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Chemotaxonomy of *Plantago*. Iridoid glucosides and caffeoyl phenylethanoid glycosides

Nina Rønsted [a], Edith Göbel [a,1], Henrik Franzyk [a], Søren Rosendal Jensen [a,*], Carl Erik Olsen [b]

[a] *Department of Organic Chemistry, The Technical University of Denmark, DK-2800 Lyngby, Denmark*
[b] *Department of Chemistry, The Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark*

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Abstract

Data for 34 species of *Plantago* (Plantaginaceae), including subgen. *Littorella* (= *Littorella uniflora*), have been collected with regard to their content of iridoid glucosides and caffeoyl phenylethanoid glycosides (CPGs). In the present work, 21 species were investigated for the first time and many known compounds were found together with three new iridoid glucosides. Of these, arborescoside and arborescosidic acid, both of the uncommon type with an 8,9-double bond, were present in several species, while 6-deoxymelittoside was found only in *P. subulata*. The known compounds deoxyloganic acid, caryoptoside and rehmannioside D were isolated from the genus for the first time. The earlier reported occurrence of sorbitol in the family was confirmed, and this compound was shown by NMR spectroscopy to be the main sugar in the three species investigated for this. The combined data show that CPGs are present in all species investigated. With regard to the iridoids, the distribution patterns showed a good correlation with the classification of Rahn. Thus, aucubin is typical for the whole genus, while bartsioside and catalpol as well as 5-substituted iridoids are each characteristic for a subgenus in the family. Finally, the close relationship between *Plantago* and *Veronica* suggested by chloroplast DNA sequence analysis, could be corroborated by the common occurrence of the rare 8,9-unsaturated iridoids in these two genera.

Keywords: *Plantago*; Plantaginaceae; Chemotaxonomy; Iridoid glucosides; Bartsioside; Aucubin; Catalpol; Arborescoside; Arborescosidic Acid; 6-Deoxymelittoside; Verbascoside; Sorbitol

1. Introduction

In recent years, a considerable number of different iridoids have been isolated from the genus *Plantago*, and these include aucubin and compounds biosynthetically related to it. The early work on the genus concerns mainly aucubin and catalpol and has been reviewed by Hegnauer (1969). A number of taxonomic works primarily by Swiatek (1977) and by Andrzejewska-Golec (1997), Andrzejewska-Golec and Swiatek (1984) and Andrzejewska-Golec et al. (1993) have shown that the iridoids can be used as valuable taxonomic markers. In addition, caffeoyl phenylethanoid glycosides (CPGs) have been shown to be widely distributed in the genus and have similarly been suggested to be taxonomic markers (Andary et al., 1988) in the Plantaginaceae.

As part of an ongoing study of the water-soluble compounds in the genus *Plantago* (Damtoft et al., 1994a; Jensen et al., 1996; Franzyk et al., 1998), we have now investigated a larger number of species for iridoids and CPGs. Previously, we have elucidated the biosynthesis of aucubin and catalpol, mainly in *Scrophularia* (Jensen, 1991, 1992; Damtoft et al., 1993, 1994b; Damtoft, 1994), but also more recently in *Plantago major* (Jensen et al., unpublished) to be that shown in Fig. 1.

2. Results

In the present work, frozen plant material was homogenized with ethanol and the water-soluble part was subjected to reverse phase chromatography. The fractions were monitored by NMR spectroscopy and the isolated compounds identified by comparison with known data. Structures of new compounds were elucidated by spectroscopy. In the following, we will go through the species

* Corresponding author. Tel.: +45-452-52103; fax: 45-459 33968.
E-mail address: oksrj@pop.dtu.dk (S.R. Jensen).
[1] Nee Falkesgaard.

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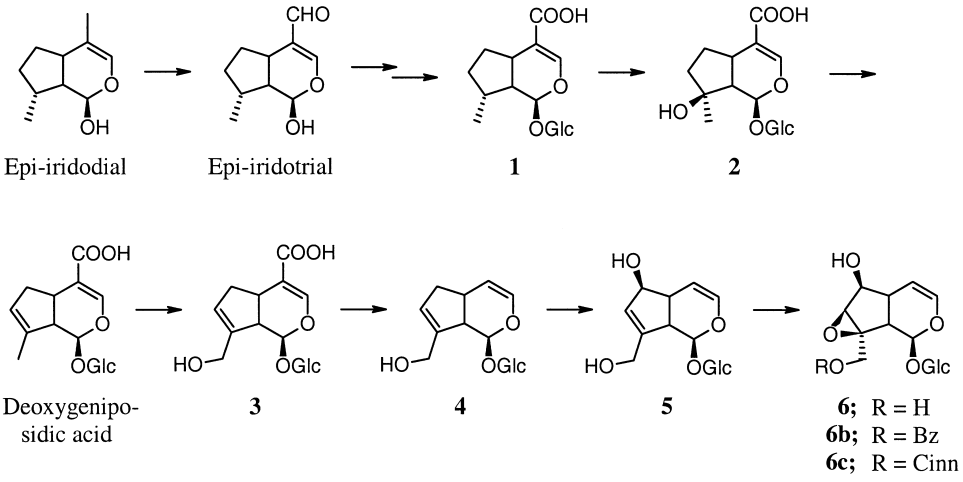

Fig. 1. Biosynthetic pathway to aucubin (**5**) and catalpol (**6**).

investigated during this work, in alphabetical order, followed by the species reported earlier.

2.1. *Plantago afra* (= *P. psyllium*)

Mediterranean — This species has been investigated several times by chromatographic methods. Thus, Popov et al. (1981) were the first to detect plantarenaloside (**7**). Later, Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) together with **7**, and a spot showing a compound which was later (Andrzejewska-Golec et al., 1993) identified as bartsioside (**4**). Finally, Andary et al. (1988) showed that verbascoside (**19a**) was a constituent in the plant. In the present work, we have isolated all the above-mentioned compounds, the main ones being **7** and **19a**.

2.2. *P. amplexicaulis*

Mediterranean — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) and catalpol (**6**). We isolated the same iridoids and detected also the presence of two CPGs by NMR spectroscopy; however, the structures of the latter were not definitely proved in this case.

2.3. *P. arborescens*

Macaronesia — Andrzejewska-Golec and Swiatek (1984) detected bartsioside (**4**) and aucubin (**5**). We isolated these iridoids together with the mussaenosidic acid (**2**), 8-epiloganic acid (**9**), gardoside (**14**) and arborescoside (**12a**), as well as the CPGs verbascoside (**19a**) and plantamajoside (**19b**).

2.4. *P. atrata*

Europe to W. Asia — Aucubin (**5**) and catalpol (**6**) as well as dihydroaucubin (**5c**) were isolated by Handjieva et al. (1991a). In the present work, we could isolate **5** and **6** as well as 8-epiloganic acid (**9**), gardoside (**14**), arborescosidic acid (**12**) and verbascoside (**19a**), the latter being the main constituent.

2.5. *P. australis*

Warm Americas — Verbascoside (**19a**) has been shown to be present in this plant (Andary et al., 1988). We isolated aucubin (**5**) as the only iridoid glucoside, together with **19a**, isoverbascoside and salidroside (**22**).

