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ISOFLAVONOID GLYCOSIDES FROM *ERIOSEMA TUBEROSUM*

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Key Word Index—*Eriosema tuberosum*; Leguminosae; roots; genistein; 5-*O*-methylgenistein; isoflavonoid glycoside.

Abstract—Five isoflavonoid glycosides together with the corresponding aglycones have been isolated from the *n*-BuOH-soluble fraction of a methanol extract of the roots of *Eriosema tuberosum*. One compound is new and its structure has been established by spectroscopic analyses and chemical methods as 5-*O*-methylgenistein 7-*O*-β-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The root of *Eriosema tuberosum* is used to treat diarrhoea, orchitis and hydrophobia, as well as a detoxifying medicine, by Miao, Tai, Wa and Yi ethnic people living in the mountains of Yunnan province, P.R. China. Antifungal phenolic constituents from the dichloromethane extract of this species have been reported in our previous papers [1–3]. Due to the presence of antifungal compounds in the methanolic extract, a study of water-soluble constituents of *E. tuberosum* was carried out. The present paper deals with the isolation and structural elucidation of five isoflavonoid glycosides, together with the corresponding two aglycones.

RESULTS AND DISCUSSION

Repeated chromatographic purifications of the *n*-BuOH-soluble fraction of the methanolic extract of *E. tuberosum* afforded one novel isoflavonoid glycoside (**1**), four known isoflavonoid glycosides (**2**–**5**) and two known isoflavonones (**6** and **7**) as their aglycones.

The structures of known compounds were established as genistein 7-*O*-β-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside (**2**), 5-*O*-methylgenistein 7-*O*-β-D-glucopyranoside (**3**), genistin (**4**), sphaerobioside (**5**), genistein (**6**) and 5-*O*-methylgenistein (**7**), from their spectral data and chemical degradation, as well as co-TLC with authentic markers [4].

Compound **1** was obtained as a pale yellow amorphous powder that appears on HPTLC (RP-18) as a yellowish brown spot after treatment with Godin reagent. The UV spectrum of this compound in MeOH and with other usual shift reagents (see Experimental) established the presence of a monohydroxylated isoflavonone skeleton at C-4′ [5]. The chemical shift for the proton H-2 (δ 8.04) and the chemical shift for C-2 (δ 153.1) further confirmed the proposed isoflavone structure [6–7]. The FD mass spectrum of **1** exhibited an intense quasi-molecular ion at *m/z* 579 $[M+H]^+$, which is consistent with a disaccharide glycoside containing one pentose (*m/z* $[132+H]^+$), one hexose (*m/z* $[162+H]^+$) and one aglycone with M_r 284. The ^{13}C NMR spectrum of **1**, resolved by DEPT experiments, afforded a total of 27 resonance lines consisting of one CH_3, three CH_2, 14 CH and nine quaternary carbons. Summarizing these data, a molecular formula of $C_{27}H_{30}O_4$ was determined. ^1H and ^{13}C NMR spectra revealed a set of signals belonging to the sugar moieties (anomeric signals resonated at δ 5.01 (J = 7.2 Hz) for H, 101.8 for C and δ 4.95 (J = 2.0 Hz) for H, 111.1 for C, together with mass data, confirmed the nature of **1** as an isoflavone disaccharide glycoside. Indeed, acid hydrolysis with 0.1 N H_2SO_4 (refluxing for 20 min) afforded apiose and compound **3** identified by TLC. 5-*O*-methylgenistein (**7**) and glucose were the constituent units of compound **1** after acid hydrolysis with 1 N HCl (refluxing for 30 min); under such strongly acidic conditions, apiose was obviously decomposed. The 4′-hydroxy substitution pattern of ring-B and substitution pattern of ring-A were readily deduced from the proton signals forming an AA′-BB′ system (δ 7.34 J = 8.9 Hz for 2′-H, 6′-H and 6.82 J = 8.9 Hz for 3′-H, 5′-H) and two protons *meta*-related with a coupling constant of 2.0 Hz, representing H-6 (δ 6.67) and

