[Chem. Pharm. Bull. 14(9) 991~996 (1966)] UDC 547.483.04.07

136. Toshio Kinoshita,[*1,*2] Morizo Ishidate,[*3] and Zenzo Tamura[*1]: 3-Ketoglucuronic Acid. II.[*4,*5] Constitution and Chemical Properties of 3-Ketoglucuronic Acid.

(*Faculty of Pharmaceutical Sciences, University of Tokyo*[*1] *and Tokyo Biochemical Research Institunte*[*2])

The preceding report[*4] of this series described the synthesis of 3-ketoglucuronic acid which represents a novel exemplar of unsubstituted 1,3-dicarbonyl carbohydrate[*4,*5] and is a key substance for studying on the properties of 1,3-dicarbonyl carbohydrates in general. This keto-sugar exibits stronger reducing power than uronic acids and the other usual sugars[*5] and it could structurally be regarded as one of reductones.

In order to determine the structure of the sugar in solution having either the straight chain•form or hemiacetal ring form, periodate oxidation[1,2] was performed. The oxidation was appropriately conditioned to pH 2±0.1 under cooling (at 4°) to avoid unfavorable side reactions.[1,3~5]

3-Ketoglucuronic acid consumed three mole of periodate within the first ten minutes. Concomitant titration showed the presence of three moles of acid; one mole of carboxyl function from the original uronic acid, one mole of formic acid and one mole of carbon dioxide. As shown in Table I, little change on periodate consumption and acid titer were observed even after the reaction mixture was stood for further 70 minutes. If 3-ketoglucuronic acid were of straight chain structure (I) and consumed three moles of periodate, four mole of acid (each two moles of glyoxylic acid and formic acid) should be formed, but it was not the case. If it had furanose structure (II), the consumption should be two moles, because α-kato-acid structure is known to be considerably resistant to periodate oxidation[5,6] and consequently the bond between C-5 and C-6 of II should not be cleaved within a short period of ten minutes at 4°. On

Table I. Periodate Oxidation of 3-Ketoglucuronic Acid (III)

Time (min.)	Periodate consumption (mol.)	Total acid (mol.)	Formic acid (mol.)	Carbon dioxide (mol.)	Formate Ester (mol.)
10	2.8	2.9	1.1	0.8	1.0
20	2.9	3.0	—	—	1.0
40	2.9	3.2	—	—	1.1
80	3.1	3.3	1.2	0.7	1.0

*1 Hongo, Tokyo (木下俊夫, 田村善蔵).
*2 Present address: School of Pharmaceutical Sciences, Showa University, Shinagawa-ku, Tokyo.
*3 Takadaminami-cho, Toshima-ku, Tokyo (石館守三).
*4 Part I. T. Kinoshita, M. Ishidate, Z. Tamura: This Bulletin, **14**, 986 (1966).
*5 Preliminary communication appeared in: M. Ishidate, Z. Tamura, T. Kinoshita: This Bulletin, **13**, 99 (1965).

1) J. M. Bobbitt: "Advances in carbohydrate Chemistry," **11**, 9 (1956), Academic Press Inc., New York.
2) Y. Imai: Yakugaku Zasshi, **81**, 1115 (1961).
3) K. Takiura, K. Koizumi: Yakugaku Kenkyu, **30**, 809 (1958).
4) M. L. Wolfrom and J. M. Bobbitt: J. Am. Chem. Soc., **78**, 2489 (1956).
5) D. B. Sprinson, E. Chargaff: J. Biol. Chem., **164**, 433 (1946).
6) E. J. Jackson: "Organic Reactions," **2**, 341 (1944).

the contrary, the obtained results of the oxidation were in good agreement with the pyranose structure (III). Therefore, the substance is assigned as 3-keto-D-glucopyranuronic acid (III) and the oxidation process is posturated as shown in Chart 1.

