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Comparison of Rumen Microbial Inhibition Resulting from Various Essential Oils Isolated from Relatively Unpalatable Plant Species

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Essential oils were isolated from eight plant species which were relatively unpalatable to sheep and deer. The inhibitory potency of these essential oils upon sheep and deer rumen microorganisms was compared, in terms of total gas and volatile fatty acid (VFA) production, by use of an anaerobic manometric technique. Inhibitory effects of oils from the eight plant species may be placed in four groups: (i) essential oils from vinegar weed (*Trichostema lanceoletum*) and California bay (*Umbellularia californica*) inhibited rumen microbial activity most; (ii) lesser inhibition was exhibited by rosemary (*Rosmarinus officinalis*) and California mugwort (*Artemisia douglasiana*) oils, followed by (iii) blue-gum eucalyptus (*Eucalyptus globulus*) and sagebrush (*Artemisia tridentata*) oils; and (iv) oils from Douglas fir (*Psuedotsuga menziesii*) and Jerusalem oak (*chenopodium botrys*) resulted in the least inhibition, when 0.3 ml of each oil was used. A highly significant correlation coefficient ($r = 0.98^{**}$) between total gas and VFA production indicated the validity of either method to measure the activity of rumen microorganisms. Our results are discussed in relation to the hypothesis that the selectivity and voluntary consumption of ruminants are related to the characteristic odor and antibacterial action of essential oils isolated from relatively unpalatable plant species.

In spite of the fact that numerous studies of food habits of both wild and domestic ruminants have been conducted, no clear basis for forage selectivity has emerged. Some evidence indicates that plants which are high in such nutrients as readily digestible carbohydrates or protein are selected. However, the fact that many unpalatable species contain equally high levels of sugar and protein confuses the issue (H. K. Oh, *unpublished data*). On the ranges of California, in late summer, most of the herbaceous forage plants are completely dry, except for a few plant species such as vinegar weed, California mugwort, and Jerusalem oak, which have such deep taproot systems that they are able to withstand the long, dry summer seasons. In many areas, green parts of woody brush and trees are also available to animals. Both wild and domestic ruminants, deer and sheep, crave green forages during the summer. In spite of this demand for green forage, many growing species are virtually untouched by animals, and others are browsed lightly. This paper relates our attempts to extract volatile compounds from relatively unpalatable plant species, as well as our investigation of the relationship between unpalatability and the antibacterial activity of those compounds.

MATERIALS AND METHODS

The nine plant species involved in this study are shown in Table 1. All plant species were collected in the vicinity of Hopland, Calif., with the exception of sagebrush, which was collected at Sacramento Pass, 30 miles east of Ely on Interstate Highway 50, Nevada. Stems and leaves of the plants were collected just prior to the date of the experiment as listed in Table 1. Young growing tips were not separated from older plant material. The procedure used for steam distillation of essential oils from these nine species was that reported by Oh et al. (5).

Rumen microorganisms were obtained from deer shot at the Hopland Field Station. Sheep rumen fluid was obtained through permanent rumen fistulas from animals maintained on good-quality alfalfa hay. Rumen microbial activity was measured in terms of total gas and volatile fatty acid (VFA) production by use of an anaerobic manometric technique (5). VFA from the manometric fermentation fluids were

TABLE 1. *Summary of variables used in the experiments*[a]

Date of expt	Sources of essential oil tested	Substrate (0.3 g/flask)	Source of rumen microbiota	Rumen *p*H	Rumen VFA		
					C_2/C_3[b]	C_2/C_4[b]	Amt (mg/100 ml)
3/29/66	California bay (*Umbellularia californica*)	Ground alfalfa hay	Deer, ♂/2 years	—	2.49	4.40	515
4/14/67	Douglas fir (*Psuedotsuga menziesii*)	Ground alfalfa hay	Deer, ♀/3 years	5.4	2.30	4.68	890
	Redwood (*Sequoia sempervirens*)	Ground alfalfa hay					
8/9/66	Blue-gum (*Eucalyptus globulus*)	Bluegum[c]	Deer, ♂/4 years	5.6	2.89	6.24	769
	Sagebrush (*Artemisia tridentata*)	Sagebrush[d]					
1/5/67	All species above except redwood	Ground alfalfa hay	Deer, ♀/2 years	6.3	3.20	5.72	556
1/10/67	California mugwort (*Artemisia douglasiana*)	Ground alfalfa hay					
	Jerusalem oak (*Chenopodium botrys*)	Ground alfalfa hay	Sheep, ♂/3 years	6.8	2.89	4.97	649
	Vinegar weed (*Trichostema lanceoletum*)	Ground alfalfa hay					
	Rosemary (*Rosmarinus officinalis*)	Ground alfalfa hay					
1/18/67	Same as 1/10/67 (four species)	Ground alfalfa hay	Deer, ♀/4 years	6.6	7.23	10.95	1,698

[a] All deer used in this study were Columbian black-tailed deer (*Odocoileus hemionus columbianus*). The sheep was a permanently fistulated Corriedale wether.

