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Structure, Gas Chromatographic Measurement, and Function of Suberin Synthesized by Potato Tuber Tissue Slices[1]

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ABSTRACT

The polymeric material (suberin) of the wound periderm of potato tuber slices was analyzed after depolymerization with $LiAlH_4$ in tetrahydrofuran or BF_3 in methanol with the use of thin layer chromatography, chemical modification, and combined gas-liquid chromatography and mass spectrometry. Fatty acids (C_{16} to C_{26}), fatty alcohols (C_{16} to C_{26}), octadec-9-ene-1,18-dioic acid, and 18-hydroxy-octadec-9-enoic acid were identified to be the major components. Based on the structural information that the two bifunctional C_{18} molecules constituted a major portion of suberin, a gas chromatographic method of measuring suberization was developed. This method consisted of hydrogenolysis of powdered tissue followed by thin layer chromatography and gas chromatographic measurement of octadecene-1,18-diol as the trimethylsilyl ether. With this assay it was shown that the development of resistance to water loss by the tissue slices was directly proportional to the quantity of the bifunctional C_{18} molecules, thus providing evidence that a function of suberin is prevention of water loss.

Suberization is an important process because suberin plays a key role in preventing weight loss and decay, two of the major problems in the potato industry (4, 12). Suberization of potato tuber tissue has been studied by many investigators (7, 8, 11). Most of such studies on the so-called suberin layer have been restricted to microscopic examinations of stained tissues. On the basis of such staining techniques, it has been assumed that suberin is made of lipid materials. However, the exact chemical nature of the material formed is not understood. Furthermore, studies on the suberization process have been limited because no reliable chemical methods of quantitation of suberization have been available. In this paper we report the results of structural studies on the polymeric material of wound periderm of potato tuber tissue. Structural analysis by combined gas-liquid chromatography and mass spectrometry showed that the aliphatic constituents of the suberin formed on the wound surface are very similar to those found in the natural skin of potato tuber. A gas chromatographic method for quantitating suberization is also described. With this newly developed assay, suberization is shown to be correlated directly to the development of resistance to water loss from potato tuber tissue.

MATERIALS AND METHODS

Materials. Potato tubers (Russet Burbank) were grown at the Othello Experimental Farm of Washington State University. The tubers (1–2 kg) were washed free of soil and stored at 7 C until used. Cylindrical sections of tissue (2 cm long, 1 cm diameter) were cut with a No. 5 cork borer; they were rinsed with distilled water and placed on rubberized mesh in wide mouth gallon jars, through which 0.6 liter/hr of air was passed. Generally the temperature was held at 20 C unless otherwise specified, and at this temperature no bacterial growth was observed. Sources of $LiAlH_4$, pectinase, cellulase, *N*,*O*-bis(trimethylsilyl)acetamide were the same as those indicated earlier (6). OsO_4 was from National Lead Co., New York.

Preparation of the Polymeric Material from the Wound Periderm. After 7 days of suberization the tissue cylinders were cut in half with a razor blade, and the slices were treated overnight with 5 g/liter *Aspergillus niger* cellulase and 1 g/liter fungal pectinase in 0.05 M acetate buffer, pH 4.0. All the internal cells were removed by this treatment leaving behind small cuplike materials which were recovered. This residue was ground with a mortar and pestle, and the soluble lipids were removed by thorough extraction with a 2:1 mixture of chloroform and methanol. The solid recovered was treated again with cellulase-pectinase as described above, and the solid recovered by centrifugation or filtration was extracted thoroughly with a 2:1 mixture of chloroform and methanol. The solid was then extracted with chloroform overnight with a Soxhlet extractor in order to remove any remaining soluble lipids.

Depolymerization by Hydrogenolysis. One gram of the solid was refluxed with 2 g of $LiAlH_4$ in 30 ml of tetrahydrofuran for 24 to 48 hr. The excess $LiAlH_4$ was decomposed by carefully adding the reaction mixture dropwise into 75 ml of distilled water with vigorous stirring. The mixture was acidified by the addition of 5 to 10 ml of concentrated HCl. The lipid products were extracted repeatedly with chloroform (4 × 50 ml). The chloroform extract was evaporated to dryness under reduced pressure.

