Phytochemistry Vol. 21, No. 9, pp. 2401–2402, 1982.
Printed in Great Britain.

0031-9422/82/092401-02$03.00/0
Pergamon Press Ltd.

SHORT REPORTS

PEROXIDASE AND AMYLASE ISOENZYMES IN THE SAPWOOD AND HEARTWOOD OF TREES*

R. EBERMANN and K. STICH

University of Agriculture, 1180 Vienna, Gregor-Mendel-Str. 33, Austria

(*Revised received* 15 *March* 1982)

Key Word Index—Angiosperms; Gymnosperms; sapwood; heartwood; peroxidase; amylase; isozymes.

Abstract—Peroxidases and amylases have been found in the sapwood and the heartwood of both angiosperm and gymnosperm trees. Cambial and outer layers of xylem of eight trees were removed by means of a lathe and it was possible to demonstrate enzymic activities in the sapwood and heartwood. Isozymic patterns varied according to the trees studied. These enzymes seem to be quite resistant to denaturation because they are still detectable in cut wood left to stand for one year.

INTRODUCTION

It is well known that enzymes occur in the xylem cells near the cambium of trees [1–4]. Barna [5] found amylase and peroxidase in shoots of *Vitis vinifera*. To our knowledge there is no experimental evidence so far reported on isoenzymes in the inner layers of several-year-old trees.

RESULTS AND DISCUSSION

This is the first time (Figs. 1–4) that enzymic activities have been electrophoretically detected in the sapwood and heartwood of trees. Previously, enzymic activities have been only reported in the cambial layer. For example Harkin and Obst [2], who stained wood discs for peroxidase activity, found the main intensity of colour in the cambial zone; this rapidly decreased towards the center of the disc, except at the border between sapwood and heartwood where a small increase was observed. Becker [6] analysed the nitrogen content of wood at different distances from the center. He found an increase in nitrogen content at the heartwood–sapwood border. Harkin and Obst [2] denied the existence of peroxidase enzymes in the cambial zone, interpreting the histochemical stain as being due to diffusion.

In our work, 2 cm of the outer layer was removed by means of a lathe in order to avoid any possible diffusion of enzymes during extraction and staining. From the remaining sapwood and heartwood tissue, the peroxidase and amylase enzymic proteins were extracted as described. Isoenzymes of peroxidase and amylase were found in the sapwood (Figs. 1, 3 and 4). Peroxidase and amylase isoenzymes from sapwood and heartwood exhibit identical electrophoretic bands in most trees investigated. The only exception is the heartwood of *Laburnum* which differs in peroxidase pattern from that of the sapwood. Enzyme patterns are typical for a given species, through some variation within species was observed in the case of *Acer campestris* (see Fig. 2). The enzymes from sapwood and heartwood can be detected not only in wood

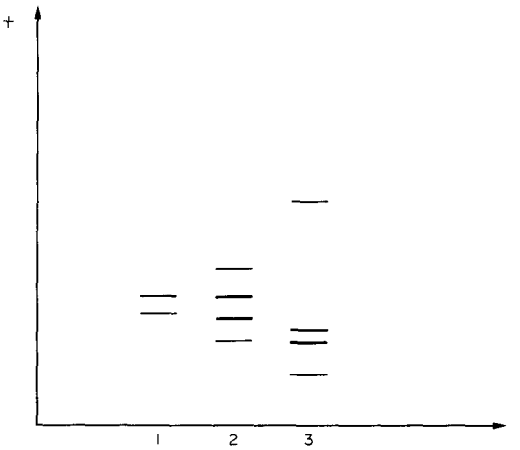

Fig. 1. Peroxidase isoenzymes of different Angiosperm trees extracted from sapwood. (1) *Acer campestris*; (2) *Acer platanoides*; (3) *Quercus robur*.

*This paper id dedicated to Professor Dr. Heribert Mirhl on the occasion of his 60th birthday.

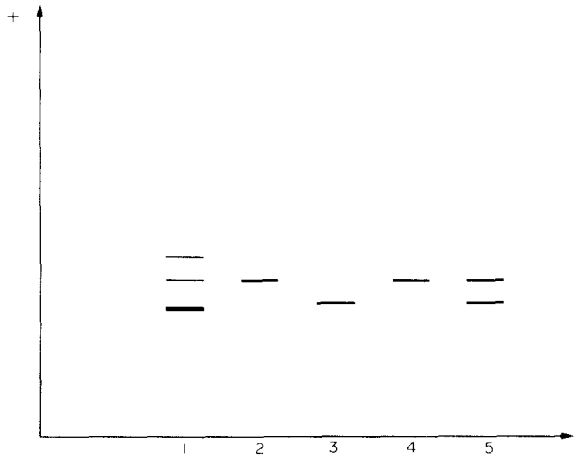

Fig. 2. Peroxidase isoenzymes of different Angiosperm trees extracted from sapwood. (1) *Acer platanoides*; (2) *Acer campestris*, 1; (3) *Acer campestris*, 2; (4) *Acer campestris*, 3; (5) *Acer campestris*, 4.

