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DIASTEREOSELECTIVE SYNTHESES OF 2-β-D-GLUCOPYRANOSYLOXY-2*H*-1,4-BENZOXAZIN-3(4*H*)-ONES FROM GRAMINEAE

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Key Word Index—*Secale cereale*; *Coix lachryma jobi*; Gramineae; diastereoselective syntheses; benzoxazinones; acetal glucosides; GDIBOA; GHMBOA.

Abstract—The first diastereoselective syntheses of the natural acetal glucosides, (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one and (2*R*)-2-β-D-glucopyranosyloxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one, are described by reaction of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) trichloroacetimidate with hemiacetalic aglucones of the 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton.

INTRODUCTION

Derivatives of the 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton are naturally occurring in the form of 2-β-D-glucosides as plant resistance factors of different species of Gramineae [1, 2], Acanthaceae [3] and Ranunculaceae [4]. The bioactive heterocyclic aglucones are set free, when β-glucosidase, a cell constituent, is put in contact with the glucosides by attack of external pests, like aphids or microbial diseases [5]. Glucosides isolated from Gramineae species like the hydroxamic acids (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (GDIBOA) (**7**) from rye [6], its 7-methoxy derivative (GDIMBOA) from maize [7] and the lactam, (2*R*)-2-β-D-glucopyranosyloxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (GHMBOA) (**6**) from *Coix lachryma jobi* [2], have been shown to have the (2*R*)-configuration. Recently, we have reported on two general approaches to the synthesis of heterocyclic aglucones [8, 9], including the hydroxamic acids, 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA) and its 7-methoxy derivative (DIMBOA), as well as their corresponding lactams, 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (HBOA) and its 7-methoxy derivative (HMBOA) (**2**). In contrast, only one example of the synthesis of 2*H*-1,4-benzoxazine glucosides has been reported. Recently, Tietze *et al.* [10] described the first synthesis and structural determination of (2*R*)-2-β-D-glucopyranosyloxy-2*H*-1, 4-benzoxazin-3(4*H*)-one (GHBOA, blepharin), isolated from *Blepharis edulis* (Acanthaceae), maize and rye (Gramineae) and its (2*S*)-diastereomer, by treatment of 2-bromo-2*H*-1,4-benzoxazin-3(4*H*)-one with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose in the presence of $Hg(CN)_2$. We report herein on the diastereoselective syntheses of the naturally occurring acetal glucosides, GHMBOA (**6**) and GDIBOA (**7**).

RESULTS AND DISCUSSION

Some recently described syntheses of several natural glycosides of steroids [11] and gangliosides [12], e.g. using trichloroacetimidates as glycosyl donors [13], prompted us to investigate the applicability of this method for the synthesis of 2*H*-1,4-benzoxazin-3(4*H*)-one acetal glucosides. Glucosylations via trichloroacetimidates are neighbour group-assisted reactions. Benzyl protected *O*-(glucosyl) trichloroacetimidates have been reported to react predominantly with inversion of the configuration at the glucosidic carbon at low temperature, while acetyl-protected trichloroacetimidates have been shown to give exclusively β-glucosides [13]. Therefore, we reacted the hemiacetal, HMBOA (**2**), with *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) trichloroacetimidate (**1**) with $BF_3 \cdot Et_2O$ as catalyst. It turned out, that protection of the lactam moiety is not necessary under these circumstances. The yield of the reaction was improved by using a two-fold excess of trichloroacetimidate. From the resulting 1 : 1 mixture of the acetylated 2-β-D-glucosides, the (2*R*)-diastereomer (**4**) could be separated by column chromatography. The β-configuration of the glucosidic bond could be assigned from the coupling constant of $J = 8$ Hz at δ4.99 for H-1′. The (2*R*)-configuration of **4** could be proven by comparing its CD spectrum, showing a positive Cotton effect at 223 nm and a negative one at 291 nm, with those of structural analogues, like natural GHMBOA [2].

