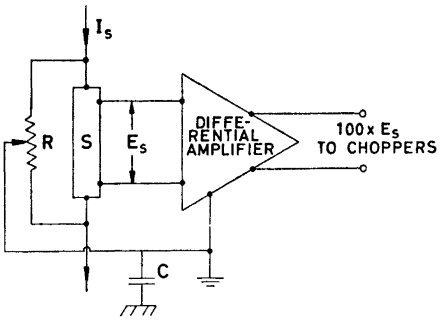

Fig. 2. Connection of differential amplifier for high common mode rejection.

With $n = 35$ c/s, an amplifier pass band of 20 c/s — 20 Kc/s is suitable, while the output impedance must satisfy the requirement $C_1 R_{out} \ll (2n)^{-1}$. ($C_1 = C_2$).

In evaluating the signal to noise ratio from the amplifier, only those noise voltages lying in the response range of the galvanometer (0 — 5 c/s approx.) need to be considered and many commercial amplifiers give satisfactory results.

A fairly high input impedance is indicated for the amplifier when Hall voltages are to be measured on specimens of higher resistance.

Acknowledgments. The authors wish to thank *Norges almenvitenskapelige forskningsråd* for financial support and Professor H. Haraldsen for the provision of experimental facilities.

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The Reaction of β-Fructosidase with the Monogalactosylsucroses Extracted from Plants

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It is generally accepted that β-fructosidase is unable to hydrolyse sucrose derivatives in which one of the hydroxyl groups of the fructose moiety is substituted. Thus, naturally occurring galactosyl-sucroses having the galactopyranosyl group linked to fructose at position 6 (planteose), position 1 (lychnose series), or position 3 (isolychnose series) are not at all attacked by yeast invertase (review articles [1-3]). This is consistent with the view that the specific role of the enzyme is to detach a fructofuranosyl group, and complete resistance towards invertase of any sucrose-containing oligosaccharide has so far been accepted as a proof of the absence of a terminal fructofuranosyl residue.

It has however been suggested [4] that also the hydroxyl group on C_3 of the glucose moiety is possibly essential for the formation of the enzyme-substrate complex between sucrose and β-fructosidase. A natural galactosyl-sucrose in which this hydroxyl group is blocked by substitution was later found (umbelliferose), and this trisaccharide proved to be extremely resistent to hydrolysis by invertase.[5] From experiments by one of us (A. W., unpublished), using umbelliferose preparations which had been carefully purified for any contamination of sucrose, it emerged that umbelliferose is not at all attacked by yeast invertase. These results are confirmed by the present work (Fig. 1).

Since a galactopyranosyl group on C_2 of the glucose moiety protects the sucrose linkage from hydrolysis by β-fructosidase, it seemed of interest to examine how much a galactosyl group on the adjacent C_3 might influence the hydrolysis rate. A natural galactosyl-sucrose of the required structure (Formula I) was detected in 1958 [6] as a constituent of the seeds of some grass species (*Festuca* and *Lolium*).

The position of the galactosyl group of this new sugar was originally studied [6] by examining the electrophoretic mobility

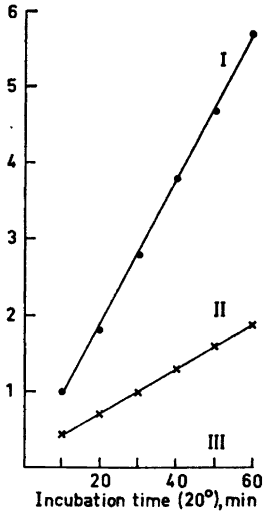
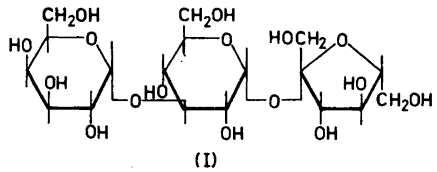


Fig. 1. Hydrolysis rates are given as mg (ordinate) of trisaccharide hydrolysed per ml of assay mixture, containing 5×10^{-5} mole of trisaccharide. Curve I, raffinose; Curve II, galactosyl-3G-sucrose; Curve III, umbelliferose. Curve III coincides with the abscissa.

of the disaccharide which remained when the fructofuranosyl group had been split off from the trisaccharide by mild acid hydrolysis or by enzymes. The assumed structure (Formula I) of the trisaccharide from *Festuca* and *Lolium* has been confirmed by means of oxidation with periodate in the present work.

In the following text we shall for practical reasons use the symbol galactosyl-3G-sucrose to designate the trisaccharide from *Festuca* and *Lolium*.

The trisaccharide under standard experimental conditions reduces 3 moles of periodate and liberates 1 mole of acid



(I)

α-D-Galactopyranosyl-(1→3)-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside. Symbol: Galactosyl-3G-sucrose. Formula I.

