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PHYTOSOCIOLOGICAL AND GEOGRAPHICAL VARIATION OF FLAVONOID GLYCOSIDES IN *CHAEROPHYLLUM AUREUM**

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Key Word Index—*Chaerophyllum aureum*; Umbelliferae; flavonoid glycosides; geographical variation; phytosociology; south-western Alps.

Abstract—Specimens of *Chaerophyllum aureum* collected from two types of alpine plant community, high altitude manured meadows (Triseto-Polygonion) and lower elevation tall grass prairies (Adenostylion), exhibited a general correlation of their glycosidic flavonoid pattern with their phytosociological origin. However, the flavonoid profiles of plants from each of the two communities varied from one collection area to another, which emphasized the geographical parameter. Thus, all the specimens from the Triseto-Polygonion growing in the Ubaye Valley and Val Grana lack lutelin 7-diglucoside, a characteristic constituent of specimens from the Adenostylion. On the other hand, in the Tinée Valley, where all *C. aureum* specimens lack luteolin 7-diglucoside, diosmetin 7-glucoside is present in *C. aureum* populations occurring in the Adenostylion but not those in the Triseto-Polygonion.

INTRODUCTION

In a previous paper [1] were reported the results of an exploratory study of the flavonoid glycoside variation occurring in six plant species, including *Chaerophyllum aureum*, collected in two types of alpine plant community growing in the south-western Alps at the subalpine level (1500–2100 m). For each of these species, the variation appeared to be correlated with the geographic origin of specimens. However, with two species, a further correlation with the phytosociological parameter was observed. In this previous study, plants were collected from only eight stations, covering a relatively wide area (*ca* 120 km diameter) of the southern Alps.

In order to eliminate the influence of the geographic parameter, the analysis presented here for *Chaerophyllum* was performed on plant material collected from a larger number of stations from a more restricted area (40 km diameter). It comprises the following three districts: Val Grana (Italy), Vallée de l'Ubaye and Vallée de la Tinée (France). Plants were again collected from two types of alpine plant community: high altitude manured meadows of Triseto-Polygonion and lower altitude tall grass prairies of Adenostylion. Data obtained for specimens of the earlier study are presented again, some with minor corrections resulting from a new chemical investigation.

The analyses were carried out on dried leaves from 2–6 plants collected from as small an area as possible (within a 1 m diameter area) and at the same stage of development (early flowering). After drying, the leaves of the specimens collected in each station were combined to form a 'sample-population' (or a 'sample-station'). After a standard extraction procedure, the flavonoid profile for the samples was obtained on a 2D-polyamide TLC [2]. Flavonoid patterns were compared directly from the plates and with the help of a photographic process [3]. The geographical and phytosociological origins of specimens are listed in Table 1.

RESULTS

The 20 *Chaerophyllum aureum* populations surveyed showed significant variation in their flavonoid patterns (Table 2). Thus, among the populations, No. 16 had the most simple pattern with only three flavonoid glycosides, whereas up to nine glycosides were observed in the other samples. In total 14 flavonoids were found. Two glycosides were universal to all populations: luteolin 7-glucoside, previously reported in a number of *Chaerophyllum* species including *C. aureum* [4], and quercetin 3-glucoside. Flavonoids **8** and **11–14** were found in insufficient quantities to allow identification and **10** (a luteolin 7-diglycoside) appeared in only a few populations. The distribution of the remaining compounds is more interesting because they appeared in some populations as major constituents but were totally absent from others; this presence or absence was also correlated with station parameters. These constituents luteolin 7-glucuronide, luteolin 4′-glucoside, apigenin 7-glucoside, diosmetin 7-glucoside, luteolin 7-diglucoside and the glycoside, **7**, which was tentatively identified as an apigenin 7-diglucoside (apigenin and glucose were found but the sugar ratio was not determined precisely) and was present in a smaller amount than other discriminating constituents. In Table 2, populations are listed in order to show the distinctions between chemical types.

The last three populations (15–17) exhibit a distinct flavonoid pattern in which luteolin 7-glucuronide, diosmetin 7-glucoside and apigenin 7-glucoside, widespread among the other populations, were totally absent, but luteolin 4′-glucoside was recorded for the first time in the survey. However, luteolin 7-diglucoside was present in populations 15 and 17 and lacking in population 16.

Based upon the distribution of some major glycosides, mainly diosmetin 7-glucoside and luteolin 7-diglucoside, the remaining populations may be divided as follows:

*The work reported in this paper formed part of the thesis to be submitted by the author for the degree of Doctorat d'Etat.

