Notes

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Hiroshi Mitsuhashi, Toshio Muramatsu, Ukon Nagai, Toshio Nakano, and Koji Ueno : Studies on the Constituents of Umbelliferae Plants. VIII.*1 Distribution of Alkylphthalides in Umbelliferae Plants.

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In the previous papers from this laboratory[*1,1,2] the isolation, purification, and chemical studies of alkylidene-, and alkylphthalides from the roots of several Umbelliferae plants were reported. In connection with taxonomic studies it would be very important to investigate the taxonomically characteristic occurrence of the constituents. The plants examined in this study were *Ligusticum acutilobum* Sieb. et Zucc., *Cnidium officinale* Makino, *Apium graveolens L.*, *Levisticum officinale* Koch and Hokkai Toki.*3 The crude drugs, "Hokkai Toki" and "Yamato Toki" (roots of *Ligusticum acutilobum*) are used equally, but their chemical constituents show some differences. According to Hikino, "Hokkai Toki" is a hybrid of *Ligusticum acutilobum* and *Angelica anomale* Laliemant.*4 If so, it might be interest to examine the distribution of alkylidene-, alkylphthalides in these three plants.

From the roots of *A. anomale*, no alkylidene-, and alkylphthalides were isolated. The compounds related to 3-butylphthalide isolated from Umbelliferae plants were summarized in the previous paper.[2] Recently, Barton and de Vries reported the isolation of sedanonic acid and butylphthalide from celery oil.[3] Gold and Wilson have reported the isolation and characterization of 3-isobutylidene-, 3-isovaleridenephthalide and related dihydro compounds from celery juice.[4] The plants specimens used in the present study are shown in Table I.

Table I.

Name of plant	Location of cultivation	Harvest time	N.B.
Ligusticum acutilobum Sieb. et Zucc.	Nara pref.	——	Commercial material
Ligusticum acutiloba var. *sugiyamae* Hikino	Hokkaido Ishikari	Sept. 1962	
Cnidium officinale Makino	Hokkaido Kitami	Sept. 1961	
Apium graveolens L.	Hokkaido Ishikari	Oct. 1962	
Levisticum officinale Koch	Drug garden Hokkaido Univ.	Sept. 1962	Seeds were obtained from Univ. of Wash. Seattle, Wash. U.S.A.
Angelica anomale Laliement	Hokkaido Teshio	July, 1962	

*1 Part Ⅶ. H. Mitsuhashi, U. Nagai : Tetrahedron, **19**, 1277 (1963).

*2 Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (三橋 博, 村松敏夫, 永井右近, 中野寿雄, 上野宏治).

*3 Dr. H. Hikino has named as "*Ligusticum acutiloba* var. *sugiyamae* Hikino." H. Hikino : Shoyakugaku Zasshi, **12**, 9 (1958).

*4 It is very difficult to distinguish *Angelica anomale* by its taxonomical description. There can be some variation in form depending on their localities. Our collected materials were compared with the type specimen in the Herbarium of Hokkaido University. The authors wish to express their gratitude to Dr. Itho, of this University for his valuable advice.

1) H. Mitsuhashi, U. Nagai, T. Muramatsu, H. Tashiro : This Bulletin, 8, 243 (1960).
2) *Ibem* : *Ibid.*, **9**, 115 (1961).
3) D.H.R. Barton, J.X. de Vries : J. Chem. Soc., **1963**, 1916.
4) H.J. Gold, C.W. Wilson Ⅲ : J. Org. Chem., **28**, 985 (1963).

The roots were dried below 60°, pulverized and extracted with hexane. The extract was treated as indicated in the previous study,[1] and separated into acid, phenolic, and neutral portions. The neutral portions were distilled under reduced pressure, and the lactone fraction (b.$p_{0.5\sim4}$ 130~150°) was collected. It was chromatographed over a column of silicic acid and eluted with chloroform (Chart 1).

