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Isolation and Characterization of 4-Acetyl-benzoxazolin-2-one (4-ABOA), a new Benzoxazolinone from *Zea mays*

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Abstract: The previously unreported 4-acetyl-benzoxazolin-2-one (4-ABOA, **1**) was isolated from corn kernels. Its structure was determined by MS, NMR and X-Ray crystallography.

In the past few decades, there has been much interest concerning the nature of disease resistance in *Zea mays*. Although a number of biologically active metabolites have been isolated from corn,[1] little is known of the implication of such metabolites in fungal resistance to species such as *Fusarium*. Such compounds may interfere with the enzyme trichodiene synthetase required for the formation of trichothecene mycotoxins. Some flavonoids and furanocoumarins are known to inhibit T-2 toxin biosynthesis in liquid cultures of *Fusarium sporotrichioides*.[2] This work describes the isolation and characterization of 4-acetyl-benzoxazolin-2-one (4-ABOA, **1**) from kernels of a *Fusarium*-resistant hybrid of *Zea mays*. Studies are in progress to determine its antifungal activity.

Results. Corn kernels (Funks 4106; Ciba-Geigy seeds) were ground through a No. 40 mesh screen. 1 kg of the corn powder was extracted with 80% aqueous MeOH following a method developed by Collins[3]. The extract was concentrated under vacuo, taken up in 2-propanol/H_2O (1:1; 500 mL) and then passed through an Octyl Sepharose CL-4B column to remove neutral lipids. Strongly acidic components were removed by passing the resulting eluant through Sephadex QAE A-25 in the acetate form. The column was then converted to the hydroxide form with 2-propanol/H_2O/NH_4OH (12:7:1) and the eluant was passed through to retain the weakly acidic components, which were then removed from the column with 2-propanol/H_2O/formic acid (5:4:1). 4-ABOA was isolated from this weakly acidic fraction by column chromatography on LH-20 (20% acetone/H_2O K_1-K_2 fraction)[3] followed by reverse phase C-18 (MeOH/H_2O/AcOH, 30:65:5). Fractions were monitored by TLC (C18 MeOH/H_2O/AcOH 50:45:5). Recrystallization from acetone/H_2O gave white needles (27 mg, m.p. 217-8°C); TLC R_f = 0.56; HPLC[4] R_t= 30.91 min.; UV (MeOH) λ_{max} (log ε) 250.2 nm (3.99), 320.2 (3.71), (MeOH + NaOH) 268.6 (3.95), 351.6 (3.87); IR (neat) 3100-3300, 1765 cm^{-1} (C=O stretching, carbamate); HRMS[5] cal'd. for $C_9H_7NO_3$ 177.0426, obs. 177.0425, m/z 43(35), 51 (12), 63 (8), 78 (10), 106 (23), 134 (8), 162 (base), 177 (M^+, 72); ^1H NMR[6] (δ) 2.60 (**CH_3**CO, 3H, s), 7.21 (H-6, dd, $J_{6,5}$= 8.2 Hz, $J_{6,7}$= 8.2 Hz), 7.41 (H-7, dd, $J_{7,5}$= 0.7 Hz, $J_{7,6}$= 8.2 Hz), 7.75 (H-5, dd, $J_{5,6}$= 8.2 Hz, $J_{5,7}$= 0.7 Hz); ^{13}C (δ) 157.0 (C-2), 131.6 (C-3a), 121.7 (C-4), 126.0 (C-5), 122.7 (C-6), 115.0 (C-7), 146.0 (C-7a), 26.0 (CH_3CO), 199.0 (**CH_3**CO) .

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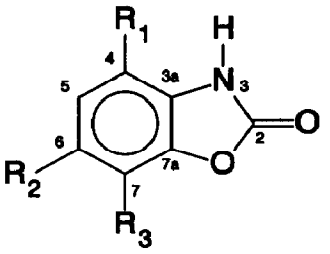
Discussion. A new compound has been isolated from the weakly acidic fraction of the extract from kernels of a *Fusarium* resistant hybrid of *Zea mays.* On TLC this material appears as a pale blue band under UV light (365 nm) and bright blue under UV light after being sprayed with a 5% solution of ethanolamine in 2-propanol. The UV spectrum (250, 320 nm) showed reversible bathochromic shifts in base (268, 351 nm), suggesting the presence of a reversible, extended conjugated system. The HRMS exhibited a molecular ion at m/z 177.0426 corresponding to a molecular formula $C_9H_7NO_3$. The base peak at m/z 162 (M-15) and a fragment ion m/z 43 suggested loss of an acetyl moiety. The ^1H NMR spectrum showed three resonances in the aromatic region and a singlet at 2.60 ppm ($COCH_3$). The aromatic resonances show ortho (J_{56}= J_{67} = 8.2 Hz) and meta (J_{57}= 0.7 Hz) coupling, confirming the adjacency of the three aromatic protons. The ^{13}C NMR spectrum contained 9 resonances, six assigned to a benzene ring, two carbonyls and one methyl function. Direct $^{13}C/^1H$ correlation established the relative positions of the methine carbon resonances at 126.0 (C-5), 122.7 (C-6) and 115.0 ppm (C-7) of the benzene ring. The juxtaposition of these carbons to the substituents at C-4, 3a and 7a was determined by inverse-detected long range $^{13}C/^1H$ correlations adjusted to emphasize $^3J_{CH}$ (~8 Hz) over $^2J_{CH}$ (~4 Hz). Thus the resonance due to H-7 at 7.41 ppm correlated to carbon resonances at 126.0 ppm (C-5) and 131.6 ppm (C-3a), the resonance at 7.21 ppm (H-6) correlated to those at 121.7 ppm (C-4) and 146.0 ppm (C-7a), while the H-5 resonance at 7.75 ppm correlated to 115.0 ppm (C-7), 131.6 ppm (C-3a) and 199.0 ppm (C=O). The methyl resonance at 2.60 ppm also showed a correlation to the carbonyl resonance at 199.0 ppm. There were no correlations to the other carbonyl resonance at 157.0 ppm. Thus the methyl and carbonyl resonances at 26.6 ppm and 199.0 ppm respectively were assigned to an acetyl moiety at C-4. The nitrogen function at C-3a and the oxygen function at C-7a are assigned on the basis of chemical shifts of those and adjacent (ortho) carbon resonances. The assigned structure (**1**) is consistent with this data.



