Isolation of a New Lipoxygenase Active Saponin and a New Triterpenoid from the Leaves of *Trachelospermum lucidum*

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A new saponin trivially named leucioside (**1**) and a new triterpenoid along with two known lignans trachelosiaside (**3**) and matairesionol (**4**) C-glycosides, were isolated from the butanolic fraction of *Trachelospermum lucidum*. Compounds **1** and **4** showed potent inhibitory activity against lipoxygenase while compounds **2** and **3** showed moderate activity against lipoxygenase.

Key words: Trachelospermum lucidum, Apocynaceae, Leucioside

Introduction

The genus *Trachelospermum lucidum* (Apocynaceae) is a genus of about thirty species distributed mainly in India, Japan and United States. In Pakistan it is represented by two species *T. lucidum* and *T. jasminoides*. *Trachelospermum* is locally called Dudhi, and the flowering season is April to May. It occurs in Pakistan from Rawalpindi to Abbotabad [1]. The plant is suitable for covering embankments, and it prefers moist and shady places. It is a shrub with milky juice. Milky juice is applied to ulcers. The plant is similar to *Alstonia scholaris*, whose bark is bitter tonic and useful in malaria, diarrhea, dysentery and snake bite.

The fruit is astringent and the ripe fruit is cooling, acidic and useful in bilious complaints. The root has the reputation of being a bitter stomachic used in conean pounded with horse urine, lime and camphor as a remedy for itch [2].

Results and Discussion

Compounds **1** – **4** were isolated from the butanolic fraction of *Trachelospermum lucidum*. Compound **1** was obtained as white amorphous solid. The UV spectrum showed absorption at 278.8 and 205 nm. The IR spectrum of **1** exhibited absorptions at 3382 – 2930 (OH) and 1727 – 1661 (C=O) cm^{-1}. The HR-EIMS showed M^+ peak at $m/z = 666.4539$ corresponding to the molecular formula $C_{35}H_{54}O_{12}$. The polar butanolic fraction showed blue to violet spots on TLC after spraying with ceric sulphate. The ^{1}H NMR spectrum of compound **1** suggested an ursane framework with a 19α-hydroxyl group, and two secondary hydroxyl groups. In ^{1}H NMR spectrum the doublet at $\delta_H = 3.72$ ($J = 5.9$ Hz, H–2) and another doublet at $\delta_H = 3.06$ ($J = 9.1$ Hz, H–3) were correlated to the carbons at $\delta_C = 69.6$ and $\delta_C = 85.9$, respectively. These two protons retained 2-α and 3-β positions; one olefinic linkage was at C–12. The doublet due to methylene of compound **1** resonated at $\delta_H = 4.04$ ($J = 11.3$ Hz) correlated with the downfield carbon signal at $\delta_C = 66.1$. The doublet of the proton H–12 appeared at $\delta_H = 5.32$ ($J = 5.9$ Hz) was correlated to the carbon at $\delta_C = 129.4$.

The ^{13}C NMR spectrum of compound **1** (Table 1) showed the presence of thirty-five carbons indicating five methyls, nine methylenes, twelve methines and eight quaternary carbons The presence of one carbonyl carbon was suggested on the basis of ^{13}C NMR at $\delta_C = 178.7$. The comparison of the ^{13}C NMR spectrum of compound **1** was with that of ployoxygenated ursolic acid glycosides [3], especially the carbon signals of the compound **1** were in good agreement. Compound **1** was methylated with diazomethane. On hydrolysis it gave D-glucose.

3β-Acetoxyolean-11-ene (**2**) was isolated from the butanolic fraction of *Trachelospermum lucidum*. The

Table 1. ^{13}C NMR (100 MHz, CD_3OD) and ^{1}H NMR (400 Mz, CD_3OD) of **1**, and ^{13}C NMR (100 Hz, $CDCl_3$) and ^{1}H NMR (400 Hz, $CDCl_3$) of **2**.

