Caragisides A – C, New Isoflavone Glucosides from *Caragana conferta* with Inhibition of Platelet Aggregation

by **Amna Nisar Khan**[a]), **Itrat Fatima**[a]), **Abdul Malik***[a]), and **Huma Rasheed**[b])

[a]) International Centre for Chemical and Biological Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan (phone: +92-21-4824926; e-mail: abdul.malik@iccs.edu)

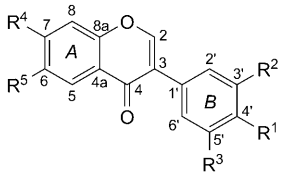
[b]) International Centre for Chemical and Biological Sciences, Dr. Panjwani Centre for Molecular Medicine and Drug Research, University of Karachi, Karachi-75270, Pakistan

Caragisides A – C (**1** – **3**, resp.), three new isoflavone glucosides, were isolated from the BuOH sub-fraction of the EtOH extract of the whole plant of *Caragana conferta*, along with ononoside (**4**), reported for the first time from this species. The structures of the new compounds were elucidated by spectroscopic techniques including MS and 2D-NMR spectroscopy. Compounds **1** – **3** showed significant inhibition of platelet aggregation.

Introduction. – The genus *Caragana* (Papilionaceae) comprises 80 species, out of which ten have so far been identified in Pakistan [1]. *Caragana conferta* is a shrub which grows in Asia, Africa, and southeastern Europe. In Pakistan, it is mainly found in the Gilgit and Kashmir vallies [1]. The whole plant is used as folk medicine in China and Korea for the treatment of neuralgia, rheumatism, arthritis, and hypertension [2]. We have previously reported a new isoflavone (= 3-phenyl-4*H*-1-benzopyran-4-one) and two 4′-hydroxyisoflavones from the hexane- and AcOEt-soluble fractions of *Caragana conferta* [3][4]. The ethnopharmacologic and chemotaxanomic importance of the genus *Caragana* prompted us to carry out further studies on *C. conferta*. An EtOH extract of this plant showed strong toxicity in the brine-shrimp-lethality test. On further fractionation, the major toxicity was observed in the BuOH sub-fraction. Pharmacologic screening of the BuOH sub-fraction revealed strong significant inhibition of platelet aggregation. The isolation studies on this sub-fraction resulted in the isolation and structural elucidation of three new isoflavone glucosides named caragisides A – C[1]) (**1** – **3**, resp.), along with ononoside (= 7-(*β*-D-glucopyranosyloxy)-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one; (**4**) [5] (see *Fig. 1*), reported for the first time from this species. The compounds **1** – **3** showed significant inhibition of platelet aggregation.

Results and Discussion. – The EtOH extract of the whole plant of *C. conferta* BENTH. was divided into sub-fractions soluble in hexane, $CHCl_3$, AcOEt, BuOH, and H_2O. The BuOH-soluble sub-fraction was subjected to a series of chromatographic

[1]) For convenience, caragiside C (**3**) is numbered like caragisides A and B (**1** and **2**); for systematic names, see *Exper. Part.*



	R^1	R^2	R^3	R^4	R^5
1	MeO	H	H	MeO	β-D-Glc–O
2	MeO	β-D-Glc–O	H	OH	H
3[1])	H	β-D-Glc–O	COOMe	MeO	H
4	MeO	H	H	β-D-Glc–O	H

Fig. 1. *Caragisides A – C* (**1**–**3**) *and ononoside* (**4**)*, isolated from* Caragana conferta

separations to obtain the compounds **1**–**4**. Their structures were established by MS, UV, IR, as well as NMR spectroscopy.

