Perillene [2-(4-methyl-3-pentenyl)furan] is somewhat unusual. It was identified by comparison of its mass spectrum with that published by Thomas and Ozainne (1970). It could be considered an oxidation product of (*E*)-β-ocimene (or of myrcene). In reviewing their previous GLC–MS data on volatiles of other plant parts such as corn, wheat, barley, and oats where (*E*)-β-ocimene had been found, we also detected a compound with the spectral and GLC retention properties of perillene but had not had the reference spectrum at that earlier date for its identification with those studies (cf. Buttery et al., 1986).

Fourth-Stage Buds and Leaves and Stems. The volatile oil from fourth-stage yellow starthistle buds was qualitatively similar to that of the third-stage buds. The relative quantitative amounts of components were also similar. The total amount of volatile oil found in the fourth-stage buds was slightly less than that of the third bud stage, but the method used was not accurate enough to be sure of the small difference.

The volatiles were also isolated from the stems and the leaves of the starthistle plant by using the same procedure as for the buds. The leaves are very small on the yellow starthistle and were not separated from the stems. The qualitative analysis of the stems and leaves identified the same compounds that were found in the buds. Quantitatively the total amount of volatile oil was only 0.5 ppm, considerably less than that found in the buds. There was also larger concentrations of the C6 compounds relative to germacrene D. (*Z*)-3-Hexenol was one-tenth the concentration of germacrene D in the stems and leaves whereas this alcohol was less than $^1/_{100}$th the concentration of germacrene D in the buds. This is not unexpected because (*Z*)-3-hexenol has long been well-known to be associated with leaves and is sometimes known as "leaf alcohol".

Biological Tests. The starthistle seed fly (*U. sirunaseva*) and the weevil (*B. orientalis*) are only available for a few weeks during the summer months, and there was not sufficient time in the present study to test the volatiles identified against these insects. Both field and laboratory bioassays to test these compounds with the insects are planned for the 1986 season.

Registry No. (*E*)-CHOCH=CHPr, 6728-26-3; $HO(CH_2)_3Pr$, 111-27-3; (*Z*)-$HO(CH_2)_2CH$=CHEt, 928-96-1; (*Z*)-$EtCO_2$-$(CH_2)_2CH$=CHEt, 33467-74-2; *o*-MeC_6H_4OMe, 578-58-5; CH_2=$CH(CH_2)_{12}Me$, 13360-61-7; myrcene, 123-35-3; limonene, 138-86-3; (*E*)-β-ocimene, 3779-61-1; *p*-cymene, 99-87-6; perillene, 539-52-6; α-copaene, 3856-25-5; caryophyllene, 87-44-5; (*E*)-β-farnesene, 18794-84-8; germacrene D, 23986-74-5; bicyclogermacrene, 24703-35-3.

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GLC–MS Analysis of the Volatile Constituents of *Panicum sp.*

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The volatiles from the leaf and stem fractions of three selections of three species of *Panicum* were analyzed by GLC–MS. Major components were 6-methyl-5-hepten-2-ol, *cis*-3-hexenol, 1-octen-3-ol, 2-nonanol, linalool, and borneol. Lavandulol, an irregular monoterpene not generally found in grasses, was isolated in significant amounts from both leaf and stem fractions.

INTRODUCTION

Grazing preference exhibited by animals for plant material has been known for sometime (Johnson-Wallace, 1937) and is thought to depend on palatability, associated plant species, climate, soil and topographical conditions, kinds of animals, and animal physiology (Heardy, 1964) as well as the olfactory cues received from a given plant species. Scehovic (1985) has recently shown that the grazing preference for tall fescue (*Festuca arundinacea* Schreb.) and ryegrass (*Lelium perenne* L.) can be reversed by spraying the juices pressed from one plant on to the other and vice versa. Further, compounds were separated by classes and the total amounts in each class determined. The classes determined most important as attractants were the volatile esters, aldehydes, ketones, and volatile phenols. Although individual compounds were not indentified, the study illustrates the importance of olfactory cues in forage preference. Switchgrass (*Panicum virgatum* L.), a subtropical, perennial grass has shown potential to provide high daily gains for steers when grazed during early and middle summer (Burns et al., 1984). Preliminary studies to establish the grazing preferences of cattle among cultivars of several *Panicum* species have been conducted. In