2.6. *P. bellardi*

Mediterranean — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) in this species. In the present work, **5**, geniposidic acid (**3**), asperuloside (**16**), verbascoside (**19a**) and chlorogenic acid (**21**) were isolated.

2.7. *P. coronopus*

Mediterranean, Europe — Of two different collections of this species, Andrzejewska-Golec and Swiatek (1984) could detect aucubin (**5**) in one while the other showed no iridoids. Andary et al. (1988) showed that verbascoside (**19a**) was present in three French collections. Our sample gave plantarenaloside (**7**) and **19a** as the main constituents, as well as salidroside (**22**).

2.8. *P. cretica*

Mediterranean — No previous work on this species. We succeeded to isolate aucubin (**5**), asperuloside (**16**), verbascoside (**19a**) and chlorogenic acid (**21**). The sugar fraction was in this case shown to consist of ca. 60% sorbitol (**20**).

2.9. *P. lundborgii*

San Ambrosio Isl — No previous work has been reported on this species. In the present work, **5** and **6** as

well as geniposidic acid (**3**), 8-epiloganic acid (**9**), gardoside (**14**), alpinoside (**12b**) and verbascoside (**19a**) were isolated; **6** and **19a** by far being the main constituents.

2.10. *P. maritima*

Cosmopolite — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) together with two unknown compounds in this species. We could isolate **5**, arborescosidic acid (**12**), melittoside (**18b**), rehmannioside D (**18c**) and the CPG echinacoside (**19e**), which is unusual in the genus.

2.11. *P. nivalis*

Spain — No previous work reported. This was one of the few species in which aucubin (**5**) could not be found. Catalpol (**6**), mussaenosidic acid (**2**), gardoside (**14**) and verbascoside (**19a**) were isolated.

2.12. *P. ovata*

Spain — No previous work reported. We isolated **5**, **6**, 8-epiloganic acid (**9**), arborescoside (**12a**), gardoside (**14**), asperuloside (**16**) as well as the CPGs verbascoside (**19a**) and plantamajoside (**19b**).

2.13. *P. patagonica*

Western USA — Bowers (1996) detected variable amounts of aucubin (**5**) and catalpol (**6**) in this species. Our specimen was in this case only analysed by ^{1}H NMR and high performance liquid chromatography (HPLC) and was shown to contain **5**, **6**, 10-benzoylcatalpol (**6b**) and verbascoside (**19a**).

2.14. *P. raoulii*

New Zealand — No previous work reported. Only aucubin (**5**) and verbascoside (**19a**) was found in this species. The sugar fraction consisted almost exclusively of sorbitol (**20**).

2.15. *P. reniformis*

Southeast Europe — No previous work reported. We could isolate **5**, geniposidic acid (**3**), 8-epiloganic acid (**9**), gardoside (**14**), and (**19a**), the latter by far being the main component.

2.16. *P. sempervirens (= P. cynops)*

Southwest Europe — Andrzejewska-Golec and Swiatek (1984) detected bartsioside (**4**) aucubin (**5**) and plantarenaloside (**7**). Later, Debrauwer et al. (1989) isolated **7** and verbascoside (**19a**) and Andrzejewska-Golec et al. (1993) isolated **4** and **7**, while **5** apparently was not present. We obtained **4** and **7**, together with mussaenosidic acid (**2**), caryoptoside (**10**), and (**19a**). Of these, **4**, **7** and **19a** were the main constituents.

2.17. *P. stauntonii*

Amsterdam and St. Paul Islands — No previous work reported. We could isolate aucubin (**5**), 8-epiloganic acid (**9**) arborescosidic acid (**12**), gardoside (**14**) and verbascoside (**19a**) from this species, the latter being the main constituent.

2.18. *P. subspathulata*

Madeira — No previous work reported. From this species, we could isolate only verbascoside (**19a**). Iridoids were apparently not present. The sugar fraction was in this case shown to consist of ca. 50% sorbitol (**20**).

2.19. *P. subulata (= carinata)*

Mediterranean — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) and melittoside (**18b**) in this species. Later, Saadi et al. (1991) isolated **5**, 10-acetylaucubin (**5a**), 3,4-dihydroaucubin (**5c**), 6-epiaucubin (**5d**), as well as monomelittoside (**18**) and **18b**. We could isolate bartsioside (**4**) in a trace amount, aucubin (**5**), arborescosidic acid (**12**), desacetylhookerioside (**12c**), 6-deoxymelittoside (**17**), **18**, **18b** and verbascoside (**19a**).

2.20. *P. uniflora (= Littorella uniflora)*

Europe — Andrzejewska-Golec (1999) has recently isolated aucubin (**5**) and catalpol (**6**) from this species. We obtained **5** and **6** as well as verbascoside (**19a**), the latter being the major component.

2.21. *P. webbii*

Macaronesia — Andrzejewska-Golec and Swiatek (1984) detected bartsioside (**4**) and aucubin (**5**). We could isolate 8-epideoxyloganic acid (**1**), mussaenosidic acid (**2**), **4**, **5**, plantarenaloside (**7**), epiloganic acid (**9**), arborescoside (**12a**), gardoside (**14**), verbascoside (**19a**) and plantamajoside (**19b**).

2.22. *P. alpina*

Europe — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) and melittoside (**18b**) in one collection, only **5** in another. Recently, Jensen et al. (1996) found mussaenosidic acid (**2**), geniposidic acid (**3**), 10-*O*-acetylgeniposidic acid (**3a**), **5**, 8-epiloganic acid (**9**), alpinoside (**12b**), gardoside (**14**), monomelittoside (**18**), **18b** and verbascoside (**19a**).

2.23. P. altissima

Europe — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) and catalpol (**6**) while Andary et al. (1988) found verbascoside (**19a**). Later, **5** and **6**, together with globularin (**6c** = 10-cinnamoylcatalpol), methyl desacetylasperulosidic acid (**15**) and asperuloside (**16**) were isolated from this species by Handjieva et al. (1991a). More recently, the plant was reinvestigated and again **5** and **6** were isolated, this time in addition to 8-epiloganic acid (**9**), desacetylhookerioside (**12c**), hookerioside (**12d**), gardoside (**14**), and **19a** (Jensen et al., 1996). The compounds **6c**, **15** and **16** were not seen in this collection.

2.24. P. arenaria

Mediterranean — Popov et al. (1981) were the first to isolate plantarenaloside (**7**) from this species. Andrzejewska-Golec and Swiatek (1984) have detected bartsioside (**4**) and plantarenaloside (**7**) while Andary et al. (1988) verbascoside (**19a**).

2.25. P. argentea

Southern Europe — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) and catalpol (**6**) and these compounds were later isolated by Handjieva et al. (1991a).

2.26. P. asiatica

South and East Asia — Oshio and Inouye (1982a) isolated aucubin (**5**) together with 3,4-dihydroaucubin (**5c**) and 6′-*O*-glucosylaucubin (**5e**) in this species, while Endo et al. (1981) isolated geniposidic acid (**3**) and **5**. Toda et al. (1985) also found **3**. Fifteen different CPGs have been isolated from this species (Ravn et al., 1990; Miyase et al., 1991; Nishibe et al., 1995) among which were verbascoside (**19a**) and plantamajoside (**19b**).

