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COUMARINS OF *ARTEMISIA TRIDENTATA* SSP. *VASEYANA**†

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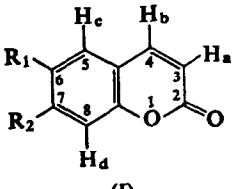
Abstract—The alcoholic leaf extract of *Artemisia tridentata* ssp. *vaseyana* was found to contain isoscopoletin, esculetin, esculin and methylesculin. These compounds were isolated and identified by spectroscopic methods.

SEVERAL attempts have been made to develop a method for the chemical taxonomy of sagebrush that obviates the problems associated with the physical variability of the individual species.[1-3] In this laboratory we have used TLC of the alcoholic leaf extract to separate the phenolic constituents that could be located as fluorescent spots.[4,5] Table 1 provides a comparison between the results obtained for several species and subspecies of sagebrush.

The phenolic extractives of *Artemisia tridentata* ssp. *vaseyana* (big sagebrush) were isolated by solvent extraction and column chromatography. The pure products which corresponded with the TLC spots, were investigated by physicochemical methods, including NMR and mass spectroscopy.[6,7] These investigations indicated that the phenolic materials are mainly coumarins (I) with various substituents in positions 6 and 7.



(I)

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TABLE 1. THIN-LAYER CHROMATOGRAM OF *Artemisia* SPECIES

	Artemisia species							
	A. nova	*A. tridentata* ssp. *tridentata*	*A. tridentata* ssp. *vaseyana*	*A. arbuscula* ssp. *arbuscula*	*A. longiloba*	*A. tripartita* ssp. *tripartita*	*A. cana* ssp. *cana*	*A. cana* ssp. *viscidula*
Fluorescent spots developed in solvent system A								
Purple 0·95*	+	−	+	−	−	−	−	+
Purple 0·93	−	−	−	−	+	+	−	−
Yellow 0·89	+	−	−	−	−	−	−	−
Purple 0·89	−	+	−	−	−	+	−	+
Green 0·87	+	−	−	−	−	−	−	−
Blue 0·86	−	−	−	+	−	−	−	+
Purple 0·86	−	−	−	−	+	−	+	−
Blue 0·84	−	+	+	+	−	+	−	−
Green 0·81	−	+	+	−	+	−	−	−
Blue 0·80	+	−	−	+	−	−	+	+
Blue 0·78	−	+	+	−	+	+	+	−
Green 0·75	−	−	−	+	−	−	−	−
Blue 0·72	+	−	−	−	−	+	−	−
Green 0·72	−	−	−	−	+	−	+	−
Green 0·67	−	−	−	−	+	−	−	−
Blue 0·67	−	−	−	−	−	−	+	−
Green 0·65	−	−	−	−	−	−	+	−
Green 0·62	+	−	−	−	−	−	−	−
Orange 0·61	−	−	−	+	+	+	−	+
Orange 0·56	+	+	+	−	−	+	−	−
Orange 0·50	+	−	−	−	−	−	−	−
Blue 0·48	−	−	−	−	+	−	−	+
Blue 0·44	−	+	+	+	−	+	+	−
Orange 0·37	−	−	−	−	−	−	+	−
Orange 0·34	−	−	−	+	−	−	−	−
Orange 0·30	−	+	+	−	−	−	−	−
Fluorescent spots developed in solvent system B								
Blue 0·98	+	+	+	+	+	+	+	+
Blue 0·82	+	+	+	+	+	+	+	+
Blue 0·60	−	−	−	+	−	+	+	+
Blue 0·34	−	−	+	+	−	+	+	+
Blue 0·24	+	+	+	+	+	+	+	+
Blue 0·21	−	+	−	+	−	+	+	−

* Color and R_f of the spots.

The first TLC spot, R_f 0·95 (see Table 1, System A), had a faint fluorescence and could not be isolated in sufficient quantities for chemical investigation. The material corresponding with the second spot (R_f 0·84), which had a strong blue fluorescence, was found to be isoscopoletin (I, $R_1 = OH$, $R_2 = OMe$).

The next strong spot (R_f 0·78), showing a blue fluorescence, was found to be due to scopoletin (I, $R_1 = OMe$, $R_2 = OH$). Despite the close chemical similarity, some of the spectroscopic characteristics of this compound were distinctly different from those of isoscopoletin.

Another strong spot (R_f 0·44), which had a blue fluorescence before spraying with the diphenylboric acid ethanolamine reagent[5] and green fluorescence after, proved to be esculetin (I, $R_1 = R_2 = OH$).

During an early investigation of *A. tridentata*, Kinney and Sugihara[8] obtained a brown amorphous powder by hot-water extraction of the leaves, which could not be further purified. However, it was shown to contain a glycoside, because on hydrolysis it provided a reducing sugar that formed D-glucose phenylosazone. Since the extract was exceedingly bitter, the glycoside was referred to as the bitter principle.

