

The Chemical Constituents of Sagebrush Foliage and Their Isolation

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Abstract

Five foliar constituents were measured seasonally from the three subspecies of big sagebrush in Montana. Monoterpene, crude terpenoid, and crude fat levels were lowest in the spring, increased through the summer with maximum quantities at flowering or in the fall and winter months thereafter. Crude protein and total nonstructural carbohydrates were at highest concentrations in the spring, decreased in the summer, and rose again in the fall. Sagebrush foliage consists of an external and internal component. The external material is glandular secondary metabolic products, primarily terpenoids, and cuticular waxes. The internal constituents are cell-wall polymers, protein, nonstructural carbohydrates, and lipids. A 5-minute chloroform extraction of fresh whole leaves removed the external material (crude terpenoids) with minimal affect on the internal components. Steam distillation extracted the epidermal terpenoids and the internal nonstructural carbohydrates leaving the cuticular waxes and protein in the dry matter residue.

Plants are capable of biosynthesizing a tremendous number of chemicals that have potential uses as food, fiber, fuels, and chemicals (Buchanan et al. 1978 a,b). Many of these resources, however, have been unutilized because of high costs, inadequate technology and limited knowledge of chemical composition. The rising cost of fossil fuels, particularly oil, has stimulated an interest in the chemicals and biomaterials that can be derived from plants. In the United States and Mexico research is being directed toward both native and introduced species that have evolved and adapted to the harsh semiarid and arid environments (Campos-Lopez and Roman-Aleman 1980, Johnson and Hinman 1980), where inadequate water supplies have limited agricultural development. On these drier sites, new crops could be established without affecting current food and fiber production.

Big sagebrush (*Artemisia tridentata*) is an abundant shrub on semiarid rangelands with considerable economic and biological impact (Gifford et al. 1979). Its distribution and population densities have increased during the last century due to overgrazing of the associated grasses, fire control, and the shrubs' inherent resistance to herbivores (Morris et al. 1976, Young et al. 1979). Improvement of sagebrush-grasslands for livestock production is a desirable management practice and requires the eradication of sagebrush. This can be accomplished by a variety of techniques with variable costs and returns (Nielsen 1979). Harvesting the shrubs for their biomaterials might be a feasible alternative to destroying them and could provide some management flexibility for sagebrush-grasslands.

Sagebrush biomass has two distinct components, wood and foliage. The stems and branches are primarily cell-wall polysaccharides held together with lignin and chemically similar to the wood of other angiosperms (Shafizadeh and Buckwa 1970). The foliage

is a mixture of cell-wall polymers, protein, nonstructural carbohydrates, lipids (Hickman 1975), and secondary metabolic products (Shafizadeh and Melnikoff 1970, Brown et al. 1975, Kelsey et al. 1978, Kelsey and Shafizadeh 1980). The objective of this study was to examine the chemical composition of big sagebrush foliage with emphasis on the secondary metabolic products and methods for their isolation. It is these compounds that have immediate potential as a renewable source of energy-rich hydrocarbons, chemical feedstocks, or specialty chemicals.

Procedures

To determine the seasonal changes in foliage constituents of big sagebrush, single populations of *Artemisia tridentata* ssp. *tridentata*, ssp. *vaseyana*, and ssp. *wyomingensis* were located in west-central Montana near Perma, Missoula, and Ramsay, respectively. Composite leaf and twig samples were gathered from numerous plants in each population on 5 dates over a 1-year period, November 1979, and February, June, September, and December, 1980. Plants sampled were always from the same geographic area within the population. The fresh tissue was sealed in a plastic bag, transported with dry ice to a freezer and stored until needed. Before analysis the leaves were frozen with liquid nitrogen and dislodged from the twigs, which were removed and discarded. The leaves were then analyzed for monoterpenes (essential oils), crude terpenoids, crude fat, total nonstructural carbohydrates (TNC), and crude protein.

Monoterpenes were quantified by steam distillation for 2 hours in an apparatus that held the leaves above the water of the steam generator. The oils were trapped over a water column in an ice cooled water condenser. After the water column was drained, the oil was transferred to a preweighed vial, where excess water was removed with a syringe. The vial was reweighed after drying in a calcium chloride desiccator for 15 hours. Crude fat contents were measured by dry ether extraction of oven-dried leaves (100°) for 16 hours in a Soxhlet extractor. The ether extracted leaf residue was oven dried to a constant weight for calculation by difference. Crude protein was assayed by microkjeldahl procedures and the methods of daSilveira et al. (1978) were used for TNC. Crude terpenoid levels, a mixture of secondary metabolic products (primarily terpenoids) and cuticular waxes on the epidermis of the leaves, were determined by extracting 10 g of fresh leaves with 240 ml of chloroform for 5 minutes. The chloroform was filtered through cotton and coarse filter paper, into a preweighed flask. Solvent was removed on a roto-evaporator with vacuum and a warm water bath. Final traces of chloroform were eliminated by gradually increasing the water bath temperature to 60–65° C and holding it within this range for 1 hour. The flask was desiccated for 30 minutes and weighed. These analyses were all conducted in duplicate, averaged, and reported as percent of tissue dry weight (100° C).

