

## Research Article



## Piperone-3 and Piperone-4: Two New Ketones Isolated from *Piper Longum* L. Dried Fruits

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### ABSTRACT

Two new chemical components PL-3 and PL-13 were isolated from petroleum ether and ethyl acetate fraction of EtOH fruit extract of *Piper longum* (Piperaceae). Their structures were elucidated by spectroscopic analysis (<sup>1</sup>H-NMR, <sup>13</sup>C NMR, DEPT, COSY, FTIR, ESI-MS spectra) and have been characterized as 8-Benzo [1,3]dioxol-5-yl-1-methoxy-octa-5,7-dien-4-one and has been designated as "Piperone-3." (PL-3) and 7-Benzo [1,3]dioxol-5-yl-1-methoxy-hepta-4,6-dien-3-one and has been designated as "Piperone-4" (PL-13) respectively.

**Keywords:** *Piper longum*, Piperone-3, Piperone-4.

### INTRODUCTION

*Piper longum* is an important medicinal plant belongs to the family Piperaceae, is used in traditional medicine in Asia and Pacific islands especially in Indian medicine.<sup>1</sup> *Piper longum* is the accepted source of drugs Pippali and Pippalimulam throughout the country; Pippali is the dried ripe fruits; Pippalimulam is the roots of this plant.<sup>2</sup> The fruits of *Piper longum* have been used traditionally for treating jaundice and allergy.<sup>3</sup> Dried unripe fruits are used as an alternative to tonic and it's also used for the treatment of respiratory tract diseases, like common cough and cold, bronchitis, asthma.<sup>4,5</sup> The characterization of plant extracts has become more important as a result of an increasing requirement for herbal remedies while remaining aware of possible side effects. Typically, the characterization of plant extracts involves purification of compounds by repeated column preparations, and then off-line identification by 1D and 2D nuclear magnetic resonance (NMR) methods and mass spectroscopy<sup>6</sup>. So in the present study we have isolated the two pure chemical components from the dried fruits of *Piper longum* and elucidated their structure by spectroscopic analysis (<sup>1</sup>H-NMR, <sup>13</sup>C NMR, DEPT, COSY, FTIR, ESI-MS spectra).

### MATERIALS AND METHODS

#### Collection of Plant Material

The fruits of *Piper longum* collected from the market Kharibawli; ChandniChawk, Delhi and authenticated at the Department of Botany, JamiaHamdard University, New Delhi. All the organic solvents used in experiments were of analytical grade, Silica gel for column chromatography and purchased from Merck, Germany.

#### Preparation of extracts and different fractions

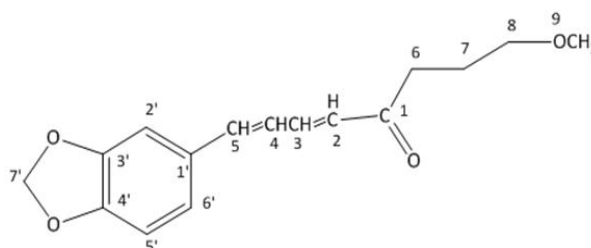
The dried fruits (5.0 kg) of *Piper longum* were crushed to coarse powder and extracted with ethanol using cold

percolation method and concentrated on water bath under reduced pressure. The ethanolic extract was dried (600 gm) and then successively fractionated into three fractions: 1. Petroleum ether (60-80°C) fraction (150 gm); 2. Ethyl acetate fraction (200 gm); 3. Methanol fraction (250 gm).

#### Isolation of Chemical Components

##### Compound PL-3

Compound 1 was eluted from petroleum ether fraction from column chromatography at polarity of petroleum ether-chloroform (25:75) yielded 30 mg as greenish crystals. Crystalline solid, R<sub>f</sub> value: 0.56 (CHCl<sub>3</sub> + MeOH= 9:1); IR ν<sub>max</sub>cm<sup>-1</sup> (KBr): 2947(CH<sub>3</sub>), 2950 (OCH<sub>3</sub>), 1707(C=O ketonic group), 1506(aliphatic=bond), 1264(C-O phenolic group), 1040(C-O aliphatic group); m/z 274[M]<sup>+</sup>(100%), 260(10%), 252(10%), 225(5%).



