SHORT REPORT

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Noropacursane: A New Nortriterpenoid from the Methanolic Extract of *Carissa opaca*

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Abstract: Aerial parts of *Carissa opaca* have been investigated for the search of new bioactive secondary metabolites and a new nortriterpenoid: noropacursane (**1**) has been isolated together with three known compounds, namely, 6-methoxy-7-hydroxycoumarin (**2**), lupeol (**3**) and 4-ketopinoresinol (**4**). The structure of the new compound was established by 1D (^{1}H-, ^{13}C-NMR), 2D-NMR (HMBC, HSQC, COSY and NOESY) and high resolution mass spectrometry (HR-EI-MS), where as the structures of the known compounds were elucidated through 1D-NMR, mass spectrometric analysis and compared with the reported data in literature. All the isolates were evaluated for their biological potential against the enzymes acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase (LOX) as well as for DPPH free radical scavenging assays. Compound **4** was found active against DPPH radical scavenging activity and BChE with IC_{50} values of 83.41±0.17 and 101.81±0.24 µM, respectively, whereas compound **2** inhibited enzyme LOX with IC_{50} value of 102.25±0.17 µM.

Keywords: *Carissa opaca*; secondary metabolites; isolation, NMR, nortriterpenoid; biological activities.

1. Plant Source

In continuous search for bioactive phytochemicals from medicinally important plants of Pakistan, herein, we report the isolation, structure elucidation and biological studies of a new nortriterpenoid, noropacursane (**1**) along with four known compounds, 6-methoxy-7-hydroxycoumerin (**2**) [1], lupeol (**3**) [2] and 4-ketopinoresinol (**4**) [3] (Figure 1), from the aerial parts of *Carissa opaca* Stapf ex Haines. The investigated plant was collected from Quaid-e-Azam University Islamabad, Islamabad, Pakistan, in June 2008 and identified by Mr. Farrukh Nisar, Plant Taxonomist, Department of Botany, University of Gujrat, Gujrat, Pakistan, where a voucher specimen (CO-46/08-BUG) is deposited in the herbarium.

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2. Previous Studies

Carissa opaca is an indigenous plant of Pakistan used in folk medicine, locally known as *Karanda* or *Kakranda* [4]. Its ethnomedicinal uses include wound healing, stimulant, purgative, hepatoprotective, aphrodisiac, cardiotonic, remedy for snakebite, chicken pox, skin diseases, respiratory diseases such as asthma, jaundice, hepatitis, cough, fever, eye disorders, diarrhea, found effective against animal horn injuries and maggot wounds [5-11]. Leaves have been reported to possess strong antioxidant and hepatoprotective activities due to the presence of high concentration of various phenolics [12-13]. Its ethanolic extract possesses antibacterial activity [14]. Previously, we reported two new 30-nortriterpenpenoids and a sphingolipid from this plant. Further phytochemical analysis of some ignored fractions from the previous work revealed some more bioactive molecules from the methanolic extract of *Carissa opaca* which are reported here.

3. Present Study

The aerial parts of *Carissa opaca* were dried under shade, ground to semi powder form and a dark green extract (350 mg) was obtained after soaking in methanol (MeOH × 3) and evaporation under vacuum. The silica gel column chromatography was performed using the eluting system *n*-hexane, *n*-hexane:dichloromethane (DCM), DCM, DCM:methanol and methanol in increasing order of polarity. As a result, four fractions (M_1-M_4) were obtained. Fraction M_1 received from the main column with *n*-hexane/DCM (7:3, v/v) on further chromatography yielded 6-methoxy-7-hydroxycoumerin (**2**, 12 mg). Fraction M_2 was collected using the eluent *n*-hexane/DCM (2:8, v/v) yielded 3*β*,27-dihydroxylup-12-ene (**3**, 16 mg) on further purification under the same conditions. The main fraction M_3 eluted with DCM/MeOH (9.8:0.2, v/v), on further silica gel column chromatography using eluent DCM/MeOH (95:05, v/v) yielded 4-ketopinoresinol (**4**, 21 mg). Other main fraction M_4 obtained with DCM/MeOH (9.4:0.6, v/v) yielded noropacursane (**1**, 23 mg) on further purification under the same conditions.

