Our microscopy findings are consistent with reports indicating that all fractions from the milling of scabby wheat are contaminated with DON and that offals have higher DON contents than flours (Scott et al., 1983; Yamazaki et al., 1983; Young et al., 1984). The micrographs clearly showed that the fungus was distributed through the kernel with generally the highest concentration of hyphae in pericarp tissues. The presence of hyphae in endosperm tissues, especially in lightly and moderately infected kernels, would appear to make it improbable to avoid DON contamination in flours from scabby wheat.

Registry No. DON, 51481-10-8; ergosterol, 57-87-4.

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Characterization of Alkenylresorcinol in Mango (*Mangifera indica* L.) Latex

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The nonvolatile composition of mango latex was investigated. The major component (Ip) was isolated by thin-layer chromatography and characterized as 5-[2(*Z*)-heptadecenyl]resorcinol by a combination of infrared, ultraviolet, proton magnetic resonance, and gas–liquid chromatography–mass spectroscopy. It was identified for the first time in mango latex and considered as mango dermatitis allergen.

The latex of mango, a transparent fluid, that oozes out instantaneously from unripe (raw) fruit, as soon as the fruit is detached from the stalk, has long been known for its ability to produce allergic contact dermatitis among workers during harvesting of mature green (raw) fruit from the tree. Keil et al. (1946) demonstrated that the exudate of freshly picked unripe mango is resinous in nature and exhibits allergenic contact dermatitis similar to poison ivy. However, structural details of the compound in mango latex responsible for the allergic action on the skin is not known. Although the constituents of allergic noxious saps of various members of the *Anacardiaceae* family, such as poison ivy, cashew nut shell, oak, and other plant families have been classified as alkyl or alkenyl derivatives of catechol, resorcinol, and phenol (Keil et al., 1945; Symes and Dawson, 1953; Cirigottis et al., 1974; Billets et al., 1976; Reffstrup et al., 1982; Yamauchi et al., 1982). Besides the allergenic properties, these phenolics act as preservatives in necrotic organs of plants against microbial infection and undergo rapid resinification (Haslam, 1979).

Polyphenolic components such as mangiferin in mango stem bark and leaves (Bhatia et al., 1967) and β-glucogallin as well as gallotannin in pennicles and in mature green fruit of mango (El Ansari et al., 1967, 1971) were reported.

Mango latex is believed to contain tannins, enzymes, resins, and terpenes (Pantastico, 1975). The odorous principles of raw mango latex were characterized as *cis*-ocimene and β-myrcene (Gholap and Bandyopadhyay, 1977). The present paper relates to the isolation and identification of mango dermatitis allergen, a nonvolatile constituent of mango latex.

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EXPERIMENTAL SECTION

Isolation of Allergen. Freshly picked, mature unripe Alphonso mangoes, a premier variety, with stalk (10–12-cm length) attached, were procured from a local market. Droplets of latex collected during destalking were extracted repeatedly with peroxide-free diethyl ether. The ether extract was washed with distilled water, dried over anhydrous sodium sulfate, and filtered. The bulk of the ether was removed in a flash evaporator at ambient temperature, and the extract was subjected to high-vacuum distillation to remove the essential oil components of the latex as described elsewhere (Gholap and Bandyopadhyay, 1977). The viscous residue left after high-vacuum distillation was light brown in color. The residue was dissolved in chloroform (10% solution), and suitable aliquots were subjected to preparative thin-layer chromatography (TLC) on silica gel G (E. Merck) plates (0.4-mm layer thickness, 20

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× 20 cm glass plate, activated at 120 °C for $1^1/_2$ h) by using petroleum ether (bp 40–60 °C)–diethyl ether (60:40 v/v) as the developing solvent at 23 ± 2 °C. After development, the major band having an R_f value of 0.45, located on the plates under UV exposure at 254 nm, was scraped off and transferred into a miniature glass column (15 × 0.5 cm), and the compound was eluted with diethyl ether (50 mL) according to the method described elsewhere (Kundu and Bandyopadhyay, 1969). Ether was removed with a stream of nitrogen, and the pale yellow oily residue, henceforth designated as isolate (Ip), was stored at −10 °C under nitrogen until further use.

Analytical Methods. The crude latex extract in chloroform solution (10%) was spotted (100 μg) on analytical TLC plates (silica gel G 250-μm layer thickness, 10 × 20 cm glass plate activated at 110 °C for $1^1/_2$ h) along with authentic standards (Sigma) of catechol and resorcinol and developed with the above solvent system. After development, the plate was sprayed with either (i) 50% aqueous sulfuric acid followed by charring at 140 °C for $^1/_2$ h or (ii) 5% vanillin in concentrated sulfuric acid and subsequently heated at 110 °C for 15 min. The major spot (Ip) having an R_f value of 0.45 along with the standard resorcinol responded immediately by turning pink with vanillin–sulfuric acid at room temperature, in contrast to catechol, which gave rise to the same pink coloration only after heating.

