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

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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-oicacid-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-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[1,2]. 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. amalago is used as anti-inflammatory agent for alleviating chest pain[4]. P. aborescens 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_3OH extract of P. lolot exhibited good inhibition of thrombocyte clustering caused by polyunsaturated ω-6 fatty acid (arachidonic acid) and PAF-acether[9]. 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[10].

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[11,12]. 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 ^{1}H, ^{13}C 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 CH_2Cl_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-enoic acid (3), octacosanoic acid (5), stigmast-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) λ_{max}: 274 nm; IR ν_{max} (film) cm^{-1}: 3550, 2919, 2850, 2359, 1638, 1462, 1377, 1265, 1028, 930, 708; ^1H NMR ($CDCl_3$): δ ppm 0.88 (t, 3H, J = 6.2 Hz, H-12′), 1.28 (brs, 6H, 3x-CH_2-), 1.63 (m, 6H, H-2′, H-5′& H-10′), 1.98 (m, 4H, H-6′ and H-9′), 2.53 (t, 2H, J = 7.6 Hz, H-1′), 5.1 (brs, 1H, -OH), 5.42 (m, 2H, H-7′ and H-8′), 6.74 (d, 2H, J = 8.5 Hz, H-2 and H-6), 7.03 (d, 2H, J = 8.5 Hz, H-3 and H-5); ^{13}C NMR ($CDCl_3$): δ ppm 14.6 (C-12′), 22.5-31.5 (C-2′ to C-5′, C-10′ and C-11′), 34.6 (C-6′ and C-9′), 34.9 (C-1′), 130.1 (C-2 and C-6), 131.3 and 131.4 (C-7′ and C-8′), 132.9 (C-3 and C-5), 138.1 (C-4), 153.5 (C-1); EIMS (*m/z*): (M^+, 260), 242 (M^+-H_2O), 232, 203 (M^+-C_4H_9), 177 (M^+-C_6H_{11}), 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; $[\alpha]_D$ + 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 ν_{max} (KBr) cm^{-1}: 2927, 1712, 1451, 1377, 1113, 757; ^1H 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_3-CO), 381, 329, 313 (M^+-C_9H_{17}), 298 (M^+-C_9H_{17}-CH_3), 286, 270 (M^+-C_9H_{17}-CH_3-CO), 243, 255, 231, 213, 138, 121, 107, 95, 81, 43.

3β-22-Dihydroxylanosta-8,24-diene-26-oicacid-δ-lactone (8): White amorphous powder; m.p. 286-288°C; $[\alpha]_D$ + 27.3 (c 0.32, $CHCl_3$); UV (MeOH) λ_{max}: 203, 252 nm; IR ν_{max} (KBr) cm^{-1}: 3531, 2930, 2862, 1709, 1457, 1373, 1261, 1141, 1091, 1036, 855, 803; Elemental analysis: C 79.22%, H 10.23%, O 10.55% (required C 79.25%, H 10.20%, O 10.56%); ^1H and ^{13}C NMR ($CDCl_3$): detected via HMBC correlations, see table 1; EIMS (m/z): 454 $[M]^+$, 439 (M^+-CH_3), 421 (M^+-CH_3-H_2O), 315, 311, 299, 281, 253, 241, 227, 215, 201, 139 (cleavage of C_{17}-C_{20} bond), 127, 111 (139-CO), 95, 74.

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**).

