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Turkey Mullein Volatile Constituents

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Extracts of the volatiles from Turkey mullein (*Eremocarpus setigerus*) were prepared from the leaves and stems by simultaneous steam distillation and extraction (SDE), both at atmospheric pressure (yield 0.057%) and at 100 torr (yield 0.005%). The extracts were analyzed by combined gas chromatography–mass spectrometry and GC retention matching. Identified constituents (with semiquantitative indications for the atmospheric-prepared extract) were β-pinene (21.5%), myrcene (21.3%), *trans*-ethyl cinnamate (12.7%), nonanal (4.2%), *trans*-2-methylbutyl cinnamate (3.7%), α-pinene (2.8%), *trans*-isobutyl cinnamate (2.7%), 2-methylbutyl hexanoate (2.6%), *cis*-ethyl cinnamate (2.2%), limonene (1.6%), *trans*-butyl cinnamate (1.5%), and citronellol (1.0%). Many other constituents also were identified, at concentrations less than 1%, to make a total of 59.

This is the third paper from this laboratory on composition of the volatile fraction from plants that are usually shunned by browsing deer. The first paper reported on constituents of the leaf oil from California bay (*Umbellularia californica*) (Buttery et al., 1974) and the second on volatiles from vinegar weed (*Trichostema lanceolatum* Benth) (Schultz et al., 1976).

Turkey mullein (*Eremocarpus setigerus* Benth) (also known as dove weed), a member of the spurge family, is an annual herbaceous plant with thick, pubescent, gray leaves and stems. It grows up to 8 in. high, but may be considerably lower, and spreads into mats 1–3 ft wide. It is abundant in generally dry, open areas, from near sea level to 4500 ft in California, Oregon, and Washington west of their principal ranges (Jepsen, 1925). (This plant is not in the same family as common mullein.)

A general correlation between palatability of plants to deer and digestibility as determined by in vitro gas production by deer rumen microbes has been reported by Longhurst et al. (1968). Such microbe activity was inhibited by essential oils from unpalatable plants. Turkey mullein is listed among the unpalatable species, but its essential oil was only moderately inhibitory. These authors state that olfaction is the primary factor in choice of plants, but they suggest that probably deer are influenced by the odor of "indicator" compounds rather than by detection of nutrients or by dislike of the odor of microbe inhibitors. We see the possibility that in some cases the indicator may be associated with harmful substances of a different kind. In a note on the chemical components of turkey mullein, with regard to toxicity to fish, Naito and Noller (1960) reported a strong vesicant action by concentrated extracts. Also they noted loss of the outer layer of skin of the palm and fingers, after collecting plants for a day, even when wearing leather gloves.

These authors isolated three crystalline products (none of them toxic to fish) which they tentatively identified as hentriacontane, hexacosanol, and β-sitosterol, but no volatile compounds were identified. A search of the literature has not revealed any other chemical studies of turkey mullein volatiles.

In the present study, the volatile fraction of turkey mullein plants was separated by simultaneous steam distillation and solvent extraction (SDE) and the extracts were analyzed by gas chromatography combined with mass spectrometry (GC–MS).

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MATERIALS AND METHODS

Source Material. Turkey mullein plants at their prime, with many of the flower buds opened up, were collected from a nearly pure stand on range land at the Hopland Field Station, University of California, Hopland, CA, in mid July, 1978. The plants were cut off near the ground with pruning shears and collected in large polyethylene bags. (Rubber gloves were used; no skin reactions were detected.) Storage was at −34 °C. Before each SDE run the desired amount of plant material was prepared by cutting the stems to give foliage pieces 2–5 in. long.

Isolation of Volatiles by SDE. Two main extracts of turkey mullein volatiles were prepared: the first by SDE at atmospheric pressure and the second at partial vacuum, ca. 100 torr, absolute. The apparatus was Flath's modification (Schultz et al., 1977) of the Likens and Nickerson (1964) distillation–extraction head, with a 12-L flask for the turkey mullein foliage and water and a 250-mL flask for the solvent. Glas-Col heaters were used. Coolant for the vacuum runs was at about 1 °C.

For each run, quantities of materials used were 1.00 kg of the foliage pieces, 4.0 L of commerical purified water, 1.0 L of 0.25 M phosphate buffer (pH 6.4), and 125 mL (more where specified) of hexane with 13 mg of Antioxidant 330 (Ethyl Corp., Baton Rouge, LA). The time of SDE was 3 h. Other details of the procedure, including bicarbonate washing, drying, and solvent removal (final water bath temperature = 90 °C), have been described (Schultz et al., 1976, 1977). The yield of extract from the run at atmospheric pressure was 5.7 g (90% residual solvent).

