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Phytochemistry 66 (2005) 1440–1447
PHYTOCHEMISTRY
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Chemotaxonomic markers in Digitalideae (Plantaginaceae)

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Received 1 March 2005; received in revised form 6 April 2005
Available online 23 May 2005

Abstract

In a chemosystematic investigation of Digitalideae (Plantaginaceae), the water-soluble part of extracts of two species of *Digitalis*, two species of *Isoplexis*, as well as *Erinus alpinus* and *Lafuentea rotundifolia* were studied with regard to their content of main carbohydrates, iridoids and caffeoyl phenylethanoid glycosides (CPGs). *Digitalis* and *Isoplexis* contained sorbitol, cornoside and a number of other phenylethanoid glycosides including the new tyrosol β-D-mannopyranoside, sceptroside but were found to lack iridoid glucosides. *Erinus* contained mainly glucose, the new 8,9-double bond iridoid, erinoside, and a number of known iridoid glucosides including two esters of 6-rhamnopyranosylcatalpol, as well as the CPG poliumoside. Finally, *Lafuentea* was characterized by the presence of glucose, aucubin and cryptamygin B but apparently lacked CPGs. The chemosystematic significance of the isolated compounds is discussed.

Keywords: *Digitalis*; *Isoplexis*; *Erinus*; *Lafuentea*; Plantaginaceae; Chemosystematics; Sorbitol; Iridoid glucosides; Erinoside; Caffeoyl phenylethanoid glycosides; Sceptroside; Cornoside

1. Introduction

Recent extensive molecular systematic investigations of the heterogeneous family Scrophulariaceae have led to significant changes in its circumscription (Olmstead and Reeves, 1995; Oxelman et al., 1999; Olmstead et al., 2001). Many former members of the family have been assigned to a largely expanded Plantaginaceae (Veronicaceae *sensu* Olmstead, 2003), now comprising approximately 92 genera and 2000 species (APG, 2003; Albach et al., 2005). Within this family, we have recently reviewed the chemotaxonomy of *Plantago* (Rønsted et al., 2000, 2003b; Taskova et al., 2002b), *Aragoa* (Rønsted et al., 2003a), *Veronica* (Taskova et al., 2002a, 2004; Jensen et al., 2005), *Paederota* (Albach et al., 2004) and *Campylanthus* (Rønsted and Jensen, 2002).

Digitalis and its allies have been found to be closely related to Veroniceae and also belong to the extended Plantaginaceae (Oxelman et al., 1999; Olmstead et al., 2001; Bello et al., 2002). Tribes Veroniceae and Digitaleae were established by Bentham (1846) but subsequently lumped into one tribe (Bentham and Hooker, 1886; von Wettstein, 1898). Pennell (1935) reestablished Digitaleae as a small tribe comprising only *Digitalis* and perhaps *Rehmannia*. Recently, Albach et al. (2005) have found *Isoplexis* and *Erinus* to be the closest relatives of *Digitalis*. *Sibthorpia*, *Lafuentea* and *Campylanthus* have also been considered as members of the tribe by different authors (Bentham and Hooker, 1886; von Wettstein, 1898; Hallier, 1903; Melchior, 1964; Olmstead, 2003).

Digitalis and *Isoplexis* have been subjects of much chemical work mainly due to their content of heart-active cardenolides (Hegnauer, 1973; Ganapaty et al., 2003). A number of caffeoyl phenylethanoid glycosides (CPGs) have been detected or isolated from *Digitalis*

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0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.04.020