[b] C_2 = acetic acid; C_3 = propionic acid; C_4 = butyric acid.

[c] Refers to 0.3 g of dried blue-gum leaves after steam-distillation.

[d] Refers to 0.3 g of dried sagebrush after steam-distillation.

analyzed by use of an Aerograph model 204 gas chromatograph, equipped with a 5 ft × ⅛ inch (152 × 0.3 cm) column containing 15% FFAP on 70/80 DMCS Chromosorb W, a hydrogen flame ionization detector, and nitrogen as a carrier gas. All of the results were subjected to analysis of variance. If the *F* value for the treatment was significant, the least significant difference of each treatment was tested against the value of the control flask within the experiment. Duncan's multiple range was computed for the data in Table 4. The coefficient of correlation between gas production and total concentration of VFA in the manometric flask was calculated to substantiate the validity of measuring the activity of rumen microorganisms by these methods.

RESULTS

The date of the experiment, the kinds and levels of substrate used, the species, sex, and age of the animals, and the *p*H and steady-state concentration of VFA in the rumen fluid are presented in Table 1. The VFA in the rumen fluid and the ratios of acetic to propionic and butyric acid were almost the same for all deer and sheep used in this study, with the exception of the deer used on 18 January 1967. This deer not only had a higher steady-state concentration of VFA in the rumen fluid (two to three times), but it also had higher ratios of acetic to propionic and to butyric acid than the rest of the deer and sheep.

Inhibitory effects of essential oils, isolated from California bay, Douglas-fir, redwood, blue-gum, and sagebrush, upon deer rumen microorganisms, measured in terms of manometric gas production, are presented in Table 2. Since rumen microbial species and their populations are dependent on the nature of plant species browsed by the host animal, all treatments were compared with the value of the control flask from the same source of rumen bacteria. In general, as levels of essential oils increased, gas production by the microbial fermentation decreased. However, there was a marked difference in the degree of inhibition among the oils from different sources as the amounts used increased. The essential oils isolated from sagebrush and California bay were the most potent inhibitors for deer rumen microorganisms, followed by blue-gum, Douglas-fir, and redwood oils, respectively. At the level of

TABLE 2. *Deer rumen microbial activity, measured in terms of gas production, as affected by increasing amounts of steam-distilled whole essential oil from California bay, Douglas-fir, redwood, blue-gum, and sagebrush*

Source of oils and date of trial	Level of oil/flask (ml)	Substrate (0.3 g/flask)	Gas[a] production (mmoles)	Relative production (%)
California bay, 29 March 1966	None	Alfalfa	2.00	100
	0.05		1.96	98
	0.1		0.95**	48
	0.2		0.50**	25
Douglas-fir, 14 April 1966	None	Alfalfa	2.51	100
	0.1		2.55	102
	0.2		2.53	101
	0.3		2.26*	90
Redwood, 14 April 1966	0.1	Alfalfa	2.71	108
	0.2		2.62	104
	0.3		2.40	96
Blue-gum, 9 August 1966	None	Blue-gum leaves[b]	1.05	100
	0.1		0.98	93
	0.2		0.87	83
	0.3		0.72**	69
Sagebrush, 9 August 1966	None	Sagebrush[c]	1.29	100
	0.05		1.22	95
	0.1		0.79**	61
	0.2		0.28**	22

[a] Mean values of two flasks.
[b] Blue-gum leaves had been steam-distilled for essential oils.
[c] Sagebrush had been steam-distilled for essential oils.
* Significant at the 5% level.
** Significant at the 1% level.

0.1 ml of oil added per flask, the relative gas production, when compared with the control value, was 48 and 61% for bay and sagebrush oils, respectively. There was no significant change from the control values for Douglas-fir and redwood oils, at the same level of oil.

Inhibitory effects of essential oils, isolated from California mugwort, Jerusalem oak, rosemary, and vinegar weed, on sheep and deer rumen microbial activity, measured in terms of manometric gas production and VFA production, are presented in Table 3. Two levels of each oil (0.2 and 0.4 ml/flask) were selected arbitrarily. Each oil manifested different degrees of inhibitory potency upon rumen microorganisms from sheep and deer. When compared to the control flask, each of the oils inhibited microbial activity significantly, with the exception of Jerusalem oak oil, added at the 0.2-ml level, using deer rumen microorganisms. When the oil levels from the other three species increased from 0.2 to 0.4 ml per flask, the inhibitory effectiveness was not increased.

