ratio of 88.5% and 11.5% respectively, which were confirmed by ultraviolet, infrared, nuclear magnetic resonance spectra and elemental analyses. Analogously *trans*-1-methylsulfinyl-2,5-diphenyl-2-penten-4-yne (Ⅲ-a) and *cis*-1-methylsulfinyl-3,1-diphenyl-2-pentin-4-yne (Ⅲ-b) at a ratio of 84% and 16% respectively, were obtained from 1,4-diphenyl-1,3-butadiyne and the carbanion. Treatment of the *trans*-sulfoxide (Ⅲ-a) with the carbanion at room temperature gave a *cis-trans* mixture at a ratio of 84% of *trans* and 16% of *cis* isomer. Furthermore, 2,3-diphenyl-1,3-butadiene (Ⅱ) was given from diphenylacetylene and methylsulfinyl carbanion at relatively high temperature. The proposed reaction mechanism affording Ⅱ would probably involve double addition of the anion to triple bond followed by elimination of two moles of methanesulfinic acid.

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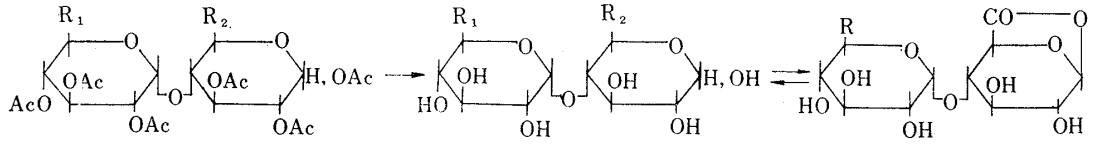
87. Yoshinobu Hirasaka and Isao Matsunaga: Studies on the α(1,4) linked Polysaccharides of D-Glucuronic Acid and D-Glucose. VIII.*1 Lactonization of the α(1,4) linked Disaccharides Containing Glucuronic Acid Residues.

(*Research Laboratory, Ukima Plant, Chugai Pharmaceutical Co., Ltd.**2)

In the previous papers,[1~4] the authors synthesized the three types of the α(1,4) linked disaccharides containing glucuronic acid residues: namely, 4-O-(α-D-glucopyranosiduronyl)-D-glucose (UG, Ⅶ), 4-O-(α-D-glucopyranosyl)-D-glucuronic acid (GU, Ⅷ) and 4-O-(α-D-glucopyranosiduronyl)-D-glucuronic acid (UU, Ⅸ) by oxidation of the primary alcohols of 1,2,3,6,2′,3′,4′-hepta-O-acetylmaltose (Ⅰ), 1,2,3,2′,3′,4′,6′-hepta-O-acetylmaltose (Ⅱ) and 1,2,3,2′,3′,4′-haxa-O-acetylmaltose (Ⅲ), followed by deacetylation, respectively (Chart 1).



Ⅰ: $R_1=-CH_2OH$, $R_2=-CH_2OAc$
Ⅱ: $R_1=-CH_2OAc$, $R_2=-CH_2OH$
Ⅲ: $R_1=R_2=-CH_2OH$
Ⅳ: $R_1=-CO_2H$, $R_2=-CH_2OAc$
Ⅴ: $R_1=-CH_2OAc$, $R_2=-CO_2H$
Ⅵ: $R_1=R_2=-CO_2H$
Ⅶ: $R_1=-CO_2H$, $R_2=-CH_2OH$ (UG)
Ⅷ: $R_1=-CH_2OH$, $R_2=-CO_2H$ (GU)
Ⅸ: $R_1=R_2=CO_2H$ (UU)
Ⅹ: $R=-CH_2OH$ (GUL)
Ⅺ: $R=-CO_2H$ (UUL)

Chart 1.

*1 Part Ⅶ. Y. Hirasaka, I. Matsunaga: This Bulletin, **13**, 176 (1965).
*2 Ukima-cho, Kita-ku, Tokyo (平坂義信, 松永 功).

