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SESQUITERPENE LACTONES OF *ARTEMISIA**: TLC ANALYSIS AND TAXONOMIC SIGNIFICANCE

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Key Word Index—*Artemisia arbuscula*; *Artemisia tridentata*; Compositae; sagebrush; sesquiterpene lactone; chemotaxonomy.

Abstract—Samples of *Artemisia arbuscula* ssp. *arbuscula*, *A. tridentata* ssp. *tridentata*, ssp. *wyomingensis*, ssp. *vaseyana* and ssp. *vaseyana* f. *spiciformis* were collected from various locations in Montana and analyzed by TLC for their sesquiterpene lactone content. *Artemisia tridentata* ssp. *tridentata* and ssp. *wyomingensis* are distinct morphologically and chemically, whereas ssp. *vaseyana* has three distinct chemical groups not yet separated morphologically. *Artemisia arbuscula* ssp. *arbuscula* and *A. tridentata* ssp. *vaseyana f. spiciformis* are easily separated by morphology but have identical TLC patterns. It has been further shown that the sesquiterpene lactones produced for a particular species or subspecies are the same regardless of the time of the year collected, although the quantity varies from winter to summer.

INTRODUCTION

THE IDENTIFICATION and classification of sagebrush (*Artemisia*) has interested taxonomists since the turn of the century. Rydberg,[1] Clements and Hall,[2] Ward[3] and Beetle[4] have each revised the classification put forth by their predecessor. Following a TLC study of the coumarins,[5] Beetle and Young recognized a third subspecies[6] in the *A. tridentata* Nutt. group to produce the taxonomic scheme accepted at present. Although there exists a comprehensible key to the sagebrush taxa it is still difficult to identify some of the species and subspecies due to morphological variation. Consequently many investigators have tried to produce additional characters through chemotaxonomic studies of the coumarins;[7–9] although there is some correlation between morphology and TLC pattern, these compounds are only marginally useful.

The program on the chemistry of sagebrush in this laboratory began with the examination of the polysaccharides of the woody tissues[10] and was extended to the identification of the

* Part VII in the series "Chemical Composition of Sagebrush". For part VI see SHAFIZADEH, F. and BHADANE, N. R. (1973) *Phytochemistry* **12**, 857.

[1] RYDBERG, P. A. (1916) *North Am. Flora* **34**, 244.

[2] HALL, H. M. and CLEMENTS, F. E. (1923) *The Phylogenetic Method in Taxonomy*, Carnegie Inst., Wash., Publication No. 326.

[3] WARD, G. H. (1953) *Contrib. Dudley Herb.* **4**, 155.

[4] BEETLE, A. A. (1960) *A Study of Sagebrush*, University of Wyoming Agricultural Experiment Station Bulletin No. 368.

[5] YOUNG, A. (1965) *Wyo. Range Mgt.* **198**, 2.

[6] BEETLE, A. A. and YOUNG, A. (1965) *Rhodora* **67**, 405.

[7] HOLBO, H. R. and MOZINGO, H. N. (1965) *Am. J. Botany* **52**, 970.

[8] WINWARD, A. H. (1970) *Taxanomic and Ecological Relationships of the Big Sagebrush Complex in Idaho*, Ph.D. dissertation, University of Idaho, Moscow, Idaho, U.S.A.

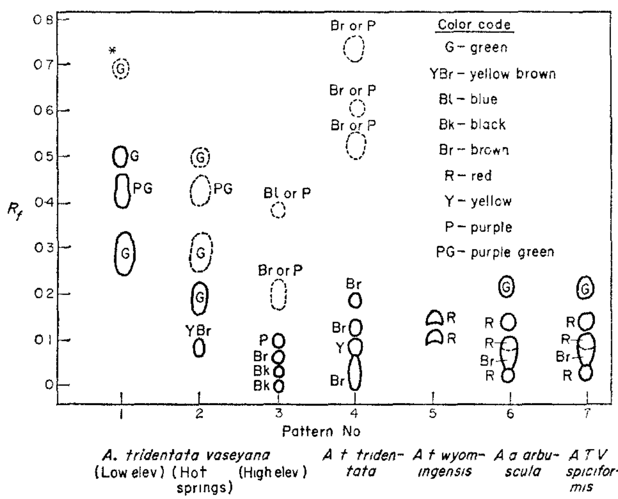
[9] BRUNNER, J. R. (1972) *J. Range Mgt.* **25**, 205.

