Isoflavonoid Glycosides from the Rhizomes of *Iris germanica*

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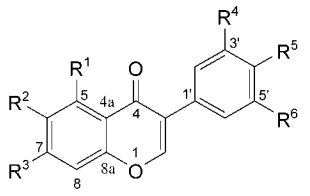
Five new di- and triglycosides, irigenin 7-[*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside] (**1**), 6-hydroxygenistein 4′-[*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside] (**2**), nigricin 4′-[*O*-*β*-D-glucopyanosyl-(1 → 6)-*β*-D-glucopyranoside] (**3**), nigricin 4′-[*O*-*β*-D-glucopyanosyl-(1 → 2)-*O*-[*α*-L-rhamnopyranosyl-(1 → 6)]-*β*-D-glucopyranoside] (**4**), and 7-{4′-{[2″-*O*-(4‴-acetyl-2‴-methoxyphenyl)-*β*-D-glucopyranosyl]oxy}-3′-(*β*-D-glucopyranosyloxy)phenyl]-9-methoxy-8*H*-1,3-dioxolo[4,5-*g*]-[1 benzopyran-8-one-] (**5**), along with a known compound, nigricin 4′-(*β*-D-glucopyranoside) (**6**), were isolated from the rhizomes of *Iris germanica*. The structures of these compounds were established by spectroscopic methods, including 2D NMR spectroscopic techniques.

Introduction. – The genus *Iris* (Irideaceae) comprises over 300 species; among them, 16 are found in Pakistan [1][2]. Plants of the genus *Iris* have been previously recognized as rich sources of secondary metabolites [3–6] and are used in the treatment of cancer, inflammation, and bacterial and viral infections. Previous phytochemical investigations on *Iris* plants have resulted in the isolation of a variety of compounds, including flavonoids, isoflavonoids and their glycosides, benzoquinones, triterpenoids, and stilbene glycosides [1][7][8]. Many of these compounds exhibited piscicidal, antineoplastic, antioxidant, antitumor, antiplasmodial, and antituberculosis properties [9–12].

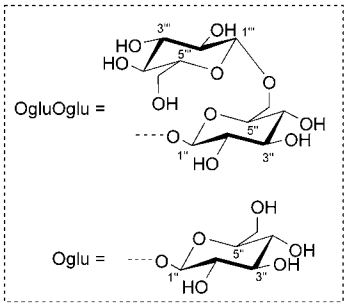
Iris germanica L., locally known as Irsa, is widely distributed in the most parts of the world. The roots are used in edema, as diuretic, and aperient. It is also used in gall bladder diseases and is violently cathartic. The juice of the rhizomes is used for the removal of freckle from the skin. Rhizomes also yield an essential oil, which is used in perfumery and cosmetics [2]. As a part of systematic phytochemical studies on medicinal plants, we have recently obtained several isoflavone glycosides from the rhizomes of *Iris germanica*.

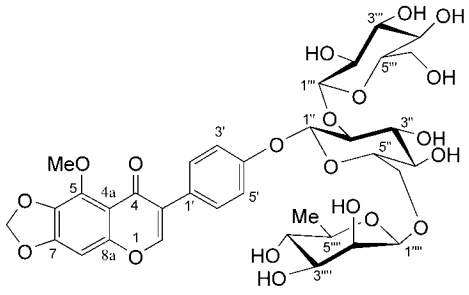
Results and Discussion. – The concentrated MeOH extract of the rhizomes of *Iris germanica* was partitioned between BuOH and H_2O. The BuOH-soluble extract was subjected to silica gel and *Sephadex LH-20* column chromatography, and prep. recycling HPLC yielded a series of isoflavonoids, *i.e.*, **1**–**6**.

Germanaism C (**1**) was obtained as an amorphous solid. IR Absorptions at 3328 (OH), 1649 (C=O), and 1559 (arom. C=C) cm^{-1} were observed. Characteristic UV absorptions at 263 and 319 nm indicated the presence of an isoflavone skeleton [13].



1 $R^1 = R^4 = OH$, $R^2 = R^5 = R^6 = MeO$, $R^3 = OgluOglu$
2 $R^1 = R^2 = R^3 = OH$, $R^4 = R^6 = H$, $R^5 = OgluOglu$
3 $R^1 = MeO$, $R^2 = R^3 = OCH_2O$, $R^4 = R^6 = H$, $R^5 = OgluOglu$
6 $R^1 = MeO$, R^2–$R^3 = OCH_2O$, $R^4 = R^6 = H$, $R^5 = Oglu$





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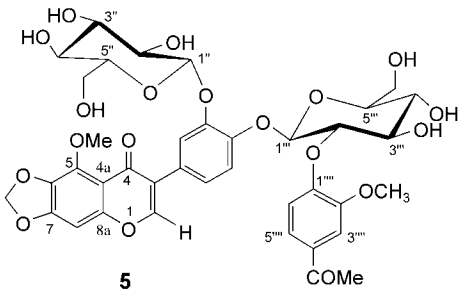

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The pseudo molecular ion $[M+H]^+$ was detected by HR-FAB-MS (positive mode) at *m/z* 685.1230, consistent with the formula $C_{30}H_{36}O_{18}$ (calc.: 685.1235). On the basis of the spectral and chemical (see below) evidence, the structure of irigenin 7-[*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside] (**1**) was deduced for the new compound.

