UDC 582.893 : 581.19

21. **Hiroshi Mitsuhashi, Ukon Nagai, and Toshio Muramatsu** : Studies on the Constituents of Umbelliferae Plants. III. Structure of Ligustilide.

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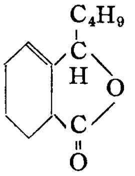
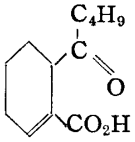
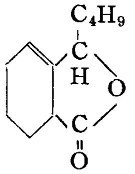
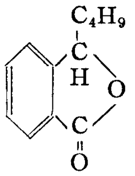
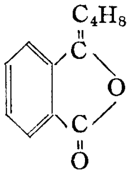
In the previous paper,[1] the isolation of a new constituent with anticholinergic activity, ligustilide, from the root of Hokkai-Tôki was reported. Ligustilide, $C_{12}H_{14}O_2$, an isomer of butylphthalide, is characterized by the strong bluish fluorescence under ultraviolet ray, the odor closely similar to that of 3-alkylidenephthalide, ultraviolet absorption maximum at 320 mμ, and infrared absorption maxima (as liquid film) at 1755, 1661, 1621, 1586, 1050, 1010, 985, 960, and 705 cm^{-1}.

From the properties mentioned above, it is reasonable to consider that ligustilide is a derivative of 3-butylidenephthalide with dihydrobenzene ring or its analog, because the molecular formula of ligustilide corresponds to the dihydro derivative of butylidenephthalide

Table I. Compounds related to 3-Butylphthalide isolated from Umbelliferae Plants

Compound		Plant	Ref.
Name	Structure		
Sedanolide		*Apium graveolens* L.	a
Sedanonic acid (possibly present in its lactonic form)		*Apium graveolens* L. *Cnidium officinale* Makino *Levisticum officinale* Koch.	a b c
Cnidium lactone (stereoisomer of sedanolide)		*Cnidium officinale* Makino	b
Butylphthalide		*Ligusticum acutilobum* Sieb. et Zucc. *Levisticum officinale* Koch.	d c
Butylidenephthalide		*Ligusticum acutilobum* Sieb. et Zucc. *Levisticum officinale* Koch.	d, e c

a) G. Ciamician, P. Silber : Ber., **30**, 492, 501, 1419, 1424, 1427 (1897).
b) T. Noguchi : Yakugaku Zasshi, **54**, 913 (1934).
c) Y. R. Naves : Helv. Chim. Acta, **26**, 1281 (1943).
d) T. Noguchi, *et al.* : Yakugaku Zasshi, **57**, 769, 783 (1937).
e) T. Kariyone, *et al.* : *Ibid.*, **56**, 662, 668 (1936).

[*1] Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (三橋 博, 永井右近, 村松敏夫).

[1] Part II : H. Mitsuhashi, *et al.* : This Bulletin, **8**, 243 (1960).

and similarity of its odor to 3-alkylidenephthalide. According to Kariyone, *et al.*,2) 3-alkylidenephthalides and their hydro derivatives all have the characteristic odor of Tôki and Berlingozzi3) reported that the odor of 3-alkylphthalides and their hydro derivatives resemble that of celery.

The strong fluorescence of ligustilide suggests the presence of a dihydrobenzene structure. In this connection, 3-butylphthalide has no fluorescence and 3-butylidenephthalide has a dark green fluorescence. Isolation of related compounds of the same skeleton from Umbelliferae plants (Table I) supports this assumption.

In order to ascertain this structure, catalytic hydrogenation with palladized charcoal was attempted and three substances were isolated from the hydrogenation product by chromatography through silicic acid-chloroform system. Saponification of each substance yielded sedanonic acid (II), 3-butylphthalide (III), and 2-valerylcyclohexanecarboxylic acid (IV), respectively.

