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THE FLAVONOIDS OF *PSORALEA* (LEGUMINOSAE)

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Abstract—The flavonoid patterns of thirty species of *Psoralea* were established by use of paper chromatography. A few compounds were identified only tentatively. Patterns of various species were generally similar and characterized by large amounts of di-*C*-glycosides, lesser amounts of 8-*C*- and 6-*C*-monoglycosides and small quantities of mixed *C*- and *O*-glycosides. Compounds not previously reported, tentatively identified as apigenin 6,8-di-*C*-glycoside, *O*-glucoside; luteolin 6,8-di-*C*-glycoside, *O*-glucoside; and chrysoeriol 6,8-di-*C*-glycoside, *O*-glucoside, occur in *Psoralea rigida*. An unidentified isoflavone occurs in two species.

INTRODUCTION

FOLLOWING a monographic study of the subgenus Pediomelum of the genus *Psoralea*[1] the flavonoids of leaves of thirty species of *Psoralea* were analyzed utilizing standard paper chromatographic techniques. The chemical data indicated a recurrent pattern of flavonoids, most of which were *C*-glycosyl flavones, to be characteristic of the genus. Isoflavones apparently occur in two species. *O*-glucosidic derivatives of vicenin, lucenin and chrysoeriol-6,8-di-*C*-glycoside are present in one species, but the locations of the *O*-glucosidic substituents are not established. Since *Psoralea* is morphologically a heterogeneous taxon (approaching the interspecific variation found in *Baptisia*), it was surprising to find the chemical interspecific variation so limited. In contrast, in the family Lemnaceae to which *Lemna* belongs, these species of duckweeds are often extremely difficult to separate morphologically, yet they are chemically distinctive. The same group of glycoflavonoids are involved in both groups of plants.[2]

RESULTS

A composite representation of the flavonoids and their tentative identifications of the thirty species (plus one variety) of *Psoralea* analyzed is shown in Fig. 1. Most of these are considered to be well established by means of spectral and chromatographic analyses after comparison with authentic standards of the compounds. Tables 1 and 2 provide data concerning the relative concentrations of all compounds listed in Fig. 1 summarized for each species from all collections analyzed. Figure 2, a two-dimensional chromatogram of a leaf extract of *Psoralea psoraloides*, illustrates the flavonoid pattern which is frequently encountered in the genus.

Compounds which are presumed to be new glycoflavonoids were detected and characterized within the limits of the techniques available with such small amounts of material.

* Part of this work was carried out while attending The University of Texas on a postgraduate NATO Studentship.

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[1] D. J. OCKENDON, *Southwestern Nat.* **10**, 81 (1965).

[2] J. W. MCCLURE and R. E. ALSTON, *Am. J. Botany*. In press (1966).

In *P. rigida* three *compounds*, 6, 16, and 23 yielded respectively lucenin, vicenin and chrysoeriol-6,8-di-*C*-glycoside upon hydrolysis with β-glucosidase. *Compounds* 6 and 16 were purified sufficiently to yield good u.v. absorption spectra (Fig. 3*a* and *b*). Benedict's reagent was sprayed onto the chromatograms of *P. rigida*, and the chromatograms were then examined in reflected u.v. light. In this test flavonoids containing ortho-dihydroxyl groups in ring B remain dark. The test indicated that *compound* 6 was not a 4′-glucoside. *Compounds* 6, 16 and 23 may therefore have a third β-*O*-glycosidic group linked directly to a *C*-glycosyl residue of lucenin, vicenin and chrysoeriol-6,8-di-*C*-glycoside respectively.