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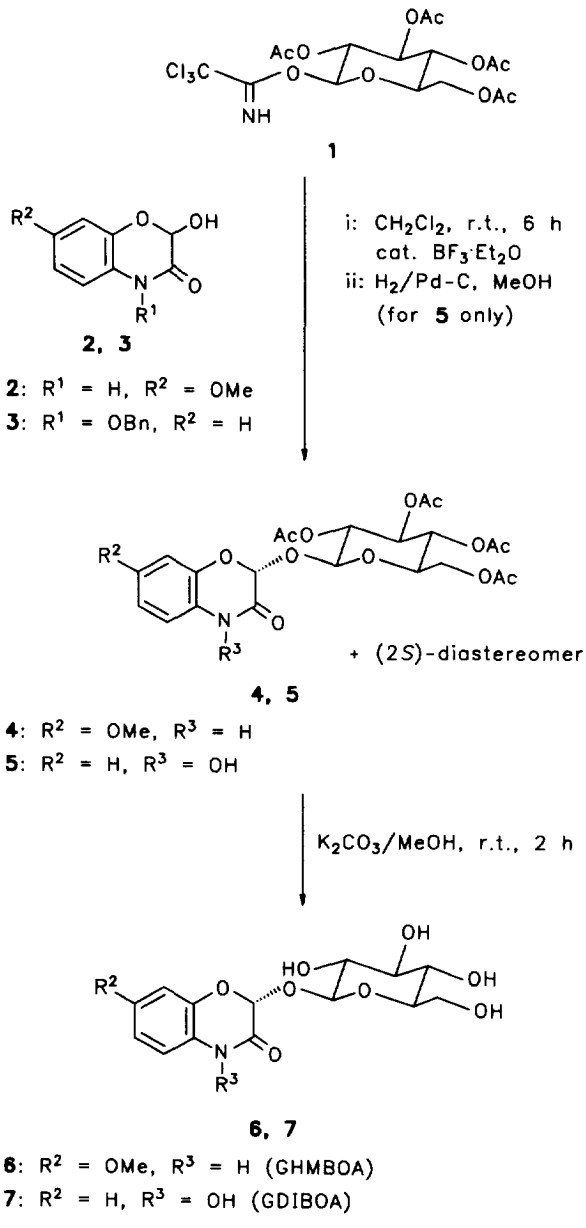

This natural product shows the same type of Cotton effects at similar wavelengths. Furthermore, Tietze's NMR rule for acetal glucosides [14], recently applied for the determination of configurations during the synthesis of blepharin [10], also gives evidence for the (2*R*)-configuration. In accordance with this rule, the chemical shift value of H-2 of **4** (δ5.61) is slightly lower than that of its (2*S*)-diastereomer (δ5.69). Finally, deprotection of **4** with K_2CO_3–MeOH yielded the pure natural lactam, GHMBOA (6).

Synthesis of the hydroxamic acid acetal glucoside, GDIBOA (7) from a heterocyclic aglucone and *O*-(glucosyl) trichloroacetimidates demands the protection of the hydroxamic acid moiety. As we have recently shown, this is owing to the synthetic lability of several 4-protected 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones, not a trivial problem [9]. Still, benzylation of DIBOA at the N-OH seemed to be the best solution. However, all attempts towards a direct benzylation of DIBOA resulted only in its degradation to benzoxazolin-2(3*H*)-one (BOA). Therefore, first of all, 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one [15] was protected by benzylation to give 4-benzyloxy-2*H*-1,4-benzoxazin-3(4*H*)-one [9]. Regioseletive bromination of this benzoxazinone at C-2 followed by Ag_2CO_3-mediated hydrolysis gave **3** as the protected hemiacetal suitable for the glucosylation procedure. Reaction of **3** with the acetyl-protected trichloroacetimidate (**1**) with $BF_3 \cdot Et_2O$ as catalyst, followed by hydrogenolysis to remove the benzyl group, resulted in a 1:1 mixture of the acetylated 2-β-D-glucosides of **5** and its (2*S*)-diastereomer. Again, it was possible to separate the (2*R*)-2-β-D-diastereomer by column chromatography to yield pure **5**. The configuration at the stereogenic centres C-1′ and C-2, of **5** could be determined by the spectroscopic methodology described above for glucoside **4**. Finally, solvolysis of **5** with MeOH–K_2CO_3 gave rise to the pure glucoside **7** with all analyses being identical to those for GDIBOA obtained from the natural source, *Secale cereale* [6].