Further studies of the inter and intra subspecies variation in crude terpenoid concentrations were conducted. In the fall of 1979 three geographically distinct populations of each big sagebrush

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subspecies were located in east-central Oregon near Squaw Butte (9 populations total). Approximately 100 g of leaf and twig material was collected from 1 randomly selected mature plant in each population. The tissue was sealed in a plastic container, frozen, and transported to the University of Montana where it was analyzed in triplicate for crude terpenoids.

Results from the seasonal analyses described above indicated that the chemical constitution of the foliage changed substantially in the spring. To monitor these changes more precisely, the leaves of *vaseyana* were sampled frequently during this period. On March 27, 1981, 3 individual plants from the Missoula population were tagged. Five branches were selected from throughout their crowns, and 3 leaves marked on each branch, 1 at the top, middle, and bottom. These leaves were all attached to the outside of the clusters and represented the oldest overwintering leaves from the previous growing season. Colored threads allowed identification for future measurements. Leaf length was taken from attachment to the longest point on the tip, and the width was recorded just beneath the lobes. Leaf and twig tissue was collected from the untagged portions of the crown and transported to the freezer on ice. Leaf measurements and samples were taken on 5 additional dates, April 9 and 30, May 14, June 4, and July 15. Terminal stem growth was measured on each marked branch through June 4. A sample of exclusively new spring leaves was also collected on this date. The leaf tissues from the 3 plants at each date were mixed and analyzed for crude terpenoids, crude protein, and TNC as described above.

Two methods for isolating the secondary metabolic products were examined, steam distillation and solvent extraction. The effect of steam distillation was tested using fresh *vaseyana* collected November 1979, and all 3 subspecies collected in June 1980. The leaves were steam distilled for 2 hours in the same apparatus used to quantify monoterpenes. The steamed leaves were air-dried and analyzed in duplicate for crude fat, crude protein, and TNC. The spring samples were also examined for crude terpenoid levels. The solvent extraction experiments were conducted with November 1979 *vaseyana* foliage. Three weighed samples of fresh leaves, approximately 115 g each, were placed into breakers and covered with 2,400 ml of either chloroform, ethanol (95%), or water, and stirred for 5 min. The solvent was filtered off and the leaves air-dried, 30 minutes for chloroform and water, overnight for ethanol. Each was analyzed for monoterpenes, crude fat, crude protein, and TNC. Complete chloroform Soxhlet extractions (16 hours) were conducted with the fresh leaves from all 3 subspecies collected December 1980.

In order to evaluate the chemical constitution of the crude terpenoid extract, it was fractionated into 3 distinct components, steam distillable oils, hexane soluble hydrocarbons, and a residual chloroform soluble fraction. Duplicate solvent free crude terpenoid extracts were prepared and quantified, as described above, from 115 and 203 g of fresh *vaseyana* leaves collected March 27, 1981. Each was fractionated separately. To determine the monoterpene content the extract was redissolved with a minimum amount of chloroform in a boiling flask. Steam was passed through the solution to remove both the chloroform and the volatile oils. After 1 hour condensed water had filled the flask. This was evaporated on a roto-evaporator with vacuum and a warm water bath. When the water appeared gone, the water bath temperature was increased to 60–65°C for 1 hour. The flask was desiccated for 30 minutes, weighed and the monoterpenes quantified by difference. The residue in the boiling flask was dissolved in 100–125 ml hot ethanol (95%) and transferred to a separatory funnel. An equal volume of hot distilled water was added. The solution was washed 4 times with 125 ml of hexanes. The washes were combined and concentrated on the roto-evaporator. Traces of solvent were removed by increasing the water bath to 60–65°C and holding for 1 hour. The extract was weighed after 30 minutes of desiccation. The ethanol-water solution in the separatory funnel was then extracted with three 125 ml portions of chloroform. These were combined and processed in the same manner as the hexanes.