Ip (50 mg) was acetylated with acetic anhydride in anhydrous pyridine at room temperature and worked out as usual. The acetylated product was analyzed by analytical TLC as stated above, and the R_f value of acetylated product was noted to be 0.75.

Ip (10 mg) was hydrogenated in the presence of 10% palladium/charcoal in methanol for 6 h at room temperature. A product was obtained after filtration followed by removal of the solvent under vacuum at room temperature. This was analyzed by analytical TLC as described above, and the R_f value was found to be 0.45.

Ip and its acetylated and hydrogenated products were subjected to spectrometric analyses. Ultraviolet (UV) spectra were recorded in methanol solution on a Shimadzu UV 240 graphical recording spectrophotometer with Graphic Printer PR-1. Infrared (IR) spectra were recorded on a Shimadzu IR 400 spectrophotometer, and samples were applied as a thin film between sodium chloride windows. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Varian A60A PMR spectrometer using carbon tetrachloride as the solvent and tetramethylsilane as an internal standard.

Ip and its hydrogenated product were analyzed by mass spectrometry using the direct probe inlet. The VG Micromass 7070F was operated at an ion source temperature of 200 °C and electron energy of 70 eV. Values for m/e with an intensity of greater than 5% of the base peak are given in the normal way. Isotope peaks are not quoted.

Ip was derivatized with hexamethyldisilazane and trimethylchlorosilane (1:2 v/v) in pyridine, and the silylated derivative was analyzed by gas–liquid chromatography (GLC) and also by gas chromatography–mass spectrometry (GC–MS). GLC analysis was performed on a BARC gas chromatograph equipped with a flame ionization detector. A stainless steel column (7 ft × $^1/_4$ in. o.d.) packed with 3% SE-30 on 60–80 mesh Chromosorb W (AW, DMCS treated) was used. The column temperature was isothermal at 220 °C with a carrier gas (nitrogen) flow rate of 25 mL/min. The peak areas of the separated components on the chromatogram were calculated by multiplying peak height by peak width at half-height. GC–MS analysis was carried out with the help of a JEOL JMSD-100 instrument. The GC was equipped with a glass column (100 × 0.2 cm) packed with 1.5% OV-1 on 100–120 mesh Chromosorb W(AW). The column temperature was maintained at 270 °C with carrier gas (helium) flow rate of 0.65 kg/cm^2. MS conditions were as follows: ionizing current, 300 μA; ionizing voltage, 28 eV; total ion monitor sensitivity, 1 × 10^{-3} A; scan time, 10 s; ion multiplier, 1.3 kV.

RESULTS AND DISCUSSION

The mango latex on treatment with diethyl ether separated into an ether-soluble clear layer and an ether-insoluble sticky mass. The yield of ether-soluble components was found to be 25% of the fresh latex. The yield, however, appeared to vary depending on the freshness of the latex.

The ether extract of latex on TLC separation resolved into seven components, among which the major one (Ip) contributed to ca. 60% of the extract as determined by preparative TLC. Both Ip and its hydrogenated product appeared to be homogeneous, as they gave rise to a single spot on analytical TLC. Similarly, the acetylated product of Ip separated out as a single spot on the TLC plate (R_f value of 0.75). The complete change in R_f value of Ip from 0.45 to 0.75 after acetylation indicated the absence of a tertiary hydroxyl group in the moiety. The R_f value of Ip resembled with that of resorcinol as well as of catechol. However, unlike catechol, the response of Ip to pink coloration with vanillin–sulfuric acid on TLC plate at room temperature revealed its structural resemblance with resorcinol rather than with catechol. The spectral characteristics of Ip are presented as follows: IR (film) 3390, 2975, 2905, 1613, 1475, 1160, 995, 840 cm^{-1}; ^{1}H NMR (CCl_4) δ 0.90 [3 H, distorted *t*, *J*, 7 Hz $CH_3(CH_2)_{13}$–], 1.30 [24 H, br s, –$(CH_2)_{12}$–], 1.80–2.20, (2 H, m, –CH_2CH=CH–), 2.25–2.60, (2 H, m, Ar–CH_2–), 5.33 (2 H, br t, –CH=CH–), 6.10 and 6.20 (3 H, br s each, Ar-*H*], 6.30 (2 H, br s, D_2O exchangeable); UV λ_{max} (methanol) 280 (sh), 274, 222 (sh), 214 nm; MS m/e (rel intensity) 346 (M$^+$, 87), 347 (76), 345 (26), 344 (17), 250 (8), 205 (8), 177 (8), 166 (13), 163 (13), 149 (11), 138 (8), 137 (72), 136 (65), 125 (54), 124 (100), 123 (44), 111 (8), 95 (8), 83 (8), 81 (13), 69 (13), 67 (19), 65 (6), 43 (8), 41 (30).

