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CHEMOSYSTEMATICS OF THE LEGUMINOSAE. FLAVONOID AND ISOFLAVONOID PATTERNS IN THE TRIBE GENISTEAE

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Abstract—Leaves of 128 species of 22 genera of the tribe of Genisteae *sensu lato* were surveyed for flavonoids and isoflavonoids. The isoflavones daidzein, formononetin, genistein and 5-*O*-methylgenistein were found variously in 73 species; they were detected in all except three of the genera of the Genisteae *sensu strictu*, but not in five associated genera. Leucoanthocyanidins were only found outside the Genisteae *s.l.* in *Hypocalyptus* and *Loddigesia.* Kaempferol, quercetin, luteolin or *C*-glycosylflavones based on luteolin or apigenin were noted in the majority of species; the distribution of the first three compounds in the genus *Genista* (46 species surveyed) largely followed sectional groupings. Luteolin was present in *Genista* mainly as the 7-glucoside but occurred exceptionally in three species of the section Spartioides as the 5-glucoside, galuteolin, earlier reported in the same family in *Galega* (Galegeae). The presence of galuteolin and 5-methylgenistein (in 17 spp.) in *Genista* and of glycoflavones in *Cytisus* (in 4 of 11 species) were the only clear-cut chemical differences between the morphologically similar genera *Cytisus* and *Genista.* The flavonoid data in general support Rothmaler's circumscription of the tribe and discount the more recent classification of Hutchinson.

INTRODUCTION

ALTHOUGH plants of the Leguminosae (or Fabaceae) are known to be extraordinarily rich, both qualitatively and quantitatively, in their flavonoid content, few deliberate surveys have been carried out in the family for chemosystematic purposes. Most phenolic studies to date (e.g. Refs. 1–3) have been confined to individual species or genera. As part of a general study of the distribution of flavonoids and isoflavonoids in the family, the phenolic constituents of the tribe Genisteae (subfamily Lotonoideae) were surveyed, principally in order to see if any chemical characters were available for use in delineating the tribal limits.

The general taxonomic treatments available of the tribe vary considerably in their circumscription; one of the most recent treatments is by Hutchinson,[4] who distributed the various genera of the Genisteae as recognized by Rothmaler,[5] into four tribes (Table 1). This treatment has already been criticized on morphological and distributional grounds[6] and chemical data thus might be particularly useful for supporting, or otherwise, these criticisms. The genera *Cytisus* and *Genista* are so closely similar morphologically that there is considerable difficulty in distinguishing their members[6] and another objective for this flavonoid study was to look for characters distinguishing these two genera.

[1] R. E. ALSTON and B. L. TURNER, *Biochemical Systematics*, Prentice-Hall, New Jersey (1963).
[2] W. F. GRANT and I. I. ZANDSTRA, *Can. J. Botany* **46**, 585 (1968).
[3] J. P. SIMON and D. W. GOODALL, *Australian J. Botany* **16**, 89 (1968).
[4] J. HUTCHINSON, *The Genera of Flowering Plants*, Vol. I, Oxford University Press, London (1964).
[5] W. ROTHMALER, *Fedde Repert* **53**, 137 (1944).
[6] P. E. GIBBS, *Notes R. Botan. Gdn. Edinb.* **27**, 11 (1966).

TABLE 1. TWO DIFFERING CLASSIFICATIONS OF THE GENERA OF BENTHAM AND HOOKER'S TRIBE GENISTEAE

After Rothmaler (1944)*

"Cytisus" group	"Genista" group
Laburnum	*Genista*
Podocytisus	*Chamaespartium* (=*Genistella*)†
Calycotome	*Echinospartum*
Lembotropis	*Lygos* (=*Retama*)†
Cytisus	*Spartium*
Chamaecytisus†	*Erinacea*
Chronanthus†	*Ulex*
Teline†	*Stauracanthos*
Petteria	

Hutchinson (1964)

