Chem. Pharm. Bull. 12 (6) 664 ~ 669

UDC 612.015.34 : 612.396.21

93. Keitaro Kato, Kazuo Yoshida, and Hisao Tsukamoto : Metabolism of Drugs. XLV.*1 Synthesis of 2-Naphthyl β-D-Glucofuranosiduronic Acid.

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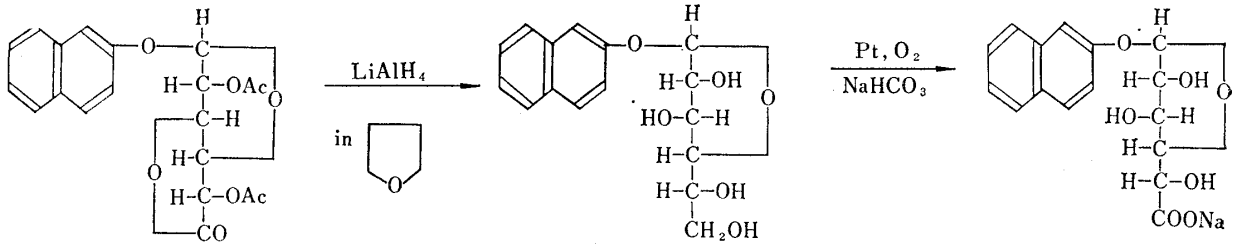
In connection with the study of the specificity of β-glucuronidase for a furanose structure of glucuronide, it is necessary to synthesize chromogenic β-D-glucofuranosiduronic acid. For this purpose, the synthesis of 2-naphthyl β-D-glucofuranosiduronic acid (V) has been undertaken. Hitherto 2-naphthyl β-D-glucofuranosiduronolactone (VII) was synthesized by Tsou and Seligman,[1] however the same authors also observed that attempts to convert VII to V through opening the lactone ring with alkali resulted in liberation of 2-naphthol even under mild conditions, catalytic oxidation of 2-naphthyl β-D-glucofuranoside (II) by gaseous oxygen in the presence of platinum catalyst was employed in this experiment. II was readily synthesized from 2-naphthyl diacetyl-β-D-glucofuranosiduronolactone (I) with lithium aluminum hydride in 52% yield. Deacetylation and reduction of a lactone were accomplished together by the application of this reducing agent.[2] The product was found not to be identical with 2-naphthyl β-D-glucopyranoside (IV) by mixed fusion method and comparison of infrared spectra. The former was more unstable to diluted acid and alkali than the latter, and partially decomposed even in hot water with liberation of 2-naphthol.

Methyl and ethyl β-D-glucofuranosides were prepared by Haworth, *et al.*[3] and by Phillips,[4] but these methods were available only for the synthesis of simple alcohol glucofuranosides. The reduction methods of furanosiduronolactone with lithium aluminum hydride might be extended to the general synthesis of β-D-glucofuranosides. Further investigations will be published later.



I II III

Chart 1.

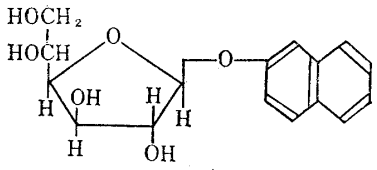
Catalytic oxidation of II by gaseous oxygen was performed according to the procedure similar to that of Marsh and Levvy.[5] A solution of II in water at pH about 8 with catalyst was oxidized at 70° for 2 hours. The glucofuranosiduronic acid was isolated as the lead salt and converted to sodium salt with hydrogen sulfide in alkaline milieu. When the lead salt was regenerated with hydrogen sulfide without sodium

*1 Part XLIV. K. Yoshida, K. Kato, H. Tsukamoto : This Bulletin, **12**, 656 (1964).
*2 Katakasu, Fukuoka (加藤敬太郎, 吉田和夫, 塚元久雄).
1) K. C. Tsou, A. M. Seligman : J. Am. Chem. Soc., **74**, 5605 (1952).
2) R. K. Ness, H. G. Fletcher. C. S. Hudson : *Ibid.*, **73**, 4759 (1951).
3) W. N. Haworth : J. Chem. Soc., **1952**, 1578.
4) D. D. Phillips : J. Am. Chem. Soc., **76**, 3598 (1954).
5) C. A. Marsh, C. A. Levvy : Biochem. J., **68**, 617 (1958).