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these studies preference appeared established after the cattle initially investigated the sample plots, but before they ingested the grass (Burns, 1985), suggesting an olfactory cue. This was confirmed in subsequent work (Burns et al., 1985) with chopped forage samples using animals that had not been fed *Panicums* prior to the trial. The preferences exhibited in this study confirmed those of the initial study and because of the way in which the forage samples were presented appeared to rule out any visual cues. The present study was conducted to determine the nature of the volatiles (odors) produced by grass selections determined by previous preference trials as preferred or nonpreferred. Differences between leaf and stem volatiles were also determined.

EXPERIMENTAL SECTION

Materials. Three cultivars from each of three species of *Panicum* (*Amarulum*, *Amarum*, and *Virgatum* for a total of nine samples), which had been established as being preferred or nonpreferred in animal preference studies at North Carolina State University, Raleigh, NC, were used in this study. Plants were grown under the same agronomic conditions. After hand harvesting, leaf and stems were separated in the field, quick-frozen in liquid nitrogen, repacked in dry ice, and air-shipped to Athens, GA. Samples were then stored at −20 °C until chemical evaluation.

Isolation of Volatiles. Whole leaf or stems (20 g) were chopped, placed in a Krups 75 grinder, ground for 30 s, and transferred to a 250-mL round-bottom flask. The flask was attached to a receiving trap cooled in liquid nitrogen. The trap was attached to a vacuum pump and the system evacuated to 1 mmHg for 1 h. The material condensed in the trap was extracted with 2 × 25 mL portions of pentane, and the combined pentane layers were washed with saturated salt solution. The pentane layer was reduced to a final volume of 5–10 μL with warming under a stream of nitrogen. To assure that no contamination occurred from the pump oil and that no material was passing through the trap uncondensed, a second trap cooled in liquid nitrogen was introduced between the primary trap and the pump. Workup of the contents of this trap revealed no volatile material. Volatiles were quantitated by adding 10 μL of a standard solution of α-ionone in hexane to the pentane layer prior to washing with saturated salt solution. Volatile concentration was determined as parts per million (ppm) of the "as is" leaf or stem.

GLC Analysis. Gas–liquid chromatographic (GLC) analysis was conducted on a Tracor MT 220 gas chromatograph that had been modified to accept a 50 m x 0.32 mm i.d. Superox 4 (Alltech Associates, Deerfield, IL) capillary column. Analysis was performed on approximately 1 μL of material with the injector at 170 °C and column oven at 45 °C. After injection, the column oven was cooled to room temperature during 20-min hold and then programmed at 4 °C/min to a final temperature of 200 °C. All values are the average of duplicate runs.

GLC–MS Analysis. Analyses were performed with a Perkin-Elmer Model 900 gas–liquid chromatograph on a sample ranging from 0.2 to 1.0 μL. The chromatograph was connected by means of an effluent splitter to a DuPont 21-490B mass spectrometer (MS) equipped with differential pumping on the analyzer section. Separations were made on a 50 m × 0.32 mm i.d. Superox 4 capillary column. GLC conditions were as follows: carrier gas inlet pressure, 0.6 kg/cm^2; injector, 170 °C; manifold, 250 °C. The column was held at room temperature for 6 min and then programmed to 200 °C at 3 °C/min.

Mass spectrometer conditions: ion source temperature, 200 °C; scan rate, 10 s/decade; ionizing voltage, 70 eV; ion source pressure, 2×10^{-5} torr. Compounds were identified by comparison of their mass spectra and GLC retention times (RT) with those of known standards. Compounds were considered to be positively identified when their mass spectra and GLC RT agreed with those of authentic samples. Authentic samples were obtained from commercial sources, and lavandulol was prepared by a published method (Grutter and Schinz, 1952).

