ISSN 0974 – 5211

Journal of Natural Products

Volume 3 (2010)

www.JournalofNaturalProducts.com

Research Paper

Flavanone Glycosides from *Gleditsia caspia*

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(Received 23 June 2009; Revised 26 June-23 July 2009; Accepted 08 August 2009)

ABSTRACT

Chemical investigation of *Gleditsia caspia* concentrate resulted in the isolation and identification of two new flavanone glycosides Gleditsin A; (2*S*)-5,7,4`-trihydroxy-flavanone-7-*O*-[*β*-D-xylopyranosyl-(1→2)]-*β*-D-glucopyranoside [1], and Gleditsin B; (2*S*)-5,7,3`,4`-tetrahydroxy-flavanone-7-*O*-[2,6-dimethyl-6-hydroxyl-2-*trans*-2,7-octadienoic acid (1→2)]-*β*-D-glucopyranosyl ester [2], as well as two known flavanone glycosides, naringenin-7-*O*-glucoside [3] and eriodictyol-7-*O*-glucoside [4]. Structures of the new compounds were established by spectroscopic analysis and chemical modification. The cytotoxic activities (ED_{50}) of these compounds on various tumor cell lines was evaluated and both compounds [1 and 2] significantly suppressed the growth of prostate carcinoma (DU 145) and colon colorectal adenocarcinoma (HRT-18), LS 174T, COLO 320DM) cell lines.

Keywords: *Gleditsia caspia*; *Leguminosae*; Cytotoxic flavanone glycosides; Gleditsin A and Gleditsin B

INTRODUCTRION

Gleditsia caspia Desf. (A subspecies of *Gleditsia horrida* Thunb., Family-*Leguminosae*) is a perennial shrub. In traditional Chinese medicine genus *Gleditsia* is used in the treatment of apoplexy, as an expectorant and for a pesticide (Jiangsu, et al., 1977). To our knowledge, this species has never been studied phytochemically. However, triterpenoidal saponins, steroids and flavonoids have been found in the genus *Gleditsia* (Zang, et al., 1999; Lim, et al., 2005; Yoshizaki, et al., 1977). We report here the isolation and structure elucidation of a new flavanone glycoside and flavanone glycoside acylated with one monoterpenic acid, along with two known flavanone glycosides. The structures of the new flavanones were established by extensive NMR studies, including DEPT, DQF-COSY, HMQC and HMBC experiments. The isolated compounds showed significant cytotoxic activity against various human tumor cell lines.

MATERIALS AND METHODS

***General Experimental Procedures*:** UV spectra were determined with a Hitachi 340 spectrophotometer, IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. CD data were obtained with a JASCO Model J-20 spectrometer. The ^{1}H- and ^{13}C-NMR measurements were obtained with a Bruker NM spectrometer operating at 600 and 400 MHz (for ^{1}H) and 100 MHz (for ^{13}C) in DMSO-d_6 solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135°). DQF-COSY, HMQC, and HMBC NMR experiments were carried out using a Bruker AMX-600 high field spectrometer equipped with an IBM Aspect-2000 processor and with software VNMR version 4.1 or NUTS program for NMR. HRFAB mass spectra were performed on a VGZAB-HF reversed geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer (MS) (VG Analytical, Inc.). ESIMS (positive and negative ion acquisition mode) was carried out on a TSQ700 triple quadrupole instrument (Finnegan, SanJose, CA, USA) mass spectrometer. Polyamide (ICN Biomedical), and Si gel (Si gel 60, Merck), were used for open column chromatography. Flash column liquid chromatography was performed using J.T. Baker glassware with 40 μm Si gel (Baker) and Sepralyte C_{18} (40 μm) as the stationary phase. TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 for 5 min, $FeCl_3$, and Pauly's reagents.

***Material and reagents used for cell culture and cytotoxic assays*:** human liver adenocarcinoma (NCI-H1755, SK-HEP-1), kidney carcinoma (A-498), kidney hypernephroma (SW 156), ovary adenocarcinoma (MDAH 2774, NIH:OVCAR-3, uterus mesodermal tumor (SK-UT-1), skin malignant melanoma (G-361, RPMI-7951, Hs 908.Sk), prostate carcinoma (DU 145) and colon colorectal adenocarcinoma (HCT-8 [HRT-18], LS 174T, COLO 320DM) cell lines were purchased from the American Type Culture Collection (ATTC). Dulbecco's Modified Eagle Medium (DMEM) was from (Gibco, Grand Island NY, USA). Eagle Minimum Essential Medium (EMEM) and Roswell Park Memorial Institute (RPMI) 1640 medium were from (Nissui Pharm. Co., Ltd., Tokyo, Japan). Flat-bottom plates, 96 well were from (Iwaki Glass Co., Ltd., Fumabashi-Chiba-Ken, Japan). (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl tetrazolium bromide) (MTT), for colorimetric assay was from Sigma (St. Louis, Mo., USA).10 % Fetal Bovine serum (FBS) was from (Gibco Br L, Rockville, MD, USA). All other chemicals used were of analytical reagent grade.

