

Isolation and characterization of piperine from the fruits of black pepper (*Piper nigrum*)

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Abstract

Black pepper (*Piper nigrum*) is a spice vine crop which is used as a food preservative and as an essential component in traditional medicines. The aim of this study was to extract, isolate and characterize the structure of piperine. The fruits of black pepper were extracted in ethanol and compounds present identified by TLC under iodine vapour. A compound (A_2) having R_f value 0.54 was isolated by column chromatography which may be responsible for the pungency of black pepper. After recrystallization of the isolate with benzene and chloroform mixture (7:1) gave pale yellow crystals of m.p. 128~129°C (reported 130°C). The IR, ^1H NMR and mass spectra of the pure crystalline compound (A_2) were recorded. The mass spectra of the compound (A_2) showed molecular ion peak M^+ at 284.5 which was similar to that of piperine (mol. wt. 285).

Keywords: Piperine, TLC, IR, NMR, Mass spectrometry

Introduction

Black pepper (*Piper nigrum*) belongs to the family Piperaceae. It is cultivated for its fruit, which is usually dried and used as a spice and seasoning. Black pepper is known as king of spices and extensively used all over the world. It is a perennial climber, climbing by means of ivy-like roots which adhere to the support tree. The sessile, small white flowers are borne in pendulous, dense, slender spikes of about 50 blossoms each. The berry-like fruits or peppercorns are round about 0.5 ~1.0 cm in diameter and contain a single seed. They become yellowish-red at maturity and bear a single seed. Spike length varies greatly based on cultivar. The young berries are green, whitish green or light purple, while mature ones are green, pale purple or pale yellow and change to red on ripening.

Actually, piperine was isolated in good yield from ground black pepper by Oerstedt H Schweigers in 1821. Pepper's pungency was found in 1821 to be due to piperine. Historically, it has been thought to cure many illnesses such as cancer, malaria and cholera (Epstein *et al.*, 1993).

Agrawal and Patwardhan (1994) reported that piperine present in black pepper fruit has antimicrobial activity. Jagella and Grosch (1999) reported that there are some compounds in black pepper fruit which are flavored and off flavored and inhibit the growth of *Sclerotium rolfsi* and *Fusarium solani* completely. Pradhan *et al.* (1999) studied the antimicrobial activity of black pepper fruit extract against some microorganisms and found that phenolic compounds present in the black pepper fruit extract. They found that black pepper fruit extract control the microorganisms. Sumathykutty and Madhusudana (1999) reported that some oil like compounds present in pepper are very effective to control *Phomopsis vsexans*. Strunz and Finlay (1994) synthesized pipericide a pesticidal unsaturated amide from black pepper fruit. Weaver and Neale (1988) studied the method for determination of piperine from black pepper fruit and they found that piperine present in black pepper fruit extract. De *et al.* (2000) reported that a chemical compound present in black pepper fruit, the name of the chemical compound is piperine. Piperine has fungicidal and insecticidal activity. Rajinder *et al.* (2002) studied the mode of action of piperine. Through human liver microsomal studies, they established that piperine, a major constituent of black pepper, inhibits human P-glycoprotein.

Materials and Methods

The work was performed at the Organic Chemistry laboratory, Department of Agricultural Chemistry, BAU, Mymensingh.

Preparation of black pepper fruit powder

Dried black pepper fruits were purchased from local market at Mymensingh, cleaned to free from stone and other undesirable matters and sun dried. Then the sun dried fruits were ground in an electrically operated grinder to fine particle size. Extraction and isolation were performed according to the method described by Lambein *et al.* (1993).

Thin layer chromatography was carried out on glass plates coated with silica gel G type 60. The different column chromatographic fractions of ethanolic extract were analysed with the help of thin layer chromatographic technique.

Results and Discussion

A column chromatographic fractions of benzene-EtOAc (3:1) were concentrated under vacuum and then recrystallized from benzene and chloroform mixture (7:1). This afforded pale yellow crystals of compound A₂ (probably piperine) (~5 g, yield 1.5%) m.p. 128~129°C (reported 130°C). The probable structure of the pure crystalline compound A₂ was established by spectroscopic analysis like IR, ¹H NMR and mass spectrometry (Madhavi *et al.*, 2009).

Infra Red (IR)

IR spectroscopy provides a fast and effective way to identify functional groups present in a molecule by looking to absorptions (bands) corresponding to the bond types present in the functional groups. The infrared absorption stretching of different bonds/functional groups of compound A₂ are tabulated in Table 1.

Table 1. The infrared stretching vibration for various bonds of compound A₂ (piperine)

Type of vibration of different bonds	Observed frequency (cm ⁻¹)	*Reference frequency (cm ⁻¹)
Aromatic C–H stretching	3008.7	above 3000
Aliphatic C–H stretching	2920	2925
-CO-N stretching	1699	1700
-O-CH ₂ -O- symmetric stretching	1253	1230
-O-CH ₂ -O- asymmetric stretching	1134	-
Symmetric stretching of conjugated diene	1633	-
asymmetric stretching of conjugated diene	1612	1610
-C-H bending	1018	1000

*P. S. Kalsi, (2004)

The presence of aromatic C–H stretching showed an absorption above 3000 cm⁻¹ and around 2925 cm⁻¹ for aliphatic C–H stretching. Our finding agreed with the reference data (Table 1).

