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STUDIES OF THE STRUCTURES OF THE CARBOHYDRATE COMPONENTS IN PLANT OLIGOSACCHARIDE GLYCOSIDES BY THE DITHIOACETAL METHOD*

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ABSTRACT

The usefulness of the dithioacetal method for studies of carbohydrate structures has been demonstrated by analyzing the constituent monosaccharides and aldehydes in the products of periodate oxidation of a number of oligosaccharide glycosides from plants.

INTRODUCTION

The constituent monosaccharides of an oligosaccharide-containing glycoside are usually analyzed, following acid hydrolysis, by gas chromatography of the derived alditol acetates[1]. Methanolysis has also served for this purpose in some instances[2]. On the other hand, the glycosidic linkage-positions are most frequently examined by methylation analysis[3].

Recently, we reported a convenient, gas-chromatographic procedure for analysis of monosaccharides as their trimethylsilylated dithioacetals[4]. This derivatization procedure is also applicable to the dialdehydes formed by oxidation of oligosuccharide glycosides with periodate. Data on the types and molar proportions of aldehydes formed provide important information on the types of interglycosidic linkage[5]. This paper is concerned with application of this method for structural studies of the carbohydrate components in a number of plant oligosaccharide glycosides.

RESULTS AND DISCUSSION

The glycosides were hydrolyzed in 2M trifluoroacetic acid, which causes less oxidative degradation than ordinary mineral acids, and the hydrolyzates were analyzed by the dithioacetal method. Because of difficulties in solubility in water, periodate oxidation of the compounds was performed in aqueous methanol, unless otherwise stated. Addition of methanol decreased the rate of oxidation.

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* Periodate Oxidation Analysis of Carbohydrates. Part XI. For Part X, see ref. 12.

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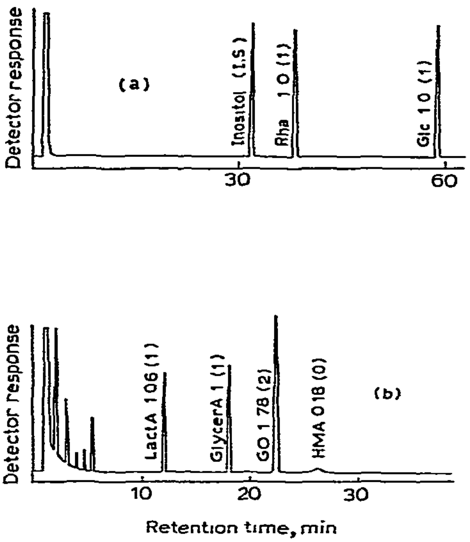

Fig. 1. Gas-chromatographic analysis of the carbohydrate residue of rutin. (*a*) Monosaccharide composition. Analytical data indicate the molar amounts of the monosaccharides per mol of the glycoside. (*b*) Distribution of the aldehydes in the product of oxidation for 48 h. Analytical data indicate the relative molar proportions of the aldehydes. The numbers in parentheses are theoretical values. Abbreviations are the same as given in Table III.

Fig. 1(a) shows the gas chromatogram for the constituent monosaccharides of rutin, a typical flavonoid glycoside. The result indicates that this compound is a glycoside of a disaccharide composed of D-glucose and L-rhamnose, which are detected as their trimethylsilylated diethyl dithioacetals. The aldehydes found in the product of periodate oxidation were D-glyceraldehyde, L-lactaldehyde, and glyoxal in the molar proportions of 1:1.06:1.78, respectively, together with a small proportion (0.18) of hydroxymalonaldehyde [Fig. 1(b)]. There are seven possible types of disaccharide glycoside containing D-glucopyranose and L-rhamnopyranose, with respect to the monosaccharide sequence and position of attachment of the interglycosidic linkage, as shown in Table I, which also includes the theoretical amounts of aldehydes

TABLE I

POSSIBLE TYPES OF QUERCETIN BIOSIDES CONTAINING D-GLUCOPYRANOSE AND L-RHAMNOPYRANOSE

Type of bioside	*Aldehydes*[a] *in the product of periodate oxidation (mol/mol)*
Glc-(1 → 2)-Rha-Q	GlycerA (1) LactA (1) GO (1) HMA (1)
Glc-(1 → 3)-Rha-Q	GlycerA (1) GO (1)
Glc-(1 → 4)-Rha-Q	GlyerA (1) GO (2) DHBA (1)
Rha-(1 → 2)-Glc-Q	GlycerA (1) LactA (1) GO (1) HMA (1)
Rha-(1 → 3)-Glc-Q	LactA (1) GO (1)
Rha-(1 → 4)-Glc-Q	LactA (1) Ery (1) GO (2)
Rha-(1 → 6)-Glc-Q	GlycerA (1) LactA (1) GO (2)

