Coumarins and a Naphthyl Labdanoate Diarabinoside from the Fruits of *Peucedanum grande* C. B. Clarke

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Phytochemical investigations of the methanolic extract of the fruits of *Peucedanum grande* C. B. Clarke (Apiaceae) led to the identification of three coumarins and a naphthyl labdanoate diarabinoside characterized as 5-hydroxy-6-isopranyl coumarin (**1**), 5,6-furanocoumarin (**2**), 7-methoxy-5,6-furanocoumarin (**3**), and labdanyl-3α-ol-18-(3'''-methoxy-2'''-naphthyl-oate)-3α-L-arabinofuranosyl-(2'→1'')-α-L-arabinofuranoside (**4**). The structures of these compounds were identified on the basis of spectral data analysis and chemical reactions. The methanolic extract and **4** showed nephroprotective activity against gentamicin-induced nephrotoxicity in Wistar rats.

Key words: Peucedanum grande, Coumarins, Naphthyl Labdanoate Diarabinoside, Nephroprotective Activity

Introduction

The genus *Peucedanum* is represented by 29 species including *Peucedanum grande* C. B. Clarke (Apiaceae) which is a succulent herb, about 1 m in height, occurring gregariously on the western sea coasts and hills of southern India. It is commonly known as baphali, duku, or wild carrot. The roots are large and perennial. The stem is fistular, emitting a strong scent on crushing. The fruits are ovate, broadly elliptical, 10–13 mm long, narrow, reddish yellow having a powerful lemon-like odour; they are used as condiment, carminative, diuretic, stimulant, and tonic. A fruit infusion is useful against gastric and intestinal disorders (National Institute of Science Communication, 2001; Mhaskar *et al*., 2000). The fruits are added to curries as a flavouring agent (Nadkarni, 2002); they contain a volatile oil composed of α-pinene, sabinene, limonene, *p*-cymene, 1,8-cineole, α-terpineol, bornyl acetate, and coumarins (Thakkar *et al*., 1987; Siddiqui and Sen, 1972; Desai *et al*., 1970).

This manuscript describes the isolation and structure elucidation of coumarins and a naphthyl labdanoate diglycoside from the fruits of *P. grande*, and nephrotoxicity of the methanolic seed extract and of the diglycoside.

Material and Methods

General

Melting points were determined using a thermoelectrically operated Perfit melting point apparatus (Ambala, India) and are uncorrected. UV spectra were measured with a Lambda Bio 20 spectrophotometer (Nicosia, Cyprus) in methanol. IR spectra were recorded, using KBr discs, with a Bio-Rad FT-IR 5000 spectrometer (FTS 135, Hongkong). ^{1}H and ^{13}C NMR spectra were scanned on Bruker Advance DRY 400 spectrospin and Bruker Advance DRX 100 spectrospin instruments (Bruker-Biospin, Rheinstetten, Germany) with tetramethylsilane (TMS) as internal standard. FAB mass spectra were obtained using a JEOL-JMS-DX 303 spectrometer (Peabody, MA, USA). Column chromatography (CC) was performed on silica gel (Qualigens, Mumbai, India), 60–120 mesh. Thin-layer chromatography (TLC) was run on silica gel G (Qualigens). Spots were visualized by exposure to iodine vapour, UV radiation, and by spraying with ceric sulfate solution.

Plant material

The fruits of *P. grande* were purchased from Khari Baoli, local market of Delhi, and authenti-

cated by Dr. H. B. Singh, National Institute of Science Communication and Information Resources (NISCAIR, CSIR), New Delhi, India. A voucher specimen (No. 3/f 3RHM/04/486/62) is kept in the herbarium of NISCAIR.

Extraction and isolation

The dried fruits of *P. grande* (2.0 kg) were coarsely powdered, defatted with petroleum ether, and then exhaustively extracted with methanol in a Soxhlet apparatus. The combined methanolic extracts were concentrated on a steam bath and dried under reduced pressure to obtain 85 g (4.25% yield) of a dark viscous brown mass. It was dissolved in a small quantity of chloroform and adsorbed on silica gel (60–120 mesh) for the preparation of a slurry. The slurry was dried and chromatographed over a silica gel column packed in petroleum ether. The column was eluted with petroleum ether, mixtures of petroleum ether and chloroform (9:1, 3:1, 1:1, 1:3, v/v), pure chloroform, and finally mixtures of chloroform and methanol (99:1, 97:3, 95:5, 90:10, v/v). Various fractions were collected separately and checked by TLC for homogeneity. Similar fractions (having the same R_f values) were combined and crystallized. The isolated compounds were recrystallized to get pure compounds.

