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A new pregnane glycoside and a furostanol glycoside from *Digitalis cariensis*

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Chemical investigation of the aerial parts of *Digitalis cariensis* Boiss. ex Jaub. & Spach resulted in the isolation of a new pregnane glycoside, cariensisoside (**1**) and a furostanol glycoside, uttroside A (**2**), along with the two known phenylethanoid glycosides, lugrandoside (**3**) and maxoside (**4**). On the basis of spectral (UV, IR, NMR, MS) and chemical methods, compounds **1** and **2** were identified as digifologenin-3-*O*-β-glucopyranosyl-(1→4)-β-oleandropyranoside and 3-*O*-(β-lycotetraosyl)-26-*O*-(β-glucopyranosyl)-(25*R*)-22α-methoxy-5-furostane-3β,26-diol, respectively.

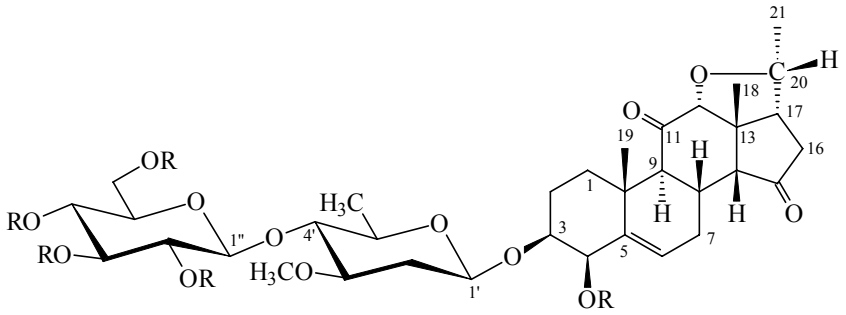
1. Introduction

The genus *Digitalis* (Scrophulariaceae) is represented by nine species in the flora of Turkey [1]. Previous phytochemical studies on *Digitalis ferruginea* ssp. *ferruginea* (syn. *D. aurea*) afforded several phenylethanoid and cardioactive glycosides [2, 3]. In continuation of this project, *D. cariensis* was investigated. This paper describes the isolation and structural elucidation of a new pregnane glycoside, cariensisoside (**1**) and a furostanol glycoside, uttroside A (**2**).

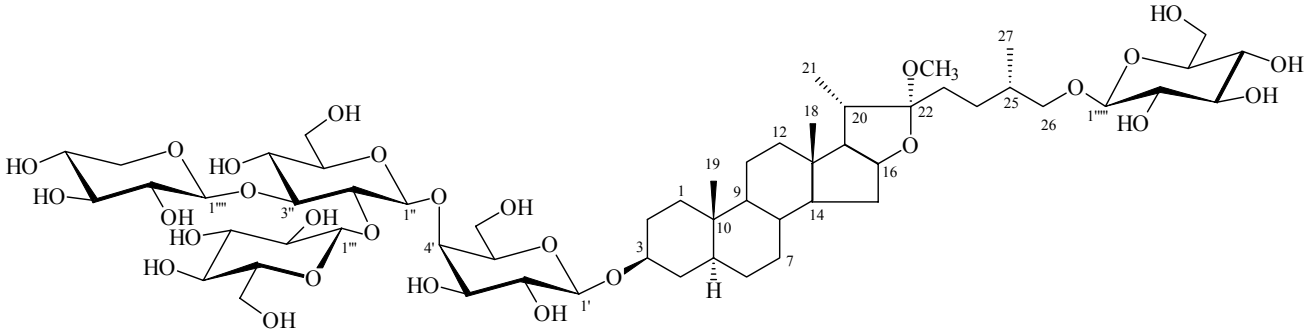
2. Investigations, results and discussion