2.27. P. cornuti

Southern Europe — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) and catalpol (**6**) in one collection, but only **5** in another. Isolation work has been performed by Handjieva et al. (1992), who found **5** and 10-hydroxymajoroside (**13b**). Recently, also majoroside (**13a**) was isolated (Taskova et al., 1999).

2.28. P. hookeriana

Southern USA — From this species aucubin (**5**), catalpol (**6**), 10-benzoylcatapol (**6b**), 8-epiloganic acid (**9**), hookerioside (**12d**) and verbascoside (**19a**) were isolated (Damtoft et al., 1994a).

2.29. P. lagopus

Mediterranean — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) and catalpol (**6**) in two collections, and Andary et al. (1988) verbascoside (**19a**). Afifi et al. (1990) have isolated **5**, plantarenaloside (**7**) and asperuloside (**16**) from this plant.

2.30. P. lanceolata

Europe — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) and catalpol (**6**) while Andary et al. (1988) found verbascoside (**19a**). Bianco et al. (1984) isolated asperuloside (**16**). Later, **5** and **6**, together with globularin (**6c** = 10-cinnamoylcatalpol), methyl desacetylasperulosidic acid (**15**) and **16** were isolated from this species by Handjeva et al. (1991a). Recently, the annual variation of **5** and **6** was investigated by Long et al. (1995). Murai et al. (1995) reported four CPGs, namely verbascoside (**19a**), isoverbascoside, plantamajoside (**19b**) and lavandufolioside (**19d**).

2.31. P. major

Europe — Andrzejewska-Golec and Swiatek (1984) detected aucubin (**5**) and melittoside (**18c**) by paper chromatography, and Bianco et al. (1984) isolated asperuloside (**16**). From an Egyptian collection was isolated **5**, melampyroside (**5b** = 10-benzoylaucubin), plantarenaloside (**7**), ixoroside (**8**) and **16** (Afifi et al., 1990). Long et al. (1995) have investigated the annual change in aucubin content, they were unable to detect any catalpol (**6**). The first compound with an 8,9-double bond in the genus, namely majoroside (**13a**), was reported by Handjeva et al. (1991b); it was found in collections from both Bulgaria and Mongolia. More recently, the Bulgarian group (Taskova et al., 1999) also reported the finding of 10-hydroxymajoroside (**13b**) and 10-acetoxymajoroside (**13c**) from this species. Murai et al. (1996) isolated geniposidic acid (**3**), **5**, **6**, gardoside (**14**) and **18c** in a collection obtained from a Swiss drughouse. However, as noted by Long et al. (1995), the drug ''Feuille de Plantain'' used here, indiscriminately consists of leaves from three different species, and we therefore regard the findings of the systematically interesting compounds **6** and **18c** in *P. major* as dubious. Andary et al. (1988) detected and Ravn and Brimer (1988) later isolated verbascoside (**19a**) in ssp. *pleiosperma* while plantamajoside (**19b**) was found in ssp. *major*.

2.32. P. media

Europe — Swiatek (1977) detected aucubin (**5**) and a compound, which was later (Swiatek et al., 1981) identified as melittoside (**18b**) while Andary et al. (1988) could detect plantamajoside (**19b**) in this species. Saadi

et al. (1990) later isolated **5**, monomelittoside (**18**) and 10-acetylmonomelittoside (**18a**) from this species.

2.33. *P. myosuros*

South America — From this species only aucubin (**5**) and the two CPGs verbascoside (**19a**) and plantalloside (**19c**) were isolated (Franzyk et al., 1998).

2.34. *P. rhodosperma*

Southern USA — Different populations of this species were investigated for aucubin (**5**) content (Johnston et al., 1997).

2.35. *Structural elucidation of new compounds*

Arborescosidic acid (**12**) was isolated from four species, namely *P. atrata, P. maritima, P. stauntonii* and *P. subulata*. The negative FAB-mass spectrum gave a M_r of 374, and this suggested the composition $C_{16}H_{22}O_{10}$. During reverse phase chromatography, **12** showed properties in agreement with being an acid, since addition of bicarbonate resulted in a very low retention time, while addition of acetic acid increased the retention time considerably. In agreement with the elemental composition found above, the ^{13}C NMR spectrum (Table 1) showed 16 signals, of which six could be assigned to a β-glucopyranosyl moiety, thus leaving 10 carbon atoms for the aglucone. The remaining signals suggested a 4-carboxylated iridoid compound, and furthermore, two low-intensity signals at δ 129.9 and 143.8 demonstrated the presence of a tetrasubstituted double bond. Comparison (Table 1) with the spectrum of alpinoside (**12b**), isolated from *P. alpina* (Jensen et al., 1996), showed a good over-all similarity, allowing for the 10-acetyl group lacking in **12**, which would be expected to bring about considerable changes for the signals arising from C-8, C-9 and C-10. Similar changes were seen when comparing **12c** and **12d** (Jensen et al., 1996). In the ^1H NMR spectrum, H-1 was seen as a singlet at an unusually low field (δ 6.26), a feature typical for iridoid glucosides with an 8,9-double bond. Therefore, we assign the structure **12** to the new compound.

Compound **12a** was isolated from *P. arborescens, P. ovata* and *P. webbii* and was named arborescoside. The positive FAB-mass spectrum gave a M_r of 388, indicating the composition $C_{17}H_{24}O_{10}$. In this case, the ^{13}C NMR spectrum (Table 1) showed 17 signals of which six as above could be assigned to a β-D-glucopyranosyl moiety, providing an aglucone with 11 carbon atoms. Altogether, comparison with the spectrum of the acid form of **12** (Table 1) showed that **12a** was the corresponding methyl ester.

Compound **17** was found in a fraction from *P. subulata* together with melittoside (**18b**), from which it was difficult to separate. However, using a high-resolution reverse phase column, we succeeded in isolating the two compounds from the mixture. The positive FAB-mass spectrum of **17** gave a M_r of 508, indicating the composition $C_{21}H_{32}O_{14}$, one oxygen atom less than **18b**. The ^1H NMR spectra of the two compounds were rather similar, except that in the spectrum of **17** a broadened AB system ($J = 17.5$ Hz) was seen at δ 2.6, a region where **18b** had no signals. In a COSY spectrum, weak interactions were seen between this system and H-7 (δ 5.85) and between the latter and another broadened AB system ($J = 14$ Hz) centered at δ 4.25, assigned to a 10-CH_2OH group. The 17.5 Hz AB system above could therefore only arise from two protons at C-6, in agreement with the fact that such a large coupling constant has previously been seen in similar systems (Jensen and Nielsen, 1985; Abe et al., 1995). The ^{13}C NMR spectrum of **17** showed 21 peaks of which 12 could be assigned to two β-glucopyranosyl moieties, leaving a nine-carbon aglucone. When comparing the spectra of **17** and **18b** (Table 1), they were also fairly similar except for the presence of a peak at δ 46.2 (CH_2) in the former which was replaced by one at δ 80.9 (CH–OH) in the latter, demonstrating that **17** was 6-deoxymelittoside.