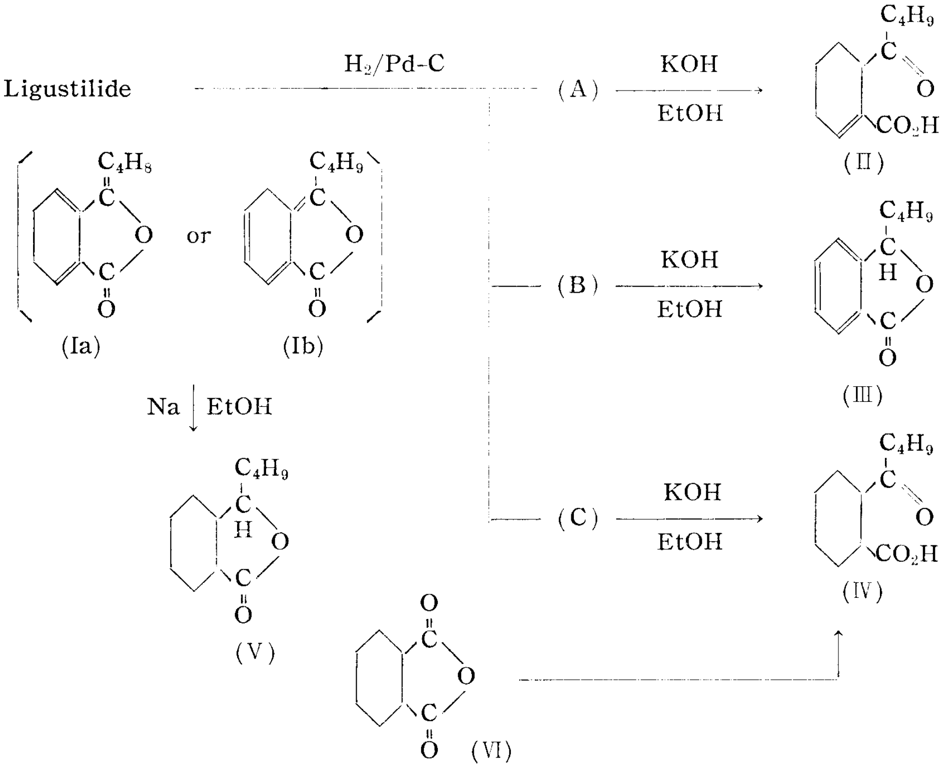


Chart 1.

Sedanonic acid and 2-valerylcyclohexanecarboxylic acid can be formed by the stepwise hydrogenation and subsequent saponification of (Ia) or (Ib). 3-Butylphthalide is considered to be formed by the displacement of double bonds to result in aromatization.

The reduction of ligustilide with metallic sodium and ethanol gave an oily substance (V) of the composition $C_{12}H_{20}O_2$, which would be diastereomeric mixture of 3-butylhexahydrophthalides.

From these data, it is evident that ligustilide is a dihydro derivative of 3-butylidenephthalide, but the position of double bonds is still uncertain. Since ligustilide has no optical rotatory power, it should have no asymmetric center.

Sedanonic acid was obtained by partial hydrogenation of ligustilide. This fact and the infrared spectral data of ligustilide suggest that one of the double bonds lies between

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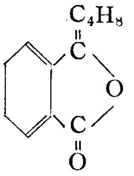
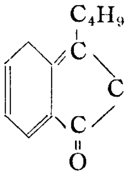
2) T. Kariyone, *et al.* : Yakugaku Zasshi, **73**, 336 (1953).
3) S. Berlingozzi : Gazz. chim. ital., **57**, 264 (1927).

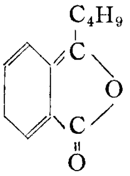
C-7 and C-7a, and another must be that of enolic lactone, if it is assumed that the migration of the double bonds from 3a—7a to 7—7a will not occur from energetic consideration. From these data, the following three structures are the most probable.