30-Nor-2α,3α,23-trihydroxyurs-12-ene (**1**): Colourless amorphous solid; $[\alpha]_D^{24}$ = + 62.0 (*c* = 0.0050, MeOH); IR ν_{max} (KBr): = 3416, 2930, 1632, 830 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) = 5.23 (1H, dd, *J* = 4.0, 3.5 Hz, H-12), 3.87 (1H, ddd, *J* = 11.5, 2.5, 2.0 Hz, H-2), 3.59 (1H, d, *J* = 2.0 Hz, H-3), 3.53 (1H, d, *J* = 11.0 Hz, H-23a), 3.39 (1H, d, *J* = 11.0 Hz, H-23b), 2.21 (1H, d, *J* = 11.0 Hz, H-18), 2.05-2.02 (1H, m, H-22), 1.98-1.95, 1.80-1.78 (each H, m, H-16), 1.96-1.93 (2H, m, H-11), 1.93-1.91, 1.64-1.60 (each H, m, H-15), 1.75-1.71 (1H, m, H-9), 1.67-1.64, 1.41-1.38 (1H each, m, H-2,7), 1.62-1.60, 1.49 (each H, m, H-20), 1.62-1.58, 1.42-1.34 (each H, m, H-6), 1.55, 1.52 (2H, m, H-21), 1.40-1.36 (1H, m, H-19), 1.60-1.57, 1.34-1.28 (each H, m, H-1), 1.28-1.27 (1H, m, H-5), 1.13 (3H, s, H-27), 1.02 (3H, s, H-25), 0.96 (3H, s, H-28), 0.89 (3H, d, *J* = 6.5 Hz, H-29), 0.84 (3H, s, H-26), 0.77 (3H, s, H-24); ^{13}C-NMR (125 MHz, CD_3OD): δ (ppm) = 139.2 (C-13), 126.6 (C-12), 78.7 (C-3), 71.3 (C-23), 67.2 (C-2), 54.4 (C-18), 48.2 (C-9), 44.1 (C-5), 43.2 (C-14), 42.5 (C-4), 42.3 (C-1), 40.8 (C-17), 40.8 (C-8), 40.4 (C-19), 39.1 (C-10), 38.1 (C-22), 33.8 (C-7), 31.8 (C-21), 30.8 (C-20), 29.2 (C-15), 25.3 (C-16), 24.4 (C-11), 24.2 (C-27), 21.5 (C-29), 18.9 (C-6), 17.8 (C-28), 17.8 (C-26), 17.7 (C-25), 17.6 (C-24); HR-EI-MS: *m/z* 444.3610 (calcd. $C_{29}H_{48}O_3$ for 444.3603).

DPPH Radical Scavenging Activity: The DPPH radical scavenging activities were examined by comparison with the standard antioxidant, quercetin using the method of Lee and Shibamoto [15]. Briefly, 100 µL reaction volume contained 90 µL of 0.1 mM methanolic solution of DPPH and 10 µL of various concentrations of the compounds **1-4** (0.5mM – 0.03 mM). The contents were mixed and incubated at 37 ^{o}C for 30 mins. The reduction in colour of DPPH was measured calculated according to the formula:

$$\text{Antiradical activity (\%)} = [(\text{A control} - \text{A sample}) / \text{A control}] \times 100$$

The samples were assayed in triplicate and mean values were calculated.

Cholinesterase and Lipoxygenase Assays: Cholinesterase (AChE, BChE) and lipoxygenase (LOX) inhibition assays were performed according to the Ellman method [16] and Tappel method [17], respectively, with slight modifications as mentioned earlier [19]. The respective positive and negative controls were included in the assay (n=3).

The dried and powdered aerial parts of *C. opaca* were extracted with MeOH. The crude extract obtained after evaporation of the solvent was subjected to conventional purification procedures that resulted in the isolation one new norterpenoid, noropacursane (**1**) (Figure 1).