5-Hydroxy-6-isopranyl coumarin (**1**): Elution of the column with petroleum ether/chloroform (1:1) furnished colourless crystals of **1**, recrystallized from acetone. – Yield: 445 mg. – R_f 0.57 (petroleum ether/acetone, 7:3). – M.p. 103–104° C. – UV (MeOH): λ_{max} = 256, 277, 366 nm (log ε 4.1, 5.8, 4.0). – IR (KBr): υ_{max} = 3350, 1709, 1615, 1542, 1441, 1337, 1270, 1122, 1040, 832 cm^{-1}. – ^{1}H NMR ($CDCl_3$): δ = 7.82 (1H, d, J = 9.6 Hz, H-4), 7.44 (1H, d, J = 8.8 Hz, H-8), 7.38 (1H, d, J = 8.8 Hz, H-7), 6.40 (1H, d, J = 9.6 Hz, H-3), 1.83 (2H, m, H_2-11), 1.75 (1H, m, H-13), 1.70 (2H, m, H_2-12), 1.26 (3H, d, J = 7.2 Hz, Me-14), 1.23 (3H, d, J = 6.8 Hz, Me-15). – ^{13}C NMR ($CDCl_3$): δ = 160.89 (C-2), 114.06 (C-3), 144.58 (C-4), 157.31 (C-5), 123.84 (C-6), 113.49 (C-7), 104.07 (C-8), 145.89 (C-9), 116.87 (C-10), 58.06 (C-11), 25.85 (C-12), 29.76 (C-13), 18.45 (C-14), 18.25 (C-15). – FAB-MS: *m/z* (rel. int.) = 232 (5.3) $[M]^+$ ($C_{14}H_{16}O_3$), 255 (40.01) $[M+Na]^+$.

5,6-Furanocoumarin (**2**): Further elution of the column with petroleum ether/chloroform (1:1) afforded colourless crystals of **2**, recrystallized from acetone/methanol (1:1). – Yield: 553 mg. – R_f 0.54 (petroleum ether/acetone, 7:3). – M.p. 131–132 °C. – UV (MeOH): λ_{max} = 254, 277, 364 nm (log ε 3.9, 4.8, 4.5). – IR (KBr): υ_{max} = 1709, 1614, 1577, 1535, 1375, 1271, 1122, 1040, 832 cm^{-1}. – ^{1}H NMR ($CDCl_3$): δ = 7.82 (1H, d, J = 9.6 Hz, H-4), 7.69 (1H, d, J = 2.0 Hz, H-12), 7.43 (1H, d, J = 8.8 Hz, H-8), 7.37 (1H, d, J = 8.8 Hz, H-7), 7.11 (1H, d, J = 2.0 Hz, H-11), 6.39 (1H, d, J = 9.6 Hz, H-3). – ^{13}C NMR ($CDCl_3$): δ = 160.85 (C-2), 114.04 (C-3), 144.90 (C-4), 157.26 (C-5), 123.83 (C-6), 113.47 (C-7), 104.04 (C-8), 148.40 (C-9), 116.53 (C-10), 108.80 (C-11), 145.99 (C-12). – FAB-MS: *m/z* (rel. int.) = 186 (4.5) $[M]^+$ ($C_{11}H_6O_3$), 202 (100) $[M+OH]^+$.