A strong absorption band in the region around 1700 cm⁻¹ is due to the C=O stretching and the presence of this band indicates that the ketonic group (>C=O) may be present in the compound. Compound A₂ showed absorption at 1699 cm⁻¹ which indicating the presence of ketonic (>C=O) group.

An ether group (methylene ether, -O-CH₂-O-) is distinctly clear from the strong band at 1230 cm⁻¹ where as the experimental data found at 1253 cm⁻¹ and 1134 cm⁻¹ for symmetric and asymmetric stretching of the same ether group respectively.

The symmetric and asymmetric stretching absorption bands at 1633 cm^{-1} and 1612 cm^{-1} , respectively were observed for conjugated diene and the reference value was 1610 cm^{-1} which indicate the presence of conjugated diene in the compound.

Besides the C–H bending for trans –CH=CH– was observed at 1018 cm^{-1} which nearly same with that of reference data at 1000 cm^{-1} .

¹H NMR

The compound A₂ isolated by column chromatography from the ethanolic extract of black pepper fruit-powder and was analysed by proton magnetic resonance (¹H NMR) spectrometry.

Every type of proton has only a limited range of δ -values over which it gives resonance. Hence the numerical value in δ -units of the chemical shift for a proton gives a clue as to the type of proton originating the signal.

The ¹H NMR spectrum in CDCl₃ was recorded at 400 MHz spectrophotometer and TMS (Tetramethylsilane) used as an internal standard.

The chemical shift values of protons in different environment of compound A₂ are tabulated in Table 2 and the spectrum shown in Fig. 1.

Table 2. The ¹H NMR data of compound A₂ (piperine) in CDCl₃

Type of proton	Number of proton (¹ H)	Observed chemical shift δ in ppm	*Reference chemical shift δ in ppm
Cycloaliphatic	10	1.54 ~ 1.62	1.2 ~ 1.4
Alkenic	4	5.7 ~ 6.7	4.6 ~ 5.9
Aromatic	3	6.65 ~ 6.93	6.8 ~ 7.3
Methylene attached to oxygen atoms	2	3.49 ~ 3.59	3.2 ~ 3.8

* P. S. Kalsi, (2004); Pavia *et al.*, (2006)

Cycloaliphatic proton (10H)

The methylene hydrogens (–CH₂–) of cycloaliphatic ring containing nitrogen atom show chemical shift at δ 1.54 ~ δ 1.62 where the reference data to those of are found δ 1.2 ~ δ 1.4 (Pavia *et al.*, 2006). The methylene proton (–CH₂–) shows resonance between methyl and methine hydrogen. Actually this shift is intermediate. The chemical shift of these protons is variable, depending not only on the chemical environment, but also on concentration, temperature and solvent.

