Synthesis of 1-O-(2',4'-Dichlorophenoxyacetyl)-D-glucopyranose

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Received May 12, 1972

1-O-Glucosyl esters of 2,4-dichlorophenoxyacetic acid (2,4-D) were easily prepared from 4,6-O-benzylideneglucose. The configuration of 1-O-ester linkage was affected by pH at the end of the reaction, that is, β -type was a major product at a neutral or acidic condition and α -type at an alkaline condition. Both of the anomers showed the same biological activities as sodium salt of 2,4-D.

Numerous studies have been accumulated about 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic plant hormone, and its derivatives. Little was known, however, about the metabolism of these compounds by plants or the degradation process in soil by bacteria. Recently it has been suggested that a part of 2,4-D is changed to its glucosyl ester¹⁾ in plant bodies, and Thomas *et al.*²⁾ identified the 2,4-D glucosyl ester separated from the stem tissue of *Avena sativa* with 1-O-(2',4'-dichlorophenoxyacetyl)- β -D-glucose (Va) as its tetraacetate without detailed chemical discussions about the identification of the structure.

On the other hand, it became clear that hydroxylation to aromatic nuclei of 2,4-D occurs at an earlier metabolic stage and the hydroxylated product is also present as 1-O- β -D-glucosyl esters in plants^{3~5)} or in a metabolite of a fungus.⁶⁾ From these results the formation of 2,4-D glucosyl ester is supposed to be the first step of detoxication. However, in the case of 1-O- β -D-glucosyl ester of indoleacetic acid (IAA), Keglevic and Pokorny⁷⁾ showed that their synthetic glucosyl ester is biologically more active than IAA itself with several auxin tests and they expressed their suspicion about a hypothesis that the com-

bination of glucose and IAA is the first step of detoxication of IAA.

In the present paper, authors describe the synthesis of 2,4-D glucosyl esters and the confirmation of their structures with an unambiguous method. Their biological activities were also tested according to the usual auxin test.

Comparing to numerous naturally occurring glucosides, the natural glucosyl ester is rare; the simplest one is periplanetin,⁸⁾ 1-O-benzoyl- β -D-glucopyranose (Vc), which had been prepared by Zervas.⁹⁾ His method was later improved by Fletscher *et al.*¹⁰⁾

Therefore, authors applied this method to the preparation of 2,4-D glucosyl ester. According to the literature 1-O-benzoyl-4,6-benzylidene- β -D-glucopyranose (IIIc) was a sole product when equimolecular amount of benzoylchloride and sodium salt of 4,6-benzylidene glucose were reacted so that the reaction results in under a weak acidic condition at the end of the process. However, if benzoylchloride was replaced by 2,4-dichlorophenoxyacetyl chloride (2,4-D chloride) and was reacted with the sodium salt under the same condition, there existed two main products, a major compound (IIIa) and a minor one (IVa). Moreover, it became clear that when excess sodium salt was used,

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FIG. 1.

IVa became the major product and only a little of IIIa was present in the reaction product. Both of IIIa and IVa was obtained as white crystal and showed reasonable carbon and hydrogen contents as an ester of 2,4-D and benzylidene-glucose. Their melting points are approximately same. The mixed melting point depressed greatly and their IR spectra were not identical in detail and R_f values of thin-layer chromatography (TLC) or solubilities in the solvent were also slightly different. Each compound was hydrogenated with palladium black as catalyst in acetic acid-dioxane to remove benzylidene group. Each product also has reasonable analytical and spectrometrical results as 2,4-D glucosyl ester. The hydrogenated product (VIa) from IVa was recrystallized from water easily and Va from IIIa does not crystallize but remains as amorphous powder.

