inhibition of the germination of lettuce seeds at 1000 ppm. The EtOAc soluble part was successively stirred with 1 l. each of hexane, C_6H_6, and then CH_2Cl_2. The each organic soluble part was separated into acidic and neutral fractions. The hexane extracts gave 110 mg, 45% germination (neutral) and 440 mg, 8% germination (acidic); C_6H_6 extracts gave 570 mg, 33% germination (neutral) and 1.43 g, 0% germination (acidic) and and CH_2Cl_2 gave 440 mg, 0% germination (neutral) and 1.49 g, 0% germination (acidic) fractions, respectively. The neutral portion of CH_2Cl_2 soluble part was eluted on SiO_2 (30 g) column with a mixed solvent of C_6H_6–EtOAc (1:1). The resultant active fraction was further separated by HPLC [column; μ-porasil, solvent; $CHCl_3$–EtOH (100:3)] to isolate pale yellow crystals (**1**). Recrystallization from C_6H_6–$CHCl_3$ afforded a pure specimen (10 mg) mp 122–125° (decomp). The germination assay with lettuce seeds showed that the (**1**, heraclenol) inhibited completely the germination of lettuce seeds at 1000 ppm after after 24 hr, while *ca* 50% of the seeds germinated at 100 ppm.

REFERENCES

1. (a) Kato, T., Tsunakawa, M., Sasaki, N., Aizawa, H. Fujita, K. Kitahara, Y. and Takahashi, N. (1977) *Phytochemistry* **16**, 45; (b) Yanagawa, H., Kato, T. and Kitahara, Y. (1973) *Tetrahedron Letters*, 1073.
2. Bandopadhyay, M. and Seshadri, R. T. (1970) *Ind. J. Chem.* **8**, 1146 and references cited therein.

Phytochemistry, 1978, Vol 17, pp 159–160 Pergamon Press Printed in England

PRODUCTION OF AN AURONE BY BRYOPHYTES IN THE REPRODUCTIVE PHASE

KENNETH R. MARKHAM and LAWRENCE J. PORTER

Chemistry Division, D.S.I.R., Petone, New Zealand

(*Received* 29 *April* 1977)

Key Word Index—*Marchantia*; *Conocephalum*; Hepaticae; aurone; aureusidin glucuronide; reproduction.

Abstract—The aurone, aureusidin 6-*O*-glucuronide, has been isolated from the antheridiophores of two liverworts, *Marchantia berteroana*, *M. polymorpha* and from *Conocephalum supradecompositum*. It occurs only in these organs in *Marchantia*. The appearance of this aurone in bryophyte reproductive structures suggests parallel evolution within the angiosperms and the bryophytes.

INTRODUCTION

The flavonoid chemistry of bryophytes has been studied intensively in recent years and a wide range of structures are now known in these plants. Flavones [1], flavonols [2], dihydroflavones [3], 3-deoxyanthocyanins [4] and biflavonyls [5] have all been identified in one or more species but chalcones, isoflavones and 3-hydroxylated anthocyanins have not. The aurone, bracteatin has recently been identified in the moss, *Funaria hygrometrica* [6] and we now report the occurrence of another aurone, aureusidin 6-*O*-glucuronide, in three liverworts.

RESULTS

In the course of our recent study of the liverwort *Marchantia berteroana* [1] we found both quantitative and qualitative seasonal variations in the flavonoid constituents. A further experiment using a single genotype of this liverwort showed that marked changes took place as the plant moved into its reproductive phase. Perhaps the most spectacular of these changes is the production of aureusidin 6-*O*-glucuronide as it appears, on PC in UV light, as a distinctive yellow fluorescent spot which turns reddish-orange in NH_3. Its absorption spectrum and reagent induced shifts were found to be identical with those of 4,6,3′,4′-tetrahydroxyaurone 6-*O*-glucoside (aureusin) isolated from *Antirrhinum majus* flowers. PC comparison in the solvents TBA and HOAc also indicated their apparent identity, but in H_2O the liverwort aurone is more mobile, which is characteristic for flavonoid glycosiduronic acid derivatives. Its identity as a glucuronide was substantiated by its resistance to acid hydrolysis, relative ease of enzymatic hydrolysis with β-glucuronidase, and GLC analysis of the sugar produced on hydrolysis. The aglycone was shown to be 4,6,3′,4′-tetrahydroxyaurone (aureusidin), thereby defining the natural product as aureusidin 6-*O*-β-D-glucuronide.

Aureusidin 6-*O*-glucuronide was found only in the antheridiophores of male plants and not in the associated thallus or in female plants. It is present in the plant for only 4–8 weeks, the life span of these reproductive structures. The same aurone was also found in the antheridiophores of *Marchantia polymorpha* and in a sample of *Conocephalum supradecompositum*.*

*Antheridiophores in this species are imbedded in the thallus in contrast to those of *Marchantia* which are erect.

