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A Chemotaxonomic Survey of the Tribe Psoraleeae in Africa

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Key Word Index—*Psoralea*; *Cullen*; *Otholobium*; *Bituminaria*; *Orbexilum*; Leguminosae; flavones; flavone *C*-glycosides; furanocoumarins; essential oils; isoflavones; biochemical systematics.

Abstract—A study of the leaf flavonoids, furanocoumarins and essential oils of 51 species (three genera) of the tribe Psoraleeae (Fabaceae) has provided chemical data to support the recent subdivision of the large worldwide genus *Psoralea* into a number of segregate genera. The flavonoid patterns in most species were very similar, consisting largely of proanthocyanidins and complex mixtures of flavone *O*- and *C*-glucosides. *Psoralea repens*, the only maritime species to be examined, is unique in containing only rutin. Three isoflavones (daidzein, formononetin and genistein) were of widespread occurrence. The genera *Psoralea* and *Otholobium*, which could not be separated on flavonoid data, were clearly demarcated by their essential oils. Furanocoumarin patterns were useful for the characterization of species.

Introduction

The Papilionoid legume tribe Psoraleeae was thought in 1981 to comprise six genera: *Psoralea, Cullen, Otholobium, Bituminarea, Hallia* and *Orbexilum* [1]. Prior to that, most modern authors recognized either a single genus *Psoralea* in the tribe Psoraleeae (Benth.) Rydb. or a number of genera in the broader-based tribe Psoralieae Hutch. These opinions however were not based on any comparative world-wide study but rested entirely on the nineteenth century classifications of Bentham [2], De Candolle [3], Meyer [4] and Ecklon and Zeyher [5].

Recent studies by one of us (C.H.S.) would indicate that the genus *Hallia* is better placed as a sub-genus of *Psoralea* and it is also apparent that the American genus *Orbexilum* should be sub-divided into a number of genera (Grimes, J., personal communication). It is evident therefore that generic limits in the tribe are still uncertain, and although the Old World genera have been studied in considerable detail their relationship to the lesser known New World genera is still unresolved. It is the purpose of this paper to show that chemical evidence can provide useful insights into the taxonomy and phylogeny of the group.

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The only detailed chemical study made so far of the tribe Psoraleeae has been that of Ockenden *et al.* [6] who surveyed the flavonoid chemistry of 30 species of North American representatives of the genus *Psoralea* sensu lato (*Orbexilum*). This work revealed the presence of a complicated pattern of *C*-glycosylflavones such as orientin, isoorientin, lucenin and vicenin, as well as *O*-glycosides of luteolin, apigenin and chrysoeriol. Their results indicated that flavonoids provided useful markers for a number of species. No attempt however was made by the authors to superimpose their data on Rydberg's [7] generic fragmentation of *Orbexilum*.

The present work on African species of the tribe has revealed a very similar flavonoid pattern to that of the North American species. The study was extended however to cover a broader range of compounds including isoflavones, proanthocyanidins, essential oils and furanocoumarins. In this way it was hoped to test whether different chemical compounds, from the same organ, would provide the same or different levels of taxonomic information. The decision to include essential oils devolves on the characteristic presence of unique leaf gland types in the genus (Turner, G., personal communication). No such chemical studies have been reported before. Furanocoumarins were studied as they were known to occur only in the tribe Psoraleeae [8]. Psoralen has been isolated from the seed coat of *Psoralea subacaulis* as a germination inhibitor [9, 10], and of *P. psoraleoides* [11]. Furanocoumarins have also been suggested in the literature to be powerful phototoxins [12].

Results

Flavonoids

A composite representation of the flavonoids (and tentative identifications) of 51 species of genera *Psoralea* and *Otholobium* analysed is shown in Fig. 1. The flavone *O*-glycosides and flavone *C*-glycosides detected on two-dimensional chromatograms are shown in Tables 1 and 2.

The genus *Cullen*, represented by *C. obtusifolia*, has no flavonoids in common with the genera *Psoralea* and *Otholobium*.

One species stands out from all the rest. *Psoralea repens* does not exhibit the characteristic pattern of flavone *O*- and *C*-glycosides typical of all the other species examined but does contain high concentrations of rutin (quercetin 3-rutinoside). This species is the only creeping dune plant of the tribe and is distributed along sandy stretches of the southern Cape coastline. The striking absence of common psoraleoid flavonoids in this genus necessitated that more samples were analysed. To this end three extra collections were obtained and analysed. The four collections sampled were therefore representative of the entire range of distribution. All the populations were found to contain rutin only. Flavonols such as quercetin were absent from the leaves of all the other species studied, including a number of American species.

The commonest flavonoids found, present in over 80% of species, were orientin, vicenin, isoorientin *O*-glycoside, compound 4, various di-*C*-glycosides of luteolin and chrysoeriol (Fig. 1, Table 2). Less common flavonoids were apigenin *O*-glycoside (25% species), isovitexin (26% species) and unidentified compound 9 (49% species). The unidentified compound 12 was found in the species *P. nodosa*, *O. caffra* and *O. wilmsii* where it replaced isoorientin. These three species are the commonest and most widespread species of the eastern summer rainfall region of southern Africa. Although this flavonoid does not occur in the isolated *O. gazense*, also from eastern parts, there is the suggestion that this compound has a geographical distribution.