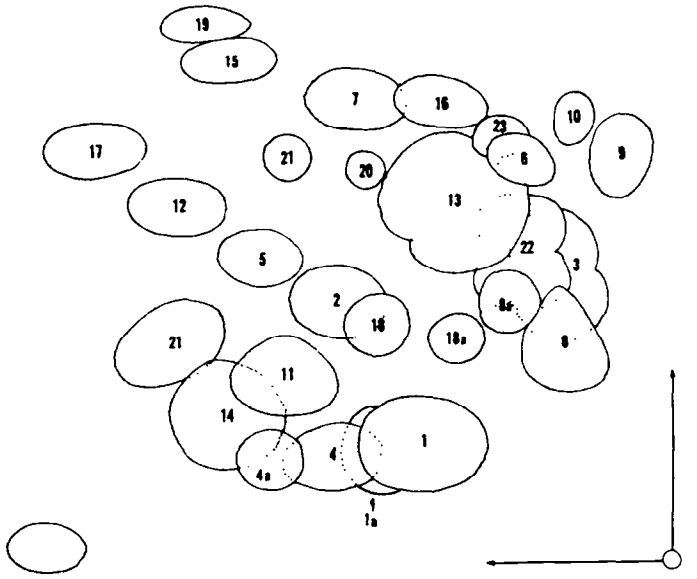

FIG. 1. COMPOSITE TWO-DIMENSIONAL PATTERN OF FLAVONOIDS FROM VARIOUS SPECIES OF *Psoralea*.

1. Orientin; 1a. scoparin; 2. homo-orientin; 3. lucenin; 4. luteolin-7-β-D-glucoside; 4a. chrysoeriol-7-β-D-glucoside; 5. isolutonarin; 6. lucenin-β-D-glucoside; 7. lutonarin (with saponarin?); 8. anthocyanin monoside (flowers only); 8a. anthocyanin monoside (flowers only); 9. petunidin-3,5-diglycoside (flowers only); 10. malvidin-3,5-diglycoside (flowers only); 11. vitexin; 12. saponaretin; 13. vicenin; 14. apigenin-7-β-D-glycoside; 15. isosaponarin; 16. vicenin-β-D-glucoside; 17. isoflavone glycoside; 18. apigenin-7-diglycoside; 18a. luteolin-7-diglycoside; 22. chrysoeriol-6,8-di-*C*-glycoside; 23. chrysoeriol-β-D-glucosyl-6,8-di-*C*-glycoside.

Many species produce *compound* 4a, which runs just ahead of orientin in the first solvent (Table 1). This compound was originally thought to be luteolin 7-β-D-glucoside since its R_f values and its absorption spectra were generally similar to that compound, and since hydrolysis with β-glucosidase yielded a compound running at the approximate position of luteolin. However, the Benedict test indicated that the compound was not luteolin since it yielded a pale-yellow color in reflected u.v. light. Furthermore, plants having *compound* 4a frequently showed (only after spraying with Benedict's solution) a double spot close to lucenin with the same peculiar pale whitish-yellow color (*compound* 22). Finally, *P. rigida* produced *compound* 23, also having the same unusual color after spraying with Benedict's solution. It has not been possible to obtain spectral data for *compounds* 22 and 23. Significantly, the u.v. spectra of *compound* 4a resemble those of luteolin with the Band I maximum in methanol shifted hypsochromically several millimicrons. *Compound* 4a was considered to be either

FIG. 2. TWO-DIMENSIONAL CHROMATOGRAM OF EXTRACT OF LEAF OF *Psoralea psoraloides* PHOTOGRAPHED IN U.V. LIGHT USED TO DETECT FLAVONOIDS.