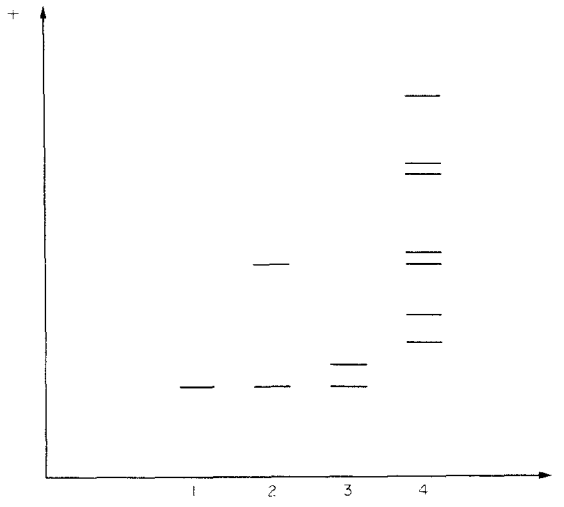

Fig. 3. Peroxidase isoenzymes of different Gymnosperm trees extracted from sapwood. (1) *Pinus nigra austriaca*; (2) *Taxus baccata*, 1; (3) *Picea excelsa*; (4) *Pinus silvestris*.

freshly cut but also in wood stored for one year or longer.

The occurrence of enzymic activities in the inner layers of wood indicates that metabolic processes are taking place in the older parts of wood. In particular, peroxidase activity may be related to lignification, to structural modification of existing lignins or to polymerization of other phenolics. Preliminary results obtained by studying the kinetics of two peroxidase isoenzymes from sapwood of *Quercus* sp. (substrate of dianisidine) show, that apparent K_m values of the two isoenzymes are different (10×10^{-4} and 2.35×10^{-4} M).

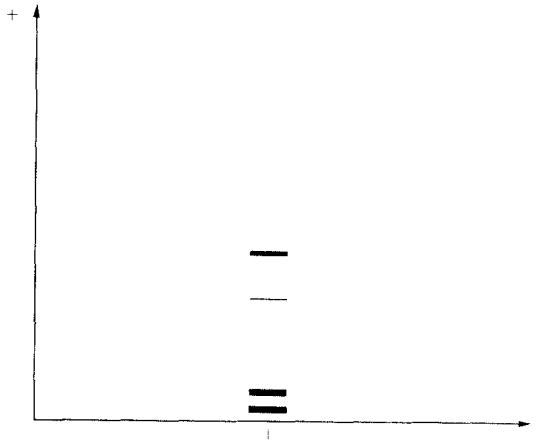

Fig. 4. Amylase isoenzymes of *Ailanthus* species extracted from sapwood. (1) *Ailanthus* sp.

EXPERIMENTAL

Branches of different trees were collected mainly in the autumn. The diameter of the wood samples obtained in most cases was *ca* 5 cm. The wood was milled with a slow-turning lathe in order to prevent excess heating. The spans from the cambium and outer xylem layers were discarded and only the spans from the inner regions were collected. The splinters were extracted overnight (1.5 g spans in 6 ml buffer). The buffer contained 1.2 g Tris, 2.0 g ascorbic acid, 2.0 g EDTA-Na_2, 3.8 g Borax, 3.6 g NaCl and 50.0 g polyethyleneglycol in one l l. water. The soln which was obtained after removing the splinters was used for electrophoretic analysis without further concn. Gel electrophoresis in polyacrylamide was performed as described earlier [1] with the pH of the separation gel changed to 6.0. Peroxidase isoenzymes were stained according to Ornstein [8]. Starch degrading enzymes were detected as transparent zones on opaque gels having inserted the starch-containing gels for this purpose into MeOH–H_2O–HOAc (5:5:1). Separation gels for starch-degrading enzymes contained 3.5% soluble starch in 10% polyacrylamide, pH 8.5, with Tris–citrate buffer, as above.

REFERENCES

1. Freudenberg, K., Reznik, H., Boesenberg, H. and Rasenack, D., (1952) *Chem. Ber.* **85**, 641.
2. Harkin, J. M. and Obst, J. R. (1973) *Science* **180**, 296.
3. Höll, W. and Meyer, H. (1977) *Holzforschung* **31**, 184.
4. Höll, W. and Lendzian, K. (1973) *Phytochemistry* **12**, 975.
5. Barna, J. (1973) Ph.D. thesis, University of Agriculture, Vienna, Austria.
6. Becker G. (1962) *Holz Roh- Werkst*, **20**, 368.
7. Ebermann, R. and Bodenseher, H. (1968) *Experientia* **24**, 523.
8. Ornstein, L. (1971) In *Disc Electrophoresis* (Maurer, H. R., ed.). Walter de Gruyter, Berlin.