96. **Daisuke Satoh, Keiji Yoshida, Hiroshi Ishii, and Yohko Nishimura:** Water-soluble Cardioglycoside from the Leaves of *Digitalis purpurea* L.*

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The known water-soluble cardioglycosides include 16-acetyldigitalinum verum and gitorin obtained from the leaves of *Digitalis lanata* by Tschesche and others¹⁾ and digitalinum verum and gitorin isolated from the leaves of *D. purpurea* by Ishidate and others²⁾. The present writers also obtained some different water-soluble glycosides from the extract of the leaves of *D. purpurea* which are described herein.

Two kinds of crystals were obtained from the dried leaves of *Digitalis purpurea* L. by the method given in the experimental part.

One of the substances was repeatedly recrystallized from a methanol-ether mixture to colorless, short rhomboprisms (A), m.p. 196~204°(decomp.). Ultraviolet absorption spectrum: $\lambda_{\max}^{\mathrm{EtOH}}$ 310 mμ (log ε=2.01) (Fig. 1, a), $[\alpha]_D^{29}$: −169.0° (c=1.476, MeOH). The analytical values corresponded to $C_{32}H_{48}O_9$, possessing one methoxyl group. This substance gives a weak Legal reaction and bluish green coloration in the acetic acid layer by the Keller-Kiliani reaction, indicating the presence of 2-desoxysugar, and brown coloration at the zone of contact with conc. sulfuric acid. Toxicity by the pigeon method is extremely weak, being 18.6 mg./kg. These properties are very similar to those of the crystals obtained from the seeds of *Digitalis lanata* by Reichstein³⁾ and the substance was found to be identical with the crystals obtained from the seeds of *D. purpurea* by Okada and others⁴⁾, giving agreeable results by mixed fusion*** and Rf values by paper chromatography. Comparison of the properties are shown in Table I. Composition of this substance is now being examined.

TABLE I.

	Crystal A	Sample by Reichstein	Sample by Okada and Yamada
Plant material	*D. purparea* leaf	*D. lanata* seed	*D. purpurea* seed
Crystal form	Short rhomboprisms	Prisms	Needles
m.p.(decomp.)	196~204°	174~214 (corr.)	205°
$\lambda_{\max}$	310 mμ		
$[\alpha]_D$	$[\alpha]_D^{29}$: −169.0°	$[\alpha]_D^{16}$: −194.0°	
Molecular formula	$C_{32}H_{48}O_9$ (assumed)	$C_{32}H_{48}O_9$	$C_{32}H_{48}O_9$
OCH_3	1	1	1
Keller-Kiliani R. (AcOH-contact zone)	Bluish green-brown	Blue-brown	Bluish green-brown
Legal Reaction	Positive		Positive
Solubility			
Chloroform	Easily sol.	Easily sol.	Easily sol.
Methanol	Easily sol.	Easily sol.	Easily sol.
Acetone	Spar. sol.	Spar. sol.	Spar. sol.
Ether	Pract. insol.	Pract. insol.	Insol.
Rf ($CHCl_3$:MeOH:H_2O =10:2:5)	0.93		0.93

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[*] A brief summarized report of this work was published as a Communication to the Editor in this Bulletin, 1, 305(1953).

[**] Imafuku, Amagasaki, Hyogo-ken (佐藤大助, 吉田珪治, 石井 宏, 西村蓉子).

[***] Sample for mixed fusion was kindly supplied by Mr. Okada.

1) R. Tschesche, G. Grimmer, F. Neuwald: Chem. Ber., 85, 1103(1952).
2) M. Ishidate, M. Okada, Y. Sasagawa: This Bulletin, 1, 186(1953).
3) K. Mohs, T. Reichstein: Pharm. Acta Helv., 24, 246 (1949).
4) M. Okada, A. Yamada: J. Pharm. Soc. Japan, 73, 525(1953).

The other was repeatedly recrystallized from a methanol-ether mixture or ethyl acetate to colorless short rhomboprisms (B), m.p. 243~246°(decomp.). Yield against dried leaves, approx. 0.024%. This substance is sparingly soluble in chloroform and ether, easily soluble in ethanol and methanol, and dissolves in about 700 volumes of water. Ultraviolet absorption spectrum: λ_{max}^{EtOH} 219 mμ (log ε=4.22) (Fig. 1, b). $[\alpha]_D^{28}$: +17.9° (c=1.232, MeOH). Toxicity by the pigeon method, 0.93 mg./kg. Legal reaction, strongly positive, Keller-Kiliani reaction, colorless acetic acid layer, red contact zone with conc. sulfuric acid. These color reactions are the same with the afore-mentioned digitalinum verum and gitorin, but the analytical values correspond to $C_{30}H_{46}O_9$, possessing one methoxyl but no acetyl group.

