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A New Reductone derived from D-Glucuronolactone by Alkali. I.[1] Isolation of 3-Keto-4,5-dideoxy-*trans*-4,5-dehydro-glucuronic Acid

Meiji Kawata, Yukiko Mizutani (née Nakagawa), Nariko Shinriki,[2a] Michiya Kimura and Morizo Ishidate[2b]

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

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An aqueous solution of D-glucuronolactone (I) produces a yellow color by alkali. This yellow substance (II) was isolated from the precipitates obtained by treating I in dimethylformamide with potassium hydroxide in methanol at 70°. It had a strong reducing character, indicating the presence of oxo-ene-diol grouping in the molecule, and gave fumaric acid (VI) and 4-oxo-glutaconic acid (IV) on periodic acid oxidation. The structure of 3-keto-4,5-dideoxy-*trans*-4,5-dehydro-glucuronic acid was proposed to this new reductone.

A yellow color developed when D-glucuronolactone (I) was dissolved in aqueous sodium or potassium hydroxide solutions,[3] while a yellow substance precipitated when it was treated with the alkali in nonaqueous solvents such as methanol, ethanol or dimethylformamide. In the alkaline solution, the yellow substance exhibited an absorption band at 420 mμ, which was relatively stable under nitrogen but faded rapidly to give a band at 280 mμ on standing under air as shown in Fig. I-A and I-B. On acidification, the yellow solution turned colorless to have a band at 340 mμ as shown in Fig. I-C, and was reversibly restored to show a band at 420 mμ on addition of the alkali. The acidic solution having a band at 340 mμ reduced promptly iodine or 2,6-dichloroindophenol sodium and produced a violet color with ferric chloride[4] to point out the presence of –CO–C(OH)=C(OH)– grouping.[5]

These properties suggest the formation of a new reductone which is different from the known reductones (λ_{max} 310 mμ in alkaline and λ_{max} 266 mμ in acidic solution) derived from pentose and hexose by alkali.[6]

Treatment of I in dimethylformamide with a methanolic potassium hydroxide at 70° gave hygroscopic yellow precipitates containing potassium glucuronate as a main product, which was removed by treating them with small amount of water. The aqueous solution of the precipitates freed from most part of the by-product was acidified to pH 5.6 to afford a deep yellow crystalline compound I, decomp. 139—140°, in 6% yield, which had the formula corresponding to $C_6H_6O_5 \cdot C_6H_5O_5K$ from the result of analysis of K and of titration as a weak acid. Treat-

1) A part of this work was published before as a Communication to the Editor. M. Ishidate, M. Kimura and M. Kawata, *Chem. Pharm. Bull.* (Tokyo), **11**, 1083 (1963).

2) Location: *Nishi-6-chome, Kita-12-jo, Sapporo*; *a*) Present address: *Government Industrial Development Laboratory, Higashi-tsukisamu, Sapporo*; *b*) Present address: *National Institute of Higienic Sciences, Tamagawayoga-machi, Setagaya-ku, Tokyo.*

