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(2*R*)-2-β-D-GLUCOPYRANOSYLOXY-4,7-DIMETHOXY-2*H*-1,4-BENZOXAZIN-3(4*H*)-ONE FROM *TRITICUM AESTIVUM*

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Key Word Index—*Triticum aestivum*; Gramineae; isolation; benzoxazinone; absolute configuration; GHDIBOA.

Abstract—(2*R*)-2-β-D-Glucopyranosyloxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one was isolated from *Triticum aestivum* for the first time. The absolute configuration was determined as the 2*R*-type by spectroscopic methods. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Acetal glucosides of derivatives of 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one have been found to occur as allelochemicals in different species of Gramineae [1, 2], Acanthaceae [3, 4], Ranunculaceae [5] and Scrophulariaceae [6]. Their aglucone hemiacetals are released by β-glucosidases which are activated on attack by external pests, like aphids or microbial pathogens and can act as plant resistance factors [7]. Recently, 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA) and its 7-methoxy derivative DIMBOA have also been proven to be allelopathic constituents of root exudates of *Agropyron repens* L. [8, 9]. We have reported on the isolation of the corresponding glucosides (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (GDIBOA) from *Secale cereale* [10] and (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (GDIMBOA) from *Zea mays* [11]. We have described the synthesis of GDIBOA with single 2-β-diastereoselectivity [12] and, most recently, a general approach to both hydroxamic acid acetal glucosides with double (2*R*)-2-β-diastereoselectivity [13]. Furthermore, we have obtained (2*R*)-2-β-D-glucopyranosyloxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (GHDBOA), for the first time synthetically by methylation of synthetic GDIMBOA [13]. Hitherto, GHDIBOA has been isolated in substance from *Zea mays* [14, 15] and *Coix lachryma-jobi* [2], however, with discrepancies regarding the correct mp (Fig. 1).

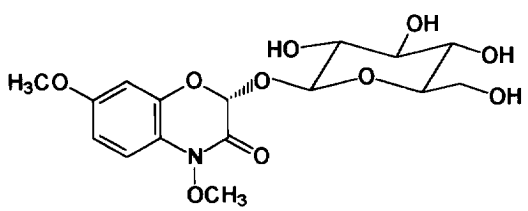

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Fig. 1. GHDIBOA isolated from *Triticum aestivum*.

A benzoxazinoid glucoside, in all probability GHDIBOA, has been reported to act as a phytoalexin in wheat leaves, because inoculation with stem rust causes a drastic increase in its biosynthesis [16]. However, the occurrence of GHDIBOA in *Triticum aestivum* could only be supported by comparison of UV and HPLC data [17]. The occurrence of benzoxazinoid hydroxamic acids in Triticeae species [18] and their potential in breeding for aphid resistance in wheat [19] has been investigated.

We report here on the first isolation of (2*R*)-2-β-D-glucopyranosyloxy-4-dimethoxy-7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one from *Triticum aestivum* shoots as a pure substance and compare it with a synthetic sample in order to prove its structure and to resolve all doubt as to the correct mp of this natural product.

RESULTS AND DISCUSSION

GHDIBOA (**1**) was reported from *Zea mays* with mp 142–144° [14] and from *C. lachryma-jobi* with mp 91–93° [2]. Our synthetic sample showed mp 143–145° [13]. We have used an isolation technique based on the denaturation of β-glucosidase by blanching the

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shoots of wheat plants in water. The lyophilized shoots were extracted with methanol and the solvent was removed from the extract. The residue was chromatographed to yield GHDIBOA as a crystalline compound.

Due to the methylated hydroxamic unit, GHDIBOA is distinctly less polar than GDIMBOA. Hence, it has a higher R_f than GDIMBOA, shows no Fe(III) reaction, but is detectable by UV_{254} quenching on the TLC plate. Due to its reduced polarity it was even possible to measure an exact HRMS spectrum under EI conditions, which is impossible for a hydroxamic acid glucoside like GDIMBOA. The glucoside isolated was proven to be pure due to its single spot in the TLC and HPTLC chromatograms.

The structure of GHDIBOA was established by spectroscopic means. The β-configuration of the glucosidic bond was assigned from the coupling constant $J = 7.7$ Hz at δ 4.68 for the H-1′–H-2′ interaction. The (2*R*) configuration could be proven by means of the CD spectrum, showing a positive Cotton effect at 231 nm and a negative one at 282 nm in aqueous solution. This feature is in coincidence with that of the closely related (2*R*)-2-β-D-glucopyranosyloxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (GHMBOA), which serves as a configurational lead, because Nagao *et al.* were able to obtain an X-ray analysis in addition to the CD spectrum [2].

