Phytochemical investigation afforded a novel Cycloartane triterpenoid from *Piper thomsoni*

Goswami Rajeev* and Jain S.C.
Department of Chemistry, University of Delhi, Delhi-110 007, INDIA

Available online at: www.isca.in, www.isca.me

Received 3rd August 2014, revised 30th August 2014, accepted 10th September 2014

**Abstract**

A Novel alkenyl phenol, 4-(7E-dodeceny1)phenol (2) and triterpenoid cycloart-23-en-3-one (4) and also first time from *Piper* genus 3β-22-dihydroxylanosta-8,24-dien-26-oic-acid-δ-lactone (8) were isolated from the leaves and stems DCM:MeOH (1:1) extracts of *Piper thomsoni*. All isolated compounds structures were defined using modern spectral techniques.

**Keywords:** *Piper thomsoni; Piperaceae; cycloart-23-en-3-one; 4-(7E-dodeceny1)phenol; anticancer.*

**Introduction**

Since ancient time, plants have been the source of medicines and the plants belong to the genus *Piper* are known for their medicinal importance. Piper species are geographically disseminated in the tropical and subtropical climatic zones and are explored as folk medicines in several ways. *P. nigrum* ripened fruit is used as white pepper, whereas as black pepper is sourced from its unripe fruit. Isobutyl amides isolated from *P. nigrum* fruits showed larvicidal effect on *Aedes aegypti*, *A. togoi* and *Culex pipiens pallens* larvae. *P. amalago* is used as anti-inflammatory agent for alleviating chest pain. *P. aborescens* stems chloroform extract exhibited good activity against P-388 lymphocytic leukemia cells and KB cell3. *P. cubeba* has long been a source of folk and herbal medicine6. The *P. longum* fruit has also been source of Indigenous medicines, which includes *Indian Ayurvedic* medicine, to treat bronchitis, diarrhea, malaria, viral hepatitis and tumors. An amide, separated from the fruit of *P. longum* L, displayed inhibitory activity against the fourth-instar larvae of *Aedes aegypti*.

Alkaloids isolated from CH3OH extract of *P. lolot* exhibited good inhibition of thrombocyte clustering caused by polynsaturated ω-6 fatty acid (arachidonic acid) and PAF-acether. Rats treated with piper methystine, abundant in *Piper* methysticum, showed increased cytosolic superoxide dismutase, cachectin mRNA expression, hepatic glutathione, CYP 1A2 and 2E1 which suggested adaptive feature to induce oxidative stress and likely drug-drug interactions.

*Piper thomsoni* is one of the forty-five species of the family *Piperaceae* and is used as traditional and folk medicines. It is well documented in the *Indian Ayurvedic* system of medicine. *P. thomsoni* leaves are the source of Pan, which also applied to wounds and swellings; whereas its aqueous root extracts is used as diuretic. Previously *P. thomsoni* phytochemical explorations has resulted in the isolation and characterization of several alkaloids and terpenoids. Here, we describe the isolation and characterization of bioactive secondary metabolites from the leaves and stems combined extract of this species, which afforded two new compounds an alkenyl phenol and one cycloartane terpenoid, additionally seven earlier reported compounds (figure-1). However, out of seven known compounds, lanostane triterpenoid (8) is first time reported from the *Piper* genus. Herein, extraction, compounds isolation and their detailed characterization are discussed.

**Material and Methods**

Solvents used for column chromatography (CC) were procured from Merck and used after distilling them. 60-120 mesh silica-gel was used for CC from Merck. Melting points were checked using Fischer Johns equipment and are uncorrected. The 1H, 13C NMR and 2D NMR spectra were recorded with tetramethylsilane as an internal standard on a Bruker-300 Spectrometer in deuterated solvents as required. Perkin-Elmer Infra-red Spectrometer was used to record IR spectra either as KBr pellets or film. The ESI mass spectra were determined using Jeol Spectrophotometer and elemental analysis was done on GmbH Vario EL V3.00 elemental analyzer.

**Plant Material:** *Piper thomsoni* leaves and stems (650g) were collated from the Botanical Survey of India, Shillong forests and identified by Dr. B.M. Wadhwa.