PL-3: 8-Benzo[1,3]dioxol-5-yl-1-methoxy-octa-5,7-dien-4-one

Figure 1: Chemical Structure of Piperone-3 (PL-3)

##### Compound PL-13

Compound 2 was eluted from petroleum ether fraction from column chromatography at polarity of petroleum ether-chloroform (25:75) yielded 32mg as greenish crystals. Crystalline solid, R<sub>f</sub> value: 0.56 (CHCl<sub>3</sub> + MeOH= 9:1); IR ν<sub>max</sub>cm<sup>-1</sup> (KBr): 3009(C-H), 2922(CH<sub>3</sub>),



2950(OCH<sub>3</sub>)1709(C=O ketonic group) 1265(C-O phenolic group), 1040(C-O aliphatic group) 1505(C=C aliphatic bond), 1555(C=C aromatic stretching); 260[M]<sup>+</sup> m/z, (100%), 250(10%), 239(10%), 215(5%)

**Table 1:** 1D and 2D NMR spectral data of PL-3

Positions	<sup>1</sup> H-NMR <sup>a</sup>	<sup>13</sup> C-NMR <sup>a</sup>	DEPT-135 <sup>b</sup>	Conclusion	COSY <sup>a</sup>
1	----	167.6	No peak	C	--
2	6.694-6.667 <i>d</i> (J= 14.8 Hz)	130.5	Positive peak	CH	H-3
3	7.454-7.389 <i>dddd</i> (J= 4.0, 14.8 Hz)	145.0	Positive peak	CH	H-2, H-4
4	6.924-6.900 <i>dd</i> (J= 1.6, 9.6 Hz)	140.3	Positive peak	CH	H-3, H-5
5	6.778-6.733 <i>dd</i> (J= 4.0, 9.6 Hz)	124.5	Positive peak	CH	H-4
6,7,8	1.21, <i>brs</i> (9H, 3x CH <sub>2</sub> )	26.1, 26.2, 26.3	Negative peak	CH <sub>2</sub>	H-6, H-7, H-8
9	3.76, <i>s</i> (3H, OMe)	50.1	Positive peak	CH <sub>3</sub>	---
1'	----	130.5	No peak	C	--
2'	6.998 <i>d</i> (J= 1.6 Hz)	123.0	Positive peak	CH	--
3'	----	148.58	No peak	C	--
4'	----	148.29	No peak	C	--
5'	6.77 <i>d</i> (J= 8.0 Hz)	105.86	Positive peak	CH	H-6'
6'	6.79 <i>d</i> (J= 8.0 Hz)	108.5	Positive peak	CH	H-5'
7'	5.988 <i>s</i>	101.4	Negative peak	CH <sub>2</sub>	--

a: Assignment were based on <sup>1</sup>H-NMR, 1H-1H COSY and <sup>13</sup>C-NMR experiments; coupling constant in hertz are given in parenthesis; s:singlet, *d*: doublet, *brs*: broad signal; b: DEPT chemical shift are presented at  $\theta=3\pi/4$  when methylene group reaches negative maximum.

## RESULTS AND DISCUSSION

### Compound PL-3

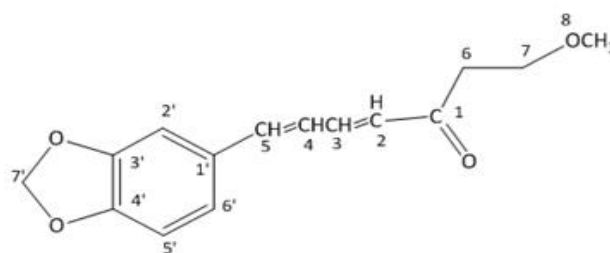
compound PL-3 was obtained as greenish crystals, gave a single spot on TLC, and exhibited a molecular formula C<sub>16</sub>H<sub>18</sub>O<sub>4</sub> as established on the basis of FAB-MS, showed a molecular ion peak at *m/z* 274.12 [M]<sup>+</sup>, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and DEPT spectra. The IR spectrum showed a sharp peak at  $\nu_{\max}$  1707 cm<sup>-1</sup> indicating the presence of ketonic group, 2947 cm<sup>-1</sup> for methyl group, 1506 and 839 cm<sup>-1</sup> for aliphatic double bond, 1264 cm<sup>-1</sup> due to C-O phenolic and 1040 cm<sup>-1</sup> due to C-O aliphatic groups. The <sup>13</sup>C-NMR and DEPT spectra showed fourteen carbon atoms including one carbonyl, three quaternary, four methylene and seven methine carbons and one methyl carbon (in total C<sub>16</sub>H<sub>18</sub>O<sub>4</sub>). The <sup>1</sup>H-NMR spectrum exhibited three aromatic signals at  $\delta_{\text{H}}$  6.77 (1H, *d*, J= 8.0 Hz, H-5'), 6.79 (1H, *d*, J=8.0 Hz, H-6') and 6.99 (1H, *d*, J= 1.6 Hz, H-2') attributable to H-5', H-6', and H-2' positions respectively. A two protons singlet displayed at  $\delta_{\text{H}}$  5.98 in <sup>1</sup>H NMR and also exhibited a -CH<sub>2</sub>- carbon signal at  $\delta_{\text{C}}$  101.4 in <sup>13</sup>C-NMR and DEPT spectra, which could be attributed to methylene group attached to oxygen atoms and thus was assigned at position 7'. Four signals of one proton each were exhibited at  $\delta$  6.67 (1H, *d*, J= 14.8 Hz, H-2,  $\delta_{\text{C}}$  130.5), 7.45 (1H, *dddd*, J= 4.0, 14.8 Hz, H-3,  $\delta_{\text{C}}$  145.0), 6.92 (1H, *dd*, J= 1.6, 9.6 Hz, H-4,  $\delta_{\text{C}}$  140.3) and 6.77 (1H, *dd*, 4.0, 9.6 Hz, H-5,  $\delta_{\text{C}}$  124.5), which were attributed to positions 2, 3, 4 and 5 respectively.

