Received Date: 08-Aug-2016

Revised Date: 03-Dec-2016

Accepted Date: 05-Dec-2016

Article Type: Full Paper

# A New Iridoid Dimer and Other Constituents from the Traditional Kurdish Plant *Pterocephalus nestorianus* Nab.

By **Fuad O. Abdullah** *[a]), **Faiq H. S. Hussain**[a]), **Marco Clericuzio**[b]), **Alessio Porta**[c]), **Giovanni Vidari***[c])

[a]) Department of Chemistry, College of Science, Salahaddin University - Erbil, Iraq (F. O. A. e-mail: fuad.abdullah@su.edu.krd)

[b]) Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Via T. Michel 11, 15121 Alessandria, Italy

[c]) Centro CEMEC and Dipartimento di Chimica, Università di Pavia, Via Taramelli 12, 27100 Pavia, Italy (G. V. phone: +39-0382987322; fax: +39-0382987323; e-mail: vidari@unipv.it)

Accompanied by other rare compounds, a new iridoid dimer, named kurdnestorianoside (**1**), showing an unprecedented secologanol stereochemistry, has been isolated for the first time from the Kurdish medicinal plant *Pterocephalus nestorianus,* which is used in Kurdistan for treating oral diseases and inflammation. The structure of **1** was established from 1D and 2D NMR spectroscopic data. Kaempferol 3-*O*-(3″,6″-di-*O*-*E*-*p*-coumaroyl) β-D-glucopyranoside (**7**) showed a remarkable antiproliferative activity against several human tumor cell lines.

Keywords: *Pterocephalus nestorianus*; Dipsacaceae; iridoid dimers; antitumor activity; Kurdish medicinal plant.

**Introduction. –** The taxonomically very complex family of Dipsacaceae contains about 300 species of perennial and annual herbs and shrubs; most of them are widely distributed over the Mediterranean region and the Middle East, with about 20% growing in Asia and Africa [1]. The genus *Pterocephalus*, one of the most important genera of this family, comprises about 30 species which are usually found in sunny, dry, rocky crevices, mostly in Europe and Western Asia (Iran, Turkey) [2-5]. Nine species are native to the Kurdistan region of Iraq. These plants are widely used in folkloric medicines,

all around the world, due to different biological activities such as anti-inflammatory, analgesic, anti-hepatotoxic, anti-oxidant, antibacterial, spasmolytic, hemostatic, antiseptic and astringent properties. Moreover, dozens of bioactive compounds, including hydroxycinnamic acid esters, iridoids, phenolic glycosides, monoterpenoid glucoindole alkaloids, and lignans [6-12], triterpenoid saponins [13, 14], and flavonoid *C*-glycosides [15] have been isolated and identified from different *Pterocephalus* species.

*P. nestorianus* Nab.**,** locally known with the Kurdish name of "Lawa", is popularly employed in the herbal medicine for the treatment of oral diseases and inflammation. No phytochemical work has been reported so far on the non-volatile components of *P. nestorianus.*

**Insert Chemical structures 1-10 about here**

**Results and Discussion. –** *Phytochemical investigation*. *P. nestorianus* flowers were collected on Safeen Mountain of Iraqi Kurdistan [16] and extracted

with MeOH. After solvent evaporation, repeated MPLC chromatographic separation of the residue on reversed-phase columns afforded a new secologanin/loganin subtype bis-iridoid compound, called kurdnestorianoside (**1**), along with known loganic acid (**2**) [17], 3,5-di-*O*-caffeoylquinic acid (**3**) [18], chlorogenic acid (3-*O*-caffeoylquinic acid) (**4**) [19], luteolin-7-*O*-glucoside (**5**) [20], kaempferol-3-*O*-(3′′-*O*-acetyl-6′′-*O*-*E*-*p*-coumaroyl)-β-glucopyranoside (**6**) [21] and kaempferol 3-*O*-(3′′,6′′-di-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside (**7**) [22]. Likewise, loganin (**8**) [23] and cantleyoside (**9**)

[24] were separated from a MeOH extract of the roots (underground parts) of the same plant. Their structures were established by extensive 1D- and 2D-NMR experiments and, for known compounds, by comparison of their spectral data with those reported in literature.

Kurdnestorianoside (**1**) was obtained as a white amorphous solid. The molecular formula $C_{33}H_{48}O_{19}$ was deduced from the HRMS analysis ($M^+$ found at *m/z* 748.2799; calcd. 748.2790), as well as from carbon and proton counting in the NMR spectra. The IR spectrum indicated the presence of OH (3400 $cm^{-1}$) and unsaturated C=O (1705 and 1640 $cm^{-1}$) groups. Accordingtly, the UV spectrum exhibited a maximum at 235 nm. Acid hydrolysis of **1** afforded D-glucose, which was –identified by the positive sign of the rotatory power and coelution with a standard sample in TLC. Moreover, the NMR signals of **1** were fully consistent with two $\beta$-configured glucopyranose units (Table 1), as indicated by the two anomeric protons resonating as doublets ($J$ = 7.9 Hz) at $\delta$ 4.63 and 4.66, resp. The $^1$H and $^{13}$C NMR spectroscopic data of **1** (Table 1) displayed two distinct parts, indicated in Figure 1 as moieties A and B, resp., corresponding to a glucosylated loganin-like and a secologanin-like iridoids, resp.. The fused dihydropyran and cyclopentane ring iridoid structure A was defined by COSY cross-peaks (Figure 1) between the proton signals at $\delta$ 5.25 [H–(C1a)], 2.00 [H–(C9a)], 3.07 [H–(C5a)] and 7.36 [H–(C3a)], and between the protons at $\delta$ 2.00 [H– (C9a)], 1.84 [H–(C8a)], 1.07 [$CH_3$(10a)], 4.01 [H–(7a)], 1.59 and 2.21 ($CH_2$(6a)], and 3.07 [H–(C5a)]. Likewise, the secoiridoid proton sequences emerged from the correlations between signals at $\delta$ 5.52 [H–(C1b)], 2.67 [H–(C9b)], 3.14 [H–(C5b)] and 7.57 [H–(C3b)],

between signals at $\delta$ 3.14 [H–(5b)], 1.65-1.80 [$CH_2$(6b)], 4.34 and 4.43 [$CH_2$(7b)], and between protons at $\delta$ 2.67 [H–(C9b)], 5.52 [H–(C8b)], 5.24 and 5.28 [$CH_2$(10b)], resp. (Figure 1). DEPT and HSQC spectra allowed the assignment of the signals of all the individual protonated carbons, including a methoxy group at $\delta_C$ 51.6 (Table 1).

