Biochemical Systematics and Ecology 42 (2012) 49–58

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Flavonoids as chemosystematic markers for the genus *Adenocarpus*

Rafaa S. Essokne [a], Renée J. Grayer [b,*], Elaine Porter [b], Geoffrey C. Kite [b], Monique S.J. Simmonds [b], Stephen L. Jury [a]

[a] Centre for Plant Diversity and Systematics, School of Biological Sciences, The Harborne Building, University of Reading, Whiteknights, Reading RG6 6AS, UK
[b] Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK

ARTICLE INFO

Article history:
Received 12 July 2011
Accepted 26 December 2011
Available online 14 March 2012

Keywords:
Adenocarpus
Leguminosae
Papilionoideae
Flavonoids
Isoflavonoids
Chemosystematics

ABSTRACT

Twenty-four species of the genus *Adenocarpus* (Leguminosae), sampled in Mediterranean and tropical African regions, were surveyed for their flavonoids by means of high performance liquid chromatography with diode array detection and atmospheric pressure chemical ionization-mass spectrometry. For ten of these species, 2–8 samples were examined to investigate a possible infraspecific variation in flavonoids. Nineteen flavonoids belonging to various different classes (flavone *C*- and *O*-glycosides, flavonol glycosides, isoflavone glycosides and flavanones) were detected. Previous DNA sequence analyses have resulted in the grouping of *Adenocarpus* species into four different clades, so that the results of the flavonoid survey could be compared with that of molecular studies. A good correlation was found in that each of the clades could also be characterised by a combination of flavonoids. For example, the species in clade 4, which are distributed in South and South-East Europe and tropical Africa, are characterised by the presence of flavonol *O*-glycosides and 5-hydroxyisoflavone *O*-glycosides. In contrast, flavonol *O*-glycosides were absent from all but one species of the other three clades, which have a mainly North Africa distribution, and these species produce flavone mono-*C*-glycosides and/or flavone 7-*O*-glycosides instead. In addition flavone 4′-*O*-glycosides were only found in species of clade 1 and flavanones were mainly restricted to clade 3. The relationships among species of *Adenocarpus* suggested by flavonoid profiles and DNA sequence analyses provide a framework that can be used as a basis for a new classification of the genus.

1. Introduction

Adenocarpus belongs to subfamily Papilionoideae of the family Leguminosae, tribe Genisteae. The genus is centred in the western Mediterranean region and presently consists of ca. 25 species. The genus was last fully revised by Gibbs (1967), who recognized 14 species; two in the Canary Islands, seven in North Africa, four in the Mediterranean region of Europe, extending into the Atlantic region (Spain, Portugal, France), and one species in Tropical Africa, *Adenocarpus mannii* Hook.f. The major floristic inventory of the Mediterranean, Med-checklist (Greuter et al., 1989), enumerated a total of 11 species and six subspecies. Since then Castroviejo (1999a) has undertaken a revision of the genus for the Iberian Peninsula for his account in Flora Iberica (Castroviejo, 1999b) and added two further species. In addition, he raised some subspecies to species status. Furthermore, Brullo and De Marco (2001) have described two new species of *Adenocarpus* from Italy. Many of the new species used to be subspecies or forms of the polymorphic circum-Mediterranean taxon *Adenocarpus complicatus* (L.) Gay, e.g. *Adenocarpus anisochilus* Boiss., *Adenocarpus aureus* (Cav.) Pau, *Adenocarpus bivonii* (Presl.)Presl., *Adenocarpus brutius* Brullo, *Adenocarpus commutatus* Guss., *Adenocarpus desertorum* Castrov., *Adenocarpus gibbsianus* Castrov. & Talavera, *Adenocarpus*

* Corresponding author. Tel.: +44 (0) 20 8332 5312; fax: +44 (0) 20 8332 5310.
E-mail addresses: r.grayer@kew.org, r.grayer@rbgkew.org.uk (R.J. Grayer).

0305-1978/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2011.12.023

lainzii (Castrov.) Castrov. and *Adenocarpus nainii* Maire. Furthermore, *Adenocarpus argyrophyllus* (Rivas Goday) Caball. used to be a subspecies of *Adenocarpus hispanicus* (Lam.) DC.