**Extraction and isolation:** Leaves and stems were dried and their powdered mixture (500 g) was extracted with cold CH3Cl2:MeOH with a Soxhlet apparatus. Solvent was evaporated from the extract under vacuum and thus obtained residue (8.0 g) was purified by CC using silica-gel. Elutions were made exploiting a linear gradient of hexane-ETOAc-MeOH and totally 101 fractions (400 ml each) were eluted and as per their TLC pattern combined to make 14 fractions (F1-F14). F1 was composed of waxy material. Compound 2 (15 mg; colourless oil) and 4 (25 mg; white amorphous powder) were obtained from fractions F2 and F4, respectively. F8 gave
compound 8 as a white amorphous powder (15 mg). Additionally, six other known compounds viz., dotriacontanol (1), octadec-10E-en-10-oic acid (3), octacosanoic acid (5), stigmasta-6-en-3β-ol (6), (2E,4E)-5-(benzod[1,3]dioxol-5-yl)-1-(piperidin-1-yl)penta-2,4-dien-1-one (7) and 7-methyl-5H-[1,3]dioxolo [4,5;4;5-b] benzo [g] quinoline-5, 6(7H)-dione (9) were also isolated from other fractions.

(E)-4-(dodec-7-en-1-yl)phenol (2): Colourless oil; [α]D -24.1 (c 0.40, CHCl3); UV(MeOH) λmax: 274 nm; IR νmax (KBr) cm⁻¹: 2927, 1712, 1451, 1377, 1113, 757; δH 7.29 (6H, 3x-CH₃), 5.29 (brs, 1H, -OH), 5.42 (m, 2H, H-7' and H-8'); 13C NMR (CDCl3): δ ppm 14.6 (C-17), 28.6, 27.0 (M⁺-HCl), 242 (M⁺-H₂O), 232, 203 (M⁺-H₂), 177 (M⁺-C₂H₁₁), 167, 149, 135, 120, 106, 95, 85, 83, 74, 57, 43.

Cycloart-23-en-3-one (4): White amorphous powder; m.p. 118-120°C; [α]D + 38.1 (c 0.41, CHCl3); Elemental analysis: C 84.83%, H 11.52%, O 3.65% (required C 84.87%, H 11.49%, O 3.65%); IR νmax (KBr) cm⁻¹: 2927, 1712, 1451, 1377, 1113, 757; 1H and 13C NMR (CDCl3): detected via HMBC correlations, see table 1; EIMS (m/z): 438(M⁺), 423 (M⁺-CH₃), 410 (M⁺-CO), 395 (M⁺-CH₂-CO), 381, 329, 313 (M⁺-C₈H₁₇), 298 (M⁺-C₉H₁₇-CH₃), 286, 270 (M⁺-C₆H₁₃-CH₂-CO), 243, 255, 231, 213, 138, 121, 107, 95, 81, 43.

Results and Discussion

The leaves and stems DCM: MeOH (1:1) extract of P. thomsoni was subjected to column chromatography on silica-gel and eluted with different polarity solvent combinations, yielded two new compounds, which include an alkenyl phenol (2) and a cycloartane terpenoid (4), and seven known compounds (1, 3, 5-9).

![Figure-1](https://example.com/f1.png)

Structures of compounds 1–9
Vol. __________, Issue __________, __________ __________