Taking into account the presence of free

OH on C₂ of the pyranose ring of the starting material (I), a rearrangement of the acid moiety of 1-O-glucosyl ester to 2-O-ester¹¹⁾ may occur during this reaction. Therefore, it became necessary first to establish that both of IIIa and IVa have 1-O-ester linkage. When 2,4-D-chloride was reacted with 2,3,4,6-tetra-O-benzyl- α -D-glucose (VII)¹²⁾ in the presence of triethylamine, a crystalline β -1-O-ester (VIII) was obtained. The β -form was confirmed by NMR spectra as described later. Debenzoylation of VIII yielded an amorphous powder which is identical with 2,4-D glucosyl ester (Va) obtained from IIIa in all respects. Therefore, Va is 1-O-(2',4'-dichlorophenoxyacetyl)-D-glucopyranose. As the pure α -form of 2,4-D 2,3,4,6-tetrabenzylglucosyl ester could not be obtained, the presence of 1-O-ester linkage in IVa molecule was proved by other method. IVa was oxidized with excess lead tetraacetate in acetic acid and the reaction was monitored by iodometric titration. The reaction stopped after the consumption of the equimolecular oxidizing reagent. If IVa is 2-O-ester, oxidation could not be observed and if hydrolysis was occurred during the process two moles of the reagent must be consumed. Therefore, IVa also has 1-O-ester linkage.

The configuration of the anomers, IIIa and IVa, was determined with NMR spectra. The anomeric proton (C₁-H) on the pyranose ring system appears at 5.5~6.0 (δ) as doublet coupled with C₂-H and their coupling constant was followed by Karplus equation, *i.e.* *trans* configuration shows the J -value near 8 Hz and *cis* near 3 Hz.¹³⁾

Chemical shifts of anomeric protons and coupling constants of the above compounds and their acetyl derivatives were summarized in Table I. The group of III and V shows lower δ -values than those of the IV and VI group and their coupling constants were always near 7.0 Hz. It is obvious that C₁-H

TABLE I. NMR SPECTRA (δ -VALUE) OF ANOMERIC PROTONES AT 60 MHz IN $(\text{CD}_3)_2\text{CO}^*$ OR CDCl_3^{**}

Series of compds.	Benzylideneglucosyl esters (III, IV)		Glucosyl esters (V, VI)	
	OH-free*	Diacetate**	OH-free*	Tetraacetate**
β -1-O-ester	5.75 $J=7.0$ Hz	5.82 $J=7.0$ Hz	5.66 $J=7.0$ Hz	5.91 $J=6.8$ Hz
α -1-O-ester	6.60 $J=4.0$ Hz	6.28 $J=3.5$ Hz	5.81 $J=3.5$ Hz	6.32 $J=3.0$ Hz

and $\text{C}_2\text{-H}$ have *trans*-configuration, in other words, they are diaxial and the III-V series must be β -1-O-ester. On the other hand *cis* (equatorial-axial) configuration or α -1-O-ester linkage was suggested about the structure of the IV-VI series.

From these results, it was established that Va is 1-O-(2',4'-dichlorophenoxy acetyl)- β -D-glucopyranose and VIa is 1-O-(2',4'-dichlorophenoxyacetyl)- α -D-glucopyranose.

Growth inhibitory activities on rice seedlings were examined by measuring the length of the second leaf sheath and growth promoting effects in *Avena* coleoptile section test was also studied. The experiment was paralleled

with equimolar amount of 2,4-D-Na-salt (Fig. 2, Fig. 3). Hydrolysis of the ester during the experiment was checked with TLC by sampling from 2.5% water solution placed under the same condition, but any detectable change of the glucosyl esters was not observed. The concentration-*versus*-growth curves of the glucosyl esters and 2,4-D-Na-salt are qualitatively same but deviations at lower or higher concentrations of β -ester were observed. These problems must be discussed from the view point of the correlation with the action of glycosyl esters in the lizosphere or the permeability to plant tissue.

FIG. 2. Elongation of *Avena* Coleoptile Sections.

Each point represents the mean for twelve sections.

■: Control, ○: Na salt of 2,4-D, △: α -Glucosyl ester, ▲: β -Glucosyl ester.

FIG. 3. Growth Inhibitory Effect to Rice Seedlings (Norin No. 21).

Each point represents the mean for ten sheaths.

