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Quantitative Estimation of the Fehling Reaction of 1-*O*-Protected Uronic Acids

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Quantitative estimation of the Fehling reaction of 1-*O*-protected uronic acid derivatives showed that the reducing power of 1-*O*-protected hexofuranuronic acid derivatives corresponds to 23—37% of that of D-glucose. The presumption that the reducing ability might be attributed to the formation of unsaturated *aldehydo*-sugars by removal of the 1-*O*-protecting group through a cupric ion-catalyzed β-elimination was supported by the fact that 2,5-dimethoxy-6-oxo-2,4-hexadienoic acid was formed by alkaline β-elimination of methyl (methyl 2,3,5-tri-*O*-methyl-α-D-glucofuranosid)uronate.

Fehling-negative charactor of *O*-glycosides has been generally recognized in the carbohydrate chemistry for a long time, and only exceptionally, the positive[1-4] or negative[1,4-6] properties of several 1-*O*-protected-D-glucuronic acid derivatives were marked by several researchers. In a previous paper,[7] we have examined qualitatively the Fehling test of nineteen kinds of 1-*O*-protected uronic acid derivatives, and found that hexofuranuronic acids having a hydrogen atom at *C*-5 position were positive, while, pentofuranuronic, hexopyranuronic, and heptofuranuronic acids were negative. The formation of unsaturated aldehydes by removal of the 1-*O*-protecting groups through cupric ion-catalyzed β-elimination was deduced to be responsible for the exposed reducing ability. In this paper, the reducing power was quantitatively estimated and an evidence for the proposed β-elimination was obtained.

Results and Discussion

In addition to the reported thirteen examples,[7] following hexofuranuronic acid derivatives showed again the positive Fehling test: *i.e.*, 5-*O*-acetyl,[4] 5-*O*-tosyl,[8] and 5-bromo-5-deoxy[9] derivatives of 1,2-*O*-isopropyli-

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[1] W. F. Goebel and F. H. Barber, *J. Biol. Chem.*, **110**, 707 (1935).

[2] E. M. Osmann, K. C. Hobbs, and W. E. Walston, *J. Amer. Chem. Soc.*, **73**, 2726 (1951).

[3] H. Weidmann, *Ann. Chem.*, **679**, 178 (1964).

[4] H. Weidmann, *Monatsch. Chem.*, **96**, 766 (1965).

[5] S. Saneyoshi, *Biochem. Z.*, **36**, 22 (1911).

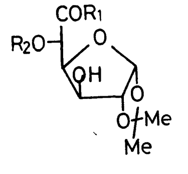
[6] F. Smith, *J. Chem. Soc.*, **1944**, 584.

[7] H. Ando, K. Ajisaka, J. Hashimoto, and J. Yoshimura, *Nippon Kagaku Kaishi*, 1161 (1972).

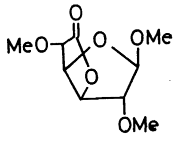
[8] L. D. Hall, L. Hough, and R. A. Pritchard, *J. Chem. Soc.*, **1961**, 1537.

[9] M. Ishidate, M. Kimura, and M. Kawata, *Chem. Pharm. Bull.* (Tokyo), **11**, 1083 (1963).

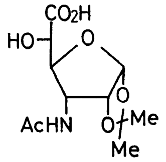
dene-α-D-glucofuranurono-6,3-lactone; 3,5-di-*O*-methyl,[10] 3,5-di-*O*-acetyl,[11] 5-*O*-acetyl[11] derivatives of methyl 1,2-*O*-isopropylidene-α-D-glucofuranuronate; 1,2-*O*-isopropylidene-α-D-glucofuranuronic acid,[12] its hydrazide (**1**), and 5-*O*-*p*-tosyl amide (**2**); (methyl β-D-glucofuranosid)urono-6,3-lactone (**3**),[12] its 2,5-di-*O*-methyl derivative (**4**),[12] and the α-anomer (**5**)[6] of **4**; methyl (methyl 2,3,5-tri-*O*-methyl-α-D-glucofuranosid)uronate (**6**),[6,13] 3-acetamido-3-deoxy-1,2-*O*-isopropylidene-α-D-allofuranuronic acid (**7**), and 5-bromo-5-deoxy-1,2-*O*-isopropylidene-α-D-idofuranurono-6,3-lactone.[9]



