Table II. Comparison of the Occurence of Components in Corn Husks, Kernels, Tomatoes, and Cotton Plant

Compound	Corn husk	Corn kernel	Tomato[b]	Cotton plant[a]
Hexanal	+	+	+	+
Hex-*cis*-3-enol	+	−	+	+
C_6–C_9 alk-2-enals	+	−	+	+
Limonene	+	+	+	+
Hepta-2,4-dienal	+	−	+	−
Deca-2,4-dienal	+	+	+	−
Deca-2,4,7-trienal	+	−	+	−
α-Pinene	+	−	−	+
α-Ylangene or α-copaene	+	+	−	+
Caryophyllene	+	+	−	+
β-Ionone	+	−	+	+
Geranylacetone	+	+	+	−

[a] Hanny et al., 1973; Hedin et al., 1971; Minyard et al., 1965; Minyard et al, 1967. [b] Buttery et al., 1971.

occurring in all four materials listed. The C_6–C_9 alk-2-enals and hex-*cis*-3-enol occur in all but the corn kernels. They are very common in most green plants. β-Ionone also occurs in husks, tomatoes, and cotton, but is generally less common in green plants. Other components occur in corn husks and either tomato or cotton but not in all three. Of these the more unusual compounds are geranylacetone, deca-2,4,7-trienal, and α-ylangene or α-copaene (very similar isomers).

Tests with the Corn Earworm Moth. It is well known that the corn ear worm is attracted to the corn plant in preference to other plants (McMillian and Wiseman, 1972). Some attempts were made to test the corn volatile oils (placed on cheesecloth as solutions in pentane containing 1% paraffin wax) with *Heliothis zea* moths in a closed chamber. Although there was some indication of preference for the corn volatile oil samples (over blank samples of cheesecloth treated only with the solvent), the results were not consistent enough to draw any valid conclusions. It was felt that in the closed chamber the volatiles probably permeated the whole chamber and that the moths could not accurately locate the concentrated source of the odor. A new chamber, being constructed which incorporates a laminar flow of air through the chamber, should eliminate the above effect.

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Termiticidal Components of Wood Extracts: 7-Methyljuglone from *Diospyros virginiana*

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The major termiticidal component of wood of the common persimmon, *Diospyros virginiana* L., was isolated and identified as 7-methyljuglone (5-hydroxy-7-methyl-1,4-naphthoquinone). Its 8-6′ dimer, isodiospyrin, was also toxic to termites but to a lesser extent. Shinanolone (4,8-dihydroxy-6-methyl-1-tetralone) and scopoletin (7-hydroxy-6-methoxycoumarin) were isolated and identified as extractive components of the wood but were not toxic to termites at the concentrations tested. Only isodiospyrin has been isolated previously from *D. virginiana*.

Environmental concern about the use of persistent chlorinated hydrocarbons as termiticides has resulted in new studies on extractives of woods resistant to termite attack (Carter, 1976). Resistant woods usually contain extractives that are toxic, repellent, or distasteful to termites. Characterization of biologically active components from resistant woods could lead to increased protection of wood from termites through treatments with some extracts or synthesized compounds with structures similar to the active components.

The genus *Diospyros* of the Ebenaceae family contains about 250 species and is widely distributed in tropical and warm temperate regions of the world. Certain species produce edible fruit known as persimmon; others are noted for their dark heartwood known as ebony. Many *Diospyros* species native to Africa and Asia are a rich source of triterpenes, naphthoquinones, and other naphthalene derivatives (Hegnauer, 1966; Thomson, 1971; Yoshihira et al., 1971; Pardhasaradhi and Sidhu, 1972; Tezuka et al., 1972, 1973; Musgrave and Skoyles, 1974; Van der Vijver and Gerritsma, 1974; and references therein).

