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SHORT COMMUNICATION

XANTHONES IN THE APPALACHIAN *ASPLENIUM* COMPLEX

DALE M. SMITH

Department of Biological Sciences, University of California at Santa Barbara,
Santa Barbara, California, U.S.A.

and

J. B. HARBORNE

Department of Botany, University of Reading

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Abstract—Four gold-fluorescent compounds earlier noted in members of the Appalachian *Asplenium* complex have now been identified from *A. montanum* as the *C*-glycoxanthones, mangiferin, isomagniferin, and their *O*-glucosides. The importance of these compounds as taxonomic markers in this group of ferns is discussed. The chromatographic profile of the plant known as *A. stotleri* is shown to be identical to *A. bradleyi*, which is further evidence that the two are conspecific.

INTRODUCTION

THE DATA from morphological,[1] cytological[1] and chemical[2] analysis of the Appalachian *Aspleniums* (Aspleniaceae) illustrate the relationships within a polyploid complex more fully and clearly than perhaps in any other plant group of comparable size. The distinctive characteristics of three diploid species are clearly evident within their diploid, triploid, and tetraploid hybrid derivatives. While the morphological features of hybrid derivatives tend to be intermediate to appropriate parents,[1] the chemical components detected in hybrids by 2-dimensional paper chromatography of alcoholic extracts of fronds show total addition of the parental attributes.[2] The respective components of all three diploid species are even detectable in the extreme cases of the trigenomic hybrids, *A.* × *kentuckiense* McCoy, and *A.* × *gravesii* Maxon. The clarity of the chemical data arises from the fact that none of the detected substances in the three diploid species of the complex is shared.

In spite of the fact that these *Asplenium* plants provide a classic example of additive inheritance of chemical characters, little is known of the chemistry of the substances present. Four compounds revealed in the diploid species, *A. montanum* Willd., are particularly outstanding in their gold-orange fluorescence under UV light, becoming fluorescent yellow when fumed with ammonia. Compounds with identical chromatographic and fluorescence characteristics are also the most readily-recognized spots on chromatograms of every hybrid derivative containing the *A. montanum* genome, viz. *A. bradleyi* D.C. Eaton, *A.* × *gravesii*, *A.* × *kentuckiense*, *A. pinnatifidum* Nutt., *A.* × *trudellii* Wherry, and *A.* × *wherryi* Smith. These substances have now been identified as xanthones.

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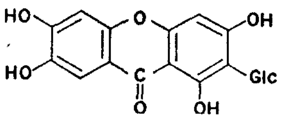
[1] W. H. WAGNER, JR., *Evolution* **8**, 103 (1954).

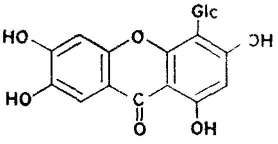
[2] D. M. SMITH and D. A. LEVIN, *Amer. J. Bot.* **50**, 952 (1963).

RESULTS AND DISCUSSION

Four fluorescent orange compounds have now been isolated from *A. montanum* and identified as the *C*-glucosylxanthones, mangiferin (I), isomangiferin (II) and their *O*-glucosides. This finding is of considerable systematic significance, since mangiferin (I) is a uniquely interesting taxonomic character in plants and both in its distribution and biogenesis, it seems to be more closely related to the flavonoids than to other xanthone derivatives.[3,4] While it has been reported in a number of different higher plant groups, it has only been reported once before in ferns, in *Athyrium mesosorum*.[5] Curiously, this fern genus was once thought to be closely related to *Asplenium* but is now generally recognized as being well separated from it.[6]



(I) Mangiferin



(II) Isomangiferin

As in several other plants (e.g. *Iris*),[7] mangiferin occurs in *Asplenium montanum* in a number of forms, including the isomer II,[8] which is formed from it by hot acid treatment. Two other related substances present here appear to be the corresponding *C*-glucosylglucosides of 1,3,6,7-tetrahydroxyxanthone (see Experimental) and are thus new compounds.

These four xanthones in *A. montanum* are apparently identical to substances previously revealed by two-dimensional chromatography[2] in *A. bradleyii*, *A.* × *gravesii*, *A.* × *kentuckiense*, *A. pinnatifidum*, *A.* × *trudellii* and *A.* × *wherryi*. *Asplenium stotleri* Wherry, examined in this study, was positive for all four xanthones and further, was identical to *A. bradleyi* in all chemical characters noted. These compounds were previously[2] noted to be absent from *A. rhizophyllum* L., *A. ebenoides* Scott, and *A, platyneuron* (L.) Oakes. Other species surveyed for xanthones in this study, *A. adiantumnigrum* L., *A. rutamuraria* L., *A. septentrionale* (L.) Hoffm., *A. trichomanes* L , *Ceterach officinarum* DC., and *Phyllitis scolopendrium* (L.) Neum. likewise proved negative. Thus, the fact that these substances are found only in *A. montanum*, among the diploid species of *Asplenium* surveyed, gives the compounds special importance in recognizing and tracing the hybrid derivaties of *A. montanum*, since the xanthones are obvious in all the hybrid derivatives of *A. montanum*, but seemingly absent from other species.

