

Milborrow B V. The metabolism of abscisic acid. *J. Exp. Bot.* 21:17-29, 1970.
 [Shell Research Ltd., Milstead Laboratory of Chemical Enzymology, Sittingbourne, Kent, England]

¹⁴C-labelled (±)-abscisic acid (ABA) was used to define the two main pathways of ABA inactivation: conjugation of both (+) and (-) forms with glucose and oxidation of the natural (+) form to an unstable "Metabolite C" that rearranged to phaseic acid. It was also used to show that the inactive 2-trans isomer occurs in leaves and so is probably formed by photoisomerisation *in vivo*. [The SCI® indicates that this paper has been cited in over 145 publications.]

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At almost every turn the chemistry and physiology of abscisic acid (ABA) reveals unexpected quirks.¹ This paper covered the as yet unrepeatable isolation of crystalline "Metabolite C."

The first sample of ¹⁴C-labelled ABA had been used to detect polar transport in petiolar segments of sycamore, and three new zones of radioactivity were detected in extracts. They were unenterprisingly named Metabolites "A," "B," and "C." "B" was identified as the recently discovered glucose ester,² which was thereby established as an inactivation product. "A" was found to be an artefact (ABA methyl ester) formed by the exchange of the glucosyl residue of "B" by methanol. "C" was a polar acid.

I gave detailed instructions for isolating the polar acid to a new research assistant, Gwilym Roberts, and went to a conference. On my return he gave me a tube containing 9 mg of acicular crystals of the metabolite, which had an intense optical rotatory dispersion spectrum, very like that of ABA. When methylated for nuclear magnetic resonance analysis, the acid rearranged to a much less optically active substance that was identified as phaseic acid: a biologically inactive compound³ whose original structure was incorrect, and the rearrangement was instrumental in establishing the correct one.⁴ Phaseic acid has since been shown to be a major, naturally occurring catabolite of ABA, but several attempts to re-

isolate the polar acid have yielded phaseic acid even though we used redistilled solvents, antioxidants, and nitrogen atmospheres and carried out the extractions in near darkness in a coldroom.

We still have the characteristic optical rotatory dispersion spectrum obtained on that first sample, but we have never managed to repeat that isolation. Perhaps we need to do the experiment again, using tomato plants grown under identical conditions at the same time of the year—plants grown in England, in January, using supplementary illumination. "Metabolite C" must be a natural intermediate in the formation of phaseic acid—it has been found as a conjugate⁵ and detected by gas chromatography/mass spectrometry⁶—but it is frustrating that we are unable to repeat the original experiment. A strange postscript to the work occurred when I moved to Australia. Customs officers chose my luggage for the 1-in-100 intensive scrutiny; just one folder was thoroughly searched and jumbled—the one bearing the presumably incriminating label, "Polar Acid."

Another topic discussed in the paper was the light-catalysed isomerism of ABA to the biologically inactive 2-trans isomer that had been detected in plant extracts but could have been formed during the workup. By exploiting the optical rotation of the natural material and ¹⁴C-labelled (±)-ABA, we showed that about 4 percent of the ABA occurred in rose leaves as the 2-trans isomer, presumably as a spurious product formed photolytically.

The paper also defined the two main pathways for the inactivation of ABA: it is either oxidised and then isomerised to phaseic acid or else it is conjugated with glucose to form a glucose ester. In addition, the article highlighted the importance of taking the two mirror-image forms of the synthetic material into consideration: phaseic acid is formed by the oxidation of only the natural (+) component of racemic ABA while the glucose esters are formed from both (+) and (-) forms.

The observations made in 1970 have been extended in a large number of papers dealing with many different plants. The details and relative amounts vary, but the overall pattern remains.⁷

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