BIOSYNTHESIS OF MUSTARD OIL GLUCOSIDES

III. FORMATION OF GLUCOTROPAEOLIN FROM L-PHENYLALANINE-C^{14}-N^{15}*

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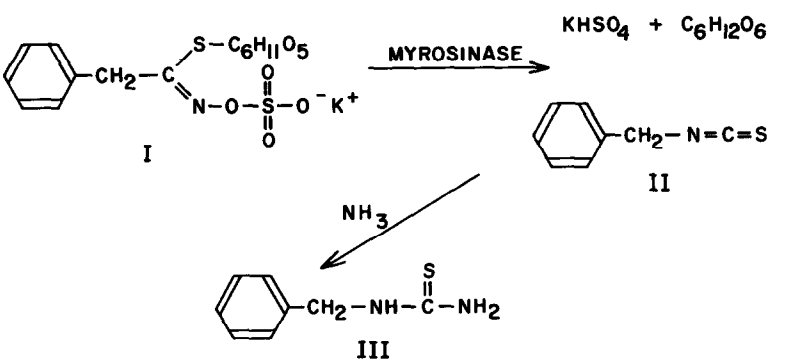
Glucotropaeolin (I), one of several mustard oil or isothiocyanate glucosides (Kjaer, 1960), when hydrolyzed by myrosinase yields potassium bisulphate, glucose and a steam volatile aglycone, benzyl isothiocyanate (II), which is isolated as benzylthiourea (III). Formation of the isothiocyanate aglycone upon enzymic hydrolysis involves a Lossen-type intramolecular rearrangement (Ettlinger and Lundeen, 1956) which also occurs in hydroxamic acids.



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The structural similarities between some isothiocyanate glucosides and amino acids has led to the speculation that these acids may be the precursors of the thioglucosides (Kjaer, 1954). In a previous publication (Underhill et al., 1962) it was shown that the C^{14} from phenylalanine-2- and -3-C^{14} as well as from phenyllactic acid-2-C^{14} was incorporated without randomization into the aglycone moiety of glucotropaeolin with high efficiency. The conversion of phenylalanine to glucotropaeolin has also been reported by Benn (1962).

This paper reports the source of the glucoside nitrogen to be the nitrogen of phenylalanine and describes attempts to elucidate the nature of the intermediates between phenylalanine and glucotropaeolin.

METHODS AND MATERIALS

Carbon-14 and nitrogen-15 labelled phenylalanine were obtained from commercial sources. The remaining C^{14}-labelled compounds were synthesized in this laboratory.

Labelled compounds were administered to the aerial portion of Tropaeolum majus L. (common garden nasturtium) by immersing the cut end of the plant stems in an aqueous solution of the tracer. The plants were allowed to metabolize under continuous light for 24 hours. The plant material was treated with myrosinase to hydrolyze the thioglucoside and the isothiocyanate was steam distilled into ammonia. The resulting benzylthiourea was recrystallized from water (Underhill et al., 1962).

Compounds were converted to carbon dioxide for measurement of C^{14} in a Nuclear Chicago, model 6000 Dynacon Electrometer. Nitrogen samples were prepared according to the method of Sprinson and Rittenberg (1949) and assayed in duplicate using an Associated Electrical Industries Ltd. mass spectrometer type MS-3.

RESULTS AND DISCUSSION

Table I shows the results of three feeding experiments using doubly-labelled L-phenylalanine-U-C^{14}-N^{15}. Since the carboxyl carbon of phenylalanine is not converted to the thioglucoside aglycone (Underhill et al., 1962), the molar specific activity of the C^{14} of L-phenylalanine-U-C^{14}-N^{15} was multiplied by eight-ninths to obtain the molar specific activity of that portion used in the synthesis of the aglycone and to calculate the appropriate C^{14}/N^{15} ratio. In addition, the atoms per cent excess N^{15} in the aglycone, benzyl isothiocyanate, was calculated from the N^{15} content of benzylthiourea by applying appropriate corrections for the nitrogen atom derived from the ammonia used in preparation of the thiourea derivative.

TABLE I

Conversion of L-phenylalanine-U-C^{14}-N^{15} into glucotropaeolin aglycone

Fresh wt. of plant (g)	L-Phenylalanine-U-C^{14}-N^{15}				Benzyl isothiocyanate**		
	Amt. fed (mg)	Specific activity (μc/mM)	Atoms % excess N^{15}	$\frac{C^{14}*}{N^{15}}$	Specific activity (μc/mM)	Atoms % excess N^{15}	$\frac{C^{14}}{N^{15}}$
73	15.7	160	46.0	3.09	4.10	1.380	2.97
40	8.9	106	46.0	2.06	4.08	1.978	2.06
52	7.9	119	46.0	2.30	2.34	0.976	2.40

* Specific activity of phenylalanine corrected for loss of one carbon atom - see text.

