IMPORTANCE OF THE 2-HYDROXY GROUP FOR THE REACTIONS OF AN ACETATE OF A NATURALLY OCCURRING PROHIBITIN, 4-ACETOXY-2-HYDROXY-2H-1,4-BENZOXAZIN-3(4H)-ONE, WITH NUCLEOPHILES

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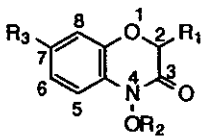
Abstract — A prohibitin, 2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-one, reacted readily with nucleophiles after 4-O-acetylation. The 2-hydroxy group is mandatory for the reactions. The plausible interpretation for the enhancing effect of the 2-hydroxy group on the benzoxazinone system's reactivity involves an acetal ring-opening and reclosure mechanism.

A prohibitin, 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one (DIBOA: 1), occurs in cereal plants,[1] and its roles in allelopathy and herbicide tolerance have been established.[2] DIBOA exists in the undisturbed plant as a biologically inactive glucoside (the 2-hydroxy group of the benzoxazinone ring is glycosylated) that, upon cell disruption, is enzymatically converted to the corresponding biologically active aglycone, suggesting the functional importance of its 2-hydroxy group.[3] Our *in vitro* study on the mutagenic toxicity of DIBOA showed that metabolic activation of the compound, such as the acetylation of the 4-hydroxy group, is also important for eliciting DIBOA's biological activity.[4] Previously, we have reported that the acetate of the 2-deshydroxy analog bearing the 7-methoxy substituent (2) can act as a biomacromolecule-alkylating agent.[5-10] However, 2 is not a good model of the prohibitin because it lacks the biologically important 2-hydroxy group. Here, the role of the 2-hydroxy group is clarified: the acetate

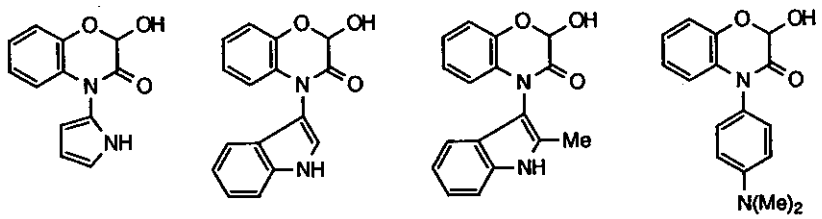
(3)[12] of DIBOA reacts readily with nucleophiles by heterolysis of the N-O bond.



R_1=OH, R_2=H, R_3=H (1: DIBOA)
R_1=H, R_2=Ac, R_3=OMe (2)
R_1=OH, R_2=Ac, R_3=H (3)
R_1=H, R_2=Ac, R_3=H (4)

The reaction of 3 with pyrrole proceeded at room temperature (1.5 h) to give 5 (60% yield), of which the structure was deduced from its ir and ^{1}H-nmr spectra (the absence of the NH hydrogen at position 4) and elemental analysis. Reactions of 3 with indole, 2-methylindole and N,N-dimethylaniline proceeded similarly to give compounds 6 (34%), 7 (38%), and 8 (30%), respectively. For all of these nucleophiles, the nitrogen atom at position 4 of the benzoxazinone ring was the reaction center.

Figure 1. Structures of 4-Substituted Benzoxazinone Adducts

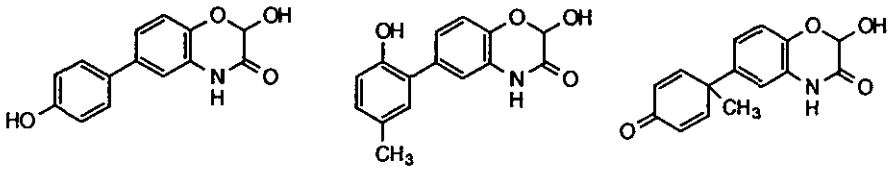


5 6 7 8

The reactivity of phenols with 3 markedly differed from that of the former nucleophiles. The reaction of 3 with phenol at room temperature gave 9 in 14% yield. The reaction site was not the nitrogen atom but the carbon atom at position 6 of the benzoxazinone, as indicated by the ir (3160 cm^{-1}; NH absorption) and ^{1}H-nmr [6.97 (d, J=8.2 Hz), 7.06 (d, J=2 Hz), 7.11 (dd, J=2, 8.2 Hz); benzoxazinone's three aromatic hydrogens] spectra and elemental analysis. The structure of 9 was conclusively decided by its conversion to 3-acetamide-4,4'-dihydroxybiphenyl. In the reaction of 3 with phenol, the presence of acid (trifluoroacetic acid, 1 equiv.) increased the yield of 9 to 74%. The reaction of 3 with p-

cresol also yielded 6-substituted benzoxazinones, 10 (17%) and 11 (16%). The structure of 11 was confirmed by ir, uv, elemental analysis and ^{1}H-nmr [6.17 and 7.03 (d, J=10.2 Hz, A_2B_2), and 1.61 (s, 3H, ipsomethyl)].

