



Chemical relationships of *Polypremum procumbens*, *Tetrachondra hamiltonii* and *Peltanthera floribunda*

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Abstract

Polypremum procumbens (Buddlejaceae) was found to contain sorbitol, cornoside, salidroside and conandroside. One collection of *Peltanthera floribunda* (Buddlejaceae) contained verbascoside, two others the cornoside-derived renygolone (= halleridone). From *Tetrachondra hamiltonii* (Tetrachondraceae) was isolated sorbitol, cornoside and verbascoside. Iridoids were not found in any of the taxa. The taxonomic significance of the different chemical constituents is discussed, and the data demonstrate a close relationship between *Polypremum* and *Tetrachondra*. They may also suggest an inclusion of *Peltanthera* in Gesneriaceae. These findings are in concert with recently published chloroplast DNA sequence data. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Polypremum procumbens*; *Peltanthera floribunda*; Buddlejaceae; *Tetrachondra hamiltonii*; Tetrachondraceae; Sorbitol; Cornoside; Verbascoside; Conandroside; Rennygolone; Chemotaxonomy

1. Introduction

During an investigation (Jensen, 1992, 1996; Jensen et al., 1998) of the chemistry of Loganiaceae (*sensu* Leeuwenberg and Leenhouts, 1980), it was found that the presence of two groups of chemical markers, namely iridoids and caffeoyl phenylethanoid glycosides (CPGs), corresponded remarkably well with some morphological and

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embryological character sets and allowed the segregation of several taxa mainly of the tribe Buddlejaceae from Loganiaceae *sensu stricto* and even from Gentianales. This is in line with recent taxonomic treatments (Cronquist, 1988; Dahlgren, 1989; Thorne, 1992), where Buddlejaceae is raised to family rank and considered to be a close relative of Scrophulariaceae. Later investigations of chloroplast DNA sequences (Bremer et al., 1994; Olmstead and Reeves, 1995; Smith et al., 1997) support this opinion.

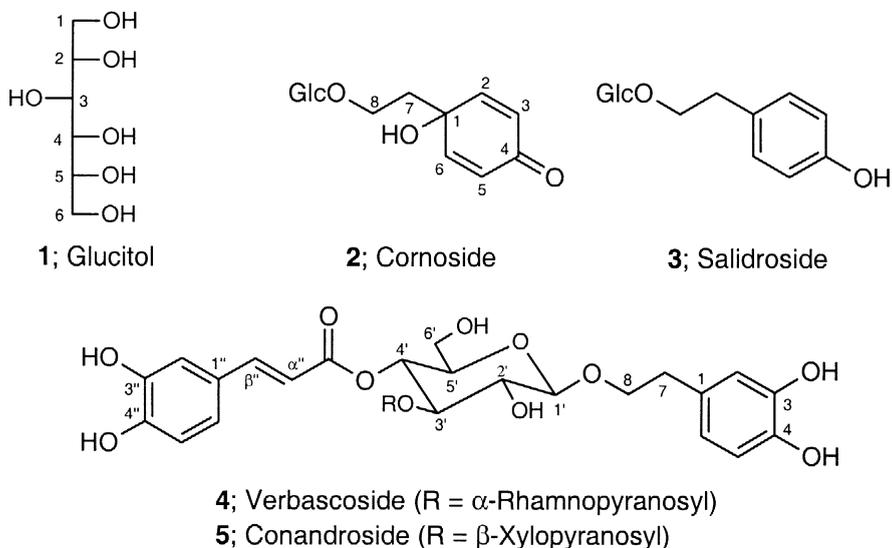
In the above investigation, most taxa in tribe Buddlejaceae contained aucubin or aucubin-like iridoids, stemming from a biosynthetic pathway different from that of the loganin-derived secoiridoids found in Loganiaceae *sensu stricto* and which are common in Gentianales. In addition, CPGs were consistently found in all the investigated species of Buddlejaceae. The CPGs were also found in *Plocosperma buxifolium* Benth. (tribe Plocospermeae) and in *Polypreum procumbens* L. (tribe Spigeliaceae), although the two latter taxa appeared not to contain iridoids. CPGs were not found in other taxa of the Loganiaceae, and they have never been reported from Gentianales at all. These results are in agreement with those of a recent chloroplast DNA (*ndhF* and *rbcL*) sequence investigation of Buddlejaceae (Oxelmann et al., 1999) where all the above taxa are found to be more related to the Scrophulariales/Lamiales complex than to Gentianales. Furthermore, *Polypreum procumbens* is inferred not to be a member of Buddlejaceae but rather to be closely related to the small aquatic genus *Tetrachondra* Petrie with one species each from New Zealand and Patagonia. Taxonomically, *Tetrachondra* has been assigned to its own family Tetrachondraceae Skottsbo. (Willis, 1973) or included in Lamiaceae (Cronquist, 1988; Thorne, 1992). The monotypic genus *Peltanthera*, formerly believed to be a member of Buddlejaceae, was also included in the above chloroplast sequence investigation, and in this case the nearest relatives were found to be members of the family Gesneriaceae. Recently, material of *Peltanthera floribunda* Benth. and *Tetrachondra hamiltonii* Petrie ex D. Oliver became available for chemical investigation, and the results are reported here together with a reinvestigation of *Polypreum procumbens*.

