A New Flavone *O*-Glycoside and Other Constituents from Wheat Leaves (*Triticum aestivum* L.)

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From leaves of *Triticum aestivum* a new O-glycosylflavone has been isolated together with chlorogenic acid and its 3′-methyl ether and 6 C-glycosylflavones. The structure of the new flavonoid was determined by 1D and 2D NMR techniques and other spectral evidence as 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone-7-O-β-rutinoside.

Introduction

The Gramineae family contains many plant species of enormous economic importance. Phytochemical investigations of Gramineae have shown that C-glycosides based on apigenin and luteolin are widespread in this family together with tricin O-glycosides (Harborne and Hall, 1964; Harborne and Williams, 1976; Kaneta and Sugiyama, 1973; Williams *et al.*, 1974). Compounds in this series have shown to be useful in taxonomic and evolutionary studies (Harborne and Williams, 1976; Harborne *et al.*, 1986; Williams *et al.*, 1974) and to be involved in plant resistance to disease pathogens (Julian *et al.*, 1971).

Wheat (*Triticum aestivum* L., Gramineae), one of the most widespread crops, has been the subject of numerous phytochemical investigations which have resulted in the isolation of several C-glycosylflavonoids and C-glycosylflavonoid O-glycosides from this plant (Feng *et al.*, 1988; Julian *et al.*, 1971; Kaneta and Sugiyama, 1973; Wagner *et al.*, 1980). Flavonoids and other phenolics are of interest because they are important both for the plant, as defensive compounds, and for humans, as antioxidants. In our efforts to determine the importance of growing conditions and genotype on contents of flavonoids and other phenolics in wheat, we have investigated a variety that is commonly used in organic and conventional farming in Denmark. The investigation afforded in addition to common phenolic acids and C-glycosylflavones a hitherto unknown flavone O-glycoside.

Materials and Methods

General

UV spectra were obtained in MeOH and standard shift reagents were added (Mabry *et al.*, 1970). FAB mass spectra (recorded on a Kratos MS50 TC double focusing mass spectrometer) were obtained in positive ion mode using glycerol (1 drop of HCl aq. was added) and/or NBA + NaI as the matrix, and ES mass spectra (recorded on a Finnigan MAT TSQ 700) were obtained in positive ion mode using MeOH + 1 drop of HCl aq. as solvent. ^{1}H NMR spectra were recorded at ambient temperature in DMSO-d_6 containing 10% TFA-*d* and with TMS as internal standard on a 300 MHz instrument (Varian gemini 300). ^{1}H, ^{1}H–^{1}H-COSY, 1D-HOHAHA, ^{1}H–^{1}H-NOESY, difference NOE, ^{13}C and ^{1}H–^{13}C-HSQC spectra were measured in DMSO-d_6 with CD_2HOD (δ 3.326 ppm) as internal standard using a 600 MHz instrument (JNM alpha 600, JEOL). Difference NOE, ^{13}C and ^{1}H–^{13}C-HSQC spectra were recorded at 23 °C, other spectra at 40 °C. All spectra were obtained using a pulse sequence supplied by JEOL.

Plant material

Leaves of organically grown wheat plants (*Triticum aestivum* L. 'Herzog') were harvested in the spring 1997 on a field located at Askov (Denmark), freeze-dried and stored at –20 °C until use.

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Extraction and isolation

Freeze-dried leaves (300 g) of *T. aestivum* were extracted twice with 50% aqueous MeCN containing 0.5% trifluoroacetic acid (TFA) at 20 °C for 5 h. The concentrated extracts (300 ml) were adsorbed on an amberlite XAD-7 column, washed with 0.5% aq. TFA and flavonoids and phenolic acids eluted stepwise with 10 to 50% aq. MeCN containing 0.5% TFA. Fractions from the XAD-7 column were further purified by prep. HPLC using a Merck L-6200 intelligent pump and a Merck L-4200 UV-VIS detector. Prep. HPLC was performed on a Develosil ODS-HG-5 (RP-18, 250 × 20 mm i.d., Nomura Chemical Co., Japan) column protected with a guard cartridge (50 × 20 mm i.d.) packed with the same material as the column. Separations were performed at 35 °C using a stepwise gradient from 10 to 50% aq. MeCN containing 0.5% TFA; flow rate 7 ml min^{-1}; monotoring at 280 nm. Evaporation of solvents *in vacuo* gave TFA salts which were stored at −80 °C. The following compounds were isolated: 6 mg 3-O-(*E*)-caffeoylquinic acid (chlorogenic acid); 22 mg 3-O-(*E*)-feruloylquinic acid; 14 mg schaftoside and isoschaftoside (isolated as a mixture); 6 mg vicenin-1; 26 mg lucenin-1 and lucenin-3 (isolated as a mixture); 25 mg isoorientin and 5 mg 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone-7-O-β-rutinoside (**1**). The identity of all known compounds was confirmed by UV spectral analysis in the presence of customary shift reagents, mass spectroscopy (FAB-MS and/or ES-MS) and 1D NMR.