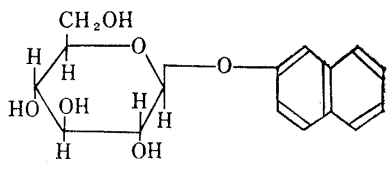
bicarbonate, it was converted to Ⅶ by the effect of acidic substances probably produced during the course of oxidation. The infrared spectrum of Ⅲ indicated a broad band due to carboxyl ion at 6.24 μ.

An attempt to obtain the free acid (Ⅴ) from the pure sodium salt was successful after convertion of Ⅲ to the lead salt followed by regeneration with hydrogen sulfide. The monohydrate of the free acid melted at 132~133° and whose infrared spectrum indicated a carboxyl band at 5.84 μ and a broad diffuse band in the 3.3~4.0 region. Ⅴ was converted to 2-naphthyl β-D-glucofuranosiduronolactone (Ⅶ) with the release of water after fusing at the melting point. This conversion was not observed with 2-naphthyl β-D-glucopyranosiduronic acid (Ⅵ). Moreover, it is interesting to note that a methyl ester of Ⅴ was easily converted to the lactone. Although the consumption of diazomethane was undoubtedly observed on the methylation of Ⅴ only the lactone was obtained after removal of the solvent. The infrared spectrum of the crystalline residue was identical with that of the lactone (Ⅶ). Acetylation of this product without further purification with pyridine-acetic anhydride gave quantitatively 2-naphthyl diacetyl-β-D-glucofuranosiduronolactone (Ⅰ). This conversion was not observed in the pyranoid type.

On the basis of these findings, it seems most likely that β-D-glucofuranosiduronic acids, if they were excreted in the urine of animals, would be converted to diacetyl-β-D-glucofuranosiduronolactones by the method of Kamil, Smith and Williams[6] which is an usual method to get urinary glucuronides in a crystalline form. Above findings



Ⅱ



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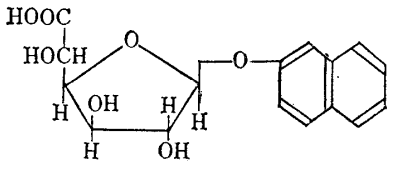

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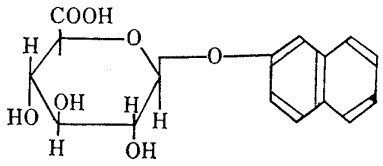

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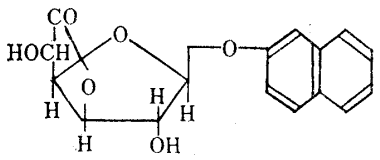


Ⅶ

Chart 2.

6) I. A. Kamil, J. N. Smith, R. T. Williams : Biochem. J., **50**, 235 (1951).

are advantageous to our previous work on the structure of *p*-aminobenzoyl β-D-glucuronide isolated from the dog urine,[7] because methyl (*p*-acetamidobenzoyl-2,3,4-tri-O-acetyl-β-D-glucopyranosid) uronate was obtained from *p*-aminobenzoyl β-D-glucuronide by the method of Kamil, Smith, and Williams. This result could be attributed to the fact that *p*-aminobenzoyl β-D-glucuronide should exist in a pyranoid type, but not in a furanoid.

