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BIOSYNTHESIS OF SINIGRIN VII.
INCORPORATION OF 4-METHYLTHIOBUTYRALDOXIME-1-^{14}C,^{15}N INTO SINIGRIN

Mitsuyoshi Matsuo

National Institute of Radiological Sciences

9-1 4-Chome Anagawa Chiba Chiba Japan

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Recently it has been reported that isobutyraldoxime-U-^{14}C was incorporated into glucoputranjivin, phenylacetaldoxime-U-^{14}C and -1-^{14}C into glucotropaeolin and phenylpropionaldoxime-1-^{14}C into gluconasturtiin respectively with high efficiency (1,2). In a previous publication (3) we showed that homomethionine was a precursor of sinigrin, and the formation of the double bond in sinigrin molecule would be caused by the elimination of methanthiol at the latest stage of biosynthesis. The similar result was obtained by Chisholm and Wetter (4). In consideration of these results, it is quite probable to assume that 4-methylthiobutyraldoxime should be an important intermediate of sinigrin biosynthesis. The present paper describes that 4-methylthiobutyraldoxime is incorporated directly into sinigrin in horseradish (Armoracia lapathifolia GILIB) leaves.

4-Methylthiobutyraldoxime-1-^{14}C, ^{15}N was prepared by a method as shown in Chart I. The labelled compounds were administered to each 204 g of fresh horseradish leaves as described in a previous paper (3). After 24 hour cultivation mustard oil was isolated from the plants and converted into allylthiourea.

The incorporation of 4-methylthiobutyraldoxime-1-^{14}C and -1-^{14}C,^{15}N into sinigrin was shown in Table I. Degradation of allylthiourea obtained in the 4-methylthiobutyraldoxime-1-^{14}C feeding experiment by a method previously described (3) showed that the aldoxime was incorporated into sinigrin without randomization (Chart II).

Chart I

The Preparation of 4-Methylthiobutyraldoxime-1-^{14}C, ^{15}N

$$CH_3SCH_2CH_2CH_2Cl \xrightarrow{K^{14}CN} CH_3SCH_2CH_2CH_2{}^{14}CN \xrightarrow{CH_3OH,\ HCl} CH_3SCH_2CH_2CH_2{}^{14}C(OCH_3)=NH\cdot HCl$$

$$\xrightarrow{CH_3OH} CH_3SCH_2CH_2CH_2{}^{14}C(OCH_3)_3 \xrightarrow{LiAlH_4} CH_3SCH_2CH_2CH_2{}^{14}CH(OCH_3)_2 \xrightarrow{3.5\%\ HCl}$$

$$\rightarrow CH_3SCH_2CH_2CH_2{}^{14}CHO \xrightarrow{{}^{15}NH_2OH\cdot HCl} CH_3SCH_2CH_2CH_2{}^{14}CH={}^{15}NOH$$

Table I

Incorporation of 4-Methylthiobutyraldoxime-1-^{14}C and -1-^{14}C, ^{15}N into Sinigrin

Precursor					Allylthiourea			
Amt. fed mg	Total act. μCi	Sp. act. μCi/mmol	Atoms% excess ^{15}N	$^{14}C/^{15}N$ a)	Sp. act. μCi/mmol	Sp. incorp. % b)	Atoms% excess ^{15}N c)	$^{14}C/^{15}N$ a)
4-Methylthiobutyraldoxime-1-^{14}C								
8.7	1.6	24.6	-	-	0.093	0.38	-	-
4-Methylthiobutyraldoxime-1-^{14}C, ^{15}N								
26.8	2.0	9.9	58	0.17	0.074	0.75	0.41	0.18

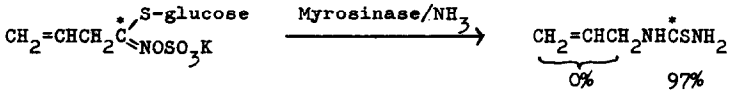
a) $^{14}C/^{15}N = \dfrac{\mu Ci/mmol \text{ of } {}^{14}C}{\text{Atoms\% excess of } {}^{15}N}$

b) Sp. incorp. $= \dfrac{\text{Sp. act. } (\mu Ci/mmol) \text{ of allylthiourea}}{\text{Sp. act. } (\mu Ci/mmol) \text{ of precursor}} \times 100$

c) The value corrected for the nitrogen atom derived from the ammonia used in the preparation of allylthiourea.

Chart II

The Incorporation of Radioactivity into Sinigrin from 4-Methylthiobutyraldoxime-1-^{14}C



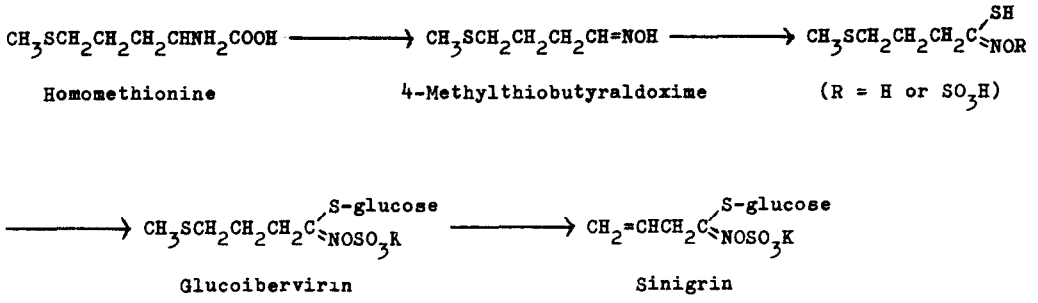
Sinigrin Allylthiourea

The ^{14}C and ^{15}N double labelled tracer experiment (Table I) showed that nitrogen atom of the aldoxime was also incorporated into the molecule of sinigrin being attached with the intact carbon chain since $^{14}C/15_N$ ratio in the precursor (0.17) was almost equal to the ratio of the metabolite (0.18). Although the specific incorporation ratio of 4-methylthiobutyraldoxime-1-^{14}C (0.38) was a little smaller than that of homomethionine (0.65) indicated in the previous paper (3) against expectation, this seems to be due to the facts that the aldoxime was a mixture of anti- and syn-form and differed from homomethionine in solubility and membrane permeability.

It appears that sinigrin is biosynthesized from homomethionine via 4-methylthiobutyraldoxime. On the other hand, the author (5) and Wetter (6) have found that the bisulphate residue in sinigrin was derived from sulphate ion and sodium thioglucoside was not a precursor of sinigrin. These findings suggest that sulphur atom of thioglucoside moiety in sinigrin would be originated from insertion of SH group into 4-methylthiobutyraldoxime or the aldoxime sulphate. On the base of these observations a possible sequence for the formation of sinigrin from homomethionine might be shown in Chart III.

Chart III

A Possible Sequence for the Formation of Sinigrin



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