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SESQUITERPENE LACTONES OF BIG SAGEBRUSH*

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Abstract—Samples of *Artemisia tridentata* ssp. *vaseyana, tridentata* and *wyomingensis* were collected from various locations throughout the state of Montana and the sesquiterpene lactone content of their leaves were analyzed by TLC. The major lactones present in each subspecies (I–VI) were isolated and chemically identified. The results showed a definite chromatographic pattern and a high degree of uniformity for each subspecies within the state of Montana. However, the comparison of these data with the analysis of a few samples obtained from the state of Wyoming failed to substantiate the general applicability of the above pattern to other ecosystems and the correlation of the chemotaxonomy with morphological identification of the plants involved.

INTRODUCTION

THE SESQUITERPENE lactones of the major subspecies of big sagebrush *Artemisia tridentata* ssp. *vaseyana, tridentata* and *wyomingensis*, growing in Montana were investigated as a part of our program on the chemical constituent of these plants[1,2] and an extension of the chemotaxonomic investigations based on composition of their coumarin compounds.[2]

It has been already shown that various species and subspecies of *Artemisia* contain a variety of sesquiterpene lactones.[3–18] This includes *A. tridentata* Nutt. ssp. *tridentata*[3] from California and *A. arbuscula* Nutt. ssp. *arbuscula*[18] from Wyoming which are referred to in the following discussions. In this study samples of sagebrush have been collected from a variety of geographical locations within the state of Montana and the resulting data is compared with the sesquiterpene lactone content of a few samples obtained from Wyoming.

* Part III in the series on Chemical Composition of Sagebrush. Part II on coumarin compounds of *Artemisia tridentata* ssp. *vaseyana* was published in *Phytochem.* **9,** 1311 (1970).

† The collection and identification of the plant materials have been conducted by M. S. Morris from the School of Forestry.

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[14] S. MATSUEDA and T. A. GEISSMAN, *Tetrahedron Letters* 2159 (1967).
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[16] K. H. LEE and T. A. GEISSMAN, *Phytochem.* **9,** 403 (1970).
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[18] M. A. IRWIN and T. A. GEISSMAN, *Phytochem,* **8,** 2411 (1969).

TABLE 1. THIN-LAYER CHROMATOGRAM

	Sample No.*	Brown 0·70†	Green 0·66	Brown 0·53	Brown 0·48	Green 0·46	Brown 0·41	Violet 0·40	Brown 0·34
A. tridentata ssp. *vaseyana*	1–18		++§			++		++	
	19–21		++			++		++	
	22–25		++			++		++	
	26–27		++			++	++		
	28–30	+		+			+		+
	31	+		+			+		+
	32‡							+++	
A. tridentata ssp. *wyomingensis*	33–36	+		+	+		+		+
	37–38	+			+		+		+
	39–49	+		+			+		+
	50–53	+		+			+		+
	54‡	+		+			+		+
A. tridentata ssp. *tridentata*	55–56	+		+	+		+		+
	57–59	+		+	+		+		+
	60–66	+		+	+		+		+
	67‡	+		+			+		+
A. arbuscula ssp. *arbuscula*	68‡		++			++		++	

* The samples are described in Table 2.
† Color and R_f value of the spots.
‡ Samples from Wyoming.
§ Relative intensity.

STRUCTURE OF THE LACTONES

Samples of *A. tridentata* ssp. *vaseyana*[19] growing in Grass Valley were collected and investigated by TLC. This showed the presence of four major spots; green spot R_f 0·66, green spot R_f 0·46, violet spot R_f 0·4 and green spot R_f 0·26 shown in Table 1.

The compound corresponding to the green spot R_f 0·26 was isolated by column chromatography and purified by preparative TLC as a colorless gum. This compound had the empirical formula of $C_{15}H_{22}O_3$. Spectroscopic investigation indicated that it had a hydroxyl group, an α–β unsaturated γ-lactone, a tertiary methyl, a CH_3—C(—)(—)—O— and a CH—O—. The hydroxyl group was assumed to be tertiary as it resisted oxidation by Jones' reagent[20] or chromium trioxide-acetic acid reagent[21] and was not acetylated at room temperature with acetic anhydride–pyridine reagent. On hydrogenation it gave a known crystalline lactone (VII),[22] which was dehydrated to provide a mixture of the two unsaturated products (VIII and IX). This showed that the compound must have the structure of arbusculin-A (I) a new crystalline sesquiterpene lactone recently isolated from *A. arbuscula* Nutt ssp.

[19] A. A. BEETLE, *A Study of Sagebrush*, University of Wyoming Agricultural Experiment Station Bulletin 368, p. 54 (1960).
[20] K. BOWDEN, I. M. HEILBRON, E. R. H. JONES and B. C. L. WEEDON, *J. Chem. Soc.* 39 (1946); A. BOWERS, T. G. HALSALL, E. R. H. JONES and A. J. LEMIN, *J. Chem. Soc.* 2555 (1953); C. DJERASSI, R. R. ENGLE and E. BOWERS, *J. Org. Chem.* **21**, 1547 (1956).
[21] W. HERZ and G. HOGENAUER, *J. Org. Chem.* **27**, 905 (1962).
[22] T. C. JAIN, C. M. BANKS and J. E. MCCLOSKEY, *Experientia* **25**, 906 (1969).

