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SESQUITERPENE LACTONES OF *ARTEMISIA ARBUSCULA* AND *A. TRIDENTATA*

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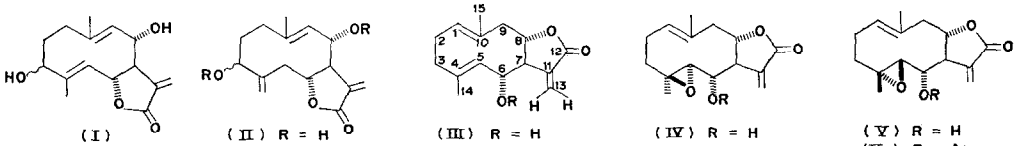
Key Word Index—*Artemisia arbuscula*; *Artemisia tridentata*; Compositae; sagebrush; sesquiterpene lactones; germacranolides; tatridins; deacetyllaurenobiolide; spiciformin.

Abstract—Chloroform extracts of *Artemisia tridentata* ssp. *vaseyana* f. *spiciformis* (Osterhout) Beetle and *Artemisia arbuscula* Nutt. ssp. *arbuscula* gave identical TLC patterns and contained the known germacranolides tatridin-A (I) and tatridin-B (II), and two new germacranolides deacetyllaurenobiolide (III) and spiciformin (IV). The structures of these lactones were shown through spectral studies and chemical transformations. Deacetyllaurenobiolide was converted to the known germacranolide laurenobiolide (IIIb) and spiciformin (IV) was synthesized from deacetyllaurenobiolide along with an isomeric epoxy lactone (V).

INTRODUCTION

THE SESQUITERPENE lactones of *Artemisia tridentata* ssp. *vaseyana* f. *spiciformis* (Osterhout) Beetle were investigated as a part of our program on chemical constituents of sagebrush in Montana.[1–5] A sample of this subspecies was collected near Red Rock lake, Montana and extracted with chloroform. TLC analysis of the extract gave a pattern which was identical to that of *Artemisia arbuscula* Nutt. ssp. *arbuscula* obtained in Montana.[4,6]



(I)

(II) R = H
(IIa) R = TMS
(IIb) R = —C(=O)—C_6H_5

(III) R = H
(IIIa) R = TMS
(IIIb) R = Ac

(IV) R = H
(IVa) R = Ac

(V) R = H
(Va) R = Ac

Extensive column chromatography of the extract gave two new non-crystalline germacranolides; deacetyllaurenobiolide (III) and speciformin (IV) in addition to the known germacranolides tatridin-A (I)[4] and tatridin-B (II).[7] The same compounds were also isolated from *A. arbuscula* Nutt. ssp. *arbuscula*.

* Part VI in the series on "Chemical Composition of Sagebrush". For Part V see F. SHAFIZADEH and N. R. BHADANE, *J. Org. Chem.* **37**, 3168 (1972).

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[3] SHAFIZADEH, F., BHADANE, N. R., MORRIS, M. S., KELSEY, R. G. and KHANNA, S. N. (1971) *Phytochem.* **10**, 2745.
[4] SHAFIZADEH, F. and BHADANE, N. R. (1972) *J. Org. Chem.* **37**, 274.
[5] SHAFIZADEH, F. and BHADANE, N. R. (1972) *J. Org. Chem.* **37**, 3168.
[6] IRWIN, M. A. and GEISSMAN, T. A. (1969) *Phytochem.* **8**, 2411.
[7] IRWIN, M. A. (1971) Ph.D. Thesis, University of California, Los Angeles, California.

TABLE 1. NMR DATA OF THE

Compound	C13–H_2	C8–H	C7–H	C6–H	C5–H
IIa	6·20, 5·96 (*dd*, 2·8, 1·5)	3·73 *mx*	*mx*	4·17 (*t*, 10)	—
IIb	6·27, 5·86 (*d*, 2·8)	~5·4 *mx*	3·2 *br*	4·30 *m*	—
IIIb	6·33, 5·87 (*dd*, 2·5, 0·5)	4·02 *m*	*mx*	4·6–5·4 *mx*	4·6–5·4 *mx*
IV	6·31, 6·05 (*dd*, 2·5, 1·5)	4·42 (*ddd*, 11 3·5, 3·5)	*mx*	4·03 (*dd*, 11·5, 3·5)	2·60 (*d*, 3·5)
IVa	6·31, 5·78 (*d*, 2·5)	4·58 (*ddd*, 11 3·5, 3·5)	3·07 *br*	5·26 (*dd*, 11·5, 3·5)	2·72 (*d*, 3·5)
V	6·40, 6·23 (*dd*, 3, 1·5)	4·00 (*ddd*, 11, 5, 2·5)	2·95 *br*	3·45 (*t*, 9·5)	2·60 (*d*, 9·5)
Va	6·37, 5·8 (*dd*, 3, 0·8)	4·15 (*ddd*, 11, 5, 2·5)	3·12 (*br*)	4·87 (*t*, 9·5)	2·72 (*d*, 9·5)

