Hexacosanol and Octacosanol, Metabolites of Disrupted Potato Tuber Tissue

The fatty alcohol fraction of stressed (sliced) and unstressed potato tuber tissue has been analyzed. Stressed tissue, on incubation, contains considerable quantities of 1-octacosanol, which was not found in the unstressed tissue. Increases in 1-hexacosanol were also found but to a much lesser extent. The function of these compounds in physiological processes has not been identified.

Major chemical changes occur when potato tuber tissue is disrupted by mechanical manipulation such as slicing. The most notable and perhaps most important chemical process to be induced is suberization (Kolattukudy and Dean, 1974; Kolattukudy and Agrawal, 1974). Other significant chemical alterations occur in protein (Click and Hackett, 1963), glycoalkaloid (Locci and Kûc, 1967), steroid (Hartmann and Benveniste, 1974), and phenolic (Tomiyama et al., 1967) composition. Although slicing is an extreme form of tissue disruption, mechanical harvesting can produce similar lesions. Microorganisms cause a different type of tissue change; however, cell disorganization under these conditions may lead to similar chemical products. The isolation of hepatotoxic furanoterpenoids from diseased or damaged sweet potatoes (Wilson et al., 1970) emphasizes the necessity to acquire more information about the chemical composition of "stressed" fruits and vegetables.

We recently have studied environmental factors which result in increased glycoalkaloid synthesis in potato tubers (Fitzpatrick et al., 1977). Slicing caused a significant increase in glycoalkaloid concentration (Locci and Kûc, 1967), the increase being approximately proportional to the initial concentration in the slice. Cholesterol is a known precursor of solanidine, the aglycon of the common potato glycoalkaloids solanine and chaconine. Cholesterol is only a minor constituent of the steroid fraction of potatoes, and this may be due to rapid turnover to solanidine. We wished to determine whether there is an increase in potato steroids in slices correlative to that observed with glycoalkaloids. The potato steroid fraction was isolated by standard procedures (Hartmann and Benveniste, 1974) and the final analysis was accomplished by gas–liquid chromatography (GLC). A dramatic change in the composition of this fraction was observed by the presence of a nonsteroidal component of this fraction. This finding is the subject of this communication.

EXPERIMENTAL SECTION

Unpeeled Katahdin potatoes were cut in half and a slice (4–5 mm thick) was taken from the exposed surface of each half. The slices, randomly selected for various incubation periods, were incubated in petri dishes at 25 °C in the dark. Samples were analyzed at 0 h, 2, 3, 4, 6, and 8 days. The samples were ground in a Waring Blendor for 2–3 min in the presence of methanol (100 mL of methanol/65 g of tissue), and the mixture was filtered on a coarse Buchner funnel under nitrogen pressure. The residue was reextracted twice with a total of 300 mL of $CHCl_3$/MeOH (2:1, v/v). The filtrates were combined and 100 mL of 0.8% Na_2SO_4 was added, the mixture was thoroughly shaken, and the two phases were allowed to separate overnight. The $CHCl_3$ layer was concentrated to dryness under N_2 at 45 °C. The residue was taken up in hexane, and insoluble material was removed by centrifugation and filtration. The hexane solution was subjected to column chromatography on a 1:1 activated MgO/Hyflo Supercel (Johns-Manville) column (1 × 7.5 cm). The column was eluted with 6 bed volumes of hexane (36 mL), followed by 10% ethyl acetate in hexane. The 10% ethyl acetate fraction was subjected to thin-layer chromatography (TLC) on silica gel plates (250 μm, Quantum LQ6) with cyclohexane/ethyl acetate (1:1) used as the mobile phase. This procedure was used for both qualitative and quantitative TLC. Further TLC purification was accomplished with 9:1 cyclohexane/ethyl acetate on silica gel plates. Plates were sprayed with 10% phosphomolybdic acid in ethanol and heated at 120 °C for 0.5 h. GLC chromatographic separation was obtained on a 4 ft × $^1/_8$ in. column packed with 1% SE-30 on Gas-Chrom Q (100–120 mesh). The column was programmed at 6 °C/min from 125–290 °C and a He flow rate of 50 mL/min. Quantitation was determined by comparison of peak areas with concentration curves derived from standard solutions of sterols and fatty acids.

