tri-O-acetyl-β-D-glucopyranosid)uronate. It was concluded that *p*-aminobenzoyl glucuronide has a pyranose configuration.

2. *p*-Aminobenzoyl glucuronide isolated from the dog urine was rapidly hydrolyzed by a rabbit-liver β-glucuronidase preparation.

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UDC 615.7[547.583.5]-092.21

206. Keitaro Kato, Kazuo Yoshida, and Hisao Tsukamoto : Metabolism of Drugs. XXXIV.*1 Studies on the Ring Structure of *p*-Aminobenzoyl Glucuronide isolated from the Urine of Dog administered with *p*-Aminobenzoic Acid. (2).*2

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In the preceding paper,[1] the present authors isolated from the urine of dog administered with *p*-aminobenzoic acid (PABA), *p*-aminobenzoyl glucuronide (PABA-GA) compound which was shown to have a pyranose configuration by comparing its acetylated and methylated derivatives with that synthesized by another method. On the other hand, Nakao, *et al.* surmised that this glucuronide might have a furanose configuration from the results obtained from their study by periodate oxidation and infrared spectra examination.[2] It was the purpose of this investigation to establish conclusively the pyranose structure of PABA-GA by actual application of Nakao's periodate oxidation procedure.

Nakao, *et al.* oxidized PABA-GA with periodic acid in aqueous solution to a dialdehyde, which was hydrolyzed in alkaline solution and then oxidized to two different products (Samples A and B) with bromine and with yellow mercuric oxide, respectively. They examined both samples by paper chromatography using Methyl Red as a spray reagent and indicated the presence of mesotartaric acid from the Rf value. According to this method, it is assumed that pyranose and furanose types of glucuronide yield tartronic acid and mesotartaric acid respectively. Therefore, the authors repeated this procedure and carefully examined Samples A and B by paper chromatography and paper electrophoresis but could not detect the presence of either mesotartaric acid or tartronic acid. Instead, it was demonstrated that hydrobromic acid and an unknown

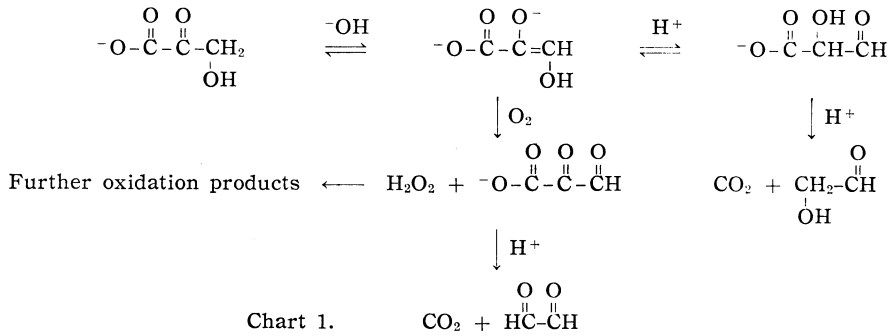


Chart 1.

*1 Part XXXIII. K. Kato, K. Yoshida, K. Tatsumi, H. Tsukamoto : This Bulletin, **10**, 1226 (1962).

*2 Presented at the Kyushu Branch Meeting of the Pharmaceutical Society of Japan, at Kumamoto, October, 1961.

*3 Katakasu, Fukuoka (加藤敬太郎, 吉田和夫, 塚元久雄).

1) K. Kato, K. Yoshida, K. Tatsumi, H. Tsukamoto : This Bulletin, **10**, 1226(1962).

2) T. Nakao, M. Nakao, T. Nakajima : J. Biochem. (Japan), **45**, 207 (1958).

acidic substance formed during the course of oxidation with bromine and yellow mercuric oxide respectively, and they interfered the detection of mesotartaric acid in the chromatography used by Nakao, *et al.* because of their similar Rf values.

Nakao, *et al.* hydrolyzed the dialdehyde by heating in alkaline solution for several minutes. If PABA-GA has a pyranose configuration, tartronic acid semialdehyde (hydroxypyruvate) should be produced in the hydrolyzate. However, Hedrik, *et al.*[3] showed that hydroxypyruvate is readily autooxidized and decarboxylated under the mild condition, and that both reactions are catalyzed by alkali. Chart 1 shows the reaction mechanism suggested by Hedrick, *et al.* Thus it should be considered that the condition used by Nakao, *et al.* is unsuitable for the hydrolysis of dialdehyde.