■: Control, ○: Na salt of 2,4-D, △: α -Glucosyl ester, ▲: β -Glucosyl ester.

EXPERIMENTAL

All melting point is uncorrected. Thin-layer chromatography was applied on 25×100 mm or 50×200 mm chromatoplate coated with silica gel H (0.25 mm) by developing with the following solvent systems: A; benzene-ethylacetate (1:1), B; benzene ethyl acetate (4:1), C; benzene-ethyl acetate (9:1), D; chloroform-methanol (4:1). NMR spectra were obtained at 60MHz with TMS as inner standard.

1-O-(2',4'-Dichlorophenoxyacetyl)-4,6-O-benzylidene-D-glucopyranose, (IIIa) and (IVa).

a) *Procedure A.* Dry sodium salt of 4,6-O-benzylidene glucose¹⁰⁾ (4.8 g 16.6 mm)* was suspended in 40 ml of dry dichloromethane and was treated with 3.8 g of 2,4-dichlorophenoxyacetylchloride (15.0 mm) dissolved in 10 ml of dichloromethane. After shaking over night at room temperature the content was concentrated under reduced pressure and 20 ml of water was added to the residual mass. The water solution was weakly acidic or neutral. The separated crude solid was dissolved in hot ethanol. The small amount of insoluble material was filtered off and β -type ester (IIIa) crystallized from ethanol. The additional crystal obtained from the condensed mother liquor contained α -type ester (IVa) which was separated with chloroform because the latter is less soluble in this solvent.

As these crystals have ethanol or water of crystallization, they were recrystallized from ethylacetate-petroleum ether respectively.

β -Type ester (IIIa): yield 3.1 g (40%), Mp 171°C, TLC (solvent A) $R_f=0.71$, $[\alpha]_D^{20}+10.5^\circ$ ($c=0.76$ CHCl₃), *Anal.* Found: C, 53.24; H, 4.31. Calcd. for C₂₁H₂₀O₈Cl₂: C, 53.51; H, 4.29%. IR $\nu_{\max}^{KBr}$ cm⁻¹: 3570, 3520 (O-H), 1760 (OC=O).

α -Type ester (IVa): yield 0.2 g, Mp 170°C, TLC (solvent A) $R_f=0.61$, $[\alpha]_D^{20}+28.8^\circ$ ($c=1.17$ CHCl₃), *Anal.* Found: C, 53.25, H, 4.03. Calcd. for C₂₁H₂₀O₈Cl₂: C, 53.51; H, 4.29%. IR $\nu_{\max}^{KBr}$ cm⁻¹: 3500, 3400 (O-H), 1730 (OC=O).

b) *Procedure B.* Excess amount of the sodium salt (14.5 g 50 mm)* suspended in 150 ml dry chloroform was reacted with 12.0 g of the acid chloride (47 mm)

* According to the literature¹⁰⁾ the sodium salt was used without any purification, therefore, its purity was variable and an accurate molar ratio to the acid chloride could not be calculated. The amount of the sodium salt in Procedure A or B was finally checked by pH at the end of each reaction.

under the same condition as procedure A. After distilling off the solvent 50 ml of water was added and the pH of the resulted water solution was between 9 and 10.

The crude crystal was recrystallized from ethanol and then ethyl acetate-petroleum ether. This crystal was a pure α -type ester (IVa) and trace amount of β -type ester (IIIa) was present in the mother liquor. Yield of IVa: 14.5 g (62%).

1-O-(2',4'-Dichlorophenoxyacetyl)-2,3-O-diacetyl-4,6-O-benzylidene-D-glucopyranose, (IIIb) and (IVb). Following the usual method, IIIa and IVa were acetylated in pyridine-acetic anhydride mixture respectively. The crude solid products were recrystallized from ethanol.