* These data were obtained with the Varian HA-60 NMR Spectrometer in $CDCl_3$ solution using TMS as *dd*—doublet of doublets; *ddd*—double doublet of doublets; *t*—triplet; *br*—broad; *m*—multiplet; and *mx*—

RESULTS AND DISCUSSION

The sample of *Artemisia tridentata* ssp. *vaseyana* f. *spiciformis* showed four major spots on TLC: grey spot R_f 0·67, red spot R_f 0·60, red spot R_f 0·36 and red spot R_f 0·30.

Deacetyllaurenobiolide (III)

The compound corresponding to the grey spot, R_f 0·67, was isolated by column chromatography and purified by preparative TLC as a colorless gum. All attempts to crystallize this material failed although GLC analysis showed that it was over 95% homogeneous. The gummy material had a tendency to decompose on standing. The following consideration provided the structure of the gummy compound that was named deacetyllaurenobiolide (III).

The trimethylsilyl derivative (IIIa) showed a parent ion at *m/e* 320 which corresponded to a monohydroxy starting compound having the empirical formula of $C_{15}H_{20}O_3$. The original compound, however, showed the last peak at *m/e* 230 which represents the M-18 fragment. The IR spectrum showed absorption bands at 3450 (OH), 1745 (γ-lactone) and 1645 (unsaturation) cm^{-1}. The NMR spectrum of III had a two proton multiplet at 6·3 ppm ($W^{1/2} = 5{\cdot}5$ Hz), typical for α-methylene lactone protons (C13–H_2).[3,4] The equivalence of these protons in III and upfield shift of one of these proton signals in the spectrum of acetate IIIb (see Table 1) suggested the presence of an α-oriented hydroxyl group at the adjacent position (*C*6).[8] There was also a broad two proton complex centered at 5·0 ppm that could be assigned to vinyl protons (Cl and C5–H). Another two proton broad complex was centred at 4·0 ppm. This signal was assigned to the lactone proton (C8–H) and the proton under the hydroxyl group (C6–H). Finally, two broadened singlets at 1·63 and 1·56 ppm

[8]YOSHIOKA, H., MABRY, T. J., IRWIN, M. A., GEISSMAN, T. A. and SAMEK, Z. (1971) *Tetrahedron* **27**, 3317.

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C1–H	C10–Me	C4–Me C4=CH_2	C9–H	C3–H	Misc.
—	1·66	5·0 *mx*	5·0 *mx*	3·73 *mx*	OTMS 0·00
—	1·96	5·4 *mx*	5·12 *br*	5·4 *mx*	7·3–8·2 (*m*) (–C–C_6H_5)$_2$ ‖ O
4·6–5·4 *mx*	1·64 *s*	1·64	—	—	2·05 OAc
5·33 (*t*, 8·5)	1·73	1·47	—	—	—
5·33 (*t*, 8·5)	1·73	1·47	—	—	2·07 OAc
5·37 (*t*, 8·5)	1·77 (*s*)	1·25 (*s*)	—	—	—
5·35 (*t*, 8·5)	1·81 (*s*)	1·32	—	—	2·05 OAc

the internal standard. Chemical shifts are quoted in δ (ppm) and the signals are denoted by *s*—singlet; mixed signal. Figures in parenthesis denote coupling constants in Hz.

assigned to vinylic methyl groups at C-4 and C10. These data suggested structure III for the lactone.

Acetylation of deacetyllaurenobiolide (III) gave a crystalline acetate (IIIb). The NMR (see Table 1) spectrum and other properties of this acetate corresponded with those of a known germacranolide laurenobiolide.[9] Finally, the identity of the two compounds was confirmed by the mixed melting point and GLC coinjection.

Spiciformin (IV)

The compound corresponding to the red spot R_f 0·60 was isolated from subsequent clutions of the chromatographic column and was purified by preparative TLC. In attempted crystallizations this compound showed a tendency to deteriorate. The freshly purified gummy compound, however, showed a sharp GLC peak. The compound had IR bands at 3460 (hydroxyl), 1755 (γ-lactone) and 1650 (unsaturation) cm^{-1}.