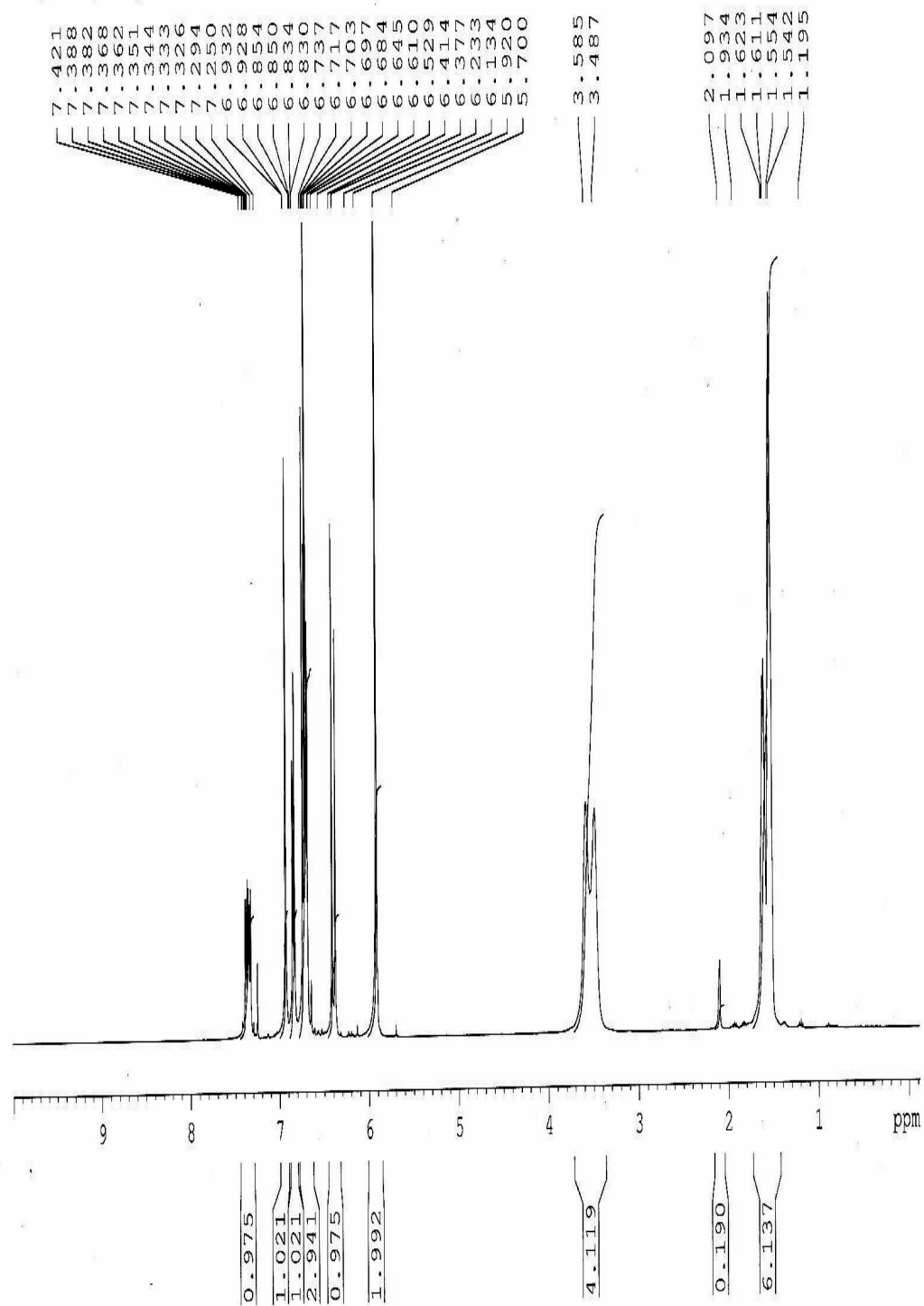
Alkenic proton (4H)

The alkenic protons show experimental chemical shift at δ 5.7 ~ δ 6.7 which is shifted to a little bit downfield in comparison to that of standard values at δ 4.6 ~ δ 5.9. It may be happened for our sample due to the effect of carbonyl (>C=O) group attached to the alkenic carbon.

Aromatic Proton (3H)

Phenyl rings are so common organic group that it is important to reveal a few facts about nmr absorptions in compounds that contain them. The ring protons of a benzenoid system have resonance, near 7.3 ppm. This value of chemical shift is diagnostic.

ARD,BCSIR,1H spectrum, PJ-1 in CDC13, Asaduzzaman, A.U

Fig. 1. 400 MHz ^1H NMR spectrum of compound A₂ (piperine)

The aromatic compound gives only one aromatic peak (a singlet) in the proton nmr spectrum but that positions are affected more strongly than others in systems with substitution patterns different from this one. Here the electron donating substituent e.g. methoxy (-OCH₃) move the resonance of the ring protons upfield.

The compound A₂ gave chemical shift value δ 6.65 ~ δ 6.93 ppm for aromatic protons which showed a little bit upfield shift than that of reference data (Table 2). This deviation of chemical shifts of aromatic protons may happen due to presence of methylene dioxy group (-O-CH₂-O-) attached to the benzene ring (Pavia *et al.*, 2006).

Methylene dioxy proton (-O-CH₂-O-)

The chemical shift at δ 3.49 ~ δ 3.59 ppm was observed for the protons of methylene dioxy (-O-CH₂-O-) group. The hydrogen on the carbon attached to the oxygen are deshielded due to electronegativity of the oxygen. It is cited from the text that in ethers (R-O-CH-), the hydrogen on the carbon next to oxygen are deshielded for the above mentioned reason and they appear in the range δ 3.2 ~ δ 3.8 ppm. The absorption of the methylene hydrogens next to the oxygen are seen at δ 3.4 ppm (Pavia *et al.*, 2006).

In methylene dioxy group, there are two hydrogens on the carbon attached to the two electronegative oxygen atoms. Therefore, the greater the electronegativity, the more it deshields protons and hence the greater is the chemical shifts of those protons.

Mass spectrometry

The mass spectra of compound A₂ clearly showed a molecular ion peak at m/z 284.5 corresponding to molecular formula, C₁₇H₁₉NO₃ which is very nearly similar to the mass spectral data of piperine (Mol. wt. 285). Thus it may be concluded that the compound A₂ is piperine and its probable structure shown in Fig. 2.

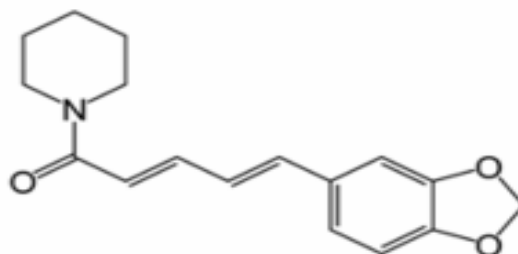


Fig. 2. Structure of piperine

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