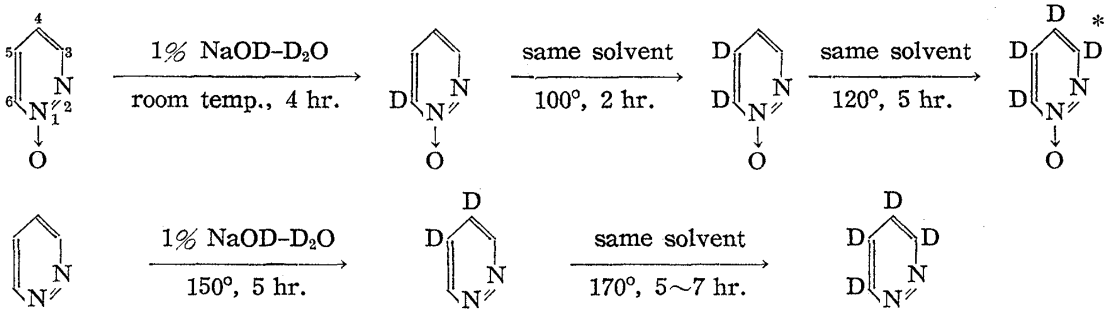

Chart 2.

* Deuteration at 3-position was a little faster than that at 4-position.

pyridazine N-oxides than at its 4 position (γ to N-oxide group). With regard to pyridazine free base, on the other hand, it is noteworthy to emphasize that 4 and 5 hydrogens more distant from the ring nitrogen were more easily deuterated in alkaline medium than 3 and 6 ones α position to the nitrogen.

The kinetic results and the mechanism of the deuteration of these compounds will be discussed in a forthcoming paper.

Deep thanks are due to professors Toshihiko Okamoto of the University of Tokyo, Chikara Kaneko of Medical and Dental University and Virgil Boekelheide of University of Oregon for their helpful discussions and thanks are also due to Dr. Sachiko Natsume of National Institute of Hygienic Sciences for a kind supply of pyridazine derivatives and to Miss Sachiko Yagishita for her technical assistance.

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Gas Chromatography of O-Glucuronides

Gas chromatography has already been applied to the separation and estimation of carbohydrates as their acetyl, methyl and trimethylsilyl derivatives, but studies on glucuronides have not hitherto been reported. The present communication deals with microscale preparation and gas chromatographic examination of some volatile glucuronide derivatives.

The carboxyl group in glucuronic acid moiety was methylated with diazomethane prior to acetylation or trimethylsilylation. Acetylation of the methyl esters was carried out with acetic anhydride and pyridine in the usual manner. Trimethylsilylation of the methyl esters was accomplished by the use of hexamethyldisilazane and trimethylchlorosilane in pyridine as reported by Sweeley, *et al.*1)

During the attempt to prepare methyl derivatives, it was found that complete methylation of glucuronides could not be effected by the method of Purdie2) or Haworth.3)

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1) C.C. Sweeley, Ronald Bentley, M. Makita, W.W. Wells : J. Am. Chem. Soc., 85, 2497 (1963).
2) T. Purdie, J.C. Irvine : J. Chem. Soc., 83, 1021 (1903).
3) W.N. Haworth : *Ibid.*, **107**, 8 (1915).

Recently, Hakomori[4] applied sodium hydride and methyl iodide to the methylation of glycolipids and polysaccharides using dimethyl sulfoxide as a solvent. Application of this procedure to some O-glucuronides gave almost satisfactory results, although attended with a small amount of a by-product. A typical gas chromatogram is illustrated in Fig. 1, where benzyl glucuronide is shown as an example. The compound with a smaller peak was collected and proved to be methyl (benzyl 4-deoxy-2,3-di-O-methyl-4,5-dehydro-β-D-glucopyranosid) uronate. However, when dimethylformamide was employed as a solvent instead of dimethyl sulfoxide, formation of the by-product was markedly suppressed.

Examinations were then made on the behavior of the three derivatives mentioned above toward columns. Acetyl derivatives generally had low volatility and were unstable at high temperature, so that their applicability to gas chromatography may be limited to glucuronides of lower molecular weight. On the other hand, methyl and trimethylsilyl derivatives were volatile as well as thermostable and were much more suitable for gas chromatography.

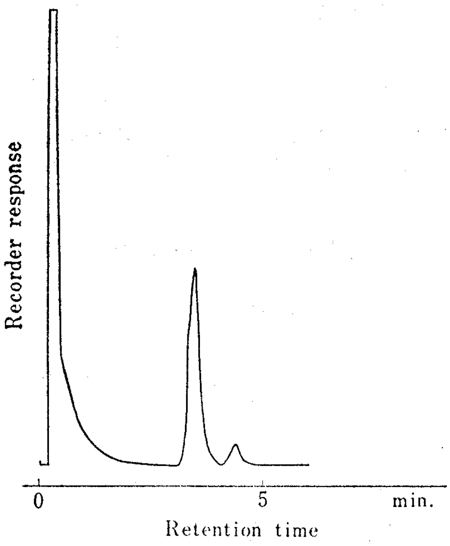
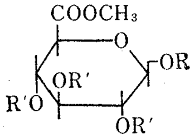
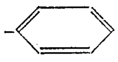
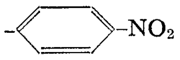
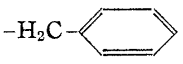
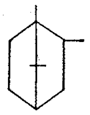
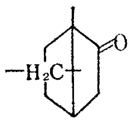


Fig. 1. Gas Chromatogram of Benzyl Glucuronide Methylated with Sodium Hydride, Methyl Iodide and Dimethyl Sulfoxide

column : 1% NGS on Anakrom, 1.5 m. ×4 mm. i.d.
temperature : column 210°
sample heater 250°
detector 230°
carrier gas : N_2 90 ml./min.
sens. 100 range 0.4 v.

Table I. Relative Retention Times of Methyl and Trimethylsilyl Derivatives

 COOCH$_3$, OR, OR′, R′O, OR′		Stationary phase				
R	R′	1.5% SE-30	1.5% SE-52	2% QF-1	1% CNSi	1% NGS
		(1.10 min.)	(1.40 min.)	(0.55 min.)	(1.67 min.)	(1.57 min.)
	Me	1.00	1.00	1.00	1.00	1.00
	TMS	2.64	2.36	1.91	2.49	0.84
 -NO$_2$	Me	4.03	3.86	7.44	5.06	8.84
	TMS	10.00	10.64	12.36	12.87	6.59
 -H$_2$C-	Me	1.32	1.29	1.24	1.31	1.27
	TMS	3.34	3.20	1.93	3.20	1.03
 H	Me	0.82	0.81	0.78	0.81	0.51
	TMS	2.32	2.11	1.25	2.13	0.51
	Me	1.64	1.61	1.55	1.62	0.83
	TMS	4.08	3.71	2.18	3.71	0.78
 —H$_2$C	Me	3.64	3.61	6.09	4.15	4.06
	TMS	8.27	8.21	8.64	9.34	3.11

Me : methyl TMS : trimethylsilyl
column : 1.5 m.×4 mm. i.d. column temperature : 220° carrier gas : N_2, 90 ml./min.

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[4] S. Hakomori : J. Biochem., **55**, 205 (1964).

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).