Pergamon

S0031-9422(96)00097-0

Phytochemistry, Vol. 42, No. 4, pp. 949–953, 1996

0031-9422/96 $15.00 + 0.00

THE PHENOLIC DOMAIN OF POTATO SUBERIN: STRUCTURAL COMPARISON WITH LIGNINS

CATHERINE LAPIERRE, BRIGITTE POLLET and JONATHAN NÉGREL*

Laboratoire de Chimie Biologique, INRA-CBAI, Institut National Agronomique, 78850 Thiverval-Grignon, France;
*Laboratoire de Phytoparasitologie INRA-CNRS, Station de Génétique et d'Amélioration des Plantes, INRA BV 1540, 21034 Dijon Cédex, France

(*Received in revised form* 17 *November* 1995)

Key Word Index—*Solanum tuberosum*; Solanaceae; potato; periderm; natural periderm; wound-healing periderm; suberin; lignin; thioacidolysis.

Abstract—Natural and wound-healing potato periderms were analysed using thioacidolysis, a specific lignin degradative method. The nature and relative frequencies of phenylpropane units and of inter-unit bonds comprising this lignin-like domain were determined. Results confirmed that this domain contains guaiacyl G and syringyl S units, similar to lignins of woody angiosperms. The recovery yield of the G and S thioacidolysis monomers was, however, about one-tenth of that obtained for wood or straw lignified cell walls. This result suggests that G and S phenylpropane units are relatively few in suberized samples. These units occur in different ratios in the natural and wound potato periderms and are assembled with the same inter-unit bonds as those found in lignins of woody angiosperms, although in different proportions. Both the scarcity of the β-O-4 and β-1 bonds, and the high terminal to internal units ratio, suggest that this lignin-like domain is highly cross-linked.

INTRODUCTION

Suberin is a complex heteropolymer comprised of aromatic and aliphatic domains [1, 2]. The composition of the lipidic domain has been determined in many plant species [3–5]. Knowledge about the ferulic derivatives of suberized tissues has made recent progress [6–9]. Studies performed by solid state ^{13}C NMR of suberin provided new insights into the composition of suberized potato cell wall [10] and, more importantly, provided clear-cut evidence of the occurrence of two hydroxycinnamic populations [11]. In spite of these various investigations, the structure of the resistant phenolic core in suberized tissues, which mimics lignins with regard to histochemical stainings and oxidation products [12], remains obscure. This intractable phenolic domain has been described as 'lignin-like', i.e. a polymer formed by oxidative coupling of p-hydroxycinnamyl alcohols. From nitrobenzene oxidation of potato periderms, Kollatukudy [2] suggested that this domain contained p-hydroxyphenyl (H), guaiacyl (G) and, to a lesser extent, syringyl (S) phenylpropane units, together with ferulic esters. In the proposed model, these various units were assembled by typical lignin inter-unit bonds, mainly labile alkyl-aryl ether bonds referred to as β-O-4 linkages, with the standard lignin labelling conventions [13]. This model was recently revised based on the results of thioacidolysis, a degradative technique which degrades β-O-4 structures into specific thioethylated monomers [14]. Whereas the occurrence of β-O-4 linked G and S phenylpropane units in potato suberin was confirmed by thioacidolysis, H analogous structures were not observed in the thioacidolysis mixture [15].

The purpose of the present work was to gain further insights into the lignin-like domain of suberized potato periderms and to establish to what extent it resembles cell wall lignins. Natural and wound-healing potato periderms were analysed using a more refined thioacidolysis protocol [16]. The nature and relative frequencies of the phenylpropane units and, for the first time, of the inter-unit bonds assembling these phenylpropane units in potato suberized periderms were determined and compared with cell wall lignins.

RESULTS AND DISCUSSION

Since the phenolic domain of suberized tissues was suggested to have similarities with lignins [1, 2], we applied a specific lignin degradation technique, thioacidolysis [16], to natural and wound-healing periderms of potato tubers. Thioacidolysis efficiently cleaves the β-O-4 bonds in lignins [16]. As shown in Fig. 1, H, G or S units involved in β-O-4 bonds specifically give rise to H, G or S thioethylated monomers, respectively.

