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Iridoid glucosides in the genus *Sutera* (Scrophulariaceae) as chemotaxonomic markers in tribe Limoselleae

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A B S T R A C T

From two species of *Sutera* (*S. foetida* and *S. cordata*) (Scrophulariaceae tribe Limoselleae) were isolated three known secoiridoid glucosides (**12-14**) as well as four iridoid congeners (**8-11**), all biosynthetically derived from iridodial glucoside (and/or deoxyloganic acid). In addition, two previously unknown compounds were found, namely a terpenoid glucoside lactone (suterolide, **21**) and the phenylethanoid glycoside 2′′′′-*O*-acetyl-angoroside A (**19**) as well as verbascoside, echinacoside and tubuloside A(**15-17**, respectively). Two other species, *Jamesbrittenia dissecta* and *Lyperia antirrhinoides*, previously considered to belong to the same genus (*Sutera*) were shown to be members of two different genera, respectively. Significantly, these two species contained iridoids derived from 8-*epi*-iridodial (and 8-epideoxyloganic acid), namely aucubin (**2**), melittoside (**3**) and acetylharpagide (4). In addition we investigated *Melanospermum transvaalense*, *Lyperia tristis* and *Microdon dubius* likewise from Limoselleae and all of these contained iridoid glucosides from the 8-*epi*-pathway. Thus, secoiridoid distribution confirms the DNA-based circumscription of *Sutera* and its sister-group relationship with *Manulea*. In addition, the results show that the clade including these two genera has a biosynthetic pathway to iridoids fundamentally different from the rest of the tribe and from the whole family Scrophulariaceae.

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1. Introduction

The circumscription of the family Scrophulariaceae *s. s.* has changed considerably in recent times, mainly due to DNA sequence results, restricting it to a group of genera mainly found in South Africa plus the large genera *Scrophularia* and *Verbascum* from the Northern Hemisphere (Olmstead and Reeves, 1995; Oxelman et al., 2005; Tank et al., 2006). With the change in taxonomic circumscription, the morphological definition and interpretation of character evolution in the family has changed (Weber 2013). Chemically, the family is characterized mainly by the presence of the iridoid glucosides: aucubin (**2**), and the epoxy analogue catalpol and derivatives of these, as well as their biosynthetic congeners, which are also a character in almost all members of the order Lamiales. These iridoids are all derived from the biosynthetic pathway recognised as route IIa through 8-*epi*-iridodial and 8-epideoxyloganic acid followed by decarboxylation (Jensen, 1991; 1992). Despite the fact that a large number of species from the family have been investigated and been shown to contain these compounds, true secoiridoids derived from the iridodial and deoxyloganic acid (**10**) pathway (route I) had only been reported in two instances within the order Lamiales, namely from *Lippia graveolens* Kunth. (Verbenaceae)(Rastrelli, et al., 1998) and *Fontanesia* (Oleaceae) (Damtoft et al., 1995). However, recently we discovered the presence of a series of secoiridoid glucosides apparently derived from the latter pathway in *Manulea corymbosa* L. f. (Gousiadou et al., 2014). Another member of the genus namely *M. altissima* L. f. (Gousiadou et al., 2015) was also found to contain secoiridoid glucosides. After this we decided to investigate the genus *Sutera* which is the immediate sister genus to *Manulea* based on previous phylogenetic analyses (Kornhall and Bremer 2004; Archibald et al. 2017) and our own investigation based on broader sampling (Fig 1) as a part in the tribe Limoselleae. Previously, aucubin (**2**) had been reported from *Sutera dissecta* (Delile) Walp. (Forgacs et al., 1986). However, *Sutera dissecta* is a synonym of *Jamesbrittenia dissecta* (Delile) Kuntze, which is the currently accepted name (Catalogue of Life; 2017). One of the authors (SRJ) made a preliminary examination about 30 years ago of a specimen considered then as *Sutera antirrhinoides* Hiern (now *Lyperia antirrhinoides* (L.f.) Hilliard, see below), but solely recorded the NMR spectra of three compounds isolated from that material, namely aucubin (**2**), melittoside (**3**) and acetylharpagide (**4**). Fortunately, we were able to access authentic *Sutera foetida* (Andrews) Roth from the Botanic Garden of Copenhagen and *Sutera cordata* (Benth.) Kuntze, a commercial ornamental sold under the common name “Bacopa.” Having realised that both *Sutera* (current work) and *Manulea* spp. (Gousiadou et al., 2014, 2015) gave neither aucubin, catalpol or their derivatives, we examined further readily available species of related genera in the Limoselleae. We now report an investigation of the available *Sutera* species together with *Melanospermum transvaalense* (Hiern) Hilliard, *Lyperia tristis* Benth., and *Microdon dubius* (L.) Hilliard as well as presenting an updated phylogeny of Limoselleae.

