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Flavonoids from *Iris spuria* (Zeal) Cultivated in Egypt

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A new 12a-dehydrorotenoid 1, 11-dihydroxy-9, 10-methylenedioxy-12a-dehydrorotenoid **(1)**, together with a new isoflavonoid glycoside tectorigenin-7-*O*-β-glucosyl-4'-*O*-β-glucoside **(3)**, were isolated and identified from the rhizomes of *I. spuria* (Zeal). In addition, 4 known compounds, tectorigenin **(2)** tectorigenin-7-*O*-β-glucosyl (1 → 6) glucoside **(4)**, tectoridin (a tectorigenin-7-*O*-β-glucoside) **(5)** and tectorigenin-4'-*O*-β-glucoside **(6)** were isolated and identified for the first time from this plant. The structures of the isolated compounds were determined by spectroscopic methods (UV, IR, ^{1}H, ^{13}C-NMR, DEPT, HMQC, NOESY, and HMBC experiments and MS spectrometry) and by comparison with literature data of known compounds. Compounds **2**, **4**, **5**, and **6** are reported for the first time from this plant through the present study.

Key words: *Iris spuria* (Zeal), Iridiaceae, Rhizomes, 12a-Dehydrorotenoid 1, 11-Dihydroxy- 9, 10 methylenedioxy-12a-dehydrorotenoid, Isoflavonoid glycoside, Tectorigenin-7-*O*-β-glucosyl-4'-*O*-β-glucoside.

INTRODUCTION

The genus *Iris* is represented in Egypt by three species of rare occurrence (El-Hadidi & Fayed, 1995). Most *Iris* plants distributed in the Egyptian gardens are introduced species or hybrids cultivated mainly for ornamental purposes. The use of underground parts of several *Iris* species in traditional European folk medicine is well documented (Wollenweber *et al.*, 2003). Earlier work on the constituents of *I. spuria* rhizomes revealed the presence of various isoflavones, a 12a-hydroxyrotenoid (irispurinol) and a 5,8,2'-trihydroxy-7-methoxy flavanone as the major secondary metabolites (Shawl *et al.*, 1984; 1988a and 1988b). Isoflavones have a wide array of biological activities including anti-inflammatory (Yasukawa *et al.*, 1989), antitumor (Elangovan *et al.*, 1994) effects and activity to reduce hepatic injury (Yamazaki *et al.*, 1997). Isoflavonoids tectorigenin and tectoridin, a glycosylated tectorigenin, have an anti-inflammatory activity by inhibiting prostaglandin (PG) E_2 production in 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)-stimulated rat peritoneal macrophages (Yamaki *et al.*, 2002; Shin *et al.*, 1999). Rotenone and related compounds were originally used to paralyze fish before being used as an insecticide (Kan, 1972). Rotenoids are not only known as toxicants, but also as candidate anticancer agents (Li *et al.*, 1993; Ito *et al.*, 2004). Our pervious publication on the entitled plant aimed to the isolation and identification of iridal glycosides; a new class of natural glycosides (Marner *et al.*, 2002). In a continuation of our pervious study on rhizomes of this plant (Marner *et al.*, 2002), this study deals with its flavonoids content. The present paper reports on the isolation and identification of two novel flavonoid compounds; 12a-dehydrorotenoid 1,11-dihydroxy-9,10-methylenedioxy-12a-dehydrorotenoid (**1**) and tectorigenin-7-*O*-β-glucosyl-4'-*O*-β-glucoside (**3**) as well as 4 known compounds, tectorigenin (**2**) tectorigenin-7-*O*-β-glucosyl (1→6) glucoside (**4**), tectoridin (a tectorigenin-7-*O*-β-glucoside) (**5**), and tectorigenin-4'-*O*-β-glucoside (**6**). Compounds **2**, **4**, **5**, and **6** have not been previously found in the plant.