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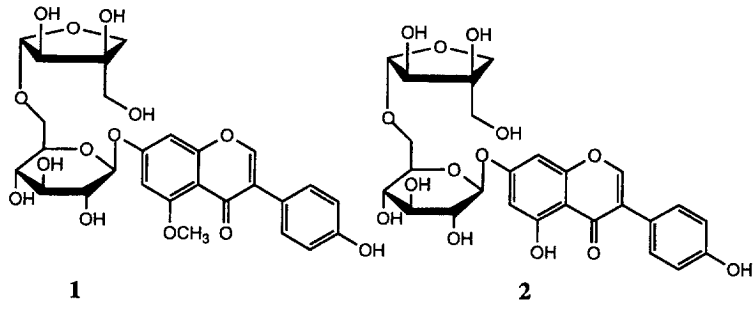

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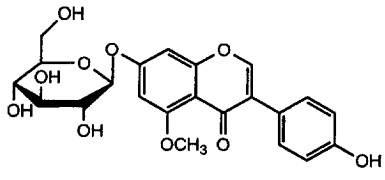

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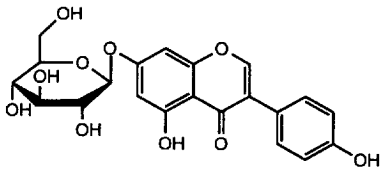

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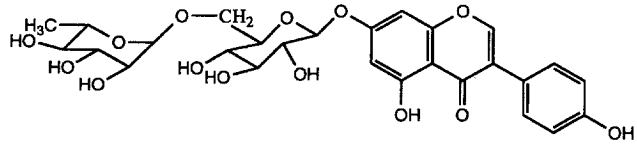

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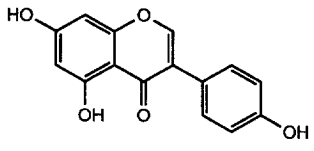

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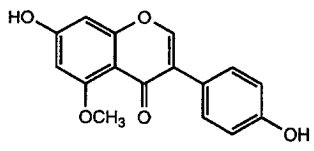

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H-8 (δ 6.84), respectively in its ^{1}H NMR spectrum. Furthermore, a large coupling constant (J = 7.2 Hz) for the anomeric proton (δ 5.01) of the glucose in the ^{1}H NMR spectrum suggested a β-configuration for the glucose unit and the β-configuration for apiose was confirmed by the shift of its anomeric carbon in the ^{13}C NMR (δ 111.1, Table 1 [8]). The configuration of the hydroxyl groups at C-2 and C-3 of apiose was determined by the coupling pattern of the C-5 protons (Table 1). In the *erythro*-form of apiose, the protons of C-5 are magnetically equivalent, while in the *threo*-form, two doublets can be observed due to the geminal coupling between these two protons [9]. As in the ^{1}H NMR spectrum, a 2H singlet immediately assignable to CH_2-5 was observed at δ 3.58; the apiose in **1** was found to be in the *erythro*-form and, therefore, the hydroxyl groups at C-2 and C-3 of apiose are *cis*-orientated. Apiose as the terminal sugar moiety in the molecule was also confirmed by the fragment ion at m/z 446 $[M-132]^+$ in its FD mass spectrum. The obvious downfield shift (>6.5 ppm) of the C-6 of the glucose unit in the ^{13}C NMR spectrum of **1** indicated that apiose was linked to the 6-OH of the glucose unit. That the disaccharide was attached to the 7-OH of the aglycone was directly deduced from the correlations observed between the anomeric proton of the glucose moiety and the C-7 position of the aglycone in the HMBC experiment (long-range heteronuclear correlation spectrum). Furthermore, the detection of an HMBC correlation between the methyl protons and C-5 of the aglycone showed that 5-*O*-methylgenistein was the aglycone of compound **1** (Fig. 1). Therefore, **1** was identified as 5-*O*-methylgenistein 7-*O*-β-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside.