The heat content and elemental composition were determined

for duplicate crude terpenoid extracts from 100 g samples *vaseyana* leaves collected in February of 1980. The heat values were measured with an automatic adiabatic calorimeter and the H, O, N, and ash analysis was performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

Results

Seasonal Changes in Foliage Constituents

The seasonal sampling included tissue from 2 growing seasons. During the first 2 collection periods, November 1979 and February 1980, the leaves harvested were produced in the spring of 1979 whereas the June 1980 material had overwintering leaves from 1979 as well as new spring leaves from 1980. The September and December 1980 foliage was all formed in the preceding spring growth period. The subspecies were flowering, or about to flower, on the September collection date, and in December the seeds had matured and were being disseminated.

The monoterpenes, crude terpenoids, and crude fats were their lowest concentrations in the spring and increased through the summer months (Fig. 1). Crude terpenoids and fats were at their maximum levels during the reproductive period in the fall and the following winter months. Monoterpene concentrations peaked near flowering, decreased in the fall, with little change during the winter. The crude terpenoids were a major constituent of the foliage leaves with quantities that differed substantially between subspecies, *tridentata* (24.8% dry matter basis), *wyomingensis* (17.9%) and *vaseyana* (14.2%).

The crude protein and TNC were high in the spring, decreased during the summer and rose again in the fall (Fig. 1). Changes were variable during winter. Protein concentrations levels did not differ much between subspecies; *wyomingensis* had the smallest amount except in the fall of 1980. It also had the least TNC at all dates.

Spring Leaf Growth and Composition

On March 27, 1981, 15 overwintering leaves were tagged and measured from throughout the crown on each of three *vaseyana* plants. No new leaf growth was visible at this time. Approximately 2 weeks later, April 9, the old leaves had expanded significantly (1% level, student's *t* test for paired samples) in length on all plants (Fig. 2). New leaves were just starting to develop, but not large enough to measure. By the end of the month, April 30, the overwintering leaves were significantly longer and wider and the new leaves averaged 1.0 cm long. New stem growth on the branches was first measurable on May 14 and increased 4 to 6 times the length in the subsequent 3 weeks. Between March 27 and June 4 the overwintering leaves had expanded an average of (5.5, 7.8 and 7 mm) in length and (1.0, 1.0 and 1.3 mm) in width, respectively, on the three plants. Visual observations indicated that new growth did not constitute a major portion of the foliage biomass before the first of June.

Physiological changes were also occurring as reflected in the changes in chemical composition. The TNC concentration went up during the period when the old leaves began to expand (Fig. 2). This remained high until the start of rapid stem elongation between May 14 and June 4, and then dropped substantially. The quantity of TNC in leaves (15.4%) was about equal to that in the mixed old and new leaf sample (16.1%) from June. Crude protein content began to rise with the development of new leaves, it peaked in June and diminished as the leaves matured (Fig. 2). New leaves from June were much higher in crude protein (17.6%) than the mixed samples containing old leaves (12.8%). Crude terpenoid concentration decreased throughout the active growth period with a subsequent increase by mid July (Fig. 2). The quantity in the June foliage (6.4%) was lower than from the mixture of new and old tissue (8.1%).

Effects of Steam Distillation and Solvent Extraction

Steam distillation extracted all the monoterpenes, 67 to 82% the crude terpenoids, and 64 to 70% of the TNC, resulting in the protein being concentrated by 12 to 38% in the residue (Tables