Caragiside A (**1**) was obtained as a white crystalline solid. The HR-FAB-MS of **1** showed a pseudomolecular-ion peak $[M-H]^-$ at *m/z* 459.1291 consistent with the molecular formula $C_{23}H_{24}O_{10}$. The IR spectrum showed the presence of OH groups (3420 cm^{-1}), a conjugated CO group (1665 cm^{-1}), a conjugated olefinic bond (1625 cm^{-1}), aromatic moieties (1540, 1500 cm^{-1}), and MeO groups (1250 cm^{-1}). The UV spectrum exhibited characteristic absorption maxima for an isoflavone at 230 and 255 nm. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra showed 23 well resolved signals comprising two Me, one CH_2, and twelve CH groups, and eight quaternary C-atoms (*Table 1*). The signals at δ(C) 152.8, 123.8, 175.4, and 120.1 were typical of C(2), C(3), C(4), and C(4a) of an isoflavone moiety. The signals of three oxygenated aromatic C-atoms were observed at δ(C) 159.9, 159.8, and 152.2. The signals of a hexose moiety appeared at δ(C) 102.2, 74.6, 79.3, 71.1, 78.4, and 62.3. The two MeO groups resonated at δ(C) 55.3 and 55.1. The EI-MS showed a peak at *m/z* 298 ($[M-162]^+$). The retro-*Diels–Alder* fragments at *m/z* 132 and 166 revealed the presence of one MeO group at ring *B* and of the remaining substituents at ring *A*. The ^{1}H-NMR spectrum of **1** showed a *s* of a conjugated olefinic H-atom at δ(H) 8.21 (*Table 2*). The H-atoms of *para*-substituted ring *B* showed an *AA′XX′* pattern with the *AA′* part (2 H) at δ(H) 7.74 (*d*, *J* = 9.0 Hz) and the *XX′* part (2 H) at δ(H) 7.03 (*d*, *J* = 9.0 Hz). The MeO group at C(4′) was evident from the HMBC spectrum showing a correlation of MeO at δ(H) 3.66 with C(4′) (δ(C) 159.8), and was further confirmed by the NOEs MeO (δ(H) 3.66)/H–C(3′) and H–C(5′). The H-atoms of ring *A* resonated as *s* at δ(H) 8.19 and 7.68. The former could be assigned to H–C(5) on the basis of the HMBC plot showing a ^2J correlations with C(6) (δ(C) 152.2) and C(4a) (δ(C) 120.1) as well as ^3J correlations with C(7) (δ(C) 159.9) and C(8a) (δ(C) 151.1) (*Fig. 2*). The H-atom at δ(H) 7.68 showed ^2J and ^3J correlations with C(7) (δ(C) 159.9), C(8a) (δ(C) 151.1), C(4a) (δ(C) 120.1), and C(6) (δ(C) 152.2) and could subsequently be assigned to H–C(8). The MeO group at δ(H) 3.64 showed ^3J correlation with C(7) (δ(C) 159.9), and its location was further confirmed by an NOE MeO/H–C(8). The anomeric H-atom was observed at δ(H) 5.90 (*d*, *J* = 7.5 Hz). The larger coupling constant allowed us to assign the β configuration to the hexose moiety.

Table 1. *^{13}C-NMR Data* (125 MHz, C_5D_5N) *of* **1**–**3**. δ in ppm.

	1	**2**	**3**[1])
C(2)	152.8	153.0	152.5
C(3)	123.8	125.0	125.9
C(4)	175.4	175.5	177.0
C(4a)	120.1	119.7	120.2
C(5)	110.2	127.7	128.3
C(6)	152.2	115.9	117.0
C(7)	159.9	162.3	163.5
C(8)	104.8	104.9	104.9
C(8a)	151.1	157.7	159.2
C(1′)	125.5	125.9	126.1
C(2′)	130.7	117.7	117.3
C(3′)	114.1	148.0	149.2
C(4′)	159.8	148.7	112.6
C(5′)	114.1	112.3	125.0
C(6′)	130.7	120.3	121.6
C(1″)	102.2	101.7	101.8
C(2″)	74.6	74.7	74.7
C(3″)	79.3	79.2	78.4
C(4″)	71.1	71.1	71.2
C(5″)	78.4	78.4	77.8
C(6″)	62.3	62.3	62.4
*Me*O–C(4′)	55.1	55.9	–
*Me*O–C(7)	55.3	–	56.2
*C*OOMe	–	–	163.4
COO*Me*	–	–	56.4

Table 2. *^{1}H-NMR Data* (500 MHz, C_5D_5N) *of* **1**–**3**. δ in ppm, J in Hz.

