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Studies on Glucuronosides of Heterocyclic Compounds. I. Studies on the Synthesis and Properties of 2-Pyridyl β-D-Glucopyranosiduronic Acid and Its O→N Rearrangement[1]

Torahiko Kishikawa, Yuji Oikawa and Shoji Takitani[2]

Faculty of Pharmaceutical Sciences, Science University of Tokyo[2]

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Condensation of methyl (tri-O-acetyl-α-D-glucopyranosyl bromide) uronate with silver 2-pyridyl oxide in toluene gave methyl (2-pyridyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)-uronate (III).

Removal of the protecting groups from III afforded the O-glucuronide of 2-hydroxy pyridine (V). The O→N rearrangement of the glucuronosyl residue in III was effected by refluxing the toluene solution of III in the presence of mercuric bromide. An attempt to hydrolyze V and VIII by the β-glucuronidase was made. It was found that whereas the O-glycoside (V) is hydrolysable by this enzyme, the N-glycoside (VIII) is completely inert to it. Acid- and alkali-catalyzed hydrolyses of V and VIII were also investigated.

Suggestion by Odell and Burt[3] that a high β-glucuronidase activity in human vaginal fluid may be indicative of cervical cancer has created a discussion and led to an extensive study of β-glucuronidases.

Up to date, the physiological significance of β-glucuronidases has remained uncertain, but it seems likely that this enzyme of a mammal plays an important role in endocrine control.[4] The structural requirements for the substrate of this enzyme have been established. The

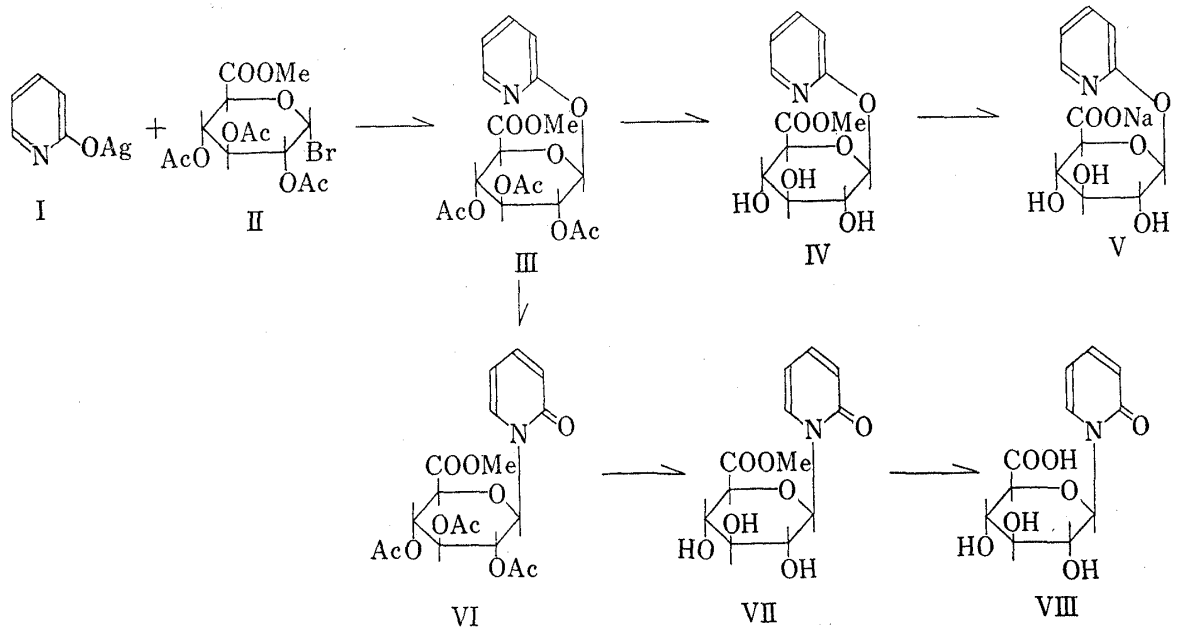

Chart 1

1) This paper was read at the 88th Annual Meeting of Pharmaceutical Society of Japan, April 5, 1968.
2) Location: *Ichigayafunagawara-machi, Shinjuku-ku, Tokyo.*
3) L.D. Odell and J.C. Burt, *J. Am. Med. Assoc.*, **142**, 226 (1950).
4) G.J. Dutton, "Glucuronic acid, Free and Combined," Academic press, New York and London, 1966, p. 301.