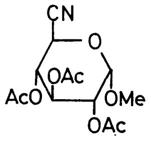
(**1**) R_1=$NHNH_2$; R_2=H
(**2**) R_1=NH_2; R_2=Ts



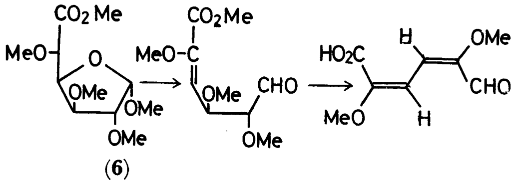
(**4**)



(**7**)



(**8**)



(**6**)

Scheme 1

On the other hand, quantitative estimation of (methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosid)urononitrile (**8**), (benzyl 2-acetamido-2-deoxy-α-D-mannopyranosid)uronic acid,[14] and (benzyl 2-acetamido-2-deoxy-α-D-glucopyranosid)uronamide (**9**),[15] in addition to previously reported Fehling-negative compounds, disclosed that the compound **9**, 1,2: 3,4-di-*O*-isopropylidene-α-D-galactopyranuronic acid (**10**),[16] and methyl (methyl α-D-galactopyranosid)uronate (**11**)[17] have a weak reducing ability.

Among the tested compounds, **1**, **2**, **4**, **7**, and **8** were newly prepared from suitable starting materials, respectively. An attempted esterification of **5** in methanolic hydrogen chloride gave **4** in 46% yield, consisting with the fact that the predominant anomer in the Fischer glycosidation of D-glucurono-6,3-lactone was β-form.[2] This anomerization implies the repulsion between C_1- and C_2-methoxy groups and an anomeric effect[18] in the α-furanose ring. The favorable conformation of **4** was deduced to be flattened T^{3}_{2} from the NMR spectrum[19] (*cf.* Experimental section).

Table 1. Reducing powers for the Fehling solution

Compounds	Oxidation-reduction equivalent
D-Glucose	5.55
Ascorbic acid	5.34
Hydrazine sulfate	3.45
Paraformaldehyde[a]	1.43
D-Glucofuranurono-6,3-lactone	5.95
1,2-*O*-Isopropylidene-[4]	1.37
3	2.04
4	1.40
5	0.46
1,2-*O*-Isopropylidene-α-D-glucofuranuronamide[12]	1.41
5-Deoxy-, hydrazide[7]	4.86 (1.41)[b]
3,5-Di-*O*-acetyl, nitrile[3]	1.16
1	4.89 (1.44)[b]
6	0.35
(Benzyl β-D-mannofuranosid)urono-6,3-lactone[7]	1.55
(Benzyl β-D-mannofuranosid)uronamide[7]	1.32
Hydrazide[7]	4.25 (0.80)[b]
7	1.27
Pectin[a]	0.97
9	0.43
10	0.40
11	0.50

a) These compounds were treated as the monomers.
b) Values in parentheses present that diminished the reducing power of the hydrazide moiety.

Quantitative estimation was carried out by the Bertrand method after refluxing for ten min, and the reducing powers were presented as oxidation-reduction equivalents (Table 1). These values of D-glucose and D-glucurono-6,3-lactone are reasonable compared with those reported.[20] Excepting fully *O*-methylated derivatives (**4** and **6**), all hexofuranuronic acid derivatives showed 23—37% reducing power of that of D-glucose. The facts that D-glucofuranuronic acid derivatives showed almost a constant reducing power and that there is an apparent difference between anomers (**4** and **5**) indicate that the reducing abilities depend on the configuration of the substrates. Lichtmyer and Hudson[21] reported that even enantiomers (D- and L-altrose) showed a different reducing power for the Fehling solution. Therefore, coordination of substrates to L-(+)-tartaric acid–Cu (II) complex will be the

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[10] K. Heyns and W. Bultes, *Chem. Ber.*, **99**, 3477 (1966).