(Lichius et al., 1995; Matsumoto et al., 1987; Baudouin et al., 1988; Brieger et al., 1995; Zhou et al., 1998; Calis et al., 1999a,b; Kirmizibekmez et al., 2002). Also, cornoside (**2**) has been reported from *Digitalis purpurea* (Jensen et al., 1975) and salidroside (**5**) from *Isoplexis chalcantha* (Gonzalez et al., 1985). In his survey for iridoid glucosides in Scrophulariaceae, Kooiman (1970) included *Digitalis*, *Isoplexis* and *Erinus*. However, only the latter was shown to contain aucubin (**15**) and some additional unidentified iridoids. Rønsted and Jensen (2002) have recently investigated *Campylanthus* and isolated mannitol and sorbitol as the major carbohydrates and a number of iridoid glucosides, similar to those found in some species of *Plantago*. Pinar (1977) has reported coumarins from *Lafuentea rotundifolia*. In the present work, we have investigated the water-soluble constituents of two species each of *Digitalis* and *Isoplexis* as well as of *Erinus alpinus* and *L. rotundifolia* and identified the isolated compounds by NMR.

2. Results and discussion

The plant material was extracted with cold or boiling ethanol and the water-soluble part of the extract was subjected to reversed phase column chromatography to give the compounds listed in Table 1. Both in the case of *D. purpurea* and *E. alpinus* fractions with additional, unidentified CPGs were collected. Unfortunately, the carbohydrate fraction was not investigated in for *D. purpurea*, but sorbitol (**1**) has previously been shown to be the main alditol present in mature leaves of this plant (Raymakers, 1973). One of us has previously isolated cornoside (**2**) from *D. purpurea* (Jensen et al., 1975), while **9** and **10** together with calceolarioside A (**8**) and forsythiaside (**13**) were reported by Matsumoto et al. (1987). With regard to the compound **9**, it was first isolated from cell-cultures of *Rehmannia glutinosa* (Shoyama et al., 1986) but not given a trivial name, then it was reported from *D. purpurea* as purpureaside A (Matsumoto et al., 1987), from *Plantago major* as plantamajoside (Ravn and Brimer, 1988), and finally from several species of *Plantago* as plantamoside (Andary et al., 1988). The name purpureaside A therefore have priority, but since plantamajoside is by far the most used name and since the compound is a characteristic for *Plantago* species (Rønsted et al., 2000, 2003b), we suggest that this name is retained for **9**.

From *D. thapsus* and *I. chalcantha* cornoside was accompagnied by **3** or **4**, repectively. We have recently

Table 1
Compounds isolated in the present work

Plant	Plant part	Extraction method	Main carbohydrate	Iridoids	Phenylethanoids
Digitalis purpurea	Fresh leaves and stems from flowering plant	Boiling EtOH	n.i.	–	Cornoside (**2**) Plantamajoside (**9**) Purpureaside B (**10**)
Digitalis thapsi	Frozen whole first year plant	Cold EtOH	Sorbitol (60%)	–	Cornoside (**2**) Cornoside agluc. (**3**) Calceolarioside A (**8**) Forsythiaside (**13**)
Isoplexis chalcantha	Fresh whole first year plant	Cold EtOH	Sorbitol (50%)	–	Cornoside (**2**) Rengyolone (**4**) Salidroside (**5**) Lugrandoside (**11**) Forsythiaside (**13**)
Isoplexis sceptrum	Fresh leaves from old plant	Cold EtOH	Sorbitol (90%)	–	Cornoside (**2**) Salidroside (**5**) Sceptroside (**6**) Dopaol glucoside (**7**) Calceolarioside A (**8**) Forsythiaside (**13**)
Erinus alpinus	Frozen whole plants	Boiling EtOH	Glucose	Aucubin (**15**) Geniposidic acid (**16**) 8-Epiloganic acid (**17**) Arborescosidic acid (**18**) Erinoside (**19**) Catalpol derivs. (**21**, **22**)	Poliumoside (**12**)
Lafuentea rotundifolia	Dry whole flowering plants	Boiling EtOH	Glucose Sucrose	Aucubin (**15**) Gardoside (**20**)	–

shown that plants in which cornoside (**2**) is present, this compound sometimes is more or less hydrolysed during work-up (Jensen et al., 2005), probably due to enzyme activity, to give the aglucone (**3**) which is unstable and depending on the conditions rearrange to rengyolone (**4**). When this effect was discovered after part of the present work was finished, we decided to test this by using hot ethanol for the work-up of *D. purpurea*, and indeed only intact **1** was isolated from this plant.

