SUMMARY

1. Considerable losses of vanillin, *p*-hydroxybenzaldehyde and syringaldehyde occur when these aldehydes are added to plant material before oxidation with alkaline nitrobenzene.

2. The recovery of added aldehyde was no better from fully extracted than from unextracted plant tissue.

3. In general, losses of aldehyde during the oxidation were greater with the young than with the older tissues.

4. The ethanol–benzene and hot-water extractions remove appreciable amounts of aldehyde-forming material.

5. The percentage of *p*-hydroxybenzaldehyde obtained on oxidation of plant material does not increase with increasing plant age; the percentage of vanillin formed increases slightly, and the percentage of syringaldehyde produced increases very markedly.

6. The yield of aldehyde in relation to Klason lignin is greater for young than for the more mature tissues.

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REFERENCES

A.O.A.C. (1950). *Methods of Analysis.* 7th ed., p. 109. Washington: Association of Official Agricultural Chemists.
Bondi, A. & Meyer, H. (1948). *Biochem. J.* **43**, 248.
Campbell, W. R. & Hanna, M. I. (1937). *J. biol. Chem.* **119**, 1.
Clark, E. P. (1938). *Industr. Engng Chem. (Anal.)*, **10**, 677.
Clark, E. P. (1939). *J. Ass. off. agric. Chem., Wash.*, **22**, 100.
Creighton, R. H. J. & Hibbert, H. (1944). *J. Amer. chem. Soc.* **66**, 37.
Creighton, R. H. J., McCarthy, J. L. & Hibbert, H. (1941). *J. Amer. chem. Soc.* **63**, 3049.
DeStevens, G. & Nord, F. F. (1953). *J. Amer. chem. Soc.* **75**, 305.
Freudenberg, K. & Bittner, F. (1953). *Ber. dtsch. chem. Ges.* **86**, 155.
Freudenberg, K., Lautsch, W. & Engler, K. (1940). *Ber. dtsch. chem. Ges.* **73**, 167.
Hibbert, H. (1942). *Annu. Rev. Biochem.* **11**, 183.
MacDougall, D. (1954). *J. Sci. Fd Agric.* **5**, 103.
Manning, K. R. & DeLong, W. A. (1941). *Sci. Agric.* **22**, 69.
Meuller, W. J. & Herrmann, W. (1926). *Papierfabrikant*, **24**, 195. [*Chem. Abstr.* (1926), **20**, 2746.]
Pepper, J. M. & MacDonald, J. A. (1953). *Canad. J. Chem.* **31**, 476.
Samsel, E. P. & McHard, J. A. (1942). *Industr. Engng Chem. (Anal.)*, **14**, 750.
Stone, J. E. & Blundell, M. J. (1951). *Analyt. Chem.* **23**, 771.
Stone, J. E., Blundell, M. J. & Tanner, K. G. (1951). *Canad. J. Chem.* **29**, 734.

The Fluorimetric Determination of β-Glucosidase: its Occurrence in the Tissues of Animals, including Insects

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In a recent paper from this Laboratory (Mead, Smith & Williams, 1955), a highly sensitive fluorimetric method of assay of β-glucuronidase, in which 4-methylumbelliferone glucuronide was used as the substrate, was described. This method has now been adapted to the determination of β-glucosidase, with 4-methylumbelliferone β-glucoside as the substrate. Previous methods of assay of this enzyme have usually depended on the use of chromogenic substrates, or upon the determination of glucose liberated by the enzyme (Viebel, 1945). The method herein described is claimed to be highly sensitive, and can be readily used for the assay of the small amounts of the enzyme found in the tissues of higher animals. In fact, few studies of the β-glucosidase content of animal tissues have been made owing to the lack of a sufficiently sensitive method of assay. This enzyme has been shown to occur in the snail (Karrer, 1930), the woodlouse (Newcomer, 1952) and the cockroach (Newcomer, 1954). Few warm-blooded animals have been examined. Neuberg & Hofmann (1935) reported its occurrence in the liver and kidney of horses and cattle, while Steensholt & Viebel (1943) showed its presence in pig intestinal mucosa.