Finally, attempts to shorten the synthesis of the natural 2-β-D-glucoside (**7**) were made by reacting the hemiacetal **3** with *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl) trichloroacetimidate in the presence of $BF_3 \cdot Et_2O$ at low temperatures. Advantageously, all protecting groups are then removable in one step by catalytical hydrogenolysis. Unfortunately, all attempts to determine the diastereoselectivity of this reaction by ^{1}H NMR spectroscopy, HPTLC or HPLC were of no avail. However, it was possible by means of high-resolution capillary electrophoresis (HPCE) to separate the reaction mixture consisting of four diastereomers [16]. The ratio of the isomeric (2*R*)-2-α-, (2*S*)-2-α-, (2*R*)-2-β- and (2*S*)-2-β-D-glucosides was 33:33:17:17, with the α-isomers being in the majority, unexpectedly.

In summary, the first syntheses of the natural acetal glucosides, (2*R*)-2-β-D-glucopyranosyloxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**6**) and (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**7**) have been achieved with β-diastereoselectivity by reaction of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) trichloroacetimidate (**1**) with the hemiacetalic aglucones, **2** and **3**, followed by chromatographic separation and deprotection.

EXPERIMENTAL

Reagents. Benzoxazinones and trichloroacetimidates were prepd according to the lit. cited, namely 2-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one [8], 4-benzyloxy-2*H*-1,4-benzoxazin-3(4*H*)-one [9], *O*-(2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl) trichloroacetimidate [17] and *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl) trichloroacetimidate [18].

4-Benzyloxy-2-hydroxy-2H-1,4-benzoxazin-3(4H)-one (**3**). A soln of Br_2 (4 g, 0.025 mol) in CCl_4 (20 ml) was

added dropwise within 30 min to a soln of 4-benzyloxy-2*H*-1,4-benzoxazin-3(4*H*)-one in CCl_4 (100 ml) under reflux and irradiation with a 500 W photolamp. After complete addition of Br_2, the mixt. was refluxed for another 15 min and cooled to room temp. The solvent was removed under red. pres. The remaining oil was dissolved in THF (30 ml) and poured at once into a vigorously stirred suspension of Ag_2CO_3 (8.3 g, 0.03 mol) in ice-H_2O (50 ml). The mixt. was stirred for 20 min, filtered and the solid washed with THF (15 ml). The filtrate was extracted with EtOAc (3 × 30 ml). The combined extract was dried (Na_2SO_4) and evapd to give **3** as crystals after recrystallization from EtOH, yield: 5.6 g (83%). Mp 161–163°, corr. (found: C, 66.41; H, 4.83; N, 5.16. $C_{15}H_{13}NO_4$ requires: C, 66.34; H, 4.73; N, 5.06%). ^{1}H NMR (400 MHz, DMSO-d_6): δ5.11 (*dd*, 2H, $PhCH_2$), 5.76 (*s*, 1H, CHCO), 6.95–7.60 (*m*, 9H, ar). EI-MS 70 eV, *m/z* (rel. int.): 271 ($[M]^+$, 3), 255 (21), 164 (12), 136 (80), 91 (71), 52 (100).

General procedure for glucosylation. To a soln of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) trichloroacetimidate (**1**) (770 mg, 2 mmol) in dry CH_2Cl_2 (20 ml), powdered molecular sieves 3 Å (500 mg) and hemiacetal **2** (for **4**) 195 mg, 1 mmol) and **3** (for **5**) (271 mg, 1 mmol), respectively, were added. A soln of $BF_3 \cdot Et_2O$ in CH_2Cl_2 (1 ml, 0.1 M) was added to the reaction mixt. via a syringe. The mixt. was stirred at room temp. for 6 hr in a sealed flask. CH_2Cl_2 (20 ml), $NaHCO_3$ (1 g) and H_2O (20 ml) were added successively. After 2 min, the organic layer was sepd, washed with H_2O (4 × 10 ml), dried (Na_2SO_4) and evapd *in vacuo*. For the synthesis of the hydroxamic acid (**5**), the remaining oil was dissolved in MeOH (50 ml) and hydrogenated over Pd-C at atm. pres.

