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PLANTEOSE, THE MAJOR SOLUBLE CARBOHYDRATE OF SEEDS OF *FRAXINUS EXCELSIOR*

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Abstract—The major soluble carbohydrate of seeds of ash has been characterized as planteose (6^F-galactosylsucrose). The taxonomic and morphological distribution of planteose is discussed

INTRODUCTION

INTACT seeds of *Fraxinus excelsior* L., the European ash tree, require both a period of maturation for the embryo fully to develop and a separate chilling period before germination will occur.[1] During analyses of the chemical changes involved in these processes, the major soluble carbohydrate was found to be a trisaccharide. This paper describes its characterization as planteose (6^F-galactosylsucrose).

RESULTS

The soluble carbohydrates of freshly collected and air-dried seeds of ash are sucrose, *myo*-inositol, a trisaccharide (the major component) and the hexoses, glucose and fructose (minor components). The trisaccharide had a lower PC mobility than melezitoze and raffinose in the 2 solvents used but co-chromatographed with planteose. Its reactions with a modified *p*-anisidine reagent[2] confirmed that it contained both ketose and aldose units. The GLC mobility of its TMS-derivative ($M_{\alpha G}$ 7·4) was greater than that of raffinose ($M_{\alpha G}$ 6·8) but identical with that of melezitoze and planteose.

Also, like planteose, it was not hydrolysed by invertase and, on mild acid hydrolysis, yielded in addition to glucose a disaccharide with identical chromatographic properties to planteobiose Despite its being a reducing sugar, planteobiose gave a single gas chromatographic peak ($M_{\alpha G}$ 3·03). When hydrolysed by strong acid or α-galactosidase, the ash trisaccharide yielded the expected products (glucose, fructose and galactose, or sucrose and

[1] VILLIERS, T A and WAREING, P F (1964) *J Exp Botany* **15**, 359
[2] LEWIS, D H, CHEN, C, WOODS, G and CULPIN, L A (1972) *J Chromatogr* **74**, 369

galactose respectively) Although these confirm its identity as planteose, the same products would also be derived from raffinose

Quantitatively, the trisaccharide represented approx. 1 3% dry wt of seeds. Dissection of embryo from rest of seed after overnight imbibition with separate analysis of seed parts showed that the trisaccharide was confined to the endosperm

DISCUSSION

Although members of the Oleaceae photosynthetically synthesize and translocate mannitol and the raffinose series of oligosaccharides in addition to sucrose,[3,4] neither mannitol nor these sucrosylgalactans are deposited in the endosperm. Instead, planteose accumulates along with the major lipid food reserve A comparable situation occurs in the genus *Plantago* (Plantaginaceae), where sorbitol (D-glucitol) substitutes for mannitol.[5-7]

Previously, planteose has been isolated from seeds of species of the Plantaginaceae,[5,6] Pedaliaceae,[8] Solanaceae,[9] and Labiatae.[10] This is of chemotaxonomic significance since, together with the Oleaceae, these are all families from closely allied orders of the Sympetalae [11] The isolation of planteose from cacao beans,[12] *Theobroma cacao* of the unrelated Sterculiaceae, does not necessarily conflict with this correlation since the sugar could have been formed during the post-harvest fermentation involved in processing beans, e.g. by an α-galactosidase transfer reaction.[13]

It may also be morphologically significant that, with the possible exception of the Labiatae, all these families have endospermic seeds so that the relatively rare trisaccharide, planteose, may be formed primarily in this genetically distinct, usually triploid, tissue The Labiatae are described as having either no or scanty endosperm[14] and planteose has been reported from rhizomes of *Teucrium canadensis*.[15]

The synthesis of planteose during development of seeds of *F excelsior*, its degradation during the various stages of embryo maturation and germination[1] and its distribution among related species are being currently investigated

EXPERIMENTAL

Material Ash fruits were collected at Thornhill, Derbyshire in March 1970 Pericarps were removed and discarded soon after collection

Extraction of soluble sugars Approximately 4500 seeds (air dry wt = 127 g) were ground in a mechanical pestle and mortar and defatted in batches using petrol (b p 40–60°) in a Soxhlet for 24 hr They were then extracted with 80% EtOH in small batches in the Soxhlet for 20 hr Extracts were filtered and divided into five portions which were re-extracted with petrol (3 × 30 ml) to ensure complete removal of lipids

Removal of fermentable sugars The extract was conc 10-fold at 40°, cooled to room temp and treated for

[3] TRIP, P., NELSON, C D and KROTKOV, G (1965) *Plant Physiol* **40**, 740
[4] TRIP, P., KROTKOV, G and NELSON, C D (1963) *Can J Botany* **41**, 1005
[5] WATTIEZ, N and HANS, M (1943) *Bull Acad Roy Med Belg* **8**, 386
[6] HÉRISSEY, H (1957) *Bull Soc Chim Biol* **39**, 1553
[7] GALKOWSKI, T T, GAUVIN, P N and SANTURRI, P R (1966) *J Agr Food Chem* **14**, 324
[8] HATANAKA, S (1959) *Arch Biochem Biophys* **82**, 188
[9] FRENCH, D (1955) *J Am Chem Soc* **77**, 1024
[10] FRENCH D., YOUNGQUIST, R W and LEE A (1959) *Arch Biochem Biophys* **85**, 431
[11] MELCHIOR, H (Ed.) (1964) *A Engler's Syllabus der Pflanzenfamilien*, Vol II, 12th Edn, Gebruder Borntraeger, Berlin-Nikolassee
[12] CERBULIS, J (1955) *Arch Biochem Biophys* **58**, 406
[13] COURTOIS, J E., PETEK F and DONG, T (1961) *Bull Soc Chim Biol* **43**, 1189
[14] CLAPHAM, A R., TUTIN T G and WARBURG, E F (1962) *Flora of the British Isles*, 2nd edn., Cambridge University Press, Cambridge
[15] WILD, G M and FRENCH, D (1952) *Proc Iowa Acad Sci* **59**, 226