Table 1

<table>
<thead>
<tr>
<th>C</th>
<th>C</th>
<th>HMBCC</th>
<th>1H</th>
<th>C</th>
<th>HMBCC</th>
<th>1H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.2</td>
<td>2, 5, 19</td>
<td>1.52 (m), 1.81 (m)</td>
<td>34.4</td>
<td>2, 3, 5, 19</td>
<td>1.71 (m), 1.26 (m)</td>
</tr>
<tr>
<td>2</td>
<td>37.5</td>
<td>1</td>
<td>2.68 (dt, J = 14.0, 6.2 Hz), 2.35 (ddd, J = 13.9, 4.3, 2.3 Hz)</td>
<td>30.7</td>
<td>1, 3</td>
<td>1.50 (m), 1.68 (m)</td>
</tr>
<tr>
<td>3</td>
<td>216.5</td>
<td>-</td>
<td>-</td>
<td>79.3</td>
<td>1, 2, 5, 29, 30</td>
<td>3.23 (dd, J = 11.3, 4.5 Hz)</td>
</tr>
<tr>
<td>4</td>
<td>50.6</td>
<td>-</td>
<td>-</td>
<td>37.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>49.1</td>
<td>1, 6, 7, 19</td>
<td>1.66 (m)</td>
<td>49.2</td>
<td>1, 6, 7, 19, 29, 30</td>
<td>1.64 (m)</td>
</tr>
<tr>
<td>6</td>
<td>22.3</td>
<td>5, 7, 8</td>
<td>1.01 (m)</td>
<td>18.0</td>
<td>5, 7</td>
<td>1.97 (m), 1.32 (m)</td>
</tr>
<tr>
<td>7</td>
<td>22.5</td>
<td>5, 6, 8</td>
<td>1.25 (m)</td>
<td>25.3</td>
<td>5, 6</td>
<td>2.15 (m), 1.35 (m)</td>
</tr>
<tr>
<td>8</td>
<td>43.1</td>
<td>6, 7, 11, 15, 19, 31, 1.58 (m)</td>
<td>134.9</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>36.7</td>
<td>-</td>
<td>-</td>
<td>134.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>36.0</td>
<td>-</td>
<td>-</td>
<td>35.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>34.4</td>
<td>8, 12, 19</td>
<td>1.39 (m)</td>
<td>19.9</td>
<td>12</td>
<td>2.26 (m), 1.53 (m)</td>
</tr>
<tr>
<td>12</td>
<td>27.1</td>
<td>11, 17, 18</td>
<td>1.34 (m)</td>
<td>29.7</td>
<td>11, 18</td>
<td>1.47 (m)</td>
</tr>
<tr>
<td>13</td>
<td>45.7</td>
<td>-</td>
<td>-</td>
<td>43.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>46.9</td>
<td>-</td>
<td>-</td>
<td>48.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>33.8</td>
<td>16, 17, 31</td>
<td>1.61 (m)</td>
<td>29.8</td>
<td>16, 17</td>
<td>1.58 (m)</td>
</tr>
<tr>
<td>16</td>
<td>34.2</td>
<td>15, 17, 20</td>
<td>2.02 (m)</td>
<td>26.6</td>
<td>15, 17, 20</td>
<td>2.04 (m)</td>
</tr>
<tr>
<td>17</td>
<td>50.6</td>
<td>15, 16, 18, 20, 21, 22</td>
<td>1.34 (m)</td>
<td>44.7</td>
<td>1215, 16, 18, 20, 21, 22</td>
<td>1.66 (m)</td>
</tr>
<tr>
<td>18</td>
<td>18.4</td>
<td>12, 17</td>
<td>0.85 (s)</td>
<td>14.3</td>
<td>12, 17</td>
<td>0.70 (s)</td>
</tr>
<tr>
<td>19</td>
<td>29.9</td>
<td>1, 5, 11</td>
<td>0.78 (d, J = 4.2 Hz), 0.58 (d, J = 4.2 Hz)</td>
<td>17.1</td>
<td>1, 5</td>
<td>0.91 (s)</td>
</tr>
<tr>
<td>20</td>
<td>48.2</td>
<td>16, 17, 21, 22</td>
<td>1.46 (m)</td>
<td>39.3</td>
<td>16, 17, 21, 22, 23</td>
<td>1.88 (m)</td>
</tr>
<tr>
<td>21</td>
<td>18.6</td>
<td>17, 20, 22</td>
<td>0.93 (d, J = 6.7 Hz)</td>
<td>12.2</td>
<td>17, 20, 22</td>
<td>1.04 (d, J = 6.6 Hz)</td>
</tr>
<tr>
<td>22</td>
<td>31.6</td>
<td>17, 20, 21, 25</td>
<td>2.20 (m)</td>
<td>80.7</td>
<td>17, 20, 21, 24</td>
<td>4.47 (m)</td>
</tr>
<tr>
<td>23</td>
<td>157.2</td>
<td>-</td>
<td>-</td>
<td>28.6</td>
<td>20, 22, 24</td>
<td>2.02 (m), 2.56 (m)</td>
</tr>
<tr>
<td>24</td>
<td>106.3</td>
<td>22, 25</td>
<td>4.67 (d, J = 15.4 Hz)</td>
<td>139.9</td>
<td>22, 23, 27</td>
<td>6.59 (dd, J = 6.4, 3.2 Hz)</td>
</tr>
<tr>
<td>25</td>
<td>30.1</td>
<td>22, 24, 26, 27, 28</td>
<td>1.95 (m)</td>
<td>128.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>37.8</td>
<td>25, 27, 28</td>
<td>1.42 (m)</td>
<td>166.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>27</td>
<td>26.2</td>
<td>25, 26, 28</td>
<td>1.10 (d, J = 5.8 Hz)</td>
<td>16.0</td>
<td>24</td>
<td>1.91 (s)</td>
</tr>
<tr>
<td>28</td>
<td>26.2</td>
<td>25, 26, 27</td>
<td>1.10 (d, J = 5.8 Hz)</td>
<td>14.5</td>
<td>15</td>
<td>0.98 (s)</td>
</tr>
<tr>
<td>29</td>
<td>21.1</td>
<td>5, 30</td>
<td>0.98 (s)</td>
<td>23.3</td>
<td>3, 5, 30</td>
<td>0.81 (s)</td>
</tr>
<tr>
<td>30</td>
<td>22.2</td>
<td>5, 29</td>
<td>1.04 (s)</td>
<td>26.8</td>
<td>3, 5, 29</td>
<td>1.00 (s)</td>
</tr>
<tr>
<td>31</td>
<td>19.6</td>
<td>8, 15</td>
<td>0.95 (s)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Compound 2**, obtained as oil, gave a molecular ion peak [M+] at m/z 260 in its EIMS and coupling this information with C and H count in its NMR suggested it to have molecular formula C<sub>18</sub>H<sub>28</sub>O. In its 13C NMR spectrum, the appearance of resonances for eight sp<sup>2</sup> hybridized carbons suggested two aromatic rings and one carbon-carbon double bond. Only twenty-seven hydrogens could be characterized through the 1H and DEPT spectra, which indicated that left out one hydrogen could be in the form of a hydroxyl group. The initial assignments of two partial skeletons were done on the bases of 1H NMR and 1H-1H COSY spectral data. Two aromatic resonances at δ 6.74 (d, 2H, J = 8.5 Hz) and δ 7.03 (d, 2H, J = 8.5 Hz) suggested that a distinctive 1,4-substitution pattern is present in the aromatic ring. The one substituent in the aromatic ring was in the form of a phenolic hydroxyl group as was indicated by its 1H NMR which displayed an exchangeable signal with D<sub>2</sub>O at δ 5.1 and also supported by its 13C NMR showed a peak at δ 153.5, characteristic for a carbon bearing hydroxyl group in the benzene ring. 1H NMR when coupled with its mass spectrum indicated that second substituent to be an alkenyl chain attached to the 4<sup>th</sup> position of the aromatic ring. The two proton multiplet at δ 5.42 suggested a disubstituted trans double bond in the side chain which was confirmed by its 13C NMR spectrum in which sp<sup>2</sup> carbons appeared at δ 131.3 and 131.4 and also by its IR spectrum as it exhibited a band at 930 cm<sup>-1</sup>. Double bond position in the chain was assigned by mass fragmentation, in which two allylic cleavages were observed as a result of a peak at m/z 203 after the loss of C<sub>6</sub>H<sub>6</sub> group and at m/z 177 after the...
loss of C₆H₁₁. This suggested that double bond placed at C-7 in the alkenyl chain. The peak at m/z 167 confirmed twelve carbon atoms in the side chain. Hence compound 2 was characterized as (E)-4-(dodec-7-en-1-yl) phenol.