OF THE COLLECTED SAMPLES OF SAGEBRUSH

Green 0·26	Gray 0·26	Brown 0·25	Green 0·17	Red 0·14	Yellowish black 0·13	Red 0·12	Brown 0·12	Yellowish black 0·08	Black 0·07	Black 0·06	Red 0·06	Black 0·03
+++					+			+				+
+++					+			+			++	+
+++			++		+			+				+
+++				++		++		+				+
		+						+++	+++			++
		+		++		++		+				+
								++	++			++
		+		+++		+++		+				++
				+++		+++		+				++
		+		+++		+++		+				++
		+		++		+++		+				++
	+++				+		+	+	+			+
		+						++	+++	++		++
		+						++	+	++		++
		+						++	+++			++
	+++				+		+	+	+			+
++					+			+				+

arbuscula by Irwin and Geissman.[18] This conclusion was verified by a direct TLC comparison with the authentic sample.

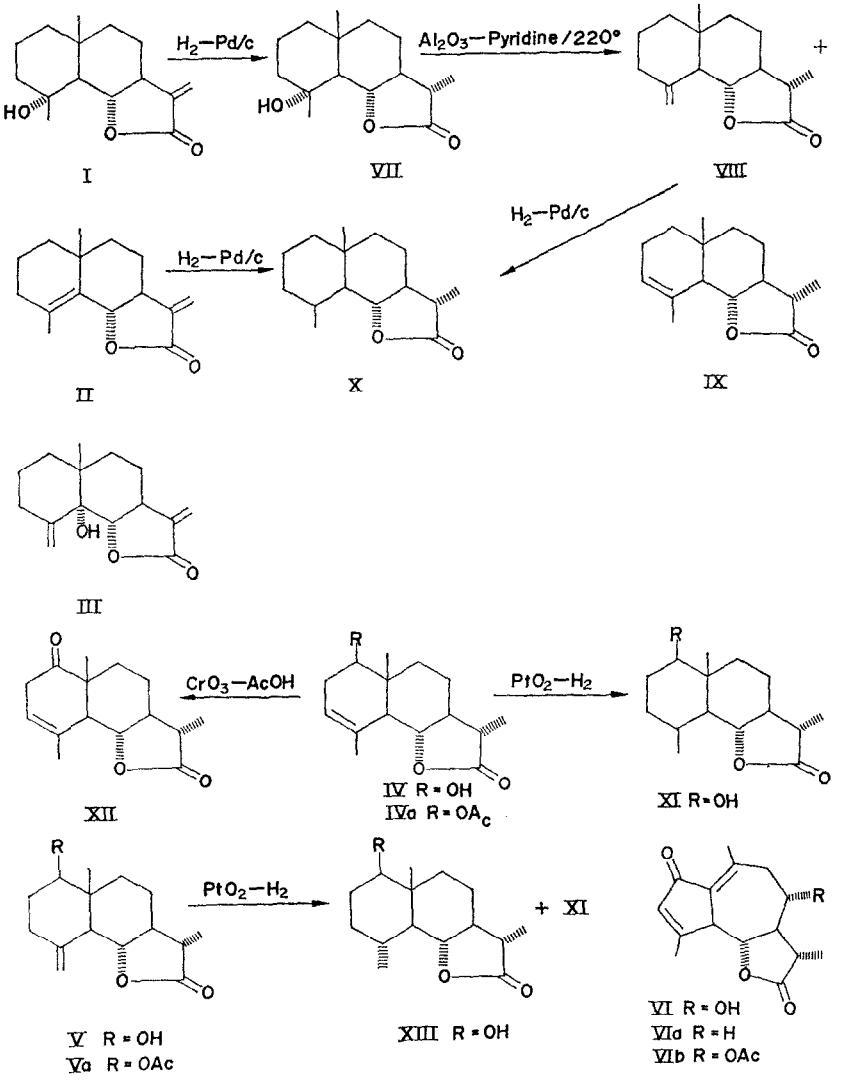
The compound corresponding to the green spot R_f 0·66 was isolated as fine needles. Spectroscopic investigation and hydrogenation to santanolide-C (X) showed the structure II for this compound, which is identical with arbusculin-B, another recently reported new sesquiterpene lactone from *A. arbuscula* Nutt. ssp. *arbuscula*.[18] Furthermore, hydrogenation of VIII to X showed the close structural relationship between arbusculin-A and -B.

The compound corresponding to the green spot R_f 0·46 was isolated as fine needles and identified as arbusculin-C[18,23] (III) in comparison with an authentic sample. The violet spot R_f 0·4 provided another crystalline lactone which appears to be related to arbusculin-C but so far has escaped complete structural determination.