The presence of the glycosides was investigated by TLC using a more polar solvent (Table 1, system B) and also by solvent extraction and column chromatography. These investigations showed that the leaves contain two coumarin glycosides, neither of which was bitter. Methylesculin (I, $R_1 = \beta$-D-glucosyl, $R_2 = OMe$), which to our knowledge has not been reported before as a natural product, was present in relatively large quantities, and esculin (I, $R_1 = \beta$-D-glucosyl, $R_2 = OH$) was present in small amounts. The structure of the former compound was proven by synthesis involving methylation of esculin followed by hydrolysis to D-glucose and isoscopoletin.

The spectroscopic data for methylesculin or 6-β-D-glucosyl-7-methoxy coumarin is given in Fig. 1. The mass spectrum was almost identical to that of isoscopoletin because the

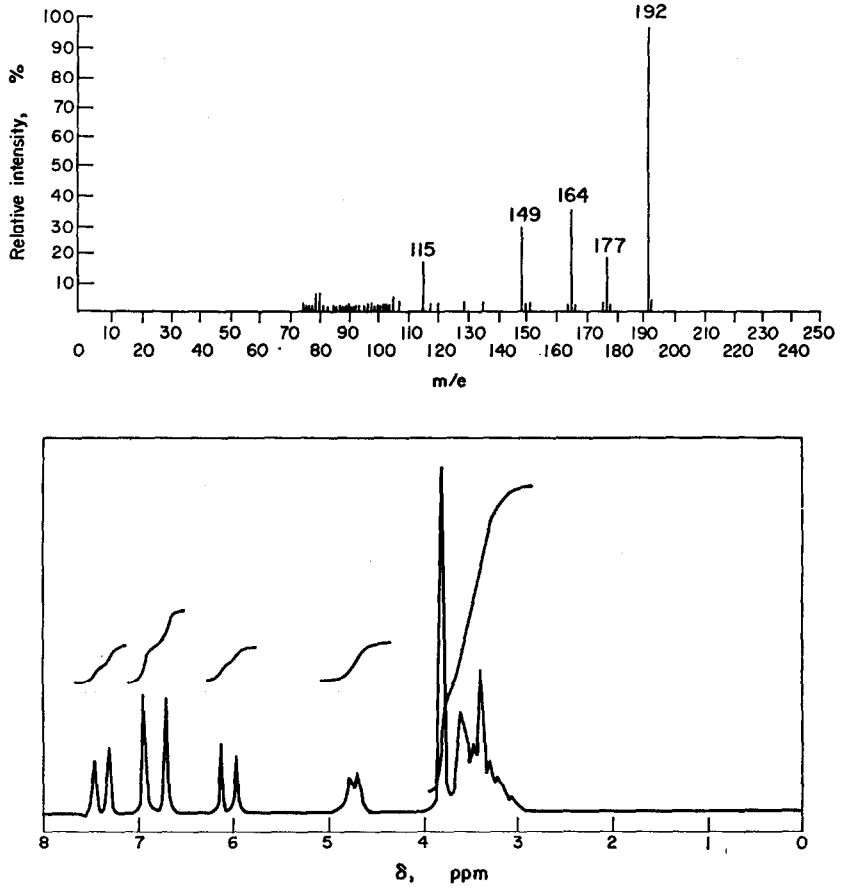

FIG. 1. MASS AND NMR SPECTRA OF METHYLESCULIN.

[8] C. R. KINNEY and J. SUGIHARA, *J. Org. Chem.* **8**, 290 (1943).

sugar moiety of the glycoside undergoes immediate decomposition in the mass spectrometer.[7] The NMR spectrum of the trimethylsilyl ether derivative of the glycoside showed the presence of a single hexose sugar with an anomeric proton at 4·67, and the CH and CH_2 protons in a broad signal centered at 3·40 ppm. The two doublets at 6·01 and 7·35 are due to H_a and H_b, the aromatic protons are at 6·64 and 6·85 and the methoxyl group at 3·73 ppm.

On this basis the major phenolic extractives of the *A. tridentata* ssp. *vaseyana* appear to be 6-β-D-glucosyl-7-methoxy coumarin (0·67%) followed by smaller quantities of its aglycone, isoscopoletin (0·037%), minor quantities of the corresponding unmethylated compounds (esculin 0·011% and esculetin 0·016%) and trace amounts of scopoletin; other compounds present, represented by the green and orange spots (Table 1, system A, R_f 0·81, 0·56 and 0·30), seem to be flavonoids.

EXPERIMENTAL

Plant Materials

The samples of *Artemisia* species were obtained from different locations within the state of Montana as shown in Table 2. Each sample gave reproducible chromatographic analysis.