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MATERIALS AND METHODS

The ^{1}H, ^{13}C-NMR and 2D-NMR spectra (δ, ppm, *J* in Hz in DMSO-d_6, TMS as int. standard) were recorded with a Varian unity Inova (500 MHz for ^{1}H and 125 MHz for ^{13}C-NMR and 2D-NMR). FAB-MS, registered in positive ion mode in glycerol matrix, HRCIMS (+ve), a high-resolution chemical ionization mass spectrometry, and EI-MS were recorded on a Hitachi M-8000 spectrometer. UV spectra were recorded in MeOH using standard procedures

(Mabry *et al.*, 1970) using Shimadzu UV-1200 spectrophotometer. The IR spectra were recorded in KBr discs using Perkin-Elmer 1720X-FTIR spectrometer. Column chromatography (CC) was performed using silica gel (60-120 mesh) and Sephadex LH-20 (25-100 µm, Pharmacia), column (100×4 cm). Preparative HPLC: Gilson-305 pump equipped with JASCO UV-970 detector and YMC-PACK R&D prep. Column (10 µm ODS 120 Å, 250×20 mm), solvent: MeOH : H_2O (1:1 & 6:4). TLC: silica 60 F_{254}, Merck, $CHCl_3$: MeOH (9:1, 8:2 and 7:3) and KC_{18} RP plates (10×20 cm 200 µm, Whatman, MeOH: H_2O (1:1) and detection was carried out by spraying TLC with 10% H_2SO_4 followed by heating.

Plant materials

Rhizomes of *I. spuria* (Zeal) were collected during the flowering season in March 2000. The plants were cultivated in the Experimental Station of Medicinal Plants at Faculty of Pharmacy, Assiut University, Assiut, Egypt. Dr. Abd El Salaam Mohamed Al-Nowiahi, Professor of Taxonomy, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt, kindly identified the plant materials. A voucher specimen (no. IR50/2002) of the authenticated *I. spuria* (Zeal) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo, Egypt.

Extraction and isolation

The fresh rhizomes (1 kg) of *I. spuria* (Zeal) were cleaned under running tap water, cut into small pieces, and extracted successively with petroleum ether (40-60 °C), $CHCl_3$, and finally with aq. MeOH (70%) at room temperature. Each extract was concentrated *in vacuo* at temperature not exceeding 45°C to give 17 g, 12 g and 52 g respectively. The chloroform extract (8 g) was fractionated by gel filtration over Sephadex LH-20 CC using $CHCl_3$: MeOH (1:1) as solvent system in 5 portions to give fourteen fractions (Fr1~Fr14). Fractions 8~14, enriched with flavonoids, were collected and repeatedly subjected to LH-20 CC eluted with methanol to afford eight fractions. Fraction 6 (70 mg) showed a crystalline deposit. It gave two major spots on TLC with R_f 0.55 and 0.23 respectively using petroleum ether: acetone (9:1) as a mobile phase. This fraction was subjected to a silica gel CC eluted with petroleum ether with gradual increasing of acetone to give 8 fractions. Fraction 3 gave one spot on TLC. It was crystallized from hot methanol to give yellow crystals (30 mg) of compound **1**. Fraction 7 was crystallized from MeOH to give yellow amorphous powder (10 mg) of compound **2**.

The 70% aq. MeOH extract (45 g) was extracted with *n*-BuOH, which was evaporated *in vacuo* at temperature below 55ºC to give yellowish brown residue 12 g. The *n*-BuOH extract (8 g) was chromatographed over Sephadex LH-20 CC, eluted with MeOH: H_2O (9:1) in 4 portions to give 10 fractions. First five fractions were enriched with iridals as described before (Marner *et al.*, 2002). Fractions 6 and 7 (850 mg) showed major two spots gave green with $FeCl_3$ on TLC (silica and RP-C_{18}) using MeOH: H_2O (1:1) as solvent system. The separation was evaluated by prep. HPLC with isocratic elution MeOH: H_2O (1:1) to give compounds **3** and **4**, each compound was finally purified over Sephadex LH-20 CC eluted with 10% aq. MeOH to give (100 mg) as colorless crystals of **3** and (65 mg) as white amorphous powder of **4**. Fractions 8 and 9 gave similar TLC pattern, were collected (450 mg) and subjected for prep. HPLC, solvent MeOH: H_2O (6:4) to give white powder from hot methanol of **5** (42 mg) and pure yellowish white amorphous powder from hot methanol of **6** (30 mg), respectively.