Several vacuum runs were made in attempts to prevent loss of most of the hexane in 1 h. Since tests for air leaks were negative, it appears that there may be a relatively large quantity of air in turkey mullein leaves. Deaeration by holding the system at 100 torr for 1 h before turning the heaters on appeared to be somewhat effective, but losses were still large. For the final run (main vacuum run) 175 mL of hexane was used at the start and 145 mL more was added near the midpoint of the 3-h run. (For vacuum runs in the future it is planned to couple a Dewar condenser, with dry ice, to the vent of the SDE head). After the usual solvent removal step the concentration of turkey mullein volatiles was still quite low; so further concentration was effected at room temperature in a vial with gentle rocking, under a moderate jet of nitrogen. The final yield of extract from the vacuum run was 0.47 g (~90% residual solvent.)

GC–MS Analysis. The separations were made with a 500-ft, 0.02-in. i.d., open tubular, stainless steel column, coated with Tween 20 (Atlas Chemical Industries, Inc.,

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Wilmington, DE) mixed with 5% Igepal CO-880 (General Aniline and Film Corp., New York). The column was operated in a Hewlett-Packard 5720 gas chromatographic oven. The mass spectrometer was a VG-Micromass MM70/70 system (Vacuum Generators, Ltd., Altrincham, Ches., England) with Nier-Johnson geometry: 70° electric sector followed by a 70°, 12.4-cm radius magnetic sector. The two instruments were interfaced through a 15-cm, 0.75-mm i.d., section of glass-lined stainless steel tubing, followed by a 4.0-mm by 0.1-mm i.d. Pyrex restrictor.

GC–MS runs on the turkey mullein extracts were made with 0.60-μL sample injections. The helium head pressure was 20 psig, which gave an average linear velocity of 31 cm/s (at the beginning column temperature). The injector temperature was 170 °C. The column temperature was initially 85 °C for 30 min and then was raised at 2 °C/min to 175 °C, where it stayed constant for the remainder of the run. The connecting tube and restrictor at the interface were held at 180 °C throughout the run. In the mass spectrometer, the acceleration voltage was 3 kV, and the ionization energy (electron impact) was 70 eV. The mass spectral resolution was approximately 1 part in 4000. The magnet was scanned to cover an m/e range of 35–367 with a 4-s cycle time and a scan rate of 3 s/decade. The chromatographic records resulting from the output were reconstructed chromatograms based on the average total ion current of each scan.

The identity of constituents found by their mass spectra was confirmed by GC retention matching, by the peak enhancement (enrichment) method. The column was the same one that was used in the GC–MS runs or a similar Tween–Igepal column for constituents in regions where the peaks were well separated. A Hewlett-Packard 5840 chromatograph with a flame ionization detector (FID) was used. Sample size of the turkey mullein extracts was usually 0.32 μL but was 0.10 μL for checking the major components, and the amount of enriching known compounds also was varied in order to obtain both unmistakable peak enhancement and high-precision matching (Schultz et al., 1970). The helium flow rate was adjusted to give about the same average linear velocity as the initial rate in the GC–MS runs.

Reference Compounds. Most of the known compounds used for GC retention matching were commercial products. Three of these (esters), in which the word "amyl" was part of the chemical name on the label, were found to be two-component mixtures. Identity of the isomers was determined by ^{13}C nuclear magnetic resonance (NMR) spectroscopy on the unseparated mixture and GC. In each case, one of the components was found to be the 2-methylbutyl isomer.

Benzyl hexanoate and *trans*-butyl cinnamate were synthesized. Each of six *cis*-cinnamate esters was obtained from the corresponding trans form by photoisomerization; 0.01 M solutions in hexane in a 100-mL Pyrex flask (24-cm neck) were irradiated for 4–7 h in a Rayonet Photochemical Reactor with 300-nm lamps. In the case of the ethyl cinnamates, both geometric isomers were isolated individually by preparative GC and their identities were confirmed by NMR and infrared (IR) spectra.

Quantitative Determinations. Constituents of both of the extracts were determined with the Hewlett-Packard 5840 system, with the Tween–Igepal columns and operating conditions noted above. A Hamilton 1-μL syringe was used, with 0.30 μL of heptane drawn in first, before the sample, to act as a chaser (flushing agent). Several replicate runs were made in order to find and use the best integration program. The area percent method was used, and results were reported on a solvent-free basis.