Attempts to obtain elementary analysis and MW by MS were not successful. Spiciformin, however, gave a crystalline acetate which had an empirical formula of $C_{17}H_{22}O_5$. The acetate had MS peaks at *m/e* 264 (M-42) and 246 (M-60). The acetate group and the lactone accounted for 4 of the 5 oxygen atoms in the molecule. The fifth oxygen atom appeared to be involved in an oxide ring since the acetate failed to show the presence of hydroxyl or ketone groups and gave a positive result in the epoxide test.[5,10,11]

In the NMR spectrum of spiciformin, the C13 protons showed two doublets of doublets

[9] TADA, H. and TAKEDA, K. (1971) *Chem. Commun.* 1391.
[10] ROSS, J. M., TARBELL, D. S., LOVETT, W. E. and CROSS, A. D. (1956) *J. Am. Chem. Soc.* **78**, 4675.
[11] LEE, K. H. and GEISSMAN, T. A. (1971) *Phytochem.* **10**, 205.

at 6·31 and 6·05 ppm (J = 2·5, 1·5 Hz) while in the spectrum of the acetate (IVa, see Table 1) these protons showed doublets at 6·31 and 5·78 ppm (J = 2·5 Hz). The upfield shift of one of the proton signals in the spectrum of the acetate suggested the presence of an α-hydroxyl group adjacent to lactone (at C6).[8]

Other features of the NMR spectrum of spiciformin included a doublet of doublets at 4·03 ppm (J = 11·5, 3·5) which represented the proton under the hydroxyl group (C6–H) and a narrow doublet at 2·60 ppm (J = 3·5 Hz). In the spectrum of acetate IVa, the C6–H doublet of doublets moved downfield to 5·26 ppm and the narrow doublet at 2·60 ppm moved to 2·72 ppm.

Irradiation at the frequency of C6–H collapsed the narrow doublet into a broad singlet and altered a broad signal at 3·07 for C7–H. Conversely, irradiation of the narrow doublet at 2·72 collapsed the C6–H doublet of doublets to a broad doublet (J = 11 Hz) leaving the rest of the spectrum unaffected. This suggests that the narrow signal at 2·72 ppm in the acetate IVa (at 2·60 in IV) is due to C5–H. Presence of a single proton at this position implies the occurrence of the epoxide group at C4–C5. This conclusion is supported by the appearance of the C4-methyl group signal at 1·47 ppm in the spectrum of both the acetate and the parent compound.

The structure of spiciformin was finally confirmed by synthesis. Deacetyllaurenobiolide on treatment with 1 mol of *m*-chloroperbenzoic acid gave a mixture of two isomeric epoxy compounds which separated as red spots on TLC. These compounds were separated by column chromatography and further purified by preparative TLC as colorless gums. The faster moving isomer corresponded to spiciformin and gave the same IR and NMR spectra as the natural product. Furthermore, acetylation of this compound gave a crystalline acetate which had identical physical and spectral properties with those of the acetate prepared from spiciformin.

The slower moving isomer also gave a crystalline acetate, Va, which analyzed for $C_{17}H_{22}O_5$ and showed the MS peaks at m/e 306 (M^+), 264 (M-42), 246 (M-60). The following considerations indicated that this compound is C4–C5 isomer of spiciformin.

NMR spectrum of V had a symmetrical triplet at 3·45 ppm for C6–H (J = 9·5 Hz, which shifted downfield to 4·87 ppm in the acetate Va) and a sharp doublet at 2·60 ppm for C–5H (J = 9·5 Hz, which shifted downfield to 2·72 ppm in the spectrum of Va). The latter assignment was confirmed by decoupling experiments with the acetate Va. Irradiation at the frequency of C6–H changed the doublet at 2·72 while irradiation at the frequency of the doublet at 2·72 ppm collapsed C6–H triplet to a broad doublet.

Studies of chemical models indicated that the observed coupling constants of C5–H and C6–H in V require C4–α and C5–β orientation of the epoxide function. Whereas, in IV the coupling constants of the same protons suggest that the epoxide group should have the reverse orientation. In IV the C4–α methyl group eclipses the C6–O bond. This situation explains the low field position of C4–Me in IV as compared to V. The epoxide orientation in the natural isomer is also responsible for the low field positions of C6–H and C8–H in IV and IVa as compared to V and Va (see Table 1).

