Biologically Active Volatile Organosulfur Compounds from Seeds of the Neem Tree, *Azadirachta indica* (Meliaceae)

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The headspace volatile constituents from freshly crushed neem (*Azadirachta indica*) seeds were purged with nitrogen, trapped onto Amberlite XAD-4 resin, concentrated into diethyl ether, and analyzed by means of GC (FID and FPD, sulfur mode), capillary GC/MS, and high-resolution MS. The volatile constituents were found to consist principally of derivatives of di-*n*-propyl- and *n*-propyl-1-propenyl di-, tri-, and tetrasulfides. A total of 25 volatile compounds were identified. Di-*n*-propyl disulfide, identified as the major volatile constituent of neem seeds (75.74%), was shown to be larvicidal to *Aedes aegypti* (yellow fever mosquito), *Heliothis virescens* (tobacco budworm), and *Heliothis zea* (corn earworm). The volatile organosulfur compounds identified (and/or their biogenetic precursors) may be responsible, at least in part, for some of the reputed insect repellent and medicinal properties of neem seeds in traditional Indian (Ayurvedic) medicine.

The neem tree, *Azadirachta indica* A. Juss. (Meliaceae), is a tropical and subtropical species indigenous to India and Southeast Asia (Kunkel, 1978). Various neem tree parts have been used by natives in cooking, in folk medicine, and as natural pesticides (Jacobson, 1986). Expressed neem seed oil has been used in traditional Indian (Ayurvedic) medicine for the treatment of leprosy, skin diseases, malaria, and other afflictions (Nadkarni and Nadkarni, 1954; Dey and Mair, 1973), and has insecticidal properties (Radwanski, 1977a,b; Radwanski and Wickens, 1981; Lewis and Elvin-Lewis, 1983). Ripening neem fruits and expressed neem seed oil give off a strong alliaceous (garliclike) odor, and some of the reputed medicinal efficacies of neem oil have been attributed to the sulfurous compounds that it contains (Nadkarni and Nadkarni, 1954; Dey and Mair, 1973; Jacobson, 1986). Although the presence of sulfur-containing compounds in neem oil has been reported previously (Nadkarni and Nadkarni, 1954; Dey and Mair, 1973; Radwanski, 1977a,b; Sinniah and Baskaran, 1981; Radwanski and Wickens, 1981), to our knowledge there are no detailed chemical studies of neem volatile organosulfur compounds. In a continuation of our studies on potentially useful biologically active agents derived from the neem tree, we have investigated the volatile principles giving neem seeds their characteristic alliaceous odor. We now report on the isolation and identification of the neem seed volatile organosulfur compounds and the biological activity of the major component, di-*n*-propyl disulfide, against three species of insects. In addition, we present a discussion of the possible pharmacological roles of neem seed volatiles (and/or their postulated biogenetic precursors) and their potential medicinal significance.

EXPERIMENTAL SECTION

Plant Materials and Isolation of Headspace Volatiles Using Amberlite XAD-4 Traps. Fresh neem seeds were collected in Rajasthan, India, by the National Forestry Research Institute of India. Neem seeds (50 g) were crushed into small pieces with a mortar and pestle, and these were placed into a 500-mL two-neck round-bottom flask. A nitrogen gas stream (purified by passage through activated charcoal and Drierite columns) was used to purge the headspace volatiles of crushed neem seeds at 1.0 L/min. Exiting N_2 gas was passed through two consecutively connected Amberlite XAD-4 porous resin traps (two polyethylene tubes (each 20 × 1.5 cm) containing 20 g of Amberlite XAD-4 each). After 36 h of purging and trapping, the XAD-4 resins were removed to a 500-mL Erlenmeyer flask. Volatiles trapped by sorption were extracted from the XAD-4 resins by gentle shaking (150 rpm, 30 min) with freshly distilled diethyl ether (200 mL). The diethyl ether extract was decanted, and the above process was repeated twice with fresh solvent. The pooled diethyl ether extracts were concentrated to approximately 0.5 mL on a Kuderna-Danish evaporative concentrator in a water bath (30 °C). The same procedure was used to purge, trap, and isolate the headspace volatiles from freshly chopped onion (*Allium cepa* L.) bulbs and garlic (*Allium sativum* L.) cloves purchased locally and to simultaneously prepare blank controls for each of the three test samples.

Gas Chromatography (GC) of the Neem Seed Volatile Concentrate. The neem seed volatile concentrate was analyzed by GC/FPD (flame photometric detection) with a Tracor MT220 gas chromatograph (FPD, sulfur mode) equipped with a U-shaped glass column (6 ft × 0.25 in. (i.d.), 1.5% OV-17/1.95% OV-210, Gas Chrom Q, 80/100 mesh). Nitrogen was used as the carrier gas at 30 mL/min. The GC was operated isothermally at 100 °C, and 1 μL of volatile concentrate was analyzed. The temperatures of the injector and detector were maintained at 220 °C. Disulfoton (Sandoz AG) was used as an external sulfur-containing standard compound (t_R = 7.2 min at 200 °C column oven temperature).

