Indole is an intermediate in the biosynthesis of cyclic hydroxamic acids in maize

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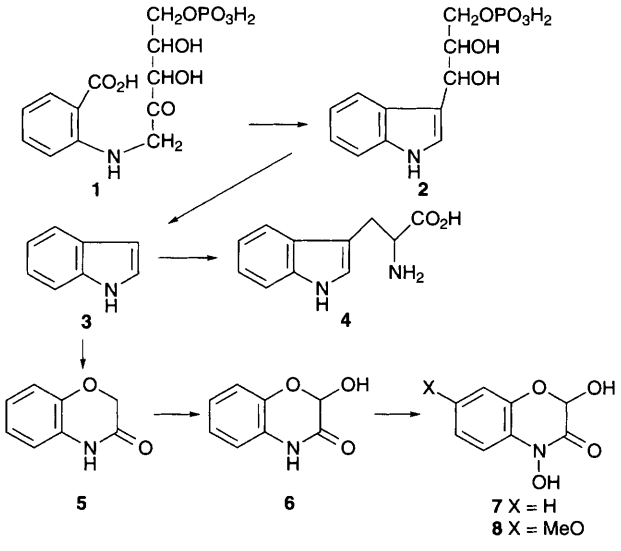
Mass spectrometry, deuterium NMR and TLC radioactivity analysis of 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) 8, recovered from maize shoots grown in the presence of indole-[$^2H_6$] and 2-[^{14}C]indole show that indole is a precursor of DIMBOA.

The cyclic hydroxamic acid 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) **8** is the major defensive metabolite of young maize and wheat plants.[1] DIMBOA is stored as the glucoside. Rye contains the glucoside of the corresponding unmethoxylated cyclic hydroxamic acid DIBOA **7**. Following wounding of maize or wheat seedlings a glucosidase releases DIMBOA, an unstable toxic compound which subsequently decomposes to the less toxic 6-methoxy-2(3*H*)-benzoxazolone (MBOA). The defensive role of DIMBOA against insects, fungi and bacteria is well documented.[1,2]

Previous biosynthetic studies showed that DIMBOA is synthesized independently in maize roots and shoots.[3] Anthranilic acid and ribose were incorporated into DIMBOA with relatively low dilution of radioactivity, suggesting that the DIMBOA pathway (Scheme 1) has some intermediates in common with tryptophan **4**.[4,5] Involvement of the tryptophan pathway was further supported by the observation that the radioactivity of 1-[^{14}C]ribose administered to maize seedlings was incorporated at C-3 of DIMBOA. The formal branch point between the DIMBOA pathway and the tryptophan pathway must occur at one of the three intermediates 2-carboxyanilino-1′-deoxyribulose-5′-phosphate **1**, indoleglycerol-3′-phosphate **2** or indole **3** because [^{14}C]tryptophan administered to maize shoots was not incorporated into DIMBOA.



Scheme 1 Biosynthetic pathways to tryptophan and cyclic hydroxamic acids

We have now demonstrated that axenic maize shoot cultures convert 2-[^{14}C]indole into [^{14}C]DIMBOA. The identity of the [^{14}C]DIMBOA was confirmed by cochromatography with authentic DIMBOA and by conversion of [^{14}C] DIMBOA to [^{14}C]MBOA by heating for 1 h at 80 °C in pH 7 buffer. Maize leaf extract has been reported to oxidize indole to *N*-formylanthranilic acid, which is subsequently hydrolysed to anthranilic acid.[6] While anthranilic acid is a precursor of DIMBOA, this route is not responsible for the introduction of radioactivity into DIMBOA because the ^{14}C-carbon atom would be lost in formic acid. Therefore indole is in the direct pathway to DIMBOA. Indole-[$^2H_6$] was administered to maize shoot cultures to confirm that indole is a precursor of DIMBOA. Mass spectral analysis indicated that 45% of the DIMBOA was derived from deuterated indole and 55% from sucrose and inorganic nitrate present in the growth medium. The deuterium NMR of recovered DIMBOA showed equal amounts of deuterium at the three aromatic carbons and at the acetal carbon of the heterocyclic ring of DIMBOA, indicating that one of the heterocyclic ring hydrogens of indole is preserved in the heterocyclic ring of DIMBOA.

These results establish indole as the substrate at the formal branch point between the tryptophan and DIMBOA pathways. Intermediates beyond the branch point have been shown to be benzoxazinone **5**, 2-hydroxybenzoxazinone **6** and DIBOA **7**.[7]

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Footnote

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