(Ia)



(Ib)



(Ic)

Calculated values of ultraviolet absorption maxima are 284 mμ for the structure (Ia), 318 mμ for (Ib), and 249 mμ for (Ic), by the method of Fieser[4] for steroids. The calculation is based upon the most bathochromic system present in each structure. Structure (Ib) shows good agreement with the observed vaule of 320 mμ for ligustilide. Such calculation is, however, not always reliable with respect to the cross-conjugated system. Although the applicability of the Woodward rule for compounds of phthalide group is questionable, such attempt cannot be excluded from the present considerations to account for the band in a very long wave-length region (320 mμ).

Trisubstituted double bond generally shows a strong absorption at 840$\sim$800 cm^{-1} due to the out-of-plane deformation of the hydrogen atom attached to the double bond; *cis*-disubstituted double bond appears near 690 cm^{-1}. Structure (Ia) contains three trisubstituted double bonds, while each of the other two structures (Ib) and (Ic) has one tetrasubstituted, one trisubstituted, and one *cis*-disubstituted double bonds. Ligustilide has no strong band at 840$\sim$800 cm^{-1}, but a weak band at 870 cm^{-1}, and has a relatively strong band at 705 cm^{-1}, which could be regarded as the absorption due to a *cis*-disubstituted double bond. This supports the structures (Ib) and (Ic) rather than (Ia). The absence of a strong band at 840$\sim$800 cm^{-1} may be explained by its shift to 870 cm^{-1} and weakening by the conjugation of the trisubstituted double bond with the lactonic carbonyl group in the structure of (Ib) and (Ic). From these spectral data, the structure (Ib) is the most possible. However, the structure (Ia) cannot be neglected because the odor of ligustilide closely resembles that of 3-alkylidenephthalides and because the compounds with double bond between C-3a and C-4 were isolated from the plants of the same family. These reasons do not exclude other possibilities.

To sum up all the considerations described above, it can be supposed that the structure (Ic) is less probable than the other two, and the structure of ligustilide is likely to be (Ia) or (Ib). In order to obtain further information, measurement of nuclear magnetic resonance spectrum is intended.

Experimental

Catalytic Hydrogenation of Ligustilide—A solution of 3.9 g. of ligustilide dissolved in EtOH was shaken with 0.5 g. of 5% Pd-C. The volume of H_2 absorbed was not determined. 4.0 g. of oily material was obtained by removal of the solvent in a diminished pressure and chromatographed over 120 g. of silicic acid (Mallincrodt for chromatography, 100 mesh) and $CHCl_3$ as an elution agent. Fractions were separated into three groups; A; 0.3 g., B; 0.7 g. and C; 1.42 g. with different pattern of ultraviolet spectra (Fig. 1).

Saponficiation of the Hydrogenation Products—i) Saponification of Fraction A: 0.3 g. of the oil was refluxed with 20 cc. of 0.5*N* EtOH-KOH solution for 1 hr. When cool, 20 cc. of water was added and EtOH was evaporated *in vacuo*. The aqueous solution was extracted with Et_2O, acidified with dil. HCl in an ice-bath, and extracted with Et_2O. Recrystallization of the Et_2O residue from benzene-hexane yielded about 50 mg. of crystals melting at 108$\sim$109° (reported[5] m.p. 110° for sedanonic

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[4] L. F. Fieser, M. Fieser: "Natural Products related to Phenanthrene," 3rd ed., 184 (1949). Reinhold Publishing Corp., Philadelphia, U. S. A.

[5] T. Noguchi: Yakugaku Zasshi, **54**, 913 (1934).

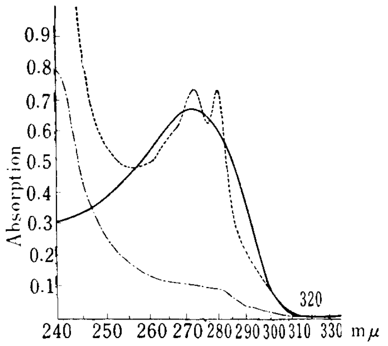

Fig. 1. Typical Ultraviolet Spectra of Hydrogenation Products

—— Fraction A
------ Fraction B
–·–· Fraction C

acid). *Anal.* Calcd. for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63. Found : C, 69.00; H, 8.76. IR ν_{max}^{KBr} cm^{-1} : 1697, 1660, 1628, 1280.