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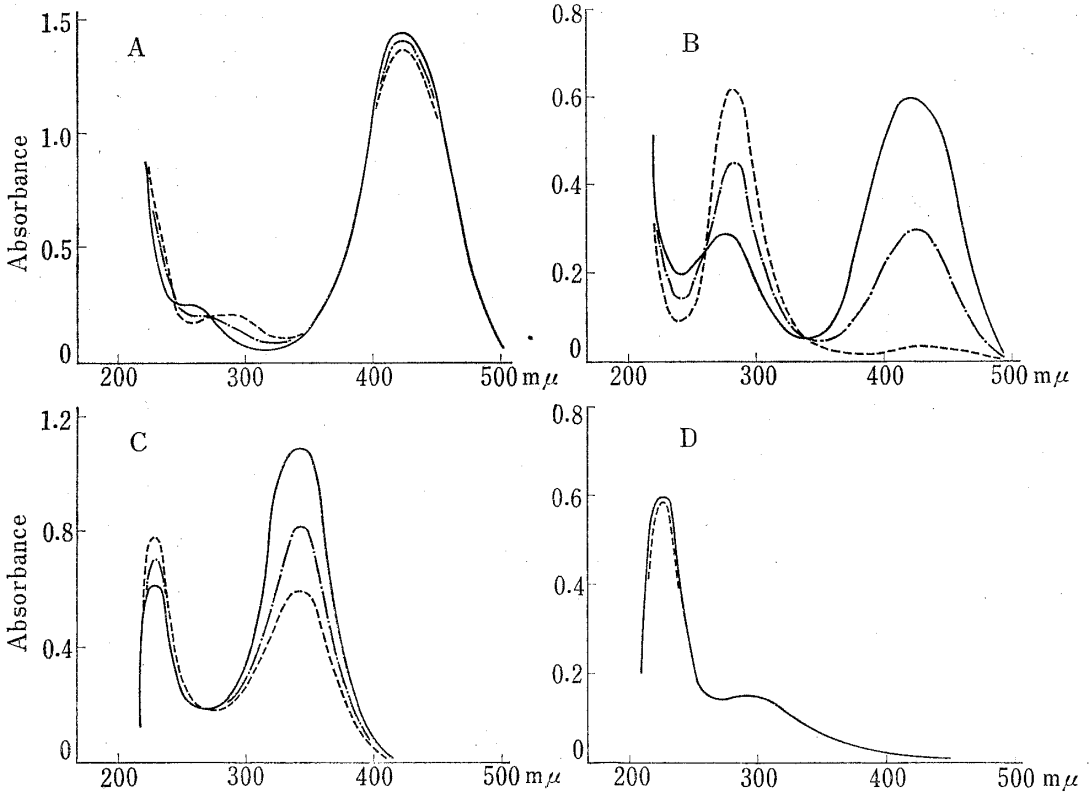

Fig. 1. Absorption Spectra of the Yellow Substance Derived from D-Glucuronolactone by Alkali

A: 5 mg of D-glucuronolactone in 3 ml of 0.1N NaOH under nitrogen (— • —: 1 min, ———: 2.5 min, ---------: 10 min).
B: 10 mg of D-glucuronolactone in 3 ml of 0.1N NaOH under air (———: 0.5 min, — • —: 1.5 min, ---------: 3.5 min).
C: 5 mg of D-glucuronolactone in 3 ml of 0.1N NaOH was kept at 20° for 10 min under nitrogen and acidified by addition of 0.05 ml of conc. HCl (———: 0.5 min, — • —: 10 min, ---------: 20 min).
D: 5 mg of D-glucuronolactone in 3 ml of 0.1N NaOH was kept at 20° for 6.5 min under air followed by addition of 0.05 ml of conc. HCl (---------: 0.5 min, ———: 6 min).

ment of the compound I with concentrated hydrochloric acid gave yellow needle crystals (II), $C_6H_6O_5$, $[\alpha]_D$ 0° (c=10, methanol), mp 163° (decomp.), which exhibited an absorption band at 420 mμ in alkaline and at 340 mμ in acidic solution, thus showing the identical absorption bands with those indicated in Fig. 1. It produced also an intensive violet color with ferric chloride. The infrared (IR) spectra of the compound I and II are shown in Fig. 2. In the regions 1600—1700 cm^{-1}, the compound I gave the absorption bands at 1624, 1655, and 1698 cm^{-1}, and II gave those at 1595, 1623, 1641, 1655 and 1682 cm^{-1}. These absorption bands are in the regions corresponding to the C=O streching vibration of α,β-unsaturated carbonyl and α,β-unsaturated carboxyl grouping or carboxylate ion respectively.

One mole of iodine or 2,6-dichloroindophenol sodium was reduced rapidly on titrating II suspended in cold water, while a far smaller amount than one

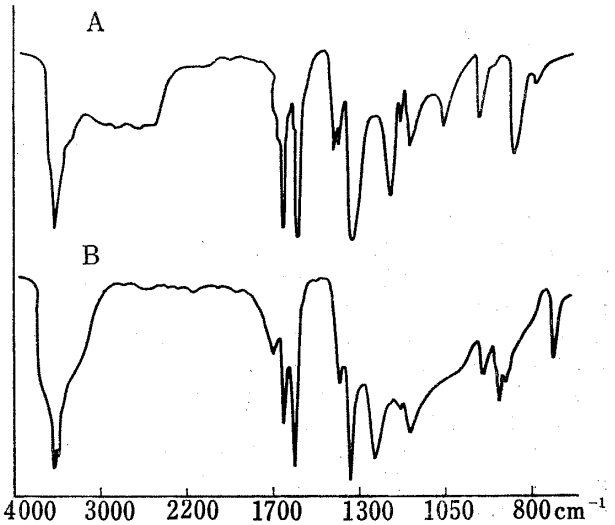

Fig. 2. Infrared Spectra of II and Compound I in KBr Disk

A: II, B: compound I

mole of these reagents was consumed when the titration was carried out after complete dissolution of II in water. Furthermore, on the titration of II suspended in cold water with sodium hydroxide, the first sharp break was observed on the addition of one equivalent of the base on account of the carboxyl group, while the second broad break appeared on adding a far less equivalent mole due to the ene–diol grouping. These facts suggest that the ene–diol form (II) is rapidly convertible into the α-ketol form (III) with a certain equilibrium in solution.