Salidroside (**5**) has previously been reported from *I. chalcantha* (Gonzalez et al., 1985), and in the present work, we could isolate this compound from both species of *Isoplexis*. From *I. sceptrum* we in addition found a new isomeric compound which we have named sceptroside (**6**).

Sceptroside (**6**) was isolated as an amorphous solid $[\alpha]_D^{20}$ −51° and the elemental composition $C_{14}H_{20}O_7$ was established by HRESIMS. The ^{13}C NMR spectrum in D_2O (see 3.3) showed the expected 14 signals of which eight could be assigned to a *p*-hydroxyphenylethyl moiety like in salidroside (**5**). The remaining six signals obviously belonged to a glycosyl moiety different from glucosyl, and comparison with the spectrum of dopaol β-D-mannopyranoside (Franzyk et al., 2004) showed almost complete coincidence with the signals from the mannopyranosyl group when allowing for the different standard used. Moreover, it was unlike the reported spectrum of methyl of α-D-mannopyranoside (Bock and Pedersen, 1983). The ^1H NMR-spectrum was also consistent with structure **6**; thus, the signals assigned to the aglycone was almost identical with those seen for **5** and the signal from the anomeric proton of **6** appeared as a singlet at δ 4.46 while that of H-2′ was seen as a doublet ($J_{2',3'}$ = 3.3 Hz) at δ 3.81, consistent with an axial position of the 2-OH group of the glycone. In conclusion, sceptroside is tyrosol β-D-mannopyranoside (**6**). Compared with galactosides and allosides, β-mannosides are rare as micromolecular natural products, we have only been able to find a few recent reports, namely of two flavonoid (Wei and Yan, 1997; Jiang et al., 2002), one anthraquinone (Rai, 1993), one saponin (Guo et al., 1991) and some alkaloid mannosides (Yamashita et al., 2002). Earlier reports on flavone mannosides from *Stachys* sp. (cf. Meremeti et al., 2004) are most likely misidentifications of allosides which are often present in this genus.

In contrast to the above species, *E. alpinus* gave glucose as the main sugar, the CPG poliumoside (**12**) and a number of known iridoid glucosides, as well as a new iridoid glucoside which we have named erinoside (**19**) and the two esters of 6-rhamnopyranosylcatalpol **21** and **22**. Erinoside was isolated as an amorphous solid which proved somewhat unstable upon isolation and therefore the optical rotation was not measured; the elemental composition was $C_{16}H_{20}O_{11}$ as established by HRESIMS. The ^{13}C NMR spectrum of **19** (Table 2) showed only 15 signals of which six could be assigned to a β-glucopyranosyl moiety and the remaining nine signals were in accordance with an iridoid aglucone containing two double bonds and two carboxylic acid groups like in ixoside (Takeda et al., 1975), although only one of the carboxylic acid groups was visible in the spectrum. This was probably due to line broadening caused by partial ionisation like in the spectrum of arborescosidic acid (**18**) (Rønsted et al., 2000). Except for the signals arising from C-8 and C-9 (δ 139.7 and 138.3), the spectrum was almost identical to that of **18** (see Table 2). Furthermore, when compared to **18**, such upfield (3 ppm) and downfield (8 ppm) shifts for C-8 and C-9, respectively, would be expected when the double bond was conjugated with a carboxylic acid group as in **19**. In the ^1H NMR-spectrum, the H-1 signal was seen at the low field position δ 6.65, characteristic for iridoids with an 8,9-double bond (Jensen et al., 1996). The assignments of the NMR spectra were partly based on the DQF-COSY, gHSQC and gHMBC spectra.