Samples of another subspecies of big sagebrush *A. tridentata* ssp. *wyomingensis*, originally identified by Beetle in Wyoming,[19] were collected from several locations throughout Eastern Montana and Gillette, Wyoming. All these plants gave a TLC containing two major red spots (R_f 0·14 and 0·12 in Table 1).

The compound corresponding to the faster moving spot was isolated as a crystalline material. It had the empirical formula of $C_{15}H_{22}O_3$ and physical constants, NMR and IR spectra identical with those of 1β-hydroxysant-3-en-6,12-olide-C (IV) obtained by transformation of santamarine[24] and douglanine.[14] Furthermore, acetylation, hydrogenation

[23] M. A. Irwin and T. A. Geissman, private communication.
[24] A. Romo de Vivar and H. Jimenez, *Tetrahedron* **21**, 1741 (1965).



and oxidation furnished the acetate (IVa),[24] 1β-hydroxysantanolide-C (XI),[24,25] and the 1-keto derivative (XII)[24] which are also known compounds.

The second lactone from the above subspecies was also isolated as a crystalline material and found to have the same empirical formula as IV and similar spectral properties except that the NMR spectrum showed the presence of an exomethylene group instead of the vinylic methyl group. Also, hydrogenation of this compound gave a mixture of two lactones, one of which was identical with tetrahydroreynosin (XIII)[25] the C4 epimer of 1β hydroxy-santanolide-C. On this basis it was identified as 1β-hydroxysant-4(14)-en-6,12-olide-C (V) which to our knowledge is a new compound.

A sample of *A. tridentata* ssp. *tridentata* from California has been investigated by Geissman, Irwin *et al.*[3] and was found to contain deacetoxymatricarin (VIa) (Leucodin[6])

[25] H. YOSHIOKA, W. RENOLD, N. H. FISCHER, A. HIGO and T. J. MABRY, *Phytochem.* **9**, 823 (1970).

TABLE 2. DESCRIPTION OF THE COLLECTED SAMPLES OF SAGEBRUSH

No.	Plant	Location	Date	Range	Township	Section	Quarter
1	*A. tridentata*	Grass Valley No. 1	April 69	R.19.W.	T.13.N.	20	S.E.
2	ssp. *vaseyana*	Grass Valley No. 2	July 69	R.19.W.	T.13.N.	20	S.E.
3		Grass Valley No. 3	Oct. 69	R.19.W.	T.13.N.	20	S.E.
4		Perma No. 1	June 69	R.23.W.	T.19.N.	19	N.E.
5		Nettleton Ranch No. 1	Aug. 69	R.6.W.	T.13.N.	14	N.W.
6		Nettleton Ranch No. 2	Oct. 69	R.6.W.	T.13.N.	14	N.W.
7		Nettleton Ranch No. 3	Oct. 69	R.6.W.	T.13.N.	14	N.W.
8		Settle Ranch	Aug. 69	R.5.W.	T.12.N.	16	S.W.
9		Scratch Gravel No. 1	Aug. 69	R.4.W.	T.11.N.	14	S.E.
10		Scratch Gravel No. 2	Oct. 69	R.4.W.	T.11.N.	14	S.E.
11		Browns Lake	Oct. 69	R.11.W.	T.14.N.	14	S.W.
12		East of Lincoln No. 1	Oct. 69	R.7.W.	T.15.N.	22	S.W.
13		Canyon Creek	Oct. 69	R.5.W.	T.12.N.	16	S.W.
14		Helena N. No. 1	Oct. 69	R.3.W.	T.12.N.	30	S.E.
15		Gates of Mountain No. 1	Oct. 69	R.2.W.	T.11.N.	18	S.W.
16		Gates of Mountain No. 2	Oct. 69	R.2.W.	T.11.N.	18	S.W.
17		Camaus Prairie	July 69	R.24.W.	T.19.N.	1	N.W.
18		LaValle	May 70	R.16.W.	T.14.N.	13	N.E.
19		Dry Cottonwood	July 69	R.8.W.	T.5.N.	17	S.E.
20		East of Lincoln No. 2	Aug. 69	R.7.W.	T.15.N.	22	S.W.
21		Flesher Pass No. 1	Oct. 69	R.6.W.	T.14.N.	10	S.W.
22		Hot Springs No. 1	March 70	R.24.W.	T.21.N.	25	S.E.
23		Hot Springs No. 2	March 70	R.24.W.	T.21.N.	25	S.E.
24		Perma No. 1	March 70	R.23.W.	T.19.N.	19	N.E.
25		Perma No. 2	March 70	R.23.W.	T.19.N.	19	N.E.
26		Helena N. No. 2	Oct. 69	R.3.W.	T.12.N.	19	N.E.
27		Hot Springs No. 3	March 70	R.24.W.	T.21.N.	25	S.E.
28		Flesher Pass No. 2	Oct. 69	R.6.W.	T.14.N.	10	S.W.
29		Emory Mine	June 70	R.8.W.	T.7.N.	2	S.E.
30		Browns Gulch	July 69	R.8.W.	T.4.N.	20	N.W.
31		Little Basin Cr. Rd.	July 69	R.8.W.	T.2.N.	22	N.E.
32		Wyoming[a]	July 70	—	—	—	—
33	*A. tridentata*	Euland Rch.-Butte	May 69	R.9.W.	T.3.N.	13	S.E.
34	ssp. *wyomingensis*	Billings	May 69	—	—	—	—
35		Iverson No. 1	Nov. 69	R.25.E.	T.12.N.	13	N.W.
36		Iverson No. 2	Nov. 69	R.25.E.	T.12.N.	13	N.W.
37		Winnet No. 1	Nov. 69	R.26.E.	T.15.N.	21	N.W.
38		Winnet No. 2	Nov. 69	R.26.E.	T.15.N.	21	N.W.
39		Winnet No. 3	Nov. 69	R.26.E.	T.15.N.	22	S.E.
40		King Area No. 1	Nov. 69	R.26.E.	T.14.N.	19	N.W.
41		E. of Badger pass No. 1	March 70	R.11.W.	T.7.S.	11	S.W.
42		E. of Badger pass No. 2	March 70	R.11.W.	T.7.S.	11	S.W.
43		S. of Glen No. 1	March 70	R.9.W.	T.4.S.	24	S.E.
44		S. of Glen No. 2	March 70	R.9.W.	T.4.S.	24	S.E.
45		Ten Miles No. 1	March 70	R.10.W.	T.7.S.	16	S.E.
46		Ten Miles No. 2	March 70	R.10.W.	T.7.S.	10	S.E.
47		McCartney Mt. No. 1	March 70	R.8.W.	T.3.S.	22	N.E.
48		King Area No. 2	Nov. 69	R.26.E.	T.14.N.	19	N.W.
49		Acton	May 70	R.24.E.	T.2.N.	12	S.E.
50		E. of Badger pass No. 2	March 70	R.11.W.	T.7.S.	10	—
51		Snowline	March 70	R.7.W.	T.14.S.	22	N.W.
52		McCartney Mt. No. 2	Jan. 70	R.8.W.	T.3.S.	23	S.E.
53		Silver Bow	July 69	R.9.W.	T.3.N.	29	—
54		Wyoming[a]	July 70	—	—	—	—