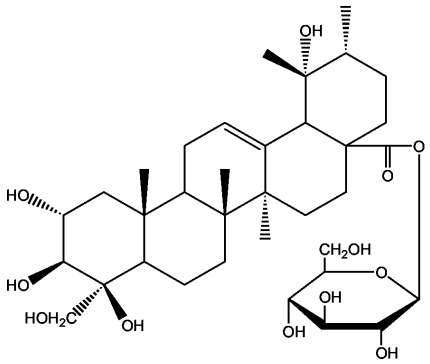
Position	^{13}C δ (ppm) **1**	^{1}H δ (ppm) **1**	J (Hz)	^{13}C δ (ppm) **2**	^{1}H δ (ppm) **2**	J (Hz)
1	48.8	2.01 d	3.9	41.5	1.95 dt	13.5, 3.3
					2.28 dt	13.5, 3.3
2	69.6	3.72 d	4.0, 9.8	31.2	1.71 dq	14.1, 3.0
					2.04 tt	14.1, 2.7
3	85.9	3.06 d	10.0	80.9	4.50 br s	–
4	82.1	–	–	46.6	–	–
5	54.8	2.5 t	5.1	47.6	1.34 dq	14.0, 2.9
6	19.8	1.06 dd	5.0, 9.7	18.2	1.27 q	3.0
7	34.3	1.25 t	5.3	39.2	–	–
8	40.8	–	–	34.2	–	–
9	48.8	1.98 d	5.1	59.5	1.93 d	9.7
10	39.1	–	–	28.7	–	–
11	38.2	1.87 dd	4.5	124.3	5.16 t	7.7
12	129.4	5.32 d	5.4, 5.1	121.6	5.10 t	7.4
13	139.5	–	–	48.6	1.97 d	7.6
14	42.6	–	–	42.2	–	–
15	29.5	1.99 t	4.5	55.2	1.81 t	3.3
16	26.4	1.78 dd	4.4, 9.7	23.2	1.40 t	3.3
17	44.3	–	–	28.7	–	–
18	54.8	2.60	s	41.5	2.21 m	–
19	78.2	–	–	38.4	1.79 t	13.0
20	42.8	1.88 t	5.3	28.9	–	–
21	27.1	1.92 m	–	38.9	1.36 t	13.5
22	38.2	1.02 t	5.4	31.2	1.37 t	13.0
23	66.1	4.04 d	11.3	25.9	0.96 s	–
24	16.5	0.69 s	–	21.3	10.97 s	–
25	17.5	1.23 s	–	25.4	1.22 s	–
26	23.7	1.19 s	–	18.3	1.55 s	–
27	24.7	1.18 s	–	17.5	0.95 s	–
28	27.1	1.31 s	–	15.5	1.18 s	–
29	178.8	–	–	16.8	0.98 s	–
30	–	–	–	16.7	1.01 s	–
31	–	–	–	170.9	–	–
32	–	–	–	23.5	2.02 s	–
1’	95.7	5.38 d	8.7	–	–	–
2’	73.7	3.31 dd	7.7, 7.9	–	–	–
3’	71.5	3.34 brt	7.6	–	–	–
4’	78.4	3.35 brt	7.8	–	–	–
5’	78.6	3.37 ddd	1.7, 7.8, 9.1	–	–	–
6’	62.3	3.69 dd	1.9, 11.7,	–	–	–
		3.59 dd	7.4, 11.7			

Table 2. In *vitro* quantitative inhibition of LOX by compounds **1 – 4**.

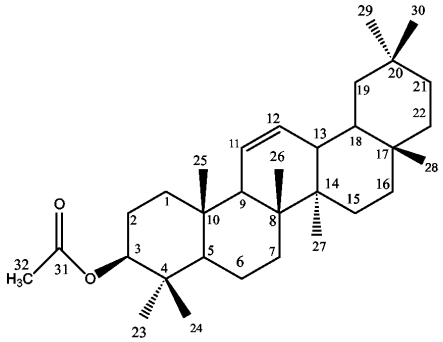
Compounds	$IC_{50} \pm$ S.E.M.[a] [μM]	Compounds	$IC_{50} \pm$ S.E.M.[a] [μM]
1	15.7 ± 0.05	**2**	22.7 ± 0.05
3	35.7 ± 0.1	**4**	9.0 ± 0.08
Baicalein[b]	22.7 ± 0.05		

[a] Standard mean error of 3 – 5 assays; [b] positive control used in assays.

