A New Lanostane Triterpene from *Skimmia laureola*

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Aerial parts of *Skimmia laureola* yielded a new (+)-lanostane-3β,24-dihydroxy-25-ene triterpene (**1**) along with fourteen known compounds. The structures were identified by spectroscopic studies.

Key words: Skimmia laureola, Activity, Rutaceae

Introduction

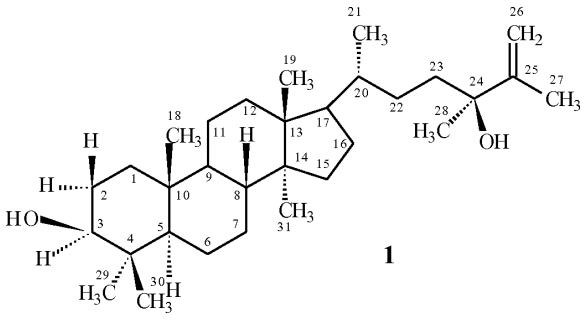
Skimmia laureola Hook (Rutaceae) is found in Kashmir and in the mountains of Northern Pakistan and is used in folklore medicine for the treatment of various ailments [1 – 3]. The quinoline alkaloids of this plant have demonstrated antifungal and immunomodulating properties [4, 5]. The ethanolic extracts of the aerial parts of *S. laureola* are active against the animal pathogen *Microsporium canis* and the plant pathogen *Fusarium solani var. lycopersici* (tomato) [5]. A number of coumarins, *e. g.* isogospherol (**2**) [6], heraclenol (**3**) [7], (+)-7-methoxy-6-(2′*R*-methoxy-3′-hydroxy-3′-methyl butyl)coumarin (**4**) [1], 5,8-dimethoxy coumarin-2*H*-1-benzopyran-2-one (**5**) [8], 7-methoxy-6-[2′-oxo-3′-methyl butyl]coumarin (**6**) [9] and (+)-ulopterol (**7**) [10, 11], were isolated, in addition to various quinoline alkaloids, *e. g.* 4-methoxy-1-methyl-3-(2′*S*-acetoxy-3′-ene butyl)-2-quinolone (**8**) [12], 4-methoxy-1-methyl-3-(2′*S*-acetoxy-3′-hydroxy butyl)-2-quinolone (**9**) [12], 3-hydroxy-2,2,6-trimethyl-3,4,5,6-tetrahydro-2*H*-pyrano[3,2-*c*]quinoline-5-one (**10**) [13], 4-methoxy-1-methyl-3-(2′-oxo-3′-methyl butyl)-2-quinolone (**11**) [15], 4-methoxy-1-methyl-3-(2′*S*-hydroxy-3′-ene butyl)-2-quinolone (**12**) [12], methyl isoplatydesmine (**13**) [2], ribalinin (**14**) [14] and dictamnine (**15**) [15 – 18].

Five known compounds, namely, *O*-methyl-cyclolaudenol, (*R*)-7-methoxy-6-(3′-hydroxy-2′*R*-methoxy-3′-methyl butyl)coumarin, (+)-(*S*)-ψ-ribalinine, (*R*)-(+)-ribalinine and methyl isoplatydesmine, previously isolated from this plant were subjected to enzymatic bioassays for the first time. Methyl cyclolaudenol and (*R*)-7-methoxy-6-(3′-hydroxy-2′*R*-methoxy-3′-methyl-butyl)coumarin were found to be prolyl endopeptidase inhibitors with IC_{50} = 8.21 ± 0.407 and 39.63 ± 1.502 μM, respectively, while ψ-ribalinine, (*R*)-(+)-ribalinine and methyl isoplatydesmine, were found to be acetyl-cholinesterase and butyryl-cholinesterase inhibitors with IC_{50} = 62.46 ± 1.58, 153.31 ± 1.9, 74.5 ± 1.05 and 150.04 ± 0.45, 12.99 ± 0.31, 78.3 ± 1.86 μM, respectively [19].

