Phytochemical investigation Afforded a novel Cycloartanetriterpenoid 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-dodecenyl)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 defining used modern spectral techniques.

Keywords: Piper thomsoni; Piperaceae; cycloart-23-en-3-one; 4-(7E-dodecenyl)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\(^1\). 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, where as black pepper is sourced from its unripe fruit. Isobutyl amides isolated from P. nigrum fruits showed larvicidal effecton Aedes aegypti, A. togoi and Culex pipiens pallens larvae\(^3\). P. amalagois used as anti-inflammatory agent for alleviating chest pain\(^4\). P. aboescens stems chloroform extract exhibited good activity against P-388 lymphocytic leukemia cells and KB cell\(^5\). P. cubeba has long been a source of folk and herbal medicine\(^6\). 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\(^7\). An amide, separated from the fruit of P. longum L. displayed inhibitory activity against the fourth-instar larvae of Aedes aegypti\(^8\).

Alkaloids isolated from CH\(_2\)OH extract of P. lolot exhibited good inhibition of thrombocyte clustering caused by polyunsaturated ω-6 fatty acid (arachidonic acid) and PAF-acether\(^7\). Rats treated with pipermethystine, 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\(^9\).

Piper thomsoni is one of the forty-five species of the family Piperaceae and is being used as traditional and folk medicines\(^2\). 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\(^11\). 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 cycloartanetriterpenoid, additionally seven earlier reported compounds (figure-1). However, out of seven known compounds, lanostanetriterpenoid (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 Fisher Johns equipment and are uncorrected. The \(^1\)H, \(^13\)C NMR and 2D NMR spectra were recorded with white trime thylsilane 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 collateral 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 CH\(_2\)Cl\(_2\):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-10z-en-10oic acid (3), octacosanoic acid (5), stigmaster-6-en-3β-ol (6), (2E,4E)-5-(benzo[d][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] benzo [1,2,3-de] 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; \([\alpha]_D-24.1\) (c 0.40, CHCl\(_3\)); UV(MeOH) \(\lambda_{max}\): 274 nm; IR \(\nu_{max}\) (film) cm\(^{-1}\): 3550, 2930, 2862, 1709, 1457, 1373, 1261, 1141, 1091, 855, 803; Elemental analysis: C 84.87%, H 11.49%, O 3.65% (required C 84.87%, H 11.49%, O 3.65%); IR \(\nu_{max}\) (KBr) cm\(^{-1}\): 3531, 2930, 2862, 1709, 1457, 1373, 1261, 1141, 1091, 855, 803; Elemental analysis: C 79.22%, H 10.23%, O 10.55% (required C 79.25%, H 10.20%, O 10.56%); \(^1\)H and \(^13\)C NMR (CDCl\(_3\)): detected via HMBC correlations, see table 1; EIMS (m/z): 454 ([M]+), 423 (M\(^+\)-CH\(_3\)), 410 (M\(^+\)-CO), 395 (M\(^+\)-CH\(_2\)-CO), 381, 329, 313 (M\(^+\)-C\(_9\)H\(_{17}\)-CH\(_3\)), 286, 270 (M\(^+\)-C\(_9\)H\(_{17}\)-CH\(_3\)-CO), 243, 255, 231, 213, 138, 121, 107, 95, 81, 43.

\(\beta\)-22-Dihydroxylanosta-8,24-diene-26-oic acid-\(\delta\)-lactone (8): White amorphous powder; m.p. 286-288°C; \([\alpha]_D + 27.3\) (c 0.32, CHCl\(_3\)); UV (MeOH) \(\lambda_{max}\): 203, 252 nm; IR \(\nu_{max}\) (KBr) cm\(^{-1}\): 3531, 2930, 2862, 1709, 1457, 1373, 1261, 1141, 1091, 855, 803; Elemental analysis: C 84.87%, H 11.49%, O 3.65% (required C 84.87%, H 11.49%, O 3.65%); IR \(\nu_{max}\) (KBr) cm\(^{-1}\): 3531, 2930, 2862, 1709, 1457, 1373, 1261, 1141, 1091, 855, 803; Elemental analysis: C 79.22%, H 10.23%, O 10.55% (required C 79.25%, H 10.20%, O 10.56%); \(^1\)H and \(^13\)C NMR (CDCl\(_3\)): detected via HMBC correlations, see table 1; EIMS (m/z): 454 ([M]+), 439 (M\(^+\)-CH\(_3\)), 421 (M\(^+\)-CO), 381, 329, 313 (M\(^+\)-C\(_9\)H\(_{17}\)-CH\(_3\)), 286, 270 (M\(^+\)-C\(_9\)H\(_{17}\)-CH\(_3\)-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 acyloartaneterpenoid (4), and seven known compounds (1,3,5–9).