Tatridin (I)

The red spot corresponding to R_f 0·36 was isolated by column chromatography and was crystallized as white needles from chloroform–ether. It was identified as the known lactone, tatridin-A, by comparing its physical constants and NMR spectrum with those of an authentic sample.[4]

Tatridin-B (II)

The lowest red spot R_f 0·30 was isolated as a gum and further purification by preparative TLC did not result in crystallization. However, the NMR spectrum of the bis-trimethylsilyl derivative (IIa) of the gummy material compared well with the spectrum of the corresponding derivative of the known lactone tatriden-B.[7] Further confirmation was obtained by preparing a crystalline benzoate (IIb) which showed identical physical and spectral properties to those of the tatridin-B dibenzoate[7] (IIb).

Badgerin[4] could also be detected in *A. tridentata* ssp. *vaseyana spiciformis*, but the trace amount was not sufficient for isolation. As in badgerin[4] tatridin-A and -B could have the lactone ring closed at C8 instead of C6 and in reality the two forms are enantiomers. Although in this paper the compounds are presented with C6 lactone closure, following the original publication,[7] the other form may be preferred on the basis of biosynthetic considerations.[12]

EXPERIMENTAL

All m.ps are uncorrected. The UV and IR spectra were recorded on Coleman-Hitachi EPS-3T and Beckman IR-33 instruments. Mass spectra were determined on Varian-Mat 111 spectrometer at 80 eV using direct insertion or GLC connection. Baker A.R. No. 3405 silica gel was used for column chromatography and silica gel G. woelm was used for TLC. The plates were developed in a solvent mixture containing C_6H_6–EtOAc–EtOH (5:4:1) and visualized by spraying with concentrated H_2SO_4 and heating. GLC analysis was carried out with a Varian Aerograph 1800 using 3% SE30 on Varaport 30 column.

Plant materials. Artemisia tridentata ssp. *vaseyana* f. *spiciformis* was collected near Red Rock Lake, Montana (T. 14 S., R. 1 W., Section 26) on 23 August 1971 and *Artemisia arbuscula* ssp. *arbuscula* was collected near Badger Pass, Montana (T. 7 S., R. 11 W., Section 11) on 22 August, 1971. Samples of these materials denoted by ATVS-1971 and AAA-1971 respectively, are kept in the laboratory files. A $CHCl_3$ extract of both species gave identical TLC patterns with four major spots of grey R_f 0·67, red R_f 0·60, red R_f 0·36 and red R_f 0·30 and a few minor spots, one of which has the R_f 0·24, corresponding to badgerin.

Isolation of deacetyllaurenobiolide. A sample of dried twigs and foliage of *Artemisia tridentata* ssp. *vaseyana* f. *spiciformis* (100 g) was extracted with 2 l. of $CHCl_3$ and processed in the usual manner.[3] The resulting yellowish syrup (9 g) was dissolved in a small amount of C_6H_6 and chromatographed on 200 g of silica gel in 4 × 60 cm column using C_6H_6 and C_6H_6–Et_2O of increasing polarity, collecting 100 ml aliquots. The-first 10 aliquots of C_6H_6 and the following 10 aliquots of mixed solvent (19:1) gave complex mixtures having menthol and camphor aroma. The next 8 fractions of mixed solvent (9:1) gave 1·2 g of almost pure deacetyllaurenobiolide (grey spot, R_f 0·67) as a gum. This was further purified by preparative TLC. Attempts to crystallize this material failed and it appeared to deteriorate on further handling or standing. Freshly purified deacetyllaurenobiolide had: $[\alpha]_D$ + 34·50° (*c* 1·603, $CHCl_3$); IR bands at 3450 (hydroxyl), 1745 (γ-lactone) and 1645 (unsaturation) cm^{-1} and MS peaks at *m/e* 230 (M-18), 215 (M-18-15). The trimethylsilyl derivative prepared in the usual manner showed parent ion at *m/e* 320. Deacetyllaurenobiolide was also isolated from *Artemisia arbuscula* ssp. *arbuscula*. However, it had an impurity of a chromatographically similar green compound which could not be separated.