[11] M. Ishidate, Z. Tamura, and T. Kinoshita, *Chem. Pharm. Bull.* (Tokyo), **10**, 1258 (1962).

[12] L. N. Owens, S. Peat, and W. J. G. Jones, *J. Chem. Soc.*, **1941**, 339; C. L. Mehltretter, B. H. Alexander, R. L. Millies, and C. E. Rist, *J. Amer. Chem. Soc.*, **73**, 2424 (1951).

[13] R. E. Reeves, *ibid.*, **62**, 1616 (1940).

[14] J. Yoshimura, H. Sakai, N. Oda, and H. Hashimoto, This Bulletin, **45**, 2027 (1972).

[15] J. Yoshimura, T. Sato, and H. Ando, *ibid.*, **42**, 2352 (1969).

[16] H. M. Sell and K. P. Link, *J. Amer. Chem. Soc.*, **60**, 1813 (1938).

[17] J. K. N. Jones and M. Stacey, *J. Chem. Soc.*, **1947**, 1340.

[18] J. D. Stevens and H. G. Fletcher, Jr., *J. Org. Chem.*, **33**, 1799 (1968).

[19] R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLauchlan, *J. Chem. Soc.*, **1962**, 3699.

[20] G. Klein, "Handbuch der Pflanzenanalyse" II. Spezielle Analyse, Wien (1932), p. 786.

[21] N. K. Richtmyer and C. S. Hudson, *J. Amer. Chem. Soc.*, **58**, 2540 (1936).

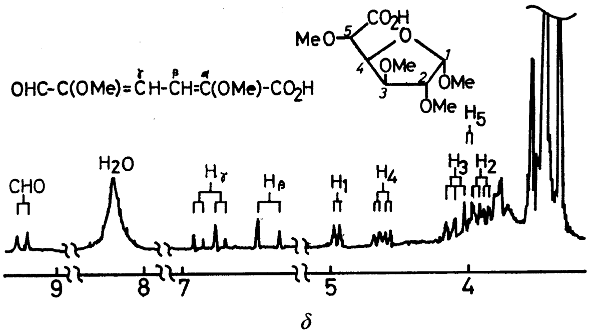

Fig. 1. NMR spectrum of the elimination products of **6** in $CDCl_3$.

first step in this oxidation-reduction, and then proceeds *via* two steps; *i.e.*, oxidation of the resulted complex with oxygen or the complex-catalyzed oxidation of substrate, as was depicted by Singh *et al.*[22]

On the other hand, no substantial change was observed in the NMR spectrum of 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone in 0.7M-sodium hydroxide at 55°C for 1 hr,[7] however, it is known that methyl (methyl α-D-glucopyranosid)uronates undergo *t*-butoxide-catalyzed β-elimination to give the corresponding 4-enoses, and the fully *O*-methylated derivative give a higher yield than others.[23] Examination of the product of such a β-elimination of **6** indicated the formation of 2,5-dimethoxy-6-oxo-2,4-hexadienoic acid in UV (λ_{max} 280 nm) and NMR (Fig. 1) spectra. The appearance of aldehyde proton (δ 9.15; d, J=7.3 Hz) and two olefinic proton signals (δ 6.81; q, J=7.3 and 16 Hz, and δ 6.38; d, J=16 Hz) supported the structure and configuration of the product shown in Scheme 1. A similar examination of methyl 1,2-*O*-isopropylidene-3,5-di-*O*-methyl-α-D-glucofuranuronate showed λ_{max} 295 nm in UV and the appearance of one olefinic proton (δ 6.20; d, J=10.6 Hz) in NMR spectrum, but aldehyde proton signal could not be detected. Thus, it was proved that the β-elimination of hexofuranosiduronic acids gave unsaturated aldehydes, accompanying with liberation of 1-*O*-protecting groups.

