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CHANGEOVER IN FLAVONOID PATTERN ACCOMPANYING REPRODUCTIVE STRUCTURE FORMATION IN A BRYOPHYTE

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Key Word Index—*Marchantia berteroana*; bryophyte; flavone glycosides; seasonal variation; reproduction; chemotaxonomy.

Abstract—Sexual reproduction in *Marchantia berteroana* is accompanied by a dramatic change in flavonoid pattern of the plant. During the sexual reproductive phase, acacetin production ceases and the predominant flavonoids are the previously absent 8-hydroxyapigenin and 8-hydroxyluteolin glycosiduronic acids. In contrast, acacetin levels reach their peak during the asexual reproductive phase. The chemotaxonomic significance of these results is discussed.

INTRODUCTION

Earlier work has established the ubiquitous distribution of flavonoid *O*-glycosiduronic acids in liverworts of the order Marchantiales [1]. Flavonoid composition has proved to be species specific and is a promising taxonomic tool for this group of plants. To date, it has been assumed that the flavonoid patterns of liverworts, in common with those of vascular plants, are independent of season and this has found some support in previous work on several liverwort species [1]. However, the current results show that this assumption is not always valid and that the flavonoid pattern may change on production of sexual branches (antheridiophores and archegoniophores). We wish to describe here a particularly dramatic example of this phenomenon resulting from a seasonal study of *Marchantia berteroana*, a thalloid liverwort native to New Zealand [2].

An earlier communication described the identification of 17 glycosiduronic acids of apigenin, luteolin, 8-hydroxyapigenin and 8-hydroxyluteolin isolated from *Marchantia berteroana* [2]. Later aureusidin 6-*O*-glucuronide was isolated from the antheridiophores of the same species [3]. The plant material used in the former investigation was collected in mid-summer and the thalli possessed sexual branches.

RESULTS

Investigation of the flavonoids of *M. berteroana* thallus tissue, collected in mid-winter*, revealed a much simpler pattern (Fig. 1) than did the mid-summer sample. The flavonoids consist of glycosiduronic acids of acacetin, apigenin and luteolin. Five of the compounds present, acacetin and its 7-glucuronide and -galacturonide and apigenin 7,4′-diglucuronide and -digalacturonide, were undetected in the previous study [2]. It was therefore evident that there is a considerable difference between the summer and winter flavonoid patterns. This prompted a detailed study of the origin of this phenomenon.

Thalli of *M. berteroana* were grown under controlled conditions, as described in the Experimental, and were analysed for flavonoid content at regular intervals throughout the year. This revealed that the flavonoid pattern in Fig. 1 was virtually invariant throughout the months February (late summer) to mid-October (mid-spring). Thereafter, numerous antheridiophores develop, and with their maturation the flavonoid composition changes dramatically.

It was evident that at the time of production of sexual branches, changes occurred in flavonoid biosynthesis in both the maturing sexual branches and in the unmodified thallus itself. The pattern of flavonoids shown in Fig. 2 was maintained in the thallus essentially unchanged throughout the period when it supported mature sexual branches (mid-October to January). The pattern differed from that of the non-reproducing thallus (Fig. 1) by the absence of acacetin and its glycosides.

The presence of large numbers of gemmae cups and gemmalings on the acacetin-rich winter thallus, and the relative lack of them on the summer thallus, raised the possibility that acacetin might be produced by these structures rather than by the thallus itself. This was discounted by a study of the flavonoids of the separated gemmae cups, associated gemmalings and remaining thallus, which revealed that although acacetin (and its 7-glycosides) occur at high levels in the gemmalings, they are also present in both the thallus and the gemmae cups. These last two possess essentially the normal winter pattern of flavonoids (Fig. 1), the slightly increased levels of acacetin in the gemmae cups presumably arising from imbedded gemmalings. It is thus clear that acacetin is produced by the thallus itself and therefore that (as discussed above) its production ceases at the time of sexual branch growth.

In contrast, a quite different pattern of flavonoids developed in the antheridiophores and archegoniophores. The pattern in each organ changed throughout its growth and culminated in the patterns shown for the mature antheridiophores (Fig. 3) and archegoniophores (Fig. 4). The pattern of flavonoids in these special branches is quite different from that of the thallus. Thus, flavonoids derived from luteolin, 8-hydroxyapigenin and 8-hydroxyluteolin

* The thalli possess gemmae cups, but not sexual branches at this time

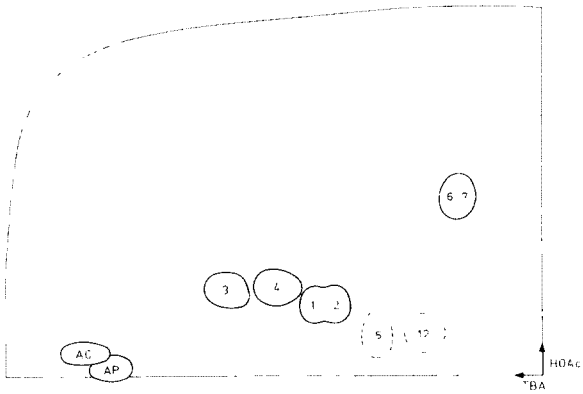

Fig. 1. Flavonoid pattern of vegetative thallus (maintained from February to mid-October). Solid outlines indicate major flavonoids and dotted outlines those present at low concentrations.