Residual solvent (hexane) in the extracts was determined with the internal standard method, using heptane as the

Table I. Constituents of Turkey Mullein Extracts Identified by GC-MS and Retention Time Matching

peak no.[a]	compd	concn, area % atm extract	vac extract
5	toluene	0.02[b]	0.12
6	α-pinene	2.8	1.4
7	2-hexanone	0.02[b]	0.21
8	hexanal	0.04	0.26
9	camphene	0.09	0.09
11	2-methylbutyl acetate	0.05[b]	0.58
12	β-pinene	21.5	13.8
14	myrcene	21.3	17.4
15	3-hexanol	0.19[b]	1.8
17	*trans*-2-hexenal	0.10[b]	1.0
18	2-hexanol	0.05[b]	0.43
19	limonene	1.6	2.3
20	β-phellandrene	0.88	0.85
21	ethyl hexanoate	0.51	0.87
23	γ-terpinene	0.05	0.07
24A	*p*-cymene	0.01[b]	0.17
26	terpinolene	0.14	0.15
27B	*cis*-3-hexenyl acetate		0.31
29	propyl hexanoate	0.05[b]	0.09
30	1-hexanol	0.03	0.43
31	*cis*-3-hexen-1-ol	0.03[b]	1.0
32	isobutyl hexanoate	0.60	0.56
34A	methyl octanoate	0.04[c]	0.06[c]
34B	2-nonanone	0.02[c]	0.12[c]
36	nonanal	4.2	6.6
38A	butyl hexanoate	0.48	0.39
38B	*trans*-2,*cis*-4-heptadienal	0.01[b]	0.80
39	ethyl octanoate	0.04	0.09
40	*trans,trans*-2,4-heptadienal	0.03	0.56
41	benzaldehyde	0.31	4.5
42	2-methylbutyl hexanoate	2.6	1.2
45	linalool	0.26	0.15
46B	1-octanol	0.03	0.29
49	methyl benzoate	0.24	1.0
54A	phenylacetaldehyde	0.23	0.60
55A	methyl decanoate	0.05	0.14
55B	2-undecanone	0.03	0.19
57	undecanal	0.14[b]	0.25
59	hexyl hexanoate	0.22	0.17
61	1-nonanol	0.02	1.8
66	α-terpineol	0.59	0.95
76	citronellol	1.0	2.3
78	myrtenol		0.18
79	nerol	0.50	1.0
86	geraniol	0.17	0.30
87	methyl dodecanoate	0.01	0.06
96	*cis*-methyl cinnamate	0.06	0.21
98	*cis*-ethyl cinnamate	2.2	4.6
104	*trans*-methyl cinnamate	0.26	0.98
106	*cis*-propyl cinnamate	0.12[b]	0.16
108	benzyl hexanoate	0.46	0.11
110	*cis*-isobutyl cinnamate	0.53[b]	0.42
112	*trans*-ethyl cinnamate	12.7	13.7
116	*cis*-butyl cinnamate	0.28	0.22
119B	*cis*-2-methylbutyl cinnamate	0.7[d]	0.3[d]
120	*trans*-propyl cinnamate	0.52	0.44
126	*trans*-isobutyl cinnamate	2.7	1.3
132	*trans*-butyl cinnamate	1.5	0.57
139	*trans*-2-methylbutyl cinnamate	3.7	0.88

[a] Peak numbers refer to chromatogram in Figure 1.
[b] Substance identified by both mass spectrum and GC retention time in the vacuum-prepared extract but by GC retention time only in the atmospheric-prepared product.
[c] Proprotions of the two components of peak 34 are roughly estimated from intensities of their mass spectra.
[d] Calculated on the assumption that, in each extract, the proportion of cinnamate ester in the cis form is about the same for all cis-trans pairs.