Hydroxycinnamic acids, ester- or amide-linked to suberized wall polymers, do not give rise to the thioetylated monomers outlined in Fig. 1. Such avoid-

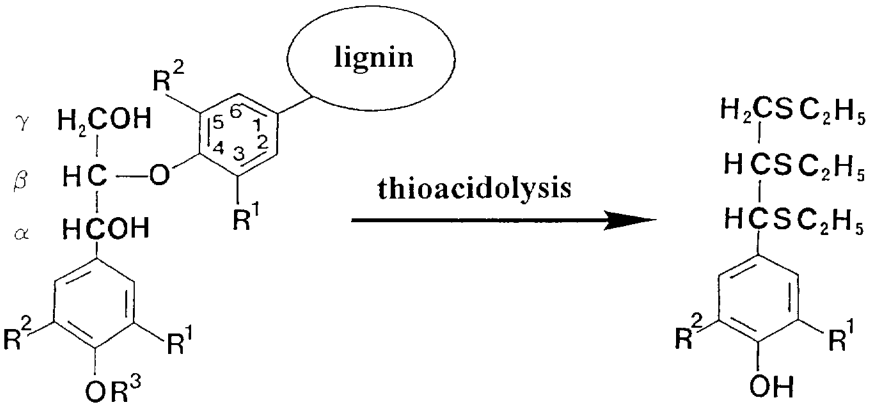

Fig. 1. Key reaction of thioacidolysis. The depolymerization of the lignin or lignin-like network proceeds by cleavage of β-O-4 bonds. The p-hydroxyphenyl ($R^1 = R^2 = H$), guaiacyl (R^1 = OMe, R^2 = H) or syringyl ($R^1 = R^2$ = OMe) phenylpropane units involved in these β-O-4 bonds specifically give rise to H, G or S thioethylated monomers, respectively. These monomers are recovered as a 50/50 mixture of *erythro*- and *threo*-isomers, with a high reaction yield which closely reflects the amount of β-O-4 substructures in the original polymer. These substructures are outlined with the standard lignin-labelling conventions. R^3 = H or C-β of lignin sidechains.

ance of interference between various phenolic populations is an advantage of thioacidolysis in contrast to the less specific nitrobenzene oxidation. Since thioacidolysis, aimed at cleavage of ether bonds, cannot quantitatively hydrolyse hydroxycinnamic amides and/or esters, these components were not characterized in the present study.

The main aromatic monomers recovered from the thioacidolysis of suberized potato periderms were identified after silylation by GC-mass spectrometry and quantified by GC [17]. Their absolute yield and relative distribution are shown in Table 1. For comparison purposes, similar data obtained for wood and straw lignified samples [16] are also reported in Table 1. These monomers are the unambiguous signature of the β-O-4 structures typically present in cell wall lignins [13]. The data show that the occurrence of β-O-4 linked G and S units is a structural feature common to the aromatic domain of potato suberized periderms and to lignins of woody angiosperms. The H analogues characteristic of reaction wood or grass lignins are not present in these suberized tissues, contrary to the model proposed from the results of nitrobenzene oxidation [2, 18]. The suberized potato periderms were also analysed by nitrobenzene oxidation. Approximately half of the amount of aromatic benzaldehydes released by nitrobenzene oxidation of suberized potato periderms was p-hydroxybenzaldehyde. In agreement with literature [15, 19], these results show that p-hydroxybenzaldehyde could not originate from β-O-4 linked H phenylpropane units, but from other phenolics [8].

The recovery yield of the thioacidolysis aromatic monomers was about one-tenth of that obtained for lignified tissues, such as wood or straw (Table 1). This indicates that the β-O-4 substructures which specifically generate these monomers are relatively few in the suberized samples. In the wound periderm, these structures occur in twice as high a concentration as in the natural periderm. This difference might reflect the stimulation of the phenylpropanoid pathway on wounding. In addition, this increase in β-O-4 structures more specifically concerned the syringyl units (Table 1).

