

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 *p*-ethoxyphenylurea N-glucuronide isolated from the rabbit urine dosed with *p*-ethoxyphenylurea, ammonium and potassium 1-[3-(*p*-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-(*p*-ethoxyphenyl)thioureido]-1-deoxy- $\beta$ -D-glucopyranuronate as starting materials, and were identical with the N-glucuronides from the rabbit urine.

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#### 4. Masuo Akagi, Isamu Aoki, Takayoshi Uematsu, and Takashi Iyanagi : Studies on Food Additives. XIII.\*<sup>1</sup> Synthesis of Arylurea N-Glucuronide from Glucuronolactone.\*<sup>2</sup>

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In the previous papers,\*<sup>1,1,2</sup>) it was reported that *p*-ethoxyphenylurea, *o*-tolylurea, *p*-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-(aryl)ureido]-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.*<sup>3)</sup> 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<sup>4~6)</sup> 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.

\*<sup>1</sup> Part XII. M. Akagi, I. Aoki, M. Haga, T. Uematsu, M. Sakata : This Bulletin, 14, 14 (1966).

\*<sup>2</sup> A part of this work was reported at the 82th Annual Meeting of Pharmaceutical Society of Japan in Shizuoka (November, 1962).

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1) M. Akagi, I. Aoki, T. Uematsu : This Bulletin, 14, 10 (1966).

2) M. Akagi, I. Aoki, T. Uematsu, T. Iyanagi : *Ibid.*, 14, 1 (1966).

3) C. Neuberg, W. Neimann : Z. physiol. Chem., Hoppe-Seyler's, 44, 97 (1905).

4) S. Takitani : This Bulletin, 9, 222 (1961).

5) K. Heyns, W. Balltes : Chem. Ber., 93, 1616 (1960).

6) K. Panagopoulos, G. Kallistratos, A. Pfau : Chim. Chronica, 26, 29 (1961).

A series of arylurea N-glucuronolactone was synthesized in a better yield in a following method: A small amount of conc. sulfuric acid was added to a pyridine solution of arylurea and I, the mixture was left at room temperature after maintaining at 50~60° for several hours. The crystalline products of m.p. (decomp.) IIa 194°, in 60%, III 187°, in 100%, IVa 191.5~192°, in 42%, V 165~168°, in 79%, VI 198°, in 62%, and VII 192°, in 36% yields were thus obtained from *p*-ethoxyphenylurea, *p*-, *o*-, and *m*-tolylureas, *p*-chlorophenylurea and anisylurea respectively. In the case of phenylurea, the product was obtained as sirup. Those reactions did not occur in the absence of conc. sulfuric acid or in the presence of water in pyridine. Those products obtained above gave a strong absorption at 1770 cm<sup>-1</sup> characteristic of O=C-O-stretching vibration.<sup>7)</sup> The ultraviolet absorption spectra of IIa were similar to those of IIb and did not exhibit less intense absorptions as compared with its aglycone, as shown in Table I, for if IIa was combined at a position of N<sup>1</sup> of ureido group with *p*-ethoxyphenylurea, it would show markedly decreased intensity of absorption as indicated by Schroeder.<sup>8)</sup> Also IIa was converted to 1-deoxy-1-[3-(*p*-ethoxyphenyl)ureido]-β-D-glucopyranuronate by treatment with potassium carbonate as mentioned below.

Accordingly, the product (IIa) should have a structure of 1-deoxy-1-[3-(*p*-ethoxyphenyl)ureido]-glucofuranuronolactone. The same conclusion might apply to other arylurea homologs in general.