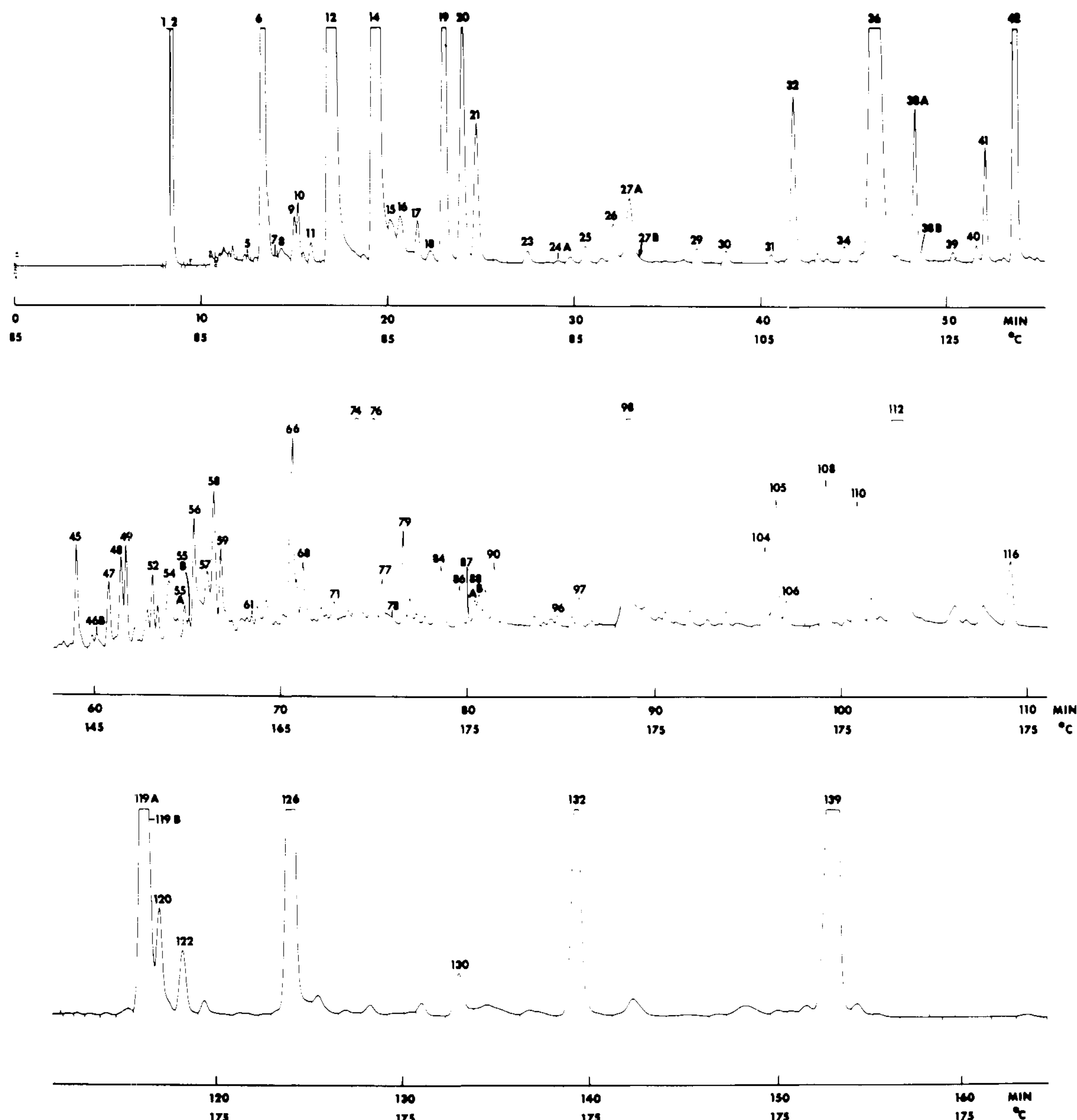

Figure 1. FID chromatogram of turkey mullein extract (atmospheric prepared): column, 500 ft, 0.02 in., stainless steel, coated with Tween 20 and 5% Igepal; sample size, 0.40 μL.

standard and acetone as chaser.

RESULTS AND DISCUSSION

A list of the identified constituents in the turkey mullein extracts is given in Table I. All of the compounds were identified in the vacuum-prepared extract by both their mass spectra and GC retention matching, and this is true for the atmospheric-prepared extract also, except for several compounds which are present at very low concentration. (See footnote *b* in Table I) Peak numbers, in the first column, refer to the GC chromatogram for the atmospheric-prepared extract, shown in Figure 1. The concentration values in the last two columns are only semiquantitative, since FID response factors were not determined.

Monoterpene hydrocarbons comprise nearly half of the atmospheric-prepared extract (solvent-free basis), the major constituents, β-pinene and myrcene, being about the same in concentration.

The most interesting group of constituents is a series of esters of cinnamic acid, the alcohol moiety varying from C_1 to C_5. Both the cis and trans isomers, 12 esters in all, were identified. The most abundant of these is *trans*-ethyl cinnamate at 12.7% and second is *trans*-2-methylbutyl cinnamate at 3.7%. The whole group adds up to about one-fourth of the atmospheric-prepared extract. The literature indicates that derivatives of cinnamic acid, notably the methyl, benzyl, and cinnamyl esters, are important constituents of a few essential oils and balsams (Guenther and Althausen, 1949). Ethyl cinnamate, however, appears to be of less importance. It occurs in oil of styrax but only at low concentration.