TABLE 1. DISTRIBUTION OF FLAVONOIDS AMONG *Psoralea* SPECIES—LEAF EXTRACTS

Psoralea species	Coll.	Compound*														
		1 or 1a	2	3	4	4a	5	6	7	11	12	13	15	17	20	22
argophylla	117	0	+	+ + +	0	0	tr	0	0	+	+ +	+ + +	+	0	+	+
aromatica	105	0	+	+ + +	0	0	0	0	0	0	+	+ + +	+	0	+	+ +
californica	100	+	+	+ + +	tr	0	tr	0	0	0	tr	+ +	tr	0	+	tr
canescens	24	+ +	+	+ + +	0	+	tr	0	0	+	+	+ + +	+	0	+	0
cuspidata	91	tr	tr	+ + +	tr	0	tr	0	0	0	tr	+ + +	0	0	+	+
cyphocalyx	45	+ +	+	+ +	tr	0	tr	0	0	+	tr	+ + +	0	0	+	+
digitata	74	+	0	+ +	+	0	0	0	+	+.	0	+ +	+ +	0	+	tr
esculenta	116	+ +	+	+	tr	0	+	0	tr	+	+	+	0	+	+	0
lanceolata	115	0	+	+ +	+	0	tr	0	0	0	tr	+ + +	+	0	+	+
latestipulata	76	+	+	+ +	tr	0	+	0	0	tr	tr	+ +	+	0	+	tr
latest. var. *appressa*	38	+ +	+	+ +	0	0	tr	0	0	+	+	+ + +	+	0	+	0
linearifolia	9	+	tr	+ +	0	+	tr	0	0	tr	0	+ +	0	0	+	+ +
lupinellus	46	+	+	+ + +	+	0	tr	0	0	0	+	+ + +	0	0	+	+
macrostachya	111	0	+ +	0	0	+	+	0	0	0	+	+ + +	+	0	+	+ +
mephitica	96	+ +	+ +	+ + +	+	0	tr	0	0	0	+	+ + +	+	0	+	tr
oligantha	103	+ + +	+	tr	+	0	0	0	0	+ +	+ +	+ + +	0	0	+	0
onybrychis	47	+	+	+ + +	tr	0	tr	0	0	0	+	+ + +	tr	0	+	tr
orbicularis	113	+	tr	0	+	0	+	0	0	0	+	+ + +	+	0	+	+
pedunculata	2	+ + +	+	0	tr	+ +	+	0	0	+ +	+ +	+	0	0	+	0
physodes	99	+	+	tr	0	tr	+	0	0	+	+ +	+ + +	+	0	+	+
psoraloides	51	+ + +	+ +	+ + +	0	+	+	0	+	+ +	+ +	+ + +	+	0	+	0
reverchoni	101	+ + +	+ +	+ +	+	0	+ +	0	tr	0	+	+ +	+	0	+	tr
rhombifolia	8	+ +	+	0	0	tr	+	0	0	+	+	+ + +	+	0	+	0
rigida	114	+ +	0	+ +	+	0	0	+	0	+	0	+ + +	0	+	+	+
scaposa	32	+ + +	+ +	0	0	0	0	0	tr	+	+ +	+	+ +	+	+	0
simplex	11	+	+	+	0	0	0	0	0	+	+	+ +	0	0	+	tr
strobilina	110	0	+ +	0	0	+	tr	0	0	0	+	+ + +	+ +	0	+	+ + +
subacaulis	49	+	+	+ +	0	0	tr	0	0	+	+	+ +	+	0	+	tr
subulata	35	+ +	+	+ +	0	tr	0	tr	0	+	+	+ + +	+	0	+	tr
tenuiflora	66	+	tr	+ + +	+	0	tr	0	0	tr	tr	+ + +	tr	0	+	+
virgata	48	tr	+	+ +	0	0	tr	0	0	0	+	+ +	tr	0	+	tr

* For identification see Figure 1; 0 = absent; tr = trace; + = small amount; + + = moderate amount; + + + = large amount. *Compound* 16 (+) and *compound* 23 (tr) in *P. rigida* only; *compound* 18 (tr) in *P. latest.* var. *appressa* only; *compound* 18a (tr) in *P. digitata* only; *compound* 19 (+) in *P. argophylla* only.

TABLE 2. FLAVONOIDS OF *Psoralea* PRESENT IN FLOWER EXTRACTS

Psoralea species	Coll.	Compound*																
		1	2	3	4	4a	5	8	8a	9	10	11	12	13	14	15	21	22
cuspidata	69	0	0	++	tr	0	+	0		+	0	tr	++	++	0	+	0	+
latestipulata	39	0	0	0	0	0	tr	0		+	0	++	++	+	0	++	0	0
rhombifolia	68	+	+	0	0		tr	+++	+	0	0	+	+	+++	0	++	0	0
simplex	10	0	0	0	+		++	0		++	0	+	++	+++	+++	0	0	0
subacaulis	63	+	+	0	0		tr	+		++	0	+	++	++	0	+	0	0
virgata	73	0	0	+++	+		+	0		+	+	0	+	+	+++	+++	++	0

* For identification see Fig. 1; 0 = absent; tr = trace; + = small amount; ++ = moderate amount; +++ = large amount.