TABLE I. Ultraviolet Spectrum Data in Water

Compound	$\lambda_{\max}$ m $\mu$ ( $\epsilon$ )	$\lambda_{\max}$ m $\mu$ ( $\epsilon$ )
<i>p</i> -Ethoxyphenylurea	235.5 (13,700)	281 (1,800)
IIa	238 (16,500)	281 (2,100)
IIb	238 (15,700)	281 (2,100)

## II. Conversion of Arylurea N-Glucuronolactone to the Corresponding N-Glucopyranuronate

Recently it was reported that arylamine N-glucofuranuronolactone was converted to arylamine N-glucofuranuronamide<sup>4,9)</sup> by treatment with ammonia-methanol, N-alkyl-1-alkylamino-1-deoxy-D-glucofuranuronamide<sup>10,11)</sup> was synthesized directly from alkylamine and I, and 1-(β-D-ribofuranosyl)urea was rapidly transformed to ribopyranosylurea in acidic or alkaline media.<sup>12)</sup>

When the compounds (IIa, IVa, and VIIIa) in aqueous solution containing methanol were treated with potassium carbonate under cooling for several days, decationized with IR-120 (H<sup>+</sup>) resin and neutralized with ammonium hydroxide, crystals of m.p. (decomp.) 135° IIb, 152~153° IVb and 165~166° VIIIb were obtained in yield of 33%, 10%, and 7% respectively.

Those products were identified by mixed fusion and infrared with authentic samples which were isolated from the urine of rabbits feeding *p*-ethoxyphenylurea, *o*-tolylurea and phenylurea.<sup>1,2)</sup>

As the anomeric center of IIb was exactly assigned as β-configuration,<sup>\*1</sup> those of IVb and VIIIb could most probably be assigned to be the same. The Mechanism of the whole reaction might be rationalized as shown in Chart 1.

7) Y. Nitta, J. Ide, A. Momose, M. Kuwada: *Yakugaku Zasshi*, **82**, 790 (1962).

8) W. A. Schroeder: *Anal. Chem.*, **23**, 1740 (1951).

9) Y. Nakajima: *Yakugaku Zasshi*, **81**, 1094 (1961).

10) J. Ide: *Ibid.*, **85**, 213 (1965).

11) Y. Nitta, J. Ide, H. Takahashi: *Ibid.*, **82**, 1563 (1962).

12) T. Ukita, A. Hamada, M. Yoshida: *This Bulletin*, **12**, 454 (1965).

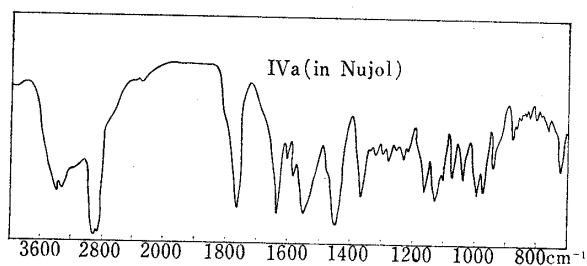


Fig. 1.

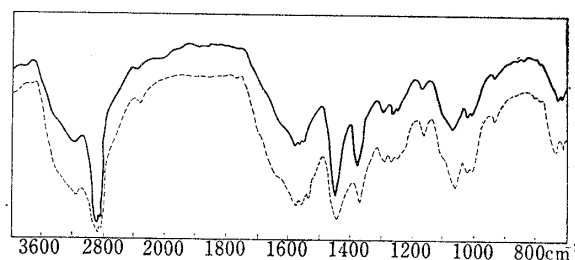


Fig. 2.

————— N-Glucuronide isolated from the rabbit urine dosed with *o*-tolylurea (in Nujol)  
 ..... Ib