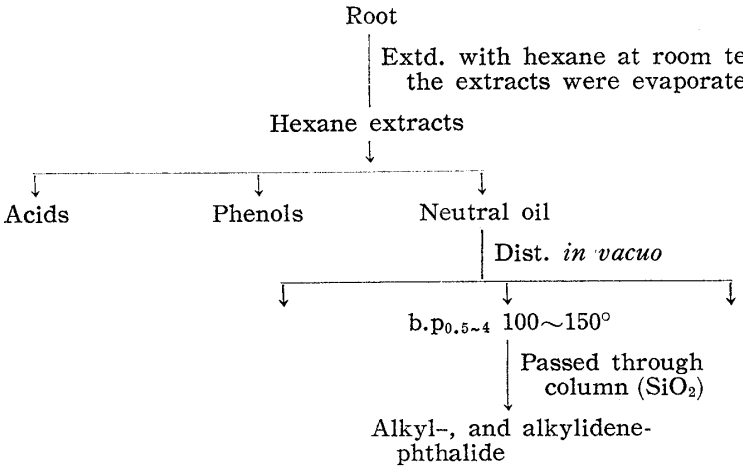
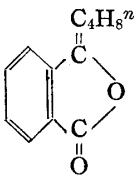
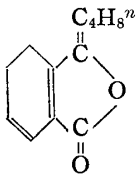
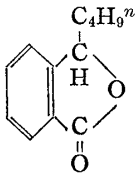
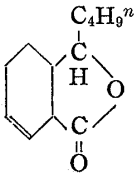
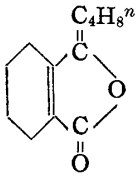
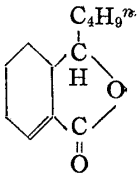


Chart 1.

The results are shown in Table II. By this method, ligustilide and several new compounds were isolated,[5~7] whereas, sedanonic acid was not obtained. It might be reasonable to assume that, sedanonic acid resists against relactonization. In the previous work, these alkylphthalide were mostly treated with alkali and naturally there were

Table II. Distribution of Phthalides

	Name	Butylidene phthalide	Ligustilide	Butyl phthalide	Cnidilide[a]	Sedanonic acid lactone	Neocnidilide[a]
Compounds	Structure						
Plants	*Ligusticum actilobum* Sieb. et Zucc.	(+) (0.0034%)[b]	(±)[c] (0.0041%)	(+) (0.0049%)	(−)	(+) (0.00085%)	(−)
	"Hokkai Toki" (*Ligusticum actiloba* var. *sugiyamae* Hikino)	(+) (0.0031%)	(+) (0.0123%)	(−)	(−)	(−)	(−)
	Cnidium officinale Makino	(±)[c]	(+) (0.42%)	(±)[c]	(+) (0.024%)	(−)	(+) (0.015%)
	Apium graveolens L.	(−)	(−)	(+) (0.0030%)	(−)	(−)	(+) (0.0017%)
	Levisticum officinale Koch	(+) (0.0077%)	(+) (0.19%)	(−)	(−)	(−)	(−)

a) The structure determination of these compounds will be published in the near future.
b) Percentage from rhizome or root.
c) These compounds were not obtained in a pure state.

5) T. Muramatsu, *et al.*, Present at the Hokkaido Branch Meeting of Pharmaceutical Society of Japan. January, 1961, Sapporo.
6) *Idem*, Reported at the 14th Annual Meeting of the Pharmaceutical Society of Japan. July, 1961, Sapporo.
7) H. Mitsuhashi, *et al.*, 5th Symposium on the Organic Chemistry of Natural Products. October, 1961, Sendai.

none the cases of isolation of sedanonic acid lactone. As our present method does not involve alkaline hydrolysis, and sedanonic acid lactone was obtained instead of sedanonic acid. It has been proved that both sedanonic acid lactone is identical with dihydroligustilide by comparison of the infrared spectra.*[1]