Semicarbazone of Sedanonic Acid : Obtained by the usual procedure with $H_2NNHCONH_2 \cdot HCl$ and AcONa. Recrystallization from EtOH yielded needles decomposing at 208~209 (reported[5]) m.p. 210° (decomp.)).

Phthalazone of sedanonic acid : Prepared by heating the EtOH solution of sedanonic acid and $NH_2NH_2 \cdot H_2O$ in a sealed tube on a boiling water bath for 25 min. When cool, water was added and the resulting crystals melted at 134~135° without recrystallization (reported[5]) m.p. 136°).

ii) Saponification of Fraction B : The chromatographic fraction with the same pattern in UV-spectrum as that of 3-butylphthalide was saponified with 0.5*N* KOH-EtOH solution in the usual way and the oily product obtained had much the same infrared spectrum as that of the synthetic 3-butylphthalide (Fig. 2).

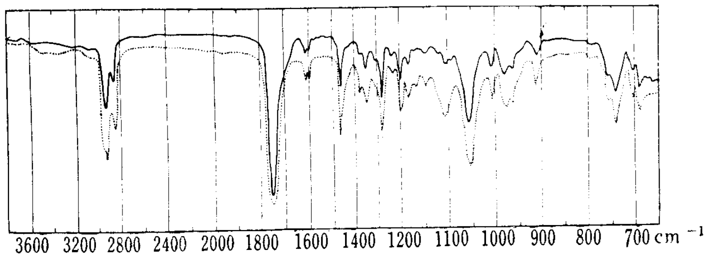

Fig. 2. Infrared Spectra of 3-Butylphthalides

—— Synthetic
------ Isolated from hydrogenation product

iii) Saponification of Fraction C : 1.2 g. of the oil was saponified by the same method as above and yielded 1.0 g. of acidic viscous oil, which on distillation (146°/2 mm. Hg) gave 0.64 g. of an oil. *Anal.* Calcd. for $C_{12}H_{20}O_3$: C, 67.89; H, 9.50. Found : C, 68.07, 67.94; H, 9.45, 9.38. IR $\nu_{max}^{liq. film}$ cm^{-1}: 1720 (shoulder), 1695, 1245.

Semicarbazone : m.p. 210° (decomp.). *Anal.* Calcd. for $C_{13}H_{23}O_3N_3$: C, 57.97; H, 8.61; N, 15.60. Found: C, 58.22, 58.65; H, 8.67, 8.64; N, 16.07.

Phthalazone : m.p. 99~101°. *Anal.* Calcd. for $C_{12}H_{20}ON_2$: C, 69.19; H, 9.68; N, 13.45. Found : C, 69.22, 68.86; H, 9.55, 9.27; N, 13.77.

Synthesis of 2-Valerylcyclohexanecarboxylic Acid—The Et_2O solution of BuMgBr was prepared in the usual way from 0.6 g. of metallic Mg and 5.1 g. of BuBr, and converted into the Cd-derivative by treating with 2.1 g. of anhyd. $CdCl_2$. The solvent was replaced with benzene. To the cold benzene solution of this reagent, 1.9 g. of *trans*-hexahydrophthalic anhydride (VI), m.p. 145° (prepared by catalytic reduction of phthalic acid),[6] was added as a fine powder with vigorous stirring and the mixture was refluxed for 1 hr., during which reddish solid material formed and made the stirring gradually difficult and stopped finally. The reaction mixture was treated with 10% H_2SO_4, benzene layer was separated, and extracted with 5% Na_2CO_3 solution. The alkaline solution was acidified with dil. HCl and extracted with Et_2O. On evaporation of Et_2O after drying over Na_2SO_4, 1.4 g. of oil was obtained. Yield, 54%. Its infrared spectrum showed a good agreement with that of the product obtained on saponification of chromatographic fraction C, from the hydrogenated ligustilide of natural source. Phthalazone : m.p. 98~101°, showing no depression on admixture with the natural product (mixed m.p. 99~101°).