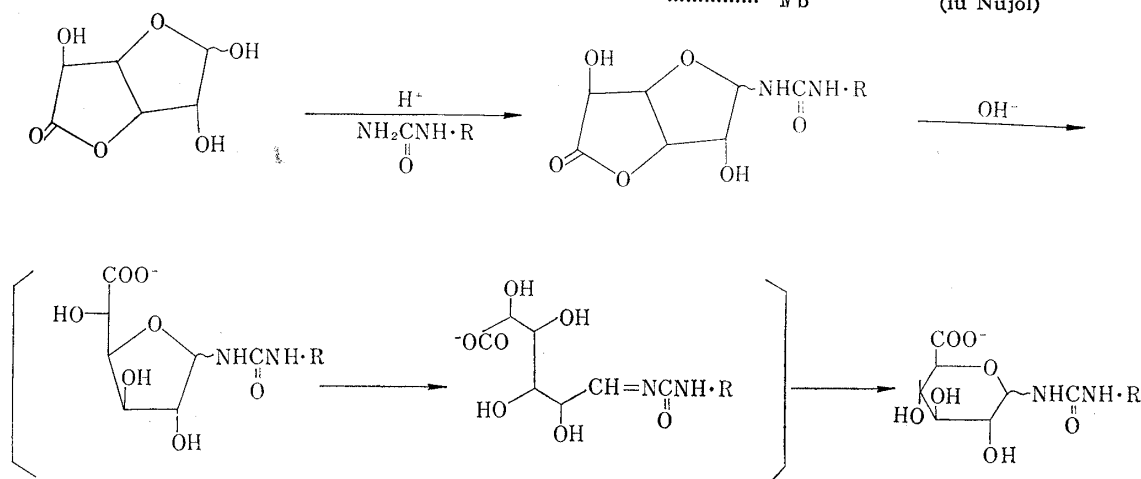


Chart 1.

The ring transformation of arylurea N-glucuronolactone from furanose to pyranose form takes place initially cleavage of the lactone ring, then that of the lactol ring and finally closure into the pyranosidic ether linkage.

#### Experimental\*4

**1-Deoxy-1-[3-(*p*-ethoxyphenyl)ureido]-D-glucofuranuronolactone (IIa)**—*p*-Ethoxyphenylurea (20 g.) and I (20 g.) were suspended in 100 ml. of pyridine, and 0.7 ml. of conc. H<sub>2</sub>SO<sub>4</sub> was added dropwise into the solution with stirring. The mixture was vigorously stirred at 50~60° for 2 hr. and allowed to stand for two days at room temperature. The crystals that separated out were recrystallized from 50% EtOH to 23 g. (60%) of colorless needles, m.p. 194°(decomp.),  $[\alpha]_D^{25}$  0.0°(c=1.00, pyridine), (Anal. Calcd. for C<sub>15</sub>H<sub>18</sub>O<sub>7</sub>N<sub>2</sub>: C, 53.25; H, 5.33; N, 8.29. Found: C, 52.97; H, 5.38; N, 8.68).

**1-Deoxy-1-[3-(*p*-tolyl)ureido]-D-glucofuranuronolactone (III)**—To a mixture of *p*-tolylurea (15 g.) and I (10 g.) in 100 ml. of pyridine, 1 ml. of conc. H<sub>2</sub>SO<sub>4</sub> was gradually added with stirring and the whole was stirred at 50° for 2.5 hr. The reaction mixture was kept at room temperature overnight, and was poured into 300 ml. of H<sub>2</sub>O. The precipitates were filtered and washed with H<sub>2</sub>O. Yield, 17 g. (100%). Several recrystallizations from 30% EtOH gave needles, m.p. 187°(decomp.),  $[\alpha]_D^{25}$  +3.0°(c=1.00, pyridine). (Anal. Calcd. for C<sub>14</sub>H<sub>16</sub>O<sub>6</sub>N<sub>2</sub>: C, 54.54; H, 5.23; N, 9.09. Found: C, 54.75; H, 5.36; N, 8.64).

**1-Deoxy-1-[3-(*o*-tolyl)ureido]-D-glucofuranuronolactone (IVa)**—Ten grams of I, 14 g. of *o*-tolylurea, 0.8 ml. of conc. H<sub>2</sub>SO<sub>4</sub> and 60 ml. of pyridine were treated in a similar manner. After standing for 3 days at room temperature, the solvent was removed by distillation *in vacuo*. The sirupy residue was solidified by addition of EtOH, and the product was recrystallized from EtOH to give 8 g. (42%) of fine needles, m.p. 191.5~192°(decomp.),  $[\alpha]_D^{25}$  0.0°(c=1.00, pyridine), (Anal. Calcd. for C<sub>14</sub>H<sub>16</sub>O<sub>6</sub>N<sub>2</sub>·C<sub>2</sub>H<sub>5</sub>OH: C, 54.23; H, 6.26; N, 7.91. Found: C, 54.17; H, 6.22; N, 7.85).

