The structure of V was established as 3β-hydroxy-5β,14β,17α-card-20(22)-enolide upon the following evidences:

(a) The presence of an α,β-unsaturated butenolide ring in V was shown by an ultraviolet absorption maximum at 218 mμ, infrared spectrum with peaks at 5.58, 5.78, 6.16 μ and positive Raymond test.

(b) The α-orientation of the butenolide side chain in V was characterized by identity of V with the hydrogenation product of β-anhydro-17α-digitoxigenin (VI) (m.p. 172~174°, $C_{23}H_{32}O_3$, UV : λ_{max}^{EtOH} 214 mμ (log ε 4.19)), derived from 17α-digitoxigenin (III).

(c) The 14β,17α-configuration of V was confirmed by transformation of V into methyl 3β-acetoxyetianate (VII) (m.p. 156~158°, $C_{23}H_{36}O_4$) which was identical with an authentic sample of methyl 3β-acetoxy-5β,14β,17α-etianate, kindly sent by Prof. Kuno Meyer.

This result shows preferred hydrogenation of the conjugated diene system in ring D rather than a double bond in the butenolide ring, and preferential attack from the front side.

Some derivatives of 14,16-dideoxy-17α-gitoxigenin (V) were obtained as follows : V was oxidized with chromic anhydride to the 3-oxo derivative (VIII) (m.p, 211~213°, $C_{23}H_{32}O_3$, UV : λ_{max}^{EtOH} 218 mμ (log ε 4.17), $[\alpha]_D$ +102.5° ($CHCl_3$)). Dehydrogenation of VIII with selenium dioxide[2] afforded the 1,4-dien-3-one (IX) (m.p. 229~231°, $C_{23}H_{28}O_3$, UV : λ_{max}^{EtOH} 221 mμ (log ε 4.38), $[\alpha]_D$ +218.5° ($CHCl_3$)), which was rearranged to a compound having a phenolic moiety in ring A (X) (m.p. 159~161°, $C_{25}H_{30}O_4$, UV : λ_{max}^{EtOH} 267 mμ (log ε 2.56), $[\alpha]_D$ +160° ($CHCl_3$), nuclear magnetic resonance : 3.13 τ (quartet of AB system)). In addition, VIII was converted to the 1,4,6-trien-3-one (XII) (m.p. 232~238°, $C_{23}H_{26}O_3 \cdot H_2O$) through the 4-en-3-one (XI) (m.p. 193~196°, $C_{23}H_{30}O_3$).

The pharmacological activities of these compounds are being examined.

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2) D. Satoh, T. Wada : Yakugaku Zasshi, **80**, 1314 (1960).

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Biosynthesis of Sinigrin

Some hypotheses[1,2] and experiments[3,4] on the biogenesis of mustard oil glucosides were reported. Underhill *et al.*[4] stated recently that the methyl carbon of acetate was predominantly incorporated into the aglucone of sinigrin, but the carboxyl carbon of acetate was not incorporated.

1) A. Kjaer *et al.* : Acta Chem. Scand., **13**, 1575 (1959). *Ibid.*, **16**, 71 (1962). *Ibid.*, **16**, 936 (1962).
2) M. G. Ettlinger, A. J. Lundeen : J. Am. Chem. Soc., **78**, 4172 (1956).
3) M. Kutáček *et al.* : Nature, **194**, 393 (1962).
4) E. W. Underhill *et al.* : Can. J. Biochem. Physiol., **40**, 1505 (1962).

