BIOSYNTHESIS AND METABOLISM OF PLANT GLYCOSIDES

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SUMMARY

The biosynthesis of selected cyanogenic glucosides and glucosinolates was examined in higher plants. Linen flax seedling shoots (Linum usitatissimum L.) were used exclusively to study linamarin biosynthesis while prunasin biosynthesis was studied in both peach shoots (Prunus persica Batsch) and cherry laurel shoots (P. laurocerasus L.). Isopropylglucosinolate and benzylglucosinolate were studied in scurvy grass seedlings (Cochlearia officinalis L.) and garden cress seedling shoots (Lepidium sativum L.) respectively.

Altogether 9 isotopically labelled compounds were prepared as part of the study and 14 were administered to plant tissue. The quantity of cyanogenic glucoside was determined by measuring hydrogen cyanide following enzymic hydrolysis. The specific activity or dilution of the labelled compound after incorporation into the glucosides was determined and used to judge the effectiveness of the administered compound as a precursor of the glucosides. Benzaldehyde from prunasin was measured as its semicarbazone and the isothiocyanates, obtained by enzyme hydrolysis of the glucosinolates, were identified by conversion to thiourea derivatives. Paper and thin layer chromatography and electrophoresis were used to separate non-volatile radioactive compounds.

Isobutyraldoxime-U-^{14}C, 2-oximinoisovaleric acid-U-^{14}C, isobutyronitrile-1-^{14}C and 2-hydroxyisobutyronitrile-1-^{14}C were all incorporated into linamarin to extents comparable to that from L-valine-U-^{14}C. By the use of ^{15}N labelled compounds the C-N bond of isobutyraldoxime and 2-oximinoisovaleric acid was shown to remain intact during the conversion to linamarin. Isobutyramide-1-^{14}C and hydrogen cyanide-^{14}C were not significantly incorporated into linamarin.

Phenylacetaldoxime-U-^{14}C, 2-oximino-3-phenylpropionic acid-2-^{14}C and phenylacetonitrile-1-^{14}C were converted to prunasin to greater extents than was L-phenylalanine-U-^{14}C. Radioactivity from D,L-mandelonitrile-1-^{14}C and, to a

lesser extent, from hydrogen cyanide-^{14}C was also incorporated into the nitrile moiety of prunasin. Phenylacethydroxamic acid-1-^{14}C was not significantly converted to prunasin.

Linen flax seedling shoots were examined for both volatile and non-volatile intermediates. Radioactive precursors of linamarin were administered in the presence of other suspected intermediates or inhibitors of linamarin biosynthesis. Both isobutyraldoxime and isobutyronitrile were shown to be formed in the shoots from L-valine-U-^{14}C.

A non-volatile compound which accumulated in the presence of a few inhibitors of linamarin biosynthesis was studied in detail. Treatment with acid under mild conditions yielded isobutyraldehyde while emulsin gave isobutyraldoxime. It was resistant to linamarase. Isobutyronitrile was a product of pyrolysis. The proposed structure for this compound is isobutyraldoxime-O-glucoside.

Isobutyraldoxime-U-^{14}C and phenylacetaldoxime-U-^{14}C were both better precursors of the corresponding glucosinolates than were L-valine-U-^{14}C or L-phenylalanine-U-^{14}C. 2-Oximinovaleric acid-U-^{14}C was not significantly incorporated into isopropylglucosinolate.

It is concluded that aldoximes are intermediates in the biosynthesis of both cyanogenic glycosides and glucosinolates. Other intermediates proposed in cyanogenic glycoside biosynthesis are nitriles and 2-hydroxynitriles in that order. N-Hydroxyamino acids may be intermediates between amino acids and aldoximes. 2-Oximino acids may also be intermediates in cyanoglycoside biosynthesis although it is possible that the observed incorporation was by way of prior non-enzymic conversion to nitriles. The experiments with labelled administered compounds have outlined a pathway of cyanogenic glycoside biosynthesis which may now be profitably studied for confirmation at the enzymic level.

PREFACE

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