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43. Hiroshi Mitsuhashi, Ukon Nagai, Toshio Muramatsu, and Hatsue Tashiro : Studies on the Constituents of Umbelliferae Plants. II.[1] Isolation of the Active Principles of Ligusticum Root.

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The root of *Ligusticum acutilobum*[*2] SIEB. ET ZUCC. (Tôki 当帰) has long been used as an important crude drug and was investigated by many workers. Sakai[2] studied it pharmacologically, and Kariyone[3] and Noguchi[5] chemically and isolated *p*-cymene, safrole, 3-butylphthalide, 3-butylidenephthalide, etc. Recently, Takahashi, Hikino, *et al.*[5] reported the chemical comparison of two crude drugs — Yamato-tôki and Hokkai-tôki.

No attempts were made, however, to concentrate the pharmacologically active principles of the crude drug and obtain the chemically pure substance. From the view point that crude drugs which have long been used in Japan and oriental countries must be re-estimated on a scientific basis, efforts were focused to find some interrelationship between their pharmacological action and chemical constituents.

What kind of action is suitable for that purpose is a very difficult problem, because in addition to an easier assay method, it must be related to the classical usage of this drug. After much consideration, anticholinergic action assayed by the Magnus method with rat intestine was adopted, and the activity of each fraction was tested at each stage of separation. For convenience in tracing the pathway of active principles in the process of separation, the activity was expressed numerically in comparison with the standard preparation of atropine sulfate.

The crude drug (root of Hokkai-Tôki) obtained from the Kitami district of Hokkaido was used. According to Hikino,[6] this plant is the hybrid species of *Angelica acutiloba* and *A. anomala*, and is named *A. acutiloba* var. *sugiyamae*. The dried commercial crude drugs were pulverized and extracted with various solvents on a small scale. Anticholinergic activity and the yield of each extract were tested (Table I).

TABLE I.

Solvent	Hexane	Benzene	Et_2O	$CHCl_3$	Me_2CO	EtOH	H_2O
Yield (%)	1.02	1.98	1.22	2.28	3.15	35.4	35.5

Relative activity: { Hexane, Benzene, Et_2O } > { $CHCl_3$, Me_2CO } ≫ { EtOH, H_2O }

These results showed that non-polar solvent is capable of extracting the active principles more effectively than polar solvents and that the yields are just in the reverse order. It was therefore considered that the total amount of the active principles extracted with various solvents is practically equal. Hexane was selected for a large-scale extraction because of the higher activity of its extract and for economical reasons. Expecting that the active principles might be a kind of volatile oil, the extract was

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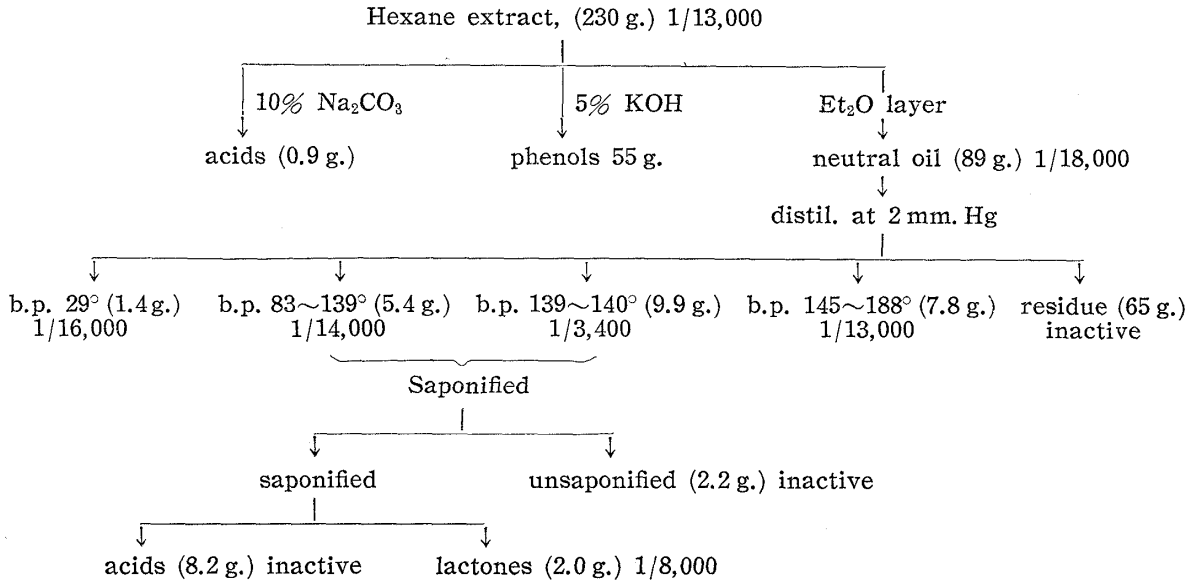
*2 The name used is that listed in Makino's "An Illustrated Flora of Nippon," 1st ed. (1940), Hokuryukan. This is listed as *Angelica acutiloba* (SIEB. ET ZUCC.) KITAGAWA by H. Hikino.[6]

1) Part I : H. Mitsuhashi, *et al.* : Yakugaku Zasshi, **78**, 539(1958).
2) W. Sakai : Tokyo Igaku Kaishi, **30**, 1943(1916).
3) T. Kariyone, *et al.* : Yakugaku Zasshi, **56**, 662, 668(1936); *ibid.*, **57**, 799(1937).
4) T. Noguchi, *et al.* : *Ibid.*, **57**, 769, 783(1937).
5) S. Takahashi, *et al.* : *Ibid.*, **78**, 1156(1958).
6) H. Hikino : Syoyakugaku Zasshi, **12**, 9(1958).