Table 1
^{13}C NMR data in D_2O for the new compounds **12**, **12a**, **17** and model compounds **12b** and **18b**[a]

Atom	**12** (acid)	**12** (salt)	**12a**	**12b** (salt)	**17**[b]		**18b**[b]	
C-1	92.2	90.6	92.3	91.5	97.4		96.2	
C-3	151.4	145.3	151.9	146.8	143.5		144.5	
C-4	n.o.	120.1	114.1	119.3	108.3		105.7	
C-5	38.2	38.3	38.0	39.2	84.5		81.8	
C-6	31.5	30.5	31.5	31.4	46.2		80.9	
C-7	34.5	34.3	34.4	34.6	127.6		128.6	
C-8	143.1	142.0	143.8	137.5	140.6		145.6	
C-9	130.2	131.5	129.9	133.6	52.7		51.6	
C-10	58.2	58.2	58.2	61.5	60.6		60.6	
C-11	n.o.[c]	176.2	170.6	n.o.				
OMe			52.6					
C-1′ (1″)	99.1	98.9	99.2	99.8	99.3	98.5	99.0	98.8
C-2′ (2″)	73.5	73.5	73.5	73.5	73.5	73.9	73.8	74.1
C-3′ (3″)	76.5	76.4	76.5	76.4	76.3	76.3	76.4	76.7
C-4′ (4″)	70.4	70.4	70.4	70.3	70.3	70.2	70.4	70.3
C-5′ (5″)	77.1	77.1	77.1	77.1	76.9	76.3	77.2	76.7
C-6′ (6″)	61.5	61.5	61.5	61.5	61.5	61.3	61.5	61.5

[a] Spectra are aligned by setting C-6′ to 61.5 ppm (Damtoft et al., 1981).
[b] Some signals in the sugar moieties may be interchanged.
[c] n.o., not observed.

3. Discussion

Altogether 34 species have now been investigated in detail for the presence of iridoid glucosides or CPGs, i.e.

the compounds have been isolated and characterized from these species. In Table 2, we have collected the data from the present investigation together with earlier reported findings of such compounds from the genus *Plantago*. The plants are listed according to the classification of Rahn (1996) and divided into subgenera and sections.

As stated in the introduction, the biosynthesis of aucubin (**5**) has been well investigated and may be represented as shown in Fig. 1. It is remarkable that most of the intermediates in the pathway to **5** have been found in one or more of *Plantago* species. Thus, deoxyloganic acid (**1**) was present in *P. webbii*, while mussaenosidic acid (**2**) was found in five of the species investigated. Compound **2** or its methyl ester is frequently found together with 8-epiloganic acid (**9**) and gardoside (**14**) or their methyl esters in plants that produce **5** (cf. Damtoft et al., 1984), and this is also the case for *Plantago*. Regarding their biosynthesis, compounds **2** and **9** are both formed by a one-step hydroxylation of **1**, while hydroxylation of deoxygeniposidic acid can give rise to either **3** or **14**, the latter after rearrangement of the double bond. In *Plantago*, **9** and **14** are more frequent than **2**, but from a few species, all three compounds have been isolated. Caryoptoside (**10**) was present only

Table 2
Iridoids and CPGs isolated from species of *Plantago*[a]

				C_{10}-iridoids[b]			C_9-iridoids[c]		
Subgenus and species	Rahn no.	Section	Voucher	'Precursors'	Δ-8, 9	Others	Normal	5-OH	CPGs
Plantago									
P. reniformis Beck	22	*plantago*	IOK-11/94	**3**, **9**, **14**			**5**		**19a**
P. cornuti Gouan	23	*plantago*			**13a**, **b**		**5**		?
P. major L.	26	*plantago*			**13a**, **b**, **c**	**7**, **8**, **16**	**5**, **5b**	(**18c**)	**19a**, **b**
P. asiatica L.	29	*plantago*		**3**			**5**, **5c**, **e**		**19a**, **b**[d]
P. media L.	41	*plantago*					**5**	**18a**, **b**, **c**	**19b**
P. raoulii Decne.	68	*mesembrynia*	IOK-9/94				**5**		**19a**
P. stauntoni Reichardt	78	*mesembrynia*	Rahn-706	**9**, **14**	**12**		**5**		**19a**
P. myosuros Lam.	91	*virginica*					**5**		**19a**, **c**
P. rhodosperma Decne.	94	*virginica*					**5**		?
P. australis Lam.	108	*virginica*	IOK-6/94				**5**		**19a**, **22**
Coronopus									
P. alpina L.	132	*maritima*		**2**, **3**, **3a**, **9**, **14**	**12b**		**5**	**18**, **18b**	**19a**
P. subulata L. (=*carinata*)	133	*maritima*	Rahn-695		**12**, **12c**		**4**, **5**, **5a**, **c**, **d**	**17**, **18**, **18b**	**19a**
P. maritima L.	135	*maritima*	IOK-10/93		**12**		**5**	**18b**, **c**	**19e**
P. coronopus L.	140	*coronopus*	Rahn-674			**7**			**19a**, **22**
P. subspathulata Pilger	142	*coronopus*	Rahn-714						**19a**
Litorella									
P. uniflora Hook. f. (=L. *uniflora* L.)	143		IOK-23/78				**5**, **6**		**19a**
Psyllium									
P. arborescens Poir.	146	*psyllium*	Rahn-677	**2**, **9**, **14**	**12a**		**4**, **5**		**19a**, **b**
P. webbii Barn.	147	*psyllium*	Rahn-676	**1**, **2**, **9**, **14**	**12a**	**7**	**4**, **5**		**19a**, **b**
P. sempervirens Crantz (=*cynops*)	149	*psyllium*	Rahn-671	**2**, **10**		**7**	**4**		**19a**
P. arenaria Waldst. & Kit. (=*indica*)	154	*psyllium*				**7**	**4**		**19a**
P. afra L. (=*psyllium* L.)	155	*psyllium*	Rahn-662			**7**	**4**, **5**		**19a**
Albicans									
P. atrata Hoppe	166	*montana*	Rahn-678	**9**, **14**	**12**		**5**, **5c**, **6**		**19a**
P. nivalis Boiss.	168	*montana*	IOK-10/94	**2**, **14**			**6**		**19a**
P. lagopus L.	169	*lanceifolia*				**7**, **16**	**5**, (**6**)		**19a**
P. lanceolata L.	170	*lanceifolia*				**15**, **16**	**5**, **6**, **6c**		**19a**, **b**, **d**
P. altissima L.	173	*lanceifolia*		**9**, **14**	**12c**, **d**	**15**, **16**	**5**, **6**, **6c**		**19a**
P. argentea Chaix.	174	*lanceifolia*					**5**, **6**		?
P. amplexicaulis Cav.	175	*bauphula*	Rahn-663				**5**, **6**		+
P. cretica L.	176	*hymenopsyllium*	Rahn-712			**16**	**5**		**19a**, **21**
P. bellardii All.	178	*hymenopsyllium*	Rahn-675	**3**		**16**	**5**		**19a**, **21**
P. ovata Forssk.	179	*albicans*	Rahn-679	**9**, **14**	**12a**	**16**	**5**, **6**		**19a**, **b**
P. lundborgii Sparre	194	*gnaphaloides*	IOK-10/95	**3**, **9**, **14**	**12b**		**5**, **6**		**19a**
P. patagonica Jacq.	211	*gnaphaloide*	Rahn-681				**5**, **6**, **6b**		**19a**
P. hookeriana Fisch. & C.A. Mey.	212	*gnaphaloides*		**9**	**12d**		**5**, **6**, **6b**		**19a**

[a] The species are arranged according to the classification of Rahn (1996).
[b] Compounds with a carboxyl group present.
[c] Compounds which have lost the carboxyl group at C-4.
[d] Many other CPGs isolated.

