Chemotaxonomy and evolution of *Plantago* L.

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Abstract. In continuation of our investigations of the genus *Plantago* L. (Plantaginaceae), sixteen species were investigated with respect to watersoluble glycosides. The iridoids auroside, strictuloside and globularicisin, as well as poliumoside, 3-[(4-β-D-glucopyranosyloxy)phenyl]propionic acid and 2-[(4-β-D-glucopyranosyloxy)phenyl]acetic acid were isolated from *Plantago* for the first time. The latter compound has not previously been isolated as a natural product. Sorbitol was the main carbohydrate in all the species investigated. The distribution of iridoids correlates well with the morphological classification of Rahn and also with a recently published molecular phylogenetic study of nuclear ribosomal and plastid DNA sequences. A new chemotaxonomic finding is an abundance of iridoid glucosides present in one of the two groups within subgenus *Coronopus* section *Coronopus*, while the other group lacks iridoids, supporting a subdivision of this section. Moreover, in subgenus *Albicans*, 10-benzoylcatalpol is a characteristic constituent of section *Gnaphaloides*, while the corresponding cinnamoyl ester globularin occurs in section *Lanceifolia*. In biosynthetic experiments, labelled epideoxyloganic acid and deoxygeniposidic acid were incorporated into aucubin and geniposidic acid in *Plantago ovata*, consistent with earlier findings, but no incorporation into asperuloside was observed. The evolution of biosynthetic pathways in *Plantago* is discussed.

Key words: Plantaginaceae, *Plantago*, chemotaxonomy, iridoid glucosides, caffeoyl phenylethanoid glycosides, sorbitol, 2-[(4-β-D-glucopyranosyloxy)phenyl]acetic acid.

*Plantago* L. (Plantaginaceae) is a genus of annual and perennial herbs and subshrubs with a worldwide distribution. The infrageneric classification of the more than 200 species has continuously been revised. Decaisne (1852) recognized about 200 species grouped in seventeen sections. In Pilger’s (1937) revision, almost 260 species were included, although some of Decaisne’s species were fused into other species. Pilger’s classification comprised two subgenera, *Psyllium* including the branched species, and *Plantago* with eighteen sections, of which many were comparable to Decaisne’s sections. The genus was later revised by Rahn (1978), who ranked the Mediterranean species of section *Coronopus* as a third subgenus with two sections, *Maritima* and *Coronopus*, sensu Dietrich (1975). Subgenus *Psyllium* was expanded by inclusion of five sections, *Oreades*, *Arnoglossum*, *Bauphala*, *Hymenopsyllium* and *Leucopsyllium*, from Pilger’s subgenus *Plantago*. This expanded subgenus *Psyllium* was subdivided into seven sections, of which the new section *Albicans* was
further subdivided into five series. In Rahn’s recent (1996) reclassification of *Plantago*, based on a parsimony analysis of 90 primarily morphological and anatomical characters, subgenus *Psyllium* has again been reduced to Pilger’s sense, and the excluded sections then comprised an additional subgenus, *Albicans*. The two small taxa *Littorella* and *Bougueria*, which were considered separate genera in Plantaginaceae by Pilger, were included as subgenera in *Plantago* (Rahn 1996).

In a recent molecular phylogenetic study (Rønsted et al. 2002) of nuclear ribosomal ITS and plastid *rrn* L-F regions, five monophyletic clades were obtained corresponding to a similar number of subgenera, namely *Plantago, Coronopus, Bougueria, Psyllium* s.l. and *Littorella*. Of these, *Psyllium* s.l. included the species from subgenus *Albicans* as in Rahn’s classification from 1978, and *Littorella* was found to be a sister group to all other *Plantago*.

Attempts at using flavonoids as taxonomic markers have shown that *Plantago* mainly contains flavones (Harborne and Williams 1971, Kawashty et al. 1994, Nishibe et al. 1995). Flavones tend to replace flavonols in Lamiales, and this is also the case in *Plantago* (Harborne and Williams 1971, Tomas-Barberan et al. 1988). Among the limited number of species investigated thus far, there is a tendency in subgenera *Plantago* and *Coronopus* to produce the flavones luteolin and 6-hydroxy-luteolin, while subgenus *Albicans* only contains luteolin. Kawashty et al. (1994) investigated eighteen Egyptian species of *Plantago* and isolated the flavonol isorhamnetin-3-glucoside from three species of subgenus *Albicans*. The taxonomic value of caffeoyl phenylethanoid glucosides (CPGs) has also been studied (Andary et al. 1988, Noro 1991, Rønsted et al. 2000), and Andary et al. (1988) consider CPGs to be useful taxonomic markers for *Plantago*, but taxonomically useful diversity seems to be limited within the genus. Verbascoside (= acetoside) (19a) is usually present, sometimes together with plantamajoside (19b). A number of other CPGs have been reported, mainly from well investigated species like *P. asiatica* (see Rønsted et al. 2000 for references). Numbers in bold refer to the structures in Schemes 1 and 2 and Table 2 and an index to compounds is shown in the caption of Table 2.

A third group of secondary metabolites, the iridoid glucosides (iridoids), has been the object of a considerable number of taxonomic studies, and the glucosides have been found to be valuable as taxonomic markers of subgenera and sections within *Plantago*. Early studies were based on thin-layer chromatographic (TLC) analysis (Andrzejewska-Golec and Swiatek 1984, Kuzmanov et al. 1984), but our recent investigation has relied on isolated compounds that were identified by nuclear magnetic resonance (NMR) spectroscopy (Rønsted et al. 2000). Andrzejewska-Golec et al. (1993) and Andrzejewska-Golec (1997) emphasized that bartsioside (4) and plantarenaloside (7) are characteristic for species of subgenus *Psyllium* s.str., and that this could be used to support the treatment of this group as a separate genus. Andrzejewska-Golec (1997) also found that catalpol (6) was present in Pilger’s sections *Oreades, Bauphula, Arnoglossum* and *Leucopsyllium*, all belonging to Rahn’s (1996) subgenus *Albicans*. In our previous study (Rønsted et al. 2000) we found that the distribution pattern of the iridoids in 34 species of *Plantago* showed a good correlation with the classification of Rahn (1996). Thus aucubin (5) was found to be typical for the entire genus, while bartsioside and plantarenaloside occur almost exclusively in subgenus *Psyllium*, as also pointed out by Andrzejewska-Golec et al. (1993). Catalpol was likewise characteristic for subgenus *Albicans*, where it was accompanied by asperuloside (16) in three of Rahn’s (1996) six sections. The subdivision of subgenus *Coronopus* was supported by the lack of iridoids in section *Coronopus* and by the presence of characteristic 5-substituted iridoids in section *Maritima*. Finally, section *Hymeno- psyllium* in Rahn’s (1996) subgenus *Albicans* also contained chlorogenic acid (21), a compound not found in other *Plantago* species. Taskova et al. (2002) have recently used TLC to study the iridoids of 44 Bulgarian collections.