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Phenylethanoid and cardioactive glycosides from *Digitalis ferruginea*

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From the aerial parts of *Digitalis ferruginea* ssp. *ferruginea* (syn. *D. aurea* Lindley), the phenylethanoid glycoside **1** was isolated in addition to three cardioactive glycosides, lanatosides A (**2**), C (**3**) and glucogitoroside (**4**). For the structure elucidation of the compounds, 1D and 2D-NMR ([LR-]COSY, HETCOR, NOESY) and MS techniques (ESI-MS and ESI-MS^2, DCI-NH_3-MS, MALDI-TOF) were used. The structure of the new compound **1** was established as 3-hydroxy,4-methoxy-β-phenylethoxy-3,6-di-*O*-(α-rhamnopyranosyl)-4-*O*-*E*-feruloyl-β-glucopyranoside.

1. Introduction

In the Flora of Turkey [1] the Genus *Digitalis* is represented by nine species. Up to now the cardenolide complex of some of them such as *D. trojana*, *D. lamarckii*, *D. schischkinii*, and *D. grandiflora* has been screened by means of chromatography (PC, TLC) as well as chemical and enzymatic degradation of both extracts and isolated compounds, while *D. ferruginea* has not received much attention [2–6]. Our previous research on one of these plants, *D. ferruginea* ssp. *ferruginea* (syn. *D. aurea* Lindley), collected from Aladag-Bolu, led to the isolation of three phenylethanoid glycosides, lugrandoside as well as ferruginosides A and B [7]. In continuation of these studies, a minor phenylethanoid was now characterized and the major cardenolides of the plant were isolated in order to achieve definitive evidence of their structure.

2. Investigations, results and discussion

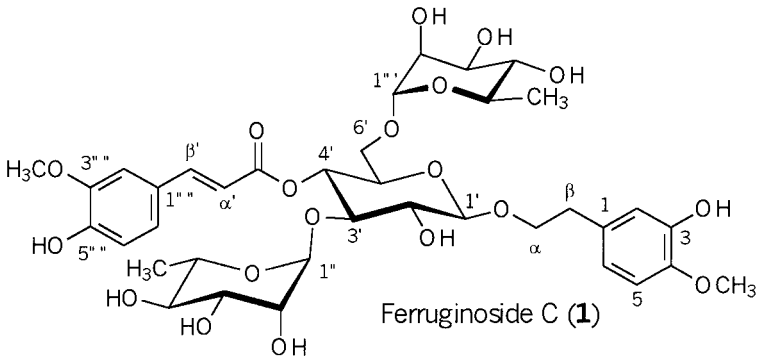
The novel phenylethanoid compound **1** was obtained as an amorphous powder. The molecular weight was established by MALDI-TOF MS (*m/z* 821 $[M + Na]^+$; solving for $C_{37}H_{50}O_{19}$). The ^{1}H NMR spectrum (see Table 1) exhibited the presence of six aromatic protons due to two ABX spin systems belonging to one ferulic acid substituent and the aglycone part. Two olefinic protons (d each, H-α′ and H-β′) were forming an AB spin system with $J_{A,B} = 15.9$ Hz indicating a *trans* geometry of the ferulic acid unit. Beside two methoxy groups, one benzylic methylene and two secondary methyl groups as typically found for rhamnose were assigned. Additionally, three anomeric protons were observed at 5.187 (J = 1.7 Hz), 4.620 (J = 1.7 Hz) and 4.370 (J = 7.9 Hz) ppm which was consistent with α configurations of both rhamnose and β configuration of the glucose unit, respectively. The assignments of all proton resonances were aided by a COSY experiment. The ^{1}H NMR spectrum of **1** also suggested that the feruloyl moiety occupies the 4-O-position of the glucose, because the H-4′ signal (δ 4.988, t, J = 9.6 Hz) was shifted to lower field. The ^{13}C NMR spectrum of **1** (see Table 1) exhibited 37 carbon resonances. The signals attributed to the aglycone and an acyl moiety were consistent with the presence of 3-hydroxy-4-methoxy-phenylethanol and ferulic acid, respectively. Because no substituent chemical shifts were observed for the rhamnose units, the two desoxy sugars were proven to be terminally linked to the glucose moiety. Thus, the carbon resonances observed at δ 81.92 and 67.70 were attributed to C-3′ and C-6′ of glucose representing the sites of rhamnosidation. These findings were supported by the excellent agreement with the glucose resonances observed in angorosides A, B and C which display a similar glycosidation pattern of the core sugar, i.e. glucose [8, 9].