Compound **1** was isolated as an optically active amorphous powder. Its UV spectrum displayed λ_{max} at 222, 298 and 314 nm. The IR spectrum of **1** contained absorption bands at 3414 (OH), 1635 (C=C), 1733, 1713 (C=O) and 1070 (C–O–C) cm^{-1}. ESIMS of **1** exhibited a quasimolecular ion $[M + Na]^+$ at m/z 689, consistent with a molecular formula of $C_{34}H_{50}O_{13}$, indicating the presence of ten degrees of unsaturation. The NMR spectra of **1** (Table 1) revealed resonances for two secondary methyls, and two tertiary methyls, a methoxyl group, four oxygenated methines, a trisubstituted double bond and two ketones. Since these last two functional groups accounted for three bond equivalents, **1** was considered as heptacyclic. The ^1H NMR spectrum of **1** also indicated that the molecule contained two sugar units, as evident from the presence of two anomeric proton signals at δ_H 4.71 and 4.45. Hence, the aglycone had to be pentacyclic. The ^{13}C NMR data, assigned by DEPT and gHSQC experiments, contained 21 carbon atoms for the aglycone. Detailed investigation of the ^{13}C NMR data showed **1** to be a highly oxygenated pregnane glycoside with an extra ring. The pregnane nucleus was established through gCOSY, TOCSY and gHMBC correlations. Analysis of the gCOSY and TOCSY spectra of **1** revealed the presence of three ^1H-^1H spin systems for the aglycone. The first one from H-1 through H-4 included two diastereopic methylene signals (δ_H 1.99 and 1.30, H_2-1; δ_H 1.92 and 1.66, H_2-2) and two deshielded signals (δ_H 3.63, H-3 and 4.28, H-4). The corresponding ^{13}C chemical shifts of the latter signals at δ_C 79.8 (d, C-3) and 75.6 (d, C-4) implied oxygen substitutions, which were best accomodated by secondary alcohol functionalities. The second spin system, extending from the olefinic proton, H-6 (δ_H 5.76) to H-9 (δ_H 1.86 d, $J = 12.9$ Hz), also incorporated H-14 (δ_H 2.45 d, $J = 3.5$ Hz). The last sequence consisted of H_2-16, H-17, H-20, and terminated with a secondary methyl group (δ_H 1.22 d, H_3-21). Connection of these substructural units was achieved by gHMBC correlations. Key long range correlations between H_2-1/C-5, H-4/C-5, H-4/C-6, H-6/C-4, H-6/C-10 and H_3-19/C-1, H_3-19/C-5 and H_3-19/C-10 allowed the closure of the A/B rings as shown. Rings C and D were assembled through $^2J_{CH}$ and $^3J_{CH}$ HMBC cross-couplings between H-12/C-13, H-12/C-14, H-12/C-18, H-14/C-12, H-14/C-13, H-14/C-18. Both H-14 and H_2-16 correlated ($^2J_{CH}$) with a ^{13}C signal at δ_C 219.8, which placed one ketone functionality at C-15. The second ketone group was assigned to C-11 (δ 214.6) based on $^2J_{CH}$ couplings from both H-9 and H-12 to C-11. Additionally, H-12 displayed a cross peak with another oxymethine carbon (δ_C 78.1, C-20). From this data, it was apparent that C-12 and C-20 formed a cycloether to generate the fifth ring. A computer search indicated this aglycone structure to be identical with digifolein, previously reported from the genus *Digitalis* [4]. To prove the relative stereochemistry of the chiral centers in **1**, a 2D NOESY experiment was performed. The nOe correlations between H-8/H_3-19, H-12/ H_3-18, H-12/ H_3-19, and H_3-18/H-20 indicated them all lie on the same (β) side of the molecule while the key nOe couplings between H-3 and H-4 revealed them to be on the opposite (α) side. The absence of any dipolar coupling from H-9 to H-8 or H_3-19 suggested its α–orientation.

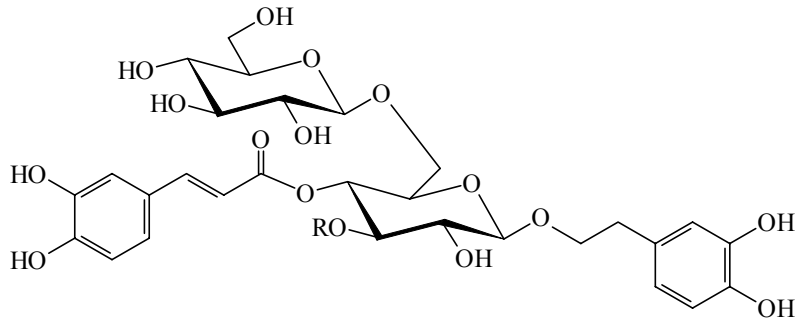
Compound **1** gave a positive test with Keller-Kliani reagent, indicating the presence of 2-desoxysugar unit(s) in the molecule. Indeed, the coupling constant analysis and the chemical shift values revealed the sugar portion of **1** to be composed of a β-oleandropyranose (δ_H 4.71 dd, $J = 1.6$, 9.4 Hz, δ_C 98.4) (Ole) and a β-glucopyranose (δ_H 4.45 d, $J = 7.8$ Hz, δ_C 104.1) (Glu). A DQF-COSY experiment allowed the establishment of the spin system sequences throughout the sugar moieties. The acetylation of **1** afforded a pentaacetyl derivative, **1a**. Four of the five acetyl signals in the ^1H NMR spectrum (Table 1) were assigned to the carbohydrate moiety, further supporting the above mentioned assignment. Since C-3 (δ_C 79.8) was deshielded *ca* + 4 ppm in comparison to C-4 (δ_C 75.6), the site of glycosylation was predicted to be C-3. Accordingly, the anomeric proton of the Ole (H-1′) was found to show HMBC cross-couplings with C-3. Further long-range correlations were observed between the anomeric proton of the Glu (H-1″) and C-4′ (δ_C 83.6) of the Ole, indicating the second sugar (Glu) was attached to C-4′ of the Ole via a β-bond. Based on all these evidences, the structure of **1** was elucidated as digifologenin-3-*O*-β-glucopyranosyl-(1→4)-β-oleandropyranoside. Final comparison of this structure with that of glucodigifolein [4] indi-