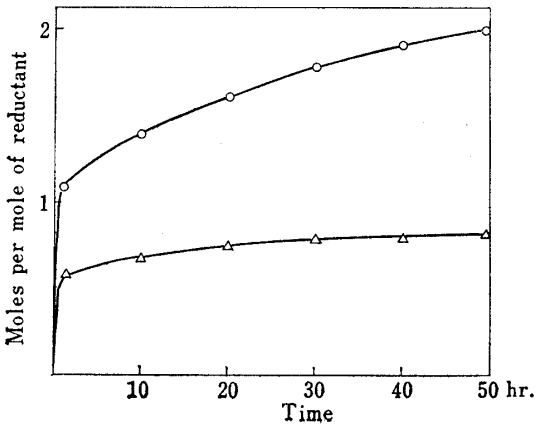


Fig. 1. Oxidation of 2-Naphthyl β-D-Glucofuranoside with Periodate in Aqueous Solution

IO_4^- consumption ○
HCHO liberation △

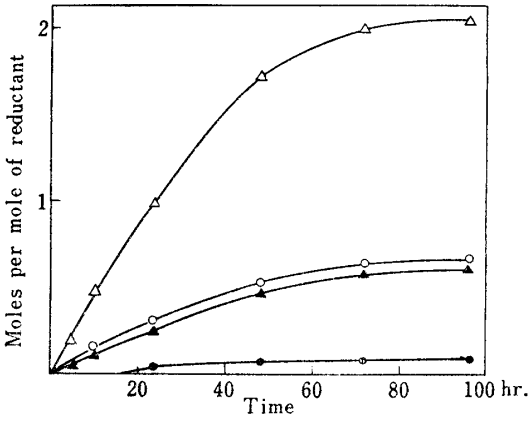


Fig. 2 Oxidation of Sodium 2-Naphthyl β-D-Glucofuranosiduronate, and Sodium 2-Naphthyl β-D-Glucopyranosiduronate with Periodate in 40% Dioxan Solution

Sodium 2-naphthyl β-D-glucofuranosiduronate
IO_4^- consumption ○
acid liberation ●
Sodium 2-naphthyl β-D-glucopyranosiduronate
IO_4^- consumption △
HCOOH liberation ▲

