



Fig. 1. Photomicrograph showing the range of size of the filaments, the mode of branching and a strand-like coating of ferric hydroxide on the largest filament. ($\times 90$.) (2) The cells contain conspicuous rounded vacuoles and minute granules. ($\times 360$.) (3) The peg-like ingrowth from one cell into the adjacent cell is clear. ($\times 360$)

being made to culture the organism on various media in the hope that further investigation will throw light on the metabolism and systematic position of this interesting plant.

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¹ Coker, W. C., and Shanor, L., *J. Elisha Mitchell Sci. Soc.*, **55**, 152 (1939).

Occurrence of Apiose in *Hevea brasiliensis*

FRUCTOSE, glucose, quebrachitol, mesoinositol, and (-)-inositol have been detected in the free state in *Hevea* latex serum, and glucose, galactose, mesoinositol, and an unknown sugar (probably a ketose) were obtained on hydrolysis of a latex lipid fraction¹. The work reported here is an examination of *Hevea* bark tissues for reducing sugars, and, in view of the common 5-carbon branched skeleton unit of the isoprenoid compounds, particular interest lay in the detection of branched-chain sugars.

The plant material used was whole bark, which had been collected fresh and dried at 90°. A powdered sample (50 gm.) was boiled under reflux for 4 hr. with 0.2 N sulphuric acid (500 ml.) and filtered. After saturating the filtrate with barium carbonate, and filtering, the solution was concentrated under reduced pressure to a syrupy residue. This was extracted with boiling 95 per cent ethanol (100 ml.), and the insoluble material removed on the centrifuge. The

ethanol was distilled off, and the resulting syrup boiled for 5 min. with water (50 ml.) and animal charcoal (2 gm.) and filtered. After concentration, and extraction with boiling ethanol, as before, the solution was finally concentrated to a thick syrup.

Chromatography of this product was carried out on Whatman No. 1 paper with a solvent system of *n*-butanol-water-ethanol (50 : 40 : 10 by vol.) in runs of up to 55-hr. duration. Eight compact and fully separated spots were stained by silver nitrate reagent. The fastest-moving component (R_F 0.28) was of greatest interest, since this agreed exactly with an authentic specimen of apiose prepared by hydrolysis² of parsley apiin (Merck). By the use of specific spray reagents, and variation of solvent system in one- and two-dimensional runs with comparative mixtures of known compounds, it was concluded that the remaining spots were due to maltose, galactose, glucose, fructose, quebrachitol, mesoinositol, and possibly xylose. Only glucose, fructose and quebrachitol could be detected in water extracts of bark. In order to characterize further the substance resembling apiose, a preparative fractionation of the sugar mixture was carried out on a column of cellulose powder³. The authentic apiose, which contained about 5 per cent glucose impurity, was fractionated in the same way. A fraction (I) isolated from the bark mixture yielded 11 mgm. of syrup. This was indistinguishable from apiose when chromatographed separately and in mixtures in a wide range of solvents. With high concentrations of fraction I a faint spot, stained red by aniline hydrogen phthalate, was visible immediately behind, but completely separated from, the suspect apiose spot. Apiose, while not yielding furfuraldehyde when treated with acid, did give faint colours (yellow-brown) when sprayed with aniline hydrogen phthalate or *p*-anisidine hydrochloride. Identical colours were given with the corresponding component of fraction I.

Phenylosazones were prepared from fraction I and apiose, and recrystallized from aqueous ethanol. These melted at 154°-156°, and the mixed melting point was unchanged. Thus, it was concluded that the main component of fraction I was apiose.

This sugar has the same carbon structure as the skeleton unit of isoprenoid substances, and it has been suggested⁴ that apiose could give rise to simple terpenes by condensation and reduction. Such a reaction seems unlikely, but the presence of apiose in *Hevea* does suggest a possible metabolic link between the sugars and the 5-carbon intermediates of isoprenoid synthesis. An interesting connexion was noted between parsley, from which apiose is most readily obtained, and several rubber-producing plants. The carbon tetrachloride extracts of *Hevea* bark, *Taraxacum kok-saghyz* root and *Taraxacum officinalis* root, were saponified, and extracted with ether. After removal of ether each of these extracts possessed the odour characteristic of fresh parsley.

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¹ Smith, R. H., *Biochem. J.*, **57**, 140 (1954).

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⁴ Stewart, A. W., in "Recent Advances in Organic Chemistry", 4th ed., 270 (Longmans, Green and Co., London, 1920).