TABLE 2 *cont.*

No.	Plant	Location	Date	Range	Township	Section	Quarter
55	*A. tridentata*	Perma No. 1	June 69	R.23.W.	T.19.N.	19	N.E.
56	ssp. *tridentata*	Gates of Mountain	Oct. 69	R.2.W.	T.11.N.	18	S.W.
57		Red Rock No. 1	Feb. 70	R.10.W.	T.11.S.	4	S.E.
58		Red Rock No. 2	Feb. 70	R.10.W.	T.11.S.	4	S.E.
59		Perma No. 2	July 69	R.23.W.	T.19.N.	32	N.E.
60		Snowline No. 1	March 70	R.7.W.	T.14.S.	22	S.E.
61		Snowline No. 2	March 70	R.7.W.	T.14.S.	22	S.E.
62		Snowline No. 3	March 70	R.7.W.	T.14.S.	22	S.E.
63		McCartney Mt.	Jan. 70	R.8.W.	T.3.S.	23	S.E.
64		Perma No. 3	March 70	R.23.W.	T.19.N.	19	N.E.
65		Perma No. 4	March 70	R.23.W.	T.19.N.	19	N.E.
66		Pipestone Pass	July 69	R.7.W.	T.2.N.	22	S.W.
67		Wyoming*	July 70	—	—	—	—
68	*A. arbuscula* ssp. *arbuscula*	Wyoming*	July 70	—	—	—	—

* These samples were obtained from Professor A. A. Beetle.

and ridentin.[12] The TLC analysis of the chloroform extracts obtained from samples of *A. tridentata* ssp. *tridentata* collected from several locations in Montana showed a pattern which was distinctly different from those of other subspecies and consisted of a major black spot ($R_f = 0{\cdot}070$) flanked by two minor black spots and some faster moving faint spots. The compound corresponding with the major spot was isolated as a pure crystalline material and identified as desacetylmatricarin (Desacetylartilesin[4]) (VI).

Desacetylmarticarin is known to occur in *A. leucodes*,[26] *A. austriaca*,[26] *A. tilesii*[4] and *Achillea lanulosa*.[27]

Unfortunately an authentic sample of desacetylmatricarin was not available for direct comparison, however the isolated sesquiterpene lactone of *A. tridentata* ssp. *tridentata* gave the reported physical properties including the formation of a monohydrate which solidifies after melting and remelts at a higher temperature;[4,27] although some of the samples directly gave the higher m.p. Furthermore, the product on acetylation gave matricarin (VIb) with the reported constants. The NMR, IR and UV spectra of the isolated lactone and its acetylated derivative, confirmed their chemical identity.

TAXONOMIC CONSIDERATION

The results obtained from TLC of the sesquiterpene lactones present in the leaf extract of a large number of samples from the three major subspecies of big sagebrush collected from different geographical locations throughout the state of Montana are given in Tables 1 and 2 and are compared with a few samples obtained from Wyoming.