Depolymerization by Transesterification. One gram of the solid was refluxed with 30 ml of 14% BF_3 in methanol for 24 to 48 hr. After the addition of 50 ml of water, the products were extracted repeatedly with chloroform (4 × 50 ml). The chloroform extract was evaporated to dryness under reduced pressure.

Chromatography. Thin layer chromatography was done on 0.5- or 1-mm layers of Silica Gel G (20 × 20 cm) activated

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overnight at 110 C. Components on the thin layer chromatograms were visualized either by the dichromate-sulphuric acid charring method, or by viewing the plate under UV light after spraying it with a 0.1% ethanolic solution of 2',7'-dichlorofluorescein. The components were recovered from the silica gel with a 2:1 mixture of chloroform and methanol. The developing solvents are indicated elsewhere in this paper.

Gas chromatography was performed with a coiled glass column (183 × 0.31 cm o.d.) packed with 5% OV-101 on 80 to 100 mesh Gas Chrom Q. Part of the effluent of the gas chromatograph was passed into a Perkin Elmer-Hitachi RMU 6D mass spectrometer with a Biemann separator interphase. Mass spectra were recorded at the apex of the gas chromatographic peaks with 70 ev ionizing voltage. In order to make sure that the peak did not represent an incompletely resolved mixture, mass spectra were also recorded at either side of the apex.

Preparation of Derivatives. Trimethylsilyl ethers were prepared by heating the material with excess (0.25 ml) of *N,O*-bis(trimethylsilyl)acetamide at 90 to 100 C for 20 min. Excess reagent was removed with a stream of N_2, and the product was dissolved in a 2:1 mixture of chloroform and methanol for gas chromatography. Introduction of a *vic*-diol function at the double bond was done by treatment of the olefin with a 0.1% solution of OsO_4 in dioxane for 2 hr. The reaction mixture was decomposed with aqueous-methanolic Na_2SO_3; the resulting precipitate was removed by centrifugation and washed once with methanol and centrifuged. Products were recovered by ether extraction of combined supernatants.

Gas Chromatographic Measurement of Suberin of the Wound Periderm. About eight cylindrical tissue slices which were suberized for varying periods of time were freeze-dried and then ground in a Wiley mill followed by a finer grinding in a Wig-L-Bug Amalgamator (Crescent Dental Manufacturing Co.). One gram of the powder was subjected to hydrogenolysis with $LiAlH_4$ for 48 hr, and the products were recovered as described in a previous section. The soluble lipid thus obtained was mixed with 80 μg of hexadecane-1,16-diol as an internal standard and was applied to 0.5-mm (20 × 20 cm) layers of Silica Gel G. The chromatograms were developed in ethyl ether-hexane-methanol (20:5:1, v/v). The components were visualized with 2',7'-dichlorofluorescein and recovered as indicated elsewhere. The recovered diol and alcohol fractions were transferred into narrow-bottomed graduated centrifuge tubes and evaporated to dryness with a stream of N_2, taking precautions to keep the solid in the narrow portion of the centrifuge tube. To each tube, 0.3 ml of *N,O*-bis(trimethylsilyl)acetamide was added and heated in an oil bath at 90 C for 20 min. Excess reagent was evaporated with a stream of N_2 and the products were made up to 300 μl with a 2:1 mixture of chloroform and methanol. Aliquots (generally 2 μl) were injected into a Varian gas chromatograph equipped with a flame ionization detector. The internal standard (C_{16} diol) was used to quantitate the C_{18} diol derived from the wound periderm, and the identity of the gas chromatographic peak of C_{18} diol was established by its mass spectrum. The alcohol fractions were also similarly analyzed.