(The numbers under each fraction are the yield and relative activity to atropine.)

Chart 1.

treated by the method usually employed for volatile oils. Chart 1 shows the process of separation and distribution of activity of each fraction.

These data indicate the decomposition of active principles during hydrolysis of the distilled fractions. Chromatographic separation of the active fractions with silicic acid–chloroform system was very efficient and two active principles were isolated. One is a 3-butylidene phthalide which was detected but not isolated by Kariyone[3] and Noguchi,[4] and the other is a new and the most active component, which was named ligustilide. Its purity was determined by the ultraviolet and infrared spectra, and glass-strip chromatography[7] of each fraction eluted.

Ligustilide has the molecular formula of $C_{12}H_{14}O_2$, i.e. an isomer of butyl phthalide, and has the following constants : No optical rotatory power, b.p_6 168~169°, and n_D^{25} 1.5649. It has also a characteristic odor of ligusticum root, remarkable bluish fluorescence, and a strong absorption band at 320 mμ ($\log \varepsilon$ 3.8) which is a good mark for separation. Fig. 1 shows its infrared absorption spectrum. It is decomposed rapidly on exposure to air and the absorption band at 320 mμ is displaced to 280 mμ but remains fluorescent. Its instability made the study difficult and may cause some ambiguity in the constants described above.

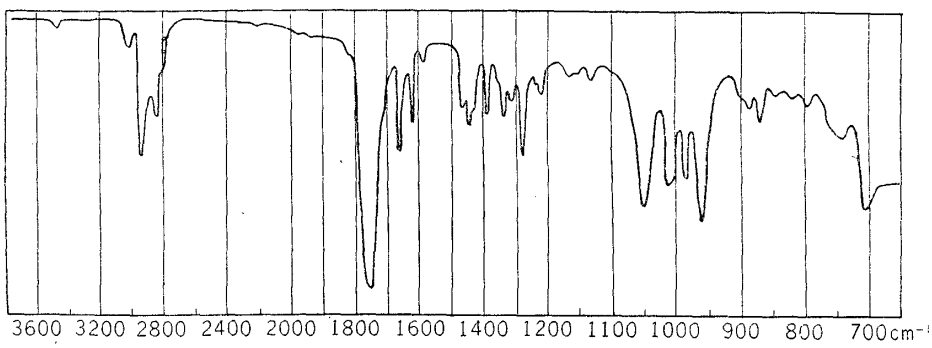


Fig. 1. Infrared Spectrum of Ligustilide

7) J. C. Kirschner, *et al.* : Anal. Chem., **23**, 423(1951).

Experimental

Determination of Anticholinergic Activity—The quantity of atropine sulfate added as 0.5 γ/cc. aqueous solution necessary to cause 50% inhibition of the spasm of female rat jejunum (Wister Ma, 180~230 g. in weight) induced with acetylcholine bromide (0.02 cc. of 0.2 mg./cc. into a 50-cc. chamber) was measured. A similar quantity was measured for the sample which was added as EtOH solution of about 5 mg./cc. and compared with that of atropine. The ratio is recorded as "relative activity to atropine" in Chart 1.

Isolation—Ten kg. of pulverized crude drug, previously dried with a forced stream of hot air (60°), was percolated with about 50 L. of hexane. 200 g. of the extract so obtained was dissolved in Et_2O, treated with 5% NaOH solution, washed with water, and dried over Na_2SO_4. On evaporation of Et_2O, about 100 g. of neutral oil was obtained which was distilled under a diminished pressure and the fractions boiling between 135° and 155° at 3 mm. Hg were collected. Yield, 10 g. It was chromatographed over a column of 500 g. of silicic acid (Mallincrodt, 100 mesh, for chromatography) and $CHCl_3$ as an eluting solvent. At first some terpene-like substances eluted. After a little interval 3-butylidenephthalide and ligustilide eluted partially overlapping in this order. Their yields were 1 g. of 3-butylidenephthalide and 3 g. of ligustilide, excepting the overlapped fractions.

Identification of 3-Butylidenephthalide—A mixture of 0.5 g. of the sample, 0.5 cc. of EtOH, and 0.5 g. of $N_2H_4 \cdot H_2O$ placed in a test tube was sealed and heated in a boiling water bath for 1 hr. When cool, 4-butylphthalaz-1-one crystallized out. Recrystallization from EtOH gave prisms, m.p. 160~163°, undepressed when mixed with the sample, m.p. 161~162°, prepared from synthetic 3-butylidenephthalide by the method of Bromberg.[8] Mixed m.p. 160.5~163°(Kofler block).

Determination of Constants of Ligustilide—*Anal.* Calcd. for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42; mol. wt., 190.23. Found: C, 76.20, H, 7.77; mol. wt. (Akiya method[9]), 184~230. $[\alpha]_D^{22}$ −0.59°±1.77° (c=3.38, d=0.5, α=0.01°±0.03°). Boiling point was determined by the micro-method and refractive index with Abbe's apparatus. UV and IR spectra were measured with Beckmann Model DK-II spectrophotometer and Koken DS-301-type infrared spectrophotometer, respectively.

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Summary

Isolation of active principles in the ligusticum root (Hokkai-Tôki) was attempted. Anticholinergic principles were traced by the Magnus method with rat intestine. Ligustilide, which is a new and the most active constituent was isolated. It is an isomer of butylphthalide. It has marked bluish fluorescence and decomposes rapidly on exposure to air.

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8) O. Bromberg: Ber., **29**, 1434(1896).
9) S. Akiya: Yakugaku Zasshi, **57**, 967(1937).