Compound 4, obtained as a white amorphous solid, was assigned the chemical formula using elemental analysis as C₃₈H₆₀O₂, which was supported by its molecular ion peak m/z M⁺438 in its EIMS. It passes Liebermann Burchard test and yellow colour with tetranitromethane, characteristic for a tetracyclic triterpenoid. Its ¹H NMR showed two doublets at δ 0.58 (J = 4.2 Hz) and δ 0.78 (J = 4.2 Hz) and characterized for C-9& C-10 cyclopropyl methylene group of a cyclooct-3-one triterpenoid. The HMBC correlations confirmed the presence of a carbonyl group, which was suggested the presence of a carbonyl group, which was further confirmed by its ¹³C NMR spectrum which displayed a peak at δ 198.5. The HMBC correlation of –C=O with carbons at δ 33.2 (–CH₂) and 36.0 (–CH₃) and the carbon at δ 37.5 (CH₂) was also attached with the carbons at δ 33.2 (–CH₂) and 37.5 (CH₂).

Absorption band in its IR spectrum displayed at 1710 cm⁻¹ suggested the presence of a carbonyl group, which was confirmed by its ¹³C NMR spectrum which displayed a peak at δ 198.5. The HMBC correlation of –C=O with carbons at δ 33.2 (–CH₂), 37.5 (–CH₃), 50.6 (q) and 49.1 (>CH-) confirmed the presence of carbonyl group at C-3, which was also akin to the basic unit of cyclooct-3-one.