The IR spectrum of Ip showed strong hydroxyl and aromatic absorption bands at 3390 and 1613 cm^{-1}, respectively. The characteristic color development with vanillin–sulfuric acid at ambient temperature on the TLC plate together with the UV absorption spectrum was similar to that of resorcinol and its alkyl/alkenyl derivative compared to that of catechol. The presence of two olefinic protons in the ^{1}H NMR spectrum at δ 5.33 and the absence of the strong 970-cm^{-1} band for the *E* geometry in IR suggested the occurrence of the *Z* geometry of the disubstituted double bond.

The presence of only one double bond in the side chain was confirmed from the MS data of the hydrogenated product of Ip, which showed only a 2 mass unit increase in molecular ion peak (M$^+$ 348). The identical UV absorption bands of Ip and its hydrogenated product suggested the absence of a conjugated double bond in Ip. The presence of a phenolic hydroxyl group was evidenced by the IR data of the acetylated product, where the strong absorption at 3390 cm^{-1} originally exhibited by Ip was absent with the appearance of a characteristic phenolic acetate band at 1785 cm^{-1}. The ^{1}H NMR data of the acetate derivative of Ip showed strong signal at δ 2.20 accounting for 6 H. This suggested the presence of two acetate groups in the moiety and hence two phenolic hy-

droxyl groups in Ip. These data coupled with the characteristic ^{1}H NMR signals at δ 6.10 and 6.20 for Ip indicated that the Ip is 5-alkenyl-substituted resorcinol. The multiplet signals between δ 1.80–2.20 and 2.25–2.60 accounting for two protons each suggested the presence of a Ph-$CH_2CH{=}CHCH_2$– group in Ip.

MS of Ip revealed four remarkable stable fragments at *m/e* 124, 125, 123, and 137, accompanied by a prominent molecular ion peak at *m/e* 346. Peaks *m/e* 124 and 123 had earlier been assigned to be derived from alkenyl-substituted resorcinol (Cirigottis et al., 1974; Reffstrup et al., 1982). The hydrogenated product of Ip on MS analysis resulted in a stable molecular ion peak at *m/e* 348 and three stable fragments at *m/e* 124, 125, and 123, indicating the incidence of a single double bond in the side chain of Ip. The position of the double bond, in this case, could be assigned from the MS of Ip by considering that the odd-electron ion *m/e* 124, $C_7H_8O_2$, was highly significant in conjunction with *m/e* 137, $C_8H_9O_2$, indicating the presence of a double bond between carbons 2 and 3 (Van Aller et al., 1983). Further, the absence of the characteristic stable peak at *m/e* 137 in the MS fragmentation pattern of hydrogenated Ip substantiated the evidence of double bond position between carbons 2 and 3 in the hydrocarbon chain. Thus, from the spectral data the structure of Ip could be assumed to be 5-[2(*z*)-heptadecenyl]resorcinol.

The Me_3Si ether derivative of Ip, however, on GLC analysis resolved into one major (Ip^i) and two minor (Ip^{ii} and Ip^{iii}) components. The Ip^i, which was of interest in the present investigation, was found to contribute 85% of Ip, and its MS recorded with the aid of GC–MS is presented as follows: 490 (M^+, 83), 491 (33), 489 (85), 488 (13), 474 (21), 352 (6), 310 (20), 283 (8), 282 (27), 281 (93), 269 (54), 268 (100), 267 (93), 266 (37), 254 (8), 252 (16), 83 (6), 75 (7), 73 (35), 69 (11), 57 (8), 55 (17), 43 (7), 41 (6). Here also the Ip^i component of Ip exhibited a similar fragmentation pattern with almost identical relative abundance of major *m/e* peaks to that of Ip isolated by preparative TLC. Hence, the minor two components of Ip seemed to have negligible effect on various spectral data presented here. Therefore, the MS data of Ip and its silylated derivative strongly supported the above proposed structure.

Keil et al. (1945) in their investigation on the relation of hypersensitive to noxious sap of poison ivy plant and cashew nut shell demonstrated that in a person sensitive to poison ivy the fundamental structural requirement for group activity is the presence of a long-chain (C_{15} and C_{17}) unsaturated hydrocarbon in the position meta to at least one hydroxyl group attached to a benzene ring. Though the allergic action of the presently identified nonvolatile major compound in mango latex could not be clinically tested, its structural resemblance with known compounds having allergic properties suggests that it is a mango dermatitis allergen.

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Registry No. 5-[2(*Z*)-Heptadecenyl]resorcinol, 94751-34-5.

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