The peak at *m/z* 360 ($C_{18}H_{16}O_8$) in the EI-MS of **1** was due to the loss of both sugar units from M^+. The fragments at *m/z* 182 and 178 were due to *retro-Diels–Alder* cleavage of ring C of the molecule and indicated the presence of two OH and one MeO groups at ring A and one OH and two MeO groups at ring B of the

Table 1. *^{1}H-NMR Chemical Shifts* δ [ppm] *of Compounds* **1**–**5**[a])[1])

	1 (CD_3OD)	**2** (C_5D_5N)	**3** (C_5D_5N)	**4** (C_5D_5N)	**5** (CD_3OD)
H–C(2)	8.24 (*s*)	7.85 (*s*)	7.94 (*s*)	7.93 (*s*)	8.00 (*s*)
H–C(8)	7.03 (*s*)	6.36 (*s*)	6.74 (*s*)	6.76 (*s*)	6.65 (*s*)
H–C(2′)	6.73 (*d*, *J*(2′,6′) = 1.9)	7.24 (*d*, *J*(2′,3′) = 8.5)	7.73 (*d*, *J*(2′,3′) = 8.6)	7.64 (*d*, *J*(2′,3′) = 8.6)	7.22 (*d*, *J*(2′,6′) = 1.9)
H–C(3′)	–	7.40 (*d*, *J*(3′,2′) = 8.5)	7.51 (*d*, *J*(3′,2′) = 8.6)	7.49 (*d*, *J*(3′,2′) = 8.6)	–
H–C(4′)	–	–	–	–	–
H–C(5′)	–	7.40 (*d*, *J*(5′,6′) = 8.5)	7.51 (*d*, *J*(5′,6′) = 8.6)	7.49 (*d*, *J*(5′,6′) = 8.6)	7.25 (*d*, *J*(5′,6′) = 8.5)
H–C(6′)	6.71 (*d*, *J*(6′,2′) = 1.9)	7.24 (*d*, *J*(6′,5′) = 8.5)	7.73 (*d*, *J*(6′,5′) = 8.6)	7.64 (*d*, *J*(6′,5′) = 8.6	6.90 (*dd*, *J*(6′,2′ = 1.9, *J*(6′,5′) = 8.5)
H–C(1″)	5.09 (*d*, *J*(1″,2″) = 7.0)	5.46 (*d*, *J*(1″,2″) = 7.5)	5.58 (*d*, *J*(1″,2″) = 7.2)	5.55 (*d*, *J*(1″,2″) = 7.5)	4.90 (*d*, *J*(1″,2″) = 7.5)
H–C(2″)	3.41 (*m*)	4.08 (*m*)	4.32 (*m*)	4.05 (*m*)	3.44 (*m*)
H–C(3″)	3.49 (*m*)	4.21 (*m*)	4.30 (*m*)	4.22 (*m*)	3.51 (*m*)
H–C(4″)	3.34 (*m*)	4.40 (*m*)	4.23 (*m*)	4.51 (*m*)	3.55 (*m*)
H–C(5″)	3.30 (*m*)	3.81 (*m*)	3.80 (*m*)	3.81 (*m*)	3.47 (*m*)
H_a–C(6″)	4.15 (*dd*, *J*(6″,5″) = 3.0, *J*(6″a,6″b) = 10.0)	4.78 (*dd*, *J*(6″,5″) = 5.5, *J*(6″a,6″b) = 12.0)	4.79 (*dd*, *J*(6″,5″) = 2.0, *J*(6″a,6″b) = 11.5)	4.90 (*dd*, *J*(6″,5″) = 2.0, *J*(6″a,6″b) = 10.0)	3.93 (*dd*, *J*(6″,5″) = 4.4, *J*(6″a,6″b) = 12.0)
H_b–C(6″)	3.85 (*dd*, *J*(6″,5″) = 3.0, *J*(6″b,6″a) = 10.0)	3.71 (*dd*, *J*(6″,5″) = 5.5, *J*(6″b,6″a) = 12.0)	4.55 (*dd*, *J*(6″,5″) = 2.0, *J*(6″b,6″a) = 11.5)	4.70 (*dd*, *J*(6″,5″) = 2.0, *J*(6″b,6″a) = 10.0)	3.72 (*dd*, *J*(6″,5″) = 4.4, *J*(6″b,6″a) = 12.0)
H–C(1‴)	4.38 (*d*, *J*(1‴,2‴) = 7.5)	5.11 (*d*, *J*(1‴,2‴) = 7.5)	5.13 (*d*, *J*(1‴,2‴) = 7.6)	5.35 (*d*, *J*(1‴,2‴) = 7.5)	5.10 (*d*, *J*(1‴,2‴) = 7.5)
H–C(2‴)	3.40 (*m*)	4.06 (*m*)	4.30 (*m*)	4.65 (*m*)	3.45 (*m*)
H–C(3‴)	3.47 (*m*)	4.20 (*m*)	4.33 (*m*)	4.20 (*m*)	3.50 (*m*)
H–C(4‴)	3.33 (*m*)	4.21 (*m*)	4.29 (*m*)	4.20 (*m*)	3.54 (*m*)
H–C(5‴)	3.31 (*m*)	3.39 (*m*)	3.81 (*m*)	4.00 (*m*)	3.46 (*m*)
H_a–C(6‴)	3.65 (*dd*, *J*(6‴,5‴) = 3.0, *J*(6‴a,6‴b) = 12.0)	4.48 (*dd*, *J*(2.0, *J*(6‴,6‴) = 12.0)	4.45 (*dd*, *J*(6‴,5‴) = 2.5, *J*(6‴a,6‴b) = 11.5)	4.49 (*dd*, *J*(6‴,5‴) = 2.0, *J*(6‴a,6‴b) = 11.5)	3.91 (*dd*, *J*(6‴,5‴) = 5.0, *J*(6‴a,6‴b) = 12.0)
H_b–C(6‴)	3.45 (*dd*, *J*(6‴,5‴) = 3.0, *J*(6‴b,6‴a) = 12.0)	4.23 (*dd*, *J*(6‴,5‴) = 2.0, *J*(6‴b,6‴a) = 12.0)	4.52 (*dd*, *J*(6‴,5‴) = 2.5, *J*(6‴b,6‴a) = 11.5)	4.30 (*dd*, *J*(6‴,5‴) = 2.0, *J*(6‴b,6‴a) = 11.5)	3.70 (*dd*, *J*(6‴,5‴) = 5.0, *J*(6‴b,6‴a) = 12.0)

