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Review

Synthesis of benzoxazinoid acetal glucosides naturally occurring in *Gramineae*

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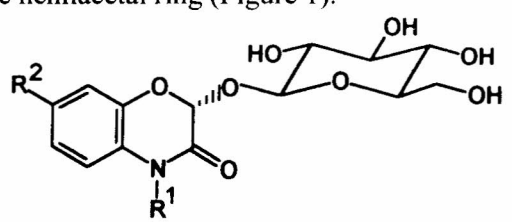
Benzoxazinoid acetal glucosides, a unique class of natural hemiacetal ethers from *Gramineae* and other species, are of interest as precursors of aglucones which exhibit high bioactivity as plant resistance factors against microbial diseases and insects, allelo chemicals and endogeneous ligands. This review gives a survey on synthetic methods which give rise to the hemiacetalic aglucones of the 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton, efficiently. As a result of investigations in the area of acetal glucosidation a double diastereoselective glucosidation method of racemic cyclic hemiacetals has been developed that affords an access to the acetal glucosides of natural configuration as well as to their enantiomers.

Almost 1000 natural products are known which due to their direct connection of two cyclic hemiacetals can be regarded as hemiacetal ethers. Though a few such hemiacetal ethers arise from the dehydration of two sugar components, like α,α-trehalose[1] or α,-β-galactobiose[2], the majority, the so called acetal glycosides, are glycosidic derivatives of hemiacetals of a great structural diversity. Nearly 100 acetal glycosides contain at least one nitrogen in the aglycone moiety. Usually, nitrogen constitutes a part of rings annelated to the cyclic hemiacetal ring of the aglucone, as in *e.g.* xylostosidine[3], pumiloside[4], or alangiside[5].

Exceptionally, only in the case of 2-β-D- glucosides of 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton the nitrogen atom forms a part of the aglyconic hemiacetal ring (Figure 1).



R^1	R^2	compound
OH	H	GDIBOA
OH	OMe	GDIMBOA
OMe	OMe	GHDIBOA

Figure 1 -- Some natural 1,4-benzoxazinone acetal glucosides

Representatives of this class have been recently found to occur as allelo chemicals in various species of *Gramineae*[6], *Acanthaceae*[7], *Ranunculaceae*[8] and *Scrophulariaceae*[9]. The biological function of such glucosides as possible endogeneous ligands in the plant cell is under investigation[10]. However, it has been shown that the hemiacetalic aglucones, enzymatically released after a pest attack, exhibit high bioactivity as plant resistance factors in maize, rye and wheat against microbial diseases and insects[11], phytotoxins of root exudates from the weed quackgrass[12], and inhibitory agents towards prostate cancer cell lines[13]. Among the acetal glucosides isolated the hydroxamic acids (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (GDIBOA),[14] its 7-methoxy derivative GDIMBOA[15] and the 4,7- dimethoxy compound GHDIBOA[16] are of utmost interest.

Various groups are investigating the biosynthesis of such acetal glucosides. Ribosyl phosphate and anthranilate have been found to be the precursors for 2*H*-1,4-benzoxazin-3(4*H*)-one as the first benzoxazine derivative of the biosynthetic pathway[17]. Recently, indole has been detected to be an intermediate of the middle part of the biosynthesis, which is not completely elucidated yet[18]. However, the final part of the pathway with possibilities and limitations for the enzymatic *O*- and *N*-hydroxylations of 2*H*-1,4- benzoxazin-3(4*H*)-one and for the stereoselective glucosylation of the aglucone hemiacetals

2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)- one (DIBOA) and its 7-methoxy derivative DIMBOA has already been investigated in detail[19,20].

The chemical reactivity of biologically active benzoxazinoids towards various types of nucleophiles has been recently reviewed in relation to their biological activity[21]. The influence and importance of *O*-functional substituents at positions 2, 4, and 7 of the 2*H*-1,4-benzoxazin-3(4*H*)-one skeleton have been investigated. The ability to form a multi-centered electrophile under biological conditions has been found to be a unique feature of the corresponding aglucones. Therefore, the biomolecule- alkylating action of a benzoxazinoid derived cationic species is regarded as the chemical source of their biological activity.

Finally, it is well known that enzymatically liberated aglucones like DIBOA and DIMBOA decompose when in solution with a half life of some hours in the neutral or even only minutes when in an alkaline medium. Formic acid and benzoxazolin-2(3*H*)-one (BOA) or its 6-methoxy derivative (MBOA) have been identified as degradation products. The structural lability of both these hemiacetals is a consequence of their oxo-cyclo tautomerism, which allows a rapid interconversion of the (2*R*)-configurated enantiomer into the (2*S*)-enantiomer and *vice versa via* the ring- opened oxo form (Scheme 1).

The mechanism of this reaction has been intensively studied[16b,22]. Donor substitution increasing the electron density of the aromatic ring has been found to accelerate the rate of decomposition[23]. This unique feature of the 2,4-dihydroxy-2*H*-1,4- benzoxazin-3(4*H*)-one skeleton is not only of interest from the mechanistic point of view, but of most importance for the planning of any synthesis, which has to keep in mind this structural lability. In fact, this paper will mainly be focussed on the syntheses of aglucones as well as of acetal glucosides.

Results and Discussion

Syntheses of aglucones. Until 1989 three syntheses leading to DIBOA or DIMBOA had been published (Scheme II).

The first synthesis affording DIBOA (Scheme I, left pathway) was reported in 1960[24], starting from a protected 2-nitrophenol. The second synthesis (Scheme I, middle pathway), subject of a patent issued in 1975 to Hoffman-La Roche, was carried out starting from an appropriately *ortho*-substituted nitrobenzene[25]. Both these syntheses utilize the reductive cyclization of nitro compounds to develop the structural unit of the cyclic hydroxamic acid. Also, in both cases the free hemiacetal is obtained by the hydrolysis of a chloride precursor. However, only the second synthesis is applicable to the preparation of the 7-methoxy compound DIMBOA. A serious disadvantage of the first synthesis is the need to handle a free donor-substituted arylhydroxylamine. In the second case also the cleavage of the 2-methoxy group requires treatment with aggressive boron trichloride solution. The third synthesis (Scheme I, right pathway) uses a different strategy[26]. In this case the hemiacetal unit is prepared by alkaline cyclization of the dichloroacetamide precursor, and then the hydroxamic acid is produced by the oxidation of the silylated lactam with $MoO_5(DMF)_2$. This procedure has been found to have serious problems in work-up. All the three procedures have unsufficient overall yields which has prompted a search for more efficient syntheses. In 1991, a first general method similar to the Hoffman-La Roche procedure was published[23],