2. Results and discussion

Fresh plant material of a species *Lyperia antirrhinoides* (previously named S*utera*) gave after reverse phase chromatography aucubin (**2**), melittoside (**3**) and 8-*O*-acetylharpagide (**4**).

S. foetida afforded cachineside I (**9**), secologanoside (**14**), sinapoyl glucoside (**20**), 5-deoxystansioside (**8**, as the main constituent), 7-deoxyloganic acid (**10**), verbascoside (**15**) and a compound named suterolide (**21**).

Compound **21** was isolated as a colourless amorphous solid. Its molecular formula was determined from the ^{13}C NMR data and HR ESIMS as $C_{16}H_{26}O_7$. The NMR data (Table 1) were assigned using 1D and 2D spectroscopic techniques. The ^{13}C NMR data showed the presence of six signals consistent with a glycopyranoside unit including an anomeric carbon (δ_C 102.7), as well as ten signals that could represent a monoterpene moiety containing a carboxyl ester group (δ_C 168.7) with a conjugated double bond (δ_C 128.7 and 144.1) together with two methyl resonances (δ_C 19.5 and 12.9) and an oxygenated methylene group (δ_C 68.5). In fact, this part of the spectrum was very similar to that of the terpenoid moiety of manuleoside G from *Manulea corymbosa* (Gousiadou et al., 2014), thus suggesting it to be a dihydrofoliamenthoate. The ^1H NMR spectrum (Table 1) was in agreement with the above information and in addition revealed that the coupling pattern of the sugar unit was consistent with a β-glucopyranoside, but with a unusually low field shift for the H-2′ signal (δ_H 4.77) suggesting that this site was acylated. The HMBC spectrum allowed us to combine the two moieties since this H-2′ signal correlates with the C-1 carboxyl group (δ_C 168.7) and the H-1′ signal (δ_H 4.46) correlates with the C-8 methylene group (δ_C 68.5). The compound **21** is therefore a 12-membered lactone and we have named it suterolide. A similar compound constituted from foliamenthoic acid and β-glucopyranose has been reported from *Swertia punicea* Hemsl. (Gentianaceae) (Ming et al., 2000), in this case as a 14-membered ring with the ester linked to C-6'.

S. cordata gave secologanoside (**14**) as the main constituent, secologanic acid (**13**), sweroside (**12**), villoside (**11**) and echinacoside (**16**) as well as several fractions (in total 300 mg) from which tubuloside A (**18**) and 2′-acetyl angoroside A (**19**), both in the semi-pure state could be isolated.
The NMR spectra of secologanic acid (**13**) were difficult to interpret directly (see SI) mainly due to the diminished proton signals and line-broadening caused by extensive H-D exchange between the hemiacetal and the solvent (CD_3OD) (Damtoft et al., 1974). However, the HRMS data showed the main peak to have

m/*z* value of 375.1284 $(M+H)^+$ which together with the NMR data proved the structure and composition to be $C_{16}H_{22}O_{10}$.

Villoside (**11**) has apparently only been reported from *Patrinia villosa* (Thunb.) DC. (now a member of the Caprifoliaceae) and with very limited NMR data of the tetraacetate (Taguchi et al., 1973). The full NMR signal assignments of the native form of **11** in CD_3OD is given in Table 1 (see also SI). The aglucone was recently found in *Valeriana wallichii* DC (Caprifoliaceae) (Glaser et al., 2015).

The semi-pure compound **19** was a colourless amorphous solid and the molecular formula was determined from the ^{13}C NMR data and HR ESIMS as $C_{36}H_{46}O_{20}$. The NMR data (Table 2) were assigned using 1D and 2D spectroscopic techniques as well as by comparison with those of model compounds. The ^{13}C NMR data showed the presence of 35 signals of which one (δ_C 72.3) was of double intensity. The spectrum was similar to that of echinacoside (**16**) (Kobayashi et al., 1987) and even more to that of angoroside A (**18**) (Calis et al., 1987; Li et al., 2011). Thus, it contained sets of signals which could be assigned to (i) a caffeoyl moiety (9C), (ii) a 3,4-dihydroxyphenyl ethyl group (8C), (iii) a central tetra-substituted β-glucopyranosyl moiety (6C), (iv) an α-rhamnopyranosyl moiety (6C) and (v) a pentosyl moiety (5C) as well as (vi) an acetyl group (2C). The observed proton couplings and the chemical shifts of the pentapyranosyl moiety (v) fitted well with the values for an α-arabinopyranosyl group esterified with acetic acid. The ^1H NMR spectrum (Table 1) likewise showed the presence of (i), (ii), (iii), (iv) and (v), mainly by the comparison with the spectrum of **18**. The assigments of the signals from the anomeric carbon atoms were the key to the elucidation of the full structure of compound **19**. Accordingly, δ_C 104.1, 103.1 and 102.7 could be assigned to C-1′, C-1″ and C-1‴, respectively, by using the HSQC and HMBC data. Thus, the H-1′ signal (δ_H 4.35) correlated with the C-8 methylene group (δ_C 72.3), the rhamnopyranosyl H-1″ signal (δ_H 5.17) correlated with the peak from the C-3′ carbon atom (δ_C 81.6) and additionally, the H-1‴ signal (δ_H 4.38) correlated with the signal of the C-6′ methylene group (δ_C 68.8) as well as with the C-2‴ signal (δ_C 73.6). In addition, the corresponding low field H-2‴ signal (δ_H 4.96) had a correlation to the carbonyl group (δ_C 172.3) of the acetyl moiety. The last link in the molecule, namely that from the central glucopyranosyl moiety to the caffeoyl group, was shown by a correlation between the other low field H-4′ signal (δ_H 4.86) and the carbonyl group (δ_C 168.0) of the ester group. In conclusion, the compound **19** is 2‴-*O*-acetyl-angoroside A.