Table 1. NMR spectral data of compounds **1** and **2** (500 MHz for ^1H and 125 MHz for ^{13}C; in CD_3OD) Chemical shifts in δ relative to TMS; J values (in Hz) in parentheses

	1		**2**	
Aglycone	δ_H	δ_C	δ_H	δ_C
2	8.04 *s*	153.1	8.13 *s*	155.4
3		127.2		125.0
4		177.8		182.5
5		163.6		163.5
6	6.67 *d* (2.3)	98.6	6.51 *d* (2.0)	101.2
7		162.6		164.7
8	6.84 *d* (2.3)	97.4	6.71 *d* (2.0)	96.1
9		161.1		159.2
10		111.2		108.1
1′		124.3		123.2
2′	7.34 *d* (8.9)	131.6	7.39 *d* (8.6)	131.4
3′	6.82 *d* (8.9)	116.1	6.85 *d* (8.6)	116.3
4′		158.6		158.9
5′	6.82 *d* (8.9)	116.1	6.85 *d* (8.6)	116.3
6′	7.34 *d* (8.9)	131.6	7.39 *d* (8.6)	131.4
OMe	3.90 *s*	56.8		
Sugar moieties				
Glucose				
1	5.01 *d* (7.2)	101.8	4.97 *d* (5.3)	101.7
2	3.47*	74.8	3.49*	74.7
3	3.45*	77.9	3.51*	77.9
4	3.34*	71.7	3.32 *t* (9.8)	71.7
5	3.69 *t* (11.1)	77.3	3.65 *t* (10.05)	77.2
6a	3.62 *dd* (12.4; 6.3)	69.1	3.61 *dd* (12.2; 5.4)	69.1
6b	4.07 *dd* (12.4; 1.5)		4.05 *dd* (12.2; 1.0)	
Apiose				
1	4.95 *d* (2.0)	111.1	4.90 *d* (2.6)	111.2
2	3.96 *d* (2.0)	78.1	3.93 *d* (2.6)	78.2
3		80.5		80.5
4a	3.76 *d* (12.4)	75.1	3.75 *d* (12.6)	75.1
4b	4.03 *d* (12.4)		4.04 *d* (12.6)	
5	3.58 *s*	65.6	3.52 *s*	65.8

* Signal pattern unclear due to overlapping.

Compound **2** was first isolated from *Neorautanenia amboensis* (Leguminosae), used as a fish poison in Central and Southern Africa [10]. However, its ^1H and ^{13}C NMR spectra data were not available in the literature; these are now summarized in Table 1.

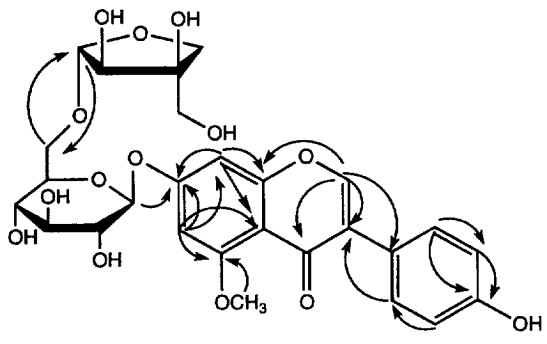

Fig. 1.

This is the first report about the water-soluble constituents of a plant belonging to the genus *Eriosema*. As expected isoflavonoid glycosides are major glycosides, as in other leguminous plants [11]. Although the water-soluble fr. of *E. tuberosum* showed antifungal activity in bioassays, the isolated isoflavonoid glycosides and their aglycones were not responsible for the positive result of bioassay. However, compound **3** was reported to have antiviral activity in *in vitro* assays [12]. It is worth mentioning that the intermediates of the biosynthetic sequence of the known compound **2**, from the aglycone genistein (**6**) through its 7-monoglucosylated precursor (**4**) and the new compound **1**, from its aglycone (**7**) *via* the derivative compound **3** have been isolated from the water-soluble fr. The aglycones **6** and **7** were not isolated from the more lipophilic fr. (CH_2Cl_2 extract) but in the hydrophilic fr.; thus, they may be degradation products from the glycosides due to the isolation procedures.