But little attention has been given to extractives of *Diospyros virginiana* L. (common persimmon), the only

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North American *Diospyros* species that is important commercially for its wood. Only one compound, isodiospyrin, has been isolated and identified from wood of *D. virginiana* (Fallas and Thomson, 1968). Because termites survived poorly when exposed to persimmon wood blocks, research was initiated to study the extractive components. This paper reports the extraction, isolation, and characterization of an antitermitic component and related compounds from wood of *D. virginiana*.

EXPERIMENTAL PROCEDURES

Preparation of Extracts. Three mature *D. virginiana* trees were cut in the Harrison Experimental Forest in southern Mississippi. Boards were cut from the heartwood and ovendried for 3 consecutive days, 1 day each at 35, 50, and 60 °C. Blocks 2.0 × 1.7 × 1.5 cm were cut from one board of each tree for bioassay with termites. Combined shavings (about 500 g) from the boards were extracted successively in a Soxhlet apparatus with *n*-pentane, 1:1 acetone–hexane (AH), and 54:44:2 acetone–hexane–water (AHW) mixtures for 24 h each. Four batches of shavings were extracted. The extracts were filtered and concentrated under reduced pressure at a temperature less than 30 °C. Extracts were stored in the dark at −15 °C when not being used.

Because photochemical modifications of certain naphthoquinones have been reported (Van der Vijver and Gerritsma, 1974, 1975), a second but small-scale extraction was carried out at room temperature and in semidarkness to check on the possibility of artifact formation during the Soxhlet extraction. Sawdust was prepared by grinding the wood shavings in a Wiley mill. A sample (50 g initial weight) was extracted successively 10 times in a Waring blender with each of the three solvents used in the Soxhlet extraction. Extracts obtained with each solvent were concentrated, and their volumes were adjusted to correspond to a given weight of sawdust. We estimated the amount of material extracted by evaporating aliquots of the three extracts and weighing the residues.

Chromatography. Analytical and preparative thin layer plates were prepared with Silica Gel G Woelm TLC absorbent at thicknesses ranging from 0.25 to 1.0 mm. Various solvents, but most frequently chloroform and mixtures of pentane and acetone (95:5 to 75:25), were used to isolate components. Components on the plates were detected by visible coloration, fluorescence or absorption under 254- and 366-nm radiation, and reaction with iodine vapor or specific reagent sprays. Isolated components were freed of silica gel by filtering them with acetone or chloroform. Isolates were rechromatographed with alternate solvents until they were considered pure.

Columns (1.27 cm i.d.) were packed with 5 g of silicic acid (Bio-Sil HA minus 325 mesh) slurried in pentane. Solvents used were: pentane, mixtures of pentane and benzene, benzene, mixtures of benzene and chloroform, chloroform, mixtures of chloroform and acetone, acetone, and ethanol. Fractions of about 5 mL were collected in test tubes with a Buchler Fractomette 200 fraction collector. Preparative columns were scaled up to 20 or 25 g of silicic acid, and larger fractions were collected. Fractions were analyzed by thin-layer chromatography (TLC) and were bioassayed with termites. Fractions containing the same component were combined; fractions containing more than one component were further purified by preparative TLC.

Elucidation of Structure of Isolates. Identification of the isolates was based on spectral data and comparison with authentic samples. Melting points were determined with a Mel-Temp Laboratory Device. Absorption spectra [ultraviolet (UV) and visible regions] of solutions in ethanol and 0.05 N potassium hydroxide in ethanol were recorded on a Perkin-Elmer 200 spectrophotometer. To resolve overlapping absorption bands, the second derivative absorbance spectra were recorded. Molar absorptivity values were determined from absorbances at selected wavelengths on either a Perkin-Elmer 200 spectrophotometer or a Gilford 240 spectrophotometer. Infrared (IR) absorption spectra of isolates in potassium bromide micropellets or carbon tetrachloride solutions were recorded on a Perkin-Elmer 337 infrared spectrophotometer. Mass spectra (MS) were obtained on a Consolidated Electrodynamics Corporation Mass Spectrometer, Model 21-110B, operated at 70 eV ionization voltage. Compounds were introduced by direct probe insertion. A source temperature of about 170 °C was used for compounds with a molecular weight of less than 200; it was increased up to 270 °C for compounds with higher molecular weights.

