*Phytochemistry*, Vol. 20, No. 9, pp. 2225–2227, 1981.
Printed in Great Britain.

0031-9422/81/092225-03 $02.00/0


# ALIPHATIC COMPOSITION OF CUTIN FROM INNER SEED COAT OF APPLE

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(*Received* 9 *January* 1981)

**Key Word Index**—*Malus pumila*; Rosaceae; apple; seed coat; cutin; suberin; hydrogenolysis.

**Abstract**—Lithium aluminum deuteride reduction released aliphatic monomers from the inner seed coat fraction but not from the outer seed coat fraction of mature apples. These monomers were identified by GC/MS and the results indicate that the inner coat of apple seed contains a cutin polymer with the major monomer acids being 18-hydroxyoctadec-9-enoic (31%), 9,10-epoxy-18-hydroxyoctadecanoic (28%) and 9,10,18-trihydroxyoctadecanoic (20%). The monomer composition of this seed coat cuticular polymer was very similar in seeds taken from freshly harvested fruit and in those taken from fruit which had been stored at 4° for 6 months.

## INTRODUCTION

The seed coats of plant seeds serve as a protective covering and frequently play a vital role in imbibition and germination [1–3]. In several cases ultrastructural analyses have shown the presence of one or more cuticular layers in the seed coat [4–6]. However, the presence of cutin polymers has been chemically shown in only a few seeds [7, 8].

The majority of cutin polymers which have been chemically analysed have been isolated from the epidermis of either leaves or fruit peels [1, 9, 10], but it has been shown that the cutin composition within the same plant may vary according to anatomical location of the polymer [7]. Such a variation may be due to the different types of protection required from one plant part to another. In this paper we report the monomer composition of a cutin polymer found in the inner seed coat of apple seed and compare this composition with those of cutin polymers from other locations in the apple plant.

## RESULTS AND DISCUSSION

The chemical depolymerization of transparent cuticular membranes, which can be isolated from the epidermal layers of various fruits and leaves, releases aliphatic monomers recoverable in per-weight yields of 60–80% [9, 11]. However, many cutin polymers, including those ultrastructurally shown to be present in seed coats, cannot be readily isolated in a pure form in quantities sufficient for chemical analysis. Nevertheless, chemical analysis of a heterogeneous tissue such as a seed coat can show that a cutin polymer (or polymers) is located within that tissue. The inner and outer seed coats of apple were carefully separated from each other and from the rest of the seed and were subjected to extensive organic solvent extraction in order to ensure that any compounds released by depolymerization were originally covalently bound in the seed coat. The wax components which were extracted by these organic solvents have been characterized previously [12–15]. Hydrogenolysis of the outer seed coat and also of the carpellary membrane released no detectable aliphatic compounds and it appears, therefore, that neither of these tissues contains a cutin polymer.

Depolymerization of the inner seed coat resulted in the release of aliphatic monomers which were derivatized and analysed by GC/MS and they gave a pattern typical of a cutin polymer [1, 9, 10]. The monomers released by hydrogenolysis of the inner seed coat isolated from apples which had been in cold storage for 6 months gave a very similar chromatogram.

$LiAlD_4$ depolymerization of a cutin polymer releases alcohols, diols, triols and tetraols by reduction of ester linkages [11]. Carboxyl moieties are reduced to OH groups and the carbons bearing these OHs can be differentiated from those carbons which were bonded to OH groups before the reductive depolymerization because two D atoms are added to the reduced carbon. This technique allowed the determination of the monomer composition of the seed coat polymer (Table 1). There were only small differences between the compositions of the polymers from the inner seed coat of seeds taken from fresh apples and those taken from storage apples. Fatty acids and fatty alcohols were minor components (<2%). The major monomer was 18-hydroxyoctadec-9-enoic acid (31%) and two other $C_{18}$ acids, 9,10-epoxy-18-hydroxyoctadecanoic and 9,10,18-trihydroxyoctadecanoic, comprised 27 and 20%, respectively, of the polymer from the seeds of fresh apples. The high proportion of polar acids in this polymer (65%) is typical of a cutin polymer [10] and the predominance of the $C_{18}$ family of cutin acids has been observed previously in the cutin polymers which serve as protective barriers in slow-growing plant tissue as opposed to the predominance of the $C_{16}$ family of acids frequently found in the cutin of fast-growing tissue [7, 16].

Table 1. Per cent composition of the aliphatic monomers of cutin polymers from the inner seed coat of seeds from fresh and stored apples

| | Inner seed coat from fresh apples | Inner seed coat from stored apples |
|---|---|---|
| 1-Alkanols | | |
| $C_{16}$ | 0.05 | 0.08 |
| $C_{17}$ | 0.56 | 0.79 |
| $C_{18}$ | 0.05 | 0.26 |
| Monobasic acids | | |
| $C_{16}$ | 0.39 | 0.30 |
| $C_{18}$ | 0.08 | 0.59 |
| $\omega$-Hydroxymonobasic acids | | |
| $C_{16}$ | 0.59 | 1.70 |
| $C_{18:1}$ (9) | 31.13 | 31.25 |
| $C_{22}$ | 1.44 | 2.13 |
| $\alpha,\omega$-Dibasic acids | | |
| $C_{16}$ | 0.46 | 1.39 |
| Polar acids | | |
| Dihydroxyhexadecanoic | 15.97 | 14.15 |
| 9,10-Epoxy-18-hydroxyoctadec-12-enoic | 1.48 | 2.63 |
| 9,10-Epoxy-18-hydroxyoctadecanoic | 27.48 | 27.35 |
| 9,10,18-Trihydroxyoctadecanoic | 20.32 | 17.38 |