1) Y. Hirasaka: Yakugaku Zasshi, **83**, 960 (1963).
2) Y. Hirasaka, I. Matsunaga, K. Umemoto, M. Sukegawa: *Ibid.*, **83**, 966 (1963).
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4) Y. Hirasaka, I. Matsunaga: This Bulletin, **13**, 176 (1965).

The present report describes one of the interesting properties of these disaccharides, that is, lactonization of the reducing glucuronic acid residues.

These disaccharides afforded the similar Rf values in paper chromatography, being about 0.08 when the upper layer of a mixture of *n*-buthanol, acetic acid and water (4:1:5) was used as a solvent.

But when the aqueous solutions of these disaccharides were stirred at room temperature with a cation exchange resin, then the unknown substances were formed GU and UU (Fig. 1), and these unknown substances with higher Rf values gave an intensive color by spraying an alkaline hydroxylamine solution followed by an acidic ferric chloride soltion, indicating the presence of lactone or ester groups.

Moreover, these unknown substances were easily converted to GU and UU by neutralization. Paper electrophoresis of these mixtures in an acetate buffer (pH, 4.64) indicated the presence of the lactone derivatives, as was shown in Fig. 2a.

But when an alkaline borate buffer (pH, 10.0) was used as a solvent, then the lactone derivatives disappeared, being similar to D-glucuronolactone (Fig. 2b). These results suggest that GU or UU was partly lactonized.

Any of disaccharides containing glucuronic acid residues in lactone form have not yet been reported in the literature.

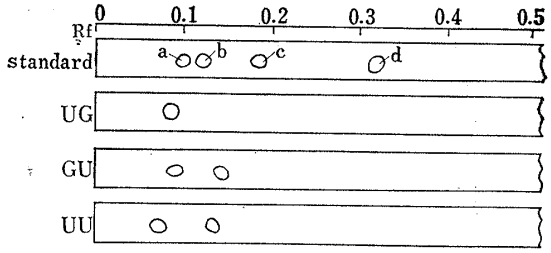
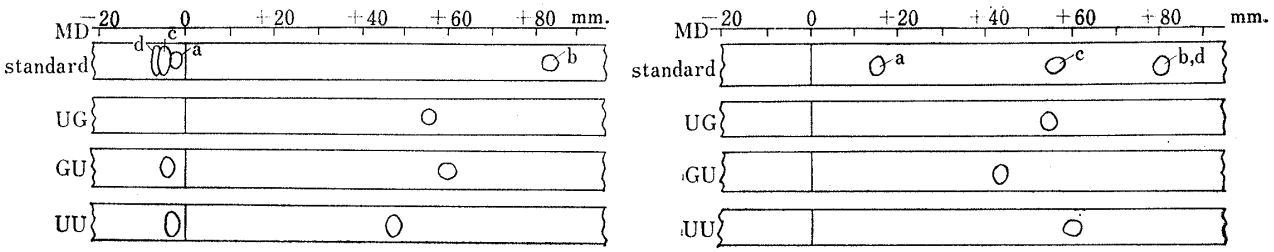


Fig. 1. Paper Chromatogram of UG, GU and UU after their Aqueous Solutions were stirred with a Cation Exchange Resin

a : maltose b : D-glucuronic acid
c : D-glucose d : D-glucuronolactone
Solvent : *n*-BuOH-AcOH-H_2O (4:1:5) upper layer
Spray reagent : Anisidine HCl



2a. Buffer solution : 0.1*M* Acetate buffer (pH 4.64)
Condition : 600 v., 0.5 mA/cm., 4 hr.

2b. Buffer solution : 0.1*M* Borate buffer (pH 10)
Condition : 600 v., 1.6 mA/cm., 4 hr.

Fig. 2. Paper Electrophoresis of UG, GU and UU

Spray reagent : Anisidine HCl
a : maltose b : D-glucuronic acid c : D-glucose d : D-glucuronolactone

It is well known that D-glucuronic acid is partly lactonized in aqueous solution to yield D-glucofuranurono-6,3-lactone, but great steric hindrance is conceivable in assuming this configuration to the $\alpha(1,4)$ linked disaccharide.