RESULTS AND DISCUSSION

When volatiles to be correlated with human or animal olfactory perceptions are isolated, preservation of the odor characteristics with a minimum of chemical degradation is imperative. Classically, steam distillation at atmospheric or reduced pressure has been used with good success to isolate volatiles from fruits and vegetables and with the preservation of their characteristic odor. However, this procedure did not work successfully for the grasses used in this study. Steam distillation produced a volatile oil, the odor of which did not resemble the odor of fresh grass or presumably the odor available to the animal in the previous preference trials. This problem was overcome by trapping the volatiles in liquid nitrogen at reduced pressure. The volatiles obtained by this procedure gave an odor that to the investigators was indistinguishable from the original plant material when compared to the odor of the volatiles isolated by steam distillation.

The volatiles isolated from leaf and stem fractions of *Panicum* are shown in Table I. The nine major components listed represent from 70 to 95% of the volatiles isolated from the leaf by the procedure described. The total volatiles isolated represent from 2 to 7 ppm of the whole leaf. Only four to five compounds represent the major volatiles isolated from the stem fraction accounting for 57–94% of the total, with the total volatiles representing a concentration of 1–2 ppm. The single largest component found in the leaf and a major component of the stem volatiles is 1-octen-3-ol. Its presence could be a result of a lipid oxidation process triggered by the rupture of the cell wall during freezing, thawing, grinding, or evacuation during the actual volatile isolation step (Buttery et al., 1982).

The majority of the volatiles identified are common to grasses; however, lavandulol is an exception. Lavandulol is an irregular monoterpene found in the Labiatia and Umbelliferae families (Epstein and Poulter, 1973). These are generally herbs and shrubs that can be grown for a variety of uses. *Panicum* is a genus of the Gramineae family consisting of annual and perenial cultivars distributed worldwide. The only other report of lavandulol having been isolated from a genera of this family was from Khavi grass (*Cymbopogon jawarancusa*) in Belgium (Saeed et al., 1978). The isolation of lavandulol from the three species of *Panicum* is quite unique since it is generally associated with herbs and shrubs.

The essential oils from Douglas fir needles have been isolated and the components separated by classes (Oh et al., 1967). Each class was evaluated for its effect on sheep and deer rumen microorganisms. All monoterpene alcohols exhibited strong antimicrobial activity. However, the concentrations used were far greater than could be obtained from a natural intake of forage, with a volatile concentration equal to that found in the present study.

Quantitative evaluation of the preference data showed that the most and least preferred within each species were 20-8 and 20-3, 20-13 and 20-19, and 20-24 and 20-23 for *Amarulum*, *Amarum*, and *Virgatum* respectively (Burns,