The acetate prepared by the application of acetic anhydride in anhydrous pyridine came as colorless needles, m.p. 223~226°(decomp.), whose analytical values corresponded to those of triacetate, $C_{36}H_{52}O_{12}$.

The Rf values in paper chromatography were 0.31 ($CHCl_3 : MeOH : H_2O = 10 : 2 : 5$) for the free compound and 0.61 ($CHCl_3 :$ Benzene $: H_2O = 1 : 9 : 2$) for the crude acetate, respectively giving only one spot. These facts indicate that this substance is a unity and clearly differs from digitalinum verum* which gives Rf 0.00 and 0.30 for the free compound and the acetate, respectively.

Hydrolysis of this substance with 3.4% hydrochloric acid (50% ethanolic) gives an aglycone as slightly yellow needles, m.p. 209~211°. Ultraviolet absorption spectrum: λ_{max}^{EtOH} 337 mμ (log ε=4.31) (Fig. 1, c). Analytical values of this aglycone correspond to $C_{23}H_{30}O_3$ and the melting point failed to show any depression on admixture with $\Delta^{14,16}$-dianhydrogitoxigenin (I), m.p. 209~211°, obtained as a by-product on the hydrolysis of gitoxin. Since the ultraviolet absorption spectrum of the original glycoside gives the maximum at 219 mμ (EtOH), the aglycone should be gitoxigenin (II) (Fig. 1, e) which had undergone dehydration by being boiled in hydrochloric acid to $\Delta^{14,16}$-dianhydrogitoxigenin.

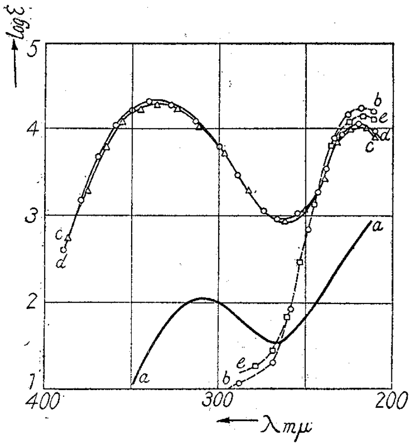

Fig. 1.
Ultraviolet Absorption Spectra
(in Ethanol)

a ———— Crystal A
b ○…○…○ Crystal B
c △—△—△ $\Delta^{14,16}$-Dianhydrogitoxigenin from Crystal B
d ○—○—○ $\Delta^{14,16}$-Dianhydrogitoxigenin from gitoxin
e □—□—□ Gitoxigenin from gitoxin

In order to confirm this theory, the Mannich hydrolysis was carried out and an aglycone was obtained as white needles, m.p. 220~223°, which failed to show any depression on admixture with gitoxigenin, m.p. 223~224°, obtained by the hydrolysis of gitoxin. These results have confirmed the aglycone of Crystal B to be gitoxigenin.

The syrupy sugar obtained by the foregoing hydrolysis gives a negative Keller-Kiliani reaction, indicating it not to be 2-desoxysugar. Since the paper chromatography gives only one spot of Rf 0.56 ($BuOH : AcOH : H_2O = 4 : 1 : 1$), it is certain that there is only one kind of sugar, and the osazone, m.p. 179~181°, as well as the analytical values, correspond to the osazone, m.p. 179~180°⁵⁾, of *d*-digitalose, $C_{19}H_{24}O_3N_4$. Comparison with the sugars (*d*-glucose and *d*-digitalose) obtained by the hydrolysis of digitalinum verum has shown the foregoing Rf value to be identical with that of *d*-digitalose. It follows, therefore, that the foregoing sugar is *d*-digitalose

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* The sample was kindly given by Prof. Ishidate.

5) O. T. Schmidt, E. Wericke: Ann., 566, 179(1944).

(III). From the content of the methoxyl group in the original glycoside, it is clear that there is only one *d*-digitalose bonded to the alycone.