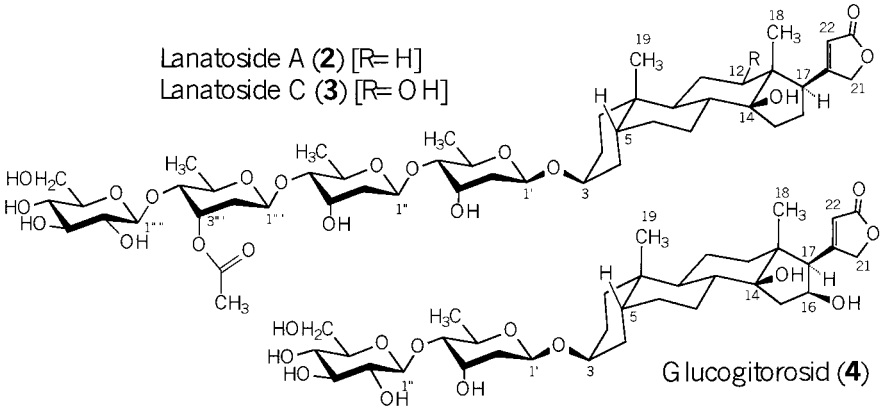
This led us to conclude that the structure of compound **1** is 3-hydroxy,4-methoxy-β-phenylethoxy-3,6-di-*O*-(α-rhamnopyranosyl)-4-*O*-*E*-feruloyl-β-glucopyranoside (**1**), for

Table 1: ^{13}C and ^{1}H NMR spectral data for the phenylethanoid glycoside 1

C/H Atom			δ_C	δ_H, Mult (J)
Aglycone				
	1	C	133.01	
	2	CH	113.14	6.724 d (2.4)
	3	C	147.89	–
	4	C	147.63	–
	5	CH	117.11	6.827 d (8.2)
	6	CH	121.18	6.687 dd (8.2, 2.4)
	α	CH_2	72.38	3.994 dddd/m
	β	CH_2	36.64	2.822 ddd/m
	OMe	CH_3	56.64	3.881 s
Glucose				
	1′	CH	104.41	4.370 d (7.9)
	2′	CH	76.01	3.368 dd (7.9, 9.0)
	3′	CH	81.92	3.84[a]
	4′	CH	70.60	4.988 t (9.6)
	5′	CH	74.77	3.60[a]
	6′	CH_2	67.70	3.468 dd (11.3, 5.6)
				3.75[a]
Rhamnose (1 → 3)				
	1″	CH	103.18	5.187 d (1.7)
	2″	CH	72.20	3.908 dd (1.7, 3.4)
	3″	CH	72.38	3.666 dd (3.4, 10.0)
	4″	CH	73.85	3.30 t (10.0)
	5″	CH	70.42	3.57 m
	6″	CH_3	18.37	1.083 d (6.4)
Rhamnose (1 → 6)				
	1‴	CH	102.31	4.620 d (1.7)
	2‴	CH	72.20	3.822 dd (1.7, 3.4)
	3‴	CH	72.38	3.571 dd (3.4, 10.0)
	4‴	CH	73.98	3.34 t (10.0)
	5‴	CH	69.91	3.58 m
	6‴	CH_3	18.00	1.191 d (6.4)
Acyl moiety				
	1⁗	C	127.30	
	2⁗	CH	112.05	7.196 d (2.0)
	3⁗	C	149.43	–
	4⁗	C	150.92	–
	5⁗	CH	116.57	6.805 d (8.0)
	6⁗	CH	124.33	7.080 dd (8.0, 2.0)
	α′	CH	115.67	6.369 d (15.9)
	β′	CH	147.89	7.660 d (15.9)
	OMe	CH_3	56.57	3.808 s

The ^{1}H NMR assignments were verified through a COSY-90 map (δ in ppm and *J* in Hz, taken at 90/360 MHz in CD_3OD)
[a] patterns unclear due to extensive signal overlap





which the trivial name ferruginoside C is proposed. During this work, a report appeared describing the same compound from *D. purpurea* together with its PKCα inhibitory activity [10].