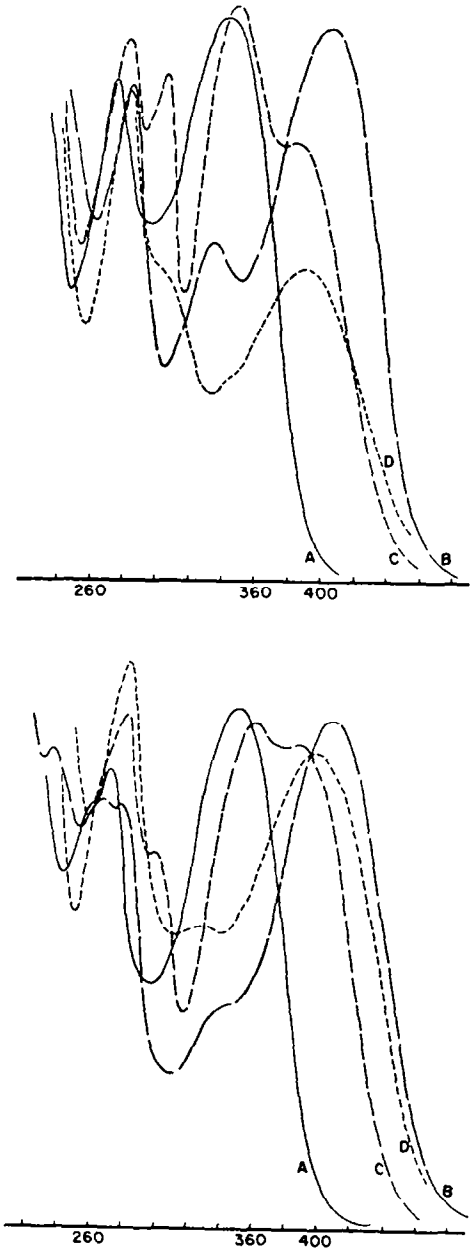

FIG. 3. ULTRA-VIOLET ABSORPTION SPECTRA OF (a) *compound* 6 AND (b) *compound* 16. Curve A obtained in spectral methanol; B obtained in sodium ethoxide; C obtained in $AlCl_3$, and D obtained in sodium acetate.

chrysoeriol (luteolin-3′-methyl ether)-7-β-D-glucoside, or diosmetin (luteolin-4′-methyl ether)-7-β-D-glucoside, but chromatographic and spectral comparison of the aglycone from *compound* 4a showed it was identical to chrysoeriol. *Compound* 4a is thus chrysoeriol-7-β-D-glucoside. Subsequently, *compound* 1a was detected and it is inferred to be chrysoeriol-8-*C*-glycoside (scoparin). *Compounds* 22 and 23 are inferred to be chrysoeriol-6,8-di-*C*-glycoside and chrysoeriol-7-β-D-glucosyl-6,8-di-*C*-glucoside by analogy to the corresponding luteolin derivatives. The chrysoeriol derivative (iso-scoparin) analogous to iso-orientin was not detected although it may have been present.

DISCUSSION

The various species of *Psoralea* exhibit a generally consistent flavonoid pattern characterized by, in every instance, the predominance of glycoflavonoids of the apigenin, luteolin, and chrysoeriol series (Tables 1 and 2). In general, the 6,8-di-*C*-glycosides such as vicenin

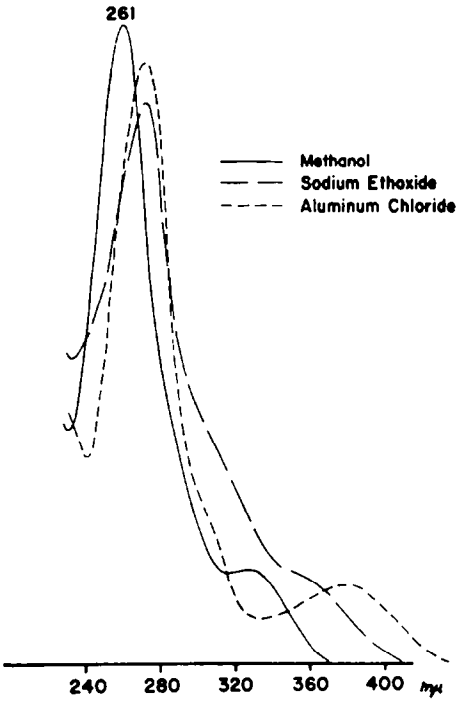

FIG. 4. ULTRA-VIOLET ABSORPTION SPECTRA OF *compound* 17 FROM *P. scaposa*.

were the most abundant flavonoids although rarely (as in *P. scaposa*) they were greatly reduced. Lucenin and chrysoeriol-6,8-di-*C*-glycoside appeared to be present generally in slightly lesser quantities than was vicenin and in a few instances lucenin was completely lacking. Often in such instances the chrysoeriol-di-*C*-glycosyl type replaced lucenin. Vicenin was never completely absent. When lucenin was absent, there was no correlation between its absence and the absence of either orientin or iso-orientin, so the distributions of these compounds shed no light on the possible sequential formation of 6-*C*-glycosides, 8-*C*-glycosides and 6,8-di-*C*-glycosides.