Results and Discussion

Compound **1** was isolated as a colorless amorphous solid. Its UV spectrum showed end absorptions only indicating the absence of any chromophoric group. The IR spectrum displayed strong absorptions at 1630 and 3300 cm^{-1} indicating the presence of C=C and OH groups, respectively [1, 2]. The HR mass spectrum of compound **1** displayed the $[M]^+$ ion at m/z = 458.4121 ($C_{31}H_{54}O_2$, calcd. 458.4123). The molecular ion $[M]^+$ was further confirmed by FDMS.

The molecular formula $C_{31}H_{54}O_2$ indicated five double bond equivalents in the molecule. The mass spectral fragmentations were characteristic of lanostane triterpenes with a $C_9H_{17}O$ side chain (m/z = 141.1276, calcd. 141.1279) with one site of unsaturation. The $[M–18]^+$ peak at m/z = 443, corresponding to the formula $C_{31}H_{52}O$, arose by the loss of one H_2O from the molecuar ion $[M]^+$. The peak at m/z = 423.3392 ($C_{30}H_{44}O$, calcd. 423.3391), which was also the base peak, could arise by the loss of a second OH group from the ion at m/z = 440 indicating the presence of two oxygen functionalities: One in ring A or B and the other one on the side chain. The peak at m/z = 141.1276 ($C_9H_{17}O$ calcd. 141.1279) could arise by



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the cleavage of the C-20, C-17 bond between ring D and the side chain indicating the presence of a second oxygen function on the side chain. The fragment at m/z = 315.2686 (calcd. 315.2687, $C_{22}H_{35}O$) indicated the attachment of the side chain at the C-17 carbon. Characteristic fragment ions were observed at m/z = 276.2452, 275.2373 and 315.2686, which is indicative of a tetracyclic lanostane-type triterpene [20].

Knowing the presence of oxygen functions, the position of the two hydroxyl groups was investigated in the tetracyclic triterpene skeleton. The ^{13}C NMR data revealed a vinylic methyl carbon resonating at δ = 19.31 and a methylene carbon at δ = 109.31 which could be part of an isopropenyl group. The first hydroxyl group was needed to be placed in such a way that it should follow the regular mass spectral fragmentation pattern. This was accomplished by placing the second hydroxyl functionality at the C-24 position. This was also in accordance with the chemical shifts of closely related triterpenes with the hydroxyl group at C-24 [21], and was further confirmed by the mass spectrum. The peak at m/z = 290 corresponding to the fragment $C_{20}H_{34}O$ may arise by the loss of a $C_{11}H_{20}O$ unit from $[M]^+$. Cleavage of the C_{17} and C_{20} bonds can yield the ion at m/z = 141.1276 (calcd. 141.1279) corresponding to the fragment $C_9H_{17}O$ for the tetracyclic part of compound **1**. This suggested the presence of a side chain on ring D and also the attachment of the second oxygen function at the side chain.

The ^{1}H NMR spectrum of **1** ($CDCl_3$, 500 MHz) showed methyl singlets resonating at δ = 0.79, 0.81, 0.92, 1.67, 1.92, 1.01, 0.94, and 0.76 which were assigned to the C-18, C-19, C-21, C-27, C-28, C-29, C-30, and C-31 methyl protons, respectively, on the basis of comparison with those reported in the literature for closely related triterpenes [22, 23]. Two downfield 2H doublets resonating at δ = 4.54 ($J_{26\alpha,26\beta}$ = 2.5 Hz) and 4.66 ($J_{26\beta,26\alpha}$ = 2.5 Hz) were assigned to the C-26 methylene protons. A well separated doublet, at δ = 0.92 ($J_{21,20}$ = 7.0 Hz), was attributed to the C-21 methyl protons. The vinylic methyl signal at δ = 1.67 and the olefinic H-26 signals at δ = 4.54 and 4.66 suggested the presence of an isopropenyl group. A downfield 1H double doublet in the aliphatic region at δ = 3.18 was assigned to the C-3 proton. The equatorial orientation (β) of the hydroxyl group was inferred from the chemical shift and the coupling pattern of the geminal C-3 proton at δ = 3.18 (dd, $J_{3\alpha,2\alpha}$ = 5.07 Hz, $J_{3\alpha,2\beta}$ = 11.13 Hz). The β-orientation of the OH group was assigned on the basis of a comparison with data reported in the literature [24, 25]. The ^{1}H NMR chemical shifts of (24*R*) cyclolaudenol, (24*R*) cyclolaudenone, (24*R*) cyclomargenol, and cyclolaudenyl acetate [30, 31] were also considered.