While the cardenolide complex of *Digitalis* plants has been well established for prominent members of the genus, the phenylethanoid pattern of *Digitalis* species has not been investigated in detail [11–13]. Investigations of the cardenolide pattern of rare species like *D. subalpina* [14] and *D. ferruginea* are essentially limited to comparative chromatographic studies and only very few are based on isolated compounds that were spectroscopically characterized [15]. Therefore, we have tracked down the main cardenolides of *D. ferruginea* to yield compounds **2**, **3** and **4** by following a positive Kedde reaction during the isolation of the phenolic constituents. The positive and negative ion ESI-MS and the positve MALDI-TOF MS of the isolates revealed the molecular weights of 968 amu for **2** (m/z 967 $[M-H]^-$ and m/z 991 $[M+Na]^+$, calc. for $C_{49}H_{76}O_{19}$), 984 amu for **3** (m/z 983 $[M-H]^-$ and m/z 1007 $[M+Na]^+$, calc. for $C_{49}H_{76}O_{20}$), and 682 amu for **4** (m/z 681 $[M-H]^-$ and m/z 705 $[M+Na]^+$, calc. for $C_{35}H_{54}O_{13}$) suggesting their tetra- and diglycosidic nature, respectively.

Under ESI-MS^2 conditions deacetylation was the initial step in the fragmentation pathway (m/z 907 $[M-HOAc-H]^-$ and 925 $[M-(HOAc-H_2O)-H]^-$) followed by the subsequent loss of two of the sugar residues starting with the terminal Glc unit (m/z 763 $[M-(HOAc-H_2O)-(Glc-H_2O)-H]^-$ and 633 $[M-([Glc\text{-}Dgx\text{-}(HOAc-H_2O)]-H_2O)-H]^-$). The ^1H NMR spectrum of **2** (see Table 2) exhibited the typical profile of a steroid glycoside. A low-field double doublet at δ 5.889 coupled with two AB-type doublets of doublets resonating at δ 5.027 and 4.905 corresponded to H-22 and H_2-21, respectively, and indicated the presence of the typical butenolide ring of a

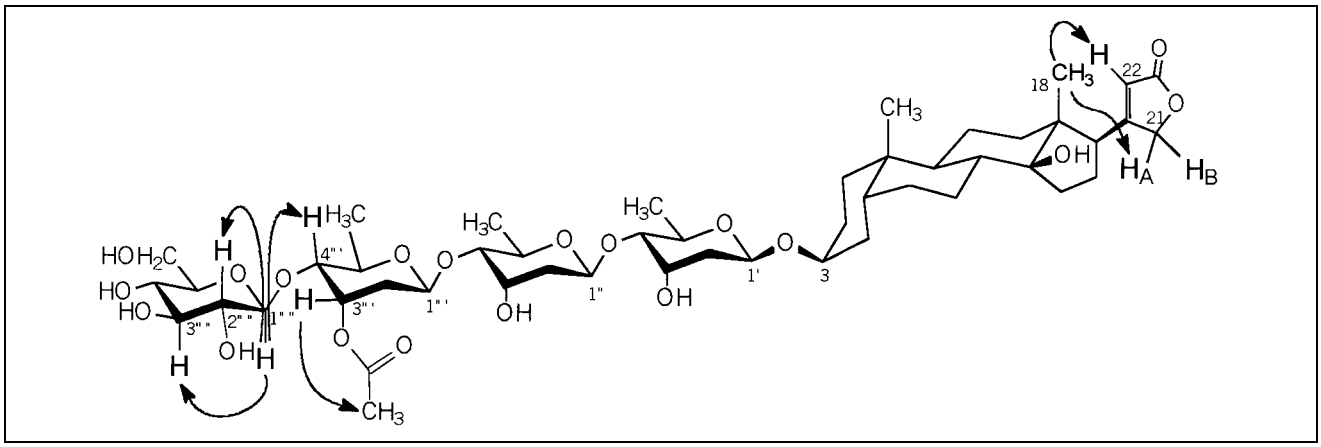


Fig.: Key NOE contacts in **2** shown as arrows providing proof for the site of acetylation (left part) and the rotameric conformation of the butenolide sidechain as well as the prochiral assignment of the methylene protons H_2-21

