고구마樹脂중 잘라핀의 糖構成에 관한 硏究

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Sugar Constituents of Jalapin from Sweet Potato Tubers

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ABSTRACT

Jalapin purified from the tubers of the sweet potato (Ipomoea batatas) was deacylated and subjected to structural educidation. Complete and degraded acid hydrolyses indicated the presence of L-rhamnose, D-fucose and D-glucose in the molar ratio of 1:1:1 and in the increasing order of acid-stability. While two moles of periodate were consumed per mole of the product, D-glucose survived in the oxidation. The following structure was, therefore, proposed tentatively for the deacylated jalapin: L-Rha$f$-(1→4)-D-Fuc$p$-(1→3)-D-Glu$p$-(1→11)-jalapinolic acid.

要 約

고구마중 잘라핀의 糖構成을 究明하기 위하여 고구마塊根에서 정제한 잘라핀을 脫아실化한 후 가수분해한 결과 L-rhamnose, D-fucose, D-glucose 가 1:1:1의 分子比 및 上記順序의 酸安定度를 가짐을 알았다. 脫아실化한 잘라핀 1몰은 2몰을 過沃素酸에 의하여 산화되었으나 D-glucose 는 산화되지 아니하였다. 따라서 고구마중 脫아실化한 잘라핀의 가능한 化學構造를 다음과 같이 제안하였다.
L-Rha$f$-(1→4)-D-Fuc$p$-(1→3)-D-Glu$p$-(1→11)-jalapinolic acid.

Introduction

It has been known that the sweet potato (Ipomoea batatas) contains jalapin, as a major component of milky exudate from cut surfaces of the tubers. Jalapin, also called "jalap resin", had been used as a drug for centuries in early times by its cathartic action[1]. This resin is known to become darker upon standing in air and is generally regarded as a troublesome constituent in the processing of sweet potato tubers.

It was reported that, on alkaline hydrolysis, jalapin ($C_{49}H_{90}O_{23}$) produces jalapic acid ($C_{34}H_{66}O_{20}$) which consists of three molecules of glucose and one molecule of jalapinolic acid ($C_{16}H_{23}O_3$, 11-hydroxyhexadecanoic acid)[2,3]. Resinous glycolipids called under different names are widely distributed among other members of the sameplant family Convolvulaceae[4]. They vary considerably in the components which they yield on complete hydrolysis, but they have definite unifying features in structure[5,6]. These glycolipids may be

called acylated glycosides of hydroxylated fatty acid, mostly consisting of short-chain aliphatic acids, jalapinolic acid, D-glucose, D-fucose and L-rhamnose.

In the light of chemical composition of resins from the plant family Convolvulaceae, the presently known structure of jalapin from sweet potato tubers seems quite ambiguous, particularly in the sugar components. Present investigation was, therefore, undertaken to elucidate the chemical structure of the glycosidic acid portion of jalapin.

Experimental and Results

1. Paper Chromatography of Sugars

For the identification, quantitation and purification of sugars, the general procedure[7,8] of paper chromatography was employed using the descending technique, Whatman No. 1 filter paper, the solvent systems, (a) 1-butanol: ethanol: water(4:1:5), (b) 1-butanol: acetic acid: water (4:1:5), and the spray reagents, (a) silver nitrate-sodium hydroxide in ethanol, (b) aniline phthalate, (c) p-anisidine. A R_g1 value refers to the rate of mobility relative to glucose.

2. Thin-Layer Chromatography

Qualitative identification of resinous materials was performed according to the thin-layer chromatography as described by Stahl[9], using silica gel G (Merck), the solvent system chloroform: methanol (9:1) and the spray reagent 50% sulfuric acid or diphenylamine.

3. Isolation of Jalapin

Two Kg of dried sweet potato strips prepared from Suwon No. 147, a recommended variety in Korea, were ground to 40-mesh powder and extracted with 6 liters of 90% ethanol for two hours at 50-60°C. The alcohol extract separated by centrifugation was concentrated to a small volume under reduced pressure, leaving 175g of yellow-brownish syrup with gelatinous consistency, which was then extracted with ethyl ether overnight in a continuous liquid-liquid extractor. The ether extract was dried over anhydrous sodium sulfate and evaporated to 27.4g of syrupy material. This syrup was then separated into the acetone soluble fraction(17.3g) and the acetone insoluble portion which is composed of monoaminophospholipids, thus being discarded.