7-Methoxy-5,6-furanocoumarin (**3**): Elution of the column with petroleum ether/chloroform (2:3) furnished colourless crystals of **3**, recrystallized from acetone. – Yield: 225 mg. – R_f 0.51 (petroleum ether/acetone, 7:3). – M.p. 178–179 °C. – UV (MeOH): λ_{max} = 257, 277, 366 nm (log ε 4.1, 5.3, 5.5). – IR (KBr): υ_{max} = 1704, 1619, 1581, 1476, 1459, 1431, 1356, 1282, 1257, 1214, 1162, 1127 1099, 982, 895, 864, 816 cm^{-1}. – ^{1}H NMR ($CDCl_3$): δ = 8.14 (1H, d, J = 10.0 Hz, H-4), 7.59 (1H, d, J = 2.4 Hz, H-12), 7.09 (1H, brs, H-8), 7.02 (1H, d, J = 2.4 Hz, H-11), 6.26 (1H, d, J = 10.0 Hz, H-3), 4.26 (3H, brs, OMe). – ^{13}C NMR ($CDCl_3$): δ = 160.70 (C-2), 114.17 (C-3), 144.32 (C-4), 157.76 (C-5), 138.74 (C-6), 152.05 (C-7), 104.51 (C-8), 148.96 (C-9), 112.09 (C-10), 93.10 (C-11), 144.20 (C-12), 59.45 (OMe). – FAB-MS: *m/z* (rel. int.) = 217 (36.8) $[M+H]^+$ ($C_{12}H_9O_4$), (100) 201.

Labdanyl-3α-ol-18-(3'''-methoxy-2'''-naphthyloate)-3α-L-arabinofuranosyl-(2'→1'')-α-L-arabinofuranoside (**4**): Elution of the column with chloroform gave pale yellow crystals of **4**, recrystallized from chloroform/methanol (1:1). – Yield: 122 mg. – R_f 0.49 (petroleum ether/chloroform, 1:1). – M.p. 65–66 °C. – UV (MeOH): λ_{max} = 256, 287, 335 nm. – IR (KBr): υ_{max} = 3425, 3360, 2925, 2845, 1725, 1635, 1551, 1360, 1235, 935, 839 cm^{-1}. – ^{1}H NMR and ^{13}C NMR ($CDCl_3$): see Table I. – FAB-MS: *m/z* (rel. int.) = 744 (15.2) $[M]^+$ ($C_{41}H_{60}O_{12}$).

*Hydrolysis of **4***

Compound **4** (35 mg) was dissolved in ethanol (5 ml), 1 M sodium hydroxide solution (2 ml) was added and the reaction mixture heated for 1 h on a steam bath. The solvent was evaporated under

reduced pressure and the residue dissolved in chloroform to separate 3-methoxy-2-naphthol.

M.p. 112–114 °C. – IR (KBr): υ_{max} = 3345 cm^{-1}. – ^1H NMR ($CDCl_3$): δ = 7.66 (1H, brs, H-1), 7.05 (1H, brs, H-4), 7.98 (1H, d, J = 8.3, 2.8 Hz, H-6), 6.25 (1H, m, H-7), 6.16 (1H, m, H-8), 8.02 (1H, dd, J = 9.2, 2.7 Hz, H-9). – +ve FAB-MS: m/z (rel. int.) = 175 (2.7) $[M+H]^+$ ($C_{11}H_{10}O_2$).

The residue, after separation of the naphthol derivative, was dissolved in water (5 ml), acidified with HCl to pH 3, and extracted with chloroform to separate labdanyl-3α-ol-18-oic acid.

M.p. 135–136 °C. – IR (KBr): υ_{max} = 3410, 3280, 1695 cm^{-1}. – ^1H NMR ($CDCl_3$): δ = 3.71 (1H, dd, J = 4.8, 5.3 Hz, H-3β), 1.05 (3H, brs, Me-16), 1.07 (3H, brs, Me-17), 0.89 (3H, brs, Me-19), 1.75 (1H, m, H-9), 1.22 (3H, d, J = 7.5 Hz, Me-20), 0.85 (3H, t, J = 6.3 Hz, Me-15). – +ve FAB-MS: m/z (rel. int.) = 325 $[M+H]^+$ (2.5) ($C_{20}H_{37}O_3$).

The solution after separation of labdanyl compounds was concentrated and chromatographed on a silica G TLC plate with a standard sample of arabinose using n-butanol/acetic acid/water (4:1:5, top layer) as a developing solvent. – R_f 0.18, co-TLC comparable.

Animals

Adult albino rats (Wistar strain) of either sex weighing 150–200 g (8 to 12 weeks old) were obtained from Central Animal Facility, Jamia Hamdard, New Delhi, India. The animals were housed in polypropylene cages with dust-free rice husk as bedding material renewed every 24 h, under 12 h/12 h light/dark cycles at (25 ± 2) °C and at (55 ± 5)% relative humidity; they were provided with standard pellet diet (Lipton Feed Ltd., New Delhi, India) and water *ad libitium* at all time. The study was conducted after approval by the Institutional Animal Ethical Committee.