β -Type ester, IIIb: Mp 132°C, TLC (solvent B) $R_f=0.66$, *Anal.* Found: C, 53.82, H, 4.26. Calcd. for C₂₅H₂₄O₁₀Cl₂: C, 54.01, H, 4.34%. IR $\nu_{\max}^{NaJol}$ cm⁻¹: 1780, 1740 (OC=O). NMR (10% in CDCl₃) δ : 2.00 (3H, s, COCH₃), 2.04 (3H, s, COCH₃), 4.68 (2H, s, OCH₂CO), 5.43 (1H, s, PhCH), 6.50~7.42 (8H, m, aromatic H).

α -Type ester, IVb: Mp 179°C, TLC (solvent B) $R_f=0.56$, *Anal.* Found: C, 53.67, H, 4.20. Calcd. for C₂₅H₂₄O₁₀Cl₂: C, 54.01, H, 4.34%. IR $\nu_{\max}^{NaJol}$ cm⁻¹: 1760, 1730 (OC=O). NMR (10% in CDCl₃) δ : 2.02 (3H, s, COCH₃), 2.19 (3H, s, COCH₃), 4.66 (2H, s, OCH₂CO), 5.45 (1H, s, PhCH), 6.50~7.34 (8H, m, aromatic H).

1-O-(2',4'-Dichlorophenoxyacetyl)-D-glucopyranose, (Va) and (VIa). Palladium black (0.1 g) was suspended in methanol and was shaken under hydrogen gas for 1 hr. Methanol was decanted and the catalyst was added to 1.0 g of the benzylidene derivatives, IIIa or IVa, dissolved in 30 ml of dioxane containing a few drop of acetic acid. The content was shaken with hydrogen until the theoretical amount of hydrogen was absorbed (10~15 hr at room temperature). After removal of the catalyst with filtration the filtrate was concentrated *in vacuo* and residual viscous oil was once dissolved in ethyl acetate. The solution was washed, dried and distilled off the solvent and the residue was treated as crude product.

a) β -Type ester, Va: The crude product was purified by column chromatography. Through 5 g of silica gel column, Va was eluted with chloroform-methanol(9:1). Amorphous white powder; Yield 550 mg (70%) $[\alpha]_D^{20}+7.50$ ($c=1.12$ MeOH), *Anal.* Found: C, 43.56, H, 4.51. Calcd. for C₁₄H₁₆O₈Cl₂: C, 43.88, H, 4.20%. IR $\nu_{\max}^{KBr}$ cm⁻¹: 3400 (O-H), 1760 (OC=O). NMR (10% in hexadeuteroacetone)

δ : 5.0 (2H, s, OCH₂CO), 7.0~7.5 (3H, m, aromatic H).

b) α -Type ester, VIa: The crude product was easily solidified and was recrystallized from water. Yield 700 mg (84%), Mp 132°C, TLC (solvent D) R_f =0.50, $[\alpha]_D^{20} + 58.9^\circ$ ($c=1.29$ MeOH). Anal. Found: C; 43.43, H; 4.01. Calcd. for C₁₄H₁₆O₆Cl₂: C; 43.88, H; 4.20%. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3400 (O-H), 1750 (OC=O). NMR (10% in hexadeuteroacetone) δ : 4.92 (2H, s, OCH₂CO), 7.0~7.5 (3H, m, aromatic H).

1-O-(2',4'-Dichlorophenoxyacetyl)-2,3,4,6-O-tetraacetyl-D-glucopyranose, (Vb) and (VIb). Va and VIa were dissolved in pyridine-acetic anhydride mixture respectively and were left for overnight at room temperature. Separation and purification of the acetyl derivatives were followed in the usual way.

a) β -Type ester, Vb: Mp 124°C (recrystallized from EtOH), TLC (solvent B) R_f =0.42, Anal. Found: C; 47.46, H; 4.16. Calcd. for C₂₂H₂₄O₁₂Cl₂: C; 47.91, H; 4.35%. IR $\nu_{\text{max}}^{\text{NaJol}}$ cm⁻¹: 1760, 1740 (OC=O), NMR (10% in CDCl₃) δ : 2.06, 2.10, 2.15 (3H each, s, COCH₃), 4.80 (2H, s, OCH₂CO), 6.9~7.5 (3H, m, aromatic H).