GC/FID (flame ionization detector, 270 °C) analyses of the neem seed volatile concentrate were performed on a Varian Vista 6000 Series gas chromatograph equipped with a split/splitless capillary injector. Separations were achieved with a fused silica wall-coated open tubular (WCOT) capillary column (30 m × 0.32 mm (i.d.)) coated with bonded-phase DB-5 (0.25-μm film thickness; J & W Scientific, Inc.) using N_2 as the carrier gas (column head pressure 8–10 psi (0.56–0.70 kg/cm^2)). GC analyses (1-μL samples in diethyl ether) were carried out in both the split (split ratio 1:25) and splitless (30 s) modes with the injector (all-glass-lined inlet system) temperature set at 200 °C. The oven temperature program was set as follows: 40 °C for 10 min, increased at 4 °C/min to 200 °C, and held for 15 min. Peaks were recorded and peak areas were calculated on a Varian (Spectra-Physics) Model SP4270 computing recorder/integrator.

Capillary Gas Chromatography/Mass Spectrometry (GC/MS) of the Volatile Concentrates. GC/MS analyses of the trapped headspace volatiles from freshly crushed neem seeds and freshly chopped onion bulbs and garlic cloves were carried out on a Hewlett-Packard (HP) Model 5890A/5970B capillary/quadrupole GC/MSD

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(mass selective detector) system equipped with a split/splitless capillary injector and a capillary-direct interface. The MSD provided EI mass spectra at 70 eV, taking mass spectral scans from mass 40 to mass 400 (1.2 scans/s; electron multiplier, 1600 V). The system was controlled via an HP Model 216/9816S computer with an HP 59970A GC/MSD data system. Chromatographic separations were achieved with an HP high-performance (HP-5) fused silica WCOT capillary column (cross-linked 5% phenylmethyl silicone (SE-52 equivalent); 25 m × 0.20 mm (i.d.); 0.33-μm film thickness). Helium was used as the carrier gas with the column head pressure maintained at 13 psi (0.91 kg/cm^2). The temperatures of the injector (all-glass-lined inlet system) and the transfer line from the capillary column to the MSD were maintained at 200 and 270 °C, respectively. The column oven was temperature programmed to maintain an initial temperature of 40 °C for 10 min and then to increase linearly to 250 °C at 4 °C/min. Samples (0.1–1 μL, in diethyl ether) were injected in both the split (1:35) and splitless (1 min) modes. Compound identifications were based on retention times and comparison of mass spectra with those in the National Bureau of Standards (NBS) Mass Spectral Data Base Library in the GC/MSD data system and the EPA/NIH Mass Spectral Data Base (Heller and Milne, 1978, 1980). Compound identifications were further confirmed by comparison (cochromatography (spiked samples) and mass spectra) with authentic reference compounds obtained either from commercial sources (di-*n*-propyl disulfide and diallyl disulfide from Pfaltz & Bauer, Inc.; 2-nonanone and 2-undecanone from Fluka Chemical Corp.) or from plant extracts of known composition (the trapped headspace volatiles from *A. cepa* and *A. sativum*).

High-Resolution Mass Spectrometry (HR-MS). HR-MS data of the major constituent of the neem seed volatile concentrate were acquired on a Varian MAT 731 double-focusing high-resolution mass spectrometer operating at an ionization potential voltage of 70 eV. The exact molecular mass was calculated by high-resolution peak matching with the m/z 142.9920 reference peak of perfluorokerosene (PFK), which was used as the standard matching compound.

Insect Bioassays. Authentic di-*n*-propyl disulfide and diallyl disulfide (di-2-propenyl disulfide) (Pfaltz and Bauer, Inc.) were tested against *Aedes aegypti* (L.) (yellow fever mosquito), *Heliothis zea* (Boddie) (corn earworm), and *Heliothis virescens* (Fabr.) (tobacco budworm).

Third-instar *A. aegypti* (Rockefeller strain), originating from the Vector Biology Laboratories of the University of Notre Dame, were transferred with a 2.5 × 2.5 cm circle of ordinary window screen to 1-oz. plastic cups (8–10 larvae/cup) containing a graduated concentration series of 0.1% acetone-diluted test compounds in 10 mL of distilled water. Care was taken to remove excess water before the larvae were entered into the test solutions. The larvae were fed dog chow and maintained at 28 °C with 24-h daily illumination. Following 72 h of exposure to the treated water, the concentrations causing 50% mortality were estimated with 95% confidence limits by the method of Litchfield and Wilcoxon (1949). Each compound was assayed six times with four treatments using 8–10 larvae/treatment.

First-instar *H. zea* and *H. virescens*, originating from USDA laboratories, were transferred with a camel hair brush onto artificial diet inside 4-dram plastic vials. The test compounds were incorporated into the diet as previously described (Chan et al., 1978; Kubo and Klocke, 1983). The larvae were maintained at 28 °C, 80% relative humidity, and 24-h daily illumination. Following 12 days of exposure to the treated diet, the concentrations causing 50% mortality and 50% growth inhibition were estimated with 95% confidence limits by the method of Litchfield and Wilcoxon (1949). Each compound was assayed three times with four treatments using 10 larvae/treatment.