EXPERIMENTAL

Materials

Preparation of 4-methylumbelliferone β-glucoside. This glucoside was prepared according to the general method of Glazer & Wulwek (1924). 4-Methylumbelliferone (11·5 g., m.p. 180°) was dissolved in acetone (140 ml.), and acetobromoglucose (19 g.) added, followed by a solution of NaOH (3·6 g.) in water (90 ml.). After the mixture had been kept at room temp. overnight the acetone was

evaporated under reduced pressure, and the crude product recrystallized four times from ethanol (charcoal). The *4-methylumbelliferone tetraacetyl β-D-glucoside* (5 g.) obtained formed white needles and had m.p. 144° and $[\alpha]_D^{20} -40 \pm 2°$ (*c*, 0·5 in $CHCl_3$). (Found: C, 56·9; H, 5·2. $C_{22}H_{26}O_{12}$ requires C, 56·9; H, 5·2%.) The tetraacetate (5 g.) was deacetylated in dry methanol solution (25 ml.) by adding a few drops of a 10% solution of barium methoxide in dry methanol. Deacetylation occurred almost immediately with the production of a precipitate (3·5 g.), which, on repeated recrystallization from ethanol and finally from water, yielded *4-methylumbelliferone β-D-glucoside hemihydrate* as colourless needles, with m.p. 211° and $[\alpha]_D^{20} -89·5 \pm 2°$ (*c*, 0·5 in water). (Found: C, 55·4; H, 5·3; H_2O, 2·4. $C_{16}H_{18}O_8, 0·5H_2O$ requires C, 55·4; H, 5·5; H_2O, 2·6%.) Before use as a substrate, the glucoside was recrystallized repeatedly from ethanol and water until its fluorescence was negligible.

Enzyme preparations. The Taka diastase, emulsin and β-glucuronidase were obtained commercially (L. Light and Co.).

Assay of β-glucosidase

Enzyme solutions. Samples of tissue from freshly killed animals, or, with small insects, the whole animals, were ground in distilled water in a Potter–Elvehjem all-glass homogenizer. After a trial incubation all tissue preparations were diluted with distilled water until 1 ml. liberated approximately 1 μg. of 4-methylumbelliferone in 30 min., with the fluorimetric method of estimation at pH 10·3 (Mead *et al.* 1955).

Method of assay. A 0·005 M solution of 4-methylumbelliferone glucoside in water was prepared freshly each day and stored in the refrigerator until needed, since it was found to decompose appreciably overnight at room temp. For assay, 0·5 ml. of substrate solution and 3·5 ml. of 0·2 M acetate buffer at the optimum pH were added to each of three tubes and all solutions were incubated at 37°. Enzyme solution (1 ml.) was added to two of the tubes, and 1 ml. of boiled enzyme solution to the third (control) tube, and the incubation continued for exactly 30 min., when the reaction was stopped and the fluorescence was developed by adding 15 ml. of 0·2 M glycine buffer, pH 10·3. In inhibition experiments the 3·5 ml. of acetate buffer were replaced by 3 ml. of buffer and 0·5 ml. of inhibitor solution in acetate buffer.

The intensity of the fluorescence was measured against a quinine standard (4 μg. of quinine sulphate/ml. in 0·1 N-H_2SO_4), by means of a Spekker absorptiometer with fluorimetry attachment, as described by Mead *et al.* (1955). Where control values were too low to be measured directly, the solution after incubation was diluted with 14 ml. of glycine buffer and 1 ml. of a 4-methylumbelliferone solution (1 μg./ml.) was added to bring the fluorescence within the range of the fluorimeter.

Optimum pH *and substrate concentration*

Estimations of β-glucosidase activity from various sources were made by the method described above over the pH range 3·7–5·5 with 0·2 M acetate buffer and 4·75–6·8 with 0·25 M succinate buffer; no alteration of pH optimum with buffer was noted in any case. The pH optima of β-glucosidase from a number of sources are shown in Table 1.

Table 1. *Optimum* pH *of some β-glucosidases*

Substrate, 0·5 mM 4-methylumbelliferone glucoside.

Source of β-glucosidase	Optimum pH in 0·2 M acetate or 0·125 M succinate buffers
Locust	
Crop fluid	5·1–5·4
Gut	5·4
Cockroach	
Gut	5·4
Rat	
Kidney	5·2–5·7
Guinea pig	
Liver	5·15–5·4
Duodenum	5·4
Rabbit	
Liver	5·2
Taka diastase	4·5–4·8
Glucuronidase	4·5–4·7

Under standard conditions the degree of hydrolysis with a given enzyme solution was found to be proportional to time of incubation for periods of up to 4 hr.