Other constituents in the turkey mullein extracts include a series of esters of hexanoic acid with the same alcohols involved with the cinnamates, and the addition of hexyl and benzyl hexanoate. There are 11 aldehydes and ketones, notably nonanal at 4.2%, 6 monoterpenoid alcohols, and various other compounds to make a total of 59 identified constituents.

The yield of volatile oil from the turkey mullein foliage was found to be 0.057% (solvent free/wet weight basis) from the SDE run at atmospheric pressure but only about 0.005% from the vacuum run. Since good recovery of volatiles by SDE at 100 torr was found in a quantitative study with a model system (Schultz et al., 1977), the low

yield from the vacuum run on turkey mullein foliage is believed to be due mainly to slow diffusion of the volatiles within the foliage, at the low temperature of distillation, about 52 °C.

Relatively unstable compounds which appear to have been largely lost chemically during SDE at atmospheric pressure include *cis*-3-hexen-1-ol (peak 31) and its acetate (peak 27B) and two geometric isomers of 2,4-heptadienal (peaks 38B and 40). Benzaldehyde and *trans*-2-hexenal also were found at considerably higher concentrations in the vacuum-prepared extract than in the atmospheric-prepared extract. However, several aliphatic alcohols (peaks 15, 18, 30, and 46B), which would be expected to be stable, also were found at concentrations about 10 times higher in the vacuum-prepared extract. Perhaps these alcohols have higher permeation rates within the turkey mullein foliage and thus were extracted more fully than most of the other volatiles in the vacuum SDE run. An exception is 1-nonanol, which was about 100 times more concentrated in the vacuum-prepared extract. For this we have no explanation.

As related in the introduction, the essential oils from plants unpalatable to deer are inhibitory to deer rumen microbes. Oxygenated monoterpenes, notably the alcohols, have been implicated as the inhibitory components (Oh et al., 1968). The six monoterpenoid alcohols found in turkey mullein, in the atmospheric-prepared extract, add up to only 2.5%, a relatively low concentration compared to 14% in California bay and 56% in vinegar weed oil, both strongly inhibitory. The low monoterpenoid alcohol content of turkey mullein extract agrees with its reported moderate inhibitory effect (Longhurst et al., 1968). However, since it is unpalatable, it would be of interest to find the effect of the odor of other turkey mullein volatiles, in particular the cinnamate esters, on the behavior of deer.

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Analysis of Ergot Alkaloids in Flour

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A method has been developed for the analysis of flour for ergot alkaloids using liquid chromatography (LC) with fluorescence detection. Recoveries of ergometrine added to wheat flour at concentrations of 3.1 and 9.4 μg/kg were 66–72%, while recoveries of ergotamine, α-ergokryptine, ergocristine, ergosine, and ergocornine at levels of 14–16 and 41–48 μg/kg were 73–93%. Ergocristine (up to 62 μg/kg) was the major alkaloid detected in commercial wheat and rye flour, and LC patterns were similar to those of a sample of wheat ergot sclerotia.

Large-scale epidemics of human poisonings due to consumption of bread prepared from ergot-contaminated grain no longer occur. Strict grading standards, as applied in Canada and many other countries, do not permit grain containing ergot or more than a very small percentage of ergot to reach commercial food channels (Lorenz, 1979). Furthermore, cleaning and milling grain remove most of the ergot that might otherwise end up in flour (Shuey et al., 1973). Nevertheless, localized outbreaks of human ergotism may still happen due to negligence, as in Pont St. Esprit, France, in 1951 (Lorenz, 1979). Outbreaks of poisoning due to ergoty bajra (pearl millet) have been reported from the State of Maharashtra, India, during 1958, 1973, 1974, and 1975 (Bhat et al., 1976; Krishnamachari and Bhat, 1976). The alkaloids found in the bajra samples were clavine alkaloids and not alkaloids derived from lysergic acid and isolysergic acid. The method of analysis used by Krishnamachari and Bhat (1976) was capable of detecting 0.2 μg of total alkaloids/g using thin-layer chromatography (TLC) and colorimetry, but no quantitative results for individual alkaloids were given. Robbers et al. (1975) proposed a colorimetric assay for total ergot alkaloids in *Triticale* grain, but the lowest level tested was 7.6 μg/g, corresponding to 0.35% ergot in the grain. Colorimetric determination without separation has the

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