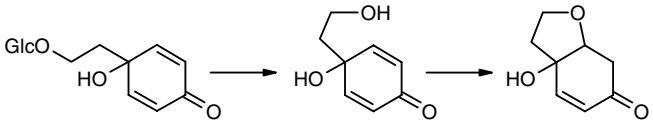
Table 2
NMR data for erinoside (**19**) and the model compound **18** recorded in D_2O

Atom	Erinoside (**19**)			Arborescosidic acid (**18**)[a]
	^1H (500 MHz)	^{13}C	HMBC correlations	^{13}C
Aglucone				
1	6.65 (s)	92.1	1′, 3, 5, 8	92.2
3	7.34 (br s)	150.4	1, 4, 5, 11	151.4
4		115.4		ca. 120
5	3.62 (m)	38.8	4, 9	38.2
6	1.49 (ax) (m)	31.2	4, 5, 7	31.5
	2.52 eq (m)		8, 9	
7	2.68 (m)	34.5	5, 6, 8, 9	34.5
	2.72 (m)		6, 8, 9	
8		139.9[b]		143.1
9		138.1[b]		130.2
10		n.o. [c]		58.1
11		172.7		n.o. [c]
Glc				
1′	4.80 (d, 8.1)	99.1	1,5′	99.1
2′	3.24 (dd, 8, 9.5)	73.5	1′, 3′	73.5
3′	3.48 (t, 9.5)	76.2	2′, 4′	76.5
4′	3.37 (t, 9.5)	70.4	3′, 5′, 6′	70.4
5′	3.47 (m)	77.0		77.1
6′	3.70 (dd, 12.4, 5.8)	61.5	4′, 5′	61.5
	3.88 (dd, 12.4, 1.2)		4′	

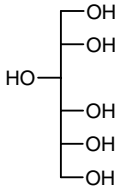
[a] Rønsted et al. (2000).
[b] Interchangeable.
[c] n.o. – not observed.

Dried plants of *L. rotundifolia*, collected in the natural habitat in Spain, had glucose and sucrose as the main carbohydrates. It contained two iridoid glucosides as well as the compound cryptamygin B (dihydromelilotoside; **23**) as one of the main constituents. Compound **23** has previously been reported from *Cinnamomum cassia* (Tanaka et al., 1989) and *Cryptocarya amygdalina*

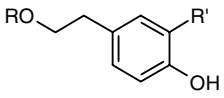
(Chan et al., 2002), both Lauraceae, and from *Mussaenda arcuata* (Ranarivelo et al., 1990), Rubiaceae.



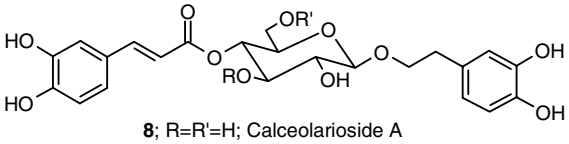
2; Cornoside **3**; Cornoside aglucone **4**; Rengyolone



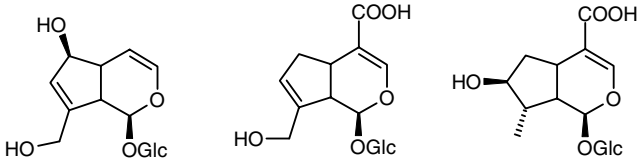
1; Sorbitol



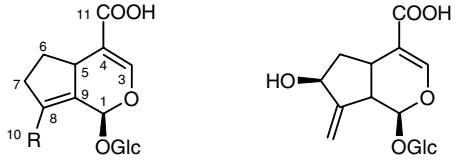
5; R=Glc; R'=H; Salidroside
6; R=Mann; R'=H; Sceptroside
7; R=Glc; R'=OH



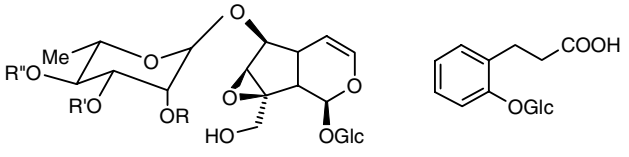
8; R=R'=H; Calceolarioside A
9; R=Glc, R'=H; Plantamajoside
10; R=Glc, R'=Rha; Purpureaside B
11; R=H; R'=Glc; Lugrandoside
12; R=R'=Rha; Poliumoside
13; R=H; R'=Rha; Forsythiaside
14; R=Rha; R'=H; Verbascoside



