Phytochemistry, 1975, Vol. 14, pp. 1083-1084. Pergamon Press. Printed in England.

PHENOLIC CONSTITUENTS OF *ARTEMESIA TRIDENTATA* spp. *VASEYANA*

DENNIS BROWN, R. OWEN ASPLUND* and VERN A. MCMAHON

Department of Chemistry and Division of Biochemistry, University of Wyoming, Laramie, WY 82071, U.S.A.

(*Revised Received* 27 *September* 1974)

Key Word Index—*Artemesia tridentata*; Compositae; coumarins; flavonoids.

Abstract—Ten coumarins and four flavonoids have been isolated from a single collection of *Artemesia tridentata* ssp. *vaseyana* (Rydb.) Beetle. The coumarins are 7-methylesculin, esculin, umbelliferone, skimmin, cichoriin, isoscopoletin, scopoletin, scoparon, esculetin and a new natural product, artelin (5,6,7,8-tetramethoxy coumarin). The flavonoids are luteolin, luteolin-7-glucoside, axillarin and eupafolin.

Artemesia tridentata Nutt. ssp. *vaseyana* (Rydb.) Beetle is reportedly the most widespread taxon of the complex of plants known as the Western Sagebrushes (group Tridentatae, Section Seriphidium of this genus) [1]. Its extensive distribution makes it an excellent candidate for the study of the effect of location upon chemical composition. Previous studies of specific components of this taxon have been reported. In one of these [2], collections from Montana, U.S.A. were found to contain five coumarins; esculetin, scopoletin, isoscopoletin, esculin and 7-methylesculin. A California, U.S.A. collection was examined for flavonoids [3] and found to contain axillarin, penduletin and quercetagetin 3,6,7-trimethyl ether.

In this paper, we report 10 coumarins and four flavonoids isolated from a single Wyoming, U.S.A. collection of this taxon. All five of the coumarins reported from the Montana collections and one of the flavonoids (axillarin) reported from the California collection were found in the Wyoming collection. In addition, five coumarins and three flavonoids not previously reported from this taxon were isolated. The coumarins are; umbelliferone (7-hydroxycoumarin), skimmin (umbelliferone-glucoside), cichoriin (esculetin-7-glucoside), scoparon (6,7-dimethoxycoumarin) and a new natural product, 5,6,7,8-tetramethoxycoumarin, for which we suggest the trivial name artelin. The flavonoids are; luteolin (3′,4′,5,7-tetrahydroxyflavone), luteolin-7-*O*-glucoside, and eupafolin (6-methoxyluteolin). Criteria for identification were mp, mmp NMR, MS and UV (including spectral shifts induced by acids and bases) [4]. Of particular importance were the NMR chemical shifts. For the compounds having free phenolic groups, these were determined using the trimethylsilyl (TMS) ether derivatives in CCl_4. Since the chemical shifts for these derivatives of coumarins have not been previously reported, they are shown in Table 1 and are comparable to the shifts reported for underived coumarins [5].

The compounds reported represent roughly 80% of the mass of the phenolic fractions (*vide infra*). The remainder is mainly accounted for by two complex fractions; a non-polar one with a UV spectrum suggesting 6,7-disubstituted coumarins [6] and a polar one with a UV spectrum suggesting flavonoid glycosides [7].

The proposed structure of 5,6,7,8-tetramethoxy coumarin for the newly discovered natural product is based upon the NMR, mass and UV spectra. The NMR (Table 1) supports the suggested positions of methoxyl substitution. The proton signal which is assigned to H_3 has a chemical shift more like that usually assigned to H_5 in these compounds. However, its clear doublet character (J 14 Hz) would seem to accord more with an H_3 signal

* To whom correspondence should be addressed.

Table 1. NMR spectra of coumarins and coumarin-TMS ethers

Compound	Proton‡ H_3	H_4	H_5	H_6	H_8	MeO	Glycoside
Isoscopoletin*	6·05*d*	7·17*d*	6·93		6·71	3·81	3·36–3·69*m* 4·73*d*
Umbelliferone–TMS ether†	6·05*d*	7·46*d*	7·19*d*	6·55*dd*	6·57*d*		
Isoscopoletin–TMS ether†	6·04*d*	7·35*d*	6·70		6·68		
Scopoletin–TMS ether†	6·04*d*	7·40*d*	6·68		6·64	3·75	
Scoparon*	6·22*d*	7·57*d*	6·80		6·79	3·88,3·85	
Artelin*§	6·80*d*	7·50*d*				3·76,3·80 3·86,3·92	
Esculetin–TMS ether†	6·04*d*	7·35*d*	6·70		6·64		

