Pergamon

PII: S0031-9422(97)00798-X

*Phytochemistry*, Vol. 48, No. 6, pp. 995–1001, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

# THE DISTRIBUTION OF ISOFLAVONOIDS IN *CICER*

PHILIP C. STEVENSON*† and NIGEL C. VEITCH‡

† Natural Resources Institute, Chatham Maritime, Kent, ME4 4TB, U.K.; ‡ Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, U.K.

(*Received in revised form* 20 *August* 1997)

**Key Word Index**—*Cicer*; Leguminosae; chickpea; chemotaxonomy; isoflavonoids; isoflav-3-enes; 2-arylbenzofuran; cicerfuran; judaicin; 2-methoxyjudaicin.

**Abstract**—Fifteen annual and perennial species of *Cicer* have been analysed for the presence of isoflavonoids. The pterocarpans medicarpin and maackiain and the isoflavonoids biochanin A and formononetin were present in the roots of all species and also occurred widely as glucosides or malonylated glucosides. Five recently identified isoflav-3-enes and a 2-arylbenzofuran were detected exclusively in three species, *C. bijugum*, *C. judaicum* and *C. pinnatifidum*, and their occurrence was restricted to the roots. Conversely, 9-*O*-methylcoumestrol was recorded in all species except for *C. bijugum*, *C. judaicum* and *C. pinnatifidum*. These species have been placed previously in series *Cicer*, a taxonomic grouping of 6 species within section *Cicer* of subgenus *Cicer*. The present results indicate that they should be considered as taxonomically distinct from other species in this series, a proposal supported by a number of other recent studies. 

## INTRODUCTION

The genus *Cicer* L. (Leguminosae; Papilionoideae; tribe Cicereae) contains 43 species of annual and perennial herbs of which *Cicer arietinum*, the chickpea, is the most well studied and the only cultivated species [1]. *C. arietinum* and the genus as a whole affords considerable research interest since the former provides a staple food source in resource poor regions of the semi-arid tropics, where its production is concentrated [2]. Understanding the interspecific relationships between the wild species is critical if agronomically useful characters, such as disease resistance [3], are to be of value in improving the cultivated species.

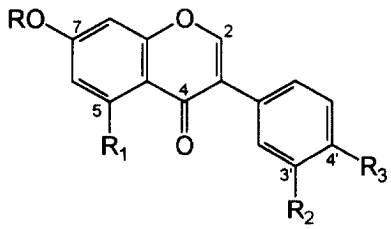
The taxonomy of the genus was first established by Popov (1929) [4] and subsequently supplemented by Linczevski (1948) [5] and van der Maesen (1972) [6] (Table 1). Previous work has focused on the annual species in subgenus *Cicer* (*Pseudononis* Popov nom. inval.) particularly those from section *Cicer* (*Monocicer* Popov nom. inval.), series *Cicer* (*Arietina* Lincz. nom. inval.) which contains species most closely related to the cultivated species (Table 1) [7].

Most phytochemical studies of *C. arietinum* have been concerned with the phytoalexins medicarpin and maackiain because of their potential value in disease management [8–12]. In contrast, only two papers had been published on the phytochemistry of other species within the genus up to 1996. Biochanin A, formononetin, medicarpin and maackiain were identified in the stems of 14 species and cuneatin (7-hydroxy-2′-methoxy-4′,5′-methylenedioxyisoflavone) was identified as a phytoalexin in one species, *C. cuneatum*, although no other qualitative differences were recorded between species [13]. In a second study, seven common isoflavonoids already known from *C. arietinum* were identified in *C. mogolatvicum* [14]. More recently, a new isoflav-3-ene, judaicin, has been identified in the roots of *C. judaicum* with its 7-*O*-glucoside and 7-*O*-malonylglucoside [15], and a derivative, 2-methoxyjudaicin, isolated from the roots of *Cicer bijugum* with its glycoside [16]. A new 2-arylbenzofuran, cicerfuran (2-(2′-methoxy-4′,5′-methylenedioxyphenyl)-6-hydroxybenzofuran) has also been isolated from the roots of *C. bijugum* [17]. This paper extends the scope of these studies with an analysis of the distribution of 17 isoflavonoids in 15 *Cicer* species. The results strongly support recently proposed new taxonomic groupings within the genus.

