and carried out as described above. Recrystallization from EtOH gave 1.9 g. (10%) of colorless needles, m.p. 188~190°C, [α]D2 -9.7° (c=1.03, CHCl3). (Anal. Calcd. for C23H25O11N2·H2O·N2: C, 54.08; H, 5.57; N, 6.01. Found : C, 54.13; H, 5.55; N, 6.00).

**Some Properties of N-Glycosidic Linkage of I and II**—Qualitative analysis: I and II synthesized and isolated from the urine did not exhibit mutarotation in aqueous solution during 48 hr. at room temperature. They reduced Fehling’s solution but not Benedict’s solution on warming, gave a rapid naphthoresorcinol reaction, a brown color with aniline-HCl and a yellow color with Ehrlich’s reagent. Quantitative analysis: Each solution (1.0 mg/ml) of I was prepared and liberated p-ethoxyhexylylurea was determined on aliquots by spectrophotometry described by Akagi, et al.9)

Thanks are due to Chugai Pharmaceutical Co., Ltd. for their supply of glucuronolactone. This work was supported in part by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education, to which the authors are indebted.

Summary

In order to determine the structure of \(\beta\)-ethoxyphenylurea N-glucuronide isolated from the rabbit urine dosed with \(\beta\)-ethoxyphenylurea, ammonium and potassium 1-[3-(\(\beta\)-ethoxyphenyl)ureido]-1-deoxy-\(\beta\)-D-glucopyranuronates were synthesized from 2,3,4,6-tetra-O-acetyl-\(\beta\)-D-glucopyranosylisocyanate, isothiocyanate, or methyl 1-[3-(\(\beta\)-ethoxyphenyl)thiourea]-1-deoxy-\(\beta\)-D-glucopyranuronate as starting materials, and were identical with the N-glucuronides from the rabbit urine.

(Received June 3, 1965)

4. Masuo Akagi, Isamu Aoki, Takayoshi Uematsu, and Takashi Iyanagi:


(Faculty of Pharmaceutical Sciences, Hokkaido University)

In the previous papers, it was reported that \(\beta\)-ethoxyphenylurea, o-tolylurea, \(\beta\)-chlorophenylurea, and phenylurea N-glucuronides were isolated from the urine of rabbits dosed with the corresponding arylureas orally and to their structure were assigned 1-[3-(arylureido)-1-deoxy-\(\beta\)-D-glucopyranuronates.

In this paper, the reaction products of glucuronolactone (I) with several arylureas and the preparation of the corresponding N-glucopyranuronate from them are described.

I. Condensation of Arylurea and I

In 1905, Neuberg, et al. obtained a condensation product by heating an aqueous solution of urea and I in the presence of sulfuric acid as catalyst. However, the application of this method to the synthesis of arylurea N-glucuronolactone was crowned with unsuccess.

It was shown that arylamine and I were generally able to form the corresponding N-glucuronolactone in a medium of polar solvent in a good yield. It would be that weaker basic compound such as arylurea could not react with I under the above condition.

\[\text{A part of this work was reported at the 82th Annual Meeting of Pharmaceutical Society of Japan in Shizuoka (November, 1962).} \]
\[\text{Nishi-7-chome, Kita-15-jo, Sapporo, Hokkaido (赤木満洲雄, 赤木 参, 根本孝悦, 井川 奥)。} \]
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