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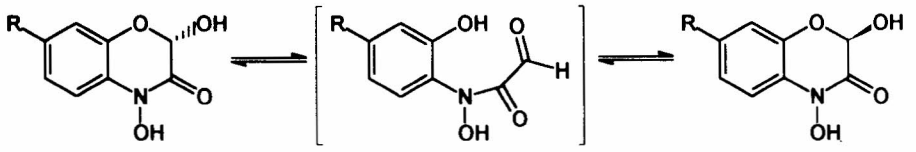
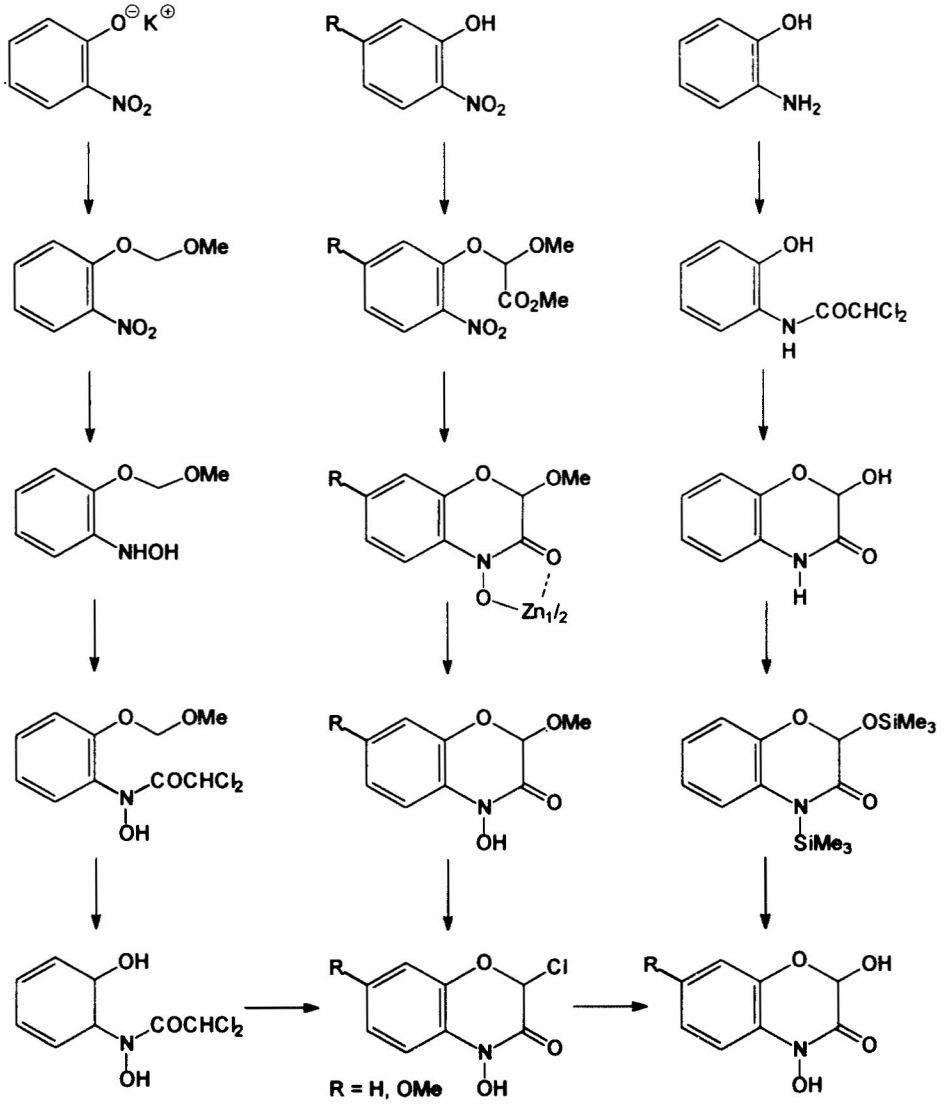


R = H: DIBOA; R = OMe: DIMBOA

Scheme I – Oxo-cyclo tautomerism of natural hemiacetalic hydroxamic acids

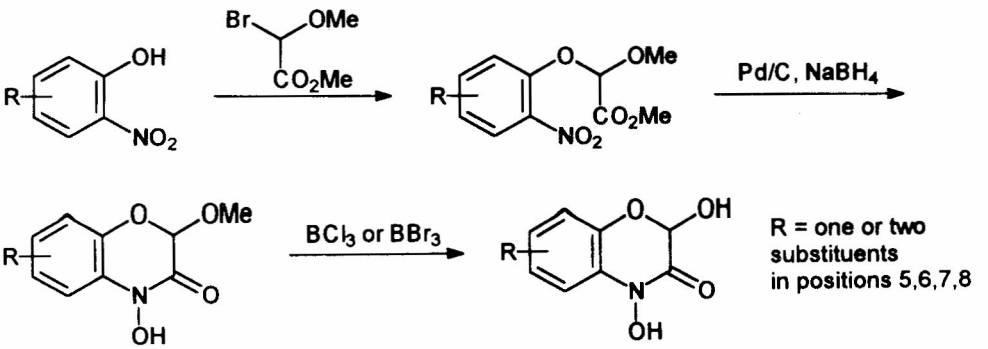


Scheme II—Literature syntheses leading to the 2,4-dihydroxy-2*H*-1, 4-benzoxazin-3(4*H*) -one skeleton

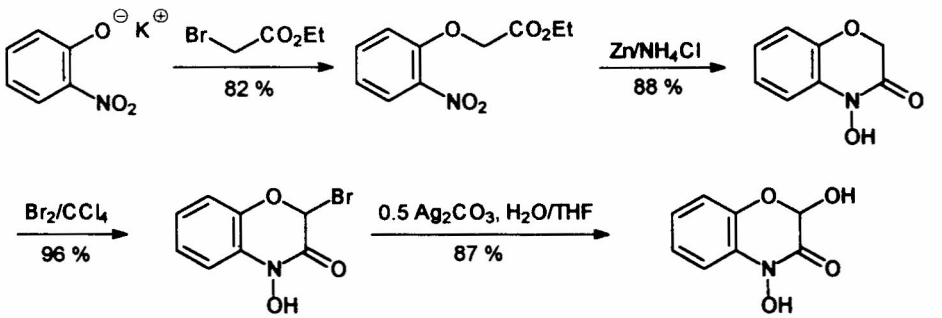
which allowed the preparation of a series of 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)- ones with substituents in positions 5, 6, 7 and 8 of the aromatic ring (Scheme III). An improvement in this method consists in making use of a catalytic transfer hydrogenation[27] (CTH) for the reductive cyclization step.

A general evaluation of the influence of substituents on the demethylation step of this method and on the other methods mentioned above briefly has been the subject of a first review on the synthesis of benzoxazoid aglucones[28].

In 1989, we have reported a selective synthesis for DIBOA[29] that avoids some disadvantages of previous methods and allows the synthesis of this aglu-



Scheme III—A literature synthesis tolerating various R substituents using a CTH for reductive cyclization



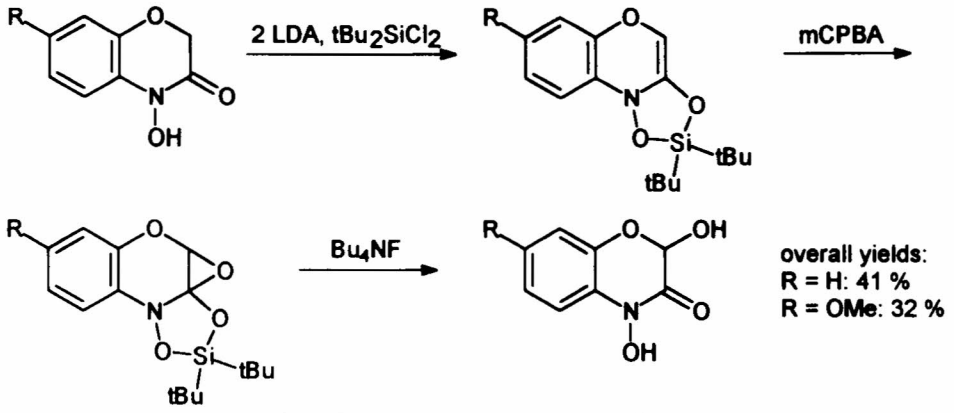
Scheme IV—A selective synthesis for DIBOA

cone at the multigram scale (Scheme IV). The readily available ethyl 2-nitrophenoxyacetate is cyclized by zinc dust in ammonium chloride solution to form 4-hydroxy-2*H*-1,4- benzoxazin-3(4*H*)-one, which is brominated regioselectively at C- 2. In contrast to a former method[25] that uses excess silver carbonate for the hydrolysis of the analogous 2-chloro derivative obtained by cleavage of the 2-methoxy precursor, the 2-bromo compound is highly reactive and affords DIBOA on treatment with only the stoichiometric amount of silver carbonate in water-THF.