Table 1. Origin of the surveyed specimens

(a) Collection on 2/7/79
- (1) Val Grana (Italy), under Chiappi Village, Triseto Polygonion, 1730 m
- (2) Val Grana (Italy), under Chiappi Village, fragmentary Adenostylion [*ca* 20 m distant from (1)], 1730 m
- (3) Val Grana (Italy), under Chiappi Village, Adenostylion, 1730 m
- (4) Vallée de l'Ubaye, between Col de Larche and the Larche Village, Triseto-Polygonion, 1660 m
- (5) Vallée de l'Ubaye, Vallon du Crachet, 'Bois de la Traverse', Adenostylion, 1970 m
- (6) and (7) Vallée de l'Ubaye, front of Melezein Village, along the Riou Mounal brook, Triseto-Polygonion, 1720 m. This meadow is very large (*ca* 4000 m^2); (6) and (7) represent two distinct points within the station, *ca* 50 m apart
- (8) Vallée de l'Ubaye, front of Melezein Village, Triseto-Polygonion, 1700 m
- (9) Vallée de l'Ubaye, Col de Restefond road from Jausiers, front of 'Halte 2000' Bar and above the 'Terres Pleines' brook, Adenostylion, 1960 m
- (10) Vallée de l'Ubaye, Col de Restefond road from Jausiers, close to 'Halte 2000' Bar, Triseto-Polygonion, 2000 m
- (11) Vallée de la Tinée, front of Bouzieyas, Rio Bas place, Triseto-Polygonion, 1870 m
- (12) Vallée de la Tinée, front of Bouzieyas, Rio Bas place, Adenostylion, 1890 m
- (13) Vallée de la Tinée, Le Pra place, along the Tinée river, Adenostylion, 1660 m
- (14) Vallée de la Tinée, above St. Dalmas le Salvage village, Triseto-Polygonion, 1690 m

(b) Collection on 4-6/7/76
- (15) French Mercantour, Vallée de la Gordolasque, 'Les Cluots' place, Adenostylion, 1750 m
- (16) French Mercantour, Vallée du Boréon, Adenostylion, 1750 m
- (17) French Mercantour, Vallée des Merveilles, vallon du Valmasque: where Valmasque and Anielle rivers join, Adenostylion, 1830 m
- (18) Vallée de l'Ubaye, Vallon du Lauzanier, Adenostylion, 1900 m
- (19) Vallée de l'Ubaye, exactly the same place as (5), Adenostylion, 1970 m
- (20) Vallée de l'Ubaye, *ca* 100 m from station (10), Triseto-Polygonion, 2000 m

Table 2. Distribution of the 14 flavonoid glycosides in *Chaerophyllum aureum* populations

Station (population) *Ubaye/Val Grana*	Glycosides†													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1 (TP)*	+	−	+	+	+	+	−	−	−	−	−	−	−	−
4 (TP)	+	−	+	+	+	+	+	−	−	+	−	−	+	+
6 (TP)	+	−	+	+	+	+	−	+	−	−	−	−	−	−
7 (TP)	+	−	+	+	+	+	−	−	−	−	−	−	−	−
8 (TP)	+	−	+	+	+	+	−	−	−	+	+	+	−	−
10 (TP)	+	−	+	+	+	+	−	−	−	−	−	−	−	−
20 (TP)	+	−	+	+	+	+	−	−	−	−	−	−	−	−
2 (Ad)*	+	−	+	+	+	+	+	−	+	−	−	−	−	−
3 (Ad)	+	−	+	+	+	+	+	−	+	−	−	−	−	−
5 (Ad)	+	−	+	+	+	+	+	+	+	−	−	−	−	−
9 (Ad)	+	−	+	+	+	+	+	+	+	−	−	−	−	−
19 (Ad)	+	−	+	+	+	+	+	−	+	+	−	−	+	−
18 (Ad)	+	−	+	+	+	+	−	−	+	−	−	−	−	−
Vallée de la Tinée														
11 (Ad)	+	−	+	+	+	+	+	+	−	+	−	−	−	−
14 (Ad)	+	−	+	+	+	+	−	−	−	−	−	−	−	−
12 (TP)	+	−	+	+	+	−	−	−	−	−	−	−	−	−
13 (TP)	+	−	+	+	+	−	−	−	−	−	−	−	−	−
French Mercantour														
15 (Ad)	−	+	+	+	−	−	−	−	+	−	−	−	−	−
16 (Ad)	−	+	+	+	−	−	−	−	−	−	−	−	−	−
17 (Ad)	−	+	+	+	−	−	−	−	+	−	−	−	−	−

*TP, Triseto-Polygonion; Ad, Adenostylion.