1 R = H
1a R = Ac



2



3 R = H
4 R = β-D-Glucopyranose

cated that these compounds were similar, except glucodigifolein contains β-diginopyranose, instead of β-oleandropyranose in the disaccharide moiety. To the best of our knowledge, **1** is being isolated for the first time from nature. We propose the trivial name cariensisoside for **1**.

Compound **2** was isolated as an optically active amorphous powder and was proposed to be a furostanol saponin on the basis of formation of red colour with Ehrlich reagent (TLC) [5]. The molecular formula was determined as $C_{57}H_{96}O_{28}$ based on the pseudomolecular ions appeared in the positive ESIMS (*m/z* 1251 $[M+Na]^+$ and 1267 $[M+K]^+$) and 1D NMR data (Table 2). The ^{13}C NMR spectrum of **2** showed resonances for 16 methylene, 33 methine and 5 methyl groups (one of which could be assigned to a methoxyl function), and 3 quaternary carbon atoms. The complete assignments of all proton and carbon resonances, based on the results of DQF-COSY, gHSQC, TOCSY and gHMBC experiments, indicated that **2** was a bidesmosidic glycoside of the 3,22,26-trioxygenated furostanol-type steroidal sapogenin [6]. Thus, the carbon resonance at δ_C 113.9 was readily assigned to the characteristic ketal carbon atom (C-22) [7]. The methoxyl function was attached to C-22 due to an HMBC coupling between C-22 and the 3H proton singlet at δ_H 3.13 (δ_C 47.6, OCH_3). Thus, C-3 and C-26 were identified as the sites of glycosidation. The ^1H NMR spectrum of **2** contained five anomeric proton resonances at δ_H 4.37 (d, $J = 7.8$ Hz), 4.58 (d, $J = 8.2$ Hz), 4.87 (d, $J = 7.8$ Hz), 4.60 (d,

$J = 8.2$ Hz), and 4.23 (d, $J = 7.8$ Hz), which correlated with the ^{13}C resonances at δ_C 102.6, 104.9, 104.8, 104.9 and 104.6, respectively. Complete assignments of each sugar proton system were achieved by considering DQF-COSY and TOCSY spectra, while the carbons were assigned from gHSQC and gHMBC spectra. Evaluation of spin-spin couplings and chemical shifts indicated the presence of a β-galactose (Gal), a β-xylose (Xyl), and three β-glucose (Glu) units within **2**. One of the glucose moieties (T-Glu-2) was attached to the furostane aglycone at C-26 since an HMBC cross peak was observed between its anomeric proton (H-1‴″) and C-26 (δ_C 76.0). The ^{13}C chemical shift values as well as the absence of any further HMBC correlations from this sugar unit indicated the terminal position of T-Glu-2, thereby the tetrasaccharidic nature of the second carbohydrate unit. The key pairs of HMBC correlations observed between H-1‴″ (Xyl)/C-3″ (Glu-I); H-1‴ (T-Glu-I)/C-2″ (Glu-I); H-1″ (Glu-I)/C-4′ (Gal) confirmed the interglycosidic linkages, and so, the identification of a second sugar moiety as lycotetrose [8]. The gHMBC spectrum displayed couplings between the H-1′ (Gal) and C-3 (δ_C 79.4) and revealed the lycotetrose group to be attached to the common position, C-3, of the aglycone. From these data, **2** was determined to be 3-*O*-(β-lycotetraosyl-26-*O*-(β-glucopyranosyl)-(25*R*)-22α-methoxy-5α-furostane-3β,26-diol. This structure is identical to that of uttroside A, previously isolated from *Solanum nigrum* (Solanaceae) whose structure was established by chemical means and IR spectroscopy [9]. We report here the complete NMR data of **2**.