Termite Survival Tests. Externally undifferentiated termites (workers) beyond the third instar were selected from field-collected colonies of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). Until used, the insects were maintained in segments of colonized logs.

Wood blocks placed on a sand substrate were tested against termites. Plastic containers 5.0 cm diameter by 3.5 cm high were packed with 50 g of sand which had been moistened with distilled water to maintain relative humidity near saturation (Carter and Smythe, 1974). One block was placed on the sand in each container, and 100 termites were added. Termites were maintained at 25 °C, and their condition was checked weekly. After 8 weeks, surviving termites were counted. The test was replicated with six blocks from each wood source.

In a second series of tests, wood extracts and aliquots of fractions from TLC or column chromatography were applied to absorbent paper pads (47 mm diameter, Gelman Instrument Co.) and allowed to dry. One-milliliter aliquots provided the most even application of test material on pads. Each pad was moistened with 1 mL of distilled water and placed in the bottom of the plastic container; 25 termites were then placed on the pad. Condition of termites was determined at 24, 48, and 72 h, and then biweekly for 2 to 4 weeks. This bioassay procedure was also used to determine the toxicity of isolates or authentic samples where sufficient material was available. Stock solutions were prepared by dissolving weighed quantities of the test material in chloroform. Appropriate dilutions were prepared and aliquots were bioassayed.

RESULTS AND DISCUSSION

Termite Survival. Survival of termites in the 8-week test was 8, 0, and 0% for the three sources of wood. The corresponding survival of termites on control blocks of loblolly pine (*Pinus taeda* L.) was 75%. The great contrast in survival of termites on blocks of persimmon and pine indicated the antitermitic properties of persimmon.

From the sawdust taken from the original boards, approximately 1.14% of extractives were removed by solvent extraction in the Waring blender at room temperature: 0.13% by pentane, 0.34% by AH, and 0.67% by AHW. Corresponding yields from a Soxhlet extraction were: 0.12, 0.50, and 0.20% for the three solvents. The extracts were compared by TLC (Figure 1).

All termites died in less than 48 h when exposed to absorbent paper pads treated with 1-mL aliquots of either the concentrated pentane or AH extracts from the Soxhlet extraction. Because the AHW extract had a lower concentration of toxic material, about 16% (mean of three replicates) of the termites survived a 2-week exposure to

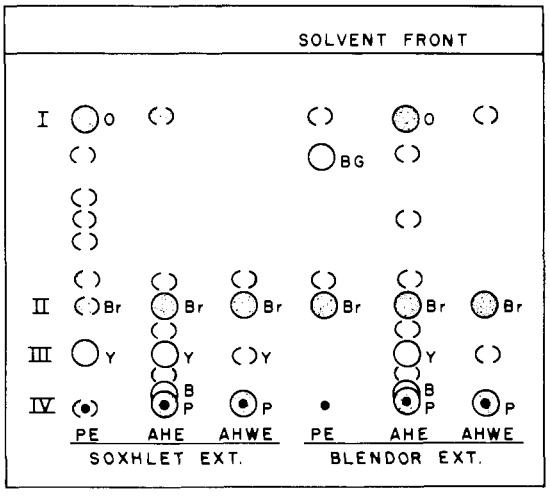

Figure 1. Thin-layer chromatography of persimmon wood extracts on silica gel G plate, developed in 92.5:7.5 pentane–acetone; shaded spots were detected visibly; others were apparent under UV radiation; ( ) indicates faint spots; O, orange-red (component I); BG, blue-green; Br, dark brown (component II); Y, yellow (component III); B, blue; P, purple-blue (component IV).

pads treated with the AHW extract. When extracts were separated into fractions by preparative TLC, dominant bands apparent visibly or under UV radiation were marked off separately. When the fractions were freed of silica gel and bioassayed with termites, only fractions obtained from two dominant bands (components I and II) were toxic to termites (Figure 1).

