Tricin was found to occur in the free form along with three of its glycosides. It was identified through its chromatographic and u.v. characteristics and demethylation which gave the corresponding 5,7,3′,4′,5′-pentahydroxyflavone which in turn was found to be identical in properties with those reported in the literature.[2]

Both tricin 5-glucoside and 7-glucoside along with the 7-glucuronide were identified through the standard methods of identification, along with chrysoeriol 7-glucoside, chrysoeriol 7-glucuronide and chrysoeriol 7-rutinoside. The identity of chrysoeriol was confirmed through u.v. and chromatographic properties along with demethylation which gave luteolin. A number of these glycosides have been previously reported in grasses.[3]

The presence of kaempferol and quercetin glycosides is rare in grasses and only one report of their glucosides in *Panicum bulbosum* and *Lolium perrene* is recorded.[2] Both glycosides appear to be polyglycosides giving kaempferol 3-glucoside and quercetin 3-glucoside as intermediates. The u.v. data indicate that glycosylation occurs at position 3 only; however insufficient material prevented any further studies. The possibility is that they are acylated derivatives, as they failed to co-chromatograph with authentic samples of 3-monoglucosides or 3-diglucosides.

Two other flavonoids of minor concentration were present but could not be identified due to lack of material.

EXPERIMENTAL

Source of Plants. 6 miles west of Cle Elum, Kittitas Co., Washington, Collection No. Maze and Bohm 501. Vouchers have been deposited in the U.B.C. Botany Department Herbarium. Plants were maintained in cultivation in the U.B.C. Botany Department gardens until used.

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FRUCTOSYLRAFFINOSE, A TETRASACCHARIDE IN WHEAT BRAN

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Abstract—A new tetrasaccharide has the structure *O*-α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl-(1→2)-[*O*-β-D-fructofuranosyl-2→1]-β-D-fructofuranoside.

INTRODUCTION

DURING an investigation into the sugars of wheat bran a tetrasaccharide was found which appeared to be a fructosylraffinose.[1] Previously a fructosylraffinose has been reported in wheat flour[2] and in wheat aleurone cells,[3] and as one of the products of a raffinosefructosyltransferase interaction.[4–6] The chemical structure of these compounds has not been elucidated although structures have been proposed. This report establishes the structural identity of the bran component (Fig. 1).

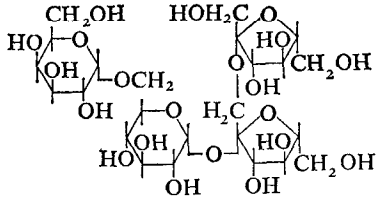

FIG. 1.

RESULTS AND DISCUSSION

Isolation of melibiose (*O*-α-D-galactopyranosyl-(1→6)-α-D-glucopyranose) after acid hydrolysis, and isolation of 1-kestose (*O*-α-D-glucopyranosyl-(1→2)-*O*-β-D-fructofuranosyl-β-D-fructofuranoside) after selective removal of D-galactose by α-galactosidase or periodate/base treatment indicates the assigned structure. From the mobility of this material and other oligosaccharides on paper chromatography it is apparent that this fructosylraffinose belongs to the melibiose-raffinose oligosaccharide series.[7]

This structure is the same as that proposed[4,5] for the raffinosefructosyltransferase product, but different to those proposed for the wheat aleurone material.[3]

EXPERIMENTAL

The methods used during isolation and identification of the fructosylraffinose were the same as previously described[1] although the bran was from a different hard red spring wheat. In this case after column chromatography of the component sugars, the peak containing the fructosylraffinose also contained stachyose, a second tetrasaccharide (glucose-fructose$_3$), and a pentasaccharide (glucose-fructose$_4$). These components were separated by paper chromatography on Whatman 3MM paper in *n*-BuOH–pyridine–water (6:4:3). Fructosylraffinose was obtained as a hard glass or amorphous solid. It had $[\alpha]_D^{23}$ + 56·4° (c, 1·43 in water).

Hydrolysis

Mild acid or invertase hydrolysis yielded compounds which behaved on paper chromatography like melibiose and fructose in the ratio 1:2. In a quantitative experiment. crystalline melibiose was isolated in 70 per cent yield; it was identified by m.p. and X-ray powder diffraction pattern.

Treatment with α-galactosidase

35 mg of fructosylraffinose dissolved in 10 ml of water was mixed with 5 mg of α-galactosidase and 0·5 m of 0·4 M NaOAc buffer pH 4·8 and left overnight at room temp. Sucrose, 1-ketose, raffinose, and stachyose were run as controls. The mixture was quickly washed through a mixed bed resin [Duolite A4(OH$^-$) and

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Dowex 50(H^+)] and concentrated to 1 ml volume. The product was chromatographed on Whatman 3MM paper in *n*-BuOH–pyridine–water (6:4:3) for 48 hr. Three components were present with R_fs corresponding to fructosylraffinose, 1-kestose, and galactose. The component corresponding to galactose assayed as 96 per cent galactose with galactose oxidase. The component with R_f equal to that of 1-kestose slowly crystallized from aqueous ethanol and was identified as such by m.p., mixed m.p., X-ray powder diffraction pattern and optical rotation.

In the control experiments, sucrose and 1-kestose were unaffected; raffinose and stachyose yielded sucrose and galactose.

Treatment with Ethanolic Periodate

This experiment to remove the terminal galactose was done in a manner similar to the method described by Mitra and Perlin[8] during studies on stachyose. 5 mg of fructosylraffinose in 0·7 ml of water and 1 ml of ethanol at 0° was treated with 4 mg $NaIO_4$ in 0·3 ml of water, and left at 0° for 18 hr. The solution was neutralized to phenolphthalein with hot strontium hydroxide solution and filtered. The filtrate was evaporated to dryness and the residue dissolved in 1 ml of water containing 4 mg Na_2CO_3 was heated on a steam bath for 3 hr. The solution was rapidly washed through a mixed bed resin [Dowex 1 (OH^-) and Amberlite IRC-50 (H^+)], then evaporated to dryness. Paper chromatography of the product showed only one product which behaved like 1-kestose. In control experiments, raffinose yielded a product which behaved like sucrose, and 1-kestose remained unaffected by the reaction.

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