15; Aucubin **16**; Geniposidic acid **17**; 8-Epiloganic acid



18; R=CH_2OH; Arborescosidic acid **20**; Gardoside
19; R=COOH; Erinoside



21; R=R'=H; R"=Cinnamoyl **23**; Dihydromelilotoside
22; R=Acetyl; R'=Cinnamoyl; R"=H

The isolated compounds, sorbitol (**1**), cornoside (**2**), CPGs (**8**–**14**) and iridoids (**15**–**22**) are all of potential chemosystematic interest. Sorbitol (**1**) is a carbohydrate with a limited distribution. It has been reported from many Rosaceae and found in Tetrachondraceae (Jensen, 2000a). In Plantaginaceae (*sensu* APG, 2003), it is present in all species of *Plantago* (Rønsted et al., 2003b), *Aragoa* (Rønsted et al., 2003) and *Campylanthus* (Rønsted and Jensen, 2002) investigated. However, in many other taxa of the family, mannitol appears to be the characteristic sugar (Hegnauer, 1973) and this is indeed also the case for *Veronica* (Jensen et al., 2005).

Cornoside (**2**) and its precursor salidroside (**5**) belong to the same C_6-C_2 pool that gives rise to dopaol glucoside (**7**), calceolarioside A (**8**) and the other CPGs (**9**–**14**). While salidroside is widespread in plants, cornoside has a much more limited distribution, and is found mainly within Lamiales (Jensen et al., 2005). It usually occurs in taxa where iridoids are lacking but would be expected to occur, and this is also the case for *Digitalis* and *Isoplexis*. Caffeoyl phenylethanoid glycosides (CPGs; i.e. **8**–**14**) are characteristic for most taxa in Lamiales, and the most common representative, verbascoside (**14**) has been reported from all the families of Lamiales. Only two examples are known from outside the order (Jensen, 1992).

Iridoid glucosides (i.e. **15**–**22**) are characteristic for Lamiales, but they are more widespread than CPGs. However, specific compounds can be systematically very useful. Aucubin (**15**) and/or catalpol are characteristic for most taxa within Plantaginaceae, usually accompagnied by one or more of their biosynthetic precursors (i.e. **16**) or compounds derived from such precursors (**17** and **20**) (Rønsted et al., 2000), but they are present in most families of the order. Conversely, the iridoid glucosides with a 8,9-double bond, like **18** and **19**, have only been reported from Plantaginaceae; until now, they are known from *Globularia*, *Paederota*, *Plantago*, *Veronica* and *Wulfenia* (Albach et al., 2004). Rhamnopyranosylcatalpol esters, like **21** and **22**, have so far been reported from Scrophulariaceae *s.l.* and some taxa of Lamiaceae (Jensen, 2000b; Helfrich and Rimpler, 1999, 2000); they have not previously been found in Plantaginaceae.

As expected, the genera *Digitalis* and *Isoplexis* are found to be very similar: both contain sorbitol (**1**), cornoside (**2**) and a number of CPGs, but lack iridoids. Accumulation of salidroside (**5**), the precursor of **2**, could be considered as characteristic for *Isoplexis*, since it was isolated from both species investigated. The new compound sceptroside (**6**), which was found only in *I. sceptrum*, is obviously related to **5**.

Glucose was the main carbohydrate present in *E. alpinus*. Although cornoside (**2**) was not found, some CPGs were detected and the isolated poliumoside (**12**) is probably a precursor for the main phenylethanoid glycoside in *Digitalis* and *Isoplexis*, forsythiaside (**13**). A number of iridoids were isolated from *E. alpinus*, namely aucubin (**15**), its congeners geniposidic acid (**16**) and 8-epiloganic acid (**17**), as well as two esters of 6-rhamnopyranosylcatalpol, compounds **21** and **22**. The two

iridoids with an 8,9-double bond, arborescosidic acid (**18**) and erinoside (**19**), clearly support *Erinus* to be a member of the Plantaginaceae. However, this is the first report of 6-rhamnopyranosylcatalpol esters from the expanded Plantaginaceae (*sensu* APG, 2003).