	1	**2**	**3**[1])
H–C(2)	8.21 (*s*)	8.13 (*s*)	8.20 (*s*)
H–C(5)	8.19 (*s*)	8.32 (*d*, $J = 9.0$)	8.13 (*d*, $J = 9.0$)
H–C(6)	–	7.25 (*dd*, $J = 2.0, 9.0$)	7.20 (*dd*, $J = 2.0, 9.0$)
H–C(8)	7.68 (*s*)	7.40 (*d*, $J = 2.0$)	7.24 (*d*, $J = 2.0$)
H–C(2′)	7.74 (*d*, $J = 9.0$)	7.75 (*d*, $J = 2.0$)	7.04 (*t*, $J = 1.0$)
H–C(3′)	7.03 (*d*, $J = 9.0$)	–	–
H–C(4′)	–	–	7.98 (*d*, $J = 1.0$)
H–C(5′)	7.03 (*d*, $J = 9.0$)	7.01 (*d*, $J = 8.0$)	–
H–C(6′)	7.74 (*d*, $J = 9.0$)	7.29 (*dd*, $J = 2.0, 8.0$)	6.97 (*d*, $J = 1.0$)
H–C(1″)	5.90 (*d*, $J = 7.5$)	5.78 (*d*, $J = 7.0$)	5.09 (*d*, $J = 7.5$)
H–C(2″)	4.31–4.32 (*m*)	4.38–4.40 (*m*)	3.48–3.50 (*m*)
H–C(3″)	4.22–4.23 (*m*)	4.20–4.22 (*m*)	3.54–3.55 (*m*)
H–C(4″)	4.33–4.34 (*m*)	4.32–4.35 (*m*)	3.38–3.39 (*m*)
H–C(5″)	4.35–4.44 (*m*)	4.42–4.43 (*m*)	3.40–3.41 (*m*)
CH_2(6″)	4.40 (*dd*, $J = 2.0, 11.5$), 4.59 (*dd*, $J = 6.0, 11.5$)	3.90 (*dd*, $J = 2.0, 12.0$), 3.68 (*dd*, $J = 6.0, 12.0$)	3.90 (*dd*, $J = 2.0, 12.0$), 3.68 (*dd*, $J = 6.0, 12.0$)
*Me*O–C(4′)	3.66 (*s*)	3.75 (*s*)	–
*Me*O–C(7)	3.64 (*s*)	–	3.84 (*s*)
COO*Me*	–	–	3.88 (*s*)

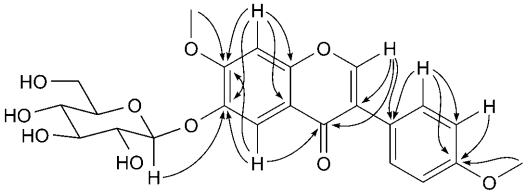


Fig. 2. *Important HMBCs of* **1**

Acid hydrolysis provided the aglycone which could be identified as 6-hydroxy-4′,7-dimethoxyisoflavone by comparison of its physical and spectral data with those reported [6]. The glycone could be isolated and characterized as D-glucose by the sign of its optical rotation and comparison of the retention time of its Me_3Si ether with a standard in the gas chromatogram (GC). The point of attachment of the glucose moiety was confirmed to be C(6) as the anomeric H-atom showed a ^3J correlation with C(6) (δ(C) 152.2). Further HMBCs and HMQCs were in complete agreement with the assigned structure of 6-(β-D-glucopyranosyloxy)-4′,7-dimethoxyisoflavone to caragiside A (**1**; *Fig. 1*).