The dialdehyde was therefore subjected to oxidation with bromine followed by acid hydrolysis or vice versa, but the detection of mesotartaric acid and tartronic acid was also unsuccessful in either cases. In the author's chromatographic study tartronic acid was found to be unstable in the acidic milieu used in the hydrolysis procedure described above. Furthermore, Dickens, *et al.*[4] studied the effect of heat (100°) on decarboxylation of hydroxypyruvate in aqueous solution and found that this compound was converted to glycolaldehyde in acidic condition. For these reasons, no tartronic acid could be detected even though PABA-GA has a pyranose configuration.

Since confirmation of the structure of the dialdehyde from PABA-GA was unsuccessful by oxidation technique, methods for reduction of periodate oxidized PABA-GA to the corresponding alcohol was tried. The dialdehyde was reduced with sodium borohydride in aqueous solution and the resulting alcohol was hydrolyzed by heating in sulfuric acid. Paper chromatographic analysis of the hydrolyzate revealed the presence of glycerol which was identified as tris-*p*-nitrobenzoate. The production of glycerol instead of glyceric acid may be described as follows: Cadotte, *et al.* reported that the so-called dialdehydes formed by periodate oxidation of methyl glycosides exist in the cyclic acetal form as shown in Chart 2.[5] Infrared and polarographic studies indicated the absence of free aldehyde in certain periodate-oxidized glycosides.[6,7] Goldstein and Smith further supported this view by methylation studies of the dialdehydes.[8]

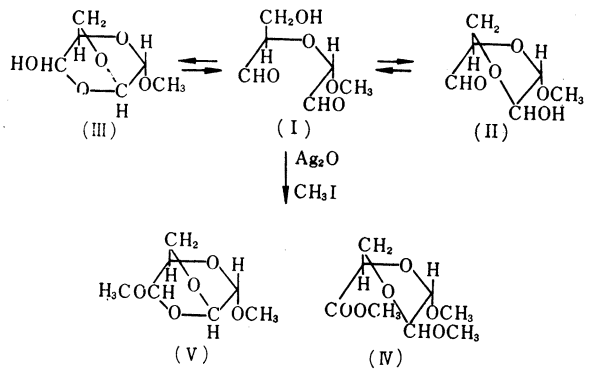


Chart 2.

From these reports, it seems most likely that the carboxylic and aldehydic groups in (VI) might be involved in the formation of ring systems as shown in (VII) or (VIII). The

3) J. L. Hedrick, H. J. Sallach: J. Biol. Chem., **236**, 1867 (1961).
4) F. Dickens, D. H. Williamson: Biochem. J., **68**, 74 (1958).
5) J. E. Cadotte, G. G. S. Dutton, J. J. Goldstein, B. A. Lewis, F. Smith, J. W. Van Cleve: J. Am. Chem. Soc., **79**, 691 (1957).
6) J. W. Rowen, F. H. Forziati, R. E. Reeves: J. Am. Chem. Soc., **73**, 4484 (1951).
7) C. D. Hurd, P. J. Baker, R. P. Holysz, W. H. Saunder: J. Org. Chem., **18**, 186 (1953).
8) I. J. Goldstein, F. Smith: J. Am. Chem. Soc., **82**, 3421 (1960).
9) J. X. Khym, W. E. Cohn: *Ibid.*, **82**, 6380 (1960).

carboxylic acid group at C_6 of the original glucuronide, then would be reduced by sodium borohydride to give (IX) which would be subsequently converted to glycerol by hydrolysis. The reduction of the dialdehyde with sodium borohydride was conducted at different pH values, i.e. acidic,[9] neutral, and alkaline conditions, but glyceric acid

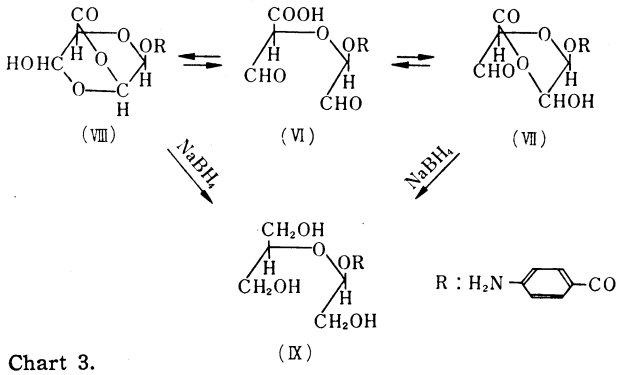
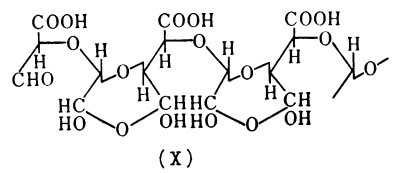