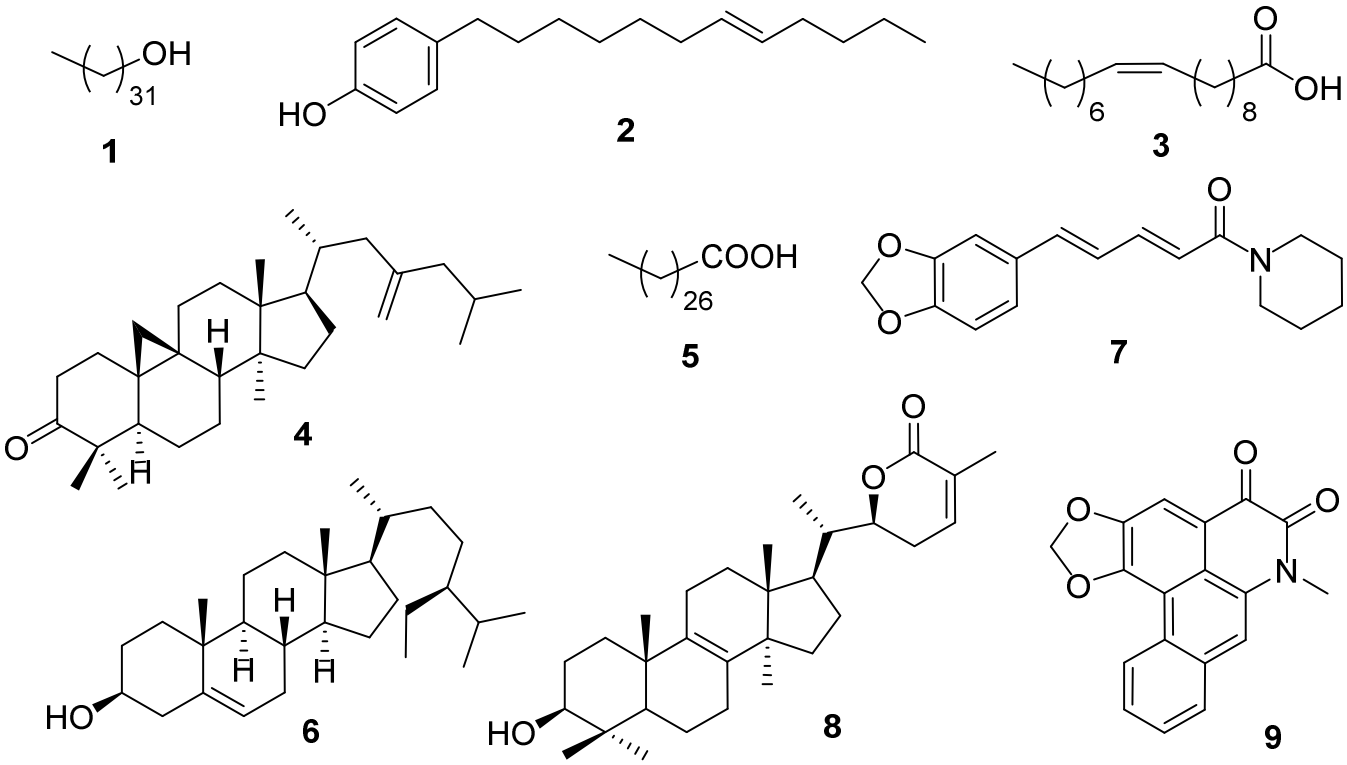

Figure-1
Structures of compounds 1–9

Table-1
^{1}H and ^{13}C NMR and HMBC spectral data for compounds 4 and 8

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

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 $C_{18}H_{28}O$. In its ^{13}C NMR spectrum,the appearance of resonances for eight sp^2 hybridized carbons suggested one aromatic ring and one carbon-carbon double bond. Only twenty-seven hydrogens could be characterized through the ^{1}H 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 ^{1}H NMR and ^{1}H-^{1}H 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 ^{1}H NMR which displayed an exchangeable signal with D_2O at δ 5.1 and also supported by its ^{13}C NMR showed a peak at δ 153.5, characteristic for a carbon bearing hydroxyl group in the benzene ring. ^{1}H NMR when coupled with its mass spectrum indicated that second substituent to be an alkenyl chain attached to the 4th 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 ^{13}C NMR spectrum in which sp^2 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_4H_9 group and at m/z 177 after the

loss of C_6H_{11}. 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 $C_{31}H_{50}O$, 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 ^{1}H NMR showed two doublets at δ 0.58 (J = 4.2 Hz) and δ0.78 (J = 4.2 Hz) and characterized for C-9& C-10 cyclopropyl methylene group of a cycloart-3-one triterpenoid[13]. The HMBC correlation studies confirmed that the protons at δ0.58 and 0.78 were cyclopropyl methylene protons and attached to carbons at δ36.7 (*q*) and 36.0 (*q*) and the carbon at δ 36.7 was also attached with the carbons at δ 33.2 (-CH_2) and 37.5 (CH_2).