EXPERIMENTAL

General

Mps: uncorr. NMR: JEOL EX-270 and Brucker-AM-500 spectrometers. Optical rotations: MeOH. IR: KBr. TLC: Merck HPTLC RP-18 WF254 plates; saccharide identification was carried out on Merck silica gel TLC plates.

Extraction and isolation

Powdered air-dried roots of *E. tuberosum* were extracted successively with CH_2Cl_2 and MeOH as described in Ref. [1]. The *n*-BuOH-soluble fr. (2.5 g) from the MeOH extract was submitted to MPLC on RP-18 (25–40 μm, Merck) and eluting with a MeOH–H_2O stepwise gradient 1:9–9:1 afforded five sub-frs. Sub-fr. 2 was analysed by Lobar RP-18 CC with MeOH–H_2O (3:7), followed by separation on Lobar Diol CC and repeated gel filtration on Sephadex LH-20 with MeOH to give compound **1** (23 mg). Sub-fr,. 3 was separated by Lobar Diol CC using $CHCl_3$–MeOH (9:1 → 7:3), followed by gel filtration on Sephadex LH-20 with MeOH, to provide compounds **2** (10 mg), **3** (50 mg), **4** (60 mg) and **5** (2.0 mg). Sub-fr. 1 was repeatedly separated by Sephadex LH-20 CC eluting with MeOH followed by silica gel CC ($CHCl_3$–MeOH, 9:1) to provide compounds **6** (10 mg) and **7** (15 mg).

Compound **1** (5-*O*-methylgenistein 7-*O*-β-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside). Yellowish amorphous powder, mp 160–163°. HPTLC RP-18 (MeOH–H_2O, 1:1) R_f 0.61. $[\alpha]_D^{21}$ −77.7° (*c* 0.26, MeOH). UV λ_{max} (MeOH) nm (log ε): 257 (4.49), 285 *sh* (3.91), 320 *sh* (3.72); + NaOMe, 252 *sh* (4.14), 273 (4.37), 309 *sh* (4.13); + $AlCl_3$, 257 (4.49), 319 *sh* (3.84); + NaOAc, 257, 319 *sh*. IR (KBr) ν_{max} cm^{-1} 3334, 1638, 1517, 1459, 1259, 1083, 972. FD-MS *m/z* 579 $[M+H]^+$, 447 $[M-132+H]^+$, 446 $[M-132]^+$, 284 $[M-132-162]^+$, 133 $[132(apiose)+H]^+$, 163 $[162(glucose)+H]^+$. ^1H and ^{13}C NMR: Table 1.

Hydrolysis of **1**. Compound **1** (5.5 mg) was refluxed in 10 ml 0.1 N H_2SO_4 for 20 min. The mixt. was cooled and 10 ml H_2O added. The aq. layer was extracted with *n*-BuOH, then neutralized with $NaHCO_3$ followed by freeze-drying. The residue obtained was dissolved in 1 ml MeOH, filtered and concd. to 0.5 ml. Comparison on TLC with authentic samples gave apiose from the MeOH fr and compound **3** from BuOH extract. (In $CHCl_3$–MeOH–H_2O, 14:6:1; R_fs.: 0.38 for apiose, 0.68 for **3**.)

Hydrolysis of **1** *with* HCl. Compound **1** (2 mg) was refluxed in 10 ml 1 N HCl for 30 min. The mixt. was cooled and then extracted with *n*-BuOH. The organic layer was evapd. to dryness and then dissolved in MeOH. The aq. layer was treated as described above. From the organic layer, compound **7** was detected and from the aq. layer glucose was identified by direct TLC comparison with authentic samples [$CHCl_3$–MeOH–H_2O, 14:6:1 for glucose (R_f 0.16); $CHCl_3$–MeOH, 9:1 for aglycone (R_f 0.45)].

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