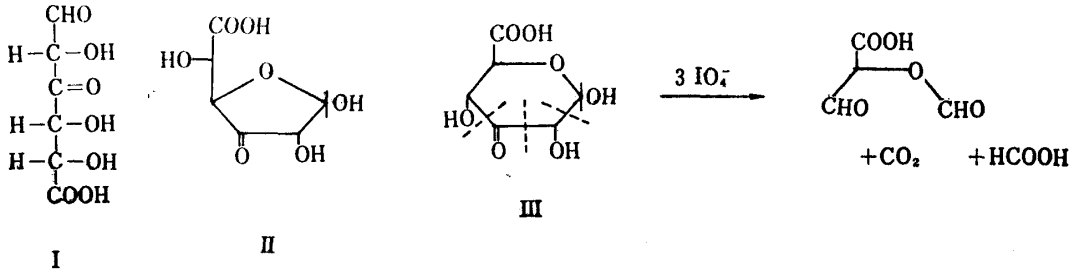


Chart 1.

When III was treated with Amberlite IR-120 (H-form) in methanol[7] there was obtained a neutral crystalline product of m.p. 119~120° corresponding to methyl ester of methyl 3-ketoglucosiduronic acid, which showed no mutarotation. The presence of carbonyl group at C-3 and methyl ester at C-6 was supported by infrare spectra. As shown in Table II, this compound consumed one mole of periodate within the first ten minutes at 5° with the concomitant formation of one mole of acid and neither further periodate consumption nor acid production was observed over a period of additional 45 hours. These results coincide with the furanoside structure. If the glycoside were of pyranoside structure, the consumption of the oxidant should exceed one mole after standing for such a long period of 45 hours. The same methyl glycosiduronate was prepared by the action of Amberlite IR-120 in methanol on a furanoside derivative of 3-ketoglucuronic acid, namely methyl 3-keto-5-O-acetyl-1,2-O-isopropylidene-D-glucofuranuronate (IV).** As a result, this methyl glycoside is assigned as methyl (methyl 3-keto-D-glucofuranosid)uronate (V) and the oxidation process might be described as shown in Chart 2. V instantly reduces ammoniacal silver nitrate solution and neutral

Table II. Periodate Oxidation of Methyl (methyl 3-keto-D-glucofuranosid)uronate (V)

Time	Periodate consumption (mol.)	Total acid (mol.)	Time	Periodate consumption (mol.)	Total acid (mol.)
10 min.	1.03	1.06	2 days	1.07	1.06
1 hr.	1.02	1.06	5 days	1.07	1.06
4 hr.	1.02	1.06			

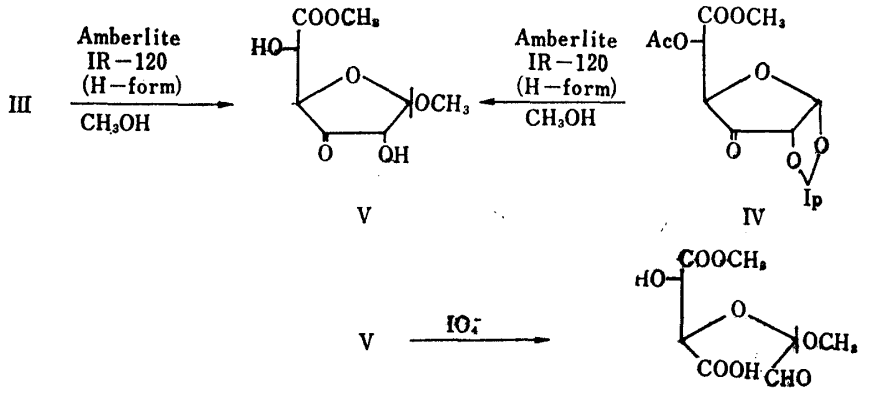
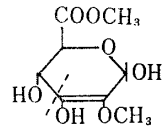
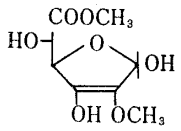
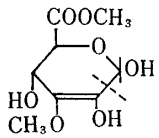
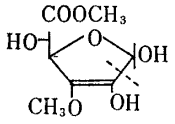
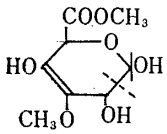
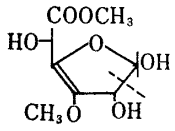
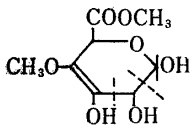


Chart 2.

