DEGRADATION STUDIES OF WHEAT HYDROXAMIC ACIDS

Macías F. A., Oliveros-Bastidas A., Castellano D., Marín D., Simonet A. M., Molinillo J. M. G.

Grupo de Alelopatía. Departamento de Química Orgánica. Facultad de Ciencias. Universidad de Cádiz, Apdo 40. 11510- Puerto Real, Cádiz- Spain

Cereal plants produce a series of benzoxazinoid compounds (cyclic hydroxamic acids). The number of this group of natural products is reduced but they exhibit a diversity of biological activity. It has been reported that these compounds are involved in the defense of plants against fungi and insects, as well as in allelopathic interactions. The most important benzoxazinoids reported are 2,4-dihydroxy-1,4-benzoxazine-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), which are present in wheat, maize and rye, and have been found in members of families Acanthaceae, Rannunculaceae and Scrophulariaceae. These compouns are in plant as glycosides and are released as aglycones by the activity of the enzyme β-glucosidase.

Moreover, these aglycones are unstable in solution and soil, being transformed to different derivatives as BOA, MBOA, and other degradation products (Figure 1). These transformations depend on the chemical and biological conditions, and must be studied, because some of these transformation products could be more active than the original ones.

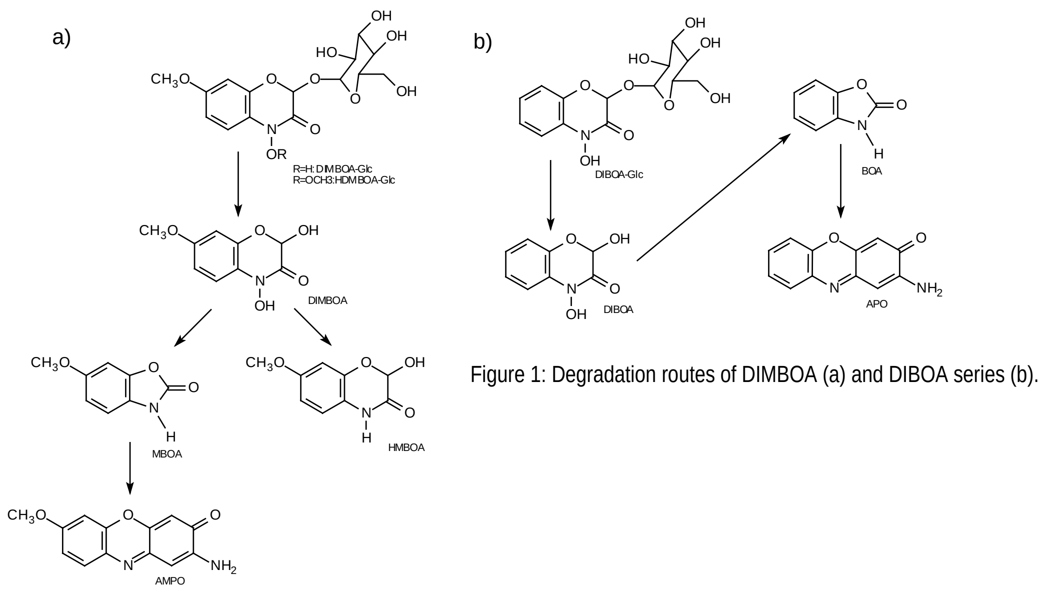

Figure 1: Degradation routes of DIMBOA (a) and DIBOA series (b).

It is necessary, therefore, the study of the physical-chemical dynamics of these allelochemicals in order to know as nature and effective concentrations of each species that are present in soil. This will be helpful for the bioactivities studies and the interpretation of the obtained data.

Here, we report the stability and degradation studies of DIBOA, DIMBOA and their derivatives in different conditions and soils used to cultivate two wheat varieties (*Triticum aestivum* varieties Astron and Ritmo). Previous publications study the isolation, characterization and biological activity of the degradation products. However, there are not the dynamic aspects of the degradative processes of these compounds as half-life, conversions in different dosage or competition for substrates.

Soils were chosen by using a double criterion: Firstly, total content of major hydroxamic acids of wheat varieties were analyzed in six varieties, being Astron, Ritmo and Portal the first ones selected. Then, a general bioactivity assay was carried out to leave lixiviates of those varieties, being the most active ones Astron, Ritmo and Portal. Then Astron and Ritmo were selected because of the higher insecticide and fungicide activity of their lixiviates. Thus, soils of these varieties were chosen for the degradation studies, in order to imitate natural conditions as accurately as possible, and to evaluate which variety could have a higher interest in order to take advantage of its defense capabilities. Thus, samples were taken from the vicinity of the roots, in order to collect the maximum density of microbial population associated to ground parts of the plant. Inoculation of hydroxamic acids and their derivatives to solutions of those soils allowed to record their conversions and evaluate their dynamics.

Degradation studies were carried out in three different systems: aqueous buffer (to discard spontaneous degradation in aqueous medium), sterile soil (in order to evaluate possible influence of metal traces or other soil elements on available concentration of hydroxamic acids) and the culture soil selected as described above.

The analysis data for degradation studies in buffered aqueous solutions were recorded by using a micropurification HPLC system with an UV-VIS spectrophotometry detector in reverse phase conditions. separation, identification and quantification of allelochemicals detected was possible by using pure standards of the compounds of interest.

The studies of degradation in soil were achieved by using an HPLC-DAD-MS system. Compounds were identified by comparing the corresponding UV-VIS and mass spectra with those of the previously prepared standards. Their structures were confirmed by monodimensional and bidimensional NMR experiments. By taking samples of soil solution at different times of incubation, and discarding effects from soil materials and spontaneous degradation processes, evolution of DIMBOA, DIBOA, its glycosides and their derivatives MBOA and BOA was recorded. Studies about influence of dose, soil type, and substract competition experiments were also achieved (Figure 2).

Conversion rates of benzohydroxamic acids to benzoxazolinones in soil
(Peak area relations vs. Time)

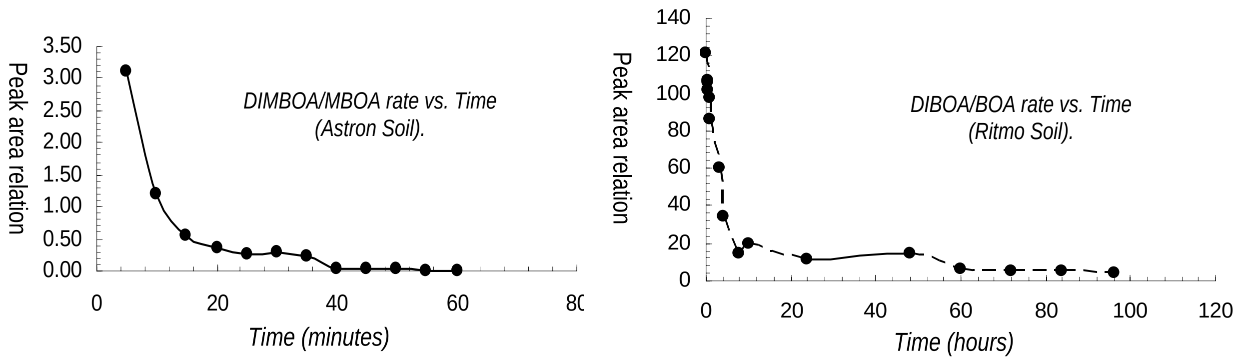

Figure 2