

Jensen 1994

**A re-examination of *Sanango racemosum*. 3.  
Chemotaxonomy.**

**Taxon 43: 619-623.**

**REFNO: 2431**

**KEYWORDS:**

**Chemotaxonomy, Sanango**

A re-examination of *Sanango racemosum*. 3. ChemotaxonomySøren Rosendal Jensen<sup>1</sup>*Summary*

Jensen, S. R.: A re-examination of *Sanango racemosum*. 3. Chemotaxonomy. - Taxon 43: 619-623. 1994. - ISSN 0040-0262.

A chemical investigation of *Sanango racemosum* for the systematically useful iridoids and verbascosides has been performed. No iridoids could be detected, but verbascosides were shown to be present by high performance liquid chromatography and by nuclear magnetic resonance spectroscopy of the crude aqueous extract. Reversed phase chromatography provided two pure verbascosides, calceolarioside C and conandroside, as well as a new caffeic acid derivative which has been named sanangoside. The presence of verbascosides in *Sanango*, combined with the lack of iridoid glycosides, suggests affinity with the family *Gesneriaceae*. A closer chemical relationship with the *Loganiaceae* (*Gentianales*) is improbable.

*Introduction*

Recently, it has been demonstrated that a group of compounds, namely the verbascosides, has systematic value (Jensen, 1992). These compounds are widespread in the *Lamianae* (sensu Dahlgren, 1989) and *Oleaceae*, with only a few scattered occurrences elsewhere. Except for the *Oleaceae* where they apparently are ubiquitous, they have not been reported from the *Gentiananae*.

Another group of compounds, the iridoids, may prove of even greater systematic value since the biosynthesis of iridoids is different in the two main groups of sympetalous plants in which they occur (Jensen, 1991). Thus secoiridoids ("Group 1", including the complex indole alkaloids) are almost exclusively found in the superorders *Cornanae*, *Loasanae*, and *Gentiananae*, but never in the *Lamianae*. On the other hand, decarboxylated iridoids (like aucubin), which result from another biosynthetic pathway ("Group 2"), are almost consistently found in *Lamianae* with a few occurrences in the *Cornanae* and *Ericanae* but none in the *Gentiananae*.

In an investigation of *Loganiaceae* (as defined by Leeuwenberg & Leenhouts, 1980), a representative number of genera (19 of 29) and species from all tribes were screened for iridoids and verbascosides (Jensen, 1992). It was found that members of the six tribes (*Spigeliaceae*, *Loganiaceae*, *Strychnaceae*, *Gelsemiaceae*, *Potaliaceae*, *Desfontainiaceae*) that contained Group 1 iridoids never contained verbascosides. On the other hand, representatives of the tribes *Buddlejeae* and *Retzieae*, which produce decarboxylated iridoids of Group 2, consistently also contained verbascosides. In the tribe *Plocospermateae* only verbascosides were isolated, and the same was true for *Polypleurum procumbens* L. (misplaced in tribe *Spigeliaceae*, of which the other representatives had iridoids of Group 1).

When comparing the chemical data with the known morphological characters, it appeared that taxa that contained Group 2 iridoids and/or verbascosides consistently had cellular endosperm formation and lacked intraxylary phloem. Conversely, the taxa with Group 1 iridoids and lacking verbascosides almost always had nuclear

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endosperm formation and intraxylary phloem. In conclusion, the latter assembly of taxa fitted well within the traditional definition of *Loganiaceae* (*Gentiananae*), while the former would fit better in the *Lamianae*.

Material of the genus *Sanango* G. S. Bunting & J. A. Duke was not available for our original investigation of *Loganiaceae*. It seemed probable that its chemical constituents would prove useful for determining its relationships.

#### Materials and methods

$^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectra were recorded at 250 and 63 Mhz, respectively, in methanol- $d_4$ .

*Isolation.* - Dry leaves (150 g) of *Sanango racemosum* (Ruiz & Pav.) Barringer (Neill 9458) were blended with 100 % ethanol (500 ml) and left for 4 days at room temperature. After filtering, the material was extracted again for 3 days with 80 % ethanol (500 ml). The combined extracts were concentrated (9.3 g), redissolved in water (50 ml) and extracted with ether ( $2 \times 150$  ml) to remove lipophilic material. The dark red aqueous extract was taken to dryness to give a foam (6.5 g). An aliquot of this (2.3 g) was separated by reversed phase chromatography on a Merck Lobar column (C<sub>18</sub> size C) eluting with water-methanol mixtures (25 : 1 to 1 : 1) and simultaneously monitoring at 206 and 254 nm with an UV detector. All fractions giving a positive response by the detector were evaporated and a NMR spectrum recorded, but no iridoids were found. However, when eluting with 1.5 : 1 and 1 : 1 a number of fractions with verbascoside-like UV-absorptions were collected. These were tested by high performance liquid chromatography and 3 fractions proved to contain only a single compound (the remaining fractions were mixtures representing about half of the mass). The above 3 fractions were taken to dryness to give (in order of elution) compounds 1 (50 mg), 2 (30 mg) and 3 (60 mg).

