POLYSACCHARIDES OF TROPICAL PASTURE HERBAGE

VI.* IDENTIFICATION OF *myo*-INOSITOL, GALACTINOL, AND RAFFINOSE IN SPEAR GRASS (*HETEROPOGON CONTORTUS*)

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[Manuscript received October 19, 1971]

We have previously[1] analysed ethanolic extracts of spear grass for sucrose, glucose, and fructose. The same extracts were also suspected to contain small amounts of raffinose.[2] Our current studies of ruminant digestion of spear grass necessitate a thorough knowledge of such components and we now report the identification of three major constituents which appear as weakly reducing substances migrating on paper chromatograms more slowly than sucrose.

The aqueous ethanolic (80%) extracts from spear grass were fractionated by preparative paper chromatography to isolate the major components migrating slower than sucrose. The products so obtained were shown to be homogeneous by paper chromatography. The fastest moving product was identified as *myo*-inositol by comparison with the authentic compound by paper chromatography in two different solvent systems and by gas chromatography as the hexaacetate, which was also crystallized.

The second component was identified as raffinose by paper chromatography in two different solvent systems. Both partial and complete hydrolysis with acid gave a range of products identical on paper chromatograms with those given by authentic raffinose.

The slowest-moving component was identified as galactinol by comparison with the authentic compound in two different solvent systems and by the following experiments. Acid hydrolysis followed by reduction, acetylation, and examination by gas chromatography showed the presence of galactitol and *myo*-inositol hexaacetates in equimolar amounts (measured by integration of the gas chromatogram peaks) together with a small amount of glucitol hexaacetate. Examination of the acid hydrolysate by paper chromatography indicated (assumed to be the D-isomer) galactose, *myo*-inositol, and smaller amounts of glucose. It was concluded that this component was galactinol contaminated with an unidentified glucoside. *myo*-Inositol, raffinose, and galactinol have been shown to be present in a wide range of plant species[3] but as far as we are aware this is the first time galactinol and *myo*-inositol have been found in a tropical species of the Graminae family.

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Experimental

Paper chromatography was carried out on Whatman papers No. 1 (analytical) and 3MM (preparative), with the following solvent systems (v/v): (*a*) ethyl acetate–pyridine–water (10 : 4 : 3); (*b*) butan-1-ol–ethanol–water (3 : 1 : 1). Alkaline silver nitrate[4] and *p*-anisidine hydrochloride[5] were used as sprays. R_{Glu} values of sugars refer to the distances moved relative to those of D-glucose. Gas chromatography was carried out as described earlier.[1]

The freeze-dried stem and leaves of vegetative spear grass (100 g) were extracted with two successive 2-l. portions of boiling 80% ethanol for 1 hr each. Extracts were filtered and concentrated (25 ml). Water (150 ml) was added and the water-insoluble components were removed by centrifugation. The solution was de-ionized by passage through Amberlite resins IRC–50 (H) and IR–45 (OH) successively and concentrated to a syrup (10 g). A portion of this extract (700 mg) was chromatographed using Whatman 3MM paper and solvent (*a*) (72 hr). The two major components (A and B respectively) were eluted from the appropriate areas of the paper with 20% ethanol and concentrated to syrups. The faster moving component A was rechromatographed using solvent system (*b*) (72 hr) and gave two components (i) and (ii) as follows:

(i) myo-*Inositol* (50 mg).—This co-chromatographed with authentic *myo*-inositol in solvent systems (*a*) and (*b*) (R_{Glu} 0·30 in solvent (*a*) and 0·45 in solvent (*b*)). The product was heated with a mixture of acetic anhydride (3 ml) and concentrated sulphuric acid (1 drop) for 1 hr at 60°. The solution was cooled, poured into a mixture of ice and solid sodium hydrogen carbonate, and extracted with dichloromethane (3 × 25 ml). The extracts were combined and evaporated to dryness. Crystallization of the residue from chloroform–light petroleum (60–80°) gave the hexaacetate, m.p. and mixed m.p. 216° (lit.[6] 217°). On gas chromatography this compound showed only one peak coincident with that of authentic *myo*-inositol hexaacetate.

(ii) *Raffinose* (5 mg).—This co-chromatographed with authentic raffinose in solvent systems (*a*) and (*b*) (R_{Glu} 0·27 in solvent (*a*) and 0·28 in solvent (*b*)). In two different experiments the oligosaccharide was hydrolysed in 0·1N sulphuric acid at 100° for 5 min and 6 hr respectively. After neutralization ($BaCO_3$) the hydrolysates were examined by paper chromatography using solvent systems (*a*) and (*b*). Meliobiose and fructose were the major products of the short-term hydrolysis, while galactose, glucose, and fructose were obtained from the longer hydrolysis in the same ratios as similar hydrolytic experiments on authentic raffinose.

The slower-moving component B was re-chromatographed on Whatman 3MM paper using solvent (*b*) (84 hr); the major component co-chromatographed with authentic galactinol in solvent systems (*a*) and (*b*) (R_{Glu} 0·20 in solvent (*a*) and 0·22 in solvent (*b*)) (30 mg). It was identified as galactinol as follows. Hydrolysis of a sample in 1N sulphuric acid for 6 hr at 100°, after neutralization ($BaCO_3$) and examination by paper chromatography in solvents (*a*) and (*b*) showed the presence of galactose and *myo*-inositol as major components plus glucose as a minor component. Reduction of the hydrolysate with sodium borohydride followed by acetylation[7] and examination by gas chromatography showed the presence of *myo*-inositol and galactitol hexaacetates (area ratios 1·0 : 0·9) together with smaller amounts of glucitol hexaacetate (*c.* 12% of the total).

Acknowledgments

An authentic sample of galactinol was kindly donated by Dr C. E. Ballou. This work was supported by grants from the Australian Meat Research Committee and the Rural Credits Development Fund of the Reserve Bank of Australia.

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