1, 11-Dihydroxy-9, 10-methylenedioxy-12a-dehydrorotenoid (1)

Yellow crystals; HRCIMS (positive) $[M+H]^+$ a protonated molecule ion peak at m/z 327.0525 (calc. for $C_{17}H_{11}O_7$); IR (KBr) ν_{max} cm^{-1}: 3350 (*br*.OH), 1650 (chelated >C=O), 1260, 925 (methylenedioxy group); UV λ_{max}^{MeOH} nm (log ε): 217 (3.52), 280 (4.56), 360 (3.55); +NaOMe, 416, 409, 293, 245, 216; +$AlCl_3$, 370, 283, 237, 220; + $AlCl_3$/HCl, 368, 280, 234, 204; +NaOAc, 448, 383, 360, 351. ^{1}H-NMR (500 MHz, DMSO-d_6, TMS, ppm); δ 5.08 (2H, s, CH_2-O), δ 6.16 (2H, s, O-CH_2-O), δ 6.77 (1H, s, H-8), δ 6.78 (1H, dd, *J*=7.33 & 1.80, H-2), δ 6.95 (1H, dd, *J*=7.55 & 1.80, H-4), δ 7.35 (1H, dd, *J*=7.33 & 7.55, H-3), exchangeable singlets at δ 10.42 (1-OH), and δ 12.75 (11-OH). ^{13}C-NMR (Table I).

Tectorigenin (2)

Yellow amorphous powder; EI/MS; *m/z* 300 $[M]^+$; UV λ_{max}^{MeOH} nm : 273, 335 (sh); +$AlCl_3$, 281, 330(sh), 335; +$AlCl_3$/HCl, 280, 315(sh), 360; +NaOAc 280, 336. ^{1}H-NMR (500 MHz, Me_2CO-d_6, TMS, ppm); δ 3.88 (3H, s, OMe), δ 6.65 (1H, s, H-8), δ 6.89 (2H, d, *J*=8.3 Hz, H-3',5'), δ 7.44 (d, *J*=8.8 Hz, H-2', 6'), δ 8.11(1H, s, H-2), exchangeable singlet at δ 12.88 (5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 180.4 (C4), 157.8 (C4'), 157.5 (C7), 154.4 (C2), 152.7 (C5), 152.5 (C9), 132.3 (C6), 131.2 (C2', C6'), 123.5 (C3), 123.0 (C1'), 116.9 (C3', C5'), 106.1 (C10), 93.1 (C8), 60.1 (OMe).

Tectorigenin-7-*O*-β-glucosyl-4'-*O*-β-glucoside (3)

Colorless needles; FAB-MS (positive); $[M+1]^+$ m/z 625; IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 1657(>C=O), 1511, 1462, 830 cm^{-1}; UV λ_{max}^{MeOH} nm (log ε): 212 (4.29), 264 (4.15), 330 (1.67); +$AlCl_3$, 274, 325(sh); +$AlCl_3$/HCl, 274, 320 (sh); +NaOAc, 266, 329. ^{1}H-NMR (500 MHz, DMSO-d_6, TMS,

ppm); δ 3.3~4.5 (sugar protons), δ 3.77 (3H, s, OMe), δ 4.91 (1H, d, *J*=7.7 Hz, H-1''), δ 5.10 (1H, d, *J*=7.7 Hz, H-1'''), δ 6.90 (1H, s, H-8), δ 7.11 (2H, *d*, *J*=8.8 Hz, H-3', 5'), δ 7.52 (2H, d, *J*=8.8 Hz, H-2', 6'), δ 8.51 (1H, s, H-2), exchangeable singlet at δ 12.88 (5-OH). ^{13}C-NMR (Table I).