Caragiside B (**2**) was obtained as a white crystalline solid which gave a violet coloration with $FeCl_3$. The molecular formula was deduced as $C_{22}H_{21}O_{10}$ by HR-FAB-MS showing a pseudomolecular-ion peak $[M-H]^-$ at *m*/*z* 445.1134. The IR spectrum was similar to that of **1**. The UV spectrum showed absorption maxima at 232 and 257 nm. On addition of AcONa, a bathochromic shift of 12 nm of the band at 257 nm was observed, suggesting the presence of an OH group at C(7) [7]. The EI-MS exhibited a prominent peak at *m*/*z* 282 due to the loss of a hexose moiety. The *retro-Diels–Alder* fragmentation gave daughter ions at *m*/*z* 136 and 148, revealing the presence of an OH group at ring *A* and the position of the remaining substituents at ring *B*. The ^{13}C-NMR spectrum showed 22 signals comprising one Me, one CH_2, and twelve CH groups, and eight quaternary C-atoms (*Table 1*). It showed common features to those of **1**, except for the presence of only one MeO group at δ(C) 55.9 in the case of **2**. The ^{1}H-NMR spectrum of **2** (*Table 2*) showed the *s* of a conjugated olefinic H-atom at δ(H) 8.13 and of a MeO group at δ(H) 3.75, respectively. The signals of the OH-substituted ring *A* were observed at δ(H) 8.32 (*d*, $J=9.0$ Hz, 1 H), 7.40 (*d*, $J=2.0$ Hz, 1 H), and 7.25 (*dd*, $J=2.0$, 9.0 Hz, 1 H). The assignments were established by means of HMBCs (*Fig. 3*). In ring *B*, the *meta*-coupled H-atoms at δ(H) 7.75 (*d*, $J=$ 2.0 Hz) and 7.29 (*dd*, $J=2.0$, 8.0 Hz) could be assigned to H–C(2′) and H–C(6′), while the comparatively high-frequency signal at δ(H) 7.01 (*d*, $J=8.0$ Hz) could be assigned to H–C(5′). The entire coupling sequence was confirmed by the ^{1}H,^{1}H-COSY and HMBC data. The presence of the MeO group at C(4′) was confirmed by the ^3J correlation MeO (δ(H) 3.75)/C(4′) (δ(C) 148.7) as well as by the NOE MeO/H–C(5′). The anomeric H-atom resonated at δ(H) 5.78 (*d*, $J=7.0$ Hz). The larger coupling constant revealed the β-configuration of the hexose moiety. Acid hydrolysis provided the aglycone which was identified as 3′,7-dihydroxy-4′-methoxyisoflavone by comparison of physical and spectral data with those reported in [8]. The glycone could be identified as D-glucose by the sign of its optical rotation and comparison of the retention time of its Me_3Si ether with a standard in the GC. The ^3J correlation of the

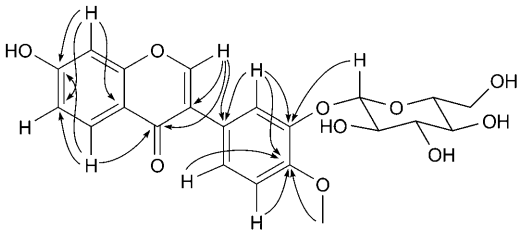


Fig. 3. *Important HMBCs of* **2**

anomeric H-atom at δ(H) 5.78 with C(3′) (δ(C) 148.0) confirmed the glucosidation of the OH group at C(3′). The structure of caragiside B (**2**) could therefore be deduced as 3′-(β-D-glucopyranosyloxy)-7-hydroxy-4′-methoxyisoflavone (*Fig. 1*).