These data indicate distinct patterns for the three subspecies within the state of Montana. Even the very few samples which showed minor variations from the general pattern still contained the characteristic spots and could be readily classified by their chemical characteristics. However, despite the wide geographical sampling of the different ecological locations

[26] K. S. RYBALKO, *Zh. Obshch. Khim.* **33**, 2734 (1963); *Chem. Abs.* **60**, 5561 (1964).
[27] E. H. WHITE and R. E. K. WINTER, *Tetrahedron Letters* 137 (1963).

within the state of Montana, the chemical pattern could not be reproduced with or applied to the few samples obtained from Wyoming.

The samples of *vaseyana* subspecies from Montana generally gave four strong, fast moving spots and three faint, slow moving spots (samples 1–18). Samples 19–21 and 22–25 in addition to these spots also had another distinct spot indicating that they may be a hybrid or an ecotype variation. Samples 26 and 27 showed the characteristic spots of the *vaseyana* as well as the *wyomingensis* subspecies. Samples 28–30 and 31 which morphologically were identified as *vaseyana* subspecies showed the general pattern of *tridentata* and *wyomingensis* subspecies respectively. A sample of the *vaseyana* subspecies from Wyoming (32) showed only one of the four major spots found in the Montana samples, whereas a sample of *A. arbuscala* ssp. *arbuscula* from Wyoming (68) showed all four characteristic spots of *vaseyana*.

All the samples of the *wyomingensis* subspecies collected over an extensive area in Montana (33–53) had two distinct red spots and some faint spots. These were again different from the pattern developed by a sample obtained from Wyoming (54). The chromatographic pattern shown by the sample of *wyomingensis* from Wyoming (54) however, was identical with the pattern obtained with a Wyoming sample of the *tridentata* subspecies (67).

The samples of *tridentata* subspecies from Montana (55–66) were not as consistent as the other Montana subspecies but generally they showed chromatographic spots on several positions that were also the same in the Wyoming sample (67). The difference between these samples seemed to be of a quantitative rather than a qualitative nature.

The above data indicate some of the problems involved in identification of the various species and subspecies of the sagebrush on the basis of morphology or chemical taxonomy alone and provides a hope that sampling of wider geographical locations and further investigations based on the combination of the two systems could provide better understanding and information about phylogeny of these plants.

EXPERIMENTAL

All m.p.'s are uncorrected. The UV and the IR spectra were recorded on Coleman–Hitachi EPS-3T and Beckman IR-5 respectively. NMR spectra were recorded with Varian HA-60 spectrometer in deuterated $CHCl_3$ with a drop of $CHCl_3$ or TMS as internal standard and the values are reported in δ ppm with $CHCl_3$ lock signal at 7·24 ppm and TMS at 0 ppm. Mass spectra were recorded on CED 21-490 single focus spectrometer.

Plant materials. Samples of *Artemisia* species and subspecies were collected from a variety of locations throughout the state of Montana. A few samples were also obtained from the state of Wyoming as listed in Table 2. The samples were identified on the basis of their morphological characteristics,[19] UV fluorescence[28] and the known ecological distribution.[19]

Extraction. 1·0 kg of dried twigs and foliage were ground and left in $CHCl_3$ for 3 days. Removal of the solvents after filtration left a dark green residue which was dissolved in hot EtOH (100 ml) and hot H_2O (200 ml) was added to the solution. After keeping at room temp. for 30 min, the milky solution was decanted from a dark tar. The process was repeated once more. The combined milky solution was treated with saturated lead acetate solution (50 ml), Celite (50 g) and a little C. It was filtered and the clear yellow filtrate extracted with $CHCl_3$ (3 × 100 ml). Removal of $CHCl_3$ gave a yellow syrup (~40 g). This material was used for TLC and isolation of the sesquiterpene lactones. For TLC analysis alone, this process was carried out with 5 g of the dried plant material.

TLC. TLC chromatograms were obtained with silica gel G (Woelm) and light petrol–$CHCl_3$–EtOAc (2:2:1). The plates were sprayed with H_2SO_4 and heated at 100° to develop spots representing the sesquiterpene lactones.

Isolation and characterization of arbusculin-B(II). Samples of *A. tridentata* ssp. *vaseyana* collected in Grass Valley, Missoula, Montana (*ca.* altitude of 3200 ft) were extracted as described before and the crude

[28] A. H. WINWARD and E. W. TISDALE, *Range Improvement Notes*, U.S.F.S., Intermountain Region **14** (3), 1 (1969).

extract (30 g) was dissolved in ~60 ml of benzene and adsorbed on a silica gel column (600 g) prepared in benzene.

