The Mis-identification of the Major Antioxidant Flavonoids in Young Barley (*Hordeum vulgare*) Leaves

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Z. Naturforsch. **58 c**, 53–56 (2003); received July 22/August 16, 2002

Several papers have appeared in the literature since 1992 which refer to a major "isoflavonoid" antioxidant in young green barley leaves (*Hordeum vulgare*) as 2″-O-glucosylisovitexin. In the present paper the original NMR data supporting this structural assignment are examined and found to have been misinterpreted. HPLC and NMR data are used to prove that the major flavonoid antioxidants in *young* green barley leaves are in fact the flavone-C-glycosides, saponarin and lutonarin.

Key words: Hordeum vulgare, Flavonoid, Mis-identification

Introduction

In 1992 a paper entitled "A novel antioxidant isolated from young green barley leaves" appeared in the literature (Osawa *et al.*, 1992) describing the isolation and structure determination of the most active antioxidant extracted. A range of data was accumulated on the purified compound including an infra-red spectrum, a mass spectrum and proton and carbon NMRs. Acid hydrolysis produced isovitexin and glucose, and analysis of the other data resulted in the definition of this component as 2″-O-glucosylisovitexin (2″-O-GIV). In subsequent papers by some of the same authors (Kitta *et al.*, 1992; Nishiyama *et al.*, 1993; Miyake *et al.*, 1997) and in the Barley Grass and Green Barley Grass Juice – Continuing Education Module (Johnson and Mokler, 2000), this compound is referred to incorrectly as an isoflavonoid rather than as a flavone or a flavone-C-glycoside.

The present paper describes the results of a re-examination of the flavonoids in *young* green barley leaves, which indicate that the major flavonoid has been mis-identified in the publications referred to above.

Materials and Methods

Plant material

A fresh commercially produced powdered extract of young green barley (*Hordeum vulgare*) leaves, which is marketed by AIM (USA) as "BarleyLife", was provided for this work by Ron Russell of NUZEAL Ltd, Edgecumbe, New Zealand. The barley leaves were harvested before the plant produced seed and when at a height of about 450–500 mm. To produce an extract, the powder (0.3 g.) was extracted overnight at room temperature with 25 mls of methanol:water (7:3 v/v). This was filtered before analysis by HPLC.

HPLC analyses

HPLC analyses were carried out using a Waters 600E solvent delivery system, Waters 996 diode array detector, and a Jasco 851-AS intelligent sampler, results being analysed using Waters Millennium^{32} version 3.05 Chromatography Manager software. An injection volume of 20 μl was used with a Merck Superspher® 100 RP-18 endcapped column (4μm, 4 × 125 mm). Elution (0.8 ml/min, 30 °) was performed using a solvent system comprising solvent A (1.5% H_3PO_4) and solvent B (HOAc-CH_3CN-H_3PO_4-H_2O (20:24:1.5:54.5 v/v) mixed using a linear gradient starting with 80% A, decreasing to 33% at 30 min, 10% A at 33 min, and 0% A at 39.3 min. Flavonoids were detected at 352 nm. Retention times and on-line absorption spectra were recorded for all major peaks.

Results and Discussion

In the course of analysing New Zealand sourced young green barley leaves for the major flavonoid

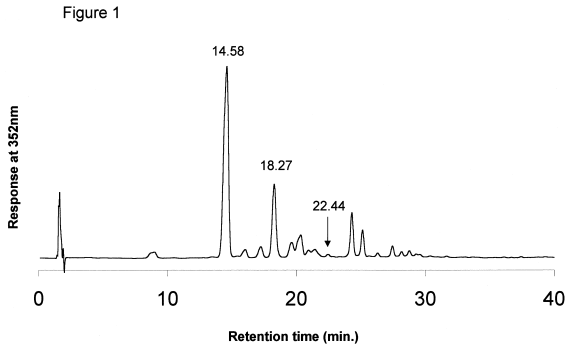

Fig. 1. HPLC profile from a methanol-water extract of young green barley leaves.

and antioxidant component, "2″-O-GIV" for a commercial client, it became evident that 2″-O-GIV, if present at all, was present at exceedingly low levels. HPLC analysis of methanol-water extracts of barley leaves with detection at 352 nm, revealed the presence of two major peaks (see Fig. 1) whose on-line spectra were typical for flavones. These had retention times (RTs) of 14.58 and 18.27 minutes. The lesser of the two (RT 18.27) possessed a spectrum with maxima at 270 and 335 nm, consistent with it being 2″-O-GIV (Mabry *et al.*, 1970), while the major component possessed a rather different spectrum (maxima at 255, 271 and 340 nm). However when authentic 2″-O-GIV isolated from *Passiflora incarnata* (Geiger and Markham, 1986) was added to the barley extract and the mixture analysed by HPLC, the authentic 2″-O-GIV failed to co-elute with either of the major flavonoids. Instead, 2″-O-GIV eluted much later (RT 22.44 min.), in a region of the chromatogram that contained only a minuscule peak (see Fig. 1).