Caragiside C (**3**) was isolated as a white crystalline solid. The HR-FAB-MS gave a pseudomolecular-ion peak $[M-H]^-$ at *m/z* 487.1240 consistent with the molecular formula $C_{24}H_{23}O_{11}$. The IR spectrum showed absorptions for OH groups (3400 cm^{-1}), an ester (1720 cm^{-1}), a conjugated CO group (1660 cm^{-1}), a conjugated olefinic bond (1625 cm^{-1}), aromatic moieties (1530, 1500 cm^{-1}), and MeO groups (1250 cm^{-1}). The ^{13}C-NMR and DEPT spectra showed 24 signals comprising two Me, one CH_2, and twelve CH groups, and nine quaternary C-atoms. The signals of rings *A* and *C* were similar to those of **2** except for the comparatively low frequency of C(7) owing to methylation of the OH–C(7) group. However, significant differences were observed for the signals of ring *B* (*Table 1*). The EI-MS gave a peak at *m/z* 326 due to the loss of a hexose moiety. The *retro-Diels–Alder* fragments appeared at *m/z* 150 and 176, revealing the presence of one MeO group at ring *A* and of all other substituents at ring *B*. The ^{1}H-NMR spectrum (*Table 2*) showed chemical shifts of various H-atoms of rings *A* and *C* and their coupling magnitudes which were very similar to those of **2**. The presence of a MeO group at C(7) was not only supported by a HMBC (*Fig. 4*) but also by the NOEs MeO (δ(H) 3.88)/H–C(6) and H–C(8). The *meta*-coupled H-atoms at δ(H) 7.04 (*t*, $J = 1.0$ Hz) and 6.97 (*d*, $J = 1.0$ Hz) of ring *B* could be assigned to H–C(2′) and H–C(6′) based on HMBCs. The *d* at δ(H) 7.98 (*d*, $J = 1.0$ Hz) was assigned to H–C(4′) based on its ^2J correlations with both C(3′) (δ(C) 149.2) and C(5′) (δ(C) 125.0) as well as ^3J correlations with C(2′) (δ(C) 117.3) and C(6′) (δ(C) 121.6), respectively. The couplings between neighboring H-atoms were further confirmed on the basis of ^{1}H,^{1}H-COSY data. The presence of a methyl ester group was evident by the ^3J

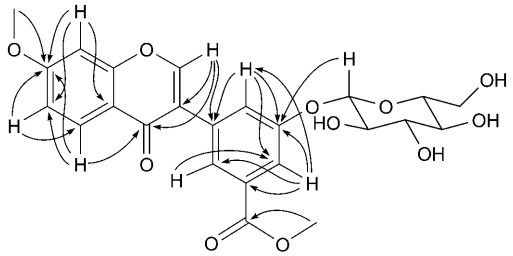


Fig. 4. *Important HMBCs of* **3**

correlation MeO (δ(H) 3.88)/ester CO (δ(C) 163.4) and could be positioned at C(5′) on the basis of the HMBC data showing ^3J correlations of both H–C(4′) and H–C(6′) with the ester CO at δ(C) 163.4. Acid hydrolysis provided a mixture of aglycone products which could not be worked up due to the paucity of material. The glycone could be identified as D-glucose by the sign of its optical rotation and comparison of the retention time of its Me_3Si ethers with a standard in the GC. The anomeric H-atom was observed at δ(H) 5.09 (d, $J = 7.5$ Hz) revealing a β-glucosidic linkage. It showed a ^3J correlation with C(3′) (δ(C) 149.2). Thus, the structure of caragiside C[1]) (**3**) was assigned as 3′-(β-D-glucopyranosyloxy)-7-methoxy-5′-(methoxycarbonyl)isoflavone (*Fig. 1*).

