Phenylethanoid Glycosides from *Digitalis ferruginea* subsp. *ferruginea* (=*D. aurea* LINDLEY) (Scrophulariaceae)

Ihsan ÇALIS,*[,a] Deniz TASDEMIR,[a] Otto STICHER,[b] and Sansei NISHIBE[c]

Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University,[a] TR-06100 Ankara, Turkey, Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich,[b] CH-8057 Zürich, Switzerland, and Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido,[c] Ishikari-Tobetsu, Hokkaido 061–02, Japan. Received March 1, 1999; accepted April 28, 1999.

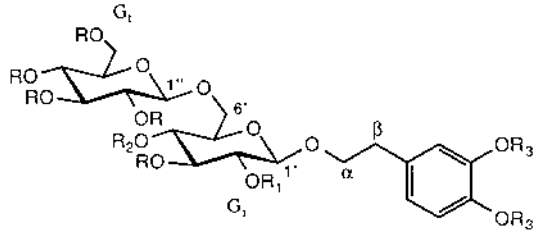
Two new phenylethanoid glycosides, ferruginoside A (3,4-dihydroxy-β-phenylethoxy-*O*-β-D-glucopyranosyl-(1→6)-2-*O*-caffeoyl-β-D-glucopyranoside) and ferruginoside B (3,4-dihydroxy-β-phenylethoxy-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside) were isolated, along with the known compounds lugrandoside and *trans*-ferulic acid, from the aerial parts of *D. ferruginea* subsp. *ferruginea*. For structural elucidation of the isolates, chemical transformation (acetylation, methylation, alkaline hydrolysis), one and two dimensional (COSY, HMQC, HMBC, ROESY) NMR techniques and FAB-MS were used.

Key words phenylethanoid glycoside; *Digitalis ferruginea*; Scrophulariaceae; lugrandoside; ferruginosides A, B; *trans*-ferulic acid

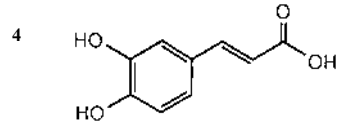
The genus *Digitalis* is reputed to be the main source of cardenolide glycosides. However, several phenylpropanoid glycosides, such as lugrandoside,[1] purpureasides A—C[2] have also been reported from some members of this genus. In a continuation of our research program on phenylethanoid glycosides from the Turkish Scrophulariaceae,[3—9] we have now examined *D. ferruginea* subsp. *ferruginea* (=*D. aurea* LINDLEY), which is the most widespread member of the nine *Digitalis* species growing in Turkey.[10] This paper describes the isolation and structural elucidation of three phenylethanoid glycosides, lugrandoside (**1**), ferruginoside A (**2**) and B (**3**), the latter two of which are new to the literature, together with a well known compound, *trans*-ferulic acid (**4**) from the aerial parts of *D. ferruginea* subsp. *ferruginea*.

Results and Discussion

The water soluble portion of the aqueous MeOH extract of the plant was fractionated on reversed-phase vacuum liquid chromatography (RP-VLC). Further separation of the resulting fractions by a combination of silica gel column chromatography (CC) and reversed-phase medium pressure liquid chromatography (RP MPLC) led to the isolation of compounds **1**—**4**.



1 R=R$_1$=H, R$_2$=*trans*-caffeoyl, R$_3$=H
2 R=H=R$_1$=*trans*-caffeoyl, R$_2$=R$_3$=H
3 R=R$_1$=R$_2$=R$_3$=H
3a R=R$_1$=R$_2$=R$_3$=COCH$_3$
3b R=R$_1$=R$_2$=H, R$_3$=Me



4

Compound **1** was identified as lugrandoside, which was formerly obtained from various *Digitalis* species, by comparison of its spectral data (UV, IR, ^{1}H-, ^{13}C-NMR, FAB-MS) with those reported in the literature.[1] Compound **4** was characterized to be *trans*-ferulic acid on the basis of its NMR data,[6] FAB-MS data and direct comparison (TLC) with the authentic sample.