The broad-band decoupled ^{13}C NMR spectrum (75 MHz, CD_3OD) of **1** yielded 31 carbon signals, as expected from the molecular formula. The DEPT spectrum [26, 27] showed the presence of eleven methylene, six methine and five methyl carbons, and hence there were six quaternary carbons. The HMQC [28] experiment established the one-bond connectivities between the carbons and the directly attached protons (Table 1). The downfield signals at δ = 79.01, 78.83, and 109.31 were due to the OH-bearing C-3, C-24 and the vinylic C-27, respectively. The three methylene carbon signals resonating at δ = 38.72, 48.32, and 20.99 were assigned to C-1, C-5 and C-11, respectively. The eight methyl carbons at δ = 16.11, 15.36, 19.32, 19.31, 27.98, 27.99, 15.98, and 14.55 were assigned to C-18, C-19, C-21, C-27, C-28, C-29, C-30, and C-31, respectively. The C-3 atom resonated at δ = 79.01. The downfield chemical shift of C-26 at δ = 109.31 was consistent with its olefinic nature. The C-3 methine proton resonating at δ = 3.18 showed cross-peaks with the C-2 methylene protons at δ = 3.18/2.38, 2.18 in the COSY-45° spectrum. In the HMBC spectrum [12, 29] the C-26 proton (δ = 4.54/4.66) showed $^3J_{CH}$ interactions with C-27 (δ = 19.31) and C-24 (δ = 78.83). The C-27 protons showed $^3J_{CH}$ interaction with C-26 (δ = 109.31) and $^2J_{CH}$ interaction with C-25 (δ = 150.96) (Fig. 1).

It was now left to define the exact position and stereochemistry of the two hydroxyl groups. The two downfield exocyclic methylene protons appearing in the COSY-45° spectrum (δ = 4.66 and 4.54) not only displayed geminal coupling interactions but also gave strong cross-peaks with the methyl protons H-27 (δ = 1.66). Moreover the chemical shift of C-23 (CH_2, δ = 40.00) adjacent to the hydroxyl-bearing C-24

Table 1. ^{13}C NMR (75 MHz) and ^{1}H (500 MHz) chemical shift assignments for compound **1**.

C atom	^{13}C NMR δ in ppm[b]	Multiplicity[a]	^{1}H NMR (δ in ppm; J in Hz)[b]
1	38.72	(CH_2)	2.29 (ddd, J = 11.0,16, 6)
			1.96 (ddd, J = 11.1, 16.6)
2	34.30	(CH_2)	2.18 (d, J = 11.15, 5.10)
			2.38 (ddd, J = 11.1, 16.6)
3	79.01	(CH)	3.18 (dd, $J_{3\alpha,2\alpha}$ = 5.07, $J_{3\alpha,2\beta}$ = 11.13)
4	40.84	(C)	–
5	48.32	(CH)	1.57 (m), 1.72 (m)
6	18.32	(CH_2)	1.57 (m), 1.72 (m)
7	29.68	(CH_2)	1.26 (m), 1.72 (m)
8	47.98	(CH)	1.30 (m)
9	50.45	(CH)	–
10	29.58	(C)	–
11	20.94	(CH_2)	1.47 (m), 1.39 (m)
12	35.59	(CH_2)	1.21 (m), 1.28 (m)
13	43.00	(C)	–
14	42.84	(C)	–
15	29.86	(CH_2)	1.35 (m),1.37 (m)
16	27.42	(CH_2)	4.18 (t, J = 6.5)
17	55.31	(CH)	1.58 (m)
18	16.11	(CH_3)	0.79 (s)
19	15.36	(CH_3)	0.81(s)
20	38.07	(CH)	4.80 (d, $J_{21a,21b}$ = 2.5)
21	19.32	(CH_3)	0.92 (d,J = 7.0)
22	25.16	(CH_2)	1.88 (m),1.90 (m)
23	40.00	(CH_2)	2.36 (m)
24	78.83	(C)	–
25	150.96	(C)	–
26	109.31	(CH_2)	4.54 (d, $J_{26a,26b}$ = 2.5)
			4.66 (d, $J_{26b,26a}$ = 2.5)
27	19.31	(CH_3)	1.67 (s)
28	27.98	(CH_3)	1.92 (s)
29	27.99	(CH_3)	1.01 (s)
30	15.98	(CH_3)	0.94 (s)
31	14.55	(CH_3)	0.76 (s)