***Plant Material*:** The fruits of *G. caspia* Desf., were collected from Al-Orman garden, Giza, Egypt in April 2002 and were identified by late professor Nabil El-Hadidy (Faculty of Science, Cairo University, Egypt). A voucher specimen (f202) has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

***Extraction and Isolation*:** The powdered fruits (2.25 Kg) of *G. caspia* were subjected to exhaustive extraction with 95% EtOH (4x8L). The alcoholic extract was concentrated (205 g), suspended in H_2O, and then partitioned successively with pet. Ether (10 g), EtOAc (8.2 g) and *n*-BuOH (34 g).The *n*-BuOH-soluble fraction was applied to a column of Polyamide and washed with H_2O and 25, 50, 75, and 100% MeOH. The 25% MeOH fraction (1.5 g) was repeatedly chromatographed over Si gel, Si gel flash and Sepralyte C_{18} flash columns to give two fractions of A (60 mg) and B (428 mg). Fraction A (60 mg) was chromatographed over Si gel flash and finally purified with Sephadex LH 20 columns to afford compound 1 (19 mg). The EtOAc

fraction (8.2 g) was applied to Si gel column to give three fractions of A (600 mg), B (1.53 g) and C (3.72 g). Fraction C (3.72 g) was repeatedly chromatographed over Si gel flash and and finally purified with Sephadex LH 20 columns to give compounds 2 (35 mg), 3 (58 mg) and 4 (1.60 g).

Gleditsin A (**1**): a pale yellow needles [MeOH]; CD (MeOH, *c* 0.1): $[\theta]_{332}$ +5650, $[\theta]_{285}$ -24.350; UV λ_{max} (MeOH) nm: 281, 330; IR ν_{max} (KBr) cm^{-1}: 3400, 1690, 1590, 1520; ^{1}H and ^{13}C NMR spectral data, see Table 1; positive HRFABMS *m/z* 589.1541 $[M+Na]^+$ (calcd for 589.1533); positive ESIMS *m/z* 567 $[M+H]^+$, 589 $[M+Na]^+$, 273 [Aglycon + H]$^+$, 1155 $[2M+Na]^+$.

Gleditsin B (**2**): a pale yellow needles [MeOH]; CD (MeOH; *c* 0.1): $[\theta]_{330}$ +6050, $[\theta]_{290}$ -36.400; UV λ_{max} (MeOH) nm: 286, 330; IR ν_{max} (KBr) cm^{-1}: 3500, 1695, 1590, 1515; ^{1}H and ^{13}C NMR spectral data, see Table 2; positive HRFABMS *m/z* 639.2060 $[M+Na]^+$ (calcd for 639.2054); positive ESIMS *m/z* 639 $[M+Na]^+$, 1255 $[2M+Na]^+$, 1871 [3M +Na]$^+$.

Naringenin-7-O-β-D-glucoside (3): A pale yellow needles [MeOH]; UV λ_{max} (MeOH) nm: 286, 332; IR ν_{max} (KBr) cm^{-1}: 3410, 1695, 1580, 1515; ^{1}H NMR spectral data (600 MHz, DMSO-d_6) aglycon δ 12.06 (1H, *brs*, 5-OH), 9.61 (1H, *brs*, 4`-OH), 7.33 (2H, *d*, *J*= 8.5 Hz, H-2`,6`), 6.81 (2H, *d*, *J*= 8.5 Hz, H-3`,5`), 6.16 (1H, *d*, *J*= 2.1 Hz, H-8), 6.14 (1H, *d*, *J*= 2.1 Hz, H-6), 5.51 (1H, *dd*, *J*= 12.7/3.0 Hz, H-2), 3.35 (1H, *dd*, *J*= 17.1/12.7 Hz, H-3_{ax}), 2.76 (1H, *dd*, *J*= 17.1/3.0 Hz, H-3_{eq}); sugar moiety δ 4.97 (1H, *d*, *J*= 7.7 Hz, H-1``), 3.68 (1H, *dd*, *J*= 11.8/3.3 Hz, H-$6_b$``), 3.46 (1H, *dd*, *J*= 11.8/5.8 Hz, H-6_a``), 3.39 (1H, *m*, H-5``), 3.28 (1H, *t*, *J*= 9.2 Hz, H-3``), 3.23 (1H, *dd*, *J*= 7.7/9.2 Hz, H-2``), 3.16 (1H, *dd*, *J*= 9.2/9.1 Hz, H-4``); ^{13}C NMR spectral data (100 MHz, DMSO-d_6) aglycon δ 197.18 (*s*, C-4), 165.28 (*s*, C-7), 162.91 (*s*, C-5), 162.71 (*s*, C-9), 157.77 (*s*, C-4` ), 128.59 (*s*, C-1`), 128.40 (*d*, C-2`,6`), 115.16 (*d*, C-3`,5`), 103.22 (*s*, C-10), 96.46 (*d*, C-6), 95.41 (*d*, C-8), 78.63 (*d*, C-2), 42.03 (*t*, C-3); sugar moiety δ 99.57 (*d*, C-1``), 77.04 (*d*, C-5``), 76.28 (*d*, C-3``), 72.98 (*d*,C-2``), 69.46 (*d*, C-4``), 60.53 (*t*, C-6``); positive ESIMS *m/z* 435 $[M+H]^+$, 457 $[M+Na]^+$, 891 $[2M+Na]^+$, 869 $[2M+H]^+$, 1325 $[3M+Na]^+$, 1341 $[3M+K]^+$, 1759 $[4M+Na]^+$, 273 [Aglycon + H]$^+$.