TABLE 1. LEAF FLAVONOIDS PRESENT IN *PSORALEA, OTHOLOBIUM* AND *CULLEN*

Voucher No.	Spp.	1	2	3	4	5	6	7	8	9	10	11	12*
8746	*P. aculeata*	+	+	+	+	+	+	+	−	+	+	−	−
9605	*P. affinis*	+	−	+	+	+	+	+	−	−	+	−	−
9623	*P. affinis*	+	+	+	+	+	+	+	−	−	+	−	−
9718	*P. affinis*	+	−	+	+	?	?	+	?	+	+	−	−
8250	*P. arborea*	+	+	+	+	+	+	+	+	−	+	−	−
9740	*P. arborea*	+	−	+	+	+	+	+	+	+	+	−	−
9736 }	*P. arborea* ×	+	−	+	+	?	?	+	−	+	+	−	−
9737 }	*P. effusa*	−	−	+	+	+	+	+	−	−	+	−	−
8420	*P. asarina*	+	−	+	+	+	+	+	+	−	−	−	−
8256	*P. axillaris*	+	+	+	+	+	+	+	−	+	+	−	−
9163	*P. connixa*	+	−	+	+	+	+	+	?	+	+	−	−
9183	*P. connixa*	+	−	+	+	+	+	+	?	+	+	−	−
9526	*P. connixa*	+	?	+	+	+	+	+	?	−	+	−	−
8220	*P. effusa*	+	+	+	+	+	+	+	−	+	+	−	−
9102	*P. effusa*	+	−	+	+	?	?	+	+	−	+	−	−
9478	*P. effusa*	+	−	+	+	+	+	+	−	+	+	−	−
9712	*P. effusa*	+	−	+	+	+	+	+	+	+	+	−	−
9739	*P. effusa*	+	−	+	+	?	?	+	−	+	+	−	−
9509	*P. exile*	+	−	+	+	+	+	+	−	+	+	−	−

TABLE 1—*CONTINUED*

Voucher No.	Spp.	1	2	3	4	5	6	7	8	9	10	11	12*
9521	*P. exile*	+	−	+	+	+	+	+	−	+	+	−	−
9602	*P. glabra*	+	−	+	+	+	+	+	−	+	+	−	−
8333	*P. imbricata*	+	−	+	−	−	−	+	+	−	−	−	−
8211	*P. laxa*	+	+	+	+	+	+	+	+	+	+	−	+
8093	*P. nodosa*	+	−	+	+	+	+	−	−	+	+	−	+
8258	*P. odoratissima*	+	−	+	+	+	+	+	+	−	+	−	−
8219	*P. oligophylla*	+	+	+	+	+	+	+	−	+	+	−	−
9184	*P. oreopolum*	+	−	+	+	+	+	+	−	−	+	−	−
9185	*P. oreopolum* × *P. ramulosa*	+	−	+	+	+	+	+	−	−	+	−	−
9186		+	−	+	+	+	+	+	+	+	+	−	−
9187		+	−	+	+	+	+	+	−	+	+	−	−
9562	*P. papillosa*	+	−	+	+	+	+	+	?	+	+	−	−
8479	*P. pinnata*	+	−	+	+	+	+	+	−	+	+	−	−
9757	*P. pinnata*	+	+	+	+	+	+	+	−	−	+	−	−
8297	*P.* sp. cf *pinnata*	+	−	+	−	+	+	+	−	−	+	−	−
9292	*P.* sp. cf *pinnata*	+	−	+	+	+	+	+	−	−	+	−	−
9742	*P.* sp. cf *pinnata*	−	−	+	+	+	?	+	−	−	+	−	−
8451	*P. pullata*	+	−	+	+	+	+	+	−	+	+	−	−
9142	*P. ramulosa*	+	−	+	+	+	+	+	?	+	+	−	−
—	*P. repens*	−	−	−	−	−	−	−	−	−	−	+	−
8413	*P. repens*	−	−	−	−	−	−	−	−	−	−	+	−
9441	*P. repens*	−	−	−	−	−	−	−	−	−	−	+	−
9681	*P. speciosa*	+	−	+	+	+	+	+	−	+	+	−	−
9709	*P. speciosa*	+	−	+	+	+	+	+	+	−	+	−	−
105	*P. tenuifolia*	+	+	+	+	+	+	+	−	−	+	−	−
9506	*P. tenuifolia*	+	?	+	+	+	+	+	−	?	+	−	−
9715	*P. tenuifolia*	+	−	+	+	+	+	+	−	+	+	−	−
9367	*P. verrucosa*	+	−	+	+	?	?	+	−	?	+	−	−
9627	*P.* sp.	+	−	+	+	+	+	+	+	+	+	−	−
9708	*P.* sp.	+	−	+	+	+	?	+	+	+	?	−	−
8445	*O. bolusii*	−	−	+	−	+	+	+	−	−	−	−	−
9301	*O. bolusii*	−	−	−	−	+	+	−	−	−	+	−	−
8403	*O. bracteolata*	+	−	+	−	+	+	+	−	−	−	−	−
8237	*O. brevibracteatum*	+	+	+	+	+	+	+	+	−	+	−	−
1579	*O. caffra*	+	−	+	−	+	+	−	−	−	+	−	+
8446	*O. candicans*	+	−	+	+	+	+	+	−	+	+	−	−
9497	*O. candicans*	+	−	−	−	+	+	+	−	+	+	−	−
8442	*O. fruticans*	+	+	+	+	+	+	+	+	−	−	−	−
10555	*O. gazense*	+	+	+	+	+	+	+	+	−	+	−	−
4832	*O. hirtum*	+	+	+	+	+	+	+	+	+	+	−	−
8425	*O. hirtum*	+	+	+	+	+	+	+	+	+	+	−	−
9360	*O. hirtum*	−	−	+	+	+	+	+	−	−	−	−	−
9089	*O. obliquum*	+	−	+	+	+	+	+	−	−	+	−	−
9090	*O. parviflorum*	+	−	+	−	+	+	+	+	+	+	−	−
9369	*O. rotundifolium*	+	−	−	−	+	+	−	−	+	+	−	−
9121	*O. salignum*	−	+	−	+	+	+	+	−	−	+	−	−
9629	*O. sericeum*	+	−	+	+	+	−	+	+	−	−	−	−
8363	*O. spicata*	+	+	+	+	+	+	+	−	+	+	−	−
9307	*O. stachydis*	−	−	+	+	+	+	+	+	−	+	−	−
9620	*O. stachyenum*	+	−	+	+	+	+	+	−	−	+	−	−
8447	*O. striata*	+	+	+	+	+	+	+	−	+	+	−	−
8444	*O. uncinata*	+	+	+	−	+	+	+	−	−	+	−	−
8913	*O. wilmsii*	+	−	+	−	+	+	−	−	+	+	−	+
9093	*O. zeyheri*	−	−	−	−	+	+	+	−	−	+	−	−
8923	*O.* sp.	+	+	+	+	+	+	+	−	−	+	−	−
9999	*Cullen obtusifolia*	−	−	−	−	−	−	−	−	−	−	−	−

