Identification of the Major Monoterpenes in the Leaf Oil of *Gossypium sturtianum* var. *nandewarense* (Der.) Fryx.

The major monoterpenes in the leaf oil of *Gossypium sturtianum* var. *nandewarense* (Der.) Fryx. have been identified by gas chromatography–mass spectroscopy to be α-pinene, β-pinene, limonene, γ-terpinene, and a mixture of β-phellandrene and *p*-cymene. The largest component in the oil is an unidentified sesquiterpene, and the data from its infrared and mass spectra are reported.

The terpenes present in the leaves of plants are generally believed to be the result of a secondary metabolism. The composition of volatile leaf oils have been widely used for taxonomic studies (Thompson et al., 1971). Although reasonable taxonomic relationships can be established by gas chromatography, confirmatory identifications of each chemical structure by an independent method, e.g., infrared absorption spectra or mass spectra, have been useful and interesting. Accidental coincidences have been observed in some oil studies (Ikeda et al., 1962). The commonly studied plants used for medicinal or flavoring purposes are moderately rich in oil with some species containing macroscopic oil glands. However, the presence of plant oils in less amount is quite widespread. The amount of terpenes present and their seasonal variation in forage or fodder is suspected to be important in the flavor of some cheeses (Dumont and Adda, 1978). The possibility that the essential oil of the cotton plant contains constituents attractive to the boll weevil, *Anthonomus grandis* Boheman, has caused some interest in the study of the composition of that oil. Eleven monoterpenes have been positively identified in the species *Gossypium hirsutum* L. cv. Deltapine Smoothleaf (Hedin et al., 1973; Minyard et al., 1965). The essential oil from the leaves of *Gossypium barbadense* L. cv. Giza 69 has been examined (Hedin et al., 1972) because it had been shown to be highly attractive to newly hatched larvae of *Spodoptera littoralis* (Boisduval) (Salama et al., 1971). We wish to report on the composition of the volatile oil from *Gossypium sturtianum* var. *nandewarense*. Both gas chromatography (GC) and GC–mass spectroscopy were used for these studies. The major monoterpene constituents were identified as α-pinene, I; β-pinene, II; limonene, IV; γ-terpinene, V; and a mixture of β-phellandrene and *p*-cymene, VI. The largest ion peak from the mass spectrograms is an unidentified sesquiterpene. Its infrared spectrum does not correspond to any of the 59 high-resolution spectra reported by Wenninger et al. (1967) and Wenninger and Yates (1970). The data from its infrared spectrum and mass spectrum are reported below.

EXPERIMENTAL SECTION

Gas Chromatography. A Varian Aerograph Series 1520 with a matrix temperature programmer was connected through a Spectra Physics autolab system I to a Beckman 10-in. recorder. It was equipped with a 10 ft × $^1/_4$ in. o.d. stainless column with a 20% LAC 446 on 60–80 mesh Chromosorb W packing. Thermal conductivity detectors were used with a helium carrier gas flow adjusted to 65 mL/min at the start of the program. The time interval for the 20-point programmer was set at a 5.9-min interval and the initial oven temperature set at 50 °C. The program was isothermal through point 5, a 6 °C/min plug in 6, isothermal plug in 7, a 4 °C/min plug in 10, isothermal plug in 11, a 2 °C/min plug in 13, and isothermal plug in 18. The injector temperature was 85 °C and detector temperature was 190 °C. Samples were condensed in 1.8-mm capillary tubes by holding a piece of dry ice on the tube and attaching it to the exhaust port with a septum as a gasket.

Infrared Spectroscopy. A Beckman IR-12 was used with a neat sample in a 0.05-mm pathlength microcell with KBr crystals.

GC–Mass Spectrometry. A Varian Aerograph Series 1400 gas chromatograph was connected to a Finnigan 1015 S/L: quadrupole mass spectrometer through a Goelke separator. The mass spectrometer was controlled by a pdp 8/m system/150 computer with a teletype input and output. Additional data processing was available through a remote terminal connection to an H.P. 3000 central computer. A 10 ft × $^1/_8$ in. o.d. stainless steel column with the same packing as above was used with helium as the carrier gas. The temperature program used for the oven was: isothermal at 50 °C for 50 min, then increased at a rate of 1 °C/min for 105 min, then isothermal at 155 °C for 25 min. The total run time was 180 min. A 5 ms/amu sweep rate was used from 10 to 140 amu and a 7 ms/amu sweep was used from 141 to 300 amu, with unit resolution for the entire range.

Open pollinated seed of *G. sturtianum* var. *nandewarense* (LA1503) was obtained from T. Richmond of Texas A&M University, and two plants were grown during 1977 on the Experimental Station at University of California, Riverside. Leaf tissue, approximately 650 g of fresh weight, was homogenized with 1.0-L of water in a carbon dioxide atmosphere with a 2-gal Waring blender. The macerated mass was transferred with a minimum of additional water to a 3.0-L, two-neck, round-bottom flask and steam distilled with a Clevenger apparatus for oils lighter than water. Refrigerated cooling solution at 0 °C was circulated through the cold finger. The steam distillation was continued for 1.5 h and the oil collected and transferred to a 1-dram screw-cap vial and stored in a freezer when not in use. A large sample (15.0 μL) was injected in the 1520 for determining the composition of the oil and trapping; and a smaller sample (1.5 μL) was used to determine the respective elution times more precisely. A 5.0-μL sample was injected in the GC–MS to obtain mass spectra. Reference materials used were: α-pinene, β-pinene, and *d*-limonene from Fritzsche Dodge and Olcott Inc.; α-terpinene and γ-terpinene from The Glidden Company; α-phellandrene and β-phellandrene from Givaudan Corp.; and *p*-cymene from Florasynth Inc.

