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Synthesis and Elimination Reaction of *O*-Methyl Derivatives of Methyl (Methyl α-D-Glucopyranosido)uronate

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Several methyl ethers [2,3,4-tri-*O*-methyl- (**1**), 4-*O*-methyl- (**2**), 3-*O*-methyl- (**3**), and 2-*O*-methyl- (**4**)] of methyl (methyl α-D-glucopyranosido)uronate were used as model compounds for the examination of β-elimination reaction. **1** was purely synthesized from methyl α-D-glucopyranoside and both **3** and **4** were newly synthesized *via* the corresponding methyl ethers of methyl α-D-glucopyranoside. The condition of β-elimination reaction was examined using **1** as a model compound. It was shown that β-elimination reaction occurred only when an alkoxide was used as a base. The yield of the unsaturated product, *i. e.*, methyl (methyl 4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosido)uronate (**13**) was about 40—50% by sodium methoxide in methanol at 60—70°C, and 70—80% by potassium *t*-butoxide in *t*-butyl alcohol even at room temperature. In both cases the reaction was completed within several minutes. The β-elimination reaction was followed by UV spectra using the molecular extinction coefficient of purely isolated **13**, and the reaction products were examined by gas-liquid chromatography. The β-elimination product of **2** was also obtained in good yield, but that of **3** in very poor yield.

The selective cleavage of a certain glycosidic bond in heteropolysaccharides is essential for the sequential elucidation of compositional monosaccharides. For this the enzymatic cleavage is often used, but the desired enzyme is not easily available. The development of some chemical method has for long been a subject of interest.

Recently the selective cleavage of glycosidic bonds around uronic acid residues has been investigated in polyuronides and polysaccharides containing uronic acids. One investigation was carried out on the selective cleavage of glycosidic bonds by utilizing the β-elimination reaction in alkali.[1,2] Another was carried out on the selective cleavage of glycuronosyl bonds by acid hydrolysis after the Hofmann degradation of uronic

1) C. W. McCleary, D. A. Rees, J. W. B. Sammel, and I. W. Steele, *Carbohyd. Res.*, **5**, 492 (1967).

2) C. J. Lawson, C. W. McCleary, H. I. Nakada, D. A. Rees, I. W. Sutherland, and J. F. Wilkinson, *Biochem. J.*, **115**, 947 (1969).

acid amide residues, derived from uronic acid esters.[3]

It is well known that polyuronides such as pectin depolymerize in neutral or alkaline solution,[4] and this was applied to obtain a few depolymerized polysaccharides.[5] Thus, the β-elimination reaction seems to be useful for the structural analysis of naturally occurring uronic acid-containing polysaccharides. However, in aqueous solution the reaction proceeds only slightly owing to competing de-esterification, and the cleavage of the glycosidic bonds might occur only when they are 1→4 linkages to hexopyranuronoside structure.

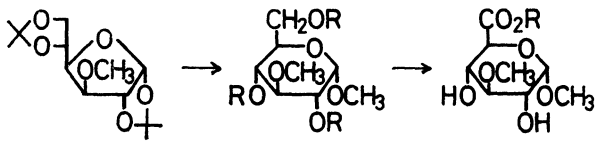
Heim and Neukom[6] reported that methyl (methyl α-D-galactopyranosido)uronate and methyl 4-*O*-(methyl α-D-galactopyranosyluronate)-(methyl α-D-galactopyranosido)uronate underwent the elimination reaction when treated with sodium methoxide in methanol, but details of the reaction were not presented. This reaction was applied by McCleary *et al.*[1] to partial depolymerization of 2-hydroxyethyl alginate, and recently by Lawson *et al.*[2] to structural analysis of colanic acid. On the other hand, Fujinaga and Matsushima[7] reported the degradation of methyl 4′,6′-*O*-ethylidene-β-pseudocellobiouronoside methyl ester by the treatment of hydrazine, conceivably due to β-elimination reaction.

For more efficient application of this β-elimination reaction to structural elucidation of uronic acid-containing polysaccharides, we examined the β-elimination reaction using several synthesized methyl ethers [2,3,4-tri-*O*-methyl- (**1**), 4-*O*-methyl- (**2**), 3-*O*-methyl- (**3**), and 2-*O*-methyl- (**4**)] of methyl (methyl α-D-glucopyranosido)uronate as model compounds. Possibility of a further cleavage of 1→2 glycosidic bond by δ-elimination reaction of once formed unsaturated compounds as shown in the case with picroclocin[8] was also examined.