*For compound key, see Table 2, + = present, − = absent.

TABLE 2. PERCENTAGE OCCURRENCE OF FLAVONOIDS IN *CULLEN, PSORALEA* AND *OTHOLOBIUM*

Spot No.	Compound	No. of records in species examined (%)	No. of records in species of *Otholobium* (%)	No. of records in species of *Psoralea* (%)
1	Luteolin 7-*O*-glucoside	62 (82)	17 (81)	26 (90)
2	Apigenin *O*-glycoside	19 (25)	9 (43)	9 (31)
3	Orientin	65 (86)	18 (86)	25 (86)
4	Flavone *O*-glycoside	60 (79)	13 (62)	27 (93)
5	Di-*C*-glycosylapigenin	64 (84)	21 (100)	28 (97)
6	Various di-*C*-glycosides of luteolin or chrysoeriol	61 (80)	20 (95)	25 (86)
7	Isoorientin	65 (86)	18 (86)	25 (86)
8	Isovitexin	20 (26)	7 (33)	10 (34)
9	Unidentified (flavone?)	37 (49)	7 (33)	20 (69)
10	Isoorientin *O*-glycoside	63 (83)	18 (86)	26 (90)
11	Rutin	3 (4)	0 (0)	3 (10)
12	Flavone *O*-glycoside	3 (4)	2 (10)	1 (0.3)

Total No. of species = 51; total No. of records = 76; total species *Otholobium* = 21; total species *Psoralea* = 29; total species *Cullen* = 1; in some species more than one accession was examined.

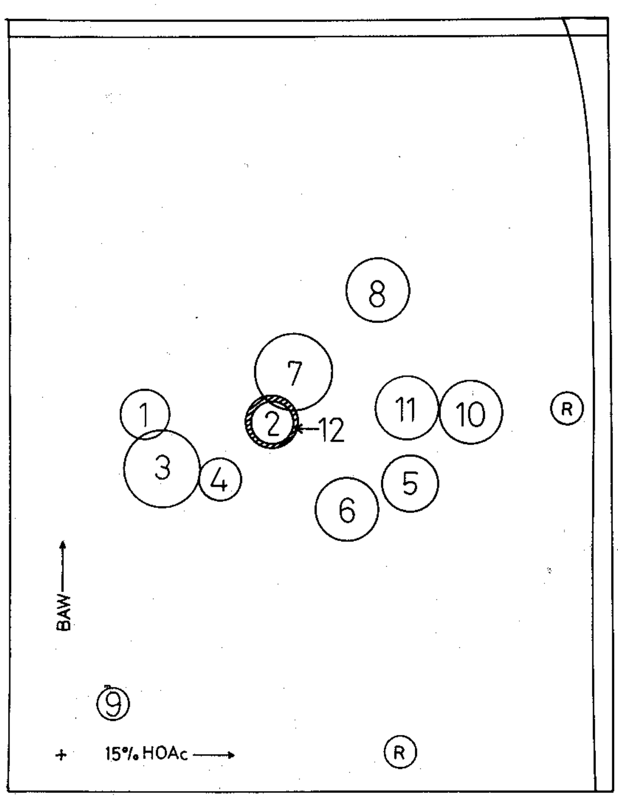


FIG. 1. COMPOSITE 2-DIMENSIONAL PAPER CHROMATOGRAM OF FLAVONOIDS IN SPECIES EXAMINED. Compound/spot No.: 1 luteolin 7-glucoside; 2 apigenin glyc?; 3 orientin; 4 flavone glyc.: 5 di-*C*-glycosylapigenin; 6 di-*C*-glycosyl-luteolin (or chrysoeriol); 7 isoorientin; 8 isovitexin; 9 unidentified; 10 isoorientin *O*-glycoside?; 11 quercetin 3-rutinoside (rutin); 12 flavone glyc.; R rutin marker. BAW (1st solvent)—*n*-butanol–HOAc–H_2O; 4:1:5 top layer. 15% HOAc (2nd solvent)—15% aqueous acetic acid. N.B. Spots 2 and 12 (shaded) occur in the same position, but are recognizable by their colour reactions (see Table 7).

The flavonoid 2D patterns of dried flowers taken from 26 species were generally rather similar, except for *P. repens* which once again had an anomalous pattern and was also the only species to contain rutin (Table 3). *Otholobium* had high concentrations of two flavonoids (9F and 12F) which were very scarce or absent in *Psoralea*. The species *O. bolusii* was characterized by an unknown flavonoid (3F). This species also has a unique leaf 2D pattern. It was not possible to identify the flavonoids as there was insufficient floral material available.

Proanthocyanidins and Isoflavones

Hydrolysed extracts of leaf material were analysed for the presence of proanthocyanidins and isoflavones. Plants which were rich in proanthocyanidins, indicated by a red colour on acid hydrolysis, were analysed further to determine which constituent anthocyanidins were present as condensed tannins. These results are shown in Table 4.