Eriodictyol-7-O-β-D-glucoside (4): A pale yellow needles [MeOH]; UV λ_{max} (MeOH) nm: 284, 335; IR ν_{max} (KBr) cm^{-1}: 3450, 1692, 1590, 1516; ^{1}H NMR spectral data (600 MHz, DMSO-d_6) aglycon δ 12.05 (1H, *brs*, 5-OH), 9.07 (2H, *s*, 3`, 4`-OH), 6.89 (1H, *brs*, H-2`), 6.79 (2H, *s*, H-5`,6`), 6.15 (1H, *d*, *J*= 2.2 Hz, H-8), 6.13 (1H, *d*, *J*= 2.2 Hz, H-6), 5.45 (1H, *dd*, *J*= 12.6/3.0 Hz, H-2), 3.29 (1H, *dd*, *J*= 17.1/12.6 Hz, H-3_{ax}), 2.75 (1H, *dd*, *J*= 17.1/3.0 Hz, H-3_{eq}); sugar moiety δ 4.99 (1H, *d*, *J*= 7.6 Hz, H-1``), 3.67 (1H, *dd*, *J*= 11.5/3.2 Hz, H-$6_b$``), 3.45 (1H, *dd*, *J*= 11.5/5.5 Hz, H-6_a``), 3.39 (1H, *m*, H-5``), 3.27 (1H, *dd*, *J*= 9.2/9.1 Hz, H-3``), 3.22 (1H, *dd*, *J*= 7.6/9.2 Hz, H-2``), 3.17 (1H, *t*, *J*= 9.1 Hz, H-4``); ^{13}C NMR spectral data (100 MHz, DMSO-d_6) aglycon δ 197.17 (*s*, C-4), 165.29 (*s*, C-7), 162.93 (*s*, C-5), 162.75 (*s*, C-9), 145.82 (*s*, C-4`), 145.21 (*s*, C-3`), 129.21 (*s*, C-1`), 118.09 (*d*, C-6`), 115.35 (*d*, C-5`), 114.44 (*d*, C-2`), 103.27 (*s*, C-10), 96.44 (*d*, C-6), 95.43 (*d*, C-8), 78.75 (*d*, C-2), 42.18 (*t*, C-3); sugar moiety δ 99.58 (*d*, C-1``), 77.08 (*d*, C-5``), 76.31 (*d*, C-3``), 73.01 (*d*, C-2``), 69.49 (*d*, C-4``), 60.57 (*t*, C-6``); positive ESIMS *m/z* 451 $[M+H]^+$, 923 $[2M+Na]^+$, 901 $[2M+H]^+$, 1373 $[3M+Na]^+$, 1823 $[4M+Na]^+$, 289 [Aglycon + H]$^+$.

Acid hydrolysis of 1 and 2: Compound **1** (5 mg) was refluxed with 2M HCl in MeOH (5 ml) at 80 ^{0}c for 2 h in a water bath. The reaction mixture was evaporated, and the hydrolysate after dilution with H_2O (10 ml) was extracted with $CHCl_3$ (10 ml x 3). The $CHCl_3$ extracts were evaporated to afford the aglycon, which was identified as

naringenin by co-TLC with authentic sample using solvent system $CHCl_3$-MeOH: 90-10. The aqueous layer was neutralized with 2N KOH solution and concentrated to 1 ml under reduced pressure. The residue was compared with standard sugars by Si gel TLC [($CHCl_3$-MeOH-H_2O: 30:12:4), 9 ml of lower layer and 1 ml of HOAc] and by PC (iso-PrOH-*n*-BuOH-H_2O: 7:1:2); detection with aniline hydrogen phthalate, which indicated the sugars of **1** to be glucose and xylose. By the same method, compound **2** (10 mg) furnished an aglycon identified as eriodictyol and the monosaccharide was also identified as glucose.