Cytiseae	Genisteae	Laburneae	Lupineae
Ulex	*Genista*	*Laburnum*	*Lupinus*
Cytisus	*Spartium*	*Podocytisus*	*Argyrolobium*
Hypocalyptus	*Petteria*	*Hesperolaburnum*	
Loddigesia	*Erinacea*	*Calycotome*	
Echinospartum		*Adenocarpus*	
Nepa			
Stauracanthos			

* Modified to include all genera recognized by Tutin, Heywood *et al.*[24] in Vol. II of *Flora Europea*. The genera are considered to fall into two groups centred around *Genista* and *Cytisus* respectively (D. G. Frodin and V. H. Heywood, unpublished) but there is no single morphological character dividing the groups and several genera are intermediate.

† These genera are regarded by Hutchinson[4] as synonymous with *Genista* or *Cytisus*.

RESULTS

The results of surveying the flavonoids in hydrolysed extracts of leaf tissue of 128 species of the Genisteae *sensu lato* are presented in Tables 2 and 3. The surveys were carried out mainly on dried leaf samples from herbarium specimens, the material being that used in a recent taxonomic study by Gibbs[6] and thus fully authenticated. It included a unique collection of *Genista* material, specimens of 46 of the 72 recognized species (65 per cent) being available for study (Table 2). Fresh leaves were also examined for those species (approx. 15) accessible in this country and the results were in good agreement with those obtained from dried leaves.

Common flavonoids were identified by routine procedures involving paper chromatographic comparisons with authentic samples in four solvents. Isoflavones, which are less easily separated on paper, were tested for both on paper and by TLC on silica gel. The results obtained (Tables 2 and 3) considerably extend previous records of isoflavones in the tribe. Thus genistein has only been reported before in five species, in leaves, flowers or twigs of *Adenocarpus complicatus*,[7] *Genista tinctoria*,[8] *Lupinus polyphyllus*,[9] *Cytisus scoparius*[9] and

7 R. R. PARIS and G. FOUGERAS, *Compt. Rend.* **261**, 1761 (1965).
8 A. G. PERKIN and P. G. NEWBURY, *J. Chem. Soc.* 830 (1899).
9 L. HÖRHAMMER and H. WAGNER, *Arzneimittel. Forsch.* **12**, 1002 (1962).

