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Syntheses of 2-Hydroxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one: A Precursor of a Bioactive Electrophile from *Gramineae*

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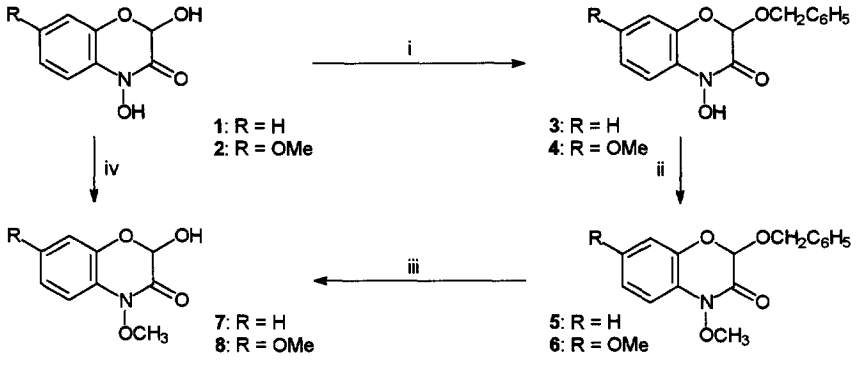
Abstract: 2-Hydroxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**8**), the hitherto undescribed free hemiacetalic aglycone of a benzoxazinoid acetal glucoside naturally occurring in wheat, has been synthesized following two pathways, independently. This cyclic hydroxamic acid methyl ester proved to be very unstable when in solution. This gives rise to the assumption that HDIBOA naturally released from its acetal glucoside is by methoxide elimination a precursor to form a multi-centered electrophile that was recently reported to be the bioactive principle of the benzoxazinoid lead.

Acetal glucosides of the 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one lead have been found to occur as allelo chemicals in *Gramineae*,[1] *Acanthaceae*,[2] *Ranunculaceae*,[3] and *Scrophulariaceae*.[4] (2*R*)-2-β-D-Glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one,[5] its 7-methoxy derivative[6] and the 4-methyl ester of the latter one[7] are of most interest. Hemiacetalic aglycones, like 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**1**) and its 7-methoxy derivative **2**, enzymatically released after a pest attack, exhibit high bioactivity as plant resistance factors against microbial diseases and insects[8] in rye, maize and wheat and occur in phytotoxic root exudates of quackgrass.[9] We have reported on syntheses of **1**[10] and **2**,[11] on a general approach to substituted 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones,[12] and on the diastereoselective glucosidation affording natural acetal glucosides.[13]

The phenomena of bioactivity of benzoxazinoids have been investigated in a variety of organisms like aphids,[14] bacteria and fungi,[15] algae,[16] as well as in single plant cells,[17] or chloroplasts.[18] **2** was shown to inactivate α-chymotrypsin[19] and acylcholinesterases.[20] Recently, all work directed on the understanding of the molecular mechanisms of the bioactivity was summarized.[21] The influence of O-functional substituents at positions 2, 4, and 7 of the 2*H*-1,4-benzoxazin-3(4*H*)-one skeleton was studied. The biomolecule-alkylating action arising from the ability to form a multi-centered electrophile under biological conditions which can react with nucleophilic biomolecules (*e.g.* DNA) was found to be the unique feature of the benzoxazinoid lead and the source of bioactivity. *In vitro*, acetylation of the 4-OH group was the crucial step to generate a cationic species by N-O bond cleavage on elimination of acetate in reactions with various nucleophiles. Depending on their

nature as C-, N-, or S-nucleophiles attacks at N-4, C-5, or C-6 have been observed. At present, a similar enzymatic acetylation is believed to be the source for the N-O heterolysis *in vivo*.

It is the aim of this paper to report on two synthetic approaches to 4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**8**), which is obviously a natural precursor for the multi-centered electrophile cited.[21]



Reagents and conditions: (i) benzyl trichloroacetimidate, CH_2Cl_2, BF_3 x Et_2O, 25°C; (ii) CH_3I, K_2CO_3, acetone, reflux; (iii) H_2/Pd-C, THF, 25°C; (iv) CH_2N_2, Et_2O/THF, 0°C.