Nephroprotective activity

Nephroprotective activity was evaluated using gentamicin induced (Wockhardt Ltd., Mumbai, India) nephrotoxicity in albino rats. Gentamicin [100 mg/(kg body weight (BW) d)] was injected subcutaneously for eight consecutive days at 8.00 a.m. to induce nephrotoxicity (Singh *et al.*, 2009). The nephritic rats were divided into five groups with six animals in each group. Drugs were administered orally using 1% carboxymethyl cellulose (CMC) as a suspending agent in distilled water. Group I (control) received 1% CMC in distilled water for 5 d. Group II (toxic) was toxicant receiving gentamicin. Group III received a lower dose (LD) of the methanolic extract of *P. grande* fruits [60 mg/(kg BW d)]. Group IV received a higher dose (HD) of the methanolic extract of *P. grande* fruits [130 mg/(kg BW d)]. Group V received naphthyl labdanoate diarabinoside **4** [5 mg/(kg BW d)] for 5 d. Serum was obtained, rapidly separated, and processed for determination of serum creatinine and blood urea nitrogen levels using commercially available kits from Span Diagnostics Ltd (Hyderabad, India) (Shirwalkar *et al.*, 2004). The data obtained was analysed using one-way ANOVA. $p < 0.01$ was considered significant.

Results and Discussion

Structure elucidation

Compound **1** was obtained as a colourless crystalline mass from petroleum ether/chloroform (1:1). It exhibited blue fluorescence and UV absorption maxima at 256, 277, and 366 nm, characteristic of coumarins. It had infrared absorption bands for a hydroxy group (3350 cm^{-1}), carbonyl function (1709 cm^{-1}), and an aromatic ring (1615, 1542, 832 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular weight of **1** was established at m/z 232 consistent with an isopranyl coumarin with the total molecular formula $C_{14}H_{16}O_3$. The ^1H NMR spectrum of **1** showed a set of doublets for α-pyron protons of the coumarin nucleus at δ_H 7.82 (H-4) and 6.40 ppm (H-3) with coupling interaction of 9.6 Hz each. Another pair of AB-type doublets at δ_H 7.44 (J = 8.8 Hz) and 7.38 ppm (J = 8.8 Hz) was ascribed to aromatic H-8 and H-7, respectively, and the remaining two positions were substituted. Two multiplets at δ_H 1.83 and 1.70 ppm, integrating for two protons each, were attributed to methylene H_2-11 and H_2-12 protons, respectively. A one-proton multiplet at δ_H 1.75 ppm was attributed to the methine H-13 proton. Two three-proton doublets at δ_H 1.26 (J = 7.2 Hz) and 1.23 ppm (J = 6.8 Hz) were due to secondary methyl C-14 and C-15 protons, respectively, indicating the presence of the isopranyl group in the molecule. The ^{13}C NMR spectrum of **1** displayed nine carbon signals for the coumarin nucleus and five carbon signals for the isopranyl chain (Shikishima *et al.*, 2001; Cruz

et al., 2001; Rao *et al.*, 2009). The presence of the C-8 signal in the upfield region at δ_C 104.07 ppm supported the existence of the isopranyl group at C-6 and the hydroxy group at C-5. The existence of the pranyl group at C-8 shifted the δ_C value of this carbon atom near 112.0 ppm (Rao *et al.*, 2009; Shikishima *et al.*, 2001). The ^{1}H-^{1}H COSY spectrum of **1** exhibited correlations of H-3 with H-4; H-7 with H-8 and H_2-11; and H-13 with Me-14, Me-15, H_2-12, and H_2-11. The HMBC spectrum of **1** showed interactions of C-6 with H_2-11, H_2-12, H-7, and H-8. The coumarins containing a 6-isoprenyl chain reported earlier are toddaculin from *Toddalia asiatica* stem bark (Vazquez *et al.*, 2012), 6-(4-acetoxy-3-methyl-2-butenyl)-7-hydroxycoumarin and 6-(2-hydroxy-3-hydroxymethyl-3-butenyl)-7-hydroxycoumarin from *Aegle marmelos* green fruits (Chakthong *et al.*, 2012), inocalophyllins from *Calophyllum inophyllum* seeds (Shen *et al.*, 2003), fatouains from *Fatoua pilosa* plant (Chiang *et al.*, 2010), isophellodenol C from *Heracleum candicans* roots (Nakamori *et al.*, 2008), balsamiferone from *Amyris balsamifera* aerial parts (Burke and Parkins, 1979), and gravelliferon from *Ruta graveolens* (Reisch *et al.*, 1968). On the basis of the foregoing discussion, the structure of **1** was elucidated as 5-hydroxy-6-isopranyl coumarin (Fig. 1).