Results and Discussion

Methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosido)uronate (**1**) was synthesized by the method of Jones[9] from D-glucurono-6,3-lactone. This was fairly contaminated with β-anomer, which was ascertained by gas-liquid chromatography and NMR spectrum. **1** was synthesized purely from methyl α-D-glucopyranoside by catalytic oxidation and methylation in the usual manner.

Methyl (methyl 4-*O*-methyl-α-D-glucopyranosido)-uronate (**2**) was synthesized by the method of Wacek.[10] Methyl (methyl 3-*O*-methyl-α-D-glucopyranosido)uronate (**3**) newly synthesized as shown in Fig. 1. 1,2:



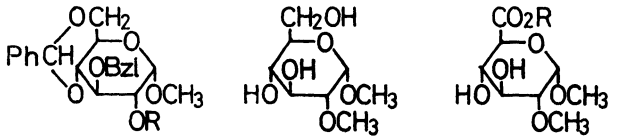
5 6, R = H 8, R = H
7, R = Ac 3, R = CH3

Ac=acetyl

Fig. 1

5,6-Di-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (**5**)[11] was converted to syrupy methyl 3-*O*-methyl-α-D-glucopyranoside (**6**) by refluxing in methanolic hydrogen chloride for 20 hr. In order to purify by distillation, acetylation of **6** was performed, and deacetylation and catalytic oxidation were carried out in one step.

Methyl (methyl 2-*O*-methyl-α-D-glucopyranosido)-uronate (**4**) was also newly synthesized from 3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose,[12] which was methanolized by refluxing in methanolic hydrogen chloride to give syrupy methyl 3-*O*-benzyl-α-D-glucopyranoside. This was converted to 4,6-*O*-benzylidene derivative (**9**), and then to 2-*O*-methyl derivative (**10**) in the usual manner. **10** was debenzylidenated and debenzylated by catalytic hydrogenolysis to give syrupy methyl 2-*O*-methyl-α-D-glucopyranoside (**11**),[13] and **11** gave methyl 2-*O*-methyl-α-D-glucopyranosidouronic acid (**12**) by catalytic oxidation, which was converted to its methyl ester (**4**) with diazomethane (Fig. 2). The



9, R = H 11 12, R = H
10, R = CH3 4, R = CH3

Ph=phenyl Bzl=benzyl

Fig. 2

anomeric configuration of all synthesized *O*-methyl derivatives of methyl (methyl D-glucopyranosido)uronate was ascertained to be α by NMR spectra and optical rotations. However, in the case of **3** and **4** slight contamination with β-anomer was observed (*ca.* 10%), because the glycosidation products could not be crystallized.

First of all, the condition of the β-elimination reaction was examined using **1** as a model compound in some basic mediums, *i. e.*, aqueous solutions containing sodium hydroxide or sodium carbonate, or alcoholic solutions containing sodium hydroxide or sodium methoxide. The expected elimination reaction was observed only when sodium methoxide was used as a base. In other cases it was observed by thin-layer chromatography

3) N. K. Kochetkov, O. S. Chizhov, and A. F. Sviridov, *Carbohed. Res.*, **4**, 362 (1967); N. K. Kochetokov, O. S. Chizhov, and A. F. Sviridov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, **1968**, 2089.

4) P. Albersheim, H. Neukom, and H. Deuel, *Arch. Biochem. Biophys.*, **90**, 46 (1960).

5) R. W. Stddart, A. J. Barrett, and D. H. Northcote, *Biochem. J.*, **102**, 194 (1967).

6) P. Heim and H. Neukom, *Helv. Chim. Acta*, **45**, 1735, 1737 (1962).

7) M. Fujinaga and Y. Matsushima, This Bulletin., **40**, 1706 (1967).

8) R. Kuhn and I. Löw, *Ber.*, **74**, 219 (1941).

9) W. J. G. Jones, L. N. Owen, and S. Peat, *J. Chem. Soc.*, **1941**, 339.