In addition, from the fact that the above lactonization was not observed in the case of UG, and that tri-O-acetyl D-glucopyranurono-6,1-lactone is derivable from 1,2,3,4-tetra-O-acetyl D-glucuronic acid, the above lactonization appears to occur between the position of carbon 6 and 1 of the reducing glucuronic acid residue.

The higher Rf substances (GUL, X and UUL, XI) were purely separated from GU and UU respectively by cellulose column chromatography and they were derived into their acetates. The result of thin-larer chromatography of these acetates was given in Fig. 3.

Furthermore the authors attempted to lactonize the acetates of GU and UU (V and VI) by refluxing in chloroform in the presence of stannic chloride according to the

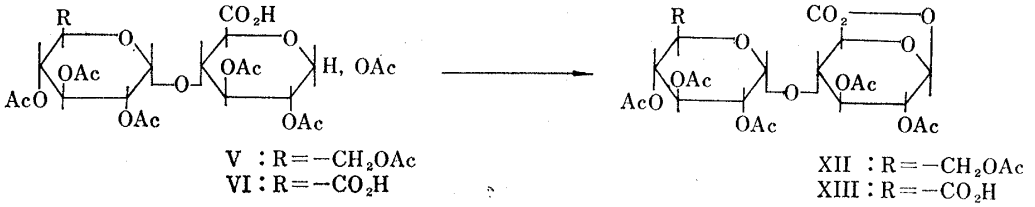


Chart 2.

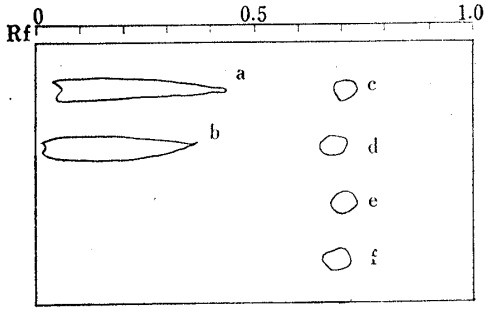


Fig. 3. Thin-layer Chromatogram of the Acetates of GUL and UUL

a) hepta-O-acetyl-4-O-(α-D-glucopyranosyl)-D-glucuronic acid (V)
b) hexa-O-acetyl-4-O-(α-D-glucopyranosiduronyl)-D-glucuronic acid (VI)
c) lactonized product of V (XII)
d) lactonized product of VI (XIII)
e) acetylated product of X
f) acetylated product of XI

Solvent: Toluene-acetone-EtOH (3:1:1)
Adsorbent: Silica gel (Merck)

Fry's procedure[5] to produce tri-O-acetyl D-glucurono-6,1-lactone, and the obtained lactones (XII, XIII) were found to be entirely identical with the acetates derived from GUL and UUL, respectively (Fig. 3).

In addition, purely separated GUL and UUL were methylated with methyl iodide and silver oxide and thereafter hydrolyzed, in order to substantiate the position of the lactone ring.

As was shown in Fig. 4, an approximately same amount of 2,3-dimethylglucuronic acid and 2,3,4,6-tetramethylglucose (or 2,3,4-trimethylglucuronic acid) was found by paper chromatography of the hydrolyzate of methylated GUL and UUL, respectively.

Thus it was confirmed that the lactonization occurred between the position of the carbon 6 and 1 of the reducing glucuronic acid residue.