** Calculated from benzylthiourea - see text.

The C^{14}/N^{15} ratios for phenylalanine and the glucoside aglycone remained unchanged within the accuracy of the analyses in each of the three experiments. Therefore, it is evident that the carbon and nitrogen of the thioglucoside aglycone are derived as a unit from phenylalanine and that the intermediates must be nitrogenous. Furthermore, it would appear that the amino nitrogen must be oxidized to the -1

oxidation state (eg. HO-N$^{=}$) to permit the formation of the glucoside N-sulphate ester. Compounds in which nitrogen is present at this level of oxidation have been known in biology for several years. Stumpf et al., (1951) have demonstrated the formation of glutamohydroxamic acid from glutamine and hydroxylamine by glutamotransferase isolated from pumpkin. Evidence for the formation of other hydroxamic acids by a similar transferase mechanism also exists (Kimura, 1959; Jones and Elliott, 1959). The occurrence of alpha-oximino acids has also been reported (Yamafuji et al., 1950). However, we are unaware of previous experimental evidence for the oxidation of the intact amino nitrogen as indicated in the experiments reported here.

Carbon-14 labelled compounds, most of which are analogous to naturally occurring products of amino acid metabolism, were administered to Tropaeolum majus (70 to 150 g fresh weight) and their efficiency as precursors of the thioglucoside aglycone compared with phenylalanine-2-C^{14}. The data in Table II summarize the results of feeding experiments. Although the compounds were administered to plants of similar weight and maturity for an individual experiment, variation in plants between experiments is reflected by the differences in per cent incorporation of phenylalanine. Relative to phenylalanine, none of the nitrogenous compounds fed were efficient precursors of the thioglucoside aglycone.

Some reservations must be held concerning the lack of incorporation of C^{14} from phenylpyruvic acid oxime and its apparent failure to serve as a precursor of the aglycone. Ahmad and Spenser (1951) have shown that alpha-keto acid oximes give the lower nitrile and carbon dioxide on warming in dilute aqueous solution. Although such a reaction is possible, it is unlikely that it would proceed to completion during the time required for uptake of phenylpyruvic acid oxime-2-C^{14}. A more important consideration concerns the conformation around the

TABLE II

Comparison of compounds as precursors of glucotropaeolin aglycone*

Expt.	Compound administered	Amount fed (mg)	Sp. act. (μc/mM)	Benzylthiourea Sp. act. (mμc/mM)	Benzylthiourea % C^{14} incorp.
I	DL-Phenylalanine-2-C^{14}	16.2	98	1580	8.58
	2-Phenylethylamine-1-C^{14}	16.2	37	4	0.05
	Phenylacetamide-1-C^{14}	6.0	208	11	0.03
II	DL-Phenylalanine-2-C^{14}	17.1	105	2340	8.97
	Benzylcyanide-1-C^{14}	3.8	188	10	0.09
III	DL-Phenylalanine-2-C^{14}	9.4	194	3850	12.10
	Phenylacethydroxamic acid-1-C^{14}	7.9	136	3	0.02
	Phenylpyruvic acid oxime-2-C^{14}	9.2	271	212	0.53

* Isolated as benzylthiourea

oxime double bond in the alpha-oximino acid and glucotropaeolin. The configuration around the C=N double bond of the mustard oil glucosides, recently determined (Waser and Watson, 1963) by X-ray diffraction patterns of crystalline sinigrin, confirms the indirect evidence (Kjaer, 1960) for the anti-configuration of the migrating side-chain (benzyl in the case of glucotropaeolin) and the sulphate group. Comparison of the infrared spectrum of phenylpyruvic acid oxime-2-C^{14} with that assumed by Ahmad and Spenser (1961) to be the syn-isomer (benzyl- -OH) indicates that the syn-isomer was in fact administered. Hence, the lack of incorporation may have been due to the administration of the incorrect geometric isomer. Methods for the synthesis of C^{14}-N^{15}-labelled anti-phenylpyruvic acid oxime are presently being pursued.

The finding that both the nitrogen-15 and the carbon-14 of L-phenylalanine-U-C^{14}-N^{15} were diluted to the same extent in the synthesis of the thioglucoside would suggest that phenylalanine and its corresponding keto acid were not readily interchangeable through transamination. Such interchange of nitrogen from phenylalanine with an unlabelled "nitrogen pool" would have resulted in C^{14}/N^{15} ratios for benzyl isothiocyanate of higher values.

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