A UDP GLUCOSE : THIOHYDROXIMATE GLUCOSYLTRANSFERASE FROM TROPAEOLUM MAJUS L.[1,2]

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Summary -- Phenylacetothiohydroximate was found to be a naturally occurring compound in Tropaeolum majus, derived from phenylacetaldoxime-1-^{14}C. An enzyme prepared from leaves of T. majus has been shown to catalyze the glucosylation of sodium phenylacetothiohydroximate by uridine diphosphate glucose (UDPG) to yield desulfobenzylglucosinolate.

Phenylacetothiohydroximic acid (II) has been suggested (1, 2, 3) as a precursor of the mustard oil glucoside, benzylglucosinolate (IV). This reaction was recently confirmed (4, 5) when sodium phenylacetothiohydroximate-2-^{14}C-^{35}S and desulfobenzylglucosinolate-^{14}C (III) were incorporated with high efficiency into benzylglucosinolate in Tropaeolum majus L. (Fig. 1). While these experiments showed the capability of the plant to transform the compounds fed into the glucosinolate, they did not confirm that phenylacetothiohydroximate is a naturally occurring intermediate in the biosynthetic pathway,

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[2]This work is part XII of a series on the biosynthesis of mustard oil glucosides. For Paper XI, see reference (5).

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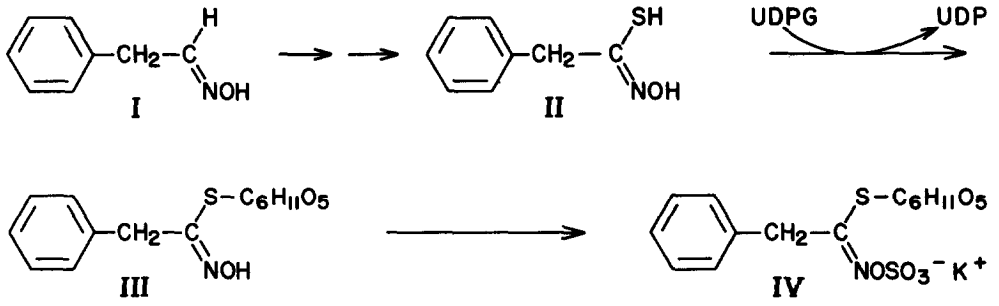

Fig. 1. Partial pathway for the biosynthesis of benzylglucosinolate.

nor did they demonstrate that the thiohydroximate is derived from phenylacetaldoxime (I), an earlier established intermediate in the biosynthetic sequence (6, 7, 8).

Experimental -- Phenylacetaldoxime-1-^{14}C (17.3 μCi, 6.2 mg) was administered to a T. majus plant (fr. wt. 39 g) via the cut stem. After 4 hrs, the plant was ground in a Waring blendor with 100 ml of water containing 204 mg of non-radioactive phenylacetothiohydroximate, and the mixture filtered through nylon. The filtrate was centrifuged at 0° and the supernatant extracted with ether. An equal volume of ethanol was added to the water layer and the precipitate removed by centrifugation. The somewhat labile thiohydroximate was then converted to its S-methyl derivative by the addition of 5 g of CH_3I (9). The mixture was kept at 22° overnight and the ethanol was removed in vacuo. The aqueous solution was extracted with ether and the ether dried over Na_2SO_4 and then concentrated. The residue was purified by thin-layer chromatography, TLC, (silica gel-G, benzene:methylethyl ketone; 5:1 v/v). Repeated recrystallization from benzene yielded S-methylphenylacetothiohydroximate of constant specific activity (1.52 x 10^5 dpm/mmole). The tri-

methylsilyl derivative of the S-methylthiohydroximate, prepared according to the procedure of Sweeley et al. (10), was recovered from the effluent of a 1/8" x 6' gas chromatographic column (4% w/w SE-30 on Chromosorb G, A/W and DMCS treated, 180°) and the specific activity was shown to be 1.50×10^5 dpm/mmole. The silyl derivative was hydrolyzed by heating with 65% methanol on a steam bath for 1 hr and the S-methylphenylacetothiohydroximate was again purified by TLC; it had a specific activity of 1.45×10^5 dpm/mmole.

No naturally occurring S-methylphenylacetothiohydroximate was detected in the plant extracts and hence the radioactive S-methylphenylacetothiohydroximate isolated was in fact formed by methylation of phenylacetothiohydroximate derived from the labelled aldoxime. These results are consistent with the conversion of phenylacetaldoxime to phenylacetothiohydroximate in T. majus.