Melanospermum transvaalense gave the known compounds: aucubin (**2**), geniposidic acid (**7**), mussaenosidic acid (**6**), bartsioside (**1**), plantarenaloside (**5**) and verbascoside (**15**).

For *Lyperia tristis* and *Microdon dubius* only a little dry material from the vouchers were available. The ^1H NMR spectra of the crude extracts showed that aucubin (**2**) and melittoside (**3**) were present in the former and geniposidic acid (**7**) in the latter. No trace of secoiridoids could be detected in any of these samples, as observed for the terminal methylene unit (CH_2-10) at δ_H ca. 5.5.

Apparently, only a few of the remaining genera of Limoselleae have been investigated for iridoid glucosides, of these, *Hebenstretia dentata* L. contains lamiide and ipolamiide (Damtoft et al., 1992), and *Zaluzianskya capensis* Walp. has catalpol as the major iridoid (Damtoft, 1994). Also *Jamesbrittenia fodina* (Wild) Hilliard contains esters of catalpol (Cogne et al., 2005). Interestingly, P. Kooiman (1970) already by paper chromatography had detected aucubin (**2**) and/or catalpol in *Lyperia* (formely *Sutera*) *antirrhinoides, Zaluzianskya capensis, Limosella aquatica* L. All these compounds as well as those from the remaining iridoid glucosides found in the Scrophulariaceae all appears to be biosynthesized from 8-*epi*-iridodial (route IIa – compounds **1-7**) while those from *Sutera* and *Manulea* as well as those from Gentianaceae are produced via iridodial (route I – compounds **8-14**) (Jensen, 1991; Jensen and Schripsema, 2002).

Consequently, the pattern of the available data revealed that the production of seco-iridoids is an autapomorphic innovation and a chemotaxonomic character for the clade formed by *Sutera* and *Manulea*. This provides additional support to the relationships found in the molecular phylogenetic analysis (Fig. 1, Kornhall and Bremer 2004) and the splitting of *Lyperia antirrhinoides* from *Sutera* (Hilliard 1991). Our phylogenetic analysis in general supports the broad circumscription of Limoselleae and the genera *Hebenstretia, Selago* and *Zaluzianskya* reported by other authors (Kornhall and Bremer 2004; Archibald et al. 2017). Thus, secoiridoid distribution confirms the DNA-based circumscription of *Sutera* and its sister-group relationship with *Manulea*. Also, the results show that this clade has a biosynthetic pathway to iridoids fundamentally different from the rest of the tribe and from the whole family Scrophulariaceae.

3. Experimental

3.1. General

One-dimensional ^{1}H, ^{13}C NMR and 2D DQF-COSY, gHSQC, gHMBC and NOESY spectra were recorded on a 400 MHz Bruker Avance III equipped with a BBO Prodigy probe or on a 800 MHz Bruker Avance III HD (compound **18**) in CD_3OD and the chemical shifts are given as δ values with reference to the solvent peaks (δ_H 3.30 or δ_C 49.0). HRMS data were obtained on a Thermo Scientific LTQ-Orbitrap XL mass spectrometer. The samples were introduced through a Thermo Accela 1250 HPLC system on a 150 mm Phenomenex Luna C_{18} column with 10–100% MeOH gradient in water (containing 0.1% formic acid throughout), via an electrospray ion source in positive mode. Chromatography was performed on a Merck Lobar RP-18 column (size C) eluting with H_2O-MeOH mixtures (1:0 to 1:1); compounds are listed in order of elution. Semi-preparative HPLC was conducted on a Waters system consisting of 600 pump, 717-plus autosampler, and 996 photodiode array detector (Milford, MA, USA), using a Genesis C_{18} column (10 mm diam. × 250 mm; Jones Chromatography, Mid Glamorgan, UK) at 30 °C at a flow rate of 4.0 ml/min. The solvent compositions were as detailed below for each of the fractions. UV absorption maxima were taken

directly from on-line LC-UV detector. The isolated known compounds were identified by comparison of the NMR data with those of authentic compounds (**1**-**4**,**6**,**7**,**12**,**14**,**15**) or the published NMR data for **11** (Taguchi et al., 1973; Glaser et al., 2015), **16**) (Kobayashi et al., 1987), (**18**) (Calis et al., 1987; Li et al., 2011) and **20** (Miyake et al., 2007).