2. Materials and methods

^1H and ^{13}C NMR spectra were recorded at 250 and 63 MHz, respectively. The solvent peaks were used as the standard, except for glucosides in D_2O , where C-6' (61.5 ppm) was used (Damtoft et al., 1981). Chromatography was performed on Merck Lobar RP18 columns size B or C.

Polypreum procumbens was collected in August, 1984 on a roadside North of Tampa, Hillsborough Co, Florida, USA and dried (voucher IOK-29/84 is deposited at The Botanical Museum, Copenhagen). Work-up: whole dry plants (25 g) were blended with EtOH (200 ml) and left for 2 days. Evaporation and partitioning between Et_2O and H_2O gave a crude aqueous extract (2.54 g) for which a ^{13}C NMR spectrum showed a 2 : 1 molar proportion between sorbitol (**1**) and cornoside (**2**). Chromatography using a size C column gave first a polar fraction containing inorganics and sugars (320 mg). The NMR spectra of this fraction showed signals for sorbitol with only a trace of glucose and sucrose being present. Fraction 2 was cornoside (**2**, 240 mg), and

fraction 3 (70 mg) was a 1 : 2 mixture of **2** and salidroside (**3**). Fr. 4 (200 mg) was a complex mixture possibly consisting of degradation products of **2**, while fr. 5 (450 mg) after rechromatography on a size-B column gave conandroside (**5**, 100 mg).



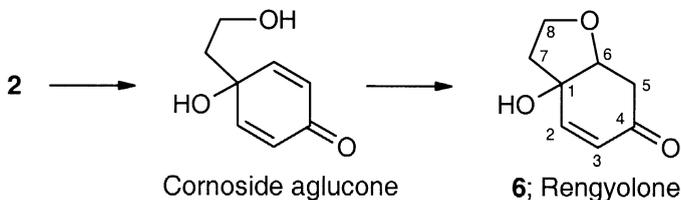
Sorbitol (**1**): Comparing with the isolated amount of **2**, the amount of sorbitol in fraction 1 could be calculated (320 mg; 1.3%). ^{13}C NMR (D_2O): δ 63.1 (C-1), 74.2 (C-2), 71.1 (C-3), 72.6 (C-4), 72.5 (C-5), 64.2 (C-6), identical to that reported (Bock and Pedersen, 1983).

Cornoside (**2**, 265 mg; 1.0%): ^1H NMR (D_2O) was identical to that of an authentic sample (Jensen et al., 1973); ^{13}C -NMR (D_2O): δ 69.2 (C-1), 154.0 (C-2 and C-6), 127.9 (C-3 and C-5), 189.3 (C-4), 39.3 (C-7), 65.8 (C-8), 102.9, 73.8, 76.5, 70.4, 76.7, 61.5 (C-1'–C-6').

Salidroside (**3**, 45 mg; 0.2%): ^1H NMR (D_2O): δ 7.16 and 6.81 (4H, AA'BB'-system, $J = 8$ Hz, arom. H's), 4.40 (d, $J = 8$ Hz, H-1'), 4.05 and 3.82 (m's, 8- CH_2), 3.15–3.85 (6H, sugar signals), 2.82 (2H, t -like, $J = 7$ Hz, 7- CH_2), identical to that of an authentic sample (Jensen et al., 1973).