The two glucosyl units were linked, through HMBC correlations (Figure 1), to the anomeric carbons C1a ($\delta_C$ 97.7) and C1b ($\delta_C$ 98.0), resp., of the iridoid and secoiridoid aglycones, resp. The position of two ester carbonyl groups at C11a and C11b of the iridoid A and secoiridoid B parts, resp., were firmly supported by the long-range correlations of H–(C3a) ($\delta$ 7.36) with C4a ($\delta_C$ 114.0) and C11a ($\delta_C$ 168.5), and those of H–(C3b) ($\delta$ 7.57*)* with C4b ($\delta_C$ 106.0) and C11b ($\delta_C$ 169.5), resp. Moreover, the methoxy group was attached to C11b thanks to the cross-peak in the HMBC spectrum between the O*Me* signal at $\delta$ 3.64 and the carbon resonance at $\delta_C$ 169.5 (C11b). Unit A was finally identified as a loganic acid ester derivative for the almost complete coincidence of its $^1$H and $^{13}$C NMR data with those of loganic acid ethyl ester [25] and, more closely, with the moiety A of the semisynthetic iridoid dimer **10** (Table 1), obtained from depresteroside by alkaline hydrolysis [26a]. This comparison, as well as the analysis of NMR coupling constants and NOESY experiments (Figure 2), fully supported the relative configuration of the moiety A in **1**, as shown in the formula. As far as the second half-unit B of **1** is concerned, the 1D NMR (Table 1) and HMBC data (Figure 1) were comparable with those of 7-acylsecologanoside derivatives [27], such as the unit B in the iridoid dimer **10** [26a]. However, significant differences observed for several $^1$H and $^{13}$C NMR

data of **1** and **10** (Table 1) strongly suggested that these two bis-iridoids were stereoisomers.

The relative configuration of unit B in **1** was eventually determined by analysis of the NMR coupling constants and confirmed by NOESY experiments (Figure 2). Specifically, a *J* (1b,9b) value of 1.6 Hz suggested a *cis*-relationship between H–(C1b) and H–(C9b). Actually, according to the literature, in the $^{1}$H NMR spectra, recorded in $CD_3OD$, of several secologanol derivatives, such as iridoid **10**, the vicinal coupling constant for *trans*-oriented H–(C1) and H–(C9) assumes a typical value in the range of 5.4 – 8.5 Hz [6, 8, 9a, 26-30]. Moreover NOESY cross-peaks (Figure 2) between H–(C5b) ($\delta$ 3.14) and H–(C9b) ($\delta$ 2.67), as well as between H–(C9b) and H–(C1b) ($\delta$ 5.52), revealed that these hydrogens were on the same side ($\beta$) of the molecular plane of **1**, while the absence of a NOESY correlation between H–(C8b) and H–(C1b) strongly suggested they were *trans*-oriented on the dihydropyran ring. The downfield chemical shift of the $CH_2$ (7b) group ($\delta$ 4.30–4.45; $\delta_C$ 69.7) indicated that the moieties A and B of **1** were linked together through an ester bond formed by the carboxylic group at C11a and the hydroxylic group at C7b. From the above data, the structure **1** was assigned to the new bis-iridoid kurdnestorianoside isolated from *P. nestorianus*.

Contrary to kurdnestorianoside (**1**), cantleyoside (**9**) [24], contained the secoiridoid moiety of the dimeric structure as the acylating group of the OH-(C7) group of the loganin unit. This bis-iridoid has been found in other extracts of *Pterocephalus* species, from which the corresponding alkyl hemiacetal and acetal artifacts have also been isolated, due to addition of the

alcohol used for extraction to the formyl group [6, 8-10]. We found that the NMR spectra of **9** were quite different in $D_2O$ and in $CD_3OD$, although the occurrence of multiple signals in both solvents revealed the presence of different chemical entities engaged in chemical equilibria [1]). In $D_2O$, the presence of about 28% hydrated form **9a** in equilibrium with about 72% free aldehyde **9** was indicated by the co-occurrence, in the $^1$H NMR spectrum, of a broad singlet at $\delta$9.57 assigned to C*H*O of **9** and a singlet at $\delta$7.43 attributable to H–(C3b) of **9a**, having intensities in a ratio of about 2.6:1. Likewise, the $^{13}$C NMR spectrum showed a series of low-intensity signals, attributed to **9a**, resonating near stronger signals assignable to **9** (Table 1). In particular, the occurrence of a *C*H signal at $\delta_C$ 90.2 was strongly indicative of the presence of the hydrated form **9a**

Similarly, analysis of the spectral data of **9** in $CD_3OD$ (Table 1) revealed an equilibrium between about 25% free aldehyde and a 75% mixture of diastereomeric hemiacetals at C7b (**9b**).

[1]) Cantleyoside (**9**) and its equilibria in $D_2O$ and in $CD_3OD$ have not yet been fully characterized spectroscopically [24].