All termites died in less than 48 h when exposed to a pad treated with the fraction containing component I from either the pentane or AH extract. Concentration of component I in the AHW extract fraction was too low to kill the termites. All termites died in less than 2 weeks on a pad treated with the AH extract fraction containing component II. Component II in the pentane extract was not so concentrated as to be toxic, but enough was present in a fraction of the AHW extract to kill 96% of the termites during the 2-week test. Because termites survived well on all other treated pads tested, the results indicated no other toxic components at the concentrations tested.

Separation of the pentane extract into 25 fractions on a silicic acid column yielded only two adjacent fractions (no. 10 and 11, in benzene) that were toxic to termites; TLC showed that these fractions consisted primarily of component I. A small amount of component II in fraction 18 was detected by TLC.

Isolation and Identification. Four components (Figure 1, I–IV) were isolated by fractionation of the extracts by preparative TLC or column chromatography. The relative amounts of these components obtained by TLC fractionation of extracts prepared with a Waring blender from 50 g of sawdust were: I, 3.0 mg; II, 5.0 mg; III, 1.2 mg; and IV, 2.5 mg (combined weights from the three extracts). Because bioassay tests indicated that the remaining extracted material was biologically inactive to the termites, fractions containing other components were discarded and not investigated further. Although some of the compounds occurred in all three extracts, components I–IV were isolated from the extracts where they could be obtained most easily in pure form.

Component I. The termiticidal component I was identified as 7-methyljuglone, 5-hydroxy-7-methyl-1,4-naphthoquinone (Figure 2, IA). Its UV-visible spectrum [λ_{max} (EtOH) 215 (log ϵ 4.54), 253 (log ϵ 4.13), 428 (log ϵ 3.61); second derivative, 217, 250, 258 nm] and its bathochromic shift in alkaline solution [λ_{max} (EtOH/OH^-) 223, 267, 287, 539; second derivative, 223, 250, 266, 290, 530 nm] are characteristic for *peri*-hydroxy-1,4-naphthoquinones (Scott, 1964). The absorption values compare favorably with those reported in the literature for 7-methyljuglone (Fallas and Thomson, 1968; Yoshihira et al., 1971; Tezuka et al., 1972; Ferreira et al., 1972). The isolate's IR spectrum is also characteristic of the proposed structure [ν_{KBr}: OH absent; 1668 and 1640 (s) (C=O), 1592 (C=C), 1345, 1305, 1282, 1232, 1158, 1104, 1054, 1020, 988, 882, 849 (s), 778, 698, 688 cm^{-1}] and agrees with the spectra reported by Ferreira et al. (1972) and Yoshihira et al. (1971). Hydroxy groups are not present in the IR spectrum because they are strongly associated with the neighboring carbonyl.

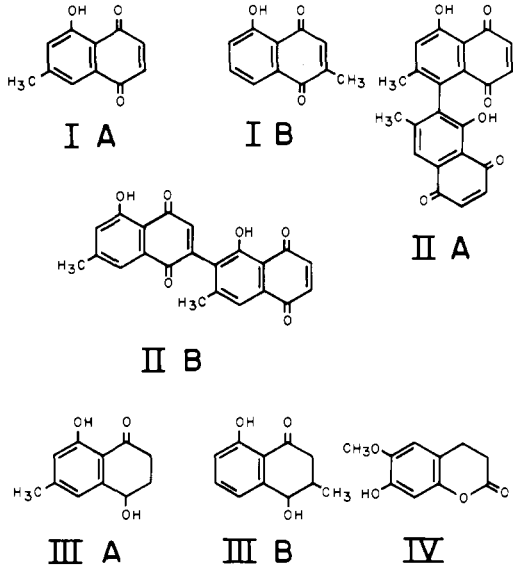

Figure 2. IA, 7-methyljuglone; IB, plumbagin; IIA, isodiospyrin; IIB, diospyrin; IIIA, shinanolone; IIIB, isoshinanolone; IV, scopoletin.