The acetone soluble fraction was concentrated to a

small volume and subjected to thin-layer chromatography which showed the presence of four components (Figure 1). Out of these, two fast-moving spots having R_f values of 0.78 and 0.44 appear to be triglycerides and the other two slow-moving components with R_f values of 0.23 and 0.10 were also positive to the diphenylamine spray reagent, indicative of glycolipids. The component having R_f value of 0.23 seems to be the major resinous glycosidic material and it was always detected distinctly.
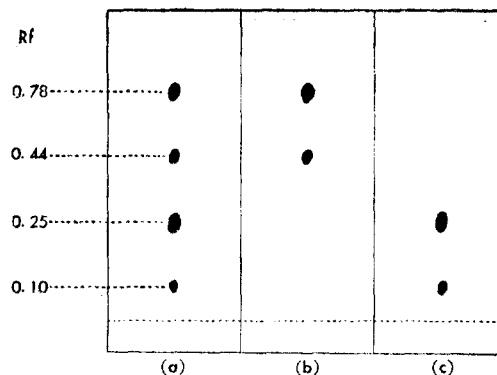

Figure 1. Thin-layer Chromatograms of Sweet Potato Resins
(a) Acetone soluble fraction, with 50% sulfuric acid spray.
(b) Petroleum ether fraction, positive with 50% sulfuric acid spray, but negative with diphenylamine reagent.
(c) 90% ethanol fraction, sprayed with diphenylamine reagent.

Since the acetone soluble extract was mixtures of neutral fats and glycolipids, it was partitioned between petroleum ether and 90% ethanol in a separatory funnel. The combined petroleum ether fraction was dried over sodium sulfate and concentrated to 6.5g of oily material which consisted of neutral fats as evidenced by the chromatographic mobilities corresponding to the R_f values of 0.78 and 0.44 and the color development. The 90% ethanol layer, after several washings with petroleum ether and concentration, yielded 10.4g of resinous glycosidic material which is positive to diphenylamine reagent on a thin-layer chromatogram. This fraction will be called "jalapin" in this paper.

4. Indentification of Sugars in Jalapin

A) Alkaline Hydrolysis of Jalapin

A portion of jalapin (7.5g) was refluxed with 1 N alcoholic potassium hydroxidefor an hour to obtain deacylated jalapin (5, 6). The reaction mixture was

then acidified with 1 N hydrochloric acid and extracted repeatedly with chloroform. The combined chloroform extract was evaporated to remove the solvent and the residue was steam-distilled to remove volatile acids. The residue after steam distillation was extracted several times with chloroform. The combined chloroform extract, after drying with sodium sulfate and concentration, yielded 4. 0 g of deacylated and partially degraded jalapin which appears to correspond to jalapic acid named by earlier investigators.

The acidic aqueous layer remaining after the first chloroform extraction was examined by paper chromatoraphy. This indicated the presence of L-rhamnose which is an acid-labile sugar in jalapin and seems to be partially liberated during the process of deacylation.

B) Acid Hydrolysis of Deacylated Jalapin

(a) Partial acid hydrolysis. —Half gram of the glycolipid was refluxed with 0. 2 N aqueous sulfuric acid solution for 15 minutes[10]. The resulting mixture was then extracted several times with chloroform. The residual aqueous layer, after neutralizing with barium carbonate and concentrating in vacuo, yielded L-rhamnose as an acid-labile sugar. The chloroform extract was evaporated to dryness (0. 4g), suspended in 30 ml of 0. 5 N sulfuric acid and allowed to stand for an hour in a boiling water bath. The mixture was then extracted with chloroform repeatedly, and the residual aqueous layer, after treating with barium carbonate (pH 7. 0), was evaporated to dryness in vacuo. D-fucose was seen faintly on the paper chromatogram and regarded as a more acid resistant sugar.

(b) Complete acid hydrolysis. —The deacylated glycolipid (1. 0g) was refluxed with 1 N sulfuric acid/ 50% dioxane for 6 hours in a boiling water bath. After extraction of this mixture with petroleum ether, the residual liquor was treated with barium sulfate and evaporated to dryness in vacuo. The resulting syrup yielded three sugars, L-rhamnose, D-glucose and D-fucose. Hydrolysis of the deacylated glycolipid by refluxing it with 1 N HCl in 50% aqueous dioxane for 2 hours gave the same result.

C) Characterization of Sugars

The tentative identification of sugars was done by paper chromatography by comparing the R_{g1} values and color of the spots as shown in Table 1 for the probale sugars encountered in this experiment. The

identity of sugars was further confirmed by preparing derivatives from the individual sugar syrup purified by the paper chromatographic techniques.