**1-Deoxy-1-[3-(*m*-tolyl)ureido]-D-glucofuranuronolactone (V)**—A solution of 20 g. of *m*-tolylurea and 20 g. of I in 60 ml. of pyridine was treated by a dropwise addition of 1.0 ml. of conc. H<sub>2</sub>SO<sub>4</sub>, stirred

\*4 All melting points were uncorrected.

and while the temperature was maintained at 50° for 2.5 hr. After standing overnight at room temperature, the precipitates were filtered off, washed with H<sub>2</sub>O, and air-dried; yield, 27 g. (79%). The crude product was recrystallized from 80% MeOH to colorless needles, m.p. 165~168° (decomp.),  $[\alpha]_D^{18} + 8.0^\circ$  (c=1.00, pyridine), (Anal. Calcd. for C<sub>14</sub>H<sub>16</sub>O<sub>6</sub>N<sub>2</sub>: C, 54.54; H, 5.23; N, 9.09. Found: C, 55.08; H, 5.59; N, 9.10).

**1-Deoxy-1-[3-(*p*-chlorophenyl)ureido]-D-glucofuranuronolactone (VI)**—This compound was obtained from *p*-chlorophenylurea (10 g.), I (8 g.), pyridine (50 ml.) and conc. H<sub>2</sub>SO<sub>4</sub> (0.5 ml.) in a similar method described above. The product, on recrystallization from EtOH gave 9.0 g. (62%) of colorless leaflets, m.p. 198° (decomp.),  $[\alpha]_D^{18} + 73^\circ$  (c=0.5, dimethylformamide), (Anal. Calcd. for C<sub>13</sub>H<sub>13</sub>O<sub>6</sub>N<sub>2</sub>Cl: C, 47.63; H, 3.80; N, 8.51. Found: C, 48.17; H, 3.80; N, 8.62).

**1-Deoxy-1-[3-(*p*-methoxyphenyl)ureido]-D-glucofuranuronolactone (VII)**—This compound was synthesized from *p*-methoxyphenylurea (10 g.), I (8 g.), and conc. H<sub>2</sub>SO<sub>4</sub> (0.5 ml.) in 50 ml. of pyridine according to the method described as above. Recrystallization from 50% MeOH gave 5 g. (36%) of crystals, m.p. 192° (decomp.),  $[\alpha]_D^{18} + 5.0^\circ$  (c=1.00, pyridine), (Anal. Calcd. for C<sub>14</sub>H<sub>16</sub>O<sub>7</sub>N<sub>2</sub>: C, 51.85; H, 4.97; N, 8.64. Found: C, 51.66; H, 4.94; N, 9.10).

**Conversion of IIa to ammonium 1-Deoxy-1-[3-(*p*-ethoxyphenyl)ureido]-β-D-glucofuranuronate (IIb)**—Five grams of IIa was suspended in 50 ml. of H<sub>2</sub>O and 10 ml. of MeOH (to avoid freezing), and 2 g. of K<sub>2</sub>CO<sub>3</sub> was added to the solution under cooling to -10°.

