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TWO ISOVITEXIN 2″-*O*-GLYCOSIDES FROM PRIMARY LEAVES OF *SECALE CEREALE*

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Key Word Index—*Secale cereale*; Poaceae; rye; *C*-glycosylflavone *O*-glycosides; isovitexin 2″-*O*-arabinoside; isovitexin 2″-*O*-galactoside.

Abstract—Two isovitexin 2″-*O*-glycosides have been isolated from primary leaves of rye and identified as isovitexin 2″-*O*-arabinoside and isovitexin 2″-*O*-galactoside. The latter is a new natural compound.

INTRODUCTION

Primary leaves of rye (*Secale cereale* L. cv. Kustro) show a tissue-specific metabolism of flavone *O*-glycosides, anthocyanins and *C*-glycosylflavone *O*-glycosides [1]. In this paper we report the isolation and characterization of the two *C*-glycosylflavone *O*-glycosides, isovitexin 2″-*O*-arabinoside and the new natural compound isovitexin 2″-*O*-galactoside, both accumulating in the epidermal tissues.

RESULTS AND DISCUSSION

From the primary leaf of *Secale cereale* L. two isovitexin glycosides (R3 and R4) were isolated as described previously [1] and their structures elucidated by means of thin layer co-chromatography and UV and mass spectrometry. Both compounds showed the chromatographic behaviour of flavone glycosides and the UV spectrum and diagnostic shifts [2] of apigenin glycosides with free hydroxyl groups at the 5,7 and 4′-positions. Acid hydrolyses of R3 and R4 gave arabinose and galactose, respectively, and isovitexin with small amounts of vitexin in both cases. TLC and HPLC were carried out in conditions where 6-*C*-glucosyl- and 6-*C*-galactosylapigenin are separated [3]. This showed R3 and R4 to be isovitexin *O*″-glycosides and the position of the *O*-glycosidic bond was determined by mass spectrometry of the permethyl (PM) derivatives [4]. The mass spectra (EIMS) of PM R3 and PM R4 exhibited the characteristic fragmentation pattern of PM 6-*C*-hexosylapigenin 2″-*O*-glycosides: absence of M − 15 and M − 31 ions replaced by the ions SO (*m/z* 515) and S (*m/z* 499) derived from the elimination of the PM 2″-*O*-glycosyl and 2″-*O*-glycosyloxy residues respectively, and presence of an intense ion *j* (*m/z* 341). The molecular ion was found at *m/z* 734 in the mass spectrum of PM R4, establishing the 6-*C*-glucosylapigenin 2″-*O*-galactoside structure of R4, but it could not be found in the EIMS of PM R3. However CIMS (NH_3) of PM R3 and PM R4 clearly showed MH^+ ions at *m/z* 691 and 735 respectively, confirming the 6-*C*-glucosylapigenin 2″-*O*-arabinoside structure of R3. The latter was found to be identical with the compound previously isolated from *Avena sativa* primary leaves [5]. To our knowledge, R4 is a new natural compound.

EXPERIMENTAL

Plant material and isolation of flavones are described elsewhere [1].

Compound R3: isovitexin-2″-O-arabinoside. TLC (silica gel) in EtOAc–MeOH–H_2O (63:12:9): R_f 0.39 (isovitexin 0.58), in EtOAc–EtCOMe–HCOOH–H_2O (5:3:1:1): R_f 0.35 (isovitexin 0.75). UV λ_{max}^{MeOH} nm: 271, 332; +NaOMe 279, 330, 399; +$AlCl_3$ 279, 303+, 352, 381; +$AlCl_3$–HCl 280, 302+, 345, 382; +NaOAc 278, 300+, 332, 386; +NaOAc–H_3BO_3 273, 306+, 323, 340, 406+; permethylation and TLC of PM ether [6]. *PM ether*: EIMS 70 eV, *m/z* > 300 (rel. int.): 559 [SO_i] (13), 545 [SO_j] (23), 529 [SO_k] (16), 515 [SO] (45), 499 [S] (100), 341 [J] (68); CIMS (NH_3) 70 eV, *m/z* > 300 (rel. int.): 691 $[MH]^+$ (100).

Compound R4: isovitexin-2″-O-galactoside. TLC (silica gel) in EtOAc–MeOH–H_2O (63:12:9): R_f 0.32 (isovitexin 0.58), in EtOAc–EtCOMe–HCOOH–H_2O (5:3:1:1): R_f 0.27 (isovitexin 0.75). UV λ_{max}^{MeOH} nm: 271, 332; +NaOMe 280, 331, 398; +$AlCl_3$ 280, 303, 350, 383; +$AlCl_3$–HCl 280, 302, 342, 382; +NaOAc 277, 299+, 335+, 389; +NaOAc–H_3BO_3 273, 308+, 324, 342, 406+; permethylation and TLC of PM ether [6]. *PM ether*: EIMS 70 eV, *m/z* > 300 (rel. int.): 734 $[M]^+$ (2), 559 [SO_i] (4), 545 [SO_j] (8), 529 [SO_k] (3), 515 [SO] (36), 499 [S] (100), 341 [J] (40); CIMS (NH_3) 70 eV, *m/z* > 300 (rel. int.): 735 $[MH]^+$ (100).