7) J. E. Cadotte, F. Smith, D. Spriestersbach: J. Am. Chem. Soc., 74, 1501 (1952).

potassium permanganate solution and the fact is reasonably ascribed to the presence of α-ketol structure.[8] It is interesting that V surely has a furanoside form whereas the original uronic acid (III) has pyranose form. Hirasaka[9] noted that methyl furanoside of glucuronolactone was predominantly produced when D-glucopyranuronic acid was treated with cation exchange resin in methanol, whereas D-galactopyranuronic acid gave mainly methyl pyranoside by the similar treatment.[7]

Table III. Possible Structures for the Product obtained by Treatment of 3-Ketoglucuronic Acid with Diazomethane and Their Presumed Results of Periodate Oxidation

	Structures	Periodate consumption (mol.)	Total acid (mol.)	Volatile acid produced by refluxing with H_2SO_4 (mol.)
VI		1	1	0
VII		0	0	0
VIII		1	1	1
IX		1	1	1
X		1	0	1
XI		1	0	1
XII		2	2	2

8) B. Lindberg, O. Theander : Acta Chem. Scand., 8, 1870 (1954); O. Theander : *Ibid.*, **11**, 1557 (1957).
9) Y. Hirasaka : Private communication.

3-Ketoglucuronic acid (Ⅲ) reacts with titanium chloride solution*6,10) to give orange-red precipitate that suggests the formation of an enediol. The precipitation, however, took place after an interval of time. This fact seems to indicate that keto-enol equilibrium of Ⅲ in solution tends dominantly to the keto form.

On treatment of 3-ketoglucuronic acid (Ⅲ) with diazomethane there was obtained a neutral crystalline substance of m.p. 158~160° in fair yield. Elemental analysis indicated that it is dimethyl derivative of Ⅲ. This product was discriminated from methyl (methyl 3-keto-D-glucofuranosid)uronate (Ⅴ) in every respect, *i.e.* in melting point and its low reactivity against silver nitrate and permanganate solution indicating the absence of α-ketol structure. Infrared spectra supported the presence of C-6 methyl ester and the formation of enol ether. Seven possible alternative structures (Ⅵ~Ⅻ) for the enediol ether are shown in Table Ⅲ.

Periodate oxidation of this compound was carried out under almost the same condition as the case of Ⅲ. As shown in Table Ⅳ, each one mole of periodate consumption, acid titer and volatile acid (formate ester) was noticed. From this fact the possible structure was limited to either Ⅷ or Ⅸ (Table Ⅲ). However, the formation of Ⅸ is most unlikely since unsaturated furanose ring exhibits far greater ring tension than in case of pyranoid ring as demonstrated by Dreiding model. Accordingly the obtained dimethyl derivative is assigned as methyl 3-O-methyl-Δ^2-D-glucopyranuronate (Ⅷ) and the oxidation sequence may well be explained as shown in Chart 3 :

Table Ⅳ. Periodate Oxidation of Methyl 3-O-methyl-Δ^2-D-glucopyranuronate (Ⅷ)

Time	Periodate consumption (mol.)	Total acid (mol.)	Volatile acid produced by refluxing oxidation mixture with H_2SO_4 (mol.)
10 min.	0.87	0.92	1.08
40 min.	1.02	1.01	1.27
1.5 hr.	1.02	1.08	—
3 hr.	1.02	1.08	—
6 hr.	1.02	1.10	—
24 hr.	1.24	1.10	—

