Carboxyl Group Methylation of Loganic Acid by a Cell-free Enzyme Preparation from *Vinca rosea*

By K. M. MADYASTHA, R. GUARNACCIA and C. J. COSCIA (E. A. Doisy Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Mo. 63104, U.S.A.)

Methylcyclopentenomonoterpene glucosides that are hydroxylated at C-7 serve as intermediates in the biosynthesis of both secoirridoids (Coscia *et al.*, 1970) and indole alkaloids (Battersby, 1970) of higher plants of the Gentianaceae, Loganiaceae and Apocynaceae families. Of the three irridoids tested, namely monotropeine, genipin and loganin, the last alone acted as precursor of the non-tryptamine moiety of the three different classes of indole alkaloids (Battersby, 1970). But Guarnaccia *et al.* (1970) have shown the occurrence and biosynthesis of the related irridoid loganic acid in the indole-alkaloid-synthesizing plants *Vinca rosea* and *Strychnos nux vomica*. In fact, the seeds of both *Vinca rosea* and *Strychnos nux vomica* were found to contain relatively large quantities of loganic acid compared with those of its methyl ester loganin. It has been demonstrated (Guarnaccia *et al.*, 1970) that [2-¹⁴C]mevalonate is incorporated into the aglucone moiety of loganic acid in both flowering *Vinca rosea* plants and germinating seedlings.

From studies *in vivo* it has been shown that the O-methyl group of loganin is derived from the methyl group of methionine (Brechtbühler-Bader *et al.*, 1968). Hence it was decided to test whether loganic acid is the precursor of loganin. Now we have shown the presence of an enzyme in *Vinca rosea* that catalyses the transfer of the methyl group of S-adenosyl[Me-¹⁴C]methionine to loganic acid to form loganin (Madyastha *et al.*, 1971). We now report the isolation, purification, substrate specificity and kinetic studies of this enzyme isolated from the tender leaves and germinating seedlings of *Vinca rosea*.

Cell-free extracts were prepared in the presence of polyvinylpyrrolidone (Polyclar AT), Na₂S₂O₅ and dithiothreitol. The enzyme was partially purified by treatment with acetone, ammonium sulphate fractionation and DEAE-cellulose chromatography. The

presence of dithiothreitol in the extracting medium was necessary to prevent inactivation of the enzyme. Inhibition studies carried out with *p*-chloromercuribenzoate, iodoacetamide and *N*-methylmaleimide indicated the active role of reduced thiol groups of the enzyme. Interestingly, thiol compounds like reduced glutathione, cysteine or mercaptoethanol did not have a significant effect on the enzyme, whereas dithiothreitol enhanced the methyltransferase activity to a remarkable extent. In the assay mixture dithiothreitol can be fully replaced by lipoic acid, whereas it can be partially replaced by a combination of NADPH and FAD. The methyltransferase lost activity in low-ionic-strength buffer. EDTA studies showed that a bivalent metal cation is apparently not involved in the reaction. The enzyme has a pH optimum between 6.9 and 7.4. Substrate-specificity studies indicated that secologanic acid is as good a substrate for the enzyme as loganic acid, whereas 7-deoxyloganic acid and geniposidic acid were not methylated to any significant extent.

To our knowledge this is the first report on a methyltransferase capable of converting the carboxyl group of a low-molecular-weight compound into its methyl ester in higher plants. There are reports on the alkylation of the carboxyl groups of pectin by S-adenosylmethionine (Kauss *et al.*, 1967). It has also been demonstrated that the extracts of *Mycobacterium phlei* convert free fatty acids into their corresponding methyl esters with S-adenosylmethionine serving as the methyl donor (Akamatsu & Law, 1970).

The results implicate loganic acid and secologanic acid as active metabolites in the biosynthesis of indole alkaloids.

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