TABLE 2. DISTRIBUTION OF FLAVONES, FLAVONOLS AND ISOFLAVONES IN THE GENUS *Genista*

Genista (sections, species)		Occurrence of: Isoflavones				Flavone	Flavonols*		
		Fm	Da	Gn	5MGn	Lu	Km	Qu	Fs
SUBGENUS GENISTA									
Genista	*G. tinctoria* L.	−	+	+	−	+	−	−	−
	G. januensis Viv	−	−	−	−	+	−	−	−
	G. lydia Boiss	−	+	+	+	+	−	−	−
	G. ramosissima (Desf) Poiret	−	−	−	−	−	+	+	+
	G. cinerea (Vill.) DC	−	+	−	−	−	−	−	+
	G. valentina (Willd ex Spreng.) Steudel	−	+	−	−	−	+	+	+
Spartioides	*G. florida* L.	−	+	−	−	−	+	+	+
	G. obtusirama Gay ex Spach	−	+	+	+	+†	−	−	+
	G. pseudopilosa Cosson	−	−	−	−	−	+	+	−
	G. teretifolia Willk.	+	+	−	−	+†	−	+	−
	G. sericea Wulf	−	+	+	+	−	+	+	+
	G. subcapitata Pančič	+	+	+	+	−	+	+	+
	G. villarsii Clementi	+	+	+	+	−	+	+	+
	G. albida Willd	−	+	+	−	−	+	+	−
	G. pilosa L.	−	+	−	−	+†	−	−	−
Erinacoides	*G. lobelii* DC	−	+	+	+	−	+	−	−
	G. pumila (Deb. & Rev.) Vierhapper	−	+	−	−	−	+	+	+
	G. baetica Spach	−	+	−	−	+	−	−	−
	G. salzmannii DC	+	+	+	+	−	+	−	−
	G. hystrix Lange	−	−	+	+	−	+	−	−
Scorpioides	*G. scorpius* (L.) DC	−	−	+	−	+	−	−	−
	G. corsica (Lois) DC	−	+	−	−	+	−	−	−
	G. carpetana Leresche ex Lang	−	+	−	−	+	−	−	−
	G. morisii Colla	−	+	+	+	+	−	−	−
	G. ferox (Poiret) Poiret	−	−	−	−	+	−	−	−
SUBGENUS PHYLLOBOTRYS									
Phyllobotrys	*G. anglica* L.	−	+	+	−	−	−	+	−
	G. falcata Brot.	−	+	−	−	−	+	+	−
Voglera	*G. micrantha* Ortega	−	−	−	+	−	−	+	−
	G. sylvestris Scop.	−	−	−	−	−	+	+	−
	G. aristata C. J. Presl.	−	+	−	−	−	−	+	−
	G. hispanica L.	−	+	+	+	−	+	−	−
	G. germanica L.	−	+	−	−	−	−	+	−
	G. hirsuta Vahl	−	+	−	−	−	+	−	−
	G. ulicina Desf	−	+	+	+	−	−	+	−
	G. tournefortii Spach.	−	−	−	−	−	+	+	
	G. triacanthos Brot.	−	−	+	+	+	−	−	−
	G. tridens (Cav.) DC	−	+	+	+	−	−	+	−
	G. cupanii Guss	−	+	+	+	−	−	−	−
SUBGENUS SPARTOCARPUS									
Spartocarpus	*G. radiata* (L.) Scop	−	+	+	+	−	+	+	−
	G. sessilifolia DC	+	+	+	−	−	−	−	+
	G. nissana Petrovic	+	+	−	−	+	−	−	−
	G. aetnensis (Rafin ex Biv) DC	+	+	+	+	−	−	−	−
	G. spartioides Spach	−	−	+	−	+	−	−	−

Table 2 — *continued*

Genista (sections, species)		Fm	Da	Gn	5MGn	Lu	Km	Qu	Fs
		Occurrence of							
		Isoflavones				Flavone	Flavonols*		
Acanthospartus	*G. acanthoclada* DC	–	+	+	–	+	–	–	–
Cephalospartus	*G. capitellata* Cosson	–	+	–	–	–	+	–	–
	G. umbellata (Desf.)Poiret	–	+	–	–	–	–	–	–

* Key: Fm, formononetin; Da, daidzein; Gn, genistein; 5MGn, 5-methylgenistein; Lu, luteolin; Km, kaempferol; Qu, quercetin; Fs, fisetin (?).

† Luteolin present in these species as the 5-glucoside, galuteolin.