Reduction with Sodium and Ethanol—To a solution of 1.0 g. of ligustilide in 30 cc. of anhyd. EtOH, 4.0 g. of metallic-Na was added over a period of 45 min. under reflux. Boiling was continued for

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[6] A. Haggis, *et al.* : J. Chem. Soc., **1953**, 393.

1 hr. The reaction mixture was treated with 15 cc. of EtOH to decompose the excess of Na, diluted with water, and EtOH was evaporated *in vacuo*. The resulting aqueous solution was acidified with 10% HCl at -10° and extracted with Et_2O which was dried over Na_2SO_4 in an ice-box and evaporated *in vacuo*. 950 mg. of the oily residue was dissolved in Et_2O and extracted with 5% Na_2CO_3. 750 mg. of acidic oil was obtained from the alkaline solution by the same procedure as above and 200 mg. of neutral oil from the Et_2O solution.

By distillation and adsorption chromatography with SiO_2 (Mallinckrodt for chromatography, 100 mesh) and $CHCl_3$ as an elution solvent, 90 mg. of slightly yellow oil was obtained from the neutral fraction. *Anal.* Calcd. for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27; mol. wt., 196.28. Found : C, 73.49; H, 10.02, mol. wt., 200, 210, 210. IR $\nu_{max}^{liq.\,film}$ cm^{-1} : 1665, 1175, 1128, 980.

Distillation of the 750 mg. of acidic fraction afforded 500 mg. of neutral oil, whose IR spectrum was in good coincidence with that of the neutral fraction (Fig. 3).

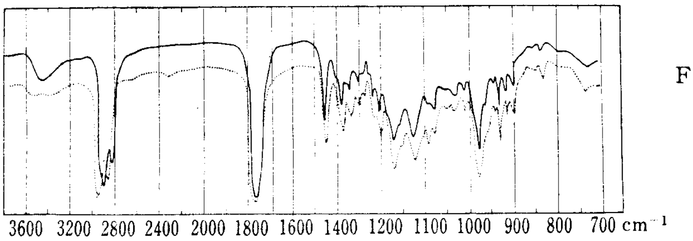

Fig. 3. Infrared Spectra of Reduction Products with Na and EtOH

—— Neutral fraction
------ Distilled from acid fraction

The authors express their gratitude to Mr. K. Narita of this Institute for the elemental analyses.

Summary

The structure of ligustilide was discussed upon the fact that sedanonic acid, 3-butylphthalide, and 2-valerylcyclohexanecarboxylic acid were obtained by catalytic hydrogenation. Tentative structures were proposed on the basis of available data. The position of double bonds, however, remains to be determined.

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UDC 577.164.11 : 547.07

22. Shun-ichi Yamada and Kazuo Achiwa : Studies on Thiamine Analogs; Synthesis of 3-[(2,4-Dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)methyl]-4-methyl-5-(2-hydroxyethyl)thiazolium Nitrate.

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Many kinds of thiamine analogs have been synthesized and the relationship between their chemical structure and physiological actions has been studied in detail. From these results,[1] it has been shown that it is essential for the action of thiamine (I) to have the amino group in 4-position of the pyrimidine ring. It is also essential that one hydrogen be present in 2-, methyl in 4-, and hydroxyethyl in 5-positions of the thiazolium ring, and

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[*1] Motofuji-cho, Bunkyo-ku, Tokyo (山田俊一, 阿知波一雄).

[1] "Vitamin B_1," compiled by The Japanese Science Council, 69 (1948). Sogensha, Tokyo.