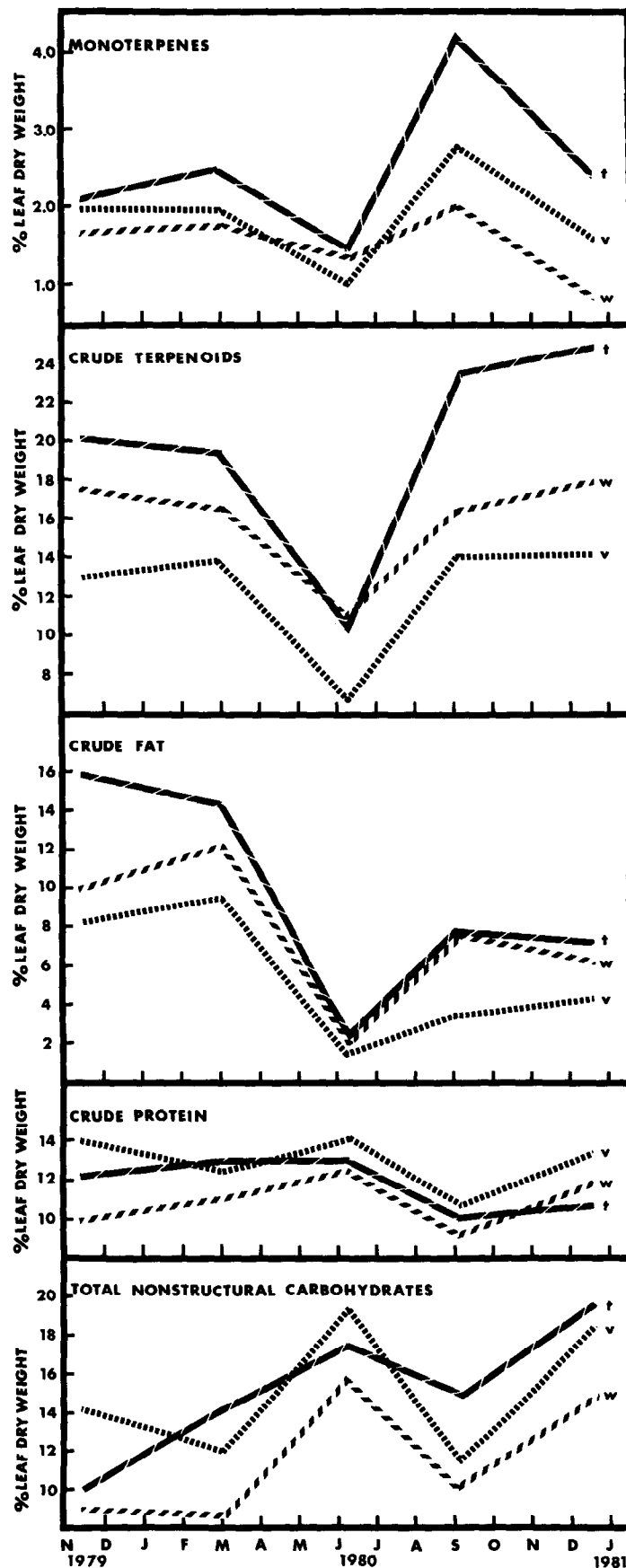


Fig. 1. Seasonal variation of the foliar constituents in the three subspecies (t, tridentata; v, vascyana; w, wyomingensis) of big sagebrush.

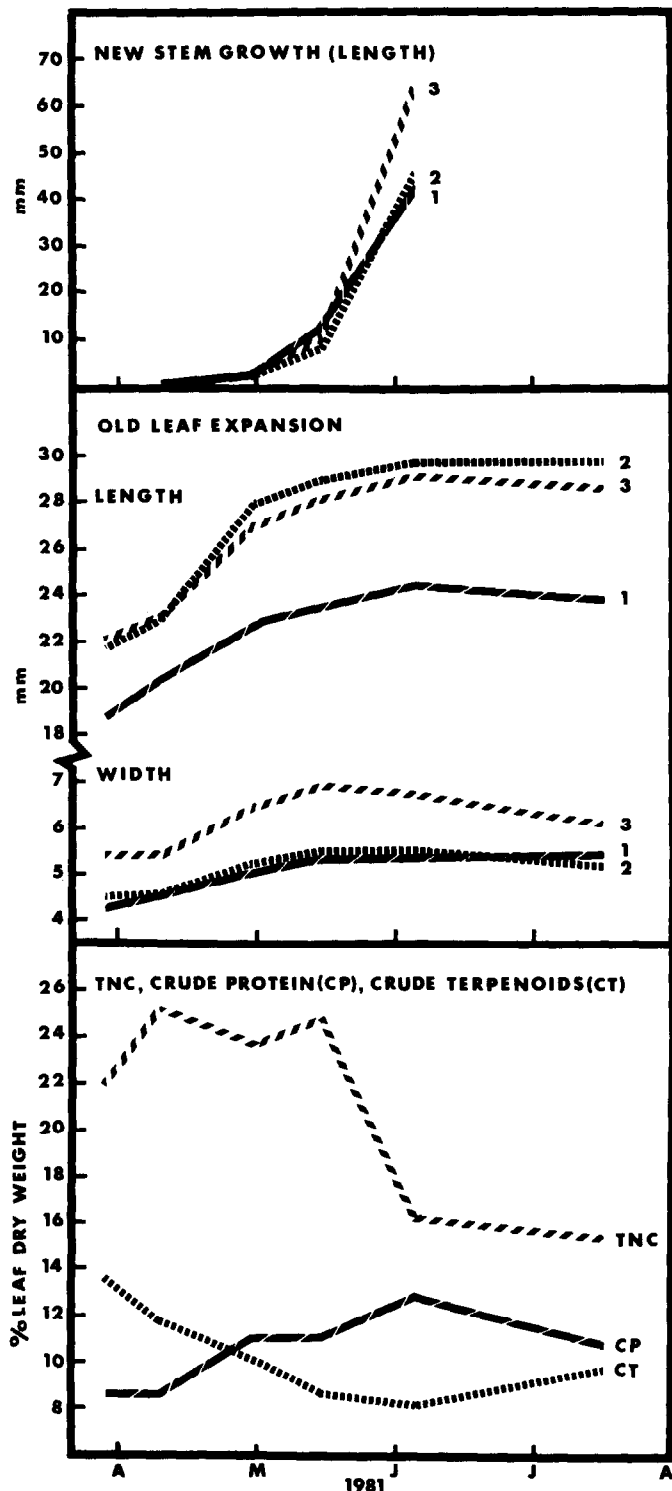


Fig. 2. Old leaf expansion, new stem growth, and changes in foliage chemical composition of vascyana during spring. Numbers correspond to individual plants.

and 2). The crude fat remaining in the steam distilled leaves increased when spring foliage was used (Table 1), but decreased in the residue from material collected in the fall (Table 2).