Table 3. Distribution of flavonoids in the tribe Genisteae sensu latu

	Isoflavone	Leucoantho cyanidin	Flavone	Flavonol*
Genisteae Proper				
Adenocarpus foliolosus	Da, Gn, 5MGn	—	—	—
Chronanthus biflorus (Desf.) Frodin & Heywood	Gn, 5MGn	—	—	Km
Cytisus scoparius L. (Link.)	Gn	—	Gf	—
C. battandieri Maire	Da, Gn	—	Gf	Km
C. triflorus L'Herit	—	—	Gf	—
C. sessilifolius L.	—	—	Lu, Ap	—
C. patens L.	—	—	Lu, Ap	
C. cantabricus (Willk.) Reich. f.	—	—	—	Fs
C. grandiflorus DC	—	—	Gf	—
C. eriocarpus R. Br.	Gn	—	Lu	Fs
C. welwitschii	Da, Gn	—	Lu, Ap	Fs
C. purgans (L.) Boiss	Da, Gn	—	—	Km
C. commutatus (Willk.) Briq.	Da, Gn	—	—	Fs
C. procumbens (Waldst. & Kit. ex Willd.) Sprengel	—	—	Gf	Fs
C. platycarpos Maire	—	—	Lu, Ap	Fs
C. proliferus L.	Da, Gn, Fm	—	Lu	Qu, Km, Fs
Chamaecytisus smyrnaeus Boiss.	Da, Gn	—	Gf	Qu
C. heuffelii Nierzb.	—	—	Gf	Fs
C. austriacus L.	—	—	Gf	—
C. ratisbonensis (Schaeffer) Rothm.	—	—	Gf	—
C. hirsutus L.	—	—	Gf, Lu	—
C. spinescens (C. Presl.) Rothm.	—	—	Gf	—
C. albus (Hacq.) Rothm.	Gn	—	Gf	Fs
Genista (46 spp.) (See Table 2)	40 spp. +ve for Da, Gn, 5MGn, Fm	all –ve	16 spp. +ve for Lu	29 spp. +ve Qu, Km, Fs
Gonocytisus angulatus (L.) Spach.	—	—	Lu	—
Laburnum anagyroides Medicus	Da, Gn, 5MGn	—	Gf	—
Lembotropis nigricans (L.) Griseb.	—	—	—	Qu, Km, Fs

TABLE 3—*continued*

	Isoflavone	Leucoantho cyanidin	Flavone	Flavonol*
Petteria ramentacea (Sieber) C. Presl.	—	—	—	Fs
Teline stenopetala Webb	Da, Gn, 5MGn	—	Lu	Qu, Fs
T. rosmarinifolia Webb & Berth.	Da, Gn, 5MGn	—	—	Km
T. monspessulana (L.) C. Koch	Da, Gn, 5MGn	—	—	My, Qu, Km
T. linifolia (L.) Webb & Berth.	Gn	—	Gf	—
T. canariensis Webb & Berth.	Da, Gn, 5MGn	—	—	Qu, Fs
T. congesta Webb & Berth.	Da, Gn, 5MGn	—	—	Fs
T. spachiana (syn. *Genista canariensis*)	Da, Gn, 5MGn	—	—	Qu, Fs
Ulex parviflorus Poirret	Da, Gn, 5MGn	—	Gf	—
U. gallii Planchon	Da, Gn, 5MGn	—	Gf	—
U. minor Roth	Da, Gn, 5MGn	—	Gf	—
U. boivinii Webb	Da, Gn, 5MGn	—	Gf	—
U. europaeus L.	Da, Gn, 5MGn	—	Gf	—
Stauracanthos genistoides (Brot) Samp.	Da, Gn, 5MGn	—	Gf	—
Erinacea anthyllis Link	Da, Gn, 5MGn	—	—	—
Podocytisus caramanicus B & H	—	—	Lu	—
Lygos monosperma (L.) Heywood	Da, Gn, 5MGn	—	Gf	—
L. raetam (Forsk) Heywood	Da, Gn, 5MGn	—	Gf	—
Chamaespartium sagittale (L.) P. Gibbs	Da, Gn, 5MGn	—	Lu, Ap	—
Calycotome spinosa (L.) Link	Gn, 5MGn	—	Gf	—
C. villosa (Poir) Link	Gn, 5MGn	—	Gf	—
Crotolarieae Type				
Hypocalyptus obcordatus Thunb	—	Cy, Dp	—	My, Qu
Loddigesia oxalidifolia Sims	—	Cy, Dp	—	My, Qu, Km
Crotalaria pumila Rafin.	—	—	Gf	—
C. anseralis	—	Cy, Dn	Gf	—
Lupineae type				
Argyrolobium 5 spp.	all−ve	all−ve	Gf in 2	Qu in 1 spp. Km in 1 spp.
Lupinus 26 spp.	all−ve	all−ve	Gf in 15 spp. Lu/Ap in 12 spp.	Km/Qu in 13 spp.

* Key: Da, daidzein; Gn, genistein; 5MGn, 5-methylgenistein; Cy, leucocyanidin; Dp, leucodelphindin; Gf, glycoflavone; Lu, luteolin; Ap, apigenin; Qu, quercetin; Km, kaempferol; Fs, fisetin; My, myricetin.