10) A. Wacek, *Monatsh. Chem.*, **90**, 562 (1957).

11) I. E. Muskat, *J. Amer. Chem. Soc.*, **56**, 2449 (1934).

12) R. Kuhn, I. Löw, and H. Trischmann, *Chem. Ber.*, **90**, 203 (1957).

13) W. N. Hawarth, E. L. Hirst, and E. G. Teece, *J. Chem. Soc.*, **1931**, 2858.

that methyl uronate were converted to the corresponding sodium uronates and no elimination was observed.

The elimination reaction was followed by measurement of the NMR and UV spectra at suitable intervals. As the elimination reaction proceeded, in NMR spectra measured in CD_3ONa/CD_3OD a signal assigned to the vinyl proton appeared, whose intensity increased to a certain maximum value and then decreased slowly. This is attributed to the exchange between hydrogen and deuterium. Thus the intensity of the vinyl proton was unsuitable for quantitative discussion. In UV spectra, the absorption of α,β-unsaturated ester appeared at 235—240 mμ, and the optical density at this wavelength increased. The elimination reaction was followed by UV spectra in a suitable solvent.

In order to determine the molecular extinction coefficient of the elimination product, *i. e.*, methyl (methyl 4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosido)uronate (**13**), its isolation was attempted. **1** was treated with sodium methoxide in methanol and refluxed for 45 min. The reaction mixture was treated with a strongly acidic resin (Amberlite IR-120 H-form) and evaporated to give a slightly yellowish syrup. **13** was obtained in about 35% yield by distillation of the syrup. The structure was ascertained by UV, IR and NMR spectra (Fig. 3).

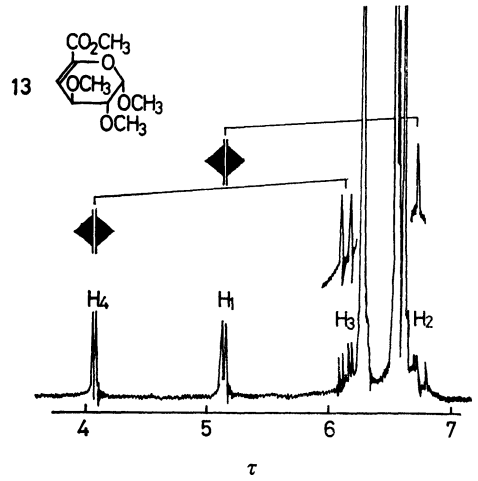


Fig. 3. NMR spectrum of **13** (100 MHz, in CCl_4).

The elimination reaction was carried out with sodium methoxide in methanol at 60—70°C and the optical density at 238 mμ was measured after partial neutralization of the reaction mixture with ethanolic hydrogen chloride. The amount of **13** was then calculated by using the molecular extinction coefficient of **13** (ε_{max}= 5500). Under these conditions the yield of **13** was about 40% (Fig. 4, curve 1). However, this increased to about 55% by direct addition of sodium to a methanol solution of **1** (Fig. 4, curve 2). The yield of the elimination product of **2** was also 50% under the same conditions (Fig. 4, curve 3). In these cases the reaction proceed to completion within 5 min. The yield could not be increased by elongation of the reaction time and elevation of the reaction temperature.

A more effective and moderate reaction condition was sought by using a stronger base, potassium *t*-butoxide. The reaction was carried out with freshly prepared potassium *t*-butoxide in freshly distilled *t*-butyl alcohol and the amount of **13** formed by the elimination reaction was determined by the optical density at 238 mμ after neutralization with ethanolic hydrogen chloride and dilution with ethanol. As shown in Fig. 4, the elimination reaction proceeded instantaneously even at room temperature and in a few minutes reached about 70—80% (curves 4 and 5). This reaction limit was not altered by elongation of the reaction time and heating. It is noteworthy that the yield decreased in about 40—50%, when stored *t*-butyl alcohol for a few days after distillation was used (curve 6). In order to investigate the nature of the reaction in detail, the reaction mixture was examined by gas-liquid chromatography after neutralization. As shown in Fig. 5, three peaks appeared in the chromatogram. The first and smallest peak was identified as the starting material (**1**),