Diffusion Resistance Measurements. The resistance of the tissue surface to water vapor loss (R_t) was calculated with the following formula (10):

$$R_t = \frac{\rho_t - \rho_a}{E} - R_a$$

where ρ_t = vapor density of the tissue in g/cm³ = $RH_{tissue} \times \rho_v$; RH_{tissue} is the relative humidity of the tissue which is 100 and ρ_v was calculated using the formula $\rho_v = 18e/RT$, where e = vapor pressure, T = temperature (°K), and R = the gas constant. ρ_a = vapor density of the air in g/cm³ = $RH_{air} \times \rho_v$, where RH_{air} is the relative humidity of the air. E = evaporation per unit surface area per unit time in g/sec·cm². E was calculated from the measured loss of weight of 20 tissue cylinders during a 3-hr exposure to the atmosphere. R_a = resistance of the air to water vapor diffusion (sec/cm). When $R_t = 0$, $R_a = (\rho_t - \rho_a)/E$ = the resistance of unsuberized tissue, and this was experimentally determined using freshly cut tissue cylinders.

RESULTS AND DISCUSSION

Identification of the Aliphatic Constituents of Potato Wound Periderm. In order to determine the structure of the monomers of the polymeric material formed on the wound periderm, a preparation of periderm-enriched material was attempted. After 7 days of suberization the cylindrical tissue slices were cut into halves to expose the internal, apparently nonsuberized, region. Treatment of these tissue slices with pectinase and cellulase removed most of the internal part of the tissue, leaving behind small cuplike structures, presumably enriched in the wound periderm material. After further treatment with the hydrolytic enzymes and thorough extraction of soluble lipids, 20% of the dry weight of the original tissue slices was left as an insoluble material (suberin-enriched). Gas-liquid chromatography of the soluble lipids isolated from the hydrogenolysate of this insoluble material showed (Fig. 1) one major component (70%) and several smaller components. The mass spectrum of the major component showed a molecular ion at *m/e* 428 and fragment ions at 413 ($M^+ - 15$), 397 ($M^+ - 31$), 338 ($M^+ - 90$), and 323 ($M^+ - 15 - 90$). These ions are indicative of octadecene-1,18-diol. Confirming this structural assignment, a fairly intense doubly charged ion and the first isotope ion were observed at *m/e* 199 and 199.5, respectively (13). The major component of the hydrogenolysate of the natural skin was also identified to be octadecene-1,18-diol (5, 9), thus sug-

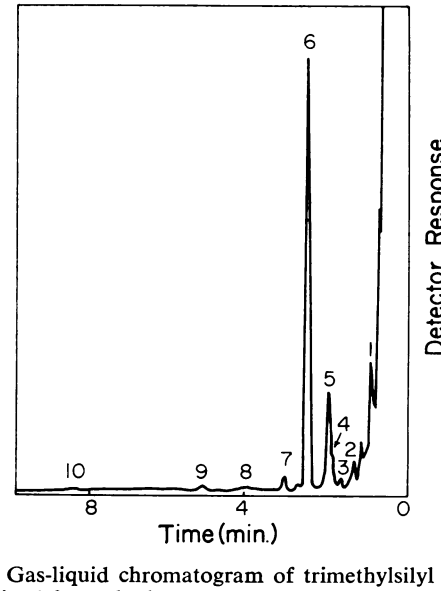

FIG. 1. Gas-liquid chromatogram of trimethylsilyl ethers of the lipids obtained from the hydrogenolysate of a suberin-rich preparation obtained from potato tuber cylinders suberized for 7 days. Gas-liquid chromatography was done as described under "Materials and Methods" with 30 psi inlet pressure of He as carrier gas with a column temperature of 252 C.

gesting that the wound periderm material is similar to the natural skin.

Components 1 and 2 were identified by their mass spectrum and retention times to be hexadecan-1-ol and a mixture of octadecan-1-ol and octadecen-1-ol, respectively. Component 3 was similarly identified to be hexadecane-1,16-diol. Component 4, which was not fully resolved from component 5, was tentatively identified as eicosan-1-ol by its mass spectrum, while component 5 could not be readily identified by its mass spectrum. The minor component between components 6 and 7 was found to be octadecan-1,18-diol, while component 7 was found to be docosan-1-ol. The minor component 8 appears to be octadecane triol, but definite structure assignment was not possible. Components 9 and 10 were identified as tetracosanol and hexacosanol, respectively. Since most of the components were present only in very small quantities, further structural studies were not attempted.