Elution with benzene gave a mixture of fast moving spots (180 mg). This mixture was separately chromatographed on a silica gel column prepared in light petrol. Elution with light petrol–benzene (1:1) of this column gave arbusculin-B that was recrystallized as fine needles from light petroleum; yield 150 mg, m.p. 84–85° alone or in admixture with an authentic sample (lit.[18] m.p. 86·5–88°), R_f 0·66 (green spot), $[\alpha]_D^{18}$ + 55·52° (c = 2·5, $CHCl_3$), λ_{max} 206 nm (log ϵ 4·2), IR bands at 1775 (γ-lactone), 1660 and 875 cm^{-1} (unsaturation), mass spectrum m/e M^+ 232 (30·5), 217 (100), 161 (20·23), 145 (20·00), 119 (20·00), 91 (30·55) and 79 (21·86). (Found: C, 77·35; H, 8·64. $C_{15}H_{20}O_2$ required: C, 77·58; H, 8·62%.) The NMR spectrum had a pair of doublets at 5·33 and 6 ppm (1 H each, J = 3 c/s, C11 = CH_2), a broad doublet at 4·4 ppm (1H, J = 10·5 c/s, C6-lactone proton), a singlet at 1·85 ppm (for C4-allylic methyl) and a singlet at 1·08 ppm (for C10-tertiary methyl).

Isolation and characterization of arbusculin-C(III). Continued elution of the main column described in the previous experiment with benzene–Et_2O (95:5) gave in earlier fractions arbusculin-C that was crystallized from $CHCl_3$–light petrol; yield 500 mg. This was purified by a single recrystallization from the same solvent, m.p. 149–150° (alone or admixed with an authentic sample),[23] R_f 0·46 (green spot), $[\alpha]_D^{18}$ + 105·2° (c = 2·5, $CHCl_3$), λ_{max} 208 nm (log ϵ 4·21), IR bands at 3508 (OH), 1769 (γ-lactone), 1650 and 895 cm^{-1} (unsaturation), mass spectrum m/e M^+ 248 (100), 233 (5·37) and 230 (9·67). (Found: C, 72·46; H, 8,23. $C_{15}H_{20}O_3$ required: C, 72·58; H, 8·06%).

The NMR spectrum had a pair of doublets at 5·97 and 5·3 ppm (1 H each, J = 3 c/s, C11 = CH_2), a triplet at 4·87 ppm (2 H, J = 1·5 c/s, C4 = CH_2), a sharp doublet at 4·17 ppm (1 H, J = 11 c/s C6-lactone proton), a broad signal at 3·27 ppm (1 H, C7-H), a sharp singlet at 2 ppm (disappears on D_2O exchange, OH) and a sharp singlet at 0·9 ppm (3 H, C10-CH_3).

Isolation of an unidentified lactone. In the following fractions a second crystalline lactone was obtained that was recrystallized from $CHCl_3$–light petrol; yield 125 mg, m.p. 162–163°, R_f 0·4 (violet spot), IR bands at 3333 (OH), 1755 (γ-lactone), 1650 (unsaturation), 902 and 813 cm^{-1}. The NMR spectrum (in deuterated DMSO) had a pair of doublets at 5·87 and 5·4 ppm (1 H each, J = 3 c/s), broad signals at 5·05 and 6·91 ppm (1 H each, W 1/2, 4·5) a singlet at 0·9 ppm (3H)) and a sharp doublet at 4·37 ppm (1 H, J = 11 c/s). Hydrogenation of 50 mg of this lactone gave a crystalline compound, yield 30 mg, m.p. 206–208°.

Isolation and characterization of arbusculin-A(I). Further elution of the same column with benzene–Et_2O (9:1) gave the lactone (4 g) as a colorless gum. Attempts to crystallize this compound failed even after rechromatography and preparative TLC. The purified material had R_f 0·26 (green spot), $[\alpha]_D^{18}$ + 18·68° (c = 3·2, $CHCl_3$), λ_{max} 208 nm (log ϵ 4·045), IR bands at 3610 (OH), 1777 (γ-lactone) 1673 and 875 (unsaturation) cm^{-1}. The NMR spectrum had a pair of doublets at 5·35 and 6 ppm (1 H each, J = 3 c/s, C11 = CH_2), a triplet at 3·97 ppm (1 H, J = 11 c/s, C6–lactone proton), a singlet at 1·3 ppm (3 H, C4-CH_3) and a singlet at 0·9 ppm (3 H, C10-CH_3).

Hydrogenation of arbusculin-A. Arbusculin A (1 g) in EtOH (30 ml) was hydrogenated at atmospheric pressure on Pd/C (5%). The absorption of H_2 ceased after 1 mole equivalent was absorbed. The resulting colorless gum was crystallized from light petroleum to give colorless plates; yield 600 mg, m.p. 102–104°. Three recrystallizations from light petrol afforded a pure material (300 mg), m.p. 109–110° alone or in admixture with an authentic sample of hydroxylactone (VII) (lit.[22] m.p. 108·5–110°), $[\alpha]_D^{18}$ + 11·28 (c = 1·375, $CHCl_3$), IR bands at 3650 (OH), 1775 (γ-lactone), cm^{-1}. Mass spectrum m/e M^+ 252 (10·5), 237 (100·00), 234 (27·34), 219 (44·2), 161 (32·63), 43 (53·68). (Found: C, 71·08; H, 9·69. $C_{15}H_{24}O_3$ required: C, 71·42; H, 9·52%.) NMR spectrum had a broad triplet at 3·91 ppm (1 H, J = 10·5 c/s, C6-lactone proton), a sharp singlet at 1·2 ppm (3 H, C4-CH_3), a doublet at 1·08 ppm (3 H, J = 7 c/s, C11-CH_3) and a sharp singlet at 0·87 ppm (3 H, C10-CH_3).