Ferruginoside A (**2**) was obtained as a colourless, amorphous powder. The positive ion FAB-mass spectrum of **2** displayed a quasimolecular ion at *m/z* 663 ([M+Na]$^+$), suggesting the molecular formula $C_{29}H_{36}O_{16}$, the same as that of **1**. The UV spectrum of **2** showed absorption maxima at 219, 249, 289 and 327 nm, indicating its polyphenolic nature. Its IR spectrum contained absorption bands for hydroxyl (3396 cm^{-1}, br), α,β-unsaturated ester [1698 (C=O), 1629 cm^{-1} (C=C)], and aromatic (1603, 1518 cm^{-1}) functionalities. The ^{1}H-NMR spectrum of **2** exhibited signals accounting for one 3,4-dihydroxyphenylethanol moiety [δ 6.49 (d, *J*=2.0 Hz, H-2), 6.44 (d, *J*=8.0 Hz, H-5), 6.32 (dd, *J*=2.0, 8.0 Hz, H-6); δ 3.49, 3.90 (m, H$_2$-α), 2.68 (t, *J*=6.7 Hz, H$_2$-β)] and one *trans*-caffeic acid unit [δ 6.94 (d, *J*=2.0 Hz, H-2‴), 6.71 (d, *J*=8.0 Hz, H-5‴), 6.82 (dd, *J*=2.0, 8.0 Hz, H-6‴); δ 5.92 (d, *J*=15.9 Hz, H-α′), 7.27 (d, *J*=15.9 Hz, H-β′)]. The disaccharidic structure of **2** was indicated by two anomeric doublets at δ 4.32 (*J*=7.8 Hz) and 4.37 (*J*=7.8 Hz), suggesting

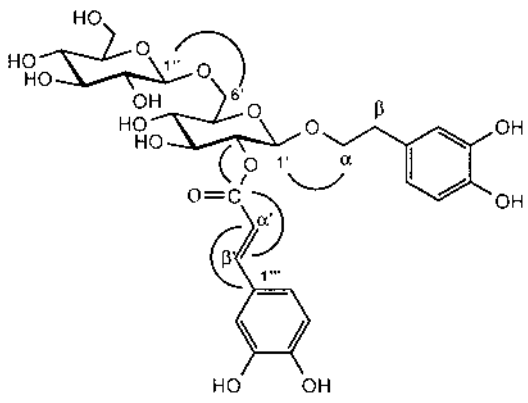


Fig. 1. The Most Significant HMBC Observed for **2**

* To whom correspondence should be addressed.

Table 1. ^{1}H-NMR Spectral Data for **1**—**4** and **3a** (300 MHz, D_2O, δ in ppm, J in Hz)

H No.	**1**	**2**	**3**	**3**[a]	**4**[b]
Aglycone					
2	6.70 d (1.6)	6.49 d (2.0)	6.68 d (2.0)	7.05 s	
5	6.72 d (8.0)	6.44 d (8.0)	6.70 d (8.0)	7.08 s	
6	6.60 dd (1.6, 8.0)	6.32 dd (2.0, 8.0)	6.57 dd (2.0, 8.0)	7.08 s	
α	3.70 m, 3.93 m	3.49 m, 3.90 m	3.72 m, 3.93 m	3.56 m, 3.68 m	
β	2.68 t (6.7)	2.68 t (6.7)	2.69 t (6.7)	2.87 t (6.9)	
Glucose (i)					
1′	4.32 d (7.8)	4.37 d (7.8)	4.32 d (8.1)	4.45 d (7.9)	
2′	3.10—3.60[c]	4.55 dd (7.8, 9.0)	3.11 dd (8.1, 9.0)	4.94 dd (7.9, 9.5)	
3′	3.10—3.60[c]	3.53[c]	3.35[c]	5.16 t (9.5)	
4′	4.81 dd (9.5, 10.4)	3.42[c]	3.25[c]	4.87 t (9.5)	
5′	3.10—3.60[c]	3.53[c]	3.45[c]	4.12 m	
6′	3.55[c]	3.85[c]	3.70[c]	3.56—3.68[c]	
	3.83 br d (11.0)	4.06 br d (11.4)	4.05 d (11.5)		
Glucose (t)					
1″	4.20 d (7.8)	4.32 d (7.8)	4.36 d (7.9)	4.55 d (8.0)	
2″	3.10—3.60[c]	3.20 dd (7.8, 8.6)	3.17 dd (8.0, 9.0)	4.98 dd (8.0, 10.0)	
3″	3.10—3.60[c]	3.30[c]	3.27[c]	5.16 t (9.4)	
4″	3.10—3.60[c]	3.42[c]	3.25[c]	5.06 t (9.6)	
5″	3.10—3.60[c]	3.30[c]	3.35[c]	3.56—3.68[c]	
6″	3.55[c]	3.78[c]	3.58 dd (12.3, 4.8)	4.12 m	
	3.70 dd (12.0, 1.5)	3.99[c]	3.75 br d (12.3)	4.26 dd (4.7, 12.4)	
Acyl					
2‴	7.00 d (2.0)	6.94 d (2.0)			7.02 d (2.0)
5‴	6.77 d (8.0)	6.71 d (8.0)			6.74 d (8.2)
6‴	6.90 dd (2.0, 8.0)	6.82 dd (2.0, 8.0)			6.90 dd (2.0, 8.2)
α'	6.20 d (15.9)	5.92 d (15.9)			6.22 d (15.9)
β'	7.50 d (15.9)	7.27 d (15.9)			7.54 d (15.9)
OMe					3.75 s