Ulex parvifolius (syn. *U. nanus*)[7] and 5-*O*-methylgenistein in two species, *Laburnum anagyroides*[10] and *Genista hispanica*.[7] Daidzein, the distinctive fluorescence and mobility of which makes it easily distinguished in chromatographic surveys, does not, in fact, appear to have been reported before but it is expectedly widespread in the tribe (in 57 species). In view

[10] J. CHOPIN, M. BOUILLANT and P. LEBRETON, *Compt. Rend.* **256**, 5653 (1963).

of the rarity of 5-*O*-methylgenistein (it has only been reported before in two of the five species mentioned above), its identification in one plant available in quantity as fresh material, namely *Teline spachiana* (syn. *Genista canariensis*), was checked by more rigorous procedures; daidzein and genistein were also isolated at the same time (see Experimental).

Since only small quantities of leaf material were available for analysis, the negative results shown in the tables only indicate those species in which isoflavones do not accumulate. These species were rechecked for isoflavones and again found to lack them, but it is possible that more thorough examination of larger samples would indicate traces of isoflavones. In addition, no attempt was made during the present work to look for the more complex isoflavonoids, which occur in a number of other legume tribes.[11,12] Parallel findings on isoflavone distribution have been reported by Francis *et al.*[13] in *Trifolium*, where only 14 of 100 species surveyed accumulated isoflavone to the extent of 0·8–5 per cent in the leaves; the remainder contained less than 0·25 per cent.

C-Glycosylflavones are not easily distinguished in plant surveys from difficultly hydrolysable flavone *O*-glycosides[14] and, in the present work, identity was based simply on lack of hydrolysis and R_f comparison with vitexin, orientin and isomers and therefore may be subject to some degree of error. However, the glycoflavone orientin 3′-methyl ether has already been found in flowers of the common broom, *Cytisus scoparius*,[15] and further support for the occurrence of glycoflavones in the Genisteae was obtained during the present work, when analysis of *Cytisus battandieri* petals yielded vitexin and isovitexin.

Unhydrolysed leaf extracts of most species were also examined chromatographically and revealed the presence of a number of glycosidic characters, which could be of taxonomic value. One particularly striking compound with blue to green fluorescence in u.v. light (without and with ammonia) was noted in three *Genista* species, all in the same section of the subgenus Genista (Table 2). It was identified as luteolin 5-glucoside, galuteolin, by direct comparison with material from *Galega officinalis* seed,[16] the only other known source in the Leguminosae. This taxonomically interesting glucoside has recently been detected in the Umbelliferae[16] but is otherwise very vare.

DISCUSSION

The chemical data obtained during this survey have some bearing on taxonomic problems in the Genisteae, although two of the five flavonoid types examined, i.e. flavonol and glycoflavone, are of sporadic occurrence and thus of little use. The three other classes have a more interesting distribution pattern and provide chemical support for the separation of *Hypocalyptus* and *Loddigesia* from the Genisteae proper, as proposed by Gibbs,[6] and their possible uniting with *Crotalaria* in the Crotalarieae. Thus, these are the only taxa giving positive leucoanthocyanidin tests, as does *Crotalaria*; furthermore, they lack isoflavones and flavones, which occur so regularly in the majority of other Genisteae species. The chemical data also show (e.g. absence of isoflavone) that *Argyrolobium* and *Lupinus*, which were regarded as

11 W. D. OLLIS, in *Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), Pergamon Press, Oxford (1962).
12 J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, London (1967).
13 C. M. FRANCIS, A. J. MILLINGTON and E. T. BAILEY, *Australian J. Agr. Res.* **18**, 47 (1967).
14 J. B. HARBORNE, *Phytochem.* **4**, 107 (1965).
15 L. HÖRHAMMER, H. WAGNER and P. BEYERSDORFF, *Naturwissenschaften* **17**, 392 (1962).
16 J. B. HARBORNE, *Phytochem.* **6**, 1569 (1967).