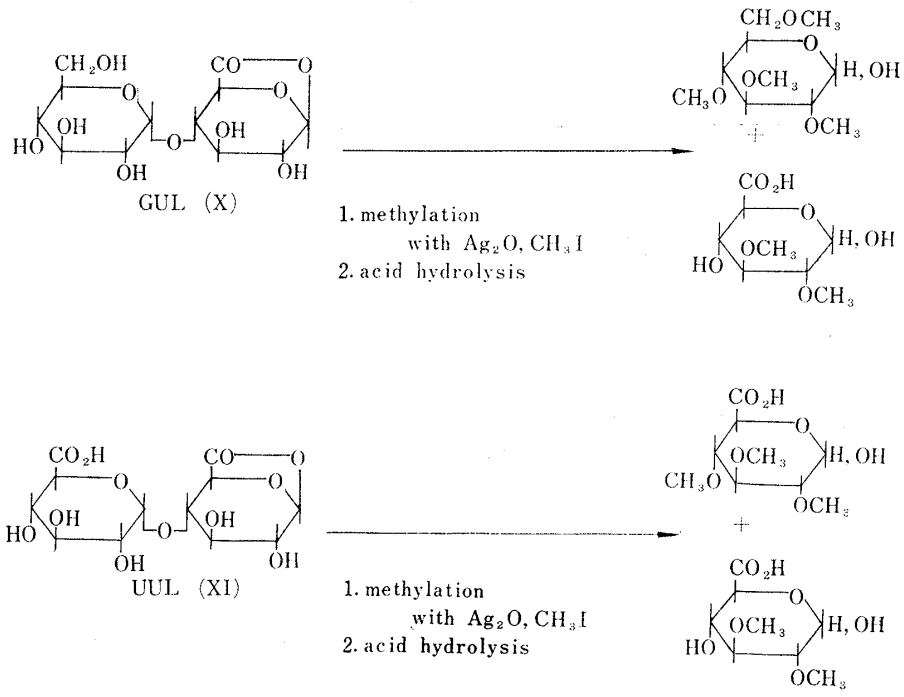


Chart 3.

5) E. M. Fry: J. Am. Chem. Soc., 77, 3915 (1955).

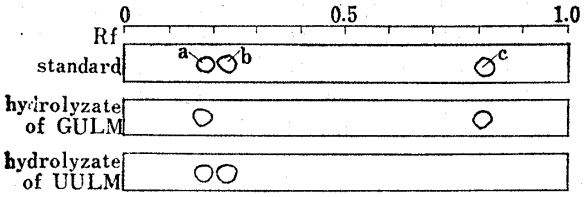


Fig. 4. Paper Chromatogram of the Methylated Sugars

a : 2,3-dimethylglucuronic acid
b : 2,3,4-trimethylglucuronic acid
c : 2,3,4,6-tetramethylglucose
Solvent : *n*-BuOH-EtOH-H_2O-conc. NH_3 (40:10:46:4) upper layer
Spray reagent : Anisidine HCl

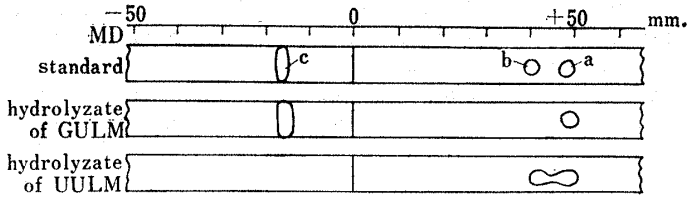


Fig. 5. Paper Electrophoresis of the Methylated Sugars

a : 2,3-dimethylglucuronic acid
b : 2,3,4-trimethylglucuronic acid
c : 2,3,4,6-tetramethylglucose
Buffer solution : 0.1*M* Borate buffer (pH 10)
Condition : 600 v., 1.6 mA/cm., 4 hr.
Spray reagent : Anisidine HCl

Experimental

All melting points are not corrected. All evaporations were carried out under reduced pressure, keeping the bath temperature below 40°.

n-BuOH-AcOH-H_2O, 4:1:5 (solvent A) and *n*-BuOH-EtOH-H_2O-conc. NH_3, 40:10:46:4 (solvent B) were used for paper chromatography and 0.1*M* borate buffer (pH 10.0) and 0.1*M* acetate buffer (pH 4.64) for paper electrophoresis (on Toyo Roshi No. 50 filter paper). The spray reagent used was anisidine HCl.