3.2 Plant material

Lyperia (Sutera) antirrhinoides (voucher IOK-68/1977) was cultivated in our private garden from seeds (3374) from The Botanical Garden of Copenhagen. *Sutera foetida* (IOK-1/2014) whole plants was grown by the staff of The Botanical Garden of Copenhagen. *Sutera cordata* (IOK-2/2014) was obtained from a local market ("Bacopa") and grown in our private garden. *Melanospermum transvaalense* (IOK-2/2009) was collected by SRJ in its natural habitat on sandy loam on a roadside ca. 30 km W. of Mokopane, close to Hanglip Rock, Entabeni Game Reserve, S. Africa. *Lyperia tristis* and *Microdon dubius* were both collected by Per Kornhall in sand at Olifantsboosbaai, Simon's Town, Western Cape, SA in 2001 (vouchers PK41 and PK42, respectively as in Oxelman et al., 2005). Vouchers are kept at herbarium OLD.

3.3 Lyperia (Sutera) antirrhinoides

Frozen plants (100 g) were blended with EtOH (300 ml) filtered and taken to dryness, and partitioned between H_2O and Et_2O. The aqueous phase was concentrated (1.6 g) and subjected to C_{18} reverse-phase chromatography (Lobar size C), eluting with MeOH–H_2O mixtures. Aucubin (**2**) and melittoside (**3**) was eluted with 25:1 while 8-*O*-acetylharpagide (**4**) was eluted with 3:1. Unfortunately the amounts of the isolated compounds were not recorded. NMR spectra of the compounds are given in SI.

3.4 Sutera foetida

Fresh plants (81 g) were blended with EtOH (300 ml) and treated as above to give after concentration 2.23 g residue. Chromatography gave: sugars (1.1 g), a fraction with mainly cachineside I (**9**, 14 mg), secologanoside (**14**, 18 mg), sinapoyl glucoside (**20**, 24 mg), 5-deoxystansioside (**8**, 230 mg), 7-deoxyloganic acid (**10**, 35 mg), verbascoside (**15**, 65 mg) and a fraction (90 mg) which was further separated with repeated C_{18} HPLC first with linear gradient of MeOH–H_2O, 40:60–100:0 over 20 min, t_R 17.3 min, then with MeCN-H_2O, 35:65, isocratic, t_R 12.3 min, to give pure suterolide (**21**, 2.3 mg).

3.5 Sutera cordata

Fresh plants (43 g) were blended with EtOH (200 ml) and treated as above to give after concentration 1.57 g residue. Chromatography gave: a fraction containing mainly secologanoside (**14**, 520 mg), a fraction with mainly secologanic acid (**13**, 70 mg), a mixture (1:1) of sweroside (**12**) and villoside (**11**) (33 mg), echinacoside (**16**, 22 mg), followed by fractions (total 300 mg) which were repeatedly chromatographed

over C_{18} HPLC (MeCN-H_2O) to yield: tubuloside A (**18**, MeCN–H_2O 15:85, 2 mg) and 2′′′′-acetyl angoroside A (**19**, MeCN–H_2O 21:79, 6 mg), both in a semi-pure state. Villoside (**11**, 5.9 mg) and sweroside (**12**, 6.0 mg) were separated by HPLC with MeCN-H_2O (20:80). Pure samples of (**13**, 20.8 mg) and (**14**, 5.2 mg) were also obtained with MeCN-H_2O gradient (17:83 → 85:15, v/v, over 15 min).

3.6 Melanospermum transvaalense:

Dry whole plants (11 g) were blended with hot EtOH (100 ml), left to stand for 11 days, and treated as above to give after concentration 1.90 g residue. Chromatography gave: sugars (140 mg), aucubin (**2**, 10 mg), impure geniposidic acid (**7**, 20 mg), impure mussaenosidic acid (**6**, 10 mg), bartsioside (**1**, 25 mg), plantarenaloside (**5**, 380 mg) and verbascoside (**15**, 40 mg).

3.7 Lyperia tristis

Dry material from the voucher (1080 mg) was blended with hot EtOH (10 ml), left to stand for 8 days, and treated as above to give after concentration 67 mg residue. The ^{1}H NMR data were obtained directly from this extract, re-dissolved in CD_3OD (see SI). The presence of aucubin (**2**) and melittoside (**3**) could be confirmed.

3.8 Microdon dubius

Dry material from the voucher (720 mg) was treated as under 3.7 to give after concentration 47 mg residue. The ^{1}H NMR data (see SI) were obtained directly from this extract, re-dissolved in CD_3OD

.

*3.9 Villoside (**11**)*

^{1}H and ^{13}C NMR data: see Table 1; HR ESIMS *m*/*z*: 347.1697 $[M+H]^+$ (calcd for $[C_{16}H_{26}O_8 + H]^+$, 347.1700).

