NOTES

Studies of the Mucilage of the Root of "Tororo-aoi" (Abelmoschus manihot, MEDIC). IV.[1] *An Aldotriouronic Acid Isolated from the Mucilage*

By Saburo INOKAWA, Ryozo GOTO, Toshiro BAN, Takane UCHIDA and Kiyoaki WAKAIZUMI

(Received March 2, 1964)

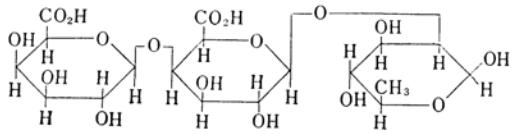
There have been many studies[2] of the mucilage of "Tororo-aoi" (*Abelmoschus manihot*, MEDIC), and the purified mucilage has been found to be composed of rhamnose and galacturonic acid, but the chemical structure of the mucilage has not yet been clarified in detail.

Oshibuchi[3] has suggested the presence of an aldotriouronic acid (rhamno-di-galacturonic acid) in the hydrolysate of the mucilage. In the present paper, the chemical structure of this aldotriouronic acid will be determined.

Hydrolysis of the mucilage with 4 percent sulfuric acid, followed by the precipitation of the neutralized hydrolysate with ethanol, gave a mixture of barium salts, from which an aldotriouronic acid was isolated as white crystals $[\alpha]_D^{13}$ +74° (*c* 1, water) by de-ionizing the mixture with ion-exchange resins and using cellulose column chromatography. The aldotriouronic acid was found to be composed of rhamnose and galacturonic acid by hydrolysis and paper chromatography. The equivalent weight of the aldotriouronic acid, as determined by titration with alkali, was 260, which shows that the aldotriouronic acid consists of rhamnose and galacturonic acid in a mole ratio of 1 : 2.

The aldotriouronic acid was converted into a neutral trisaccharide by the reduction of the methyl ester of the methyl glycoside with sodium borohydride. The methylation of the trisaccharide gave a derivative which, on acid hydrolysis, gave 2, 3, 4, 6-tetra-*O*-methylgalactose, 2, 3, 6-tri-*O*-methylgalactose, and 3, 4-di-*O*-methylrhamnose.

The structure of the aldotriouronic acid is, therefore, determined to be *O*-α-D-galacturonosyl-(1→4)-*O*-α-D-galacturonosyl-(1→2)-*O*-L-rhamnose, the anomeric configuration of which is supposed from the optical rotation:



Experimental

Paper-partition chromatography was carried out with the upper layer of butanol - acetic acid - water (4 : 1 : 5). Paper ionophoresis was carried out in a 0.2 M borate buffer at pH 10. R_{gal} and R_G (or M_G) values are proportional to the rates of the migration of galactose and 2, 3, 4, 6-tetra-*O*-methylglucose respectively. The *R* values observed were compared with those of authentic samples.

The Preparation of Purified Mucilage.—After the roots of "Tororo-aoi" had been ground and soaked in distilled water for 2 days at 0°C, the mucilage was filtered through a cotton cloth. The crude mucilage was then homogenized with a mixer, filtered through a cellite-bed, and precipitated with ethanol-ether (8 : 2), and the precipitate was redissolved in water, re-precipitated with ethanol, washed with absolute ethanol and dry ether, and dried in vacuum.

The Partial Hydrolysis of Purified Mucilage with Dilute Sulfuric Acid.—Dry purified mucilage (10 g.) was heated in 4 percent sulfuric acid 100°C for 11 hr. The filtered solution was neutralized with barium carbonate and then filtered, and the filtrate was concentrated under reduced pressure to a light syrup (100 ml.), which was then stirred into ethanol (400 ml.). The precipitate was washed thoroughly with 80 percent ethanol, absolute ethanol, and dry ether, and dried in a vacuum. The precipitate was redissolved in water (100 ml.) and the solution was poured into ethanol (400 ml.), washed, and dried similarly to give a white powder

1) Part III: S. Inokawa, This Bulletin, **34**, 29 (1961).
2) See S. Inokawa and R. Goto, *Wood Research* (Japan), No. 17, 50 (1956); S. Inokawa, This Bulletin, **33**, 1473 (1960).
3) T. Oshibuchi and H. Kusunose, *J. Agr. Chem. Soc. Jaapan* (*Nippon Nogei-Kagaku Kaishi*), **31**, 481 (1957).

(4.1 g.). On a paper chromatogram after having been de-ionized with IR-120 resin, the product gave two spots, namely, galactose and R_{gal} 0.32.

The Isolation of Aldotriouronic Acid. — The white powder (2 g.) described above was de-ionized with IR-120 resin, and a portion corresponding to R_{gal} 0.32 was separated on a cellulose column (5× 40 cm.) with butanol - acetic acid - water (12 : 3 : 5) to give white crystals (0.40 g.), $[\alpha]_D^{13}$ +74° (*c* 1, water). On titration with a 0.005 N sodium hydroxide solution, the equivalent weight of the material was 260 (calc. for rhamno-di-galacturonic acid; 258). The crystals were then completely hydrolysed with 2 N sulfuric acid in a sealed tube at 100°C for 24 hr. The neutralized solution, de-ionized with IR-120 resin, gave rhamnose and galacturonic acid on a paper chromatogram.

The Identification of the Aldotriouronic Acid. —The aldotriouronic acid (300 mg.) was refluxed with 1.8 percent methanolic hydrogen chloride (50 ml.) for 7 hr., neutralized with silver carbonate, and filtered. The filtrate was evaporated to dryness, the residue dissolved in water (6 ml.), and the solution added slowly to a solution of sodium borohydride (200 mg.) in water (3 ml.). After 2 hr., the excess borohydride was destroyed by adding dilute acetic acid, and the solution was de-ionized with IR-120 and IR-45 resins and evaporated under reduced pressure to dryness to give white prisms (120 mg.), $[\alpha]_D^{13}$ +2° (*c* 1, water). The crystals were completely hydrolysed, and the neutralized solution gave only rhamnose and galactose on a paper chromatogram.

The crystals were methylated three times with methyl sulfate and sodium hydroxide, and, after the extraction of the acidified mixture with chloroform, the product was further methylated three times with methyl iodide and silver oxide to give a pale yellow oil (100 mg.).

The oil was hydrolysed with N sulfuric acid (10 ml.) at 100°C for 18 hr. in a sealed tube. The hydrolysate was neutralized with barium carbonate and extracted with chloroform. The chloroform solution was evaporated to give a syrup (30 mg.) which gave an intensive spot (R_G 0.89) and a faint spot (R_G 0.74) on a paper chromatogram. The aqueous residual solution was de-ionized with IR-120 resin and evaporated to give a syrup (10 mg.) which gave an intensive spot (R_G 0.74), a faint spot (R_G 0.89), and a very faint spot (R_G 0.58) on a paper chromatogram. The spots at R_G 0.74 and 0.58 corresponded to 2,3,6-tri-*O*-methylgalactose and 4-*O*-methylrhamnose respectively. The spot at R_G 0.89, by paper ionophoresis,[4] was separated into two spots, $M_G \fallingdotseq 0$ and M_G 0.39, which corresponded to 2,3,4,6-tetra-*O*-methylgalactose and 3,4-di-*O*-methylrhamnose, respectively.

This work was partially supported by a grant from the Hattori Hokokai, to which the authors' thanks are due.

Department of Chemistry
Faculty of Science
Kyoto University
Sakyo-ku, Kyoto

4) A. B. Foster, *J. Chem. Soc.*, **1953**, 982.