Tectorigenin-7-*O*-β-glucosyl (1 → 6) glucoside (4)

White amorphous powder; FAB-MS (positive); $[M+1]^+$ m/z 625; IR (KBr) ν_{max} cm^{-1}: 3450 (OH), 1652 (>C=O), 1511, 1462, 1019, 816 cm^{-1}; UV λ_{max}^{MeOH} nm (log ε): 216 (4.20), 265 (4.15), 329 (1.67); +$AlCl_3$, 270, 359sh; +$AlCl_3$/HCl, 268, 330sh; +NaOAc, 271, 331(sh). ^{1}H-NMR (500 MHz, DMSO-d_6, TMS, ppm); δ 3.25-4.04 (sugar protons), δ 3.75 (3H, s, OMe), δ 4.23 (1H, d, *J*=7.77 Hz, H-1''), δ 4.88 (1H, d, *J*=7.32 Hz, H-1'''), δ 6.51 (1H, s, H-8), δ 7.15 (2H, d, *J*=8.7 Hz, H-3', 5'), δ 7.49 (2H, d, *J*=8.7 Hz, H-2', 6'), δ 8.37 (1H, s, H-2), exchangeable singlets at δ 10.79 (4'-OH), and δ 13.01 (5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 180.3 (C4), 157.5 (C4'), 156.3 (C7), 154.4 (C2), 153.1 (C5), 152.6 (C9), 131.4 (C6), 130.1 (C2', C6'), 124.4 (C3), 121.5 (C1'), 116.0 (C3', C5'), 106.7 (C10), 103.1 (C1'''), 100.3 (C1'') 93.9 (C8), 76.7 (C5''), 76.5 (C5'''), 76.3 (C3''), 75.9 (C3'''), 73.4 (C2''), 73.0 (C2'''), 69.9 (C4'''), 69.6 (C4''), 68.1 (C6''), 60.9 (C6'''), 59.8 (OMe).

Tectoridin (tectorigenin-7-*O*-β-glucoside) (5)

White powder; FAB-MS (positive); $[M+H]^+$ m/z 463; IR (KBr) ν_{max} cm^{-1}: 3369 (OH), 1658 (C=O), 1519, 1461, 1088, 813 cm^{-1}; UV λ_{max}^{MeOH} nm (log ε): 266.4 (4.29), 325(sh); +$AlCl_3$, 277, 315(sh), 380; +$AlCl_3$/HCl, 278, 322(sh), 388; +NaOAc 266, 331(sh). ^{1}H-NMR (500 MHz, DMSO-d_6, TMS, ppm); δ 3.8-4.5 (sugar protons), δ 3.76 (3H, s, OMe), δ 5.44 (1H, d, *J*=7.0 Hz, H-1'), δ 6.89 (1H, s, H-8), δ 6.83 (2H, d, *J*=8.8 Hz, H-3',5'), δ 7.41 (d, *J*=8.8 Hz, H-2',6'), δ 8.44 (1H, s, H-2), exchangeable singlets at δ 9.63 (4'-OH) and δ 12.93 (5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 180.6 (C4), 157.3 (C4'), 156.5 (C7), 154.5 (C2), 152.7 (C5), 152.3 (C9), 132.3 (C6), 130.1 (C2', C6'), 121.9 (C3), 120.9 (C1'), 114.9 (C3', C5'), 106.4 (C10), 100.0 (C1''), 93.9 (C8), 77.1 (C5''), 76.5 (C3''), 72.2 (C2''), 69.5 (C4''), 60.5 (C6''), 60.1 (OMe).