Although the hydrogenolysis technique readily gives easily identifiable products, the structural details of suberin monomers are lost because the method converts both ω-hydroxy acids and dicarboxylic acids into indistinguishable diols. For a detailed structural analysis we resorted to a transesterification technique with 14% BF_3 in methanol. The thin layer chromatogram of the lipids (6% of the suberin enriched material) obtained by this technique showed four major components (Fig. 2). Comparison of the R_F values of fractions A (about 10%) and C (about 15%) with those of authentic standards indicated that they were fatty acid methyl esters and fatty alcohols, respectively. Combined gas-liquid chromatography and mass spectrometry confirmed that fractions A and C were fatty acids (methyl esters) and fatty alcohols (analyzed as trimethylsilyl ethers). The percentage compositions of these two fractions are shown in Table I. In both fractions C_{16} and C_{18} were the major components. This predominance was not observed in the earlier studies on the natural skin of potato tubers (5, 9). It is possible that internal lipids which were incompletely removed by the extraction procedures contributed to this fraction. The most important feature of the acid and alcohol fractions is that both of them contained significant proportions of C_{20} to C_{26} carbon chains. Such very long chain acids and alcohols were also observed in the natural skin (5, 9). The previous observation that aged but not fresh potato slices had the ability to synthesize C_{20} and longer acids (14) can be best explained as due to induction of suberin-synthesizing enzymes.

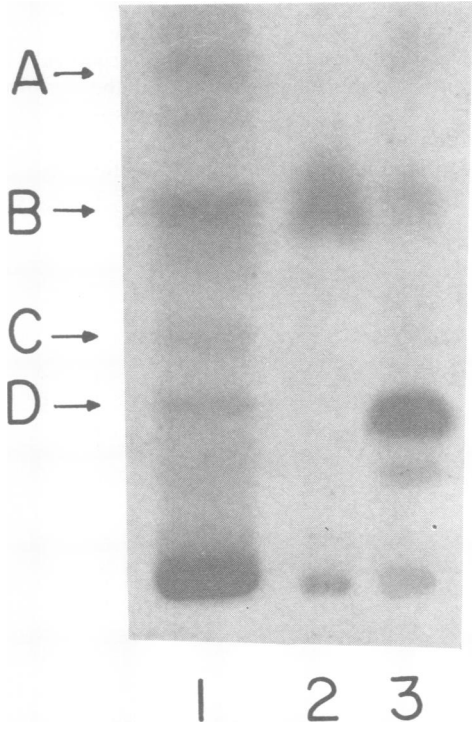

FIG. 2. Thin layer chromatogram of the soluble lipids derived by BF_3-CH_3OH treatment of a suberin-rich preparation obtained from potato tuber cylinders suberized for 7 days. Chromatography was done on 0.5-mm layers of Silica Gel G with hexane-ethyl ether-formic acid (65:35:2, v/v) as the solvent system. A: Fatty acid methyl esters; B: dicarboxylic acid dimethyl esters; C: fatty alcohols; D: ω-hydroxy acid methyl esters; 2: dimethyl hexadecane-1,16-dioate; 3: methyl ω-hydroxyhexadecanoate.

Table I. *Chain Length Distribution of Fatty Acids and Fatty Alcohols of the Wound Periderm Polymer of Potato Slices*

Chain Length	Fatty Acid	Fatty Alcohol
	%	%
C_{16}	24.7	25.0
C_{18}	56.8	35.0
C_{20}	12.8	14.4
C_{22}	4.6	22.4
C_{24}	1.2	1.8
C_{26}	Trace	0.9