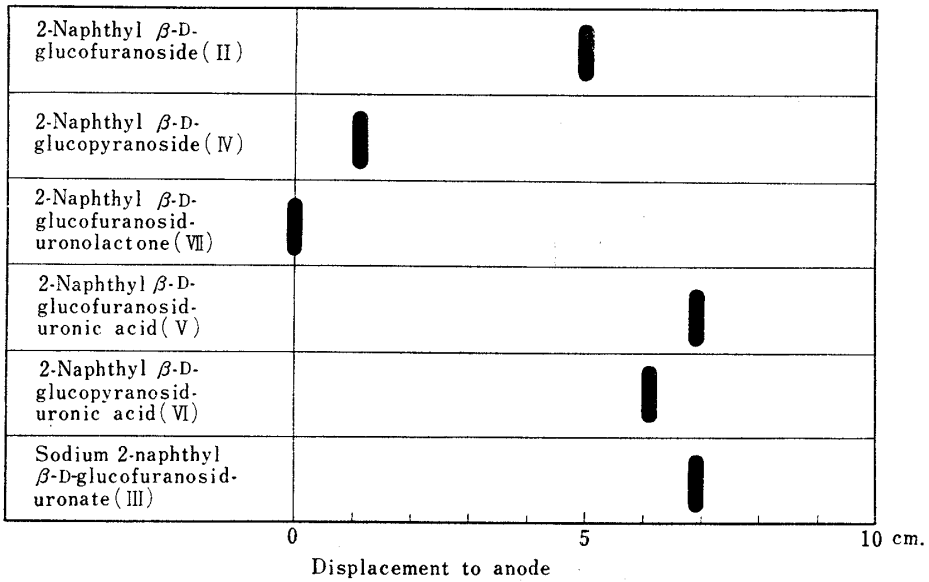
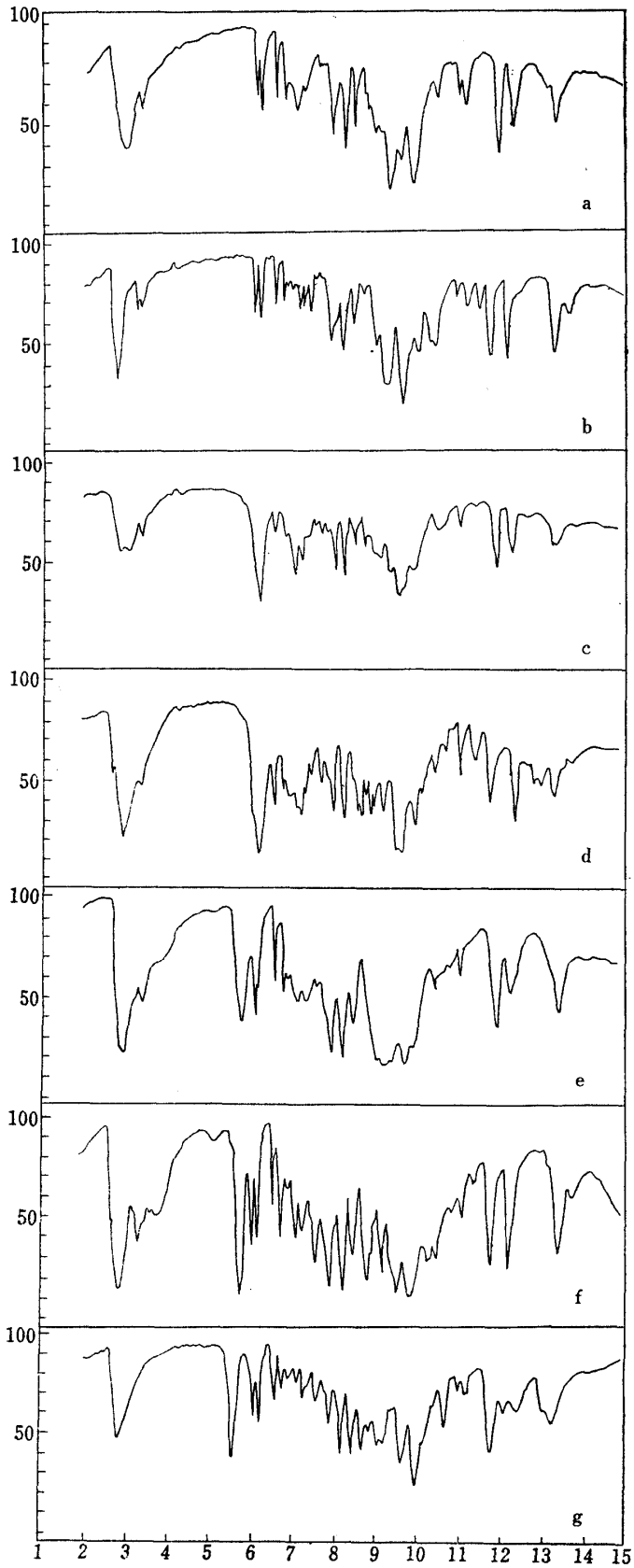


Fig. 3. Paper Electrophoresis of the Pyranoid and Furanoid Types of 2-Naphthyl β-D-Glucosides, the Both Types of 2-Naphthyl β-D-Glucuronides and 2-Naphthyl β-D-Glucofuranosiduronolactone

The conditions were described in the section of paper electrophoretic method.

