# Starch Gel Electrophoresis of Basic Water-Soluble Barley Grain Proteins

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We have earlier reported the fractionation of water-soluble proteins of barley grain by ion-exchange chromatography on DEAE-cellulose [1,2]. The basic proteins (about 30 % of the total) passed unretarded through the column in 5 mM phosphate buffer of pH 7.5 and the neutral and acid proteins were resolved into 15 components with gradient elution.

The basic protein fraction gives four components on paper electrophoresis at pH 8.6 (Fig. 1). These are apparently identical with the four cathodic components which we have found in the paper electrophoresis of unfractionated barley extracts [3]. Apparently they correspond to the three cathodic fractions which Mourgue and his collaborators have found in the paper electrophoresis of *Ricinus* [4] and *Jatropha* [5] seed extracts.

*Fig. 1.* Paper electrophoresis at pH 8.6 of basic barley proteins.

We have developed a preparative method for the purification of the basic proteins and resolved the material obtained into two anodic and fourteen cathodic components by starch-gel electrophoresis at pH 8.4.

*Preparation of basic proteins.* 500 g of finely ground and acetone treated barley (Pirkka) was extracted for 2 h with 1 000 ml of water [6] with occasional stirring at 4°C. The slurry was centrifuged at 5 000 r.p.m. for 10 min and the extract was left standing at 4°C for two days. During this period most of the phytic acid and water-soluble polysaccharides are hydrolyzed [7]. The small precipitate which forms on standing was removed by centrifugation. The proteins were then separated from interfering small-molecular substances and simultaneously transferred to 5 mM sodium phosphate buffer of pH 7.5 by gel filtration on Sephadex G-25 equilibrated with this buffer. The solution obtained was run through a 20 g preparative column of DEAE-cellulose (Schleicher & Schüll), equilibrated with the 5 mM buffer, to remove neutral and acid proteins. The solution containing the unretarded basic proteins and polysaccharides was acidified to pH 4.5 and made 0.1 M in respect to acetate with cautious addition of 1 M acetic acid and sodium acetate. The proteins were absorbed on a 20 g column of CM-cellulose (Schleicher & Schüll) at pH 4.5 and, after washing with the starting buffer, eluted with 0.3 M sodium phosphate pH 8.0. The sodium phosphate was removed from the proteins by gel filtration on Sephadex G-25 equilibrated with a 10 mM ammonium formate buffer. The volatile salt was required to suppress the ion-exchange properties of Sephadex G-25. After lyophilization the basic proteins weighed about 700 mg. 60—80 mg of this dry protein material was dissolved in 0.5 ml of water and this solution was used for electrophoretic analyses.

*Electrophoretic conditions.* The electrophoretic separation was performed by the vertical method described by Smithies [8], with minor alterations. The gels were prepared in plastic trays 30 × 12 × 0.6 cm from starch supplied by *Connaught Medical Research Laboratories, Toronto.* The starch was used at a concentration of 11.3 g/100 ml of buffer solution (for

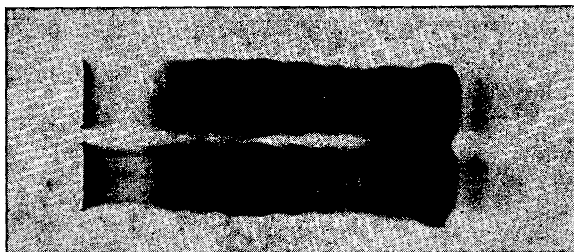

*Fig. 2.* Starch gel electrophoresis at pH 8.4 of basic barley proteins.

serum 12.3 g/100 ml was recommended). The buffer for gels contained 0.026 mole of $H_3BO_3$ and 0.014 mole of NaOH per litre, giving a final pH of 8.4 in the settled gel. The bridge solution contained 0.2 mole of $H_3BO_3$ and 0.04 mole of NaOH per litre. The potential gradient employed was 10 V/cm the positive electrode being in the upper vessel and the basic proteins migrating downwards to the cathode. All runs were made at 4°C. The cut gels were stained for 10 min. with Amido Black B and washed 4−5 times.

*Results.* The result obtained is shown in Fig. 2, in which 14 cathodic zones can be seen. On the anodic side there is one very diffuse region and one strongly stained band near the start which apparently corresponds to the least basic of the four bands in paper electrophoresis. At pH 7 all the protein material migrated towards the cathode. Thus the two anodic bands at pH 8.4 can also be considered to be basic proteins.

Under the conditions described here, the basic fraction of water-soluble barley grain proteins can be resolved reproducibly into 16 bands of significant intensity, which appear to be native components of barley grain.

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# A Lignan Xyloside from the Sapwood of *Sorbus aucuparia* L.

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The isolation of two biphenyl derivatives of unusual structure, aucuparin and methoxyaucuparin from the heartwood of mountain ash (*Sorbus aucuparia* L.) has recently been reported from this laboratory.[1] The sapwood contains a lignan xyloside (1−3 % of the dry wood). This xyloside is identical with the lignan xyloside from *Alnus glutinosa* [2] and a *Lyonia* species [3] as shown by a comparison of the physical constants recorded in Table 1.

The investigation of the *Alnus* xyloside [4] showed that the aglycone has the *structure* I (X = H).

The *Sorbus* aglycone was obtained by the hydrolysis of the xyloside with acid as well as emulsin. This observation and the small difference in optical rotation between the xyloside and the aglycone indicates that the xyloside is a *β*-xyloside. Permanganate oxidation of the aglycone dimethyl ether in acetone afforded galloylgallic acid hexamethyl ether, m.p. 190−191°, and gallic acid trimethyl ether. Mild oxidation of the aglycone dimethyl ether with chromic anhydride in pyridine gave a lactone ("*α*-lactone"), $C_{18}H_{10}O_2(OCH_3)_6$, m.p. 200−201°, $[\alpha]_D -103°$ (a), −122° (c) and a cyclohemiacetal, $C_{18}H_{11}O(OH)(OCH_3)_6$, m.p. 180−181°, $[\alpha]_D -6°$ (c) [monoacetate, $C_{18}H_{11}O(OAc)(OCH_3)_6$, m.p. 158−160°, $[\alpha]_D -13°$ (c)] which on further oxidation furnished the *α*-lactone. The *α*-lactone gave the aglycone dimethyl ether on reduction with lithium aluminium hydride. On dehydration with potassium hydrogen sulphate the latter aglycone derivative afforded the anhydro-compound, $C_{18}H_{10}O(OCH_3)_6$, m.p. 145−146°, $[\alpha]_D -41.6°$ (a) as does the *Alnus* aglycone dimethyl ether.

The *Sorbus* xyloside dimethyl ether was oxidised with chromic anhydride in pyridine to an amorphous acid which on hydrolysis furnished the *Sorbus* "*α*-lactone". The sugar residue must be attached to the methylol group not suffering oxidation and the problem of elucidating the structure of the xyloside is therefore reduced to a determination of the structure of the *α*-lactone.