Fraction B had an R_F identical to that of dimethyl hexadecanedioate, and its gas chromatogram showed one major (99.2%) component and one minor component (0.8%) (Fig. 3). The mass spectrum of the major component showed a molecular ion at *m/e* 340 and a fairly intense ion at *m/e* 308 ($M^+ - CH_3OH$). The other significant ions at the high mass region were at *m/e* 290 (M-50) and 276 (M-64) with a smaller ion at 248 (M-92). This spectrum is quite characteristic of dimethyl octadecenedioate (2). The molecular ion of the minor component was extremely weak at *m/e* 314. The major ions in the high mass region were at *m/e* 283 (M-31), 241 (M-73), 222 (M-92), 209 (M-105), and 191 (M-123). This pattern is characteristic of dimethyl hexadecane-1,16-dioate, and the spectrum was identical to that obtained in this laboratory with authentic dimethyl hexadecanedioate. These identifications were further confirmed in the following manner: treatment with $LiAlH_4$ followed by combined gas-liquid chromatography and mass spectrometry of the products showed one major component and one minor component which were identified by their mass spectra as descirbed before (13) as octadecene-1,18-diol and hexadecane-1,16-diol, respectively.

The technique described above does not allow us to locate the double bond in the C_{18} dioic acid. Therefore, a vicinal diol function was introduced at the dobule bond by means of OsO_4 treatment. The gas chromatogram of the product (as trimethylsilyl ether) showed one very large component (Fig. 3). The mass spectrum of this compound showed an extremely weak molecular ion at *m/e* 518 and a weak derivative ion at *m/e* 503 ($M-CH_3$). A fairly prominent ion was observed at 487 ($M-CH_3O$). The other significant ions at the high mass region were at *m/e* 428 [$M-(CH_3)_3SiOH$], 413 [$M-CH_3-(CH_3)_3SiOH$], and 397 [$M-(CH_3)_3SiOH-CH_3O$]. These are the ions expected from a di(trimethylsiloxy)octadecane-1,18-dioic acid dimethyl ester. That the two trimethyl siloxy functions were in the middle of the molecule was shown by the fact that the base peak of the spectrum was at *m/e* 259, the ions produced by cleavage between the two trimethylsiloxy functions (Fig. 3). The α-cleavage on the other side of the substituted carbons was also observed (ion at *m/e* 361). The expected trimethylsilyl re-

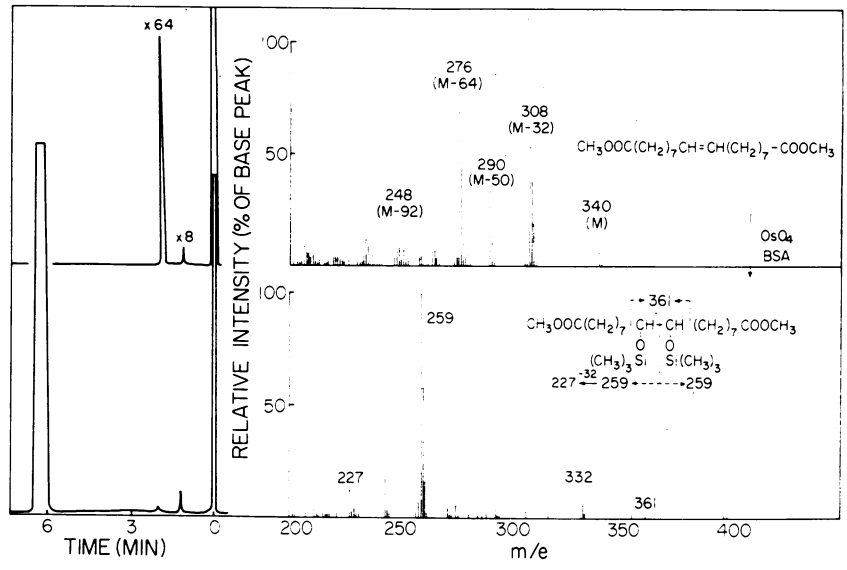

FIG. 3. Gas-liquid chromatogram of fraction B of Figure 2 (top left), partial mass spectrum of the major component (top right), the gas-liquid chromatogram of the trimethylsilyl ether of OsO_4 treatment product of fraction B (bottom left), and partial mass spectrum of the major OsO_4 treatment product (bottom right). The column temperature and inlet pressure for the upper left chromatogram were 240 C and 23 p.s.i., respectively. All attempts to detect longer dicarboxylic acids failed. The lower left chromatogram was obtained with 270 C column temperature and 23 p.s.i. inlet pressure. The two minor components in this chromatogram were tentatively identified as dimethyl hexadecane-1,16-dioate and dimethyl octadecane-1,18-dioate.