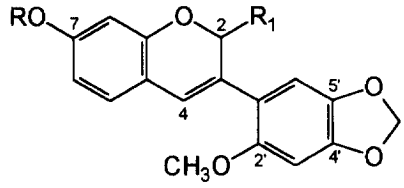
## RESULTS AND DISCUSSION

HPLC analysis with photodiode array detection of fresh methanolic root extracts of 15 species of *Cicer* revealed significant differences in their isoflavonoid profiles. Seventeen compounds were identified and are

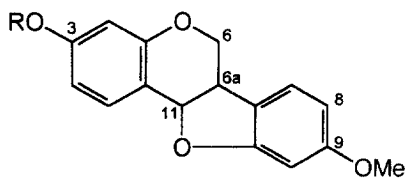
* Author to whom correspondence should be addressed.



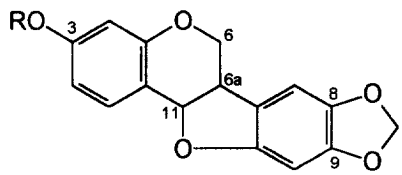
**1** R = Glc, $R_1 = R_2 = H$, $R_3$ = OMe
**5** R = Glc, $R_1$ = OH, $R_2 = H$, $R_3$ = OMe
**7** R = H, $R_1 = R_2$ = OH, $R_3$ = OMe
**10** $R = R_1 = R_2 = H$, $R_3$ = OMe
**13** $R = R_2 = H$, $R_1$ = OH, $R_3$ = OMe



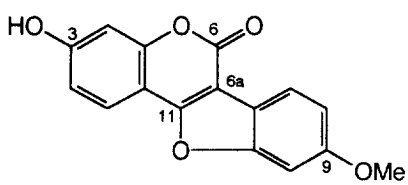
**2** R = Glycosyl, $R_1$ = OMe
**6** R = Glc, $R_1 = H$
**8** R = Malonyl-Glc, $R_1 = H$
**9** R = H, $R_1$ = OMe
**16** $R = R_1 = H$



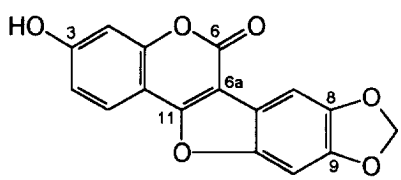
**12**



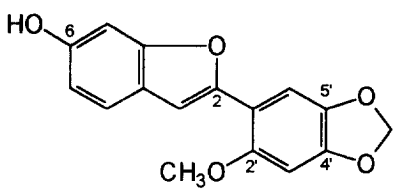
**3** R = Glc
**4** R = Malonyl-Glc
**11** R = H



**15**



**14**



**17**

listed in Table 2 together with UV/VIS spectral and HPLC retention time data. Their identity was confirmed by co-chromatography with authentic standards in two different elution programmes (Table 2). Four isoflavones **1**, **5**, **10** and **13** and three pterocarpans **4**, **11** and **12** were common to the roots of all species studied while the isoflavone **7** was recorded in all species with the exception of *C. pinnatifidum* and *C. reticulatum*. Compounds **1**, **5**, **10** and **13** were the major isoflavonoid components in all species with the exception of *C. bijugum*, *C. judaicum* and *C. pinnatifidum* in which **3**, **4**, **6**, **8**, **9**, **11** and **16** dominated as described previously for *C. judaicum* [15]. The relatively high concentration of the maackiain glycosides **3** and **4** in these three species may be indicative of their potential value as sources of improved resistance to root diseases [17]. It has been proposed that facile cleavage of glucoside units from **3** and **4** may be a more direct source for the anti-fungal aglycone **11**, a known spore germination and germ tube growth inhibitor of the wilt pathogen *Fusarium oxysporum* f.sp. *ciceri* [12, 17]. In addition, the species which contain high concentrations of the glucosides of maackiain are resistant to *Fusarium oxysporum* f.sp.