Compounds **2** and **3** were known coumarins identified as 5,6-furanocoumarin and 7-methoxy-5,6-furanocoumarin, respectively (Fig. 1).

Compound **4**, a naphthyl labdanoate diarabinoside, was obtained as pale yellow crystals from chloroform. It gave positive tests for glycosides. Its infrared spectrum showed characteristic absorption bands for a hydroxy group (3425, 3360 cm^{-1}), ester function (1725 cm^{-1}), and aromatic rings (1635, 1551, 839 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the relative molecular mass of **4** was determined at *m/z* 744 consistent with the molecular formula of a naphthyl-substituted diarabinosyl diterpenoid, $C_{41}H_{60}O_{12}$. The ^{1}H NMR spectrum (Table I) of **4** displayed two one-proton broad signals at δ_H 7.60 and 6.99 ppm assigned to *para*-coupled H-1''' and H-4''', respectively, indicating a 2,3-disubstituted naphthyl moiety. Two one-proton double doublets at δ_H 8.02 (J = 8.5, 3.0 Hz) and 8.06 ppm (J = 9.6, 3.0 Hz), and two

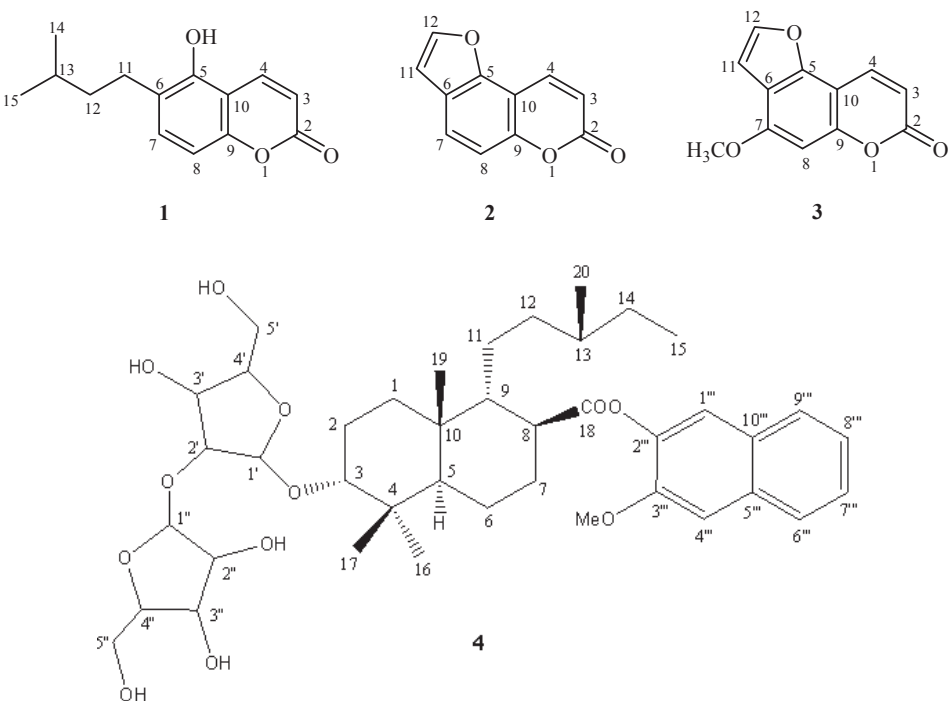

Fig. 1. Chemical structures of the isolated compounds 5-hydroxy-6-isopranyl coumarin (**1**), 5,6-furanocoumarin (**2**), 7-methoxy-5,6-furanocoumarin (**3**), and labdanyl-3*α*-ol-18-(3'''-methoxy-2'''-naphthyl-oate)-3*α*-L-arabinofuranosyl-(2'→1'')-*α*-L-arabinofuranoside (**4**).