(2R)-2-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyloxy*)-7-*methoxy*-2H-1,4-*benzoxazin*-3 (4H)-*one* (**4**). On chromatography of the reaction mixt. [silica gel 60, 60–200 μm, Merck, toluene–EtOAc (1 : 1)] a total amount of 340 mg (65%) crude product containing 50% of each (2*R*)- and (2*S*)-2-β-D-glucosides was isolated, from which 110 mg (21%) of the pure diastereomer **4** (mp 181–185°) were sepd by a second chromatography and recrystallization from EtOH. Frs containing **4** were identified by HPTLC [silica gel 60 F_{254}, Merck; eluent: toluene–EtOAc (1 : 1)], R_f (**4**) 0.33, R_f [(2*S*)-diastereomer] 0.36 (found: C, 52.47; H, 5.32; N. 2.73. $C_{23}H_{27}NO_{13}$ requires: C, 52.57; H, 5.18; N, 2.66%). FAB-MS *m/z* (rel. int.): 525 ($[M]^+$, 46), 331 (100), 271 (10), 229 (9), 178 (41), 169 (62). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 262 (2.93), 288 (2.72). $[\alpha]_{589}^{23}$ + 33° ($CHCl_3$; *c* 1.5). CD $\Delta\varepsilon_{235}$ + 15.0, $\Delta\varepsilon_{291}$ − 1.7 ($CHCl_3$; *c* 0.033). ^{1}H NMR (400 MHz, $CDCl_3$): δ1.99, 2.03, 2.04, 2.11 (4 *s*, 12H, Ac), 3.77 (*s*, 3H, OMe, 3.80 (*ddd*, 1H, $J_{5',4'}$ = 10 Hz, $J_{5',6b'}$ = 5 Hz, $J_{5',6a'}$ = 3 Hz, H-5'), 4.19 (*dd*, 1H, $J_{6a',5'}$ = 3 Hz, ^2J = 12 Hz, H-6a'), 4.25 (*dd*, 1H, $J_{6b',5'}$ = 5 Hz, ^2J = 12 Hz, H-6b'), 4.93 (*dd*, 1H, $J_{2',1'}$ = 8 Hz, $J_{2',3'}$ = 9.5 Hz, H-2'), 4.99 (*d*, 1H, *J* = 8 Hz, H-1'), 5.06 (*dd*, 1H, $J_{4',5'}$ = 9.5 Hz, $J_{4',3'}$ = 9.5 Hz, H-4'), 5.23 (*dd*, 1H, $J_{3',4'}$ = 9.5 Hz, $J_{3',2'}$ = 9.5 Hz, H-3'), 5.61 (*s*, 1H, H-2), 6.56–6.79 (*m*, 3H, ar), 9.10 (*s*, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ20.6, 20.8 (4 COMe), 55.6 (OMe-7), 61.9 (C-6'), 68.1 (C-4'), 71.1 (C-2'), 72.5 (C-5'), 72.6 (C-3'), 96.3 (C-2), 100.9 (C-1'), 104.2 (C-8), 109.1 (C-6), 116.3 (C-5), 118.4 (C-9), 141.0 (C-10), 160.0 (C-7), 169.4 (2 COMe), 170.3, 170.7 (2 COMe).