An enzyme extract was prepared by grinding 50 g of T. majus leaves with sand and 50 ml of 0.1 M Tris-HCl buffer (pH 7.4) containing 0.05 M β-mercaptoethanol and 1 g of Polyclar AT. The mixture was filtered through a nylon mesh and the homogenate was centrifuged at 32,000 x g for 30 min at 0°. The supernatant was passed through a Sephadex G-25 column (2.5 x 34 cm) equilibrated with 5 mM Tris-HCl buffer pH 7.4 containing 0.5 mM β-mercaptoethanol. A portion of the protein eluate was employed immediately in the following experiments and the remainder was freeze-dried.

To the enzyme solution (0.9 ml) were added UDPG (glucose-U-^{14}C, 1 μCi, 4 μmole), sodium phenylacetothiohydroximate (4 μmole), $MgCl_2$ (1 μmole) in a total volume of 1 ml. The mixture was incubated for 2 hr at room temperature and the reaction

terminated by the addition of 2 ml of methanol and removal of the precipitated protein by centrifugation. The supernatant was concentrated and aliquots were analyzed by paper chromatography (n-butanol:acetic acid:water, 4:1:1.8 v/v) and by TLC (n-butanol:n-propanol:acetic acid:water, 3:1:1:1 v/v) (11). In both systems the migration of the major radioactive spot corresponded with authentic desulfobenzylglucosinolate (Table I). The major radioactive spot was extracted from the paper chromatogram with methanol (total activity 3.60×10^4 dpm) and 100 mg of nonradioactive desulfobenzylglucosinolate was added. On repeated recrystallization from a methanol-acetone-ether mixture the product exhibited a constant specific activity (1.08×10^5 dpm/mmole). In addition, the pentaacetate derivative prepared by using acetic anhydride and pyridine on

Table I. The identification of the reaction products by paper and thin-layer chromatography

	R_f values	
	Paper[1] chromatogram	Thin-layer[2] chromatogram
Reaction product	0.60	0.83
Desulfobenzylglucosinolate	0.60	0.83
UDPG	0.00 - 0.02	0.05
Sodium phenylaceto-thiohydroximate	1.00	1.00
D-Glucose	0.31	0.44

[1]Solvent employed was n-butanol:acetic acid:water, 4:1:1.8 v/v, Whatman #1 paper

[2]Solvent employed was n-butanol:n-propanol:acetic acid:water, 3:1:1:1 v/v.

recrystallization from ethanol had a specific activity of 1.09 x 10^5 dpm/mmole. The major radioactive component, extracted from a paper chromatogram, was further identified as desulfobenzylglucosinolate by gas-liquid chromatography of its trimethylsilyl derivative (12).

Transglucosylation from UDPG (glucose-U-^{14}C) into desulfobenzylglucosinolate, calculated by counting the radioactivity on paper chromatograms of aliquots of the enzyme reaction mixture was 78% after a 2 hr incubation. No desulfobenzylglucosinolate could be detected when sodium phenylacetothiohydroximate was omitted from the reaction mixture or when boiled enzyme (100° for 5 min) was employed. A similar conversion of labelled UDPG into desulfobenzylglucosinolate was found when the freeze-dried enzyme was employed. Aliquots of the enzyme reaction withdrawn at 10, 30, 60, 90 and 120 min after initiation of the reaction showed that the radioactivity in desulfoglucosinolate increased in proportion to the decrease in radioactivity in UDPG.

Discussion -- In contrast to the large number of O-glucosylating enzymes isolated in recent years (13, references therein) there has been only one other report on an S-glucosylating enzyme. Gessner and Acara (14) reported that thiophenol and 5-mercaptouracil were S-glucosylated by UDPG using and enzyme obtained from an insect species. The probable function of their enzyme seems to be the detoxication of exogenous thiols. The natural occurrence of phenylacetothiohydroximate in T. majus, its formation from phenylacetaldoxime-1-^{14}C and the metabolic activity of the enzyme described in this paper, support the hypothesis that the thiohydroximate is an intermediate in the biosynthesis of benzylglucosinolate and that the thiohydroximate is converted into desulfobenzyl-

glucosinolate by transglucosylation from UDPG. It seems likely that the mustard oil glucoside is formed in vivo from the desulfo-glucosinolate by esterification between its oxime group and sulfate, probably an active sulfate such as 3'-phosphoadenosine-5'-phosphosulfate. Since the available information on the biosynthesis of mustard oil glucosides suggests a common biosynthetic pathway and since the specificity of the enzyme described in this communication has not yet been ascertained, we suggest the systematic and trivial names for the enzyme be UDP glucose:thiohydroximate glucosyltransferase and desulfo-glucosinolate-UDP glucosyltransferase, respectively.

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