The Flavonoids of *Lotus corniculatus*

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**Introduction**

Since the first studies published in the fifties and sixties (Nakaoki et al., 1956; Harney and Grant, 1964; Bate-Smith, 1965), many authors have investigated the flavonoid chemistry of *Lotus corniculatus* (Table 1 and Table 2) and demonstrated the richness and diversity of flavonoid compounds in this species. Some authors have examined the variation with altitude of the flavonoid content of *Lotus corniculatus*. Others have used flavonoids as speciation markers within the *Lotus corniculatus* complex.

**Flavonoids**

Flavonoids are a large class of secondary plant metabolites of widespread occurrence in higher plants (more than 6000 known structures; Harborne and Baxter, 1999). Of the two most frequent subclasses, flavones and flavonols (Figure 1), only derivatives of flavonols have been identified in *Lotus corniculatus* (3-OH free or substituted by a sugar).

A recent study (Sarelli et al., 2003) has revealed that *Lotus corniculatus* also contained insignificant amounts of two isoflavonoids at budding and flowering stages: formononetin and biochanin A (Figure 2). These two phytoestrogens are in too small a quantity to have adverse effects on reproductive functions.

**Figure 1.** Structure of flavones and flavonols

![Flavones](image1)

Flavones: $R_3=H$
Flavonols: $R_3=OH$

**Figure 2.** Structure of isoflavonoids

![Isoflavonoids](image2)

Formononetin: $R_1=H$, $R_2=OH$, $R_3=Ome$
Biochanin A: $R_1=R_2=OH$, $R_3=OMe$
Aglycones (Table 1)

As in most plants, flavonoids of *Lotus corniculatus* are not present as free aglycones: in the different studies reported in Table 1, the aglycones were obtained after acid hydrolysis of the plant material (leaves or flowers). The 10 compounds mentioned in the different studies are all derivatives of kaempferol and quercetin. The species is particularly rich in 5-desoxyflavonols (structures characteristic of the polyphenoic profile of *Fabaceae*) and, in flower material only, in 8-hydroxy or 8-methoxy flavonols. A LC-MS (Liquid Chromatography-Mass Spectrometry) study with different detection modes recently published by de Rijke et al. (2004) did not reveal the presence of these methoxy and desoxy derivatives as free aglycones.

**Table 1.** Flavonoid aglycones identified in *Lotus corniculatus* (after acid hydrolysis of the plant material).
**Monosides and Diosides (Table 2)**

*Lotus corniculatus* is particularly characterized by the great diversity of its flavonol glycoside content (12 monosides and 10 diosides have been reported to date). In 1969, Harborne reported on the presence of 7-O-methyl-gossypetin, but the information was inaccurate and was further rectified; in 1978, the same author corrected the identification to 8-O-methylgossypetin (or 8-methoxyquercetin). Although the presence of isorhamnetin has been reported in the literature (Hasan, 1976; Jay et al., 1978), no glycoside based on this molecule has been evidenced to date. The plant seeds are particularly rich in flavonol glycosides (5 monosides and 6 diosides). The recent study performed by de Rijke et al. (2004) has shown that the 2 major compounds present in *Lotus corniculatus* are two isomers of 3-O-rhamnoglusosyl-kaempferol.

**Flavonoids and flower color**

For Jay and Ibrahim (1986), the predominant flavonoids (present as glycosides) in the flower buds of *Lotus corniculatus* are kaempferol and quercetin. Small amounts of gossypetin are also present. The yellow coloration of flower petals is concomitant with the accumulation of large amounts of gossypetin and corniculatusin and much smaller amounts of sexangularetin. For these authors, gossypetin and corniculatusin are mostly responsible for the intensity of the yellow color during flower development.

In some individuals, the flowers have entirely yellow keel petals ("light-keeled Lotus"). In other, less common individuals, the keel petals are red-brown ("dark-keeled Lotus"). Several authors, like Jones and Crawford (1977), have shown a cline in keel color frequencies in different parts of Western Europe (England and Wales, Denmark, West Germany, the Netherlands, Austria, France, Spain and Sweden). These authors have also shown the lack of relationship between the color of keel petals and cyanogenesis.

A study by Jones et al. (1986) of the relation between keel color, insect visits and reproductive output has indicated that "keel color does not influence pollinator foraging behavior nor colonization by flower insects". Their data show that the phenotypes do not differ in pod and seed production.