Unfortunately, this method cannot be used in the synthesis of the 7-methoxy compound DIMBOA. Again, bromination of the corresponding 4-hydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one analogue takes place regioselectively, but due to the donor effect of the methoxy group at C-6 rather than at C-2.

Therefore, we became interested in a different approach to the 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton independent of the substituent at the aromatic ring. The initial idea was to develop the lactol unit not by a known means of substitution from a 2-halogen precursor, but by the oxidation of the unsubstituted methylene function (Scheme V)[30].

The starting 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones were prepared by catalytic transfer hydrogenation of the appropriate 2- nitrophenoxyacetate precursors with the sodium borohydride/Pt-C method. The oxidative transformation requires a need for the protection of the hydroxamic acid moiety. Several methods for protecting the 4-position have been taken into consideration to yield precursors for oxidizable silyl enol ethers. First, acetoxy derivatives have been excluded due to their ability to form an electrophile under heterolysis of the N-O bond[31]. Protection of the hydroxamic acid unit as 4-benzyloxy derivatives has been possible[30], however all attempts to transform them into the corresponding silyl enol ethers by means of LDA/TBDMSCl led to unsuitable decomposition products only. Also, an attempt to silylate the acidic N-OH with TBDMSCl/imidazole was unsuccessful. Finally, two equivalents of LDA allowed the enolisable hydroxamic acids to react with di-*tert*-butyldichlorosilane in absolute toluene to give the desired stable cyclosilyl enol ethers. Therefrom, the intermediate epoxides have been formed by oxidation with *m*-chloroperbenzoic acid in methylene chloride. Finally, treatment of both epoxides with tetra-n-butylammonium fluoride in THF effected the

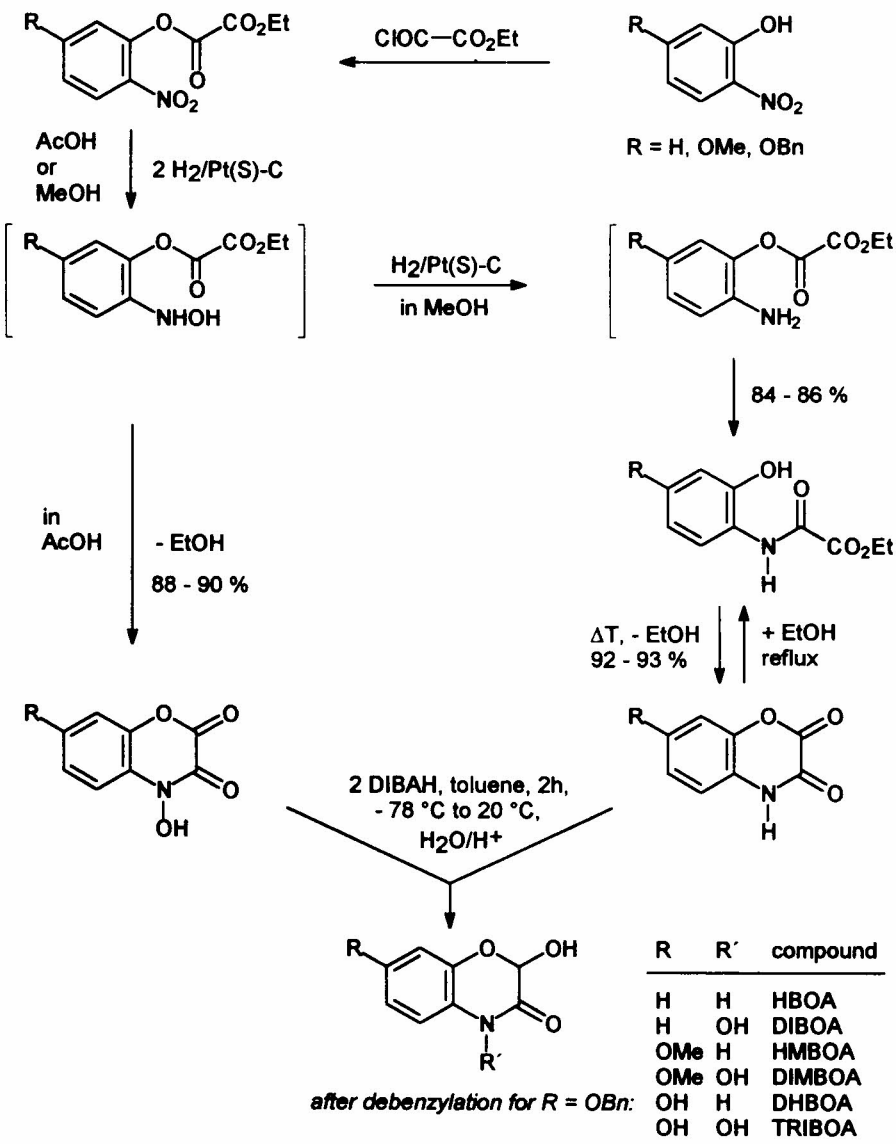


Scheme V—Oxidative generation of the lactol unit by α-hydroxylation of a cyclic hydroxamic acid

deprotection of the silyl enol ethers and ring opening of epoxides to yield DIBOA and DIMBOA, respectively, after separation from the reaction mixture by column chromatography. The whole sequence of reactions shown in Scheme V has been run in a one-pot procedure. It is the first α-hydroxylation of cyclic hydroxamic acids reported and ensures independence from substitution in the aromatic ring. However, it is to be noted that the overall yields of aglucones are not as good as the targets of a search for a better synthetic pathway.