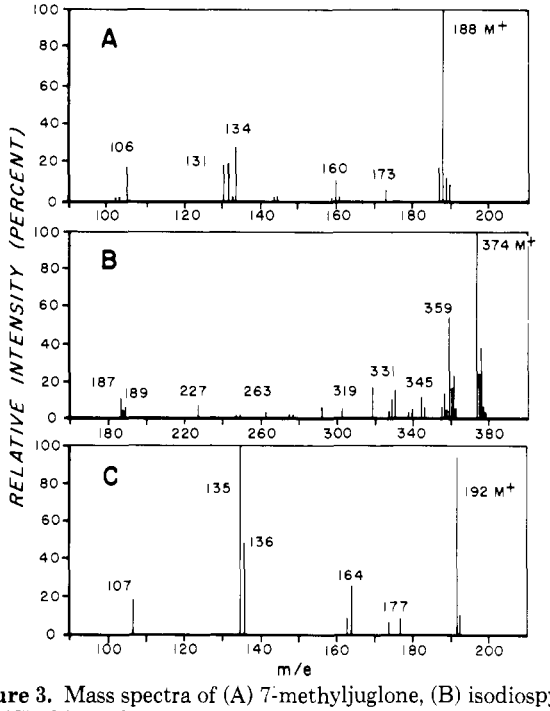

Figure 3. Mass spectra of (A) 7-methyljuglone, (B) isodiospyrin, and (C) shinanolone.

The mass spectrum (Figure 3A) gives a molecular weight of 188. The fragmentation ions suggest the 7-methyl-

juglone structure: 173 (M – CH_3), 160 (M – CO), 134 (M – C_2H_2 – CO), 132 (M – 2CO), 106 (132 – C_2H_2), or (134 – CO). The doublet at 134 and 132 corresponds to the doublet, M – 54 and M – 56, which is characteristic of a 2,3-unsubstituted naphthoquinone (Bowie et al., 1965). This differentiates the isolate from plumbagin, 2-methyl-5-hydroxy-1,4-naphthoquinone (Figure 2, IB), an isomer to which the poisonous and vesicant properties of many tropical *Diospyros* species have been attributed (Thomson, 1971). The fragmentation of component I corresponds with that reported previously for 7-methyljuglone (Fallas and Thomson, 1968).

Authentic samples of 7-methyljuglone and several of its dimers were obtained for direct comparison with the isolates. On TLC plates our isolates of component I corresponded to the authentic 7-methyljuglone. The spots were yellowish visibly but red-orange (Van der Vijver and Gerritsma, 1975) under 366-nm radiation; R_f's were: benzene, 0.62; pentane–acetone (92.5:7.5), 0.82; chloroform, 0.82. The UV-visible spectrum and the MS of the authentic sample also corresponded to the purified isolate. 7-Methyljuglone was first isolated from leaves of *D. hebecarpa* by Cooke and Dowd in 1952; they reported its synthesis in 1953. Since then, it and closely related compounds, especially dimers, have been identified in many Ebenaceae species.