Compound (Trivial Name)		R_1	R_2	R_3	Ref.
1	(4-ABOA)	CH_3CO	**H**	**H**	**this work**
2	(BOA)	H	H	H	8
3	(MBOA)	H	OMe	H	9
4	(DMBOA)	H	OMe	OMe	10

The proposed structure (**1**) for 4-ABOA was confirmed by X-ray crystallographic analysis. The ORTEP drawing of 4-ABOA is shown in Figure 1.[7]

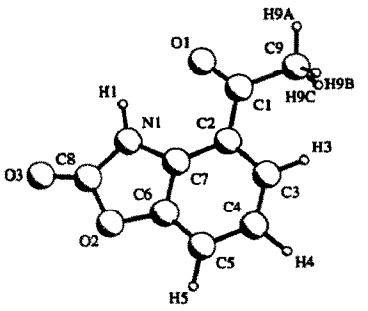


Figure 1.

Benzoxazolin-2-one (BOA, **2**) was first isolated from rye seedlings.[8] Together with the 6-methoxy (MBOA, **3**) and the 6,7-dimethoxy (DMBOA, **4**) analogues, these are the only benzoxazolin-2-ones to be isolated from natural sources.[8-10] The 5- and 6-acetyl derivatives and other analogues have been synthesized and exhibit antiinflammatory properties.[11] Benzoxazolin-2-ones are known to be degradation products of 1,4-benzoxazin-3-ones.[12] Benzoxazinones occur in cereals as the 2-β glucosides. During initial extraction, the glucoside is cleaved by released plant enzymes and in aqueous solutions the aglycon quickly degrades to form the benzoxazolinone.[13] Although the glucosides have been shown to exhibit relatively little biological activity, both the aglycones and benzoxazolin-2-ones are known to be quite active.[14] They have been shown to play a role in the resistance of plants to a number of insect species,[15] bacteria[16] and fungi.[17] These observations raise the possibility that 4-ABOA arises from the corresponding 4-acetyl-1,4-benzoxazin-3-one. A number of methods are now being examined to detect both the glycoside and its aglycon.

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4. **High Pressure Liquid Chromatography (HPLC)**. 4-ABOA analysis was carried out on a Varian Vista 5000 HPLC system, equipped with a UV200 detector (set at 320nm) and a Varian DS402 data system. 4-ABOA was injected onto a Merck HiBar RP-18 5μm column (0.3 x 25cm) using the solvent program: 0-15 min., 20% MeOH in H_2O isocratic; 15-45 min., 20-70% MeOH in H_2O; 45-47 min., 70-100% MeOH in H_2O; 47-52 min., 100% MeOH; 52-57 min., 100-20% MeOH/H_2O and a flow rate of 1ml/min.
5. **High Resolution Mass Spectrometry (HR/MS)**. Mass spectra were obtained on a Finnigan-MAT 90 system with an ICIS data system. 4-ABOA was analysed by solid probe at a resolution of 3000.

6. **Nuclear Magnetic Resonance (NMR).** ^{1}H and ^{13}C NMR spectra were run on a Bruker AM500 NMR spectrometer. Chemical shifts were referenced to deuteromethanol at 3.30 and 49.0 ppm for ^{1}H and ^{13}C respectively, and reported relative to tetramethylsilane (Me_4Si). ^{13}C chemical shift assignments were made with $^{1}H/^{13}C$ long range correlation spectra, acquired by inverse detection.
7. A crystal of **1** was mounted on a glass capillary. All the measurements were made on a Rigaku diffractometer with Mo Kα radiation. Cell constants and an orientation matrix for data collection, were obtained from least squares refinement using the setting angles of 25 reflections in the range $40<2\theta<50$ corresponded to a monoclinic cell with the dimensions: a = 8.7388 (19), b = 10.8035 (24), c = 16.652 (4), β = 101.028 (20). For Z = 8 and FW = 177.16, the calculated density is 1.525 g/cm^3. Based on the systematic absences, the space group was determined to be $P2_1/c$. The data were collected at a temperature of -150°C using the $\Phi/2\theta$ scan technique to a maximum 2θ value of 49.9. A total of 2897 reflections was collected. The unique set contains only 2711 reflections. The standards were measured after every 150 reflections. No crystal decay was noticed. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods. All the atoms were refined anisotropically except the hydrogen. The hydrogen atoms were found by differences Fourier maps. The final cycle of full matrix least-squares refinement was based on 2201 observed reflections ($I>2.5\ \sigma(I)$) and 292 variable parameters. Weights based on counting statistics were used. The maximum and minimum peaks on the final differences Fourier map corresponded to 0.290 and -0.320 a/a3, respectively. All the calculations were performed using the NRCVAX crystallographic software package.

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