Therefore, an alternative procedure was established in which the crucial lactol unit was synthesized from a lactone (Scheme VI). In this procedure, appropriate 5-substituted 2-nitrophenols are transformed by acylation with chloroformylformate into (substituted) ethyl 2-nitrophenyl oxalates. We have found these oxalates to be the reactive building blocks for the synthesis of the 2,3-dioxo-1,4-benzoxazine skeleton[32]. Any procedure for the reductive cyclization of 2-nitrophenyl oxalates has to take into account their extreme sensitivity towards hydrolysis as a feature of a nitrophenyl ester. Water has definitely to be excluded when performing the reduction of the nitro group. Hence, a transfer hydrogenation was impossible as a method for the synthesis of a hydroxamic acid. However, the hydroxamic acids could be obtained by catalytic hydrogenation of nitro oxalates in glacial acetic acid over 3% Pt(S)-C in the presence of 2,2-dimethoxypropane added for the chemical transformation of the water resulting from the partial hydrogenation of the nitro group. Also, precautions were made to ensure that really dry hydrogen gas was used for hydrogenation. Surprisingly, a simple preparative trick gave access to the corresponding lactam series. Alone changing the solvent of the hydrogenation to methanol gave rise to the ethyl N-(4-substituted-2-hydroxyphenyl)oxalamides. Mechanistic aspects of these selective heterogeneous hydrogenation have been discussed in detail[32]. The oxalamides underwent lactonization to form the required lactones on heating in a Kugelrohr apparatus to their melting points. Lactone-lactams of this type cannot be recrystallized from protic solvents like ethanol, because this causes ring-opening and formation of the oxalamide precursors. The lactone unit in both substituted 4-hydroxy- and 4*H*-2,3-dioxo- 1,4-benzoxazines has been chemoselectively reduced by means of diisobutylaluminium hydride in toluene to form 7-substituted-2- hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones and 7-substituted-2, 4- dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones, respectively[33]. We have found the use of two equivalents of the reducing agent most suitable, the first one for deprotonation of the acidic functionality, and the second one for the lactone-lactol reduction. Attempts to make use of a third equivalent of the hydride for simultaneous unmasking of the 7-hydroxy group in case of a 7-benzyloxy substitution proved to be unsuccessful. It was shown by TLC monitoring of the reaction mixture that cleavage of the benzyl ether under this condition did occur only to a very low degree, which was detectable but not of preparative value.

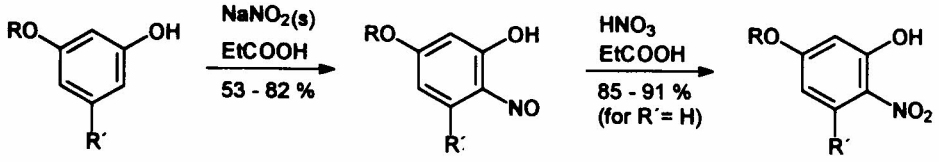
Therefore, the aglucones DHBOA and TRIBOA with 7-hydroxy substitution have been prepared[34] in which this OH group was liberated by standard hydrogenolyses of their benzylated precursors over Pd-C in acetic acid. In contrast to the substitutive syntheses of the lactol unit (Schemes II, III, and IV) or to its oxidative generation (Scheme V) all steps of the reductive pathway (Scheme VI) are accompanied



Scheme VI—A general synthesis for 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones by lactone-lactol reduction

by good to excellent yields and the method provides a general access to the 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one as well as to the 2,4- dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeletons as shown by the synthesis of six natural aglucones. In summary, the pathway of first forming the hydroxamic acid by reductive cyclization and then developing the hemiacetal unit by reduction from a lactone precursor is at present the best synthetic entry into the field of natural benzoxazinoids.

It has to be mentioned here that our attempts to prepare 7- hydroxy derivatives required the hitherto unknown 5-benzyloxy-2-nitrophenol. In turn, the synthesis of this nitrophenol was only possible by developing a novel regioselective nitrosation procedure for resorcinol monoethers[35]. Monobenzyl resorcinol was used as starting material. It is much better accessible in two steps by complete benzylation of resorcinol (85 % yield) followed by selective catalytic mono-hydrogenolysis over Pd-C in acetic acid (81 % yield) than by a direct monobenzylation of resorcinol (30 % yield only). It was known to us from earlier attempts directed on the synthesis of the related 5-methoxy-2- nitrophenol[36], that a selective direct nitration of such electronically overheated aromatics is impossible even with dilute aqueous nitric acid. In such a case using a weaker N- electrophile like the nitrosyl cation should be helpful because this can give rise to a nitroso compound which can finally be oxidized to a nitro compound. However, in the



combinations: R = Me, R' = H; R = Me, R'= OMe; R = Bn , R' = H.

Scheme VII—Regioselective nitrosation of resorcinol monoethers and oxidation to 2-nitrophenols

case of monobenzyl resorcinol further problems arose from the insolubility of this compound in water which prevented a conventional nitrosation procedure in an aqueous medium. All these problems were solved by using an unconventional nitrosation procedure for resorcinol monoethers (Scheme VII).

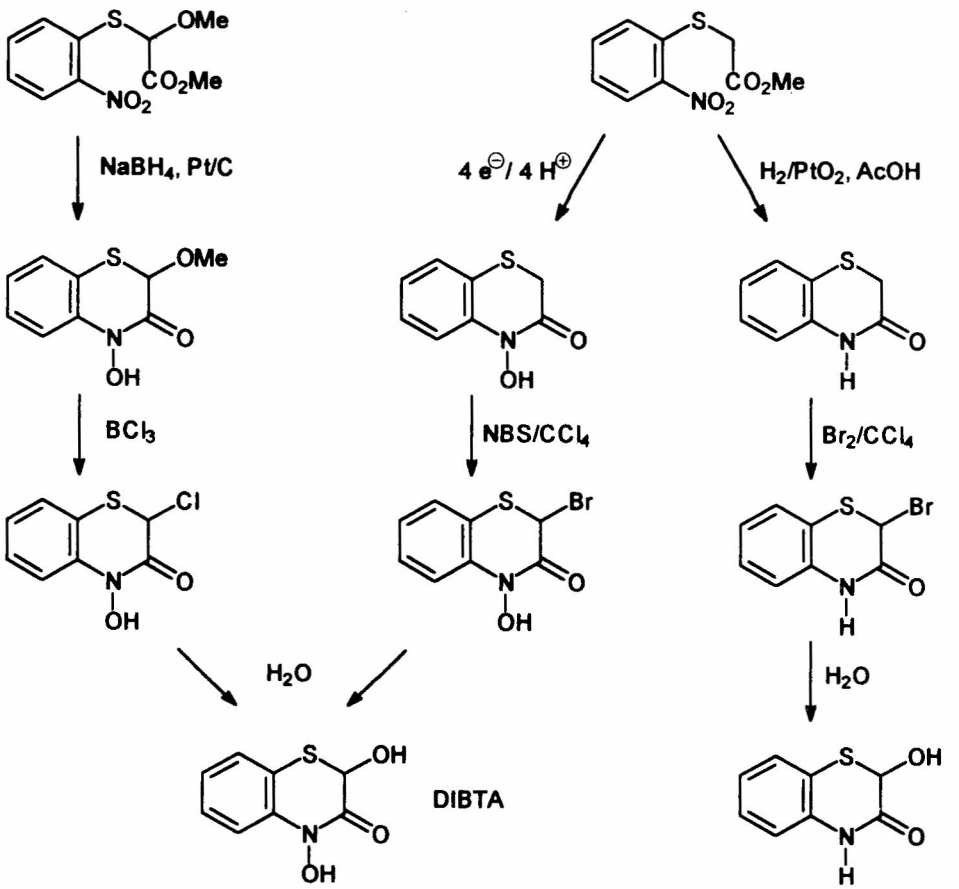
Solid sodium nitrite in anhydrous propionic acid as solvent was found to be an effective system for the completely regioselective nitrosation of the subject compounds[35]. High yields of pure 2- nitroso products are obtained rapidly and efficiently. Attack at the 4-position becomes only competitive if water is present in the medium. Absence of water was ensured by addition of one weight percent of propionic anhydride. The observed excellent regioselectivity may be related to the apparent presence of an acyl nitrite whose *in situ* formation in solutions of sodium nitrite in anhydrous carboxylic acids is known[37]. Mesomeric structures of propionyl nitrite show the O-NO bond with the NO unit having a partially positive charge. Therefore, propionyl nitrite, the mixed anhydride of propionic and nitrous acid formed under the reaction conditions, attacks at the electron rich monoalkyl resorcinol like nitrosyl propionate, *i.e.* as a polarized covalent source of a nitrosyl cation. Hence, the regioselectivity of this reaction can be understood in terms of the weak electrophilicity of the NO^+-generating species. Finally, the 5-alkoxy- (but not the 3,5-dialkoxy)-2-nitrosophenols can be easily oxidized with nitric acid to yield the desired 2-nitro compounds.