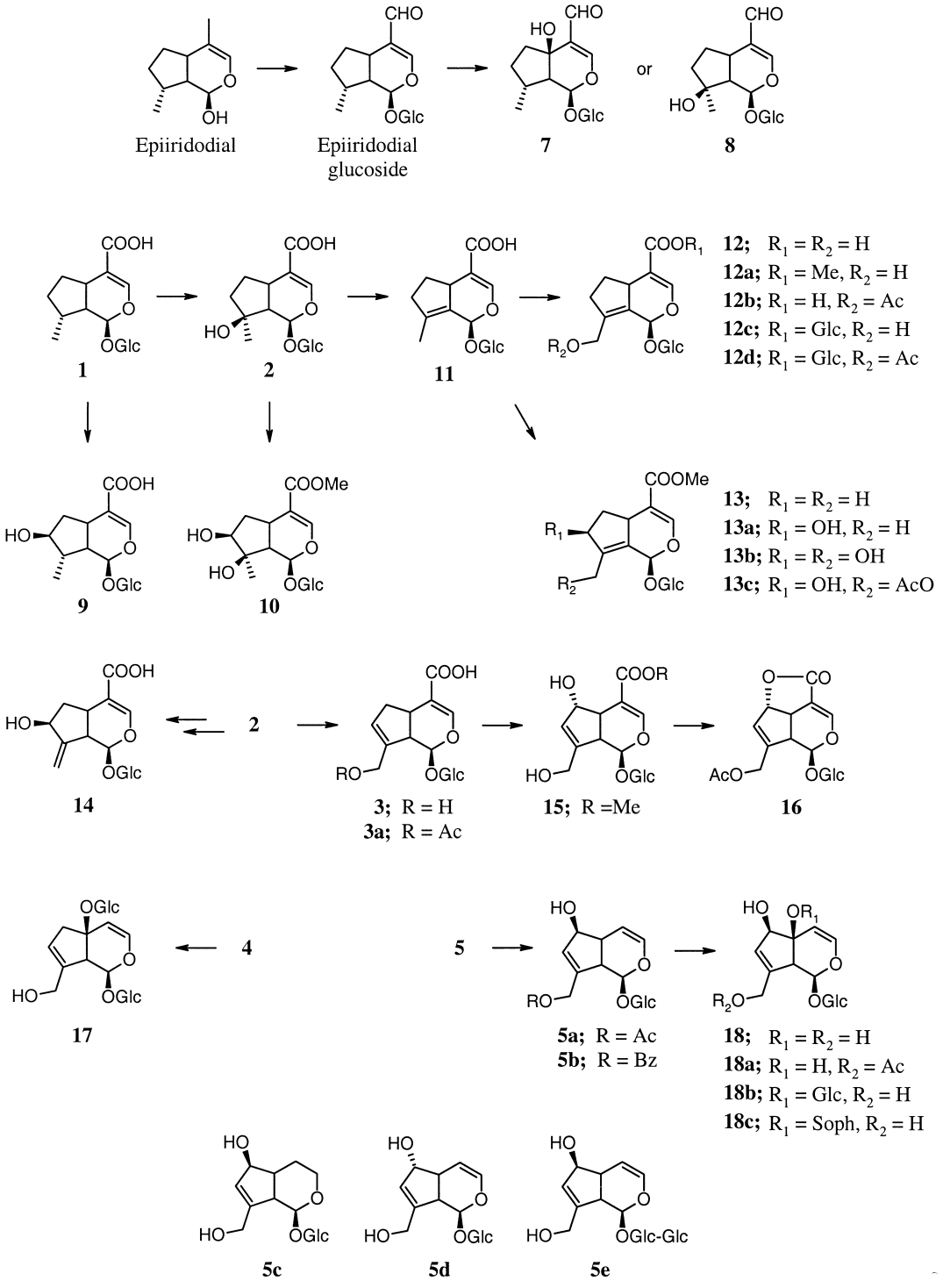

Fig. 2. Iridoid glucosides from *Plantago* with inferred biosynthetic pathways.

in a single species, *P. sempervirens*, and this compound could be biosynthetically derived from either **2** or **9**. The next compound on the biosynthetic pathway, deoxygeniposidic acid, is only rarely found in plants, and it was not detected in the present work either. On the other hand, geniposidic acid (**3**), the immediate precursor for bartsioside (**4**) was present in five of the species. The distribution of these 4-carboxylated iridoid glucosides seems erratic in the genus and hence, they are not useful for systematic purposes.

Two of the new compounds isolated and characterized here were arborescosidic acid (**12**) and arboresco-

side (**12a**). These two compounds are representatives for iridoid glucosides with the rare 8,9-unsaturation. Such iridoids are so far only known from the genus *Plantago* (Table 2) and from *Veronica anagallis-aquatica* var. *anagalloides* (Lahloub, 1992) which contains anagalloside (**13**), as well as *Veronica cymbalaria* with alpinoside (**12b**) (Taskova et al., 1999). The biosynthesis of this group of compounds has not been investigated, but it seems reasonable to suggest (Fig. 2) that they are formed from mussaenosidic acid (**2**). This is in analogy with the formation of deoxygeniposidic acid (Fig. 1), but with an alternative direction of the elimination. However, it must be noted that such an elimination might be energetically disfavoured since the hydrogen atom at C-9 is in the *cis*-position to the leaving group at C-8. The systematic value of the compounds with an 8,9-double bond appear to be limited on the genus level since they are present in some members of all subgenera and most sections of *Plantago* investigated (Table 2).

The aldehyde plantarenaloside (**7**) and the lactone asperuloside (**16**) may both have some taxonomic potential. As pointed out by Andrzejewska-Golec et al. (1993), **7** is very often present in subgen. *Psyllium*, although not consistently, together with bartsioside (**4**) which is present in all the species investigated. On the other hand, it is also found in single instances in some of the other subgenera. Similarly, **16** is found in seven species, of which six belong to subgen. *Albicans*, where it is apparently restricted to three of the five sections investigated. It has also been reported from *P. major* from subgen. *Plantago*.