It is interesting that small amounts of the 7-β-D-glycosides of chrysoeriol and luteolin are present as are occasionally traces of the 7-diglycoside of luteolin, but the corresponding *O*-glycosides of apigenin are absent except in the flowers of *P. simplex* and *P. virgata* in which large quantities of apigenin 7-β-D-glucoside occur (Table 2).

Compound 5, which yields first iso-orientin, then a mixture of this and orientin upon hydrolysis, is still unidentified. It is always present in lower concentration than iso-orientin and it is yellow in u.v. light following Benedict's spray. It may be isolutonarin.

The designation of *compound* 17 as an isoflavone although tentative is firmly based on chromatographic and u.v. spectral data (Figure 4). The position of the compound on the chromatograms, its appearance under u.v. light and its u.v. spectra in various reagents are similar to but not identical to sphaerobioside.[3]

The presence of large quantities of apigenin 7-β-D-glucoside in the flowers, only, of *P. simplex* and *P. virgata* is interesting because of the otherwise limited occurrence of *O*-glycosides in the genus. *Psoralea rigida* is chemically the most distinctive species since in addition to the presence of *compounds* 6, 16 and 23 it lacks the otherwise extremely typical group of *compounds*; 2 (iso-orientin); 5 (possibly isolutonarin); and 12 (isovitexin).

The flavonoids of *Psoralea* represent an additional group of taxonomic characters which may characterize the genus, and which occasionally are extremely useful in delimiting species (as in *P. rigida*).

The degree of intraspecific variation in *Psoralea*, on the basis of our limited sampling, is quite similar to intraspecific variation in *Baptisia leucophaea*.[4]

METHODS

Dried material as prepared for herbarium specimens was available for the chromatographic analyses. Bulk material was available for a few species but in many instances the material was limited to plants of a single collection. *Psoralea reverchoni* occurs, for example, in a single county in Oklahoma. For most collections it was possible to analyze several plants from the same collections. In these instances only extremely minor differences were encountered. Intraspecific variation within those species in which several collections were available was limited to relatively minor quantitative variation.

Standard compounds were available for comparison of the major *C*-glycosyl flavones (e.g. vitexin, isovitexin, vicenin, orientin, iso-orientin and lucenin). The leaf material was extracted overnight on a shaker in absolute methanol and chromatographed in two directions by the descending method, first in tert-butanol–acetic acid–water (3:1:1 v/v) and then in 15% aq. acetic acid. This solvent system gives excellent separation of most *C*-glycosyl flavones. For spectral analyses the individual spots were cut out, eluted and rechromatographed variously as required for their separation from neighboring spots. The purified compounds were eluted with spectral methanol and analyzed with a Beckman Model DB Spectrophotometer in methanol, in sodium ethoxide, in sodium acetate and in aluminum chloride after the methods described by Jurd.[5]

For further confirmation of the glycosidic nature of *compound* 14 present in large amounts in the flowers of certain species, the glycoside was rechromatographed as a streak in tert-butanol–acetic acid–water (3:1:1 v/v), eluted with absolute methanol, filtered carefully

[3] H. RÖSLER, T. J. MABRY and J. KAGAN, *Chem. Ber.* **98**, 2193 (1965).
[4] B. G. BREHM and R. E. ALSTON, *Am. J. Botany* **51**, 644 (1964).
[5] L. JURD, *The Chemistry of Flavonoid Compounds* (Edited by T. W. Geissman). Macmillan, New York (1962).

through celite and hydrolyzed by prolonged heating over steam in methanol containing 0·5 N HCl. The reaction mixture was evaporated to dryness and the gas chromatographic analysis of the trimethylsilyl ethers of the sugar derivative completed by the method of Kagan and Mabry.[6] Enzymatic hydrolysis of this compound and *compounds* 6 and 16 was effected by the method of Harborne[7] using β-glucosidase purchased from Sigma Chemical Co., New York.

Acknowledgements—We wish to thank Dr. Heinz Rösler for the synthesis of chrysoeriol and Dr. Peter Raven for the collection of material.

[6] J. KAGAN and T. J. MABRY, *Anal. Chem.* **37**, 288 (1965).
[7] J. B. HARBORNE, *Phytochem.* **4**, 107 (1965).