1034. *The Composition of* Afraegle paniculata *Mucilage.*

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Afraegle paniculata mucilage gives on hydrolysis D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid. Partial hydrolysis of the mucilage gives an aldobiouronic acid, 6-O-β-D-glucuronosyl-D-galactose.

Afraegle paniculata Engl. (family Rutaceae) is an evergreen tree occurring in savannah country near the sea in Ghana. It bears fruits resembling grapefruit in size and appearance. The insides of the fruits are divided into segments like those of an orange, each segment holding several seeds immersed in a sticky mucilage.

Purified *Afraegle paniculata* mucilage has a small positive specific rotation and an equivalent weight of 4000, indicating the presence of a small proportion of acidic residues. On complete hydrolysis, D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid are obtained.

Paper chromatography of the products of hydrolysis by 0·02N-sulphuric acid at 95° showed the liberation within 2 hours of arabinose, and after 6 hours of galactose and rhamnose, indicating that arabinose is present in the furanose form or is located at peripheral positions in the polysaccharide molecule.

Partial hydrolysis of the mucilage gave a product containing an aldobiouronic acid which was shown to be homogeneous by prolonged chromatography in an acid solvent and migrated at the same rate on the chromatogram as 6-*O*-β-D-glucuronosyl-D-galactose isolated from gum arabic.

The aldobiouronic acid was shown to be 6-*O*-β-D-glucuronosyl-D-galactose by oxidation of the galactose moiety with bromine, and by isolation of 2,3,4-tri-*O*-methyl-D-galactose from the hydrolysis products of the fully methylated form.

The optical rotation of the aldobiouronic acid indicates that a β-glucuronosyl junction. The mucilage thus bears a superficial resemblance to *Acacia* gums.[1,2]

Experimental

Paper chromatograms were run in the solvent systems (A) butan-1-ol–ethanol–water (5 : 1 : 4 v/v, upper phase), (B) butan-1-ol–ethanol–water (20 : 1 : 3) with added cetylpyridinium bromide (3 g./100 c.c.), (C) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), (D) butanol–benzene–pyridine–water (5 : 1 : 3 : 3), and (E) ethyl methyl ketone saturated with water. Spots were detected with an aqueous aniline oxalate spray.

Optical rotations were determined for water solutions.

Purification of the Mucilage.—The fruits were opened and the segments containing the seeds and mucilage were separated from the brown pulp. The segments were split and the mucilage pressed out, care being taken to avoid contamination with other parts of the fruit.

The mucilage from 30 fruits was made up with distilled water to about 1 l. and added to methanol (3·5 l.). The precipitated material, collected on filter paper, dried to a thick gel (180 g.) in air.

A portion (20 g.) of the crude gel was purified by precipitation: solutions, twice in dilute hydrochloric acid and then once in water, were poured with stirring into methanol. The product, after drying at 30° in a partial vacuum ($CaCl_2$) for 48 hr., was a white amorphous powder (11 g.), $[\alpha]_D^{23}$ +2·5° (*c* 0·3) [Found: equiv., 4000 (by titration); ash, 0].

Total Hydrolysis.—The purified mucilage was completely hydrolysed by heating a 0·5% solution in 2N-sulphuric acid in a sealed tube in a boiling-water bath for 24 hr. The solution was neutralised with barium carbonate, filtered, and concentrated under reduced pressure to small volume. On chromatograms with reference substances run in several solvent systems, the sugars galactose, arabinose, and rhamnose were detected. The mucilage was extensively decomposed during the hydrolysis, but an approximate estimate of the proportions of sugars present was obtained by comparing the areas of spots on a chromatogram with those formed by solutions of sugars of known concentration. This gave the galactose : arabinone : rhamnose ratio of 4 : 3 : 2 (very approximate values owing to the prior decomposition).

Isolation of the Aldobiouronic Acid.—Purified mucilage (75 g.) was heated in N-sulphuric acid (600 c.c.) at 100° for 24 hr. The filtered solution was neutralised with barium carbonate and again filtered and the filtrate was concentrated under reduced pressure to a syrup (100 c.c.). This was poured into methanol (1 l.), and the precipitated barium salt purified by reprecipitation in methanol from aqueous solution, giving a cream solid (4·5 g.), $[\alpha]_D^{23}$ +2° ± 5° (*c* 2·5) (Found: Ba, 16·9. Calc. for $C_{12}H_{17}Ba_{0\cdot5}O_{12}$: Ba, 16·2%). Prolonged chromatography (104 hr.) of the barium aldobiouronate in solvent D gave only one spot, indicating homogeneity. The rate of migration in solvent C ($R_{gal.}$ *ca.* 0·25) was the same as that of 6-*O*-β-D-glucurosonyl-D-galactose, from gum arabic, run on the same paper.