In the present work, we can confirm that bartsioside (**4**) is consistently present in subgen. *Psyllium*, for which it may be considered obligatory, as earlier shown by Andrzejewska-Golec et al. (1993). However, we found it also (although not as a main constituent) in *P. subulata*, which belongs to subgen. *Coronopus*, and this is the first report of **4** outside subgen. *Psyllium*.

Aucubin (**5**) has always been considered characteristic for the genus *Plantago*, and this is indeed true since it was found in all but five species. In two species from subgen. *Psyllium*, its precursor bartsioside (**4**) may be considered to replace it, while in *P. nivalis* (subgen. *Albicans*), its biosynthetic successor catalpol (**6**), is present instead. As noted above, the report on isolation of **6** from *P. major* is dubious. Catalpol is the characteristic compound in subgen. *Albicans* and *Littorella*, and it is apparently restricted to these two subgenera. Notably, however, catalpol is lacking in two species of subgen. *Albicans*, namely *P. cretica* and *P. bellardi*, the only members of sect. *hymenopsyllium* investigated here. The chemistry of these two species is also otherwise distinctive since they both contain chlorogenic acid (**21**), a compound found only in these two species within the genus.

Two other species, namely *P. coronopus* and *P. subspathulata* from subgen. *Coronopus* sect. *coronopus* are also peculiar since any of the compounds **4**, **5** and **6**, otherwise characteristic for different subgenera in the family, are completely missing. In fact, the latter is completely devoid of iridoids, while the former only contains plantarenaloside (**7**).

In contrast, section *maritima* of subgen. *Coronopus* contains an abundance of iridoids. This taxon is characterized by 5-substituted iridoids like monomelittoside (**18**) and its analogues. Among these are the new compound 6-deoxymelittoside (**17**) from *P. subulata* as well as rehmannioside D (**18c**), which was present in *P. maritima*. The last compound has so far only been found in *Rehmannia glutinosa* (Schrophulariaceae; Oshio and Inouye, 1982b). Such compounds have also been reported from *P. major* and *P. media*, but although the report on the former is dubious due to the source of the plant material, the occurrence in the latter shows that these compounds are not only confined to subgen. *Coronopus* sect. *maritima*.

We can confirm that verbascoside (**19a**, Fig. 3) and its analogues are consistently present in all members of Plantaginaceae investigated, as first suggested by Andary et al. (1988).

Sorbitol (**20**) has been reported to be a common constituent in Plantaginaceae (Wallaart, 1981). In the present work, we have found **20** to be the main carbohydrate constituent in all the three species representing three different subgenera investigated for this. Otherwise, sorbitol is comparatively rare as a natural substance, except in members of Rosaceae (Plouvier, 1963; Wallaart, 1980). Recently, it was also found to be the main sugar in *Polypremum procumbens* and in *Tetrachondra hamiltonii* (Jensen, 2000), but neither of these taxa appear to be related to Plantaginaceae.

Traditionally, the genus *Littorella* with only three species has been considered to be distinct from *Plantago*, but Rahn in his recent classification included the former as a subgenus of the latter. This is fully consistent with the chemistry since *P. uniflora* (= *L. uniflora*) contains both aucubin (**5**) and catalpol (**6**) as well as the CPG verbascoside (**19a**), all compounds which are typical for many members of the genus *Plantago*.

While the occurrence of compounds with the 8,9-double bond did not seem to be useful for the taxonomy of Plantaginaceae itself, the common presence of such compounds in *Plantago* and *Veronica* may be indicative of the close relationship between the two genera as pointed out by Taskova et al. (1999), despite the fact that these taxa traditionally have been considered members of two different families. Recent information from chloroplast DNA sequence data (Reeves and Olmstead, 1998; Oxelman et al., 1999) has shown that part of Scrophulariaceae (Scroph II) including *Digitalis, Veronica* and *Antirrhinum* may reasonably be imbedded

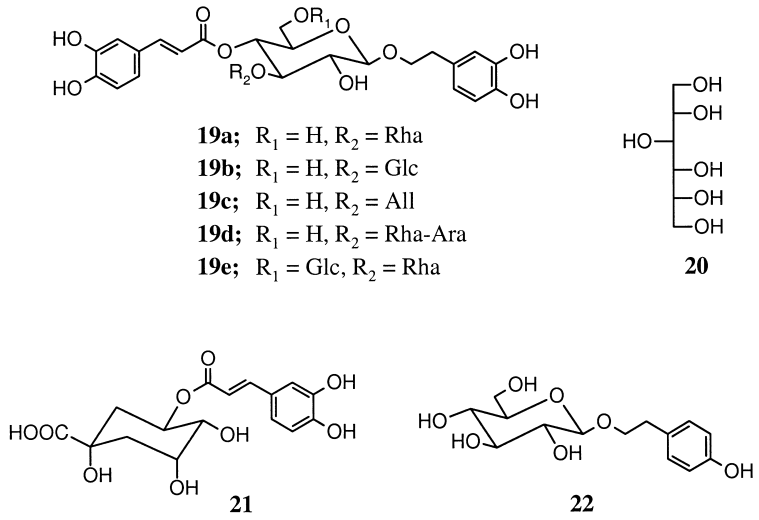

Fig. 3. Other compounds isolated from *Plantago* species.

in Plantaginaceae. In fact, this information has shown that *Veronica* is more closely related to *Plantago* than it is to *Scrophularia* and *Verbascum* (Scroph I).

4. Experimental

4.1. General

Most of the plant material was mainly grown from seeds at the experimental field of The Botanical Garden in Tåstrup, Sealand, Denmark. *P. maritima* was collected on the beach at Gniben, NW Sealand, while *P. uniflora* was collected on Öland, Sweden. Vouchers have been deposited at The Botanical Museum of Copenhagen (C). The freshly collected plants were frozen in polyethylene bags and kept at −23°C until use. Each species was homogenized twice with EtOH and the concentrated extract partitioned in Et_2O–H_2O. The aqueous phase was taken to dryness, redissolved in MeOH, and filtered through a thin layer of act. C to give the crude extract. Preparative chromatography was performed on Lobar® RP-18 columns from Merck (crude extracts on size C and fractions on size B) eluting with the H_2O–MeOH mixtures specified in each case. Compounds are listed in order of elution. ^{1}H NMR spectra (300 MHz) were recorded in D_2O using the solvent peak (δ 4.75) as the standard. In the ^{13}C NMR spectra (75 MHz), the C-6′ shift was set to 61.5 ppm (cf. Damtoft et al., 1981). For FAB MS spectra were used HEDS matrix in the positive mode, except for **7**, which was recorded in the negative mode.

4.2. Work-up of P. afra

The frozen plants (300 g) gave 4.4 g of crude extract. Of this, 3.7 g was chromatographed (1:0 to 1:1). Many small fractions contained iridoids, but only the following were identified: aucubin (**5**; 80 mg, 0.03%), a fraction with mainly bartsioside (**4**; 30 mg), plantarenaloside (**7**; 315 mg, 0.1%) and verbascoside (**19a**; 220 mg, 0.1%).

