

Isolation of Bulk Amount of Piperine as Active Pharmaceutical Ingredient (API) from Black Pepper and White Pepper (*Piper nigrum* L.)

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Abstract

In the pharmaceutical world the majority of the active pharmaceutical ingredients (API) have been obtained from the natural products. Piperine is such naturally occurring alkaloid which can be considered as major bioactive phytochemical having broad spectrum of pharmacological activities. It is obtained from the most valuable ethnomedicinal spices peppercorns *i.e.* black pepper and white pepper, which are the fruits of the Asian vine *Piper nigrum* L. Because of the widespread traditional uses of this medicinal compound, present article reveals a simple and effective isolation method of bulk piperine. The novelty of this investigation is to provide an idea for utilizing such natural method of large scale commercial piperine production as API drug in spite of chemical synthesis. Piperine was isolated in a pure crystal form and characterized by its melting point, X-Ray diffraction (XRD) studies and spectral data, including two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy. Chromatographic techniques like Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were applied to determine the purity of the yielded piperine. It was found that piperine yield from black pepper was within 2.5% - 3.0% and from white pepper within 4.0% - 4.5% and the purity of the yielded piperine was found to be up to 98.5% for black pepper and 98.2% for white pepper. Considering this yield value and purity it is indicated that, such effective isolation method can be successfully utilized for industrial large-scale production commercially. According to the result, it can be claimed that, as a natural product the isolated piperine can also be utilized as API drug like other expensive chemically synthesized piperine in different drug formulation.

Keywords

Piper nigrum L., NMR Spectroscopy, X-Ray Diffraction Studies, Bulk Piperine

1. Introduction

Piperine is the pungent chemical constituent that imparts an excellent medicinal value to pepper spices, most commonly used in folk medicine e.g. in “Trikatu”, a classic ayurvedic herbal formulation containing different kinds of pepper spices like black pepper, long pepper etc. traditionally used to support digestion, the overall gastric function and also the respiratory function due to its strong pungent qualities caused by piperine [1]. Piperine has tremendous role in pharmacotherapeutics as it has been recognized as potential bioavailability enhancer by promoting rapid absorption and also having the effect on inhibiting metabolizing enzyme responsible for biotransformation of drugs or nutrients and thus preventing their inactivation and elimination [2] [3]. Piperine also possesses physiological effects like increased salivation, enhanced secretion of gastric juice which lead to better digestion and bioavailability of nutritional constituents [4]. Piperine is generally consumed through black pepper or white pepper and by using these peppers to our daily consuming meal is the convenient way to increase nutrient absorption and metabolism.

Piperine possesses a broad spectrum of pharmacological activities and it is mentioned through several experiments that piperine can be used as antioxidant, antibacterial, antifungal, anti-colon toxin, antidepressant, antidiarrhoeal, anti-inflammatory, antimutagenic, anticancer, antispasmodic, insecticidal activity etc. [5]. It is reported that piperine has an antimicrobial effect and thus protects the seeds in which it is placed from the attack of microbes [6]. Having this antimicrobial activity of piperine, pepper spices were traditionally used in folk medicine for the treatment of many respiratory infectious diseases such as cough, bronchitis, tonsillitis, sinusitis, tuberculosis etc. and also some Gastrointestinal Tract (GIT) disorder like gastritis, peptic ulcers etc. [7] [8]. Due to these remarkable biological roles, researchers are now regarded piperine as a valuable natural bio-active compound and day by day new research is documenting many health benefits regarding of piperine.

Natural products have played a key role in pharma research, as many medicines are either natural products or derivatives thereof [9]. Modern chemistry has ushered in a new era for the study and use of natural products. Analytical and structural chemistry have provided the tools to purify various compounds and to determine their structures, which, in turn, has given insights into their action on the human body. The structural analysis of natural compounds and the ability to synthesize them allowed chemists to modify these compounds in order to suppress or enhance certain characteristics such as solubility, efficiency or stability in the human body [10] [11]. But, many non-natural, synthetic drugs

cause several side effects that were not acceptable and sometimes it is difficult to synthesize structurally complex metabolites; which can be overcome by the use of metabolites discovered in medicinal plants and other natural products [12]. The present study was aimed to isolate piperine in a pure form from these natural sources like black pepper and white pepper indigenous to Bangladesh in a bulk amount to be used in drug formulation as an API.

