adsorbed the blue matter, was formed by the addition of the small portion of EtOH. After filtration, EtOH was further added to the filtrate and 10.4 g. of magnesium salt or 11.4 g. of calcium salt was obtained. Barium salt of the L-isomer and calcium salt of the D-isomer were prepared in a similar way.

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Hans Bohrmann, Egon Stahl,*1 and Hiroshi Mitsuhashi*2 : Studies of the Constituents of Umbelliferae Plants. XIII.*3 Chromatographic Studies on the Constituents of *Cnidium officinale* MAKINO.

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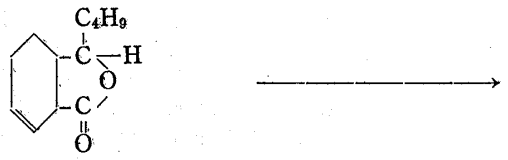
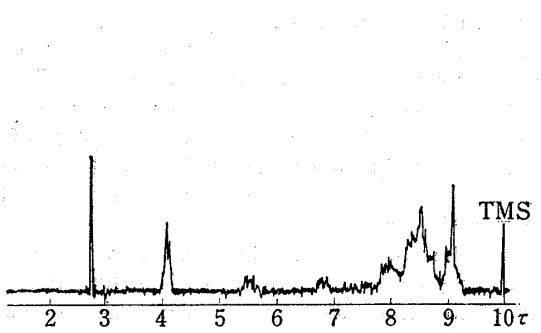
The structure determination of cnidilide and neocnidilide, phthalides from *Cnidium officinale* MAKINO, was reported in a previous paper of this series.[1] Since the repeated chromatography of the lactone mixture always gave cnidilide with a contamination of about 10% of butylphthalide, it was suggested, that cnidilide might aromatize spontaneously (Chart 1).

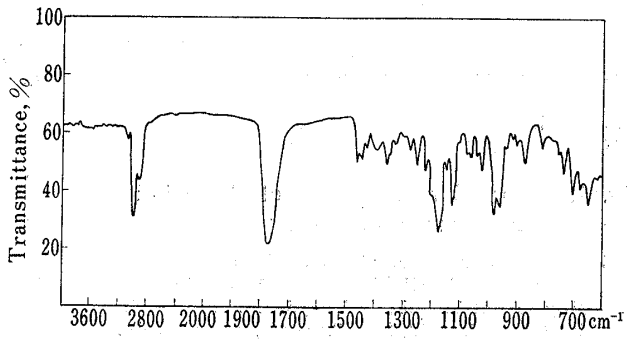


cnidilide butylphthalide

Chart 1.



Nuclear Magnetic Resonance Spectrum of Cnidilide (in $CDCl_3$)



Infrared Spectra of Cnidilide (neat)

Fig. 1.

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This work is considered to be a part of the thesis by H. Bohrmann. The authors are indebted to the DAAD which partly supported this work.

*3 Part XII : This Bulletin, **14**, 777 (1966).

1) H. Mitsuhashi, T. Muramatsu : Tetrahedron, **20**, 1921 (1964).

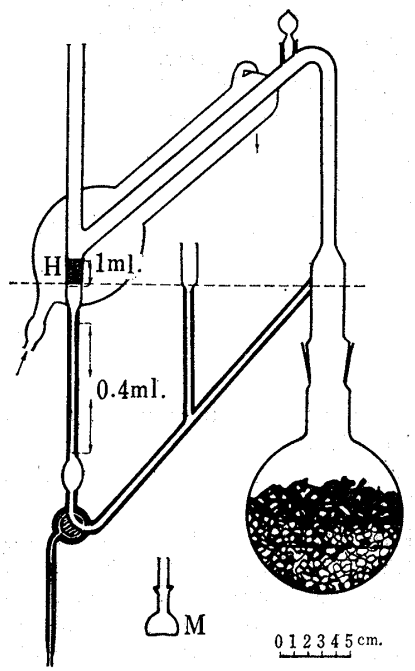

Fig. 2. Karlsruhe Apparatus

A careful study now shows that pure cnidilide can be obtained by using a combination of chromatographic methods. Pure cnidilide appears to be completely stable. After standing some days in the atmosphere, no evidence for aromatic compounds could be obtained by infrared (IR) or nuclear magnetic resonance (NMR) spectra (Fig. 1).