Experimental

Ligusticum acutilobum

Extraction and Isolation——The pulverized crude drug (3.5 kg.), previously dried under the stream of hot air (60°), was percolated with hexane about 22 L. The extract (80 g.) so obtained was dissolved in Et_2O, treated with 10% Na_2CO_3, and 10% NaOH solution, washed with H_2O and dried over Na_2SO_4. On evaporation of the solvent a neutral oil (23.5 g.) was obtained, which was distilled under reduced pressure and the fractions boiling between 80~120° at 4 mm. Hg collected. Yield 1 g. The distillate was chromatographed over a column of silicic acid (Mallincrodt, 100 mesh, for chromatography) (50 g.) with $CHCl_3$ as an eluting solvent. The product was eluted in the following order. Butylidenephthalide; UV λ_{max} mμ : 237, 262, 272, 312 (log ε 3.97, 3.99, 3.85, 3.40), IR ν_{max} cm^{-1} : 1780 (five membered lactone), 1690 (shoulder, double bond), 1610 (aromatic), phthalazone, m.p. 153~154°. Yield 122 mg. Ligustilide; UV : λ_{max} 320 mμ (log ε 3.90), IR ν_{max} cm^{-1} : 1755, 1661, 1621, 1586. Yield 155 mg. Sedanonic acid lactone; UV : λ_{max} 273 mμ (log ε 4.02), IR ν_{max} cm^{-1}: 1770, 1680, 1640, phthalazone, m.p. 131~132°. Yield 30.4 mg. Butylphthalide; UV λ_{max} mμ: 226, 275, 283 (log ε 3.10, 2.27, 2.27), IR ν_{max} cm^{-1} : 1770, 1610, 1595. Yield 170 mg.

"Hokkai Toki"——An extract (160 g.) was obtained from 13 kg. of pulverized crude drug, and from this extract the neutral oil 40.5 g. was isolated. The oil was distilled at $b.p_{1.6}$ 115~134°, yield 2.96 g. Butylidenephthalide (403 mg.) and ligustilide (801 mg.) were obtained by chromatography over silicic acid with $CHCl_3$, as above.

Cnidium officinale——The pulverized crude drug (11.1 kg.) was percolated with hexane 30 L. The extracts (620 g.) so obtained, were separated to acids, phenols, and neutral oily fraction.
400 g. of the resulting neutral oil was distilled and fractions between $b.p_{2.5}$ 147~150° collected. Yield 91 g. By chromatography (SiO_2, $CHCl_3$), butylidenephthalide (1 g.), ligustilide (40 g.), cnidilide (3 g.),[5] neocnidilide (1.6 g.)[5] and an unknown lactone (1.2 g.) were obtained. Cnidilide; IR ν_{max} cm^{-1}: 1775, 1665. Neocnidilide; UV : λ_{max} 218 mμ (log ε 3.30), IR ν_{max} cm^{-1} : 1770, 1690.

Apium graveolens——Dried and milled roots of *Apium graveolens* (5.3 kg.) were treated according to the procedure as shown in Chart 1. 44 g. of neutral oil was distilled ($b.p_4$ 100~117°) and finally butylphthalide (156 mg.), and neocnidilide (90 mg.) were obtained after chromatography.

Levisticum officinale—— 3.3 kg. of dried powdered roots were extracted, and 27.3 kg. of resulting oil was distilled ($b.p_1$ 130~135°). Ligustilide (6.3 g.), and butylidenephthalide (255 mg.) were obtained.

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Summary

The distribution of alkylphthalides in the roots of *Ligusticum acutilobum* Sieb. et Zucc., *Ligusticum acutiloba* var. *sugiyamae* Hikino, *Cnidium officinale* Makino, *Apium graveolens* L., *Levisticum offiicinale* Koch, and *Angelica anomale* Laliement have been surveyed. From the roots of *Angelica anomale* no alkylphthalides could be found. The results are summarized in Table II.

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