2. Materials and Methods

2.1. Instrumentation

The extract was condensed in rotary vacuum evaporator (Heidolph Instruments GmbH & Co. KG, Germany). Melting point was determined on Stuart melting point apparatus SMP30 (UK). Crystalline structure was observed by XRD study (D8 Advance, BRUKER, Germany). NMR spectra were recorded on Bruker WP AM 400 spectrometer (Switzerland). For purity analysis Hitachi HPLC system equipped with La-chrome C₁₈ column (5 μm, 4.6 mm × 250 mm) and UVGL-58 Handheld UV Lamp (UK) was used for TLC analysis.

2.2. Chemicals and Reagents

Black pepper and white pepper were purchased from a local retail market. Potassium hydroxide (Calbiochem, Merck, Germany) and CDCl₃ (Sigma-Aldrich, USA) were used in this study. All other chemicals were purchased from Active Fine Chemicals Ltd., Bangladesh.

2.3. Isolation and Purification

The black pepper and white pepper fruits were washed, dried and mechanically reduced to coarse powder form. 100 g of dried powder of black pepper and white pepper was successively extracted to exhaustion in a soxhlet using 400 ml of 95% ethanol. For condensing the extract, the solvent was evaporated on the rotary evaporator where we can get the oleoresin, the true essence of spices. From the concentrated extracts yellow colored needles were formed which were collected through filtration and from the remaining filtrate the crystals were isolated by desinification with 10% alcoholic KOH solution through the precipitation with water which was further collected by filtration. In order to get the compound in a more pure form the isolated crystals were recrystallized by dissolving in a dichloromethane where few drops of n-Hexane was applied and allowed the solution to settle undisturbed for few hours. Rod-like, light yellow crystals were formed which were collected through filtration and purified by treatment with solvent mixture (n-Hexane/1% Dichloromethane) to remove the undesired components. Finally, the resulting crystals were weighed to know the exact yield.

2.4. Characterization of the Isolated Compound

The isolated compound **1** was characterized by its melting point, XRD studies and spectral data including 2D-NMR.

2.4.1. Melting Point Studies

Piperine can be characterized by its melting point which is within range of 129°C - 130°C [12]. In the present study the melting point of the compound **1** from both of black pepper and white pepper were determined.

2.4.2. XRD Studies

XRD studies of powdered crystals were carried out to study crystalline nature of the isolated compound **1** from both of black pepper and white pepper.

2.4.3. NMR Spectra Studies

NMR spectra were recorded (¹H at 400 MHz and ¹³C at 100 MHz, CDCl₃) for the isolated compound **1** obtained from both of black pepper and white pepper to elucidate the structure and chemical shifts were reported in ppm.

2.5. Purity Analysis of the Isolated Piperine

Chromatographic techniques like TLC and HPLC were applied to determine the purity of the isolated piperine.

2.5.1. TLC Analysis

Thin layer chromatographic technique was used for the initial screening of the extracts and checking the purity of isolated compound using toluene/20% ethyl acetate and visualized under UV light at 254 nm and 365 nm.

2.5.2. HPLC Analysis

Piperine obtained from both of black pepper and white pepper was dissolved in methanol to obtain a concentration of approximately 0.2 mg/ml. The samples were analyzed by a Hitachi HPLC system with UV visible detector. The mobile phase consists of acetonitrile, water and acetic acid at ratio of 60:39.5:0.05 and 1 ml/min flow rate. 10 µl of sample was injected into the column at ambient temperature. Chromatogram was monitored at 340 nm. Peak area for piperine was observed at retention time of approximately 8.55 min. Purity of piperine was calculated by area percent method.

3. Results

3.1. Quantification of the Isolated Compound

The content of the isolated compound **1** in 100 g raw materials were found to be higher in white pepper (4.1 g) compared to black pepper (2.9 g).

3.2. Characterization of the Isolated Compound

3.2.1. Melting Point Studies

The compound **1** was characterized by its melting point which showed that it undergoes melting at 129°C for white pepper and at 130°C for black pepper which is close to the standard range (129°C - 130°C) [12].