Oxidation of II with periodic acid at room temperature gave fumaric acid (VI), while at −10°, 4-oxo-glutaconic acid (IV) which was titrated as a dibasic acid and afforded phenylhydrazone (V) and (VI) on treatment with phenylhydrazine and hydrogen peroxide respectively. On the other hand, II reacted with three moles of hydroxylamine in acidic solution affording a colorless crystalline compound, $C_6H_{11}O_6N_3$, mp 149°, which was titrated as a monobasic acid. This compound is assumed to have the formula (VII) by the fact that an α,β-unsaturated carbonyl grouping is known to react with two moles of hydroxylamine to give a β-oxyamino-oxime.[7] The formation of fumaric acid on the oxidation of II suggests the *trans*–form about the ethylenic linkage. From the results obtained above it is concluded that the structure of II would be 3-keto-4,5-dideoxy-*trans*-4,5-dehydro-glucuronic acid. A possible reaction process involved in the formation of II from I by alkali is shown in Chart 1.

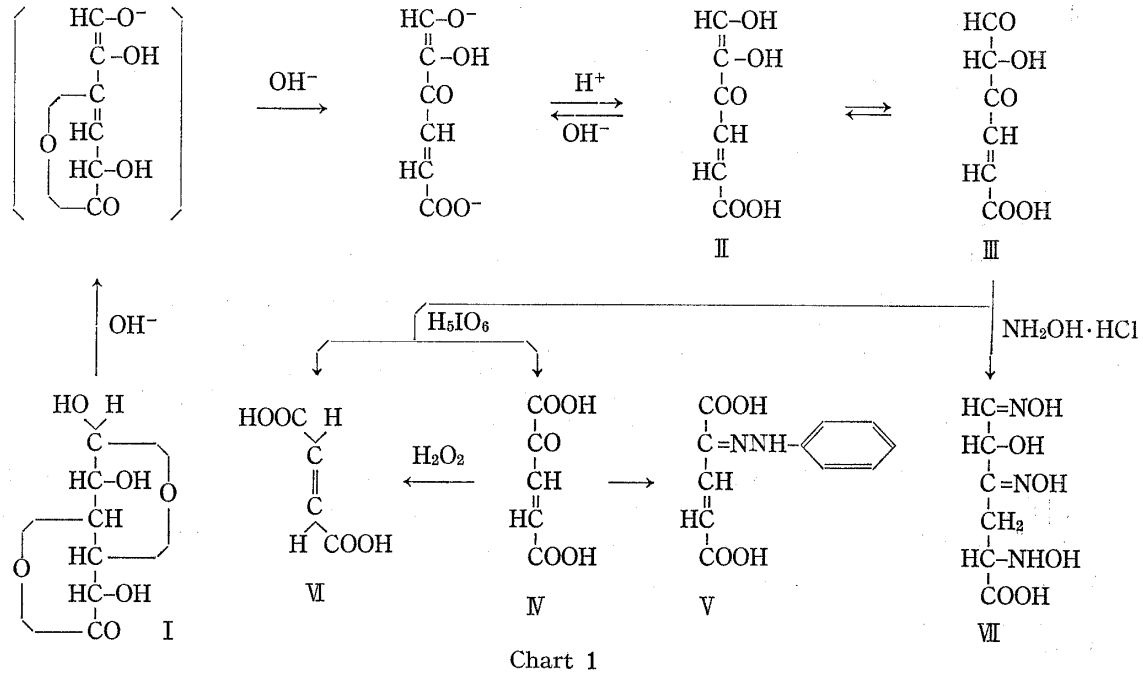

Chart 1

Experimental

Preparation of the Compound I——100 ml of 15% methanol solution of KOH was placed in 200 ml flask, and kept at 70°. 50 ml of 25% dimethylformamide solution of I was heated to 70°, and added to the above alkaline solution under stirring with the Hershberg's wire stirrer by which precipitates were finely crushed out immediately to minimize the decomposition of the compound 1. A few minutes later, the flask was cooled by an ice–salt bath. The resulting yellow precipitates were filtered, washed with MeOH, and rinsed with 20 ml of H_2O. Most potassium glucuronate included in the precipitates was removed to the aqueous layer. The remaining gum was dissolved in 50 ml of H_2O under nitrogen. On dropwise addition of concentrated HCl at 0°, the yellow substance was separated as fine needle crystals of deep yellow metallic luster, decomp. 139—140°, hardly soluble in cold H_2O and MeOH, washed with cold H_2O, and dried under reduced pressure. The yield was 0.7 g. Recrystallization from various solvents was failed due to marked decomposition. Two samples of the different preparation gave the following analyses.

7) C. Harries and F. Lehmann, *Ber.*, **30**, 320, 2726 (1897); C. Harries and L. Jablonski, *Ber.*, **31**, 1371 (1898).

1) Analysis of K. *Anal.* Calcd. for $C_{12}H_{11}O_{10}K$ ($C_6H_6O_5 \cdot C_6H_5O_5K$): K, 11.02. Found: No. 1, 11.23; No. 2, 11.39.