5,7-Dihydroxy-3′,4′,5′-trimethoxyflavone-7-O-β-rutinoside (**1**). UV λ_{max} (nm) 270, 311sh, 331; + NaOMe: 279, 300sh, 366; + $AlCl_3$ 279, 300, 345, 382sh; + $AlCl_3$/HCl: 279, 299sh, 339, 380sh. FAB-MS (glycerol): *m/z* 653 $[M + H]^+$ ($C_{30}H_{37}O_{16}^+$), 507 $[(M + H) - \text{rhamnosyl}]^+$, 345 $[(M + H) - \text{rhamnosyl} - \text{glucosyl}]^+$. FAB-MS (NBA + NaI): *m/z* 675 $[M + Na]^+$. ^{1}H NMR (600 MHz, DMSO-d_6) (aglycone): δ 12.80 (1H, s, 5-OH), 7.36 (2H, s, H-2′, H-6′), 7.11 (1H, s, H-3), 6.86 (1H, d, *J* = 2.2 Hz, H-8), 6.50 (1H, d, *J* = 2.2 Hz, H-6), 3.91 (6H, s, 3′- and 5′-OMe), 3.76 (3H, s, 4′-OMe). 7-O-β-D-Glucopyranosyl: δ 5.07 (1H, d, *J* = 7.3 Hz, H-1″), 3.28 (1H, t, *J* = 8.8 Hz, H-2″), 3.31 (1H, t, *J* = 8.8 Hz, H-3″), 3.17 (1H, br t, *J* = 9.2 Hz, H-4″), 3.59 (1H, ddd, *J* = 1.8, 6.2, 9.5 Hz, H-5″), 3.47 (1H, dd, *J* = 6.2, 11.4 Hz, H-6″), 3.83 (1H, dd, *J* = 1.8, 11.4 Hz, H-6″). (1→6)-O-α-L-Rhamnopyranosyl: δ 4.54 (1H, d, *J* = 1.2 Hz, H-1‴), 3.63 (1H, m, H-2‴), 3.43 (1H, dd, *J* = 3.3, 9.5 Hz, H-3‴), 3.13 (1H, t, *J* = 9.5 Hz, H-4‴), 3.41 (1H, m, H-5‴), 1.07 (3H, d, *J* = 6.2 Hz, H-6‴). ^{13}C-NMR (150 MHz, DMSO-d_6) (aglycone): δ 182.2 (C-4), 163.5 (C-2), 163.0 (C-7), 161.2 (C-5), 156.9 (C-9), 153.2 (C-3′, C-5′), 143.1 (C-4′), 125.8 (C-1′), 105.5 (C-10), 105.2 (C-3), 104.3 (C-2′, C-6′), 99.4 (C-6), 95.4 (C-8), 60.2 (4′-OMe), 56.3 (3′- and 5′-OMe). 7-O-β-D-Glucopyranosyl: δ 99.8 (C-1″), 73.1 (C-2″), 76.3 (C-3″), 69.6 (C-4″), 75.6 (C-5″), 66.0 (C-6″). (1→6)-O-α-L-Rhamnopyranosyl: δ 100.5 (C-1‴), 70.3 (C-2‴), 70.7 (C-3‴), 72.0 (C-4‴), 68.3 (C-5‴), 17.8 (C-6‴).

Results and Discussion

The compounds of the aqueous acetonitrile extract of the leaves of *T. aestivum* were separated and purified by column chromatography on amberlite XAD-7 and preparative HPLC, yielding chlorogenic acid and its 3′-methyl ether (Herrmann, 1978), 6 C-glycosylflavones and a new O-glycosylflavone (**1**) (Fig. 1). The known C-glycosylflavones were identified as 6-C-β-D-glucopyranosylluteolin (isoorientin), 6-C-β-D-xylopyranosyl-8-C-β-D-glucopyranosylluteolin (lucenin-1), 6-C-β-D-glucopyranosyl-8-C-β-D-xylopyranosylluteolin (lucenin-3), 6-C-β-D-glucopyranosyl-8-C-α-L-arabinopyranosylapigenin (schaftoside), 6-C-α-L-arabinopyranosyl-8-C-β-D-glucopyranosylapigenin (isoschaftoside) and 6-C-β-D-xylopyranosyl-8-C-β-D-glucopyranosylapigenin (vicenin-1), which have previously been isolated from *T. aestivum* (Julian *et al.*, 1971; Wagner *et al.*, 1980) and other plant species (Chopin and Bouillant, 1975). Although flavone O-glycosides with no glycosylated carbons have been isolated from other members of the genus *Triticum* (Harborne and Williams, 1975; Harborne *et al.*, 1986) this is to the best of our knowledge the first report of a O-glycosylflavone from *T. aestivum.*