Tectorigenin-4'-*O*-β-glucoside (6)

Yellowish white amorphous powder; FAB-MS (positive); $[M+H]^+$ m/z 463; IR (KBr) ν_{max} cm^{-1}: 3469 (OH), 1657 (C=O), 1581, 1511, 1462, 1187, 1019, 843 cm^{-1}; UV λ_{max}^{MeOH} nm (log ε): 216 (4.29), 272 (4.15), 320(sh); +$AlCl_3$, 280, 325(sh); +$AlCl_3$/HCl, 280, 315(sh); +NaOAc, 280, 315. ^{1}H-NMR (500 MHz, DMSO-d_6, TMS, ppm); δ 3.38-5.1 (sugar protons), δ 3.75 (3H, s, OMe), δ 5.34 (1H, d, *J*=7.0 Hz, H-1''), δ 6.51 (1H, s, H-8), δ 7.09 (2H, d, *J*=8.9 Hz, H-3', 5'), δ 7.49 (d, *J*=8.9 Hz, H-2', 6'), δ 8.51 (1H, s, H-2), exchangeable singlet at δ 13.01 (5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 180.2 (C4), 157.1 (C7), 156.5 (C4'), 154.4 (C2), 152.9 (C5), 152.3 (C9), 132.5 (C6), 130.1 (C2', C6'), 121.9 (C3), 120.9 (C1'), 115.9 (C3', C5'), 106.5 (C10), 100.1 (C1''), 93.8 (C8), 76.9 (C5''), 76.5 (C3''), 72.1 (C2''), 69.5 (C4''), 60.1 (C6''), 59.8 (OMe).

Acid hydrolysis of 3~6

Each compound (3 mg) was refluxed in 0.1 N H_2SO_4 (10 mL) for 4 h. The aglycones were extracted with diethyl ether and identified by comparison with authentic samples on silica gel TLC, petroleum ether : acetone (9:1) and petroleum ether: EtOAc (95:5). The aq. layer was neutralized with $NaHCO_3$ and freeze dried. The sugar was extracted with pyridine, the solvent evapd. The residue dissolved in a small amount of MeOH and run on silica gel TLC in EtOAc: MeOH: H_2O: HOAc (57:13:13:17) using p-anisidine phthalate as detection reagent.

RESULTS AND DISCUSSION

The chloroform extract of *I. spuria* (Zeal) was fractionated and purified by Sephadex LH-20 and silica gel columns chromatography followed by crystallization to afford compounds **1** and **2**.

Compound **1** was isolated as yellow crystals. It gave green color with alcoholic $FeCl_3$ solution indicating the possibility of phenolic hydroxy function. This finding was confirmed by IR bands at 3350 cm^{-1} (OH) and 1650 cm^{-1} for (a chelated >C=O). This functionality was further confirmed by bathochromic shift observed in the UV spectrum in the presence of different shifting reagents (Mabry *et al.*, 1970). The IR spectrum also showed a band at 925 cm^{-1} characteristics for methylenedioxy group (Agarwal, 1989), which was confirmed by presence of a sharp singlet in ^{1}H-NMR spectrum, integrated for two protons at δ 6.16, correlated with methylene carbon at δ 102.7 in HMQC experiment. HRCIMS (ev+), a high resolution chemical ionization mass spectrometry, of **1** exhibited $[M+H]^+$ at *m/z* 327.0525 (calcd. 327.0505), which was in accordance with a molecular formula $C_{17}H_{11}O_7$. Two fragments at *m/z* 181 and *m/z* 146 originated from typical retro-Diels-Alder fragmentation of rotenoid (Messana *et al.*, 1986), suggested the presence of a methylenedioxy group in ring D. ^{1}H-NMR spectrum showed another sharp singlet at δ 5.08 integrated for two methylene protons characteristic for (CH_2-6) of rotenoid system. The absence of ^{1}H-NMR signals for an ABC system attributed to sequence of (O-CH_2-CH-O) (Shawl *et al.*, 1988a) suggested the 12a-dehydrorotenoid system. ^{1}H-NMR also exhibited other characteristic signals at δ 6.77 (s, H-8), δ 6.78 (dd, *J*=7.33 & 1.80, H-2), δ 6.95 (dd, *J*=7.55 & 1.80, H-4), δ 7.35 (dd, *J*=7.33 & 7.55, H-3) and two deuterium exchangeable

