penultimate step in normal anthocyanin bisoynthesis in *Pisum*, where it can be recognized by failure to convert to anthocyanin in *am* genotypes.

EXPERIMENTAL

The acid test. Single wing petals were removed from individual flowers and submerged in 50% HCl. A positive reaction appeared within 1–2 min.

pH measurements. Made directly using an Orion specific ion meter fitted with a combined glass electrode. pH changes were made using microlitre additions of conc. HCl.

Visible spectra. Recorded using a Pye–Unicam SP8-100 spectrophotometer. Absorption curves in H_2O (Fig. 1) show some inconsistencies in absorbance values in the 420–500 nm region due to turbidity, which could not be eliminated by centrifugation. Repeat expts in MeOH confirmed that there was a consistent increase in colour with reducing pH from 6.0 to 1.9.

Paper chromatography. Conducted using Whatman No. 1 paper and the solvent systems BAW, 5% HOAc and 1% HCl.

REFERENCES

1. de Haan, H. (1930) *Genetica (the Hague)* **12**, 321.
2. Wellensiek, S. J. (1947) *Genetica (the Hague)* **24**, 74.
3. Lamprecht, H. (1954) *Agric. Hort. Genet.* **12**, 38.
4. Crowden, R. K. (1982) *Pisum Newsletter* **14** (in press).
5. Reznik, H. and Neuhausel, R. (1959) *Z. Bot.* **47**, 471
6. Werckmeister, P. (1955) *Der Zuchter* **25**, 315.
7. Statham, C. M., Crowden, R. K. and Harborne, J. B. (1972) *Phytochemistry* **11**, 1083.

Phytochemistry, Vol. 21, No. 12, pp. 2990–2991, 1982.
Printed in Great Britain.

0031–9422/82/122990–02$03.00/0

THE FLAVONOIDS OF *TRICHOPHORUM CESPITOSUM*

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(*Received* 1 *March* 1982)

Key Word Index—*Trichophorum cespitosum*; Cyperaceae; *C*-glycosylflavones; flavones; flavonols; 6-*C*-glucosyl-8-*C*-arabinosylchrysoeriol; 6-*C*-arabinosyl-8-*C*-glucosylchrysoeriol.

Abstract—Fifteen flavonoids were isolated from *Trichophorum cespitosum*, including two new di-*C*-glycosylflavones, 6-*C*-arabinosyl-8-*C*-glucosylchrysoeriol and its Wessely–Moser rearrangement isomer. The known compounds are isorhamnetin 3, 7-dimethyl ether, kaempferol 3, 7-dimethyl ether, chrysoeriol 7-methyl ether, chrysoeriol, sudachitin, tricin, isorhamnetin 3-*O*-galactoside, 6,8-di-*C*-glucosylchrysoeriol, isoschaftoside, schaftoside, vicenin-2, isoscoparin and neoisoschaftoside.

INTRODUCTION

Trichophorum (Cyperaceae), a genus of three species, is also often included in the genus *Scirpus*. These sedges grow mostly in damp peaty areas in boreal coniferous forests in Europe, Asia and North America. Flavonoid chemistry has been used previously in taxonomic studies on sedges [1, 2] but this is the first detailed report of flavonoids isolated and identified from a member of this genus. In this paper we report the flavonoids of *Trichophorum cespitosum* (L.) Hartman (*Scripus cespitosus* L.) including two new di-*C*-glycosylflavones, 6-*C*-arabinosyl-8-*C*-glucosylchrysoeriol and its Wessely–Moser isomer.

RESULTS AND DISCUSSION

Stems of *Trichophorum cespitosum* were analysed for their flavonoid content using standard procedures [3]. The dichloromethane fraction contained isorhamnetin 3,7-dimethyl ether, kaempferol 3,7-dimethyl ether, chrysoeriol 7-methyl ether, sudachitin, tricin, isorhamnetin 3-*O*-galactoside and chrysoeriol. Tricin (*ca* 40 mg) was the major flavonoid component of this fraction. This compound, although relatively rare in the dicotyledons, is a common aglycone in the Cyperaceae [4]. The remaining compounds occurred in trace amounts (*ca* 1 mg each).

The water fraction contained a mixture of *C*-gly-

cosylflavones. The R_f values and UV spectra with standard reagents indicated the presence of both mono- and di-*C*-glycosylflavones of the apigenin and chrysoeriol types. After permethylation it was possible to separate the PM-derivatives. By this procedure the water fraction was found to contain 6,8-di-*C*-glucosylchrysoeriol, 6-*C*-arabinosyl-8-*C*-glucosylchrysoeriol, 6-*C*-glucosyl-8-*C*-arabinosylchrysoeriol, isoschaftoside, schaftoside, vicenin-2, isoscoparin and neoisoschaftoside [5].

EXPERIMENTAL

Plant material. Plants were collected from the bog Raimansuo, Finland. Voucher specimen (*Trichophorum cespitosum* (L.) Hartman subsp. *cespitosum* (Kukkonen 10261) is deposited in the Botanical Museum of the University of Helsinki.

