Since all of the samples which we examined were from mature or nearly mature plants (all samples contained germinatable seeds), it appears that Phenotype III marijuana is unable to convert significant quantities of cannabidiol to *trans*-THC as 'normal' marijuana plants do. This suggests that the presence of *cis*-THC in these samples may be related to the blockage of this biosynthetic path, allowing material which normally would be channeled into eventual conversion to *trans*-THC to be diverted more easily at some early point in the biosynthetic sequence toward the production of *cis*-THC than in Phenotype I plants.

EXPERIMENTAL

Dried plant material from seized contraband (460 g) was sieved to remove seeds and stalks, and the remaining material (240 g) extracted thoroughly with 3 l. of petrol. The residue (3.9 g) after flash evaporation of the solvent was taken up in a minimal vol. of CHCl$_3$, to which was added a large excess of MeOH. The resulting mixture was filtered to remove insoluble waxes, and solvent removed by flash evaporation. The oily residue (3 g) was passed through a small Florisil column [1], and the cannabinolic fractions (monitored by GLC) thus obtained were combined and subjected to repeated preparative-TLC on Si gel using C_6H_6. The band corresponding to *trans*-THC was visualized by spraying a small portion of the plate with aq. Fast Blue B, and then removed and extracted with CHCl$_3$–Me$_2$CO (1:1). After 3 developments 86 mg of residue was obtained, which by GLC was found to consist primarily of 3 compounds, cannabidiol, *trans*-THC, and a compound having a R_f midway between those of cannabidiol and *trans*-THC. Final purification was effected by subjecting the residue from TLC to repeated HPLC using a chromatograph fitted with a variable wavelength detector (243 nm). The separation was carried out on a 0.5 m × 2.6 mm i.d. Sil-X or Phenyl Sil-X column at 25–42° using 2.5% CHCl$_3$, 2.5% *iso*-propyl ether, and 0.05% MeOH in hexane with a flow rate of 0.5 ml/min. Three passages through the column were necessary to give *ca* 1 mg (0.0002% overall yield) of a colorless oil which was at least 95% pure by GLC. GLC R_t 240°, 2m 3% OV-101 on HP Chromosorb W, 0.89 relative to *trans*-THC; IR (neat), 3410 (OH), 2930, 1624 (—C=C—), 1580 (—C=C—), 1510 (aromatic), 1428 (—CH$_2$—), 1160, 1055, and 1039 cm^{-1}; MS (GC–MS; 70 eV); *m/e* (rel. intensity), 314 (48), 299 (52), 271 (30), 258 (22), 243 (43), and 231 (100); PMR (CDCl$_3$), δ6.23 (*br*-2H; olefinic and aromatic H), δ6.13 (*br*-1H; aromatic), δ4.76 (*s*-1H; phenol), δ3.55 (*br*-1H; C-3 methine), δ1.69 (*s*-3H; olefinic CH$_3$), δ1.39 (*s*-3H) and δ1.27 (*s*-3H; *gem*-diMe), and δ0.88 (*t*-3H; ω-Me) [11].

Plant assays. *Ca* 100 mg samples of dry, unmanicured plant material from 20 different sources were soaked in CHCl$_3$ (1 ml) and the relative concns of the pentyl cannabinoids in these solns were determined by GLC (conditions as above). Although this procedure readily yielded relative concns of cannabidiol and total THC, those samples containing *cis*-THC generally had such high concns of cannabidiol that determining the relative amounts of *cis*- and *trans*-THC by this method proved impractical. In these cases *ca* 1 g samples of plant material were extracted with CHCl$_3$ and the extract subjected to preparative-TLC (*vide supra*). The band corresponding to *trans*-THC was then removed and examined by GLC as above.

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FERULATES FROM CORK LAYERS OF *SOLANUM TUBEROSUM* AND *PSEUDOTSUGA MENZIESII*

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato tuber; *Pseudotsuga menziesii*; Pinaceae; Douglas fir; ferulates; high pressure liquid chromatography; suberization.

INTRODUCTION

The process of natural wound healing of the cut surface of potato tubers has been well documented [1, 2]. The initial step in wound healing is the suberization of a layer of intact cells at the surface of the wound. Any agent which enhances suberin formation may also be directly involved in the exclusion of pathogens [3]. Investigators have found that phenolic compounds are an essential part of the wound healing process in potato tubers. Simonds, *et al.* [4], found that 7 of the 8 phenolic compounds they tested on potato tubers promoted a thicker suberized layer than that found in the non-treated control tubers. Consequently, Johnson [5], treated seed potato tissue with the cork-rich fraction of the whole bark from Douglas Fir, *Pseudotsuga menziesii*, which contains *ca* 2% dihydroquercetin. Anatomical studies of the treated seed potato tissue showed that the treatment increased the thickness of the suberized layer formed below the cut surface. However, the data also showed that treatment of the seed potato tissue with 2% dihydroquercetin in an inert medium did not significantly affect suberization. This discrepancy suggested the presence of other suberization factors in Douglas Fir bark, which prompted this investigation.