These results show that the Crystal B must be gitoxigenin monodigitaloside (IV) and this substance corresponds to strospeside (desgluco-digitalinum verum) (IV), $C_{30}H_{46}O_9$, obtained from the seeds of *Strophanthus speciosus* and *Str. Boivinii* by Reichstein and others[6,7]. Comparison of the data of Crystal B with those of strospeside given in litearture[6] is as follows:

	m.p. (decomp.)	$[\alpha]_D$
Crystal B	243~246°	$[\alpha]_D^{28}$: +17.9°
Strospeside	248~255° (corr.)	$[\alpha]_D^{14}$: +17.6°
Triacetate of Crystal B	223~226°	
Strospeside triacetate	227~230° (corr.)	

The samples of strospeside and its triacetate, kindly given by Dr. T. Reichstein, were compared with Crystal B and its triacetate, and gave following results of mixed fusion, paper chromatography, and infrared absorption spectrum.

	Crystal B	Strospeside	Mixed
m.p.(decomp.)	243~246°	243~246°	243~246°
Triacetate m.p.(decomp.)	223~226°	224~227°	223~226°
Rf ($CHCl_3$: MeOH : H_2O = 10 : 2 : 5)	0.31	0.31	

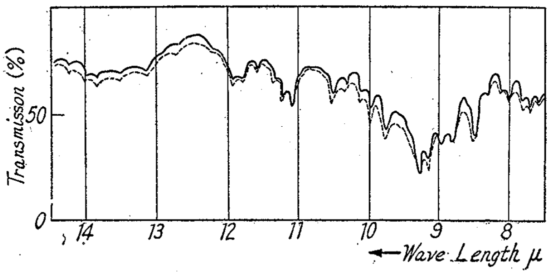
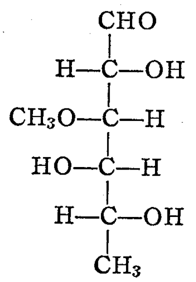
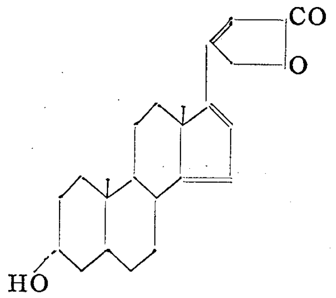


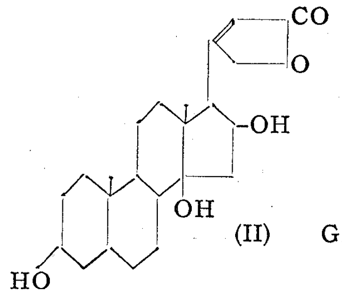
Fig. 2.
Infrared Absorption Spectra
(in Nujol)



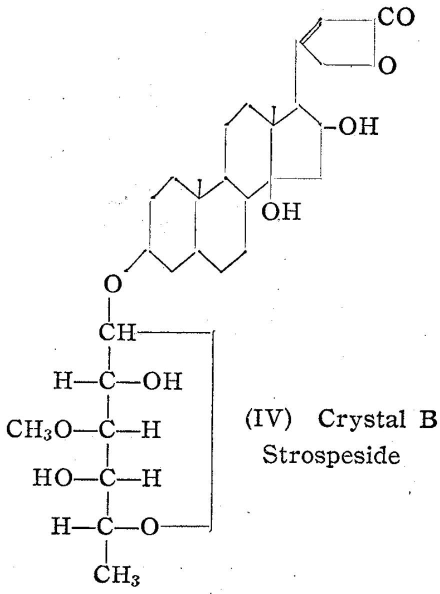
(III) *d*-Digitalose



(I) $\Delta^{14,16}$-Dianhydrogitoxigenin



(II) Gitoxigenin



(IV) Crystal B
Strospeside

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6) W. Rittel, A. Hunger, T. Reichstein: Helv. Chim. Acta, 35, 434(1952).
7) O. Schindler, T. Reichstein: Helv. Chim. Acta, 35, 442(1952).

Infrared spectra of both agreed well, as shown in Fig. 2.

The foregoing results have shown that one (Crystal B) of the water-soluble cardioglycosides isolated from the leaves of *Digitalis purpurea* L. by the present writers is identical with strospeside obtained by Reichstein and others. This is the first time, however, that strospeside had been isolated from the Digitalis leaves.

