Analysis of Phthalides from Umbelliferae by Combined Liquid-Solid and Gas-Liquid Chromatography

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Key Words

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Summary

A liquid-solid chromatographic pre-fractionation of naturally occurring phthalides has been developed. The LSC was carried out on a column of silica applying a 1–50% gradient elution of diethyl ether (containing 2% methanol) in pentane. The enrichment in the fractions led to better possibilities for a gas chromatographic separation and isolation allowing further studies by spectroscopic methods. Ligustilide may easily isomerize during GC as a result of ageing of the column.

Introduction

Phthalides are present in the essential oils of some umbelliferous plants. A survey of these compounds, their structural formulas, their occurrence and the pharmacological activities attributed to them was given in a previous paper [1]. Problems may arise in the separation and identification of these phthalides, because of their structural similarity. Yamagishi and Kaneshima [2] applied column chromatography and preparative thin-layer chromatography for isolation of the phthalides present in *Cnidium officinale* Makino. However, their method was time and solvent consuming.

For the analysis of monoterpenes from essential oils we described a relatively simple procedure consisting of gas chromatography (GC) following prefractionation by liquid-solid chromatography (LSC) [3, 4]. The enrichment of monoterpenes in the fractions collected during LSC led to an improved GC analysis, that is, it permitted or facilitated a GC identification of the various components present in essential oils [5–8]. In many cases it also enabled an identification by GC-MS or a preparative GC isolation of components allowing further investigations by spectroscopic methods. However, the procedure proved to be unsatisfactory for phthalides.

In view of the results obtained for monoterpenes we developed a corresponding, again relatively rapid and simple, method for the analysis of mixtures consisting of naturally occurring phthalides. In the present paper we describe this method on applying it to an extract from roots of *C. officinale*, as this species could provide us with a mixture of already known phthalides.

Experimental

Material

Dried roots (1 kg) of *Cnidium officinale* Makino (in Japanese: "Senkyu") were supplied by the Mikuni Company, Osaka, Japan. The roots were minced by an Ultra-Turrax top drive macerator (Janke & Kunkel K.G., Staufen i.Br., F.R.G.) using 2 l redistilled pentane as extraction liquid. After maceration for 14 h at 20°C and filtration a further 1 l pentane was added to the plant material. The mixture was stirred for 2 h and subsequently the solvent was again filtered. After combining both amounts of liquid extract evaporation of pentane yielded an oily liquid, 0.2 ml of which was submitted to LSC.

Liquid-Solid Chromatography

A chromatographic column (18 mm i.d.) with cooling jacket to keep the temperature at 10°C was packed with a slurry of 40 g adsorbent in redistilled pentane. As adsorbent we used Silica gel 60, 70–230 mesh ASTM, for column chromatography (Merck, Darmstadt, F.R.G.). To prevent isomerization during LSC the silica was purified, and deactivated by addition of 5% water as described before [9]. 0.2 ml of the oily liquid to be analyzed (see above) was chromatographed by elution with a step-

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wise 1%–50% gradient of a diethyl ether-methanol mixture (98 + 2) in pentane. Flow rate: 3 ml/min. In detail: the elution was commenced with 100 ml pentane containing 1% diethyl ether-methanol (98 + 2) and continued by 6 portions each of 100 ml with an increasing diethyl ether-methanol (98 + 2) content, *viz.* 2%, 4%, 7%, 10%, 20% and 50% respectively. The eluate was collected as follows: 1 fraction of 100 ml; 9 fractions each of 20 ml; 42 fractions each of 10 ml. All fractions were concentrated under reduced pressure at 0°C in a rotary evaporator and subsequently analyzed by GC.

Gas-Liquid Chromatography

Packard gas chromatograph (Model 427; Packard-Becker B.V., Delft, The Netherlands) equipped with FID was used. Conditions were as follows: column: copper 8 m x 2.1 mm i.d.; solid support: Chromosorb W 60–80 mesh acid-washed; stationary phase: 10% SF 96; oven temperature: 180°C; carrier gas: nitrogen.

Gas Chromatography — Mass Spectrometry

For identification of the phthalides an LKB 2091–2130 (EI/CI) gas chromatograph - mass spectrometer - computer system (LKB-Instrument AB, Bromma, Sweden) was used. The GC was equipped with solid injector. Conditions were as follows: column, SCOT, glass, 8 m x 0.5 mm i.d.; solid support, benzyltriphenylphosphonium chloride deactivated Cab-O-Sil; stationary phase, mixture of OV 17 and PPE 21; oven temperature, 130°C; carrier gas, helium; ion source temperature, 200°C; separator temperature, 210°C; electron energy, 70 eV; accelerating voltage, 3.5 kV; trap current, 50 μA.

Results and Discussion

Fig. 1 shows a typical gas chromatogram obtained with the SF 96 column for the pentane extract from roots of *Cnidium officinale* Makino. After evaporation of pentane from a part of the extract the remaining oily liquid was submitted to LSC as described under Experimental. GC analysis of each of the LSC fractions and comparison of the gas chromatograms with that given in Fig. 1 showed that a marked enrichment of the various components in different fractions was achieved by the LSC procedure. In Table I the fractions are mentioned in which each component reached its maximum amount. From the data given in Table I and the chromatogram shown in Fig. 1 it is obvious that the elution sequences during LSC and GC (on SF 96) were completely different. So we easily detected by GC analysis of the fractions that the peak numbered 5+6 was made up of two components – maximum amounts of the constituents 5 and 6 in fractions 10 and 29 respectively.