3 hr with an equal vol of aerated, washed, 5% w/v suspension of baker's yeast [16] The yeast was removed by centrifugation and the solution cleared with 10% $Al(OH)_3$ suspension [17]

Isolation of trisaccharide After removal of fermentable sugars, the solution was passed through a charcoal–Celite column,[18] which was successively eluted with 800 ml H_2O, 1 5 l 5% EtOH and 1 5 l 15% EtOH Fractions (100 ml) of the last eluant giving a positive Molisch test were combined and, after concentration to a low volume, the trisaccharide was further purified by streaking on to washed Whatman No 1 paper and chromatography using PrOH–EtOAc–H_2O (7 1 2) The trisaccharide was eluted with H_2O and the eluate evaporated to dryness Portions were than characterized by PC and GC and by identification of products of acid and enzymic hydrolysis

Mild acid hydrolysis A portion of the purified trisaccharide was dissolved in 5 ml 0 1 N H_2SO_4 and heated at 100° for 30 min After cooling, the solution was neutralized with excess $BaCO_3$, filtered, de-ionized by shaking with a mixture of Amberlite 1R-120 (H) and 1R-45 (OH)[19] and concentrated by evaporation The neutralized hydrolysate was then characterized by PC and GC and by comparison with authentic raffinose and planteose

Strong acid hydrolysis A portion of the purified trisaccharide was dissolved in 5 ml 1·5 N H_2SO_4 and heated at 100° for 1 hr After cooling, the solution was neutralized by addition of excess (20 ml) 20% v/v *N,N*-dioctylmethylamine in $CHCl_3$, vigorous shaking and centrifugation [20] The $CHCl_3$ layer was withdrawn and the H_2O layer washed by successive shaking with 5 ml $CHCl_3$ and centrifugation The neutralized hydrolysate was then characterized as above

Hydrolysis with invertase (β-fructofuranosidase) A portion of the purified trisaccharide was dissolved in 3 ml H_2O and incubated with mechanical shaking at 28° for 43 hr with 2 ml 0 05 phthalate buffer, pH 4 5, and 1 ml 2% B.D H invertase The digest was deproteinized with 0 3 N $Ba(OH)_2$ and 5% $ZnSO_4$[21] and centrifuged After de-ionization as above, the products of the reaction were characterized as above

Hydrolysis with emulsin (α-galactopyranosidase) This was effected as for the invertase hydrolysis using almond emulsin

Paper chromatography Sugars were separated on Whatman No 1 paper using (i) EtOAc–HOAc–H_2O, 14.3.3 and (ii) PrOH–EtOAc–H_2O, 7 1 2[22] and detected using silver nitrate–sodium ethoxide[23] or a modified *p*-anisidine technique[2] which permits initial identification of fructose and oligosaccharides containing fructose and subsequent identification of free and combined aldoses

Gas chromatography Trimethylsilyl (TMS) derivatives were prepared and quantitatively estimated using a Pye Model 64 instrument, fitted with flame ionization detectors and 5 ft × 1/4 in glass columns containing 2% SE52 on Chromosorb W (80–100 mesh) [24] N_2 was used as the carrier gas at a flow rate of 45 ml/min The temperature programme was 180–290° at 4°/min + an isothermal period of 20 min at 290° when only the trisaccharide was being determined When products of acid and enzymic hydrolysis were being characterized, the initial temperature was 140°

Identity of compounds In both GC and PC, the identity of sugars was checked by co-chromatography with authentic standards

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[16] YEMM, E W (1935) *Proc Roy Soc* **B117,** 483
[17] HARLEY, J L and JENNINGS, D H (1958) *Proc Roy Soc* **B148,** 403
[18] BELL, D J (1955) *Modern Methods of Plant Analysis* (PAECH, K and TRACEY, M V, eds), Vol II, pp 1–54, Springer, Berlin
[19] LEWIS, D H and HARLEY, J L (1965) *New Phytol* **64,** 224
[20] HOUGH, L, JONES, J S. C and WUSTEMAN, P (1972) *Carbohyd Res* **21,** 9
[21] SOMOGYI, M (1945) *J biol Chem* **160,** 69
[22] LEWIS, D H and SMITH, D C (1967) *New Phytol* **66,** 185
[23] TREVELYAN, W E., PROCTOR, R D. P and HARRISON, J S (1950) *Nature, Lond.* **166,** 444
[24] HOLLIGAN, P M and DREW, E A (1971) *New Phytol* **70,** 271