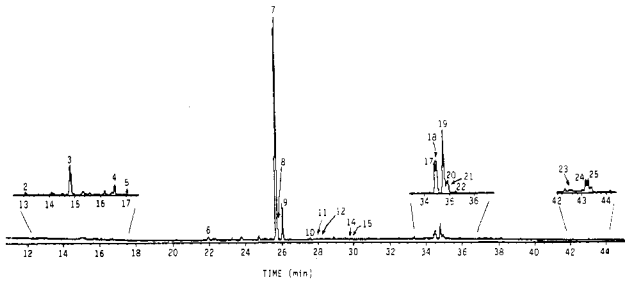

Figure 1. Typical capillary GC/MS total ion chromatogram (TIC) of the headspace volatiles from crushed neem seeds. Organosulfur compounds are indicated by peak numbers; for identification, see Table I.

RESULTS AND DISCUSSION

The previously reported alliaceous odor of neem seed oil has been attributed to sulfur-containing compounds (Nadkarni and Nadkarni, 1954; Dey and Mair, 1973; Radwanski, 1977a,b; Jacobson, 1986). However, there have been no detailed analytical studies done to characterize the sulfur-containing volatile constituents of neem seeds. In our studies on the biologically active constituents from the neem tree, we noticed that, immediately after crushing, fresh neem seeds exhibit a strong alliaceous odor without eliciting any lachrymatory effect. The alliaceous odor of crushed neem seeds diminishes slowly over time. Attempts to isolate the volatile constituents from neem seeds by steam distillation or solvent (CH_2Cl_2) extraction followed by sample concentration in vacuo at room temperature considerably diluted and diminished the alliaceous odor. Because of the extreme volatility of the odor principles, the headspace volatiles from crushed fresh neem seeds were, therefore, trapped on the Amberlite polymeric adsorbent XAD-4 (Woodrow and Seiber, 1978).

Both the XAD-4 resin traps containing volatiles from neem seeds and the neem seed volatiles eluted from the XAD-4 resin exhibited the same strong alliaceous odor as freshly crushed neem seeds. Our initial organoleptic evaluation of the neem seed volatiles was confirmed by qualitative GC analysis using a flame photometric detector (FPD) operated in the sulfur-specific mode. The neem seed volatile concentrate contained two FPD-detectable peaks: a major peak (t_R = 12.8 min) and a minor peak (t_R = 13.6 min) when the GC was operated isothermally at 100 °C. This GC analysis positively demonstrated the presence of volatile organosulfur compounds of relatively simple composition in the trapped headspace volatiles from crushed neem seeds. Similar chromatographic separation patterns were also obtained by GC/FID (t_R = 21.73 and 22.10 min, respectively, for the major and minor constituent peaks).

A GC/MS total ion chromatogram (TIC) of the volatile concentrate of fresh neem seeds is shown in Figure 1. From this GC/MS trace, the relative simplicity of the neem seed volatile concentrate and the prominence of its two major components, di-*n*-propyl disulfide and *n*-propyl *trans*-1-propenyl disulfide (i.e., peaks 7 and 9), are evident. A list of identified and tentatively identified GC/MS-detectable neem seed volatiles, their retention times, percent areas, and mass spectral data (ions) is shown in Table I. Three closely eluting peaks, i.e., 7–9, constitute 88.17%

of the total area, while all of the detected compounds together (25 in all) account for 98.74% of the TIC area.

Characteristic sulfur isotope (P + 2) peaks (Beynon et al., 1968; Shrader, 1971) were observed in the mass spectra of most of the volatile compounds from neem seeds, thus confirming the presence of this element in most of the trapped volatiles. Furthermore, most of the mass spectral fragment ions and fragmentation patterns observed were consistent with those known for the organosulfur compounds (polysulfides) listed in Table I or their homologous derivatives (see supplementary material) (Spåre and Virtanen, 1963; Brodnitz et al., 1969; Nishimura et al., 1970, 1973; Boelens et al., 1971; Schreyen et al., 1976; Heller and Milne, 1978, 1980; Gupta et al., 1981; Wu et al., 1982; Gupta and Joshi, 1983).

Of the 25 volatile neem constituents identified, 22 were organosulfur compounds. Most of the sulfur-containing compounds identified were either aliphatic disulfides (eight in all) or higher polysulfides (nine trisulfides and three tetrasulfides). A number of the di- and polysulfides present in the neem seed volatile concentrate were mixed unsymmetrical aliphatic alkyl (methyl, propyl, butyl) and alkenyl (propenyl) containing derivatives. However, no monosulfides or dimethyl polysulfides (often observed in onion and garlic extracts) were found among the neem seed volatiles (Table I).

