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PHENOLICS OF SUBERIZED ENVELOPES GENERATED BY ISOLATED TOMATO LOCULE PROTOPLASTS

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; protoplasts; suberin; phenolics; vanillin; *p*-coumaric acid; *p*-hydroxybenzaldehyde; syringaldehyde.

Abstract—The phenolic compounds of the suberized multilamellar wall secreted during a 7-day culture period by protoplasts isolated from tomato fruit locule tissue were studied. Phenols constituted 25% of the total monomers obtained from the suberin extract. In descending quantitative order, the major phenols were vanillin, *p*-coumaric acid, *p*-hydroxybenzaldehyde and syringaldehyde.

INTRODUCTION

In culture, protoplasts isolated from the locule tissue of immature tomato fruit secrete a wall which appears multilamellated when examined by electron microscopy [1], and which recently has been shown to contain suberin [2]. We have suggested that this system may prove to be a valuable experimental tool in the study of the secretion and deposition of suberin and of its associated products [2].

Suberin is a complex polymeric material composed of a variety of aliphatic monomers (review [3]). In some instances, it has been shown that polyphenolic compounds may also be covalently attached to the suberin polymer [4]. In our earlier analysis of the envelope secreted by tomato fruit protoplasts [2], we found that more than 60% of the analysate was a highly polar brown material which remained close to the origin after Chromarod/Iatrosan TLC [5]. Since this might indicate that aromatic compounds such as polyphenols were present, a detailed analysis for phenols was performed with the results reported here.

RESULTS AND DISCUSSION

TLC on silica gel Chromarods with flame ionization detection by Iatroscan (TLC-FID) analysis showed that phenolic compounds constituted 24.9% of the total area of the depolymerized suberin. We had previously reported that aliphatic monomers constituted 37% of the depolymerized suberin from tomato protoplasts; thus the quantitative ratio of phenolic to aliphatic monomers in the multilamellar walls (mlw) of tomato protoplasts is less than the extreme ratio of 7:3 reported for some corks [6].

Upon GC analysis four phenolic compounds were identified using authentic standards. Vanillin was the major phenolic compound followed by lesser quantities of *p*-coumaric acid, *p*-hydroxybenzaldehyde and syringaldehyde (Table 1).

In previous studies, vanillin and *p*-hydroxybenzaldehyde have been shown to be major components of the phenolics of potato wound suberin [7] and of the suberized cell wall of green cotton fibre [8]. *p*-Coumaric acid has been identified in cutins obtained from fruits such as tomato, apple, pear and peach [4], but has not previously been reported in suberins. The present study shows that *p*-coumaric acid could be a polyphenol common to both suberin and cutin.

Other components, including ones at the origin, which separated from phenols on the silica gel of Chromarods were more polar than phenols and made up 23.5% of the Iatroscan FID response for the mlw extract. Their identity is still to be determined.

EXPERIMENTAL

Details of the methods for isolation and culture of the tomato fruit protoplasts and for obtaining enriched suberin have been

Table 1. Phenolic compounds from the depolymerized mlw extract of tomato protoplasts as determined by GC

Phenol	Wt%
Vanillin	74.9
p-Coumaric acid	14.9
p-Hydroxybenzaldehyde	6.6
Syringaldehyde	3.6

described previously [2]. The enriched suberin was depolymerized with $LiAlH_4$ in THF for 48 hr [4]. The reaction was quenched by the addition of H_2O (50 ml), the mixture extracted with Et_2O and the aq. layer discarded. A part of the Et_2O extract which contained both the aliphatic and the aromatic components of suberin was concd under N_2 and analysed quantitatively by Chromarod/Iatroscan TLC with FID detection using a Iatroscan TH-10 Analyser (Iatron Labs, Tokyo, Japan) with a development in hexane–Et_2O–MeOH (25:20:4). The remaining portion of the initial Et_2O extract was further extracted with 5% aq NaOH to obtain an alkali extract which contained the phenolics. The alkali extract was acidified and extracted with Et_2O and the Et_2O layer separated, concd under N_2 and examined on Adsorbosil-5 Precote TLC plates (Appl. Sci. Labs) activated at 110° for 30 min. The plates were developed in hexane–Et_2O–MeOH (5:4:2) to verify the presence of phenols using authentic standards.

Phenolic acetates were prepared by mixing 100 mg of the sample with Ac_2O (2 ml), 3 N NaOH (0.5 ml) and crushed ice (1–2 g). The mixture was thoroughly shaken and H_2O (3 ml) was added. The soln was acidified with 3 N HCl and extracted with Et_2O. The Et_2O extract was dried (Na_2SO_4) and concd under N_2. The phenolic acetates were analysed by GLC equipped with FID on a bonded methyl silicone fused silica capillary column (50 m × 0.24 mm, Quadrex Corporation, Connecticut, U.S.A.) at 180° with He (60 psig) as carrier gas.

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