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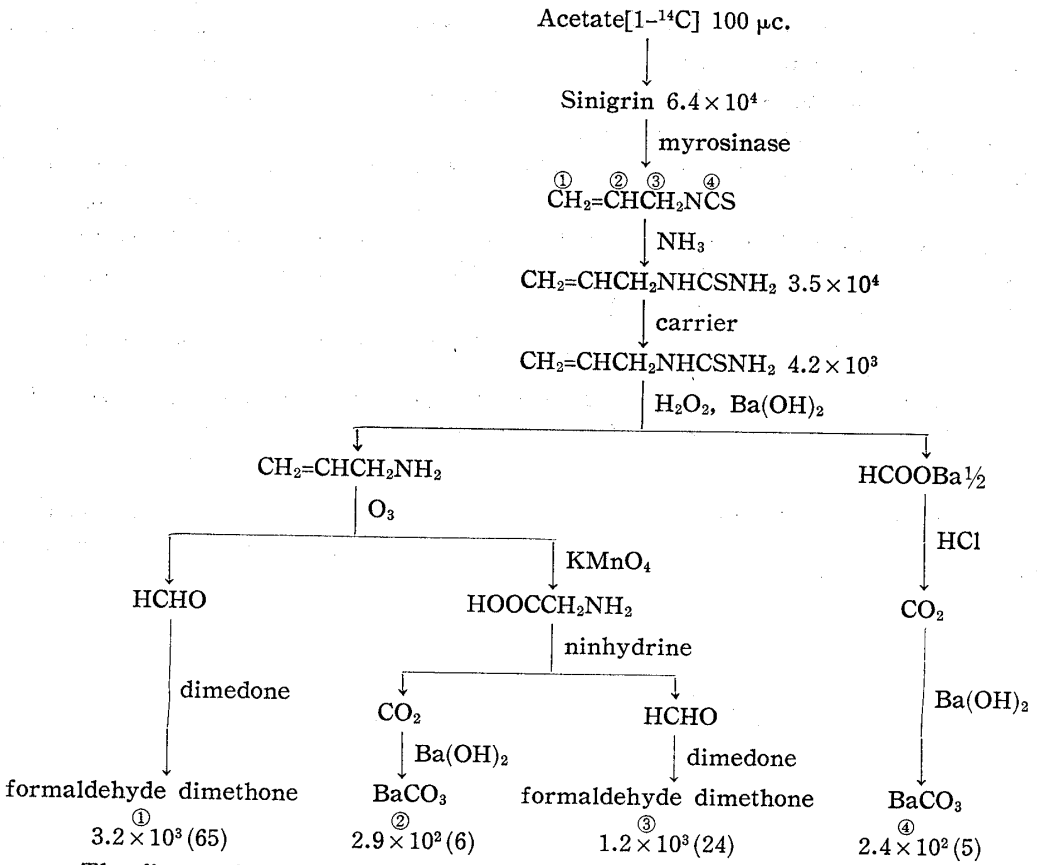
In the present work, it has been found that the carboxyl carbon of acetate is incorporated into allyl isothiocyanate derived from sinigrin in *Brassica juncea* Cosson and the location of the labeled carbon in sinigrin has been determined.

Sodium acetate [1-^{14}C] (100 μc) was administered into the stem of 6 months old Brassica plant by the cotton-thread method before the flowering period, and the seeds were harvested 6 weeks after administration.

Sinigrin and allyl isothiocyanate were obtained from the seeds, and allyl isothiocyanate was converted into allyl thiourea. Sinigrin and allyl thiourea were purified by paper chromatography (the solvent systems for sinigrin; PhOH-H_2O-NH_4OH=160:40:1, BuOH-Pyridine-H_2O=6:4:3,[5] for allyl thiourea; H_2O saturated $CHCl_3$[6]). Their specific activities were determined, and then allyl thiourea diluted with the non-radioactive carrier was degradated to determine the location of radioactivity as follows.[7,8]

Chart 1. The Specific Radioactivites (c.p.m./mM)



The figures in the parentheses indicate the distribution ratio (%) of radioactivities of degradation products; those in the circle are the corresponding carbon number of original allyl isothiocyanate.