Table 1 (cont.)

H–C(1‴′)	–	–	–	5.60 (br. *s*)	–
H–C(2‴′)	–	–	–	4.21 (*m*)	–
H–C(3‴′)	–	–	–	3.65 (*m*)	7.56 (*d*, $J(3‴′,5‴′) = 1.9$)
H–C(4‴′)	–	–	–	4.49 (*m*)	–
H–C(5‴′)	–	–	–	4.30 (*m*)	7.64 (*dd*, $J(5‴′,3‴′) = 1.9$, $J(5‴′,6‴′) = 8.5$)
Me(6‴′) or H–C(6‴′)	–	–	–	1.88 (*d*, $J(6‴′,5‴′) = 6.1$)	7.20 (*d*, $J(6‴′,5‴′) = 8.5$)
MeO–C(2‴′)	–	–	–	–	3.95 (*s*)
Ac–C(4‴′)	–	–	–	–	2.56 (*s*)
MeO–C(5)	–	–	4.09 (*s*)	4.11 (*s*)	4.01 (*s*)
MeO–C(6)	3.87 (*s*)	–	–	–	–
MeO–C(4′)	3.81 (*s*)	–	–	–	–
MeO–C(5′)	3.86 (*s*)	–	–	–	–
6,7-(OCH_2O)	–	–	6.05 (*s*)	6.05 (*s*)	6.01 (*s*)

[a]) The spectra were recorded at 25°, with $SiMe_4$ as internal standard; *J* in Hz.

Table 2. *^{13}C-NMR Chemical Shifts* δ [ppm] *of Compounds* **1**–**5**[a])[1])

	1 (CD_3OD)	**2** (C_5D_5N)	**3** (C_5D_5N)	**4** (C_5D_5N)	**5** (CD_3OD)
C(2)	156.2	153.2	151.3	151.5	154.7
C(3)	124.4	123.0	126.5	126.5	125.0
C(4)	184.5	180.1	174.8	174.8	177.2
C(5)	154.6	151.6	142.0	142.2	142.5
C(6)	134.2	134.0	136.5	136.7	136.5
C(7)	157.9	150.9	153.1	153.0	154.5
C(8)	95.9	90.0	93.8	93.8	93.0
C(8a)	156.2	157.0	158.4	154.8	157.0
C(4a)	108.5	106.3	114.8	114.0	113.0
C(1′)	127.9	127.0	127.0	128.5	127.5
C(2′)	106.1	131.0	131.1	131.2	115.1
C(3′)	151.6	116.6	117.0	117.0	148.3
C(4′)	138.0	158.0	154.8	158.2	152.5
C(5′)	154.5	116.6	117.0	117.0	122.5
C(6′)	111.2	131.0	131.1	131.2	116.3
C(1″)	101.7	101.9	102.2	102.2	101.6
C(2″)	71.6	74.8	74.8	75.5	74.4
C(3″)	78.0	77.4	78.4	78.6	77.7
C(4″)	75.1	70.8	71.2	72.7	70.9
C(5″)	77.4	78.0	78.3	79.3	77.9
C(6″)	70.6	69.3	69.7	69.6	62.3
C(1‴)	105.1	104.9	105.2	105.3	102.5
C(2‴)	71.7	74.4	74.7	71.7	78.6
C(3‴)	78.0	77.4	77.9	78.3	77.3
C(4‴)	75.2	71.3	71.7	72.4	70.8
C(5‴)	74.8	78.0	78.4	78.4	77.7
C(6‴)	62.0	62.4	62.7	62.5	62.2
C(1⁗)	–	–	–	100.2	152.0
C(2⁗)	–	–	–	71.4	150.0
C(3⁗)	–	–	–	71.3	112.5
C(4⁗)	–	–	–	74.2	132.6
C(5⁗)	–	–	–	69.8	124.2
C(6⁗)	–	–	–	18.8	117.5
*Me*O–C(2⁗)	–	–	–	–	56.3
*Me*CO–C(4⁗)	–	–	–	–	26.4
Me*C*O	–	–	–	–	199.0
*Me*O–C(5)	–	–	61.2	61.2	61.0
*Me*O–C(6)	62.0	–	–	–	–
*Me*O–C(4′)	60.7	–	–	–	–
*Me*O–C(5′)	56.5	–	–	–	–
6,7-(OCH_2O)	–	–	102.9	102.9	101.9