7) K. Kato, K. Yoshida, K. Tatsumi, H. Tsukamoto : This Bulletin, **10**, 1238 (1962).



a: 2-Naphthyl β-D-glucopyranoside
b: 2-Naphthyl β-D-glucofuranoside
c: Sodium 2-naphthyl β-D-glucopyranosiduronate
d: Sodium 2-naphthyl β-D-glucofuranosiduronate
e: 2-Naphthyl β-D-glucopyranosiduronic acid
f: 2-Naphthyl β-D-glucofuranosiduronic acid
g: 2-Naphthyl β-D-glucofuranosiduronolactone

Fig. 4. Infrared Absorption Spectra (KBr disk)

For the purpose to confirm the ring structure of II and III, the periodate oxidation of these compounds was undertaken. The oxidation of II consumed 2.0 moles of periodate and produced 0.8 moles of formaldehyde in aqueous solution. Formaldehyde was determined and identified as methylenebismethone. As shown in Fig. 1, II rapidly consumed 1 mole of periodate, followed by a slower, secondstage oxidation. This behavior shows the primary oxidation of the glycol linkage outside of the ring and the secondary slow oxidation of the *trans*-glycol within the ring. The oxidation of III, as compared with that of sodium 2-naphthyl β-D-glucopyranosiduronate (VIII), is shown in Fig. 2. Periodate oxidation was carried out for these two compounds under the same condition in 40% dioxane solutions. VIII consumed 2.07 moles of periodate and produced 0.6 moles of titratable acid within 72 hours. On the other hand, III consumed 0.63 moles of periodate and produced a little amount of acid in the same period of time. The pyranoid type has been recognized to react rapidly with 2 moles of oxidant and the furanoid type will consume 1 mole of oxidant over a long period. These reaction types are consistent with those of 2-naphthyl β-D-glucopyranosiduronamide and 2-naphthyl β-D-glucofuranosiduronamide described in an earlier paper of

this laboratory.[8] Therefore, above experiments indicated the presence of a furanose ring in II and III.

In Fig. 3, the paper electrophoretic patterns of the pyranoid and furanoid types of 2-naphthyl β-D-glucosides, the both types of 2-naphthyl β-D-glucuronides, and 2-naphthyl β-D-glucofuranosiduronolactone were shown. Electrophoresis was carried out with 1% borax solution. As shown in Fig. 3, II showed the higher mobility toward anode than IV. This behavior indicated that the negatively charged borate complex would easily be formed between the hydroxyl groups on C_5 and C_6 outside of the ring. The slightly higher mobility of V than of VI might be contributed by the effect of α-hydroxyl acid in V.

In the following report, 2-naphthyl β-D-glucofuranosiduronic acid is used as a substrate for β-glucuronidase in order to determine the specificity of the enzyme for the furanose ring structure. For this purpose, the stability of V in aqueous solution was examined. The specific rotation value of V did not change in water for 48 hours (the specific rotation values of V, VI, and VII in water were $[\alpha]_D^{25}$ −144°, −134°, and −87° respectively) and neither VI nor VII were detected in this solution by paper electrophoresis. After 72 hours, the measured specific rotation was −120° and VII but not VI was detected in the solution by paper electrophoresis. This rotation value did not change until 120 hours. In the following paper, the enzyme assay was usually carried out for 1 hour and even in the longest case the incubation time was 5 hours, therefor, the glucofuranosiduronic acid can be used as a suitable test compound to study the specificity of β-glucuronidase for the furanose ring structure.

Experimental

2-Naphthyl β-D-Glucofuranosid (II)——The suspension of 15 g. of 2-naphthyl diacetyl-β-D-glucofuranosiduronolactone in 50 ml. of tetrahydrofuran was added over a period of 30 min. to 6.64 g. of $LiAlH_4$ suspended in 200 ml. of tetrahydrofuran with cooling, stirring and protecting from atmospheric moisture. The flask was then placed in a water bath at 50°. After stirring for 1.5 hr., the reaction mixture was cooled in an ice-bath, and excess $LiAlH_4$ decomposed by the gradual addition of H_2O. The precipitate was removed by centrifugation at 0° and washed twice with 50% EtOH. The combined supernatant and washings were treated with IR-120 and adjusted to pH about 5.5. The deionized solution was concentrated to a small volume under reduced pressure and yielded 6.8 g. of white crystals, m.p. 110~127°. The crystalline product was extracted twice with hot benzene to remove 2-naphthol. Recrystallization from H_2O yielded 6.2 g. (52%) of white crystals, m.p. 140~141°; $[\alpha]_D^{20}$ −184° (c=0.25, H_2O). *Anal.* Calcd. for $C_{16}H_{18}O_6$: C, 62.75; H, 5.88. Found: C, 62.61; H, 6.17.