(2R)-2-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyloxy*)-4-*hydroxy*-2H-1,4-*benzoxazin*-3(4H)-*one* (**5**). First, chromatography of the reaction mixt. [silica gel 60, 60–200 μm, Merck, toluene–EtOAc (1 : 2)] yielded a total amount of 380 mg (74%) of a 1 : 1 mixt. of the (2*R*)- and (2*S*)-2-β-D-glucosides as an oil after evapn of solvent. Then, 61 mg (12%) of pure **9** (mp 223–224°) were sepd by further chromatography. TLC [silica gel 60_{F254}, Merck; eluent–toluene–EtOAc (1 : 2)], R_f (**5**) 0.39, R_f [(2*S*)-diastereomer] 0.46 (found: C, 51.69; H, 5.01; N, 2.66. $C_{22}H_{25}NO_{13}$ requires: C, 51.66; H, 4.93; N, 2.73%). FAB-MS *m/z* (rel. int.): 512 ($[M + H]^+$, 34), 460 (16), 405 (10), 354 (25), 331 (38), 252 (40), 169 (78), 164 (95), 107 (100, matrix). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 258 (3.38), 227 (3.32), 282 (3.23). $[\alpha]_{589}^{23}$ + 54° ($CHCl_3$; *c* 1.0). CD $\Delta\varepsilon_{225}$ + 4.05, $\Delta\varepsilon_{282}$ − 0.4 ($CHCl_3$; *c* 0.033). ^{1}H NMR (400 MHz, $CDCl_3$): δ1.95, 1.96, 1.98, 2.06 (4 *s*, 12H, Ac), 3.68 (*ddd*, 1H, $J_{5',4'}$ = 9.8 Hz, $J_{5'6b'}$ = 4 Hz, $J_{5',6a'}$ = 2 Hz, H-5'), 4.14 (*dd*, 1H, $J_{6b',5'}$ = 4 Hz, ^2J = 12.5 Hz, H-6b'), 4.18 (*dd*, $J_{6a',5'}$ = 2 Hz, ^2J = 12.5 Hz, H-6a'), 4.86 (*dd*, 1H, $J_{2',1'}$ = 8 Hz, $J_{2',3'}$ = 9.3 Hz, H-2'), 4.92 (*d*, 1H, *J* = 8 Hz, H-1'), 5.01 (*dd*, 1H, $J_{4',5'}$ = 9.8 Hz, $J_{4',3'}$ = 9.8 Hz, H-4'), 5.16 (*dd*, 1H, $J_{3',4'}$ = 9.8 Hz, $J_{3',2'}$ = 9.3 Hz, H-3'), 5.76 (*s*, 1H, H-2), 7.0–7.39 (*m*, 4H, ar), 9.37 (*s*, 1H, N-OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ20.5, 20.6, 20.8 (4 COMe), 61.7 (C-6'), 68.0 (C-4'), 71.0 (C-2'), 72.4 (C-3', C-5'), 97.0 (C-2), 100.6 (C-1'), 113.6 (C-5), 117.4 (C-8), 123.7 (C-7), 125.2 (C-6), 126.4 (C-9), 140.3 (C-10), 155.0 (C-3), 169.4, 169.7, 170.3, 170.9 (COMe).

Deprotection of glucosides **4** *and* **5**. Glucosides **4** (for **6**) (51 mg, 0.1 mmol) and **5** (for **7**) (53 mg, 0.1 mmol), respectively, were dissolved in dry MeOH (10 ml) and, after addition of activated K_2CO_3 (50 mg), stirred for 2 hr at room temp. The mixt. was acidified to pH 1 with a few drops of 12 M HCl. Evapn *in vacuo* to dryness and purification of the residue by CC [silica gel 60, 60–200 μm, Merck, $CHCl_3$–MeOH (3 : 2)] gave glucoside **7** as pure crystals and **6** as a foam after evapn of solvents. Glucoside **6** was then recrystallized from H_2O.

(2R)-2-β-D-*Glucopyranosyloxy*-7-*methoxy*-2H-1,4-*benzoxazin*-3 (4H)-*one* (**6**). Yield: 27 mg (80%). Mp 237–240°, corr. (lit. [2] 245°, lit. [19] 228–235°). All spectroscopic and chiroptical data were identical to those of GHMBOA isolated from *Coix lachryma jobi* by Nagao *et al.* [2] and determined to have the (2*R*)-configuration.

(2R)-2-β-D-*Glucopyranosyloxy*-4-*hydroxy*-2H-1,4-*benzoxazin*-3 (4H)-*one* (**7**). Yield: 28 mg (82%). Mp 255–257°, corr. (lit. [6] mp 256–257°). All spectroscopic and chiroptical data were identical to those of GDIBOA isolated from rye seedlings [6] and determined to have the (2*R*)-configuration.

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