Table 1. Rgl values and Color Reactionsof Sugars in Jalapin

		D-Glucose	D-Fucose	L-Rhamnose
Color reagent	(a)	dark brown	dark brown	dark brown
"	(b)	brown	brown	brown
"	(c)	light brown	green	green
Rgl in solvent	(a)	1.00	1.57	1.79
"	(b)	1.00	1.38	1.54

(a) Sugar suggesting D-glucose—To 25 ml of glacial acetic acid solution containing 300mg of the sugar was added 0. 5g of p-nitrophenylhydrazine, and the mixture refluxed for 2 hours. On dilution and allowing to cool, p-nitrophenylhydrazone was crystallized out and it was recrystallized from acetic acid, whose melting point was 181-2°C and the mixed m. p. with D-glucose p-nitrophenylhydrazone, 181-2°C; reported m. p. , 181. 5°C[5]. The sugar was therefore identified as D-glucose.

(b) Sugar suggesting D-fucose. —About 200mg of the sugar was dissolved in 10 ml of water and 10 ml of 7% toluene p-sulfonylhydrazone solution was added to it. The mixture was then refluxed for 30 minutes, cooled to 0°C and kept at this temperature for 24 hours. The resulting precipitate was recrystallized from methanol. The melting point was 169-170°C and the mixed m. p. with D-fucose toluene p-sulfonylhyd-razide, 168-9°C; reported m. p. , 168-9°C(5). The sugar was therefore identified as D-fucose.

(c) Sugar suggesting L-rhamnose. —About 250 mg of sugar dissolved in 25 ml of glacial acetic acid was converted to the p-nitrophenylhydrazone having m. p. 189-190°C, mixed m. p. , with L-rhamnose p-nitro-phenylhydrazone, 188-9°C; reported m. p. , 188-9°C[5]. The sugar was therefore confirmed as L-rhamnose.

(D) Evaluation of Molar Ratio of Sugars in Jalapin

Jalapin was subjected to the direct acid hydrolysis, followed by the separation of sugars using the paper chromatographic techniques and determination of total carohydrates by the phenol-sulfuric acid method[11], using the Coleman Universal Model 14 spectrophotometer.

For the acid hydrolysis, 0. 3 g of purified jalapin

was dissolved in 100 ml of 50% dioxane, and HCl was added to make 1 N concentration. After refluxing the mixture for 4 hours, solvent was removed under reduced pressure and residue was steam-distilled. The aqueous residue containing sugars and aglycone portion was extracted with petroleum ether to remove the aglycone portion. The sugar fraction was treated with silver carbonate, concentrated to a syrup and used for the molar ratio determination of sugars in jalapin. The results indicated the molar ratio of 1.0, 1.3 and 1.3 for D-glucose, D-fucose and L-rhamnose, respectively.

5. Periodate Oxidation of Deacylated Jalapin

A) Measurement of Periodate Consumption

Quantitative periodate oxidation of the deacylated jalapin was carried out after modification from the procedure of Fleury and Lange[12] A portion of deacylated glycoside (0.3g) was dissolved in 100 ml of 95% ethanol and to this was added 50 ml of 28 mM periodate solution in 0.05 M acetate buffer (pH 4.0). Two ml aliquot withdrawn from the reaction mixture in the dark at various time intervals was mixed with 10 ml of saturated sodium bicarbonate, 10 ml of 0.017 N arsenious acid, and 2 ml of 20% potassium iodide solution. After storing the mixture for 15 minutes in the dark, it was titrated with 0.02 M iodine solution. The calculation was based on the proposed structure of deacylated jalapin as given in Discussion. As shown in Figure 2, approximately 2 moles of periodate were consumed per mole of the compound.
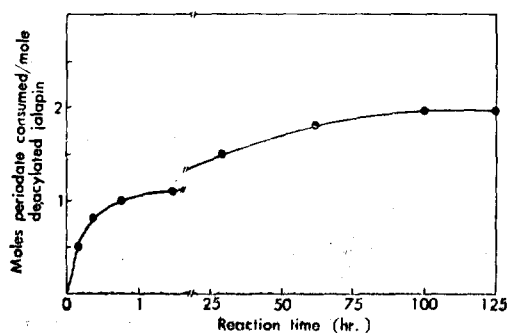

Figure 2. Time Course of Periodate Oxidation with Deacylated Jalapin

B) Characterization of Sugars Surviving in the Periodate Oxidation

The reaction mixture of periodate oxidation (120 hours) in the absence of buffer was neutralized with barium hydroxide and the resulting precipitates of barium iodate and barium periodate were removed by centrifigation. The supernatant was evaporated to dryness and then hydrolyzed with 1 N sulfuric acid for an hour. The hydrolyzate was neutralized with barium carbonate, filtered, and the resulting solution was evaporated to a syrup which, upon paper chromatographic analysis, was shown to contain only D-glucose and other fragments suspected as dialdehyde compounds.