The BuOH-soluble sub-fraction showed significant inhibition of platelet aggregation. This prompted us to test the isolated compounds **1**–**3**. All of these showed significant inhibition of platelet aggregation (*Table 3*), but compound **3** was the most potent.

Table 3. *The Effect of Compounds* **1**–**3** *on Arachidonic Acid* (1.73 mM) *Induced Platelet Aggregation*

Inhibitor	IC_{50} [μM] ± s.e.m.[a])
BuOH fraction	201 ± 3.8
1	210 ± 5.4
2	185 ± 4.2
3	178 ± 4.4
Aspirin	150 ± 4.4

[a]) The data is the mean ± s.e.m. ($n = 8$–10) and is indicated as half-maximal inhibitory concentration (IC_{50}) of the inhibitor.

We gratefully acknowledge the *Higher Education Commission of Pakistan* for financial support.

Experimental Part

General. Arachidonic acid (=(5*Z*,8*Z*,11*Z*,14*Z*)-eicosa-5,8,11,14-tetraenoic acid) and sodium citrate were purchased from *Sigma Chemical Co.* (St. Louis, Mo., USA). All other chemicals used were of the highest purity grade available. Column chromatography (CC): silica gel (SiO_2; 250–400 mesh; *E. Merck*, Darmstadt, Germany), *Diaion HP-20* (*Nippon Rensui Co.*, *Mitsubishi Chemical Corporation*, Tokyo, Japan), and polyamide *6* powder 0.05–0.16 mesh (*Serva*, Heidelberg, Germany). TLC: silica-gel-*60-F*$_{254}$ plates (*E. Merck*, Darmstadt, Germany). Prep. HPLC: *LC-908W-C-60* (*Japan Analytical Industry Co. Ltd.*, Tokyo, Japan); *ODS-M-80* column (4 μM, 250 × 20 mm; *Japan Analytical Industry Co. Ltd.*, Tokyo, Japan); t_R in min. GC: *Shimadzu* gas chromatograph (*GC-9A*); *3%OV-1* silanized *ChromosorbW* column; column temp. 180°; injection port and detector temp. 275–300°; flow rate 35 ml/min; flame-ionization detector; t_R in min. Optical rotations: *Jasco-DIP-360* digital polarimeter. UV Spectra: *Hitachi-UV-3200* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco-302-A* spectrophotometer; in KBr; in cm^{-1}. NMR Spectra: *Bruker-500* instrument; δ in ppm, J in Hz. EI-, and HR-FAB-MS: *Jeol-JMS-HX-110* and *-JMS-DA-500* mass spectrometers; glycerol as matrix.

Plant Material. The whole plant of *Caragana conferta* BENTH. was collected from Gilgit (Pakistan) in July 2007 and identified by Dr. *Rubina Ashraf* of the National Agriculture Research Center, Islamabad, Pakistan. A voucher specimen has been deposited with the Herbarium of the Department of Botany, University of Karachi (voucher no. 319).

Extraction and Isolation. The shade-dried whole-plant material (15 kg) was extracted with MeOH (3×30 l, 10 d each) at r.t. The combined extract was concentrated to a thick gummy mass (700 g) which was subjected to liquid/liquid partitioning to obtain fractions soluble in hexane (200 g), AcOEt (150 g), and BuOH (80 g). The BuOH-soluble fraction (40 g) was subjected to CC (*Diaion HP-20*, H_2O, then H_2O/MeOH in decreasing order of polarity). The *Fraction B* obtained from MeOH/H_2O 1:1 (3 g) was subjected to CC (polyamide, $CHCl_3$ and MeOH in increasing order of polarity) major *Frs. B1–B3*. *Fr. B1* (eluted with $CHCl_3$/MeOH 9.8:0.2; 1 g) was subjected to recycling HPLC (MeOH/H_2O 1:1, flow rate 3.5 ml/min) ononoside (**4**; 20 mg; t_R 47). *Fr. B2* (35 mg) (eluted with $CHCl_3$/MeOH 9.6:0.4) was a binary mixture which was separated by HPLC (MeOH/H_2O 1:1, flow rate 3 ml/min): **1** (21 mg; t_R 35) and **2** (9 mg; t_R 43), resp. *Fr. B3* (1.2 g; eluted with $CHCl_3$/MeOH 9:1) was subjected to recycling HPLC (MeOH/H_2O 1:1, flow rate 3 ml/min): **3** (8 mg; t_R 29). Ononoside (**4**) identified by comparison of physical and spectral data with those reported in [5].