Proanthocyanidins occur in 37 of the species investigated. Prodelphinidin was present in all 37 species. Those species which consistently stained dark red on acid hydrolysis nearly always had traces of procyanidin. Larger concentrations of procyanidin were found in *Psoralea asarina, P. imbricata* and *P.* sp. (accession No. 9627). These oligomers are absent from *Cullen obtusifolia* and all Otholobiums, except for the distinctive *O. sericeum.*

TABLE 3. FLAVONOIDS PRESENT IN FLOWER PETAL EXTRACTS OF *PSORALEA* AND *OTHOLOBIUM* SPECIES

Voucher No.	Spp.	Compound No. 1F	2F	3F	4F	5F	6F	7F	8F	9F	10F	11F	12F	13F	14F	15F	Anthocyanins present
8746	*P. aculeata*	+	+	−	+	+	+	+	−	+	−	−	−	−	−	−	—
9718	*P. affinis*	+	+	−	+	+	+	−	+	+	+	−	−	−	−	−	Dp, Pt
8250	*P. arborea*	+	+	−	+	+	−	+	−	−	+	−	−	−	+	−	Dp, Pt
9736 }	*P. arborea* ×	+	+	−	+	+	−	+	−	−	−	−	−	+	−	−	Dp, Pt
9737 }	*P. effusa*	+	+	−	+	+	−	+	−	−	+	−	−	+	−	−	Dp, Pt
8256	*P. axillaris*	+	+	−	+	+	−	+	−	−	+	−	−	−	+	−	Pt
8258	*P. odoratissima*	+	+	−	+	+	−	+	+	−	+	−	−	−	+	−	Dp, Pt
8219	*P. oligophylla*	+	+	−	+	+	+	+	−	?	−	−	−	−	−	−	Dp, Pt
8297	*P.* cf *pinnata*	+	+	−	+	+	+	+	?	+	+	−	+	−	−	−	Dp, Pt
9742	*P.* sp. cf *pinnata*	+	+	−	+	+	−	+	−	−	+	+	−	−	−	−	Dp, Pt
—	*P. repens**	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	Dp, Pt
9681	*P. speciosa*	+	+	−	−	+	+	+	+	−	+	−	−	−	+	−	Pt
9301	*O. bolusii*	+	+	+	−	−	−	+	−	+	−	−	+	−	−	+	Pt
8403	*O. bracteolata*	+	+	−	+	+	−	+	−	+	+	+	+	+	−	−	Pt
8237	*O. brevibracteatum*	+	+	−	+	+	+	+	−	+	+	+	+	−	−	−	Dp, Pt
1579	*O. caffra*	+	+	−	+	−	−	+	−	+	−	−	+	−	−	−	—
8446	*O. candicans*	+	+	−	+	+	?	+	−	+	+	−	+	−	−	−	Dp, Pt
8422	*O. fruticans*	+	+	−	+	+	−	+	−	−	+	+	−	+	−	−	Dp, Pt
4832	*O. hirtum*	+	+	−	+	+	−	+	−	+	+	+	+	−	−	−	Dp, Pt
8425	*O. hirtum*	+	+	−	+	+	−	+	−	+	+	−	+	−	−	−	—
8447	*O. striata*	+	−	−	?	−	?	+	−	+	−	−	+	−	−	−	Pt
8444	*O. uncinata*	+	+	−	−	−	+	+	−	+	−	−	+	−	−	−	Dp, Pt
8913	*O. wilmsii*	+	+	−	+	+	−	+	−	+	+	+	−	−	−	−	Dp, Pt

Key: + = present; − = absent; Dp, delphinidin glycosides present; Pt, petunidin glycosides present; 1F luteolin 7-glucoside; 2F chrysoeriol glycoside.

*Flowers of *P. repens* also contained rutin.

Three isoflavones which occur widely in the Papilionoideae were detected in hydrolysed leaf extracts: daidzein, genistein and formononetin. Daidzein has previously been reported in *P. corylifolia* roots along with several isopentenyl isoflavonoids [13], but genistein and formononetin have not been described before from *Psoralea* s.l. Since these isoflavones were generally present in all those taxa examined (Table 4), they did not appear to have any taxonomic significance so they were not further considered.

Furanocoumarins

The results of examining hydrolysed extracts of leaf material for furanocoumarins is given in Table 5. Psoralen and angelicin were the only two compounds identified, but other furanocoumarins were also present (cf. Table 6). Psoralen occurs in all species of *Otholobium* and *Psoralea*, except for *P. repens* and *P. tenuifolia*. It does not occur in *Cullen*. Angelicin is present only in *P. affinis* and *O. rotundifolium*.

Essential Oils

Many of the plants surveyed have recognizably different odours and appear to contain volatile oils in special leaf glands. Results from a preliminary survey of essential oils in the tribe using TLC would suggest that the chemicals present provide good generic markers (Table 5 and Fig. 2). *Psoralea* is characterized by the combined presence of oils C, D and E. *Otholobium*, on the other hand, has a richer pattern of essential oils (compounds A–G) and although more types of oil are produced than in *Psoralea* it is still unclear whether there are any meaningful patterns within the genus. The genus warrants a broader study. The essential oil D, it should be pointed out, occurs in all