[a]) The spectra were recorded at 25°, with $SiMe_4$ as internal standard.

aglycone. The ^{1}H- and ^{13}C-NMR spectra of **1** (*Tables 1* and *2*, resp.) exhibited characteristic signals for isoflavone and sugar moieties. Thus, the ^{1}H-NMR spectrum showed signals at δ 8.24 (H–C(2)) and 7.03 (H–C(8) characteristic of an isoflavone skeleton, whereas two 1-H *d* at δ 6.73 (J(2′,6′) = 1.9 Hz) and 6.71 (J(6′,2′) = 1.9 Hz) were due to protons of a trisubstituted unsymmetrical aromatic ring B[1]). The two anomeric-proton *d* at δ 5.09 (J(1″,2″) = 7.0 Hz) and 4.38 (J(1‴,2‴) = 7.5 Hz) suggested the presence of two sugar units

[1]) For convenience (see *Tables 1* and *2*), the numbering of **1**–**5** is partly arbitrary; for systematic names, see *Exper. Part*.

along with three MeO substituents (δ 3.81, 3.86, and 3.87). Comparison with reported data revealed that the aglycone and its monoglycoside is a known compound [6][14]. The broad-band-decoupled ^{13}C-NMR spectrum of compound **1** showed resonances for all 30 C-atoms, with 3 Me, 2 CH_2, 14 CH, and 11 quaternary C-atoms. The ^{1}H,^{1}H and direct one-bond ^{1}H,^{13}C correlations were determined by the COSY-45°, TOCSY, and HMQC experiments. The downfield shift of the CH_2 group of one glucose moiety indicated the attachment of a second glucose unit at C(6″) (δ 70.6) [15–17]. The structure of **1** was confirmed by the HMBC experiments. The interaction of the anomeric H–C(1″) (δ 5.09) with C(7) (δ 157.9) of the aglycone unit indicated that this sugar moiety was connected to C(7). The second anomeric proton at δ 4.38 (H–C(1‴)) showed HMBC interaction with C(6″) (δ 70.6). Similarly, the H–C(2′) (δ 6.73) showed HMBC interactions with C(3′) (δ 151.6) and C(6′) (δ 111.2), while H–C(6′) (δ 6.71) showed interactions with C(2′) (δ 106.1), and C(4′) (δ 138.0), and C(3) (δ 124.4). The position of the sugar moiety was finally confirmed by the NOESY correlation between H–C(1″) (δ 5.09) and H–C(8) (δ 7.03), and the interaction between the second anomeric proton H–C(1‴) (δ 4.38) and H_a–C(6″) (δ 4.15) further supported the structure.

Germanasim D (**2**) was isolated as an amorphous solid. The pseudo molecular ion $[M+H]^+$ in the HR-FAB-MS (positive mode) appeared at *m*/*z* 611.0679, consistent with the formula $C_{27}H_{30}O_{16}$ (calc. 611.0685). The positive $FeCl_3$ test indicated the presence of free OH groups in **2**. From the spectral data, the structure of 6-hydroxygenistein 4′-[*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside] (**2**).