Scheme 1. Two independent syntheses for **8** and its analogue **7**

8 has been reported as present in corn whorl surface waxes.[22] A structural assignment was given, however, some doubt remains, at least in the purity of the sample described. We decided to follow two pathways for the preparation of the hydroxamic acid methyl esters **7** and **8** from their precursors **1** and **2**, respectively.

The hemiacetals **1** and **2** have been subjected to benzylation with benzyl trichloroacetimidate[23] using the knowledge gained from the investigation of their glucosidation.[13] Thus, boron trifluoride etherate used in excess added to the 2-OH and 4-OH groups, acted as a noncovalent protecting group for the 4-OH group and a promoter for the regioselective benzyl transfer to the more nucleophilic hemiacetalic function, only. 2-Benzyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (**3**) and its analogue **4** have been obtained as novel derivatives of the lead.[24] The hydroxamic acids **3** and **4** have been methylated by using the standard K_2CO_3/CH_3I method in refluxing acetone to afford the hydroxamic acid methyl esters **5** and **6**.[25] Finally, hydrogenolysis over Pd-C in dry THF was used to liberate the hemiacetalic function of **7** and **8**.[26] Hydrogenation of **6** must be carried out in THF because polar solvents cause a rapid decomposition of **8**. On the contrary, **5** can also be hydrogenated in methanol to form stable **7** without problems. An independent synthesis for **7** starting from 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one has been described in a patent.[27] Alternatively, the 2,4-dihydroxy-substituted aglycones **1** and **2** in a THF solution have been methylated with a solution of diazomethane in diethyl ether at their more acidic hydroxamic acid unit, regioselectively.[28]

Interestingly, hydroxamic acid ester **7** is stable in solution, whereas **8** when dissolved in methanol or DMSO undergoes a degradation to form 6-methoxy-2*H*-benzoxazolin-2(3*H*)-one. Spectroscopy of **8** without degradation was only possible in THF-d_8. The transformation of **8** was found to proceed *via* an orange coloured

intermediate detectable by TLC some minutes after dissolution in methanol. Its nature could not be established yet. After 12 hours the solution contains 6-methoxy-2*H*-benzoxazolin-2(3*H*)-one as the only aromatic compound. **8** is the first natural compound representing the principles found for the generation of a multi-centered electrophile as the bioactive principle of the benzoxazinoids,[21] by a methoxide elimination from 4-N-OCH_3. The 7-methoxy group present enhances the N-O heterolysis. Therefore, at least in plants containing the glucoside of **8**, after release of **8** by a β-glucosidase, methoxide elimination is assumed to be the initial step during the defence of the plant. Indeed, a high bioactivity of maize lines containing **8** was reported.[22]

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24. General Procedure: Hemiacetal **1** (or **2**) (0.5 mmol), benzyl trichloroacetimidate (252 mg, 1.0 mmol), and BF_3 x Et_2O (0.5 ml) in absolute CH_2Cl_2 (15 mL) were stirred until complete consumption of **1** (or **2**). After hydrolysis and extraction with EtOAc the organic phase was evaporated *in vacuo*. The residue was purified by silica gel CC (eluent: toluene/EtOAc 1/1 v/v) to yield colourless foams of **3** (110 mg, 81 %) or **4** (142 mg, 94 %). Analytical data for **3** and **4**. **3**: ^{1}H NMR (200 MHz, $CDCl_3$), δ (ppm): 4.76 (2H, dd, ^{2}J=11.8 Hz, OCH_2), 5.58 (1H, s, H-2), 6.97-7.46 (9H, m, ar.), 9.64 (1H, s, NOH); ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 71.3 (OCH_2), 96.7 (C-2), 114.5 (C-5), 117.7 (C-8), 123.8 (C-7), 125.6 (C-6), 127.6 (C-4a), 128.7 (C-2', C-6'), 128.8 (C-4'), 129.0 (C-3', C-5'), 136.3 (C-1'), 141.0 (C-8a), 156.9 (C-3); EI MS, m/z (%): 271 (M^+,10), 43 (100). **4**: ^{1}H NMR (200 MHz, $CDCl_3$), δ (ppm): 3.78 (3H, s, OCH_3), 4.77 (2H, dd, ^{2}J=11.8 Hz, OCH_2), 5.58 (1H, s, H-2), 6.53-6.81 (3H, m, ar.), 7.16-7.36 (5H, m, ar.); ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 56.1 (OCH_3), 71.3 (OCH_2), 97.0 (C-2), 104.1 (C-8), 108.9 (C-6), 115.3 (C-5), 121.1 (C-4a), 128.7 (C-2', C-6'), 129.0 (C-3', C-5'), 129.3 (C-4'), 136.4 (C-1'), 142.1 (C-8a), 157.9 (C-3), 164.7 (C-7); EI MS, m/z (%): 301 (M^+,35), 91 (100).