Upon hydrolysis of the barium salt with 2N-sulphuric acid in a sealed tube at 100° for 16 hr., the neutralised and de-ionised [I.R.-120(H^+)] solution was examined on a chromatogram. Galactose, glucuronic acid, and glucurone were identified.

Isolation of Galactose, Arabinose, and Rhamnose.—Concentration of the methanolic mother liquors from the precipitation of the barium aldobiouronate, and storage at 0°, yielded crude crystalline galactose. Purified by recrystallisation from methanol, it had m. p. and mixed

[1] Hirst and Perlin, *J.*, 1954, 2622.
[2] Charlson, Nunn, and Stephen, *J.*, 1955, 169.

m. p. 162—165°, $[\alpha]_D^{23}$ +80° (*c* 0·5), and gave mucic acid, m. p. and mixed m. p. 212—214°, on oxidation with nitric acid.

A portion of the syrup left after the removal of galactose was separated on a large paper chromatogram, and L-arabinose, m. p. and mixed m. p. 156°, $[\alpha]_D^{22}$ +104° (*c* 0·5) [benzoylhydrazone, m. p. and mixed m. p. 206—207° (from ethanol)], and α-L-rhamnose hydrate (from ethanol), m. p. and mixed m. p. 93—94°, $[\alpha]_D^{23}$ +10° (*c* 1·5), were obtained.

Identification of Uronic Acid.—Barium aldobiouronate (0·400 g.) was heated with 2N-sulphuric acid (5 c.c.) in a sealed tube at 100° for 24 hr. The neutralised solution was evaporated to a light syrup and poured into methanol. The precipitated barium uronate was removed on the centrifuge and purified by two reprecipitations in methanol from aqueous solution. The product was a cream powder (0·083 g.), $[\alpha]_D^{21}$ +15° (*c* 0·3) (Found: Ba, 26·7. Barium hexouronate requires Ba, 26·2%).

When de-ionised with I.R.-120(H^+) resin, it gave glucuronic acid and glucurone on chromatograms run in several solvents with authentic reference substances. Complete lactonisation was effected by heating it in a vacuum at 70° for several hours; the product was converted into the *p*-nitroanilide,[3] m. p. and mixed m. p. 129—130°, of glucuronic acid.

Structure of the Aldobiouronic Acid.—A specimen of barium aldobiouronate was oxidised with bromine water for a week and the bromine was removed by aeration. The product was hydrolysed with 1·75N-sulphuric acid at 100° for 24 hr., neutralised, and de-ionised with I.R.-120(H^+) resin. On a paper chromatogram run in solvent (B) only uronic acid was detected.

Barium aldobiouronate (1·7 g.) was methylated four times with dimethyl sulphate and alkali. The product, after acidification of the reaction mixture, was isolated by continuous extraction with chloroform and then methylated twice with methyl iodide and silver oxide. The product, a viscous syrup (1·0 g.), was distilled, giving a main fraction (0·5 g.), b. p. 180°/0·01 mm., n_D^{26} 1·4655 (Found: C, 50·8; H, 7·7; OMe, 50·1. Calc. for $C_{20}H_{36}O_{12}$: C, 51·3; H, 7·8; 8OMe, 53·0%). The syrup (0·450 g.) was heated with 2N-sulphuric acid in a sealed tube at 100° for 24 hr. The filtered solution was neutralised with sodium hydroxide solution and extracted in a continuous extractor with ethyl acetate. The extract, on evaporation, gave a syrup (0·090 g.), which on the chromatogram gave a spot corresponding to 2,3,4-tri-*O*-methylgalactose as well as a trace of tetra-*O*-methylgalactose. The syrup was separated on a large paper chromatogram, 2,3,4-tri-*O*-methylgalactose, $[\alpha]_D^{23}$ +95° (*c* 0·5), being obtained. Its identity was confirmed by preparation of the crystalline *N*-phenyl-D-galactosylamine trimethyl ether, m. p. and mixed m. p. 162—163°, and by oxidation with sodium periodate, giving formaldehyde (dimedone compound, m. p., and mixed m. p., 192°).

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[3] Hamilton, Spriestersbach, and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 443.