The three solvents, chloroform, ethanol, and water, used to extract the leaves had similar effects on the chemical composition, but in different degrees (Table 2). All reduced the monoterpenes, crude fat (except water), and the crude protein; TNC were concentrated. Chloroform caused the greatest changes, removing all monoterpenes and 81% of the crude fat. The crude protein was reduced

Table 1. The chemical composition of fresh and steam distilled spring (June, 1980) big sagebrush leaves.

Subspecies	Leaf components (% leaf dry weight)			
	Crude terpenoid	Crude fat	Crude protein	Total nonstructural carbohydrates
<i>tridentata</i>				
Fresh	10.6	2.1	13.1	17.6
Steam distilled	1.9 (18.1) ¹	3.5 (166.8)	18.0 (138.1)	5.9 (33.7)
<i>vaseyana</i>				
Fresh	6.6	1.5	14.0	19.4
Steam distilled	2.2 (32.9)	2.2 (146.7)	18.0 (128.4)	6.7 (34.2)
<i>wyomingensis</i>				
Fresh	11.0	2.0	12.5	15.7
Steam distilled	2.4 (21.4)	2.6 (134.0)	16.4 (131.6)	5.6 (35.9)

¹The number in parenthesis is the percentage remaining in the steam distilled sample relative to what was present in the fresh material.

by 9% whereas TNC were concentrated by 19%. Neither ethanol nor water were effective in extracting the organic constituents (monoterpenes and crude fat), they decreased the crude protein 17% and 20% and concentrated the TNC 3% and 35%, respectively.

Chloroform Crude Terpenoid Extraction and Extract Composition

The efficiency of the 5-minute chloroform crude terpenoid extraction was tested by comparing these quantities with a complete (16 hours) chloroform Soxhlet extraction of fresh leaves, (Fig. 3). The rapid procedure was quite thorough and took out 79%, 81%, and 87% of the total chloroform soluble constituents in *vaseyana*, *wyomingensis*, and *tridentata*, respectively. Cutting the extraction time from 5 minutes to 1 minute resulted in only minor reductions of extract (Fig. 4).

Near Squaw Butte, Ore., the *tridentata* had a significantly higher concentration of crude terpenoid (5% level, nested classification ANOVA and Duncan's multiple range test) than *vaseyana* or *wyomingensis* (Table 3). There was no difference between the latter two. The variation within each subspecies was the same, with a 4% range between the lowest and highest values, *wyomingensis* (10.9 to 14.8%), *vaseyana* (13.6 to 17.6%) and *tridentata* (18.6 to 22.9%).

Solvent fractionation of the crude terpenoid extract revealed that 16.2% was steam-distillable monoterpenes. The distilled residue contained a 16.0% (based on the total extract) nonpolar hexane fraction and a 66.7% residual chloroform soluble portion. Elemental composition of the complete extract was C (69.9%), H

Table 2. The chemical composition of sagebrush leaves subjected to various types of extraction (*vaseyana*, November, 1979).

Extraction procedure ¹	Leaf components (% leaf dry weight)			
	Monoterpenes	Crude fat	Crude protein	Total nonstructural carbohydrates
Fresh	2.0	8.2	14.0	14.2
Steam distilled	0.0 (0.0) ²	5.4 (66.3)	15.6 (111.8)	4.3 (30.2)
Chloroform	0.0 (0.0)	1.6 (19.2)	12.7 (90.8)	16.9 (118.7)
Ethanol	1.6 (79.1)	7.0 (85.9)	11.6 (83.1)	14.6 (102.8)
Water	1.8 (93.4)	8.3 (102.0)	11.2 (80.2)	19.2 (135.1)

¹Steam distillation was 2 hours, solvent extractions were 5 minutes.

²The number in parenthesis is the percentage remaining in the treated sampled relative to what was present in the fresh material.