Chart 3.

was barely detected in the hydrolyzate of the reduced dialdehyde by paper chromatography and paper electrophoresis.*4 The detection of a 2-carbon fragment, the other component of (IX), was unsuccessful. Paper chromatography showed the presence of an unknown component which had a Rf value similar to that of glyoxal and whose *p*-nitrobenzoate melted at 143°. This evidence, showing that the 3-carbon fragment was obtained from the reduced dialdehyde, inferred that PABA-GA had a pyranose configuration.

For further confirmation, the consumption of periodate and formation of acid during PABA-GA oxidation were determined. The oxidation of PABA-GA consumed 2.07 moles of periodate and produced 1.05 moles of titratable acid in aqueous solution. Under the same condition, synthetic 2-naphthyl-*β*-D-glucopyranosiduronic acid consumed 2.07 moles of periodate and produced 1.09 moles of acid. So the types of reaction are entirely the same in both cases. On the other hand, there is a marked difference between the periodate oxidation behavior of synthetic 2-naphthyl-*β*-D-glucopyranosiduronamide and that of 2-naphthyl-*β*-D-glucofuranosiduronamide. Periodate oxidation was carried out for these two compounds under the same condition in 40% dioxane solutions. The former consumed 2.0 moles of periodate and produced 0.6 moles of acid in 48 hours. In the same period of time, the latter consumed 0.2 moles of periodate but no titratable acid was produced. In the case of the latter, 1.0 mole of the theoretical amount of periodate was consumed after 360 hours of reaction. The former reacted very rapidly with 2 moles of oxidant and the latter consume 1 mole of oxidant over a long period. Since

*4 In the experiment reported by Mihashi,[10] oligogalacturonic acid was oxidized with periodate and the product was reduced with sodium borohydride. From the hydrolyzate of the resulting alcohol, glyceric acid was obtained as a calcium salt. In this laboratory, glyceric acid was not obtained from PABA-GA under the same reaction conditions described in Mihashi's paper. So his finding is in conflict with the present result. For oligogalacturonic acid, however, it is assumed that the 1,4-dioxane ring formation would take place between the aldehydic group at C_2 derived from terminal galacturonic acid and the aldehydic group at C'_3 derived from adjacent galacturonic acid as shown in (X). So in the terminal aldehyde, the cyclization of the carboxylic acid group at C_6 and the aldehydic group at C_2 might be prohibited leaving the carboxylic acid exposed and unsusceptible to reduction with sodium borohydride. The structure(X) seems to fit Mihashi's postulation.



10) Y. Mihashi : Yakugaku Zasshi, **81**, 1003 (1961).

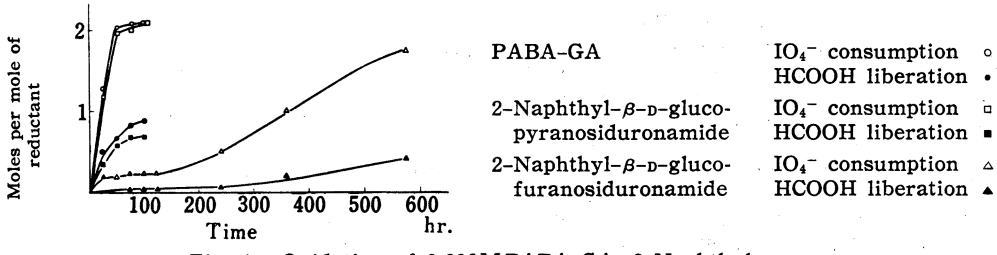
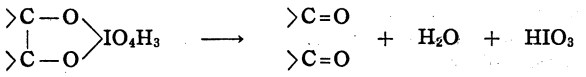