part of the Genisteae by Bentham and Hooker,[17] should be separated from the tribe, as proposed by both Rothmaler[5] and Hutchinson.[4]

The flavonoid characteristics of the Genisteae, assuming the above genera are removed, become very uniform. They are: high concentration of isoflavones, absence of leuco-anthocyanidin, regular occurrence of glycoflavones and the flavonols kaempferol and quercetin (but not myricetin, cf. *Hypocalyptus*, etc. in Table 2) and presence of the flavone, luteolin, which in phylogenetic terms is a more advanced character than flavonol. The only individual substance of note is 5-*O*-methylgenistein, which, since it has not yet been detected elsewhere in the Leguminosae, may be said to characterize the tribe.

How far accumulation of leaf isoflavone is useful for distinguishing the Genisteae from members of other tribes is not yet clear, since isoflavonoids have been recorded in all but one of the eleven tribes of the subfamily Lotonoideae.[12] A limited survey of genera outside the Genisteae has, however, indicated that the ability to accumulate simple isoflavone varies considerably and does not, as yet, characterize any other tribe. For example, while *Ougenia dalbergioides* leaves are known to contain isoflavanones,[18] a survey of other Hedysareae (13 spp. *Hedysarum*, 3 spp. *Desmodium* and 18 spp. *Onobrychis*) failed to yield any positive record for leaf isoflavone. Again, the occurrence of orobol (5,7,3′,4′-tetrahydroxyisoflavone) in the root of *Lathyrus montanus* Bernh. (syn. *Orobus tuberosus*) of the Viceae is well recorded[19] but an examination of the leaves of other *Lathyrus* species and of representative members of *Pisum*, *Vicia* and *Cicer* failed to show any other isoflavone accumulators. Even the Pod-alyrieae, which contains the genus *Baptisia* most species of which are rich in isoflavones,[1,20,21] has genera without these substances; thus, a survey showed the presence of daidzein (and/or formononetin) in *Pickeringia montana*, *Piptanthus nepalensis* and in five *Thermopsis* species, but there were negative records for *Phyllota* (1 sp.), *Pultenea* (3 spp.), *Brachysema* and *Daviesia* (2 spp.). Finally, in the Trifolieae, which has *Trifolium* with isoflavones in 14 of 100 species,[13] a survey showed *Ononis* with 28 species all positive but also *Medicago* with 24 species all negative.

The definition of generic limits within the Genisteae and particularly the distribution of species between *Cytisus* and *Genista* has caused considerable trouble to taxonomists.[6] The chemical results as regards the flavonoids are not unexpectedly of little help here. There are a number of differences between the genera in their flavonoids (i.e. glycoflavones only in *Cytisus*, 5-*O*-methylgenistein and luteolin 5-glucoside only in *Genista*) but none of these characters is consistent in its distribution. The chemical data are perhaps of more value at the intrageneric level; certainly, the present survey of *Genista* shows a number of correlations between flavonoid distribution and sectional groupings (see Table 1). The flavone vs. flavonol pattern is particularly striking in the subgenus Genista with luteolin occurring in all three species of section Genista and all five species of section Scorpioides, but being replaced with only few exceptions by kaempferol and/or quercetin in other sections.

While the flavonoid data are of some assistance in distinguishing *Cytisus* and *Genista*, they are of less value for separating the two main generic groupings in the tribe, as recognized by Frodin and Heywood (unpublished) (See Table 1). The taxa of the Genisteae indeed are very difficult to treat by conventional methods and a multivariate taxonomic approach, which

[17] G. Bentham and J. D. Hooker, *Genera Plantarum* (1862).
[18] V. K. Ahluwalia, G. P. Sachdev and T. R. Seshadri, *Indian J. Chem.* **4**, 250 (1966).
[19] C. Charaux and J. Rabate, *Bull. Soc. Chim. Biol.* **21**, 1330 (1939).
[20] K. R. Markham, T. Mabry and T. W. Swift, *Phytochem.* **7**, 803 (1968).
[21] K. R. Markham and T. J. Mabry, *Phytochem.* **7**, 791 (1968).

has already been applied to *Cytisus* and its allies,[22] is called for. It is to be hoped that the flavonoid data here reported and the equally extensive alkaloidal data of Fougeras and Paris,[23] will be incorporated with morphological and cytological features in such an approach.