Discussion

Sweet potato resin consists of mixtures of several components which can be separated by means of selective solvent extraction. The crude jalapin which was obtained by successive extraction with alcohol, ether and acetone by early investigators was shown to be mixtures of at least 4 components by thin-layer chromatography. The use of the solvent system, petroleum ether and ethanol provided the chance to remove neutral fat portion from the crude jalapin. Thus purified jalapin which showed one major component and another minor component on thin-layer chromatogrm was subjected to structural elucidation of its glycosidic constituents.

The presence of three sugars L-rhamnose, D-fucose and D-glucose in jalapin was proved by their isolation and characterization. The previous observation that glucose is the sole sugar in jalapin should be due to the unsatisfactory method of separating sugars in the old time. The presence of the three sugars in jalapin seems quite probable in the light of sugar composition of other resins from the same plant family Convolvulaceae. The molar ratio of L-rhamnose, D-fucose and D-glucose in jalapin was estimated to be 1.3, 1.3 and 1.0, respectively. In order to know the possible structure of glycosidic constituents of jalapin, it was subjected to alkaline hydrolysis to obtain deacylated jalapin, followed by partial and complete acid hydrolyses and periodate oxidation.

By mild acid hydrolysis, only L-rhamnose was released, indicating that L-rhamnose is attached by a furanosidic linkage at the nonreducing end. D-fucose

was liberated by stronger acid hydrolysis. In view of the greater stability of pyranosidic linkage than furanosidic[13, 15], D-fucose and D-glucose should be linked in pyranose form. Since it is known that very bulky secondary alkyl groups as aglycone may cause steric hindrance to the protonation stage involved in the hydrolysis of glycosidic linkage[6], the sugar directly joined to jalapinolic acid moiety should be quite acid-resistant. The present result that D-glucose was released only under complete acid hydrolysis indicates the possibility that D-glucose is directly attached to jalapinolic acid. Consequently, D-fucose is likely to exist in between L-rhamnose and D-glucose. The deacylated jalapin consumed two moles of periodate per mole of the compound; the first one mole was taken up very rapidly while the second was consumed much slowly. It was also observed that only D-glucose was not oxidized by periodate under the experimental conditions and isolated from the reaction mixture while no other sugars, formic acid nor formaldehyde were observed in the reaction mixture. The formation of formaldehyde would be possible only from the primary alcohol group of D-glucose when it possesses a free hydroxyl group at C-5. The production of formic acid in non-reducing oligosaccharides should be possible only if a monosaccharide unit consumes two moles of periodate, thus leaving two sugar molecules intact in case of deacylated jalapin. The present observation that only D-glucose survived in the periodate oxidation should indicate that L-rhamnose and D-fucose consumed one mole each of periodate.
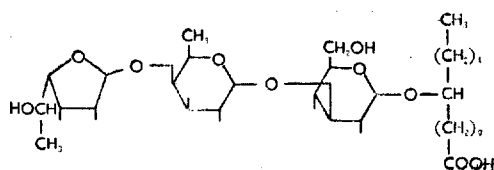
The above experimental results suggest the formula shown below to be the most likely structure of deacylated jalapin from sweet potato tubers, though the assignment of anomeric configuration was not possible yet. It is also possible that the terminal L-rhamnose is joined to D-fucose by a 1→2 linkage, rarely encountered in nature. The slow uptake of second mole of

periodate in the oxidation of deacylated jalapin may be explained by the fact that adjacent hydroxyl groups in the trans position as in the case of D-fucose are more resistant to periodate oxidation[16].

Since it has been shown that the sugar hydroxyl groups of Convolvulaceous resins are esterified with volatile acids[5,6], as supported by the I. R. spectra and gas chromatographic analysis of the various glycoside fraction and degradation products, it is quite probable that jalapin is also acylated with various aliphatic acids whose composition has not been identified yet in this experiment. It may be possible that various aliphatic acids might have been attached to deacylated jalapin to give different components. These might have revealed as trace amounts of glycolipids in addition to one major component on thin-layer chromatogram of purified jalapin. However, the proposed formula is most likely to represent the chemical structure of deacylated major component of jalapin.

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