Fig. 1. Oxidation of 0.006*M* PABA-GA, 2-Naphthyl-β-D-glucopyranosidournamide, and 2-Naphthyl-β-D-glucofuranosiduronamide with 0.013*M* Periodate in 40% Dioxane Solution

the reaction type differs for these compounds, it was referred for use in the confirmation of the ring structure of PABA-GA. PABA-GA consumed 2.0 moles of periodate and produced 0.68 moles of titratable acid in 48 hours under the same condition. Therefore, there is a good agreement in the type of periodate oixdation between PABA-GA and 2-naphthyl-β-D-glucopyranosiduronamide (Fig. 1). This experiment indicated the presence of a pyranose ring in PABA-GA. The marked difference in the rate of oxidation of five-membered ring as compared to six-membered ring glycols depends on the relative steric arrangement of the vicinal hydroxyl groups. The oxidation of 1,2-glycols by periodate involves a cyclic intermediate and the rate-determining step is the ring closure to form the intermediate. *Trans*-1,2-diols in the chair conformation of the



six-membered ring are favorable for the formation of the cyclic intermediates and the resistance of *trans*-1,2-diol in the five-membered ring to formation of the cyclic intermediate markedly decreases the rate of oxidation.

Experimental and Results*5

Paper Chromatographic Method——Ascending development was employed with Toyo Roshi No. 50. Solvent systems employed were (A) BuOH-AcOH-H_2O (4:1:5), (B) AcOEt-AcOH-H_2O (3:1:1), (C) Et_2O-AcOH-H_2O (13:3:1), (D) PhOH-HCOOH-H_2O (75:1:25), (E) AcOEt-HCOOH-H_2O (5:2:2), (F)AcOEt-pyridine-H_2O (70:25:15), (G) BuOH-EtOH-H_2O (40:11:19). Products were detected on the chromatogram by spraying the following reagents: (1) glucose-aniline (approx. 2% of each) in EtOH-H_2O (2:8);[11] (2) $NaIO_4$-benzidine;[12] (3) 2% $FeCl_3$. Identification of spots was made by reference to standard compounds rather than by measurement of Rf values.

Paper Electrophoretic Method——Toyo Roshi No. 50 filter parer (12×24 cm.) was used with a buffer solution of the following composition: 0.5*M* AcOH adjusted with pyridine to pH 4.0. The paper was freely suspended in a horizontal plane and supported by a removable plastic frame work. The entire system consisting of electrode vesseles and the paper and its holder were placed in a closed chamber. Electrophoresis was run for 1 hr. with a potential across the electrodes of 300 v. Upon completion, the paper was dried at room temperature and sprayed with the reagents.

Paper Chromatography and Paper Electrophoresis of Samples A and B——31.2 mg. of PABA-GA dissolved in 30 cc. of H_2O was oxidized with 42.8 mg. of $NaIO_4$ in darkness at room temperature for

Table I. Rf Values of T, M and HBr

Compd.	Solvent System				
	A	B	C	D	E
T	0.45	0.45	0.31	0.28	0.54
M	0.30	0.36	0.12	0.24	0.44
HBr	0.42	0.32	0.09	0.23	0.51

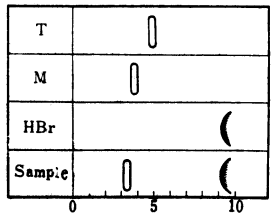
The compounds were detected with the reagent (1) and (2).

*5 All melting points are uncorrected.

11) D. Gross: Nature, **184**, 1298 (1959).
12) D. F. Mowery: Anal. Chem., **29**, 1560 (1957).

45 hr. After oxidation, the procedure of Nakao, *et al.* was repeated and Samples A and B were examined by paper chromatography and paper electrophoresis. Table I shows Rf values of standard compounds namely, tartronic acid (T), mesotartaric acid (M), and HBr. Solvent systems of A, B, C, D were employed as in Nakao's experiments.