The composition of apple cutins has been previously shown to vary, dependent upon anatomical location [7, 17]. Hydroxylated $C_{18}$ acids comprised 45 and 73%, respectively, of the leaf and fruit peel cutins, but only 12 and 14%, respectively, of the flower petal and stigma cutins. These $C_{18}$ acids total 80% of the inner seed coat cutin, the composition of which is somewhat similar to that of the polymer from the fruit peel. A major difference is that 18-hydroxyoctadec-9-enoic acid is 31% of the seed coat polymer, but only 10% of the fruit-peel cutin [7]. This high proportion of 18-hydroxyoctadec-9-enoic acid is unusual for a cutin polymer, but this component was also the most dominant monomer (51%) in the cutin polymer from the seed coat of maize [7].

Dihydroxyhexadecanoic acid comprised 21, 58, 77 and 77% of the fruit peel, leaf, flower petal and stigma apple cutins, respectively [7], but only 14–16% of the inner seed coat cutin (Table 1). This acid normally occurs as a mixture of positional isomers with the mid-chain OH on either carbon 7, 8, 9 or 10 [11, 18, 19]. In the dihydroxyhexadecanoate from the seed coat cutin, the distribution of the mid-chain OH was: C-8 (6%), C-9 (16%) and C-10 (78%). These values are comparable to those previously reported for the dihydroxyhexadecanoates from apple fruit peel (8, 21 and 69% for C8, C9 and C10, respectively) [18].

The present results indicate that there is a cutin polymer in the inner seed coat of apple. Although suberin has been reported to be present in several seed coats on the basis of ultrastructural analysis [20–23] and in the chalazal region of the inner seed coat of grapefruit on the basis of both chemical and ultrastructural studies [8], the monomers listed in Table 1 indicate that the amount of suberin in the apple seed coat must be very low. The most characteristic components of suberin are usually long-chain $\alpha,\omega$-dibasic acids and $\omega$-hydroxy acids ranging from $C_{16}$ to $C_{24}$ in chain length [24]. The 22-hydroxydocosanoic and 1,16-hexadecanedioic acids (Table 1) are two such monomers but are both present in small amounts. In both instances, however, the quantities of these acids are slightly greater in the seed coat polymer isolated from cold storage apples. It is possible that small areas of the seed coat are suberized and are not readily detected when the entire seed coat is depolymerized. Such regions might be more easily detected by an ultrastructural analysis.

Although apple seeds must undergo stratification in order to obtain optimal germination rates, it is not known what chemical changes occur during this cold treatment [25]. The present results support the previous finding [26] that although the seed coat of apple is a major barrier to germination, the seed coat itself does not undergo significant chemical changes during the 'after ripening' process.

## EXPERIMENTAL

*Tissue isolation.* Seeds and carpellary membranes were isolated from mature apples (*Malus pumila* cv Tetovka) which had just been harvested and from those which had been kept in cold storage (4°) for 6 months. The brown outer seed coat and the translucent inner seed coat were isolated and these two fractions and the carpellary membranes were lyophilized and extracted with petrol, $C_6H_6$ and MeOH as described before [27]. The seed coat fractions and carpellary membranes were treated with 0.03 M ammonium oxalate and 0.17 M oxalic acid, pH 4, at 37° for 48 hr, washed with $H_2O$, air-dried, ground in a Wig-L-Bug amalgamator (Crescent Dental Manufacturing Co., Chicago, Il.) and the resultant powders Soxhlet extracted with $CHCl_3$ for 48 hr [11].

*Depolymerization and fractionation.* Portions (50–100 mg) of the final tissue fractions were depolymerized by refluxing in THF for 24 hr with excess $LiAlD_4$ [11]. Each reaction mixture was cooled, added slowly to $H_2O$, acidified with 6 M HCl and extracted with $CHCl_3$ (3 × 100 ml). The $CHCl_3$-soluble material was subjected to TLC on Si gel G (1 mm thick, 20 × 20 $cm^2$) using $Et_2O$–hexane–MeOH (8:2:1). All components moving

from the origin were recovered from the adsorbent by elution with $CHCl_3$–MeOH (2:1).

*GC separation of components and MS.* Eluted fractions were derivatized with *N,O*-bis(trimethylsilyl)acetamide for 10 min at 90°. The derivatized fractions were analysed by GC/MS equipped with a Biemann separator. A coiled glass column (190 cm × 0.3 cm) packed with 5% OV-1 on Gas-Chrom Q was used with temp programming. Peak areas were estimated by triangulation and components were identified from their MS as previously described [28].

*Acknowledgements*—We thank Professor P. E. Kolattukudy, in whose laboratory a portion of this work was completed, for guidance and Dr. David Coahran for assistance with GC/MS. Dr. Espelie thanks, for a partial support of this work, the National Science Foundation Grant PCM 77-00927 and the Washington State Tree Fruit Commission for grants. Scientific Paper No. 5730, Project 2001, College of Agriculture Research Center, Washington State University, Pullman, WA 99164, U.S.A.

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