Air-dried ground stems (0.5 kg) were extracted with 85% aq. MeOH (2 × 10 l.) and with 50% aq. MeOH (1 × 8 l.). The combined extracts were evaporated under red. pres. and the concentrate (45 g) was then partitioned against CH_2Cl_2 and EtOAc. The CH_2Cl_2 fraction (12.5 g) was chromatographed over a Polyclar column (40.5 × 5 cm) eluted with modified Egger's solvent (CH_2Cl_2–MeOH–MeCOEt–Me_2CO, 20:10:5:1), with the polarity of the solvent being gradually increased. The isolated fractions were further separated on microcrystalline cellulose columns (15% HOAc). Compounds were cleaned over Sephadex LH-20 columns prior to spectral analysis. The EtOAc fraction (1.5 g) contained a mixture of flavonoids also found in the CH_2Cl_2 and H_2O fractions and was, therefore, not further analysed. The material from the H_2O fraction was chromatographed over a Polyclar column (27 × 5 cm) eluted with 50% aq. MeOH. The fractions collected contained mixtures of several *C*-glycosylflavones; these mixtures were further separated on microcrystalline cellulose columns (15% HOAc). Final separation of the compounds was achieved by TLC following permethylation.

Known compounds were identified by comparison of UV and mass spectral data with those for standard compounds and by co-TLC of permethylated derivatives with permethylated standards. 6-*C*-Glucosyl-8-*C*-arabinosylchrysoeriol (**1**) and 6-*C*-arabinosyl-8-*C*-glucosylchrysoeriol (**2**) were identified by comparison of their PM-derivatives with the PM-derivatives of the corresponding di-*C*-glycosylluteolins, carlinoside and isocarlinoside respectively [6], after the MS of PM-1 and PM-2 had shown molecular ions at *m/z* 734 and the characteristic fragmentation patterns [7] of a PM 6-*C*-hexosyl-8-*C*-pentosylluteolin ($[M-175]^+ > [M-131]^+$) for PM-1 and of a PM 6-*C*-arabinosyl-8-*C*-hexosylluteolin ($[M-131]^+ > [M-175]^+$; $[M-131]^+ > [M-119]^+ > [M-145]^+$) for PM-2.

6-C-*Glucosyl*-8-C-*arabinosylchrysoeriol* (**1**). TLC (cellulose) R_f 0.29 (TBA), 0.51 (15% HOAc). EIMS (70 eV) of PM ether, *m/z* > 400 (rel. int.): 734 $[M]^+$ (17), 719 $[M-15]^+$ (33), 703 $[M-31]^+$ (100), 689 $[M-45]^+$ (12), 687 $[M-47]^+$ (10), 673 $[M-61]^+$ (18), 671 $[M-63]^+$ (7), 631 $[M-103]^+$ (13), 603 $[M-131]^+$ (13), 573 $[M-161]^+$ (15), 559 $[M-175]^+$ (48), 545 $[M-189]^+$ (12), 529 $[M-205]^+$ (12), 515 $[M-219]^+$ (4). Co-TLC (Si gel) with PM carlinoside, R_f 0.29 ($CHCl_3$–EtOAc–Me_2CO, 5:4:1), 0.42 ($CHCl_3$–Me_2CO, 8:2).

6-C-*Arabinosyl*-8-C-*glucosylchrysoeriol* (**2**). TLC (cellulose) R_f 0.22 (TBA), 0.43 (15% HOAc). EIMS (70 eV) of PM ether, *m/z* > 400 (rel. int.): 734 $[M]^+$ (16), 719 $[M-15]^+$ (28), 703 $[M-31]^+$ (100), 689 $[M-45]^+$ (11), 687 $[M-47]^+$ (13), 673 $[M-61]^+$ (19), 671 $[M-63]^+$ (6), 645 $[M-89]^+$ (8), 615 $[M-119]^+$ (30), 603 $[M-131]^+$ (38), 589 $[M-145]^+$ (16), 571 $[M-163]^+$ (11), 559 $[M-175]^+$ (19), 545 $[M-189]^+$ (5). Co-TLC (Si gel) with PM isocarlinoside: R_f 0.15 ($CHCl_3$–EtOAc–Me_2CO, 5:4:1), 0.67 ($CHCl_3$–EtOAc–Me_2CO, 5:1:4).

Acknowledgements—This work was supported at the University of Texas by the Robert A. Welch Foundation (Grant F-130) and the National Institutes of Health (Grant HD-04488).

REFERENCES

1. Kukkonen, I. (1971) *Mitt. Bot. Staatssamml. Munchen* **10**, 622.
2. Toivonen, H. (1974) *Ann. Bot. Fenn.* **11**, 225.
3. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
4. Harborne, J. B. (1971) *Phytochemistry* **10**, 1569.
5. Osterdahl, B. G. (1979) *Acta Chem. Scand.* **33**, 400.
6. Linard, A., Delaveau, P., Paris, R. R., Dellamonica, G. and Chopin, J. (1982) *Phytochemistry* **21**, 797.
7. Bouillant, M. L., Favre-Bonvin, J. and Chopin J. (1975) *Phytochemistry* **14**, 2267.