Actually, the ESIMS (positive ion mode) spectrum of **9**, dissolved in MeOH, showed two pseudomolecolar ion peaks at *m*/*z* 769 and 801, attributed to the $[M+Na]^+$ ion peaks of free aldehyde **9** and methyl hemiacetals **9b**, resp. Moreover, the $^1$H NMR spectrum of **9** in $CD_3OD$ displayed a triplet ($J$ = 1.4 Hz) at $\delta$9.70, attributable to the aldehydic proton of **9**, and a double doublet ($J$ = 6.5, 5.3 Hz) at $\delta$4.65, assignable to the hemiacetalic proton of **9b** [8]. The

$^{13}$C NMR spectrum showed a series of low intensity peaks, including a quaternary carbon for a formyl group ($\delta_C$ 200.0), attributable to the free aldehyde **9**, and several pairs of higher peaks, in a ratio of about 1:1, due to the presence of diastereomeric hemiacetals **9b** (Table 1). In addition, the NMR spectra were fully consistent with that of cantleyoside methyl-hemiacetal reported in the literature [8].

*Tumor cell antiproliferative activity of kaempferol derivatives* **6** *and* **7.** The effects of kaempferol glucosides **6** and **7** on the proliferation of human cells were evaluated in comparison to the well-known chemotherapeutic agent *cis*-diamminedichloroplatinum (II) (cisplatin) by MTT assay. In particular, MCF7 and SkBr3 breast, IST-MES1 mesothelioma, A549 lung, BG-1 ovarian and Ishikawa endometrial cancer cells and human mammary MCF-10A epithelial cells were treated for 48 h with increasing concentrations of tested samples. The resulting $IC_{50}$ values (μM) (Table 2) were calculated by probit analysis ($P<0.05$, $\chi 2$ test). These data clearly indicated that the two flavonoids exhibited an antitumor activity from moderate, in case of **6**, to excellent for the dicoumaroyl derivative **7**. The latter activity was comparable, or even higher than that of cisplatin on several tumor cell lines. The remarkable higher activity of compound **7**, compared to **6**, was clearly due to the presence of two coumaroyl units instead of the only one present in **6**.

**Conclusions. –** Bis-iridoids characterize the Dipsacaceae family and may be considered chemotaxonomic markers also for the genus *Pterocephalus*. However, to our knowledge, this is the first report of a secoiridoid having a glycosylated anomeric OH–(C1) group which is *trans*-

oriented with respect to H–(C9). Moreover, all the mixed iridoid-secoiridoids previously isolated from *Pterocephalus* species [6, 8-10], have the secoiridoid moiety as the acylating group; in contrast, the iridoid moiety is the acylating unit in kurdnestorianoside **1,** which is characteristic of a few bis-iridoids isolated from *Gentiana* species [26, 30]. To the best of our knowledge luteolin-7-*O*-glucoside (**5**), kaempferol-3-*O*-(3′′-*O*-acetyl-6′′-*O*-*p*-coumaroyl)-β-glucopyranoside (**6**), and kaempferol-3-*O*-(3′′,6′′-di-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside (**7**) have been isolated for the first time from a *Pterocephalus* species. The presence of flavonoids and caffeoyl derivatives may well support the use of *P. nestorianus* as anti-inflammatory herbal remedy in Kurdish traditional medicine. In addition, kaempferol 3-*O*-(3′′,6′′-di-*O*-*E*-*p*-coumaroyl) β-D-glucopyranoside (**7**) showed a remarkable antiproliferative activity against several human tumor cell lines.

This study of the secondary metabolites of *P. nestorianus* is one of the first ones on medicinal plants growing on Safeen mountain [16] and represents an important step for more intensive investigations on the phytochemistry and pharmacology of the principles of the rather unexplored Kurdish traditional medicine [31].

**Acknowledgement. -** This work was supported by a generous grant from the Salahaddin University/Erbil-Iraq and the University of Pavia-Italy, in the framework of a split-side Ph.D. program between the two universities. The authors are grateful to Abdullah Shakur Sardar for plant identification.

## Experimental Part

*General*. For most general experimental techniques and procedures see reference [32]. $^{1}$H-NMR and $^{13}$C_NMR chemical shifts (δ, ppm) are relative to deuterated MeOH signals at $\delta$ 3.27 (central line of a quintuplet) and at $\delta_C$ 49.0 (central line of a septuplet), resp. Preparative MPLC separations were carried out on a Biotage Isolera instrument.

*Plant material.* Flowers and roots of *P. nestorianus* Nab., collected in June 2014 from Safeen Mountain [16] located about 50 kilometers northeast of Erbil-Iraq (GPS coordinates: latitude 36° 22' 24; longitude 44° 18' 25"), were authenticated by Al-Khayat (Department of Biology, Salahaddin University). A voucher specimen (n$^{o}$ 6253) has been deposited at the Herbarium of the Department of Biology, Salahaddin Salahaddin at Erbil.

*Extraction and isolation from flowers*. Freshly collected flowers were air-dried in the shade at room temperature for one week, and then ground, using an electrical mill. The powder (100 g) was defatted and freed of most chlorophyll by soaking in *n*-hexane (3× 350 ml) with occasional shaking in an ultrasonic bath for 30 min; subsequently, the mixture was left in the same solvent at rt for 24 h, and then it was filtered. The residue was macerated in EtOAc (3×400 ml) with occasionally stirring in an ultrasonic bath for 30 min, then it was left in the same solvent at rt for 24 h. The combined EtOAc extracts were filtered from some insoluble material and then evaporated *in vacuo* at 25-30$^{o}$C to give a yellowish residue (A, 3.75 g). The material insoluble in EtOAc was then taken on in MeOH (3×450 ml); filtration and