Absorption band in its IR spectrum displayed at 1710 cm^{-1} suggested the presence of a carbonyl group, which was confirmed by its ^{13}C NMR spectrum which displayed a peak at δ 216.5. The HMBC correlation of –C=O group with carbons at δ 33.2 (-CH_2), 37.5 (-CH_2), 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[13,14].

The mass fragmentation displayed a prominent peak at *m/z* 313 (M+-C_9H_{17}) due to the loss of 125 amu thereby showing one site of unsaturation in the side chain. Further its ^{13}C NMR and DEPT spectrum showed two sp^2 carbons at δ 157.2 (q) and 106.3 (-CH_2) and indicated the presence of an exocyclic methylene group which was confirmed by its ^{1}H NMR spectrum as a characteristic doublet appeared at δ4.67 (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*[15](figure-2), in the absence of other olefinic proton and the vinylic methyl in the molecule.

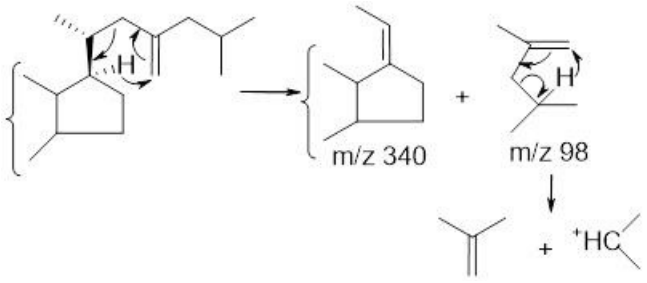

Figure-2
McLafferty-type rearrangement of the side chain of compound 4

The ^{1}H 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 formosanum* fern[16]. 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 (2a*R*, 3*R*, 5a*S*, 5b*S*, 7a*R*, 11a*R*, 12a*S*)-2a, 5a, 8, 8-tetramethyl-3- ((*R*)-6-methyl-4-methyleneheptan-2-yl)tetradecahydro-9*H*,12*H*-cyclopenta [*a*] cyclopropa [*e*] phenanthren-9-one (which shortly termed as cycloart-23-en-3-one).

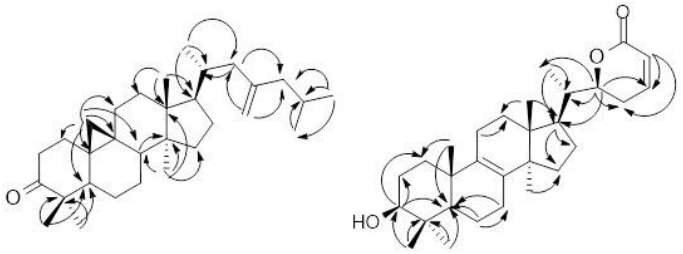

Figure-2
HMBC (H→C) correlation of compounds 4 and 8

Compound **8** was purified as a white amorphous solid and its chemical formula was confirmed as $C_{30}H_{46}O_3$ by its elemental analysis and EIMS. Its DEPT and ^{1}H-^{13}C COSY spectrum showed signals for seven methyl, nine methylene and five methine groups. The ^{1}H NMR spectrum displayed among other resonances, the two low field protons attached to carbons bearing oxygen at δ3.23 (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 δ79.3 for C-3 in ^{13}C NMR and also by a characteristic double doublet at δ3.23 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 ^{13}C NMR spectrum which displayed characteristic resonances at δ167.13, 140.29, 127.96 and 80.51. The HMBC correlation (figure 2 and table-1) with respect to –CH_3 group at δ 1.91 (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 δ4.47 (m) correlations with δ167.13 (>C=O), 127.96 (=CH-), 39.3 (>CH-), 28.6 (>CH_2), 12.2 (-CH_3) carbons clearly 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 ^{13}C 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-lactones[17,18]. Based on that we have assigned the streochemistry as 22*S*. On the basis of above spectral analysis and also comparing with reported data[19],

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.

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