On the reason for the weak reducibility of a few hexopyranuronic acids (**9**—**11**), examination of the Fehling test of 4-enoses might give some suggestions, and moreover, it will be worthy to note that the reaction is also stereospecific.

Experimental

All melting points are uncorrected. The solutions were evaporated under diminished pressure at a bath temperature not exceeding 45 °C. Specific rotations were measured in a 0.5-dm tube, with a Carl Zeiss LEP-A1 Polarimeter. The IR spectra were recorded with a Hitachi Model EPI-G2 grating IR spectrophotometer. The NMR spectra were taken in deuteriochloroform with a JNM-4H-100 MHz Spectrometer using tetramethylsilane as an internal standard. Chemical shifts and coupling constants were recorded in δ and Hz units, and frequencies in cm^{-1}.

1,2-O-Isopropylidene-α-D-glucofuranuronohydrazide (1). A solution of 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone (5.0 g) in hydrazine (5 ml) was refluxed for 1 hr, and evaporated to give a syrup which was crystallized from ethanol–petroleum ether. Yield, 3.2 g (56%); mp 143—146 °C; $[\alpha]_D^{23}$ −13.0° (*c* 1.08, water). IR: 3400—3300 (OH), 3220—3100 (NH), 1670 and 1520 (CONH), 1615 (NH_2), 1380—1370 (C–CH_3).

Found: C, 43.31; H, 6.17; N, 11.20%. Calcd for $C_9H_{16}N_2O_6$: C, 43.54; H, 6.50; N, 11.29%.

1,2-O-Isopropylidene-5-O-(p-tosyl)-α-D-glucofuranuronamide (2). To a suspended solution of 1,2-*O*-isopropylidene-5-*O*-(*p*-toluenesulfonyl)-α-D-glucofuranurono-6,3-lactone[7] (4.5 g) in methanol (100 ml) was added at −50 °C saturated methanolic ammonia (70 ml), and the resulted solution was kept at this temperature for 1.5 hr, neutralized with 60% acetic acid, evaporated to 15 ml, and then extracted with chloroform. Evaporation of the extracts gave a syrup from which crystals (250 mg, 5%) was obtained by standing the ether solution overnight in a refrigerator. Mp 155—157 °C; $[\alpha]_D^{23}$ +19.4° (*c* 0.99, acetone); IR: 3400 (OH), 3370—3180 (NH_2), 1680 and 1540 ($CONH_2$), 1585, 670, and 760 (benzene).

Found: C, 49.34; H, 5.21; N, 3.49; S, 8.36%. Calcd for $C_{16}H_{21}NO_8S$: C, 49.61; H, 5.47; N, 3.62; S, 8.28%.

(Methyl 2,5-di-O-methyl-β-D-glucofuranosid)urono-6,3-lactone (4). A solution of the α-anomer of **4**[6] (0.3 g) in methanolic hydrogen chloride (5%, 5 ml) was refluxed for 1 hr, and evaporated to give a syrup which was crystallized by standing overnight its methanol solution in a refrigerator. Yield, 46% (139 mg); mp 95.5—97 °C; $[\alpha]_D^{23}$ −19.1° (*c* 1.00, chloroform). IR: 1790 (lactone); NMR: 5.02 (H_1; s, $J_{1,2}\approx0$), 4.99 (H_4; q, $J_{3,4}$=4.9, $J_{4,5}\approx6.4$), 4.85 (H_3; d, $J_{2,3}\approx0$), 4.03 (H_5; d), 3.91 (H_2; s), 3.66 (OMe), 4.45 and 4.36 (2×C–CH_3).

Found: C, 49.25; H, 6.49%. Calcd for $C_9H_{14}O_6$: C, 49.54; H, 6.47%.