evaporation of the combined alcoholic solutions afforded a dark-brown residue (B, 17.5 g). A and B were stored at -20°C. Chlorophyll was then completely removed from A and B by dissolving separate batches (1 g each) of the two residues in 50 ml of MeOH-$H_2O$, 90:10, and passing the solution through a SPE column, fitted with RP-18 phase (100 g). Elution was carried out with 50 ml of MeOH-$H_2O$, 90:10, and then with 100 ml of $Me_2CO$ before the successive separation. A total of 3.26 g and 16.5 g of chlorophyll-free extracts A1 and B1 were recovered. Subsequently, a sample of A1 (1 g) was separated (column A) by MPLC on a RP-18 column (100 g), eluted with a MeOH-$H_2O$ gradient, from 40:60 to 100:0 in 67.4 min; flow rate: 30 ml/min; volume of each collected fraction: 15 ml. After TLC analysis, original fractions were collected in 15 main fractions (EF1-EF15). Purification of fraction EF4 by exposing the residue to MeOH, followed by $Me_2CO$, gave luteolin-7-*O*-glucoside (**5**) (8 mg), identical with the literature [20]. A sample (10 g) of residue B1 was initially fractionated by repartition between $H_2O$ and, in the order, $CH_2Cl_2$, EtOAc, and *n*-BuOH to afford, after evaporation of the organic solutions, three residues $B1_A$ (0.2 g), $B1_B$ (1.44 g), and $B1_C$ (2.98 g), resp. $B1_B$ was further repartitioned between $H_2O$ and $CH_2Cl_2$, followed by $Et_2O$, to give, after evaporation, residues $B1_B$-1 (48 mg) and $B1_B$-2 (165 mg), resp. MPLC separation of $B1_B$-2 on a RP-18 column (25 g), eluted with a MeCN-$H_2O$ gradient, from 0:100 to 100:0, afforded fifteen fractions (Et1- Et15). Separate crystallisations of Et12 and Et12 from MeOH gave kaempferol 3-*O*-(3’’-*O*-acetyl-6’’-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside (**6**) (7.9 mg) [22] and kaempferol 3-*O*-(3″, 6″-di-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside (**7**) (13.3 mg) [23], resp., identical with the literature. MPLC chromatography of Et6

(27.3 mg on a RP-18 column (12 g), eluted with a MeOH-$H_2O$ gradient afforded 3, 5-di-*O*-caffeoylquinic acid (**3**) (19 mg), identical with the literature [18]. $B1_C$ was further partitioned between $H_2O$ and, in the order, $CH_2Cl_2$, EtOAc, and *n*-BuOH to afford, after evaporation of the organic solvents, residues $B1_C$-1 (30 mg), $B1_C$-2 (557 mg) and $B1_C$-3 (1.203 g), resp. MPLC separation of a sample of $B1_C$-2 (90 mg) on a RP-18 column (12 g), eluted with a MeOH gradient in $H_2O$ afforded chlorogenic acid (**4**) (10 mg), identical with the literature [19]. MPLC separation of a sample (620 mg) of $B1_C$-3 on a RP-18 column (100 g), eluted with a MeOH-$H_2O$ gradient, from 10:90 to 100:0, afforded 13 fractions ($B1_C$-3/1 – $B1_C$-3/13). MPLC separations of $B1_C$-3/7 (36.3 mg) and $B1_3$-3/10 (20 mg) on two different RP-18 columns (12 g each), eluted with a MeOH-$H_2O$ gradient, from 05:95- to 100:0, gave loganic acid (**2**) (3 mg), identical with the literature [17] and kurdnestorianoside (**1**) (5 mg), resp.

***Kurdnestorianoside (1)***. Pale-yellow powder; $[\alpha]^{23}{}_D = -57$ (c, 0.002, MeOH); $R_f$ 0.61 (MeOH: $H_2O$, 1:1); $\lambda_{max}{}^{MeOH}$ nm (log ε) 235 (4.15); IR (KBr) 3400, 1705, 1640, 1435, 1305, 1070, 1035, 855 $cm^{-1}$. Dark reddish brown spot on TLC plate under UV light; positive reaction to vanillin-sulphuric acid reagent. $^1H$ NMR (300 MHz, $CD_3OD$) and $^{13}C$ NMR (75 MHz $CD_3OD$) spectra: Table 1. MS (ESI, positive ion mode): *m/z* 771.17 [M + $Na^+$]. HRMS: [$M^+$] calcd for $C_{33}H_{48}O_{19}$: *m/z* 748.2790; found: *m/z* 748.2799.

*Acidic hydrolysis of **1***. Compound **1** (1.5 mg) was dissolved in 2 M HCl in a sealed vial and heated at 100°C for 45 min. After cooling to rt and extraction with $CHCl_3$, the aqueous layer was repeatedly evaporated to dryness

with the aid of MeCN. The residue, which showed positive optical rotation, was identified as glucose by TLC [33], upon comparison with an authentic sample of D-(+)-glucose.

*Extraction and isolation from underground parts*. Powdered roots (underground parts) of *P. nestorianus* (100 g) were defatted by soaking in *n*-hexane (3×400 ml) with occasional shaking in an ultrasonic bath for 30 min; then the biomass was left in the same solvent at rt for 24 h. After filtration, the biomass was macerated in EtOAc (3×500 ml) with occasional stirring in an ultrasonic bath for 30 min, then it was left at rt for 24 h. Filtration of the combined EtOAc extracts from some insoluble material and evaporation *in vacuo* at 25-30$^{o}$C gave a yellowish residue (C, 0.63 g). The fraction insoluble in EtOAc was then taken on in MeOH (3×600 ml); filtration and evaporation of the alcoholic solution afforded a dark-brown residue (D, 9.25 g). C and D were stored at -20$^{o}$C. Subsequently, residue D was fractionated by repartition between $H_2O$ and, in the order, $Et_2O$, EtOAc, and *n*-BuOH to afford, after evaporation of the organic solvents and $H_2O$, residues $D_A$ (0.4 g), $D_B$ (0.28 g), $D_C$ (4.45 g), and $D_D$ (4.1 g), resp. MPLC separation of a sample of $D_C$ (1 g) on a RP-18 column (100 g), eluted with a MeOH-$H_2O$ gradient, from 0:100 to 100:0, gave 12 main fractions ($D_C$/1–$D_C$/12), after TLC analysis of initially collected fractions (12 ml each). MPLC separation of $D_C$/7 (150 mg) on a RP-18 column (12 g), eluted with a MeOH-$H_2O$ gradient, from 0:100 to 100:0, afforded 7 subfractions ($D_C$/7-1 – $D_C$/7-7). $D_C$/7-2 was constituted by loganin (**8**) (5 mg), identical with the literature [23]. Preparative TLC of a sample of $D_C$/7-6 (30 mg) on an analytical C-18 plate, eluted with MeOH-$H_2O$, 1:1,

afforded cantleyoside (**9**) (20 mg), identical with the literature [24].