cardiac glycoside. This was confirmed by the carbon resonances in the ^{13}C NMR spectrum of **2** (see Table 3) at δ 178.47 (C-20), 75.36 (C-21), 117.79 (C-22), and 177.26 (C-23). Two three-proton singlets at δ 0.870 and 0.933 suggested the presence of two methyl groups on the steroid nucleus (Me-18 and Me-19, respectively) and according to their exact chemical shifts pointed towards the substitution pattern of digitoxigenin [16]. Furthermore, a three-proton singlet at δ 2.076 and the corresponding carbon resonances at δ 21.22 (CH_3) and δ 172.65 (C=O) indicated the presence of an acetoxy substituent. The ^1H NMR spectrum of **2** further exhibited the signals of three Me doublets at high field (δ 1.187, 1.192 and 1.326, J = 6.2 Hz) and of four anomeric protons three of which due to their dd appearance must belong to 2,6-didesoxy sugars. Their relative stereochemistry was determined by establishing the connectivities through a 2D-COSY experiment followed by the full interpretation of all spin systems and J values of the sugar units. As a result, all desoxy sugars were identified as digitoxose, while the hexose unit was determined as glucose (Glc), all with anomeric β configuration. The most downfield sugar proton being observed as a ddd at δ 5.475 (J = 6.1, 3.1 and 2.9 Hz) was assigned to H-3 of one of three digitoxose (Dgx) units and indicated the site of acetylation. While the remaining sugar proton signals of this moiety could be assigned in the COSY map, the particular position of this acylated sugar within the sugar chain had to be estab-

Table 2: ^1H NMR spectral data of the cardenolides 2–4 (360 MHz in CD_3OD)