Cytotoxicity assays (Hosny and Rosazza, 2002**;** Goren, et al., 1996) **:** Cell lines NCI-H1755, SK-HEP-1, A-498, SW 156, MDAH 2774, NIH: OVCAR–3, SK-UT-1, HEC-1-A, G-361, RPMI-7951 and Hs 908. Sk were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% heat-inactivated Fetal Bovine Serum (FBS). The DU 145 cells were cultured in Eagle Minimum Essential Medium (EMEM) containing Earle's salts heated and supplemented with amino acids and 10% heat inactivated Fetal Bovine Serum (FBS). The HCT-8 (HRT-18), LS 174T and COLO 320 DM) cell lines were maintained in Roswell Park Memorial Institute (RPMI) 146 Medium containing 10% heat inactivated Fetal Bovine serum (FBS). All cell lines were cultivated in an incubator at 37ºC in humidified air containing 5% CO_2. For routine cytotoxicity assays, all cell lines were adapted to one single medium RBMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, penicillin (100 unites/1 ml), and streptomycin (100 µg/ 1ml). For the micro assay, the growth medium was supplemented with 10 mM HPES (1-[2-hydroxy ethyl] piprazine-4-ethane sulfonic acid) buffer pH 7.3 and incubated at 37ºC in CO_2 incubator. These methods are similar to those used by Hosny *et al* [18] and Gore *et al* [19]. Cellular viability in the presence and absence of experimental reagents was determined using the standard MTT (3-[4, 5-dimethyl thiazole-2-yl]-2, 5-diphenyl tetrazolium bromide) colorimetric assay. The assay is based on reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product that can be measured spectrophotometrically. In brief, exponentially growing cells were harvested and 200 µL cells suspension were seeded in 96-well microplates and preincubated for 24 h at 37ºC under 5% CO_2 to allow cell attachment. After attachment 10 µL of an EtOH : H_2O (1 : 1 solution) containing varying concentrations of test samples (isolated compounds, **1-4**) were added in wells in duplicate, and 10 µL EtOH : H_2O (1 : 1) was added into wells as a control. Sample containing microplates were further incubated for 6 days. Cell survival was evaluated by adding 10 µL of 5 mg/ml MTT in 0.1 mM, pH 7.4 phosphate buffered saline to each well, and reincubating plates in 5% CO_2 /air for 4 hrs at 37ºC. Plates were then centrifuged at 1500 x *g* for 5 minutes to precipitate cells and the reduced product of MTT (blue formazan). An aliquot of 100 µL of the supernatant was removed, and DMSO (100 µL) was added to dissolve precipitated, reduced blue formazan. The plate was mixed on a microshaker for 10 minutes, and the absorbance was determined at 550 nm with a multiwell scanning spectrophotometer (Dynex MR 5000) Chaltilly VA, USA). The ED_{50} values, which reduce the viable cell number, was defined as the concentration of test samples resulting in a 50% reduction of absorbance compared to untreated controls (Hosny and Rosazza, 2002; Goren, et al., 1996). The 50% effective dose (ED_{50}) obtained by measuring growth inhibition with MTT, are shown in Table-3.

Statistical Analysis (Woodson, 1987)**:** All cytotoxic data were expressed as Mean±SE. Student's *t*-test was applied for detecting the significance of difference between each sample; $P < 0.05$ was taken as the level of significance.