*Antiproliferative activity*. *Cell cultures*. MCF-7 breast and A549 lung cancer cells were maintained in DMEM/F-12 supplemented with 10% fetal bovine serum (FBS), 100 μg/ml penicillin/streptomycin (Life Technologies, Milan, Italy). SkBr3 breast and BG-1 ovarian cancer cells were cultured in RPMI-1640 and DMEM medium resp., without phenol red supplemented with 10% FBS and 100 μg/ml penicillin/streptomycin (Life Technologies, Milan, Italy). Ishikawa endometrial cancer cells were maintained in MEM supplemented with 10% FBS, 100 μg/ml penicillin/streptomycin, 2mM L-glutamine and 1% Non-Essential Amino Acids Solution (Life Technologies, Milan, Italy). IST-MES1 malignant mesothelioma cells were grown in Nutrient Mixture F-10 Ham (Ham's F-10) medium supplemented with 10% FBS and 100 μg/ml penicillin/streptomycin. Human mammary MCF-10A epithelial cells were cultured in DMEM/F-12 medium supplemented with 5% Horse Serum (Eurobio, Les Ullis, Cedex, France) and 0.1 nmol/l nonessential amino acid, 2 mmol/l L-glutamine, and 50 units/ml penicillin/streptomycin. All cell lines were obtained by ATCC and used less than 6 months after resuscitation, except IST-MES1 cells which were kindly provided by Dr. Orengo (Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy).

*Inhibtion of cell proliferation*. The effects of kaempferol derivatives **6** and **7** on cell viability were determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, which is based on the conversion of MTT to MTT-formazan by mitochondrial enzyme [34]. Cisplatin was used as the reference compound. Cells were seeded in quadruplicate in 96-well

plates in regular growth medium and grown until 70% confluence. Cells were washed once they had attached and then treated with increasing concentrations of each compound for 48 h in regular medium supplemented with 1% FBS. Relative cell viability was determined by MTT assay according to the manufacturer's protocol (Sigma-Aldrich, Milan, Italy). Mean absorbance for each sample dose was expressed as percentage of the cells treated with vehicle absorbance and plotted versus sample concentration. $IC_{50}$ values were calculated by probit analysis ($P < 0.05$, $\chi 2$ test) and are expressed in μM.

## References


[1] a) R. Verlaque, 'A biosystematic and phylogenetic study of the Dipsacaceae', in 'Plant Biosystematics', Ed. W. Grant, Academic Press, Toronto, 1984, pp. 307-320, 1984; b) S. E. Carlson, V. Mayer, M. J. Donoghue, *Taxon* **2009**, *58*, 1075.

[2] K. A. Shekha, 'Systematic Study of the Genus *Pterocephalus* Vaill ex Adans (Dipsacaceae) in Kurdistan of Iraq', M.Sc. thesis, Salahaddin University at Erbil, Iraq, 2007.

[3] A. Ghahreman, 'Flora of Iran'. Research Institute of forest and rangelands, Tehran University Press, Tehran, 1995.

[4] V. Hooshang, N. Maliha, L. Jalil, H. Majid, *J. Med. Plants Res.* **2011**, *5*, 5646.

[5] A. Fahem, M. Maha, A. Ahmed, K. Adel, *J. Arab Soc. Med. Res.* **2008**, *3*, 83.

[6] K. Graikou, N. Aligiammis, I. B. Chinou, C. Harvala, *Z. Naturforsch. C Bio. Sci.* **2002**, *57*, 95.

[7] K. Graikou, N. Aligiannis, I. B. Chinou, *Biochem. Syst. Ecol.* **2006**, *34*, 438.

[8] D. Gülcemal, M. Masullo, O. Alankus-Caliskan, T. Karayildirim, S. G. Senol, S. Piacente, E. Bedir, *Magn. Reson. Chem.* **2010**, *48*, 239.

[9] (a) Y. Wu, Y. Yin, Y. Li, F. Guo, G. Zhu, *Magn. Reson. Chem.* **2014**, *52*, 734; (b) Y.-C. Wu, C.-X. Guo, Y.-Z. Zhu, Y.-M. Li, F.-J. Guo, G.-F. Zhu, *Fitoterapia* **2014**, *98*, 104.

[10] J. Tian, F. Wu, M. Qiu, R. Nie, *Yunnan Zhi Wu Yan Jiu* **1995**, *17*, 108.

[11] J. Zhang, S. Huang, L. Shan, L. Chen, Y. Zhang, X. Zhou, *Youji Huaxue* **2015**, *35*, 2441.

[12] Y.-C. Wu, Y.-J. Ying, F.-J. Guo, G.-F. Zhu G-F, *Biochem. Syst. Ecol.* **2014**, *56*, 209.

[13] J. Tian J, F. E. Wu, M. H. Qiu, R. L. Nie, *Phytochemistry* **1993**, *32*, 1535.

[14] J. Tian, F. E. Wu, M. H. Qiu, R. L. Nie RL, *Phytochemistry* **1993**, *32*, 1539

[15] F. A. Ahmed, A. A. Shahat AA, *Nat. Prod. Commun* **2006**, *1*, 457.

[16] F. O. Abdullah, F. H. S. Hussain, A. S. Sardar, P. Vita-Finzi, G. Vidari, *Nat. Prod. Commun*. **2016**, in press.

[17] a) H. Tomita, Y, Mouri, *Phytochemistry* **1996**, *42*, 239; b) I. Calis, M. F. Lahloub, O. Sticher, *Helv. Chim. Acta* **1984**, *67*, 160.

[18] T. Tatefuji, N. Izumi, T. Ohta, S. Arai, M. Ikeda, M. Kurimoto, *Biol. Pharm. Bull*. **1996**, *19*, 966.