The mass fragmentation displayed a prominent peak at m/z 313 (M+–C₆H₁₁) due to the loss of 125 amu thereby showing one site of unsaturation in the side chain. Further its ¹³C NMR and DEPT spectrum showed two sp³ carbons at δ 157.2 (q) and 106.3 (-CH₃) and indicated the presence of an exocyclic methylene group which was confirmed by its ¹H NMR spectrum as a characteristic doublet appeared at δ 4.67 (J = 15.4 Hz). This exocyclic double bond was fixed at C-23 because this was the only best suited position available in the side chain which was also supported by its McLafferty-type fragmentation (figure-2), in the absence of other olefinic proton and the vinylidin methyl in the molecule.

The ¹H NMR spectral pattern as well as the mass fragmentation of 4 was compared to those reported for cyclooct-25-en-3-one isolated from Polypodium formosanum fern. The HMBC spectrum correlations (figure-2) were deduced to cyclooct-23-en-3-one, the constitution assigned for compound 4. HMBC spectra finally assisted in the assignment of the protonated carbons (table-1). On the basis of spectral discussion, compound 4 was defined as (2aR, 3R, 5aS, 5bS, 7aR, 11aR, 12aS)-2a, 5a, 8, 8-tetramethyl-3-[(R)-6-methyl-4-methyleneheptan-2-yl]tetradecahydro-9H,12H-cyclopenta [α] cyclopropan [e] phenanthren-9-one (which shortly termed as cyclooct-23-en-3-one).

**Figure-2**

HMBC (H→C) correlation of compounds 4 and 8

Compound 8 was purified as a white amorphous solid and its chemical formula was confirmed as C₃₀H₄₀O₂ by its elemental analysis and EIMS. Its DEPT and ¹H,¹³C COSY spectrum showed signals for seven methyl, nine methylene and five methine groups. The ¹H NMR spectrum displayed among other resonances, the two low field protons attached to carbons bearing oxygen at δ 3.23 (dd, J = 5.1 Hz and 11.3 Hz) and at δ 4.47 (m). The presence of 3β-hydroxy was confirmed by a peak at δ 79.3 for C-3 in ¹³C NMR and also by a characteristic doublet at δ 3.23 for 3α-hydrogen. The IR absorption at 1707 cm⁻¹ and UV absorption at 252 nm indicated the presence of a six membered α, β-unsaturated δ-lactone ring which was supported by its ¹³C NMR spectrum which displayed characteristic resonances at δ 167.13, 140.29, 127.96 and 80.51. The HMBC correlation (figure 2 and table-1) with respect to –CH₃ group at δ 1.91 (s) showed correlation at δ 140.29 (q), 167.13 (q) and 127.96 (s) carbons suggesting it attached to lactone ring at C-25 position. HMBC correlation suggested that –CH=O group at δ 4.47 (m) correlations with δ 167.13 (q), 127.96 (s), 39.3 (>CH-), 28.6 (>CH₂), 12.2 (>CH₃) carbons clearly suggested that it is 22 carbon from lactone ring and confirmed the presence of six-membered α, β-unsaturated δ-lactone ring and is attached at δ 39.3 (C-20) carbon with lanostane unit. The ¹³C NMR spectra also revealed tetra substituted olefinic carbons at δ 134.1 and 134.4 positioned between C-8 and C-9. By comparison of these spectral features with steroids containing α, β-unsaturated δ-lactone indicated that 8 has lanostane skeleton which was further confirmed by the HMBC spectrum (figure-2) and its Mass fragmentation pattern. Also δ-lactone carbon resonances of 8 was identified with those reported for steroids containing δ-lactone. Based on that we have assigned the stereochemistry as 22S. On the basis of above spectral analysis and also comparing with reported data.
compound 8 was assigned as 3β-22-dihydroxylanosta-8,24-dien-26-oicacid-δ-lactone.

Conclusion

We have performed a phytochemical exploration of Piper thomsoni and isolated two novel secondary metabolites of (E)-4-(dodec-7-en-1-yl) phenol (2) and cycloart-23-en-3-one (4). However, 3β-22-dihydroxylanosta-8, 24-dien-26-oicacid-δ-lactone (8) was first time reported from the Piper genus.

Acknowledgement

We thank Danish International Development Agency (DANIDA), Denmark for the financial assistance.

References

1. Kirtikar K.R. and Basu B.D., Indian Medicinal Plants, III, 2128 (1933)
4. Dominguez X.A. and Alcorn J.B., Screening of medicinal plants used by Huastec Mayans of Northeastern Mexico, J Ethnopharmacol., 13, 139-156 (1985)