

## Constituents of Peppers.

### I. Qualitative Analysis of Piperine Isomers

#### Die Bestandteile von Pfeffern.

### I. Qualitative Analyse von Piperin-Isomeren

#### Les composants des poivres.

### I. Analyse qualitative des isomères de la pipérine

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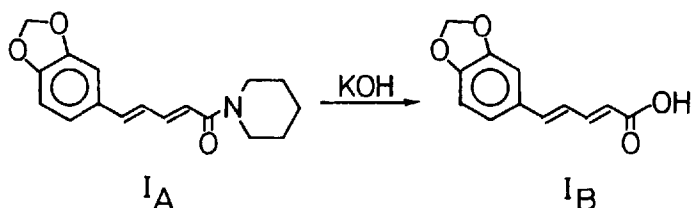
**Summary:** Thin layer and high pressure liquid chromatography techniques are described for the analysis of piperine and its trans-cis isomers isopiperine, isochavicine and chavicine. U. V. irradiation of piperine produces isochavicine. Synthesis of piperine and its isomers by reacting piperidine with the corresponding acid chlorides leads to mixtures of the isomers.

**Zusammenfassung:** Es wird die dünn-schicht-chromatographische und die Hochdruck-LLC-Analyse von Piperin sowie seinen cis-trans-Isomeren iso-Piperin, iso-Chavicin beschrieben. Durch UV-Bestrahlung entsteht aus Piperin iso-Chavicin. Bei der Synthese von Piperin und seinen Isomeren durch Umsetzung von Piperidin mit den korrespondierenden Säurechloriden entstehen Isomerengemische.

**Résumé:** L'analyse par chromatographie sur couche mince et par chromatographie en phase liquide sous haute pression de la pipérine et de ses isomères l'isopiperine, l'isochavicine et la chavicine est décrite. La pipérine est transformée en isochavicine par irradiation en lumière ultra-violette. La réaction de la pipéridine avec chacun des chlorures acides isomères donne un mélange d'isomères.

#### 1. Introduction

Piperine ( $I_A$ ) was isolated from black pepper as long ago as 1820, later also from other pepper varieties [1]. The structure of piperine [1] was elucidated around 1850, its most important reaction being the alkaline hydrolysis to



piperidine and piperinic acid ( $I_B$ ). The trans-trans configuration of piperine was proved by *Doebner* [2] and by *Ladenburg* and *Scholtz* [3]. At this time it became obvious that several geometrical isomers of piperine and of piperinic acid were possible. These isomers although unknown at that time were given the names shown in table I.

Table I		Configuration
Name		
piperine	piperinic acid	trans - trans
isochavicine	isochavicinic acid	trans - cis
isopiperine	isopiperinic acid	cis - trans
chavicine	chavicinic acid	cis - cis

The double bond next to the carbonyl is named first.

Although claims were made [4] about the isolation of chavicine, this was later proved to be wrong [5, 6] and the challenge of isolating the isomers was therefore still there at the start of our investigation. Another important point prompting us to take up this work was that, allegedly [4], chavicine had a more pungent taste than piperine. This idea was based on the fact that pure crystalline piperine is hardly pungent at all compared to crude pepper extract. If true this could have important economic aspects.

Recently [5] the synthesis of the four acids and amides of table I was reported. These preparations can however be criticised because they involve reconstituting the amides from the acid chloride and piperidine, and in our hands this invariably led to mixtures. The present paper is mainly about the separation of the mixtures either synthetic or obtained from natural sources.

#### 2. Extraction and Purification of Piperine

Freshly milled black pepper (70 g) of the Lampong variety was extracted in a Soxhlet type apparatus, and therefore at the solvent boiling temperature. The results for different solvents are collected in table II.

The crude vacuum evaporated extract as obtained with methylene chloride is washed several times with cold ether and the residue (m. p. 123–125°) is crystallised five times from ethanol. This affords pure piperine m. p. 129°.