(formic), Table 1. After complete oxidation followed by hydrolysis, intact glucose was found in high yield. A glucopyranose

Table 1. Oxidation by periodate. The figures represent mean values from two series of experiments using a) raffinose or b) galactosyl-3G-sucrose.

Reaction time at room temperature (h)		1	4	24	51	72
Moles of periodate reduced per mole of trisaccharide	a)	2.43	3.33	4.37	4.56	4.70
	b)	2.03	2.56	2.92	3.08	3.12
Moles of monovalent acid liberated per mole of trisaccharide	a)	0.56	0.95	1.64	1.87	1.96
	b)	0.41	0.60	0.96	1.07	1.11

residue with no adjacent hydroxyl groups, capable of reducing periodate, must be linked through positions 1 and 3. The galactopyranosyl group is apparently linked in the α-form in galactosyl-3G-sucrose, since this trisaccharide was hydrolysed by α-galactosidase at about the same rate as raffinose.

A galactopyranosyl group which is undoubtedly located in position 3 of the glucose residue does not prevent hydrolysis by invertase of the trisaccharide's sucrose linkage, but the hydrolysis proceeds at a considerably lower rate than the hydrolysis of raffinose, whose galactopyranosyl group is linked to the more distant carbon 6 of the glucose unit, see Fig. 1. In umbelliferose the galactopyranosyl group is nearer to the sucrose linkage, and hydrolysis by β-fructosidase does not occur under standard experimental conditions.

The hydrolysis rate of raffinose before and after the admixture of umbelliferose was also examined, and the results obtained are given in Fig. 2. Admixture of umbelliferose in proportions representing a wide range of concentrations did not appreciably alter the rate of hydrolysis of raffinose by yeast invertase. It is therefore concluded that umbelliferose exhibits no competitive effect upon the enzymatic hydrolysis of the sucrose linkage of raffinose, and also that the umbelliferose sample examined does not contain inhibitors of yeast invertase as important impurities. These results indicate that

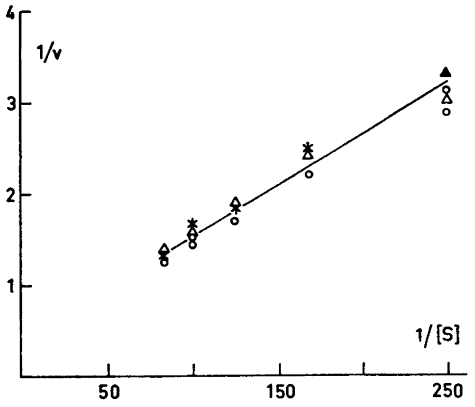


Fig. 2. Hydrolysis by yeast invertase of raffinose with or without admixture of umbelliferose. Reaction time 30 min at 20°. [S] = molar concentration (0.004—0.012) of the substrate, raffinose. v = velocity of the reaction. The concentrations of umbelliferose are indicated by the following symbols: O no umbelliferose; ∗ 0.002 M; △ 0.01 M; ▲ 0.04 M.

umbelliferose does not combine with yeast invertase and that the presence of the bulky galactopyranosyl group on C_2 of the glucose unit constitutes a steric hindrance which protects the sucrose linkage from hydrolysis by β-fructosidase.

Knowledge of the action of invertase upon oligosaccharides which contain sucrose as a part of the molecule, is useful within structural carbohydrate chemistry. It also is of interest within agricultural chemistry, especially for the evaluation of analytical procedures based upon the action of invertase upon plant extracts.[7]

Experimental. Materials. Crystalline raffinose (Fluka), $[\alpha]_D^{20}$ +103°, anhydrous umbelliferose [5] $[\alpha]_D^{20}$+125° (*c* 2,0, water), anhydrous galactosyl-3G-sucrose $[\alpha]_D^{22}$ +122° (*c* 2.0, water). The latter trisaccharide was extracted by boiling ethanol (80 %) and purified by means of charcoal columns as described for umbelliferose.[5] Seeds of *Lolium perenne* L. and *Festuca pratensis* Huds. gave identical samples of galactosyl-3G-sucrose (average yield 0.3 g from 250 g of the seeds).