H-Atom	**2** δ_H, Mult (J)	**3** δ_H, Mult (J)	**4** δ_H, Mult (J)
	Dtg	Dgg	Gtg
H-3α	4.001 dddd/m (see [10])	4.002 dddd/m (see [10])	4.01 dddd/m (see [10])
H-9		1.600	1.694
H-11eq		1.597	
H-11ax		1.295	
H-12α		3.372 dd (4.2, 11.7)	
H-15A			2.604 dd (8.3, 14.9)
H-15B			1.691
H-16A=α	2.167	2.130	4.630 dt (2.1, 8.0, 8.0)
H-16B	1.869	1.925	–
H-17α	2.823 dd (8.6, 5.8)	3.328 †	3.113 d (7.8)
H_3-18	0.870 s	0.775 s	0.908 s
H_3-19	0.933 s	0.941 s	0.923 s
H-21A	5.027 dd (18.6, 1.7)	4.972 dd (18.7, 1.7)	5.089(!) dd (18.5, 1.7)
H-21B	4.905 dd (18.6, 1.8)	4.887 dd (18.7, 1.9)	5.158(!) dd (18.5, 1.7)
H-22	5.889 dd (1.7, 1.8)	5.898 dd (1.7, 1.9)	5.928 dd (1.7, 1.7)
	Dgx	Dgx	Dgx
H-1′	4.912 dd (9.2, 1.8)	4.912 dd (9.2, 1.8)	4.907 dd (9.1, 2.1)
H-2′eq	2.013	2.013	1.903
H-2′ax	1.792	1.747	1.698
H-3′	4.257 ddd (6.2, 3.1)	4.257 ddd (6.2, 3.1)	4.286 ddd (6.2, 3.1)
H-4′	3.272 dd (3.1, 9.7)	3.261 dd (2.9, 9.6)	3.245 dd (2.9, 9.6)
H-5′	3.826 dq (6.2, 9.7)	3.829 dq (6.2, 9.7)	3.852 dq (6.2, 9.7)
H_3-6′	1.187 d (6.2)	1.189 d (6.2)	1.288 d (6.2)
	Dgx	Dgx	
H-1″	4.907 †	4.907 †	
H-2″eq	1.924	1.936	
H-2″ax	1.715	1.701	
H-3″	4.228 ddd (6.2, 3.1)	4.228 ddd (6.2, 3.1)	
H-4″	3.217 dd (3.1, 9.7)	3.214 dd (3.0, 9.6)	
H-5″	3.779 dq (6.2, 9.7)	3.781 dq (6.2, 9.7)	
H_3-6″	1.192 d (6.2)	1.195 d (6.2)	
	Dgx	Dgx	
H-1‴	4.907 †	4.907 †	
H-2‴eq	2.002	2.023	
H-2‴ax	1.869	1.845	
H-3‴	5.475 ddd (6.1, 3.1, 2.9)	5.476 dd (6.3, 3.0)	
H-4‴	3.424 dd (3.1, 9.7)	3.417 dd (3.1, 9.7)	
H-5‴	3.939 dq (6.2, 9.7)	3.937 dq (6.2, 9.7)	
H_3-6‴	1.326 d (6.2)	1.326 d (6.2)	
	Glc	Glc	Glc
H-1⁗/″	4.349 d (7.8)	4.347 d (7.8)	4.367 d (7.7)
H-2⁗/″	3.109 dd (7.8, 9.1)	3.114 dd (7.8, 9.1)	3.114 dd (7.8, 9.1)
H-3⁗/″	3.316 t (9.1)	3.34 m †	3.34 †
H-4⁗/″	3.201 t/dd (9.2, 9.8)	3.206 t/dd (9.0, 9.5)	3.283
H-5⁗/″	3.257 ddd (1.9, 5.5, 9.8)	3.30 m †	3.294 †
$H\text{-}6''''/''_A$	3.823 dd (1.9, 12.0)	3.822 dd (1.9, 12.0)	3.815 dd (1.9, 12.0)
$H\text{-}6''''/''_B$	3.592 dd (5.5, 12.0)	3.549 dd (5.5, 12.0)	3.688 dd (5.5, 12.0)
$OCOCH_3$	2.076 s	2.078 s	

Abbrs.: Dgx: Digitoxose; Glc: Glucose; Dtg: Digitoxigenin; Dgg: Digoxigenin; Gtg: Gitoxigenin
† coinciding with solvent signal or pattern unclear due to severe signal overlap

lished independently; the MS^2 measurements cited above could not create definitive evidence. Therefore, the spatial proximity of the relevant substituents was determined in a 2D NOESY experiment: The anomeric proton of Glc correlated with the co-axial H-3(Glc) but also with the axial H-4 of the proceeding Dgx unit. The latter by COSY could be associated with the acyl-shifted H-3 (Dgx) and though a NOESY contact subsequently led to the acetyl group. Thus, the acylated digitoxose had to be the preterminal sugar as in the C_{26} carbohydrate chain of the lanatosides. Finally, the remaining 23 out of the total of 49 carbon signals of **2** could be unambiguously assigned to a 3-O glycosidated skeleton of digitoxigenin. Consequently, the structure of **2** was determined as lanatoside A.

Compared to **2**, compound **3** revealed one more oxygen atom due to a molecular formula of $C_{49}H_{76}O_{20}$ which was also determined in a positive-ion MALDI-TOF MS (m/z 1007 $[M + Na]^+$). The ^{1}H- and ^{13}C NMR spectra of **3** (see Tables 2 and 3) supported the presence of the same sugar moiety as in compound **2**. However, important NMR spectroscopic differences between **2** and **3** were observed for the carbon resonances of C-11, C-12 and C-13 and in the region around 3.30 ppm of the ^{1}H NMR spectrum. In addition, the typical dd arising from H-17α (2.823 ppm in **2**) was subjected to a remarkable downfield shift to 3.328 ppm in **3** as following from the cross correlations of H_2-16 in the 2D COSY. At the same time, another dd-type signal at δ 3.372 was recorded which had to be attributed to the additional hydroxylated site. This resonance showed COSY correlations with two methylene protons which in turn were coupled to a methine signal at 1.600 ppm. The latter in agreement with recent studies of steroid core protons [17] could be assigned to the H_2-11 methylenes and the axial bridgehead proton H-9, respectively. Taking also into consideration the carbon substituent chemical shifts centered at C-12, the presence of an OH group at this position was proven. Accordingly, the structure of the aglycone of **3** was established as digoxigenin and thus the chemical structure of **3** be determined as lanatoside C.