The other carbon signals in the aromatic region exhibited signals at  $\delta_{\text{C}}$  123.0 (C-2'), 108.5 (C-6'), 105.8 (C-5'), due to

methine carbon atoms of the aromatic ring. In addition, carbon signals at  $\delta$  130.5 (C-1'), 148.3 (C-4') and 148.5 (C-3') were assigned to quaternary carbon atoms of the aromatic ring. These assignments were also substantiated further by correlating the proton peaks in COSY experiment and have been presented in Table-1.

The IR spectrum of PL-3 also exhibited peaks at 2950 cm<sup>-1</sup> 1050 cm<sup>-1</sup> due to a methoxyl (OCH<sub>3</sub>) group which was confirmed by three proton signal due to methoxyl group at  $\delta_{\text{H}}$  3.76 (3H, *s*, OMe,  $\delta_{\text{C}}$  50.1) attributed to at position 9 and three peaks at  $\delta_{\text{H}}$  1.21 (9H, *brs*, 3x CH<sub>2</sub>) and  $\delta_{\text{C}}$  26.1, 26.2, 26.3 due to three methylene group indicating the presence of (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>) group attributed at positions 6,7 and 8 linked to carbonyl group.

Thus, on the basis of above observation the structure of isolated chemical component PL-3 has been elucidated as 8-Benzo[1,3]dioxol-5-yl-1-methoxy-octa-5,7-dien-4-one and designated as "Piperone-3".

**PL-13:** 7-Benzo[1,3]dioxol-5-yl-1-methoxy-hepta-4,6-dien-3-one**Figure 2:** Chemical Structure of Piperone-4 (PL-13)

**Table 2:** 1D and 2D NMR spectral data of PL-13

Positions	<sup>1</sup> H-NMR <sup>a</sup>	<sup>13</sup> C-NMR <sup>a</sup>	DEPT-135 <sup>b</sup>	Conclusion	COSY <sup>a</sup>
1	-----	167.6	No peak	C	--
2	6.694-6.667 <i>d</i> (J= 14.8 Hz)	130.5	Positive peak	CH	H-3
3	7.454-7.389 <i>dddd</i> (J= 4.0,14.8Hz)	145.0	Positive peak	CH	H-2, H-4
4	6.923-6.899 <i>dd</i> (J= 1.6,9.6 Hz)	140.3	Positive peak	CH	H-3, H-5
5	6.797-6.777 <i>dd</i> (J= 4.0, 9.6 Hz)	124.5	Positive peak	CH	H-4
6,7	1.28, <i>brs</i> (4H, 2 x CH <sub>2</sub> )	25.1, 25.2	Negative peak	CH <sub>2</sub>	H-6, H-7
8	3.76, <i>s</i> (3H, OMe)	51.4	Positive peak	CH <sub>3</sub>	---
1'	-----	130.5	No peak	C	--
2'	6.997 <i>d</i> (J= 1.6 Hz)	123.0	Positive peak	CH	--
3'	-----	148.58	No peak	C	--
4'	-----	148.29	No peak	C	--
5'	6.79 <i>d</i> (J= 8.0 Hz)	105.86	Positive peak	CH	H-6'
6'	6.82 <i>d</i> (J= 8.0 Hz)	108.5	Positive peak	CH	H-5'
7'	5.986 <i>s</i>	101.4	Negative peak	CH <sub>2</sub>	--

a: Assignment were based on <sup>1</sup>H-NMR, 1H-1H COSY and <sup>13</sup>C-NMR experiments; coupling constant in hertz are given in parenthesis; s:singlet, *d*: doublet, *brs*: broad signal; b: DEPT chemical shift are presented at  $\theta=3\pi/4$  when methylene group reaches negative maximum.