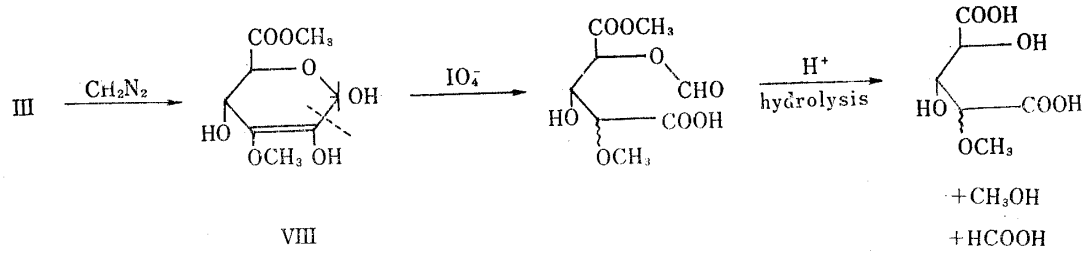


Chart 3.

3-Ketoglucuronic acid is able to reduce 2,6-dichlorophenol indophenol (Tillman reagent) and gives oxidation wave on a polarogram (pH 2.5) as do L-ascorbic acid[11] and other reductones.[12] However, the reactivities were found very low as compared with aci-reductones which assume completely enediolic form in solution. Although the

*6 0.1% titanium trichloride in methanol-pyridine (3:1). This solution was mixed with equivolume of methanolic solution of 3-ketoglucuronic acid. of T. Momose : "Qualitative Organic Analysis," 217 (1961), Hirokawa Publishing Co., Tokyo (in Japanese).

10) F. Weygand, E. Csendes : Chem. Ber., **85**, 45 (1952).

11) S. One, M. Takagi : Bull. Chem. Soc. Japan, **31**, 356 (1958).

12) *Idem* : *Ibid.*, **31**, 364 (1958).

gradual increase of wave height was observed during the first ninety minutes, the wave height was only one-fortieth of that of L-ascorbic acid even at its maximum (Fig 1). These facts, together with the behavior of 3-ketoglucuronic acid to titanium chloride solution, indicates again that contribution of enediol form in solution is very small under acidic conditions.

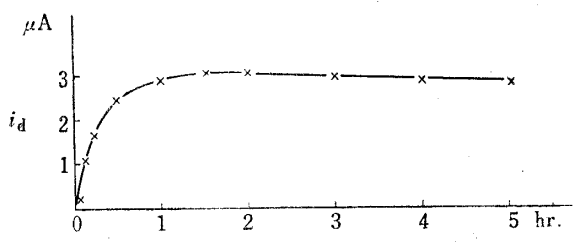


Fig. 1. Oxidation Wave-height of D-3-Ketoglucuronic Acid in Solution on Polarogram Measured in Time Intervals
(1.93×10^{-2} mol./L., pH 2.5, 25±0.1°)

Experimental

All of the experiments were carried out using crystalline 3-ketoglucuronic acid which contains 1 mole of dioxane as a crystallization solvent, m.p. 97~98°.*4 All evaporations were done under reduced pressure.

Periodate Oxidation of 3-Ketoglucuronic Acid (III) (Table I)——i) Conditions employed the oxidation: III (196 mg., 1.01 mmol.) was dissolved in 0.5*N* H_2SO_4 (9.0 ml.) and the resulting solution was cooled to 4°. A volume of 0.05*M* sodium metaperiodate (140 ml., previously cooled to 4°) was added and the solution was adjusted to 200 ml. with cold distilled water. The resulted mixture was allowed to stand in the dark at 4°. Under these conditions, pH value of the reaction mixture was maintained within the range of 2.0±0.1 in the course of the oxidation.