The ^{1}H- and ^{13}C NMR data of **4** which are given in Tables 2 and 3, respectively, revealed the anomeric resonances of two sugar units at δ 4.907 (dd, J = 2.1, 9.0 Hz, H-1′ of Dgx) and 4.367 (d, J = 7.7 Hz, H-1″ of Glc). Starting from the anomeric protons, a COSY experiment clarified all the spin systems and thus the relative stereochemistry of the sugar units as follows: Glc must be the hexose sugar because the spin system of its hexanopyranoid ring exclusively yielded large trans-diaxial J values. The stereochemistry of the 2,6-didesoxy sugar originated from the C-3 epimer mannose and exhibited an equatorial position of H-3 with only small J's to its neighbors. The carbon resonance observed at δ 84.21 was assigned to C-4′ of the Dgx unit and showed the site of interglycosidic linkage, and also the remaining set of Dgx resonances was in good accordance with those of the preterminal sugar unit in **2** and **3** (see Table 3). Taking into account the molecular formula of the diglycoside **4** being deduced as $C_{35}H_{54}O_{13}$, the aglycones of **4** and **3** were expected to have the same molecular weight. However, the site of hydroxylation must be different as could clearly be seen from the presence of a well resolved doublet for H-17α observed at δ 3.113 (J = 8.0 Hz). Accordingly, the latter only had one coupling partner, i.e., H-16α resonating at 4.630 ppm (dt, J = 2.1 and 2 × 8.0 Hz) which in turn was coupled to a methylene pair (δ 2.604 [dd, J = 8.4 and, 11.9 Hz, H-15α] and 1.691 [overlapped br d, H-15β). This already gave evidence for 16β-hydroxylation. Further proof came from three significant alterations of the H_2-21 resonances of the butenolide ring: Due to their close proximity to the hydroxylation site in ring D (i) both signals are shifted towards lower field, (ii) their assignment has to be reversed with respect to the chemical shift order (A/B reversal), and (iii) due to a decreased δν the intensities of the dd-type signals were considerably shifted towards the center and resulted in a remarkable roof effect for the H-22 signal which is usually not observed. Finally,

Table 3: ^{13}C NMR spectral data including DEPT multiplicity of the cardenolides 2–4 (90 MHz in CD_3OD)