Table 1: ^{13}C and ^{1}H NMR data for 1 (CD_3OD) and 1a, ($CDCl_3$) (^{13}C NMR, 100 MHz; ^{1}H NMR, 400 MHz)*

C/H Atom	**1**			**1a**	
	δ_C ppm	DEPT	δ_H ppm, *J* (Hz)	δ_C ppm	δ_H ppm, *J* (Hz)
Digifologenin					
1	38.9	CH_2	1.99 m	39.6	2.06 †
			1.30 m		1.36 †
2	24.1	CH_2	1.92 m	25.6	1.87 m
			1.66 m		1.74 m
3	79.8	CH	3.63 m	76.1	3.66 †
4	75.6	CH	4.28 d (2.7)	70.5	5.51 br s
5	142.9	C		138.0	
6	129.1	CH	5.76 dd (5.5, 1.2)	130.8	5.86 br s
7	30.6	CH_2	2.72 ddd (17.6, 11.7, 1.2)	29.5	2.78 dd (11.0, 18.3)
			2.16 ddd (17.6, 5.5, 4.7)		2.17 br d (11.0)
8	32.1	CH	2.59 dddd (12.9, 11.7, 4.7, 3.5)	30.7	2.47 br t (11.0)
9	60.3	CH	1.86 d (12.9)	60.0	1.92 †
10	40.0	C		38.6	
11	214.6	C		214.0	
12	90.5	CH	3.96 s	89.1	3.90 s
13	56.7	C		55.3	
14	57.7	CH	2.45 d (3.5)	48.7	2.27 br s
15	219.8	C		217.6	
16	40.4	CH_2	2.24 ddd (19.2, 9.0, 1.2)	36.8	2.20 †
			1.75 dd (19.2, 10.5)		1.92 †
17	49.8	CH	2.47 ddd (5.1, 9.0, 10.5)	56.7	2.34 m
18	25.4	CH_3	1.57 s	23.8	1.53 s
19	22.5	CH_3	1.20 s	21.4	1.09 s
20	78.1	CH	4.59 dq (6.3, 5.1)	76.6	4.59 dq (7.0, 7.3)
21	17.3	CH_3	1.22 d (6.3)	17.2	1.25 d (7.0)
Ole					
1′	98.4	CH	4.71 dd (9.4, 1.6)	97.1	4.50 dd (9.0, 2.0)
2′	37.9	CH_2	2.36 ddd (12.5, 5.5, 1.6)	35.8	2.34 †
			1.44 ddd (12.5, 9.4, 9.8)		1.30 †
3′	80.3	CH	3.42 ddd (9.8, 5.5, 2.5)	79.4	3.36 m
4′	83.6	CH	3.36 dd (9.0, 2.5)	84.0	3.14 t (7.3)
5′	72.7	CH	3.40 dq (6.3, 9.0)	73.1	3.26 m
6′	18.7	CH_3	1.37 d (6.3)	17.9	1.21 d (7.0)
OCH_3	58.1	CH_3	3.46 s		3.34 s
Glu					
1″	104.1	CH	4.45 d (7.8)	101.0	4.82 d (8.0)
2″	75.6	CH	3.16 dd (7.8, 9.0)	72.0	4.94 dd (8.0, 9.0)
3″	78.1	CH	3.32 t (9.0)	74.9	5.14 t (9.0)
4″	71.8	CH	3.27 t (9.0)	68.3	5.08 t (9.0)
5″	78.3	CH	3.24 m	71.8	3.66 m
6′	63.0	CH_2	3.86 dd (11.3, 2.0)	62.0	4.24 dd (11.0, 5.0)
			3.65 dd (11.3, 5.5)		4.12 br d (11.0)
$COCH_3$				20.7	2.05 s
				20.7	2.02 s
				20.7	2.00 s
				20.6	1.99 s
				20.6	1.97 s

Additional carbon signals for **1a**: $\underline{CO}CH_3$; 170.6, 170.2, 170.2, 169.3, 169.3.
*: All proton and carbon assignments are based on 2D NMR (DQF-COSY, TOCSY, gHSQC and gHMBC). †: Multiplicity of the signal is unclear due to overlapping.