TABLE 4. PRESENCE OF PROANTHOCYANIDINS AND ISOFLAVONES IN HYDROLYSED EXTRACTS OF *CULLEN, OTHOLOBIUM* AND *PSORALEA*

Voucher No.	Spp.	Presence/absence of proanthocyanidins	Type of proanthocyanins present	Isoflavones present (where examined)
8746	*P. aculeata*	++	Dp	Da, Gen
9605	*P. affinis*	++	Dp	N.E.
9623	*P. affinis*	+	Dp	N.E.
9718	*P. affinis*	tr	Dp	N.E.
8250	*P. arborea*	++	Dp	Da, Gen
9740	*P. arborea*	+++	Dp, Cy	N.E.
9736	*P. arborea* × *P. effusa*	++	Dp, Cy	N.E.
9737	*P. arborea* × *P. effusa*	++	Dp, Cy	N.E.
8420	*P. asarina*	++	Dp, Cy	Da
8256	*P. axillaris*	+	N.E.	Gen
9163	*P. connixa*	tr	Dp	N.E.
9183	*P. connixa*	+	Dp	N.E.
9526	*P. connixa*	+	Dp	N.E.
8220	*P. effusa*	++	Dp	Da, Gen
9102	*P. effusa*	++	Dp, Cy	N.E.
9478	*P. effusa*	++	Dp	N.E.
9712	*P. effusa*	++	Dp, Cy	N.E.
9739	*P. effusa*	+++	Dp, Cy	N.E.
9509	*P. exile*	+	Dp	N.E.
9521	*P. exile*	++	Dp	N.E.
9602	*P. glabra*	+++	Dp, Cy	N.E.
8333	*P. imbricata*	++	Dp, Cy	Da
8211	*P. laxa*	++	Dp	Da, Gen
8093	*P. nodosa*	+	N.E.	Da
8445	*O. bolusii*	tr	N.E.	Da, Gen
9301	*O. bolusii*	+	Dp	N.E.
8403	*O. bracteolata*	–	–	Da, Gen, F
8237	*O. brevibracteatum*	–	–	Da, Gen, F
1579	*O. caffra*	tr	N.E.	Da?
8446	*O. candicans*	–	–	Da, Gen
9497	*O. candicans*	–	–	N.E.
8422	*O. fruticans*	–	–	Da
10555	*O. gazense*	tr	N.E.	N.E.
4832	*O. hirtum*	–	–	Da, Gen
8425	*O. hirtum*	–	–	Da
9360	*O. hirtum*	–	–	N.E.
9999	*Cullen obtusifolia*	–	–	N.E.
8258	*P. odoratissima*	tr	–	Gen
8219	*P. oligophylla*	++	Dp	Da, Gen
9184	*P. oreopolum*	tr	Dp	N.E.
9818	*P. oreopolum* × *P. ramulosa*	tr	Dp	N.E.
9185	*P. oreopolum* × *P. ramulosa*	tr	Dp	N.E.
9186	*P. oreopolum* × *P. ramulosa*	tr	Dp	N.E.
9562	*P. papillosa*	+	Dp	N.E.
8479	*P. pinnata*	++	Dp	Da
9757	*P. pinnata*	+	Dp	N.E.
8297	*P.* sp. cf *pinnata*	+	N.E.	Da, Gen, F
9292	*P.* sp. cf *pinnata*	++	Dp, Cy	N.E.
9742	*P.* sp. cf *pinnata*	+++	Dp	N.E.
9451	*P. pullata*	++	Dp, Cy	N.E.
9142	*P. ramulosa*	+	Dp	N.E.
–	*P. repens*	tr	Dp	N.E.

TABLE 4—*CONTINUED*

Voucher No.	Spp.	Presence/absence of proanthocyanidins	Type of proanthocyanins present	Isoflavones present (where examined)
8413	*P. repens*	–	–	Da, Gen
9441	*P. repens*	tr	Dp	N.E.
9681	*P. speciosa*	+	Dp	N.E.
9709	*P. speciosa*	++	Dp, Cy	N.E.
105	*P. tenuifolia*	+	N.E.	Da, Gen, F
9187	*P. tenuifolia*	tr	Dp	N.E.
9506	*P. tenuifolia*	+	Dp	N.E.
9715	*P. tenuifolia*	+	Dp	N.E.
9367	*P. verrucosa*	++	Dp, Cy	N.E.
9627	*P.* sp.	++	Dp, Cy	N.E.
9708	*P.* sp. nov.	tr	Dp	N.E.
9089	*O. obliquum*	–	–	N.E.
9090	*O. parviflorum*	–	–	N.E.
9369	*O. rotundifolium*	–	–	N.E.
9121	*O. salignum*	–	–	N.E.
9629	*O. sericeum*	++	Dp, Cy	N.E.
8363	*O. spicata*	tr	N.E.	Da, Gen, F
9307	*O. stachydis*	–	–	N.E.
9620	*O. stachyerum*	–	–	N.E.
8447	*O. striata*	–	–	Da, Gen
8444	*O. uncinata*	+	N.E.	Da, Gen, F
8913	*O. wilmsii*	–	–	Da, Gen
9093	*O. zeheri*	++	N.E.	N.E.
8923	*O.* sp.	–	–	Da, Gen

Key: + = presence; – = absence; tr = trace present; N.E. = not examined; Dp = prodelphinidin; Cy = procyanidin; Da = daidzein; Gen = genistein; F = formononetin.

species of the tribe studied so far, except in *O. hirtum*.

Discussion

The significance of the taxonomic value of chemical data for the identification and classification of the African Psoraleeae can be seen in Fig. 3. The two main genera *Psoralea* and *Otholobium* form distinct groups. The diagram also highlights the distinctiveness of *P. repens*. Unfortunately only *Cullen obtusifolia*, one of the six African *Cullen* species, was available for study. This genus is widespread in south-east Asia and Australasia, and given the apparent chemical distinctness of *C. obtusifolia* would merit a detailed chemical study.