EXPERIMENTAL

Plant Material

Dried leaf tissue was obtained from specimens in the University of Liverpool herbarium by kind permission of the Curator. The identity of almost all specimens of the Genisteae has recently been checked by P. E. Gibbs[6] and plants used have been labelled by the author. Full details of plant specimens examined outside the Genisteae are not given here, for the sake of brevity, but these will be furnished on request by the author. Fresh leaf and flower were obtained from plants growing at the University of Liverpool botanic gardens. Material of *Teline*, *Loddigesia*, *Hypocalyptus* and *Crotalaria* was kindly provided by Dr. P. E. Gibbs.

Flavonoid Identifications

Leaf tissue was immersed in 2 N HCl, hydrolysed for 0·5 hr at 100° and the cooled solution extracted into ethyl acetate; coloured solutions were further extracted with amyl alcohol. Flavonols, flavones, glycoflavones and anthocyanidins were identified by chromatographic comparison with authentic material in four solvents. Trace amounts of a yellow fluorescent flavonol with R_f similar to fisetin were noted in many extracts (see Tables 2 and 3) but the presence of this flavonol in the tribe has yet to be confirmed.

The presences of isoflavones were noted on paper chromatograms of hydrolysed extracts as bright blue fluorescent spots (daidzein derivatives) in u.v. light with ammonia or as dark absorbing spots in short u.v. light (genistein derivatives). They were clearly separated from other flavonoids on papers run in 50 per cent HOAc or in 5 per cent HOAc. Presence of isoflavones was confirmed in all cases by TLC on silica gel using a range of solvents, the most useful being 11 % MeOH in $CHCl_3$. The isoflavones appeared on plates as dark-brown or purple absorbing spots in short u.v. light, giving a blue colour in visible light when sprayed with Folin–Ciocalteau reagent. Genistein derivatives also gave a bright blue colour in u.v. light when sprayed with alcoholic $SbCl_3$. Typical R_f in 11% MeOH in $CHCl_3$ are: daidzein, 0·47; genistein, 0·54; 5-methylgenistein, 0·39; formononetin, 0·73. Other simple isoflavones chromatographed in this system had different values.

Genistein, daidzein and 5-*O*-methylgenistein were isolated in crystalline form from a hydrolysed extract of *Teline spachiana* leaf by preparative TLC in 11% MeOH in $CHCl_3$. Genistein and daidzein were identified by detailed chromatographic and spectral comparison with authentic samples. Molecular wt. by mass spectra of daidzein was 254 (required for $C_{15}H_{10}O_4$ 254). The isolated 5-methylgenistein had m.p. 310–312° decomp. (lit m.p. 316° (decomp)) (mol. wt. by mass spectra 284, $C_{16}H_{12}O_5$ requires 284) and its spectral properties were identical to those reported in the literature (λ_{max}^{EtOH} 256, inf. 282; λ_{max}^{EtONa} 267, 303 nm; $+AlCl_3$, no shift). On demethylation with pyridinium chloride, it gave genistein and on reductive cleavage, it yielded phloroglucinol monomethyl ether.

Luteolin 5-glucoside was isolated from 70 per cent EtOH extracts of *Genista pilosa* leaf samples (present in all of twelve specimens examined) and identified by colour reactions, spectral and chromatographic comparison with authentic galuteolin.[16] Vitexin and isovitexin were obtained from hydrolysed petal extract of *Cytisus battandieri* by chromatography on paper in and identified by direct comparison with authentic samples.

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