In Digitalideae, the chemical profile of *Erinus* distinguishes it from the studied representatives of *Digitalis* and *Isoplexis* and this is in contrast to the morphological evidence and recent molecular systematic results (Albach et al., 2005). Evidently, the ancestors of the *Digitalis-Erinus* clade must have been able to produce iridoids and the blockage of iridoid biosynthesis happened in a branch which gave rise to *Digitalis* and its closest relative, *Isoplexis*. Here, biosynthesis of cardenolides has replaced that of the iridoids and reduction of glucose to sorbitol took place. Indeed, the iridoid pattern in *Erinus* with aucubin and compounds with an 8,9-double bond is characteristic for the remaining genera in Veroniceae-*Plantago* clade, the sister to Digitalideae (Albach et al., 2004).

Chemically, *L. rotundifolia* is unusual in the family since it lacks mannitol and sorbitol, displays a low content of aucubin (**15**) and no CPGs. *Lafuentea* has been included in Digitaleae (von Wettstein, 1898; Olmstead, 2003), in Rehmanniae-Ourisieae (Rouy, 1909) and most recently, it has been found to be a sister to tribe Antirrhineae (Albach et al., 2005). The chemical profile of the genus, in accordance with the floral and nuclear inclusions morphology (Bigazzi, 1993; Albach et al., 2005), do not corroborate the molecular evidence. Thus, (i) mannitol has been found as the main sugar in some Antirrhineae (Toth et al., 1978; Khan and Aqil, 1993); (ii) CPGs have been reported from *Linaria* (Lahloub, 1992; Otsuka, 1993), *Kickxia* (Amer, 1993) and *Antirrhinum* (Franzyk et al., 1998), although usually in low concentrations; (iii) all investigated genera of the tribe are characterised by the iridoid glucoside antirrhinoside and/or its derivatives. However, it could be assumed that the presence of aucubin in *Lafuentea* reflects a primitive condition for the tribe and change to antirrhinoside production happened after the separation of Antirrhineae.

3. Experimental

3.1. General

Fresh or frozen (−23 °C) plant material was blended with EtOH (4 × weight) and filtered. The concentrated extracts were partitioned in Et_2O–H_2O. The aqueous phase was taken to dryness, dissolved in 10% aq. acetic acid (in order to give retention to acidic compounds) and separated by reverse phase preparative chromatography. Merck Lobar RP-18 columns (size B and C) were eluted with H_2O–MeOH mixt. (1:0 to 1:1); compounds are listed in order of elution; the amount of sorbitol (**1**) was estimated from the ^{13}C NMR spectrum of the crude sugar fraction. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova-500 MHz or Mercury-300 MHz instruments in D_2O or MeOH-d_4 using the solvent peak (δ 4.75, 3.31 or 49.0) as the internal standard. In the cases where ^{13}C NMR spectra were recorded in D_2O the C-6′ shift was set to 61.5 ppm (Damtoft et al., 1981). 2D DQF-COSY, gHSQC and gHMBC spectra were aquired using standard pulse sequences. LC-HR ESIMS was performed on an Agilent HP 1100 Liquid Chromatograph equipped with a BDS-C18 reversed phase column running a water–acetonitrile (50 ppm TFA in water) gradient. The LC was coupled to a LCT of a TOF MS (Micromass, Manchester, UK) operated in the negative electrospray ion mode using 5-leucineenkephalin as lock mass. The known compounds isolated were identified by their NMR data: sorbitol (**1**), cornoside and congeners (**2**–**4**) (Jensen, 2000a); salidroside (**5**), plantamajoside (**9**) and iridoids (**15**–**20**) (Rønsted et al., 2000); dopaol glucopyranoside (**7**) (Sakurai and Kato, 1983); calceolarioside A (**8**) (Nicoletti et al., 1986), purpureaside B (**10**) (Matsumoto et al., 1987); lugrandoside (**11**) (Calis et al., 1999a); poliumoside (**12**) (Zhou et al., 1998); forsythiaside (**13**) (Nishibe et al., 1982); 6-α-L-(4″-*O*-*trans*-cinnamoyl)-rhamnopyranosylcatalpol (**21**) (Helfrich and Rimpler, 1999), scrospioside B (**22**) (Zhang et al., 1992).