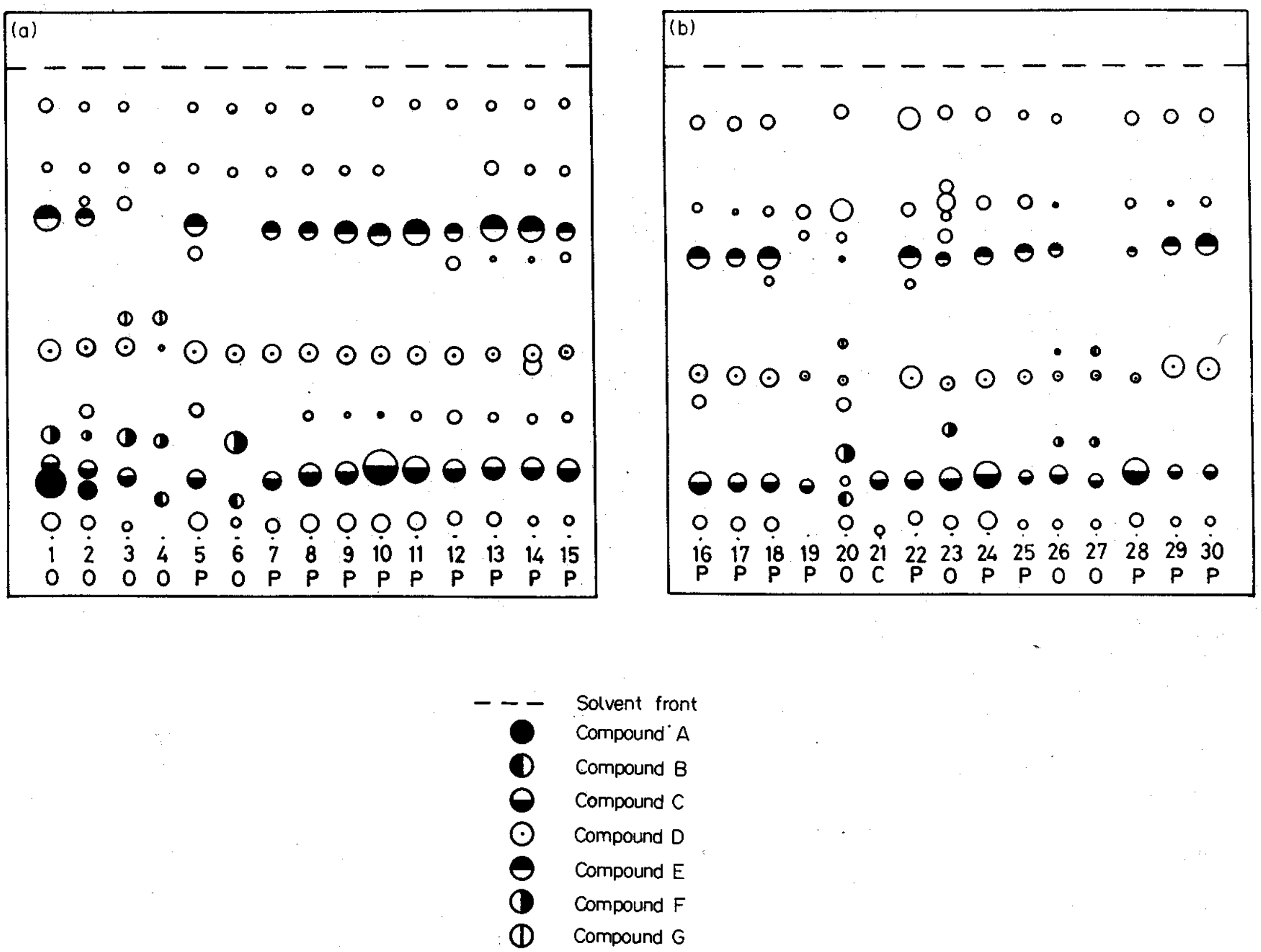
The most striking chemical differences between the genera lie in their as yet unidentified essential oils. The essential oils found in *Psoralea* occur universally in that genus, quite unlike the situation in *Otholobium* where there is a greater diversity of different oils. These need to be studied in more depth across the genus as they promise some valuable taxonomic markers and could be useful in unravelling the phylogeny of the group.

Another important chemical difference between the genera is the almost total absence of proanthocyanidins in *Otholobium*. This is a particularly interesting finding as one of us (C.H.S.) has observed in the field that *Psoralea* is rarely eaten by herbivores whereas *Otholobium* is. There is already evidence to suggest that proanthocyanidins are anti-feedants. Two examples are the inhibition of feeding on winter moth larvae in oak leaves [14] and cattle