The major compound, peak 7 (75.74% area, t_R = 25.71 min), was identified as di-*n*-propyl disulfide ($C_6H_{14}S_2$; $M^{\bullet+}$, m/z 150) by GC/MS and by comparison (cochromatography) with an authentic standard and the trapped onion volatiles. Peaks 8 and 9 were identified by GC/MS as cis and trans isomers of *n*-propyl 1-propenyl disulfide, respectively. Other minor organosulfur compounds were tentatively identified by GC/MS as derivatives of di-*n*-propyl-, *n*-propyl 1-propenyl-, and di-1-propenyl trisulfides and tetrasulfides, and *n*-propyl butyl disulfide (see Table I). These identifications were further supported by comparison of these GC/MS data with those obtained from the trapped onion and garlic volatiles and literature data (Carson and Wong, 1959, 1961; Spåre and Virtanen, 1963; Bernhard, 1968; Brodnitz et al., 1969; Brodnitz and Pollock, 1970; Nishimura et al., 1970, 1971b, 1973; Boelens et al., 1971; Johnson et al., 1971; Schreyen et al., 1976; Yagami et al., 1980; Gupta et al., 1981; Wu et al., 1982; Gupta and Joshi, 1983).

The exact molecular mass of peak 7 was determined unequivocally by HR-MS. When the neem seed volatile concentrate was analyzed by HR-MS, a single measured molecular mass of 150.052 60 was observed. This peak was computer correlated to the calculated molecular mass of 150.053 71, corresponding to the molecular formula $C_6H_{14}S_2$ (di-*n*-propyl disulfide).

Except for the absence of the lachrymatory factors and certain other minor constituents, the GC/MS chromatograms of the neem seed volatiles generally closely resemble those of the onion volatiles, both qualitatively and quantitatively. Furthermore, the presence of di-*n*-propyl disulfide (the major neem seed volatile constituent), the *n*-propyl 1-propenyl disulfides, and 2-methyl-2-pentenal, together with the absence of diallyl disulfide, indicate that the neem seed volatile concentrate is much more like that of onion (*A. cepa*) (Brodnitz et al., 1969; Boelens et al., 1971; Johnson et al., 1971; Yagami et al. 1980; Block, 1985) than that of garlic (*A. sativum*) (Johnson et al., 1971; Block, 1985). The similarity between onion and neem seed volatiles extends to details such as the ratios between the 2,4- and 3,4-dimethylthiophenes (Boelens et al., 1971) and the cis and trans isomers of the 1-propenyl derivatives. However, unlike onions, neem seeds do not exhibit lachrymogenic effects even though they contain volatile organosulfur chemicals presumably derived biosynthetically from a lachrymatory precursor (Yagami et al., 1980).

The detection of small amounts of dimethylthiophenes upon GC/MS analysis of the neem seed volatile concentrate suggests that some thermal decomposition of alkenyl (propenyl) disulfides (with a loss of hydrogen sulfide) occurs during the analysis (Boelens et al., 1971; Schreyen et al., 1976; Wu et al., 1982). It is possible that some of the other trace constituents identified among the neem seed volatiles may also be heat-generated artifacts produced upon GC/MS analysis of thermolabile precursors. Polysulfides and other sulfurous compounds are easily split by temperatures greater than about 180 °C. Therefore, injector and transfer line temperatures of 200 and 270 °C, respectively, are likely to cause molecular rearrangement and the production of artifacts (Boelens et al., 1971; Hiley and Cameron, 1975; Wajon et al., 1985).

Both neem oil (Radwanski, 1977a,b; Schmutterer et al., 1982; Schmutterer and Ascher, 1984) and garlic oil (Greenstock, 1970) have been reported to exhibit insecticidal activities. For example, neem oil (Jotwani and Srivastava, 1981 and references cited therein) and garlic oil (Amonkar and Reeves, 1970) have both been found to be mosquito larvicides. The mosquito larvicidal principles of garlic oil were identified as diallyl disulfide (the major garlic volatile oil constituent) and trisulfide (Amonkar and Banerji, 1971). We have identified the major neem seed volatile constituent, di-*n*-propyl disulfide, as a mosquito larvicidal principle of neem seed oil. However, di-*n*-propyl disulfide was about 10-fold less active as a larvicide against *A. aegypti* (Table II) and about 3- or 4-fold less active as a larvicide and growth inhibitor against *H. zea* and *H. virescens* than diallyl disulfide (Table III). A more potent effect of diallyl disulfide compared to di-*n*-propyl disulfide was also reported by Amonkar and Banerji (1971), who found that a concentration of 5 ppm of the former compound killed *Culex pipiens quinquefasciatus* larvae but that a concentration of 200 ppm of the latter compound was inactive.