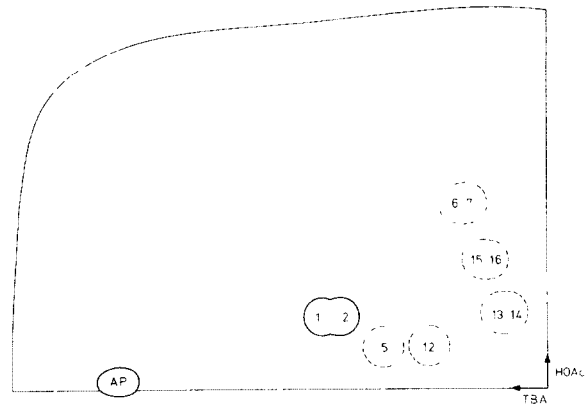

Fig. 2 Flavonoid pattern of thallus supporting mature sexual branches (maintained from mid-October to January)

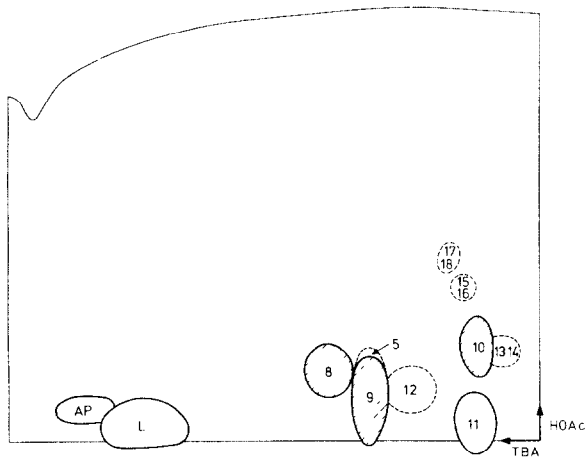

Fig 3. Flavonoid pattern of mature antheridiophores

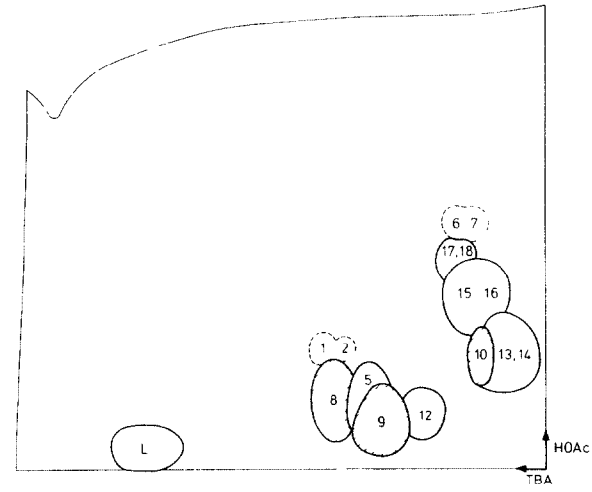

Fig. 4. Flavonoid pattern of mature archegoniophores.

Key to Figs 1–4

AC = acacetin aglycone;
AP = apigenin aglycone;
L = luteolin aglycone;
1 = apigenin 7-glucuronide;
2 = apigenin 7-galacturonide;
3 = acacetin 7-glucuronide,
4 = acacetin 7-galacturonide;
5 = luteolin 7-glucuronide;
6 = apigenin 7,4′-diglucuronide;
7 = apigenin 7,4′-digalacturonide;
8 = 8-hydroxyapigenin 8-glucuronide;
9 = 8-hydroxyluteolin 8-glucuronide.
10 = 8-hydroxyluteolin 8,4′-diglucuronide;
11 = aureusidin 6-glucuronide,
12 = luteolin 7-galacturonide;
13 = luteolin 7,3′-diglucuronide;
14 = luteolin 7,3′-digalacturonide,
15 = luteolin 7,4′-diglucuronide;
16 = luteolin 7,4′-digalacturonide;
17 = luteolin 3′,4′-diglucuronide;
18 = luteolin 3′,4′-digalacturonide

predominate. Additionally the antheridiophore alone contains aureusidin 6-*O*-glucuronide, a flavonoid type previously unknown in liverworts [3].