[10] SHAFIZADEH, F. and BUKWA, W. (1970) *Phytochemistry* **9**, 871.

coumarins[11] and investigation of the sesquiterpene lactones. The study of the three subspecies of *A. tridentata*[12] showed that the sesquiterpene lactones isolated from a Montana sample of *A. tridentata* ssp. *vaseyana* (Rydb.) Beetle were the same as those reported for *A. arbuscula* Nutt. ssp. *arbuscula* collected in Wyoming.[13,14] However, the compounds in Wyoming *A. arbuscula* ssp. *arbuscula* differed substantially from those isolated from the same species in Montana.[15,16] These observations prompted a more detailed study, some of the results of which are reported in this article.

RESULTS

The TLC patterns of the sesquiterpene lactones of the three subspecies of *A. tridentata* have been discussed before.[12] Further sampling and investigation of these patterns have resulted in additional interesting information. *Artemisia tridentata* ssp. *tridentata* is easily identified by its morphology and produces a brown TLC pattern which is quite different from the patterns of the other two subspecies (Fig. 1, pattern 4). *Artemisia tridentata* ssp. *wyomingensis*, however, can be difficult to separate morphologically from *A. tridentata* ssp. *vaseyana*. It shows a distinct TLC pattern with two red spots (Fig. 1, pattern 5), the upper one being 1β-hydroxysant-3-en-6,12-olide-C (I) and the lower one 1β-hydroxysant-4(14)-en-6,12-olide-C (II).[12] All samples of this subspecies contained both of these compounds, except for two that contained only the lower spot. *Artemisia tridentata* ssp. *vaseyana* proved to be of the greatest taxonomic interest. Plants from this species produced three distinct and consistent TLC patterns without having discernible morphological differences in the field.



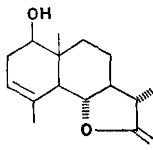
* Dotted lines indicate the spots vary quantitatively; also only diagnostic spots are shown.

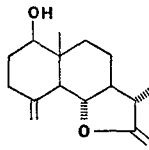
FIG. 1. TLC PATTERNS OF *Artemisia tridentata* AND *A. arbuscula* SESQUITERPENE LACTONES.

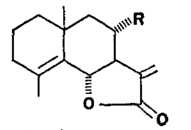
[11] SHAFIZADEH, F. and MELNIKOFF, A. B. (1970) *Phytochemistry* **9**, 1311.
[12] SHAFIZADEH, F., BHADANE, N. R., MORRIS, M. S., KELSEY, R. G. and KHANNA, S. N. (1971) *Phytochemistry* **10**, 2745.
[13] IRWIN, M. A. and GEISSMAN, T. A. (1969) *Phytochemistry* **8**, 2411.
[14] IRWIN, M. A. and GEISSMAN, T. A. (1971) *Phytochemistry* **10**, 637.
[15] SHAFIZADEH, F. and BHADANE, N. R. (1972) *J. Org. Chem.* **37**, 274.
[16] SHAFIZADEH, F. and BHADANE, N. R. (1973) *Phytochemistry* **12**, 857.

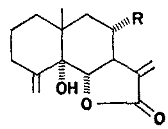
The first pattern contained four characteristic spots[12] for arbusculin-*B* (III) at the top, followed by arbusculin-*C* (IV), an unidentified compound and a large spot of arbusculin-*A* (V) at the bottom (Fig. 1, pattern 1). This pattern has been found in plants ranging in elevation form 860 to 1950 m (Table 1) and has been referred to as low elevation pattern.

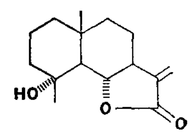
The second pattern (Fig. 1, pattern 3) contained a blue spot of variable intensity and four brown, purple or black spots at the base. These compounds are currently under investigation and appear to differ structurally from those previously isolated from this species. This pattern occurs in plants collected from approx. 1830–2740 m in elevation. Both patterns occur together in a transition zone at an approximate height of 1830–1950 m. Above this zone the high elevation type occur and below it, plants exhibiting the low elevation pattern.

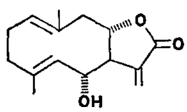

(I)

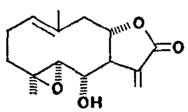

(II)

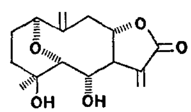

(III) R = H
(VI) R = OH

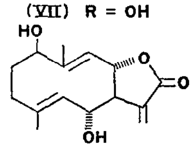

(IV) R = H
(VII) R = OH

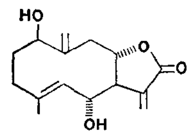

(V)


(VIII)


(IX)


(X)


(XI)[16]


(XII)[16]

The third pattern (Fig. 1, pattern 2) was originally observed and reported[12] as a variation of the generally observed low elevation pattern in Montana. Chemical studies since then have shown that these plants contain variable amounts of arbusculin-*B* (III) and -*C* (IV), a medium quantity of arbusculin-*A* (V) and heavy concentration of rothin-*A* (VI) and rothin-*B* (VII). The latter two compounds were initially discovered in *A. rothrockii*[14] from Wyoming and have been observed for the first time in Montana plants. The only plants exhibiting this pattern have been found in the Hot Springs Valley of northwestern Montana at an elevation of 800–1000 m, in a dry environment. This group is associated with the low elevation subspecies discussed before (Fig. 1, pattern 1).