3.2. Plant material

Digitalis purpurea L. (IOK-3/2004) was grown in a private garden, *Digitalis thapsi* L. (IOK-4/2004), *Isoplexis chalcantha* Svent. & O'Shan. (IOK-5/2004) and *Erinus alpinus* L. (IOK-19/2003) were all grown from seeds in the experimental fields of The Botanic Garden, University of Copenhagen. *Isoplexis sceptrum* (L. fil.) Loudon (S1993/374) was obtained from a greenhouse of the same institution. *Lafuentea rotundifolia* L. (IOK-16/2004) was collected in the wild in Spain: Almería, Vícar, barranco de Vícar. The voucher specimens were deposited in the Herbarium of Vienna.

3.2.1. Digitalis purpurea

Fresh leaves and stems (88 g), after being blended in hot EtOH, gave 4.4 g of crude extract; chromatography (Lobar column C) gave a sugar fraction (1.0 g, not further investigated), cornoside (**2**; 35 mg), pure plantamajoside (**9**; 20 mg), a mixture containing mainly **9** (140 mg), purpureaside B (**10**; 75 mg) and a mixt. of CPGs (140 mg).

3.2.2. Digitalis thapsi

Frozen vegetative plants (50 g) gave crude extract (1 g); chromatography (Lobar column B) gave a sugar fraction (580 mg) in which the main sugar was sorbitol (**1**; ca. 60% of total sugars) followed by cornoside (**2**;

10 mg), impure cornoside aglucone (**3**; 60 mg), calceolarioside A (**8**; 30 mg) and forsythiaside (**13**; 180 mg).

3.2.3. Isoplexis chalcantha

Fresh vegetative plants (50 g) gave 2.2 g of crude extract; chromatography (Lobar column B, 1:0 to 1:1) gave a sugar fraction (550 mg) consisting of mainly sorbitol (**1**; ca. 50%); then came a mixture of ethyl β-glucopyranoside and cornoside (**2**; 70 mg), pure **2** (120 mg), a fraction with mainly rengyolone (**4**; 30 mg), salidroside (**5**; 170 mg), lugrandoside (**11**; 80 mg), forsythiaside (**13**; 200 mg). The last fraction eluted (420 mg) consisted apparently of saponins acc. to ^{1}H NMR.

3.2.4. Isoplexis sceptrum

Fresh leaf material (26 g) gave 1.6 g of crude extract; chromatography (Lobar column B, 1:0 to 1:1) gave a sugar fraction (280 mg) with mainly sorbitol (**1**; ca. 90%), cornoside (**2**; 55 mg), followed by dopaol β-glucopyranoside (**7**; 15 mg), salidroside (**5**; 35 mg), sceptroside (**6**; 35 mg), calceolarioside A (**8**; 20 mg), and forsythiaside (**13**; 100 mg).

3.2.5. Erinus alpinus

Frozen whole plants (88 g) in hot EtOH gave crude extract (2.4 g); chromatography (Lobar column C, 1:0 to 1:1) gave a sugar fraction (2.3 g) consisting of mainly α- and β-glucopyranose, aucubin (**15**; 230 mg), geniposidic acid (**16**; 60 mg), 8-epiloganic acid (**17**; 15 mg), impure arborescosidic acid (**18**; 15 mg), erinoside (**19**; 40 mg), a fraction A with CPGs (70 mg), poliumoside (**12**; 40 mg), a fraction B with iridoids and CPGs (230 mg) and finally 4″-cinnamoyl-rhamnopyranosylcatalpol (**21**; 40 mg). Fraction A was not further investigated, but separation of fraction B gave 2″-acetyl-3″-cinnamoyl-rhamnopyranosylcatalpol (**22**; 20 mg).