Even though *A. montanum* has hybridized with *A. platyneuron* and *A. rhizophyllum*, it does not appear to be nearly related to these species. This is to be seen in its very different morphology, the failure of chromosome pairing in its hybrids, and now, its production of xanthones which are lacking in both *A. platyneuron* and *A. rhizophyllum*. When wider chemical surveys of other *Asplenium* species can be made, it will be interesting to see whether or not some will be found to contain xanthones and possibly suggest a relationship to *A. montanum*.

The fern, *A. stotleri*, until recently known from a single colony in West Virginia (and that now extinct), has been rediscovered in Arkansas, and kindly made available for this

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[3] E. C. BATE-SMITH, *Bull. Soc. Bot. Fr.* 16 (1965).

[4] I CARPENTER, H. D. LOCKSLEY and F. SCHEINMANN, *Phytochem.* **8**, 2013 (1969).

[5] A. UENO, *Yakaguku Zasshi* **82**, 1482 (1962).

[6] I. MANTON, *Problems of Cytology and Evolution in the Pteridophyta*, Cambridge University Press (1950).

[7] E C. BATE-SMITH and J. B. HARBORNE, *Nature* **198**, 1307 (1963).

[8] M. ARITOMI and T. KAWASAKI, *Tetrahedron Letters* 941 (1969)

study. This fern has been considered the hybrid, *A. pinnatifidum* × *platyneuron*,[10] the hybrid, *A. pinnatifidum* × *trichomanes*,[11] and most recently,[9] a minor morphological form of the well-known amphidiploid species, *A. bradleyi*. The chromatographic data clearly favour the latter interpretation, since *A. stotleri* is chromatographically indistinguishable from *A. bradleyi*. It does not possess any of the substances characteristic of *A. rhizophyllum*, which would be expected to be inherited through the *A. pinnatifidium* parent if either of the first two hypotheses were true.

Work is now in progress to identify the characteristic substances present in the other diploid species in the Appalachian *Asplenium* complex, since some or all of these compounds appear to be flavonoid in nature.

EXPERIMENTAL

Plant material. Both fresh and dried specimens were utilized in this study. The geographical origin and the herbaria where vouchers are located follows:

A. adiantumnigrum; Astorias, Spain (RU); *A. montanum*; Kentucky, U.S.A. (UCSB); *A. rutamuraria*; Dunbarton, Scotland (UCSB): *A. septentrionale*; USSR (RU): *A. stotleri*; Arkansas, U.S.A. (UCSB): *A. trichomanes*; Dunbarton, Scotland (UCSB): *Ceterach officinarum*; Oxfordshire (UCSB): *Phyllitis scolopendrium*; Berkshire (UCSB).

Identification. The four xanthones were extracted, separated, purified, and identified by the same procedures used for flavonoids.[12] Two compounds agreed precisely with authentic mangiferin and isomangiferin from *Mangifera*. The other two differed from the standards in having lower R_f values in BAW, but higher R_f's in H_2O and 15% HOAc—properites of more highly glycosylated compounds. On acid hydrolysis, they yielded glucose and mangiferin or isomangiferin. The mangiferin *O*-glucoside yields a trace of isomangiferin, and isomangiferin *O*-glucoside yields a trace of mangiferin, presumably through isomerization during hydrolysis. The similarity of the neutral spectra of the *O*-glucosides to mangiferin and isomangiferin and their similar spectral behaviour on the addition of inorganic salts (i.e. they gave bathochromic shifts with NaOEt, $AlCl_3$, NaOAc and H_3BO_3–NaOAc) suggest that the hydrolysable sugar is attached to the *C*-glycosyl group. These characteristics are analogous to the *O*-glycosyl 6- or 8- *C*-glycosides of flavones on similar treatment. The R_f values observed in H_2O, HOAc, BAW and phenol were: mangiferin, 0·16, 0·43, 0·46, 0·39; isomangiferin, 0·08, 0·23, 0·32, 0·19; mangiferin glucoside, 0·42, 0·61, 0·24, 0·38; isomangiferin glucoside, 0·33, 0·50, 0·27, 0·21.

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[9] W. H. WAGNER. JR., and F. WAGNER, *Brittonia* **21**, 178 (1969).

[10] E. T. WHERRY, *Amer. F. J.* **15**, 46 (1925).

[11] E. T. WHERRY, *The Fern Guide: Northeastern and Midland United States and Adjacent Canada*, New York (1969).

[12] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London (1967).