enzyme acts on both ether– and ester–linked β-D-glucopyranosiduronic acids, while their α-anomers are not attacked. On the other hand, 2-naphthyl β-D-glucofuranosiduronic acid recently synthesized was shown to be a substrate for the β-glucuronidase,[5] though it had a low affinity for the enzyme and was slowly hydrolyzed. The N-glucuronides of strongly basic compounds are not acted on, however, there are some evidences[6] for slow hydrolysis of sulfonamide-N^1-glucuronides and meprobamate-N-glucuronide by the β-glucuronidase. In order to obtain further evidences on the substrate specificity of β-glucuronidase and the glucuronic acid conjugation of heterocycles, the authors have been engaged in the study of the glucuronides of heterocyclic compounds. So far as the literatures are concerned, the authors are not aware of the O-glucuronides of these compounds, while the glucosides of them have been prepared by Wagner, *et al.*[7]

This paper deals with the synthesis of O-glucuronide of 2-hydroxypyridine, its O→N rearrangement and the difference in properties of the O- and N-glucuronides toward chemical or enzymatical hydrolyses.

On treatment of silver salt of 2-pyridinol (I) with methyl (2,3,4-tri-O-acetyl-α-D-glucopyranosyl bromide) uronate (II) in toluene (Chart 1), methyl (2-pyridyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid) uronate (III) was obtained in a reasonable yield (70%).

The compound (III) was de–acetylated with catalytic amount of sodium methoxide in absolute methanol to afford IV. De–esterification of IV by treatment with an equimolar amount of sodium methoxide in aqueous methanol gave 2-pyridyl β-D-glucopyranosiduronic acid (V), which was isolated as sodium salt. On the other hand, the rearrangement of the glucuronosyl residue in compound III was effected by refluxing the toluene solution of III in the presence of mercuric bromide and resulted in formation of the corresponding N-glucuronide (VI).

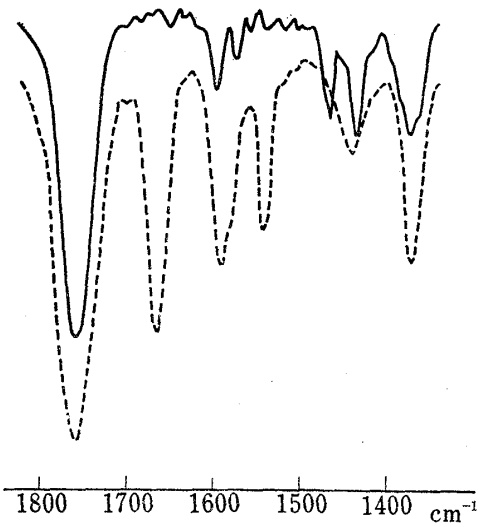

Fig. 1. IR Spectra of III and VI
——— III – – – – – VI

The analogous rearrangement of the sugar portion in O-glycosyl heterocyclic compounds have been observed by several workers.[8]

Removal of protecting groups from compound VI was made by the same procedures as described above and crystalline 1-deoxy-1-(2-oxo-1,2-dihydro-1-pyridyl)-β-D-glucopyranuronic acid (VIII) was obtained.

The infrared spectrum of VI had absorption at 1665 cm^{-1} (amide carbonyl), while that of III lacked the amide carbonyl absorption at this region (Fig. 1).