ii) Estimation of periodate consumption[2]: An aliquot of the above reaction mixture (2.0 ml.) was pipetted into a flask containing 10 ml. of a borateboric acid buffer solution (containing 40 mg. of $Na_2B_2O_7$ and 30 mg. of H_3BO_3 per ml.). To the mixture 20%. KI (5 ml.) was added and the resulting solution was allowed to stand in the dark for 15 min. at room temperature. Finally, I_2 produced was determined by titration with 0.1*N* $NaAsO_2$.

iii) Estimation of total acid[2]: To an aliquot of the above oxidation mixture (5 ml.), ethylene glycol (0.4 ml.) was added and the solution was allowed to stand for 30 min. at room temperature and was titrated with 0.1*N* NaOH using phenolphthalein as an indicator. Total acid was given by subtracting the amount of added H_2SO_4 from the acid found by the titration.

iv) Estimation of volatile acid: To a volume of the above oxidation mixture (40 ml.) ethylene glycol (3.2 ml.) was added. The resulting mixture was allowed to stand for 30 min. at room temperature and then submitted to distillation under reduced pressure (1 mm. Hg; bath temperature 55°) with continuous addition of water (100 ml.) into the flask from a dropping funnel and the distillate was collected in a flask containing 0.1*N* NaOH (20 ml.). Finally the remaining alkaline was back-titrated with 0.1*N* HCl. The amount of formic acid was given by subtracting the amount of CO_2 from the amount of volatile acid.

v) Estimation of formate ester: Carried out according to Wolff, *et al.*.[13]

vi) Estimation of carbon dioxide: CO_2 was swept from the reaction mixture with a stream of N_2 into *N*/5 $Ba(OH)_2$[4] which thereafter back-titrated with *N*/10 HCl.

Methyl(methyl 3-keto-D-glucofuranosid)uronate (V)——i) From 3-ketoglucuronic acid (III): To a solution of III (1.4 g.) in absolute MeOH (15 ml.) added an amount of Amberlite IR-120 (H-form, 4 g.) and the resulting mixture was heated under reflux with vigorous stirring over a period of 80 min. The resin was filtered off from the mixture and the obtained pale yellow solution was evaporated to a thick syrup which crystallized on scratching. The deposited crystals were collected by filtration with the aid of a minimum amount of ether. On recrystallization from acetone 0.5 g. of V was obtained; m.p. 119~120°, $[\alpha]_D^{25}$ +302° (c=1, H_2O), IR cm^{-1} (KBr): $\nu_{C=O}$ 1775 (carbonyl at C-3); δ_{C-H} 1440 (methyl ester at C-6). Rf 0.73 on paperchromatogram using *n*-BuOH-EtOH-H_2O (2:1:1) as a solvent (*Anal.* Calcd. for $C_8H_{12}O_7$: C, 43.64; H, 5.49. Found: C, 43.64; H, 5.60).

ii) From methyl 3-keto-5-O-acetyl-1,2-O-isopropylidene-D-glucofuranuronate (IV): To a solution of IV (10 g.) in absolute MeOH (50 ml.) was added 5 g. of Amberlite IR-120 (H-form) and the resulting mixture was heated under reflux with vigorous stirring over a period of 3 hr. The resin was filtered off from the mixture, the obtained yellow solution was decolorized with charcoal and evaporated to a thick syrup. On trituration of the syrup with ether, there deposited a crystalline material which, after recrystallization from acetone, was identified with V by mixed melting point measurement, paperchromatography using *n*-BuOH-EtOH-H_2O (2:1:1) as a solvent and infrared spectroscopy as KBr-tablet; yield 5.0 g.

13) J. A. Wolff, B. T. Hofreiter, P. R. Watson, W. L. Detherage, M. M. MacMasters: J. Am. Chem. Soc., **77**, 1654 (1955).

Periodate oxidation of V (Table II)——The procedures are similar with that employed in the oxidation of III.