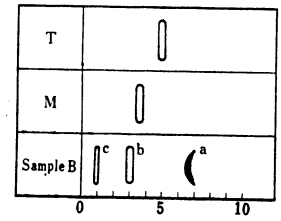
In the case of Sample A, HBr formed during the course of oxidation with Br_2 showed nearly the same Rf values as those of M in the three solvent systems (B, C, D). (In Nakao's experiment, HBr was not removed from Sample A). In solvent A, the Rf value of HBr differed from that of M. However, when Sample A was chromatographed, HBr showed tailing and covered the spot of M. Therefore it is difficult to distinguish between M and HBr with the solvents described in Nakao's paper. In order to distinguish M from HBr, paper chromatography with Solvent System E and paper electrophoresis were used. Solvent E was useful in separating these two substances but the presence of M in Sample A was not revealed. M and HBr had different mobilities in paper electrophoresis, but the contamination of large quantities of HBr interferred the migration rate of M. Sample A was therefore developed on a sheet of filter paper (40×40 cm.) using Solvent A. The area corresponding to M was cut, extracted with H_2O and after removal of H_2O, the residue was submitted to paper electrophoresis. Fig. 2 shows the electrophoretic pattern of this Sample. This technique revealed no M in the eluate and neither could T be detected in Sample A.



Displacement to anode (cm.)

Fig. 2. Paper Electrophoresis of T, M, HBr, and the extracted Sample

The Conditions were described in the Section of Paper Electrophoretic Method.



Displacement to anode (cm.)

Fig. 3. Paper Electrophoresis of T, M, and Sample B

The Conditions were described in the Section of Paper Electrophoretic Method.

The Sample B was also analysed by paper electrophoresis. Fig. 3 shows the electrophoretic pattern of the Sample B.

Paper electrophoresis did not show the presence of both M and T in Sample B. The three areas (a, b, c) on the three sheets of paper were cut out, extracted with H_2O and after removal of H_2O the residue was submitted to paper chromatography. Among the three extracted samples, (a) was superimposed on the spot of M in the four different solvent systems (A, B, C, E) (Table II). The sample extracted from the area (c) showed no spot on the paper chromatogram because the amount was too small.

Table II. Rf Values of T, M, (a) and (b)

Compd.	Solvent System			
	A	B	C	E
T	0.45	0.45	0.31	0.54
M	0.30	0.32	0.12	0.44
(a)	0.32	0.35 (0.12)*	0.08	0.40
(b)	0.06	0.15 (0.07)*	0.10	0.22

* In the solvent B, (a) and (b) showed two spots respectively.
The compounds were detected with the reagent (1) and (2).

Reducotin of the Dialdehyde with Sodium Borohydride——General procedure : 0.5 g. of PABA-GA dissolved in 28 cc. of H_2O was oxidized with 0.716 g. (2.1 mol. equiv.) of $NaIO_4$ in darkness at about 7° for 3 hrs. After periodate oxidation the yellow dialdehyde was precipitated. To the reaction mixture saturated $Ba(OH)_2$ solution was added. The precipitated dialdehyde was redissolved, and white precipitates of $Ba(IO_3)_2$ and $Ba(IO_4)_2$ were discarded by filtration (solution I).

Solution I was reduced with $NaBH_4$. After reduction the reaction mixture was acidified with H_2SO_4 or HCOOH, and then heated at 100°. After acid hydrolysis, a precipitate was removed by filtration, and the filtrate was passed through a column of Amberlite IR 120. To the effluent saturated aqueous lead acetate was added untill precipitation was complete. The precipitate was removed by filtration. The filtrate was adjusted to pH 7 with NH_4OH, and saturated aqueous basic lead acetate

was added in excess. The basic lead precipitate was filtered off, washed with H_2O, and suspended H_2O. From this suspension Pb was removed by saturation with H_2S. After removal of PbS by filtration, the filtrate was evaporated to dryness in a reduced pressure (Fraction I). The filtrate obtained by filtration of the basic lead precipitate, after removal of excess Pb with H_2S, was evaporated to dryness in a reduced pressure (Fraction II). In order to remove H_3BO_3, each fraction was dissolved in MeOH to form volatile methyl borate and the solvent was evaporated in a reduced pressure. Fraction I was examined by paper chromatography and paper electrophoresis for glyceriec acid and Fraction II was examined by paper chromatography for glycerol.

Table III. Rf Values of Ethyleneglycol, Glycolaldehyde, Glyoxal, Glycerol and Erythritol

Compd.	Solvent System				
	A	F	C	B	G
Ethyleneglycol	0.68	0.77	0.81	0.73	0.74
Glycolaldehyde	0.62	0.40	—	—	—
Glyoxal	0.56	0.61	0.72	0.61	0.64
Glycerol	0.41	0.43	0.59	0.45	0.53
Erythritol	—	0.29	0.36	—	—

The compounds were detected with reagent (2).