Table I. Major Volatiles from Leaf and Stem of *Panicum*

sample (code)[c]	pref rating[b]	total volatile, ppm	6-methyl-5-hepten-2-ol	hexanol	*cis*-3-hexenol	*trans*-2-hexenol	1-octen-3-ol	2-nonanol	linalool	lavandulol	borneol
					Leaf Volatiles[a]						
Amarulum											
(20-3)	–	6.6 ± 0.1	0.8 ± 0.1	1.2 ± 0.2	12.6 ± 1.0	--	40.2 ± 1.6	1.9 ± 0.6	6.6 ± 0.8	3.9 ± 0.6	18.8 ± 3.6
(20-7)	+	2.4 ± 0.2	13.1 ± 1.5	0.9 ± 0.6	3.3 ± 0.3	1.4 ± 0.1	43.5 ± 1.7	3.4 ± 0.8	5.2 ± 0.3	3.2 ± 0.7	8.6 ± 1.5
(20-8)	+	3.5 ± 0.7	12.7 ± 0.4	4.7 ± 1.1	11.4 ± 0.5	2.7 ± 0.4	24.8 ± 1.3	1.9 ± 0.0	11.7 ± 0.4	14.4 ± 0.5	5.9 ± 3.1
Amarum											
(20-11)	–	7.4 ± 0.4	0.9 ± 0.1	1.1 ± 0.1	7.1 ± 0.5	1.4 ± 0.1	74.3 ± 3.2	7.0 ± 0.7	1.4 ± 0.3	0.9 ± 0.2	3.5 ± 0.5
(20-13)	+	2.7 ± 0.3	4.2 ± 2.6	5.0 ± 0.4	9.5 ± 3.6	1.6 ± 0.3	51.2 ± 9.0	4.2 ± 0.6	1.0 ± 0.1	5.0 ± 1.2	6.0 ± 2.4
(20-19)	–	3.7 ± 0.1	T	T	2.7 ± 0.2	--	69.6 ± 3.4	5.4 ± 0.2	2.3 ± 0.4	1.2 ± 0.5	1.3 ± 1.8
Virgatum											
(20-22)	+	3.2 ± 0.5	3.9 ± 1.0	T	6.4 ± 0.3	--	59.0 ± 5.3	3.9 ± 0.7	2.0 ± 0.2	3.3 ± 0.5	13.8 ± 4.7
(20-23)	–	2.7 ± 0.3	3.4 ± 1.5	1.1 ± 0.1	17.6 ± 6.0	1.1 ± 0.1	51.5 ± 1.9	2.9 ± 0.6	2.8 ± 0.7	--	15.8 ± 6.0
(20-24)	+	4.5 ± 0.2	T	T	8.6 ± 0.1	--	47.0 ± 5.5	3.1 ± 0.5	2.0 ± 0.7	--	13.3 ± 3.1
					Stem Volatiles[a]						
Amarulum											
(20-3)	–		2.3 ± 0.2	--	4.1 ± 0.3	--	14.1 ± 2.0	--	36.3 ± 2.0	--	--
(20-7)	+		1.8 ± 0.2	--	--	--	7.0 ± 3.2	--	30.2 ± 3.2	4.5 ± 0.5	33.2 ± 11.4
(20-8)	+		4.4 ± 0.3	--	1.7 ± 0.2	--	10.8 ± 5.3	--	7.4 ± 1.4	2.2 ± 0.1	54.2 ± 2.6
Amarum											
(20-11)	–		T	--	T	--	48.1 ± 5.7	--	4.4 ± 0.3	--	27.4 ± 1.1
(20-13)	+		2.4 ± 0.3	--	1.2 ± 0.1	--	20.7 ± 5.2	--	7.3 ± 4.1	3.0 ± 0.3	59.3 ± 9.5
(20-19)	–		--	--	T	--	41.9 ± 6.1	--	37.5 ± 0.7	T	11.7 ± 0.3
Virgatum											
(20-22)	+		6.4 ± 0.4	--	6.4 ± 0.2	--	17.2 ± 3.0	--	7.1 ± 0.5	1.5 ± 0.2	52.0 ± 2.7
(20-23)	–		10.9 ± 1.0	--	--	--	15.4 ± 4.0	--	12.6 ± 1.2	--	46.2 ± 6.0
(20-24)	+		14.4 ± 1.1	--	7.2 ± 0.3	--	37.7 ± 8.0	--	3.8 ± 0.2	--	22.0 ± 3.0

[a] Percent volatiles are expressed as relative area percent and are the average of duplicate analysis (standard deviation to nearest 0.1%). -- indicates the compound is not present; T indicates compound is present in trace amounts. The difference between the total for each selection and 100% represents the material not identified. [b] Plus indicates preferred; minus indicates nonpreferred. [c] Code refers to the specific selections within each species and was used for identification purposes only.

1985). Examination of the compositional data shows that for *Amarulum* and *Amarum* the most preferred selections have higher percentages of lavandulol. Further animal studies are needed and will be conducted to establish the significance of these data.

