D. HORMONE ANALYSIS

D1. Instrumental Methods of Plant Hormone Analysis.

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INTRODUCTION

From information presented in previous chapters it will be clear to readers that plant hormones are, as a rule, present at very low levels in most plant tissues. Whilst relatively high levels of some hormones are found in immature seeds of certain species (e.g., GAs in developing pea seeds (10)) even these levels are low when compared with the levels of most plant secondary metabolites. Thus while many alkaloids, terpenoids and phenolics may be present at levels of mgs. per gm. dry weight of plant material, plant hormones are usually present at several hundred to several thousand fold lower levels. It is not surprising therefore that knowledge of the chemical identity of plant hormones has been limited by the techniques available for their isolation in a pure state and by the sensitivity of the spectroscopic techniques required to elucidate their chemical structure.

In the last ten years there have been spectacular improvements in the sensitivity of spectroscopic methods of structure determination and corresponding increases in performance in chromatographic techniques, principally via the development of high performance liquid chromatography (HPLC) and capillary column gas-liquid chromatography (GLC). This improvement in methodology can be clearly seen if one compares the isolation and identification of zeatin by Letham in 1963 (15), where 60 kg of plant material had to be extracted and purified by traditional chromatographic methods to yield the mg. of material needed for spectroscopic studies, with the identification of 1’-deoxy ABA as a precursor of abscisic acid (ABA) in the fungus Cercospora rosicola (25) where, after purification by HPLC, identification was possible at the μg level.

This chapter is concerned with the application of modern instrumental techniques to the isolation, identification and quantitation of plant hormones. Clearly the theoretical background to these
techniques is beyond the scope of this work and readers are referred to suitable textbooks for this information (e.g., 9, 28, 19). However, it is very important for a critical understanding of the methods used in the identification of plant hormones to appreciate the inherent limitations of the various techniques and so these will be touched upon in the relevant sections. In particular it is necessary to appreciate the importance of sample purity to the interpretation of spectroscopic data. Even with the most sophisticated instrumentation, correct identifications can only be made if the spectroscopic data obtained is relevant to the hormone under investigation. Two examples of mistaken identities provide informative reading on this point (33,5).

The chapter is organised in the chronological order in which a real analysis of a plant hormone would probably proceed. First, the compound would have to be isolated in a sufficiently pure form, second, its structure would have to be determined by appropriate methods, and finally a strategy would need to be devised for its quantitative measurement.

ISOLATION AND PURIFICATION OF PLANT HORMONES

Extraction and Preliminary Purification

The methods of extraction and preliminary purification of plant hormones using traditional methods such as solvent partitioning, ion exchange chromatography and, paper and thin layer chromatography will not be discussed in this chapter as strictly speaking they fall outside the area of instrumental methods and many of these methods are being superseded by HPLC based methods. Nevertheless it is often necessary to revert to older methods particularly with plant extracts that are too large for the initial use of HPLC. The readers attention is drawn to the extremely comprehensive treatment of these methods by Yokota et al. (34).

Bioassays

Although the bulk of this chapter is concerned with the use of physical methods for the detection of plant hormones it should be noted at this point that the primary detection of any novel plant hormones is dependent on biosassay. Bioassays are also necessary when studying the hormone content of novel plant materials particularly with regard to gibberellins and cytokinins. Because of the trace nature of plant hormones in most extracts, direct physico-chemical detection is impossible during the early stages of purification. In the case of the gibberellins even detection at the latter stages of purification is difficult due to the low wavelength and low extinction coefficient of UV absorption by these compounds. In these situations bioassays have to be used to detect the compounds of interest and to monitor the purification process. The choice of suitable bioassays