1) To solution I, adjusted to 12.5, was added 0.3 g. of $NaBH_4$. After 17 hr. at about 20°, the reation mixture was neutralizedwi th N H_2SO_4, and then 0.15 cc. of conc. H_2SO_4 was added. The acidic solution was heated at 100° for 30 min. and treated by the procedure described above. By this method glycerol and an unknown compound (x) which showed a Rf value similar to that of glyoxal were detected in Fraction II.

2) Solution I was saturated with CO_2 gas, and after removal of a precipitate the filtrate was adjusted to pH 8.5. To this solution 33 mg. of $NaBH_4$ was added in small portions over a 20 min. period at 0°. Then reaction mixture was brought to pH 3.0 with HCOOH, heated at 100° for 10 min., and treated by the standard procedure. As a result, glycerol and (x) were detected in Fraction II and a trace of glyceric acid was detected in Fraction I by paper chromatography and paper electrophoresis.

Table IV. Comparison of the Rf Values and the Displacement of DL-Glyceric Acid with those obtained from the Sample of Fraction I

Compd.	Solvent System		Displacement towards the anode (cm.)
	A	E	
DL-Glyceric acid	0.42	0.70	3.8
Fraction I	0.40	0.70	3.8

The compounds were detected with the reagent (1), (2) and (3). The conditions of paper electrophoresis were described in the section of paper electrophoretic method.

3) To solution I, after saturation with CO_2 gas and removal of a precipitate, 20 cc. of saturated H_3BO_3 and 50 cc. of 1M AcOH was added. To this solution 1.5 g. of $NaBH_4$ dissolved in 20 cc. of H_2O was added drop by drop over a period of 1 hr. at 15° with mechanical stirring. After reduction, the reaction mixture was adjusted to pH 3.0 with HCOOH, heated at 100° for 10 min., and treated by standard procedure. As a result, glycerol and (x) were detected in Fraction II.

4) Solution I was saturated with CO_2 gas. After removal of a precipitate, the filtrate showed pH 6.0. To the filtrate 33 mg. of $NaBH_4$ was added in small portions over 50 min. at 15° with mechanical stirring. During the reaction, the solution was kept at pH 7~8 by adding N H_2SO_4. After an additional 10 min., the reaction mixture was adjusted to pH 1.8 with conc. H_2SO_4, heated at 100°, and treated by the standard procedure. In this case glycerol and (x) were detected in Fraction II.

5) After periodate oxidation, 0.6 g. of $NaBH_4$ was added to the reaction mixture (pH 2.2). The solution, after standing for 5 hr. at about 20°, was neutralized with N H_2SO_4 and then 0.27 cc. of conc. H_2SO_4 was added. Then the acidic solution was heated at 100° for 20 min. After hydrolysis, saturated $Ba(OH)_2$ solution was added to the solution untill precipitation was complete. The precipitate was discarded by filtration. The filtrate was saturated with CO_2 gas, and after removal of a precipitate passed through a column of Amberlite IR 120. The effluent was treated by the standard procedure and divided into two fractions. In this case glycerol and (x) were detected in Fraction II.

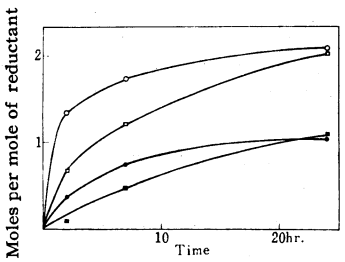
Identification of the Glycerol Moiety——1.0 g. of PABA-GA dissolved in 60 cc. of H_2O was oxidized with 1.4 g. of $NaIO_4$ in darkness at about 7° for 5 hr. The precipitated dialdehyde was separated