Because heteroanalogues of natural leads are of interest in investigations for their structure-activity relationship we have synthesized 2,4-dihydroxy-2*H*-1,4-benzothiazin-3(4*H*)-one (DIBTA) as the thioanalogous hemiacetal of DIBOA (Scheme VIII).

Reductive cyclization of methyl 2-methoxy-2-(2-nitrophenylthio)acetate by means of a catalytic transfer hydrogenation or of methyl 2-(2-nitrophenylthio)acetate by electrochemical reduction has been used as a key step in the synthesis of DIBTA and the corresponding lactam of this hydroxamic acid[38]. The hemiacetal function has been developed in all the three pathways by the substitutive approach from a halogen precursor. Surprisingly, the sulfur compound DIBTA in contrast to its natural counterpart DIBOA from rye did not undergo any degradation by extrusion of formic acid. Therefore, benzothiazol-2(3*H*)-one, which might have been expected as a degradation product was not observed. On the contrary, DIBTA proved to be stable even in aqueous solution forming a crystalline monohydrate from this solution on crystallization. Of course, the structural reason for this different behaviour is the sulfur atom. We assume that due to the larger size of S atom in comparison to O atom recyclization of the ring-opened oxo form (compare Scheme II) exclusively takes place to form the six- membered hemiacetal DIBTA, whereas in case of degradation a competitive five-membered lactol is discussed as intermediate[22d].

From the viewpoint of physical organic chemistry, hemiacetals like DIBOA, DIMBOA or HMBOA are interesting due to the oxo-cyclo tautomerism (see Scheme I). We have been the first to isolate DIMBOA in a pure form[39]. The natural compound as well as a synthetic sample are a racemic mixture of both enantiomers. We have been interested in a possibility to separate or at least to prove the existence of both enantiomers. Therefore, some analytical investigations have been undertaken on the free hemiacetals DIBOA and DIMBOA and their racemic methyl acetals. The latter ones are of course stable towards inversion of configuration in contrast to the hemiacetals.

First, a HPLC procedure on a 5 μm LiChroCART column using chemically bonded β-cyclodextrin (ChiraDex) as chiral selector was developed for the enantiomeric separation of the methyl acetals of DIBOA and DIMBOA[40]. Also, the corresponding lactam methyl acetals could be separated. However, the racemic DIBOA or DIMBOA could not be separated under similar conditions.This is obviously due to the



Scheme VIII—Synthesis of 2-hydroxy-2*H*-1,4-benzothiazin-3(4*H*)- ones –Thioanalogues of natural hemiacetals

racemization caused by the oxo-cyclo tautomerism of the hemiacetal unit, which occurs very rapidly all the time during the separation procedure. Therefore, only a single peak has been observed for both natural aglucones.

Cyclic hydroxamic acid derivatives are due to their remarkable acidity (*e.g.* pK_a of DIBOA and DIMBOA *ca.* 6.9) able to form anions in alkaline buffers (*p*H 8-10), which have been the subject of studies with high performance capillary electrophoresis (HPCE)[41]. An HPCE procedure for the enantioseparation of the methyl acetals of DIBOA and DIMBOA could be developed using borate buffers (*p*H 9-10 range), α-, -β- or γ-cyclodextrins as chiral additives to the mobile phase and addition of up to 20% methanol. The size of the chiral additive necessary depends on the shape of the methyl acetal. Again, free hemiacetals could not be separated due to the same effect as described above. However, HPCE proved to be an excellent method also for the separation of benzoxazinoid acetal glucosides, as shown by the base line separation of an artificial mixture of natural GDIBOA[14b] from rye and GDIMBOA[15b] from maize.

Finally, if enantioseparation proves to be impossible for DIBOA and DIMBOA, we expected at least for their enantiodifferentiation by means of a chiral NMR technique, because NMR is based upon a rapid measuring and differentiation process in comparison to the separation processes in chromatography. The discrimination of enantiomeric cyclic hemiacetals and methyl acetals has not been described yet by means of an NMR method. Among the methods known for chiral NMR measurements the best for our purpose was the chiral solvating agent (CSA) method. It is based on the principle that a pair of enantiomers on addition of a chiral solvating agent can be able to form a pair of diastereomeric solvation complexes. If this happens (which is not self-evident) one can observe a differentiation of signals which have been equivalent under achiral measuring conditions. The size of the so called chemical nonequivalence $\Delta\delta$ arising thereby depends very much on the structure of the (suitable) chemical solvating agent

used, but also on solvent, temperature and concentration. We required for our acidic hydroxamic acid a basic chiral solvating agent that should form such diastereomeric solvation complexes and lead to chemical nonequivalence of hitherto equivalent protons. The discrimination of enantiomeric cyclic hemiacetals and methyl acetals derived from hydroxamic acids and lactams having 2*H*-1,4-benzoxazin-3(4*H*)-one skeleton was investigated using (*S*)-(-)-phenylethylamine, (-)-quinine, β-cyclodextrin, and for the first time, (5*R*,11*R*)-(+)- 2,8-dimethyl-6*H*,12*H*-5,11-methano-dibenzo[*b*,*f*][1,5]dia-zocine, a Troegers base enantiomer, as chiral solvating agents (CSA)[42].

Indeed, DIBOA and DIMBOA have been the first cyclic hemiacetals that could be differentiated by means of ^{1}H NMR into enantiomers, despite their oxo-cyclo tautomerization. (-)-Quinine has been the most effect chiral solvating agent. Of course, enantioseparation of their methyl acetals has also been possible. The influence of the structure of enantiomers, CSA, temperature and concentration on the size of chemical shift anisochrony (Δδ up to 0.05 ppm was achieved) has been investigated in detail.