Table 1. Taxonomic groupings of 15 *Cicer* L. species [6] with ID/collector numbers and source

| *Cicer L.* SPECIES | | SUB GENUS | SECTION | SERIES | ID | SOURCE |
|---|---|---|---|---|---|---|
| *C. arietinum* L. | a | *Cicer* Popov* | *Cicer* Popov** | *Cicer* Lincz.*** | ICC4951 | ICRISAT |
| *C. reticulatum* Ladiz. | a | *Cicer* Popov* | *Cicer* Popov** | *Cicer* Lincz.*** | ICCW48 | ICRISAT |
| *C. echinospermum* P. H. Davis | a | *Cicer* Popov* | *Cicer* Popov** | *Cicer* Lincz.*** | ICCW44 | ICRISAT |
| *C. bijugum* Rech.f. | a | *Cicer* Popov* | *Cicer* Popov** | *Cicer* Lincz.*** | ICCW42&41 | ICRISAT |
| *C. judaicum* Boiss. | a | *Cicer* Popov* | *Cicer* Popov** | *Cicer* Lincz.*** | ICCW73, 89, 92 | ICRISAT |
| *C. pinnatifidum* Jaub. et Spach | a | *Cicer* Popov* | *Cicer* Popov** | *Cicer* Lincz.*** | ICCW38&88 | ICRISAT |
| *C. chorassanicum* (Bunge) Popov | a | *Cicer* Popov* | *Chamaecicer* Popov | *Annua* m. | ICCW29 | ICRISAT |
| *C. cuneatum* Hochst. ex A. Rich | a | *Cicer* Popov* | *Cicer* Popov** | *Cirrhifera* m. | ICCW47 | ICRISAT |
| *C. yamashitae* Kitam. | a | *Cicer* Popov* | *Cicer* Popov** | *Macro-aristae* m. | ICCW1 | ICRISAT |
| *C. anatolicum* Alef. | p | *Viciastrum* Popov | *Polycicer* Popov | *Anatolo-persica* Lincz. | PI 383626 | Pullman |
| *C. microphyllum* Benth. in Royle | p | *Viciastrum* Popov | *Polycicer* Popov | *Microphylla* Lincz. | PAK52900 1 2 | PGRI. PARC |
| *C. nuristanicum* Kitam. | p | *Viriastrum* Popov | *Polycicer* Popov | *Flexuosa* Lincz. | PAK 52889 | PGRI. PARC |
| *C. oxyodon* Boiss. & Hohen. | p | *Viciastrum* Popov | *Polycicer* Popov | *Persica* Popov | PI561084 | Pullman |
| *C. macracanthum* Popov | p | *Viciastrum* Popov | *Acanthocicer* Popov | *Macracantha* Lincz. | PAK 52913 4 5 | PGRI. PARC |
| *C. canariensis* Guerra & G. P. Lewis | p | *Stenophylloma* | ? | ? | PI 557453 | Pullman |

* formerly subgenus *Pseudononis* Popov nom. inval.; ** formerly section *Monocicer* Popov nom. inval.; *** formerly series *Arietina* Lincz. nom. inval., after [7].
a = annual; p = perennial.

*ciceri* [3]. Maackiain 3-*O*-glucoside (trifolirhizin), **3**, was recorded in all species except *C. echinospermum*, *C. microphyllum*, *C. nuristanicum*, *C. oxyodon*, *C. reticulatum* and *C. yamashitae*. The absence of **3** from these species is unlikely to be taxonomically significant since the corresponding malonylated glucoside, **4**, occurs in all of them albeit in trace amounts. It is possible that **3** was not detected in these species because it occurs at very low concentrations.

The occurrence of **10**–**13** in all species was anticipated since the compounds have been reported previously in stems of *Cicer* [13] although in this previous study **11** and **12** occurred only as phytoalexins. The 7-*O*-glucosides of formononetin (**1**) and biochanin A (**5**) have also been identified previously in the foliage of *C. arietinum* together with their malonylated glucosides [9].