The effect of substrate concentrations varying between 2×10^{-5} M and 5×10^{-4} M at pH 4·5 for Taka diastase and 5·2 for locust-crop fluid showed almost maximal activity at 5×10^{-4} M. Above this concentration the fluorescence of the substrate caused appreciable blank values, and a 5×10^{-4} M concentration was used for all subsequent assays.

β-Glucosidase activity of various tissues

The hydrolytic activity at pH 5·2 for animal tissues, and at the observed optima for other sources, was measured by the method described. The results (Table 2) show a widespread distribution of β-glucosidase at low concentrations in animal tissues, with higher values in the insects. Rabbit tissues were somewhat higher in activity than those of other warm-blooded animals examined.

Identity of β-glucosidase in locust-crop fluid

β-Glucuronidase has been shown to be present in locust-crop fluid (Robinson, Smith & Williams, 1953), and the presence of β-glucosidase in the same material led us to inquire whether the two activities might not be due to a single enzyme of low substrate specificity. Karunairatnam & Levvy (1951) found that mouse-liver β-glucuronidase was completely inhibited by a 0·01 M saccharate solution, the inhibition being later shown to be due to the presence of saccharo-1:4-lactone (Levvy, 1952), which is formed in the solution on boiling. Robinson *et al.* (1953) showed that at this concentration

Table 2. *β-Glucosidase activity of various sources*

Source	Activity at optimum pH in acetate buffer (μg. of 4-methylumbelliferone liberated/hr./g. wet wt.) Average	Range	No. of samples
Locust (*Locusta migratoria*)			
Crop fluid*	371 000	10 900–960 000	9
Fore-gut	1 610	1 480–1 740	2
Mid-gut	6 030	2 600–9 140	4
Hind-gut	10 900	1 780–25 600	4
Cockroach (*Periplaneta americana*)			
Fore-gut	160 000	34 600–316 000	5
Mid-gut	171 000	15 200–630 000	5
Hind-gut	104 000	2 000–458 000	5
Flour beetle (*Tenebrio*)			
Whole larva	35 800	20 900–54 200	3
Greater water boatman (*Notonecta*)			
Whole insect	732	605–815	5
Black aphis (*Aphis fabae*)			
Whole insect	1 660	—	Pooled, 84 mg.
Rat			
Liver	442	202–623	6
Kidney	3 710	1 270–8 650	6
Spleen	500	151–980	6
Duodenum	998	420–1 290	6
Heart	161	18–333	4
Muscle	50	0–50	3
Guinea pig			
Liver	1 710	1 000–2 900	4
Kidney	401	182–685	5
Spleen	391	150–578	5
Duodenum	13 400	9 750–18 100	5
Heart	279	71–550	5
Muscle	Too low to be detected		
Ferret			
Liver	249	202–290	3
Kidney	256	239–278	3
Spleen	179	77–301	3
Duodenum	438	290–605	3
Heart	63	27–112	3
Muscle	Too low to be detected		
Rabbit			
Liver	68 400	59 600–82 300	3
Kidney	20 200	14 900–27 200	3
Spleen	562	572–630	3
Duodenum	105 000	49 200–202 000	3
Heart	1 105	226–1 590	3
Muscle	Too low to be detected		
Mouse			
Liver	586	370–765	5
Kidney	312	194–485	5
Spleen	699	335–865	5
Duodenum	721	600–945	5
Heart	223	139–384	4
Muscle	Too low to be detected		
Cress seedlings	933	232–1 824	8
Emulsin	36 000	—	1
Taka diastase	64 000	—	1
β-Glucuronidase†	180 000	—	1

* Values expressed as μg. liberated/hr./ml. of fluid.

† The glucuronidase activity was 46 600 μg./g. expressed as 4-methylumbelliferone liberated/hr.

Table 3. *Separate identity of β-glucosidase in locust-crop fluid*

Equal portions of diluted crop fluid incubated with substrate in acetate buffer, pH 4·5.*

Substrate	Wt. of 4-methyl-umbelliferone liberated/hr. (μg.)
4-Methylumbelliferone β-glucoside (0·5 mM)	0·35
4-Methylumbelliferone β-glucuronide (0·1 mM)	0·13
4-Methylumbelliferone β-glucoside (0·5 mM) + 4-methylumbelliferone β-glucuronide (0·1 mM)	0·49

* At this pH, the activity of β-glucuronidase is at its optimum, but the β-glucosidase activity (optimum pH 5·1–5·4) is reduced to the same order as that of β-glucuronidase.