Isolation of spiciformin. The next 5 fractions (29–33) eluted from above column with the same solvent composition gave a mixture of deacetyllaurenobiolide and another compound corresponding to the red spot R_f 0·60. The next 5 fractions (34–38) eluted with the same solvent mixture gave 200 mg of homogeneous spiciformin (red spot, R_f 0·60) as a transparent gum. This was further purified by preparative TLC. Attempts to crystallize failed and the compound showed a tendency to deteriorate on standing. Freshly purified samples had: $[\alpha]_D$ + 81·70° (*c* 1·63, $CHCl_3$); IR bands at 3460 (hydroxyl), 1755 (γ-lactone) and 1650 (unsaturation) cm^{-1} and the NMR as listed in Table 1. Similar processing of 650 g of *A. arbuscula* ssp. *arbuscula* gave 300 mg of spiciformin.

Isolation of tatridin-A. Continued elution of the above column with 8:2 and 7:3 solvent mixtures eluted fractions 39–65 from which no homogeneous compound could be isolated. Subsequent elution with C_6H_6–Et_2O (6:4) gave a colorless gum which crystallized as needles from $CHCl_3$–Et_2O to give 75 mg tatridin-A: m.p. 159–160° alone or in admixture with the authentic sample;[4] $[\alpha]_D$ −54° (*c* 1·2, MeOH); IR bands at 3333 (hydroxyl), 1762 (γ-lactone) and 1666, 1647 and 890 (unsaturation) cm^{-1}. Tatridin-A was also isolated from the collection of *A. arbuscula* ssp. *arbuscula*.

Isolation of tatridin-B. Further elution of the main column with C_6H_6–Et_2O (6:4) gave 400 mg of tatridin-B as a colorless gum which could not be crystallized and was converted to bis-trimethylsilyl derivatives in the usual manner. A crystalline dibenzoate obtained from the gummy tatridin-B had: m.p. 220–224° (lit.[7]

[12] Communication from the referee.

218–220°); IR bands at 1765 (γ-lactone), 1710 O–CO–Ar and 706 (aromatic) cm^{-1}; NMR data given in Table 1.

Laurenobiolide. A solution of deacetyllaurenobiolide (200 mg) in pyridine (4 ml) and Ac_2O (4 ml) was allowed to stand 16 hr at room temp. The solvents were removed under reduced pressure. The dark residue was passed through silica gel column to yield a colorless gum which crystallized from light petrol.–Et_2O to give 175 mg of crystals: m.p. 99–100° (97–98° in admixture with authentic laurenobiolide[9] which had a m.p. of 97°); $[\alpha]_D$ + 14·50° (*c* 1·55, EtOH); single GLC peak on coinjection with authentic laurenobiolide; MS peaks at *m/e* 290 (M^+), 248 (M-42), 230 (M-60) and 215 (M-60-15); IR bands at 1760 (γ-lactone), 1730 and 1235 (acetate) and 1650 (unsaturation) cm^{-1}; NMR spectrum described in Table 1 (Found: C, 70·05; H, 6·73. $C_{17}H_{22}O_4$ required: C, 70·34; H, 6·89%).

Spiciformin acetate. Spiciformin (100 mg) was acetylated in the manner described above to give 80 mg of crystalline acetate: m.p. 154–155°; $[\alpha]_D$ + 57·00 (*c*. 1·51, $CHCl_3$); IR bands at 1750 (γ-lactone and acetate), 1225 (acetate) and 1650 (unsaturation) cm^{-1}; MS peaks at *m/e* 264 (M-42), 246 (M-60); NMR spectral data given in Table 1 (Found: C, 66·28; H, 7·20. $C_{17}H_{22}O_5$ required: C, 66·66; H, 7·18%).

Epoxidation of deacetyllaurenobiolide. A solution of 310 mg of deacetylaurenobiolide in 15 ml of $CHCl_3$ was treated with a solution of 265 mg of *m*-chloroperbenzoic acid in 5 ml of $CHCl_3$ at room temp. After 30 min the reaction mixture was poured in H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with $NaHCO_3$ solution and H_2O. Removal of the solvent gave a gummy mixture of spiciformin and an isomeric epoxide which were separated by column chromatography and purified by preparative TLC to give colorless gums resisting crystallization. The synthetic spiciformin (110 mg) had the same spectral properties as the natural product, gave a similar TLC spot and the same acetate. The isomeric compound (V) showed the NMR spectrum described in Table 1 and gave a crystalline acetate, Va; m.p. 196–197°; $[\alpha]_D$ + 26·74° (*c* 0·92, $CHCl_3$); IR bands at 1750 (γ-lactone), 1725 and 1230 (acetate) and 1650 (unsaturation) cm^{-1}; the NMR data given in Table 1 (Found: C, 66·75; H, 7·12. $C_{17}H_{22}O_5$ required: C, 66·66; H, 7·18%).

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