Table 2: ^{13}C and ^{1}H NMR data of 2 (CD_3OD, ^{13}C NMR, 100 MHz; ^{1}H NMR, 400 MHz)

Aglycone				Sugar moiety			
C/H Atom	δ_C ppm	DEPT	δ_H ppm, *J* (Hz)	C/H Atom	δ_C ppm	DEPT	δ_H ppm, *J* (Hz)
1	38.2	CH_2	0.98** and 1.72**	Gal			
2	30.4	CH_2	1.51** and 1.86**	1′	102.6	CH	4.37 d (7.8)
3	79.4	CH	3.67**	2′	72.9	CH	3.66**
4	33.5	CH_2	0.93** and 1.70**	3′	75.3	CH	3.48**
5	46.0	CH	1.09**	4′	80.1	CH	4.00 br d (3.1)
6	29.9	CH_2	1.32 m	5′	75.7	CH	3.49**
7	31.4	CH_2	1.62** and 1.81**	6′	61.0	CH_2	3.58** and 3.89**
8	36.5	CH	1.57**	Glu-I			
9	55.8	CH	0.68 m	1″	104.9	CH	4.58 d (8.2)
10	36.8	C		2″	80.9	CH	3.79 dd (8.2, 8.6)
11	22.1	CH_2	1.30** and 1.53**	3″	87.5	CH	3.70**
12	41.1	CH_2	1.13** and 1.72**	4″	70.5	CH	3.86**
13	42.1	C		5″	74.8	CH	3.46**
14	57.5	CH	1.12**	6″	62.4	CH_2	3.70** and 3.93**
15	32.7	CH_2	1.23** and 1.95**	T-Glu-1			
16	82.4	CH	4.35 dd (14.5, 7.4)	1‴	104.8	CH	4.87 d (7.8)
17	65.2	CH	1.70**	2‴	73.4	CH	3.55**
18	17.0	CH_3	0.81 s	3‴	77.5	CH	3.30**
19	12.8	CH_3	0.85 s	4‴	71.0	CH	3.50**
20	41.2	CH	2.16 m	5‴	78.1	CH	3.24**
21	16.1	CH_3	0.99 d (7.0)	6‴	63.2	CH_2	3.55** and 3.87**
22	113.9	C		Xyl			
23	35.4	CH_2	1.29** and 1.65**	1⁗	104.9	CH	4.60 d (8.2)
24	28.9	CH_2	1.13** and 1.59**	2⁗	75.2	CH	3.23**
25	35.0	CH	1.73**	3⁗	77.2	CH	3.56**
26	76.0	CH_2	3.37** and 3.70**	4⁗	70.5	CH	3.25**
27	17.3	CH_3	0.94 d (6.6)	5⁗	67.2	CH_2	3.25** and 3.90 dd (11.9,5.4)
OCH_3	47.6	CH_3	3.13 s	T-Glu-2			
				1⁗′	104.6	CH	4.23 d (7.8)
				2⁗′	75.2	CH	3.18 dd (7.8, 8.5)
				3⁗′	77.9	CH	3.32**
				4⁗′	71.7	CH	3.25**
				5⁗′	78.3	CH	3.33**
				6⁗′	62.8	CH_2	3.65** and 3.85 **

*: All proton and carbon assignments are based on 2D NMR (DQF-COSY, TOCSY, gHSQC and gHMBC).
**: Multiplicity of the signal is unclear due to overlapping

The NMR and MS data for the phenylethanoid glycosides lugrandoside (**3**) [10] and maxoside (**4**) [11] were in agreement with previously published data.

The phenylethanoid glycosides were tested for their radical scavenging activity using 2,2-diphenyl-1-picryllhydrazyl (DPPH) radical as a TLC spray reagent [12, 13]. Both compounds showed the ability to efficiently scavenge free radicals.

3. Experimental

3.1. Apparatus

Optical rotations were measured on a JASCO DIP-370 digital polarimeter using a sodium lamp. UV spectra (MeOH) were recorded on a Shimadzu UV-160A spectrophotometer. IR spectra (KBr) were measured on a Perkin Elmer 2000 FT-IR spectrometer. NMR spectra were recorded using a Varian instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) equipped with a Nalorac MDBG 3 mm probe head. Negative and positive mode ESIMS were recorded on a Finnigan TSQ 7000 instrument. TLC analyses were carried on silica gel 60 F_{254} precoated plates (Merck, Darmstadt). Compounds were detected by spraying with 1% vanillin/H_2SO_4. MPLC separations were performed on LiChroprep C_{18} (Merck) material, using Büchi columns (2.6×46 cm, i.d. and 1.8×35 cm, i.d.) with a LKB 17000 Minirac fraction collector, a Rheodyne injector, and a Lewa M5 pump. Silica gel 60 (0.063–0.200 mm; Merck, Darmstadt) was used for open CC.