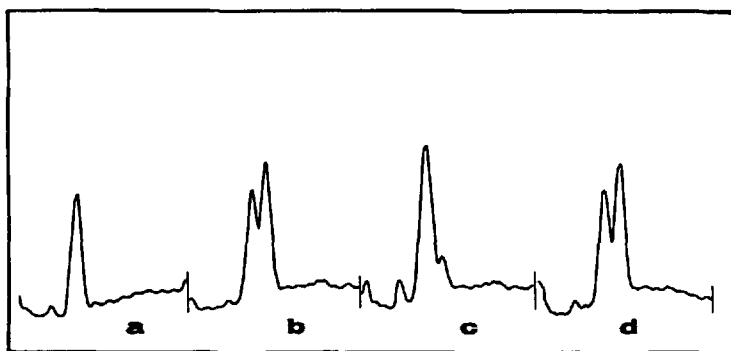


Fig. 1

- Densitograms of TLC on silver nitrate impregnated plates: (a) pure piperine, (b) the same after 2 hours exposure to light with  $\lambda = 350$  nm, (c) crude pepper extract, (d) crude pepper extract after 2 hours exposure to light with  $\lambda = 350$  nm. Other details in text.
- Densitogramme von DSC auf Platten mit Silbernitrat-Imprägnierung: (a) reines Piperin, (b) dasselbe nach 2 Stunden Bestrahlung mit Licht von 350 nm Wellenlänge, (c) Rohpfeffer-Extrakt, (d) Rohpfeffer-Extrakt nach 2 Stunden Bestrahlung mit Licht von 350 nm Wellenlänge. Weitere Einzelheiten im Text.
- Densitogrammes des chromatogrammes obtenus sur couches minces imprégnées de nitrate d'argent: (a) pipérine pure, (b) même échantillon de pipérine après irradiation, pendant 2 heures, sous la lumière de longueur d'onde 350 nm; (c) extrait de poivre brut, (d) extrait de poivre brut après irradiation, pendant 2 heures (350 nm). Autres conditions voir le texte.

Table II. Percent weight extracted from black pepper

Solvent	Extraction time at boiling temperature	
	4 h	12 h
ethanol	12.6	12.8
acetone	13.0	13.6
methylene chloride	13.7	13.9
n hexane	—	11.0

### 3. Thin Layer Chromatography (TLC) of piperine

Several techniques for TLC analysis of piperine have been published [6, 7, 8]. The aim is qualitative and quantitative analysis of piperine in pepper and in pepper extracts. In those techniques based on U. V. photometry, the isomers of piperine are overlooked while, as will be shown, they are nearly always present and have U. V. absorption characteristics very different from those of piperine. TLC separation of these isomers was not possible with silica gel G or H, and no improvement was obtained with buffered or specially activated plates. The successful technique of *Van Sumere* [9] for coumarines and plant phenols was also ineffective. Partial separation was achieved on silver nitrate impregnated plates with ethyl acetate as solvent. This influence of complex formation by the silver cation on the separation of trans cis unsaturated acids is well known [10] and is thus once more confirmed.

Fig. 1 shows some TLC analyses on such silver nitrate treated plates with ethyl acetate as eluent. The traces (densitograms) are obtained by scanning the plates with a Zeiss PMQ II *Chromatogramm Spektralphotometer* at  $\lambda = 343$  nm.

Trace a is practically pure piperine, b is the same sample solution after 2 hours (light with  $\lambda = 350$  nm) irradiation, c is crude pepper extract and d the crude pepper extract irradiated for 2 hours. Scanning TLC plates with the Zeiss