Table 1. Yield and relative distribution of the main H, G and S monomers issued from the thioacidolysis of suberin-enriched potato periderms and of lignified cell walls. The yield in monomers outlined in Fig. 1 is expressed in μmol g^{-1} of sample. The lignin contents of the extractive-free poplar wood, pine reaction wood and wheat straw are 23.4, 37.7 and 17% (% Klason lignin, w/w), respectively. Values are means of three replicate experiments (5% standard error)

Samples	H	G	S	H + G + S	Rel. distribution (H/G/S)
Potato suberin-enriched periderms					
Natural	ND	11	6	17	ND:65:35
Wound-healing	ND	16	20	36	ND:45:55
Lignified cell walls					
Poplar wood	ND	221	319	540	ND:41:59
Pine reaction wood	77	353	TR	430	18:82:TR
Wheat straw	9	87	81	177	5:49:46

ND, not detected; TR, traces.

Contrary to thioacidolysis, the nitrobenzene oxidation technique yielded no distinction between natural and wound periderms.

Permethylation of the samples before thioacidolysis makes it possible to recover further structural information on the relative distribution of the thioacidolysis *β*-*O*-4 target structures inside the phenolic network. These structures may be either terminal units with free phenolic groups or internal units with etherified phenolic groups. Only the former structures can be methylated at their phenolic group by diazomethane and thus give rise to methylated monomers upon thioacidolysis [20]. Using this strategy, we found that about 90% of the *β*-*O*-4 linked S units were internal units, whatever the cell wall sample. In contrast, 53 and 48% of the *β*-*O*-4 linked G units were terminal units with free phenolic groups, in the natural and wound periderms, respectively. These values were about twice as high as those obtained for wood lignins [20]. Such a high terminal to internal G unit ratio in suberized tissues argues for the occurrence of a phenolic network marked by a higher cross-linking degree and/or a lower molecular weight than that of wood lignins.

In the thioacidolysis mixture, there are thioethylated dimers which have retained a resistant carbon–carbon or diaryl ether bond of the polymer [17]. Their direct GC–mass spectrometric determination is impeded by the thioethyl substituents that make their molecular weight excessive. To quantify these dimers, a desulphurization step is performed on the thioacidolysis mixture. The structures of the main aromatic dimers recovered from woody angiosperm lignins or from suberized potato periderms are shown in Fig. 2. In agreement with the results of Table 1, the main dimers found comprised G and S units only, and not H units. These G–G, G–S or S–S dimers were representative of various resistant bonds, namely the *β*-1, *β*–*β*, *β*-5, 4-*O*-5 and 5–5 linkages (Fig. 2). Their relative amounts are reported in Table 2.

The main resistant inter-unit bonds characteristic of woody angiosperm lignins [16,21] were also observed in potato suberized periderms, but their representative dimers were recovered with different relative frequencies. From the data of Table 2, it can be assumed that *β*-1 bonds are relatively less frequent in these suberized tissues than in woody angiosperm lignins. It is noteworthy that the relative importance of *β*-1 bonds in lignins has been a matter of conflicting results. While acidolytic degradations provided substantial amounts of *β*-1 dimers from natural lignins [21, 22], two-dimensional NMR experiments applied to various lignin samples either failed to [23, 24] or succeeded in [25, 26] providing signals assignable to *β*-1 structures. By subjecting a series of *β*-*O*-4, *β*-5, *β*–*β*, *β*-1 and 5–5 dimers to thioacidolysis and desulphurization, we verified that no artefact *β*-1 bond was formed during thioacidolysis or desulfurization. Accordingly, the *β*-1 dimers recovered as major dimers from native lignins and as trace dimers from synthetic lignins [16, 21] are representative of *β*-1 bonds pre-existing in the polymer, whatever the native structure carrying these *β*-1 bonds (diarylpropane-1,3-diols or their cyclohexadienone precursors formed according to ref. [27]).

With regard to the relatively low content of *β*-*O*-4 and *β*-1 bonds, the lignin-like moiety of potato suberized periderms resembles synthetic lignins prepared by peroxidative polymerization of *p*-hydroxycinnamyl alcohols supplied in high local concentration [16], re-