the β-glucuronidase of locust-crop fluid was also completely inhibited. By means of the method described above, it was found that the β-glucosidase present was unaffected by a 0·01 M concentration of boiled saccharate solution. Under similar conditions, a 0·01 M concentration of glucono-1:4-lactone in the solutions during incubation completely inhibited the β-glucosidase activity of locust-crop fluid, 60 % inhibition being observed with an inhibitor concentration of 10^{-4} M. No inhibitory effect on β-glucuronidase was observed at these concentrations. These latter results, which are comparable with those of Conchie (1954) for sheep-rumen-liquor β-glucosidase, suggest that the two activities are not related to the same enzyme. This conclusion was confirmed by an experiment with mixed substrates. If a single enzyme is involved, two substrates presented together at their optimum concentrations should compete for the active centres, whereas the presence of two enzymes would result in both substrates being hydrolysed at their maximum rates.

The β-glucuronidase activity of a diluted sample of locust-crop fluid was estimated by the method of Mead *et al.* (1955), and the β-glucosidase activity of the same sample measured by the present method. In a third experiment, both substrates were presented simultaneously; here, the amount of 4-methylumbelliferone liberated proved to be equal to the sum of the amounts liberated from the two substrates when treated separately, showing that no competitive inhibition had taken place (Table 3).

Paper electrophoresis of locust-crop fluid

Erdman & Jorpes (1941) have been able to purify β-glucosidase by electrophoresis, and by a similar method Jermyn (1952) showed the β-glucosidase of *Aspergillus oryzae* to be a complex mixture of enzymes.

The β-glucosidase of locust-crop fluid could be separated from the β-glucuronidase present by paper electrophoresis in the following manner. A strip of filter paper (Whatman no. 3MM) 8 cm. × 50 cm. was soaked in buffer of the desired pH, diluted so that a potential difference of 10 v/cm. could be obtained across the paper with a current of 4–5 mA. Surplus moisture was removed and a thin line of the crop fluid drawn centrally across the strip, which was then clamped between two silicone-treated glass plates 10 cm. × 40 cm., and the exposed ends were allowed to hang in Perspex vessels containing buffer and fitted with carbon electrodes. A potential difference of 500 v was applied across the electrodes for 2·5 hr., when the paper was removed, allowed to dry and cut into longitudinal strips.

β-Glucosidase could be detected on the paper by spraying with a 1 % solution of 4-methylumbelliferone β-glucoside in 0·2 M acetate buffer, pH 5, and placing the damp paper in an air incubator at 37° for 10 min. On exposure of the paper to ammonia vapour the position of the enzyme could be observed by the fluorescence, under ultraviolet light, of the liberated aglycone. β-Glucuronidase could be detected by treating another strip analogously with a solution of 4-methylumbelliferone β-glucuronide.

A quantitative estimation of the distribution of the enzymes was made by performing the electrophoresis as already described. The paper was then cut laterally into 1 cm. strips, each strip being placed immediately into a separate test tube with 4·5 ml. of acetate buffer, pH 5·2 for β-glucosidase and pH 4·5 for β-glucuronidase. The tubes were heated to 37°, and 0·5 ml. of the appropriate substrate solution was added. After incubation for 30 min. the fluorescence was developed and measured in the usual way.

The results are shown in the form of histograms in Fig. 1. Electrophoresis was carried out at different pH values over the range 3–10·3, and in each case β-glucuronidase showed a different distribution on the paper from β-glucosidase, suggesting that two different enzymes are involved.

At pH 6·8, β-glucosidase appeared in two locations. After 2·5 hr., approximately 75 % of the total activity was found 8–13 cm. from the origin in the direction of the anode, while the remainder had moved only 1–4 cm. in the same direction. This