Dehydration of hydroxylactone (VII). The hydroxylactone VII (200 mg) was heated with Al_2O_3 (400 mg, Alcoa chromatographic alumina F20) and pyridine (2 drops) at 220° for 6 hr. The reaction mixture was cooled and extracted with $CHCl_3$. The extract was washed with 2 N HCl (25 ml) and dried (Na_2SO_4). Removal of solvents left a colorless gum (165 mg). TLC analysis showed the absence of the starting material. NMR analysis showed that the lactone VIII was present in 85–90% and lactone IX was present in 10–15%. Repeated crystallization from light petrol afforded pure lactone VIII, as needles; yield 80 mg, m.p. 136–137·5° alone or in admixture with an authentic sample (lit.[22] m.p. 137–140°). NMR spectrum of this lactone had broad signals at 4·88 and 4·73 ppm (1 H each, W 1/2 = 4, C4=CH_2), a broad triplet at 3·95 ppm (1 H, J = 10 c/s, C6-H), a doublet at 1·2 ppm (3 H, J = 7 c/s, C11-CH_3) and a singlet at 0·87 ppm (3H, C10-CH_3). The isomer (IX) however was not isolated in pure state.

Hydrogenation of lactone VIII. The unsaturated lactone VIII, (40 mg) was hydrogenated at atmospheric pressure on Pd/C (5%). Removal of the solvent and crystallization from light petrol–Et_2O gave santanolide-C (X), m.p. 147–149° (lit. m.p. 153–154°[15] and 137–138°[29]).

[29] O. KOVACS, V. HEROUT, M. HORAK, and F. SORM, *Colln Czech. Chem, Commun.* **21**, 225 (1956).

Hydrogenation of arbusculin-B The lactone (40 mg) was hydrogenated at atmospheric pressure on Pd/C (5%). Removal of solvents and crystallization of the product furnished exclusively santanolide-C (X) m.p. 146–149° alone or in admixture with the compound obtained from arbusculin-A.

Isolation and characterization of 1β-*hydroxysant*-3-*en*-6,12-*olide-C* (IV). Samples of *A. tridentata* ssp. *wyomingensis* were collected within a small area near Acton on May 19, 1970. 900 g of dried leaves were ground and extracted in the same manner as described earlier to yield a dark yellow syrup (24 g).

This syrup was loaded on silica gel column (~500 g) prepared in benzene. Benzene–Et_2O (9:1) eluates afforded crystalline 1β-hydroxysant-3-en-6,12-olide-C (IV) which was recrystallized from Et_2O–light petroleum; yield 400 mg, m.p. 132–133° (lit.[14,24] m.p. 133·5–134·5°), R_f 0·14 (red), $[\alpha]_D^{27}$ + 78° (*c* = 2·07, $CHCl_3$), IR bands at 3550 (OH) and 1750 (γ-lactone) cm^{-1}. The NMR spectrum showed a singlet at 0·9 ppm (3 H, C10-CH_3), a singlet at 1·83 ppm (3 H, C4-CH_3), a doublet at 1·21 ppm (3 H, *J* = 6 c/s, C11-CH_3), a broad signal at 5·31 ppm (1 H, C3-H), a broad triplet at 3·93 ppm (1 H, *J* = 10·5 c/s, C6-H) and a quartet at 3·61 ppm (1 H, *J* = 11,6 c/s, Cl-H). (Found: C, 71·93; H, 9·08. $C_{15}H_{22}O_3$ required: C, 72·00; H, 8·80%.)

1β-*Hydroxysant*-4(14)-*en*-6,12-*olide-C* (V). Subsequent elution of the column in the previous experiment provided 1β-hydroxysant-4(14)-en-6,12-olide-C which was recrystallized twice from Et_2O–light petrol; yield 300 mg, m.p. 130–131°, R_f 0·12 (red), $[\alpha]_D^{31}$ + 137° (*c* = 2·07, $CHCl_3$) IR bands at 3525 (OH), 1754 (γ-lactone), 1652 and 892 (unsaturation) cm^{-1}. The NMR spectrum had a singlet at 0·83 ppm (3 H, C10-CH_3), a doublet at 1·20 ppm (3 H, *J* = 6 c/s, C11-CH_3), a quartet at 3·48 ppm (1 H, *J* = 10,5 c/s, C1-H), a broad triplet at 4·02 ppm (1 H, *J* = 10·5 c/s, C6-H) and two broad signals at 4·95 and 4·85 ppm (1 H each, W 1/2 = 3·5, C4 = CH_2). (Found: C, 71·56; H, 8·73. $C_{15}H_{22}O_3$ required: C, 72·00; H, 8·80%.)