The method of extraction was the same as described previously.[1] The lactone mixture was rechromatographed using a graduated (0~30%) hexane–methylene chloride solvent mixture. Finally repeated micropreparative thin layer chromatogram (TLC)[2] on 500 mμ layers of Silicagel, developed twice with hexane–ether (80:20) afforded pure butylidenephthalide, ligustilide,[3] butylphthalide, cnidilide and neocnidilide in the order given.

A new universal spraying reagent has been very effective for detecting the phthalides on the chromatograms. It is a combination of anisaldehyde–H_2SO_4–phosphomolybdic acid, and can also be used with very

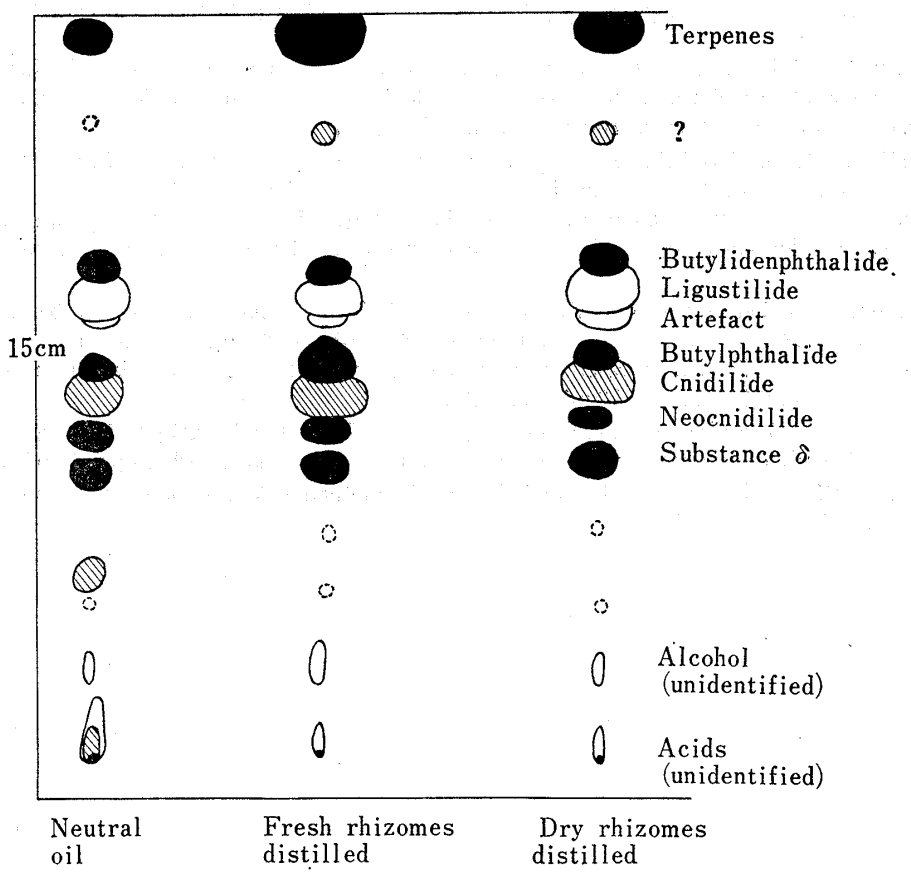

Fig. 3.

● UV Absorption at 254 μm. ○ Fluorescending under UV 366 μm.
▨ Appearing after spraying and heating.