TABLE 2. LOCATION OF *Artemisia* VARIETIES

Variety	Town	Range	Township	Section	Quadrant
A. nova	E. of Bannack	R.11.W.	T.8.S.	5	S.E.
	E. of Dillon	R.7.W.	T.7.S.	27	N.W.
	S. of Virginia City	R.38.E.	T.9.S.	20	N.E.
A. cana ssp. *viscidula*	E. of Dillon	R.7.W.	T.7.S.	27	N.W.
	S. of Dillon	R.36.E.	T.9.S.	8	N.E.
	S. of Dillon	R.36.E.	T.9.S.	8	N.E.
A. longiloba	S. of Virginia City	R.38.E.	T.9.S.	2	S.W.
A. arbuscula ssp. *arbuscula*	N.E. of Bannack	R.11.W.	T.8.S.	26	N.W.
	N.W. of Dillon	R.11.W.	T.6.S.	36	S.E.
A. tridentata ssp. *tridentata*	E. of Bannack	R.11.W.	T.8.S.	5	S.E.
	N.E. of Bannack	R.11.W.	T.8.S.	26	N.W.
	W. of Dillon	R.10.W.	T.7.S.	16	S.E.
A. tridentata ssp. *vaseyana*	N.W. of Missoula	R.20.W.	T.14.N.	13	S.W.
	W. of Missoula	R.20.W.	T.13.N.	10	S.W.
	S. of Stevensville	R.20.W.	T.8.N.	13	S.W.
A. tripartita ssp. *tripartita*	S. of Virginia City	R.38.E.	T.9.S.	2	S.W.
	S.W. of Bannack	R.13.W.	T.9.S.	29	S.W.
A. cana ssp. *cana*	N. of Malta	R.30.E.	T.32.N.	5	N.W.

Thin-Layer Chromatography

2 g of the leaves were refluxed in 100 ml of methanol for 30 min and the extract concentrated on a rotary evaporator to 10 ml. The concentrate was extracted with 2 vol. of pet. ether to remove chlorophyll; acetone was then added to precipitate the waxy materials and remaining acetone–methanol solution was then concentrated to about 10 ml.

The concentrated solution was spotted on Gelman type SG instant TLC sheets with a capillary tube and developed with benzene–Et_2O–HOAc (89:10:1) (solvent system A, Table 1). The chromatogram was then air-dried and sprayed with diphenylboric acid ethanolamine complex and examined in u.v. light.[4,5] The coumarins showed blue fluorescence without the complexing reagent.

For the separation of the glucosides the solvent system was changed to $CHCl_3$–MeOH–HOAc (94:5:1) (solvent system B, Table 1).

Instrumental Analysis

All m.p's are uncorrected; the NMR spectra were recorded in deuterated DMSO with TMS as internal standard. The glycosides (50 mg) were treated with TRI-SIL (5 ml). The excess reagent was removed under reduced pressure. The trimethylsilyl ether derivative of the glycoside was extracted with CCl_4. The extract was filtered, concentrated (0·25 ml) and used for NMR studies.

Isoscopoletin

1·4 kg air-dried leaves of *A. tridentata* ssp. *vaseyana* were extracted with 10 l. of methanol. The methanolic solution was concentrated, dissolved in hot benzene and placed on twenty silicic acid columns (4·3 × 10 cm). The columns were developed with benzene–ether (6:4) to give three u.v. fluorescent bands. Elution of the first band and subsequent concentration and crystallization from acetone gave crude isoscopoletin (220 mg). Recrystallization from acetone yielded pure material (54 mg), m.p. 185° (lit.[9] m.p. 183–185°), R_f 0·84, λ_{max} 227 and 349 (major bands), and 258 and 292 nm (minor bands). Mass spectrum: *m/e* 192 (100), 177 (10·5), 164 (25·5), 149 (39·7), 121 (6·0). NMR: ppm doublets at 6·10 (1H) and 7·80 (1H) (J = 10 c/s) and singlets at 3·80 (3H), 6·92 (2H) and 9·52 (1H). Found: C, 62·11; H, 4·27. Calc. for $C_{10}H_8O_4$: C, 62·50; H, 4·17%. This compound was also obtained by water extraction of dried leaves (340 g), concentration of the aqueous extract, re-extraction of the concentrate with benzene and evaporation of the solvent. This gave a crude material (126 mg) that was purified by recrystallization from acetone. Alternatively the methanolic leaf extract was re-extracted with $CHCl_3$ and the $CHCl_3$ extract was processed as before.