The enrichment of the various components in different fractions due to such a separation by LSC facilitated or even permitted an identification by GC-MS. By applying GC-MS to the extract and to some of the fractions, and comparing the mass spectra with those given by Yamagishi and Kaneshima [2] the identity of the phthalides numbered 1–6 could be ascertained. The compounds we identified were identical with those isolated before [2]; their structural formulas are shown in Fig. 2. However, one more phthalide seemed to be present in the pentane extract we prepared, that is, the compound corresponding to peak 7 in the gas chromatogram given below.

During a study on the presence of phthalides in *Levisticum officinale* Koch [10] we detected a component with the same retention time as compound 7 – also on stationary phases of different polarity. LSC of the essential oil of *L. officinale* showed that the component was eluted after ligustilide, whereas in the study described here compound 7 was always present in LSC fractions together with ligustilide. Further, the maximum amount of 7 was found in

Table I. Fractions in which amount of phthalide was maximum

Phthalide	Fraction	Eluent (ml)[1]	No[2]
Butylidenephthalide	9	260	*2*
Ligustilide	10	280	*5*
Artefact[3]	10	280	*7*
Butylphthalide	23	410	*1*
Cnidilide	26	440	*3*
Senkyunolide	29	470	*6*
Neocnidilide	34	520	*4*

[1] Total amount of eluent (in ml) corresponding to fraction concerned;
[2] number of compound in gas chromatogram;
[3] isomer of ligustilide formed during GC.

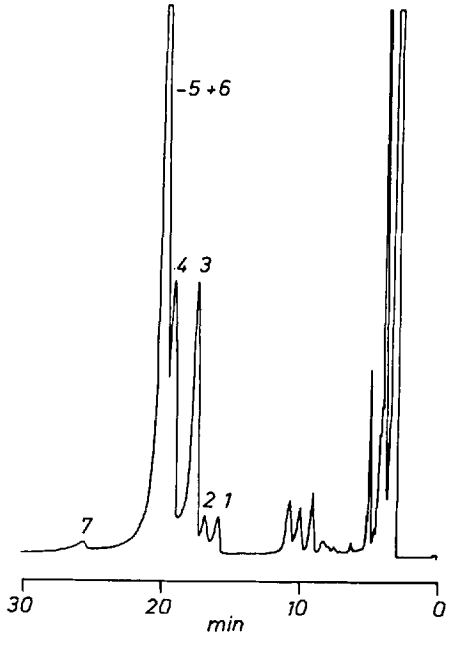

Fig. 1
Gas chromatogram obtained with SF 96 column for pentane extract from roots of *Cnidium officinale* Makino. For numbering of peaks see Table I; for conditions see under Experimental.

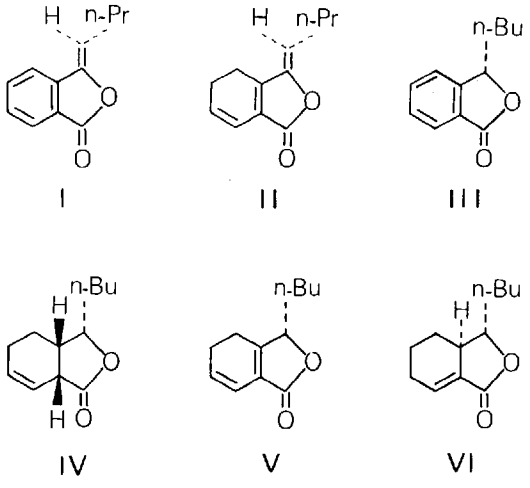

Fig. 2

Phthalides of *Cnidium officinale* Makino. I = butylidenephthalide; II = ligustilide; III = butylphthalide; IV = cnidilide; V = senkyunolide; VI = neocnidilide.

the fraction where ligustilide also reached its maximum. We submitted this fraction to GC-MS, which revealed that the MS of 7 was identical with that of ligustilide. Therefore we assumed that compound 7 was an isomer of ligustilide formed during GC, probably as a result of ageing of the column. Once again we prepared an SF 96 column and – as we expected – no compound 7 could be detected. However, when a 10 cm long column filled with Chromosorb without any coating was inserted between the injection port of the GC and the new SF 96 column, compound 7 was found in relatively large amounts. We concluded compound 7 was an artefact formed during GC of ligustilide, although it might be a genuine component of *L. officinale*.

We applied the procedure to essential oils containing phthalides, that is, the oil of *L. officinale*, and noticed that interference between the monoterpenes and the phthalides of the oil was totally avoided, because during LSC all monoterpenes were eluted before the phthalides [10]. On the other hand, when the pre-fractionation procedure developed for monoterpenes [4] was applied to those essential oils, some phthalides were eluted together with oxygen-containing monoterpenes, while some other were not eluted at all.

Conclusion

A pre-fractionation of a naturally occurring mixture of phthalides by LSC leads to better possibilities for separation of the components by GC. Therefore, an identification by GC or GC-MS is facilitated or even permitted. The LSC procedure also enables a preparative GC isolation of components allowing further studies. When the pre-fractionation is applied to essential oils containing phthalides, interference between monoterpenes and phthalides is totally avoided.

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