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This Week's Citation Classic

Milborrow B V. The identification of (+)-Abscisin II [(+)-Dormin] in plants and measurement of its concentrations. *Planta* **76**:93-113, 1967. [Shell Res. Ltd., Milstead Lab. Chem. Enzymol., Sittingbourne, Kent, England]

The optical rotatory dispersion (ORD) of (+)-abscisic acid (ABA) was used to identify the hormone in plant extracts. A number of plants were known to contain a growth-inhibitory chromatographic fraction 'inhibitor β.' Quantitative bioassay of 'β' and ORD showed that the activity was attributable to ABA. [The *SCI*® indicates that this paper has been cited over 235 **times since 1967.]**

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"This research was carried out because several observations suddenly interrelated. Our group had isolated the 'dormancy hormone' from sycamore and found that it was intensely optically active. It was identical with Abscisin II (now called abscisic acid —ABA) isolated from cotton by F.T. Addicott's group and we had just synthesised their proposed structure. The optical rotatory dispersion (ORD) spectrum could be used to identify and measure the natural hormone's concentration in partially purified extracts (stress was placed on the need for corroborative chromatographic and physiological data).

"The ORD method showed that most of the growth-inhibitory material, ubiquitous in plant extracts, long known as 'inhibitor β,' was abscisic acid. Repetition of the published extractions gave solutions of the various inhibitors and it was then possible to assay their growth-inhibitory activity and their content of (+)-abscisic acid. Most of the activity could be accounted for by the weight of (+)-abscisic acid present. In private we referred to a 'Tower of Babel' syndrome because the various groups had used their own chromatographic system, to isolate their own inhibitory extract, from their own plant. No attempts had been made to compare the various inhibitory fractions by using a reference chromatographic system or including other species. Furthermore, few groups had used quantitative bioassays. 'Manhattan diagrams' (semi-quantitative histograms of chromatograms) lacked serial dilutions and were seldom accompanied by standard.

"The 'Racemic Dilution Method' was devised to measure the concentration of the natural, optically-active (+)-abscisic acid within the plant by adding a known amount of the optically-inactive, synthetic (±)-abscisic acid to a fresh plant extract. After careful purification the total ABA could be measured by, for example, UV absorption, and then the amount of optically-active material by ORD. It was a simple task to calculate the (+)-ABA originally present from the ratio of optically-active to total ABA, and the weight of (±)-ABA added. This was the first measurement of the concentration of a plant hormone uncomplicated by losses during extraction. It was something of a surprise to find that about 60% to 70% was lost during the conventional isolation procedure.

"One unfortunate incident occurred because several eminent chemists advised me that the RD method wouldn't work. However, I carried out the experiments as a sideline. Another worker had the same idea several months later and he also sought, and received, the same advice from the chemists. He didn't pursue the topic and assumed that his idea had been pirated when the paper appeared a few months afterwards. Discovery of the bad feeling occurred by chance but when notebooks, even dated receipts for the cabbages used, were scrutinised the misunderstanding was cleared up.

"Glc now provides a quicker, more accurate method of analysis and [^{14}C]ABA can be used to monitor extractions. The paper's main value, when it was written, was to show that there was just one major growth inhibitor."