Component II. Compound II was identified as isodiospyrin, the 8-6′ dimer of 7-methyljuglone (Figure 2, IIA). The purified isolate consisted of dark red crystals (mp 226–227.5 °C dec). Isodiospyrin was first isolated from *D. chloroxylon* by Sidhu and Prasad (1967, 1970) and is the only compound previously identified as a component of *D. virginiana* (Fallas and Thomson, 1968). A molecular ion of 374 from the MS and absorption similar to 7-methyljuglone in both infrared and UV-visible regions indicate that the compound is a dimer of 7-methyljuglone. The major absorption peaks in the IR spectrum are: ν_{KBr}: OH absent; 1665 and 1640 (C=O), 1605 (C=C), 1440, 1420, 1365, 1283, 1242, 1210, 1204, 1100, 1050, 854, 750 cm^{-1}. The UV-visible spectrum shows the characteristic bathochromic shift in alkaline solution for a *peri*-hydroxynaphthoquinone: [λ_{max} (EtOH) 217 (log ϵ 4.67), 253 (log ϵ 4.40), 430 (log ϵ 3.89); second derivative, 217, 250 nm; ($EtOH/OH^-$) 226, 290, 560; second derivative, 226, 289, 549, 593 nm]. Our values compare favorably with those reported by Yoshihira et al. (1971) for isodiospyrin isolated from *D. lotus* and by Ferreira et al. (1972) for the dimer isolated from *D. lycioides*. Similar UV-visible spectra were obtained for ethanol solutions of authentic samples of isodiospyrin [λ_{max} (EtOH) 217, 253, 431] and another dimer, diospyrin (Figure 2, IIB) [λ_{max} (EtOH) 217, 251, 431 nm].

The mass spectrum (Figure 3B) indicates a molecular weight of 374, and the fragmentation ions suggest the methyljuglone dimer structure: 376 (M + 2), 374 (M^+), 359 (M – CH_3), 345 (359 – CH_2), 331 (359 – CO), 319 (M – C_3H_3O), 303 (331 – CO), 189, 187 (Fallas and Thomson, 1968). The spectrum consists primarily of low-intensity peaks with a prominent M + 2 peak. The M/2e ions are relatively abundant in the lower mass region. The spectrum corresponded with that obtained for an authentic sample of isodiospyrin; significant differences were observed for the spectrum of an authentic sample of diospyrin.

Our isolates of component II also corresponded to isodiospyrin when compared by TLC with authentic samples of diospyrin and isodiospyrin. Both dimers appeared brown-orange visibly on the plates, but under 366-nm radiation, the diospyrin spot was orange-red, whereas the isodiospyrin was very dark brown. Furthermore, the R_f's varied for diospyrin and isodiospyrin as follows: benzene, 0.27 and 0.18; pentane–acetone (92.5:7.5), 0.34 and 0.27; and chloroform (1% ethanol), 0.78 and 0.70.

Component III. When isolated by TLC, a dominant component (III) of the pentane extract (Figure 1) appeared bright yellow under UV radiation. The spectra were characteristic of shinanolone (4,8-dihydroxy-6-methyl-1-tetralone) (Figure 2, IIIA), which was first isolated from roots of *D. japonica* (Kuroyanagi et al., 1971). An acetophenone chromophore was suggested by the UV-visible spectrum [λ_{max} (EtOH) 218 (log ϵ 4.22), 229 (sh), 268 (log ϵ 4.05), 333 (log ϵ 3.53); second derivative, 218, 220, 229, 260, 270, 320, 327 nm]; a bathochromic shift was not obtained for the component in alkaline ethanol. The 268 band indicates an aromatic ketone with the hydroxy group ortho (8 position) to the acetophenone group and the methyl group para (6 position) (Scott, 1964). The IR absorption spectra indicate the presence of a secondary alcohol group (ν_{CCl_4}, OH 3615 cm^{-1}) and a hydrogen bond between a carbonyl group and an *ortho*-hydroxy group (ν_{KBr}, C=O 1635 cm^{-1}). The major IR bands are: ν_{KBr}, 3420, 2955, 2930, 1635, 1385, 1338, 1310, 1265, 1213, 1197, 1097, 844, 818 cm^{-1}. The mass spectrum (Figure 3C) gives the molecular ion as 192 and the following major fragmentation ions: 177 (M – CH_3), 174 (M – H_2O), 164 (M – CO), 136 (M – 2CO), or (M – CO – C_2H_4), 135 (M – C_3H_5O), 107 (136 – CHO), or (135 – CO).