The most significant phytochemical differences were recorded among annual species in subgenus *Cicer*. The isoflav-3-enes **2**, **6**, **8**, **9** and **16** and the 2-arylbenzofuran **17** were detected only in *C. bijugum*, *C. judaicum* and *C. pinnatifidum* and with the pterocarpans **3**, **4** and **11** constitute the major components of their phenolic profiles. This observation affords a significant distinction between these species and all others in this subgenus. Moreover it indicates that *C. bijugum*, *C. judaicum* and *C. pinnatifidum*, are more distantly related to the other species in series *Cicer* (*C. arietinum*, *C. echinospermum* and *C. reticulatum*) than published taxonomy suggests [6]. Previous studies have also distinguished these two groups (groups I and II) in series *Cicer* according to other genotypic and phenotypic characters. For example one significant qualitative distinction is that a unique post-zygotic reproductive barrier mechanism exists between members of Group II and those in Group I [18, 19]. *C. bijugum*, *C. judaicum* and *C. pinnatifidum* have also been distinguished from other species in series *Cicer* according to other characters including seed storage protein profiles [19], characteristics of trypsin and chymotrypsin inhibitors [20] and isozyme data using Nei's genetic distance analysis [21] a study further supported by earlier isozyme analyses [22, 23]. Recent chromosome banding pattern analysis supports this distinction in part in that *C. judaicum* and *C. pinnatifidum* have small chromosomes and similar banding patterns [24]. However, the same study grouped *C. bijugum* and *C. cuneatum* together according to their relatively high heterochromatin content. *C. cuneatum*, however, has a very distinct isozyme profile [25] and morphology and is also the only species known to produce the phytoalexin cuneatin [13]. The present study provides no evidence to support a closer genetic relationship between *C. bijugum* and *C. cuneatum* than between *C. cuneatum* and any other *Cicer* species.

The coumestan **15** (9-*O*-methylcoumestrol), was common to all species except *C. bijugum*, *C. judaicum* and *C. pinnatifidum*. A second coumestan **14** (medicagol) was found in all species except *C. echi-*

Table 2. Chromatographic and UV/VIS spectral properties of *Cicer* isoflavonoids and their identification

| Isoflavonoid | Method 1* $R_t$ (min) | Method 2* $R_t$ (min) | UV/VIS spectral† maxima (nm) | Identification |
|---|---|---|---|---|
| **1** | 3.16 | 14.06 | 257, 302 sh | Formononetin 7-*O*-glucoside (Ononin)[1] |
| **2** | 3.35 | 14.53 | 290 sh, 326 | 2-Methoxyjudaicin 7-*O*-glycoside[2] |
| **3** | 3.76 | 15.18 | 285 sh, 310 | Maackiain 3-*O*-glucoside (Trifolirhizin)[3] |
| **4** | 4.28 | 16.20 | 285 sh, 309 | Maackiain 3-*O*-(6′-*O*-malonylglucoside)[3] |
| **5** | 4.38 | 16.33 | 261, 328 sh | Biochanin A 7-*O*-glucoside (Sissotrin)[4] |
| **6** | 5.76 | 17.78 | 294 sh, 337 | Judaicin 7-*O*-glucoside[3] |
| **7** | 6.42 | 18.11 | 226, 260, 328 sh | Pratensein[4] |
| **8** | 6.74 | 18.86 | 294 sh, 337 | Judaicin 7-*O*-(6″-*O*-malonylglucoside)[3] |
| **9** | 8.10 | 19.63 | 290 sh, 325 | 2-Methoxyjudaicin[2] |
| **10** | 9.61 | 21.17 | 256, 302 sh | Formononetin[4] |
| **11** | 11.47 | 22.77 | 285 sh, 310 | Maackiain[3] |
| **12** | 12.73 | 24.10 | 285 | Medicarpin[4] |
| **13** | 15.56 | 27.74 | 261, 328 sh | Biochanin A[4] |
| **14** | 15.93 | 28.11 | 245, 310, 348, 362 sh | Medicagol[4] |
| **15** | 17.27 | 29.08 | 242, 265 sh, 304, 342, 356 sh | 9-*O*-Methylcoumestrol[4] |
| **16** | 17.56 | 29.14 | 295 sh, 337 | Judaicin[3] |
| **17** | 19.08 | 29.90 | 251 sh, 275 sh, 284 sh, 300 sh, 309 sh, 338, 354 sh | Cicerfuran[5] |

* For details of HPLC solvents see experimental section.