2) Potentiometric titration with 0.1N NaOH as a monobasic acid. 54.9 mg (No. 1) and 51.5 mg (No. 2) of the compound 1 consumed 1.517 and 1.294 ml of 0.1N NaOH respectively; Molecular weight: Calcd. for $C_{12}H_{11}O_{10}K$: 354.3. Found: 362 (No. 1) and 398 (No. 2).

Isolation of the compound 1 from both aqueous and nonaqueous alkaline solution at room temperature was failed due to its extremely low yield and rapid decomposition.

Isolation of 3-Keto-4,5-dideoxy-*trans*-4,5-dehydro-glucuronic Acid (II)——0.8 g of the compound 1 was suspended in 10 ml of concentrated HCl and kept at 0° for 10 min under stirring. The deep yellow crystals of the compound 1 changed to pale yellow. The resulting fine needle crystals were collected and washed with cold H_2O and dried. Recrystallization from dioxane gave 0.5 g of II as yellow needles, mp 163° (decomp.). $[\alpha]_D^{23}$ 0° (c=10, MeOH). *Anal.* Calcd. for $C_6H_6O_5$: C, 45.58; H, 3.83. Found: C, 45.54; H, 3.76.

Titration of II with Iodine——a) The following samples were weighed into 50 ml flasks, suspended in 20 ml of H_2O at 0°, and titrated with 0.1N iodine solution using 5% starch solution as an indicator. 50.3, 51.0 mg of II consumed 6.49, 6.22 ml of 0.1N iodine; Found mole equivalent: 1.02 and 0.99 respectively.

b) In the case that II was dissolved in H_2O or in a buffer solution of pH 7.0 before titration, 0.4 to 0.7 mole of iodine was consumed, depending on the experimental conditions, such as solvent system, temperature and titration time, indicating no distinct titration end point.

c) 50 mg of II was weighed into a glass-stoppered flask, suspended in 30 ml of H_2O, and dissolved by adding 2 ml of 2N NaOH. After addition of 15 ml of 0.1N iodine, the solution was acidified with 2 ml of 2N HCl, and titrated with 0.1N sodium thiosulfate. Mole of iodine consumed was 1.5. On addition of 0.1N iodine in this case, yellow precipitates appeared rapidly. Recrystallization from EtOH gave crystals of mp 120° which decomposed with evolution of iodine. The IR spectrum was identical with that of iodoform.

Titration of II with 2,6-Dichloroindophenol Sodium——a) Each of the following samples was suspended in 20 ml of H_2O at 0° and titrated with 0.01M 2,6-dichloroindophenol sodium which was standardized with pure L-ascorbic acid. 6.21 mg, 5.73 mg of II consumed 4.31, 3.81 ml of 0.01M 2,6-dichloroindophenol; Found mole equivalent: 1.10 and 1.05 respectively.

b) In the case that II was dissolved in H_2O or in a buffer solution of pH 7.0 before titration, 0.4 to 0.7 mole of 2,6-dichloroindophenol sodium was consumed, according to the experimental conditions as in the case with iodine.