As discussed previously, both neem seeds and expressed neem seed oil (margosa oil) possess a strong, sharp, garliclike odor (Nadkarni and Nadkarni, 1954; Dey and Mair, 1973). Other parts of the neem tree also possess alliaceous odors. For example, neem leaflets exhibit a noticeable onionlike odor (Kunkel, 1978), and the steam-distilled leaf oil possesses a highly penetrating odor (Chopra et al., 1952). In addition, the moistened inner bark of the tree produces a garliclike odor as a result of enzymatic activity leading to the formation of volatile organosulfur compounds (Dey and Mair, 1973). Neem timber and heartwood also exhibit unpleasant and offensive odors when they are fresh and/or worked (Irvine, 1961; Kunkel, 1978). Thus, it appears that almost all of the above-ground parts of the neem tree may possess the disagreeable odor of volatile organosulfur compounds.

The presence of volatile organosulfur compounds may explain, at least in part, the insect repellent effects of neem leaves and seeds. Neem leaves are known to repel numerous insects and arachnids, including book mites, locusts, white ants (Nadkarni and Nadkarni, 1954; Irvine, 1961; Dey and Mair, 1973; Radwanski, 1977b; Kunkel, 1978), and cockroaches (Prakash, R. K., personal communication). A commercial neem seed extract preparation was found to be strongly repellent to American cockroaches (Adler and Uebel, 1985). In Sri Lanka, neem leaves and seed oil are used as an insect repellent against flies on

Table I. Capillary GC/MS Analysis of the Headspace Volatiles from Crushed Neem Seeds[a]