[a] Ery = D-erythrose, Q = quercetin. Other abbrevations are the same as given in Table III.

expected in the product of oxidation of each type of glycoside. Obviously, the type of glycoside that gives the closest aldehyde distribution is L-rhamnopyranosyl-(1→6)-D-glucopyranosylquercetin, which should give rise to one mol each of D-glyceraldehyde and L-lactaldehyde and two mol of glyoxal. Thus, unequivocal determination of the monosaccharide sequence and the linkage-type of this glycoside was achieved by analyzing its carbohydrate component by the dithioacetal method. The small proportion of hydroxymalonaldehyde detected may be attributed to incomplete oxidation of the D-glucose residue because of weak hydrogen-bonding between the carbonyl group of the flavonol aglycon and the 2-hydroxyl group of the D-glucosyl residue, because the C-2′–C-3′ bond in robinin, which has a similar structure, was also difficult to cleave (see Table III).

Fig. 2 shows the second example of structure elucidation. Tomatin is known to be a tetrasaccharide glycoside of a steroid whose structure[6] is given in Chart I. Both the analytical data for the constituent monosaccharides [1.0 mol for D-xylose, 1.0 mol for D-galactose, and 2.1 mol for D-glucose, Fig. 2(a)] and those for the relative molar proportions of the conjugated aldehydes in the products of oxidation [glycolaldehyde: D-glyceraldehyde: glyoxal = 0.93:1;2.80, Fig 2(b)] support this structure. The

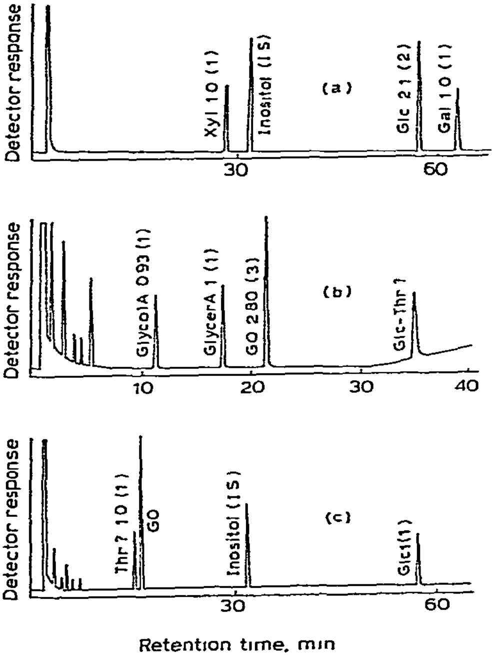

Fig. 2. Gas-chromatographic analysis of the carbohydrate moiety of tomatin. (*a*) Monosaccharide composition. (*b*) Distribution of the aldehydes in the product of oxidation for 48 h. (*c*) Monosaccharide composition of the product of oxidation for 48 h. The analytical data (*a*) indicate the molar amounts of the monosaccharides per mol of the glycoside, and (*b*) and (*c*) indicate the relative molar proportions of the aldehydes and monosaccharides, respectively. The numbers in parentheses are theoretical values. Thr = D-threose. Other abbreviations are the same as given in Table III. Presumed D-threose was estimated by use of the molar response-factor (0.77) of the D-erythrose derivative.