- *Sanangoside (1).* - Selected  $^1\text{H}$  NMR data:  $\delta$  5.67 (br. t,  $J = c. 3$  Hz, All- $\text{H}_3$ ); 4.76 (d,  $J = 8$  Hz, All- $\text{H}_1$ ), 3.59 (dd,  $J = 3$  and 8 Hz, All- $\text{H}_2$ ), 2.83 (t,  $J = 7.5$  Hz,  $\beta$ - $\text{CH}_2$ ).

-  $^{13}\text{C}$  NMR data:  $\delta$  131.5, 116.4, 144.6, 146.1, 117.0, 121.2, (aglycone C-1 through C-6), 71.9, 36.6 (aglycone C- $\alpha$  and C- $\beta$ ), 102.1, 71.0, 74.6, 67.6, 76.1, 62.7 (allosyl C-1 through C-6), 127.8, 115.4, 146.7, 149.4, 116.2, (caffeoyl C-1 through C-6), 115.1, 146.9, 169.1 (caffeoyl C- $\alpha$ , C- $\beta$  and CO).

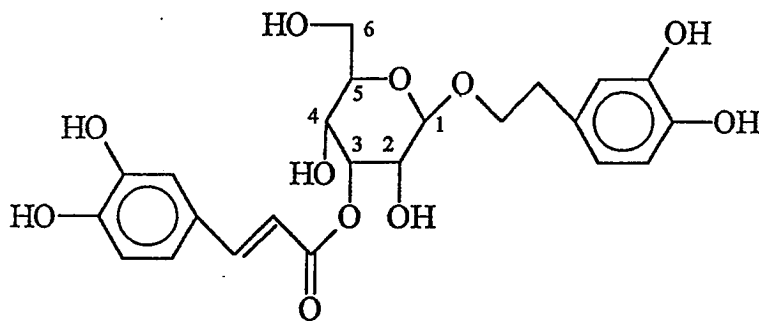


Fig. 1. Structural formula of Sanangoside.

- *Calceolarioside C* (2). - Selected  $^1\text{H}$  NMR data (250 MHz, methanol- $d_4$ ; using solvent suppression technique):  $\delta$  4.95 (t,  $J = 9.5$  Hz, Glu- $\text{H}_4$ ), 4.42 (d,  $J = 8$  Hz, Glu- $\text{H}_1$ ), 4.29 (d,  $J = 7.5$  Hz, Xyl- $\text{H}_1$ ), 2.83 (t,  $J = 7.5$  Hz,  $\beta\text{-CH}_2$ ).
- *Conandroside* (3). - Selected  $^1\text{H}$  NMR data (250 MHz, methanol- $d_4$ ; using solvent suppression technique):  $\delta$  4.97 (t,  $J = 9.5$  Hz, Glu- $\text{H}_4$ ), 4.47 (d,  $J = 7.5$  Hz, Xyl- $\text{H}_1$ ), 4.46 (d,  $J = 8$  Hz, Glu- $\text{H}_1$ ), 2.84 (t,  $J = 7.5$  Hz,  $\text{-CH}_2$ ). -  $^{13}\text{C}$  NMR spectra of 2 and 3 were also obtained but are not reported here.

An extract of fresh foliage of *Gesneria pedicellaris* Alain (17 g) gave, after similar work-up, sanangoside (1, 10 mg) and conandroside (3, 10 mg). Extracts from dried samples of *G. leucomalla* (Hanst.) Kuntze (10.7 g) and *G. ventricosa* Sw. (9.4 g) also yielded 3 as the only isolable compound (c. 10 mg from each).