The reverse situation to the above has been observed for *A. arbuscula* ssp. *arbuscula* and *A. tridentata* ssp. *vaseyana* form *spiciformis*, which have the same TLC pattern but distinctly different morphology. It should be noted, however, that these groups have a rather restricted distribution in Montana. The common pattern (Fig. 1, patterns 6 and 7) is characterized by a green spot, deacetyllaurenobiolide (VIII), and a red spot, spiciformin (IX),[16] a brown spot with a red tip, currently unidentified, and a red spot at the very base composed of badgerin (X), tatridin-*A* (XI) and -*B* (XII).[15,16] This pattern is quite different from those of the other species and subspecies of sagebrush. Morphologically, the two taxa can be separated from each other as well as from the three subspecies of *A. tridentata*. Both taxa match quite well with the description provided by Beetle.[4] Furthermore, extraction of an authentic sample of *A. tridentata* ssp. *vaseyana* form *spiciformis* from Wyoming showed a pattern identical to that of the Montana plants.

It has been demonstrated for *Artemisia douglasiana*[17–19] Bess. that in the spring the plant

[17] Matsueda, S. and Geissman, T. A. (1967) *Tetrahedron Letters* 2013.
[18] Matsueda, S. and Geissman, T. A. (1967) *Tetrahedron Letters* 2159.
[19] Lee, K. H., Matsueda, S. and Geissman, T. A. (1971) *Phytochemistry* **10**, 405.