Scopoletin

Elution of the second band from silicic acid columns, concentration and recrystallization of the resulting material from acetone gave scopoletin (40 mg). This was recrystallized from acetone (yield 8 mg), m.p. 203–204°, alone or in admixture with a known sample (lit.[10] m.p. 204°), R_f 0·77, λ_{max} 225 and 350 (major bands), 260 and 292 nm (minor bands). Mass spectrum: *m/e* 192 (29·3), 177 (27·5), 164 (24·0), 149 (42·4), 121 (18·2), 117 (7·0), 115 (17·3), 103 (13·0), 105 (8·9), 102 (9·0), 91 (40·1), 89 (100), 87 (28·9). NMR: ppm doublets 6·10 (1H) and 7·80 (1H) (J = 10 c/s) and singlets 3·80 (3H), 6·72 (1H) and 7·13 (1H), 9·52 (1H).

Esculetin

Elution of the third band from the silicic acid column and subsequent processing gave crude esculetin that could not be purified by fractional recrystallization. The alcoholic extract of the dried leaves (1·4 kg) was concentrated and re-extracted with EtOAc. Concentration and crystallization of this extract gave crude esculetin (226 mg, m.p. 234–297° decomp.). Recrystallization from acetone gave pure material (40 mg), m.p. 263–263·5° (decomp.), alone or in admixture with a known sample (lit.[11] m.p. 268°), R_f 0·44, λ_{max} 231 and 354 (major bands), 250 and 302 nm (minor bands). Mass spectrum: *m/e* 178 (60·5), 150 (100), 149 (54·1), 131 (31·0), 121 (10·9), 119 (8·8), 104 (9·2), 100 (16·7), 76 (26·0), 75 (21·6). NMR: ppm doublets 7·70 (1H) and 5·97 (1H) (J = 10 c/s) and singlets 6·56 (1H), 6·82 (1H), 9·25 (1H) and 10·05 (1H).

Esculin

Methanolic extract of air-dried leaves (610 g) was concentrated, extracted with petroleum ether and the remaining methanolic extract re-extracted with $CHCl_3$ and then EtOAc. The EtOAc extract when concentrated gave a crude mixture of esculin and methylesculin as determined by TLC. The concentrated material was placed on a column (4·3 × 12 cm) of Woelm polyamide mixed with Celite 545 (7:3) and developed with water. This gave two u.v. fluorescent bands. The first band was eluted from the column, concentrated and crystallized from methanol to yield esculin (65 mg), m.p. 208° alone or in admixture with a known compound (lit.[12] m.p. 205° decomp.), R_f 0·24 (see Table 1). The u.v. and i.r. spectra of the product were also identical with the spectra of the known compound.

6-β-D-Glucosyl-7-methoxycoumarin

Elution and processing of the second band provided crude methylesculin. The pet. ether extract (4 l.) in the previous experiment gave a strongly fluorescent aqueous layer that settled out on standing for 3 days. This was evaporated and the dry residue (5·23 g) extracted with methanol. The methanolic extract was concentrated to provide crude 6-β-D-glucosyl-7-methoxycoumarin (3·68 g), m.p. 224–228°. Recrystallization from dilute methanolic solutions gave pure material (137 mg), m.p. 234–234·8° (lit.[12] m.p. 229°), $[\alpha]_D^{26}$ − 35·1° (in pyridine *c.* 0·304) same as esculin. R_f 0·34, λ_{max} 227 and 337 (major bands), 259 and 296 nm (minor bands). Mass spectrum: *m/e* 192 (100), 177 (16·4), 164 (33·8), 149 (27·2), 115 (15·0). NMR of trimethylsilyl ether derivative: ppm doublets 6·01 (1H) and 7·35 (1H) (J = 10 c/s), singlets 3·73 (3H), 6·64 (1H) and 6·85 (1H), and broad bands 3·40 (6H) and 4·67 (1H).

[9] V. A. Zagorevskii and Z. D. Sovzenko, *Zh. Obshch. Khim.* **34** (12), 3987 (1964), C.A. **62**, 9095 (1965).
[10] J. A. Goodson, *J. Biochem.* **16**, 489 (1922).
[11] L. Gattermann and M. Köbner, *Ber.* **32**, 287 (1899).
[12] A. K. MacBeth, *J. Chem. Soc.* 1288 (1931).

The above product was also prepared from EtOAc extraction of a concentrated aqueous leaf extract.

Methylesculin (8 mg) was hydrolyzed in a sealed tube with 3 ml of 2 N CF_3CO_2H at 124° for 1·5 hr. Isoscopoletin crystallized on evaporation of the hydrolyzate. This had the same m.p., R_f and mass spectrum as the authentic compound. The mother liquor was evaporated to dryness. The residue was treated with TRI-SIL and found to contain D-glucose by GLC analysis. For the synthesis of methylesculin,[12] 605 mg esculin and 614 mg KOH were dissolved in 100 ml methanol and the pH brought to 6·2 by adding MeI. Refluxing for 16 hr gave crude methylesculin which was purified by recrystallization; yield 168 mg, m.p. 234–234·8°. The synthetic product had the same m.p., specific optical rotation, R_f and NMR spectrum as the natural product.

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