The correlation coefficients between gas and VFA produced by sheep and deer rumen microorganisms were 0.97** and 0.92**, respectively. The coefficient of correlation between sheep and deer rumen microorganisms was 0.99** for gas production and 0.89** for VFA production.

A comparison of deer rumen microbial inhibition, as affected by essential oils isolated from eight relatively unpalatable plant species, is presented in Table 4. Arbitrarily, 0.3 ml of each oil per flask was chosen to compare the inhibitory potency of all of the plant species previously tested (redwood oil was not included in this study because of the limited supply of oil). When the reduced gas and VFA productions were compared with the control flasks, all treatment values were significantly different at the 1% level, with the exception of Jerusalem oak and Douglas-fir oils which showed no significant differences.

Based on their inhibitory effectiveness, the eight essential oils can be grouped within four categories. Essential oils from vinegar weed and California bay exhibited the strongest inhibitory effect upon deer rumen microorganisms, fol-

TABLE 3. *Effect of various essential oils on the sheep and deer rumen microbial activity measured in terms of manometric gas production and total VFA production*[a]

Source of oils	Levels of oil/flask	Sheep rumen microbial activity 10 January 1967				Deer rumen microbial activity 18 January 1967			
		Amt of gas/0.3g[b]	Relative effectiveness	VFA[b]	Relative production	Amt of gas/0.3 g[b]	Relative effectiveness	VFA[b]	Relative production
	ml	*mmoles*	%	*mg/100 ml*	%	*mmoles*	%	*mg/100 ml*	%
Alfalfa alone	—	1.92	100	442	100	1.93	100	521	100
California mugwort	0.2	0.41**	21	147**	33	0.45**	23	189**	36
	0.4	0.31**	16	141**	32	0.43**	22	176**	34
Jerusalem oak	0.2	1.64**	85	353**	80	1.88	97	564*	123
	0.4	1.53**	80	291**	66	1.75*	91	467*	90
Rosemary	0.2	0.38**	20	161**	36	0.59**	31	200**	39
	0.4	0.33**	17	215**	49	0.52**	27	231**	44
Vinegar weed	0.2	0.24**	13	161**	36	0.30**	16	131**	25
	0.4	0.20**	10	215**	49	0.38**	20	116**	22

[a] Each flask received 0.3 g of ground alfalfa hay which contained 15% crude protein. Correlation coefficient (r) between gas production and VFA was $r = 0.97$** for sheep rumen microorganisms, and $r = 0.92$** for deer rumen microorganisms. Correlation coefficient of gas production (as affected by these 4 oils) between sheep and deer rumen microorganisms was 0.99**, and 0.89** for VFA production.

[b] Mean value of two flasks.

* Significant at 5% level.

** Significant at 1% level.

TABLE 4. *Comparison of deer rumen microbial inhibition, in terms of gas and VFA production, as affected by essential oils distilled from eight relatively unpalatable plant species (run on 6 January 1967)*[a]

Source of oils	Levels of oil/flask	Amt of gas/0.3 gm substrate[b]	Relative production	VFA production[b]			
				C_2/C_3	C/C_4	Total	Relative production
	ml	*mmoles*	%			*mg/100 ml*	%
Alfalfa alone	None	2.15 A	100	2.31	6.09	464 A	100
Jerusalem oak	0.3	1.96 A	91	2.35	5.19	447 A	96
Douglas-fir	0.3	1.96 A	91	2.47	4.81	426 A	91
Blue-gum	0.3	1.22 B	57	2.53	4.48	230 B	49
Sagebrush	0.3	1.16 B	54	2.90	4.27	201 B	43
Rosemary	0.3	0.69 C	32	1.34	4.93	217 C	45
California mugwort	0.3	0.66 C	31	2.11	3.57	140 C	30
California bay	0.3	0.33 D	15	2.88	4.30	97 D	21
Vinegar weed	0.3	0.30 D	14	3.05	4.51	99 D	21

[a] Each flask received 0.3 g of ground alfalfa hay which contained 15% crude protein. Correlation coefficient between gas and VFA production was $r = 0.98$**.

[b] Mean value of two flasks. Values followed by the same letter are not significantly different at the 1% level.

lowed by rosemary and California mugwort, blue-gum, and sagebrush. Oils from Jerusalem oak and Douglas-fir showed the weakest effect.

A highly significant correlation coefficient of 0.98** was obtained between manometric gas and VFA productions. This result further substantiated the validity of measurement of rumen microbial activity in terms of manometric gas production or analysis of VFA production from the manometric fermentation fluid.