a) Measured in $CDCl_3$. Additional signals for **3a** at δ 2.26 and 2.28 (each 3H, aromatic OAc), and 1.92, 1.95, 1.98, 1.99, 2.01, 2.02, 2.08 (each 3H, aliphatic OAc). *b*) Measured in CD_3OD. *c*) Multiplicity of the signals is unclear due to overlapping.

the presence of two β-D-glucose moieties. The sugar sequence within **2** was determined to be ($G_t1\rightarrow G_i6$) with the aid of ^{1}H–^{13}C long range correlations (Fig. 1) observed in the heteronuclear multiple bond correlation (HMBC) spectrum between a deshielded C-6′ atom (δ 68.4) and H-1″, as well as by a significant dipolar coupling traced from the rotating-frame overhauser spectroscopy (ROESY) experiment of **2** between H-6′ and H-1″. From the comparison of these data with those of **1**, it was apparent that **2** bore close resemblance to **1**. However, H-2′, which was intercoupling with H-1′ of the core glucose unit, was abnormally shifted downfield (δ 4.55, dd, J=7.8, 9.0 Hz), indicating the involvement of a caffeoyl function at this position, instead of at C-4′ as in **1**. An HMBC cross peak between H-2′ and the carbonyl function of the acyl moiety (δ 168.2) further confirmed **1** and **2** to be positional isomers. All other structural assignments were substantiated by the results contained in the two dimensional homo- and heteronuclear NMR measurements. Thus, the structure of **2** is 3,4-dihydroxy-β-phenylethoxy-*O*-β-D-glucopyranosyl-(1→6)-2-*O*-caffeoyl-β-D-glucopyranoside.

Ferruginoside B (**3**) was also obtained as a colorless, amorphous powder with a molecular weight of 478, $C_{20}H_{30}O_{13}$ (FAB-MS: *m/z* 501 $[M+Na]^+$). The UV spectrum of **3** exhibited absorption bands at 226, 284 and 331 nm, and its IR spectrum contained significant absorption bands typical of hydroxyl (3387 cm^{-1}) and aromatic ring (1603, 1528, 1500 cm^{-1}) functionalities. The ^{1}H- and ^{13}C-NMR spectra of **3** were very similar to those of **1** and **2**, containing three aromatic protons (ABX system at δ 6.68, 6.70, 6.57, H-2, H-5, H-6, respectively) and a benzylic methylene proton signal at δ 2.69 (t, J=6.7 Hz, H_2-β) indicative of the same aglycone moiety found in both **1**, **2**, and two anomeric protons at δ 4.32 (J=8.1 Hz) and δ 4.36 (J=7.9 Hz), but the signals of the acyl moiety were absent. Indeed, the acetylation of **3** in the usual manner yielded a nonaacetate **3a** exhibiting only two aromatic and seven aliphatic acetoxyl resonances in its ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2). These results led to the conclusion that **3** is deacyllugrandoside (=deacylferruginoside A). In order to confirm this assumption, compounds **1**—**3** were separately methylated with CH_2N_2. The following alkaline hydrolysis of **1** and **2** yielded **3b** (=deacylglycoside dimethylether), on TLC, which was identical to the partially methylated derivative of **3** by CH_2N_2. On the basis of this evidence, the structure of ferruginoside B (**3**) was determined to be 3,4-dihydroxy-β-phenylethoxy-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Experimental