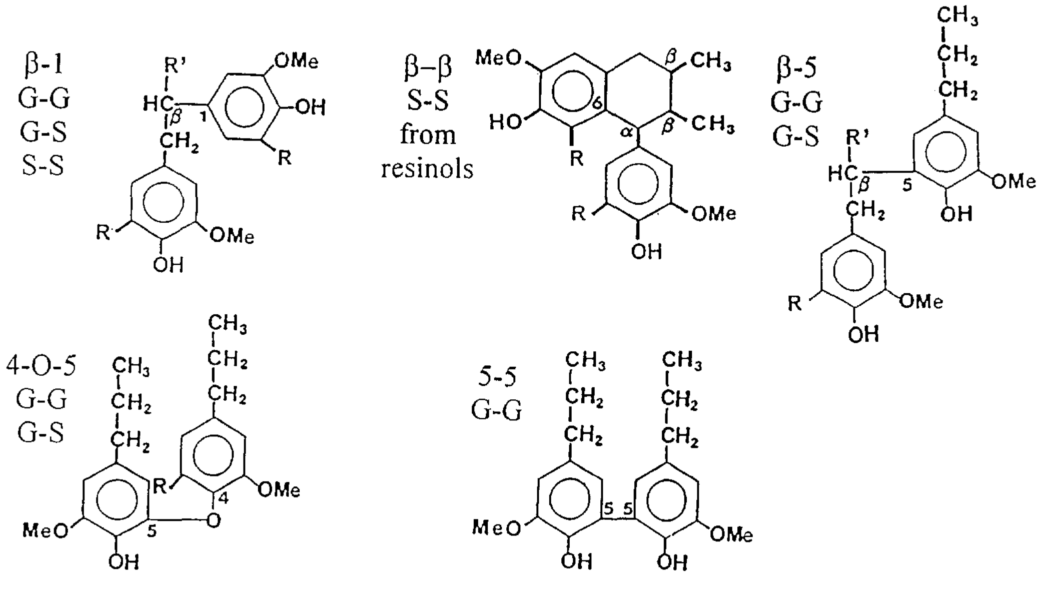

Fig. 2. Chemical structures of the main guaiacyl–guaiacyl (G–G) (R = H), mixed guaiayl–syringyl (G–S) (R = H or OMe), and syringyl–syringyl (S–S) (R = OMe) dimers obtained after thioacidolysis and subsequent desulphurization of suberin-enriched potato periderms and of woody angiosperm lignins. These dimers represent the 5–5, *β*-5, 4-*O*-5, *β*-1 and *β*–*β* inter-unit bonds of the original phenolic network. The *β*–*β* S–S dimer essentially stems from syringaresinol structures. R′ = H or CH_2OH.

Table 2. Relative frequencies of the main G-G, G-S and S-S dimers recovered from natural and wound-healing potato periderms or from poplar wood, subjected to thioacidolysis, then Raney nickel desulphurization. The main dimers are identified by GC-MS and outlined in Fig. 2. They represent the various inter-unit bonds usually met in lignins (β-1, β–β, β-5, 4–*O*-5 and 5–5). Their relative frequencies were determined by selected ion monitoring (SIM) and compared to the data similarly obtained from polar lignin. For each dimer, the most specific ion was selected and the surface of the corresponding signal was measured on the SIM chromatogram. Values are means of three replicate experiments (10% standard error)

Sample	β-1 G–G G–S S–S	β–β S–S	β-5 G–G G–S	4–*O*-5 G–G G–S	5–5 G–G
Potato natural periderm	8	11	25	13	43
Potato wound periderm	11	43	28	3	15
Poplar wood lignin	24	31	31	5	9

ferred to as the bulk polymerization process [28]. In this respect, the suberin phenolic core contrasts with xylem lignins rich both in β-*O*-4 and β-1 bonds [16], and formed with low local concentration of *p*-hydroxycinnamyl alcohols. The analysis of the thioacidolysis monomers revealed a relative increase of S units in the wound periderm, compared to the natural one (Table 1). The dimer analysis corroborated this observation: the relative importance of the β–β dimer involving only S units was four-fold higher in the wound periderm, compared to the natural one. Conversely, the relative importance of the 5–5 dimer that exclusively assembles G units decreased in the wound periderm (Table 2). These marked structural differences between the aromatic domains of wound and natural potato periderms contrast with the previously reported similarities of their aliphatic domains [3].

Taken together, our results confirm that suberized potato periderms contain a phenylpropane polymer which exhibits both similarities with and differences from xylem lignins. This polymer is a minor component of these suberin-enriched tissues, in agreement with recent literature data [29]. These results cannot address whether the G and S phenylpropane units, released by thioacidolysis, originate from suberin *per se* or stem from a distinct lignin-like domain present in the cell walls of the suberin-enriched preparations.