2) E. Stahl: Laboratory Practice, **13**, 496 (1964).
3) H. Mitsuhashi, U. Nagai, T. Muramatsu, H. Tashiro: This Bulletin, **8**, 243 (1960). H. Mitsuhashi, U. Nagai, T. Muramatsu: *Ibid.*, **9**, 115 (1961). H. Mitsuhashi, U. Nagai: Tetrahedron, **19**, 1277 (1963).

sensitive effects on other classes of natural substances such as alkaloids, steroids etc. to give dark blue and other colored spots on a white background.

A comparison has also been made between the neutral oil of *Cnidium officinale* obtained according to the previous report,[1] and the essential oil of dried and fresh rhizomes obtained by continuous steam distillation by means of the "Karlsruhe Apparatus" (Fig. 2).[4] It is evident, that the essential oils obtained by the latter process contain more phthalides. This shows that the alkaline treatment during the process of isolation causes a loss of product by lactone ring opening (Fig. 3).

Experimental

Silicic acid of Mallinckrodt R 100 mesh for chromatography was used for column chromatography. Silicagel GF_{254} Merck acc. to Stahl was used for TLC and micropreparative TLC.

IR spectra were measured with a Shimadzu IR Spectrophotometer-IR-27, and NMR spectra were taken on a Hitachi-H-60 Mc. spectrometer with tetramethylsilane as an internal standard.

Extraction and Isolation——1.3 kg. of dried and ground rhizomes were percolated with hexane (3.5 L.) and the extract (77 g.) dissolved in ether, carefully treated with 5% $NaHCO_3$, 5% Na_2CO_3 and 5% NaOH, washed with water and dried over Na_2SO_4. After evaporation of the solvent, the neutral oil (46 g.) obtained was distilled and a constant boiling fraction 121°/1 mm. Hg collected; yield 14 g. Eight gramme of this oil was chromatographed over a column of Silicic acid (250 g.) with 100 ml. aliquots of hexane : CH_2Cl_2 95:5, 90:10 to hexane-CH_2Cl_2 70:30. 20 ml. fractions were collected and monitored by TLC (plates prepared according to the standard method).[5] Fractions 10 and 11 contained pure butylidenephthalide (23 mg.), fractions 16~20 pure ligustilide (1.3 g.), other fractions contained all other phthalides which were impure and overlapped each other.

Rechromatography of 150 mg. of the latter impure fractions was done on a smaller column (30 g.). The separation was not complete and the purification was made by micropreparative TLC[2] which afforded pure cnidilide (21 mg.), butylphthalide (7 mg.) and neocnidilide (18 mg.).

Essential Oil by Steam Distillation——a) Two hundred and thirty gramme of fresh rhizomes were cut into small pieces and continuously steam distilled in a "Karlsruhe Apparatus" (Fig. 2) with 500 ml. of water for 48 hr. The essential oil dissolved in the hexane layer (Fig. 2 H) was separated from the water phase and transferred into a microflask (Fig. 2 M). After evaporation of the solvent 429 mg. of essential oil (0.17%) was obtained.

b) Fifty gramme of dried and ground rhizomes were distilled in the same way as before with 500 ml. of water, and 629 mg. (1.25%) of essential oil was obtained. About 200 μg. of essential oil in CH_2Cl_2 was spotted on a TLC plate and developed twice with hexane-ether for 15 cm. The plate was sprayed with the reagent described below and heated for 10 min. at 110°.

Preparation of the Spraying Reagent——Reagent 1; 4.5 g. of anisaldehyde is dissolved in 90 ml. of glacial acetic acid, 860 ml. of methanol is added, and 45 ml. of H_2SO_4 is added dropwise with stirring.

Reagent 2; A 10% solution of phosphomolybdic acid in methanol. 10 ml. of Reag. 2 are added to Reag. 1, the mixture is stable for several days.

4) E. Stahl: Microchimica acta, **40**, 367 (1953).
5) E. Stahl: Dünnschichtchromatographie Ein Laboratoriumshandbuch. Springer Verlag, Berlin. 1962.