RESULTS AND DISCUSSION

Compound 1**,** obtained as a pale yellow amorphous powder and gave on TLC greenish-brown and yellowish-brown with $FeCl_3$ and Pauly`s reagents (Jork, et al., 1994), respectively. It showed an $[M+Na]^+$ ion at *m/z* 589.1541 in its positive HRFABMS, an $[M+H]^+$ ion at *m/z* 567 and an $[M+Na]^+$ ion at *m/z* 589 in the positive ESIMS, corresponding to the molecular formula $C_{26}H_{30}O_{14}$. This was corroborated by the ^{13}C NMR DEPT spectrum which showed signals for all the twenty six carbons of the molecule. The UV spectrum of 1 showed a maximum at 281 nm and a shoulder at 330 nm which was typical for flavanone (Markham, 1982). Free hydroxyl group at C-4` and C-5 indicated by bathochromic UV shifts induced by NaOMe (72 nm) and $AlCl_3$\HCl (26 nm), respectively. No UV bathochromic shift was observed with NaOAc, suggested the absence of free C-7 hydroxyl group (Markham, 1982). The IR spectrum of 1 indicated the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1690 cm^{-1}) groups. After an extensive 2D NMR study, the aglycon was identified as naringenin (Table 1). The characteristic signals by ^1H NMR analysis of 1, appeared as a doublet of doublet at δ 5.49 ($J_{2,3eq}$= 2.9 Hz, $J_{2,3ax}$= 12.4 Hz, H-2) and the equatorial and axial protons appeared at δ 2.71 and 3.30 (each 1H, each *dd*, J_{AB}=17.0/2.9 Hz, H-3_{eq} and 17.0/12.4 Hz, H-3_{ax}) revealed the characteristic chemical shifts and coupling patterns for flavanone skeleton(Harborne, 1994). The chemical shifts of the C-2 (78.64 ppm) and C-3 (42.06 ppm) carbon atoms were characteristic of flavanone (Agrawal, 1989; Wagner, et al., 1976). In the ^1H NMR spectrum, two doublets at δ 6.10 and 6.13 with a coupling constant of 2.2 Hz, typical of two *meta*-coupled protons, were assigned to H-6 and H-8, respectively. The disubstituted aromatic B-ring appeared as AA`BB` spin system at δ 6.80 (H-3`, H-5`) and δ 7.33 (H-2`, H-6`), each 2H, J_{AB}= 8.5 Hz. The ^1H and ^{13}C NMR of 1 exhibited two sugar anomeric protons at δ_H 5.10 (1H, *d*, *J*= 7.2 Hz,) and δ_H 4.43 (1H, *d*, *J*= 7.2 Hz,) and carbons at δ_C 97.95 and δ_C 104.96, respectively. On acid hydrolysis, compound 1 gave glucose and xylose as the component sugars based on GLC analysis, and an aglycon identified as naringenin by co-TLC analysis with an authentic sample. The identities of the monosaccharides were determined by a combination of DEPT, DQF-COSY, HMQC and HMBC experiments. Starting from the anomeric proton of each sugar unit, all the hydrogens within each spin system were assigned using DQF-COSY spectrum. On the basis of the assigned proton signals by DQF-COSY, a HMQC experiment then gave the corresponding carbon assignments, and these were further confirmed by an HMBC experiment. After the assignment of the protons and protonated carbons were established (Table 1), the two sugar units were identified as glucose and xylose and further confirmed by TLC and PC analysis of the acid hydrolysate, indicating the diglycosidic flavanone structure. The two monosaccharides were determined to be in the pyranose form from their ^{13}C NMR data. Chemical shifts and coupling constants in the ^1H NMR spectrum (Table 1) indicated anomeric *β*-configurations for glucopyranosyl and xylopyranosyl (J_{H1-H2}= 7.2 Hz) units (Harborne, 1994). The linkage of xylose unit to C-2`` of glucose was established from the following HMBC correlations: H-1``` (4.43) of xylose with C-2`` (82.15) of glucose and H-2`` (5.23) of glucose with C-1``` (104.96) of xylose. Significant downfield shift of the methin proton (H-2``) and carbon (C-2``) indicated that the glucose unit was glycosylated at this position. The placement of the disaccharide moiety at C-7 of the aglycon was confirmed by the long-range correlation between H-1`` (5.10) of glucose and C-7 (162.85) of the aglycon. Further confirmation was obtained from the downfield shift of H-6 and H-8 and by downfield shifts of the *ortho*-related C-8 and C-6 and *para*-related C-10 with respect to naringenin(Agrawal, 1989; Markham,et al., 1978).

The fragmentation pattern at *m/z* 273 [aglycon+ H]$^+$ in the ESIMS, suggest the loss of a pentose (132 mass unit) and a hexose (162 mass unit), supported the presence of the diglycosidic moiety in 1. A negative Cotton effect $[\theta]_{285}$ -24.350, was observed in the CD spectrum of 2, thereby indicating that the absolute configuration on C-2 is *S* in agreement with the literature (Gaffield, 1970). Consequently, the structure of 1 was established as (2*S*)-5,7,4`-trihydroxy flavanone-7-*O*-[*β*-D-xylopyranosyl-(1→2)]-*β*-D-glucopyranoside, and was named Gleditsin A.