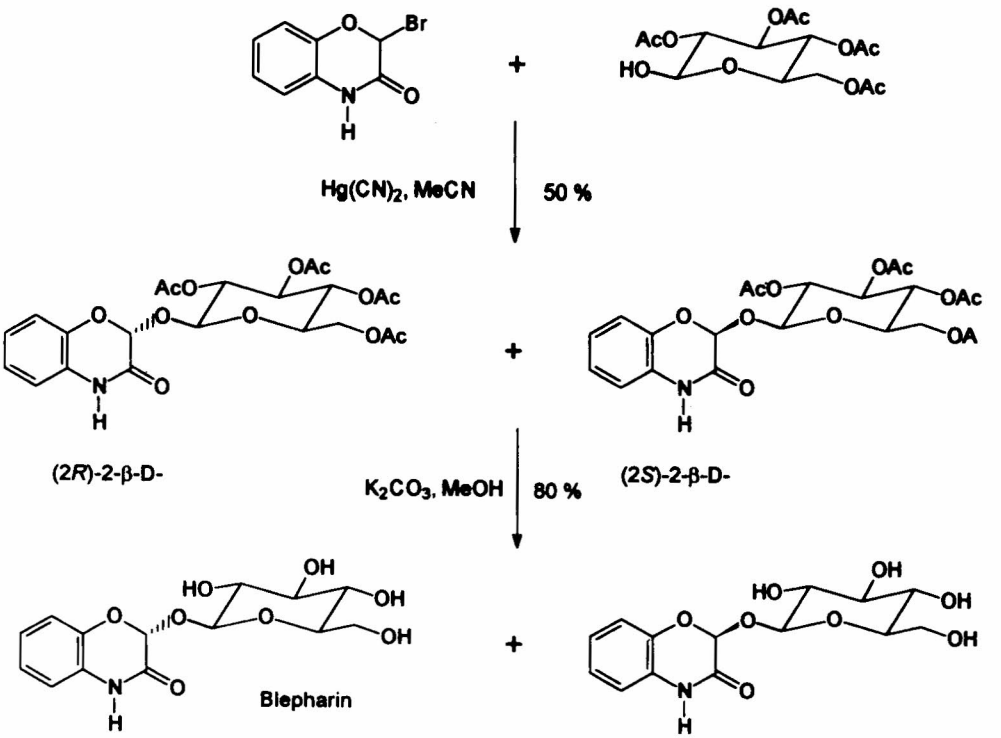
Diastereoselective Glucosidations. A multitude of glycosidation methods have been described for alcohols or phenols. Stereochemically, the synthesis of such glycosides deals with the problem of how to achieve a high single diastereoselectivity either for the formation of an α- or a β-linked glycoside, whereas the configuration of an alcoholic glycosyl acceptor is transferred unchanged to the product. In contrast, glycosidations of cyclic hemiacetals have seldom been reported. Most likely, this is caused by the necessity to control now the two configurational possibilities that can be formed at the anomeric centers of both the glycosyl donor and the acceptor. Therefore, in principle a set of four diastereomers is to be expected. Nevertheless, a total synthesis of a natural acetal glycoside with stereogenic centers in both the aglycone and the glycosidic unit can proceed with high diastereoselectivity due to the support by the asymmetric induction of both partners. An excellent example is the synthesis of iridoid glucosides, described by Tietze *et al.* using a trimethylsilyl β-glucopyranoside as glucosyl donor in the presence of catalytic amounts of trimethylsilyl triflate[43].

The benzoxazinone glucosides of interest offer the same stereogenic challenge, but with a reduced kind of support because no asymmetric induction results from a 2-hydroxy-2*H*-1,4- benzoxazin-3(4*H*)-one skeleton. Blepharin, [(2*R*)-2-β- D-glucopyranosyloxy-2*H*-1,4-benzoxazin-3(4*H*)-one], a natural product found in *Blepharis edulis* Pers[44], was the first benzoxazinone glucoside synthesized again by Tietze's group[45] by an inverse Koenigs-Knorr technique using the aglucone equipped with the bromine function (Scheme IX).

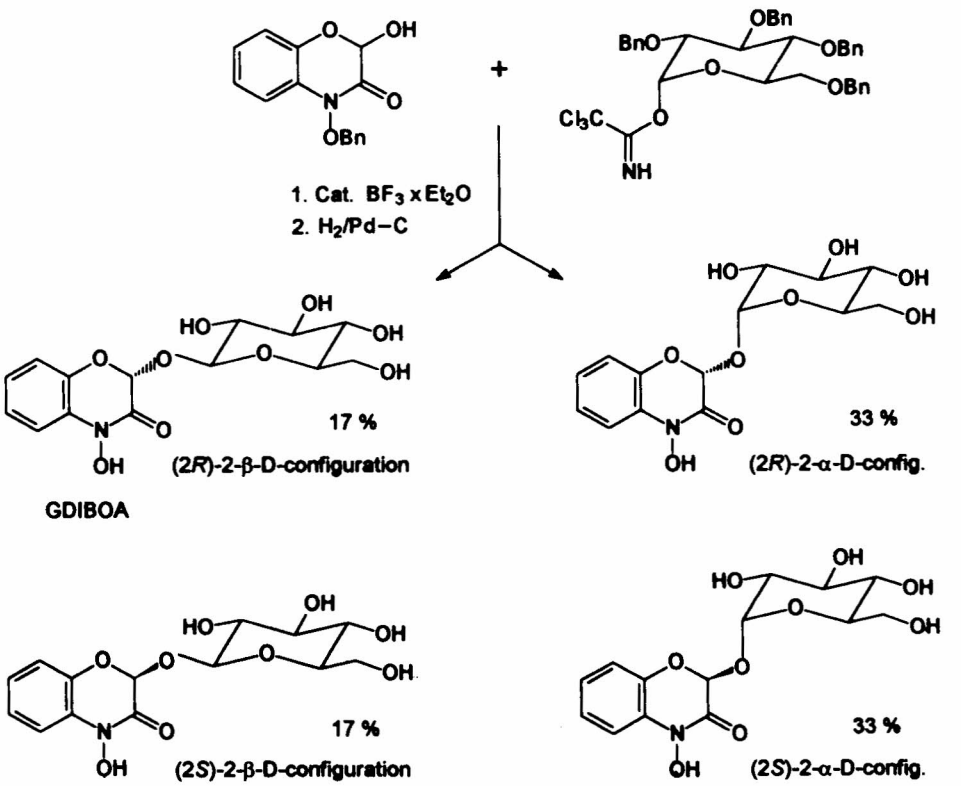
The glucosidation step yielded diastereoselectively two of the four isomers, (2*R*)-2-β- and (2*S*)-2-β- isomers (ratio 1:2), which have been separated by chromatography. The glucosidation of a benzoxazinoid compound of the hydroxamic acid type, *i.e.* a 2,4-dihydroxy-substituted benzoxazinone derivative, has not been reported yet.

Interested in the effects influencing the diastereoselectivity of the glucosidation reaction we started our work in this field with an attempt to synthesize GDIBOA. Using 4-benzyloxy-2-hydroxy-2*H*- 1,4-benzoxazin-3(4*H*)-one[30] as 4-protected glucosyl acceptor and *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl) trichloroacetimidate[46] as glucosyl donor (Scheme X). The reaction was performed in methylene chloride in the presence of a catalytic amount of boron trifluoride etherate. A mixture of all the four diastereomers has been obtained[47]. Advantageously, all protecting groups are removable in this case in one step by catalytic hydrogenolysis. However, due to similarity of the diastereomers all attempts to determine the diastereoselectivity by means of HPLC or NMR spectroscopy were of no avail. Fortunately, it was again possible by means of HPCE to separate the mixture of the four diastereomers. 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 2:2:1:1, with the α-isomers being in the majority[41]. Synthetic GDIBOA contained 17% of (2*R*)-2-β-isomer in the product mixture as determined by the addition of natural GDIBOA from rye seedlings[14b].