C-atom	Mult.	**2**	Mult.	**3**	Mult.	**4**
		Dtg		Dgg		Gtg
1	CH_2	31.00	CH_2	30.77	CH_2	30.95
2	CH_2	28.06	CH_2	28.32	CH_2	27.73
3	CH	74.49	CH	74.34	CH	74.40
4	CH_2	31.38	CH_2	30.93	CH_2	31.30
5	CH	37.97	CH	37.89	CH	37.87
6	CH_2	27.51	CH_2	27.41	CH_2	27.48
7	CH_2	22.36	CH_2	22.74	CH_2	22.04
8	CH	42.69	CH	42.12	CH	42.80
9	CH	36.86	CH	36.91	CH	37.76
10	C	36.95	C	37.83	C	36.20
11	CH_2	22.58	CH_2	31.55	CH_2	22.38
12	CH_2	40.97	CH	75.62	CH_2	40.93
13	C	51.07	C	57.23	C	51.28
14	C	86.46	C	86.76	C	85.60
15	CH_2	33.39	CH_2	33.49	CH_2	51.28
16	CH_2	27.87	CH_2	27.72	CH	73.01
17	CH	52.13	CH	46.99	CH	59.64
18	CH_3	16.40	CH_3	9.86	CH_3	17.05
19	CH_3	24.29	CH_3	24.20	CH_3	24.26
20	C	178.47	C	178.43	C	177.28
21	CH_2	75.36	CH_2	75.40	CH_2	75.62
22	CH	117.79	CH	117.70	CH	120.54
23	C	177.26	C	177.30	C	173.65
		Dgx		Dgx		Dgx
1′	CH	98.89	CH	96.89	CH	96.80
2′	CH_2	38.49	CH_2	38.41	CH_2	38.41
3′	CH	68.26	CH	68.18	CH	68.18
4′	CH	83.49	CH	83.64	CH	84.21
5′	CH	69.45	CH	69.45	CH	69.59
6′	CH_3	18.45	CH_3	18.45	CH_3	18.53
		Dgx		Dgx		Glc
1″	CH	100.53	CH	100.53	CH	105.75
2″	CH_2	38.95	CH_2	38.86	CH_2	75.01
3″	CH	68.48	CH	68.37	CH	77.84
4″	CH	83.77	CH	83.72	CH	71.08
5″	CH	69.45	CH	69.45	CH	77.68
6″	CH_3	18.45	CH_3	18.45	CH_3	62.92
		Dgx		Dgx		
1‴	CH	100.49	CH	100.39		
2‴	CH_2	36.33	CH_2	36.14		
3‴	CH	70.63	CH	70.63		
4‴	CH	81.30	CH	81.24		
5‴	CH	71.46	CH	71.46		
6‴	CH_3	18.62	CH_3	18.59		
		Glc		Glc		
1⁗	CH	105.80	CH	105.71		
2⁗	CH	75.16	CH	75.09		
3⁗	CH	77.95	CH	77.85		
4⁗	CH	71.78	CH	71.68		
5⁗	CH	77.91	CH	77.85		
6⁗	CH_2	62.92	CH_2	62.88		
$\underline{C}OCH_3$	C	172.65	C	172.59		
$\overline{C}O\underline{C}H_3$	CH_3	21.22	CH_3	21.24		

Abbrs.: Dgx: Digitoxose; Glc: Glucose; Dtg: Digitoxigenin; Dgg: Digoxigenin; Gtg: Gitoxigenin

interesting observations were made in a 3 Hz long-range COSY map: Firstly, ^{4}J couplings affected H-17α/H-22 and H-17α/H-21A indicating the complex coupling pattern arising from the unsaturated butenolide. Secondly, the protons of the five-membered ring D are also involved into long-range coupling as shown by cross-peaks between H-17α and H-15β as well as H-17α and H-18. Finally, the second angular Me H-19 also showed a ^{4}J correlation to a dt-like signal at 1.694 ppm which can be assigned to H-9. In summary, clear evidence was gathered for the presence of a secondary hydroxyl group at C-16. Consequently, the aglycone of **4** was determined as gitoxigenin and thus **4** was elucidated as glucogitoroside.

The elucidated cardenolide structures are in agreement with the chromatography screening of various *Digitalis* plants including *D. ferruginea* ssp. *ferruginea* [19]. Nevertheless, to our best knowledge this is the first structure assignment of isolated cardenolides from this plant. Furthermore, although being previously established through chemical methods [7, 8], independent proof for the site of acylation of the lanatoside tetrasaccharide moiety relying to NMR evidence is also reported for the first time. At the same time, comprehensive ^{1}H NMR datasets are presented for the cardenolides including the analysis of overlapping resonances by the use of 2D techniques. Concerning the bioactivity of the constituents, it was shown very recently that phenylethanoid glycosides develop an interesting chronotropic cardioactive potential in the Langendorff isolated rat heart [18]. Since these phenolic constituents have been essentially neglected in most phytochemical studies of *Digitalis* species in the past, their contribution to the cardioactivity of traditional *Digitalis* remedies and possible interactions have to be considered. Finally it should be mentioned that the structures of the isolated digitaloids are in agreement with the HPLC chromatographic assignments in the literature [19].