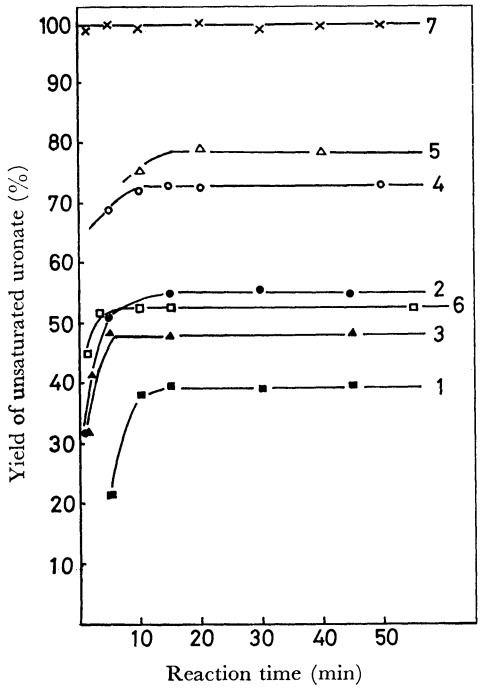


Fig. 4. Elimination reaction of **1**, **2**, and **13**.
1: **1**, CH_3ONa, 70°C
2: **1**, Na, 64°C
3: **2**, Na, 64°C
4: **1**, *t*-C_4H_9OK
5: **1**, *t*-C_4H_9OK
6: **1**, *t*-C_4H_9OK (stored *t*-butyl alcohol was used.)
7: **13**, *t*-C_4H_9OK

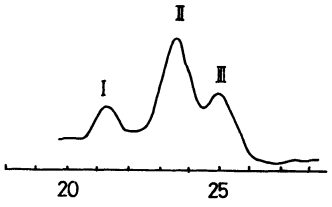


Fig. 5. Gas-liquid chromatogram of reaction mixture of **1**.
I: **1**
II: de-esterified **1** and **13**
III: **13**

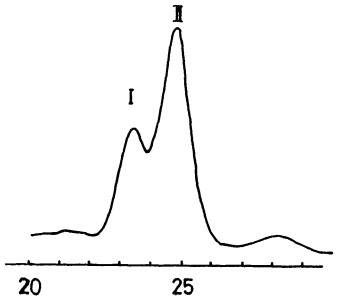


Fig. 6. Gas-liquid chromatogram of reaction mixture of **13**.
I: de-esterified **13**
II: **13**

the second and largest one as a mixture of methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosidouronic acid and methyl 4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosidouronic acid (de-esterified **1** and **13**), and the last as **13**. It is remarkable that the ester groups of both the unchanged starting material and reaction product were partially hydrolyzed during the reaction. Comparing the result of the treatment of **13** with potassium *t*-butoxide in *t*-butyl alcohol (Fig. 6), it is suggested that the elimination reaction did not proceed completely, because of hydrolysis of a certain part of the starting material. Such hydrolysis took place probably by the moisture introduced during the operation.

No marked peak, other than the above mentioned three peaks, was detected, which suggests that the δ-elimination reaction did not occur. Both at room and elevated temperature no component other than **13** and its de-esterified derivative was detected in the reaction mixture by gas-liquid chromatography (Fig. 6). This was also ascertained by the fact that the optical density at 238 mμ of **13** was not altered by treatment with potassium *t*-butoxide (Fig. 4, straight line 7).

The elimination reaction of the synthesized model compound **2** and **3** was then attempted. It is predicted that water liberated as the reaction proceeds inhibits the elimination reaction by de-esterification. After treatment with potassium *t*-butoxide in *t*-butyl alcohol for 10 min at room temperature the reaction solution was passed through the column packed with an acidic resin. The syrup obtained by evaporation of the solvent was purified once or twice on an alumina column. However, a small amount of impurity detected in NMR spectra was involved in both products. Although methyl (methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosido)uronate (**14**) was obtained from **2** in very good yield (80%), from **3** de-esterified methyl 4-deoxy-3-*O*-methyl-β-L-threo-hex-4-enopyranosidouronic acid (**15**) was obtained in every low yield (a few %). The low yield of **15** will be attributed to adsorption on the alumina column, since its formation in about 20—30% was estimated by UV spectra.