3.2.6. Lafuentea rotundifolia

Dry flowering plants (14 g) was brought to boiling with EtOH (100 ml), homogenized and left to stand for 2 weeks. Work-up gave a crude extract (0.40 g); chromatography (Lobar column size B; 1:0 to 1:1) gave first a sugar fraction (180 mg) consisting mainly of glucose and sucrose, aucubin (**15**; 20 mg), gardoside (**20**; 5 mg) and dihydromelilotoside (**23**; 25 mg).

3.3. Sceptroside (6)

Amorphous solid: $[\alpha]_D^{20} = -51^\circ$ (MeOH; *c* 0.5); LC-HR ESIMS *m/z* : 299.1171 $[M-H]^-$; ($C_{14}H_{19}O_7$ requires 299.1131); ^{1}H NMR (500 MHz, CD_3OD): δ 7.05 and 6.68 (2 × 2H; AA′BB′-system, J = 8.5 Hz, H-2/6 and H-3/5), 4.46 (s, H-1′), 4.04 (dt, J = 9.6 and 7.2 Hz, H-αa), 3.85 (dd, J = 2.3 and 11.8 Hz, H-6a′), 3.81 (d, J = 3.3 Hz, H-2′), 3.70 (dd, J = 5.8 and 11.8 Hz, H-6b′), 3.69 (dt, J = 9.6 and 7.2 Hz, H-αb), 3.54 (*t*, J = 9.5 Hz, H-4′), 3.40 (dd, J = 3.3 and 9.5 Hz, H-3′), 3.17 (ddd, J = 2.3, 5.8 and 9.5 Hz, H-5′), 2.80 (2H; t-like, J = 7.3 Hz, β-CH_2); ^{13}C NMR (75 MHz, D_2O): δ 131.2 (C-1), 130.7 (2C; C-2 and C-6), 115.8 (2C; C-3 and C-5), 154.3 (C-4), 34.8 (β-CH_2), 70.9 (α-CH_2), 100.3 (C-1′), 70.9 (C-2′), 73.4 (C-3′) 67.3 (C-4′), 76.7 (C-5′), 61.5 (C-6′); ^{13}C NMR (50 MHz, CD_3OD): δ 131.0 (C-1), 131.0 (2C; C-2 and C-6), 116.1 (2C; C-3 and C-5), 156.8 (C-4), 36.4 (β-CH_2), 72.5 (α-CH_2), 101.7 (C-1′), 71.8 (C-2′), 73.4 (C-3′) 68.6 (C-4′), 78.3 (C-5′), 62.9 (C-6′).

3.4. Erinoside (19)

Amorphous solid: LC-HR ESIMS *m/z*: 387.0902 $[M-H]^-$; ($C_{16}H_{19}O_{11}$ requires 387.0927); NMR data in Table 2.

3.5. Dihydromelilotoside (23)

Amorphous solid: ^{13}C NMR (50 MHz, CD_3OD): δ 131.6 (C-1), 157.0 (C-2), 116.3 (C-3), 128.6 (C-4), 123.4 (C-5), 131.0 (C-6), 35.8 (C-a), 27.2 (C-b), 178.2 (CO), 102.6 (C-1′), 75.0 (C-2′), 78.1 (C-3′), 71.4 (C-4′), 78.1 (C-5′), 62.5 (C-6′); very similar to that reported (in DMSO; Chan et al., 2002).

Acknowledgements

We thank the staff of The Botanic Garden, The University of Copenhagen, and Dr. M. Montserrat Martínez-Ortega, University of Salamanca, Spain, for providing the plant material, Kristian Fog Nielsen, BioCentrum, DTU, for recording the mass spectra, and Dr. Dirk Albach, Institut für Botanik der Universität Wien, for authenticating the plants. R.M. Taskova is grateful for the grant by the Danish Ministry of Foreign Affairs and Cirius Foundation.

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