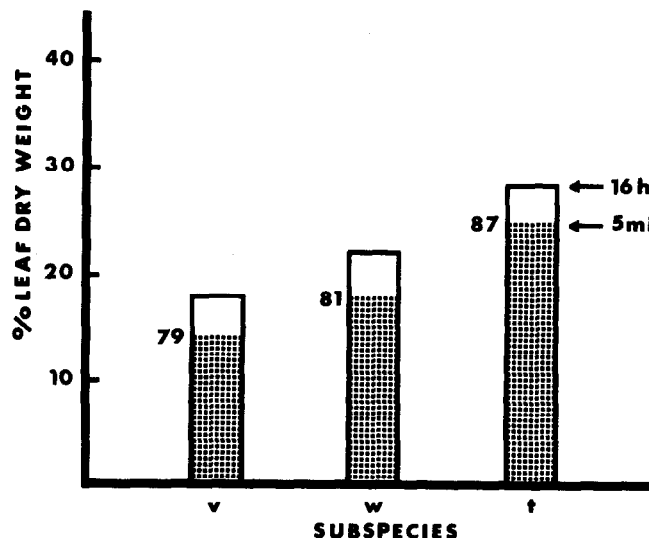


Fig. 3. Crude terpenoid yields from 5-minute and 16-hour (Soxhlet chloroform extractions of fresh whole sagebrush leaves. Numbers at top of bars are the 5-minute yields as a percentage of the 16 hour yield. Subspecies: t, *tridentata*; v, *vaseyana*; w, *wyomingensis*.

(8.9%), O (21.1%), N (<0.05), and ash (0.56%). The heat content was high at 32.6 megajoules per kilogram (7790 calories per gram

Discussion

The terpenoids, monoterpenes (Welch and McArthur 1981), and sesquiterpene lactones (Kelsey and Shafizadeh 1979, 1980) are the major secondary metabolic products in sagebrush, with less quantities of coumarins (Shafizadeh and Melnikoff 1970, Brown et al. 1975) and flavonoids (Rodriguez et al. 1972, Brown et al. 1975). All of the sesquiterpene lactones and some of the monoterpenes are stored in glandular trichomes on the epidermis of the leaves (Kelsey and Shafizadeh 1980). Consequently, these compounds are readily accessible to solvent extraction with minimal processing of the tissue. The glands can be burst and their terpenoid content released by washing for 60 seconds in chloroform (Kelsey and Shafizadeh 1980). This solvent is also very effective in removing the cuticular waxes (Silva Fernandes et al. 1964).

To get an approximate measure of the readily accessible chemicals on the surface of sagebrush foliage (crude terpenoids), fresh whole leaves were extracted with chloroform for 5 minutes. The short extraction time and the physical barriers, due to cell wall

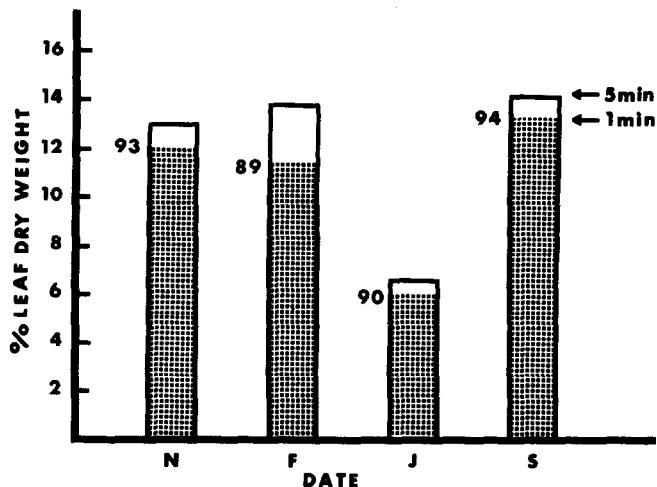


Fig. 4. Crude terpenoid yields from 5- and 1-minute chloroform extractions of fresh whole *vaseyana* leaves. Numbers at top of bars are the 1-minute yields as a percentage of the 5-minute yields. Dates: N November 1979; F, J, S are February, June, and September 1980.

and water in the fresh whole tissue, helped restrict the extraction to the epidermal surface. This simple procedure was quite efficient, removing 79 to 87% of the chloroform soluble material in the foliage (Fig. 3). Yields from 1-minute extractions were similar (Fig. 4), further confirming the accessibility of the compounds. Chloroform was the best solvent for isolating monoterpenes and crude fat (Table 2); and 5-minute washes with fresh whole leaves extracted more material than Soxhlet extracting dry tissue 16 hours with ether (Fig. 1, compare crude terpenoid and crude fat values). Dichloromethane was about as effective (11.8%) as chloroform (12.7%) for isolating crude terpenoids. It would be a desirable substitute commercially because it is less expensive, has a lower boiling point, and is less toxic.