Not only are the flavonoids in the sexual branches quite different from those in the thallus, but their concentration is far higher, up to nine times the concentration of those in the supporting thallus. A predominance of flavonoids containing *ortho*-dihydroxyl functions over those of the apigenin type is also evident. This predominance reaches a maximum mole ratio of 33 in the antheridiophore in mid-October, whereas in the antheridial thallus the mole ratio is less than 0.001.

DISCUSSION

The observation that a plant possesses more than one distinct flavonoid composition, differing *both* qualitatively and quantitatively at different seasons, is apparently unique. In seasonal studies of vascular plants, mainly

quantitative changes have been noted (e.g. beech leaf flavonoids [5]). The phenomenon noted for *M. berteroana* however is not unique amongst liverworts of the order Marchantiales. We have also noted seasonal changes with *Marchantia foliacea* and *Asterella australis* [6] and the antheridial aurone is known to be produced by *Marchantia polymorpha* [3]. Although no change was detected in the summer and winter patterns of European *Conocephalum conicum* [7], it should be pointed out that the thalli were not supporting sexual structures.

The above results have important consequences regarding the use of flavonoid data for chemotaxonomic purposes in this group of plants. It is clear that one must ensure that chemotaxonomic data are obtained only from vegetative thalli (i.e. those not supporting sexual structures). However, this is not a disadvantage as it is at the vegetative stage that thalloid liverwort species are the most difficult to identify from morphological features alone.

A particularly interesting observation is the complete disappearance of acacetin during the sexual reproductive phase. Its disappearance coincides with the presence of a high overall relative concentration of flavonoids possessing *ortho*-dihydroxyl functions. Borchardt and Huber [8] and Gugler and Gengler [9] have shown that flavonoids possessing *ortho*-dihydroxyl functions are especially effective inhibitors of catechol *O*-methyl transferase enzyme. Admittedly the methylation of acacetin will not proceed via this specific enzyme and may occur at the cinnamic acid stage [10] but almost certainly it would involve an *S*-adenosylmethionine intermediate and be subject to similar substrate control.

The markedly different patterns of flavonoids produced by the sexual branches and thallus suggest that the change from apigenin and luteolin glucoside production may play a role in sexual reproduction in the plant. For example, the confinement of the aurone to the antheridiophore, and its rapidly increasing level with antheridiophore maturation, could imply its action as an antheridiogen with a function comparable to that of the terpenoid antheridium-inducing compound detected in some fern families [11]. We are currently investigating the possibility that these flavonoids may induce gametogenesis.

EXPERIMENTAL

The samples of *Marchantia berteroana* L. & L. (voucher specimen, Massey University Herbarium, MPN 17001) were collected from a single site near Upper Hutt. The gemmalings were cultured at Plant Physiology Division, DSIR, Palmerston North.

Method of culturing. Thalli, derived from gemmalings from a single genotype of *M. berteroana*, were sub-cultured on to soil in trays and grown under natural light conditions in a greenhouse. The thalli grew very vigorously, producing numerous gemmae cups and subsequently produced both antheridiophores and archegoniophores approximately 9 months after sub-culturing.

Sampling. The thalli flavonoids were determined at monthly intervals, from February 1976 to April 1977, by extraction of the crushed thalli with Me_2CO–H_2O (1 : 1) and analysis of extract by 2D PC. Chromatograms of the extract from both 50 and 100 mg of dried thallus were run for each sampling. At the inception of sexual branch growth the thalli and sexual structures were extracted separately. In a separate experiment gemmalings were collected by suction (Pasteur pipette and H_2O) and washed with H_2O, dried and extracted. Parent thalli were dissected and the gemmae cups and residual thalli extracted separately.

Chemical methods. The chromatographic and spectroscopic techniques were as described previously [2]. The new compounds, unreported in the earlier paper [2], acacetin and its 7-glucuronide and -galacturonide, apigenin 7,4′-diglucuronide and -digalacturonide were all identified by techniques previously described [2]. Quantitative data were obtained by eluting individual flavonoid spots with MeOH–H_2O (2:3) and estimation spectrophotometrically by measurement of the absorbance of the maximum near 270 nm. Extraction coefficients were obtained from appropriate lit. values [12], with due allowance for extraction efficiency (demonstrated to be 80% by the method used). The overall concn of the flavonoids in the thallus was estimated to be constant at 7×10^{-7} mol per gram of dried thallus, except at the stage near the death of the thallus itself. The concn rose to about 8×10^{-6} mol per gram in the mature sexual branches. Levels of flavonoids were also measured in antheridiophores of increasing maturity (as evidenced by the diameter of the heads). The antheridiophores were divided into groups with 0–5, 5–8 and 8–12 mm diameter heads for analysis. Quantification of compounds 11 and 15/16 indicated a six-fold increase in the level of 11 and a near-constant level of 15/16 with increasing head size.

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