Compound 2, obtained as a pale yellow powder and gave on TLC greenish-brown and yellowish-brown with $FeCl_3$ and Pauly`s reagents (Jork, et al., 1994), respectively. The molecular formula of 2 was determined by positive HRFABMS and positive ESIMS to be $C_{31}H_{36}O_{13}$ from the pseudomolecular ions at *m/z* 639.2060 [M +Na]$^+$ and 639 [M+Na]$^+$, respectively. The UV spectrum of 2 exhibited maxima at 286 and 330(sh) nm, indicated 2 was also a flavanone (Markham, 1982). The bathochromic shifts induced by $AlCl_3$ (70 nm) and NaOMe (74 nm), are typical of flavanone with two hydroxyl groups at C-3` and C-4`. No UV bathochromic shift was observed with NaOAc suggested the absence of free 7-hydroxyl group in 2 as with 1 (Markham, 1982). The complete assignments of the protons and carbons of 2 were achieved by a combination of DQF-COSY, DEPT, HMQC and HMBC experiments (Table 2). The ^{1}H NMR spectrum of 2 revealing the presence of two sets of ABX-spin systems, one reminiscent to the flavanone heterocyclic C-ring at [δ 5.42 (1H, *dd*, *J*= 12.5/3.0 Hz, H-2), δ 2.73 (1H, *dd*, *J*= 17.1/3.0 Hz, H-3$_{eq}$), δ 3.28 (1H, *dd*, *J*= 17.1/12.5 Hz, H-3$_{ax}$)], and the other corresponding to B-ring, at δ 6.78 (1H, *s*, H-2`), δ 6.74 (2H, *s*, H-5` and H-6`). The remaining two aromatic proton signals at δ 6.03 and 6.09 (each 1H, each *d*, *J*=2.1 Hz,), were assigned to H-6 and H-8, respectively. These signals indicated the presence of eriodictyol aglycon **(**Harborne **,** 1994). The ^{1}H and ^{13}C NMR spectra of **2** revealing the resonance of an anomeric proton and carbon signals at δ_H 5.33, d, *J*= 8.0 Hz, δ_C 97.30, consistent with the presence of an *β*-D-glucose unit. Acidic hydrolysis furnished eriodictyol as an aglycon identified by co-TLC analysis with an authentic sample, and the monosaccharide component was detected as glucose based on TLC and PC analysis. The ^{1}H NMR data showed signals attributed to one monoterpenoic acid moiety; [two singlet methyl groups at δ 1.71 (Me-9```) and 1.13 (Me-10```)]. A typical ABX-spin system corresponding to the vinyl hydrogen signals at δ 4.95 (1H, *dd*, *J*= 10.6/1.9 Hz, H-8$_a$```), 5.14 (1H, *dd*, *J*= 17.2/1.9 Hz, H-8$_b$```) and 5.85 (1H, *dd*, *J*= 17.2/10.6 Hz, H-7``). Moreover signal of *β*-vinyl proton of *trans*-2-methyl-2-decanoic acid appears downfield at δ 6.65 (*t*, *J*= 7.6 Hz, H-3```), in comparison with that appearing at δ 6.06 of a *cis*-isomer (Okada,, et al., 1980). From these results, and in conjunction with the ^{13}C NMR, DEPT DQF-COSY, HMQC and HMBC spectra (Table-2) indicated **2** to be consistent with an monoterpenoic acid represented as being 2,6-dimethyl-6-hydroxy-2-*trans*-2,7-octadienoic acid. By ^{13}C NMR of **2**, 15 carbon signals were assigned to eriodictyol, 6 carbon signals to glucose moiety and the remaining 10 carbon signals were assigned to a monoterpenoic acid. As observed in the HMBC spectrum, the long-range correlation of H-2`` (4.80) of glucose with C-1``` (166.17) of the monoterpene unit established that, the monoterpene unit was attached to C-2`` of glucose. The downfield shift of H-2``of glucose in comparison with that of naringenin-7-*O*-glucoside (Harborne, 1994), in addition to the downfield shift of C-2`` and the upfield shifts of C-1`` and C-3`` of glucose (Yamasaki, et al., 1977), confirmed the site of acylation. The attachment of the acylated sugar moiety to C-7 was deduced from the long-range correlation between the anomeric proton of glucose (5.33) and C-7 (164.54) of the aglycon, as observed in the HMBC spectrum. Which was also

confirmed by the following: lack of bathochromic shift with NaOAc and by downfield shifts of the *ortho*-related protons (Harborne, 1994) and *para*-related carbon (Agrawal, 1989; Markham, et al., 1978), with respect to eriodictyol? The absolute configuration at C-2, was assigned as 2*S* from the CD curve; $[\theta]_{290}$ -36.400. Therefore, by the above mentioned discussion the structure of 2 was proposed as (2*S*)-5,7,3`,4`-tetrahydroxyflavanone-7-*O*-[2,6-dimethyl-6-hydroxy-2-*trans*-2,7-octadienoic acid (1→2)-*β*-D-glucopyranosyl ester, and was named Gleditsin B.

For this work, several cell lines were used: human liver adenocarcinoma (NCI-H1755, SK-HEP-1), kidney carcinoma (A-498), kidney hypernephroma (SW 156), ovary adenocarcinoma (MDAH 2774, NIH:OVCAR-3, uterus mesodermal tumor (SK-UT-1), skin malignant melanoma (G-361, RPMI-7951, Hs 908.Sk), prostate carcinoma (DU 145) and colon colorectal adenocarcinoma (HCT-8 [HRT-18], LS 174T, COLO 320DM) cell lines (Smith, 1979; Amanda, ,et al., 1997; Kenneth, et al., 1978; Julius, et al., 1983) to evaluate the cytotoxic activities of isolated compounds [1-4] obtained in this work. The 50% effective dose (ED_{50}) obtained by measuring growth inhibition with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Hosny and Rosazza, 2002; Goren, et al., 1996), are shown in Table 3. Flavanones 1 and 2 were generally more potent than flavanones 3 and 4, possess the most cytotoxic activities on prostate carcinoma (DU 145) and colon colorectal adenocarcinoma (HCT-8[HRT-18], LS 174T, COLO 320 DM) cell lines.

Acknowledgements: Thanks are due to Prof. John, P. N. Rosazza, Head, Division of Medicinal and Natural Products Chemistry and Director of the Center for Biocatalysis and Bioprocessing, College of Pharmacy, University of Iowa, Iowa City, lA 52242 USA, for financial support of spectroscopic data and cytotoxic activity.