Experimental

Melting points are uncorrected. Specific rotations were measured with a Carl Zeiss LEP Al polarimeter and a 0.2 dm tube. The IR spectra were recorded with a Hitachi Model EPI-GS grating IR spectrophotometer. The NMR spectra were recorded at 100 MHz with a Japan Electron Optics JNM 4H-100 spectrometer for solutions in suitable solvents such as $CDCl_3$, CCl_4, and CD_3OD containing tetramethylsilane as the internal standard. The UV spectra were recorded with a Hitachi Perkin-Elmer 139 spectrophotometer and a Hitachi Model EPS-3T recording spectrophotometer. Thin-layer chromatography was performed with Wako gel G-O and the solvent system [A: ethyl acetate - acetic acid - formic acid - water (18 : 3 : 1 : 4), B: *n*-butanol - acetic acid - water (4 : 1 : 5), C: chloroform - methanol - acetic acid (9 : 1 : 1)]. Gas-liquid chromatography was performed with a Hitachi Model K-53 gaschromatograph using a column (1 m) packed with butanediol succinate (20%) on Chromosorb WAW (60—80 mesh) at 150—190°C.

General Method of Methylation. Methylation was carried out by the methods of Purdue and Irvine,[14] and Kuhn *et al.*[15] To a solution of starting material in chloroform or dimethylformamide was added silver oxide (1.2 parts), Drierite (15—20 g/100 m*l* of solvent), and finally methyl iodide dropwise under stirring and cooling in an ice-bath. Stirring was continued at room temperature for 2—4 days in the dark and the undissolved materials were filtered off. Evaporation of the filtrate gave methylated derivative.

General Method of Catalytic Oxidation. Into a suspended solution of starting material and the same amount of 10% platinum-charcoal or palladium-charcoal in water was bubbled oxygen (1.5—2.0 atm) at 80—100°C under vigorous stirring. The pH-value of the solution was maintained between 6 and 8 by addition of a sodium hydrogencarbonate solution during the reaction. After disappearance of the spot of the starting material on thin-layer chromatography, *i. e.*, after 20—30 hr, the catalyst was filtered off and the filtrate was evaporated to give the corresponding sodium uronate. The free uronic acid derivative was obtained by treatment of this salt on a column packed with Amberlite IR-120 or Dowex 50 W×8 (H-form).

Methyl (Methyl 2,3,4-Tri-O-methyl-α-D-glucopyranosido)uronate (1). *Method (A)*: This compound was synthesized by the method of Jones *et al.*[9] from D-glucurono-6,3-lactone. Bp 88—90°C/0.05 mmHg, $[\alpha]_D^{28}$ +87° (*c* 1.0, methanol); lit,[9] bp 120°C/0.02 mmHg, $[\alpha]_D$ +84° (*c* 1.0, methanol).

Method (B): Methyl α-D-glucopyranoside (12.4 g) was catalytically oxidized to give methyl α-D-glucopyranosidouronic acid, which was methylated by the method of Kuhn *et al.*[15] to give **1** in 35% yield (5.6 g). Bp 94—97°C/0.18 mmHg, $[\alpha]_D^{23}$ +140° (*c* 1.2, methanol).

Found: C, 50.33; H, 7.46%. Calcd for $C_{11}H_{20}O_7$: C, 49.99; H, 7.63%.

Methyl (Methyl 4-O-Methyl-α-D-glucopyranosido)uronate (2). This compound was synthesized by the method of Wacek.[10] Bp 140°C/0.15 mmHg, $[\alpha]_D^{23}$ +135° (*c* 1.0, methanol); lit,[10] bp 130—140°C/0.6 mmHg, $[\alpha]_D$ +145° (methanol).

Methyl (Methyl 3-O-Methyl-α-D-glucopyranosido)uronate (3). Methyl 2,4,6-tri-*O*-acetyl-3-*O*-methyl-α-D-glucopyranoside[16] (**7**, 5.0 g) was converted by catalytic oxidation to syrupy methyl 3-*O*-methyl-α-D-glucopyranosidouronic acid (**8**), which was treated with methanolic hydrogen chloride overnight. After evaporation of the solvent, the residual syrup was distilled to give **3** in 68% yield (2.4 g). Bp 137°C/0.07 mmHg,

14) T. Purdue and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).
15) R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
16) R. W. Jeanloz and M. Gut, *J. Amer. Chem. Soc.*, **76**, 5793 (1954).