3-Acetamido-3-deoxy-1,2-O-isopropylidene-α-D-allofuranuronic acid (7). To a suspended solution of 3-acetamido-3-deoxy-1,2-*O*-isopropylidene-α-D-allofuranose[24] (4.8 g, 14 mmol) and platinum-charcoal (10%, 6 g) in water (150 ml) was bubbled oxygen in the rate of 10 ml/sec, under adjusting the pH at 7—7.8 with aqueous sodium bicarbonate (8%). When calculated amount of alkali was consumed, the solution was filtered, and the filtrate was evaporated to give a syrup. A solution of the syrup in acetone–water (1:1 in v/v, 300 ml) was treated four times with IR-120 (30 ml, H-form), and evaporated to give a syrupy product. Yield, 3.65 g (72%); $[\alpha]_D^{23}$ +48.8° (*c* 1.00, methanol); IR: 3350 (OH), 3100 (NH), 1725 (COOH), 1640 and 1550 (NHCO).

Found: C, 47.75; H, 6.40; N, 4.81%. Calcd for $C_{11}H_{17}NO_7$: C, 48.00; H, 6.23; N, 5.09%.

(Methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)urononitrile (8). A solution of (methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosid)-uronamide[25] (3.32 g, 10 mmol) and *p*-tosyl chloride (5.7 g, 30 mmol) in pyridine (15 ml) was heated at 80 °C for 3 hr, and then poured into ice-water. The precipitate was filtered and recrystallized from methanol. Yield, 70% (2.19 g); mp 156—157 °C; $[\alpha]_D^{23}$ +140° (*c* 0.4, methanol); IR: 1755 (ester).

Found: C, 49.38; H, 5.32; N, 4.37%. Calcd for $C_{13}H_{17}NO_8$: C, 49.52; H, 5.44; N, 4.44%.

This compound was directly obtained from D-glucurono-6,3-lactone in 17% yield, by successive glycosidation, amida-

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[22] S. V. Singh, O. C. Saxena, and M. P. Singh, *J. Amer. Chem. Soc.*, **92**, 537 (1970).

[23] H. Hashimoto, T. Sekiyama, H. Sakai, and J. Yoshimura, This Bulletin, **44**, 235 (1971).

[24] H. Ando and J. Yoshimura, *ibid.*, **43**, 2966 (1970).

[25] E. Hardegger and D. Spitz, *Helv. Chim. Acta*, **32**, 2165 (1949).

tion, acetylation, and dehydration.

Quantitative Analysis. A mixture of the Fehling solution (A and B, each 25 ml) and sample (20 mg) was boiled for just 10 min, cooled with ice-water, and filtered (Toyoroshi No. 2 filter-paper). Cuprous oxide was dissolved in ferric sulfate solution with boiling, and then the solution was titrated with 0.1M-potassium permanganate. Analysis was repeated two or three times, and blank-test value was reduced from each mean value. Oxidation-reduction equivalent was calculated from the consumed potassium permanganate as follows;

$$\frac{(0.1\text{M-KMnO}_4,\ \text{ml}) \times \text{factor} \times \text{molecular weight}}{(\text{Sample weight, mg})}$$

*Elimination of Methyl (Methyl 2,3,5-tri-*O*-methyl-α-*D*-glucofuranosid)uronate.* The following sample was used. Bp 63—73 °C/0.017 mmHg (bath temperature, 114—130 °C); $[\alpha]^{23}_{D}$ +109° (*c* 0.99, chloroform) [lit,[6] +122° (*c* 0.7, water)]; IR: 1740 (ester), 1450—1430 (C–CH_3); NMR: 4.92 (H_1; d, $J_{1,2}$=4.0), 4.52 (H_4; t, $J_{4,5}$=6.3), 4.02 (H_3; t, $J_{3,4}$=6.4), 3.94 (H_5; d), 3.88 (H_2; q, $J_{2,3}$=6.5), 3.77 (CO_2-CH_3), 3.47, 3.45, and 3.41 (3 × OCH_3).

A mixed solution of **7** (100 mg) in *t*-butyl alcohol (0.5 ml) and potassium (30 mg) in *t*-butyl alcohol (0.5 ml) was stood for 30 min at room temperature, neutralized with IR-120 (H^+), and evaporated to give a syrup, which was examined with UV and NMR spectrum, as mentioned in the before section.