GHDIBOA is unique among the benzoxazinoid acetal glucosides due to its methylated hydroxamic acid unit. Its aglucone 2-hydroxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (HDIBOA) has not been fully characterized yet. However, it was identified by EI-MS and NMR data as present in corn whorl surface waxes and shown to be toxic to the south-western corn borer [20]. Though successful in the elaboration of two general syntheses for the 2,4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton [21, 22] we have hitherto been unable to synthesize HDIBOA. Undoubtedly, the possibility to study the properties of HDIBOA will lead to a better understanding of the bioactivity of the benzoxazinoids in general. The 4-methoxy group is the feature of HDIBOA. Due to the leaving group properties of a methoxide ion, HDIBOA can be much faster transformed into the phytotoxic 6-methoxy-benzoxazolin-2(3*H*)-one (MBOA) than DIMBOA [17]. Hitherto, investigations towards the mode of action of synthetic 4-acetyl-DIBOA [23] and 4-acetyl-DIMBOA [24] have been undertaken. Whereas the former could be prepared in substance, the latter was only assumed as an intermediate. It can be pointed out that a donor in the 7-position (methoxy) supports the heterolysis of the N–O bond and causes the extrusion of the N-substituent as anion if a suitable leaving group (acetate, methoxide) can be formed.

EXPERIMENTAL

Plant material. Seeds of *Triticum aestivum* cv. 417/65 were germinated under greenhouse conditions at 20–22° for 14 days with a 14L:10D photoperiod.

Extraction. From 250 g of wheat seedlings, shoots were sepd from the roots and put immediately into 1 l of boiling H_2O for 3 min. An amount of 150 g of blanched shoots was lyophilized to yield 14 g of dry material, which was crushed under 1 l MeOH in a Waring Blendor for 10 min. The mixture was filtered to yield a deep green extract and colourless shoot particles, which were extracted a second time with 400 ml MeOH. The combined extracts were reduced to dryness *in vacuo* to yield 2.0 g of a green waxy residue. The residue was mixed with silica gel (10 g) in a mortar, placed at the top of a chromatographic column (silica gel 60, 60–200 μm, Merck) and chromatographed with $CHCl_3$–MeOH (4:1). All frs containing GHDIBOA were identified by TLC (Kieselgel 60, Merck; eluent; $CHCl_3$–MeOH (3:1), R_f 0.56), combined and evapd to dryness, yielding 40 mg of a pale green oily residue, which was chromatographed again under identical conditions. Frs containing GHDIBOA as the only UV visible spot were combined, evapd to dryness, yielding 28 mg of oil. A solution of this oil in 5 ml water was centrifuged for 10 min at 13000 rpm to separate an insoluble colloidal impurity. The aqueous phase was lyophilized to yield 20 mg of pure (2*R*)-2-β-D-glucopyranosyloxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one as crystals, mp 138–140° (dec.), corr. (lit. [2] 91–93°, lit. [14] 142–144°, lit. [13] 143–145°). EI-HRMS *m/z* [M^+] 387.1186 (Calc. for $C_{16}H_{21}NO_{10} = 387.1165$). UV $\lambda_{max}^{H_2O}$ nm (lg ε): 264 (1.7). IR ν_{max}^{KBr} cm^{-1}. 1696, 1624, 1508, 1075. $[\alpha]_{589}^{21} = +22°$ (H_2O; *c* 0.25). CD $\nu\Delta\varepsilon_{231} + 25.9$, $\Delta\varepsilon_{282} - 11.9$ (H_2O; *c* 0.776). ^{1}H-NMR (199.975 MHz, CD_3OD): δ 3.12–3.45 (4H, *m*, H-2′, H-3′, H-4′, H-5′), 3.68 (1H, *m*, 6′-H_a), 3.79 (3H, *s*, 7-OCH_3), 3.85 (1H, *m*, 6′-H_b), 3.95 (3H, *s*, 4-OCH_3), 4.68 (1H, *d*, $J_{1',2'} = 7.7$ Hz, H-1′), 5.89 (1H, *s*, H-2), 6.73 (1H, *dd*, $J_{6,8} = 2.5$ Hz, $J_{6,5} = 8.8$ Hz, H-6), 6.79 (1H, *d*, $J_{6,8} = 2.5$ Hz, H-8), 7.19 (1H, *d*, $J_{6,5} = 8.8$ Hz, H-5). ^{13}C-NMR (50.289 MHz, CD_3OD): δ 56.5 (7-OCH_3), 62.9 (C-6′), 63.7 (4-OCH_3), 71.4 (C-4′), 75.0 (C-2′), 78.2 (C-5′), 78.8 (C-3′), 98.7 (C-2), 104.4 (C-1′), 105.6 (C-8), 110.4 (C-6), 114.8 (C-5), 120.9 (C-4a), 143.8 (C-8a), 157.1 (C-3), 159.4 (C-7).

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