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Aneugenic 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) in sprouts of *Triticum aestivum* cultivars – A 'safety health food'?

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ABSTRACT

The concentrations of the benzoxazinoids (BAs), 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) were determined in four selected *Triticum aestivum* cultivars. The impact of the cultivation period on the overall concentrations in the cultivars was determined by HPLC. Furthermore, the distribution of the two BAs was analysed in aerial and subterranean parts of the seedlings after different time periods. The highest levels were detected after 3 days. Subsequently, the levels of DIMBOA declined in all cultivars (reduction between 84% and 93%), while DIBOA was not detectable after 7 days. The distribution between aerial parts and roots varied substantially in the different cultivars. In addition, DIMBOA amounts, expressed in per cent, as well as in mg per seedling, were determined in one selected cultivar during the entire time course for better understanding of eventual dilution effects during plant growth. Our findings indicate that the consumption (up to several 100 g) of fresh sprouts (as suggested by suppliers) may already lead to exposure levels of up to 470 mg DIMBO and 190 mg of DIBOA per 100 g each. The potential adverse health effects of these doses are discussed.

1. Introduction

Benzoxazinoids (BAs) are major secondary metabolites found in Poaceae, including major agricultural crops, such as wheat, maize and rye, but also in the dicotyledons, Acanthaceae, Lamiaceae, Ranunculaceae and Scrophulariaceae (Sicker, Frey, Schulz, & Gierl, 2000; Sicker & Schulz, 2002). These allelochemicals hinder the germination and growth of competitive plants (Perez, 1990; Wu, Haig, Pratley, Lemerle, & An, 2001). In addition, BAs also act as phytoalexins (Sicker et al., 2000; Sicker, Hao, & Schulz, 2004). Due to their multifunctional toxicity they are regarded as natural pesticides against pathogens, microorganisms, fungi, insects, and weeds (Silva, Copaja, Bravo, & Argandona, 2006; Soltoft, Joergensen, Svensmark, & Fomsgaard, 2008). For decades, research investigations have been performed to benefit from the potentials of BAs and their degradation products in plant breeding and agriculture (Fomsgaard, 2006; Krogh et al., 2006; Macias et al., 2007, 2008). BAs are stored in intact plants in biologically inactive forms, as (2R)-2-β-D-glucosides, in vacuoles. The active aglycones are formed by enzymatic deglucosylation, after wounding or tissue damage (Esen, 1992; Nikus, 2003; Nikus & Jonsson, 1999). The intrinsic regulation of aglycone formation is triggered by age, exogenous factors (e.g. light intensity, drought), and stress (Aahman & Johansson, 1994).

Besides studies on the acute toxicity of BAs in insects and bacteria (Sicker et al., 2000), genotoxic effects have also been described; i.e. weak mutagenic activities of certain BAs were found in Salmonella/microsome assays (Hashimoto et al., 1979). Furthermore, *in vitro* reaction of BAs (e.g. 4-acetyl-1,4-benzoxazin-3-one) with calf thymus DNA was observed, and it was postulated, that the BAs might be activated via *N*-acetylation in the same way as aromatic and heterocyclic aromatic amines (Ishizaki, Hashimoto, Shudo, & Okamoto, 1982). In a recent study, potent clastrogenic properties were detected in mammalian cells: the aneugenic effects of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA, Fig. 1a), which were found in human-derived liver cells (HepG2) (Buchmann et al., 2007), can be taken as an indication that consumption of sprouts might cause adverse health effects in humans.

With respect to these findings, the increasing promotion of wheat sprouts and sprout-derived products in health foods requires sound information about the exposure of humans to the BAs. DIMBOA is mainly contained in wheat sprouts, but also in other species, such as maize, whereas DIBOA is the most abundant

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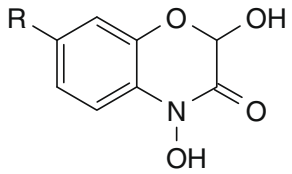

DIMBOA R = -OMe
DIBOA R = -H

Fig. 1a. Chemical structures of the two benzoxazinoid aglycones.

BA in rye, but also detected in barley (Sicker et al., 2000). BAs are not found in the seeds themselves, but are detected in seedlings as early as two to three days after germination. After reaching a maximum within the first days of germiantion, their amounts decrease subsequently (Argandona, Niemeyer, & Corcuera, 1981; Copaja, Nicol, & Wratten, 1999; Wolf, Spencer, & Plattner, 1985). Earlier investigations showed that their amounts in *Triticum* differ between the species, as well as within different cultivars of one species (Wu et al., 2001). Data about their concentrations in *T. aestivum* L. are controversial and incomplete. In addition, cultivars with high BA contents seem to be interesting candidates for plant development and may gain increasing importance in agriculture.

As the two Bas, DIMBOA and DIBOA, are considered the most abundant benzoxazinoids in wheat seedlings after enzymatic degradation (Sicker & Schulz, 2002), the objectives of the present study were 3-fold. The first was the determination of their contents in various *T. aestivum* cultivars after different days of germination. The cultivars were chosen with respect to their importance in agriculture and their assumed role in the preparation of wheat seedling products. The second was the determination of the contents in subterranean and aerial parts of the seedlings. The third was to correlate the DIMBOA amounts expressed in per cent as well as in mg per seedling of one selected cultivar during the entire time course, for better understanding of eventual dilution effects during plant growth.