3.2.2. XRD Studies

Structural properties of the isolated compound **1** from both of black pepper and

white pepper were investigated by XRD with $\text{CuK}\alpha$ ($\lambda = 1.54$) radiation and 2θ ranges from 4° to 60° . The XRD pattern of the compound **1** (Figure 1) reveals that the pattern matched with the standard pattern of piperine (JCPDS Card no: 00-043-1627) and its chemical formula $\text{C}_{17}\text{H}_{19}\text{NO}_3$ having monoclinic structure (space group: $\text{P}21/n$ (14), $a = 8.69500\text{\AA}$, $b = 13.60200\text{\AA}$, $c = 13.15800\text{\AA}$, $z = 4$).

3.2.3. NMR Spectra Studies

The compound **1** was assigned piperine (Figure 2) by 1D- and 2D-NMR spectroscopic methods. The ^{13}C NMR spectrum (100 MHz, CDCl_3) of **1** displayed 17 carbon resonances, while HSQC experiment indicated that 13 out of 17 carbons were attached to proton. ^1H NMR (400 MHz, CDCl_3) and DEPT 135 (100 MHz, CDCl_3) spectra of **1** revealed the presence of five methylene groups which belongs to the piperidine ring, a methylene dioxy group and seven olefinic/aromatic methines of which three are aromatics and four are olefinics. The ^1H NMR and ^{13}C NMR chemical shifts are shown in Table 1.

The resonances at $\delta = 3.62$ ppm and 3.51 ppm in the ^1H NMR and $\delta = 43.3$ ppm and 46.9 ppm in the ^{13}C NMR spectra, in conjunction with the DEPT 135 spectrum could be attributed for the hydrogens and carbons [Pip-(2) and Pip-(6), respectively] of the piperidine ring, which are next to the nitrogen. The resonances at $\delta = 1.58$ ppm, 1.65 ppm and 1.58 ppm in the ^1H NMR and $\delta = 26.8$ ppm, 24.7 ppm and 25.7 ppm, respectively, in the ^{13}C NMR spectra, in conjunction