4.3. Work-up of P. amplexicaulis

The frozen plants (200 g) gave 7.0 g of crude extract. Of this, 3.5 g was chromatographed (1:0 to 2:1) to give catalpol (**6**; 215 mg, 0.2%) and aucubin (**5**; 180 mg, 0.2%). Some minor acid constituents and two CPG-like compounds were also present as seen by ^{1}H NMR, but not identified in this case.

4.4. Work-up of P. arborescens

The frozen plants (200 g) gave 3.2 g of crude extract. Of this, 2.0 g was chromatographed (1:0 to 1.5:1) to give a fraction with mainly aucubin (**5**; 20 mg), gardoside (**14**; 30 mg, 0.03%), a 1:1 mixture (30 mg) of 8-epiloganic acid (**9**) and mussaenosidic acid (**2**), bartsioside (**4**; 70 mg, 0.06%), arborescoside (**12b**, 40 mg, 0.03%), plantamajoside (**19b**; 10 mg, 0.01%) and verbascoside (**19a**; 100 mg, 0.1%).

4.5. Work-up of P. atrata

Frozen plants (122 g) gave 3.0 g of crude extract, which was dissolved in dil. AcOH (5%) and chromatographed (1:0 to 1:1) to give catalpol (**6**; 30 mg, 0.02%), aucubin (**5**; 100 mg, 0.08%), gardoside (**14**; 15 mg, 0.01%), 8-epiloganic acid (**9**; 10 mg, 0.01%), arborescosidic acid (**12**; 15 mg, 0.01%) and verbascoside (**19a**; 640 mg, 0.5%).

4.6. Work-up of P. australis

The frozen plants (275 g) gave 7.1 g of crude extract. Chromatography (1:0 to 2:1) of 3.5 g gave aucubin (**5**;

25 mg, 0.02%), salidroside (**22**; 50 mg, 0.04%) verbascoside (**19a**; 90 mg, 0.07%) and isoverbascoside (65 mg, 0.05%).

4.7. Work-up of P. bellardii

Frozen plants (250 g) gave 6.1 g of crude extract. Chromatograpy (1:0 to 2:1) gave a fraction A (150 mg) containing a mixture of acids, followed by aucubin (**5**; 310 mg, 0.2%), asperuloside (**16**; ca. 250 mg, 0.15%) and verbascoside (**19a**; 1.3 g, 0.8%). To fraction A was added dil. AcOH (5%) and rechromatography (1:0 to 5:1) gave geniposidic acid (**3**; 20 mg, 0.01%) and chlorogenic acid (**21**; 40 mg, 0.02%).

4.8. Work-up of P. coronopus

Frozen plants (300 g) gave 2.5 g of crude extract. Chromatography (1:0 to 1.5:1) gave salidroside (**22**; 50 mg, 0.02%), plantarenaloside (**7**; 50 mg, 0.02%) and verbascoside (**19a**; 500 mg, 0.2%).

4.9. Work-up of P. cretica

Whole, frozen plants (185 g) gave 7.3 g of crude extract. An aliquot (4.0 g) was chromatographed (1:0 to 2:1) to give first a polar fraction (1.03 g) consisting mainly (ca. 60%) of sorbitol (**20**), aucubin (**5**; 50 mg, 0.05%), a 2:1 mixture of asperuloside and chlorogenic acid (**16** and **21**; 70 mg, 0.04 and 0.03%) and verbascoside (**19a**; 880 mg, 0.9%).

4.10. Work-up of P. lundborgii

Frozen plants (100 g) gave 3.9 g of crude extract. Chromatography (1:0 to 1.5:1) of an aliquot (3.2 g) gave catalpol (**6**; 550 mg, 0.7%), aucubin (**5**; 170 mg, 0.2%), gardoside (**14**; 35 mg, 0.04%), geniposidic acid (**3**; 35 mg, 0.04%), 8-epiloganic acid (**9**; 45 mg, 0.05%), alpinoside (**12b**; 10 mg, 0.01%) and verbascoside (**19a**; 500 mg, 0.6%).

4.11. Work-up of P. maritima

The frozen plants (mainly leaves; 322 g) gave 6.6 g of crude extract. An aliquot (3.0 g) was chromatographed (1:0 to 1:1) to give aucubin (**5**; 50 mg, 0.02%), fraction B (135 mg) containing a mixture of **12**, **18b** and **18c**, and finally echinacoside (**19e**; 30 mg, 0.01%). To fraction B was added sat. $NaHCO_3$ soln. (2 ml), and rechromatography (10:1 to 7:1) gave the sodium salt of arborescosidic acid (**12**; 37 mg) followed by a 1:1 mixture of **18b** and **18c** (54 mg). Prep. thin layer chromatography (TLC) of this mixture on a 1 mm SiGel plate ($CHCl_3$–MeOH; 1:1) gave as the faster moving band almost pure rehmannioside D (**18c**) followed by melittoside (**18b**).

4.12. Work-up of P. nivalis

The frozen plants (145 g) gave 3.8 g of crude extract. Chromatography (1:0 to 1:1) gave gardoside (**14**; 110 mg, 0.1%), catalpol (**6**; 50 mg, 0.03%), mussaenosidic acid (**2**; 90 mg, 0.06%) and verbascoside (**19a**; 380 mg, 0.3%).

4.13. Work-up of P. ovata

The frozen plants (170 g) gave 2.7 g of crude extract. Chromatography (1:0 to 2:1) gave a 5:1 mixture (40 mg) of catalpol (**6**) and gardoside (**14**), aucubin (**5**; 190 mg, 0.1%), δ-epiloganic acid (**9**; 20 mg, 0.01%), asperuloside (**16**; 20 mg, 0.01%), arborescoside (**12b**; 30 mg, 0.02%), plantamajoside (**19b**; 90 mg, 0.05%) and verbascoside (**19a**; 800 mg, 0.5%).

4.14. Work-up of P. patagonica

The frozen plants (89 g) gave 1.39 g of crude extract. An initial analysis by ^1H NMR and HPLC on an analytical reverse phase column demonstrated that catalpol (**6**), aucubin (**5**), 10-benzoylcatalpol (**6b**) and verbascoside (**19a**) were present, and that the composition was similar to that of *P. hookeriana* (Damtoft et al., 1994a,b).

4.15. Work-up of P. raoulii

The frozen plants (115 g) gave 2.9 g of crude extract. Chromatography (1:0 to 2:1) gave first a polar fraction (850 mg) consisting mainly (ca. 90%) of sorbitol (**20**). Later was eluted aucubin (**5**; 175 mg, 0.05%) and verbascoside (**19a**; 350 mg, 0.3%).

4.16. Work-up of P. reniformis

The frozen plants (140 g) gave 4.6 g of crude extract, which was dissolved in aq. AcOH (10%) and chromatographed (1:0 to 1.5:1) to give aucubin (**5**; 150 mg, 0.1%), gardoside (**14**; 20 mg, 0.01%), geniposidic acid (**3**; 30 mg, 0.02%), δ-epiloganic acid (**9**; 30 mg, 0.03%) and verbascoside, (**19a**; 1.14 g, 0.8%).