DISCUSSION

The occurrence of aureusidin as a glucuronide in *M. berteroana*, *M. polymorpha* and *C. supradecompositum* is in accord with earlier chemical studies which show that these [1, 7] and other [8] liverworts of the order Marchantiales possess flavonoids which are predominantly glycosylated with either glucuronic or galacturonic acid. However, the occurrence of aurones in bryophytes is unexpected in view of their apparent restricted distribution in the plant kingdom. To date they have been found in only 8 angiosperm families [9], where they are generally, but not always associated with flowers. The present finding, and that of bracteatin in *Funaria* [6] are therefore further examples of the occurrence in bryophytes of a chemical feature which had, until recently, been considered characteristic of higher plants. As such it would seem to strengthen the view [10] that bryophytes have evolved biochemically parallel with the higher plants to reach, in some cases, the level of biosynthetic sophistication achieved by the advanced angiosperms.

EXPERIMENTAL

Voucher specimens of *M. berteroana* and *M. polymorpha* are held at Massey University, Palmerston North (MPN 17001 and 8575 respectively). The sample of *Conocephalum supradecompositum* was supplied fresh in November 1976 by Professor S. Hattori from the Hattori Botanical Laboratory, Japan. 2DPC was carried out on Whatman 3MM paper using *t*-BuOH–HOAc–H_2O (3 1.1) (TBA) and 15% HOAc (HOAc) and UV-visible spectra and reagent induced shifts were measured as previously described [11].

Isolation procedure. Male and female plants from the same clone of *M. berteroana* were sampled throughout the growing season and the flavonoids were evaluated by 2DPC Extract derived from 50 mg dried plant material was found to be an appropriate loading for a single PC. On the appearance of antheridiophores larger samplings of male plants were made and PC analysis was carried out on both antheridiophore and thallus material Later, archegoniophore material was examined. A typical mature sample of male plants collected in early November yielded 1.05 g antheridiophore material from 2.6 g sample. A total of 32 g fresh antheridiophores (4 g dry wt) was extracted with Me_2CO–H_2O (1 1) and the aurone glycoside was isolated by 2DPC Small samples of *M. polymorpha* antheridiophores and *C. supradecompositum* were treated in the same way. Similar extraction and chromatography yielded authentic aureusin from yellow *Antirrhinum majus* flowers. The *M. berteroana* aurone glycoside had PC properties, dark, UV absorbing spot (R_f 0.08 (TBA), 0.04 (HOAc)) turning red-orange in NH_3 and brilliant reddish-orange with Naturstoffreagenz A; and UV spectra identical to those published for aureusin. Their PC mobility in H_2O however was different, aureusin (0.02), liverwort aurone (0.12)

Hydrolyses. (*1*) *Acid.* Aureusin was completely hydrolysed to its aglycone, after 30 min in 2N HCl at 100°. The liverwort aurone glycoside was incompletely hydrolysed after 2 hr under the same conditions. The aglycones possessed identical UV spectra and were chromatographically indistinguishable (R_f 0.31 (TBA), 0.41 (BAW), 0.03 (C_6H_6–HOAc–H_2O, 125:72:3). (*2*) *Enzyme.* Both aurone glycosides were treated separately in H_2O at 20° with β-glucosidase and β-glucuronidase. Aureusin was hydrolysed only by β-glucosidase and the liverwort aurone only by β-glucuronidase. The sugar produced from liverwort aurone was shown to be glucuronic acid by PC (*n*-BuOH–Py–H_2O, 2:2:1) and GLC (TMS ethers on 3% OV-1, 170°) comparison with authentic samples of glucuronic and galacturonic acids.

Acknowledgements—The authors wish to acknowledge the assistance of Dr K Giles of Plant Physiology Division, DSIR, Palmerston North for growing and sampling the *M berteroana* and Prof. S. Hattori of the Hattori Botanical Laboratory, Obi-Honmachi, Nichinan-shi, Miyazaki-ken, 889-25, Japan, for the sample of *C. supradecompositum*.

REFERENCES

1. Markham, K. R. and Porter, L. J (1975) *Phytochemistry* **14**, 1093.
2. Markham, K. R., Porter, L. J., Campbell, E. O., Chopin, J. and Bouillant, M-L. (1976) *Phytochemistry* **15**, 1517
3. Markham, K. R. and Porter, L. J. (1975) *Phytochemistry* **14**, 199.
4. Bendz, G., Martensson, O and Terenius, L. (1962) *Acta Chem. Scand.* **16**, 1183.
5. Lindberg, G., Osterdahl, B-G. and Nilsson, E. (1974) *Chem. Scrip.* **5**, 140.
6. Weitz, S. and Ikan, R. (1977) *Phytochemstry* **16**, 1108.
7. Markham, K. R. and Porter, L J. (1974) *Phytochemistry* **13**, 1937.
8. Markham, K. R., Porter, L. J and Miller, N. G. (1976) *Phytochemistry* **15**, 151.
9. Bohm, B. A (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H eds) p 442. Chapman and Hall, London
10. Markham, K. R. and Porter, L. J. (1973) *Phytochemstry* **12**, 2007; *idem*, *Progress in Phytochemistry* **5** in press; Steere, W. C. (1969) in *Current Topics in Plant Science* (Gunckel, J. E ed.) p. 134. Academic Press, New York.
11. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *Systematic Identification of Flavonoids* p 35. Springer-Verlag, New York.