TIC peak no.	compound	t_R,[d] min	area %	major characteristic MS ions, m/z (rel intens)[f]
1	2-methyl-2-pentenal[b]	9.97	tr	98 ($M^{\bullet+}$, $C_6H_{10}O$, 39%), 97 (M − H, 5), 83[g] (14), 79 (M − H − H_2O, 5), 69 (34), 67 (4), 55 (36), 53 (14), 43 (25), 41 (100)
2	2,4-dimethylthiophene	13.07	tr	112 ($M^{\bullet+}$, C_6H_8S, 63%), 111 (M − H, 100), 97[g] (40), 53 (1), 45 (56)
3	3,4-dimethylthiophene	14.83	0.10	112 ($M^{\bullet+}$, C_6H_8S, 83%), 111 (M − H, 100), 97[g] (54), 77 (22), 71 (14), 69 (13), 67 (10), 57 (8), 53 (8), 51 (9), 45 (56)
4	methyl *n*-propyl disulfide	16.68	tr	122 ($M^{\bullet+}$, $C_4H_{10}S_2$, 64%), 93[h] (14), 80[i] (100), 64 (23), 47 (24), 45 (62), 43 (86), 41 (65)
5	methyl 1-propenyl disulfide	17.15	tr	120 ($M^{\bullet+}$, $C_4H_8S_2$, 62%), 105[g] (5), 87[j] (8), 80 (24), 75 (27), 73 (27), 72 (46), 64 (12), 61 (10), 59 (14), 47 (28), 45 (100), 41 (48)
6	methyl butyl disulfide	21.92	tr	136 ($M^{\bullet+}$, $C_5H_{12}S_2$, 28%), 121[g] (6), 107[h] (10), 89[k] (86), 79[l] (12), 73 (20), 61 (42), 57 (26), 55 (34), 47 (37), 45 (61), 41 (100)
7	di-*n*-propyl disulfide[c]	25.71	75.74	150 ($M^{\bullet+}$, $C_6H_{14}S_2$, 38%), 108[i] (26), 79 (6), 73 (10), 66 (18), 64 (5), 45 (24), 43 (100), 41 (56)
8	*n*-propyl *cis*-1-propenyl disulfide	25.81[e]	2.76	148 ($M^{\bullet+}$, $C_6H_{12}S_2$, 72%), 106[i] (58), 78 (21), 73 (25), 72 (26), 64 (25), 45 (71), 43 (53), 41 (100)
9	*n*-propyl *trans*-1-propenyl disulfide	26.04	9.67	148 ($M^{\bullet+}$, $C_6H_{12}S_2$, 65%), 106[i] (54), 78 (20), 73 (27), 72 (28), 64 (26), 61 (14), 59 (18), 47 (22), 45 (83), 43 (59), 41 (100)
10	methyl *n*-propyl trisulfide	27.52	tr	154 ($M^{\bullet+}$, $C_4H_{10}S_3$, 99%), 112[i] (64), 79 (30), 78 (15), 73 (14), 64 (36), 47 (90), 45 (72), 43 (100), 41 (95)
11	methyl *cis*-1-propenyl trisulfide	27.92[e]	tr	152 ($M^{\bullet+}$, $C_4H_8S_3$, 24%), 88[m] (50), 45 (100)
12	methyl *trans*-1-propenyl trisulfide	28.16	tr	152 ($M^{\bullet+}$, $C_4H_8S_3$, 28%), 105[k] (8), 88[m] (60), 79 (17), 73 (35), 71 (36), 64 (15), 59 (15), 57 (34), 47 (24), 45 (100), 43 (28), 41 (38)
13	*n*-nonan-2-one (2-nonanone)[c]	29.24	tr	142 ($M^{\bullet+}$, $C_9H_{18}O$, not obsd), 71 ($CH_3CO^+(CH_2)_2$, 31%), 58 ($(CH_3)_2CO^{\bullet+}$, 62), 43 (CH_3CO^+, 100)
14	*n*-propyl butyl disulfide	29.77	0.38	164 ($M^{\bullet+}$, $C_7H_{16}S_2$, 34%), 122[i] (6), 108[n] (30), 57 (56), 45 (26), 43 (96), 41 (100)
15	1-propenyl butyl disulfide	29.90	tr	162 ($M^{\bullet+}$, $C_7H_{14}S_2$, 12%), 105[l] (3), 89[o] (100), 73 (16), 61 (34), 59 (10), 57 (11), 55 (18), 47 (30), 45 (56), 41 (66)
16	*n*-hendecan-2-one (2-undecanone)[c]	33.12	tr	170 ($M^{\bullet+}$, $C_{11}H_{22}O$, not obsd), 71 ($CH_3CO^+(CH_2)_2$, 30%), 58 ($(CH_3)_2CO^{\bullet+}$, 68), 43 (CH_3CO^+, 100)
17	di-*n*-propyl trisulfide	34.41[e]	2.19	182 ($M^{\bullet+}$, $C_6H_{14}S_3$, 54%), 150[p] (7), 140[i] (5), 117 (4), 108 (5), 98 (11), 75 (93), 73 (18), 64 (16), 47 (23), 45 (34), 43 (100), 41 (93)
18	*n*-propyl *cis*-1-propenyl trisulfide	34.48[e]	2.20	180 ($M^{\bullet+}$, $C_6H_{12}S_3$, 56%), 151[h] (4), 116[m] (24), 115 (30), 106 (33), 83 (29), 74 (72), 73 (44), 64 (42), 59 (27), 45 (91), 41 (100)
19	*n*-propyl *trans*-1-propenyl trisulfide	34.78	4.22	180 ($M^{\bullet+}$, $C_6H_{12}S_3$, 60%), 151[h] (6), 116[m] (26), 115 (28), 106 (34), 83 (30), 74 (75), 73 (53), 64 (41), 59 (26), 47 (22), 45 (95), 41 (100)
20	*n*-propyl 2-propenyl trisulfide[b]	34.92[e]	0.62	180 ($M^{\bullet+}$, $C_6H_{12}S_3$, 29%), 116[m] (27), 74 (43), 73 (66), 64 (18), 47 (25), 45 (100), 43 (56), 41 (84)
21	di-*cis*-1-propenyl trisulfide[b]	35.04[e]	0.42	178 ($M^{\bullet+}$, $C_6H_{10}S_3$, 22%), 114[m] (49), 105[o] (22), 73 (33), 71 (27), 61 (25), 59 (18), 47 (22), 45 (100), 41 (68)
22	di-*trans*-1-propenyl trisulfide[b]	35.25	0.23	178 ($M^{\bullet+}$, $C_6H_{10}S_3$, 18%), 146[p] (2), 114[m] (37), 105[o] (20), 74 (28), 73 (26), 71 (22), 61 (22), 59 (17), 47 (17), 45 (100), 41 (53)
23	di-*n*-propyl tetrasulfide	42.49	tr	214 ($M^{\bullet+}$, $C_6H_{14}S_4$, 14%), 147 (19), 108 (10), 73 (100), 71 (10), 47 (26), 43 (46), 41 (62)
24	*n*-propyl *cis*-1-propenyl tetrasulfide	43.16	0.11	212 ($M^{\bullet+}$, $C_6H_{12}S_4$, 15%), 147 (22), 138 (15), 115 (7), 106 (4), 74 (51), 73 (100), 64 (11), 59 (14), 45 (58), 41 (57)
25	*n*-propyl *trans*-1-propenyl tetrasulfide	43.25	0.10	212 ($M^{\bullet+}$, $C_6H_{12}S_4$, 15%), 147 (22), 138 (14), 115 (4), 106 (6), 74 (52), 73 (100), 64 (9), 59 (12), 57 ($HC{\equiv}CS^+$, 36), 45 (60), 41 (74)
			98.74	

[a] See text for GC/MS conditions. [b] Tentative compound identification based on MS and t_R data. [c] Compound identification confirmed by cochromatography and spectral comparison (GC/MS) with an authentic standard. [d] $T_R \pm 0.04$ min (SD obtained from nine GC/MS runs). [e] Poorly resolved by capillary GC; compounds identified by means of extracted ion chromatograms and mass spectra obtained by subtraction. [f] See supplementary material for identification of many of the mass spectral fragment ions. [g] M − 15 = M − $CH_3^{\bullet}$. [h] M − 29 = M − $(CH_3CH_2^{\bullet})$. [i] M − 42 = M − $(CH_3CH{=}CH_2)$. [j] M − 33 = M − $SH^{\bullet}$. [k] M − 47 = M − $CH_3S^{\bullet}$. [l] M − 57 = M − $(CH_3(CH_2)_3^{\bullet})$. [m] M − 64 = M − S_2. [n] M − 56 = M − $(CH_3CH_2CH{=}CH_2)$. [o] M − 73 = M − $(CH_3CH{=}CHS^{\bullet})$. [p] M − 32 = M − S.