### Compound PL-13

Compound PL-13 was obtained as greenish crystals, gave a single spot on TLC, and exhibited a molecular formula C<sub>15</sub>H<sub>16</sub>O<sub>4</sub> as established on the basis of FAB-MS, showed a molecular ion peak at *m/z* 260.10 [M]<sup>+</sup>, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and DEPT spectra. The IR spectrum showed a sharp peak at  $\nu_{\max}$  1709 cm<sup>-1</sup> indicating the presence of ketonic group, 2922cm<sup>-1</sup> for methyl group, 1505 and 839 cm<sup>-1</sup> for aliphatic double bond, 1265 cm<sup>-1</sup> due to C-O phenolic and 1040 cm<sup>-1</sup> due to C-O aliphatic groups. The <sup>13</sup>C-NMR and DEPT spectra showed fifteen carbon atoms including one carbonyl, three quaternary, three methylene and seven methine carbons and one methyl carbon (in total C<sub>15</sub>H<sub>16</sub>O<sub>4</sub>). The <sup>1</sup>H-NMR spectrum exhibited three aromatic signals at  $\delta_{\text{H}}$  6.77 (1H, *d*, J= 8.0 Hz, H-5'), 6.78 (1H, *d*, J=8.0 Hz, H-6') and 6.98 (1H, *d*, J= 1.6Hz, H-2') attributable to H-5', H-6', and H-2' positions respectively. A two protons singlet displayed at  $\delta_{\text{H}}$  5.97 in <sup>1</sup>H NMR and also exhibited a -CH<sub>2</sub>- carbon signal at  $\delta_{\text{C}}$  101.4 in <sup>13</sup>C-NMR and DEPT spectra, which could be attributed to methylene group attached to oxygen atoms and thus was assigned at position 7'. Four signals of one proton each were exhibited at  $\delta$  6.69 (1H, *d*, J= 14.8 Hz, H-2,  $\delta_{\text{C}}$  130.5), 7.45 (1H, *dddd*, J= 4.0, 14.8 Hz, H-3,  $\delta_{\text{C}}$  145.0), 6.92 (1H, *dd*, J= 1.6, 9.6 Hz, H-4,  $\delta_{\text{C}}$  140.3) and 6.79 (1H, *dd*, 4.0, 9.6 Hz, H-5,  $\delta_{\text{C}}$  124.5), which were attributed to positions 2, 3, 4 and 5 respectively.

The other carbon signals in the aromatic region exhibited signals at  $\delta_{\text{C}}$ 123.0 (C-2'), 108.5 (C-6'), 105.8 (C-5'), due to methine carbon atoms of the aromatic ring. In addition, carbon signals at  $\delta$  130.5 (C-1'), 148.3 (C-4') and 148.5 (C-3') were assigned to quaternary carbon atoms of the aromatic ring. These assignments were also substantiated

further by correlating the proton peaks in COSY experiment and have been presented in Table-2.

The IR spectrum of PL-13 also exhibited peaks at 2922cm<sup>-1</sup> and 1041 cm<sup>-1</sup> due to a methoxyl (OCH<sub>3</sub>) group which was confirmed by three proton signal due to methoxyl group at  $\delta_{\text{H}}$  3.76, *s* (3H, OMe),  $\delta_{\text{C}}$ 51.4 attributed at position 8 and four peaks at  $\delta_{\text{H}}$ 1.28, *brs* (4H, 2 x CH<sub>2</sub>) and  $\delta_{\text{C}}$ 25.1, 25.2 due to two methylene group indicating the presence of (CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>) group attributed at positions 6 and 7 linked to carbonyl group.

Thus, on the basis of above observation the structure of isolated chemical component PL-13 has been elucidated as 7-Benzo [1,3]dioxol-5-yl-1-methoxy-hepta-4,6-dien-3-one and designated as "Piperone-4".

### CONCLUSION

Here both of the isolated chemical components "Piperone-3" and "Piperone-4" isolated from the dried fruits of *Piper longum* are reported first time.

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