After solvent removal the extract is a complex mixture of steam distillable monoterpenes (16.2%), cuticular compounds (16%, long chain hydrocarbons, esters, and alcohols) soluble in hexane (Thomas 1976) and a residual fraction (66.6%) with sesquiterpene lactones and other more polar compounds including coumarins and flavonoids (these were detected in the chloroform fraction by thin-layer chromatography).

The chemical composition and harvestable yields of plant materials are strongly associated with the phenological stage of the plants. The yearly cycle for sagebrush can be broken down into four periods, the primary growth of spring, secondary growth and differentiation in the summer, reproduction in the fall and a winter quiescence. The monthly timing of these stages may vary depending on species and subspecies, environmental, and climatic conditions (Wright 1970, DePuit and Caldwell 1973, Daubenmire 1975).

The winter dormancy is broken by the expansion of overwintering leaves and new leaf development (Fig. 2). The formation and rapid growth of new stems, both vegetative and reproductive, then begins with their subsequent new leaf growth. There is a transition into summer as the vegetative growth slows and expanded overwintering leaves begin to drop (Daubenmire 1975, Rickard and Warren 1981). During summer, primary growth of vegetative and reproductive stems stops, followed by secondary growth and differentiation into woody tissue. New leaf development continues but at a reduced rate. Overwintering and ephemeral spring leaves are shed. Flower buds form on the inflorescence, these mature and open, marking the end of this period. The reproductive stage in the fall is initiated by flowering and lasts until the seed is mature and shed from the plant. Very little vegetative growth is observed and all tissue differentiation is completed. During the winter the shrubs are physiologically dormant relative to the other seasons.

Crude terpenoids were most concentrated on the foliage in the fall and winter (Fig. 1), during which time subspecies differences were most pronounced. Subspecies *tridentata* produced the greatest quantities of epidermal chemicals in both the Montana and Oregon populations (Fig. 1, Table 3). Harvesting the shrubs late in the growing season would provide the best yields per unit of dry matter, but it may not give the greatest yields potentially available from an individual plant. Overwintering leaves remain on the plants through the primary spring growth period so that maximum foliage biomass occurs just prior to leaf shedding. Although the crude terpenoid concentrations decrease on a dry matter basis (Fig. 2) this is most likely the result of leaf expansion and a dry weight increase rather than an actual loss of crude terpenoids. Between March 27 and April 30, the foliage on *vaseyana* was predominately overwintering leaves expanding in size (Fig. 2). During this time the crude terpenoid concentration dropped but there were too few new leaves present with their low crude terpenoid levels to cause a dilution effect. These compounds could have been used in metabolism as a source of carbon and energy, but this seems unlikely since TNC levels were at their peaks (Fig. 2) and should have been sufficient to meet these needs. If the quantity of crude terpenoid on each overwintering leaf remains unchanged during spring growth, then harvesting the shrubs at maximum foliage biomass, before leaf shed in early summer, will provide the greatest crude terpenoid yields on a per plant basis because of the additional material on the new leaves. By early June the new leaves had a crude terpenoid

Table 3. The crude terpenoid content of the three big sagebrush subspecies at Squaw Butte, Oregon.

Plant sample ¹	Subspecies (% of leaf dry weight)		
	<i>wyomingensis</i>	<i>tridentata</i>	<i>vaseyana</i>
1	14.8 (0.2) ²	18.6 (0.4)	13.5 (0.5)
2	10.9 (0.3)	19.4 (0.6)	15.8 (0.2)
3	14.4 (0.4)	22.9 (0.6)	17.6 (0.4)
Subspecies avg.	13.4 ³ (2.2)	20.3 ³ (2.3)	15.7 ³ (2.0)

¹Each plant represents a distinct and separate population.

²The number in parenthesis for each plant is 1 standard deviation from triplicate analysis; for subspecies it is the standard deviation for all 9 analyses.

³Subspecies averages followed by the same letter were not significantly different at the 5% level according to Duncan's multiple range test.

had a crude terpenoid content of 6%. The concentration of the individual chemicals in these extracts could vary between spring and fall, but this is probably not significant unless a particular compound was being sought.