Confirmation of the structure of galactosyl-3G-sucrose. (a) *Hydrolysis.* Complete hydrolysis liberated fructose, glucose, and galactose detected by paper chromatography. When exposed to α-galactosidase from coffee seeds at pH 4.8 and 37° as described in a previous paper,[5] galactosyl-3G-sucrose was hydrolysed at approximately the same rate as raffinose in parallel experiments. The hydrolysis was followed for 48 h and the amounts of galactose and sucrose were evaluated by paper chromatography (propanol-water 78/22 v/v). (b) *Oxidation by periodate.* Details of the experimental conditions employed have been described previously.[5] The results obtained are given in Table 1 and at the same time compared with the oxidation of raffinose. (c) *Determination of intact glucose after oxidation of galactosyl-3G-sucrose by periodate.* A) Raffinose (10^{-4} mole) was dissolved in a 0.01 M sodium meta-periodate solution (50 ml) and kept in the dark for two days to ensure complete oxidation. The reaction was stopped by adding an excess of barium acetate (1 ml, 0.5 M) and then glucose (10^{-4} mole) was dissolved in the filtrate, which was further purified by means of ion exchange resins (Amberlite IR 120 and IR 4B). The solution (after being concentrated under reduced pressure) was hydrolysed by sulphuric acid (acidity 1 N, 2 h, 100°) worked up in the usual way (barium carbonate) and finally concentrated under reduced pressure to 6.5 ml. A semi-qantitative paper chromatographic analysis demonstrated that about 50 % of the glucose was recovered. B) The above sequence of experiments was then repeated using galactosyl-3G-sucrose instead of raffinose and without adding glucose. Glucose was, however, found by paper chromatography of the final reaction mixture and its quantity was evaluated to about one mole of glucose per mole of the trisaccharide.

Hydrolysis by yeast invertase.[8] Experimental conditions were chosen as to give reactions of zero order kinetics. The assay mixture (10 ml) contained 5×10^{-4} mole of trisaccharide, sodium acetate buffer of pH 4.8 (2 ml, 0.01 M), and BDH invertase concentrate diluted by 100 parts of water (0.5 ml). The temperature was maintained at 20° by means of a water thermostat. Aliquots of 1 ml were removed at intervals, the enzyme destroyed by barium hydroxide (1 ml, 7.5 %) and zinc sulphate (1 ml, 5 %), and the quantity of reducing sugars in a suitable volume of the diluted filtrate were determined by the colorimetric method of Nelson-Somogyi [9,10] using a Beckman DU spectrophotometer. The amount of trisaccharide split by invertase was determined by use of an individual standard curve for each trisaccharide based upon an equimolecular mixture of the corresponding galactosyl-glucose and fructose, which may be obtained by mild acid hydrolysis of the trisaccharide

(0.01 N sulphuric acid, 40 min in a boiling water bath). The results obtained are shown in Fig. 1.

Hydrolysis of raffinose by yeast invertase in the presence of umbelliferose. The experimental procedure given above was followed and the total volume of the assay mixture reduced to 2 ml. Results and further information are given in Fig. 2.

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A New Synthesis of Thelephoric Acid

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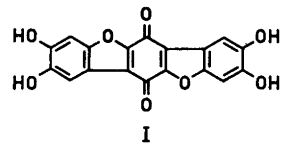
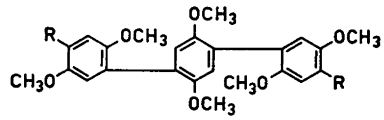
The structure (I) of thelephoric acid has been supported by two independent syntheses.[1,2] However, neither of them can be regarded as completely unambiguous and a third synthesis is described here, which finally removes any doubts that might be cast on the correctness of formula (I).

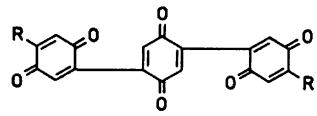


I



II, R = OCH_3

V, R = H



III, R = OH

IV, R = H

The starting material, 2,4,5,2′,5′,2″,4″,5″-octamethoxy-*p*-terphenyl (II), was prepared by standard Ullmann coupling. Its conversion into the triquinone (III) is analogous to the synthesis of (IV) described by Erdtman *et al.*[3] The triquinone (III) is rather unstable and its complete purification has not been possible to achieve.

That diquinones can undergo photochemical and/or thermal rearrangement to dibenzofuranquinones is known.[4-9] A triquinone such as (III) should in the same way give a benzobisbenzofuranquinone. In fact, when (III) was boiled with acetic anhydride it gave thelephoric acid tetra-acetate and in the presence of zinc dust thelephoric acid leuco-acetate, both of which have earlier been converted into thelephoric acid.[1] The triquinone (IV), on the other hand, failed to undergo any conversion to a benzobisbenzofuranquinone.

Experimental. U. V. spectra were measured with a Beckman DK-2 spectrophotometer, and I.R. spectra with a Perkin-Elmer 125 spectrophotometer. Microanalyses were done by Dr. A. Bernhardt, Mülheim, Germany.

2, 4, 5, 2′, 5′, 2″, 4″, 5″-Octamethoxy-p-terphenyl (II). 2,5-Diiodohydroquinone dimethylether[3,10] (1.8 g), 5-iodohydroxyhydroquinone trimethylether[11] (10.8 g) and copper

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