† The spectral maxima in this column are those recorded for compounds as they were eluted from the column using the HPLC conditions for method 1, an aqueous acetonitrile solution acidified with 2% acetic acid. UV/VIS spectra for these compounds in neutral methanol may be slightly different. Sources of chemical standards: [1] Apin Chemicals Ltd., [2][16], [3][15], [4]Plantech UK Ltd, [5][17].

*nospermum*, *C. macracanthum*, *C. microphyllum* and *C. reticulatum*. This character, however, does not correlate with any others known to the authors or to previous classifications although it is noteworthy that these four species also contain only trace amounts of **4** and **11**, the structurally related pterocarpan and its malonylated glucoside.

Eight other related compounds, daidzein, astraciceran (7-hydroxy-2′-methoxy-4′,5′-methylenedioxyisoflavan), cuneatin, liquiritigenin, liquiritigenin 7-methyl ether, liquiritigenin 4-methyl ether, pseudobaptigenin and coumestrol were not detected in the roots of any of the species analysed. It is noteworthy that the leaves, seeds and stems of *C. bijugum*, *C. judaicum* and *C. pinnatifidum* did not contain **2**, **3**, **4**, **6**, **7**, **8**, **9**, **14**, **15**, **16** and **17**, (Table 3) (data for *C. bijugum* and *C. pinnatifidum* not shown) and only the leaves contained **11** and **12**.

An overview of likely biosynthetic relationships between the isoflavonoids discussed in this study is presented in Fig. 1, with particular emphasis on those compounds whose presence is unique to *C. bijugum*, *C. judaicum* and *C. pinnatifidum*. In this scheme, a 2′-hydroxyisoflavanol, the immediate precursor of isoflavans, isoflav-3-enes and pterocarpans, is shown as the first intermediate for convenience. The generally accepted pathway leading to its formation has been discussed in a recent review [26]. Compounds **9**, **11**, **14**, **16**, and **17** all possess equivalent A-ring hydroxy and methylenedioxy groups. These have been included on all structures in Fig. 1, as the precise stage of their addition is not a determining factor for the transformations envisaged here. *C. bijugum*, *C. judaicum* and *C. pinnatifidum* are the only species to contain isoflav-3-enes and the 2-arylbenzofuran, **17**. This distinctive feature can be related in biosynthetic terms to the activity of a C-2′ methyltransferase, the regulation of which may be important in allowing compounds of these classes to accumulate. Two routes have been suggested previously for the biosynthesis of 2-arylbenzofurans, either loss of C-6 from a coumestan such as **14**, or loss of one carbon from the phenylalanine-derived A-ring (C-2 of the immediate precursor of **17** in Fig. 1) [27]. Investigation of the biosynthesis of the phytoalexin vignafuran (2-(2′-methoxy-4′-hydroxyphenyl)-6-methoxybenzofuran)) through labelling studies supported the latter route in the cowpea *Vigna unguiculata* [28]. Consideration of the isoflavonoids present in *C. bijugum*, *C. judaicum* and *C. pinnatifidum*, also supports this biosynthetic route for

Table 3. Distribution of seventeen isoflavonoids in the roots of fifteen species of *Cicer* L.