Steam distillation had a different effect on the epidermal chemicals than solvent extraction. The apparatus used for distillation held the leaves above the steam generator and all the monoterpenes were volatilized and trapped as an oil over a water column in a condenser. Steam distilled-air-dried leaves were free of their characteristic terpenoid odor. During distillation steam condensed on the leaves and dripped back into the generator, carrying with it nonvolatile water soluble compounds such as sesquiterpene lactones, coumarins, flavonoids, sugars, and starch. The crude terpenoid constituents in the water were recovered by washing with chloroform. A total of 67 to 82% of the crude terpenoids were taken from the tissue (Tables 1 and 2) and the residual left behind was probably the nonpolar, water insoluble cuticular waxes. This is supported by the high crude fat concentrations remaining in the steam distilled tissue, 66% in fall leaves (Table 2), and 134 to 167% in the spring foliage (Table 1). Unlike solvent extraction, steam distillation fractionated the various chemical groups that make up the crude terpenoids, but the total yield of material (monoterpenes plus the chloroform extract from the steam generator) was much lower 2.4%, 2.3%, and 2.4% than the solvent-extractable crude terpenoids, 10.6%, 6.6%, and 11.0% from June 1980 *tridentata*, *vaseyana* and *wyomingensis*, respectively.

Seasonal changes of the crude protein and TNC concentrations in the foliage followed the same basic pattern (Fig. 1). They were high in the June tissue, decreased during the summer and rose again in December as the plants entered their winter dormancy. Hickman (1975) reported similar changes for crude protein but with a greater increase in spring, because his sample was exclusively new growth, and a smaller increase in the fall because he included low protein stem tissues in the clippings. The proportion of crude protein in the total foliage dry matter does not change a great amount throughout the year (Fig. 2). New tissues have more protein (17.6% *vaseyana*, June 4, 1981) but if all the leaves were harvested in early summer at peak biomass this would be offset by the lower quantities in the overwintering leaves (12.8% in a mixed new and old leaf sample from *vaseyana*, June 4, 1981). The amount of crude protein in the new leaves also drops quite rapidly after it peaks (Hickman 1975). TNC accumulate in the previous years twigs (Coyne and Cook 1970) and old leaves (Fig. 2) as new growth begins. This reserve is utilized and depleted during the period of rapid stem development. These low TNC levels remain until the need for carbon and energy decreases when metabolic activity slows in the fall. Solvent extraction and steam distillation affected these two tissue constituents differently. Chloroform reduced protein levels slightly and concentrated the TNC (Table 2). Steam distillation fractionated them, isolating 64 to 70% of the carbohydrates (Tables 1 and 2) into the water of the steam generator, and concentrating the protein in the leaf residue (Tables 1 and 2).

The foliage biomass of sagebrush can be viewed as a 2-component (external and internal) system. The external portion is composed of secondary metabolic products (monoterpenes and

sesquiterpene lactones) and cuticular waxes; whereas the internal component is cell-wall polymers, protein and nonstructural carbohydrates. Rapid solvent extraction with chloroform is an effective method for separating these two groups. The extract provides a complex mixture of oxygenated hydrocarbons and the dry matter residue retains its protein and carbohydrates. The extract could be further processed for chemicals while the leaves could be used for feed. Steam distillation also changes the composition of sagebrush foliage but in a much different manner. The secondary metabolic products and nonstructural carbohydrates were isolated and partially fractionated. The monoterpenes were recovered as an oil and the sesquiterpene lactones and carbohydrates were mixed in water solution. These latter two were separable by organic solvent extraction of the water. The cuticular waxes remained on the epidermis of the tissue and the protein was concentrated as the other constituents were removed. The terpenoid fractions could be used for their chemicals, the carbohydrates are fermentable, and the dry matter, high in protein, has value as feed. Steam distillation is certainly a less efficient method than solvent extraction for isolating the external organic chemicals from the tissue.

The plant species chosen and commercially developed for botanical chemicals and biomaterials will depend on a variety of botanical, chemical, technical and economic considerations. Sagebrush is of interest because it is naturally abundant on semiarid rangelands and has little value, at least in terms of livestock production. Furthermore, it is representative of many semiarid plants that produce substantial quantities of secondary metabolic products and leaf resins in association with epidermal glands and glandular trichomes (Dell and McComb 1978). These species of plants, with external chemicals, should be examined very thoroughly, since this characteristic allows easy access to a wide variety of desirable compounds with minimal processing.

High concentrations of epidermal secondary metabolic products in glandular trichomes may also have considerable biological significance. Many glandular constituents, particularly terpenoids, including some of those in sagebrush, are biologically active (Kelsey et al. in press), being phytotoxic, cytotoxic, antimicrobial, and insect pheromones and feeding deterrents. Therefore the potential exists for these chemicals to function as a defense mechanism. As a physically dominant component of rangeland communities, in both time and space, sagebrush appears to be grazed by a disproportionately small number of insect herbivores, and ungulate consumption is only common from native species, mule deer and antelope, that have coevolved with the shrubs. The primary defense of the epidermal secondary metabolic products could be against herbivores. Escape of phytotoxic chemicals into the environment (Kelsey et al. 1978) through volatilization, leaching and litter deposition might provide secondary benefits by reducing excessive competition from their own seedlings and the associated plant species.

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