Titration of II with 0.1N NaOH——Each of the following samples was dissolved in 30 ml of 50% MeOH. The molecular weight was estimated as a monobasic acid at the first sharp break on the titration curve with 0.1N NaOH. 30.5, 30.2 mg of II consumed 1.894 and 1.875 ml of 0.1N NaOH respectively; Molecular weight: Calcd. for $C_6H_6O_5$: 158.1. Found: 161.1 and 161.0.

Oxidation of II with Periodic Acid to Fumaric Acid (VI)——2 g of II was suspended in 10 ml of H_2O and 10 ml of 25% aqueous periodic acid solution was added dropwise at room temperature. A vigorous reaction proceeded under production of iodine vapor. After standing overnight, separated iodine was filtered off, and the resulting solution was evaporated to dryness under reduced pressure. The residual solid was recrystallized from H_2O to give 0.8 g of colorless needles sublimating at about 270°. The IR spectrum was identical with that of fumaric acid (VI). *Anal.* Calcd. for $C_4H_4O_4$: C, 41.37; H, 3.47. Found: C, 41.35; H, 3.45.

Oxidation of II with Periodic Acid to 4-Oxo-glutaconic Acid (IV)——1.5 g of finely powdered II was added to 50 ml of 3% aqueous periodic acid solution under stirring at −10° for 30 min. Separated iodine was filtered off, and the resulting solution was extracted with ether, dried over anhyd. Na_2SO_4 and evaporated under reduced pressure at room temperature. The residual solid was recrystallized from ether to give 0.2 g of IV as yellow needles, mp 125° (decomp.). *Anal.* Calcd. for $C_5H_4O_5$: C, 41.68; H, 2.80. Found: C, 41.83; H, 2.89.

Titration of IV with 0.1N NaOH: 21.3 mg of IV was dissolved in 30 ml of H_2O and titrated with 2.939 ml of 0.1N NaOH. There was only one sharp break in the titration curve, but the molecular weight was calculated as a dibasic acid, in the consideration of the elemental analysis result. Molecular weight:Calcd. for $C_5H_4O_5$: 144.08. Found: 144.9.

Reaction of IV with Phenylhydrazine——A solution of IV (0.5 g) in 10 ml of H_2O was added to 10 ml of 3% aqueous phenylhydrazine hydrochloride solution. After standing for 30 min at room temperature, the resulting precipitates were filtered off and dried. Recrystallization from 75% EtOH gave 0.5 g of V as orange-yellow needles, mp 165° (decomp.). *Anal.* Calcd. for $C_{11}H_{10}O_4N_2$: C, 56.41; H, 4.30; N, 11.96. Found: C, 56.67; H, 4.51; N, 11.98.

Titration with 0.1N NaOH: The following samples of V in 30 ml of a mixture of dimethylformamide and H_2O (1:1) were titrated with 0.1N NaOH. The molecular weight was calculated as a dibasic acid. 30.9, 33.2 mg of V consumed 2.570 and 2.723 ml of 0.1N NaOH respectively; Molecular weight: Calcd. for $C_{11}H_{10}O_4N_2$: 234.2. Found: 240.5 and 243.8.

Reaction of IV with Hydrogen Peroxide to Fumaric Acid (VI)——20 mg of IV was suspended in 5 ml of H_2O and added to 0.3 ml of 30% H_2O_2 at room temperature. A colorless product precipitated rapidly.

Recrystallization from H_2O gave needles sublimating at 270°. The IR spectrum was identical with that of fumaric acid.

Reaction of II with Hydroxylamine Hydrochloride——0.3 g of II was suspended in 10 ml of H_2O and added to a hydroxylamine hydrochloride solution (1 g in 5 ml of H_2O) under nitrogen. A clear solution was obtained in 5 min under stirring. A crystalline product was separated on standing at room temperature for 1 hr. Recrystallization from 50% EtOH gave 0.2 g of VII as colorless granular crystals, mp 149° (decomp.). The ethanol solution of VII had a blue fluorescence. *Anal.* Calcd. for $C_6H_{11}O_6N_3$: C, 32.58; H, 5.01; N, 19.00. Found: C, 32.72; H, 5.12; N, 18.34.

Titration with 0.1N NaOH: The samples of VII were dissolved 30 ml of 50% EtOH, and titrated with 0.1N NaOH as a monobasic acid. 50.8, 52.3 mg of VII consumed 2.303 and 2.383 ml of 0.1N NaOH respectively. Molecular weight: Calcd. for $C_6H_{11}O_6N_3$: 221.7. Found: 220.6 and 219.4.

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