The EI-MS of **2** showed the fragment at *m*/*z* 286 resulting from the loss of two sugar units from the molecule. The ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) showed close similarities with those of **1**. The 2 2-H *d* of **2** at δ 7.24 (J(2′,3′) = J(6′,5′) = 8.5 Hz) and 7.40 (J(3′,2′) = J(5′,6′) = 8.5 Hz) were assigned to H–C(2′) and H–C(6′) and to H–C(3′) and H–C(5′), respectively, while H–C(2) and H–C(8) resonated at δ 7.85 and 6.36, respectively. The presence of a diglycoside moiety in **2** was inferred from the resonance of two anomeric protons at δ 5.46 (*d*, J(1″,2″) = 7.5 Hz) and 5.11 (*d*, J(1‴,2‴) = 7.5 Hz). The broad-band decoupled ^{13}C-NMR spectrum showed resonances for all 27 C-atoms. The structure of **2** was finally deduced by the HMBC technique. The anomeric proton H–C(1″) (δ 5.46) showed HMBC interactions with C(4′) (δ 158.0) indicating that the sugar unit was connected to C(4′) of the aglycone. The second anomeric proton H–C(1‴) (δ 5.11) showed HMBC interactions with C(6″) (δ 69.3). The position of the sugar moiety was confirmed by NOESY correlations between δ 5.46 (H–C(1″)) and 7.40 (H–C(3′), H–C(5′)) and 7.24 (H–C(2′), H–C(6′)). Interaction of the second anomeric proton H–C(1‴) (δ 5.11) with H_a–C(6″) (δ 4.47) further supported this inference.

Germanasim E (**3**) was isolated as a white amorphous solid. Its pseudo molecular ion $[M+H]^+$ in the HR-FAB-MS (positive mode) appeared at *m*/*z* 637.3130, in agreement with the formula $C_{29}H_{32}O_{16}$ (calc. 637.3135). From the spectral data, compound **3** was identified as nigricin 4′-[*O*-β-D-glucopyanosyl-(1 → 6)-β-D-glucopyranoside]. Compound **3** was previously isolated from *Iris florentina* but the nature of glycosidic linkage was not reported [18].

The ^{1}H- and ^{13}C-NMR spectra of **3** closely resembled those of compound **2** (*Tables 1* and *2*), the main differences being the appearance of ^{1}H-NMR signals for the OCH_2O and MeO groups at δ 6.05 and 4.09, respectively. Spectral comparison suggested the presence of a diglycoside corresponding to a known monoglycoside, which was recently isolated from this plant [6], with the aglycone nigricin [19][20]. The ^{13}C-NMR spectra of **3** showed resonances for all 29 C-atoms. The anomeric proton H–C(1″) (δ 5.58) showed HMBC interactions with C(4′) (δ 154.8) indicating that the sugar unit was connected to C(4′) of the aglycone. The position of the second glucose moiety was deduced from the downfield chemical shifts of the CH_2 protons [15–17]. The position of the sugar moiety was confirmed by the NOE correlations between δ 5.58 (H–C(1″)) and δ 7.51 (H–C(3′), H–C(5′)) indicating that one of the glucose moiety was adjacent to H–C(3′) and H–C(5′). Similarly, the irradiation of the second anomeric proton H–C(1‴) (δ 5.13) showed enhancement of the H_a–C(6″) signal (δ 4.79) indicating that the second sugar moiety was connected to C(6″) of the first sugar unit.

Germanasim F (**4**) was isolated as a white amorphous solid. In the HR-FAB-MS (positive mode), the pseudo molecular ion $[M+H]^+$ appeared at *m/z* 783.1420, in agreement with the formula $C_{35}H_{42}O_{20}$ (calc. 783.1427). From the spectral data, compound **4** was identified as nigricin 4′-[*O*-*β*-D-glucopyanosyl-(1 → 2)-*O*-[*α*-L-rhamnopyranosyl]-(1 → 6)-*β*-D-glucopyranoside].

The ^{1}H- and ^{13}C-NMR spectra of **4** closely resembled those of **3** (*Table 1* and *2*), the main differences being the appearance of the signals of three anomeric H-atoms at *δ* 5.55 (*d*, *J*(1″,2″) = 7.5 Hz), 5.35 (*d*, *J*(1‴,2‴) = 7.5 Hz), and 5.60 (br. *s*) along with a 3-H *d* at *δ* 1.88 (*d*, *J*(6⁗,5⁗) = 6.1 Hz). The ^{13}C-NMR spectra of **4** showed resonances for all 35 C-atoms and confirmed the presence of a triglycosidic unit, exhibiting three anomeric C-atoms resonating at *δ* 100.2, 102.2, and 105.3, along with signals for OH-bearing CH groups and two CH_2 (*δ* 62.5 and 69.6) and one Me group (*δ* 18.8) which was assigned to C(6⁗) of a rhamnose unit. The locations of the glycosidic linkages were deduced from a comparison of the ^{13}C-NMR spectra with those of known glycosides [15–17][21]. The anomeric proton H–C(1″) (*δ* 5.55) showed HMBC interactions with C(4′) (*δ* 158.2) indicating that the sugar unit was connected to C(4′). The attachment of the second glucose moiety was deduced from the downfield chemical shift of *C*H(2″) (*δ* 75.5). On the other hand, connectivity of the third sugar unit was deduced from the downfield shift of *C*H_2(6″) (*δ* 69.6) and from the HMBC interaction of H–C(1⁗) (*δ* 5.60) with C(6″) (*δ* 69.6). The position of the sugar moiety was confirmed by the NOE between *δ* 5.55 (H–C(1″)) and *δ* 7.49 (H–C(3′), H–C(5′)) indicating that one of the glucose moiety was adjacent to H–C(3′) and H–C(5′).