Cycloartan-23-en-3-one (4): White amorphous powder; m.p. 118-120°C; \([\alpha]_D + 38.1\) (c 0.41, CHCl\(_3\)); Elemental analysis: C 84.83%, H 11.52%, O 3.65% (required C 84.87%, H 11.49%, O 3.65%); IR \(\nu_{max}\) (KBr) cm\(^{-1}\): 2927, 1712, 1451, 1377, 1113, 757; \(^1\)H and \(^13\)C NMR (CDCl\(_3\)): detected via HMBC correlations, see table 1; EIMS (m/z): 438(M\(^+\)), 423 (M\(^+\)-CH\(_3\)), 410 (M\(^+\)-CO), 395 (M\(^+\)-CH\(_2\)-CO), 381, 329, 313 (M\(^+\)-C\(_9\)H\(_{17}\)-CH\(_3\)), 286, 270 (M\(^+\)-C\(_9\)H\(_{17}\)-CH\(_3\)-CO), 243, 255, 231, 213, 138, 121, 107, 95, 81, 43.

Figure 1

Structures of compounds 1–9
Vol. 4(9), 7-11, September (2014)  

Table-1

<table>
<thead>
<tr>
<th>Compound</th>
<th>1H and 13C NMR and HMBC spectral data for compounds 4 and 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>HMBC</td>
</tr>
<tr>
<td>4</td>
<td>2, 5, 19</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1, 6, 7, 19</td>
</tr>
<tr>
<td>7</td>
<td>5, 7, 8</td>
</tr>
<tr>
<td>8</td>
<td>5, 6, 8</td>
</tr>
<tr>
<td>9</td>
<td>6, 7, 11, 15, 19, 31</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>8, 12, 19</td>
</tr>
<tr>
<td>12</td>
<td>11, 17, 18</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>16, 17, 31</td>
</tr>
<tr>
<td>16</td>
<td>15, 17, 20</td>
</tr>
<tr>
<td>17</td>
<td>15, 16, 18, 20, 21, 22</td>
</tr>
<tr>
<td>18</td>
<td>12, 17</td>
</tr>
<tr>
<td>19</td>
<td>1.5, 11</td>
</tr>
<tr>
<td>20</td>
<td>16, 17, 21, 22</td>
</tr>
<tr>
<td>21</td>
<td>17, 20, 22</td>
</tr>
<tr>
<td>22</td>
<td>17, 20, 21, 25</td>
</tr>
<tr>
<td>23</td>
<td>22, 25</td>
</tr>
<tr>
<td>24</td>
<td>22, 25</td>
</tr>
<tr>
<td>25</td>
<td>22, 24, 26, 27, 28</td>
</tr>
<tr>
<td>26</td>
<td>25, 27, 28</td>
</tr>
<tr>
<td>27</td>
<td>25, 26, 28</td>
</tr>
<tr>
<td>28</td>
<td>25, 26, 27</td>
</tr>
<tr>
<td>29</td>
<td>5, 30</td>
</tr>
<tr>
<td>30</td>
<td>5, 29</td>
</tr>
<tr>
<td>31</td>
<td>8, 15</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_{18}H_{23}O. In its 1^3C NMR spectrum, the appearance of resonances for eight sp^3 hybridized carbons suggested one aromatic ring 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_{2}O at δ 5.1 and also supported by its 1^3C 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^th position of the aromatic ring. The two proton multiplet at δ 5.42 suggested a disubstitutedtrans double bond in the side chain which was confirmed by its 1^3C NMR spectrum in which sp^3 carbons appeared at δ 131.3 and 131.4 and also by its IR spectrum as it exhibited a band at 930 cm^{-1}. 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_{4}H_{6} 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 
C₃₁H₆₀O₄, 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.

Compound 4 was purified as a white amorphous solid and its 
IR absorption at 1710 cm⁻¹ suggested the presence of a carbonyl group, which was 
confirmed by its ¹H NMR spectrum displayed a prominent peak at δ 
216.5. The HMBC correlation of –C=O group with carbons at δ 
33.2 (-CH₃), 37.5 (-CH₂), 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 cycloart-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 sp² carbons at δ 157.2 (q) and 
106.3 (-CH₂) andindicated the presence of an exocyclic 
methine 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 vinylic methyl in 
the molecule.

The ¹H NMR spectral pattern as well as the mass fragmentation of 
4 was compared to those reported for cycloart-25-en-3-one 
isolated from Polypodium formosanumأتثن. The HMBC 
spectrum correlations (figure-2) were deduced to cycloart-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-

\[
\text{H} \quad \text{HMBC (H→C) correlation of compounds 4 and 8}
\]

Compound 8 was purified as a white amorphous solid and its 
chemical formula was confirmed as C₃₀H₄₆O₇ 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 double 
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 
nine 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 9.91 (s) showed correlation at δ 140.29 (q), 
167.13 (q) and 127.96 (>CH-) 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 (>C=O), 
127.96 (=CH-), 39.3 (>CH-), 28.6 (>CH₂), 12.2 (>CH₂) carbons 
strongly suggested that it is C-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 
tetrasubstituted 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.

\[
\text{Figure-2}
\]

McLafferty-type rearrangement of the side chain of 
compound 4

\[
\text{Figure-2}
\]

HMBC (H→C) correlation of compounds 4 and 8
compound 8 was assigned as 3β-22-dihydroxylanosta-8,24-dien-26-oic acid-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-oic acid-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)