The above finding necessitated a re-evaluation of the data published by Osawa *et al.* (1992). When the ^{13}C-NMR data for authentic 2″-O-GIV (Geiger and Markham, 1986) are compared with those of the barley flavonoid with RT 18.27 (see Table I), it is clear that the spectra do not match, in particular in the region of the glucose carbons.

Table I. Comparison of ^{13}C-NMR data (DMSO-d_6) on isovitexin glucosides.

Carbon No.	2″-O-GIV ex *Passiflora* (Geiger and Markham, 1986)	Barley flavonoid (Osawa *et al.*, 1992)	"7-O-GIV" = saponarin (Markham and Chari, 1982)
Isovitexin			
2	163.4[a]	164.2	164.4
3	102.7	103.2	103.3
4	181.9	182.1	182.1
5	161.1[a]	159.4	159.9
6	107.9	110.6	110.9
7	160.9[a]	162.5	162.3
8	93.4	93.8	94.1
9	156.4	156.4	156.5
10	102.7	104.9	105.4
1′	121.1	120.9	121.2
2′	128.4	128.7	128.5
3′	115.9	116.1	116.1
4′	161.1[a]	161.5	161.3
5′	115.9	116.1	116.1
6′	128.4	128.7	128.5
Glucoses			
1″	71.1	72.7	73.8
1‴	105.3	101.2	101.7
2″	81.5	70.9	71.0
2‴	74.6	73.8	73.0
3″	78.3	78.9	79.2
3‴	76.3	75.8	76.2
4″, 4‴	70.3, 69.3	69.6, 69.5	70.5, 70.1
5″	80.8	81.0	81.0
5‴	76.3	77.2	77.3
6″, 6‴	61.3, 60.3	60.7, 60.3	61.1, 61.1

[a] Assignments in the same column may be rearranged.

When glucose is substituted at the 2-position with another glucose, a downfield shift of about 10 ppm is brought about in the chemical shift of the (substituted) C-2 signal. This is clearly evident in the spectrum of the *Passiflora* compound which exhibits its C-2″ signal at 81.5 ppm, 10.1 ppm downfield from its position in an unsubstituted C-glucosyl moiety (Agrawal and Bansal, 1989; Markham and Chari, 1982). Such a shift is not seen in the C-2″ of the barley compound which appears in the normal position for an unsubstituted C-2″. The chemical shift of the C-1‴ in the barley compound at 101.2 ppm indicates instead that the second glucose is attached directly to a flavonoid hydroxyl (Markham and Chari, 1982), and the shift of the *para*-related C-10 signal to 104.9 ppm from its 102.7 ppm position in authentic 2″-O-GIV, suggests that it is attached to the 7-hydroxyl. 7-O-Glucosyl-isovitexin (7-O-GIV) is a known compound, saponarin, and its ^{13}C-NMR spectrum is available from literature sources (Agrawal and Bansal, 1989; Markham and Chari, 1982). One example of this spectrum is presented in Table I, and a good match with the spectrum of the barley compound is evident.

When authentic saponarin was added to the barley extract and the mixture chromatographed by HPLC, the peak at RT 18.27 min. was enhanced. Furthermore, the original peak at RT 18.27 min. had an absorption spectrum identical with that of saponarin. From this evidence, it is concluded that the ‘ Novel antioxidant isolated from young green barley leaves is saponarin (“7-O-GIV”) and not “2″-O-GIV” as claimed. It follows that this compound, like 2″-O-GIV, is not an isoflavonoid and thus would not possess the estrogenic activity associated with some isoflavones.

Reference to the literature reveals that saponarin has previously been isolated from mature barley leaves (*Hordeum vulgare*), and that it is commonly associated with lutonarin, its 3′-hydroxylated analogue (Chopin and Bouillant, 1975; Reuber *et al.*, 1996). Cochromatography of the barley extract with lutonarin from barley (ex collection of the late Margaret Seikel), co-eluted with the peak at RT 14.48 min. and possesses an identical absorption spectrum. Lutonarin is thus defined as the major flavonoid accompanying saponarin in young green barley leaves. In subsequent work we have observed that the relative proportions of saponarin and lutonarin can be affected by levels of UV radiation, but that these two components are always the only two major flavones evident on the HPLC.

Flavones and flavone-C-glycosides are known to be effective free-radical scavengers and antioxidants, with the B-ring *ortho*-dihydroxylated flavonoids such as lutonarin being superior (Yokozawa, *et al.*, 1997; Burda and Oleszek, 2001). The structural features important in determining the antioxidant properties of flavones were defined by Yokozawa *et al.*, (1997), and it is evident from these that the activity observed for the “2″-O-GIV” from barley could be accounted for by the saponarin structure.

Acknowledgements

The authors acknowledge financial support from the N. Z. Foundation for Research Science and Technology (Contract No CO8X0209) for part of this work.

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