by centrifugation, washed with H_2O and dissolved in N NaOH. The solution was adjusted to pH 6.5 and the volume was brought to 10 cc. To this solution, 0.57 g. of $NaBH_4$ dissolved in 2 cc. of H_2O was added. After standing at 15° for 45 hr., the reaction mixture was neutralized with N H_2SO_4. To the neutralized solution, 0.2 cc. of conc. H_2SO_4 was added, and heated at 100° for 3 hr. The hydrolyzate was neutralized with 10% NaOH and after removal of a precipitate evaporated to dryness in a reduced pressure. The residue was extracted with EtOH, and the solvent distilled off in a reduced pressure. After several treatment with EtOH, the residue was dissolved in H_2O and passed through Amberlite IR 120 and IR 45 columns successively. The effluent was evaporated to dryness in a reduced pressure. 23 mg. of the neutral residue was dissolved in 5 cc. of pyridine and treated with 410 mg. of *p*-nitrobenzoyl chloride at 80° for 30 min. When the reaction mixture was poured into $NaHCO_3$ solution, 0.15 g. of a precipitate was obtained. The dried precipitate was extracted with 7 cc. of benzene and recrystalized from AcOEt, m.p. 188~190°. Yield, 18 mg. *Anal.* Calcd. for $C_{24}H_{17}O_{12}N_3$: C, 53.43; H, 3.15; N, 7.79. Found : C, 53.24; H, 3.43; N, 8.04. This compound showed no depression of melting point on admixture authentic glycerol tris-*p*-nitrobenzoate, and this infrared spectrum was identical with that of the authentic sample.

From the benzene extract an unknown compound was obtained, m.p. 142~143°(30 mg.). After debenzoylation with MeONa in MeOH, this compound showed a Rf value similar to that of glyoxal. The debenzoylated compound was unchanged by acid hydrolysis, oxidation with Br_2, or reduction with $NaBH_4$.

Determination of Formic Acid Liberation and Oxidant Consumption

1) Oxidation of PABA-GA and 2-naphthyl-β-D-glucopyranosiduronic acid in aqueous solution : To a solution of 0.15 g. of PABA-GA in 45 cc. of H_2O 0.215 g. of $NaIO_4$(2.1 mol. equiv.) was added. The reaction mixture was kept in darkness at about 7°. 0.15 g. of 2-naphthyl-β-D-glucopyranosiduronic acid was treated with 0.210 g. of $NaIO_4$(2.1 mol. equiv.) in the same manner as that of PABA-GA. HCOOH was determined by titration with 0.01N NaOH after destruction of the excess of periodate with ethylenelycol. Oxidant consumption was determined by the method of Fleury and Lange.[13]



PABA-GA — IO_4^- consumption ○ HCOOH liberation •

2-Naphthyl-β-D-glucopyranosiduronic acid — IO_4^- consumption □ HCOOH liberation ■

Fig. 4. Oxidation of 0.01M PABA-GA and 2-Naphthyl-β-D-glucopyranosiduronic Acid with 0.022M Periodate

2) Oxidation of PABA-GA, 2-naphthyl-β-D-glucopyranosiduronamide, and 2-naphthyl-β-D-glucofuranosiduronamide in 40% dioxane solution : To a solution of 0.20 g. of PABA-GA in 100 cc. of 40% dioxane 0.286 g. of $NaIO_4$(2.1 mol. equiv.), was added. The reaction mixture was kept in darkness at 20°. The determination of HCOOH liberation and oxidant consumption was carried out by the method described above.

0.20 g. each of 2-naphthyl-β-D-glucopyranosiduronamide and furanosiduronamide was treated with 0.281 g. of $NaIO_4$(2.1 mol. equiv.) in the same manner as that of PABA-GA.

The authors are indebted to Miss Indo for microanalysis, and to Mr Matsui for infrared absorption spectra measurement.

Summary

PABA-GA was oxidized with periodate to identify the ring structure of this compound. The dialdehyde from PABA-GA was examined by reducting it to the corresponding alcohol. The presence of glycerol instead of glyceric acid in the hydrolyzate of the resulting alcohol was established by paper chromatography and the glycerol was identified as the crystalline tris-*p*-nitrobenzoate. The presence of glycerol in the

13) P. F. Fleury, T. Lange : J. Pharm. Chim. [8] **17**, 107, 196 (1933).

hydrolyzate suggested that the dialdehyde from PABA-GA exists in the cyclic form.

Periodate oxidation of synthetic 2-naphthyl-β-D-glucopyranosiduronamide and 2-naphthyl-β-D-glucofuranosiduronamide was conducted to compare the two compounds in the rate of periodate consumption and the production of acid. There is a good agreement between PABA-GA and 2-naphthyl-β-D-glucopyranosiduronamide with respect to the type of periodate oxidation. In view of above findings it was surmised that PABA-GA has a pyranose configuration.

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