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Table-1: 1D and 2D NMR Spectral Data for Gleditsin A (1).

(DMSO-d_6, 600 MHz for ^{1}H, and 100 MHz for ^{13}C NMR)

Position	^{1}H (*J* in Hz)	^{13}C	DEPT	COSY	HMBC
Aglycon					
2	5.49 dd 12.4, 2.9	78.64	CH	H-3_{ax}, 3_{eq}	C-3,4,1`,2`,6`
3_{eq}	2.71 dd 17.0, 2.9	42.06	CH_2	H-3_{ax}, 2	C-2,4,1`
3_{ax}	3.30 dd 17.0, 12.4			H-3_{eq}, 2	C-2,4
4	-	197.23	C	-	-
5	12.04 brs [OH]	162.80*	C	-	-
6	6.10 d 2.2	96.48	CH	-	C-5,7,8,10
7	-	162.85*	C	-	-
8	6.13 d 2.2	95.43	CH	-	C-7,9,10
9	-	162.72	C	-	-
10	-	103.24	C	-	-
1`	-	128.55	C	-	-
2`	7.33 d 8.5	128.39	CH	H-3`	C-2,1`,4`
3`	6.80 d 8.5	115.16	CH	H-2`	C-1`,4`
4`	9.65 s [OH]	157.81	C	-	-
5`	6.80 d 8.5	115.16	CH	H-6`	C-1`,4`,6`
6`	7.33 d 8.5	128.39	CH	H-5`	C-2,4`
Glucose					
1``	5.10 d 7.2	97.95	CH	H-2``	C-7, 3``,5``
2``	4.59 dd 7.2, 9.9	82.15	CH	H-1``, 3``	C-1``,3``,1```
3``	3.68 t 9.9	76.86†	CH	H-2``, 4``	C-2``,4``
4``	3.15 dd 9.9, 10.2	69.48	CH	H-3``, 5``	C-3``,6``
5``	3.27 m	76.80†	CH	H-4``, $6``_{a,b}$	C-3``,6``
$6``_a$	3.48 dd 12.0, 5.3	60.36	CH_2	H-5``, $6``_b$	C-5``
$_b$	3.61 dd 12.0, 2.3			H-5``, $6``_a$	C-4``
Xylose					
1```	4.43 d 7.2	104.96	CH	H-2```	C-2``, 3```
2```	3.32 dd 7.2,9.3	74.13	CH	H-1```, 3```	C-1```, 3```
3```	3.37 t 9.3	75.82	CH	H-2```, 4```	C-2```, 4```
4```	3.51 m	69.00	CH	H-3```, 5```	C-3```, 5```
$5```_a$	3.25 dd 13.0,10.0	65.36	CH_2	H-4```, $5```_b$	C-4```
$_b$	3.67 m			H-4```, $5```_a$	-

* † Assignments with the same superscript may be reversed.

Table-2: 1D and 2D NMR Spectral Data for Gleditsin B (2).

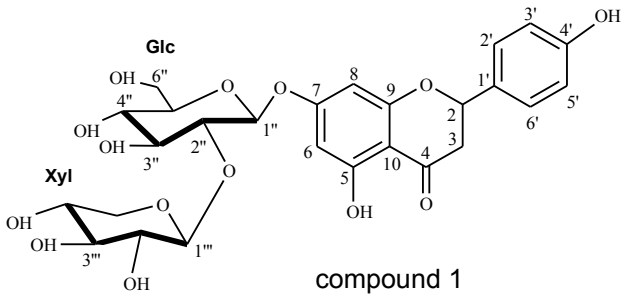
(DMSO-d_6, 600 MHz for ^{1}H, and 100 MHz for ^{13}C NMR)