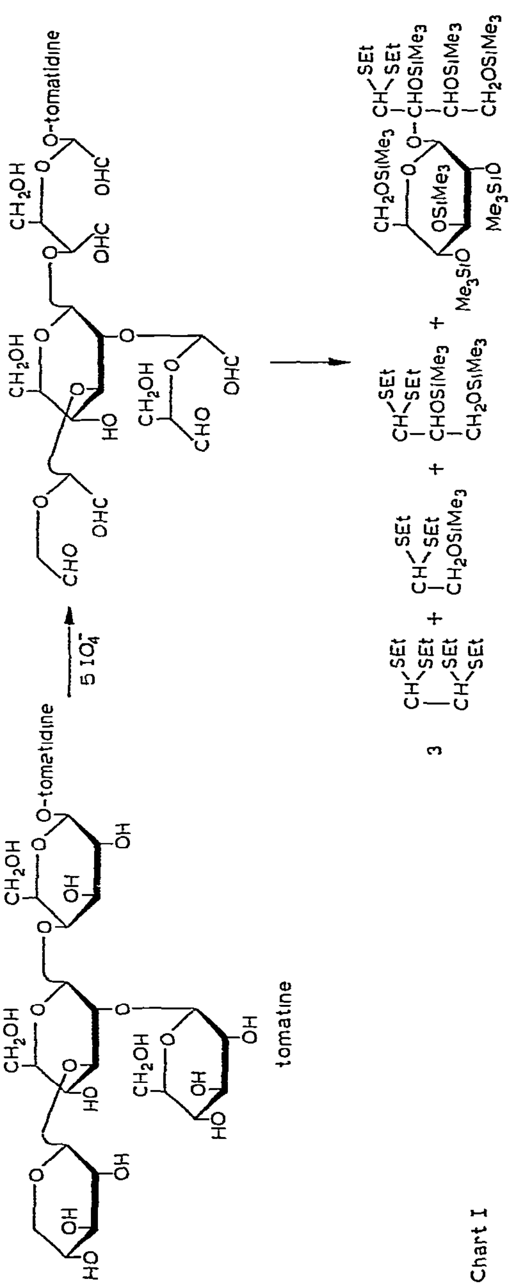

Chart I

slowest eluted, intense peak at ~35 min [Fig. 2(b)] may be assigned to the trimethylsilylated dithioacetal of *O*-D-glucosyl-D-threose, arising from the branching point that survived oxidation.

One certain result from the analysis of this tetrasaccharide glycoside is that D-xylose is located at the nonreducing terminal, because glycolaldehyde was found, but no trace of hydroxymalonaldehyde was detected. D-Xylose residues substituted at O-2 would produce not only glycolaldehyde but also hydroxymalonaldehyde. Another important item of information is the presence of the partial structure of a substituted *O*-D-glucosyl-(1→4)-unsubstituted hexopyranoside, as evidenced by the presence of a peak for the D-glucose derivative and a peak in the tetrose region from the hydrolyzate of the product of oxidation [Fig. 2(c)]. It is not known whether the hexose in this partial structure is D-galactose or D-glucose, as an authentic specimen of the D-threose derivative was not available. The positions of attachment of the terminal D-xylose and the third hexose residue are also uncertain from the present data alone. However, this analysis by the dithioacetal method was quite helpful for partial confirmation of the reported structure.

Table II gives analytical data for the monosaccharide compositions of other plant glycosides examined. The molar proportions of pentoses, hexoses, and a 6-deoxyhexose (L-rhamnose) agreed well with the theoretical values in all instances. The determination of apiose and D-glucuronic acid was difficult, because of their instability to acid. Further studies on the conditions for hydrolysis are necessary for resolution of this problem.

Table III summarizes the data on aldehyde distribution in the products of periodate oxidation. Naringin was oxidized rather more slowly than ordinary oligosaccharide glycosides, presumably because of formation of a less-soluble product upon oxidation of the phenolic hydroxyl groups. Part of the primary oxidation-product separated as a precipitate, and the resultant, heterogeneous system underwent slower, further oxidation. However, the data obtained after 96 h of oxidation were in good agreement with the theoretical values. The apiose residue in apiin seems to be unstable and was partially hydrolyzed under the conditions used, thus exposing the D-glucose residue for further oxidation. The degree of over-oxidation became more prominent on prolonged reaction. The incomplete oxidation of the D-galactose residue in robinin has already been pointed out. Hesperidine was almost insoluble in aqueous methanol, and so it was oxidized with periodic acid in aqueous pyridine according to conditions

TABLE II

MONOSACCHARIDE COMPOSITIONS[a] OF PLANT GLYCOSIDES

Glycoside	*Ara*	*Xyl*	*Glc*	*Gal*	*Rha*	*Api*	*GlcA*
Hesperidin	–	–	1(1)	–	1.1(1)	–	–
Naringin	–	–	1(1)	–	1.1(1)	–	–
Apiin	–	–	1(1)	–		detected(1)	–
Robinin	–	–	–	1 (1)	2.1(2)	–	–
Digitonin	–	0.9(1)	2(2)	2.1(2)	–	–	–
Desacyl-jego-saponin, ethyl ester	–	–	1(1)	1.1(1)	–	–	detected(1)
Mi-saponin A	1.1(1)	1 0(1)	1(1)	–	2.0(1)	–	–
Sakuraso-saponin	–	–	1(1)	1.1(1)	2.1(2)	–	detected(1)

[a]Abbreviations are the same as given in Table III.