CONCLUSION

The present work has demonstrated the capability of thioacidolysis to provide detailed structural insights into the lignin-like domain of potato suberized tissues, from a few milligrams of sample. Our results confirm that this domain comprises the same G and S phenylpropane units as lignins of woody angiosperms. The recovery yield of these units from suberized periderms is, however, about one-tenth of the yield obtained from xylem lignin, which shows that these β-*O*-4 linked G and S units are a minor component of these suberized samples. In contrast, H units could not be found in this phenolic network. The G and S units within the phenolic network of suberized potato samples occur in different proportions in the natural and wound periderms. These phenylpropane units are assembled with the same inter-unit bonds as the lignins of woody angiosperms, but in different proportions. Both the scarcity of the β-*O*-4 and β-1 bonds, and the high terminal to internal units ratio, suggest that this polymer is highly cross-linked [30].

EXPERIMENTAL

Plant material. Potato tubers (*Solanum tuberosum* L., cv. Bintje) were surface sterilized with NaClO for 30 min and washed with sterile H_2O. The tubers were then peeled and the natural periderm isolated by incubation for 24 hr at room temp. on a rotary shaker in a mixt. of cellulase and pectinase (10 and 1 mg ml^{-1}, respectively, CAYLA, Toulouse, France), in 0.1 M K-Pi buffer pH 6. The suberized sheets were collected and ground in liquid N_2. The residue was treated again with the enzymic mixt., washed with H_2O and then extracted successively with MeOH and then $CHCl_3$ for 24 hr in a Soxhlet extractor. For the prepn of wound-healing periderm, tuber discs (5 mm thick) were cut and allowed to wound-heal in Petri dishes at 25°, in darkness, under sterile conditions. After 14 days of suberization, the tissue cylinders were cut and treated as described for natural periderm. The natural and wound-healing potato periderms were thoroughly permethylated by repeated addition of an Et_2O soln of CH_2N_2. Between each addition of reagent, the solvent was evapd under a N_2 stream, in a hood. Once the yellow colour of CH_2N_2 had been observed to stay for 24 h, the methylation was considered to be complete and the solvent was evapd.

Thioacidolysis. The thioacidolysis reagent was prepd as previously described [17]. Original or permethylated suberin-enriched periderms (*ca.* 20 mg) were added to 10 ml thioacidolysis reagent in a glass tube closed with a Teflon-lined screw-cap. Thioacidolysis was performed at 100° (oil bath) for 4 hr. The cooled reaction mixt., together with 3 × 10 ml H_2O, was then poured into 30 ml CH_2Cl_2 containing 0.25 mg docosane, the GC int. standard. The pH of the aq. phase was adjusted to 3–4 with 0.4 M aq. $NaHCO_3$. The aq. phase was extracted with 3 × 30 ml CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and then evapd under red. pres. The residue was redissolved in *ca.* 0.5 ml CH_2Cl_2. An aliquot (10 μl) was silylated with 100 μl bistrimethyslyltrifluoroacetamide and 10 μl pyridine for 30 min at room temp.

Desulphurization. Half the thioacidolysis soln was placed in a screw-capped glass tube, with 2 ml Raney nickel aq. slurry (Aldrich) and 5 ml dioxane. Desulphurization was run at 50° (oil bath) for 4 hr, behind a safety shield. The ice-cooled reaction mixt., together with 20 ml H_2O used to rinse the tube, was poured into 30 ml CH_2Cl_2. The aq. layer where Raney nickel remained was adjusted to pH 4 and extracted 3 more times with CH_2Cl_2. The organic extracts, dried over Na_2SO_4, were evapd to dryness and the final residue redissolved in 0.2 ml CH_2Cl_2. Further silylation was performed as described above.

Analysis of the thioacidolysis reaction products. GC quantitative analysis of the TMSi reaction products was performed as previously described [17, 21]. GC-MS identification of the TMSi reaction products was performed with a quadrupole mass spectrometer working in positive electron impact mode (70 eV). The relative frequencies of the main phenolic dimers recovered from cell walls were estimated as follows. For each TMSi dimer identified from its characteristic mass spectrum [17] and retention time in the total ion chromatogram, the most specific ion was selected. A SIM GC-MS analysis was then performed and signals of selected ions were measured on the SIM chromatogram. By so doing, we avoided interference from a series of lipid components of the reaction mixt. (not quantitatively considered herein, as thioacidolysis is not aimed at ester cleavage) and we increased the signal-to-noise ratio for better quantitative evaluations.