Table I. ^{1}H and ^{13}C NMR spectral data of naphthyl labdanoate diarabinoside **4**.

Position	δ_H (J in Hz)	δ_C
1	2.01 dddd (6.4, 7.2, 6.4, 3.5) 1.97 dddd (6.0, 6.4, 5.3, 4.5)	31.87
2	1.79 m, 1.67 m	34.24
3	4.27 dd (5.2, 5.9)	77.59
4	----	36.94
5	1.70 dd (5.1, 6.2)	26.95
6	1.41 m, 1.45 m	29.70
7	1.63 m, 1.65 m	29.53
8	1.95 m	26.81
9	1.73 m	26.38
10	--	38.93
11	1.40 m, 1.37 m	27.20
12	1.43 m, 1.35 m	24.55
13	1.48 m	26.27
14	1.33 m, 1.37 m	22.62
15	0.84 t (6.4)	14.06
16	1.02 brs	25.74
17	1.06 brs	18.90
18	--	173.43
19	0.92 brs	22.83
20	1.24 d (8.4)	17.86
1’	5.32 d (6.0)	105.33
2’	4.94 dd (6.9, 5.9)	75.86
3’	4.49 m	72.81
4’	4.27 m	81.22
5’	4.15 d (6.1)	72.44
1”	5.23 d (6.5)	105.23
2”	4.72 dd (6.5, 6.3)	75.59
3”	4.43 m	73.82
4”	4.29 m	80.13
5”	4.11 d (6.6)	71.65
1’’’	7.60 brs	139.40
2’’’	--	145.10
3’’’	---	160.30
4’’’	6.99 brs	142.92
5’’’	--	126.76
6’’’	8.02 dd (8.5, 3.0)	130.35
7’’’	6.22 m	112.67
8’’’	6.19 m	112.55
9’’’	8.06 dd (9.6, 3.0)	126.70
10’’’	--	150.19
OMe	4.04 brs	60.64

one-proton multiplets at δ_H 6.22 and 6.19 ppm were ascribed correspondingly to *ortho*-, *meta*-coupled H-6’’’, H-9’’’ and to H-7’’’, H-8’’’ aromatic protons, respectively. A three-proton triplet at δ_H 0.84 ppm (J = 6.4 Hz), three broad signals at δ_H 1.02, 1.06, and 0.92 ppm integrating for three protons each, and a three-proton doublet at δ_H 1.24 ppm (J = 8.4 Hz) were associated with the primary methyl H_3-15, tertiary methyl H_3-16, H_3-17, and H_3-19, and the secondary methyl H_3-20 protons, respectively, supporting a labdane-type diterpenic moiety linked to the naphthyl ring. A one-proton double doublet at δ_H 4.27 ppm with coupling interactions of 5.2 and 5.9 Hz was attributed to an oxygenated methine H-3β proton. Two one-proton doublets at δ_H 5.32 (J = 6.0 Hz) and 5.23 ppm (J = 6.5 Hz) were assigned to α-oriented anomeric H-1’ and H-1’’ protons, respectively. Six one-proton signals from δ_H 4.94 to 4.27 ppm were due to oxygenated methine protons of the sugar moieties. Two two-proton doublets at δ_H 4.15 (J = 6.1 Hz) and 4.11 ppm (J = 6.6 Hz) were assigned to hydroxymethylene H_2-5’ and H_2-5” protons, respectively. The shifting of the H-2’ proton in the deshielded region at δ_H 4.94 ppm suggested attachment of the second sugar moiety at the C-2’ position. A three-proton broad signal at δ_H 4.04 ppm was due to methoxy protons. The other methine and methylene protons of the labdane moiety appeared between δ_H 2.01 and 1.33 ppm. The ^{13}C NMR spectrum of **4** exhibited signals for an ester carbon atom at δ_C 173.43 ppm (C-18), aromatic signals from δ_C 160.30 to 112.55 ppm, anomeric carbon atoms at δ_C 105.33 (C-1’) and 105.23 ppm (C-1”), other sugar carbon atoms from δ_C 81.22 to 71.65 ppm, an oxygenated methine carbon atom at δ_C 77.59 ppm (C-3), a methoxy carbon atom at δ_C 60.64 ppm, and labdane carbon atoms from δ_C 38.93 to 14.06 ppm. The shifting of the sugar carbon atoms in the deshielded region at δ_C 75.86 (C-2’), 81.22 (C-4’), 75.59 (C-2”), and 80.13 ppm (C-4”) suggested the furanic form of the sugar units. The DEPT spectrum of **4** showed the presence of six methyl, nine methylene, and nineteen methine groups, as well as seven quaternary carbon atoms. The NMR spectral data of the labdanyl unit were compared with those of the related diterpenes (Ali, 2001; Wu *et al.*, 2011). The ^{1}H-^{1}H COSY spectrum of **4** showed correlations of H-3 with H_2-1, H_2-2, Me-17, and H-1’; H-2’ with H-1’, H-3’, and H-1”; H-4” with H-3” and H_2-5”; H-8 with H_2-7, H_2-6, and H-9; H-4’’’ with OMe and H-6. The HMBC spectrum of **4** exhibited interactions of C-3 with H_2-2, Me-17, and H-1’; C-2’ with H-1’, H-3’, and H-1”; C-4” with H-2”, H-3”, and H_2-5”; C-18 with H-8, H_2-7, and H-9; C-2’’’ with H-1’’’ and H-4’’’; and C-5’’’ with H-4’’’, H-6’’’, H-7’’’, and H-8’’’. Hydrolysis of **4** with 1 M NaOH solution yielded 3-methoxy-2-naphthol, labdanyl-3α-ol-18-oic acid, and arabinose. On the basis of spectral data analysis, the structure of **4** was established