HRMS of compound **2** showed peak M^+ at $m/z =$ 468.39672 calcd. for $C_{32}H_{52}O_2$. There was an intense IR absorption at 1710 cm^{-1} indicating the presence of (C=O) group while the band at 1300 – 1000 cm^{-1} was due to (C–O) group. The ^{1}H NMR spectrum showed the presence of one double bond by signals (C–11) at $\delta_H = 5.16$ ($J = 7.7$ Hz), and (C–12) at $\delta_H = 5.10$ ($J = 7.7$ Hz). The protons of the methine carbons interacting with this double bond were C–9 at $\delta_H = 1.40$ ($J = 3.3$ Hz) and C–13 at $\delta = 1.97$ ($J = 7.7$ Hz), respectively. The downfield proton at $\delta_H = 4.50$ ($J =$ 7.7 Hz) was attached to the carbon at $\delta_C = 80.9$. The downfield proton at $\delta_H = 4.50$ ($J = 7.7$ Hz) was interacting with the protons of the methyls C–23 at $\delta_H =$ 1.13 and C-24 at $\delta_H = 0.95$. The singlet at $\delta_H = 2.02$ was for the methyl attached to carbonyl carbon. The carbonyl carbon appeared at $\delta_C = 170.9$ in ^{13}C NMR



Leucioside **1**



3-β–Acetoxyolean–11–ene **2**

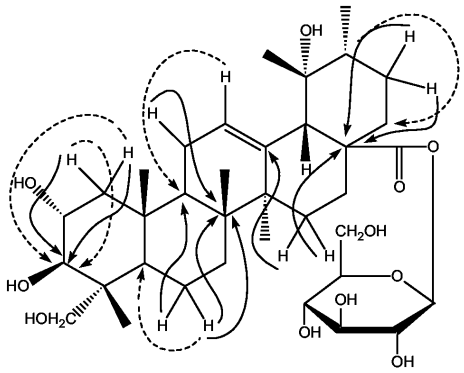


Fig. 1. Selective HMBC ($\rightarrow$) and NOESY ($\dashrightarrow$) correlations of leucioside **1**.

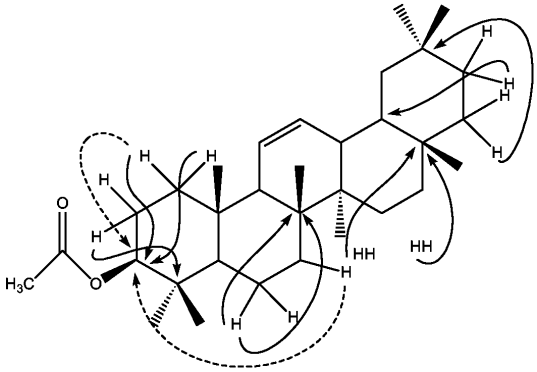


Fig. 2. Selective HMBC ($\rightarrow$) and NOESY ($\dashrightarrow$) correlations of **2**.

spectrum. The presence of seven quaternary, seven methine, and nine methylene carbons was confirmed through BB and DEPT spectra. The ester substituent was placed at C–3 due to the downfield proton appearing at $\delta_{\mathrm{H}} = 4.50$. The relative configuration of H–15, H–16 and other spatial information of compound **2** were further supported by NOESY and HMBC experiments. Therefore, based on all the evidences, the structure of the new compound **2** was assigned as 3-β-acetoxyolean-11-ene. The signals for the double bond C–11 were at $\delta_{\mathrm{C}} = 124.3$ and C–12 at $\delta_{\mathrm{C}} = 121.6$, respectively. The assignment of the methyl signals, the remaining protons and carbon signals was performed through the analysis of HMBC, HMQC and COSY experiments and the results were consistent with oleanan type triterpenoids [4].

Compounds **1** and **4** showed potent inhibitory activity against lipoxygenase (LOX) as compared to baicalein standard having $\mathrm{IC}_{50}(22.7 \pm 0.05)$ while compounds **2** and **3** showed moderate inhibitory activity (Table 2).

Experimental Section

General: Column chromatography (CC): silica gel, 70 – 230 mesh. TLC: pre-coated silica gel G–*25*–UV_{254} plates, detection at 254 nm, and by ceric sulphate and *Kedde* reagent. Optical rotations: *Jasco*-DIP–*360* digital polarimeter. UV and IR spectra: *Hitachi*–UV–*3200* and *Jasco-320*-A spectrophotometers, resp. ^{1}H NMR and ^{13}C NMR, COSY, HMQC and HMBC: *Bruker* spectrophotometers operating at 400 and 500 MHz; chemical shifts δ in ppm, and coupling constants J in Hz. EI–CI–MS: *JMS–HX–110* spectrometer with a data acquisition system.