DISCUSSION

Magnitudes of inhibitory potency vary greatly among the oils of the relatively unpalatable species tested. These differences can be explained by considering the different kinds and amounts of compounds in the oils from each species.

More than 25 individual compounds have been identified in Douglas-fir oil by Sakai et al. (J. Agr. Food Chem., *in press*), by use of infrared

spectra and relative retention time, in conjunction with the enhancement technique with known pure compounds. Most of the Douglas-fir oil components identified thus far have been purified by preparative gas chromatography, and the effect of individual components on rumen microorganisms of deer and sheep has been reported by Oh et al. (5). These individual components may be grouped into three categories: monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpenes. The monoterpene hydrocarbons from Douglas-fir enhanced microbial fermentation slightly, oxygenated monoterpenes inhibited microbial activity, and the sesquiterpene group from Douglas-fir promoted microbial activity. In the oxygenated monoterpene group, all monoterpene alcohols, α-terpineol, terpinen-4-ol, linalool, citronellol, and fenchyl alcohol, strongly inhibited the rumen microbial activity of both sheep and deer.

Since certain groups of compounds in the essential oils enhance microbial activity and others inhibit such activity, it is evident that the concentration of each group of compounds and the ratio of each group to the other is an important factor in determining the net effect of the whole oil. By fractionation of the whole oil from Douglas-fir, the ratio of monoterpene hydrocarbon to oxygenated monoterpenes to sesquiterpenes was found to be approximately 5:1:0.05, on a volume basis. Approximately the same ratio was found for redwood oil, based on the sum of the relative peak heights on a gas chromatogram. In contrast, the bay oil had a ratio of 3:4:1.

The data in Tables 2 and 4 substantiate the conclusion that a high ratio of oxygenated monoterpenes was important in inhibiting rumen microbial activity. In Table 2, 0.1 and 0.2 ml of Douglas-fir or redwood oils did not have a significant effect upon gas production by rumen microbes, but, as the concentration of bay oil was increased from 0.1 to 0.2 ml, gas production was reduced 22%. In Table 4, rumen microbial activity was not significantly reduced by the addition of 0.3 ml of Douglas-fir oil per flask, but the same amount of bay oil drastically reduced rumen microbial activity. One can estimate, on the basis of the in vitro results, that approximately 35 ml of the California bay oil ingested may cause inhibitory effects upon rumen microorganisms of intact sheep and deer (this is based on a 3-liter rumen volume). To ingest such a level of oil, the animal would have to consume at least 3 to 4 kg of fresh bay leaves per day, which is rather unlikely. Investigation of the effect of various oils upon rumen microorganisms in living animals is needed to determine whether the above assumptions are valid. Living plants may also contain compounds which affect palatability and digestibility but are not found in the essential oils obtained by steam distillation of the plant material.

Guenther (3) reported that bay oil contained up to 60% umbellulone and that 70% of the blue-gum oil was cineol, both oxygenated monoterpenes. The gas chromatogram of these two oils (obtained with an Aerograph model 204) showed that there were 20 to 30 different components which were depicted as employing 5% Carbowax 20M and 60/80 mesh Chromosorb W.

Ward and Nagy (*unpublished data*) reported that *d*-camphor and cineol were the most abundant compounds in sagebrush oil.

Even though the plant species used in this study were browsed to some extent, they were generally of low palatability. In 1966, in order to study preference, a cafeteria-type feeding trial, using nine browse species, was conducted at the Hopland Field Station with two sheep and two captured deer. The results showed that California bay, Douglas-fir, redwood, and Monterey pine (*Pinus radiata*) were the least palatable species in terms of dry matter consumption per day, whereas lichen (*Ramalina reticulata*) and mistletoe (*Phorodendron villosum*) were the most palatable ones for both sheep and deer. The seedlings of blue-gum were subject to deer browsing, as were the Douglas-fir seedlings, but the mature blue-gum tree was relatively unpalatable although high in essential oil. Bissel et al. (1) reported that sagebrush was a relatively unpalatable species for deer, and only a small amount was consumed in a digestion trial when sagebrush was the only feed given. However, these investigators also pointed out that, under natural conditions, sagebrush is sometimes a major component of the diet of the Rocky Mountain mule deer.

The relationship between the lack of voluntary consumption of the plant species, from which essential oils were extracted, and their antibacterial chemical constituents can be explained on the basis of certain recognized facts (2, 4): (i) antibacterial chemical compounds cause a reduction in microbial activity, and (ii) reduced microbial activity results in prolonged rumen retention time of ingesta. The selection of browse species for ruminants, however, may be dependent on physiological responses, such as the olfactory, gustatory, or tactile senses, or a combination of these senses.

Another important area in ruminant food selection which should be studied is the acquired food selection habits learned while the animals are young.

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