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 αβ-unsaturated butenolide ring in V was shown by an ultraviolet absorption maximum at 218 μν, infrared spectrum with peaks at 5.58, 5.78, 6.16 μν 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°, C23H35O5, UV : λ_max 214 μν (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°, C25H35O4) 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°, C25H37O5, UV : λ_max 218 μν (log ε 4.17), [α]_D -102.5°(CHCl3)). Dehydrogenation of Ⅷ with selenium dioxide afforded the 1,4-dien-3-one (Ⅸ) (m.p. 229~231°, C23H35O3, UV : λ_max 221 μν (log ε 4.38), [α]_D +218.5°(CHCl3)), which was rearranged to a compound having a phenolic moiety in ring A (Ⅹ) (m.p. 159~161°, C23H35O3, UV : λ_max 267 μν (log ε 2.56), [α]_D +160°(CHCl3)), nuclear magnetic resonance : 3.13 τ (quartet of AB system). In addition, Ⅷ was converted to the 1,4,6-trien-3-one (Ⅺ) (m.p. 232~238°, C23H35O3·H2O through the 4-en-3-one (Ⅻ) (m.p. 193~196°, C23H35O3).

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 hypotheses1,2) and experiments3,4) on the biogenesis of mustard oil glucosides were reported. Underhill et al.5) 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.

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 [1-¹⁴C] (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-H₂O-NH₄OH=160:40:1, BuOH-Pyridine-H₂O=6:4:3,⁹ for allyl thiourea; H₂O saturated CHCl₃). Their specific activities were determined, and then allyl thiourea diluted with the non-radioactive carrier was degraded to determine the location of radioactivity as follows,⁷ ⁸

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

Acetate[¹⁴C] 100 μC.

\[
\begin{align*}
\text{Sinigrin} & \quad 6.4 \times 10^4 \\
\text{myrosinase} & \quad \text{NH}_3 \\
\text{CH}_2=\text{CHCH}_2\text{NCS} & \quad 3.5 \times 10^4 \\
\text{CH}_2=\text{CHCH}_2\text{NHCSNH}_2 & \quad 4.2 \times 10^4 \\
\text{H}_2\text{O}_2, \text{Ba(OH)}_2 & \quad \text{HCOOBa}^{1\frac{1}{2}} \\
\text{CH}_2=\text{CHCH}_2\text{NH}_2 & \quad \text{HCl} \\
\text{O}_3 & \quad \text{CO}_2 \\
\text{HCHO} & \quad \text{CO}_2 \\
\text{KMnO}_4 & \quad \text{Ba(OH)}_2 \\
\text{HOOCCH}_2\text{NH}_2 & \quad \text{BaCO}_3 \\
\text{ninyhydrine} & \quad \text{formaldehyde dimethone} \quad (65) \\
\text{dimedone} & \quad \text{CO}_2 \\
\text{formaldehyde dimethone} & \quad \text{BaCO}_3 \\
\text{Ba(OH)}_2 & \quad \text{formaldehyde dimethone} \quad (24) \\
\text{Ba(OH)}_2 & \quad \text{BaCO}_3 \\
\text{BaCO}_3 & \quad \text{HCHO} \\
\text{Ba(OH)}_2 & \quad \text{dimedone} \\
\end{align*}
\]

The figures in the parentheses indicate the distribution ratio (% of radioactivity 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 C³ and about 24% at C⁵.

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⁸ C. Harries, P. Reichard : Ber., 37, 612 (1904).
Chart 2. The Position of the Labelled Carbon Atom in Sinigrin

\[
\begin{align*}
\text{Acetate}^{[1-1^4C]} & \quad \text{Sinigrin} \\
\text{CH}_2\text{COOH} & \quad \text{CH}_2=\text{CHCH}_2\text{C}=\text{N}-\text{OSO}_3\text{K} \\
100 \mu\text{c} & \quad B. \text{ juncea} \\
\end{align*}
\]

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Structure of Kessane

The sesquiterpenoid oxide, kessane, has newly been isolated from several kinds of Japanese valerian roots.\(^1\)\(^3\) 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, \(\text{C}_{13}\text{H}_{20}\text{O}\)\(^6\), b.p. \(110 \sim 112^\circ\), \(d_f^\circ 0.970\), \(n_f^\circ 1.491\), \((\alpha)_D -7.2^\circ\) (\(\text{CHCl}_3\)), whose infrared spectrum (liquid) exhibited no band associated with hydroxyl or carbonyl group and a band at \(1095 \text{ cm}^{-1}\) which could be assigned to an ether bridge. The nuclear magnetic resonance spectrum of I showed a doublet (3H) at \(9.23\tau\) \((\tau=6.0 \text{ c.p.s.})\) due to the methyl group in \(\text{CH}_3\text{CH}\) type and two singlets at \(8.98\tau\) (3H) and \(8.82\tau\) (6H) attributed to the methyl groups in \(\text{CH}_3\text{C} \leq \text{O-}\) 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 \(\alpha\)-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 \sim 149^\circ.

All these data permitted the hypothesis that I might be the deoxy-compound of II.

In an attempt to find support for this hypothesis by synthesis, 2-\(\text{epi-}^\alpha\)-kessyl alcohol (III; \(R=\text{H}\))\(^4\) was converted with tosyl chloride in pyridine to the corresponding tosylate (III; \(R=\text{Ts}\)), \(\text{C}_{22}\text{H}_{37}\text{OsS}\), m.p. \(96 \sim 97^\circ\), infrared bands (KBr) at 1602, 1355, 1172 (tosylate) \text{ cm}^{-1}, which by reduction with lithiumaluminium hydride furnished I, \(\text{C}_{13}\text{H}_{20}\text{O}\), b.p. 105\(^\circ\), \(d_f^\circ 0.969\), \(n_f^\circ 1.492\), \((\alpha)_D -4.9^\circ\) (\(\text{CHCl}_3\)), together with small amount of III \((R=\text{H})\). 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 \(\text{CCl}_4\) vs. \(\text{Me}_2\text{Si}\) as internal reference.

4) unpublished data.