The mixture was mechanically stirred while it was maintained at 0° to -10° on an ice-bath until dissolved completely for a week. The reaction mixture was treated with Amberlite IR-120 (H form) to remove potassium ion, and neutralized with NH<sub>4</sub>OH, and then evaporated to dryness *in vacuo*. MeOH was added to the residue and the crystals that formed were recrystallized from MeOH (containing NH<sub>4</sub>OH, 1 ml. in 100 ml.) to 1.8 g. (33%) of colorless needles, m.p. 135° (decomp.),  $[\alpha]_D^{18} - 43^\circ$ , (c=1.82, H<sub>2</sub>O), (Anal. Calcd. for C<sub>15</sub>H<sub>23</sub>O<sub>8</sub>N<sub>3</sub>: C, 48.25; H, 6.00; N, 11.26. Found: C, 48.07; H, 6.27; N, 11.15). The obtained product showed no depression on admixture with the authentic sample reported by authors.<sup>\*1, 1, 2)</sup>

**Conversion of IVa to ammonium 1-Deoxy-1-[3-(*o*-tolyl)ureido]-β-D-glucofuranuronate (IVb)**—To a suspension of IVa (2 g.) in 30 ml. of H<sub>2</sub>O and 10 ml. of MeOH, K<sub>2</sub>CO<sub>3</sub> (1 g.) was added and stirred at 0° to -10° for 72 hr. until it become homogeneous. After the reaction mixture was treated with IR-120, the decationized solution was neutralized with NH<sub>4</sub>OH, concentrated to dryness *in vacuo* and a small amount of MeOH was added to the sirup. The precipitates were filtered and washed with MeOH. Recrystallization from CHCl<sub>3</sub>-MeOH containing a trace of NH<sub>4</sub>OH gave 0.25 g. (10%), of colorless needles, m.p. 152~153° (decomp.),  $[\alpha]_D^{18} - 53^\circ$  (c=1.25, H<sub>2</sub>O), (Anal. Calcd. for C<sub>14</sub>H<sub>21</sub>O<sub>7</sub>N<sub>3</sub>·1/2H<sub>2</sub>O: C, 47.72; H, 6.22; N, 11.93. Found: C, 47.53; H, 6.07; N, 11.84). This compound showed no depression of melting point with the substances, synthesized and isolated from the rabbit urine dosed with *o*-tolylurea.

**Ammonium 1-Deoxy-1-[3-(phenyl)ureido]-β-D-glucofuranuronate (VIIIb) from Glucuronolactone and Phenylurea**—To a solution of phenylurea (6 g.) and I (5 g.) in pyridine (30 ml.), conc. H<sub>2</sub>SO<sub>4</sub> (0.3 ml.) was added and stirred at 50~60° for 3 hr. After standing at room temperature for 2 days, the solvent was removed by distillation *in vacuo*. Being not obtained as crystals, without further purification this oil (VIIa) was used as the starting material for the next reaction. The sirup was dissolved to a solution containing K<sub>2</sub>CO<sub>3</sub> (5 g.) in MeOH (20 ml.) and H<sub>2</sub>O (80 ml.).

The mixture was stirred for 50 hr. under cooling at -5° to 5°.

After the precipitates that separated out were discarded, the supernatant was treated with IR-120 resin, made alkaline with NH<sub>4</sub>OH and evaporated *in vacuo* to a sirupy residue. The sirup was solidified by addition of MeOH on standing for several days.

After recrystallization from MeOH containing a small amount of NH<sub>4</sub>OH, VIIIb was obtained as 0.6 g. (7%) of colorless leaflets, m.p. 165~166° (decomp.),  $[\alpha]_D^{18} - 60^\circ$  (c=1.00, H<sub>2</sub>O), (Anal. Calcd. for C<sub>13</sub>H<sub>19</sub>O<sub>7</sub>N<sub>3</sub>: C, 47.41; H, 5.82; N, 12.76. Found: C, 47.50; H, 6.12; N, 12.87). This compound showed no depression on admixture with the products prepared from glucuronic acid and isolated from the rabbit urine.<sup>2)</sup>

The authors wish to thank Chugai Pharmaceutical Co., Ltd., for a 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

1-[3-(Aryl)ureido]-1-deoxy-D-glucofuranuronolactones were synthesized from arylureas and glucuronolactone. Some of them were converted to ammonium 1-[3-(aryl)ureido]-1-deoxy-β-D-glucofuranuronates by treatment with alkaline, which were identical with metabolites from the rabbit urine dosed with the corresponding arylureas.

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