1β-*Hydroxysantanolide-C* (XI). Lactone IV (100 mg) in EtOH (20 ml) was hydrogenated on Pd/C (40 mg, 5%). Removal of the catalyst and solvent left a white residue which was crystallized from $CHCl_3$–light petrol; yield 65 mg, m.p. 162–163° (lit.[24,25] 164–166°).

Hydrogenation of 1β-*hydroxysant*-4(14)-*en*-6,12-*olide-C* (V). Lactone V (100 mg) in EtOH (20 ml) was hydrogenated in the presence of Pt_2O (40 mg). Removal of the catalyst and solvent left a residue which was crystallized twice from $CHCl_3$–light petrol; yield 30 mg, m.p. 162–163°, the m.p. of this compound was however depressed when mixed with lactone XI, prepared in the previous experiment, indicating that this compound may be a mixture of XI and its C4 epimer (XIII). Repeated crystallizations of this mixture finally gave the lactone XIII, m.p. 165–171° (lit.[25] 171°). This had the same IR spectrum as that of lactone XI, the C4 isomer except in the fingerprint region.[25]

1β-*Hydroxysant*-3-*en*-6,12-*olide-C-acetate* (IVa). Lactone IV (100 mg) was dissolved in anhydrous pyridine (2 ml) and Ac_2O (2 ml) and kept overnight. Removal of solvents in vacuo and crystallization from Et_2O–light petrol gave the acetate IVa, m.p. 132–134°, IR bands at 1233, 1745 (acetate) and 1773 (γ lactone) cm^{-1}.

1β-*Hydroxysant*-4(14)-*en*-6,12-*olide-C-acetate* (Va) This acetate was prepared in the same manner, m.p. 169–171°, IR bands at 1239, 1739 (acetate) and 1779 (γ lactone) cm^{-1}.

1-*Ketosant*-3-*en*-6,12-*olide-C* (XII). The lactone IV (100 mg) was dissolved in HOAc (2 ml) and oxidized with a solution of CrO_3 (40 mg) in HOAc (2 ml). The oxidation product (XII) was obtained in the usual manner, m.p. 130–133° (lit.[24] 134–136).

Isolation and identification of desacetylmatricarin (VI). Samples of *A. tridentata* ssp. *tridentata* were collected north of Perma (Western Montana, approx, altitude of 2600 ft) on 20 June 1969. 500 g of dried twigs and foliage were ground and extracted in the same manner as described for ssp. *vaseyana* to yield a yellowish syrup (about 10 g).

This syrup was dissolved in a minimum amount of benzene and adsorbed on a column of silica gel. The column was developed with benzene (10 × 100 ml), followed by $CHCl_3$ (10 × 100 ml) and eluted with $CHCl_3$–EtOAc (4:1), followed by (2:1) and pure EtOAc.

A crystalline material was obtained only from the $CHCl_3$–EtOAc elutions. This material was recrystallized from $CHCl_3$–Et_2O; yield 400 mg. It melted at 130–131°, then solidified and remelted at 152–153° (lit.[4,27] 130–132° and 154°), R_f 0·07 (brownish black), $[\alpha]_D^{18}$ + 14·28° (*c* = 2·1, CH_3OH), λ_{max} 255 nm (log ε 4·18), IR bands at 3500 (OH), 1775 (γ-lactone), 1690 (cyclopentenone), 1640 and 1620 (unsaturation) cm^{-1}. The NMR spectrum showed a vinylic proton at 6·02 (1 H, C3-H), a triplet (*J* = 9 c/s) at 3·6 (1 H, C6-H), two sharp singlets at 2·31 (3H) and 2·2 (3H) (two methyl groups on double bonds β to carbonyl group) and a doublet (*J* = 6 c/s) at 1·35 (3 H), C11-CH_3) ppm.

In one experiment when the total sesquiterpene syrup in $CHCl_3$–Et_2O was kept in refrigerator for a long time, the crude desacetylmatricarin separated out. This could be purified by repeated crystallization.

Matricarin (VIb). Desacetylmatricarin (100 mg) was dissolved in anhydrous pyridine (1 ml) and Ac_2O (1 ml); and the solution was left overnight at room temp. Removal of the solvents under reduced pressure and crystallization from $CHCl_3$–light petrol gave matricarin (75 mg), m.p. 192–193°, (lit.[4,27] 190–191°), $[\alpha]_D^{18}$ + 25·4° (*c* = 1·1, $CHCl_3$), λ_{max} 255 nm (log ε 4·17), IR bands at 1780 (γ-lactone), 1745 (acetate), 1688 (cyclopentenone) 1642 and 1620 (unsaturation) cm^{-1}. NMR spectrum showed a broad signal at 6·05 (1 H), a triplet of doublets (*J* = 10 and 3 c/s) at 4·75 (1 H), a triplet (*J* = 9 c/s) at 3·66 (1 H), three sharp singlets (3 H each) at 2·35, 2·22 and 2·02 and a doublet (*J* = 6 c/s) at 1·25 (3 H) ppm.

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