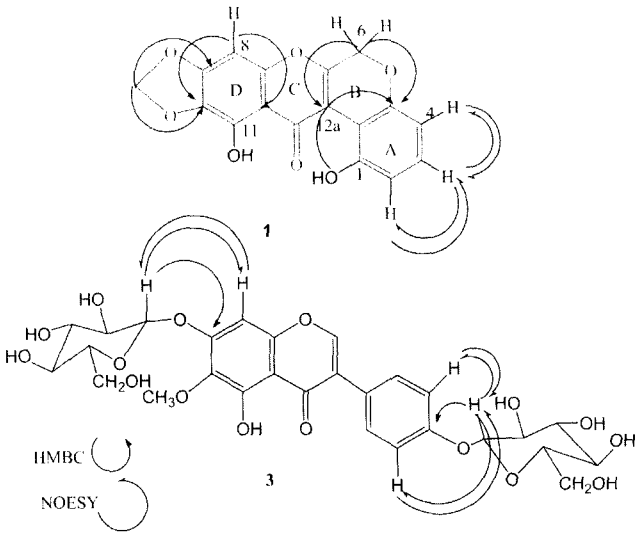

Fig. 1. Structures of compounds **1** & **3**

singlets at δ 10.42 & δ 12.75 which were assigned for (C-1 and C-11) hydroxy groups. The assignment of the three aromatic protons located in ring A was determined by 2D-NOESY correlation experiments (Fig. 1). All protons and carbon resonances were assigned from ^{13}C-NMR (Table I), DEPT, HMQC, 2D-NOESY and HMBC experiments. The HMBC spectrum of 1 showed significant correlations between H-2/C-1a/C-4 (δ 6.95/110.4/115.6), H-3/C-1/C-4a (δ 7.35/154.6/135.1), H-4/C-1a/ C-2 (δ 6.78/110.4/117.1), CH_2-6/C-4a/C-12a (δ 5.08/135.1/140.5), H-8/C-10/C-11a (δ 6.77/129.1/107.4) and O-CH_2-O/C-9/C/10 (δ 6.16/153.6/129.1). In the light of the above evidences, the structure of **1** was thus unequivocally determined to be 1,11-dihydroxy-9,10-methylenedioxy-12a-dehydrorotenoid, a new rotenoid derivative, which is reported here for the first time from natural source. The possibility of **1** as an artifact was ruled out by the fact that TLC showed the presence of **1** in the extract from the outset.

Compound **2** was isolated as pale yellow amorphous powder and identified as tectorigenin by comparison of its spectral data with literature data (Agarwal *et al.*, 1984).

Fractionation of the *n*-butanol rhizomes extract on Sephadex LH-20 CC followed by prep. HPLC analysis resulted in the isolation of four isoflavonoid glycosides **3**~**6**.

Compound **3** was obtained as colorless needles. The positive-ion FAB mass spectrum showed a peak $[M+1]^+$ at m/z 625, consistent with the molecular formula $C_{28}H_{32}O_{16}$. Fragments at m/z 463 and 301 suggested the presence of 2 hexose units. The IR spectrum displayed bands at 3400 cm^{-1}(OH) and 1657 cm^{-1} (a chelated >C=O group). Acid hydrolysis followed by TLC comparison with authentic samples revealed the presence of glucose and tectorigenin (Farag *et al.*, 1999). The UV spectrum exhibited

Table I. ^{13}C-NMR spectral data of compounds **1** & **3** (125 MHz, DMSO-d_6, ppm from TMS as internal standard)*

C #	δ ppm	C#	δ ppm
Compound **1**		Compound **3**	
		Aglycone	
1	154.6	2	152.4
2	117.1	3	121.6
3	133.1	4	180.2
4	115.6	5	152.8
4a	135.1	6	132.4
6	67.8	7	156.7
6a	134.4	8	93.9
7a	150.5	9	152.6
8	89.5	10	106.5
9	153.6	1'	123.9
10	129.1	2', 6'	129.9
11	151.0	3', 5'	115.9
11a	107.4	4'	157.4
12	174.3	**Glc (C-7)**	
12a	140.5	1''	100.0
1a	110.4	2''	73.1
O-CH_2-O	102.7	3''	75.8
		4''	69.9
		5''	76.7
		6''	60.4
		Glc (C-4')	
		1'''	100.1
		2'''	73.4
		3'''	76.5
		4'''	69.6
		5'''	76.9
		6'''	60.5
		OMe	60.0