Studies are now being made to see whether this substance is contained, *per se*, in the fresh leaves, or whether the true glycoside is digitalinum verum which underwent loss of a glucose by enzymatic action during drying to form strospeside.

The writers take this opportunity to offer their deep gratitude to Prof. Dr. T. Reichstein and to Prof. Ishidate and Mr. Okada of the University of Tokyo for giving them valuable samples. The writers are also grateful to Dr. K. Takeda, Director of this Laboratory, for his unfailing guidance, to Messrs. Miyahara, Ieki, Hirai, Morita, Nakai, Matsui, and Tohri for carrying out the elemental analyses and absorption spectra measurements, to the members of the Pharmacological Section of this Laboratory for carrying out the animal tests, and to the members of the Akoh Plant of this firm for the Digitalis materials offered.

Experimental8)

I. Isolation of Crystals A and B from Dried Leaves of *Digitalis purpurea*—Leaves of *Digitalis purpurea* were dried as soon as possible after harvest by blowing air of 60°, and 1.200 g. of such dried leaves was extracted twice with 15 L. of 50% methanol at 30° for 8 hours each. Basic lead acetate solution was added to the extract to precipitate chlorophyll and other impurities and hydrogen sulfide was passed through this filtrate to remove the excess of lead. The filtrate therefrom was neutralized and concentrated at a low temperature, under a reduced pressure, to about one-third the original volume. The sparingly soluble glycoside that separated out was removed by suctional filtration, and the filtrate was extracted with ether to remove the impurities. The aqueous layer was then extracted with chloroform, and chloroform, after drying over sodium sulfate, was distilled off at a low temperature from which crude, water-soluble glycoside was obtained as pale yellowish brown powder. Yield, approx. 1.2 g., about 0.1% against the dried leaves.

Ten g. of this substance was dissolved in about 20 volumes of chloroform, poured through an adsorption column filled with neutral alumina, the column was developed with chloroform and a 60:1 mixture of chloroform and methanol, and fractionated according to the Keller-Kiliani reaction.

1) Chloroform Fraction: The pale yellowish brown powder obtained on the removal of chloroform at a low temperature was dissolved in a small amount of ethyl acetate with application of heat, and the crystals that separated out on cooling were collected by suctional filtration to 0.6 g. of crude crystals. These crystals were washed with a small amount of ethyl acetate and repeatedly recrystallized from a methanol-ether mixture to colorless, short rhomboprisms, m.p. 196∼204°(decomp.). These crystals were designated as Crystal A, properties of which are given in the main text. *Anal.* Calcd. for $C_{32}H_{48}O_9$: C, 66.64; H, 8.41; OCH_3, 5.39. Found: C, 67.08; H, 8.06; OCH_3, 5.64; $COCH_3$, 0.09.

2) Chloroform-Methanol (60:1) Fraction: The pale yellowish brown powder obtained on the removal of the solvents was dissolved in a small amount of ethyl acetate with warming, and 2.4 g. of crystals that separated out on cooling were collected by suctional filtration and repeatedly recrystallized from a methanol-ether mixture or ethyl acetate to colorless, short rhomboprisms, m.p. 243∼246°(decomp.), which were designated as Crystal B. The properties of this substance are as given in the main text.

The Rf value by paper chromatography by the descending method, at 26∼27°, developed for 1.5 hrs., with a mixture of chloroform-methanol-water=10:2:5, was 0.31 for Crystal B and 0.00 for digitalinum verum. *Anal.* Calcd. for $C_{30}H_{46}O_9$: C, 65.43; H, 8.42; OCH_3, 5.63. Found: C, 65.26; H, 8.73; OCH_3, 5.34; $COCH_3$, 0.12.

II. Acetylation of Crystal B—To a solution of 100 mg. of Crystal B dissolved in 1 cc. of dehydrated pyridine, 1 cc. of acetic anhydride was added and the mixture was allowed to stand for 43 hours at a room temperature. After warming the mixture for 1 hour at 80°, pyridine and excess of acetic anhydride were removed under a reduced pressure, and addition of water to the residue with stirring yielded 105 mg. of crude acetate. In order to prove the unity of the Crystal B, this crude acetate was submitted to paper chromatography by the descending method at 29°, developed for 1.75 hrs., with a mixture of chloroform-benzene-water (1:9:2) and Rf 0.61

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8) All m.p.s are uncorrected unless otherwise noted.

was obtained for the acetate of Crystal B. The same was carried out with the hexaacetate of digitalinum verum for comparison and Rf 0.30 was obtained.