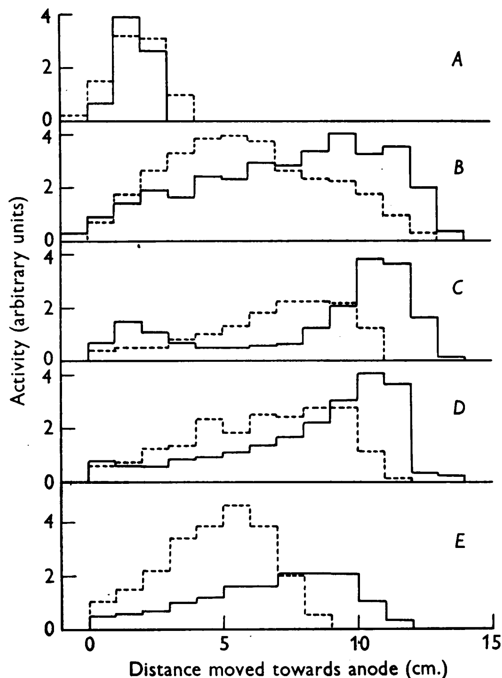

Fig. 1. Distribution of β-glucuronidase and β-glucosidase activities after paper electrophoresis at 10 v/cm. for 2·5 hr. - - - -, β-Glucuronidase activity; ——, β-glucosidase activity. *A*, pH 3·0 in 0·02 M acetate–HCl buffer; *B*, pH 5·0 in 0·02 M acetate buffer; *C*, pH 6·8 in 0·02 M phosphate buffer; *D*, pH 8·6 in 0·025 M veronal buffer; *E*, pH 10·3 in 0·02 M glycine buffer.

may not be a true representation of the relative concentrations of the two fractions, since it has been shown that the β-glucosidase fractions may vary in substrate specificity (Jermyn, 1952). Measurement of optimum pH in 0·125 M succinate buffer showed that the fast-moving band had maximum activity at pH 4·9, whereas the band remaining close to the origin had an optimum pH of 5·4.

DISCUSSION

The fluorimetric method described is rapid and simple in operation. The extreme sensitivity allows small amounts of β-glucosidase to be detected and estimated; stronger sources can be diluted so extensively that interference by coloured substances and by tissue particles is eliminated. In no case was it found necessary to deproteinize the solution before the final measurements.

The results shown in Table 2 indicate that the enzyme is widespread in low concentrations in animal tissues, the most active organs in the warm-blooded animals being liver, kidney and duodenum, and it is perhaps significant that the activity is lowest in the only carnivorous animal examined, the ferret. A similar relationship occurs in the insects, where the carnivorous *Notonecta* appears to have a lower concentration than the locusts. However, strict comparison is not possible, since the whole insect was used in the former case owing to difficulties of dissection. The results throw light on the earlier observations of Hunt (1923) that rabbits and guinea pigs are susceptible to poisoning by amygdalin, an effect ascribed to decomposition by the gut contents with release of HCN. The snail (*Helix pomatia*), which is known to contain a powerful β-glucosidase, is also poisoned, but the carnivorous dog appears to be relatively immune.

The occurrence of both β-glucuronidase and β-glucosidase in the same material is not unusual. β-Glucuronidase is widespread in the tissues of warm-blooded animals, and has been shown to occur in high concentrations in the digestive juice of the snail (Jarrige & Henry, 1952). Both enzymes have also been shown to occur in sheep-rumen contents (Karunairatnam & Levvy, 1951; Conchie, 1954), and it has been suggested that they may be associated with the digestion of polysaccharides. The enzymes may have a similar role in the locust, but it is not known whether they are secreted, or whether they are of microbial origin as in the sheep. The β-glucosidase of locust-crop fluid has the same optimum pH (5·1–5·4) as the enzyme from animal tissues, and the enzyme occurring in a bacterial glucuronidase preparation has a different optimum, at pH 4·5–4·7.

SUMMARY

1. 4-Methylumbelliferone β-D-glucoside has been prepared, and its use as a fluorigenic substrate in the assay of β-glucosidase is described.

2. The enzyme has been found to be present in the five species of insects and the five laboratory animals examined.

3. The pH optima for animal tissues lie within the range 5·1–5·7.

4. The β-glucosidase of locust-crop fluid has been shown to be different from the β-glucuronidase occurring in this fluid, and the two enzymes behave differently on electrophoresis.

5. The results are discussed in relation to the nutritional habits of the animals concerned.

I am grateful to Dr E. Cameron of this school, and to the Anti-Locust Research Centre, Natural History Museum, for supplies of cockroaches and locusts respectively, and to Professor R. T. Williams for his interest in this work. The expenses of this work were in part defrayed by a grant to Professor Williams from the Medical Research Council.

REFERENCES

Conchie, J. (1954). *Biochem. J.* **58**, 552.
Erdman, P. & Jorpes, E. (1941). *Acta physiol. scand.* **2**, 41.
Glazer, E. & Wulwek, W. (1924). *Biochem. J.* **145**, 514.