* In $CDCl_3$.
† In CCl_4.
‡ Aromatic splittings are in agreement with standard coupling constants.
§ Average of 75 scans by computer of average transients.

which has been deshielded by oxygen substitution at H_5. The UV spectrum exhibits maxima at 355, 259 and 209 nm and a shoulder at 275 nm. No shifts are observed as a result of added base. This spectrum is consistent with those of other highly substituted coumarins [4, 6]. The MS exhibits peaks (relative intensity) at; 267 (2·4%, M + 1), 266 (9·8, M+), 251 (6·0, M − 15), 237 (3·5, M − 29), 146 (72), 120 (26), 105 (100, base) [8]. The observed molecular ion requires a tetramethoxyl-substituted coumarin. Physically, the compound is an amorphous solid.

Three classes of chemical compounds have been studied in this taxon at two or more of the above locations. The sesquiterpane lactones are different at each location [9–11]. There is but one flavonoid common to the Wyoming and California sites [3]. Major coumarin components are identical from the Wyoming and Montana collections [2]. Additional coumarins observed from the Wyoming plants were minor and may also be present in the Montana plants.

EXPERIMENTAL

Artemesia tridentata ssp. *vaseyana* was collected 17 June 1972 from the Happy Jack Area, Albany County, Wyoming [voucher specimen 262378 (RM)] and freshly extracted (100 g) in a Soxhlet extractor with MeOH. The residue from evaporation was taken up in 2 l. H_2O and filtered. The aq soln was passed through a column of Amberlite IRC-50(H) (2·5 × 35) which was subsequently washed with 2 l. H_2O. The combined column effluent was reduced to 1 liter by evaporation and extracted 3 × with 500 ml of *n*-BuOH which was evaporated *in vacuo* to yield phenol Fraction 1(1·3 g). The column was next eluted with 1 liter EtOH (95%) which was evaporated *in vacuo* to yield phenol Fraction 2 (1·9 g). Phenol Fraction 1 was chromatographed over polyvinylpyrolidone (PVP) (2·5 × 40 cm) with a logarithmic gradient of H_2O–EtOH (95%) at a flow rate of 30 ml/min; 90% solvent replacement occurred at 7 hr. Effluent was monitored for fluorescence and the compounds appeared in the following order; isoscopeletin, esculin, umbelliferone, skimmin.

Phenol Fraction 2 was chromatographed in the same manner as above; but when solvent replacement was essentially complete, the column was eluted with AcOH (glacial)–EtOH (95%) 1 : 1. The order of elution was cichoriin, isoscopoletin, scopoletin, scoparon and artelin, esculetin, luteolin-7-*O*-glucoside, axillarin, eupafolin, luteolin. Scoparon and artelin were eluted together and subsequently separated by prepartive TLC on silica.

Instrumentation used has been previously described [9, 12].

REFERENCES

1. Beetle, A. A. (1960) *A Study of Sagebrush*, Univ. of Wyoming Ag. Expt. Sta. Bull. 368.
2. Shafizadeh F. and Melnikoff, A. B. (1970) *Phytochemistry*, **9**, 1311.
3. Rodrigues, E., Carman, N. J., Vander Velde, C., McReynolds, J. H., Mabry, T. J., Irwin, M. A. and Geirsman, T. A. (1972) *Phytochemistry* **11**, 3509.
4. Horowitz, R. M. and Gentili, B. (1960) *J. Org. Chem.* **25**, 2183.
5. Steck, W. and Mazurek, M. (1972) *Lloydia* **35**, 418.
6. Kuznetsova, G. A. (1967) *Natural Coumarins and Furanocoumarins*, Moscow
7. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, New York.
8. Budzikiewicz, H., Djerassi, C. and Williams, D. M. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, vol. 2, Holden-Day, San Francisco.
9. Asplund, R. O., McKee, M. and Balsubramaniyan, P. *Phytochemistry*, **11**, 3542.
10. Irwin, M. A. (1971) Ph.D. dissertation, Univ. of California, Los Angeles.
11. Shafizadeh, F., Bhodane, N. R., Morris, M. S., Kelsey, R. G. and Khanna, S. N. (1971) *Phytochemistry*, **10**, 2745.
12. Brown, D. (1973) Ph.D. dissertation, Univ. of Wyoming, Laramie.