The ultraviolet (UV) absorption spectrum of V and VIII closely resembled 2-methoxypyridine and N-methyl-2 (IH)-pyridone, respectively. The NMR spectra[9] of III and VI

5) K. Kato, K. Yoshida and H. Tsukamoto, *Chem. Pharm. Bull.* (Tokyo), **12**, 664 (1964).
6) T. Uno and M. Kono, *Yakugaku Zasshi*, **82**, 1660 (1962); M. Takeda, H. Yoshimura and H. Tsukamoto, *ibid.*, **86**, 1191 (1966); J. Bridges, M. Kibby and R. Williams, *Biochem. J.*, **96**, 829 (1965).
7) G. Wagner and H. Pischel, *Arch. Pharm.*, **295**, 373 (1962).
8) G. Wagner and H. Pischel, *Arch. Pharm.*, **296**, 699 (1963); **298**, 62, 297 (1965); **300**, 737, 783, 856 (1967); G. Schmidt and J. Farkas, *Coll. Czechoslov. Chem. Commun.*, **31**, 4442 (1966); G. Schmidt and J. Farkas, *Tetrahedron Letters*, **1967**, 4252; T. Ukita, H. Hayatsu and Y. Tomita, *Chem. Pharm. Bull.* (Tokyo), **11**, 1068 (1963); T. Ulbricht, *Proc. Chem. Soc.*, **1962**, 298; T. Ulbricht and G. Rogers, *J. Chem. Soc.*, **1965**, 6125, 6130; H. Garg, T. Ulbricht, *ibid.*, **1967**, 51; D. Thacker and T. Ulbricht, *Chem. Commun.*, **1967**, 122.
9) The NMR spectra were taken on JNM4H–100 analytical spectrometer, manufactured by the Japan Electron Optics Co. TMS was used as an internal reference in $CDCl_3$.

showed the anomeric proton at δ=6.33, 6.55 (ppm), and $J_{H'_1,H'_2}$ of 6.8 and 8.9 HZ indicated the di-axial orientation[10] of the C'_1 and C'_2 protons, which established the β-configuration at the glycosidic linkage of these compounds. The acid lability of V and stability of VIII were in good agreement with proposed structures.

Generally, the aromatic or aliphatic O-glucopyranosiduronic acids are comparatively stable to both acid and alkali. However, compounds in which the aglycon is conjugated to D-glucuronic acid through an enolized keto group are susceptible to acid and alkali hydrolysis and undergo rapid decomposition even at incubation temperature.[11] Though 2-pyridinol-O-glucuronide synthesized above is a compound of this type, it was relatively stable at pH 4.5 (acetic acid–acetate buffer solution), pH 8.0 (phosphate buffer solution) at 37°. But in the 0.1 N HCl and 1 N NaOH, as can be seen from Fig. 2 and Table I, hydrolysis of V smoothly took place and 2-pyridinol and D-glucuronic acid were released, whereas the D-glucuronosyl-N bond in the O→N rearranged product (VIII), was resistant to hydrolytic cleavage and more vigorous conditions were required for the hydrolysis of the glucuronide. Fig. 2 indicates that the hydrolysis of 2-pyridinol-O-glucuronide is a pseudo first order reaction, the detailed kinetic data for the acid- and alkali-catalyzed hydrolysis of the D-glucopyranosiduronic acids having been presented.[12]

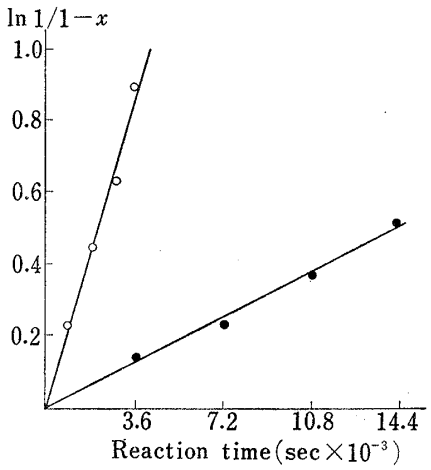

Fig. 2. Plots of ln 1/1-x against Reaction Time

—○— with 0.1N HCl at 37°
—●— with 1N NaOH at 60°

The compounds V and VIII were also studied as substrates for the bacterial or mammalial β-glucuronidase. The compound (V) was acted upon by the enzyme and it smoothly liberated 2-hydroxypyridine, while VIII was not hydrolyzed in spite of the longer period of treatment with the enzyme.

This is in contrast to the fact that the sulfonamide (-NH-SO_2-) and carbamate (-NH-COO-)-N-glucuronides[6] are slowly hydrolyzed by the β-glucuronidase preparations.