This compound when mixed with IV showed a marked depression of melting point and its IR spectrum was not identical with that of IV. The compound gave 2-naphthol and D-glucose after hydrolysis with 5% HCl (1 hr. at 80°), and also partially hydrolyzed by warming at 50° for 1 hr. in H_2O.

Sodium 2-Naphthyl β-D-Glucofuranosiduronate (III)——The reaction vessel was a slender glass cylinder with a diameter of 7 cm. and a height of 34 cm. Oxygen was introduced through two gas bubblers fitted with fritted-glass balls at the bottom of the cylinder. A solution of 2-naphthyl β-D-glucofuranoside (2 g.) and $NaHCO_3$ (0.18 g.) in H_2O (200 ml.) with 0.5 g. of Pt black was oxidized at 70° with mechanical vigorous stirring. The reaction mixture was maintained at pH 8 with frequent addition of satd. $NaHCO_3$ solution (total 0.36 g. of $NaHCO_3$). Two or three drops of ethyl silicate was added at times to prevent the reaction mixture from bubbling over. The reaction was complete in 2 hr. The amber colored solution was filtered, cooled, and satd. $Pb(OAc)_2 \cdot Pb(OH)_2$ solution was added to precipitate the Pb salt of the glucofuranosiduronic acid. The Pb salt was collected by centrifugation, washed with H_2O, and dried over $CaCl_2$. The Pb salt was made into a fine suspension in H_2O with two thirds amount of equimolar $NaHCO_3$, and Pb was removed by saturation with H_2S. After removal of PbS, the filtrate was evaporated to dryness under reduced pressure. When the filtrate was concentrated to a small volume, a flocculated precipitate appeared in the solution. This was filtered off and discarded. The residue was disolved in a small volume of 50% EtOH and crystallized after standing overnight in a refrigerator. The crystalline product was collected and recrystallized from 50% EtOH, m.p. 216~216.5° (decomp.). Yield, 0.31 g. (14.4%). *Anal.* Calcd. for $C_{16}H_{15}O_7Na \cdot H_2O$: C, 53.33; H, 4.72. Found: C, 53.25; H, 5.03.

8) K. Kato, K. Yoshida, H. Tsukamoto: This Bulletin, **10**, 1242 (1962).

This compound showed a marked depression of melting point when mixed with the Na salt of VI (m.p. 297~298° (decomp.)). The IR spectrum of III indicated a broad carbonyl band due to carboxyl ion at 6.24 μ, and was not identical with that of the Na salt of VI. The compound showed a positive naphthoresolcinol test and gave 2-naphthol, glucuronolactone and glucuronic acid after hydrolysis with 5% HCl (1 hr. at 80°).

2-Naphthyl β-D-Glucofuranosiduronic Acid (V)——To a solution of sodium 2-naphthyl β-D-glucofuranosiduronate (0.49 g.) in H_2O (20 ml.) was added the satd. $Pb(OAc)_2 \cdot Pb(OH)_2$ solution. The Pb salt was collected by centrifugation, washed with H_2O, and made into a fine suspension in H_2O. Pb was precipitated by saturation with H_2S. This mixture was warmed in a water bath and then the precipitate was removed by filtration. The precipitate was washed twice with 15 ml. of hot H_2O. After cooling, V was crystallized from the combined filtrate and washings. Yield, 0.25 g. (54.3%). m.p. 132~133° (decomp.); $[\alpha]_D^{25}$ −144° (c=0.25, H_2O), −124° (c=1.00, EtOH). *Anal.* Calcd for $C_{16}H_{16}O_7 \cdot H_2O$: C, 56.80; H, 5.36. Found: C, 56.80; H, 5.53.

