2. Clark, W. D. and Wollenweber, E. (1984) *Z. Naturforsch.* **39c**, 1184.
3. Bohlmann, F. and Zdero, C. (1976) *Chem. Ber.* **109**, 2021.
4. Bohlmann, F. and Zdero, C. (1979) *Phytochemistry* **18**, 1185.
5. Bohlmann, F., Zdero, C., Robinson, H. and King, R. M. (1979) *Phytochemistry* **18**, 1675.
6. Bohlmann, F. and Mailahn, W. (1981) *Chem. Ber.* **114**, 1091.
7. Bohlmann, F., Gupta, R. K., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 2982.
8. Bohlmann, F. and Giencke, W. (1983) *Tetrahedron* **39**, 443.
9. Lincoln, D. E. and Lawrence, B. M. (1984) *Phytochemistry* **23**, 933.
10. Clark, W. D., Urbatsch, L. E., Hartman, R. L., Mayes, R. A. and Mabry, T. J. (1980) *Biochem. Syst. Ecol.* **8**, 257.
11. Urbatsch, L. E., Mabry, T. J., Miyakado, M., Ohno, N. and Yoshioka, H. (1976) *Phytochemistry* **15**, 440.
12. Wollenweber, E. (1982) in *The Flavonoids, Advances in Research* (Harborne, J. B. and Mabry, T. J., eds). Chapman & Hall, London.

Phytochemistry, Vol. 24, No. 9, pp. 2131–2132, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

5,7,3′-TRIHYDROXY-4′,5′-DIMETHOXYFLAVONE AND OTHER PHENOLICS FROM *POA HUECU*

RICARDO D. ROFI and ALICIA B. POMILIO*

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

(*Received* 2 *January* 1985)

Key Word Index—*Poa huecu*; Gramineae; 5,7,3′-trihydroxy-4′,5′-dimethoxyflavone; tricin; selagin.

Abstract—Whole plants of *Poa huecu* have yielded a new flavone characterized as 5,7,3′-trihydroxy-4′,5′-dimethoxyflavone as well as tricin, selagin, umbelliferone and scopoletin.

INTRODUCTION

In continuation of our research on *Poa huecu* Par. [1] (Gramineae), an Argentinian plant toxic to cattle, we now report the isolation and identification of the flavones: 5,7,3′-trihydroxy-4′,5′-dimethoxy flavone (**1**), 5,7,3′,4′-tetrahydroxy-5′-methoxyflavone (selagin, **2**), 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone (tricin, **3**) and the coumarins umbelliferone and scopoletin.

RESULTS

Upon column chromatography of the methanolic percolate of *Poa huecu* a fraction rich in flavonoids was obtained. Further chromatography on Sephadex LH-20 led to the five components mentioned above. Tricin was the main component. The UV spectrum of the new flavone **1** showed maxima at 271 and 329 nm. The presence of a chelated hydroxyl was supported by a bathochromic shift of 26 nm with aluminium chloride–hydrochloric acid in UV. The shift with sodium acetate ($\Delta\lambda$ 7 nm) indicated the presence of a hydroxyl at the 7-position. The aluminium chloride–hydrochloric acid as well as sodium acetate–boric acid spectra ruled out an *o*-dihydroxyl in the B-ring. The shift with sodium methoxide ($\Delta\lambda$ 21 nm) indicated the absence of a free hydroxyl group at the 4′-position. By contrast, tricin and selgin with the same agent, showed a shift higher than 50 nm, indicating a free hydroxyl group in that position.

The ^{1}H NMR spectrum of **1** showed two singlets at δ4.01 and 4.02 due to the presence of two methoxyl groups. The 5,7-disubstitution pattern of A-ring was indicated by the two doublets at δ6.27 (H-6) and δ6.59 (H-8) with a *meta* coupling constant of 2 Hz. The singlet at δ6.82 (H-3) integrating for one proton supported the flavone skeleton, whilst the singlet at 7.44 (2H) accounted for H-2′ and H-6′ that gave this signal instead of a doublet as has been also observed in selagin. The presence of a free hydroxyl at 5- position was confirmed by the signal at δ12.94.

A retro-Diels–Alder fragmentation pattern was observed in the MS leading to the fragments $A_1 + H$, B_2 and B_1. The results supported the presence of one hydroxyl and two methoxyl groups in ring B and two hydroxyls in ring A.

*Research member of the National Research Council of Argentina (CONICET). To whom correspondence should be addressed.