| SPECIES Compound | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. anatolicum* | x | | x | x | x | | x | | | x | x | x | x | x | x | | |
| *C. arietinum* | x | | x | x | x | | x | | | x | x | x | x | x | x | | |
| *C. bijugum* | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | | **x** | **x** |
| *C. canariensis* | x | | x | x | x | | x | | | x | x | x | x | x | x | | |
| *C. chorassanicum* | x | | x | x | x | | x | | | x | x | x | x | x | x | | |
| *C. cuneatum* | x | | x | x | x | | x | | | x | x | x | x | x | x | | |
| *C. echinospermum* | x | | | x | x | | x | | | x | x | x | x | | x | | |
| *C. judaicum* | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | **x** | | **x** | **x** |
| *C. macracanthum* | x | | x | x | x | | x | | | x | x | x | x | | x | | |
| *C. microphyllum* | x | | | x | x | | x | | | x | x | x | x | | x | | |
| *C. nuristanicum* | x | | | x | x | | x | | | x | x | x | x | x | x | | |
| *C. oxyodon* | x | | | x | x | | x | | | x | x | x | x | x | x | | |
| *C. pinnatifidum* | **x** | **x** | **x** | **x** | **x** | **x** | | **x** | **x** | **x** | **x** | **x** | **x** | **x** | | **x** | **x** |
| *C. reticulatum* | x | | | x | x | | | | | x | x | x | x | | x | | |
| *C. yamashitae* | x | | | x | x | | x | | | x | x | x | x | x | x | | |
| *C. judaicum* ROOT | x | x | x | x | x | x | | x | x | x | x | x | x | x | | x | x |
| *C. judaicum* LEAF | x | | | | x | | | | | x | x | x | x | | | | |
| *C. judaicum* STEM | x | | | | x | | | | | x | x | x | x | | | | |
| *C. judaicum* SEED | x | | | | x | | | | | x | | | x | | | | |

**1** = Formononetin 7-*O*-glucoside, **2** = 2-Methoxyjudaicin 7-*O*-glycoside, **3** = Maackiain 3-*O*-glucoside, **4** = Maackiain 3-*O*-(6′-*O*-malonylglucoside), **5** = Biochanin A 7-*O*-glucoside, **6** = Judaicin 7-*O*-glucoside, **7** = Pratensein, **8** = Judaicin 7-*O*-(6″-*O*-malonylglucoside) **9** = 2-Methoxyjudaicin, **10** = Formononetin, **11** = Maackiain, **12** = Medicarpin, **13** = Biochanin A, **14** = Medicagol, **15** = 9-*O*-Methylcoumestrol, **16** = Judaicin, **17** = Cicerfuran.