Conandroside (**5**, 100 mg; 0.3%): ^1H NMR and ^{13}C -NMR spectra (methanol- d_4) were identical to those of the sample earlier isolated from *Sanango racemosum* (Jensen, 1996).

Peltanthera floribunda was collected in June, 1998 in Alajuela: Canton de Atenas; Cuesta del Aguacate, entre Atenas y San Mateo, Costa Rica (voucher: Hammel 20144; INB). Freshly dried leaves (16.5 g) were blended with EtOH and left to stand for 4 days. The extract was treated as above to give the crude extract (350 mg). Chromatography on the size B column eluting with H_2O –MeOH (25 : 1–1 : 1) gave three fractions. The sugar fraction (7 mg) appeared devoid of sorbitol. Next came



Scheme 1

verbascoside (**4**, 22 mg) followed by a fraction (30 mg) which, according to the ¹H NMR spectrum, contained 3 unidentified, verbascoside-like compounds; these were not further characterized.

Another collection of *P. floribunda*, from May, 1995 at San Jose: Cantón de Turrubares, Valle del Tárcoles, Cuesta Carrara, along Río Carrara, Costa Rica. (Voucher: Hammel 19855; INB). Dry leaves (10 g) treated as above, provided a crude (Scheme 1) extract (100 mg). The ¹H NMR spectrum showed that rengyolone (**6**) was present. Chromatography as above gave **6** (5 mg) as the only pure compound.

A third specimen was collected in July 1995 at San Martin, Rioja, Rio Negro, Peru; (voucher: I. Sanchez Vega and M. O. Dillon 8069, deposited at The Field Museum in Chicago). From dry leaves (1.4 g) was obtained a crude extract (12 mg) and the ¹H NMR spectrum was very similar to the above and showed distinct signals to prove that **6** was present. Due to the small amount, no further characterisation was attempted.

Verbascoside (**4**): ¹H NMR and ¹³C-NMR spectra (methanol-d₄) were identical to those of an authentic sample (Jensen, 1996).

Rengyolone (**6** = halleridone): ¹H NMR (D₂O): δ 6.90 (dd, *J* = 1.5 and 10 Hz, H-2), 6.04 (d, *J* = 10 Hz, H-3), 4.27 (ddd, *J* = 1.5, 5 and 6 Hz, H-6), 4.02 and 3.91 (m's, 8-CH₂), 2.82 (dd, *J* = 5 and 17 Hz, H-6a), 2.63 (dd, *J* = 6 and 17 Hz, H-6b), 2.28 (*t*-like, *J* = 7.5 Hz, 7-CH₂); ¹³C-NMR (D₂O): δ 152.1 (C-2), 129.8 (C-3), 82.9 (C-6), 68.5 (C-8), 41.4 (C-5), 39.9 (C-7), virtually identical to those reported (Endo and Hikino, 1984; Messina et al., 1984), allowing for the different solvents used, and for the missing signals from the quaternary carbon atoms (C-1 and C-4) which were not seen due to the small amount isolated here.

Tetrachondra hamiltonii was collected by Dr. Peter Johnson at Lake Te Anau, New Zealand (1992) and cultivated in pots at the experimental gardens of Landcare Research, Lincoln, Canterbury, New Zealand. The voucher (P.N. Johnson, 236/92) is deposited at Lincoln, New Zealand (cf Wagstaff and Olmstead, 1997). Work-up: Fresh plants (43 g) were homogenized with EtOH (2 × 200 ml). The extract was concentrated and partitioned in Et₂O–H₂O. Separation and evaporation of the aq. phase gave a crude extract (820 mg). A ¹³C NMR spectrum of the latter showed that sorbitol and cornoside were present in a molar proportion of 4.5 : 1. Chromatography on the size B column eluting with H₂O–MeOH (25 : 1–1 : 1) gave three fractions. The first (340 mg) showed only the signals of sorbitol with a trace of sucrose. The next fraction was pure cornoside (**2**, 70 mg, 0.16%), followed by pure verbascoside (**4**, 60 mg,

0.14%). The amount of sorbitol (**1**, based on isolated **2**) in the first fraction was calculated to be 180 mg (0.4%), the remaining mass probably being inorganic salts not detected by NMR.