TABLE 1. DESCRIPTION OF SAMPLING LOCATIONS*

Date collected	Sample Nos.	Quantity	Location	Township	Range	Section	Elevation (m)
Artemisia tridentata ssp. *vaseyana* (low elevation)							
8/14/70	2–4	3	Wisdom	T.2 S.	R.16.W.	26	1859
2/15/71	1	1	Drummond	T.10.N.	R.12.W.	10	1219
2/15/71	7–8	2	Helmville Road	T.13.N.	R.11.W.	6	1372
2/15/71†	10–11	2	Flesher Pass	T.14.N.	R 6.W.	15	1890
2/15/71†	13–14	2	Flesher Pass turn off	T.15 N.	R.7.W.	27	1512
2/15/71†	18–20	3	Clearwater Bridge	T 14.N.	R.14.W.	4	1158
2/17/71†	1–4	4	Lavalle Creek	T.14.N.	R.20.W.	13	1091
2/22/71†	14	1	Hot Springs Pass	T.21 N.	R.24.W.	25	1012
6/23/71	14, 16, 17	3	Steel Creek Campground	T.3 S.	R.14.W.	4	1939
6/23/71	41	1	Above Sagecreek	T.2.S	R.17.W.	18	1926
7/15/71	6, 9, 11	3	Camas Prairie	T.20 N.	R.24.W.	36	869
Artemisa tridentata ssp. *vaseyana* (high elevation)							
2/15/71†	9	1	Flesher Pass	T.14.N.	R.6 W.	15	1890
2/15/71†	12, 15	2	Flesher Pass turn off	T.15.N.	R.7 W.	27	1512
6/22/71	1–11	11	Sage Creek	T.2.S.	R.17.W.	17	1951
6/23/71	15,18, 20, 22	4	Steel Creek Campground	T.3.S.	R.14.W.	4	1939
6/23/71	44–45	2	Lost Trail Pass	T.2.S.	R.18.W.	8	1975
8/24/71	6–17	12	Montana-Idaho Border	T.16.S.	R.10.W.	9	2499
8/25/71	56–59	4	Continental Divide	T.14.S.	R.12.W.	10	2743
8/25/71	67–68	2	Indian Creek	T.14 S.	R.12.W.	13	2438
8/27/71	102–107	6	Below Elk Lake	T.13.S.	R.1.E.	31	2048
8/28/71	111–112	2	Upper Red Rock Lake	T.14.S.	R.1.W.	25	2042
8/28/71	119,120, 122	3	Red Rock Pass	T 14.S.	R 2.E.	19	2173
Artemisia tridentata ssp. *vaseyana* (Hot Springs)							
2/22/71†	15	1	Hot Springs Pass	T.21.N.	R.24.W.	25	1012
7/15/71	3–5	3	Camas Prairie	T.20.N.	R.24.W.	36	869
7/15/71	13–19	7	Hot Springs Pass	T.21.N.	R.24.W.	25	1012
7/15/71	20–21	2	East of Hot Springs	T.21.N.	R.23.W.	6	829
9/25/71	2–4	3	Garden Creek	T.22.N.	R.24.W.	20	914
Artemisia tridentata ssp. *wyomingensis*							
8/14/70	7	1	Polaris turn off	T.6.S.	R.13.W.	24	1878
8/14/70	9	1	Above Bannack	T.8.S.	R.11.W.	8	1783
8/14/70	12–13	2	Bannack Flats	T.7.S.	R.12.W.	25	1829
8/14/70	26, 30	2	Ingersoll Mine	T.8.S.	R.11.W.	3	1838
8/25/71	44–47	4	Bannack Pass Road	T.15.S.	R.10.W.	16	2134
Artemisia tridentata ssp. *tridentata*							
8/14/70	8	1	Bannack Ranch	T.8.S.	R.12.W.	1	1768
8/15/70	32, 35	2	Red Rock	T. 11.S.	R.10.W.	16	1783
2/22/71†	1–3	3	Old Mulick Place	T.19.N.	R.23.W.	32	817
2/22/71†	4–6	3	Phillips Ranch	T 19.N.	R.23.W.	19	814
8/25/71	34–36	3	Deadman Creek	T 15.S.	R.10.W.	10	2060
8/27/71	93, 95, 97	3	Game Refuge	T.13.S.	R.2.W.	24	2036
Artemisia tridentata ssp. *vaseyana* form *spicoformis*							
8/28/71	108–110						
	113–114	5	Upper Red Rock Lake	T.14.S.	R.1.W.	26	2057
8/5/70	5	1	Wyoming, Bettle				
Artemisia arbuscula ssp. *arbuscula*							
8/14/70	10–11	2	Above Bannack	T.8.S.	R.11.W.	8	1783
8/14/70	14–18	4	Badger Pass	T.7.S.	R.11.W.	11	1926
8/14/70	19–24	6	Badger Pass	T.7.S.	R.11.W.	11	1926
8/26/71	69–73	5	Four Eyes Canyon	T.15.S.	R.10.W.	2	2048

* This is a partial list of over 200 samples collected. A full list of sampling locations is available on application to the authors.

† Samples that were tagged and collected to check seasonal variation.

produces different sesquiterpene lactones from those in the fall. To investigate the possible confusing effect of this phenomena in sagebrush, plants of *A. tridentata* ssp. *tridentata* and *A. tridentata* ssp. *vaseyana*, low elevation, high elevation and Hot Springs types were tagged and samples removed in February, May, July and September. As shown in Table 2, the patterns contained the same chemicals in different seasons and the observed changes were only due to quantitative differences. Although an identifiable pattern could be obtained in any month of the year, except for high elevation type in winter, the best analysis was from samples collected from May through September.

TABLE 2. EXAMPLES OF SEASONAL VARIATION IN TLC ANALYSIS

Compound	Color*	R_f	February	May	July	September
A. tridentata ssp. *vaseyana* (low elevation) Sample 2/15/71-19						
Arbusculin-*B*	G	0·69		+	++	+
Arbusculin-*C*	G	0·49	−+†	+	+	+
Unknown	PG	0·42	−+	+	++	+
Arbusculin-*A*	G	0·29	+++	+++	++++	++++
A. tridentata ssp. *vaseyana* (high elevation) Sample 2/15/71-9						
Unknown	Bl or P	0·36		++	+++	+++
	Br or P	0·22			++	++
	P	0·10		+	+	+
	Br	0·06		+	++	++
	Bk	0·04		+	+	+
	Bk	0·02	+	+	+	+
A. tridentata ssp. *vaseyana* (Hot Springs) Sample 2/22/71-15						
Arbusculin-*B*	G	0·69				
Arbusculin-*C*	G	0·49				
Unknown	PG	0·42	−+	−+	−+	−+
Arbusculin-*A*	G	0·29	−+		−+	−+
Rothin-*A*	G	0·17	++	++	++	+++
Rothin-*B*	YB	0·09	++	+	+	+
A. tridentata ssp. *tridentata* Sample 2/22/71-1						
Unknown	Br or P	0·74		−+	++	−+
	Br or P	0·59		−+	+	−+
	Br or P	0·54	−+	−+	+	+
	Br	0·19	+	+	+	+
	Br	0·13	+	+	−+	+
	Y	0·09	+	+	+	+
	Br	Below 0·09	+++	+++	+++	+++

* See Fig. 1 for color code.