[a] Multiplicity assignments based on DEPT experiments; [b] one-bond heteronuclear correlations determined by HMQC experiment.

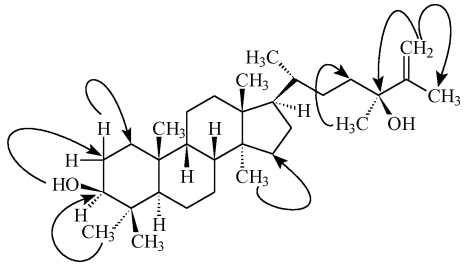

Fig. 1. Select HMBC correlations.

is in close agreement with the values reported by Pascual [21]. In addition, the two downfield protons (H-23) also exhibited weak interactions with H-28 (δ = 1.92) in the same 2D experiment, indicating the vicinity of the methylene protons and the hydroxyl-bearing C-24, thereby confirming the position of the second hydroxyl group.

The relative stereochemistry of **1** at various asymmetric centers was consistent with that reported for related triterpenes. Thus through a combination of these considerations all the ^{13}C and ^{1}H NMR resonances could be assigned (Table 1). On the basis of the above spectroscopic studies, the compound was deduced to be the (+)-lanostane-3β,24-dihydroxy-25-ene triterpene.

Experimental Section

General experimental procedures

Mass spectra were recorded on a Jeol HX-110 instrument. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 500 and 75 MHz, respectively, on a Bruker AM-500 NMR spectrometer. UV and IR spectra were recorded on Shimadzu UV-240 and JASCO A-320 spectrophotometers. Optical rotations were measured on a Polatronic D polarimeter. The purity of the compounds was checked on TLC (Silica-gel, Merck PF_{254}, 0.25 mm thickness). Melting points were determined in glass capillary tubes using a Buchi 535 and a Gallenkamp 30/MF-370 apparatus.

Plant material

The aerial parts of *S. laureola,* Hook (20 kg) were collected from Azad Kashmir. A voucher specimen (KUH # 58106) was deposited in the Herbarium of Department of Botany, University of Karachi.

Extraction and isolation

Air-dried aerial parts of *S. laureola* (20 kg dry weight) were dried and extracted with EtOH (100 L) [30 – 33]. The EtOH extract was concentrated to a gum (822 g), dissolved in distilled water and extracted thoroughly with petroleum ether (45 L). The petroleum ether-soluble portion was evaporated under reduced pressure to yield a gum (66.92 g) which was chromatographed on a silica-gel column (Merck, 70 – 230 mesh, 2025 g). The elution of the column was initiated with petroleum ether. The combined column sub-fractions 1 – 8 (5.91 g) obtained by elution with 1 : 9 acetone-petroleum ether, which showed similar TLC behavior upon spraying with ceric sulfate reagent, were combined and again subjected to CC using silica-gel (type 60, 70 – 230 mesh, 200 g), and the column was eluted with petroleum ether-acetone (9 : 1). The sub-fractions 6 – 30 (1.86 g), which showed similar TLC behavior, were combined and further purified on preparative TLC plates developed in petroleum ether-acetone (97 : 3) to afford pure compound **1** (19.5 mg). Elution of the major column which was loaded with 66.92 g of petroleum ether-soluble material with 50 % acetone-petroleum ether yielded an impure