The FAB mass spectrum of **1** showed a molecular ion peak at *m/z* 653 $[M + H]^+$, in good agreement with the mass calculated for $C_{30}H_{37}O_{16}^+$. The fragments at *m/z* 507 $[(M + H) - 146]^+$ and *m/z* 345 $[(M + H) - 146 - 162]^+$ indicated the subsequent losses of one deoxyhexose and one hexose unit. The fragment at *m/z* 345 corresponds to the aglycone 5,7-dihydroxy-3′,4′,5′-tri-

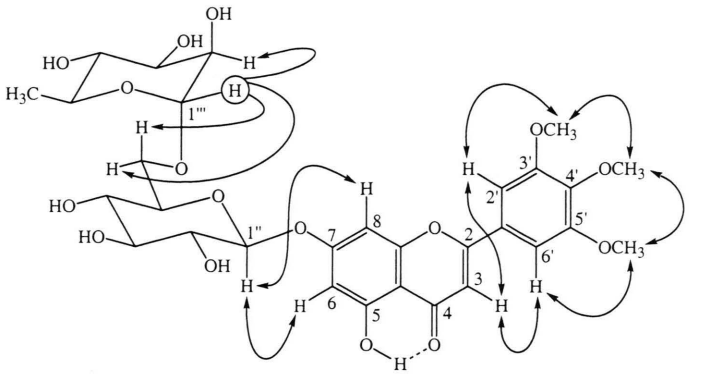

Fig. 1. NOEs of compound **1**. NOESY (↔) and difference NOE (○→).

methoxyflavone. A singlet at δ 12.8 in the ^{1}H NMR spectrum of **1** clearly indicated the presence of a free hydroxyl group at C-5, in accordance with the UV spectral analysis showing a bathochromic shift by addition of $AlCl_3$ and $AlCl_3$/HCl (Mabry *et al.*, 1970). The presence of 3 methoxy groups in ring B was confirmed by the ^{1}H NMR signals at δ 3.76 (3H, s, 4′-OMe) and δ 3.91 (6H, s, 3′- and 5′-OMe) which were shown to be correlated with the carbon signals at δ 60.2 and δ 56.3, respectively, in the ^{1}H–^{13}C-HSQC spectrum. The position of the methoxy groups at C-3′, C-4′ and C-5′ was deduced from the ^{1}H–^{1}H-NOESY spectrum (Fig. 1) with NOE cross-peaks from the C-4′ methoxyl to the C-3′ and C-5′ methoxyl, respectively, and from H-2′ to the C-3′ methoxyl, and from H-6′ to the C-5′ methoxyl. Two additional doublets at δ 6.50 and δ 6.86 with a meta coupling (2.2 Hz) were assigned to H-6 and H-8, respectively, supporting the 5,7-disubstitution in ring A, hence a disaccharide must be attached to the hydroxyl group at C-7.

The presence of one hexose and one deoxyhexose unit in the structure of **1** was confirmed by 1D-HOHAHA, ^{1}H–^{1}H-COSY and ^{1}H–^{13}C-HSQC spectra. The sugar units were identified as D-glucopyranose and L-rhamnopyranose (see Materials and Methods). The presence of a rhamnopyranosyl unit was evidenced by a doublet at δ 1.07 (3H, J = 6.2 Hz) and a carbon signal at δ 17.8. The doublet at δ 4.54 was assigned to H-1 of L-rhamnopyranose (H-1‴) and a diequatorial coupling constant of 1.2 Hz between H-1‴ and H-2‴ indicated an α-configuration for the anomeric proton. Similarly, the doublet at δ 5.07 was assigned to H-1 of D-glucopyranose (H-1″) and a diaxial coupling of 7.3 Hz between H-1″ and H-2″ indicated β-configuration.

The positions of the glycosidic linkages of **1** were determined by ^{1}H–^{1}H-NOESY and NOE difference spectra (Fig. 1). NOE cross-peaks were observed from H-6 to H-1″ and from H-8 to H-1″ which clearly indicated that the β-D-glucose moiety is directly linked to the hydroxyl group at C-7. By irridation of H-1‴ negative NOEs were observed on H-6″ and on H-2‴ clearly indicating that the rhamnopyranosyl unit is linked to the hydroxyl group at C-6″. Thus the disaccharide unit of **1** is rutinose. From the above data the structure of **1** was established as 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone-7-O-β-rutinoside.

Flavone O-glycosides closely related to **1** e.g. tricin 7-rutinoside have been isolated from other Gramineae species (Harborne and Williams, 1975). The free aglycone 5,7-dihydroxy-3′,4′,5′-trimethoxyflavone (tricin-4′-methyl ether) has so far only been isolated from dried grass silage (Stelzig and Qasim, 1973), *Hordeum vulgare* var. *hexastichon*, *Festuca myuros* and *Bromus pauciflorus* (Gramineae) (Kaneta and Sugiyama, 1973) and shown to have a pronounced metabolic rate-stimulating effect when fed to male rats (Stelzig and Qasim, 1973). Biological studies of **1** could not be performed due to the small amounts available of this compound. However, it is not unlikely to assume that **1** has a similar activity as its active aglycone also considering that **1** may be hydrolyzed into this compound when ingested.

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