The crude acetate was recrystallized from diluted methanol to 80 mg. of colorless needles, m.p. 223～226°(decomp.). *Anal.* Calcd. for $C_{36}H_{52}O_{12}$: C, 63.89; H, 7.75. Found: C, 64.06; H, 7.34. These analytical values correspond to those of the triacetate of Crystal B.

III. Hydrolysis of Crystal B—1) Hydrolysis with 3.4% hydrochloric acid (50% ethanolic)—A solution of 300 mg. of Crystal B dissolved in 30 cc. of 50% ethanolic solution of 3.4% HCl was refluxed for 6 hours in a carbon dioxide stream by which the solution colored pale yellow. This solution was concentrated under a reduced pressure in carbon dioxide stream and 150 mg. of pale yellow fine needles, m.p. 197～202°, separated out. The filtrate was extracted with chloroform and, after washing and drying over sodium sulfate, the chloroform was removed and only a minute amount of residue remained.

The foregoing crystals were repeatedly recrystallized from a methanol-ether mixture to practically colorless needles, m.p. 209～211°. *Anal.* Calcd. for $C_{23}H_{30}O_3$: C, 77.97; H, 8.47. Found: C, 78.20; H, 8.60. Ultraviolet absorption spectrum: λ_{max}^{EtOH} 337 mμ (log ε=4.31). There was no depression of the melting point on admixture of this substance with $\Delta^{14,16}$-dianhydrogitoxigenin, m.p. 209～211°, obtained by the hydrolysis of gitoxin, and the absorption maxima were also identical.

The aqueous solution obtained by the removal of the aglycone was stirred with freshly prepared silver carbonate and filtered to remove hydrochloric acid, and hydrogen sulfide was passed through the filtrate chilled to 0° to remove the excess of sliver ion. The filtrate therefrom was again concentrated to a syrupy state in a carbon dioxide stream, acetone added to remove the minute amount of impurities, and the filtrate was again concentrated in a carbon dioxide stream from which 50 mg. of colorless syrup was obtained. This substance reduces the Fehling solution.

2) Chromatography of the sugar (Comparison with the sugar from digitalinum verum)—The sugar was obtained from 10 mg. of digitalinum verum by the same method as above. The paper chromatography was carried out on both sugars by the descending method at 30～34°, developed for 6 hours, with a 4:1:1-mixture of butanol, acetic acid, and water.

	Rf	
	d-Glucose	*d*-Digitalose
Sugar from Crystal B		0.56
Sugar from Digitalinum verum	0.22	0.57
d-Glucose	0.22	

3) Osazone of the sugar—A mixture of 90 mg. of the foregoing syrupy sugar, 180 mg. of phenylhydrazine, and 1.5 cc. water was warmed on a boiling water bath for 4 hours in a carbon dioxide stream. The product thereby obtained was collected by suctional filtration, washed twice with benzene, and recrystallized from 50% ethanol to yellow needles, m.p. 179～181°. *Anal.* Calcd. for $C_{19}H_{24}O_3N_4$: C, 64.02; H, 6.79; N, 15.06. Found: C, 63.72; H, 6.29; N, 15.40.

4) Hydrolysis by the Mannich method—To a solution of 300 mg. of Crystal B dissolved in 26 cc. of acetone with warming, 0.26 cc. of 38% hydrochloric acid was added and the mixture was allowed to stand at a room temperature for 13 days. The pale yellowish brown solution was added with 20 cc. water and concentrated under a reduced pressure, in a carbon dioxide stream, by which white turbidity appeared. This mixture was extracted with chloroform, and after washing with water and drying over sodium sulfate, chloroform was removed. The residue was repeatedly recrystallized from a methanol-ether mixture to colorless needles, m.p. 220～223°, which gave a mixed m.p. of 221～223° when admixed with gitoxigenin, m.p. 223～224°, obtained by the hydrolysis of gitoxin.

IV. Comparison of Crystal B and Strospeside—Mixed fusion of Crystal B, m.p. 243～246° (decomp.), and its triacetate, m.p. 223～226°(decomp.), with samples of strospeside, m.p. 243～246° (decomp.), and its triacetate m.p. 224～227°(decomp.), sent from Dr. T. Reichstein, showed no depression of the melting point.