Experimental[13]

The Enzyme——Bacterial β-glucuronidase and bovine liver β-glucuronidase were purchased from Sigma Chemical Co. and Tokyo Kasei Co., respectively. The assay of the enzyme activity was made according to the method described by Fishman.[14]

Acid and Alkali Hydrolysis of V, and a Procedure for the Separatory Estimation of V and Liberated 2-Pyridinol——The ultraviolet absorption spectra of V and 2-hydroxypyridine in weakly alkaline solution (V: $\lambda_{max}^{pH8.0}$ 268 mμ; 2-pyridinol: $\lambda_{max}^{pH8.0}$ 295 mμ) were sufficiently different to allow the estimation of the concentration of each compound. One percent solutions (w/v) of V in 0.1N HCl and 1N NaOH were heated at 37° and 60°. At an interval, an aliquot (0.02 ml) from the reaction mixture was added to 4.98 ml of phosphate buffer solution (0.2M KH_2PO_4–0.2N NaOH, pH 8.0) and the absorbances of the solution were measured at 268 and 295 mμ. The concentration of V and 2-pyridinol was calculated according to the following equations.

$$C_V = \frac{E^{268}/\varepsilon_{py}^{268} - E^{295}/\varepsilon_{py}^{295}}{\varepsilon_v^{268}/\varepsilon_{py}^{268} - \varepsilon_v^{295}/\varepsilon_{py}^{295}} \qquad C_{PY} = \frac{E^{268}/\varepsilon_v^{268} - E^{295}/\varepsilon_v^{295}}{\varepsilon_{py}^{268}/\varepsilon_v^{268} - \varepsilon_{py}^{295}/\varepsilon_v^{295}}$$

C_V and C_{PY}: Concentrations of V and 2-pyridinol (mole per liter),
E^{268} and E^{295}: Absorbance at 268 and 295 mμ of the diluted reaction mixture,
ε_v^{268} and ε_{py}^{268}: Molar extinction coefficients at 268 mμ of V and 2-pyridinol,
ε_v^{295} and ε_{py}^{295}: Molar extinction coefficients at 295 mμ of V and 2-hydroxypyridine.

10) R. Lemieux, R. Kullnig, H. Bernstein and G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).
11) J. Mead, J. Smith and R. Williams, *Biochem. J.*, **68**, 61 (1958); M. Wakabayashi, H. Wotiz and W. Fishman, *Biochim. Biophys. Acta*, **48**, 198 (1961).
12) E. Tomita, *Yakugaku Zasshi*, **87**, 479 (1967).
13) All melting points were not corrected.
14) W.H. Fishman, *J. Biol. Chem.*, **173**, 449 (1948).

Table I. Hydrolysis of V with Acid,[a] Alkali[b] and Enzyme[c]

a)	With 0.1N HCl at 37°		Kinetic data		
	Time (min)	15	30	45	60
	Hydrolysis rate (%)	20.0	36.2	46.7	59.2
	$k \times 10^4$ (sec^{-1})	2.46	2.50	2.33	2.48
		Mean $k = 2.44 \times 10^{-4} \cdot sec^{-1}$			
b)	With 1N NaOH at 60°		Kinetic data		
	Time (min)	60	120	180	240
	Hydrolysis rate (%)	11.7	20.0	30.7	39.4
	$k \times 10^5$ (sec^{-1})	3.44	3.10	3.39	3.48
		Mean $k = 3.35 \times 10^{-5} \cdot sec^{-1}$			
c)	With β-Glucuronidase at 37°		Kinetic data		
	Time (min)	15	30	45	60
	Hydrolysis rate (%)	22.4	39.4	54.8	64.4
	$k \times 10^4$ (sec^{-1})	2.82	2.78	2.72	2.87
		Mean $k = 2.80 \times 10^{-4} \cdot sec^{-1}$			

c) The incubation mixture consisted of 0.9 ml of acetate buffer solution (pH 4.5) containing 2.9 mg of V and 0.1 ml of an aqueous solution of the enzyme (2000 Fishman unit/ml).