mixture (7.83 g) which was again subjected to CC (diameter 4 cm, silica-gel, 70 – 230 mesh, 60.20 g). The fractions which were eluted with 20 : 80 acetone-petroleum ether showed identical TLC behavior upon spraying with ceric sulfate reagent. These fractions were combined and subjected to preparative TLC using 20 : 80 acetone-petroleum ether to afford pure **2** (17.51 mg). The fractions which were eluted from the same column with 30 : 70 acetone-petroleum ether were also combined and further purified by silica-gel preparative TLC plates using 35 : 65 acetone-petroleum ether to afford pure **5** (19.82 mg). Elution of the same column with 40 : 60 acetone-petroleum ether yielded an impure compound **4**, which was further purified by preparative TLC using a system of 40 : 60 acetone-petroleum ether to obtain **4** (20 mg). Further elution of the same column with 40 : 60 acetone-petroleum ether yielded fractions 160 – 175 with similar TLC behavior (75 mg) containing mainly compound **3**. These fractions were combined and further purified by preparative TLC using 45 : 55 acetone-petroleum ether to afford **3** (30.26 mg). Further elution of the same column with 50 : 50 acetone-petroleum ether gave semipure fractions (1 g) containing compounds **14** and **6**. Compound **6** was purified by preparative TLC using 45 : 55 acetone-petroleum ether, to afford **6** (18.15 mg). Compound **14** was purified by preparative TLC using 30 : 70 acetone-petroleum ether, to afford **14** (28.62 mg).

The remaining aqueous layer was acidified with acetic acid to pH = 3, and the aqueous acidic layer was then extracted with $CHCl_3$. The aqueous acidic layer was made alkaline with NH_4OH to pH = 12 and extracted with $CHCl_3$ (40 L). The $CHCl_3$ soluble portion was dried over Na_2SO_4, filtered and evaporated to dryness in a vaccum to afford a crude alkaloidal mixture (224 g) which was chromatographed on a silica-gel column. Elution of this column with 96 : 4 $CHCl_3$-MeOH yielded an impure mixture containing compounds **7** – **13** and **15** which were chromatographed on a silica-gel column (Merck, 70 – 230 mesh) and first eluted with $CHCl_3$. Fractions 1 – 15 were found to contain **7** and **8** which were purified by preparative TLC plates using $CHCl_3$-MeOH (99 : 1) to afford pure **7** (19.81 mg) and **8** (19.22 mg).

Fractions 30 – 42 were found to contain **9**, **10**, and **12** which were purified by preparative TLC plates using 98 : 2 $CHCl_3$-MeOH to afford pure **9** (19.81 mg), **10** (19.51 mg), and **12** (15.12 mg).

Fractions 55 – 90 were found to contain **11**, **13**, and **15** which were purified by preparative TLC plates using 98 : 2 $CHCl_3$-MeOH to afford pure **11** (15.31 mg), **13** (18.13 mg), and **15** (8.31 mg).

*(+)-Lanostane-3β, 24-dihydroxy-25-ene triterpene (**1**)*

White amorphous substance (19.5 mg). – $[\alpha]_D^{29} = +62°$ (c = 0.04, $CHCl_3$). – UV (MeOH): only terminal absorption. – IR ($CHCl_3$): ν_{max} = 3397 (OH), 1721, 1615 (C=C), 1125 (OC) cm^{-1}. – EIMS: m/z (%) = 443, $C_{31}H_{25}O$ [M–18]$^+$, 423 (100) $C_{30}H_{44}O$, 315 (50) $C_{22}H_{35}O$, 141 (2) $C_9H_{17}O$. – HRMS: m/z = 458.4121 ($C_{31}H_{54}O_2$, calcd. 458.4123, [M]$^+$), 423.3392 (calcd. 423.3391 for $C_{30}H_{44}O$), 315.2686 (calcd. 315.2687 for $C_{22}H_{35}O$), 141.1276 (calcd. 141.1279 for $C_9H_{17}O$). – ^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (75 MHz): see Table 1.

*Isogospherol (**2**)*

Brown gum, 17.51 mg. – $[\alpha]_D^{29} = 40°$ (c = 0.05, $CHCl_3$). – UV (MeOH): λ_{max} = 300, 248, 218 nm. – IR ($CHCl_3$): ν_{max} = 3395 (OH), 1716 (C=O), 1628 (C=C), 1590, 1580 (C=C conju.) cm^{-1}. – ^{1}H, ^{13}C NMR ($CDCl_3$, 125 MHz) reported in the literature [6].