This distribution is an expression of the preference of the anomeric effect that favours the formation of α- glucosides due to the non-participating effect of the benzyl protecting groups. Nevertheless, it is surprising in a certain respect, because one might have expected a higher amount of β-glucosides when an α-linked trichloroacetimidate is reacted according to the S_N2 conditions. Obviously, this has either not



Scheme IX—Synthesis of Blepharin using an inverse Koenigs- Knorr technique



Scheme X—A synthesis of GDIBOA using benzyl protecting groups in the glucosyl donor and acceptor

been the case or equilibration governed by the anomeric effect has occurred to a certain extent.

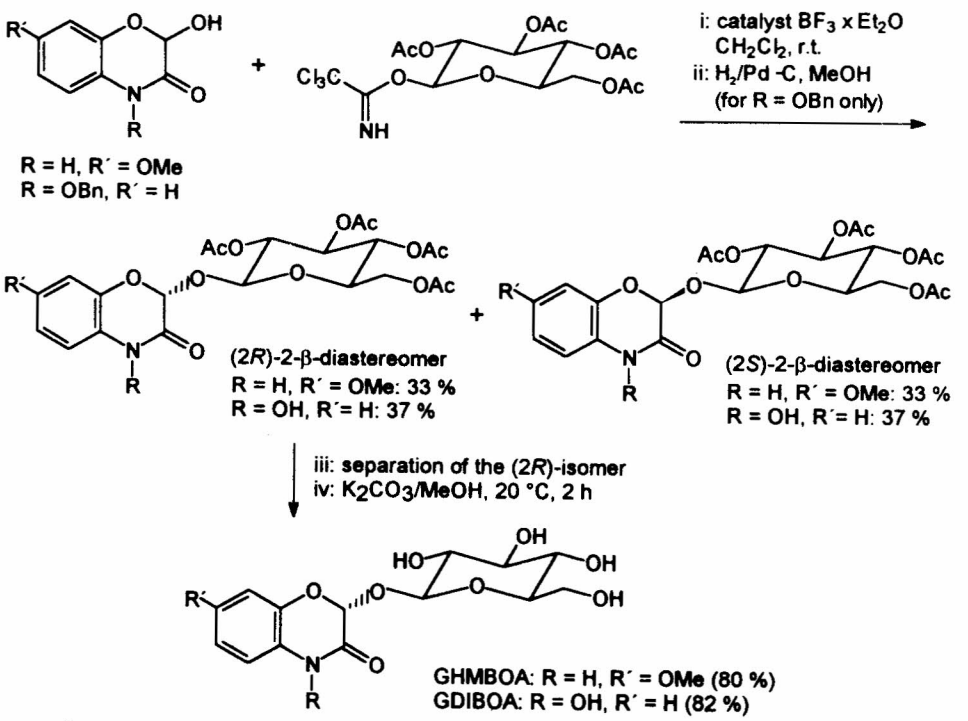
Of course, from the synthetic point of view a better diastereoselectivity has to be achieved. We have reported the first synthesis of GDIBOA[47] with full β-isomer, but not (*2R/2S*)-diastereoselectivity using *O*-(2,3,4,6-tetra-*O*-acetyl- β-D-glucopyranosyl) trichloroacetimidate[48] catalysed by boron trifluoride etherate for the glucosidation of 4-benzyloxy-2*H*-1,4-benzoxazin-3(4*H*)-one according to the Schmidt method[48] (Scheme XI). Acetyl-protected trichloroacetimidates like the one mentioned have been shown to give exclusively β-glucosides due to neighbouring group assistance of the acetyl groups[49]. The same glucosyl donor has been used for glucosidation of the lactam hemiacetal 2-hydroxy-7-methoxy-2*H*-1, 4-benzoxazin-3(4*H*)-one (HMBOA) to yield GHMBOA[47] after separation of its tetraacetate precursor from the (2*S*)-epimer.

However, all attempts to use this methodology for the synthesis of GDIMBOA, the by far most interesting acetal glucoside, have failed due to the strong influence of 7-methoxy group on the properties of the aglucone, which has been discussed most recently in a general context[21].

A successful synthesis of GDIMBOA has to meet the following requirements: (i) The racemic aglucone DIMBOA has to undergo the reaction without a need for a preceding covalent protection of the 4-position, because it proved to be impossible to achieve the protection of 4-position of DIMBOA by a typical protecting group such as a benzyl or trimethylsilyl group. This behaviour is obviously consistent with the 7-methoxy promoted ability to expel a substituent from the 4-position which is known for close analogues, for example derivatives of 4-hydroxy-7-methoxy-2*H*-1,4- benzoxazin-3(4*H*)-one[50]. (ii) A selective access should give rise to the natural (*2R*)-2-β-configuration exclusively. (iii) The method should be applicable to the synthesis of other benzoxazinoid hydroxamic acid glucosides like GDIBOA too.

Our double diastereoselective glucosidation procedure[51] for racemic cyclic hemiacetals containing a hydroxamic acid unit as well is shown in Scheme XII.

The essential feature of the new method of glucosidation consists in using an excess of boron trifluoride etherate. This Lewis acid accomplishes several functions. An interaction with the aglucone was proven by a comparison of the ^{1}H NMR spectra of DIMBOA in DMSO-d_6 in the absence and the presence of BF_3Et_2O. Both the signals for 4-OH and for 2-OH groups disappeared on the addition of BF_3Et_2O. Obviously, the anions of the hydroxamic



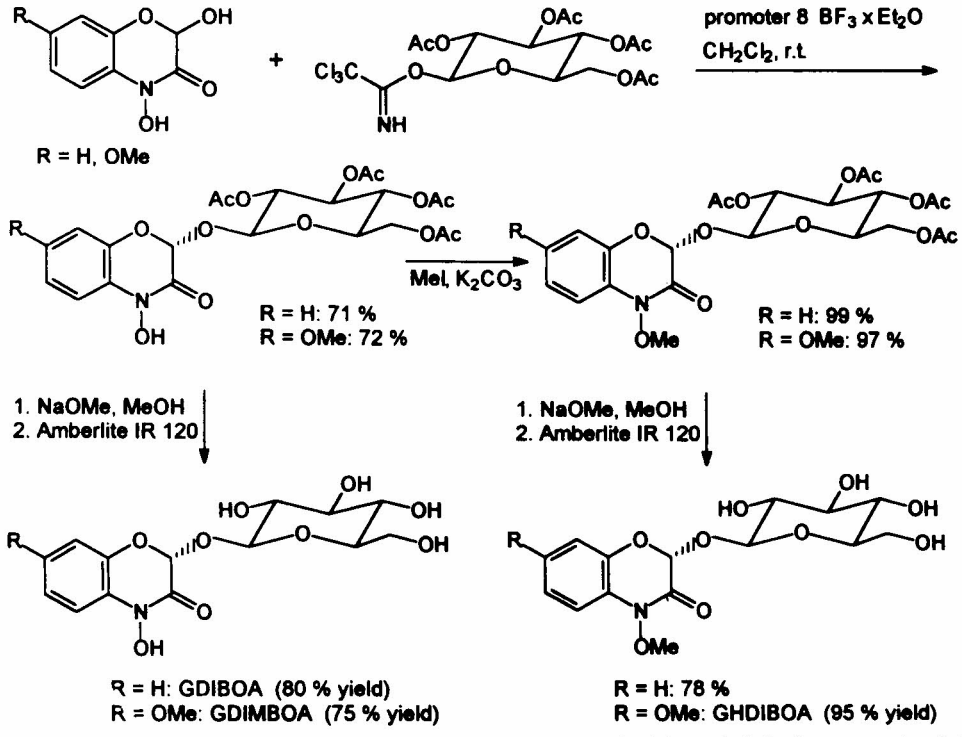
Scheme XI -- A synthesis of GDIBOA and GHMBOA with single β-diastereoselectivity