25. In a typical procedure, after filtration, evaporation and chromatographic purification (eluent toluene/ EtOAc 1/1 v/v) **5** (134 mg, 94 %) and **6** (144 mg, 91 %) were obtained as colourless oils. Analytical data for **5** and **6**. **5**: ^{1}H NMR (200 MHz, $CDCl_3$), δ (ppm): 3.98 (3H, s, $NOCH_3$), 4.79 (2H, dd, ^{2}J=11.9 Hz, OCH_2), 5.54 (1H, s, H-2), 7.03-7.31 (9H, m, ar.); ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 63.3 ($NOCH_3$), 71.3 (OCH_2), 97.1 (C-2), 113.3 (C-5), 118.3 (C-8), 123.9 (C-7), 125.3 (C-6), 127.0 (C-4a), 128.7 (C-2', C-6'), 128.8 (C-4'), 129.0 (C-3', C-5'), 136.4 (C-1'), 141.1 (C-8a), 156.3 (C-3); EI MS, m/z (%): 285 (M^+, 34), 91 (100). **6**: ^{1}H NMR (200 MHz, $CDCl_3$), δ (ppm): 3.78 (3H, s, OCH_3), 3.97 (3H, s, $NOCH_3$), 4.80 (2H, dd, ^{2}J=11.9 Hz, OCH_2), 5.54 (1H, s, H-2), 6.58-6.75 (3H, m, ar.), 7.12-7.37 (5H, m, ar.); ^{13}C NMR (50 MHz, $CDCl_3$), δ (ppm): 56.2 (OCH_3), 63.2 ($NOCH_3$), 71.4 (OCH_2), 97.7 (C-2), 104.6 (C-8), 109.0 (C-6), 114.0 (C-5), 120.7 (C-4a), 128.7 (C-2', C-6'), 129.0 (C-3', C-5'), 129.5 (C-4'), 136.4 (C-1'), 143.1 (C-8a), 157.7 (C-3), 157.9 (C-7); EI MS, m/z (%): 315 (M^+,8), 91 (100).

26. **7** (40 mg, 82 %) was identical with the literature.[27] Analytical data for **8** (44 mg, 78 %): mp. 101-104°C (THF); ^{1}H NMR (200 MHz, THF-d_8), δ (ppm): 3.73 (3H, s, OCH_3), 3.88 (3H, s, $NOCH_3$), 5.54 (1H, d, J= 6.2 Hz, H-2), 6.60-6.63 (2H, m, ar.), 7.00 (1H, d, J=6.2 Hz, OH), 7.09 (1H, d, J=9.4 Hz, H-5); ^{13}C NMR (50 MHz, THF-d_8), δ (ppm): 55.7 (OCH_3), 62.5 ($NOCH_3$), 94.2 (C-2), 104.8 (C-8), 108.4 (C-6), 113.6 (C-5), 121.7 (C-4a), 143.2 (C-8a), 157.0 (C-3), 157.9 (C-7); EI MS, m/z (%): 225 (M^+,53), 166 (100).

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28. **7** (84 mg, 86 %) and **8** (89 mg, 79 %) obtained were identical with the literature.[26]

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