*Heraclenol (**3**)*

Light-brown gum, 30.26 mg. – $[\alpha]_D^{29} = 11°$ (c = 2.0, $CHCl_3$). – UV (MeOH): λ_{max} = 300, 248, 218 nm. – IR ($CHCl_3$): ν_{max} = 3400 – 3500 (br. OH), 1720 (C=O), 1625 (C=C) cm^{-1}. – ^{1}H, ^{13}C NMR reported in the literature [7].

*(+)-7-Methoxy-6-(2′R-methoxy-3′-hydroxy-3′-methyl butyl) coumarin (**4**)*

White powdery mass, 20 mg. – $[\alpha]_D^{29} = 40°$ (c = 0.05, $CHCl_3$). For further spectroscopic data see [1].

*5,8-Dimethoxy coumarin-2H-1-benzopyran-2-one (**5**)*

White powdery compound, 19.82 mg. – $[\alpha]_D^{29} = 20°$ (c = 0.05, $CHCl_3$). – UV (MeOH): λ_{max} = 207, 246, 274, 322 nm. – IR ($CHCl_3$): ν_{max} = 1719 (C=O), 169 (C=C), 1119 (OCH_3) cm^{-1}. – ^{1}H NMR ($CDCl_3$, 300 MHz) reported in the literature [8].

*7-Methoxy-6-[2′-oxo-3′-methyl butyl] coumarin (**6**)*

Yellowish-brown oily mass, 18.15 mg. – UV (MeOH): λ_{max} = 300, 223 nm. – IR ($CHCl_3$): ν_{max} = 1720 (C=O), 1100 (OCH_3), 1610 (C–H) cm^{-1}. – ^{1}H NMR reported in the literature [9].

*(+)-Ulopterol (**7**)*

Light-brown gummy substance, 19.81 mg. – $[\alpha]_D^{29} = 10°$ (c = 0.10, $CHCl_3$). – UV (MeOH): λ_{max} = 223, 327 nm. – IR ($CHCl_3$): ν_{max} = 3400 (OH), 1720 (six-membered lactone carbonyl carbon), 1615 (C=C) cm^{-1}. – ^{1}H NMR reported in the literature [10, 11].

*4-Methoxy-1-methyl-3-(2′S-acetoxy-3′-ene butyl)-2-quinolone (**8**)*

19.22 mg. – For further spectroscopic data see [9].

*4-Methoxy-1-methyl-3-(2′S-acetoxy-3′-hydroxy butyl)-2-quinolone (**9**)*

19.81 mg. – For further spectroscopic data see [12].

*3-Hydroxy-2,2,6-trimethyl-3,4,5,6-tetrahydro-2H-pyrano[3,2-c]quinoline-5-one (**10**)*

White amorphous substance, 19.51 mg. – $[\alpha]_D^{29} = -57^\circ$ ($c = 0.138$, MeOH). – For further spectroscopic data see [13].

*4-Methoxy-1-methyl-3-(2′-oxo-3′-methyl butyl)-2-quinolone (**11**)*

15.31 mg. – For further spectroscopic data see [9].

*4-Methoxy-1-methyl-3-(2′S-hydroxy-3′-ene butyl)-2-quinolone (**12**)*

15.12 mg. – For further spectroscopic data see [12].

*Methyl isoplatydesmine (**13**)*

White crystalline compound, 18.13 mg, m. p. = 73 – 75 °C. – $R_f = 0.32$. – $[\alpha]_D^{29} = 40^\circ$ ($c = 0.10$, $CHCl_3$). – For further spectroscopic data see [2].

*Ribalinin (**14**)*

The compound gave a red color test with Dragendorff's reagent. Pale-yellow gummy substance, 28.62 mg. – $[\alpha]_D^{29} = 10^\circ$ ($c = 1$, $CHCl_3$). – For further spectroscopic data see [14].

*Dictamnine (**15**)*

White crystalline compound, 8.31 mg. – $[\alpha]_D^{29} = 40^\circ$ ($c = 0.10$, $CHCl_3$). – For further spectroscopic data see [15 – 17].

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