Mass spectra were obtained on an LKB-9000 mass spectrometer via GLC introduction and conditions for GLC described above. Spectra were obtained at 70 eV and a source temperature of 290 °C.

Table I. Steroid Fraction Composition of Fresh and Aged Potato Slices

compound	μg/g of fresh weight: fresh	μg/g of fresh weight: aged 4 days
cholesterol	0.1	0.42
1-hexacosanol	<0.1	0.87
1-octacosanol	<0.1	3.60
stigmasterol	0.38	1.50
β-sitosterol	0.56	1.60

RESULTS AND DISCUSSION

While the increase in steroids in potato slices incubated for 4 days was substantial, a more dramatic increase was observed in a nonsteroidal component of the steroid fraction (Table I). This compound was identified as 1-octacosanol by GLC and TLC cochromatography and mass spectrometry. We could not confirm the presence of this compound in fresh tissue. Gas chromatograms showed a small peak with the same retention time as 1-octacosanol but insufficient quantities of this fatty alcohol were available from fresh potato tissue for mass spectral confirmation. 1-Hexacosanol was also found in incubated tuber slices at a concentration about one-third that of 1-octacosanol. After observing this dramatic change, we verified, by using standard long-chain alcohols that our isolation procedure would include all the saturated aliphatic alcohols from C_{18} to C_{35}. Thus, 1-octacosanol, and to a much lesser extent 1-hexacosanol, are the only free fatty alcohols that are synthesized in this stress system. These alcohols represent only trace components of suberin formed on tissue slices (Kolattukudy and Dean, 1974); however, the C_{22} to C_{26} homologues make up the major portion of the suberin fraction derived from tuber skin. The free alcohols may arise from deesterification of suberin, although this would have to be unusually specific to include only C_{26} and C_{28} alcohols.

The occurrence of 1-octacosanol in such large concentrations on tuber slices is particularly interesting in light of the report by Ries et al. (1976) that triacontanol is a

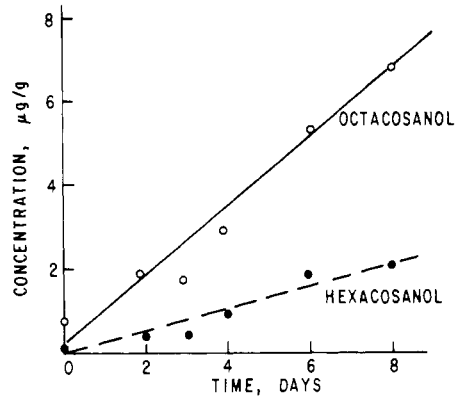

Figure 1.

naturally occurring plant growth regulator. 1-Octacosanol may have a physiological function in the wound-healing process. We have tested the effects of prior topical applications of 1-octacosanol on potato slices infected with the fungus *Phytophthora infestans*. Some inhibition of the compatible (susceptible) interaction was observed; however, further work is needed to verify these observations.

The rate of formation of the C_{26} and C_{28} alcohols on potato slices is summarized in Figure 1. Two common varieties, Kennebec and Katahdin, showed similar rates of 1-octacosanol accumulation. The greatest increase in 1-octacosanol formation occurred between 2 and 4 days of aging. Time studies on suberin development showed that the most significant increases occurred between 5 and 8 days (Kolattukudy and Dean, 1974); therefore, 1-octacosanol apparently accumulates at a faster rate.

In summary, the free alcohol 1-octacosanol was found to occur at a relatively rapid rate in incubated potato slices to levels 30 times that observed in fresh tissue (Table I); 1-hexacosanol accumulates at a slower rate. In contrast, the fatty alcohol fraction of suberin has been reported to contain none or only trace amounts of these alcohols. Therefore, it is possible that these compounds are required for some function other than suberin formation.

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Received for review August 10, 1978. Accepted September 26, 1978. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.