TABLE III

DISTRIBUTION OF THE ALDEHYDES IN THE PRODUCTS[a] OF PERIODATE OXIDATION OF PLANT GLYCOSIDES

Glycoside and its structure	*Reaction time (h)*	*Molar proportion of aldehydes*[b]					
		GlycolA	GlycerA	LactA	GO	HMA	*Other aldehydes*
Hesperidin[c] α-Rha-(1 → 6)-β-Glc-hesperetin	48	0.00(0)	1(1)	1.06(1)	2.10(2)	0.00(0)	–
Naringin α-Rha-(1 → 2)-β-Glc-naringenin	48	0.00(1)	1(1)	1.10(1)	0.87(1)	1.26(1)	–
	96	0.00(0)	1(1)	1.01(1)	0.87(1)	1.08(1)	–
Apiin α-Apif-(1 → 2) → β-Glc-apigenin	48	0.00(0)	1(1)	0.00(0)	1.42(1)	0.88(1)	–
	96	0.00(0)	1(1)	0.00(0)	1.71(1)	0.24(1)	–
Robinin α-Rha-(1 → 6)-β-Gal- α-Rha-kaempferol	48	0.00(0)	1(1)	1.82(2)	2.56(3)	0.42(0)	–
	96	0.00(0)	1(1)	1.86(2)	2.83(3)	0.16(0)	–
β-Xyl-(1 → 3) Digitonin β-Glc-(1 → 3)-β-Gal-(1 → 2) β-Glc-(1 → 4)-β-Gal-digitogenin	48	0.94(1)	1(1)	0.00(0)	2.94(3)	0.00(0)	–
Desacyl jego-saponin ethyl ester β-Gal-(1 → 4) β-Glc-(1 → 2) β-GlcA Et ester-barringtogenol	48	0.00(0)	2(2)	0.00(0)	2.00(2)	0.00(0)	–
Misaponin A α-Rha-(1 → 3)-β-Xyl-(1 → 4)-α-Rha-(1 → 2)-α-Ara- β-Glc-protobassic acid	24	0.94(1)	1(1)	0.88(1)	2.49(3)	1.25(1)	Xyl-DHBA
	48	0.92(1)	1(1)	0.88(1)	2.59(3)	1.13(1)	Xyl-DHBA
	96	0.90(1)	1(1)	0.89(1)	2.97(3)	0.98(1)	Xyl-DHBA
Sakuraso-saponin α-Rha-(1 → 2)-α-Rha-(1 → 2)-β-Gal-(1 → 4) β-Glc-(1 → 2) β-GlcA-protoprimulagenin A	48	0.00(0)	2(2)	2.08(2)	1.96(2)	1.74(2)	–

[a] Abbreviations: Ara = L-arabinose, Xyl = D-xylose, Gal = D-galactose, Glc = D-glucose, Rha = L-rhamnose, Apif = apiofuranose, GlcA = D-glucuronic acid, GlycolA = glycolaldehyde, GlycerA = D-glyceraldehyde, LactA = L-lactaldehyde, GO = glyoxal, HMA = 2-hydroxymalonaldehyde, DHBA = 2,3-dihydroxybutanaldehyde. [b] The numbers in parentheses are theoretical values. [c] Oxidized in aqueous pyridine.

given in the previous paper[7]. Oxidation then proceeded in normal Malapradian fashion.

Another steroid oligosaccharide glycoside, digitonin, was oxidized as for tomatine; the aldehyde distribution observed was consistent with that expected, but the partial structure that survived oxidation (a D-galactose–D-glucose–D-threose fragment) was not detected, probably because of its low volatility.

All of the data for the triterpenoid glycosides were in good agreement with the theoretical values, but it was noted that the oxidation of such higher oligosaccharide glycosides as Mi-saponin A required a longer reaction-time.

The foregoing results demonstrate the usefulness of the dithioacetal method for structural studies of oligosaccharide glycosides from plants. As this method requires only small samples, it should be especially useful for pre-examination and confirmation of structure of the carbohydrate component.

EXPERIMENTAL

Materials. — The samples of flavonoid and steroid glycosides were from commercial sources. Of these samples, apiin, naringin, and robinin were gifts from Professor K. Takaishi of this faculty. The samples of triterpenoid glycosides, desacyl jegosaponin ethyl ester, Mi-saponin A, and sakuraso-saponin, which had been isolated from *Styrax japonica* SIEB et ZUCC (pericarps)[8], *Madhuca longifolia* (L.) MACBRIDE (seed kernels)[9], and *Primula sieboldi* E. MORREN (roots)[10], respectively, were supplied by Professor I. Kitagawa of Osaka University. All of these samples were used as obtained, except for digitonin, which was purified by chromatography on a column of silica gel (Wako Silica Gel C-200) with 13:12 (v/v)chloroform–methanol.