TABLE 5. DISTRIBUTION OF FURANOCOUMARINS AND ESSENTIAL OILS IN LEAF EXTRACTS

Voucher No.	Spp.	Furanocoumarins a	b	c	d	Essential Oils A	B	C	D	E	F	G
9605	*P. affinis*	+	+	+	+	–	–	+	+	+	–	–
9623	*P. affinis*	++	++	+	+	–	–	+	+	+	–	–
9718	*P. affinis*	+	–	+	+	–	–	+	+	+	–	–
9740	*P. arborea*	++	+	–	+	–	–	+	+	+	–	–
9736 }	*P. arborea* ×	+	+	–	++	–	–	+	+	+	–	–
9737 }	*P. effusa*	+	+	–	+	–	–	+	+	+	–	–
9163	*P. connixa*	++	+	+	–	–	–	+	+	+	–	–
9183	*P. connixa*	++	++	+	+	–	–	+	+	+	–	–
9526	*P. connixa*	++	+	+	–	–	–	+	+	+	–	–
9102	*P. effusa*	++	++	–	+	–	–	+	+	+	–	–
9478	*P. effusa*	++	+	–	+	–	–	+	+	+	–	–
9712	*P. effusa*	+	+	–	++	–	–	+	+	+	–	–
9739	*P. effusa*	++	++	+	+	–	–	+	+	+	–	–
9509	*P. exile*	+	+	+	–	–	–	+	+	+	–	–
9521	*P. exile*	+	+	–	–	–	–	+	+	+	–	–
9602	*P. glabra*	++	++	++	–	–	–	tr	+	tr	–	–
9184	*P. oreopolum*	+	+	+	+	–	–	+	+	+	–	–
9185 } 9186 } 9187 }	*P. oreopolum* ×	+	+	+	+	–	–	+	+	+	–	–
	P. ramulosa	++	+	+	–	–	–	+	+	+	–	–
9562	*P. papillosa*	+	+	+	++	–	–	+	+	+	–	–
9757	*P. pinnata*	–	+	+	+	–	–	+	+	+	–	–
9292	*P.* sp. cf *pinnata*	++	+	+	–	–	–	+	+	+	–	–
9742	*P.* sp. cf *pinnata*	+	+	–	+	–	–	+	+	+	–	–
9451	*P. pullata*	+	+	++	–	–	–	+	+	+	–	–
9142	*P. ramulosa*	+	+	+	++	–	–	+	+	+	–	–
—	*P. repens*	–	+	++	+	–	–	+	+	+	tr	–
9441	*P. repens*	–	+	+	+	–	–	+	tr	tr	–	–
9681	*P. speciosa*	+	+	+	–	–	–	+	+	+	–	–
9709	*P. speciosa*	++	+	+	–	–	–	tr	+	+	–	–
9187	*P. tenuifolia*	+	+	+	+	–	–	+	+	+	–	–
9506	*P. tenuifolia*	+	+	+	–	–	–	+	+	+	–	–
9715	*P. tenuifolia*	+	/+	+	–	–	–	+	+	+	–	–
9367	*P. verrucosa*	–	+	+	–	–	–	+	+	+	–	–
9627	*P.* sp.	++	+	+	+	–	–	tr	tr	tr	–	–
9708	*P.* sp.	++	+	+	+	–	–	+	+	+	–	–
9301	*O. bolusii*	++	+	++	++	–	–	+	+	+	tr	–
9497	*O. candicans*	–	+	++	++	–	tr	–	+	tr	+	+
10555	*O. gazense*	+	+	++	++	–	–	+	+	+	?	–
9360	*O. hirtum*	+	+	++	–	–	–	?	–	+	+	tr
9089	*O. obliquum*	+	+	+	+	+	–	+	+	tr	+	–
9090	*O. parviflorum*	++	+	–	–	–	–	+	tr	tr	+	+
9369	*O. rotundifolium*	++	–	–	++	+	–	+	+	+	+	–
9121	*O. salignum*	+	+	++	++	–	tr	–	+	–	+	tr
9629	*O. sericeum*	++	++	+	–	–	tr	–	tr	–	–	–
9307	*O. stachydis*	+	+	+	+	–	+	–	tr	–	tr	tr
9620	*O. stachyenum*	+	+	+	+	–	–	+	+	–	–	–
9093	*O. zeyheri*	+	+	+	–	–	tr	–	tr	–	+	+
9999	*Cullen obtusifolia*	–	+	++	+	–	–	+	tr	–	–	–

Key: + = present; ++ = present as major component; – = absent; tr = trace.



No.		No.	
1	*O. rotundifolium*	16	*P. effusa*
2	*O. obliqua*	17	*P. arborea*
3	*O. parviflorum*	18	*P.* sp. cf *pinnata*
4	*O. zeyheri*	19	*P.* sp.
5	*P. effusa*	20	*O. hirtum*
6	*O. salignum*	21	*C. obtusifolia*
7	*P. ramulosa*	22	*P. pinnata*
8	*P. verrucosa*	23	*O. gazense*
9	*P. connixa*	24	*P. repens*
10	*P. connixa*	25	*P.* sp. cf *pinnata*
11	*P. oreopolum*	26	*O. bolusii*
12	*P. oreopolum* x *P. ramulosa*	27	*O. stachydis*
13	" "	28	*P. repens*
14	" "	29	*P. pullata*
15	*P.* sp. cf *pinnata*	30	*P. effusa*

FIG. 2. DIAGRAMMATICAL REPRESENTATION OF ESSENTIAL OIL RESULTS ON TLC PLATES. P, *Psoralea*; O, *Otholobium*; C, *Cullen*.

TABLE 6. PROPERTIES OF FURANOCOUMARINS AND ESSENTIAL OILS IN LEAF EXTRACTS

(i) Furanocoumarin data

Spot on TLC plate	Colour on spraying with 5% KOH in MeOH	Identity (if known)
a	green	angelicin
b	purple	psoralen
c	yellow	—
d	blue-green	—

(ii) Essential oil data

Spot on TLC plate	~ R_f×100 (in hexane:chloroform 3:2)	Colour with Vanillin/H_2SO_4 spray
A	10	dark purple
B	10	blue
C	15	green
D	40	pink
E	60	blue
F	30	purple
G	42	blue

(See also Fig. 2).

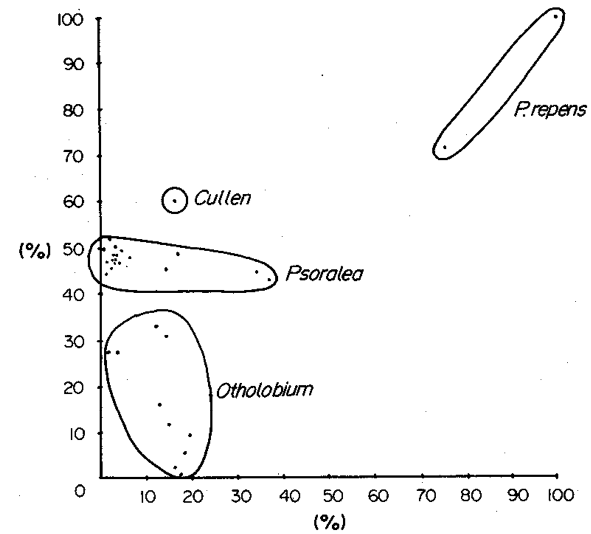


FIG. 3. RECIPROCAL AVERAGING OF CHEMICAL DATA.

browsing in *Lespedeza cuneata* [15], both of which were affected by variations in tannin levels.