Germanasim G (**5**) was isolated as a white amorphous solid. The HR-FAB-MS (positive mode) showed a pseudo molecular ion $[M+H]^+$ at *m/z* 801.2130, consistent with the formula $C_{38}H_{40}O_{19}$ (calc. 801.2135). The negative $FeCl_3$ test showed that **5** contains no free phenolic OH groups. Based on the spectral data, its structure was finally deduced as 7-{4-{[2-*O*-(4-acetyl-2-methoxyphenyl)-*β*-D-glucopyranosyl]oxy}-3-(*β*-D-glucopyranosyloxy)phenyl}-9-methoxy-8*H*-1,3-dioxolo[4,5-g][1]benzopyran-8-one (**5**).

In the FAB-MS of **5**, a peak at *m/z* 639 showed the loss of a sugar unit from M^+. The peak at *m/z* 328 in the EI-MS ($C_{17}H_{12}O_7$) indicated that the benzene ring was attached to a sugar unit. The ^{1}H-NMR spectrum of **5** (*Table 1*) showed characteristic *s* of the isoflavone skeleton at *δ* 8.00 (H–C(2)) and 6.65 (H–C(8)). Similarly, H–C(2′) of the aromatic ring B appeared as a *d* at *δ* 7.22 (*d*, *J*(2′,6′) = 1.9 Hz) showing *meta* coupling with H–C(6′) at *δ* 6.90 (*dd*, *J*(6′,5′) = 8.5 Hz, *J* (6′,2′) = 1.9 Hz). H–C(6′) was *ortho*-coupled to H–C(5′) at *δ* 7.25 (*d*, *J*(5′,6′) = 8.5 Hz). The ^{1}H-NMR showed another aromatic *ABX* system in which H–C(6⁗) (*d* at *δ* 7.20 (*J*(6⁗,5⁗) = 8.5 Hz)) was *ortho*-coupled with H–C(5⁗) (*dd* at *δ* 7.64, (*J*(5⁗,6⁗) = 8.5 Hz, *J*(5⁗,3⁗) = 1.9 Hz). H–C(3⁗) at *δ* 7.56 (*d*, *J*(3⁗,5⁗) = 1.9 Hz), on the other hand, showed *meta*-coupling with H–C(5⁗). Two MeO *s* at *δ* 3.95 and 4.01, a 2-H *s* of a OCH_2O group (*δ* 6.01) and a 3-H *s* (*δ* 2.56) suggested that two MeO, one OCH_2O, and an Ac group(s) were also present in the molecule. Two anomeric protons resonated as *d* at *δ* 5.10 (*J*(1‴,2‴) = 7.5 Hz) and 4.90 (*J*(1″,2″) = 7.5 Hz). The ^{13}C-NMR spectra of **5** (*Table 2*) showed all 38 resonances with 2 Me, 3 CH_2, and 18 CH groups, besides 13 quaternary C-atoms. The anomeric H–C(1″) (*δ* 4.90) showed HMBC interaction with H–C(3′) (*δ* 148.3) indicating that the sugar moiety was connected to C(3′) of the aglycone moiety. The second anomeric H–C(1‴) (*δ* 5.10) showed interaction with C(4′) (*δ* 152.5). The presence of another substituted benzene moiety in **5** was revealed by the ^{13}C-NMR spectrum, which showed the resonances of a carbonyl (*δ* 199.0) and *Me*CO group (*δ* 26.4) and of 6 sp^2 C-atoms, besides those due to the isoflavone moiety. H–C(3⁗) (*δ* 7.56) showed HMBC interactions with C(1⁗) (*δ* 152.0) and C(4⁗) (*δ* 132.6), and H–C(6⁗) (*δ* 7.20) with C(1⁗) (*δ* 152.0) and C(4⁗) (*δ* 132.6); MeCO (*δ* 2.56) showed interactions with CO (*δ* 199.0) and C(4⁗) (*δ* 132.6) which further supported the structure. The location of this benzene moiety at a glucose moiety was deduced from a comparison of the ^{13}C-NMR spectra of known glycosides [22].

For all compounds **1**–**5**, the sugar moieties and their configurations were deduced as *β*-D-glucopyranose and *α*-L-rhamnose by comparison with ^{13}C-NMR chemical shift data reported for various sugars [23][24]. The nature of the sugars was further confirmed by hydrolysis and co-TLC with authentic samples of glucose and rhamnose

as well as by GLC experiments [25] (see *Exper. Part*). The aglycones, after hydrolysis, were also identified by co-TLC with authentic samples.