Apparatus. — Gas chromatography was performed in a Shimadzu 4BMPF instrument equipped with a hydrogen flame-ionization detector. Peaks were integrated by using a Shimadzu Chromatopak E1A integrator.

Analysis of constituent monosaccharides. — The procedure was essentially the same as that described in the previous paper[4]. A glycoside sample (0.1–1 μmol) was dissolved in a solution (100 μL) of myo-inositol (internal standard, 1 μmol) in 2M trifluoroacetic acid contained in a small ampoule, which was flushed with nitrogen for a few min, shielded, and heated for 5 h on a boiling-water bath. The ampoule was then opened, and the solution was transferred to a small reaction-tube and evaporated to dryness under diminished pressure in a desiccator containing sodium hydroxide. A 2:1 (v/v) mixture (20 μL) of ethanethiol and trifluoroacetic acid was added to the residue, the reaction vessel was tightly closed with a polyethylene stopper, and the residue was dissolved completely by gentle swirling. The solution was kept for 10 min at 25°, and then pyridine (50 μL) was added, followed by hexamethyldisilazane (100 μL) and chlorotrimethylsilane (50 μL). This mixture was incubated for 30 min at 50° with occasional shaking, and then centrifuged. A 1–10 μL sample of the supernatant solution was injected into the gas-chromatography column. A Scot SF-96 capillary column (0.28 mm i.d., 50 m, Gasukuro Kogyo, Shinjuku, Tokyo) was used at 225°, and the carrier gas (nitrogen) was regulated at a rate of 1.5–2.0 mL/min by use of a

100:1 splitter. The eluate was continuously mixed with a scavenger gas (nitrogen) at a flow rate of 50 mL/min, and the mixture was introduced into the detector. Peaks were identified and the relative molar proportions of monosaccharides were determined by referring to a mixture of authentic specimens of aldoses.

Periodate oxidation. — A sample of a glycoside (0.1–1 µmol) was dissolved in methanol (100 µL), and to the resultant solution was added a 0.1M aqueous solution (100 µL) of sodium metaperiodate. The mixture was kept in the dark for the specified time given in Figs. 1, 2, and Table III. In the case of hesperidine, a sample was dissolved in 0.05M periodic acid in a 1:1 (v/v) mixture (200 µL) of pyridine and water, and the solution was kept for 48 h at 25° in the dark. A 1% methanolic solution (100 µL) of ethylene glycol was added, the mixture was kept for 15 min in the dark, and then evaporated to dryness under diminished pressure in a desiccator. The residue was extracted with ethanol (500 µL), the extract was evaporated to dryness in the same manner, and the residue was subjected to component analysis as shown next.

Analysis of the conjugated aldehydes in the products of periodate oxidation. — This procedure follows that described in the previous papers [5,7,11]. The residue obtained in the foregoing experiment was mercaptalated with a 10:1 (v/v) (20 µL) of ethanethiol and trifluoroacetic acid. The reaction was stopped by adding a solution (50 µL) of xylitol (internal standard) in pyridine, and the mixture was immediately trimethylsilylated with hexamethyldisilazane (100 µL) and chlorotrimethylsilane (50 µL) for 30 min at 50°. This series of operations was the same as that described for the analysis of constituent monosaccharides, except that the conditions for mercaptalation were changed slightly. Gas chromatography of the derivatized products was performed on a glass column (3.0 mm i.d., 2 m) packed with 3% of silicone OV-1 on Chromosorb W. The carrier gas (nitrogen) was regulated at a rate of 50 mL/min. A temperature gradient of 100–250° (5°/min) was applied to the column immediately after injection. Peaks were identified by referring to authentic specimens of the dithioacetal derivatives. Glycoladehyde, D-glyceraldehyde, and L-lactaldehyde were detected as their trimethylsilylated diethyl dithioacetals having molar response-factors relative to trimethylsilylated xylitol (r.m.r.f.) of 0.46, 0.64, and 0.51, respectively. Glyoxal was converted into its bis(diethyl dithioacetal) having r.m.r.f. 0.52. Hydroxymalonaldehyde was observed as its trimethylsilylated bis(diethyl dithioacetal) having r.m.r.f. 0.75.

Analysis of the constituent monosaccharides in the products of periodate oxidation. — The product of oxidation just described was hydrolyzed in 2M trifluoroacetic acid and the hydrolyzate was analyzed as described for the analysis of constituent monosaccharides in the starting glycosides.

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