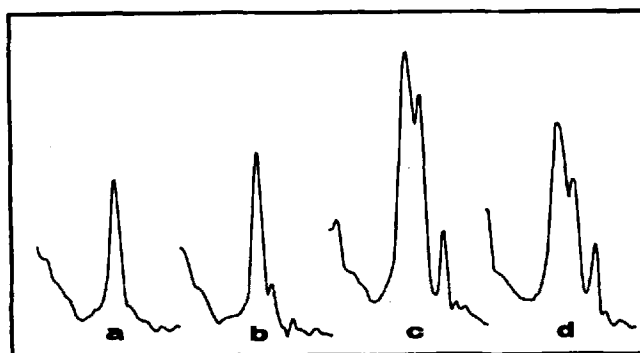


Fig. 2

- Densitograms of TLC on silver nitrate impregnated plates of the reaction mixture of piperidine with the acid chloride of: (a) piperine, (b) isochavicine, (c) chavicine and (d) isopiperine
- Densitogramme von DSC auf Platten mit Silbernitrat-Imprägnierung der Reaktionsgemische von Piperidin mit dem Säurechlorid von: (a) Piperin, (b) iso-Chavicin, (c) Chavicin und (d) iso-Piperin
- Densitogrammes des chromatogrammes obtenus sur couches minces imprégnées de nitrate d'argent, avec le produit de la réaction de la pipéridine avec le chlorure acide de la pipérine (a), de l'isochavicine (b), de la chavicine (c) et de l'isopipérine, (d)

*Chromatogramm-Spektralphotometer* usually gives a better base line but silver nitrate plates turn brown to black even on very short light exposure and this darkening of the plates apparently does not occur evenly enough, thus causing the unstable base line. The analyses of fig. 1 (also fig. 2) only give one spot or peak on plates without silver nitrate.

Fig. 2 shows the TLC analyses, under the same conditions as for fig. 1, of the mixtures obtained by reacting piperidine with the acid chloride of the four acids of table 1.

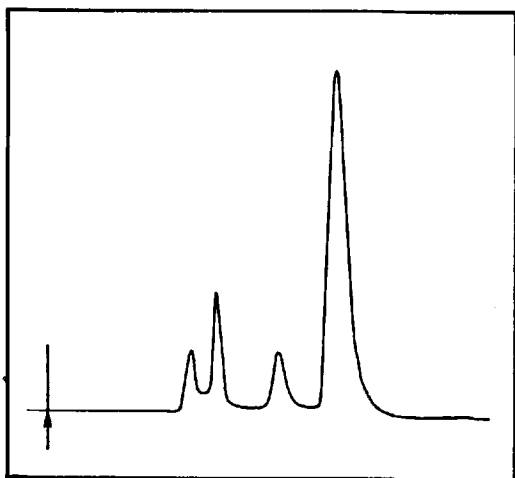


Fig. 3

- High pressure LC of the mixture obtained on reacting irradiated piperinic acid chloride with piperidine. In order of appearance peaks are chavicine, isopiperine, isochavicine and piperine. Silica gel H with ethyl acetate as solvent. Other details in text.
- Hochdruck-LC des Gemisches nach Reaktion von bestrahltem Piperinsäurechlorid mit Piperidin. Die Peaks in der Reihenfolge ihres Erscheinens sind Chavicin, iso-Piperin, iso-Chavicin und Piperin. Silicagel H mit Äthylacetat als Lösungsmittel. Weitere Einzelheiten im Text.
- Chromatogramme en phase liquide sous haute pression du mélange obtenu avec les produits de réaction du chlorure d'acide pipérinique et de la pipéridine. Dans l'ordre de leur apparition, les pics correspondent à la chavicine, à l'isopipérine, à l'isochavicine et à la pipérine. Gel de silice H et acétate d'éthyle comme solvant. Autres conditions voir le texte.

These acids are the subject matter of another paper in this series. Except for piperine (a) the others, viz: isochavicine (b) chavicine (c) and isopiperine (d) all show extensive isomerisation. Piperine is the main reaction product in all cases. As mentioned above the synthesis in this way of the four amides was reported [5] but it is obvious we cannot confirm these results.

#### 4. Liquid Chromatography (LC) under High Pressure

As separation by TLC was considered insufficient and also less suitable for later quantitation we turned to other techniques. GC was not applicable as isomerisation occurs at the high temperatures necessary for the elution of the substances. High pressure LC, however, proved effective. This is fortunate as high pressure LC has reached a stage where commercial instruments have become available. This technique is also fast and sensitive and can be carried out technically like a gas chromatographic separation. Two instruments were used, one was the commercial Varian 4020LC and the other a combination of commercial and self-made equipment; the details are reproduced in table III.

As an example of the results, fig. 3 shows the separation of the mixture obtained on reacting piperidine with the acid chloride of U. V. irradiated piperinic acid (40 h at  $\lambda = 350$  nm). In order of appearance the peaks are chavicine, isopiperine, isochavicine and piperine. According to molecular models this is in the order of decreasing steric hindrance.