*3.10 2′′′′-Acetyl angoroside A (**19**)*

^{1}H and ^{13}C NMR data: see Table 2; HR ESIMS *m*/*z*: 816.2916 $[M+NH_4]^+$ (calcd for $[C_{36}H_{46}O_{20} + NH_4]^+$, 816.2921).

*3.11 Suterolide (**21**)*

$[\alpha]^{21}_D$ -37 (*c* 0.1; MeOH); ^{1}H and ^{13}C NMR data: see Table 1; HR ESIMS *m*/*z*: 331.1743 $[M + H]^+$ (calcd for $[C_{16}H_{26}O_7 + H]^+$, 331.1751).

3.12. Phylogenetic analysis

DNA sequences of the nuclear ribosomal ITS region for Limoselleae were downloaded from GenBank (www.ncbi.nih.gov; Table 3). Additionally, DNA was extracted from *Melanospermum transvaalense*

(Voucher: IOK-2/2009, OLD) and *Lyperia antirrhinoides* (IOK-68/1977, OLD) herbarium material in commercial kits according to the manufacturer's instruction (innuPREP Plant DNA-Kit; Analytik Jena; Jena; Germany). Finally, DNA from and *Glekia krebsiana* (Voucher: M.Hirst, D.Webster, R. Dunbar and S.Harley 165, K) was received from the DNA Bank at RBG Kew (http://apps.kew.org/dnabank/). The ITS region was amplified using primers ITS-A (Blattner 1999) and ITS-4 (White et al. 1990) and the PCR program as in Ellmouni et al. (2017). PCR products were sequenced by GATC (Konstanz, Germany on an ABI capillary sequencer. Assembled sequences were manually edited using Phyde (www.phyde.de). The final dataset included 130 sequences including six species from tribes in Scrophulariaceae related to Limoselleae (see SI p, 21). Indel characters were coded using the simple method according to Simmons and Ochoterena (2000) as implemented in SeqState (Müller 2005) resulting in a final dataset of 1005 nucleotide characters and 283 coded indels. Maximum likelihood analysis was conducted using RaxML v. 8.0 (Stamatakis 2014) with a GTR+Γ model chosen according to AIC model testing in in jModeltest v.2 (Darriba et al. 2012). Support for the phylogeny was assessed by 400 fast bootstrap replicates and Bayesian inference using MrBayes 3.2 (Ronquist et al., 2012). Analyses were run for three million generations, sampling every 1,000th generation. Stationarity was checked by controlling PSRF. The first 25% of the generations were discarded as burnin.

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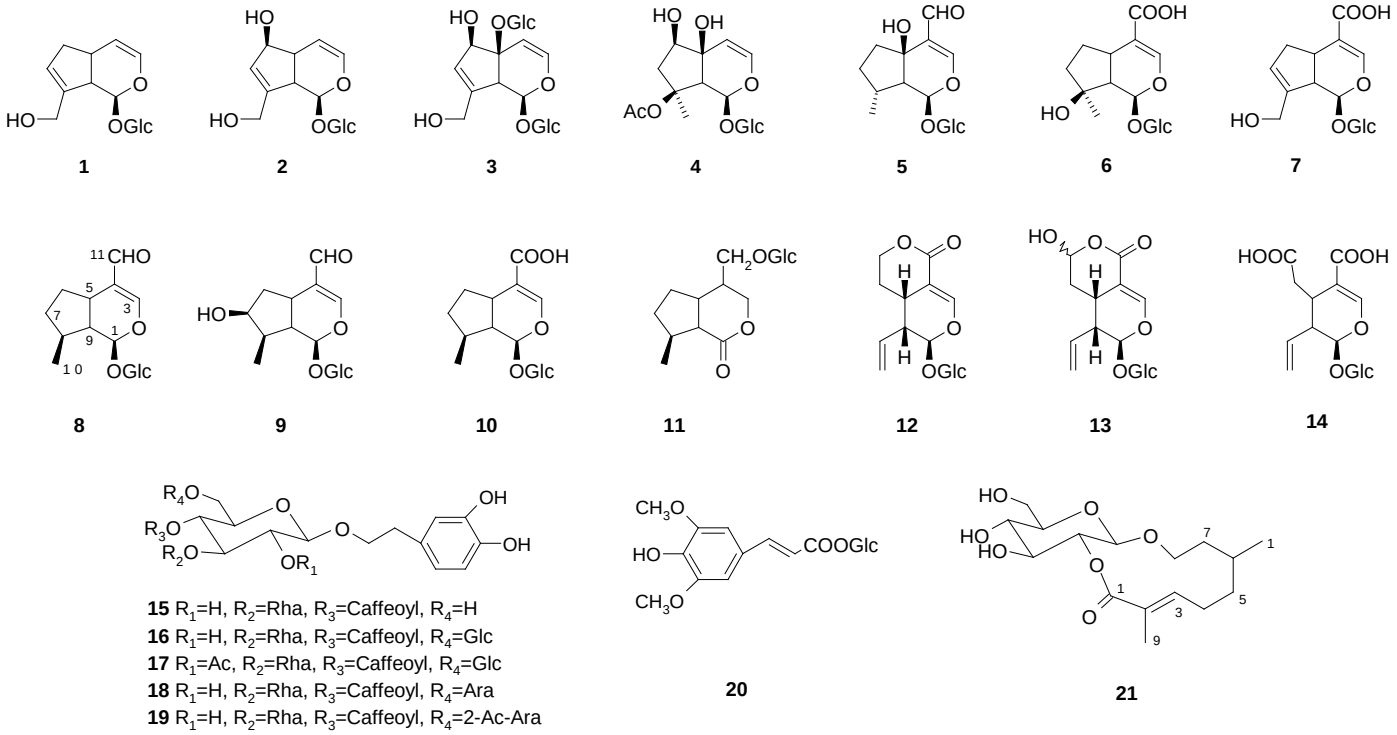