[19] I. Berregi, J. I. Santos, G. del Campo, J. I. Miranda, J. M. Aizpurua, *Anal. Chim. Acta* **2003**, *486*, 269.

[20] T. Iwashina, H. Setoguchi, J. Kitajima, *Bull. Natl. Mus. Nat. Sci., Ser. B* **2011**, *37*, 87.

[21] Y.-Q. Wu, Y. Li, X.-F. Lin, Z.-L. Liu, Y. Li, *Pharmazie* **2003**, *58*, 833.

[22] H. Liu, J. Orjala, O. Sticher, T. Rali, *J. Nat. Prod*. **1999**, *62*, 70.

[23] a) H. Kawai, M. Kuroyanagi, A. Veno, *Chem. Pharm. Bull*. **1988**, *36*, 3664; b) S. J. Qin, H. J. Li, P. Li, D. Tang, *Chin. Pharm. J*. **2008**, *43*, 662; c) D. Prasad, V. Juyal, R. Singh, V. Singh, G. Pant, M. S. M. Rawat, *Fitoterapia* **2000**, *71*, 420; d) S. Damrofr, S. R. Jensen, B. J. Nielsen, *Phytochemistry* **1981**, *20*, 2717; e) reference [18b].

[24] S. R. Jensen, S. E. Lyse-Petersen, B. J. Nielsen, *Phytochemistry* **1979**, *18*, 273.

[25] D. Ji, C. Zhang, J. Li, H. Yang, J. Shen, Z. Yang, *Molecules* **2012**, *17*, 1419.

[26] a) A. J. Chulia, J. Vercauteren, M. Kaouadji, *Phytochemistry* **1994**, *36*, 377; b) A. J. Chulia, J. Vercauteren, A. M. Mariotte, *Phytochemistry* **1996**, *42*, 139.

[27] E. Mpondo Mpondo, J. Garcia, J. Lestani, *J. Nat. Prod.* **1989**, *52*, 1146.

[28] L.-C. Zou, T. F. Zhu, H. Xiang, L. Yu, Z.-H. Yan, S.-C. Gan, D.-C. Wang, S. Zeng, X.-M. Deng, *Molecules* **2008**, *13*, 2049, and references cited therein.

[29] Tomassini L, Foddai S, Serafini M, Cometa MF, *J. Nat. Prod.* **2000**, *63*, 998.

[30] I. Calis, T. Ersöz, A. J. Chulia, P. Rüedi, *J. Nat. Prod.* **1992**, *55*, 385.

[31] H. I. M. Amin, M. F. Ibrahim, F. H. S. Hussain, A. S. Sardar, G. Vidari, *Nat. Prod. Commun*. **2016**, *11*, 291.

[32] G. Gilardoni, X. Chiriboga, P. Vita Finzi, G. Vidari, *Chem. Biodiv.* **2015**, *12*, 946.

[33] S. A. Hansen, *J. Chromatogr*. **1975**, *107*, 224.

[34] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.