Plant material: The aerial parts of the plant *Trachelospermum lucidum* (Apocynaceae) were collected form Mountain Eelum in Swat, Pakistan during July 2003. Taxonomic identification was done by Dr. Habib Ahmad (taxonomist), Department of Botany, Postgraduate Jahan Zeb College, Saidu Sharif, Swat, Pakistan. A voucher specimen (# JCH-205) has been deposited in the herbarium of the Botany Department, Postgraduate Jahan Zeb College, Saidu Sharif, Swat, Pakistan.

Extraction and purification: The air-dried ground material (10 kg) was extracted with methanol at room temperature; the extract was evaporated to yield the residue (627 g). The whole residue was extracted with hexane, chloroform and butanol. The TLC of butanolic fraction showed several blue to violet spots after spraying with ceric sulphate (characteristic for lignan glycosides). The butanolic fraction (20 g) was subjected to CC over silica gel column using $CHCl_3$ with a gradient of MeOH up to 100%. Thirty fractions were collected. Fractions 10 – 20 were showing two spots on TLC which were recombined and purified by CC using flash silica 230 – 400 mesh, and eluted with MeOH/$CHCl_3$ (5:95). Gradually increasing the percentage of MeOH. In 80% MeOH/$CHCl_3$ 10 sub-fractions were obtained showing three spots on TLC in butanol/acetic acid/water (12:3:5) as a solvent system. These fractions were combined and VLC was performed using flash silica gel (230 – 400). Ten fractions were collected and the fractions containing same spots were combined and passed through sephadex (mesh lipophilic LH 20 – 100). Fraction no 1 at MeOH/H_2O (80:20) containing two spots (BAW) was subjected to recycling HPLC using water and methanol as a solvent system with a ratio of 1:1, compound **1** was obtained as white amorphous solid at RI = 150, UV = 0.1, with a flow rate of 4 ml/min while compounds **3** and **4** were also obtained from the same fraction with RI = 200, UV = 0.5 with a flow rate of 4 ml/min.

The chloroform extract (20 g) was subjected to CC over a silica gel column using hexane with a gradient of $CHCl_3$ up to 50%. By using a percentage of $CHCl_3$/Hexane (95:5), compound **2** was obtained as white powder (30 mg).

*2α,3β,19,23-Tetrahydroxy 24-nor urs-28-oic acid 28–O–β–D glucopyransoyl ester (**1**):* Amorphous white solid, m.p. 265 °C (decomposed). $[\alpha]_D^{25}$ + 46.15 (*c*, 0.0013, MeOH); UV λ_{max} (MeOH): $(\log \varepsilon)$: 278.8 (6.7), 205.4 (7.5) nm. IR (KBr) ν_{max}: 3382.1 (OH), 1727.7 – 1661.2 (C=O) cm^{-1}; HR–EI–MS: *m/z* 666.4539 [M+1] (calcd. for $C_{35}H_{54}O_{12}$: 666.4436) For ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) Table 1.

*3-β–Acetoxyolean–11–ene (**2**):* White powder. m.p. 220 °C. $[\alpha]_D^{25} - 60.8°$ (*c*, 0.0 07, $CHCl_3$). UV λ_{max} (MeOH) $(\log \varepsilon)$: 260 (3.3), 250 (4.4) nm; IR ν_{max}: 1710 – 1600 (C=O), 1300 – 1000 (C–O) cm^{-1}; EI–MS: *m/z*: $[M + 1]$ 468.39169 (calcd. for $C_{32}H_{52}O_2$: 468.39672), 393 (M–CH_3CO_2H), 190 (20.6), 302 (82), 249 (7.4), 218 (100), 205 (8.3), 189 (36.8).

For ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Table 1.

Acid hydrolysis of compound **1**

Compound **1** (20 mg) was hydrolysed with 2M HCl in aqueous methanol (10 ml) at 100 °C for 3 h. The methanol was evaporated under reduced pressure and the mixture was diluted with water and extracted with EtOAc. The EtOAc and water were evaporated under reduced pressure. The EtOAc contained aglycone and aqueous phase was concentrated and sugar was identified by the sign of its optical rotation $[\alpha]_D^{25} = +52.5°$. It was also confirmed based on the retention time of its TMS ether with a standard. The sugar was identified as glucose by comparison of the TLC with authentic samples of glucose and galactose using solvent system as EtOAc/MeOH/HOAc/H_2O (11:2:2:2).

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