Deacetylation of Hepta-O-acetyl-4-O-(α-D-glucopyranosiduronyl)-D-glucose (IV), Hepta-O-acetyl-4-O-(α-D-glucopyranosyl)-D-glucuronic Acid (V) and Hexa-O-acetyl-4-O-(α-D-glucopyranosiduronyl)-D-glucuronic Acid (VI)——The sugar (IV, V or VI, 1.9 g.) was dissolved in dry MeOH (20 ml.) and 0.56*N* $NaOCH_3$ methanolic solution (7.8 ml.) was added under stirring at room temperature. After standing in a refrigerator overnight, the solution was neutralized by addition of Amberlite IR-120 (H) under stirring, decolorized and concentrated. Yield, 1 g. (almost quantitatively).

The Rf values of the obtained sugars in paper chromatography (solvent A) were as follows : UG, 0.07 (red); GU, 0.08, 0.14 (red); UU, 0.06, 0.13 (reddish brown) (Fig. 1).

The moving distances (mm.) of the obtained sugars in paper electrophoresis when an acidic or alkaline solvent was used were as follows :

(a) an acetate buffer (pH, 4.64), 600 v., 0.5 mA/cm., 4 hr. : UG, +57 (red); GU, +60, −4 (red); UU, +48, −4 (reddish brown).

(b) a borate buffer (pH, 10.0), 600 v., 1.6 mA/cm., 4 hr. : UG, +54 (red); GU, +43 (red); UU, +60 (reddish brown). (The used spray reagent was anisidine HCl).

Separation of GU (VIII) from its Lactone (X), and UU (IX) from its Lactone (XI) by Cellulose Column Chromatography——The sugar (about 500 mg.) obtained by deacetylation of V, was dissolved in a small amount of EtOAc-AcOH-H_2O (3:1:1) and adsorbed on cellulose column (5 g.). The column was eluted with the same solvent. The first fraction was found to be X (about 100 mg.) alone. The next fraction was found to be a mixture of VIII and X (about 300 mg.), and VIII (about 100 mg.) alone was separated in the last fraction.

IX and XI were separated similarly in the case of VIII and X.

The obtained amorphous powder, X or XI, was chromatographically pure and positive to the hydroxamic acid test.

Preparation of Hexa-O-acetyl-4-O-(α-D-glucopyranosyl)-D-glucurono-6,1-lactone (XII) from V——V (1.3 g.) was dissolved in $CHCl_3$ (30 ml.) and $SnCl_4$ (0.3 ml.) was added at 30°. A small amount of flocculent precipitate was formed. After left to stand for 40 min., the reaction mixture was poured into ice-H_2O. The $CHCl_3$ layer was neutralized with $NaHCO_3$ aq. solution, dried over Na_2SO_4, concentrated to a sirup and powdered by addition of ether-petr. ether. Yield, about 0.6 g. (about 50%). m.p. 80~85°. $[\alpha]_D^{25}$ +48.3° (c=5.46, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1} : 1814 (C=O), 1750 (C=O). *Anal.* Calcd. for $C_{24}H_{30}O_{17}$: C, 48.82; H, 5.12. Found : C, 48.64; H, 4.95.

The unreacted (V) was recovered from the H_2O layer.

Preparation of Penta-O-acetyl-4-O-(α-D-glucopyranosiduronyl)-D-glucurono-6,1-lactone (XIII) from VI——VI (1.2 g.) was dissolved in $CHCl_3$ (30 ml.) and $SnCl_4$ (0.3 ml.) was added at 30°. After left to stand for 40 min., the reaction mixture was poured ice-H_2O. The $CHCl_3$ layer was washed with H_2O, dried over Na_2SO_4 and concentrated.

The separation of produced XIII from a small amount of unreacted material (VI) was carried out by column chromatography, using silica gel (Merck) as an adsorbent and toluene-acetone-EtOH (3:1:1) as a solvent. Then XIII was separated chromatographically pure.