*Table 1. NMR data of compounds* **1**, **9**, **9a**, **9b** and **10**

| H/C | **1** | **10*** | **1** | **10*** | **9** | **9a** | **9b** |
|---|---|---|---|---|---|---|---|
| | $^{1}$H-NMR$^{\dagger,\ddagger}$ | | $^{13}$C-NMR$^{\P,\phi}$ | | $^{13}$C-NMR$^{\S,\phi}$ | $^{13}$C-NMR$^{\P,\phi,\pm}$ | |
| *Iridoid moiety* A | | | | | | | |
| Aglycone | | | | | | | |
| 1a | 5.25 *d* (4.5) | 5.29 *d* (4) | 97.7 | 97.7[a] | 97.2 | 97.2 | 97.5 |
| 3a | 7.36 *d* (1.1) | 7.38 *d* (1.5) | 152.1 | 152.0 | 152.0 | 152.0 | 152.6 |
| 4a | — | — | 114.0 | 114.3 | 113.2 | 113.3 | 113.3 |
| 5a | 3.07 *dddd* (9.2, 7.9, 7.6, 1.1) | 3.10 *m* (9.5, 8, 7.7, 1.5) | 32.2 | 32.0 | 31.0 | 30.9 | 32.6 |
| 6a | β 2.21 *ddd* (13.9, 7.9, 1.3) α 1.59 *ddd* (13.9, 7.6, 5) | 2.23 *m* (14, 8, 1.5) 1.66 *m* (14, 7.5, 5) | 42.7 | 42.8 | 39.1 | 37.5 | 40.3 |
| 7a | 4.01 *br t* (4.6, 1.3) | 4.04 *ddd* (5, 5, 1.5) | 75.1 | 75.1 | 79.1 | 79.1 | 78.3[a] |
| 8a | 1.84 *m* (9.2, 6.9, 4.6) | 1.86 *m* (9, 7, 5) | 42,2 | 42.1 | 40.0 | 40.0 | 40.8 |
| 9a | 2.00 *td* (9.2, 4.5) | 2.04 *ddd* (9.5, 9, 4) | 46.5 | 47.7 | 46.1 | 46.1 | 47.1 ; 47.2 |
| 10a | 1.07 *d* (6.9) | 1.09 *d* (7) | 13.4 | 13.2 | 12.9 | 12.9 | 13.8 ; 13.9 |
| 11a | — | — | 168.5 | 169.1 | 170.7 | 170.7 | 169.4 |
| OMe | — | — | — | — | 52.6 | 52.6 | 51.8 |
| Sugar | | | | | | | |
| 1a’ | 4.63 *d* (7.9) | 4.66[a] *d* (8) | 99.7 | 100.2 | 99.4[a] | 99.4[a] | 100.2[b] |
| 2a’ | 3.16 *dd* (8.9 7.9) | *ca* 3.20-3.40 *m* | 74.7 | 74.9 | 73.3[b] | 73.3[b] | 74.7[c] |
| 3a’ | 3.25-3.40 *m* | | 77.9[a] | 79.1 | 76.3[c] | 76.3[c] | 77.9 |
| 4a’ | | | 71.5[b] | 71.8 | 70.3 | 70.3 | 71.6 |
| 5a’ | | | 78.4 | 79.4 | 77.1 | 77.1 | 78.4[a] |
| 6a’ | 3.88 *dd* (12, 1.7) 3.66[a] dd (12, 5.7) | 3.91[b] *dd* (12, 2) 3.69[c] *dd* (12, 6) | 62.8[c] | 62.8 | 61.4 | 61.4 | 62.7[d] |
| *Secoiridoid moiety* B | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Aglycone | | | | | | | |
| 1b | 5.52 *d* (1.6) | 5.54 *d* (6) | 98.0 | 97.8[a] | 97.7 | 98.1 | 97.8 ; 97.9 |
| 3b | 7,57 *d* (2,5) | 7.46 *d* (1) | 153.9 | 153.6 | 154.3 | 153.4 | 153.1 ; 153.3 |
| 4b | — | — | 106.0 | 111.5 | 109.7 | 111.4 | 112.2 |
| 5b* | 3.14 *m* | 2.92 *m* (7.5, 6.5, 5.5, 1) | 28.4 | 30.8 | 28.0 | 29.3 | 29.6 ; 29.8 |
| 6b | 1.65-1.80 *m* | 2.09 *m* (14, 6.5. 6,.5) 1.77 *m* (14, 7.5, 7.5, 6) | 25.9 | 30.0 | 45.0 | 45.0 | 37.5 : 37.8 |
| 7b | 4.43 *ddd* (11.1, 4.0, 2.4) 4.34 *td* (11,1, 3.0) | 4.20 *ddd* (11, 6, 5) 4.09 *ddd* (11, 7.5, 6) | 69.7 | 63.4 | 207.3 | 90.2 | 98.2 ; 98.5 |
| 8b | 5.52 *ddd* (17, 10, 9.5) | 5.76 *ddd* (16, 10.5, 8.5) | 133.3 | 135.6 | 133.7 | 134.4 | 135.8 ; 135.9 |
| 9b | 2.67 *ddd* (9.5, 5.5, 1.6) | 2.68 *ddd* (8.5, 6, 5.5) | 43.8 | 45.2 | 44.4 | 44.1 | 45.4 ; 45.5 |
| 10b | 5.28 *dd* (17, 1.7) 5.24 *dd* (10, 1.7) | 5.27 *dd* (16, 1.5) 5.25 *dd* (10.5. 1.5) | 120.8 | 119.6 | 121.5 | 120.7 | 119.7 |
| 11b | — | — | 169.5 | 169.0 | 169.2 | 169.7 | 168.4 |
| MeO | 3.64 *s* | 3.67 *s* | 51.6 | 51.8 | — | — | — |
| $CD_3O$ | — | — | — | — | — | — | 49.6 ; 49.9 |
| Sugar | | | | | | | |
| 1b' | 4.66 *d* (7.9) | 4.69[a] *d* (8) | 100.0 | 100.2 | 99.5[a] | 99.5[a] | 100.1[b] |
| 2b' | 3.16 *dd* (8.9 7.9) | *ca* 3.20-3.40 *m* | 74.7 | 74.9 | 73.4[b] | 73.4[b] | 74.4[c] ; 74.8[c] |
| 3b' | 3.25-3.40 *m* | | 78.0[a] | 79.1 | 76.4[c] | 76.4[c] | 77.9 |
| 4b' | | | 71.6[b] | 71.8 | 70.3 | 70.3 | 71.6 |
| 5b' | | | 78.4 | 79.4 | 77.1 | 77.1 | 78.4[a] |
| 6b' | 3.88 *dd* (12, 1.7) 3.64[a] *dd* (12, 5.7) | 3.88[b] *dd* (12, 1.5) 3.64[c] *dd* (12, 5.5) | 62.7[c] | 62.8 | 61.4 | 61.4 | 62.8[d] |

nce [26a]; [†]in $CD_3OD$ ($\delta$ 3.27); [‡]at 300 MHz for **1**; at 400 MHz for **10**; [¶]in

$CD_3OD$ ($\delta$ 49.0); [§]in $D_2O$ and 1,4-dioxane-$d_8$; [ϕ]at 75 MHz for **1**, **9**, **9a**, **9b**; at 50 MHz for **10**; [a-d]values with the same superscript in the same column are interchangeable; [±]doubled signals are due to the presence of two diastereomeric hemiacetals at C-7b.

*Table 2. Antiproliferative activity ($IC_{50}$)[a] of kaempferol derivatives* **6** and **7** *against human cell lines*.

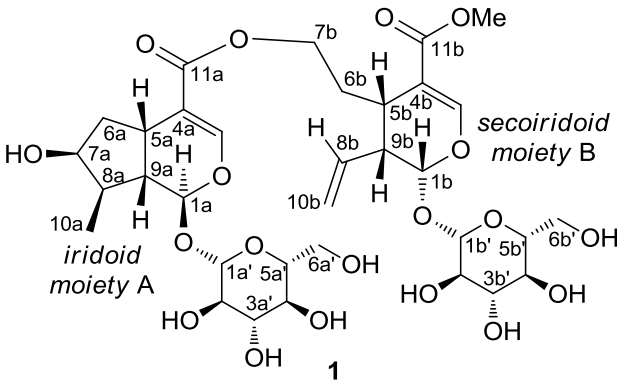
| Tested sample | MCF-7 | SkBr3 | IST-MES1 | A-549 | BG-1 | Ishikawa | MCF-10A |
|---|---|---|---|---|---|---|---|
| Compound **6** | 20 (±4) | 22 (±3) | 20 (±2) | 27 (±3) | 25 (±5) | 15 (±3) | >50 |
| Compound **7** | 2 (±1) | 4 (±1) | 3 (±2) | 6 (±2) | 3 (±1) | 4 (±2) | 5 (±2) |
| Cisplatin | 19 (±3) | 4 (±3) | 7 (±3) | 10 (±2) | 15 (±4) | 8 (±1) | 39 (±4) |

[a]$IC_{50}$ ± SD values are expressed in μM.