Experimental Part

General. Column chromatography (CC): silica gel, 70–230 mesh, *Sephadex LH-20* (*Pharmacia*, Uppsala, Sweden). Recycling prep. HPLC: instrument from *Japan Analytical Industry Co., Ltd.; LC-908* column. TLC: detection at 254 and 366 nm, and by staining with ceric sulfate spraying reagent. UV Spectra: *Hitachi U-3200* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco A-302* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. Optical rotations: *Schmidt & Haensch Polartronic-D* polarimeter. NMR Spectra: *Bruker AM-400* and *AMX-500* spectrometers with the UNIX data system; ^1H at 300 and 500 MHz, resp., ^{13}C at 75 and 125 MHz, resp.; in (D_6)DMSO, CD_3OD, or (D_5)pyridine at r.t. with $SiMe_4$ as internal standard, δ in ppm, J in Hz. MS: FAB in the positive-ion mode, with Ar (8000 eV); ion-source energy 70 eV and ion-source temp. 250° for HR-EI; *Jeol JMS-600* and *HX-110* mass spectrometers with the data system DA 5000; m/z (rel. %).

Plant Material. The rhizomes of *Iris germanica* L. were collected from Reyhanli, Hatay, Turkey, in July 1999, and air-dried. An authentic voucher specimen (GUE# 2229) was deposited in the Herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey. The plant was identified by one of us (*B. S.*).

Extraction and Isolation. Air-dried roots of *I. germanica* (1 kg) were extracted with MeOH (15 l) for 15 days at 25°. After evaporation of the solvents, a crude extract (130.2 g) was obtained which was dissolved in distilled H_2O and defatted with hexane. The defatted aq. extract was further fractionated with $CHCl_3$ (5 l), AcOEt (5 l), and then with BuOH (5 l). The BuOH fraction was evaporated and the residue (60.0 g) subjected to CC (silica gel; 0 → 100% MeOH/$CHCl_3$ each 3 l): *Fr. 1–10*. *Fr. 4*, eluted with 20% MeOH/$CHCl_3$ (500 mg), was resubmitted to CC (silica gel), elution with 18% MeOH/$CHCl_3$ yielded **5** (25 mg), whereas elution with 8% MeOH/$CHCl_3$ afforded compound **6** (15.0 mg). *Fr. 5* of the first CC, eluted with 25% MeOH/$CHCl_3$ (1 g), was rechromatographed (*Sephadex LH-20*, H_2O/MeOH). *Fr. 5.6*, eluted with H_2O/MeOH 1:1, was subjected to prep. HPLC (H_2O/MeOH 1:1), 4 ml/min, det. at 254 nm, *RI* 500): *Fr. 5.6–5.7*. Repeated prep. HPLC (H_2O/MeOH 1:1, 4 ml/min, detection at 254 nm, *RI* 500, R^t 22 ml/min) of *Fr. 5.6.1–5.6.4* yielded **1**. *Fr. 5.1*, obtained with 100% H_2O, afforded *Fr. 5.1.1–5.1.5*. *Fr. 5.1.3* was also subjected to prep. HPLC (H_2O/MeOH 1:1, 4 ml/min, det. at 254 nm, *RI* 500, R^t 32 ml/min) to give **2**. *Fr. 5.1.7* was subjected to prep. HPLC (H_2O/MeOH 1:1, 4 ml/min, det. at 254 nm, *RI* 1000, R^t 21 ml/min) to give **3** and **4** (H_2O/MeOH 1:1, 2.5 ml/min, det. at UV 254 nm, *RI* 1000, R^t 26 ml/min).

Hydrolyses. Compounds **1**–**6** were dissolved in MeOH (2 ml) and 3% HCl soln. (2 ml). Each mixture was heated at 100° for 2 h. The soln. was neutralized with Na_2CO_3 and extracted with AcOEt. The H_2O-soluble compounds were identified as glucose and rhamnose by co-TLC (AcOEt/BuOH/H_2O 2:7:1 and BuOH/AcOH/H_2O 12:3:5) with authentic samples of glucose and rhamnose [16][25].

To a soln. of the sugar (2.0 mg) in a 5-ml flask in dry pyridine (3.0 ml), hexamethyldisilazane (3.0 ml) was added by syringe. Then chlorotrimethylsilane (3.0 ml) was added and the mixture left for 30 min. After evaporation, the residue was washed with dry heptane (10.0 ml) and the silylated product analyzed by GC: product identical to that obtained from authentic β-D-glucose [26].

Germanasim C (= *7-{[*O-β-D-*Glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]oxy}-5-hydroxy-3-(3-hydroxy-4,5-dimethoxyphenyl)-6-methoxy-4*H-*1-benzopyran-4-one*; **1**). White amorphous solid (7.0 mg, $0.7 \cdot 10^{-3}$ % yield. R_f (H_2O/MeOH 1:1) 0.1. $[\alpha]_D^{24} = +61.6$ ($c = 0.83$, MeOH). UV (MeOH): 319 (3.423), 263 (3.986). IR (KBr): 3328 (OH), 1649 (C=O), 1559 (arom. C=C). ^1H- and ^{13}C-NMR ($CDCl_3$): *Tables 1* and *2*. HR-FAB-MS (pos.) 685.1230 ($C_{30}H_{36}O_{18}^+$, $[M+H]^+$; calc. 685.1235). FAB-MS: 685 ($[M+H]^+$), 361 ($[M+H-2\,(glu)]^+$. EI-MS: 360 (100), 345 (85), 182 (20), 178 (25).

