As shown in Table I, methyl derivatives were more volatile than trimethylsilyl derivatives and may be favorable for other glucuronides of higher molecular weight.

Details of this work will be reported in the near future.

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Biosynthesis of Sinigrin. II[*1]

Recently the incorporation of some ^{14}C-labeled compounds into sinigrin has been studied.[1,2] However, there have been no evidences for the direct precursor of isothiocyanate moiety of sinigrin. This paper reports the three carbons of isothiocyanate moiety of sinigrin in *Brassica juncea* COSSON are derived from aspartic acid and the one carbon from malonic acid, while 2-amino-4-pentenoic acid is also incorporated in it.

Some ^{14}C-labeled compounds were administered into the stem of 7 months old Brassica plant by the cotton wick method after the flowering period, and the seeds were harvested 3 weeks after administration. Allyl isothiocyanate was obtained from the ground seeds and converted into 1-allyl-2-thiourea with ammonia. The specific radioactivities of 1-allyl-2-thiourea were determined by the sample purified by paper chromatography (the solvent system; H_2O-saturated $CHCl_3$). 1-Allyl-2-thiourea diluted with the non-radioactive carrier was degraded to determine the location of radioactivity. The degradation method was shown in the previous paper.[2]

Table I and II show the results of the feeding experiments and the degradation reactions of 1-allyl-2-thiourea obtained from mustard seeds. Underhill, *et al.*[1] have demonstrated that on the short time feeding experiment of horseradish leaves acetate [2-^{14}C] is easily incorporated into allyl isothiocyanate moiety. However, according to our data of the long time feeding experiment, malonic acid is more effective as the precursor than acetic acid. The incorporation of 2-amino-4-pentenoic acid into isothiocyanate moiety suggests the possibility that 2-amino-4-pentenoic acid is one of the precursors. The results of degradations show that the methylene carbon of malonic acid or the methyl carbon of acetic acid is incorporated into C-4 in allyl isothiocyanate moiety, and δ-carboxyl carbon of aspartic acid into C-1 (the numbering of allyl isothiocyanate moiety is the same as 1-allyl-2-thiourea in Table II). On feeding aspartic acid [2-^{14}C] the distribution of radioactivity on C-2 and C-3 of allyl isothiocyanate moiety is seemed to be resulted by the interconversion between aspartic acid and succinic acid (or fumaric acid) in plant. The data suggest that allyl group in sinigrin is derived from the three carbons of aspartic

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[*1] Some part of this paper was presented at the 83rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, November, 1963.

1) E.W. Underhill, *et al.* : Can. J. Biochem. and Physiol., **40**, 1505 (1962).

2) M. Matsuo, M. Yamazaki : This Bulletin, **11**, 545 (1963).

Table I. The Specific Radioactivity of 1-Allyl-2-thiourea obtained from Yellow Mustard Seeds

Compound, administered	Amount, fed (μc.)	Sp. act. (mc./mM)	1-Allyl-2-thiourea sp. act. (μc./mM)	Dilution ratio[a] ($\% \times 10^{-2}$)
Sodium acetate [2-^{14}C]	100	6.0	0.81	1.3 (1.3)[b]
Sodium malonate [2-^{14}C]	100	6.1	0.93	1.5 (1.5)
Sodium formate [^{14}C]	100	4.0	0.11	0.5 (0.5)
Succinic acid [1,4-^{14}C]	100	3.0	0.21	0.7 (1.4)
DL-Methionine [CH_3-^{14}C]	100	2.3	0.08	0.3 (0.6)
DL-Aspartic acid [2-^{14}C]	100	1.7	0.23	1.4 (2.8)
DL-Aspartic acid [4-^{14}C]	50	1.5	0.13	0.9 (3.6)
DL-Glutamic acid [2-^{14}C]	100	1.4	0.14	1.0 (2.0)
DL-2-Amino-4-pentenoic acid [2-^{14}C]	1	2.9×10^{-1}	9.3×10^{-4}	3.2×10^{-2} (6.4)

[a] Dilution ratio $= \dfrac{\text{sp. act. of 1-allyl-2-thiourea}}{\text{sp. act. of administered compound}} \times 100$

[b] The figures in the parentheses indicate the dilution ratio in the case that ^{14}C-labeled compounds of 100 μc. are administered and D-amino acid is not metabolized in the plant.

Table II. The Distribution of ^{14}C in 1-Allyl-2-thiourea obtained from Yellow Mustard Seeds

Compound, administered	1-Allyl-2-thiourea sp. act. (d.p.m./m$M \times 10^4$)	Each carbon sp. act. (d.p.m./m$M \times 10^3$)				Degradation yield (%)
		C-1	C-2	C-3	C-4[a]	
Sodium acetate [2-^{14}C]	3.0	2.3 (8)	2.5 (9)	4.8 (19)	17 (63)[b]	90
Sodium malonate [2-^{14}C]	7.7	4.5 (6)	5.0 (7)	15 (20)	50 (67)	98
Sodium formate [^{14}C]	0.59	1.6 (29)	0.4 (8)	1.2 (22)	2.2 (40)	92
Succinic acid [1,4-^{14}C]	1.7	12 (67)	0.4 (2)	5.2 (28)	0.5 (3)	105
DL-Methionine [CH_3-^{14}C]	0.54	0.5 (11)	0.2 (4)	0.4 (10)	3.7 (75)	91
DL-Aspartic acid [2-^{14}C]	3.3	3.9 (13)	8.9 (30)	14 (47)	2.7 (9)	92
DL-Aspartic acid [4-^{14}C]	1.1	9.2 (84)	1.1 (9)	0.3 (3)	0.4 (4)	100
DL-Glutamic acid [2-^{14}C]	3.2	2.5 (76)	0.1 (3)	0.5 (15)	0.2 (5)	101

[a] The numbering of 1-allyl-2-thiourea carbon as follows.

$$\overset{1}{C}H_2=\overset{2}{C}H\overset{3}{C}H_2NH\overset{4}{C}SNH_2$$

[b] The figures in the parentheses indicate the distribution percent of radioactivity of degradation products.

acid except α-carboxyl carbon, and the carbon linked with allyl group arises from the methylene carbon of malonic acid. Although methionine [CH_3-^{14}C] gave a very low dilution ratio, the ^{14}C was incorporated specifically into C-4 of allyl isothiocyanate moiety. It appears that glutamic acid and succinic acid are incorporated into sinigrin *via* TCA cycle.

When we have completed this experiment, Chisolm and Wetter[3] have reported that methionine was a very predominant precursor of allyl isothiocyanate moiety. We suppose it would be possible that allyl isothiocyanate moiety of sinigrin is biosynthesized *via* 2-amino-4-pentenoic acid which is derived from aspartic acid or methionine and malonic acid.

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[3] M.D. Chisolm, L.R. Wetter: Can. J. Biochem. and Physiol., **42**, 1033 (1964).