arrangement ion was observed at *m/e* 332 (3). Thus, it is clear that the two trimethylsiloxy functions were at the C-9 and C-10 positions and, therefore, the double bond in the original dioic acid was at the C-9 position.

Fraction D had an R_F identical to that of authentic ω-hydroxy hexadecanoic acid methyl ester. The gas chromatogram of this fraction showed one major component and a few minor components (Fig. 4). The mass spectrum of the major component showed a substantial molecular ion at *m/e* 384. The other major ions at the high mass region were at 369 ($M-CH_3$), 353 ($M-CH_3O$), and 337 ($M-CH_3-CH_3OH$). This pattern suggests that this component is 18-trimethylsiloxy-octadecenoic acid methyl ester. The metastable ion representing the transition [M-15] → [M-47] was also observed. Confirming the identification, $LiAlH_4$ treatment followed by analysis of the products by combined gas chromatography and mass spectrometry showed that the major product was octadecene-1,18-diol.

In order to determine the position of the double bond, the ω-hydroxy acid fraction was treated with OsO_4 which introduces a *vic*-diol function at the double bond. The gas chromatogram of the products showed one major component (Fig. 4). The mass spectrum of this compound showed significant

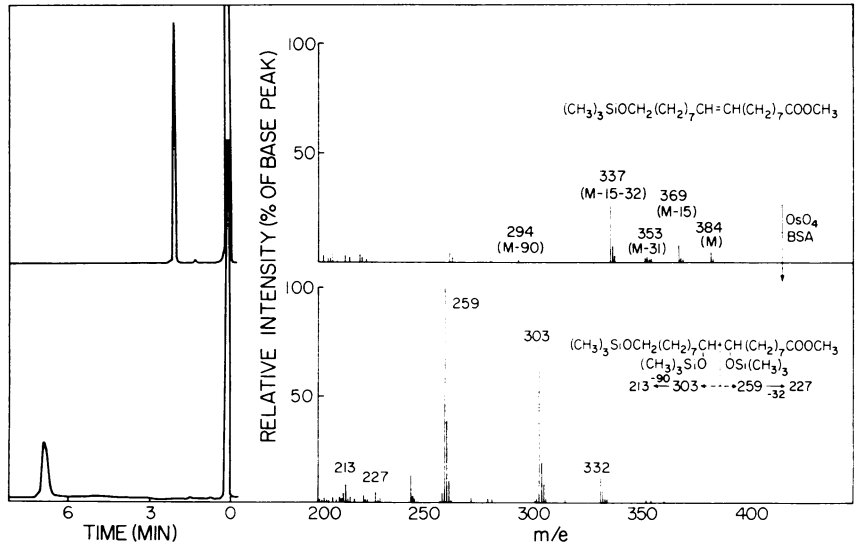

FIG. 4. Gas-liquid chromatogram of the trimethyl silyl ether of fraction D (ω-hydroxy acid methyl ester) of Figure 2 (upper left), partial mass spectrum of the major component (upper right), gas-liquid chromatogram of the OsO_4 treatment product (as trimethylsilyl ether) of fraction D of Figure 2 (lower left), and a partial mass spectrum of the major product of the OsO_4 treatment (lower right). The upper left chromatogram was obtained with 240 C column temperature and 23 psi inlet pressure of He as carrier gas. Injection of larger quantities of fraction D revealed the presence of small quantities of C_{20}-C_{26} ω-hydroxy acids, but they are not visible at the conditions used in this chromatogram. The lower left chromatogram was obtained with a column temperature of 270 C and an inlet pressure of 23 p.s.i.