1 **2** **3** **4** **5** **6** **7**

8 **9** **10** **11** **12** **13** **14**

15 R_1=H, R_2=Rha, R_3=Caffeoyl, R_4=H
16 R_1=H, R_2=Rha, R_3=Caffeoyl, R_4=Glc
17 R_1=Ac, R_2=Rha, R_3=Caffeoyl, R_4=Glc
18 R_1=H, R_2=Rha, R_3=Caffeoyl, R_4=Ara
19 R_1=H, R_2=Rha, R_3=Caffeoyl, R_4=2-Ac-Ara

20

21

Compounds **1-7** are derived from route IIa and compounds **8-14** are from route I.

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Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra of suterolide (**20**) and villoside (**11**).

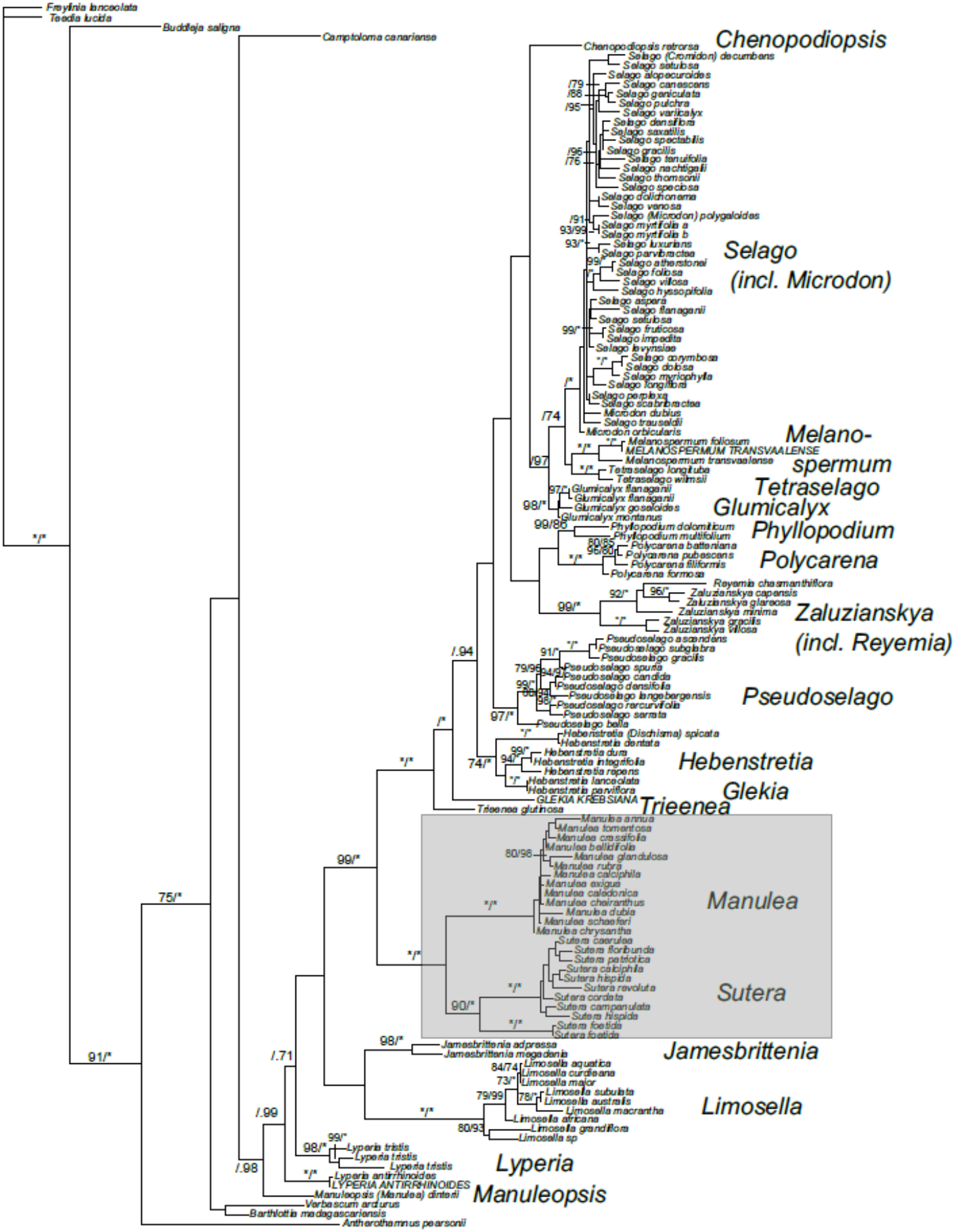
Atom	Suterolide (**20**)		Villoside (**11**)	
Agluc	^{1}H	^{13}C	^{1}H	^{13}C
1		168.7		178.2
2		128.7		
3	6.79 br. *t* (7.5)	144.1	4.45 *dd* (3.2, 10.9) 4.17 *dd* (9.2, 10.9)	70.6
4	2.16 2H *m*	27.6	2.40 *m*	40.0
5	1.40 *m*, 1.23 *m*	37.4	1.86 *m*	42.3
6	1.54 m	30.5	2.06 *m*, 1.31 *m*	33.3
7	1.62 *m*, 1.26 *m*	37.4	1.87 *m*, 1.19 *m*	35.7
8	3.97 *m*, 3.48 *m*	68.5	2.23 *m*	40.1
9	1.84 3H br. *s*	12.9	2.45 *dd* (8.2, 10.8)	50.0
10	0.85 3H *d* (6.5)	19.6	1.17 3H *d* (6.5)	20.4
11			3.44 *dd* (8.0, 10.2) 4.02 *dd* (4.4, 10.2)	70.3
Glc'				
1'	4.46 *d* (8.0)	102.7	4.22 (7.8)	104.9
2'	4.77 *dd* (9.1, 8.0)	75.5	3.16 *dd* (7.8, 10.2)	75.1
3'	3.57 *d* (9.1)	76.1	3.33 *m*	77.9
4'	3.37 *d* (9.1)	71.8	3.26 obsc.	71.6
5'	3.31 *m*	78.1	3.26 obsc.	78.0
6'	3.88 *dd* (11.8, 1.6) 3.69 *dd* (11.8, 5.5)	62.7	3.85 *dd* (11.2, 1.0) 3.65 *m*	62.7