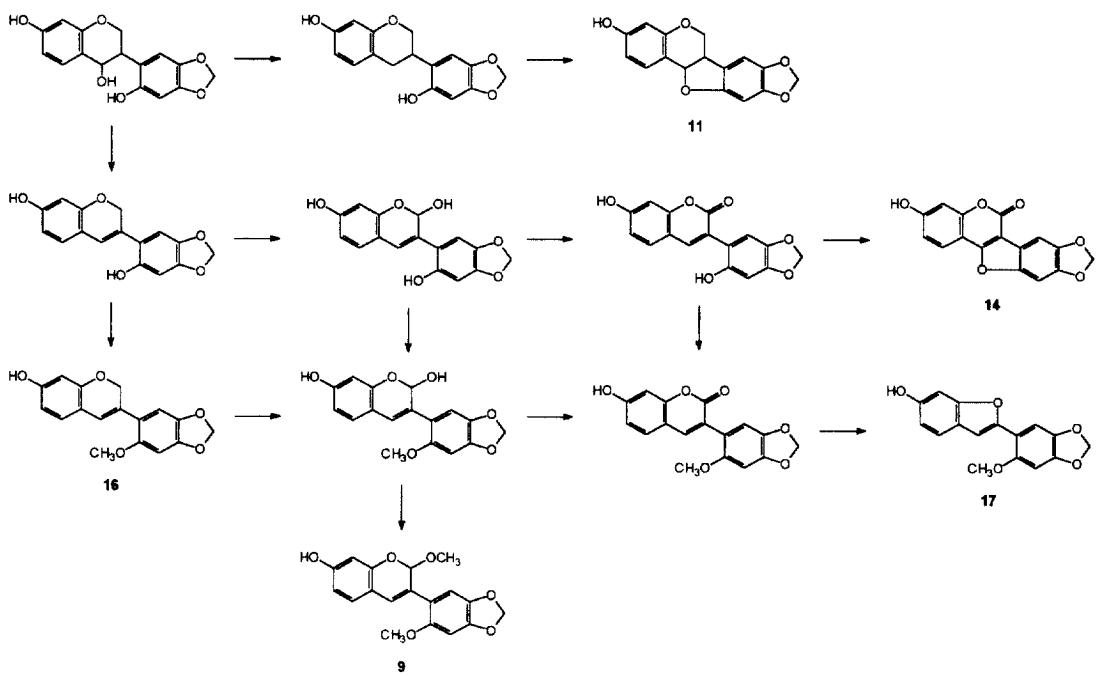



Fig. 1. Summary of proposed biosynthetic relationships among isoflavonoids found in the roots of *Cicer* species. The scheme commences with a 2′-hydroxyisoflavanol, the precursor of isoflavans, isoflav-3-enes and pterocarpans [26]. Pathways for the formation of pterocarpan (**11**), isoflav-3-ene (**9** and **16**), coumestan (**14**) and 2-arylbenzofuran (**17**) derivatives are indicated by arrows, although it should be noted that some of the steps may be reversible. The presence of **9**, **16** and **17** is unique to three species, *C. bijugum*, *C. judaicum* and *C. pinnatifidum*.

the formation of **17**, as loss of C-6 from the coumestan **14** would give the 2-arylbenzofuran, 2-(2′,4′-dihydroxyphenyl)-5,6-methylenedioxybenzofuran.

## CONCLUSIONS

The unique occurrence of two isoflav-3-enes **9** and **16**, their glycosylated forms **2**, **6** and **8** and the 2-arylbenzofuran **17** in *C. bijugum*, *C. judaicum* and *C. pinnatifidum* indicates that these species are closely related to each other but not to *C. arietinum*, *C. echinospermum* and *C. reticulatum* from the same series. This conclusion, taken with those of previous studies drawn from a variety of different phenotypic and genotypic characters [18, 19, 21], emphasises the need for a re-grouping of species within *Cicer*. In a recent revision a new series *Pinnatifida* has been proposed within Section *Cicer* containing *C. judaicum* and *C. pinnatifidum* [29]. It is clear that *C. bijugum* should also be included in this series.

## EXPERIMENTAL

### *Plant material*

Seeds of all wild species of *Cicer* were obtained from the Genetic Resources Unit of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) India, Dr W. Kaiser, University of Portland, WA, U.S.A. and Mr. S. Bhatti, Pakistan Agricultural Research Council, Islamabad, Pakistan. Plants were grown under greenhouse conditions at the Royal Botanic Gardens, Kew, U.K. [see Table 1 for Accession numbers]. Voucher specimens are held at the Royal Botanic Gardens, Kew. Root material was freeze dried at the flowering stage (60 days after sowing) and milled.

### *Analytical and isolation procedures*

Freeze dried root material from approximately 5 g fr. wt per species was ground with a minimum vol. of MeOH using a mortar and pestle. A further 250 ml of MeOH was added and the root material allowed to extract at 4° for 18 h. The resulting slurry was filtered and the filtrate evapd to dryness under red. pres. This material was re-dissolved in MeOH to give an extract corresponding to 1 g $ml^{-1}$ of original plant material. Each filtered extract (0.45$\mu$m Millipore filters) was injected in 10 $\mu$l aliquots directly onto a Spherisorb 5 ODS analytical column, 4.6 mm (i.d.) × 250 mm. A Waters HPLC system consisting of a 717 autosampler, an LC 600 pump and 996 photodiode array detector was used in gradient elution mode. Two different analytical gradient elution programmes were used with the solvents 2% acetic acid (A) and 2% acetic acid in acetonitrile (B). Method 1: A = 50% at $t$ = 0 min; A = 40% at $t$ = 20 min and A = 20% at $t$ = 30 min at 1 ml $min^{-1}$. Method 2: A = 90% at $t$ = 0 min; A = 50% at $t$ = 20 min: A = 20% at $t$ = 25 min and A = 0% at $t$ = 30 min at 1 ml $min^{-1}$. **1**–**17** were identified by comparison of UV/VIS spectra and retention times with authentic standards (Table 2).

*Acknowledgements*—This work was funded by the Darwin Initiative of the Department of the Environment. The authors would like to thank Mr Clive Foster for growing plant material, Dr W. Kaiser at Washington State University, Pullman, Dr. Pundir at ICRISAT Asia Centre, India and Mr S. Bhatti, PARC, Islamabad, Pakistan, for provision of seed material.