RESULTS AND DISCUSSION

After chromatography, the total wax extract of the cork fraction of *P. menziesii* yielded behenyl ferulate and lignoceryl ferulate in a 1:1 ratio. These were identified by PMR, IR, UV, MS, saponification and HPLC coinjection of their acetylated derivatives with synthesized acetylated behenyl ferulate and acetylated lignoceryl ferulate. Neither of the ferulates or their acetates could be resolved or eluted on the following GLC columns: SE30, OV-17, QF-1, Apiezon L, Carbowax 20M and Silicar 10C.

IR and UV together with TLC in 3 systems indicated that the suberin layer of the potato contained ferulates similar to those found in *P. menziesii*. HPLC of the acetylated ferulates indicated that it contained at least 7 ferulates, two of which (behenyl and lignoceryl ferulate) were identified by R_t and coinjection. The remaining 5 ferulates are given the following tentative structures, hexadecyl ferulate, octadecyl ferulate, eicosyl ferulate, hexacosyl ferulate and octacosyl ferulate based on symmetry of the HPLC chromatogram and GLC of the alcohol components after saponification. The relative composition was octadecyl (5), behenyl (4), eicosyl (4), hexadecyl (2), lignoceryl (1), hexacosyl (1) and octacosyl (1).

Ferulic acid has been reported as covalently bonded to the suberins of various vegetable root crops [6] and in the case of potato suberin after depolymerization the alcohols reported ranged from C_{16}–C_{28} [7]. This suggests that the ferulates we isolated may be precursors of the suberin polymer.

Another function of these ferulates is suggested by the work of Wheeler and King [8], who were able to show that numerous phenols, including ferulic acid and one of its unidentified esters from *Phaseolus vulgaris*, when incubated in the presence of polyphenolase, an enzyme found on the cut surface of potatoes, catalyzed the conversion of tryptophan to auxin (IAA).

A method developed by Kolattukudy [7] for quantitating suberization involves hydrogenolysis of powdered tissue followed by TLC and GLC analysis. Since the quantity of ferulate appears to be a function of suberization, the HPLC technique we have developed would simplify this quantitation.

EXPERIMENTAL

PMR spectra were recorded in CCl_4 on a 60 MHz instrument and reported as ppm from TMS as internal standard. The ferulates were saponified with EtOH–KOH at 100° for 75 min. The reaction mixture was evaporated to dryness and the residue triturated with Et_2O. Evaporation of the Et_2O yielded alcohols which were identified by MS and comparison with authentic compounds (in some cases their OMe derivatives) [9] by GLC (5% SE30). The EtOH residue, upon treatment with aq. acid, yielded ferulic acid, which was identical to an authentic sample by IR and PMR. Ferulates were synthesized by two different methods. Schotten–Baumann condensation of acetyl feruloyl chloride with the appropriate alcohol to yield acetyl ferulates and Wittig reaction [10] of 3,4-dimethoxybenzaldehyde with the appropriate ylid to yield the 4-methoxy ferulates. The latter procedure gave higher yields (30%).

HPLC analyses were performed on a Waters Associates ALC301 with a 254 nm UV detector and a pressure of 175 kg/cm^2. Two μ-Porasil columns were used in series with CH_2Cl_2 as eluent and a flow rate of 2.4 ml/min. The acetylated ferulates gave the following R_t (CNo alcohol) 1 hr 51 min (26), 1 hr 54 min (24), 1 hr 57 min (22), 2 hr (20), 2 hr 3 min (18), 2 hr 6 min (16), 2 hr 9 min (14).

Isolation of ferulates. A total wax $CHCl_3$ extract of the ground cork fraction of the bark from *P. menziesii* (Mirb.) Franco was supplied by Weyerhaeuser Lumber Company. This fraction was triturated with CCl_4 and filtered. The filtrate was evaporated and chromatographed by LPLC (Si gel (ICN, 0.03–0.06 mm), $CHCl_3$–hexane (1:1) 6.3 kg/cm^2, 20 ml/min). The fractions with R_f 0.2 on Si gel F_{255} ($CHCl_3$–hexane, 1:1) which fluoresced light blue under UV and gave a positive test with diazotized sulfanilic acid were collected and evaporated to give a white solid; PMR (CCl_4, δ) 1.20 (*br. s*, $-(CH_2)_n-$), 3.96 (3H, *s*, OMe), 4.13 (2H, *m*, CH_2CO), 5.65 (1H, *s*, OH (exchangeable with D_2O)), 6.17 (1H, *d*, $J = 16$ Hz, α-H), 7.03 (3H, *m*, aromatic H's), 7.53 (1H, *d*, $J = 16$ Hz, β-H); IR (film) 1730 cm^{-1} (C=O); UV λ_{max}^{MeOH} nm: 325, 235, 218. 5 g of the CCl_4 residue yielded 0.5 g of ferulates. Tubers of *S. tuberosum* L. cu Burbank were cut longitudinally through the central axis of the tuber. The cut surface of the potato tubers were allowed to suberize at 21° under high humidity for 7 days. *Ca* 1 mm section of the cut surface was extracted with 95% EtOH, H_2O added to the residue, extracted with $CHCl_3$, and chromatographed on Si gel plates with $CHCl_3$–hexane (1:1). A band at R_f 0.2–0.3 which was visualized as above was removed from the plate and extracted with $CHCl_3$. Evaporation yielded a white solid with IR and UV essentially the same as observed for the isolated ferulate mixture from *P. menziesii*. *Ca* 10 mg of ferulates mixture was obtained from 20 g of 1 mm sections.

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