Germanasim D (= *3-{4-{[*O-β-D-*Glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]oxy}phenyl}-5,6,7-trihydroxy-4*H-*1-benzopyran-4-one*; **2**). Amorphous solid (10.0 mg, $1.0 \cdot 10^{-3}$%. R_f (H_2O/MeOH 1:1) 0.14. $[\alpha]_D^{24} = +50.2$ ($c = 0.57$, MeOH). UV: 319 (3.808), 262 (3.287). IR: 3345 (OH), 1665 (C=O), 1565 (arom. C=C). ^1H- and ^{13}C-NMR (C_5D_5N): *Tables 1* and *2*. HR-FAB-MS (pos.): 611.0679 ($C_{27}H_{30}O_{16}^+$, $[M+H]^+$; calc. 611.0685). FAB-MS (pos.): 611 ($[M+H]^+$), 287 ($[M+H-2\,(glu)]^+$. EI-MS: 286 (90), 168 (30), 118 (15).

Germanasim E (= *7-{4-{[*O-β-D-*Glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]oxy}phenyl}-9-methoxy-8*H-*1,3-dioxolo[4,5-*g*][1]benzopyran-8-one*; **3**). White amorphous solid (30.3 mg, $3.0 \cdot 10^{-3}$ %). R_f (H_2O/MeOH 1:1) 0.17. $[\alpha]_D^{24} = +47.6$ ($c = 0.63$, MeOH). UV (MeOH): 271 (3.556). IR: 3340 (OH), 1674 (C=O), 1570 (C=C), 923 (OCH_2O). ^1H-and ^{13}C-NMR (CD_3OD): *Tables 1* and *2*. HR-FAB-MS (pos.): 637.3130 ($C_{29}H_{32}O_{16}^+$, $[M+H]^+$; calc. 637.3135). EI-MS: 312 (100), 298 (50), 194 (25), 118 (18).

Germanasim F (= *7-[4-{[*O-*β*-D-*Glucopyranosyl-(1 → 2)*-O-*[6-deoxy-α*-L-*mannopyranosyl-(1 → 6)]-β*-D-*glucopyranosyl]oxy}phenyl]-9-methoxy-8*H-*1,3-dioxolo[4,5*-g*][1]benzopyran-8-one*; **4**). White amorphous solid (5.2 mg, $0.52 \cdot 10^{-2}$ %). R_f (H_2O/MeOH 1:1) 0.14. $[\alpha]_D^{24} = +52.6$ ($c = 0.54$, MeOH). UV (MeOH): 273 (3.635). IR: 3325 (OH), 1656 (C=O), 1565 (C=C), 936 (OCH_2O). ^{1}H- and ^{13}C-NMR (C_5D_5N): *Tables 1* and *2*. HR-FAB-MS (pos.) 783.1420 ($C_{35}H_{42}O_{20}^+$, $[M+H]^+$; calc. 783.1427). EI-MS: 312 (100), 298 (46), 194 (22), 117 (19).

Germanasim G (= *7-[4-[[2-*O-*(4-Acetyl-2-methoxyphenyl)-β*-D-*glucopyranosyl]oxy}-3-(β*-D-*glucopyranosyloxy)phenyl]-9-methoxy-8*H-*1,3-dioxolo[4,5*-g*][1]benzopyran-8-one*; **5**). White amorphous solid (25.0 mg, $3.0 \cdot 10^{-3}$ %). R_f ($CHCl_3$/MeOH 82:18) 0.17. $[\alpha]_D^{24} = +48.2$ ($c = 0.42$, MeOH). UV: 271 (3.556). IR: 3340 (OH), 1674 (C=O), 1570 (C=C), 923 (OCH_2O). ^{1}H- and ^{13}C-NMR (CD_3OD): *Tables 1* and *2*. HR-FAB-MS (pos.): 801.2130 ($C_{38}H_{40}O_{19}^+$, $[M+H]^+$; calc. 801.2135). FAB-MS (pos.): 639 ($[M+H]^+$). EI-MS: 328 (98).

Germanasim B (= *7-[4-(β*-D-*Glucopyranosyloxy)phenyl]-9-methoxy-8*H-*1,3-dioxolo[4,5*-g*][1]benzopyran-8-one*; **6**). Amorphous solid (12.0 mg, $1.2 \cdot 10^{-3}$ %). R_f ($CHCl_3$/MeOH 92:8) 0.14. $[\alpha]_D^{24} = +50.2$ ($c = 0.57$, MeOH). UV: 319 (3.808), 262 (3.287). IR: 3345 (OH), 1665 (C=O), 1565 (arom. C=C), 935 (OCH_2O). HR-FAB-MS (pos.): 475.1235 ($C_{23}H_{22}O_{11}^+$, $[M+H]^+$; calc. 475.1240). EI-MS: 312 (89), 298 (100), 194 (20), 118 (15).

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