Table 2. ^{1}H (800 MHz) and ^{13}C NMR (100 MHz) spectra of **18** and model compounds.

Atom	2‴″-*O*-Acetyl-angoroside A (**19**)		Angoroside A(**18**)[a]	Tubuloside A (**17**)	Echinacoside (**16**)
Aglucone	^{1}H	^{13}C	^{13}C	^{13}C	^{13}C
1		131.5	131.8	131.6	131.6
2	6.71 (*d* 2.0)	117.2	117.3	117.4	117.3
3		146.1	146.3	147.1	146.2
4		144.7	144.9	145.0	144.8
5	6.67 (*d* 8.1)	116.3	116.5	116.6	116.4
6	6.57 (*dd* 8.1/2.0)	121.3	121.5	121.6	121.4
7	2.79 (*m*)	36.5	36.8	36.8	36.7
8	4.01/3.71 (*m*)	72.3	72.5	72.6	72.5
Centr. Glc					
1′	4.35 (*d* 8.0)	104.1	104.4	103.3	104.3
2′	3.37 (*dd* 8.0/9.3)	76.1	76.4	75.0	76.3
3′	3.79 (*obsc*)	81.6	81.8	81.9	81.7
4′	4.86 (*obsc*)	70.5	70.7	70.8	70.7
5′	3.67 (obsc)	75.0	75.1	76.3	74.9
6′	3.50/3.80 (*obsc*)	68.8	69.2	69.3	69.5
3'-Rha					
1″	5.17 (*d* 1.7)	103.1	103.3	102.6	103.1
2″	3.90 (*dd* 1.7/3.4)	72.3	72.3	71.7	72.5
3″	3.59 (*dd* 3.4/9.5)	72.0	72.6	72.3	72.2
4″	3.29 (*obsc*)	73.8	74.0	73.9	73.9
5″	3.57 (*obsc*)	70.4	70.7	70.7	70.5
6″	1.07 (*d* 6.2)	18.5	18.6	18.7	18.5
6'-Glyc	(Ara)	(Ara)	(Ara)	(Glc)	(Glc)
1⁗	4.38 (*d* 7.2)	102.7	105.2	104.3	104.8
2⁗	4.96 (*dd* 7.2/9.0)	73.6	72.5	75.4	75.2
3⁗	3.62 (*obsc*)	72.3	74.3	76.3	77.9
4⁗	3.78 (*obsc*)	69.8	69.7	71.7	71.6
5⁗	3.48/3.83 (*obsc*)	67.0	66.9	78.2	78.0
6⁗				62.7	62.8
Caffeoyl					
1‴		127.7	127.8	127.9	127.8
2‴	7.05 (*d* 2.0)	115.2	115.5	115.1	115.4
3‴		146.9	147.1	146.4	146.9
4‴		149.8	150.1	150.0	149.9
5‴	6.77 (*d* 8.1)	116.5	116.8	116.7	116.6
6‴	6.95 (*dd* 8.1/2.0)	123.2	123.5	123.4	123.3
β‴	7.55 (*d* 15.9)	148.0	148.4	148.1	148.3
α‴	6.25 (*d* 15.9)	114.7	114.8	115.4	114.9
CO‴		168.0	168.5	168.2	168.6
Acetyl					
CH_3	1.98 (*s*)	21.2		21.5	
CO		172.3		172.3	