*All carbons were assigned by DEPT and 2D NMR (HMQC).

absorption maxima at 212, 264, 330 nm indicating an isoflavonoid moiety (Mabry *et al.*, 1970). A bathochromic shift after addition of $AlCl_3$ confirmed the presence of 5-OH, while the absence of significant bathochromic shifts after addition of other shifting reagents suggested the substitution at C-7 and C-4' of tectorigenin moiety (Mabry *et al.*, 1970). The ^{1}H-NMR spectrum showed a singlet proton at δ 8.51 characteristic for H-2, one proton singlet at δ 6.90 assigned for H-8 and a sharp singlet at δ 3.77 integrated for one methoxyl group of an isoflavonoid moiety. The ^{1}H-NMR spectrum also showed a pair of doublets at δ 7.11 (d, *J*=8.8, 2 H, C-3', C-5') and at δ 7.52 (d, *J*=8.8, 2 H at C-2', C-6') characteristic of a *p*-substituted B-ring of aglycone moiety. The glucosidic nature of **3** was

established from the appearance of two anomeric protons as pair of doublets at δ 5.10 (d, *J*=7.7) and δ 4.91 (d, *J*=7.7) indicated a β-sugar linkage (Harborne *et al.*, 1975). The ^{13}C-NMR spectra (Table I) were in good agreement with the reported substitution values in tectorigenin glycosides (Farag *et al.*, 1999; Agarwal, 1989). All protons and carbon resonances were assigned according to the data obtained from DEPT and HMQC experiments. The ^{13}C-NMR chemical shifts of the sugar carbons were consistent with two glucose units (Breitmaier & Voelter, 1987). The normal chemical shifts of C-6'' and C-6''' of two glucose moieties at δ 60.4 and 60.5 suggested the absence of interglucosidic linkage. The precise determination of the site of attachment of the glucose units at aglycone moiety was achieved after the interpretation of HMBC and 2D-NOESY spectral data. The recorded HMBC data (Fig. 1), revealed a diagnostic correlation between H-1''/C-7 (δ 5.10/156.7), H-1'''/C-4' (δ 4.91/157.4) suggested the sites of attachment of sugars to aglycone moiety at C-7 and C-4' respectively. This finding was further confirmed from the correlation between H-1'''/H-3', 5' (δ 4.91/7.11) and H-1''/H-8 (δ 5.10/6.90) in 2D-NOESY experiment. Based on the above data, the structure of **3** was assigned as tectorigenin-7-*O*-β-D-glucopyranosyl-4'-*O*-β-D-glucopyranoside, a new isoflavonoid glycoside.

Other known compounds were identified as tectorigenin-7-*O*-β-glucopyranosyl (1 → 6)-*O*-β-glucopyranoside (**4**), tectoridin (the 7-*O*-β-D-glucopyranoside of tectorigenin) (**5**), and tectorigenin-4'-*O*-β-D-glucopyranoside (**6**) respectively. Their structures were evaluated by comparison of their spectral data with those reported in literature (Shawl *et al.*, 1992; Farag *et al.*, 1999; Nagarajan & Narayanan, 1988) and co-chromatography with authentic samples. The co-occurrence of the isoflavone and rotenoid compounds in the rhizomes of *I. spuria* (Zeal) is consistant with the biogenetic scheme proposed for rotenoids (Dewick, 2001).

It is worthy to note that no natural 12a-dehydrorotenoid derivatives and/or tectorigenin isoflavonids were isolated before from *I. spuria* (Shawl *et al.*, 1984, 1988a, 1988b). This may be due to the ecological effect. Biological study will be necessary in future to explore the medicinal value of the rhizomes of this plant as a rich source of bioactive compounds.

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