$[\alpha]_D^{23}$ +98.5° (*c* 1.1, methanol).

Found: C, 45.97; H, 7.09%. Calcd for $C_9H_{16}O_7$: C, 45.76; H, 6.83%.

Methyl 3-O-Benzyl-4,6-O-benzylidene-α-D-glucopyranoside (9). 3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose[12] (30 g) was dissolved in methanol containing 3% hydrogen chloride (200 m*l*) and the solution was refluxed for 20 hr. After neutralization with barium carbonate, undissolved material was filtered off and the filtrate was evaporated to dryness. The resulting syrup was benzylidenated with benzaldehyde and anhydrous zinc chloride in the usual manner. The crude product was crystallized from ethanol. Yield, 9.2 g (29%), mp 172—174°, $[\alpha]_D^{23}$ +30.4° (*c* 1.1, methanol).

Found: C, 67.55; H, 6.58%. Calcd for $C_{21}H_{24}O_6$: C, 67.73; H, 6.50%.

Methyl 3-O-Benzyl-4,6-O-benzylidene-2-O-methyl-α-D-glucopyranoside (10). **9** (9.0 g) was methylated by the method of Purdue and Irvine.[14] Yield, 7.8 g (83%), mp 103—104°C, $[\alpha]_D^{23}$ +16.7° (*c* 1.5, ethanol).

Found: C, 68.63; H, 7.00%. Calcd for $C_{22}H_{26}O_6$: C, 68.63; H, 6.78%.

Methyl (Methyl 2-O-Methyl-α-D-glucopyranosido)uronate (4). Methyl 2-*O*-methyl-α-D-glucopyranoside[13] (**11**, 4.4 g), which was obtained by catalytic hydrogenolysis of **10**, was catalytically oxidized in the presence of platinum-charcoal to give syrupy methyl 2-*O*-methyl-α-D-glucopyranosidouronic acid (**12**). **12** was esterified with diazomethane in ether - methanol. After evaporation of the solvent the residual syrup was distilled to give **4** in 33% yield (1.6 g). Bp 152°C/0.095 mmHg, $[\alpha]_D^{23}$ +98° (*c* 1.0, methanol).

Found: C, 45.17; H, 6.69%. Calcd for $C_9H_{16}O_7$: C, 45.76; H, 6.83%.

Methyl (Methyl 4-Deoxy-2,3-di-O-methyl-β-L-threo-hex-4-enopyranosido)uronate (13). **1** (5.2 g) was dissolved in methanol containing 1.5 N sodium methoxide (30 m*l*). After refluxing for 45 min the reaction mixture was treated with Amberlite IR-120 (H-form) and evaporated to dryness. The residual syrup was distilled to give **13** in 35% yield (1.6 g). Bp 75—82°C/0.03 mmHg, $[\alpha]_D^{23}$ +189° (*c* 1.0, methanol), IR (NaCl, cm^{-1}): 2940, 1725, 1645, UV (ethanol, mμ): 238 (ε=5500), NMR (CCl_4, τ): 4.07 (d, H_4), 5.13 (d, H_1), 6.13 (q, H_3), 6.68 (q, H_2), 6.27 (s, CO_2CH_3), 6.54, 6.55, 6.61 (three s, OCH_3), $J_{1,2}$ 2.0 Hz, $J_{2,3}$ 7.5 Hz, $J_{3,4}$ 2.5 Hz (see also Fig. 3).

Found: C, 51.24; H, 6.84%. Calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94%.

Methyl (Methyl 4-Deoxy-β-L-threo-hex-4-enopyranosido)uronate (14). To a solution of **2** (3 g) in *t*-butyl alcohol (20 m*l*) was added quickly potassium (680 mg) in *t*-butyl alcohol (50 m*l*) under nitrogen stream. After being shaken gently for 10 min the reaction mixture was neutralized with methanolic hydrogen chloride and evaporated under reduced pressure at room temperature. The residual syrup was dissolved in a small amount of methanol and evaporated. The resulting syrup was then dissolved in ethanol and undissolved potassium chloride was filtered off. Evaporation of the filtrate gave crude **14**. Purification was performed on an alumina column (30 g) by elution with petroleum ether - ethanol (95 : 5, 400 m*l*) and then with methanol - water (7 : 3, 50 m*l*). Evaporation of the latter eluate gave **14** in about 80% yield (2.2 g). $[\alpha]_D^{23}$ +184° (*c* 1.0, methanol), IR (NaCl, cm^{-1}): 3400, 1725, 1645, UV (ethanol, mμ): 238 (ε=4600), NMR ($CDCl_3$, τ): 3.97 (d, H_4), 4.97 (d, H_1), 6.22 (s, CO_2CH_3), 6.51 (s, OCH_3).