Table II. Mortality in Third-Instar *Aedes aegypti* Immersed in Aqueous Suspensions of Diallyl Disulfide (Di-2-propenyl Disulfide) and Di-*n*-propyl Disulfide

test compound	LC_{50},[a] ppm
diallyl disulfide	6 (5–7)[b]
di-*n*-propyl disulfide	66 (61–72)

[a] LC_{50} values are the concentrations causing 50% mortality. [b] Numbers in parentheses are the 95% confidence limits determined by the method of Litchfield and Wilcoxon (1949).

Table III. Mortality and Growth Inhibition in First-Instar *H. zea* and *H. virescens* Fed Diallyl Disulfide (Di-2-propenyl Disulfide) and Di-*n*-propyl Disulfide in Artificial Diet

insect sp.	test compound	LC_{50},[a] ppm	EC_{50},[b] ppm
H. zea	diallyl disulfide	280 (236–333)[c]	240 (174–332)
	di-*n*-propyl disulfide	980 (717–1339)	940 (785–1126)
H. virescens	diallyl disulfide	340 (266–434)	230 (174–304)
	di-*n*-propyl disulfide	1000 (887–1127)	850 (716–1010)

[a] LC_{50} values are the concentrations causing 50% mortality. [b] EC_{50} values are the concentrations causing 50% growth inhibition. [c] Numbers in parentheses are the 95% confidence limits determined by the method of Litchfield and Wilcoxon (1949).

cattle, postharvest insect pests, and stored-products pests (Ganesalingam, 1986). Neem seeds and seed oil are reportedly burned to repel mosquitoes and flies, and neem timber has a characteristic odor reported to prevent insect attack (Islam, 1984; Ganesalingam, 1986). The odor of burning powdered neem leaves is reportedly fatal to insects (Radwanski, 1977a). Furthermore, in parts of India, neem trees planted around villages are believed to promote a healthy environment by acting as a prophylactic against malaria (Nadkarni and Nadkarni, 1954; Dey and Mair, 1973). Both neem seed kernels and their volatiles (i.e., the distillate prepared in vacuo from crushed neem kernels) exhibited strong olfactory repellency (i.e., at a distance, without contact) against adult female *Heliothis armigera* (Hübn.) moths (Saxena and Rembold, 1984). The white-backed and brown female rice planthoppers (*Sogatella furcifera* (Horváth) and *Nilaparvata lugens* (Stål), respectively) were repelled by the odor of neem oil (Stål), (Heyde et al., 1984). Saxena et al. (1982) report that neem seed oil repels first-instar larvae of the rice leaf folder (*Cnaphalocrocis medinalis* (Guenée)) and that the strong, garlicky odor of neem oil may be an ovipositional repellent to the female moths and/or a repellent to the parasites of this insect species.

Neem organosulfur compounds and/or their biogenetic precursors may also play a significant role in the purported pharmacological properties and folk medicinal efficacy of neem preparations. The neem tree, which is indigenous to India, has been used as a traditional remedy in Hindu (Ayurvedic) medicine since antiquity. Neem seed (margosa) oil has been used in India (and elsewhere) as an antimalarial, febrifuge, anthelmintic, and vermifuge (for the expulsion of intestinal worms), for its antiseptic and antimicrobial properties against leprosy, scrofula, consumption (tuberculosis), and bronchitis, and externally as a healing agent against various skin diseases, erysepelas, wounds, boils, and ulcers (Chopra et al., 1952; Nadkarni and Nadkarni, 1954; Irvine, 1961; Dey and Mair, 1973; Radwanski, 1977a; Kunkel, 1978; Sinniah and Baskaran, 1981). Neem leaf paste also has been used externally in the treatment of smallpox (Nadkarni and Nadkarni, 1954). In Sri Lanka, neem is utilized in the manufacture of antimicrobial bath soap used to prevent topical skin infections (Ganesalingam, 1986).

Neem has been reported to exhibit antibiotic activity (Lewis and Elvin-Lewis, 1977, 1983). Both neem seed oil and steam-distilled leaf oil have been shown to exhibit marked antibacterial activity against *Staphylococcus aureus* and other pathogenic bacteria, including the causative (etiologic) agents of tuberculosis (*Mycobacterium tuberculosis*), typhoid fever (*Bacillus typhosus*), cholera (*Vibrio cholerae*), and plague (*Pasteurella pestis*) (Chopra et al., 1952). It is noteworthy that the causative agent of leprosy, against which neem seed oil is purportedly effective (i.e., *Mycobacterium leprae*), is closely related to *M. tuberculosis* (i.e., both are mycobacteria). Furthermore, certain organosulfur compounds, such as the sulfone dapsone, have been found to be potent antileprotic agents useful as drugs, and sulfones have also been used recently as antimalarial agents (Gupta and Joshi, 1983). In addition, compounds such as diethyl disulfide have been reported to exhibit significant antituberculous effects (Virtanen, 1965).