Paper chromatography was carried out on these two samples by the descending method developed at 27～28° for 1.3 hrs., with a 10:2:5-mixture of chloroform, methanol, and water, and the Rf values obtained were 0.31 for Crystal B and 0.31 for strospeside.

Summary

Two kinds of water-soluble glycosides were isolated from the dried leaves of *Digitalis purpurea* L. One gave the melting point of 196～204°, physiologically almost inactive, and

was similar to the glycoside obtained from the seeds of *Digitalis lanata* by Reichstein and others, and identical with the glycoside obtained from the seeds of *D. purpurea* by Okada and others.

The other glycoside of m.p. 243~246° possessed a stronger cardiac action and was found to be gitoxigenin-monodigitaloside. This substance was found to be identical with strospeside obtained from the seeds of *Strophanthus speciosus* and *Str. Boivinii* by Reichstein, and this is the first instance in which this substance had been found from Digitalis leaves. It is not yet known whether this substance is contained, *per se*, in the fresh leaves.

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97. **Hiroyuki Inouye**: Über die Bestandteile von *Pirola japonica* Sieb. IV.[1] Die Konstitution des Pirolatins. (3).

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In der vorangegangenen Mitteilung bewies der Verfasser zuerst aus der Tatsache, dass das Tetrahydropirolatin bei der Oxydation mit $KMnO_4$ 4,8-Dimethylnonansäure liefert, dass das Pirolatin eine Diisopentenyl-Seitenkette besitzt. Ferner vermutete er an Hand der bekannten Naturstoffe wie Osthrutin, Chlorophorin und dgl. für das Pirolatin die Konstitutionsformel (I) oder (II).

In dieser Arbeit wurde nun die Lage der zwei Doppelbindungen in der Seitenkette behandelt. Die weiteren Untersuchungen ermöglichten, den Schluss zu ziehen, dass höchstwahrscheinlich die Formel (I) für das Pirolatin gilt.

Zum Studium dieses Problems wurde der Ozonabbau benützt. In Anbetracht der Löslichkeit der Substanzen in Lösungsmitteln, wurden als Ausgangsmaterial das Pentaacetylpirolatin gewählt (das unter Eiskühlung mittels Einwirkung von Essigsäureanhydrid auf die Pirolatinlösung in Pyridin gewonnen wurde) und das Pirolagenin (gewonnen durch Hydrolyse das Pirolatins mittels Emulsin). Diese beiden Substanzen blieben sirupös und liessen sich trotz aller Versuche nicht in Kristallform gewinnen. Das erstere lieferte jedoch durch katalytische Hydrierung Pentaacetyltetrahydropirolatin vom Schmp. 77~79°, womit erwiesen war, dass es sich um die Pentaacetylverbindung handelt.

Nun muss man beim Ozonabbau von Pentaacetylpirolatin bzw. Pirolagenin als Zersetzungsprodukt entweder Aceton und Lävulinaldehyd, falls die Formel (I) für Pirolatin gilt, oder Formaldeyd und Heptandion-2,6, falls die Formel (II) für dasselbe gilt, erhalten.

Beim Ozonabbau dieser beiden Substanzen in üblicher Weise wurden aber nur Lävulinaldehyd und eine äusserst kleine Menge Acetaldehyd in Form ihrer 2,4-Dinitrophenylhydrazone von dem Schmelzpunkt 234~235° bzw. 160~162° gewonnen. Das erstere wurde weiter durch die Mischprobe mit dem 2,4-Dinitrophenylhydrazon des Lävulinaldehyds, das durch den Ozonabbau von Geraniol erhalten wurde, und das letztere durch die Mischprobe mit dem des authentischen Acetaldehyds bestimmt. Was das Acetaldehyd betrifft, so ist dessen Entstehungsmechanismus zwar noch nicht geklärt, scheint aber für die Erkenntnis der Konstitution der Seitenkette nicht von Bedeutung zu sein, wenn man ausser seiner eben sehr kleinen Menge, auch noch die Tatsache in Betracht zieht, dass durch die Oxydation von Tetrahydropirolatin 4,8-Dimethylnonansäure erhalten wurde.

Obgleich beim Ozonabbau weder Aceton noch Formaldehyd gewonnen wurde, muss

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* Yoshida-konoe-cho, Sakyo-ku, Kyoto (井上博之).

[1] III. Mitteil.: J. Pharm. Soc. Japan, 72, 731(1952)