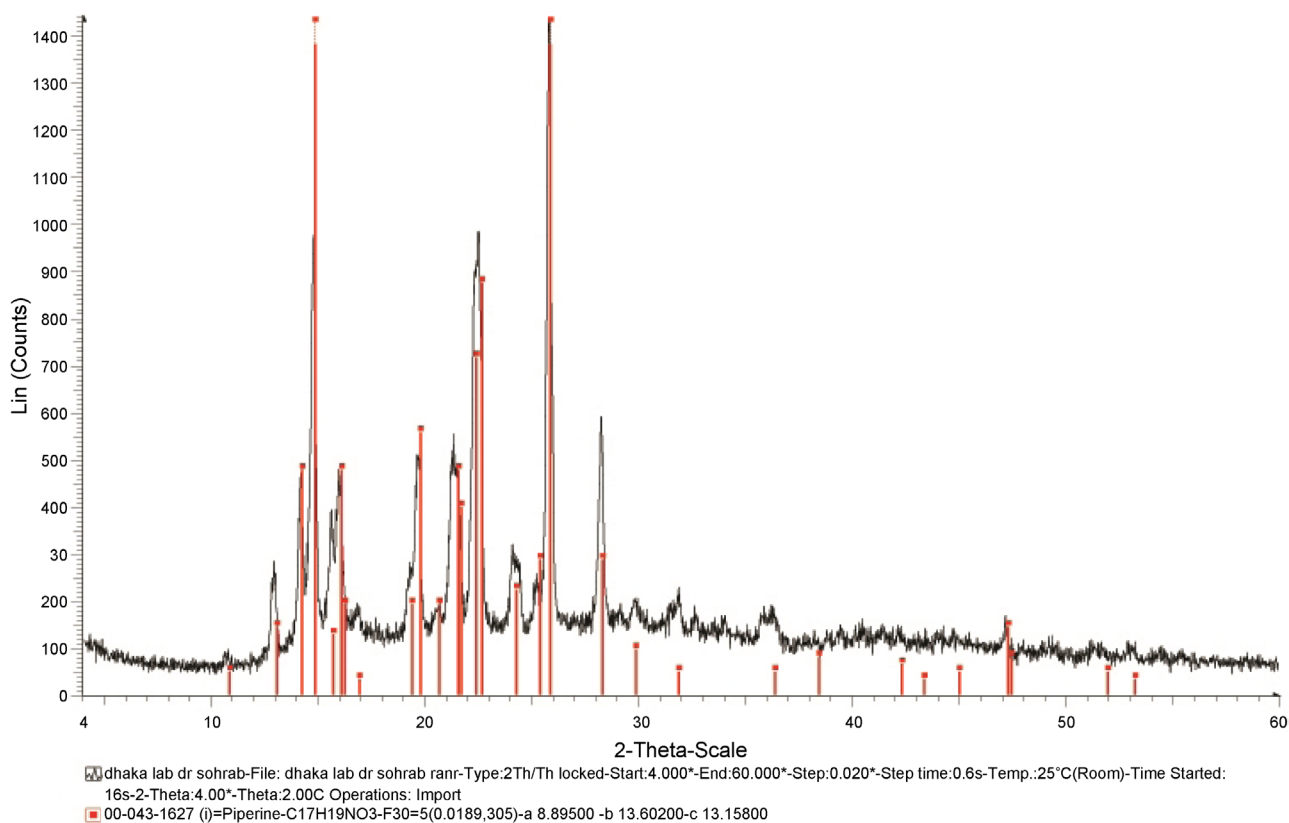


Figure 1. X-ray diffractogram of compound 1.

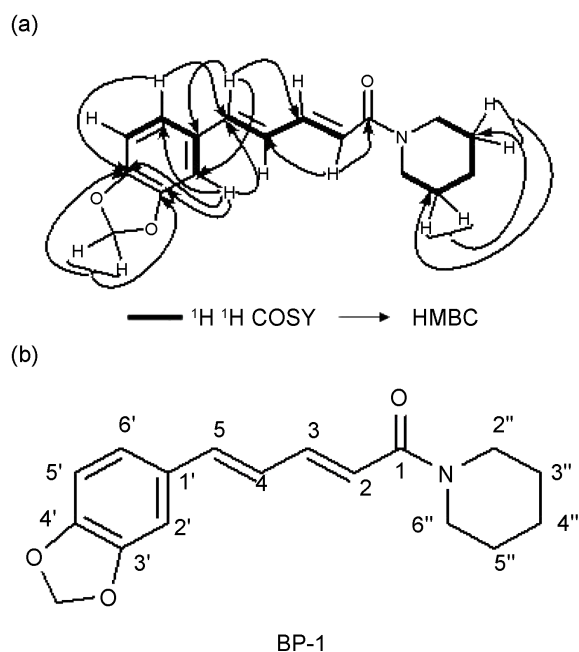


Figure 2. (a) connectivities of 1 and (b) structure of 1.

Table 1. ^1H (400 MHz, CDCl_3) and ^{13}C (100 MHz, CDCl_3) NMR data for compound 1.

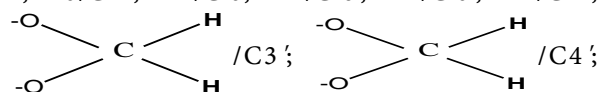
Position	1				Piperine [13]	
	δ_{H} (mult., J in Hz)	δ_{C}	COSY	HMBC	δ_{H} (mult., J in Hz)	δ_{C}
1	---	165.5	---	---	---	165.6
2	6.42, d, $J = 14.8$ Hz	120.1	H3	C1, C4	6.38, d, $J = 14.7$ Hz	119.7
3	7.38, ddd, $J = 14.3, 8.4, 1.8$ Hz	142.5	H2, H4	-	7.36, ddd, $J = 14.7, 8.3, 1.9$ Hz	142.8
4	6.74, m	125.4	H3	C3, C1'	overlapping	125.2
5	6.74, m	138.2	H3	C3, C1', C2'	overlapping	138.4
1'	---	131.1	-	-	---	130.9
2'	6.96, s	105.7	H6'	C5, C3', C4', C6'	6.93, d, $J = 1.5$ Hz	105.6
3'	---	148.2	-	---	---	148.06
4'	---	148.1	-	---	---	148.09
5'	6.76, d, $J = 8.0$ Hz	108.5	H6'	C1', C3', C4'	overlapping	108.4
6'	6.88, d, $J = 8.0$ Hz	122.5	H2', H5'	C5, C2', C4'	6.84, dd, $J = 6.0, 1.5$ Hz	122.5
Methylenedioxy	5.96, s	101.3	-	C3', C4'	5.9, s	101.2
2''	3.62, m	43.3	H3''	---	3.48, brs	43.3
3''	1.58, m	26.8	H2'', H4''	C5''	1.54, m	26.6
4''	1.65, m	24.7	H3'', H5''	---	1.54, m	24.5
5''	1.58, m	25.7	H4'', H6''	C3''	1.54, m	25.5
6''	3.51, m	46.9	H5''	---	3.57, brs	46.9