4.17. Work-up of P. sempervirens

The frozen plants (300 g) gave 12.6 g of crude extract. Of this, 4.0 g was chromatographed (1:0 to 1.5:1) to give gardoside (**14**; 40 mg, 0.04%), a 3:1 mixture (50 mg) of mussaenosidic acid (**2**) and geniposidic acid (**3**), bartsioside (**4**; 375 mg, 0.4%), caryoptoside (**10**; 195 mg, 0.2%), plantarenaloside (**7**; 485 mg, 0.5%) and verbascoside (**19a**; ca. 0.5 g, 0.5%).

4.18. Work-up of P. stauntonii

Frozen plants (97 g) gave 2.8 g of crude extract. Chromatography (1:0 to 1:1) gave gardoside (**14**; 40 mg, 0.04%), aucubin (**5**; 100 mg, 0.1%), 8-epiloganic acid (**9**;

10 mg, 0.01%), a fraction with mainly arborescosidic acid (**12**; 25 mg, 0.03%) and verbascoside (**19a**; 1.17 g, 1.2%).

4.19. Work-up of P. subspathulata

Frozen plants (260 g) gave 6.3 g of crude extract of which 3.3 g was chromatographed (10:1 to 1.5:1) and gave a polar fraction (not weighed), of which sorbitol (**20**) was the main (ca. 50%) sugar, followed by verbascoside (**19a**; 150 mg, 0.1%).

4.20. Work-up of P. subulata

Frozen plants (310 g) gave 4.4 g of crude extract. Chromatography (1:0 to 2:1) gave gardoside (**14**; 20 mg, 0.006%), aucubin (**5**; 18 mg, 0.006%), fraction C (144 mg) containing a mixture, desacetylhookerioside (**12c**; 20 mg, 0.006%), bartsioside (**4**; 9 mg, 0.003%), and verbascoside (**19a**; 1500 mg, 0.5%). The above fraction C was rechromatographed (1:0 to 20:1) to give arborescosidic acid (**12**; 12 mg, 0.04%), monomelittoside (**18**; 25 mg, 0.008%) and a 3:1 mixture of 6-deoxymelittoside and melittoside (, 60 mg). Finally, **17** and **18b** was separated on a Merck HiBar column (250-25) packed with LiChrosorb RP-18 (7 μm) in H_2O–MeOH (6:1).

4.21. Work-up of P. uniflora

Frozen plants (140 g) gave 2.0 g of crude extract. Chromatography (1:0 to 1.5:1) gave a 3:1 mixture (100 mg) of aucubin (**5**) and catalpol (**6**) followed by verbascoside (**19a**; 400 mg).

4.22. Work-up of P. webbii

The frozen plant (210 g) gave 3.1 g of crude extract which was chromatographed (1:0 to 2:1) to give gardoside (**14**; 20 mg, 0.01%), aucubin (**5**; 20 mg, 0.01%), mussaenosidic acid (**2**; 70 mg, 0.03%), δ-epiloganic acid (**9**; 35 mg, 0.02%), bartsioside (**4**; 150 mg, 0.07%), plantarenaloside (**7**; 420 mg, 0.2%), of arborescoside (**12a**; 40 mg, 0.02%), 8-epideoxyloganic acid (**1**; 60 mg, 0.03%), plantamajoside (**19b**; 20 mg, 0.01%) and verbascoside (**19a**; 70 mg, 0.03%).

4.23. Arborescosidic acid sodium salt (*12*)

^{1}H NMR (500 MHz, D_2O): δ 6.98 (*d, J* = 1.5 Hz, H-3), 6.26 (*s*, H-1), 4.87 (*d, J* = 8.1, H-1′), 4.34 (*dd, J* = 13.6 and 1.3 Hz, H-10b), 4.26 (*dd, J* = 13.6 and 2.1 Hz, H-10a), 3.96 (*dd, J* = 12.4 and 2.1 Hz, H-6b′), 3.67 (*dd, J* = 12.4 and 5.5 Hz, H-6a′), 3.59 (*m*, H-5), 3.54 (*t, J* = 9.5 Hz, H-3′), 3.53 (*m*, H-5′), 3.44 (*dd, J* = 9.5 and 9.0 Hz, H-4′), 3.30 (*dd*; *J* = 9.5 and 7.9 Hz, H-2′), 2.55 (3H, H-6b/H-7a/H-7b), 1.46 (*m*, H-6a). FAB MS: $[M-H]^-$ at *m/z* 373. ^{13}C NMR data in Table 1. Rechromatography after addition of excess acetic acid changed the retention time drastically and gave the acid form of **7**. The ^{1}H NMR spectrum (300 MHz, D_2O) of this differed little from the above except for one signal: δ 7.43 (*d*, 1.5 Hz, H-3). However, the ^{13}C NMR spectrum was more different (see Table 1).

4.24. Arborescoside (*12a*)

^{1}H NMR (500 MHz, D_2O): δ 7.47 (*d, J* = 1.5 Hz, H-3), 6.37 (*s*, H-1), 4.89 (*d, J* = 8.0 Hz, H-1′), 4.34 (*dd, J* = 13.5 and 1.5 Hz, H-10b), 4.28 (*dd, J* = 13.7 and 2.0 Hz, H-10a), 3.97 (*dd, J* = 12.5 and 2.0 Hz, H-6b′), 3.78 (*s*, 3H, OMe), 3.77 (*dd, J* = 12.5 and 5.5 Hz, H-6a′), 3.61 (*m*, H-5), 3.54 (*t, J* = 9.5 Hz, H-3′), 3.53 (*m*, H-5′), 3.45 (*t, J* = 9.5 Hz, H-4′), 3.29 (*dd*, H = 9.5 and 8.0 Hz H-2′), 2.57 (3H, H-6b/H-7a/H-7b), 1.51 (*m*, H-6a). FAB MS: $[M+Na]^+$ at *m/z* 411. ^{13}C NMR data in Table 1.

4.25. 6-Deoxy-melittoside (*17*)

^{1}H NMR (500 MHz, D_2O): δ 6.53 (*dd, J* = 6.4 Hz, H-3), 5.85 (*m*, H-7), 5.31 (*d, J* = 6.8 Hz, H-1), 5.20 (*d, J* = 6.4 Hz, H-4), 4.79 (*d, J* = 8.1 Hz, H-1′), 4.60 (*dd, J* = 8.1 Hz, H-1″), 4.28 (*br. d, J* = 14.1 Hz, H-10b), 4.22 (*d, J* = 14.1 Hz, H-10a), 3.89 (2H, H-6b′/H-6b″), 3.72 (2H, H-6a′/H-6a″), 3.35–3.54 (7H, H-2″/H-3′/H-3″/H-4′/H-4″/H-5′/H-5″), 3.32 (*br. d, J* = 6.4 Hz, H-9), 3.24 (*dd, J* = 9.4 and 8 Hz, H-2′), 2.79 (*br. d, J* = 17.5 Hz, H-6b), 2.66 (*d, J* = 17.5 Hz, H-6a). FAB MS: $[M+Na]^+$ at *m/z* 531. ^{13}C NMR data in Table 1.

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