Caragiside A (=*6-(β-*D*-Glucopyranosyloxy)-4′,7-dimethoxyisoflavone* = (*6-(β-*D*-Glucopyranosyloxy)-7-methoxy-3-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one*; **1**): White crystalline solid. M.p. 217°. $[\alpha]_D^{20} = +23.1$ ($c = 0.02$, C_5H_5N). IR (KBr): 3420, 1665, 1625, 1540, 1500, 1250. UV (MeOH): 230 (4.2), 255 (3.8). ^{13}C- and ^{1}H-NMR: *Tables 1* and *2*. EI-MS: 298 (100, $[M-162]^+$), 283 (20), 166 (45), 132 (50). HR-FAB-MS (neg.): 459.1291 ($[M-H]^-$, $C_{23}H_{23}O_{10}^-$; calc. 459.1280).

Caragiside B (=*3′-(β-*D*-Glucopyranosyloxy)-7-hydroxy-4′-methoxyisoflavone* = *3-[3-(β-*D*-Glucopyranosyloxy)-4-methoxyphenyl]-7-hydroxy-1*H*-1-benzopyran-4-one*; **2**): White crystalline solid. M.p. 236°. $[\alpha]_D^{20} = +26.3$ ($c = 0.02$, C_5H_5N). IR (KBr): 3420, 1665, 1625, 1540, 1500, 1250. UV (MeOH): 232 (4.0), 257 (4.1). ^{13}C- and ^{1}H-NMR: *Tables 1* and *2*. EI-MS: 284 (100, $[M-162]^+$), 269 (20), 148 (25), 136 (34). HR-FAB-MS (neg.): 445.1134 ($[M-H]^-$, $C_{22}H_{21}O_{10}^-$; calc. 445.1125).

Caragiside C (=*3′-(β-*D*-Glucopyranosyloxy)-7-methoxy-5′-(methoxycarbonyl)isoflavone* = *3-(β-*D*-Glucopyranosyloxy)-2-methoxy-5-(7-methoxy-4-oxo-4*H*-1-benzopyran-3-yl)benzoic Acid Methyl Ester*; **3**): White crystalline solid. M.p. 238°. $[\alpha]_D^{20} = +15.7$ ($c = 0.02$, C_5H_5N). IR (KBr): 3400, 1720, 1660, 1625, 1530, 1500, 1250. ^{13}C- and ^{1}H-NMR: *Tables 1* and *2*. EI-MS: 326 (100, $[M-162]^+$), 312 (18), 176 (34), 150 (50). HR-FAB-MS (neg.): 487.1240 ($[M-H]^-$, $C_{24}H_{23}O_{11}^-$; calc. 487.1227).