Extraction and Isolation The plant material was collected from Aladag-Bolu, Turkey, in July 1996. Voucher specimens (HUEF 96105) are deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey. The air-dried and powdered aerial parts of the plant (500 g) were extracted twice with a MeOH–H_2O (8 : 2) mixture. The water soluble part of the extract was fractionated by VLC (stationary phase: LiChroprep C-18), and eluted with H_2O and H_2O–MeOH mixtures. An aliquot (770 mg) of the H_2O eluate (1.90 g) was subjected to RP MPLC [Buchi 688 pump, Sepralyte C-18, MeOH–H_2O (3 : 7)] to give **1** (55 mg). Silica gel CC of a 25 g quantity of 25% MeOH eluate (50 g) using $CHCl_3$–MeOH–H_2O (80 : 20 : 2→60 : 40 : 4) yielded 5 fractions. Of these, combined fractions 4, 5 were further chromatographed by RP MPLC using a gradient of MeOH in H_2O (5% to 50% MeOH) to afford **3** (39 mg), **2** (27 mg) and **4** (14 mg), respectively.

Lugrandoside (**1**): Amorphous, colourless compound. UV (MeOH; λ_{max})

Table 2. ^{13}C-NMR Spectral Data for **1**—**4** and **3a** (75.5 MHz, D_2O)

C No.	Multipl.	**1**	**2**	**3**	**3a**[a]	**4**[b]
Aglycone						
1	C	131.3	131.2	130.9	137.5	
2	CH	116.2	116.2	117.6	123.9	
3	C	143.8	143.6	144.7	141.8	
4	C	142.2	142.1	142.9	140.6	
5	CH	116.7	116.3	116.6	123.2	
6	CH	121.1	120.8	120.5	127.2	
α	CH_2	71.1	70.8	71.0	69.9	
β	CH_2	34.4	34.1	34.4	35.3	
Glucose (i)						
1′	CH	102.2	100.5	102.2	100.4	
2′	CH	72.9	73.6	73.0	71.1	
3′	CH	73.8	74.9	75.6	72.7	
4′	CH	69.5	69.3	69.4	68.3	
5′	CH	72.6	73.4	74.9	71.9	
6′	CH_2	67.8	68.4	68.5	68.2	
Glucose (t)						
1″	CH	102.6	102.7	102.7	100.8	
2″	CH	73.0	73.0	73.0	71.1	
3″	CH	75.8	75.8	75.8	73.3	
4″	CH	70.6	69.5	69.6	69.2	
5″	CH	75.5	75.6	75.6	72.7	
6″	CH_2	60.6	60.7	60.7	61.9	
Acyl						
1‴	C	126.7	125.7			127.0
2‴	CH	113.7	114.7			114.2
3‴	C	144.3	145.1			147.4
4‴	C	147.3	149.4			151.0
5‴	CH	115.1	115.8			116.6
6‴	CH	122.8	123.0			123.1
α'	CH	116.2	112.8			114.8
β'	CH	146.9	146.6			147.2
C=O	C	168.3	168.2			169.9
OMe	CH_3					51.9

a) Measured in $CDCl_3$. Additional signals for **3a** at δ 168.3—170.6 (9×O<u>C</u>OCH$_3$) and 20.5—20.7 (9×OCO<u>C</u>H$_3$). *b*) Measured in CD_3OD.

220, 248, 290, 331 nm. IR (KBr; ν_{max}) 3410, 1695, 1630, 1605, 1520 cm^{-1}. ^{1}H-NMR (300 MHz, D_2O) Table 1. ^{13}C-NMR (75.5 MHz, D_2O) Table 2. FAB-MS (positive, NOBA) *m/z* 663 $[M+Na]^+$; Calcd for $C_{29}H_{36}O_{16}$.