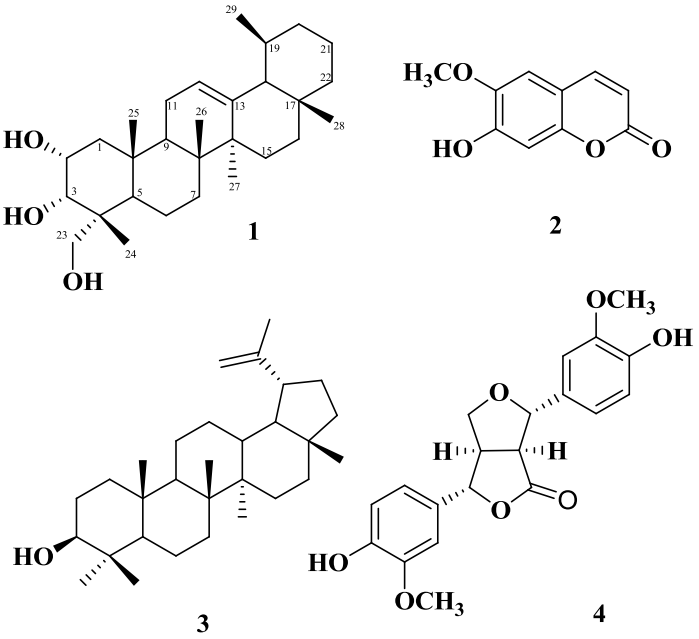

Figure 1. Compounds **1-4** isolated from *C. opaca*

Compound **1** was purified as colorless amorphous solid. The HR-EI-MS spectrum showed its molecular ion peak at *m/z* 444.3610 which corresponded to the molecular formula $C_{29}H_{48}O_3$ with six double bond equivalence (DBE). Its IR spectrum displayed the absorption bands due to secondary hydroxyl (3416 cm^{-1}) and olefinic (1632 cm^{-1}) functions.

The ^{1}H-NMR spectrum of **1** showed five resonances due to tertiary methyls at δ 1.13, 1.02, 0.96, 0.84 and 0.77, which were correlated with the carbon signals at δ 24.2, 17.7, 17.8, 17.8 and 17.6, respectively, in HSQC spectrum. The total 29 carbon signals were appeared in the ^{13}C-NMR spectrum of **1** were distinguished as six methyl, ten methylene, seven methine and six quaternary carbon atoms, based on the DEPT experiments. The resonance for an olefinic proton was showed its presence at δ 5.23 (1H, dd, J = 4.0, 3.5 Hz), attributed to a double bond between C-12 and C-13 with respective carbon shifts at δ 126.6 and 139.2. In addition, the signal of a secondary methyl (δ 0.89, d, J = 6.5 Hz, H-29) and a methine doublet (δ 2.21, J = 11.0 Hz, H-18) suggested compound **1** could be a nor-pentacyclic triterpenoid of ursane series [18, 19]. Further analysis of the ^{1}H-NMR and COSY spectra revealed a 2,3-dihydroxy system in **1** due to the oxymethine signals at δ 3.59 (1H, d, J = 2.0 Hz, H-3) and 3.87 (1H, ddd, J = 11.5, 2.5, 2.0 Hz, H-2), which were correlated with the carbon resonances at δ 78.7 and 67.2. The ^{1}H-signal of an oxymethylene at δ 3.53 (1H, d, J = 11.0 Hz, H-23a) and 3.39 (1H, d, J = 11.0 Hz, H-23b) exhibited HMBC correlation (Figure 2) with C-3 (δ 78.7), C-4 (δ 42.5) and C-24 (δ 17.6). This observation indicated that Me-23 has been oxidized to a primary alcohol. H-19 (δ 1.40-1.36) was correlated in COSY spectrum with a methylene (δ 1.62-1.60, 1.49, H-20), H-18 (δ 2.21) and methyl-29 (δ 0.89), which finally confirmed the position of secondary methyl and revealed that Me-30 is missing in compound **1**. HMBC interactions of H-29 (δ 0.89) with C-20 (δ 30.8), C-19 (δ 40.4) and C-18 (δ 54.4) (Figure 2) substantiated the above deduction, and therefore, compound 1 was found to be a 30-nortriterpenoid. Retro Diels-Alder (RDA) fragment at *m/z* 240.1724 ($C_{14}H_{24}O_3$)

and 204.1877 ($C_{15}H_{24}$) observed in HR-EI-MS spectrum further confirmed 30-nortriterpenoid skeleton.