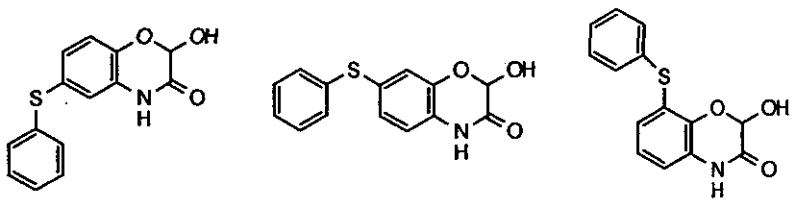
Figure 2. Structures of Reaction Adducts with Phenols



9 10 11

A thiol nucleophile, thiophenol, attacked the 6-position of 3 giving 12 (18%), together with 7- and 8-phenylthiobenzoxazinones (13 and 14, isolated after acetylation, total yield: 19%). The sites of substitution on the benzoxazinone moiety were deduced from the ^{1}H-nmr spectra (all proton signals were unequivocally assigned).

Figure 3. Structures of Reaction Adducts with Thiophenol



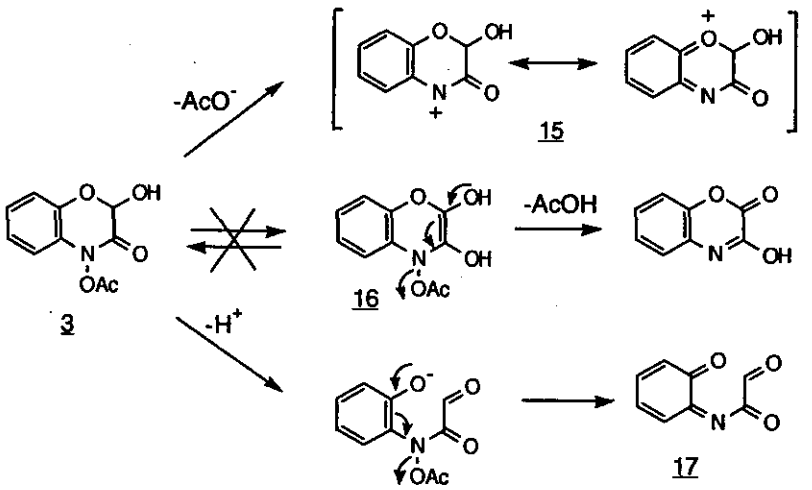
12 13 14

The reactions of 3 with these nucleophiles can be superficially interpreted by the participation of the cation 15 formed by the heterolysis of the N-O bond. One of the canonical forms of 15 is an o-benzoquinone monoimine, of which the multi-centered electrophilic reactivity has been well discussed.[9,12]

Previously we have reported the reactions of the 2-deshydroxy analogs of DIBOA with nucleophiles[9]: the acetate (2) reacted with nucleophiles such as phenols, indoles, amino acids, and nucleic acids at various positions (the 2-, 4-, 5-, and 6-positions) according to the nature of the nucleophiles and reaction conditions. On the other hand, the reactions

of the acetate without the methoxy group (4) hardly proceeded under similar or even more severe reaction conditions. It appeared that for the heterolytic reactions with the nucleophiles, the electron-donating 7-methoxy group is required. However, the acetate (3) of DIBOA reacted with nucleophiles even though 3 lacks the electron-donating 7-methoxy group: the presence of the 2-hydroxy group enhanced the reactivity. As a possible explanation for the enhancing effect of the 2-hydroxy group on the reactivity of the benzoxazinone system, we suspected the participation of the enol form (16). However, no deuterium incorporation of the methyne hydrogen at position 2 was observed in the reaction of 3 with pyrrole to give the adduct (5) in D_2O/DMSO, excluding any mechanism involving a proton exchange at position 2. Moreover, the participation of 16 can not account for the formation of the 6- and 8-substituted benzoxazinone adducts. Another possible mechanism considered is the stereo-electronic effect[13] of the 2-hydroxy group, which enhances the electron-donating effect of the ether oxygen, and facilitates the N-O bond heterolysis. But, this does not explain the lack of reactivity of the glucoside. A more plausible mechanism involves a ring-opening and reclosure. The opening of the acetal ring of 3 followed by N-O bond

Figure 4. Possible Reaction Pathways



heterolysis would result in formation of the o-benzoquinone monoimine (17), which might be an electrophile.[12] This would explain the requirement of the free hydroxy group, and the acid-promotion of the reaction. A proton activates the electrophilicity of 17 as well as activating the heterolysis of the N-O bond.

In conclusion, a plausible metabolite of naturally occurring DIBOA, i.e., compound (3), reacted with nucleophilic compounds under mild conditions. The importance of the 2-hydroxy group was shown. As the nucleophiles used in the experiments can be regarded as representatives of nucleophilic moieties of biomacromolecules such as proteins and nucleic acids, the reactions we have reported in this short paper should afford information concerning the chemical basis for the molecular mechanism of biological actions elicited by DIBOA.

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11. Compound (1) was acetylated with ketene gas in a cold ethereal solution to give crystalline 3 (mp 98-99℃ , y: 93%), which can be stored in a refrigerator for a few weeks without decomposition.
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