Position	^{1}H (*J* in Hz)	^{13}C	DEPT	COSY	HMBC
Aglycon					
2	5.42 dd 12.5, 3.0	78.77	CH	H-3_{ax}, 3_{eq}	C-3,1`,2`,6`
3_{eq}	2.73 dd 17.1, 3.0	42.17	CH_2	H-3_{ax}, 2	C-2,4
3_{ax}	3.28 dd 17.1, 12.5			H-3_{eq}, 2	C-2,4
4	-	197.25	C	-	-
5	12.02 brs [OH]	162.91	C	-	-
6	6.03 d 2.1	96.43	CH	-	C-5,7,8,10
7	-	164.54	C	-	-
8	6.09 d 2.1	95.94	CH	-	C-7,9,10
9	-	162.73	C	-	-
10	-	103.48	C	-	-
1`	-	129.08	C	-	-
2`	6.87 s	114.39	CH	-	C-2,1`,3`,4`,6`
3`	9.05 s [OH]	145.17	C	-	-
4`	9.05 s [OH]	145.80	C	-	-
5`	6.74 s	115.30	CH	-	C-1`,3`,4`,6`
6`	6.74 s	118.01	CH	-	C-2,1`,4`,5`
Glucose					
1``	5.33 d 8.0	97.30	CH	H-2``	C-7, 3``,5``
2``	4.80 dd 8.0, 9.4	73.41	CH	H-1``, 3``	C-1``, 3``, 1```
3``	3.54 t 9.4	73.71	CH	H-2``, 4``	C-2``, 4``
4``	3.25 dd 9.4, 9.0	69.63	CH	H-3``, 5``	C-3``, 6``
5``	3.36 m	76.96	CH	H-4``, $6``_{a,b}$	C-3``,6``
$6``_a$	3.50 dd 12.0, 4.2	60.37	CH_2	H-5``, $6``_b$	C-5``
$6``_b$	3.69 dd 12.0, 1.8			H-5``, $6``_a$	C-4``
Monoterpene					
1```	-	166.17	C	-	-
2```	-	126.61	C	-	-
3```	6.65 t 7.6	142.92	CH	H-4```	C-1```,4```,5```,9```
4```	2.10, 2.17 m	23.06	CH_2	H-3```, 5```	C-2```,3```,5```,6```
5```	1.42, 1.46 m	40.42	CH_2	H-4```	C-3```,7```,10```
6```	-	71.23	C	-	-
7```	5.85 dd 17.2, 10.6	145.66	CH	H-$8```_{a,b}$	C-5```,8```,10```
$8```_a$	4.95 dd 10.6, 1.9	111.23	CH_2	H-7```, $8```_b$	C-6```,7```
$8```_b$	5.14 dd 17.2, 1.9			H-7```, $8```_a$	C-6```,7```
9```	1.71 s	12.21	CH_3	-	C-1```,2```,3```
10```	1.13 s	27.70	CH_3	-	C-5```,6```,7```

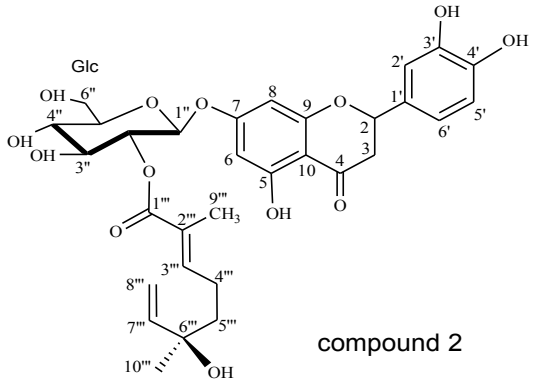
Table-3: Cytotoxicity of Isolated Flavonoids (1-4) Against Selected Liver, Kidney, Ovary, Uterus, Skin, Prostate and Colon Tumor Cell Lines*.

Cell lines	Compounds ED_{50} (μg/ml)			
	1	**2**	**3**	**4**
Liver adenocarcinoma NCI-H1755	> 100	48.10 (± 0.25)	> 100	66.90 (± 0.41)
SK-HEP-1	> 100	73.25 (±0.60)	> 100	77.20 (± 0.63)
Kidney carcinoma A-498	> 100	82.78 (± 0.70)	> 100	> 100
Kidney hypernephroma SW 156	> 100	75.18 (± 0.55)	73.12 (± 0.22)	74.23 (± 0.55)
Ovary adinocarcinoma MDAH 2774	60.47 (± 0.36)	45.37 (± 0.22)	57.80 (± 0.26)	46.50 (± 0.25)
NIH:OVCAR	62.00 (± 0.33)	57.80 (± 0.26)	66.53 (± 0.35)	65.16 (± 0.30)
Uterus mesodermal tumor SK-UT-1	45.75 (± 0.25)	35.53 (± 0.16)	48.65 (± 0.27)	43.70 (± 0.21)
Skin malignant melanoma	59.25 (± 0.28)	34.95 (± 0.15)	52.35 (± 0.23)	38.50 (± 0.18)
G. 361	75.00 (± 0.32)	60.05 (± 0.24)	76.23 (± 0.35)	62.56 (± 0.28)
RPMI-7951	69.80 (± 0.35)	70.42 (± 0.48)	63.50 (± 0.32)	65.03 (± 0.34)
Hs 908. SK				
Prostate carcinoma DU 145	15.73 (± 0.14)	12.52 (± 0.12)	21.55 (± 0.17)	17.00 (± 0.15)
Colon colorectal adenocarcinoma	48.70 (± 0.25)	21.16 (± 0.19)	44.35 (± 0.20)	27.52 (± 0.20)
HCT-8 (HRT-18)	49.35 (± 0.25)	13.84 (± 0.10)	56.60 (± 0.30)	23.74 (±0.18)
LS 174T	8.57 (± 0.05)	8.10 (± 0.08)	18.75 (± 0.15)	25.43 (± 0.20)
COLO 320 DM				

- *Values are presented as mean ± SE of 2 test sample observations, compared with that of control group ($p < 0.05$) for all values.



compound 1



compound 2