3. Results

From an extract of dried whole plants of *P. procumbens* collected in Florida the reduced sugar sorbitol (**1**) and cornoside (**2**) were isolated as the main components while salidroside (**3**) and conandroside (**5**) were less abundant. Iridoids could not be detected, and neither was the earlier reported (Scogin and Romo-Contreras, 1992) verbascoside found. This could be due to the chromatographic method used, where **4** and **5** might have identical elution times and thus not be distinguished.

Three different collections of *Peltanthera floribunda* were investigated. Two of these were from Costa Rica, and from one of them was isolated verbascoside (**4**) together with a mixture of three similar, unidentified compounds. The other had rengyolone (**6**) as the only identifiable compound. The third specimen from Peru also had (**6**) present. Iridoids could not be detected in any of the collections.

A living specimen of *Tetrachondra hamiltonii* cultivated in New Zealand was investigated. Fractionation of the water soluble extract provided sorbitol (**1**) as the major constituent, while cornoside (**2**) and verbascoside (**4**) were also isolated. No iridoids could be detected.

4. Discussion

The presence of large amounts of sorbitol (**1**) in *P. procumbens* and in *T. hamiltonii* was immediately evident by recording a ^{13}C NMR spectrum of the crude extracts. Sorbitol is widely used in the food industry, since it is readily obtained by reduction of glucose. As a natural substance, however, it is comparatively rare. It is fairly common in members of Rosaceae and was first isolated from *Sorbus aucuparia* which gave rise to the name (Plouvier, 1963; Wallaart, 1980). Otherwise, it appears to be a common constituent only in Plantaginaceae (Wallaart, 1981) with a few, scattered occurrences in other plant taxa.

Cornoside (**2**) is also a compound with a limited distribution in plants. It has been reported from many species of *Cornus* (Jensen et al., 1975a) and in *Forsythia* (Jensen et al., 1975b), but has otherwise only been reported from a few genera within the Scrophulariales/Lamiales complex (Jensen, 1992), particularly in the family Gesneriaceae (Jensen, 1996). Rengyolone (**6** = halleridone) is usually co-occurring with **2** and can be considered to be an artifact (Scheme 1) formed from cornoside aglucone either in vivo or during work-up of the plant material (cf. Bianco et al., 1993).

Salidroside (**3**) is the precursor for **2**. It is widespread in plants and thus of little taxonomic significance. Verbascoside (**4**) and conandroside (**5**), however, both belong to the CPGs which are common in Oleaceae and in Scrophulariales/Lamiales and hardly anywhere else (Jensen, 1992).

The combined presence of sorbitol, cornoside and CPGs together with a lack of iridoids would in itself suggest a strong probability for a close relationship between *Polypremum* and *Tetrachondra*, since such a combination of chemical characters is unique for these two taxa. Moreover, in this case we have the added evidence from the chloroplast DNA sequence data (Oxelman et al., 1999) which also suggest such a close relationship. The chemical data do not give any clear indications of relationships to other taxa. One possibility could be Plantaginaceae since this family do contain sorbitol and also CPGs (cf Franzyk et al., 1998), but in more than 50 species of *Plantago* investigated in this laboratory, iridoids are almost obligatory constituents and cornoside has never been found. Another prospect are the Gesneriaceae which combine the presence of CPGs and (sometimes) cornoside with lack of iridoids. However, sorbitol has never been reported from that family, and the DNA sequence data do not indicate a close relationship either.

The chemistry of *Peltanthera*, with presence of CPGs and the cornoside-derived renygolone (**6**) taken together with the lack of iridoids, is not like the chemical profile for Buddlejaceae. In the latter, iridoids appear always to be present (Jensen, 1999) and cornoside has not so far been detected. The chemical markers would rather point to Gesneriaceae, although with some reservation, since this combination of compounds is found sporadically elsewhere within the Scrophulariales/Lamiales complex. However, in the light of the chloroplast DNA sequence data where *Peltanthera* appears in a distinct Gesneriaceae lineage also including *Sanango* (Oxelman et al., 1999), the chemical data would fit nicely with such a taxonomic position.

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