Ferruginoside A (**2**): Amorphous, colorless powder. UV (MeOH; λ_{max}) 219, 249, 289, 327 nm. IR (KBr; ν_{max}) 3396, 1698 , 1629 , 1603, 1518 cm^{-1}. ^{1}H-NMR (300 MHz, D_2O) Table 1. ^{13}C-NMR (75.5 MHz, D_2O) Table 2. FAB-MS (positive, NOBA) *m/z* 663 $[M+Na]^+$; Calcd for $C_{29}H_{36}O_{16}$.

Ferruginoside B (**3**): Amorphous, colorless powder. UV (MeOH; λ_{max}) 226, 284, 331 nm. IR (KBr; ν_{max}) 3387, 1603, 1528, 1500 cm^{-1}. ^{1}H-NMR (300 MHz, D_2O) Table 1. ^{13}C-NMR (75.5 MHz, D_2O) Table 2. FAB-MS (positive, NOBA) *m/z* 501 $[M+Na]^+$; Calcd for $C_{20}H_{30}O_{13}$.

Trans-Ferulic Acid (**4**): Amorphous powder. ^{1}H-NMR (300 MHz, MeOD) Table 1. ^{13}C-NMR (75.5 MHz, MeOD) Table 2. FAB-MS (positive, NOBA) *m/z* 195 $[M+H]^+$, 217 $[M+Na]^+$; Calcd for $C_{10}H_{10}O_4$.

Acetylation of Ferruginoside B (3) Treatment of **3** (10 mg) with Ac_2O (1 ml) and pyridine (1 ml) at room temperature overnight, followed by CC over silica gel using C_6H_6–Me_2CO (5 : 1) yielded 9 mg of ferruginoside B nonaacetate (**3a**) as an amorphous powder. The ^{1}H-NMR (300 MHz, $CDCl_3$) and ^{13}C-NMR (75.5 MHz, $CDCl_3$) data of **3a** are given in Tables 1 and 2.

Partial Methylation of 1—3 Followed by Alkaline Hydrolysis of 1—2 A solution of each compound (10 mg) in MeOH was treated with excess CH_2N_2 and kept 1 h at room temperature. The reaction mixture was then evaporated to dryness. The residues of **1** and **2** were further subjected to alkaline hydrolysis (5% KOH in MeOH) to afford the same product, 3,4-dimethoxy-β-phenylethoxy-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside. This compound was identified by TLC comparison with **3a**, which was obtained by the partial methylation of **3** with excess CH_2N_2, as described above.

Acknowledgements The authors are thankful to Dr. E. Zass, ETH-Z Chemistry Department, for performing a literature search, and Mr. R. Haefliger and Dr. Walter Amrein, ETH-Z Chemistry Department Mass Spectral Service, for recording FAB mass spectra.

References

1) Baudouin G., Skaltsounis A.-L., Tillequin F., Koch M., *Planta Med*., **48**, 321 (1988).
2) Matsumoto M., Koga S., Shoyama Y., Nishioka I., *Phytochemistry,* **26**, 3225 (1987).
3) Çalis I., Gross G.-A., Sticher O., *Phytochemistry* , **26**, 2057 (1987).
4) Çalis I., Gross G.-A., Sticher O., *Phytochemistry* , **27**, 1465 (1988).
5) Çalis I., Saracoglu I., Kitagawa S., Nishibe S., *Doğa Tip ve Ecz. D.* **12**, 234 (1988).
6) Çalis I., Tasdemir D., Wright A. D., Sticher O., *Helv. Chim. Acta*, **74**, 1273 (1991).
7) Akdemir Z., Çalis I., Junior P., *Planta Med*., **57**, 584 (1991).
8) Akdemir, Z., Çalis, I., Junior P., *Phytochemistry* **30**, 2401 (1991).
9) Akdemir Z., Çalis I., *DOGA Turkish J. Pharm*., **1**, 67 (1991).
10) Davis, P. H. "Flora of Turkey and the East Aegean Islands," Vol. 6, University Press, Edinburgh, 1978, p. 680.