acid and the hemiacetal units of the aglucone are accepted as fourth ligands at boron atoms. For a further reaction with glucosyl donor the hemiacetalic function is clearly expected to be more nucleophilic than a hydroxamate ion which is stabilized by mesomerism. Thus, for the aglucones DIBOA and DIMBOA interaction with BF_3Et_2O means a noncovalent protection of their hydroxamic acid unit. Furthermore, in the case of DIMBOA it acts as a tool to receive the enhanced electron density which is supplied to the N- substituent by the 7-MeO group. In contrast to many unsuccessful attempts to stabilise DIMBOA by a covalent 4-protecting group, the ion pair now formed by this Lewis acid interaction proved to be stable.

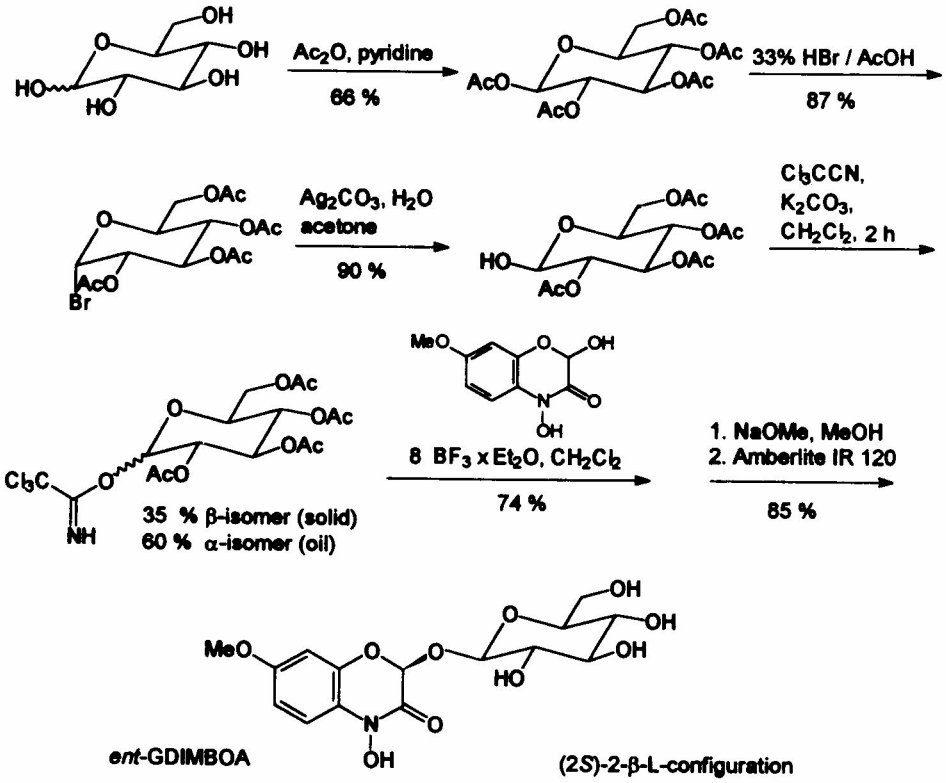
Improving the diastereoselectivity of the glucosidation reaction meant finding a way to distinguish between the natural (*2R*)-2- β- and the diastereomeric (*2S*)-2-β- configuration. We have found that changing from a catalytic amount (10%) of BF_3Et_2O hitherto used during glucosidation[47], to an 8-fold excess, results in generating conditions which promote the glucosyl transfer and allow equilibration to the thermodynamically more stable diastereomer, which proved to be that with the natural configuration.

This means it was possible for the first time to obtain in a generally applicable glucosidation reaction the tetraacetates of GDIBOA and GDIMBOA as the only reaction products. Therefore, a chromatographic separation of diastereomers was no longer necessary. Finally, these precursors were deprotected to give the acetal glucosides GDIBOA and, for the first time, to synthetic GDIMBOA. Furthermore, their tetraacetates were methylated with methyl iodide in acetonitrile in the presence of K_2CO_3 to afford the corresponding 4-methoxy derivatives. Deprotection of these compounds liberated the 4-methylated hydroxamic acid acetal glucosides GHDIBOA and its desmethoxy derivative, respectively. Whereas the latter one is an artefact which has not been described as a natural product yet, GHDIBOA, a known constituent of some *Gramineae* species[16], is of special interest because its aglucone 2-hydroxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (HDIBOA) is expected to show a very high bioactivity due to the ease of formation of a reactive electrophile that should be possible by extrusion of a methylate ion from the 4-position[16b]. This aglucone could not be isolated in pure form as yet. However, GHDIBOA is a possible precursor. A chemical *de novo* synthesis of HDIBOA will be reported in due course[52].

The structures of all (2*R*)-2-β-configurated acetal glucoside derivatives were established by spectroscopic methods. The β-configuration of the glu-



Scheme XII—A synthesis of GDIMBOA and GDIBOA with double (2*R*)-2-β-diastereoselectivity



Scheme XIII—Stereoselective synthesis of *ent*-GDIMBOA

cosidic bond could be assigned from the value of the coupling constant, (*J*), of about 8 Hz for the H-1′-H-2′ interaction. The (*2R*)-configuration could be proven by means of CD spectra, showing a positive Cotton effect at about 230 nm and a negative one at about 280 nm. This behaviour is analogous with the CD spectrum of natural GHMBOA[6c], for which also an X-ray structure exists. Furthermore, Tietze's rule for acetal glucosides[53], recently applied for the determination of configurations during the synthesis of Blepharin and 1′-Epiblepharin[45], also provides evidence for the (*2R*)-configuration.

As mentioned earlier, analogues of natural leads are of interest for structure-activity investigations. We have therefore decided to synthesize *ent*-GDIMBOA as the first enantiomer of a natural acetal glucoside. The synthesis of enantiomers of natural products is a field of increasing interest because such compounds find utility in structural studies and as probes for the elucidation of biological processes, a recent example being *ent*- enterobactin[54]. Therefore, the results obtained from the glucosidations discussed above were used to accomplish a stereoselective L-glucosidation of racemic DIMBOA affording *ent*-GDIMBOA[55] (Scheme XIII).

As can be seen from Scheme XIII the same principles discussed above have been followed in the synthetic pathway.

Summary

Benzoxazinoid acetal glucosides, a unique class of natural hemiacetal ethers, are of interest as biological precursors of a multi-centered electrophile the bioactivity of which arises from its biomolecule-alkylating action. The biological activity of these compounds as plant resistance factors against microbial diseases, allelo chemicals and endogeneous ligands is under continued investigation. These natural products are also interesting targets for a total synthesis. This review has given a survey on synthetic methods which give rise to the hemiacetalic aglucones of the 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one skeleton efficiently. Furthermore, a method for double diastereoselective glucosidation of racemic cyclic hemiacetals has been developed that affords an access to the acetal glucosides of natural configuration as well as to their enantiomers.

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