† Plus signs indicate relative concentrations, −+ indicate the spot is just visible on the TLC plate.

DISCUSSION

The present results show a high degree of correlation between morphology of the plants and the TLC analysis of sesquiterpene lactones produced. These compounds not only aid in the separation of species, but are sufficiently specific to reliably differentiate the subspecies. Since each TLC pattern could be indicative of a genetically distinct group, it should provide a useful guide for detailed morphological and cytological investigations that could resolve the complex taxonomy of these plants. It is interesting to note the environmental arrangement of the three chemical types of *A. tridentata* ssp. *vaseyana*. The Hot Springs type is found at the elevations of 760–1040 m with scattered occurrence of the low elevation type, which extended from 800 to 1950 m. Above this limit a transition occurs, giving way to the high elevation group which extends to as high as 2740 m. Undoubtedly the environmental conditions associated with each group are different.

The limited analysis of samples from Montana could not provide a general solution to the chemical variation found in *A. arbuscula* ssp. *arbuscula* from Wyoming and Montana. Further sampling is needed. It is now clear that the chemical variation is not due to seasonal changes in biosynthesis as reported for *A. douglasiana*[17–19] but may stem from ecological factors.

EXPERIMENTAL

Plant materials. All plants were identified in the field based on morphological characteristic provided by Beetle.[4] Approx. 15 g of leaf materials was collected and placed in a paper bag marked with the species, date collected, sample number and location. At the same time a sample was collected and placed in a plant press for future reference.

Sesquiterpene lactone extraction.[12] Leaves (10 g) were crushed, covered with $CHCl_3$ (100 ml) and allowed to stand for a minimum of 48 hr. The leaves were filtered off and the solvent removed to give a green residue. Hot EtOH (10 ml) was added to the residue followed by hot H_2O (15 ml) which was allowed to cool to room temp. This was then treated with PbOAc solution (5 ml, 1 g PbOAc per 5 ml H_2O) and charcoal. It was filtered and the yellow solution extracted with $CHCl_3$ (2 × 25 ml). The $CHCl_3$ was removed and the yellow syrup stored.

TLC analysis. This was carried out on fresh plates of silica gel G (Woelm), preheated in an oven set at 110° and developed in light petrol–$CHCl_3$–EtOAc (2:2:1). The plates were sprayed with conc. H_2SO_4 and heated at 100–110° for 5 min.

Characterization of rothin-A (VI). The leaves of *A. tridentata* ssp. *vaseyana* (Hot Springs type) were dried and ground to yield 2481 g of materials which was then extracted with $CHCl_3$ as described above. This gave 81·3 g of yellow syrup which was dissolved in C_6H_6 and placed on a silica gel column prepared with C_6H_6. Elution of the column with Et_2O–C_6H_6 yielded a mixture of arbusculin-*A* (V) and rothin-*A*. Rechromatography of 3 g of this mixture on a small silica gel column failed to separate these compounds. The mixture was then run against an authentic sample of rothin-*A*[20] on a TLC plate in three solvents. The R_fs and color of the rothin-*A* and the compound in question were identical in light petrol.–$CHCl_3$–EtOAc (2:2 1), C_6H_6–EtOAc–EtOH (5:4:1) and light petrol.–$CHCl_3$–EtOH (4:5:1).

Isolation and characterization of rothin-B (VII). Continued elution of the column in the above experiment with increasing amounts of Et_2O in C_6H_6 yielded a crystalline material. It was recrystallized once from EtOH and a second time from MeOH producing crystals of rothin-*B* (250 mg), m.p. 254–257°, alone or on admixture with the authentic sample.[20] This compound and the authentic rothin-*B* were identical by cochromatography in the three solvents mentioned above. The NMR in pyridine-d_5 was also identical to that reported by Irwin.[21]

Acknowledgements—The authors thank the Forest and Conservation Experiment station of the University of Montana and the Hoerner-Waldorf Corporation of Montana for financial support. They also thank Professor T. A. Geissman for authentic samples of rothin-*A* and rothin-*B*.

20 The authentic sample of rothin-*B* and -*A* were obtained from Professor T. A. GEISSMAN.

21 IRWIN, M. A. (1971) Sesquiterpene Lactones of Artemisia, Ph.D. Dissertation from the Dept. of Chemistry, UCLA, California, U.S.A.