### Results

From the NMR data of 1-3, it could at once be established that the compounds all contained a 3,4-dihydroxy-phenylethyl moiety and a caffeoyl moiety like in the verbascosides. However, in compound 1 only six additional signals were present in the  $^{13}\text{C}$  NMR spectrum, suggesting the presence of only a single sugar moiety. In the  $^1\text{H}$  NMR spectrum, the signals for H-1, H-2 and H-3 could be assigned by decoupling experiments, showing the high field triplet ( $J = 3$  Hz) at  $\delta$  5.66 to be the point of attachment for the caffeoyl group at an allopyranosyl moiety, and thus establishing the structure of 1 (Fig. 1). Plantainoside A, a similar compound, but with glucose as the central sugar, has recently been isolated from *Plantago asiatica* L. (Miyase & al., 1991), and except for the signals arising from the sugar moieties, the  $^{13}\text{C}$  NMR spectra were almost coincident. The structure of 1 appeared to be new in nature, and the compound was named sanangoside. Three analogous compounds have been isolated from *Magnolia obovata* Thunb., namely magnolioside A, B, and C (Hasegawa & al., 1988a, b). However, in the latter compounds additional sugar moieties (rhamnosyl and/or glucosyl) were attached to the central allose unit.

Compound 2 was found to be identical to calceolarioside C (Fig. 2) by comparison with the published NMR data (Nicoletti & al., 1988). This compound is known from *Calceolaria hypericina* Poepp. ex Benth. (*Scrophulariaceae*) and also from *Cassinopsis madagascariensis* Baill. (*Icacinaceae*; Rasoanaivo & al., 1990).

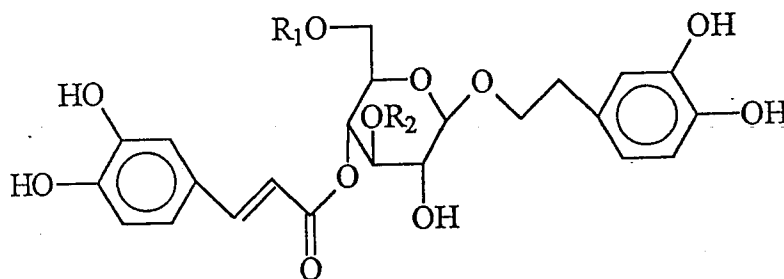


Fig. 2. Structural formulas of Calceolarioside C ( $\text{R}_1 = \text{Xyl}$ ;  $\text{R}_2 = \text{H}$ ) or Conandroside ( $\text{R}_1 = \text{H}$ ;  $\text{R}_2 = \text{Xyl}$ ).

Compound 3 appeared to be identical to conandroside (Fig. 2) from *Conandron ramondioides* Sieb. & Zucc. (*Gesneriaceae*; Nonaka & Nishioka, 1977), although the published NMR data are not of the genuine compound. In our own laboratory, we have also isolated this compound from *Polypremum procumbens* L. (Jensen, 1992). The recently published (Scogin & Romo-Contreras, 1992) occurrence of verbasco-side (acteoside) from this taxon could not be confirmed.

### Discussion

The chemical profiles support the recognition of the *Buddlejaceae* (including *Polypremum*), *Plocospermataceae*, and *Retziaceae* as families distinct from *Loganiaceae* (*Gentiananae*), and their transfer to the *Lamianae* (sensu Dahlgren, 1989). For the same reasons, also *Sanango* should definitely belong to the latter superorder.

Morphological and anatomical data (Norman, 1994; Dickison, 1994) indicate a relationship of *Sanango* with the *Gesneriaceae*. The lack of iridoids seems therefore significant, since the *Gesneriaceae* are the only family in the *Lamianae* where these compounds appear to be consistently absent. The finding of sanangoside (1) and conandroside (3) in one species, and of 3 as the main component in the two other species of *Gesneria* investigated in the present work, lend further support to that postulated link. Moreover, in a preliminary survey of about twenty additional species of *Gesneriaceae*, NMR spectra of crude extracts have shown the presence of sanangoside in at least three of them, namely *Episcia cupreata* (Hook.) Hanst., *Gesneria christii* Urb. and *Koellikeria erinoides* (DC.) Mansf.

At present, about 160 species (mainly belonging to the *Lamianae* and *Oleaceae*) have been reported to contain verbascoside or verbascoside-like compounds. Sanangoside is similar in structure to these, but has apparently not so far been encountered except in *Sanango* and the above-mentioned four species of *Gesneriaceae*. This strengthens the argument that *Sanango* may also belong to that family.

Despite the fact that verbascoside and its analogues appear to be ubiquitous in *Oleaceae* and that many different such compounds have been reported from the family, we have not so far encountered examples of verbascosides like 2 and 3 with a sugar moiety different from rhamnose in the C-3 position of the core glucopyranose unit. Conversely, such compounds are not uncommon in *Lamianae*, (Jensen & al., unpublished). The presence of conandroside in *Sanango* and *Polypremum* shows that neither is closely related to *Oleaceae*.

### Acknowledgements

I thank Dr. Hans Wiehler, Gesneriad Research Foundation, Sarasota, Florida, and the staff of the Botanical Garden of Copenhagen for gifts of plant material.

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