ions at *m/e* 547 (M-CH_3) and 531 (M-CH_3O), indicating that the compound was tri(trimethylsiloxy) C_{18} acid methyl ester. The *vic*-diol function was clearly shown to be at the 9,10-position by the two dominant ions at *m/e* 259 and 303, which were produced by cleavage between C-9 and C-10. The expected trimethylsilyl migration was also obvious by the presence of a moderately strong ion at *m/e* 332. These results clearly show that the original compound was ω-hydroxy octadec-9-enoic acid. Small quantities of ω-hydroxy C_{16} acid, ω-hydroxy C_{20} acid, and ω-hydroxy C_{22} acid were also detected in the ω-hydroxy acid fraction by combined gas chromatography and mass spectrometry.

The results discussed thus far show that ω-hydroxyoctadec-9-enoic acid, octadec-9-ene-dioic acid, C_{18}-C_{26} alcohols are the major aliphatic components of the polymeric material contained in the wound periderm of potato tuber slices. These components were also found to be the major components of the natural skin of potato tuber (5, 9). The relatively minor components, such as 18-hydroxy-9,10-epoxystearic acid and 10,16-dihydroxypalmitic acids, identified in the natural skin of potato tubers (5) were not detected in the present investigation of the wound periderm. However, this difference might be attributed to the relative ease of detection of components present in the large quantities of the natural skin to those present in the relatively small quantities of the polymer present in the wound periderm. Since BF_3-CH_3OH treatment of crude material is known to produce artifacts which remain in the origin, this method is not suitable for detection of minor components and quantitation.

Gas Chromatographic Measurement of Suberization. Since the cytochemical methods of measuring suberization are neither direct nor quantitative, we used the structural information discussed above to design a gas chromatographic method for quantitation of wound suberin. Since ω-hydroxy octadecenoic acid and octadec-9-ene-dioic acid are the major compounds which distinguish suberin from other cellular components, the quantity of these two compounds should be a reliable measure of suberization. Since hydrogenolysis of the polymer would convert both these acids into octadecene-1,18-diol, which can be easily measured, we chose this technique. This compound also eliminated the necessity for measuring two different components, and it increased the sensitivity of the method. Hydrogenolysis of powdered dry tissue can be done either directly or after extraction of soluble lipids with a Soxhlet extractor. In the former case, the large quantities of C_{16} and C_{18} alcohols, derived from the usual internal lipids, make it more difficult to quantitate the C_{18} diol (derived from suberin) which is only a relatively minor component in the mixture. Therefore, preliminary TLC is advisable, if not necessary. Since C_{16} diol is only a very minor component of suberin, we chose to add C_{16} diol standards prior to TLC. This procedure not only aided quantitation but also enabled us to visualize the diol fraction readily on the thin layer chromatograms. Gas chromatography of the diol fraction recovered from such thin layer chromatograms (Fig. 5) showed two major components, which were identified by mass spectrometry to be octadecene-1,18-diol and hexadecane-1,6-diol. The octadecene-1,18-diol which originated from the wound periderm was quantitated by comparison of the area under its gas chromatographic peak with the area under the C_{16} diol peak obtained from the known quantity of the added C_{16} diol.

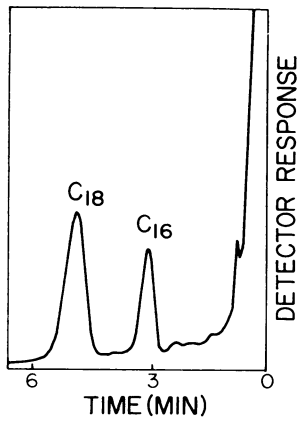

FIG. 5. A typical gas-liquid chromatogram of the trimethylsilyl ether of the diol fraction isolated by TLC from the hydrogenolysate of suberized (7 days) tissue slice used in the gas chromatographic assay for suberin. The C_{16} diol was added as a standard. In this assay a coiled stainless steel column (⅛ in × 4 ft) packed with 5% OV-1 on 80–100 mesh Gas Chrom Q was used with a column temperature of 230 C and an inlet pressure of 20 psi of N_2 as the carrier gas.