with the DEPT 135 spectrum could be attributed for the other methylene protons in the piperidine ring [Pip-(3), Pip-(4) and Pip-(6), respectively]. The singlet at $\delta = 5.96$ ppm in the ^1H NMR and at $\delta = 101.3$ ppm in the ^{13}C NMR spectra, in conjunction with the DEPT 135 spectrum could be attributed for methylene-

dioxy of dioxolane ring. The resonances at $\delta = 165.5$ ppm in the ^{13}C NMR spectrum was characteristic for the presence of the amide carbonyl atom.

The most deshielded signal at $\delta = 7.38$ ppm in the ^1H NMR spectrum could be assigned to H-3, as its chemical shifts is typical for β -proton in α/β -unsaturated carbonyl compounds. The sharp doublet at $\delta = 6.42$ ppm with the spin coupling of 14.8 Hz in the ^1H NMR spectrum could be assigned to H-2. The singlets at $\delta = 6.96$ ppm, doublets at $\delta = 6.76$ ppm ($J = 8$ Hz) and 6.88 ppm ($J = 8$ Hz) in the ^1H NMR spectra constitute the very typical pattern of a 1, 2, 4-trisubstituted aromatic compound.

Analysis of one- and two-dimensional NMR spectra including COSY, HSQC and HMBC led to the assignment of structure "a" (Figure 2). In this structure, the C2-C3-C4-C5 portion, C2'-C1'-C6'-C5' portion and C2''-C3''-C4''-C5''-C6'' portion were assigned by tracing of cross peaks in COSY spectrum. The structure of piperine (Figure 2) was disclosed by HMBC correlations (H-2/C-1; H-2/C-4; H-5/C-3; H-5/C-2; H-5/C-1; H-2'/C-5; H-2'/C-6; H-2'/C-3; H-2'/C-4; H-6'/C-4; H-6'/C-5; H3''/C5''; H5''/C3'') in 1.



The ^1H NMR and ^{13}C NMR data of 1 was found to be identical to those reported previously for the piperine [13] [14].

3.3. Purity Analysis of the Isolated Piperine

3.3.1. TLC Analysis

During primary purity screening of the isolated piperine, it was appeared as a dark quenching spot on the TLC plate ($R_f = 0.5$, toluene/20% ethyl acetate) under UV light at 254 nm and blue fluorescent spot at 365 nm.

3.3.2. HPLC Analysis

Purity of the isolated piperine was determined by HPLC. Area percent method was used for calculation where major peak at retention time 8.55 min was considered as the peak of piperine (Figure 3). Piperine yield from black pepper was 2.5% - 3.0% and from white pepper 4.0% - 4.5% complying with the previous report [15]. Purity of the yielded piperine found to be up to 98.5% and 98.2% obtained from black pepper and white peeper respectively (Table 2).

4. Discussion

Currently in spite of the presence of huge available synthetic drug materials, many pharmaceutical companies are now focused on the development of plant-derived active ingredients in order to avoid the costs and complexities. In fact, around 50% of pharmaceuticals are derived from compounds first identified or isolated from herbs/plants, including organisms, animals, and insects, as active ingredients e.g. Artemisinin, Taxol, Vincristine etc. These naturally obtained drugs are now attempted to be isolated in bulk amount for the development of newly approved therapeutic agents as API [16]. Piperine is the naturally occurring