Component IV. The mass spectrum of another isolate (IV) also gives a molecular ion of 192: 192 (M^+), 177 (M – CH_3), 164 (M – CO), 151 (177 – HC_2O), 149 (164 – CH_3), 121 (149 – CO). The major fragmentation ions differ from those for shinanolone (component III) and for its isomer, isoshinanolone (Figure 2, IIIB), which has been isolated from *D. ferrea* (Tezuka et al., 1973). The UV-visible spectrum [λ_{max} (EtOH) 228, 252, 260, 287, 298, 345; second derivative, 229, 250, 257, 340 nm] suggests a coumarin structure (Scott, 1964) and compares well with the spectrum of an authentic sample of scopoletin, 7-hydroxy-6-methoxycoumarin (Figure 2, IV). When cochromatographed on TLC plates, an authentic sample of scopoletin and isolate IV appeared as blue fluorescent spots with identical R_f values (75:25 pentane–acetone, 0.30; chloroform plus 2% ethanol, 0.21). This coumarin was previously isolated from the bark of *D. maritima* (Meijer, 1947). The infrared spectrum shows the following major absorption bands: ν_{KBr}, 3350, 1700, 1610, 1564, 1291, 1260, 1136, 1015, 858, 818, 595 cm^{-1}.

Artifact Formation. Van der Vijver and Gerritsma (1975) found that 7-methyljuglone tended to undergo conversion reactions when adsorbed on silica gel coated TLC plates and exposed to air. Under the same conditions, however, they found no transformational products from isodiospyrin. Tezuka et al. (1973) found that when 7-methyljuglone was refluxed with silica gel in methanol for 20 h, low yields of two methoxy derivatives and mamegakinone, a dimer of 7-methyljuglone, were obtained as artifacts. Under the same conditions, they found that isodiospyrin yielded small amounts of its dimer, bisisodiospyrin. When chloroform was substituted for methanol, they found no formation of dimers. In our experiments we tried to minimize the time that the extracts and isolates on silica gel were exposed to air and light and where possible, used chloroform over methanol. We substituted ethanol for methanol as the solvent for the UV absorption spectra. The major components were present in extracts

obtained either by Soxhlet (hot) or Waring blender (cold) extraction (Figure 1). We believe that our isolates were not artifacts but compounds occurring naturally in our wood samples of *D. virginiana*.

Toxicity of 7-Methyljuglone and Isodiospyrin. In a 2-week test where termites were exposed to absorbent paper pads treated with different concentrations of 7-methyljuglone and isodiospyrin, the monomer was considerably more toxic than the dimer. No termites died during the test when exposed to 200 ppm (0.10 mg) of isodiospyrin; all died within 24 h on the same concentration of 7-methyljuglone. For the monomer, all termites died within 48 h on 160 ppm and on 120 ppm and within 72 h on 80 ppm. Approximately 50% of the termites died within 1 week on pads treated with 0.02 mg (40 ppm). For the dimer, 28% of the termites survived the 2-week test on 500 ppm. The termites died in 10 days on 1000 ppm and in 9 days on 1500 ppm.

Termiticidal Properties of Persimmon Wood. Sandermann and Dietrichs (1957) tested resistance to termites [*Reticulitermes lucifugus* (Rossi) and *R. flavipes*] of three *Diospyros* species. Only macassar ebony, the heartwood of *D. celebica*, proved very resistant. Its major component, macassar II, was later identified as 1,8-dimethoxy-6-methyl-2-naphthol (Brown et al., 1965). The sample of *D. virginiana* tested by Sandermann and Dietrichs was not resistant.

Several factors can explain why samples of a selected *Diospyros* species may vary considerably in resistance to termites. First, the quantity of an extractive component often varies, not only for samples taken from different trees, but also for those taken from different areas within a single tree. Also, an extractive component is often stabilized within the wood but undergoes rapid chemical change when exposed to air. Because of the reactivity of 7-methyljuglone, it may be transformed into dimers or other products which have no or decreased termiticidal properties.

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