Table II. Effect of the methanolic extract of the fruits of *Peucedanum grande* and compound **4** on blood urea nitrogen and serum creatinine levels in gentamicin-induced nephrotoxicity in rats.

Group	Treatment	Dose	Blood urea nitrogen [mg/100 mL]	Creatinine [mg/100 mL]
I (Control)	Vehicle	10 ml/kg BW	11.54 ± 1.17	0.203 ± 0.007
II (Toxicant)	Gentamicin	100 mg/kg BW	22.25 ± 2.05	0.265 ± 0.013
III	*P. grande* (LD)	60 mg/kg BW	16.45 ± 1.19 (54.25%)	0.253 ± 0.011 (96.7%)
IV	*P. grande* (HD)	130 mg/kg BW	12.82 ± 1.81 (88.05%)	0.268 ± 0.024 (no inhibition)
V	**4**	5 mg/kg BW	13.16 ± 1.23 (75.18%)	0.249 ± 0.03 (86.6%)

Values are expressed as means ± SEM. One-way ANOVA Tuckey Kramer post hoc test; $p < 0.001$.
Values given in parentheses indicate percent of inhibition.

as labdanyl-3α-ol-18-(3'''-methoxy-2'''-naphthyloate)-3α-L-arabinofuranosyl-(2'→1'')-α- L-arabinofuranoside (Fig. 1). This is a new labdanyl diarabinosidic naphthyl ester.

Nephrotoxicity study

The effect of the methanolic extract of *P. grande* fruits on gentamicin-induced renal toxicity in rats and normal rats was investigated. The renal effects of the extract were studied by monitoring blood levels of urea nitrogen and creatinine. The effect of an 8-day treatment of the methanolic extract of *P. grande* fruits (130 mg/kg BW) on the renal function was investigated in normal rats. No significant variation was determined in the test drug-treated animals compared with control animals.

Aminoglycoside antibiotics including gentamicin are widely used in the treatment of infections with Gram-negative bacteria. A major complication of the use of these drugs is nephrotoxicity. The effect of the extract of *P. grande* fruits on renal functions was examined in the gentamicin nephrotoxicity model. The daily subcutaneous administration of gentamicin at 100 mg/kg BW for 5 days caused renal dysfunction as evidenced by an increase in blood urea (192.8%) and serum creatinine (130.5%). Administration of a low dose (60 mg/kg BW) (oral route) of *P. grande* fruit extract along with gentamicin subcutaneously prevented the rise in blood urea nitrogen (54.25%) and serum creatinine (96.7%). On the other hand, a higher dose retarded the rise of blood urea nitrogen (88.05%), but there was no alteration in serum creatinine. Naphthyl labdanoate diarabinoside **4** reduced the blood urea nitrogen by 75.18% and serum creatinine levels by 86.6% (Table II).

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