From this result, it was shown that the carboxyl carbon of acetate was incorporated into the glucose and aglucone of sinigrin, and the distribution of radioactivity was in the same extent in the glucose and aglucone moieties, and that about 65% of radioactivity of labeled allyl isothiocyanate was located at C① and about 24% at C③.

5) O. Schulz, W. Wagner: Z. Naturforschg., **11b**, 73 (1956).
6) A. Kjaer, K. Rubinstein: Acta Chem. Scand., **7**, 528 (1953).
7) D.S. Hector: J. prakt. Chem., (2) **43**~**44**, 492 (1891).
8) C. Harries, P. Reichard: Ber., **37**, 612 (1904).

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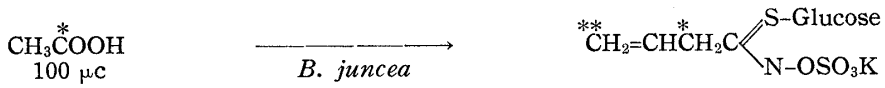
Chart 2. The Position of the Labelled Carbon Atom in Sinigrin



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Structure of Kessane

The sesquiterpenoid oxide, kessane, has newly been isolated from several kinds of Japanese valerian roots.[1,2] In the present communication the authors wish to propose structure (I) for kessane on the basis of the following experimental evidences.

Kessane (I) is a colorless liquid, $C_{15}H_{26}O$*[1], b.p_6 110~112°, d_4^{25} 0.970, n_D^{25} 1.491, $[\alpha]_D$ −7.2° ($CHCl_3$), whose infrared spectrum (liquid) exhibited no band associated with hydroxyl or carbonyl group and a band at 1095 cm^{-1} which could be assigned to an ether bridge. The nuclear magnetic resonance spectrum of I showed a doublet (3H) at 9.23 τ (τ=6.0 c.p.s) due to the methyl group in CH_3-CH< type and two singlets at 8.98 τ (3H) and 8.82 τ (6H) attributed to the methyl groups in CH_3-C< O- type. In the mass spectrum of I, there were the molecular ion peak at m/e 222, but no other dominant peaks at the heavy end which would be useful for structural elucidation. Although the prominent peaks at m/e 126 and below were less indicative of the structure, they were present in that of α-kessyl alcohol (II)[3] without exception, and thus both ion spectra or lower m/e bore a striking resemblance. Dehydrogenation of I with palladium-carbon or sulfur gave S-guaiazulene, characterized as its 1,3,5-trinitrobenzene adduct, m.p. 147~149°. All these data permitted the hypothesis that I might be the desoxy-compound of II.

In an attempt to find support for this hypothesis by synthesis, 2-*epi*-α-kessyl alcohol (III; R=H)[4] was converted with tosyl chloride in pyridine to the corresponding tosylate (III; R=Ts), $C_{22}H_{32}O_4S$, m.p. 96~97°, infrared bands (KBr) at 1602, 1355, 1172 (tosylate) cm^{-1}, which by reduction with lithiumaluminium hydride furnished I, $C_{15}H_{26}O$, b.p_5 105°, d_D^{25} 0.969, n_D^{25} 1.492, $[\alpha]_D$ −4.9° ($CHCl_3$), together with small amount of III (R=H). The identity was established by gas chromatography, infrared spectrum, and nuclear magnetic resonance spectrum.

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*1 All analytical values are in good agreement with molecular formulae shown. Melting points and boiling points are uncorrected. NMR spectra were measured at 60 Mc. in CCl_4 vs. Me_4Si as internal reference.

1) H. Hikino, Y. Hikino, H. Kato, Y. Takeshita, T. Takemoto: Yakugaku Zasshi, 83, 219 (1963).
2) H. Hikino, Y. Hikino, Y. Takeshita, Y. Isurugi, T. Takemoto: *Ibid.*, (1963). in press and references cited therein
3) cf. J. Simonsen, P. de Mayo: "The Terpenes," V 564 (1957), Cambridge University Press, London,
4) unpublished data.

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