Acid Hydrolysis. A soln. of **1**, **2**, or **3** (each 4 mg) in MeOH (5 ml) containing 1N HCl (2 ml) was refluxed for 4 h. Then, the mixture was concentrated, diluted with H_2O, and extracted with AcOEt. The org. phase from **1** provided the aglycone as colorless gummy solid which was identified as 6-hydroxy-7,4′-dimethoxyisoflavone by comparison of IR, UV, and NMR data with those reported in [6]. The org. phase from **2** provided the pure aglycone which crystallized from MeOH as colorless plates (m.p. 235–236°), and was identified as 3′,7-dihydroxy-4′-methoxyisoflavone by comparison of physical and NMR data with those reported in [8]. The org. phase from **3** was a mixture of aglycone products which could not be worked up due to paucity of material. The aq. phase in each case was concentrated to obtain the glycone which could be identified as D-glucose by the sign of its optical rotation ($[\alpha]_D^{23} = +51.8$) from **1**; $[\alpha]_D^{23} = +51.5$ from **2**; $[\alpha]_D^{23} = +51.4$ from **3**). The presence of D-glucose was further confirmed by comparing the GC retention time of the corresponding Me_3Si ethers (α-anomer t_R 4.1, β-anomer t_R 7.8) with that of a standard sample. The method of preparation of Me_3Si ethers was similar to those reported previously in [9][10].

Inhibition of Platelet Aggregation. Blood was drawn by vein puncture from healthy human subjects reported to be free of medication for one week. Blood samples were mixed with 3.8% (*w*/*v*) aq. sodium citrate soln. (9:1) and centrifuged at 260 *g* for 15 min at 20° to obtain platelet-rich plasma (PRP). The platelet count was determined by phase-contrast microscopy, and all aggregation studies were carried out at 37° with PRP having platelet counts between 2.5 and $3.0 \cdot 10^8$ ml^{-1} of plasma [11]. Aggregation was monitored by a dual-channel *Lumi-Aggregometer*, model *400* (*Chronolog Corporation*, Chicago, USA), with 0.45 ml aliquots of PRP. The final volume was made up to 0.5 ml with the test drug dissolved either in normal saline or an appropriate vehicle known to be devoid of any effect on aggregation. Aggregation was induced with arachidonic acid (1.73 mM), and the inhibition of platelet aggregation by **1**–**3** was studied by pretreatment of PRP with various concentrations of the inhibitor for 1 min followed by the addition of the sub-threshold concentrations of arachidonic acid. The resulting aggregation was recorded for 5 min after challenge by the change in light transmission as a function of time. Once, the inhibition of

platelet aggregation by **1**–**3** against agonists was established, dose-response curves were constructed to calculate the IC_{50} values of the agonists and inhibitor. Aspirin was taken as a reference drug.

REFERENCES

[1] S. I. Ali, M. Qaiser, 'Flora of Pakistan', Department of Botany, University of Karachi, Vol. 100, Ferozesons Publishers, Karachi, 2001, p. 98.
[2] S. Kitanaka, M. Takido, K. Mizoue, H. Kondo, S. Nakaike, *Chem. Pharm. Bull.* **1996**, *44*, 565.
[3] R. Khan, A. Malik, A. Adhikari, M. I. Qadir, M. I. Choudhary, *Chem. Pharm. Bull.* **2009**, *57*, 415.
[4] R. Khan, I. Fatima, N. Ahmad, A. Malik, *J. Asian Nat. Prod. Res.* **2008**, *10*, 823.
[5] A. A. Drenin, E. K. Botirov, E. V. Petrulyak, *Chem. Nat. Compd.* **2008**, *44*, 24.
[6] H. C. Jha, F. Zilliken, E. Breitmaier, *Can. J. Chem.* **1980**, *58*, 1211.
[7] T. J. Mabry, K. R. Markham, M. B. Thomas, in 'The Systematic Identification of Flavonoids', Springer-Verlag, New York, 1970, p. 48.
[8] X. Du, Y. Bai, H. Liang, Z. Wang, Y. Zhao, Q. Zhang, L. Huang, *Magn. Reson. Chem.* **2006**, *44*, 708.
[9] S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.* **1987**, *35*, 501.
[10] X. Zhou, X. He, G. Wang, H. Gao, G. Zhou, W. Ye, X. Yao, *J. Nat. Prod.* **2006**, *69*, 1158.
[11] H. Rasheed, S. A. Saeed, *Exp. Mol. Med.* **2004**, *36*, 220.

Received May 28, 2010