Onion and garlic oils are well-known to exhibit antibiotic activity against pathogenic bacteria such as *S. aureus* and *Streptococcus* spp., as well as the causative agents of bacillary dysentery and shigellosis (*Bacillus dysenteriae*, *Shigella dysenteriae*), gastroenteritis (*Bacillus enteritidis*), typhoid fever (*B. typhosus*), and cholera (*V. cholerae*) (Kohman, 1947; Virtanen and Matikkala, 1959; Virtanen, 1962, 1965; Al-Delaimy and Ali, 1970; Block, 1985). Johnson and Vaughn (1969) found that garlic and onion preparations were lethal to cultures of *Salmonella typhimurium* and *Escherichia coli* and that di-*n*-propyl disulfide exhibited either bacteriostatic or bactericidal effects against *S. typhimurium* depending upon culture conditions. Onion and garlic oils and extracts also exhibit significant antifungal activity against various zoopathogenic fungi and yeasts (Conner and Beuchat, 1984; Block, 1985). Neem seed oil, cake, and leaf extract also have been shown to exhibit significant antifungal activity (Singh et al., 1980, 1984; Radwanski and Wickens, 1981), this activity being attributed, at least in part, to their sulfur-containing compounds (Singh et al., 1980; Radwanski and Wickens, 1981). Dey and Mair (1973) also attribute the general antiseptic activity of neem seed oil, bark, and leaves to their sulfur-containing compounds. Thus, it is possible that the strong antibiotic activity of neem seed oil may be partly attributable to its organosulfur constituents, thereby substantiating some of its reputed uses in traditional Hindu (Ayurvedic) medicine.

The similarity in composition of neem seed and onion volatiles suggests that similar biosynthetic pathways and biochemical processes may be operating in both plant species. The neem seed volatiles analyzed here by GC/MS are probably artifacts generated by enzymatic activity when the seeds are crushed. The enzyme(s) involved may be of the allinase/C-S lyase type acting upon nonvolatile precursor substrates related to *trans*-*S*-(1-propenyl)-L-cysteine sulfoxide and other S-substituted L-cysteine sulfoxide derivatives (alliin-type sulfoxide amino acids) (Virtanen, 1962, 1965; Spåre and Virtanen, 1963; Bernhard, 1968; Nishimura et al., 1970, 1971a; Johnson et al., 1971; Yagami et al., 1980; Block, 1985). As in the case of onion, neem seed precursor substrates may also include *S*-methyl- and *S*-*n*-propyl-L-cysteine sulfoxides (Virtanen and Matikkala, 1959; Block, 1985). However, it is noteworthy that no lachrymatory factors or lachrymogenic effects were detected among the neem seed volatiles. This suggests a situation that may be analogous to that of American wild onions, which have been reported to possess a typical onion aroma without exhibiting any lachrymatory properties (Johnson et al., 1971).

The most probable chemical–ecological explanation for the presence of biologically active volatile organosulfur compounds in neem seeds is that these compounds, like many other plant secondary metabolites, may play a role in chemical defense mechanisms against invading microorganisms, herbivorous insects, and higher animals. Due to their extreme volatility and pungency, these compounds may serve as a repellent to attacking insects (Block, 1985) before they can cause significant injury to neem seeds.

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Registry No. 2-Methyl-2-pentenal, 623-36-9; 2,4-dimethylthiophene, 638-00-6; 3,4-dimethylthiophene, 632-15-5; methyl *n*-propyl disulfide, 2179-60-4; methyl 1-propenyl disulfide, 5905-47-5; methyl butyl disulfide, 60779-24-0; di-*n*-propyl disulfide, 629-19-6; *n*-propyl *cis*-1-propenyl disulfide, 23838-20-2; *n*-propyl *trans*-1-propenyl disulfide, 23838-21-3; methyl *n*-propyl trisulfide, 17619-36-2; methyl *cis*-1-propenyl trisulfide, 23838-24-6; methyl *trans*-1-propenyl trisulfide, 23838-25-7; 2-nonanone, 821-55-6; *n*-propyl butyl disulfide, 72437-64-0; 1-propenyl butyl disulfide, 115321-79-4; 2-undecanone, 112-12-9; di-*n*-propyl trisulfide, 6028-61-1; *n*-propyl *cis*-1-propenyl trisulfide, 23838-26-8; *n*-propyl *trans*-1-propenyl trisulfide, 23838-27-9; *n*-propyl 2-propenyl trisulfide, 33922-73-5; di-*cis*-1-propenyl trisulfide, 115321-80-7; di-*trans*-1-propenyl trisulfide, 115321-81-8; di-*n*-propyl tetrasulfide, 52687-98-6; *n*-propyl *cis*-1-propenyl tetrasulfide, 115321-82-9; *n*-propyl *trans*-1-propenyl tetrasulfide, 115321-83-0.

Supplementary Material Available: Identification of some selected mass spectral fragment ions of neem organosulfur compounds (polysulfides) observed by GC/MS (3 pages). Ordering information is given on any current masthead page.

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