Found: C, 46.73; H, 6.14%. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.92%.

Methyl 3-O-Methyl-4-deoxy-β-L-threo-hex-4-enopyranosidouronic Acid (15). To a solution of **3** (5 g) in *t*-butyl alcohol (20 m*l*) was added potassium (160 mg) in *t*-butyl alcohol (6 m*l*) under nitrogen stream. After standing at room temperature for 10 min the reaction mixture was treated with Amberlite IR-120 (H-form) to remove potassium ions. The syrup obtained by evaporation of the solvent was purified on an alumina column (100 g) by elution with petroleum ether - ethanol (95 : 5, 300 m*l*). Evaporation of the eluant gave crude **15** (40 mg), which was purified once more on the alumina column to give **15** in very low yield (22 mg). Elemental analysis could not be carried out but the structure was ascertained by spectral data. $[\alpha]_D^{23}$ +170° (*c* 1.0, ethanol), IR (NaCl, cm^{-1}): 3450, 1725, 1645, UV (ethanol, mμ): 238 (ε=4100), NMR ($CDCl_3$, τ): 3.97 (d, H_4), 5.03 (d, H_1), 6.42, 6.51 (two s, OCH_3).

Examination of β-Elimination Reaction in Some Basic Mediums. 1) By UV Spectra: The following three kinds of alkali solution of **1** were heated in sealed tubes at 60—95°C for about 1 hr. (a) 0.02N and 2.0N sodium carbonate aqueous solution, 0.02N, 0.5N and 2.0N sodium hydroxide aqueous solution, (b) 1.3N sodium hydroxide in ethanol, (c) 1.5N sodium methoxide in methanol. After neutralization with 1N hydrochloric acid or ethanolic hydrogen chloride the UV spectrum of the reaction solution was measured.

2) By NMR spectra: NMR spectrum was measured only in the case of (c). The reaction was carried out in an NMR measuring tube using CD_3ONa in CD_3OD as a base.

β-Elimination Reaction of 1. 1) By Sodium Methoxide in Methanol: The elimination reaction was carried out in two different ways. *Method (A)*: A solution of **1** (1.6×10^{-2}mol/*l*) and sodium methoxide (2.7N) in methanol were prepared separately. 1 m*l* of each solution was mixed in a glass tube and sealed quickly. After heating in a water bath at 70°C the tube was cooled with tap water and the reaction solution was acidified and diluted to exactly 5 m*l* with ethanolic hydrogen chloride (about 0.4N). The solution was centrifuged with 7000—8000 rpm for 10 min at 0°C and the optical density at 238 mμ was measured after dilution of the supernatant with ethanol (Fig. 4, curve 1).

Method (B): To 1 m*l* of a methanol solution of **1** (1.6×10^{-2} mol/*l*) in a glass tube (0.8×20 cm), which was previously heated at 60°C in a mantle heater, a piece of sodium (18 mg) was added. While the piece of sodium dissolved in the methanol, the reaction solution was refluxed. The reaction temperature was then maintained at 64°C by refluxing in an open tube or by heating in a sealed tube. The UV spectra were measured in the same manner as above mentioned.

2) By Potassium t-Butoxide in t-Butyl Alcohol: A solution of **1** and **13** (1.4×10^{-1} mol/*l*) and potassium *t*-butoxide (0.37N; curves 4, 6 and straight line 7 and 0.56N; curve 5) in *t*-butyl alcohol were prepared separately. The same amount of each solution was mixed as quickly as possible. 0.01 m*l* of the reaction solution was pipetted at suitable intervals and the UV spectra were measured after dilution with ethanolic hydrogen chloride to 5 m*l*.

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