

**DEVELOPMENT OF A GC/MS METHODOLOGY TO RE-INVESTIGATE THE CALVIN
PATHWAY IN PHOTOSYNTHESIS**

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Earlier studies of CO₂ uptake during photosynthesis utilized ¹⁴CO₂ to measure the positional identity of the radiolabel incorporation into selected sugar phosphates. The results led Calvin to propose a pathway for assimilation of CO₂ in plants¹ for which he was awarded the Nobel Prize in Chemistry in 1961. More recent evidence, which includes the identification of labelled octulose phosphates in chloroplasts exposed to ¹⁴CO₂, indicates that the classical Calvin Pathway may be an incomplete description and some modification could be necessary.

The original methods for determination of the radiolabelling patterns in the selected sugar phosphates involved tedious separation, purification and degradation of the individual sugar phosphates. In order to re-investigate the path of carbon in photosynthesis in a rapid and more comprehensive fashion, we have developed a new methodology based on GC/EIMS of the methoxime-TMS derivative of the mixture of dephosphorylated sugar phosphates. Analysis of the isotope patterns of various fragment ions in the EI mass spectra of the derivatised individual sugars, using selected ion monitoring, has allowed us to calculate the incorporation of ¹³C during photosynthesis into single or small clusters of carbon atoms in the C₄ to C₈ sugar phosphates identified in isolated chloroplasts exposed to ¹³CO₂.

A general outline of the methodology and significance of the results obtained will be discussed.

1. Calvin, M., J. Chem Soc. (London), 1956, 1895.