As expected, this MS was coincident with that of tricin except in the relative intensities. Thus **1** is 5,7,3′-trihydroxy-4′,5′-dimethoxyflavone, a novel plant flavone. As far as we know this is the first report on the occurrence of selagin (**2**) in the Gramineae. This compound has been previously isolated from *Huperzia selago* [2], two species of *Isoetes* [3] and *Artemisia ludoviciana* [4].

EXPERIMENTAL

Plant material. Whole plants of *Poa huecu* were collected in Bariloche and Zapala (Argentina) by INTA. Voucher specimen are deposited in Instituto de Botánica Darwinion (Buenos Aires, Argentina) under the Nr SI 14036.

Isolation and identification of the flavones and coumarins. Dried ground plants were successively extracted in a Soxhlet with petrol and MeOH. The methanolic extract (6.8% of dry plant) was percolated on polyamide with $CHCl_3$ (3.3% of dry plant), H_2O (3.1% of dry plant) and finally MeOH (0.4% of dry plant). The methanolic percolate was chromatographed on a silica gel H column with CH_2Cl_2 and gradients of CH_2Cl_2–MeOH as eluents. Five fractions were obtained. The first one was further chromatographed on a Sephadex LH-20 column using MeOH.

5,7,3′-trihydroxy-4′,5′-dimethoxyflavone (**1**), selagin (**2**), tricin (**3**), umbelliferone and scopoletin were subsequently obtained.

HPLC (Waters; UV detection at 254 nm) was performed on μ Bondapak C_{18} (30 cm × 4 mm, 10 μm). R_t flavones (eluent: MeOH–H_2O–HOAc, 1:1:0.01; 1.7 ml/min): **1**: 15.25 min; **2**: 8.20 min; **3**: 14 min. R_t coumarins: (eluent: MeOH–H_2O, 3:7; 2 ml/min) umbelliferone: 5.30 min; scopoletin: 6.00 min.

5,7,3′-Trihydroxy-4′,5′-dimethoxyflavone (**1**). UV λ_{max}^{MeOH} nm: 271, 284 (sh), 329; +NaOMe: 278, 298 (sh), 350; +$AlCl_3$: 278, 305 (sh), 355; +$AlCl_3$–HCl: 277, 303 (sh), 354; +NaOAc: 278, 302 (sh), 350; +NaOAc–H_3BO_3: 270, 329. ^{1}H NMR (100 MHz, Me_2CO-d_6): δ4.01–4.02 (2H, *s*, OMe-4′ and 5′), 6.27 (1H, *d*, J = 2 Hz, H-6), 6.59 (1H, *d*, J = 2 Hz, H-8), 6.82 (1H, *s*, H-3), 7.44 (2H, *s*, H-2′ and H-6′), 12.94 (1H, *s*, HO-5). MS *m/z* (rel. int.): 331 $[M+1]^+$ (21.0), 330 $[M]^+$ (100.0), 329 (2.4), 315 $[M-Me]^+$ (3.0), 302 $[M-CO]^+$ (3.2), 178 $[B_1]^+$ (7.8), 181 $[B_2]^+$ (5.0), 153 $[A_1+H]^+$ and $[B_2-CO]^+$ (11.0), 152 $[B_2-HCO]^+$ (2.7), 148 $[B_1-2\times Me]^+$ (19.2), 147 $[B_1-OMe]^+$ (23.8).

Selagin (**2**). UV and MS data were in agreement with those previously reported [2, 4]. ^{1}H NMR (100 MHz, Me_2CO-d_6): δ4.0 (3H, *s*, OMe-5′), 6.27 (1H, *d*, J = 2 Hz, H-6) 6.56 (1H, *d*, J = 2 Hz, H-8), 6.64 (1H, *s*, H-3), 7.25 (2H, *s*, H-2′ and H-6′), 13.01 (1H, *s*, HO-5). The spectral data (UV, IR, MS, ^{1}H NMR) of tricin (**3**), umbelliferone and scopoletin were identical with those of standards.

Acknowledgements—We thank CONICET and SECYT (Argentina) for financial support; INTA (Argentina) for supplying plant material and UMYMFOR (CONICET, Argentina) for performing mass and ^{1}H NMR spectra. One of us (RDR) also thanks CIC (Argentina) for a research fellowship.

REFERENCES

1. Rofi, R. D. and Pomilio, A. B. (1982) *Fitoterapia* **53**, 183.
2. Voirin, B., Jay, M. and Hauteville, M. (1976) *Phytochemistry* **15**, 840.
3. Voirin, B. and Jay, M. (1978) *Biochem. Syst. Ecol.* **6**, 95.
4. Liu, Y.-L. and Mabry, T. J. (1982) *Phytochemistry* **21**, 209.