Hunt, R. (1923). In *Experimentelle Pharmakologie*, vol. 1, p. 702. Ed. by Heffter, A. Berlin: Springer.
Jarrige, P. & Henry, R. (1952). *Bull. Soc. Chim. biol., Paris*, **34**, 872.
Jermyn, M. A. (1952). *Aust. J. sci. Res.* B, **5**, 433.
Karrer, P. (1930). *Kolloidschr.* **52**, 304.
Karunairatnam, M. C. & Levvy, G. A. (1951). *Biochem. J.* **49**, 210.
Levvy, G. A. (1952). *Biochem. J.* **52**, 464.
Mead, J. A. R., Smith, J. N. & Williams, R. T. (1955). *Biochem. J.* **61**, 569.
Neuberg, C. & Hofmann, E. (1935). *Biochem. Z.* **281**, 431.
Newcomer, W. S. (1952). *Anat. Rec.* **113**, 536.
Newcomer, W. S. (1954). *J. cell. comp. Physiol.* **43**, 79.
Robinson, D., Smith, J. N. & Williams, R. T. (1953). *Biochem. J.* **53**, 125.
Steensholt, G. & Viebel, S. (1943). *Acta physiol. scand.* **6**, 62.
Viebel, S. (1945). *Die Methoden der Fermentforschung*, p. 1775. Ed. by Bamann, E. & Myrbäck, K. New York: Academic Press.

Enzyme Systems in Marine Algae. The Carbohydrase Activities of Unfractionated Extracts of *Cladophora rupestris, Laminaria digitata, Rhodymenia palmata* and *Ulva lactuca*

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Present knowledge of the metabolism of terrestrial plants and freshwater algae is considerable (cf. Bonner, 1950; Fogg, 1953) and a large number of enzymes and enzyme systems have been detected in plant tissues and extracts; subsequently, many of these have been isolated and purified. In addition, certain freshwater algae (e.g. *Chlorella* and *Scenedesmus*) have been widely used in studies of photosynthesis and intermediary carbohydrate metabolism. By contrast, similar information on marine algae is lacking, and few investigations of the enzyme systems of marine algae have been reported, although the chemical structure of many of the end-products of anabolism has been investigated (cf. Black, 1953).

In co-operation with the Institute of Seaweed Research, a survey of the enzyme systems of marine algae has been commenced, and, in view of our previous interest in the enzymic hydrolysis of glucosides and glucosans (Manners, 1952, 1955), our preliminary experiments have been directed towards the detection of carbohydrases in extracts of marine algae. In the present paper, evidence for the presence of a number of soluble carbohydrases in a member of the Phaeophyceae (*Laminaria digitata*), a member of the Rhodophyceae (*Rhodymenia palmata*) and two species of Chlorophyceae (*Cladophora rupestris* and *Ulva lactuca*) is recorded. A preliminary account of part of this work has already been published (Duncan, Manners & Ross, 1954).

METHODS AND MATERIALS

Analytical methods

Paper chromatography. (*a*) *Sugars.* Descending chromatograms were carried out at room temperature with Whatman no. 1 paper and *n*-butanol–pyridine–water–benzene (5:3:3:1, by vol.) as solvent (De Whalley, Albon & Gross, 1951). An alkaline silver nitrate reagent (Trevelyan, Procter & Harrison, 1950) or aniline oxalate reagent (Partridge, 1949) was used to detect the sugars on the chromatograms. The rate of movement of sugars (R_G) was determined by dividing the distance moved by the sugars from the starting line by the distance moved by D-glucose (R_G 1·0) under identical conditions. The R_G value of a particular sugar was found to vary on different chromatograms, e.g. laminaribiose had R_G 0·65–0·76; hence, preliminary identification of a sugar was carried out by placing the sugar and the appropriate reference compound on the same chromatogram, and not by calculation of the R_G value.

(*b*) *Phosphate esters.* Development and detection was effected by the method of Hanes & Isherwood (1949), with *n*-propanol–ammonium hydroxide–water (6:3:1, by vol.) and glucose 1- and 6-phosphates as reference compounds.

Reducing sugars. Reducing sugars were determined by (*a*) the iodometric Shaffer & Somogyi (1933) reagent as modified by Hanes & Cattle (1938), (*b*) the iodometric Somogyi (1945*a*) reagent, or (*c*) the colorimetric Nelson (1944) reagent as modified by Somogyi (1952). The reagents were calibrated, as required, against glucose and maltose. Deproteinization, when necessary, was effected by $ZnSO_4$–$Ba(OH)_2$ (Somogyi, 1945*b*).

Glucose 1-phosphate. A slight modification of the method of Allen (1940) was used.