Separation of Acetates having Carboxylic Acid from that Lactone Groups by Thin-layer Chromatography——On separation of the acetates having free carboxylic acid and lactone groups by thin-layer chromatography, a mixture of toluene-acetone-EtOH (3:1:1) afforded a satisfactory result. As was illustrated in Fig. 3, the acetates having free carboxylic acid group (V and VI) were found to afford lower, somewhat tailing Rf values. On the contrary, the acetates having lactone group afforded higher Rf values (XII, 0.71 and XIII, 0.68).

Acetylation of X and XI——A reaction mixture of X or XI (several mg.), Ac_2O (30 drops) and conc. H_2SO_4 (2 drops) was heated at 50~60° for 10 min., poured into ice-H_2O and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, $NaHCO_3$ aq. solution, H_2O, dried over Na_2SO_4 and concentrated. As was shown in Fig. 3, the obtained acetates of X and XI were found to be entirely indistinguishable from XII and XIII respectively by thin-layer chromatography.

Identification of the Structure of X and XI——A reaction mixture of X or XI (about 100 mg.), DMF (1 ml.), CH_3I (3 ml.) and freshly prepared Ag_2O (1 g.) was stirred at 10° for 20 hr. in the dark. The filtrate was concentrated to a sirup, and the $CHCl_3$ (3 ml.) soluble part was washed with H_2O, dried over Na_2SO_4 and concentrated to a sirup. The second methylation was carried out under the same conditions. The obtained sirup (about 100 mg.) was refluxed with 4% HCl (2 ml.) for 3 hr. After precipitates were removed, the filtrate was treated with excess amount Ag_2CO_3 and then Amberlite IR-120 (H), decolorized with charcoal and concentrated to a sirup.

The Rf values in paper chromatography (solvent B) of the sirup were as follows: X, 0.82 (reddish brown, indicating the presence of 2,3,4,6-tetramethylgucose, Rf 0.81) and 0.18 (red, indicating the presence of 2,3-dimethylglucuronic acid, Rf 0.18); XI, 0.23 (red, indicating the presence of 2,3,4-trimethylglucuronic acid, Rf 0.23) and 0.17 (red, indicating the presence of 2,3-dimethylglucuronic acid, Rf 0.18).

The moving distances (mm.) in paper electrophoresis (600 v., 1.6 mA/cm., 4 hr.) of the sirup were as follows: X, +48 (red, indicating the presence of 2,3-dimethylglucuronic acid, +48) and —17 (reddish brown, indicating the presence of 2,3,4,6-tetramethylglucose, —17); XI, +40 to +47 (red, indicating the presence of 2,3,4-trimethylglucuronic acid, +40 and 2,3-dimethylglucuronic acid, +48).

The authors express their gratitude to Prof. Emeritus M. Ishidate of the University of Tokyo for his valuable advices, and to Dr. S. Hayashi, a Managing Director of the Company, for his encouragements during the work. The authors are also grateful for Miss M. Ishii of the Central Analysis Room for the elementary analysis.

Summary

The two α(1,4) linked disaccharides containing the reducing glucuronic acid residue (GU and UU) were found to tend to lactonize in aqueous solution. 4-O-(α-D-Glucopyranosyl)-D-glucurono-6,1-lactone (X) and 4-O-(α-D-glucopyranosiduronyl)-D-glucurono-6,1-lactone (XI) were purified by chromatographic separation and identified by methylation with silver oxide and methyl iodide followed by acid hydrolysis.

Moreover hepta-O-acetyl-4-O-(α-D-glucopyranosyl)-D-glucurono-6,1-lactone (XII) and penta-O-acetyl-4-O-(α-D-glucopyranosiduronyl)-D-glucurono-6,1-lactone (XIII) were obtained from hepta-O-acetyl-4-O-(α-D-glucopyranosyl)-D-glucuronic acid (V) and hexa-O-acetyl-4-O-(α-D-glucopyranosiduronyl)-D-glucuronic acid (VI), respectively.

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