Harborne (1969) carried out a comprehensive survey of the flavonoids and isoflavonoids in the tribe Genisteae. He studied the acid-hydrolysed leaf extracts of 128 species belonging to 22 genera and found in the majority of Genisteae species the isoflavones, daidzein, genistein and 5-methylgenistein (=isoprunetin). Flavonols were also common, such as kaempferol, quercetin and fisetin, but the flavones luteolin and apigenin occurred less frequently. Glycoflavones were common in some genera, e.g. *Ulex* and *Chamaecystus*, but absent or rare in others. In this study of the Genisteae, Harborne (1969) also investigated one species of *Adenocarpus*, *Adenocarpus foliolosus*, and reported the isoflavones, daidzein, genistein and 5-methylgenistein from this species, but no flavonols or flavones. Most other chemical studies of the genus *Adenocarpus* have involved alkaloids rather than flavonoids, and many of these investigations were carried out more than five decades ago (summarized in Mears and Mabry, 1971). Sparteine-type quinolizidine alkaloids were found in *A. argyrophyllus*, *Adenocarpus decorticans* and *A. hispanicus*; the pyrrolizidine alkaloid, decorticasine, in the same three species and in *Adenocarpus grandiflorus* and the ammodendrine-hystrine alkaloids, adenocarpine, isoorensine and santiaguine in *A. grandiflorus*, *Adenocarpus intermedius* and *A. mannii*. In addition, adenocarpine and santiaguine were also detected in *Adenocarpus anagyrus* (=*Adenocarpus viscosus*) and *A. foliolosus*.

In the present work, flavonoids and isoflavonoids were surveyed in 24 species of *Adenocarpus*, collected in nine countries or geographic areas (Morocco, Algeria, Canary Islands, Spain, Portugal, Italy, European Turkey, Malawi and Tanzania). For ten of the species two or more accessions were investigated, sometimes from different geographic regions, to study possible infraspecific chemical variation. These included eight accessions of *Adenocarpus telonensis* (six from Morocco, one from Portugal and one from Spain), four accessions of *A. decorticans* (two from Morocco, one from Algeria and one from Spain), six accessions of *A. complicatus* (one from Morocco, two from Spain, one from Portugal and two from Turkey) and four accessions of *A. mannii* (two from Malawi and two from Tanzania). The results of the flavonoid and isoflavonoid survey were projected onto dendrograms based on a DNA sequence analysis of several genes by Cubas et al. (2010) and of the ITS region by one of us (Essokne, 2011). This paper forms part of a study to provide a new monographic treatment of *Adenocarpus* that reflects the morphology, geographic distribution, phytochemistry and DNA sequences of the genus.

2. Material and methods

2.1. Plant material

In order to revise and understand the variation in *Adenocarpus* species, field collections were made from the end of May until mid June 2006 in Morocco, GPS readings for all the sites were recorded and the specimens transported to the Herbarium of the University of Reading (RNG), where they were dried ready for morphological and phytochemical analysis. Permission to collect and export the material was obtained from Professor Sherif Harouni (IAV-Agadir) and Professor Mohammed Fennane (RAB), respectively. A total of 23 specimens were collected at each site by two of us (S.L.J. & R.S.E.) from natural habitats in Morocco. Voucher specimens have been deposited in RNG. In addition, for the other European and Tropical African *Adenocarpus* species, herbarium specimens were used from the Herbaria of RNG and E. Details of the specimens studied are presented in Table 1.

2.2. Extraction

Dried leaves of each *Adenocarpus* species (ca. 0.5–1.0 g) were crushed and extracted with ca. 10 ml of 80% MeOH in a test tube. The plant material was boiled for 10 min in a heating block at 85 °C. The samples were left to stand overnight at room temperature. After filtration, 3 ml of the solvent from each sample was removed to a small beaker to dry. The dried extract was redissolved in 1 ml of 80% MeOH in preparation for analysis by HPLC.

2.3. HPLC

The HPLC system consisted of a Waters LC 600 pump and 996 photodiode array detector. A Merck LiChrospher 100RP-18 (5 μm) column was used; the dimensions were 4.0 mm (i.d) × 250 mm. Two solvent solutions, denoted A and B, were used for elution. Solvent A consisted of 2% HOAc and solvent B of MeOH:HOAc:H_2O, 18:1:1. The gradient pogramme started at 75% A and 25% B, and progressed with a linear gradient reaching B = 100% at $t = 20$ min. This was followed by isocratic elution (B = 100%) to $t = 24$ min, after which the programme returned to the initial solvent composition. Column temperature was maintained at 30 °C and a flow rate of 1.0 ml/min was used. Injections of 20 μl were made by autosampler. Spectral scanning took place over the range 200–400 nm and chromatograms were printed at 340 nm. Retention times and UV spectra of flavonoids were compared with those of standards and published data.

2.4. LC–MS

Positive ion Atmospheric Pressure Chemical Ionization (APCI)-mass spectra were obtained with a quadrupole ion-trap instrument (Thermo Scientific LCQ 'Classic') using a vaporiser temperature of 550 °C, sheath and auxiliary nitrogen