**Relation between flavonoids and altitude**

An article published in 1972 by Ceruti et al. investigated the total flavonoids of *Lotus corniculatus* flowers collected at various altitudes in Northern Italy. After extraction, they quantified their flavonoid content by measuring the Optical Density (OD) at 350 nm (the wavelength corresponding to maximum absorption of kaempferol and quercetin glycosides). Their measures revealed that variations of the flavonoid content of the plant (OD, maximum value = 1) correspond to 3 areas:

* from 230 to 600m, OD increased from 0.2 to 0.4
* from 600 to 1600m, OD remained stable at approximately 0.4
* from 1600 to 2600m, OD increased from 0.4 to 0.7
Table 2. Flavonoid glycosides identified in *Lotus corniculatus*

<table>
<thead>
<tr>
<th>Plant organs and references</th>
<th>Seeds</th>
<th>Leaves</th>
<th>Flowers</th>
<th>Aerial parts</th>
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<tr>
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<td>Glucosyl-3-Kaempferol</td>
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(*) for these diosides, the aglycone and the sugars have been identified but the exact positions of the sugars on the aglycone skeleton remain to be determined (3 or 7).

References for Table 1 and Table 2.

1 Harney and Grant, 1964       6 Waleska and Strzelecka, 1984
2 Bate-Smith, 1965            7 Gorski *et al.*, 1975
3 Jay *et al.*, 1978           8 Harborne, 1969
4 Hasan, 1976                 9 Nielsen, 1970
5 Reynaud *et al.*, 1982       10 Nakaoki *et al.*, 1956
From these findings, they concluded that the upregulation of the flavonoid content is related with the quantity and the quality of sun radiations received by Lotus corniculatus individuals as a function of altitude. For these authors, the stability observed between 600 and 1600m would be due to the fact that individuals were collected in forest habitats.

Several years ago, in my thesis work, I assessed the ratio of flavonoid diosides to monosides (D/M) in Lotus corniculatus samples collected at various altitudes in two French regions (Massif Central and Alps). Variations of the D/M ratio were not similar in the two areas. In plants collected between 600 and 1400m in the Massif Central (ancient hercynian massif), the D/M ratio varied from 2.3 at 600m to 14.8 at 1600m, whereas in the Alps (a recent mountain range) the ratio varied from 5.5 at 1200m to 0.6 at 1800m. My conclusion was that Lotus corniculatus populations of the Massif Central correspond to early plant settlements, probably all tetraploids, with more evolved flavonoid chemistry and a strong capacity to synthesize diosides. In the Alps, the plant populations are more recent (recolonization after the last ice age), with tetraploid individuals at lower altitudes and diploid individuals (sometimes named Lotus alpinus) at higher altitudes. The capacity of these high altitude diploid populations to synthesize diosides is reduced.

**Flavonoids as speciation markers**

We have studied 412 individuals collected from diploid and tetraploid populations of Lotus corniculatus growing in the Southern French Alps (Mercantour, Ventoux and Lure Mountain). After extraction and HPLC analysis of their polyphenolic content, a polyphenolic "fingerprint" of each individual was obtained. A statistical analysis of the 412 HPLC profiles led us to the following conclusions:

- in this geographic area, at low altitudes, there are tetraploid plants with a rich and diversified polyphenolic content.
- at higher altitudes, where conditions are more unstable, we find two poor and homogeneous polyphenolic profiles corresponding to two types of diploid Lotus corniculatus: one type is characteristic of the inner Alps and the other one of the western Alps (Mont Ventoux, for instance).

Results of the different studies described above have been published in this journal and elsewhere (Reynaud and Jay, 1989; 1990; 1991; Jay et al., 1991; Reynaud et al., 1991).

**Conclusion**

*Lotus corniculatus*, a plant with high agronomic value in some regions of the world, is also of particular interest for more theoretical research due to its rich flavonoid content. Though only based on kaempferol and quercetin flavonols, the rich and numerous flavonoid compounds synthesized by the plant can be used to study speciation in the Lotus corniculatus complex or variations of flavonoid chemistry as a function of altitude. The increasing sensitivity of isolation and identification methods should make it possible to identify occurrences of new, yet undisclosed flavonoids in this species.
Acknowledgements
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References


**Flavonoids of Lotus corniculatus**


REYNAUD J. and JAY M. 1990. Evolution of *Lotus corniculatus* s.l. populations in the Mercantour (French Prealps). Lotus Newsletter, 21, 24-28