## REFERENCES

1. Van der Maesen, L. J. G., in *The Chickpea*, ed. M. C. Saxena and K. B. Singh. CAB International, Oxford, UK, 1987, p. 11.
2. Jodha, N. S. and Subba Rao, K. V., in *The Chickpea*, ed. M. C. Saxena and K. B. Singh. CAB International, Oxford, UK, 1987, p. 1.
3. Kaiser, W. J., Alcala-Jiminez, A. R., Hervas-Varga, A., Trapero-Casas, J. L. and Jiminez-Diaz, R. M., *Plant Disease*, 1994, **78**, 962.
4. Popov, M. G., *Bulletin of Applied Botany, Genetics and Plant Breeding*, 1929, **21**, 1.
5. Linczevski, I. A., *Cicer* L. in *Flora USSR*, ed. V. L. Komarov, B. Shishkin and E. Bobrov. Leningrad, 1948, **13**, 386.
6. Van der Maesen, L. J. G., *Cicer* L., Monograph. H. Veenman and N. V. Zonen, Wageningen, 1972.
7. Santos Guerra, A. and Lewis, G. P., *Kew Bulletin*, 1986, **41**, 459.
8. Ingham, J. L., *Phytopathologische Zeitschrift*, 1976, **87**, 353.
9. Weigand, F., Koster, J., Weltzein, H. C. and Barz, W., *Journal of Phytopathology*, 1986. **115**, 214.
10. Kessman, H., Daniel, S. and Barz, W., *Zeitschrift für Naturforschung*, 1988, **43 c**, 529.
11. Stevenson, P. C., Padgham, D. E. and Haware, M. P., *Acta Horticulturae*, 1994, **381**, 631.
12. Stevenson, P. C., Turner, H. and Haware, M. P., *Physiological and Molecular Plant Pathology*, 1997, **50**, 167.
13. Ingham, J. L., *Biochemical Systematics and Ecology*, 1981, **9**, 125.
14. Yusapova, S. S., Batirov, E. K., Kiyamitdinova, F., *Khimiya Prirodnykh Soedinenii*, 1986, 639.
15. Stevenson, P. C. and Veitch, N. C., *Phytochemistry*, 1996, **43**, 695.
16. Veitch, N. C. and Stevenson, P. C., *Phytochemistry*, 1997, **44**, 1587.
17. Stevenson, P. C. and Veitch, N. C., *Phytochemistry*, 1998 (in press, this volume).
18. Ladizinsky, G. and Adler, A., *Theoretical and Applied Genetics*, 1976, **48**, 197.
19. Ahmad, F. and Slinkard, A. E., *Theoretical and Applied Genetics*, 1992, **84**, 688.
20. Smirnoff, P., Rotman, M. and Ladizinsky, G., *Biochemical Systematics and Ecology*, 1981, **9**, 23.

21. Tayyar, R. I. and Waines, G., *Theoretical and Applied Genetics*, 1996, **92**, 245.
22. Ahmad, F., Gaur, P. M. and Slinkard, A. E., *Theoretical and Applied Genetics*, 1992, **83**, 620.
23. Kazan, K. and Muehlbauer, F. J., *Plant Systematics and Evolution*, 1991, **175**, 11.
24. Tayyar, R. I., Lukaszewski, A. J. and Waines, G., *Genome*, 1994, **37**, 656.
25. Tayyar, R. I., PhD Thesis, University of California, Riverside, 1993.
26. Dewick, P. M., in *The Flavonoids: Advances in Research since* 1986, ed. J. B. Harborne. Chapman and Hall, 1993, p. 117.
27. Dewick, P. M., in *The Flavonoids: Advances in Research*, ed. J. B. Harborne and T. J. Mabry. Chapman and Hall, 1982, p. 535.
28. Martin, M. and Dewick, P. M., *Tetrahedron Letters*, 1978, 2341.
29. Seferoba, I. V., *Botanicheskii Zhurnal*, 1996, **8**, 96.