Establishing a relationship between a given volatile or group of volatiles and grazing preference is difficult. Environmental factors (Arnold and Hill, 1972), as well as time and number of cuttings (Scehovic et al., 1985), have been shown to have an effect on the chemical composition of forage. While some trends appear, investigations are under way to establish possible correlation between the volatile profile and grazing preference in cattle using more closely controlled animal studies.

Registry No. $HO(CH_2)_5Me$, 111-27-3; *cis*-$HO(CH_2)_2CH{=}CHEt$, 928-96-1; *trans*-$HOCH_2CH{=}CHPr$, 928-95-0; $CH_2{=}CHCH(OH)CH_2Bu$, 3391-86-4; $MeCH(OH)(CH_2)_3Bu$, 628-99-9; $MeCH(OH)(CH_2)_2CH{=}CMe_2$, 1569-60-4; linalool, 78-70-6; lavandulol, 498-16-8; borneol, 507-70-0.

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Tocopherols of Soybean Seeds and Soybean Curd (Tofu)

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α-, γ-, and δ-tocopherols from soybean seeds and soybean curd (tofu) were extracted, separated, and quantitated by high-performance bonded normal-phase liquid chromatography with ultraviolet (UV) detection at 280 nm. Two successive extractions with ethanol at a solvent to dry matter ratio of about 20:1 were performed. Saponification was not necessary. The tocopherols were separated in 18 min in an amino column by using 1% isopropyl alcohol in hexane as the mobile phase. Tocopherol content varied significantly from one soybean variety to another. The amounts of α-, γ-, and δ-tocopherols in the soybeans ranged from 10.9 to 28.4, 150 to 191, and 24.6 to 72.5 μg/g (dry basis), respectively. Processing of soybeans into tofu results in a loss of vitamin E (47% and 30% for two varieties analyzed), but the tofu is a greater source of tocopherols than the soybeans on a dry basis. Storage of the curds for 15 days under commercial conditions does not affect vitamin E content.

The good nutritional properties of soybean food products rest largely on the high content and quality of the soybean protein. A number of nitrogen balance studies have suggested that the digestibility and biological value of soybean protein for humans are satisfactory and compare favorably with animal proteins (Liener, 1972). Tofu, a highly hydrated cheeselike product made by coagulation of the protein present in a soybean extract or soy milk, is a regular item in American supermarkets now. A high proportion of polyunsaturated lipids and lack of cholesterol are additional nutritional characteristics used in the marketing of tofu.

The level of vitamins and minerals in tofu is not well documented in the literature even though it is occasionally referred to in the popular literature. Miller et al. (1952) studied the retention of calcium, iron, thiamin, riboflavin, and niacin in commercially prepared soybean curd. Values for the content of some vitamins and minerals in soybeans and tofu are also listed in USDA Handbooks No. 8 (Agricultural Research Service, 1968) and No. 456 (Agricultural Research Service, 1975). These values are the averages of two samples that are made from unknown soybean varieties, by unknown processing methods, and for which older analytical methodology is employed. One of the vitamins not listed in the handbooks is vitamin E. Vitamin E activity in foods derives from two distinct series of compounds, the tocopherols and the tocotrienols. Many values for vitamin E levels in foods have been based in total tocopherol determinations based upon the Emmerie and Engel reaction (Ames, 1967). Practically, total tocopherol values thus obtained are a measure of the total reducing materials remaining after a series of purification procedures. Separation and quantitation of the individual tocopherols and tocotrienols became more common with the refinement of chromatographic techniques.

α-Tocopherol has the highest biological activity. In the past, it was the only compound considered in dietary calculations (National Research Council, 1980). Changing dietary fat patterns in the United States have resulted in soybean oil becoming the predominant dietary fat, and, consequently, average diets may have twice as much γ-tocopherol as α-tocopherol (Bieri and Evarts, 1974). In general, each one of the vitamin compounds has different vitamin E activities and antioxidant properties, making

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