Methyl 3-O-Methyl-Δ^2-D-glucuronate (VIII)——III (387 mg.) was dissolved in MeOH (20 ml.) and the resulting solution was cooled to $-10°$ in an ice-salt bath. To this solution was added an etheric solution of diazomethane. When evolution of nitrogen ceased and the solution colored yellow, the reaction mixture was evaporated to a crystalline solid which was recrystallized from acetone, giving 112 mg. of pure VIII, m.p. 158~160° (decomp.), IR cm^{-1} : $\nu_{C=O}$ 1754, δ_{C-H} 1440 (methyl ester at C-6); $\nu_{C=C}$ 1623 cm^{-1} (厂) Rf 0.60. in paper chromatography using *n*-BuOH-EtOH-H_2O (2:1:1) as a solvent (*Anal.* Calcd. for $C_8H_{12}O_7$: C, 43.64; H, 5.49. Found : C, 43.50; H, 5.50). VIII is soluble in water, methanol, ethanol and sparingly soluble in ether.

Periodate Oxidation of VIII (Table IV)——The procedures are similar with that employed in the oxidation of III. In order to hydrolyse the formate ester, 50% H_2SO_4 (3 ml.) was added to 50 ml. of the oxidation mixture and the resulting solution was heated under reflux for 10 min. Formic acid liberated was estimated the same way with that used in above described determination of volatile acid.

Polarography of 3-Ketoglucuronic Acid——A Yanagimoto Polarograph Model PB-4 was used. The electrolytic cell and the bridges were essentially the same with that employed by Tamura and Nagano.[14] The cell was thermostated in the range of $25 \pm 0.1°$. The pH was maintained at 2.5 by use of McIlvain buffer. 3-Ketoglucuronic acid gave oxidation wave at $E_{1/2} = +0.153$ V (*vs.* S.C.E.). Wave heights are as shown in Fig. 1. L-Ascorbic acid afforded oxidation wave at $+0.175$ V (*vs.* S.C.E.) and the wave height was $i_d = 9.35$ μA at the concentration of 1.70×10^{-3} mol./L.

The authors express their gratitude to Dr. Y. Hirasaka, Chugai Pharmaceutical Co., Ltd. for his useful suggestions and to Mr. K. Yamamoto, of the Company for his skilful assistance. Thanks are also due to the same Company for the supply of the materials.

Summary

Periodate oxidation of 3-ketoglucuronic acid indicated the presence of hemiacetal ring structure and in consequence the uronic acid was assigned as 3-keto-D-glucopyranuronic acid (III), which on treatment with cation resin in methanol gave methyl furanoside (V). Enolization of 3-ketoglucuronic acid between C-2 and C-3 was confirmed by conversion of the keto-acid into the corresponding enol ether, namely methyl 3-O-methyl-D-glucopyranoenediol-(2,3)-uronate (VIII). Equilibrium between keto- and enol-form of this keto-sugar in an aqueous solution was, however, found to be inclined predominantly to the keto-form (III) by means of polarography.

14) Z. Tamura, K. Nagano : This Bulletin, **11**, 793 (1963).

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137. Minoru Sekiya and Keiichi Ito : Reaction of Amide Homologs. XIII.*1 Catalytic Hydrogenolysis of N-Acylaminomethyl and N-Arylsulfonamidomethyl Compounds.

(*Shizuoka College of Pharmacy**2)

It has been established in a recent paper[1] that formic acid reduction of N-amidomethyl compound attached to aliphatic or aromatic secondary amine introduces reductive fission at the methylene carbon bond connecting to the amido nitrogen resulting in the formation of N-methylated amine and amide.

*1 Part XII : This Bulletin, **12**, 674 (1964).

*2 Oshika, Shizuoka (関屋 実, 伊藤敬一).

1) M. Sekiya, K. Ito : This Bulletin, **12**, 677 (1964).