RESULTS AND DISCUSSION

A reconstructed gas chromatogram from GC–MS is shown in Figure 1. The peaks are normalized with the total ion current of the largest peak at 100. This peak, VII, is an unidentified sesquiterpene with the following mass spectrum; *m/e*, with relative peak intensity in parentheses are: 121 (100); 93 (89); 107 (56); 91 (44); 79 (43); 105 (41); 119 (36); 81 (33); 161 (28); 77, 67 (24); 94, 41 (23); 120, 92 (18); 136, 55 (17); 122 (16); 108, 106, 53 (13); 133, 95, 80 (12); 134 (11); 204 (9). Strong infrared absorption was observed in cm^{-1} at: 3020, 2960, 2030, 2860, 1657, 1460, 1415, 1395, 1380, 1368, 1205, 1181, 1151, 1125, 1070, 997,

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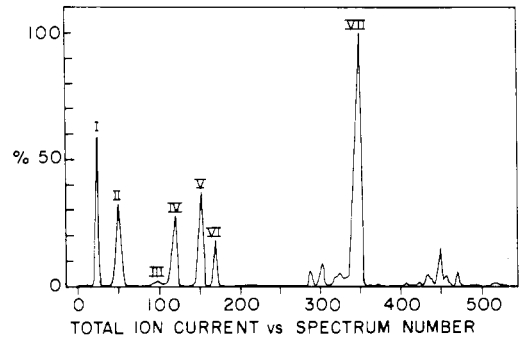

Figure 1. Reconstructed gas chromatogram. The abscissa is normalized to 100% for the ion current of the largest peak. The ordinate gives the spectrum number.

Table I. Composition of Oil

terpenes	time of elution, min	% of oil
α-pinene	21.97	8.0
β-pinene	34.50	7.2
α-terpinene	40.80	0.6
limonene	42.77	14.3
γ-terpinene	47.07	19.7
β-phellandrene + *p*-cymene	50.63	7.6
major sesquiterpene	93.93	34.6

983, 929, 887, 880, 868, 850, 832, 790, 578, 554, 510, 498, 489. In order of elution, the following monoterpenes have been identified by comparing elution times and quadrupole mass spectra with the reference compounds: α-pinene, I; β-pinene, II; α-terpinene, III; limonene, IV; γ-terpinene, V; and β-phellandrene cochromatographing with approximately 11% *p*-cymene, VI. Ikeda et al. (1962) have reported that *p*-cymene cannot be separated from terpinolene with a column using diethyleneglycol succinate as the stationary phase and we find that our LAC-446 column does not resolve *p*-cymene from β-phellandrene. All of the monoterpenes found in our leaf oil have already been reported by Minyard et al. (1965) to be present in the flower buds of Deltapine smoothleaf cotton, *Gossypium hirsutum*. α-Pinene and γ-terpinene were found in the air-dried powdered cotton leaf of *Gossypium barbadense* L. cv. Giza 69 by Hedin et al. (1972).

The composition of the oil, determined with the Varian Aerograph Series 1520, is given in Table I. Studies on the identification of our main sesquiterpene are still in progress.

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Extraction of Chlorpyrifos-methyl Residues from Aqueous Solution and Analysis by Flame Photometric Gas Chromatography

Methods for extractions and analyses of samples of natural water and basal salt medium for bacterial growth containing traces of chlorpyrifos-methyl have been developed. Aqueous solutions were extracted by high-speed stirring after addition of a small volume of hexane in the sample. Extracts of lower concentration were concentrated by evaporation prior to analyses by flame photometric gas chromatography to yield a detection limit of 1 ppb. The extracts from all aqueous solutions were found to be relatively stable for 45 days. The efficiency of the extraction methods was near 100% for the water samples and near 95% for the basal salt medium samples under the experimental conditions described.

Chlorpyrifos-methyl ($C_7H_7Cl_3NO_3PS$) is similar in chemical structure to Dursban, the former being the *O,O*-dimethyl and the latter the *O,O*-diethyl form of *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate. Its broad spectrum of insecticidal activity and low mammalian toxicity make it a promising insecticide for many uses (Dow Chemical Co., 1973). The relatively few applications described thus far have been a field evaluation for the control of the spruce budworm (Hopewell, 1977), a study as a protector for hard winter wheat and seed corn by stored grain insects (LaHue, 1976, 1977), a study of its persistence in corn silage (Johnson et al., 1974), a comparison against malathion-resistant insects in wheat (Bengston et al., 1975), an evaluation for the control of *Dermestes maculatus deb.* (Coleoptera, Dermestidae) on sheep skins (MacQuillan and Shipp, 1976). Finally, an evaluation of its biological activity in soil (Harris, 1977) and metabolism studies (Bakke and Price, 1976; Whitten and Bull, 1974) were reported. Few chemical methods of analysis have been reported so far, except for a brief

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