b) α -Type ester, VIb: Mp 120°C (recrystallized from EtOH), TLC (solvent B) R_f =0.50, Anal. Found: C; 47.88, H; 4.24. Calcd. for C₂₂H₂₄O₁₂Cl₂: C; 47.91, H; 4.35%. IR $\nu_{\text{max}}^{\text{NaJol}}$ cm⁻¹: 1735, 1760 (OC=O). NMR (10% in CDCl₃) δ : 2.04, 2.12, 2.21 (3H each, s, COCH₃), 4.65 (2H, s, OCH₂C=O), 6.6~7.4 (3H, m, aromatic H).

1-O-(2',4'-Dichlorophenoxyacetyl)-2,3,4,6-O-tetrabenzyl- β -D-glucopyranose (VIII). 2,3,4,6-Tetrabenzyl- α -D-glucose¹⁴⁾ (VII) (2.7 g 5.0 mm) was dissolved in 1.0 g of triethylamine and 3 ml of chloroform. To this solution 1.7 g of dichlorophenoxyacetylchloride (II) (6.5 mm) diluted with 3 ml of chloroform was added dropwise with stirring and outer cooling by ice-water. After the exothermic reaction was subsided the content was stirred for 15 hr at room temperature. The reaction mixture was diluted with 50 ml chloroform and washed with dil. H₂SO₄, sat. NaHCO₃ sol. and water. Distilling off the solvent the residual oil was dissolved in 100 ml of hot ethanol and crystallized at cold place.

White crystal: Yield 1.4 g (40%), Mp 111°C, TLC (solvent C) R_f =0.78, $[\alpha]_D^{20} + 6.67^\circ$ ($c=1.20$ CHCl₃), Anal. Found: C; 67.72, H; 5.17. Calcd. for C₄₂H₄₀O₈Cl₂: C; 67.83, H; 5.42%. IR $\nu_{\text{max}}^{\text{NaJol}}$ cm⁻¹: 1770 (OC=O). NMR (5% in CDCl₃) δ : 4.72 (2H, s, OCH₂CO), 5.62 (1H, d, $J=7.0$ cps, anomeric H).

Debenzylation of VIII. Tetrabenzyl derivative (VIII) was hydrogenated under the same condition as in the case of Va from IIIa. From 1.5 g of VIII 500 mg of pure amorphous powder was obtained after fractionation with column chromatography. (Yield 65%). IR Spectrum and thin-layer chromatogram of the product were completely superimposable to those of Va. Mixed melting point of the tetraacetate and Vb showed no depression.

Lead tetraacetate oxidation¹⁵⁾ of IVa. To the 20 ml acetic acid solution of IVa (400 mg 1.06 mm) was added 20 ml of 0.113 M lead tetraacetate-acetic acid solution (2.26 mm of Pb(OAc)₄). With stirring at room temperature the reaction process was monitored by sampling 1 ml of the reaction mixture which was mixed with 5 ml of 0.12 M KI-CH₃COONa solution and free I₂ was titrated with 0.02 N Na₂S₂O₃ solution. The theoretical amount of Pb(OAc)₄ was consumed for 24 hr.

Avena coleoptile section test (Fig. 2). Twelve coleoptile sections were incubated in 5 ml of 2% sucrose solution with each concentration of the glucosyl esters for 18 hr at 27°C in the dark. Each sections were initially 5 mm and the test was paralleled with equimolar of 2,4-D Na salt and was compared with untreated control.

Growth inhibitory test on rice seedling (Fig. 3). Seeds of *Oryza sativa* L. (Norin 21) were soaked for 2 days at 30°C before the use. Twelve seeds were placed on 50 ml of 0.6% agar plate in Petri dish with each concentration of the test substrate and incubated for 6 days at 28°C under light.

Acknowledgement. The authors express their thanks to Prof. K. Matsuda, Tohoku University, and Dr. H. Kuzuhara, the Institute of Physical and Chemical Research, for their valuable advices about sugar chemistry.

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