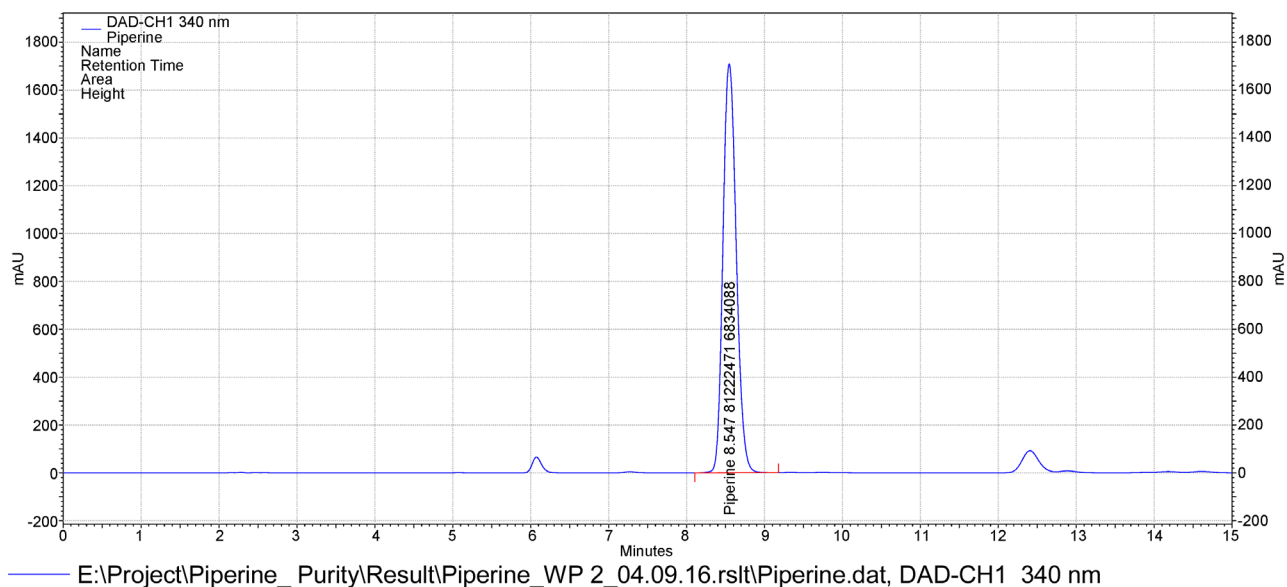


Figure 3. HPLC chromatogram of isolated piperine.

Table 2. Purity of the yielded piperine from black pepper and white pepper.

Source	Yield (%)	Purity (%)
Black pepper	2.9	98.5
White pepper	4.1	98.2

alkaloid considered as the most desirable therapeutic agent obtained from the piper spices which imparts pungency and medicinal values of that spices. In present work a short and effective method was developed to isolate bulk piperine from these natural pepper spices *i.e.* black pepper and white pepper to formulate as API.

Piperine was successfully isolated in a pure crystal form and that isolated crystals were characterized by its melting point which showed within the standard range (129°C - 130°C) indicating no impurities and were used for further phytochemical screening. While determining Structural properties, XRD pattern of the isolated compound matched with the standard pattern of piperine (**Figure 1**) and structure of piperine was confirmed as that of piperine (**Figure 2**) by 1D- and 2D-NMR spectroscopic methods. Analysis of isolated piperine by HPLC revealed its purity 98.5% and 98.2% obtained from black pepper and white pepper respectively, with a good yield value of 2% - 5% and considering the purity that was the acceptable range of the yield value of the isolated piperine to be claimed as API.

5. Conclusion

According to the literature reviews, piperine can be considered as the already established bioactive compound [5]. So following purity analysis it can be claimed that, the active pharmaceutical activity of the extracted piperine in the work will find to be similar like other expensive synthetic piperine. In that case,

a comparative bioactivity screening between naturally and chemically synthesized piperine can be investigated in future. Finally, it can be concluded that the isolation method applying in this study can be successfully followed for the commercial production of bulk piperine to be utilized as API.

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Competing Interests

The authors declare that they have no competing interests.

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Abbreviations

JCPDS: Joint Committee on Powder Diffraction Standard; HSQC: Heteronuclear Single-Quantum Correlation; MHz: megahertz; CDCl₃: Deuterated chloroform; DEPT: Distortionless enhancement by polarization transfer; ppm: parts per million; HMBC: Heteronuclear Multiple Bond Correlation; COSY: Correlation spectroscopy.



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