2. Materials and methods

2.1. General procedures

Thin-layer chromatography (TLC) analysis was performed on Polygram Polyamid-6 UV_{254} TLC plates of 0.1 mm thickness (Macherey-Nagel & Co, Dueren, Germany) with *n*-butanol (ButOH):glacial acetic acid (HAc) = 20:1 (solvent system 1) and $CHCl_3$:ButOH:HAc = 30:15:1 (solvent system 2). TLC plates were developed after chamber saturation. After drying in air for 15 min, they were sprayed with acidic $FeCl_3$-solution, as described in detail by Argandona et al. (1981).

Melting points were monitored on a LEICA-heating table microscope (Leica AG, Reichert Division; digital thermometer Testo 700). UV spectra were recorded with a Beckman DU-600 spectrophotometer in MeOH (*c* 0.001538%). EI-MS analyses were performed with a Shimadzu GCMS-QP5050 A DI 50, at 70 eV, 6×10^{-6} Torr and 250 °C in scan mode of 40–500 amu/2 s. NMR spectra were recorded at 400 and 100.6 MHz, for ^1H and ^{13}C, respectively, with a Varian Unity Inova 400 in MeOH-d_4 and DMSO-d_6. Apart from ^1H and ^{13}C NMR, DEPT, HSQC, HMBC, COSY, 1D-TOCSY, and NOE experiments were performed. HPLC analyses were performed with a Perkin–Elmer series 200 LC 250B pump, Perkin–Elmer LC 235 diode array detector and an evaporative light scattering detector, Sedex 75 ELS (Sedere, Montluçon, France) on a LiChrospher RP-18 (5 μm), 250 × 4 mm column (Hewlett–Packard, Stuttgart, Germany), equipped with a Hypersil ODS 5 μm, 4 × 4 mm precolumn (Hewlett–Packard, Stuttgart, Germany) with an isocratic programme. Solvent A was aqua dest., containing 10% HAc, solvent B was MeOH. After equilibration with 15% B at a flow rate of 1.0 ml/min for 15 min, 10 μl of the sample were injected and analysed within 20 min at 265 nm, followed by a purge at 100% B.

2.2. Reagents

HPLC-grade methanol (MeOH) was obtained from VWR International (Leuven, Belgium), and Sephadex® LH-20 from Amersham

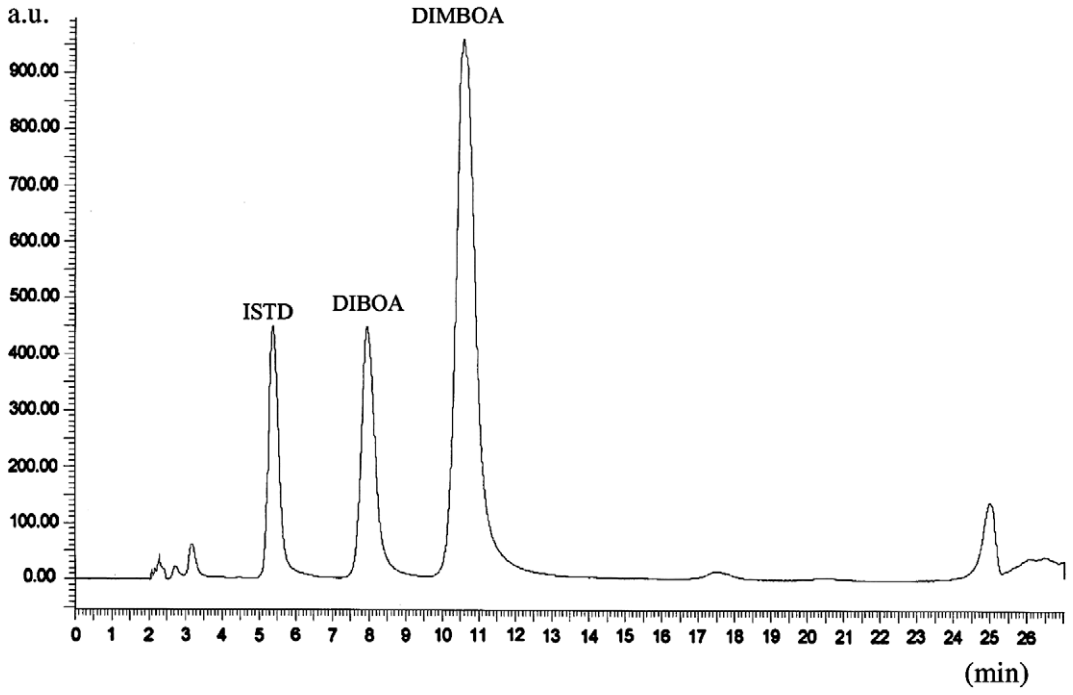

Fig. 1b. HPLC chromatogram of the *Triticum aestivum* cultivar CM82036 on day 3 of germination with theophylline as internal standard (ISTD).