3. Experimental

3.1. Plant material

The plants were collected from Aladag-Bolu, Turkey, in July 1996. A voucher specimen (No. 96105) is deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Haçettepe University, Ankara, Turkey.

3.2. Extraction and isolation

The air-dried and powdered aerial parts of the plant were extracted twice with MeOH−H_2O (4 : 1). The water soluble part of this extract was fractionated by VLC using LiChroprep C-18 as stationary phase and eluting with H_2O−MeOH mixtures to afforded ten fractions (A-J). Fractions G, H and I exhibited positive Kedde reactions and were further applied to chromatographic separations over silica gel to yield compounds **1**–**4** as follows: CC of fraction G (4 g) on silica gel (200 g) using EtOAc−MeOH−H_2O mixtures (100 : 5 : 2 → 100 : 7 : 3 → 100 : 10 : 5 → 100 : 20 : 10 → 100 : 25 : 15 → 100 : 30 : 20) yielded 102 fractions which were combined into three main fractions (fr. G_1: 1−55; fr. G_2: 56−65; fr. G_3: 66−102). Fraction G_2 (216 mg) was further subjected to a silica gel (30 g) CC using $CHCl_3$−MeOH−H_2O (90 : 10 : 1 → 80 : 20 : 1) thus affording compounds **3** (50 mg), **4** (26 mg) and **1** (10 mg), respectively. Fraction H (928 mg) upon CC on 80 g of a silica gel using $CHCl_3$−MeOH−H_2O (80 : 20 : 1) yielded five main fractions (frs. H_1−H_5). Repeated CC of fr. H_4 (131 mg) on a silica gel (14 g) column using EtOAc−MeOH−H_2O (100 : 5 : 2) afforded pure compound **2** (12 mg).

3.3. NMR Spectroscopy

The NMR spectra were recorded at a probe temperature of 300 K on a Bruker AM 360 spectrometer with a 5 mm dual probe operating at 360 MHz for ^{1}H and 90 MHz for ^{13}C, respectively. Methanol-d_4 (99.8% D, Merck, Darmstadt, No. 106002) was used as solvent with a sample volume of 0.7 ml corresponding to a filling height of 40 mm in 5 mm tubes (Norell 507). Chemical shifts are reported in ppm on the δ scale with reference to the residual solvent peak (3.300 and 49.00 ppm, respectively), the coupling constants (J) are given in Hz.

The spectra were obtained under the following conditions: ^{1}H 30 deg. pulse, delay after acquisition (D1) 1 s, processing with 0.3 Hz line broadening (LB) or with Lorentz-Gauss resolution enhancement and zero-filling. Acquisition using 16k (SI) data points in a spectral window of ca. 8 ppm yielded a digital resolution better than 0.2 Hz. Water peak suppression was achieved by presaturation setting the HDO signal on resonance (O1). ^{13}C 30 deg. pulse, D1 3 s, collecting a spectral window of 250 ppm into 32K data points and processing with 1.2...3 Hz line broadening (LB) or with Lorentz-Gauss resolution enhancement after zero-filling. Signal multiplicity was determined by APT. ^{1}H 2D COSY, presaturation of HDO off-resonance, SW ∼ 6 ppm, D 1.2 s, 1K × 512 or 256 t_1 increments, 90 deg. shifted sinebell-squared apodization, zero-filling in t_1 dimension during processing.

Offline data processing was done with the manufacturers NMR data processing software (Bruker DISNMR) as well as with the Nuts program package, Acorn NMR, Vermont (CA).

3.4. Mass spectrometry

ESI MS were run on a Finnigan LCQ System in the direct inlet mode scanning for both positive and negative ions. MALDI-TOF data were taken on a Lazarus-II instrument with DHB as matrix substance. DCI-MS came from an INCOS-50 using NH_3 as reactant gas.

Acknowledgements: The authors are grateful to Dr. U. Matthiesen, HHU Düsseldorf, for DCI-MS, and to Dr. H. Luftmann, WWU Münster, for MALDI-TOF measurements. The expertise technical assistance of Mrs. K. Voß, WWU Münster, is thankfully acknowledged.

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Received April 23, 1999
Accepted June 8, 1999

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