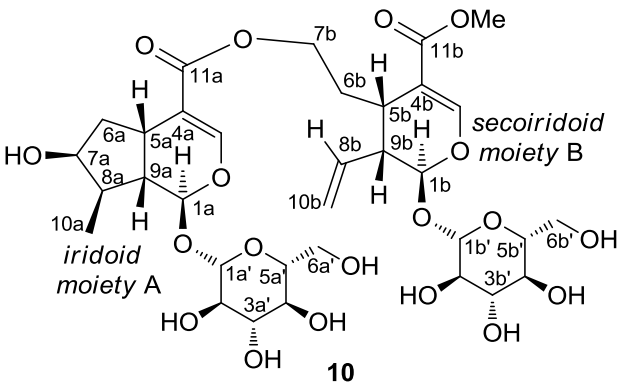
***Legends to Figures***

***Figure 1****. COSY and HMBC correlations of kurdnestorianoside (**1**).*

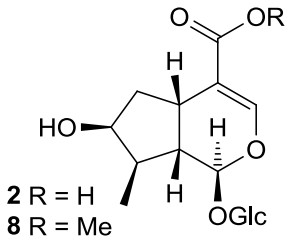
***Figure 2****. Significant NOESY correlations of kurdnestorianoside (**1**).*



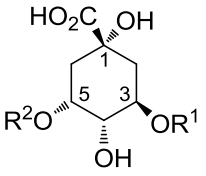

**1**



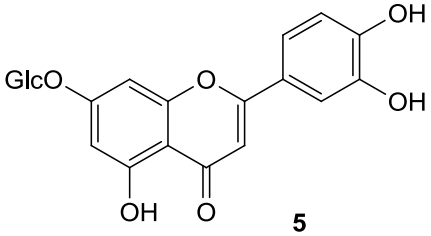

**10**



**2** R = H
**8** R = Me

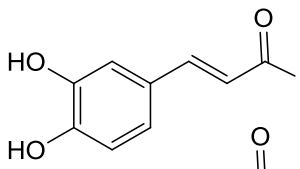


**3** $R^1 = R^2 = X$
**4** $R^1 = X$ $R^2 = H$

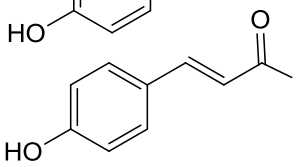


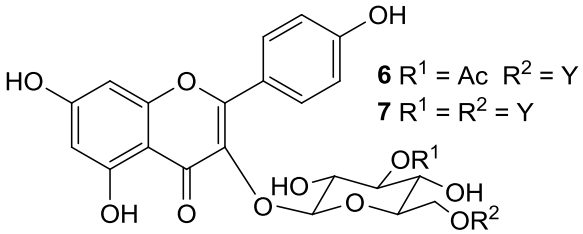
**5**

X =

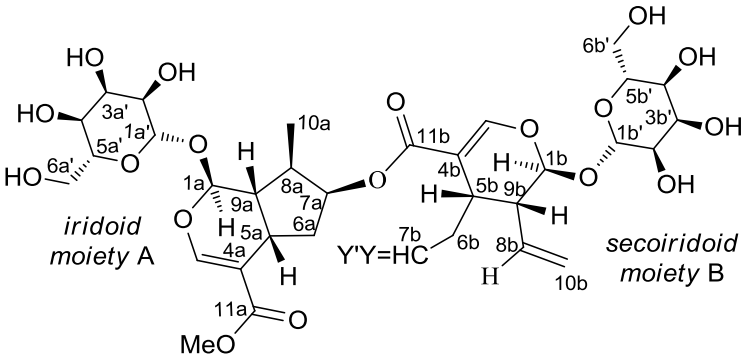


Y =





**6** $R^1$ = Ac $R^2 = Y$
**7** $R^1 = R^2 = Y$




**9** Y,Y' = O
**9a** Y = Y' = OD
**9b** Y= $OCD_3$ Y' = OD

***Chemical structures* 1-10**

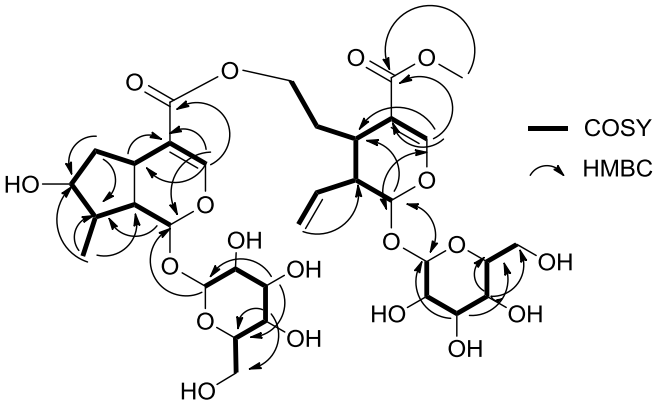



***Figure 1***

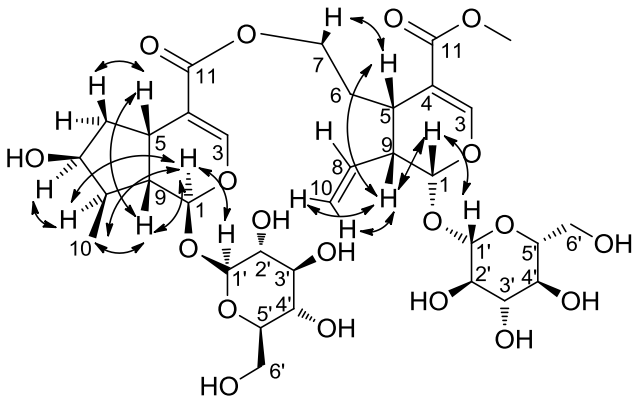


***Figure 2***