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Biosynthesis of Sinigrin. V.[1] On the Origin of Thioglucoside Moiety of Sinigrin

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Mustard oil glucoside contains two atoms of sulphur, one in the thioglucoside moiety and the other in the bisulphate residue. Wetter showed that the bisulphate residue in sinigrin was derived from a sulphate ion in horseradish leaves[3] and we obtained the same result in horseradish.[4] Kindl observed that sodium sulphate(^{35}S) was incorporated into the bisulphate residue of sinalbin in white mustard.[5] Kutáček, *et al.* also described the incorporation of sodium sulphate(^{35}S) into glucobrassicin in cabbage.[6] On the other hand, Wetter found that sulphur of methionine was transferred to the thioglucoside moiety in sinigrin,[3] and Meakin reported recently that the thioglucoside moiety of glucotropaeolin was derived from thioglucose.[7]

We have now found that on administration of DL-methionine(^{35}S), DL-cysteine(^{35}S) hydrochloride, sodium thioglucoside(^{35}S) and sodium sulphate(^{35}S) into horseradish leaves, sulphur of cysteine is most efficiently incorporated into the thioglucoside moiety of sinigrin than the others.

The labelled compounds were fed to horseradish leaves by the method as described in the previous paper.[8] After 3 hours, the leaves were soaked into boilling ethanol, and sinigrin was separated and purified by the method previously reported.[4] Sinigrin was converted into allyl isothiocyanate with myrosinase prepared from yellow mustard seeds, and allyl isothiocyanate was purified as allyl thiourea which was readily formed from it by the action of ammonia. The specific activities of sinigrin and allyl thiourea were shown in Table I.

Table I. Incorporation of the Labelled Compounds into Sinigrin and Allyl thiourea in Horseradish Leaves (Fresh Wt. 200 g)[a]

Compounds fed	Amount (mM)	Total act. (dpm) ($\times 10^6$)	Sp. act. (dpm/mM) ($\times 10^7$)	Sinigrin	Allyl thiourea	
				Sp. act. (dpm/mM) ($\times 10^4$)	Sp. act. (dpm/mM) ($\times 10^4$)	Dilution[b] %
DL-Methionine(^{35}S)	0.18	8.4	4.9	5.3	4.8	0.10
DL-Cysteine(^{35}S) HCl	0.26	7.8	3.1	6.2	5.7	0.18
Na thioglucoside(^{35}S)	0.23	9.4	4.0	0.70	0.62	0.02
Na_2SO_4(^{35}S)	0.15	79.0	51.0	26.0	7.9	0.02

[a] Cultivated for 3 hours

[b] $\text{Dilution (\%)} = \frac{\text{Specific activity of allyl thiourea}}{\text{Specific activity of precursor}} \times 100$

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A comparison of the incorporation ratios showed that the ^{35}S of cysteine(^{35}S) was most efficient as the origin of the thioglucoside moiety in sinigrin. The activity of thioglucose(^{35}S) administered was transferred to the thioglucoside moiety of sinigrin, but the incorporation ratio was unexpectedly low. It should be doubtful to assume that thioglucose would directly be incorporated into sinigrin. However it is plausible that the SH group of cysteine would be a source of sulphur of the thioglucoside moiety in sinigrin.

Experimental

Synthesis of Labelled Compounds and Measurement of Radioactivity——DL-Methionine(^{35}S), DL-cysteine-(^{35}S) hydrochloride and sodium sulphate(^{35}S) were obtained from commercial sources.

Sodium thioglucoside(^{35}S) was synthesized by a method of Horton.[9] Acetobromoglucose (560 mg) in EtOH (1 ml) was added to a solution of potassium ethylxanthate(^{35}S) (220 mg) in EtOH (1.5 ml). The mixture was heated on a boilling water bath for 5 min. After water (15 ml) was added to the mixture, oily substance was separated and solidified by scratching it with a glass rod. Water was removed by decantation.

The residual solid was washed with a small amount of water and dried in a desiccator. The recrystallization from petr. ether and EtOH afforded tetra-O-acetyl-β-D-glucopyranosyl ethylxanthate(^{35}S) (150 mg). The xanthate (100 mg) was hydrolyzed with abs.MeOH (1 ml) containing Na (9 mg) at −15° for 10 min. The mixture was neutralized with 10% AcOH using phenolphthalein as an indicator. Ether (15 ml) was added to it and an oily substance was separated. The precipitates was acetylated with acetic anhydride in pyridine on standing overnight in icebox. The acetate was recrystallized from EtOH. Tetra-O-acetyl-S-acetyl-1-thio-β-D-glucopyranose(^{35}S) (30 mg) was obtained. mp 119° (lit. 121°). *Anal.* Calcd. for $C_{16}H_{22}O_{10}S$: C, 47.39; H, 5.34. Found: C, 47.29; H, 5.42. The acetate was hydrolyzed with abs.MeOH containing Na. and the oily sodium thioglucoside(^{35}S) was separated by the addition of ether to the mixture neutralized with 10% AcOH, which was administered into the plants as an aqueous solution.

Radioactivity was measured with Tri-Carb liquid scintillation spectrometer, series 314 EX (Packard Instrument Company, Inc.) by an ordinary method.

Administration of Labelled Compound into the Plants and Isolation of Sinigrin and Allyl thiourea—— Labelled compounds were administered to horseradish leaves as described in the previous paper.[8] Sinigrin was isolated by the method reported previously[4] and converted into allyl isothiocyanate with myrosinase which was separated from yellow mustard seeds. Allyl isothiocyanate was isolated as allyl thiourea which was prepared by addition of an ammonia solution to it.

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9) D. Horton, "Method in Carbohydrate Chemistry", 2, edited by R. L. Whistler and M. L. Wolfrom, Academic Press, Inc., New York, 1963, p. 433.

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Syntheses of 2,3-Di-O-benzyl-α-L-*arabino*-pentodialdo-1,4-furanoside and Its β-Anomer

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In a previous paper,[2] it was reported that treatment of 2,3-di-O-benzyl-5-O-mesyl-6-O-trityl-D-glucofuranose (I) with sodium methoxide under an oxide ring migration afforded

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1) Location: *Hiromachi, Shinagawa-ku, Tokyo.*

2) T. Iwashige and H. Saeki, *Chem. Pharm. Bull.* (Tokyo), **15**, 132 (1967); H. Saeki, T. Iwashige, and E. Ohki, *ibid.*, **16**, 1040 (1968).