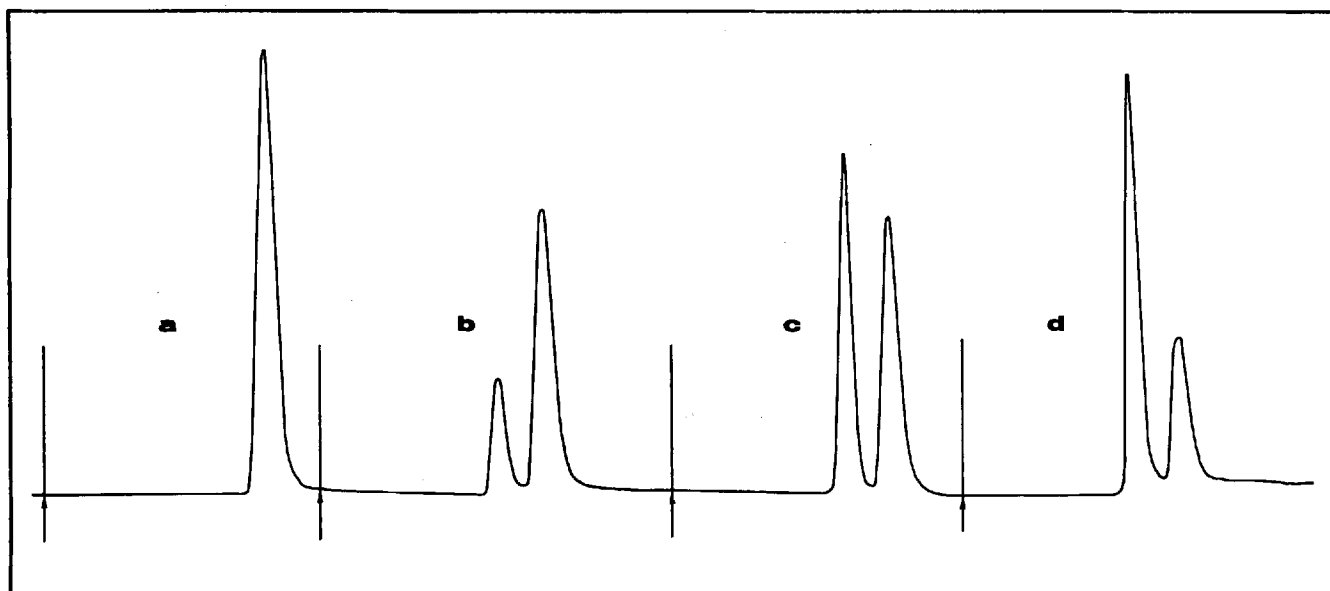


Fig. 4

- High pressure LC of irradiated piperine solutions (a) pure piperine, (b) after 1/2 hour irradiation at  $\lambda = 350$  nm, (c) after 1 hour and (d) after 4 hours
- Hochdruck-LC von bestrahlten Piperin-Lösungen. (a) reines Piperin, (b) nach halbstündiger Bestrahlung bei 350 nm, (c) nach 1 Stunde und (d) nach 4 Stunden
- Chromatogrammes en phase liquide sous haute pression de solutions de pipérine irradiées. (a) pipérine pure, (b) après irradiation, pendant 30 minutes, (350 nm); (c) après une heure, (d) après 4 heures