The IR spectrum of this compound indicated a carboxyl band at 5.84 μ and a broad diffuse band in the 3.3~4.0 region. The compound was slightly soluble in H_2O and quite soluble in EtOH. After fusing at the melting point and recrystallization from H_2O-EtOH, the compound melted at 177~178°. Its IR spectrum was identical with that of 2-naphthyl β-D-glucofuranosiduronolactone. This changed compound showed no depression of melting point when mixed with the lactone.

Methylation and Acetylation of 2-Naphthyl β-D-Glucofuranosiduronic Acid——To a solution of 2-naphthyl β-D-glucofuranosiduronic acid (0.07 g.) in a small volume of MeOH, 15 ml. of Et_2O solution of CH_2N_2 was added. The reaction mixture was allowed to stand overnight in a refrigerator. After removal of the solvent under reduced pressure, the IR spectrum of the crystalline residue was identical with that of 2-naphthyl β-D-glucofuranosiduronolactone. This product was dissolved in 1 ml. of pyridine without further purification to which 0.7 ml. of Ac_2O was added. The reaction mixture, after standing overnight in a refrigerator, was poured into ice H_2O with stirring. The crystalline precipitate was collected and recrystallized from EtOH-$CHCl_3$ to fine white needles, m.p. 229~231°. Yield, 0.076 g. (95%). This compound showed no depression of melting point when mixed with I and its IR spectrum was identical with that of I.

Periodate Oxidation of 2-Naphthyl β-D-Glucofuranoside——To a solution of 0.400 g. of 2-naphthyl β-D-glucofuranoside in 60 ml. of H_2O, 0.698 g. of $NaIO_4$ was added. The reaction mixture was kept in darkness at room temperature. HCHO was determined and identified as methylenebismethone. Oxidant consumption was determined by the method of Fleury and Lange.[9]

Periodate Oxidation of Sodium 2-Naphthyl β-D-Glucofuranosiduronate and Sodium 2-Naphthyl β-D-Glucopyranosiduronate——To a solution of 0.136 g. of sodium 2-naphthyl β-D-glucofuranosiduronate in 75 ml. of 40% dioxane was added 0.170 g. of $NaIO_4$. To a solution of 0.200 g. of sodium 2-naphthyl β-D-glucopyranosiduronate in 110 ml. of 40% dioxane was added 0.280 g. of $NaIO_4$. Each reaction mixture was kept in darkness at 20°. HCOOH was determined by titration with 0.01*N* NaOH after destruction of the excess of periodate with ethyleneglycol. Oxidant consumption was determined by the method described above.

The authors are indebted to Miss Miyawaki and Miss Nawata for their assistance in the experimental work. Thanks are also due to Mr. Matsui and Miss Soeda for spectral analysis, and to Miss Indo for microanalysis.

Summary

1. 2-Naphthyl β-D-grucofuranoside was synthesized from 2-naphthyl diacetyl-β-D-glucofuranosiduronolactone with lithium aluminum hydride, and the ring structure of this compound was examined by periodate oxidation.

2. Catalytic oxidation of 2-naphthyl β-D-glucofuranoside with gaseous oxygen and platinum black yielded 2-naphthyl β-D-glucofuranosiduronic acid. The ring structure of this compound was examined by periodate oxidation. Methyl 2-naphthyl β-D-glucofuranosiduronate was easily converted to 2-naphthyl β-D-glucofuranosiduronolactone. Methylation and acetylation of 2-naphthyl β-D-glucofuranosiduronic acid yielded 2-naphthyl diacetyl-β-D-glucofuranosiduronolactone.

(Received December 18, 1963)

9) P. F. Fleury, T. Lange: J. pharm. chim. [8] 17, 107, 196 (1933).