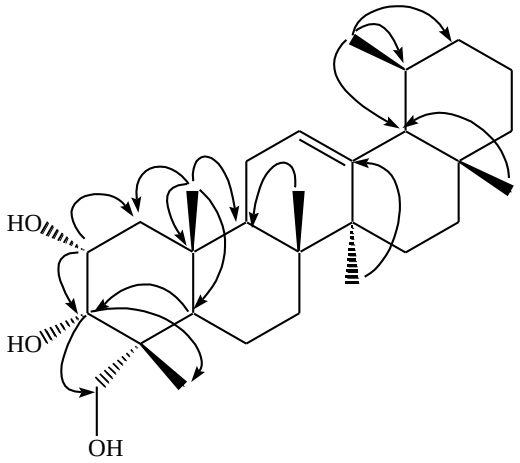

Figure 2. Important HMBC interactions of **1**

All assignments were established with COSY, HSQC and HMBC correlations and the data was compared with carisursane B [20]. The stereochemistry at C-2 and C-3 positions could be assigned by careful analysis of NOESY spectrum (Figure 3), molecular modeling, and coupling constants. The lower value of coupling constant of H-3 (2.0 Hz) and its NOESY correlation with H-24 and that of H-24 with H-25 suggested its β and equatorial orientation [20], while the splitting pattern and *J* values of H-2 (ddd, *J* = 11.5, 2.5, 2.0 Hz) and its NOESY correlation with H-25 confirmed H-2 as β and axial in orientation. All above analyses, discussion and comparing the data with carisursane B [19], compound **1** could be elucidated as 30-nor-2α,3α,23-trihydroxyurs-12-ene. It is new natural product.

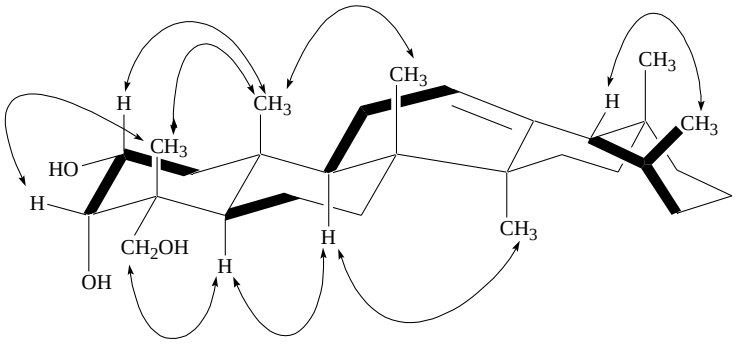

Figure 3. Diagnostic COSY (▬) and NOESY (↷) correlations observed in the spectra of **1**

Compounds **1-4** were screened for their DPPH free radical scavenging and enzyme inhibitory activities at concentration of 0.5 mM. Compound **4** was Compound **1** was found to possess weak activities with percent inhibition of 48.37±0.66, 55.51±0.58 and 61.21±0.82 against AChE, BChE and LOX, respectively. It exhibited weak antioxidant activity of 21.56±0.58% at 0.5 mM concentration as determined by DPPH radical scavenging assay. Compounds **2** and **3** displayed moderate inhibition of LOX; **2** was more active than **3** and **1** as shown by the respective IC_{50} values (Table 1). Compound **3** was inactive against AChE. Compounds **4** showed considerable activity in DPPH and BChE screening assays with IC_{50} of 83.41±0.17 and 101.81±0.24 μM, respectively (Table 1).

Table 1. Biological activity of compounds **1-4**

Compound	DPPH		AChE		BChE		LOX	
	Inhibition (%) at 0.5 mM	IC_{50} (µM)	Inhibition (%) at 0.5 mM	IC_{50} (µM)	Inhibition (%) at 0.5 mM	IC_{50} (µM)	Inhibition (%) at 0.5 mM	IC_{50} (µM)
1	21.56±0.58	-	48.37±0.66	527.48±0.73	55.51±0.58	473.65±0.74	61.21±0.82	197.46±0.39
2	24.61±0.27	-	62.35±0.14	289.31±0.48	24.61±0.72	-	78.92±0.16	102.25±0.17
3	20.31±0.33	-	39.61±0.35	-	32.15±0.54	-	62.31±0.55	178.21±0.23
4	91.11±0.18	83.41±0.17	54.63±0.14	481.62±0.58	89.53±0.13	101.81±0.24	44.58±0.46	562.37±1.72
Quercetin	93.21±0.97	16.96±0.14	-	-	-	-	-	-
Eserine	-	-	91.29±1.17	0.04±0.001	82.82±1.09	0.85±0.001	-	-
Baicalein	-	-	-	-	-	-	93.79±1.2	22.4±1.3

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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