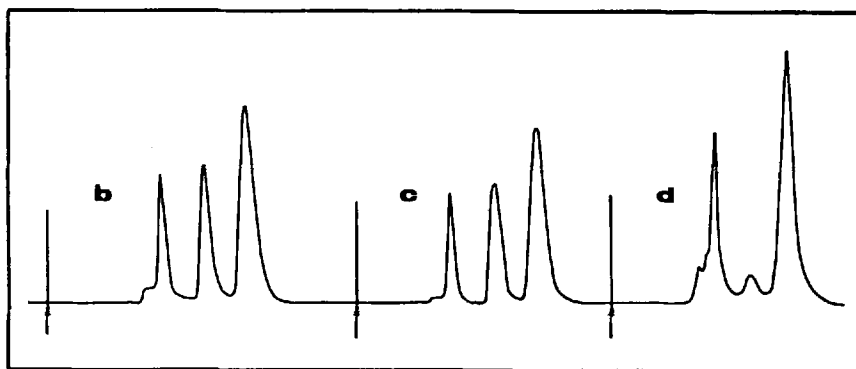


Fig. 5

- The same mixtures as b, c and d in fig. 2 but now with high pressure LC. Other details in text.
- Die gleichen Gemische wie b, c und d in Fig. 2, jedoch unter den Bedingungen der Hochdruck-LC. Weitere Einzelheiten im Text.
- Analyse des même mélanges b, c et d que dans la figure 2, par chromatographie en phase liquide sous haute pression. Autres conditions voir le texte.

Table III.

	Varian LC4020	Combination
Column:	stainless steel 80 x 2.5 cm	stainless steel 160 x 4 cm
Filling	Silica gel H	Silica gel H
Particle size	10 – 40 u	10 – 40 u
Solvent	ethyl acetate	ethyl acetate
Temp.	40°	room
Pressure	300 kg/cm <sup>2</sup> (continuous)	100 kg/cm <sup>2</sup> (pulsed)
Flow rate	50 ml/h	150 ml/h
Detector	Varian	Uvicord 4701A
Wave length	254 nm	254 nm
Sample size	10 µl 2 %	25 µl 2 %

The columns are packed using a pre-column of slightly larger diameter. A thick slurry of silica gel H and ethyl acetate (volume ratio 1 : 2) is poured in the pre-wetted, combined column and pre-column. High pressure is applied and packing is thus achieved in one operation under working pressure conditions. As can be deduced from fig. 3 the columns produce about 1000 theoretical plates.

### 5. Influence of U. V. Light on Piperine in Solution

High pressure LC analyses of the mixtures obtained by irradiating a 1 % piperine solution in methanol with U. V. light of  $\lambda = 350$  nm in a Rayonet Photoreactor are given in fig. 4. Trace a is pure piperine, b the same sample solution irradiated for 0,5 h., c the solution after 1 h. and d after 4 h. No further change is noted and d represents thus the photostationary state. It is obvious from these traces that piperine is transformed into isochavicine and not into chavicine as has been reported [10].

This photo-reaction can explain the slow disappearance with time of the pungent pepper taste. We have indeed ascertained that iso-chavicine has practically no taste. This is also the case for isopiperine and chavicine and therefore piperine is the main pungent principle of pepper. Older, ground pepper must contain large amounts of isochavicine which is determined as piperine by the usual analytical techniques.

### 6. Further Examples of LC Separation of Piperine Isomers

Some of the separations in fig. 2 are once more shown in Fig. 5, now using the LC technique. Isochavicine (b) and chavicine (c) give about the same mixtures. The traces in fig. 5 obtained on different dates show slightly different retention times. The throughput of the Orlita pump, with column attached, was not reproducible, although the pressure developed was as nearly as possible the same for all chromatograms.

### Experimental

- The pepper extracted was of the Lampong variety of piper Nigrum L.
- TLC plates were made with a suspension of 50 g silica gel G-Merck with 100 ml of a 10 % aqueous silver nitrate solution. Extreme care has to be taken while drying and activating the plates to protect them from light.
- **Piperinic acid chloride.**

Making the acid chloride of piperinic acid is difficult, as hydrolysis is very easy and all operations have to be carried out in strictly anhydrous conditions. The first to point out this difficulty was *Rügheimer* [11]. The acid chloride of piperinic acid is mentioned several times in the literature and is reported to have a melting

point of 180°. At first we obtained all sorts of melting points between 100 and 200° but after some effort it appeared that the true acid chloride melts at 104°. The recipe is as follows: —

Piperinic acid (7 g) is dissolved in dry benzene (750 ml) and thionyl chloride (110 ml) is added. The mixture is refluxed for 7 hours. The benzene and excess thionyl chloride are removed in vacuo at 20–30° bath temperature. The crystalline residue is recrystallised twice from very dry n hexane-toluene (3:1). The yield is 80 % of a substance with m. p. 104° and for which Schöniger analysis reveals 15.2 % Cl. (theor. 15.0 %).

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