Enzyme catalyzing the cleavage of the phenylpropanoid side chain was partially purified by ion exchange and gel filtration column chromatography after $(NH_4)_2SO_4$ precipitation. Enzyme activities were dependent on the concentration of dithiothreitol (DTT) or glutathione (GSH) and activated by addition of 0.5 mM Fe²⁺. Enzyme activity for ferulic acid was as high as for 4-coumaric acid in the presence of GSH, suggesting that GSH acts as an endogenous reductant in vanillin biosynthesis. Analyses of the enzymatic reaction products with quantitative NMR (qNMR) indicated that an amount of glyoxylic acid (GA) proportional to vanillin was released from ferulic acid by the enzymatic reaction. These results suggest that phenylpropanoid 2,3-dioxygenase is involved in the cleavage of the ferulic acid side chain to form vanillin and GA in *Vanilla planifolia*.

Phenylpropanoid 2,3-dioxygenase is involved in the cleavage of the ferulic acid side chain to form vanillin and glyoxylic acid in *Vanilla planifolia*.