Acid hydrolyses. Each compound (1 mg) in 2 ml MeOH-4 N HCl (1:1) is heated at 100° in a sealed tube for 1 hr. After repeated evaporation, the residue is taken up in H_2O and extracted with *n*-BuOH.

Identification of sugars. TLC on silica gel saturated with aq. 0.2 M NaH_2PO_4 in Me_2CO–H_2O (9:1); spraying with aniline malonate.

Identification of isovitexin. TLC in silica gel (Merck, freshly activated by heating at 125° during 1 hr) in EtOAc–pyridine–H_2O–MeOH (16:4:2:1); spraying with $NaHCO_3$ and bisdiazotized benzidine. HPLC: Lichrosorb RP-18 (10 μ) column (30 × 0.4 cm) in MeOH–H_2O–HOAc (40:58:2); flow

rate 2 ml/min; R_t (min), 5.3 (isovitexin), 6.2 (6-*C*-galactosylapigenin).

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PHENOLIC COMPOUNDS FROM *VEPRIS HETEROPHYLLA*

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Key Word Index—*Vepris heterophylla*; Rutaceae; *trans*-sinapic acid methyl ester; 2″-*O*-glucosylisovitexin; 2″-*O*-glucosylvitexin; isovitexin; vitexin; 7-*O*-acylscoparin; chrysoeriol 7-glucoside; chrysoeriol 7-rhamnosylgalactoside.

Abstract—From the leaves of *Vepris heterophylla*, *trans*-sinapic acid methyl ester, 2″-*O*-glucosylisovitexin, 2″-*O*-glucosylvitexin, isovitexin, vitexin, 7-*O*-acylscoparin and chrysoeriol 7-glucoside were identified. A novel compound, chrysoeriol 7-rhamnosylgalactoside, was also characterized.

Vepris heterophylla, an African member of the Rutaceae used in folk medicine as a diuretic and an antipyretic, has been examined previously for some chemical constituents [1–3]. We now report the identification of one phenolic acid ester (**1**) and seven glycosylflavones (**2**–**8**) from leaves of this plant.

The UV spectrum of **1** showed a strong base shift indicating the presence of a phenolic group. Bands at 3270, 1685, 1620 and 700–900 cm^{-1} in the IR spectrum suggested the presence of a hydroxyl group, an ester carbonyl function, an ethylene group and a substituted aromatic ring. The ^1H NMR spectrum exhibited a singlet at δ 3.80 (3H) attributable to a carbomethoxy function, a singlet at δ 3.91 (6 H) assignable to two methoxy groups and a singlet of two aromatic protons at δ 6.75 which was little affected (δ 6.76) by acetylation of **1**. Further evidence for the presence of one phenolic group was provided by the signal observed at δ 5.40 in the spectrum of **1**, which disappeared upon the addition of D_2O, and by the singlet at δ 2.35 (3H) in the spectrum of the acetate. These spectral data established **1** to be *trans*-sinapic acid methyl ester.

Five flavonoids were isolated and identified as 2″-*O*-glucosylisovitexin (**2**), 2″-*O*-glucosylvitexin (**3**), isovitexin (**4**), vitexin (**5**) and chrysoeriol 7-glucoside (**6**) by their chromatographic and spectral properties (UV [4], mass spectra of PM derivatives [5, 6]), their hydrolysis products and by direct comparison (TLC and HPLC of free compounds and TLC of PM derivatives) with authentic samples. Acid hydrolysis of **7** gave scoparin and isoscoparin which were identified from spectral data and direct comparison with authentic samples, but no sugar was formed. Alkaline hydrolysis gave scoparin in agreement with the presence of an acyl group which was assigned to the 7-position because of the absence of a bathochromic shift in band II with sodium acetate. Permethylation of **7** gave a mass spectrum similar to that of PM-orientin. Thus it was considered to be a 7-*O*-acylscoparin but the acyl group could not be identified because of an insufficient amount of the compound.

Compound **8** showed UV spectra and diagnostic shifts [4] characteristic of a 7,3′-substituted luteolin. Acid hydrolysis gave galactose, rhamnose and chrysoeriol, which was identified from its chromatographic properties and spectral data. Permethylated **8** gave the mass spectrum of a PM luteolin 7-*O*-deoxyhexosylhexoside: 720 $[M]^+$, 328 (69%), 189 (100%), 157 (70%), 125 (51%) [7,