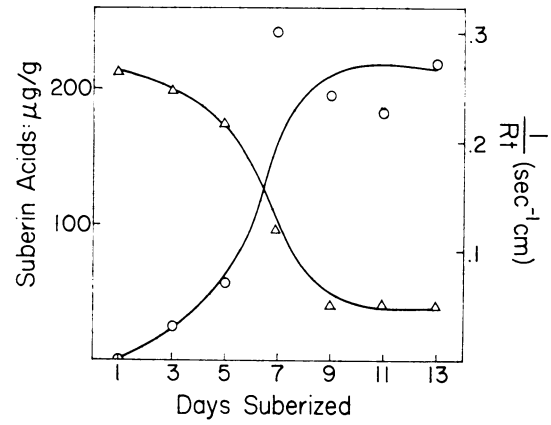

FIG. 6. Time course of development of suberin as measured by gas-liquid chromatography and the development of resistance to diffusion of water vapor. The suberin expressed as μg/g represents only the octadecene diol derived from ω-hydroxy octadec-9-enoic acid and octadec-9-ene dioic acid. The reciprocal of diffusion resistance is plotted to show the inverse relationship between water loss from the tissue and formation of the major aliphatic components of suberin.

Time Course of Suberization and Development of Resistance to Water Loss. A time course of development of suberin was determined with the gas chromatographic assay discussed above. There was very little of the suberin acids in the tissue for 3 to 5 days, after which there was a dramatic increase (Fig. 6). The quantity of these acids did not show a significant further increase after about 7 days. On the other hand, the lag period for the synthesis of the very long acids appeared to be longer, and their rate of increase leveled off later than that of the C_{18} suberin acids. The individual components showed somewhat different time courses.

If suberin plays an important role in preventing water loss, the rate of formation of the major suberin acids might show a correlation with the development of resistance to water loss. In order to investigate this possibility, the time course of development of resistance to water loss was determined as described under "Materials and Methods" (Fig. 6). The results clearly showed that up to 3 to 4 days the tissue slices had not developed much resistance to water loss. After this time, there was a dramatic increase in resistance, which reached a maximum within 2 to 3 days and beyond which no further increase in resistance was observed. This pattern is identical to that observed for the formation of 18-hydroxyoctadec-9-enoic acid

and octadec-9-ene-18-dioic acid, the major components of suberin. The other components of suberin did not show such a clear correlation in that their levels increased even after the resistance to water loss leveled off and the lag period in their development was somewhat longer than that observed for development of resistance to water loss (data not shown). Temperature dependence of development of water loss and formation of the C_{18} aliphatic constituents of suberin provided further evidence that these constituents of suberin are involved in preventing water loss. For example, suberization for 7 days at 10 C did not result in the development of significant resistance to water loss, and gas chromatographic measurement showed that little C_{18} diol was produced by hydrogenolysis of the tissue. Furthermore, under N_2 no suberization was detected as measured by the gas chromatographic technique or by the diffusion resistance technique. The effects of temperature and N_2 reported here are in agreement with previous reports (7, 12). This experimental evidence strongly suggests that one role of suberin, or at least its major aliphatic constituents, is to protect the tissue from water loss. One other important function was recently shown to be prevention of decay (4), but we have not as yet attempted to correlate this function to suberization as measured by gas-liquid chromatography. It is possible that the phenolic components of suberin prevent pathogen entry while the aliphatic constituents prevent water loss.

The time course experiments suggest that the enzymes involved in the suberization process might be induced during the first several days after wounding. From the structural studies reported in this paper, it is suggested that an ω-hydroxylating enzyme and an ω-hydroxy acid dehydrogenase, which would give rise to the dicarboxylic acids, are the two key enzymes involved in the synthesis of the major suberin acids. An enzyme which catalyzes the polymerization process must also be involved, as found earlier in cutin synthesis (1). It appears that a signal produced or activated by the wound triggers the induction of these enzymes.

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