REFERENCES

1. Kollatukudy, P. E. (1981) *Ann. Rev. Plant Physiol.* **32**, 539.
2. Kollatukudy, P. E. (1984) *Can. J. Botany* **62**, 2918.
3. Kollatukudy, P. E. and Dean, B. B. (1974) *Plant Physiol.* **54**, 116.
4. Kollatukudy, P. E., Kronman, K. and Poulose, A. J. (1975) *Plant Physiol.* **55**, 567.
5. Perra, B., Haluk, J-P. and Metche, M. (1993) *Holzforschung* **47**, 486.
6. Bernards, M. A. and Lewis, N. G. (1992) *Phytochemistry* **31**, 3409.
7. Davin, L. B. and Lewis, N. G. (1992) in *Phenolic Metabolism in Plants* (Stafford, H. A. and Ibrahim, R. K., eds), p. 325. Plenum Press, New York.
8. Negrel, J., Javelle, F. and Paynot, M. (1993) *J. Plant Physiol.* **142**, 518.
9. Lofty, S., Negrel, J. and Javelle, F. (1994) *Phytochemistry* **35**, 1419.
10. Garbow, J. R., Ferrantello, L. M. and Stark, R. E. (1989) *Plant Physiol.* **90**, 783.
11. Stark, R. E. and Garbow, J. R. (1992) *Macromolecules* **25**, 149.
12. Hammerschmidt, R. (1985) *Potato Res.* **28**, 123.
13. Higuchi, T. (1985) in *Biosynthesis and Biodegradation of Wood Components* (Higuchi, T., ed.), p. 23. Academic Press, Orlando, FL.
14. Lapierre, C., Monties, B. and Rolando, C. (1986) *Holzforschung* **40**, 113.
15. Borg-Olivier, O. and Monties, B. (1989) *C. R. Acad. Sci. Paris* **308**, 141.
16. Lapierre, C. (1993) in *Forage Cell Wall Structure and Digestibility* (Jung, H. G., Buxton, D. R., Hatfield, R. D. and Ralph, J., eds), p. 133. Amer Society of Agronomy, Madison, WI.
17. Lapierre, C., Pollet, B., Monties, B., and Rolando, C. (1991) *Holzforschung* **45**, 61.
18. Cottle, W. and Kollatukudy, P. E. (1982) *Plant Physiol.* **69**, 393.
19. Borg-Olivier, O. and Monties, B. (1993) *Phytochemistry* **32**, 601.
20. Lapierre, C. and Rolando, C. (1988) *Holzforschung* **42**, 1.
21. Lapierre, C., Pollet, B. and Rolando, C. (1995). *Res. Chem. Intermed.* **21**, 397.
22. Adler, E. (1977) *Wood Sci. Technol.* **24**, 23.
23. Ede, R. M., Brunow, G., Simola, L. K. and Lemmetyinen, J. (1990) *Holzforschung* **44**, 95.
24. Ede, R. M. and Brunow, G (1992) *J. Org. Chem.* **57**, 1477.
25. Kilpeläinen, I., Sipilä, J, Brunow, G., Lundquist, K. and Ede, R. M. (1994) *J. Agric. Food Chem.* **42**, 2790.
26. Fukagawa, N., Meshitsuka, G. and Ishizu, A. (1992) *J. Wood Chem. Technol.* **12**, 91.
27. Lundquist, K. and Miksche, G. E. (1965) *Tetrahedron Letters* **25**, 2131.
28. Sarkanen, K. V. (1973) in *Lignins: Occurrence, Formation, Structure, and Reactions* (Sarkanen, K. V. and Ludwig, C. H., eds), p. 95. Wiley-Interscience, New York.
29. Bernards, M. A., Lopez, M. T., Zajicek, J. and Lewis, N. G. (1995) *J. Biol. Chem.* **270**, 7882.
30. Kollatukudy, P. E. and Espelie, K. E. (1989) in *Natural Products of Woody Plants II* (Rowe, J. W., ed.), p. 304. Springer, Berlin.