Methyl(2-pyridyl 2,3,4-tri-O-Acetyl-β-D-glucopyranosid)uronate (III)——One gram of well powdered silver salt of 2-pyridinol[15] was suspended in 25 ml of anhydrous toluene. To this suspension, 2 g of methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)uronate[16] was added and refluxed with stirring for 45 minutes and then stored in a refrigerator overnight. The crude crystals thus precipitated were recrystallized from toluene–petr. ether in colorless needles. Yield 1.4 g (70%). mp 78—80°. $[\alpha]_D^{20}$ +6° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{21}O_{10}N$: C, 52.55; H, 5.11; N, 3.41. Found: C, 52.81; H, 5.11; N, 3.39.

Sodium(2-pyridyl β-D-Glucopyranosid)uronate (V)——III (200 mg) was dissolved in 3 ml of abs. methanol. To this solution, 1 ml of 0.2M sodium methoxide was added and the mixture was boiled for 1 minute. After cooling, the mixture was neutralized with Amberlite IR-120 (H^+). After the solvent was removed *in vacuo*, the residue (IV, mp 155—157°) was dissolved in the mixture of 3 ml of 0.2M sodium methoxide and 0.5 ml of water. The solution was boiled for 1 minute and then cooled quickly. The cold solution was adjusted to pH 7.0 with Amberlite IR-120 (H^+) and then carbon treated. Complete removal of the solvent *in vacuo* afforded a residue, which was dissolved in 2 ml of methanol. To this solution, 10 ml of chloroform was added to give V, which was washed with ether, and dried at 100°. Yield 100 mg (70%). mp 165—170°. $[\alpha]_D^{20}$ −67° (c=0.4, H_2O). UV $\lambda_{max}^{H_2O}$ mμ (ε): 268 (3430). *Anal.* Calcd. for $C_{11}H_{12}O_7NNa$: C, 45.06; H, 4.09; N, 4.78. Found: C, 45.41; H, 3.90; N, 5.12.

Methyl 1-Deoxy-1-(2-oxo-1,2-dihydro-1-pyridyl)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (VI)——To a solution of 1 g of III in 50 ml of anhydrous toluene, 3 g of mercuric bromide was added and the solution was refluxed for 5 hours. After cooling, the insoluble material was filtered off and the filtrate was washed with 50 ml portions of 30% potassium iodide solution and water, and dried over calcium chloride. The solution was concentrated to *ca.* 5 ml under reduced pressure. The concentrate was diluted with 30 ml of isopropyl ether to afford a precipitate, which on recrystallization from 20% aqueous methanol gave colorless needles. Yield 0.4 g (40%). mp 183—184°. $[\alpha]_D^{20}$ +87° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{21}O_{10}N$: C, 52.55; H, 5.11; N, 3.41. Found: C, 52.43; H, 4.90; N, 3.50.

1-Deoxy-1-(2-oxo-1,2-dihydro-1-pyridyl)-β-D-glucopyranuronic Acid (VIII)——VI (200 mg) was dissolved in 6 ml of abs. methanol. To this solution, 1 ml of 0.2M sodium methoxide in methanol was added and the mixture was boiled for 1 minute. After cooling, precipitate (VII, mp 260°) was collected and washed with methanol. A solution of (VII) in 5 ml of water was treated with 3 ml of 0.2M sodium methoxide in methanol and refluxed for 3 minutes. After cooling, the mixture was adjusted to pH 2.0—2.5 with Amberlite IR-120 (H^+) and then the solvent was completely removed *in vacuo* to dryness. The residue was dissolved in 3 ml of methanol and *ca.* 15 ml of ether was added to afford VIII. Yield 80 mg (60%). mp 245—247°. $[\alpha]_D^{17}$ +33° (c=0.3, H_2O). UV $\lambda_{max}^{H_2O}$ mμ (ε): 225 (6670), 302 (7160). *Anal.* Calcd. for $C_{11}H_{13}O_7N$: C, 48.70; H, 4.80; N, 5.16. Found: C, 48.51; H, 4.43; N, 5.10.

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15) H. Pechmann and O. Baltzer, *Ber. Dtsch. Chem. Ges.*, **24**, 3114 (1891); K. Winterfeld and H. Michael, *Chem. Ber.*, **93**, 61 (1960).
16) G.N. Bollenback, John W. Long, D.G. Benjamin and J.A. Lindquist, *J. Am. Chem. Soc.*, **77**, 3310 (1955).