This study began with the hypothesis that different classes of chemical compounds, from the same organ, should provide different levels of taxonomic information. It is clear that the chemical data obtained from this survey of the Psoraleeae supports the hypothesis. Thus, while furanocoumarins and flavonoids are most significant at the species level, essential oils and proanthocyanidins are more useful at the generic level. Isoflavones, which are entirely uniform within the group are probably only meaningful at tribal level. The importance therefore of studying a range of chemical characters in a chemotaxonomic study cannot be overstressed.

Experimental

Plant material. Dried plant material, collected by the second author (C.H.S.), was donated by the Botanical Research Institute, South Africa. Details are given in Table 1. Herbarium vouchers are housed in PRE and STE.

Flavonoids

Flavone/flavonol glycosides. These were initially examined by 2-dimensional P.C. in BAW (*n*-butanol–acetic acid–water, 4:1:5, top layer) and 15% aqueous acetic acid (see Fig. 1) using aqueous ethanolic extracts of dried leaf material. To identify the spots large scale separation of crude extracts of several species was carried out on 3 mm paper, variously in

TABLE 7. R_f, SPECTRAL AND COLOUR DATA FOR FLAVONOIDS IN *PSORALEA* (SEE ALSO FIG. 1).

Compound No.	λ_{max} (nm) in MeOH	R_f×100 in BAW	H_2O	15% HOAc	PhOH	Colour UV	UV+NH_3
1	256, 268, 349	48	—	18	—	dk	br yellow
2	272, 338	38	—	36	—	dk	yellow
3	269, 349	35	04	13	42	dk	dull yellow
4	272, 333	30	—	28	—	l blue	blue yellow
5	273, 334	35	15	40	75	dk	dull yellow
6	273, 345	32	—	45	—	dk	yellow
7	271, 349	51	07	30	65	dk	dull yellow
8	270, 333	58	—	60	—	dk	dull yellow
9	—	—	—	—	—	dk	l yellow
10	273, 335	50	46	71	—	dk	dull brown yellow
11	257, 358	46	—	55	—	dk	yellow
12	270, 342	45	—	34	—	l blue	blue yellow

br=bright; l=light; dk=dark absorbing.

BAW, 15% HOAc, CAW 1:1:0.1 ($CHCl_3$–HOAc–H_2O). Purified compounds were identified by standard methods (UV spectroscopy, R_f data, hydrolysis in 2 N HCl at 100° and analysis of products) and compared with authentic markers when available. See Table 7 for R_f data and colour reactions.

Isoflavones and proanthocyanidins. Hydrolysed extracts only were used. Dried leaf material was hydrolysed at 100° with 2 N HCl for 30 min. Isoflavones were extracted into EtOAc and run on silica gel GF 254 (Merck) TLC plates in 11% MeOH in $CHCl_3$. Identification was confirmed by running extracts as a small streak in the above system, eluting the isoflavone and comparison with authentic markers and UV spectroscopy. Proanthocyanidins were extracted from hydrolysed extracts (as above) into amyl alcohol. The presence or absence of proanthocyanidin was determined by a characteristic red colour on hydrolysis. Further analysis of species with a strong red extract was performed on microcrystalline cellulose plates in Forestal (HOAc–H_2O–HCl, 30:10:3) and co-chromatography with delphinidin and cyanidin.

Furanocoumarins. Hydrolysed extracts were partitioned with EtOAc, and the resulting extracts chromatographed on silica gel plates in Et_2O–toluene–15% HOAc (1:1:1), top layer). The colours of the spot patterns obtained were intensified by spraying with 10% KOH in MeOH. Separation of psoralen and angelicin was carried out by preparative TLC on fluorescent silica gel plates in CH_2Cl_2, followed by further purification in hexane–EtOAc–MeOH (5:5:1). These were identified by UV spectra, colour reactions and MS data [16, 17].

Psoralen, λ_{max} 242, 247, 291, 330 nm fluorescent blue in UV, intense blue+KOH, MS $[M]^+$ 186 ($C_{11}H_6O_3$ requires 186) [M−CO] 158. Angelicin λ_{max} 243, 247, 299, 325 sh nm dull absorbing in UV, bright green+KOH, MS $[M]^+$ 186 ($C_{11}H_6O_3$ requires 186) [M−CO] 158.

Essential oils. Crushed dried leaf material was extracted with Et_2O for 30 min, and the resultant extract chromatographed on silica gel plates in hexane–$CHCl_3$ (3:2). Developed plates were then sprayed with vanillin/H_2SO_4 reagent and heated to 105°. Compounds were recognized by their colour after spraying (Table 6). None of the spots corresponded with any of the commonly available mono- or sesquiterpenoids.

Further investigation of the major *Psoralea* essential oil was carried out. Compound C which gave a green colour with vanillin/H_2SO_4 spray, was purified on silica gel TLC plates in hexane–$CHCl_3$ 3:2. It had UV maximum at 262 nm, and IR spectra in $CHCl_3$ showed absorption bands at 1050, 1215, 2970, 2950 and 3030 cm^{-1}. It gave no colour reaction with 2.4 DNP, $FeCl_3$ or bromocresol blue, but reduced potassium permanganate solution. It could not be further characterized.

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