3.2. Plant material

Digitalis cariensis Boiss. ex Jaub & Spach (Scrophulariaceae) was collected from Burdur-Altınyayla, Southwest Anatolia, Turkey, in June 1999. A voucher specimen (HUEF 99025) is preserved at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University.

3.3. Extraction and isolation

Air dried and powdered aerial parts of the plant (250 g) were exhaustively extracted with 80% MeOH (2×1500 ml). The crude MeOH extract (60 g, yield 24%) was dissolved in H_2O and partitioned with petroleum ether (3×200 ml). An aliquot (27 g) of the aqueous phase was fractionated by LiChroprep C_{18}–vacuum liquid chromatography (VLC) using a 10% stepwise gradient elution from H_2O to MeOH, to yield fractions A–K. Fraction E (1.498 g) was subjected to C_{18}–MPLC eluting with increasing amounts of MeOH in H_2O (5–75%) to afford four main fractions (E_1–E_4). Fraction E_3 (410 mg) was rechromatographed using a silica gel (40 g) column with CH_2Cl_2–MeOH–H_2O (61 : 32 : 7, 500 ml) as eluent to give lugrandoside (**3**) (105 mg) and maxoside (**4**) (54 mg). Fraction F (947 mg), eluted with 45% MeOH, was subjected to silica gel (70 g) column chromatography using CH_2Cl_2–MeOH–H_2O mixtures (90 : 10 : 1 to 61 : 32 : 7, 1150 mL) to yield 40 mg of semi-pure compound **1**. Final purification of **1** (15.5 mg) was accomplished by silica gel (6 g) CC employing CH_2Cl_2–MeOH–H_2O (85 : 15 : 1, 200 mL) as eluent. Fraction J (1.220 g), eluted with 80% MeOH, was likewise applied to a silica gel (80 g) column which was eluted with CH_2Cl_2-MeOH-H_2O (80 : 20 : 2 to 61 : 32 : 7, 1350 ml) to give six fractions (J_1–J_6). Fraction J_5 (214 mg) was purified by C_{18}–MPLC (column dimensions, 1.8×35 cm) using 5% stepwise gradient from 60–80% MeOH in H_2O to obtain compound **2** (13 mg).

3.4. Cariensisoside (1)

Amorphous powder, $[\alpha]^{20}_D = -96^\circ$ (*c* 0.8, MeOH). UV (MeOH, nm): 222, 298 and 314. IR (KBr, cm^{-1}): 3414 (OH), 1635 (C=C), 1733, 1713 (C=O) and 1070 (C-O-C). ESIMS *m/z*: 689 $[M+Na]^+$. ^{1}H and ^{13}C NMR: see Table 1.

3.5. *Uttroside A* (2)

Amorphous powder, $[\alpha]^{20}_{D} = -35°$ (*c* 0.8, MeOH). UV and IR data are identical to those reported [9]. ESIMS *m/z* 1251 $[M+Na]^+$ and 1267 $[M+K]^+$. ^1H and ^{13}C NMR: see Table 2.

3.6. *Acetylation of cariensisoside* (1)

A solution of **1** (3.5 mg) in Ac_2O (1 ml) and pyridine (1 ml) was allowed to stand at room temperature overnight. The reaction mixture was diluted with H_2O and then transferred to a LiChroprep C_{18} cartridge using H_2O and CH_2Cl_2 as eluents, respectively. Compound **1a** was eluted with CH_2Cl_2 and purified by silica gel CC, using a benzol-acetone (3 : 1) mixture.

3.7. *Reduction of DPPH radical*

Methanolic solutions (0.1%) of the phenylethanoid glycosides **3** and **4** were chromatographed on a silica gel TLC plate, developing in $CHCl_3$–MeOH-H_2O (61 : 32 : 7) solvent system. After drying, the TLC plate was sprayed with a 0.2% DPPH (Fluka) solution in MeOH. Compounds showing a yellow-on-purple spot were regarded as antioxidant [13].

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