[a] Data from Li et al., 2011 (150 MHz).

Fig. 1: Phylogeny of Limoselleae based on ITS-sequences. Numbers on the branches indicate likelihood bootstrap support and Bayesian posterior probabilities, respectively. An asterisk indicates maximum support. (Terminals in capital letters were analysed in the present work).


Freylinia lanceolata
Teedia lucida
Buddleja saligna
Camptoloma canariense
Chenopodiopsis retrorsa
Selago (Cromidon) decumbens
Selago setulosa
Selago alopecuroides
Selago canescens
Selago geniculata
Selago pulchra
Selago varicalyx
Selago densiflora
Selago saxatilis
Selago spectabilis
Selago gracilis
Selago tenuifolia
Selago nachtigalii
Selago thomsonii
Selago speciosa
Selago dolichonema
Selago venosa
Selago (Microdon) polygaloides
Selago myrtifolia a
Selago myrtifolia b
Selago luxurians
Selago parvibractea
Selago atherstonei
Selago foliosa
Selago villosa
Selago hyssopifolia
Selago aspera
Selago flanaganii
Selago setulosa
Selago fruticosa
Selago impedita
Selago levynsiae
Selago corymbosa
Selago dolosa
Selago myriophylla
Selago longiflora
Selago perplexa
Selago scabribractea
Microdon dubius
Selago trauseldii
Microdon orbicularis
Melanospermum foliosum
MELANOSPERMUM TRANSVAALENSE
Melanospermum transvaalense
Tetraselago longituba
Tetraselago wilmsii
Glumicalyx flanaganii
Glumicalyx flanaganii
Glumicalyx goseloides
Glumicalyx montanus
Phyllopodium dolomiticum
Phyllopodium multifolium
Polycarena bateniana
Polycarena pubescens
Polycarena filiformis
Polycarena formosa
Reyamia chasmanthiflora
Zaluzianskya capensis
Zaluzianskya glareosa
Zaluzianskya minima
Zaluzianskya gracilis
Zaluzianskya villosa
Pseudoselago asendens
Pseudoselago subglabra
Pseudoselago gracilis
Pseudoselago spuria
Pseudoselago candida
Pseudoselago densifolia
Pseudoselago longebergensis
Pseudoselago recurvifolia
Pseudoselago serrata
Pseudoselago bella
Hebenstretia (Dischisma) spicata
Hebenstretia dentata
Hebenstretia dura
Hebenstretia integrifolia
Hebenstretia repens
Hebenstretia lanceolata
Hebenstretia parviflora
GLEKIA KREBSIANA
Trieenea glutinosa
Manulea annua
Manulea tomentosa
Manulea cassiifolia
Manulea bellidifolia
Manulea glandulosa
Manulea rubra
Manulea calciphila
Manulea exigua
Manulea caledonica
Manulea cheiranthus
Manulea dubia
Manulea schaeferi
Manulea chrysantha
Sutera caerulea
Sutera floribunda
Sutera patriotica
Sutera calciphila
Sutera hispida
Sutera revoluta
Sutera cordata
Sutera campanulata
Sutera hispida
Sutera foetida
Sutera foetida
Jamesbrittenia adpressa
Jamesbrittenia megadenia
Limosella aquatica
Limosella curdieana
Limosella major
Limosella subulata
Limosella australis
Limosella macrantha
Limosella africana
Limosella grandiflora
Limosella sp
Lyperia tristis
Lyperia tristis
Lyperia tristis
Lyperia antirrhinoides
LYPERIA ANTIRRHINOIDES
Manuleopsis (Manulea) dinteri
Verbascum arcturus
Barthlottia madagascariensis
Antherothamnus pearsonii
Chenopodiopsis
Selago
(incl. Microdon)
Melano-
spermum
Tetraselago
Glumicalyx
Phyllopodium
Polycarena
Zaluzianskya
(incl. Reyemia)
Pseudoselago
Hebenstretia
Glekia
Trieenea
Manulea
Sutera
Jamesbrittenia
Limosella
Lyperia
Manuleopsis