†**1**, Luteolin 7-glucuronide; **2**, luteolin 4′-glucoside; **3**, quercetin 3-glucoside; **4**, luteolin 7-glucoside; **5**, apigenin 7-glucoside; **6**, diosmetin 7-glucoside; **7**, apigenin 7-diglucoside?; **8**, unidentified; **9**, luteolin 7-diglucoside; **10**, luteolin 7-diglycoside; **11–14**, unidentified.

populations 12 and 13, which lacked both diosmetin 7-glucoside and luteolin 7-diglucoside (group 1); populations 1, 4, 6–8, 10, 11, 14 and 20, in which diosmetin 7-glucoside was present, but luteolin 7-diglucoside absent (group 2); and populations 2, 3, 5, 9, 18 and 19, where luteolin 7-diglucoside was detected and in most cases (83%) apigenin 7-diglucoside was present as a minor constituent (group 3).

DISCUSSION

For a proper understanding of the flavonoid glycoside distribution among the surveyed populations, both the geographical and phytosociological origins of the specimens must be considered.

As mentioned above, the first chemical group includes three populations from the French Mercantour. Their flavonoid pattern appears to be unique when compared with those of specimens from the Valiée de l'Ubaye or Vallée de la Tinée. This is what we have previously shown (and named) as the influence of geographical parameter [1]. This French Mercantour area, although only 80 km from the other collection site, is insulated from the other valleys chosen by high mountains. The same observation can be made for the remaining areas, but they are situated in a geographically more restricted and relatively more homogeneous area. Populations growing in each 'great' valley probably have been isolated for a longer or shorter time and their evolution, including chemical evolution, even if starting from an identical point, may have followed different lines. So, to emphasize the proposed influence of the phytosociological parameter, it is necessary to consider the flavonoid patterns of specimens growing only in a homogeneous geographical unit.

From this consideration, populations of chemical groups 2 and 3 must be rearranged and their flavonoid content discussed according to their native origin.

As the populations from the Vallée de l'Ubaye and Val Grana exhibit the same kind of correlation between flavonoid content and phytosociological adherence, their respective results will be discussed together. Thus, all the populations from the Adenostylion are characterized by the presence of luteolin 7-diglucoside, while this glycoside is absent from the Triseto-Polygonion specimens. Similarly, apigenin 7-diglucoside frequently occurs (83%) as a minor constituent in tall grass prairies but was found only once among meadow specimens. These chemical characters appear to be linked with the phytosociological parameter: in the case of Val Grana, the distance between stations 1 and 2 being only *ca* 20 m. It should also be noted that specimens collected in 1976 (Nos. 18–20) and 1979 (the rest of the group) in the same stations and respective communities exhibited identical flavonoid chemistry.

In the Vallée de la Tinée, no populations produced luteolin 7-diglucoside. However, the distinction between specimens from each kind of community is supported by the presence of diosmetin 7-glucoside which is found only in plants from the Adenostylion and is lacking in those from the Triseto-Polygonion.

In most of the cases examined, the flavonoid chemistry appears to allow a distinction between *Chaerophyllum aureum* specimens growing in each kind of selected plant community, even if they are morphologically identical and provided that the geographical area of the collection stations is restricted. Similar studies, presently underway with other species, namely *Centaurea montana*, *Trollius europaeus* and *Polygonum bistorta*, from the same communities, showed consistent results. So, the thesis by Guinochet [5] that specimens growing in these two different kinds of community with an identical morphology may have different genotypes is supported by the flavonoid analysis, at least in the case of *Chaerophyllum aureum*.

However, the presence of minor constituents with an apparently uncorrelated distribution shows that minute flavonoid variation may occur, even within a single community, whereas several different populations may also coexist with their own genotypic characteristics. For example, samples 6 and 7, which were collected in the same community, a vast meadow of Triseto-Polygonion of *ca* 4000 m^2, at two points only 50 m apart, did not have exactly similar flavonoid patterns in that **8**, an unidentified glycoside, was present only in population 6. An extensive survey of specimens growing in the same community is in progress and *ca* 20–50 specimens of *C. aureum*, *C. montana*, *T. europaeus* and *P. bistorta*, collected from several points of each community will be analysed one by one for their flavonoid content in order to obtain an evaluation of the intra-population variability.

Furthermore, the apparent correlation between flavonoid glycoside content and the phytosociological parameter in the species surveyed must be compared with the ecological, geographical and meteorological conditions for each collection area before a better understanding of the relationships existing between these two types of alpine plant community is possible.

EXPERIMENTAL

Dried leaves (4–10 g) were extracted with 2 × 400–600 ml MeOH–H_2O (7:3) and then 1 × 400 ml MeOH–H_2O (1:1). Evaporation of solvents gave a residue which was dissolved in H_2O and extracted with 3 × 250 ml *n*-BuOH. A short PVP column eluted first with H_2O and later with MeOH–H_2O (7:1) removed chlorophylls, resins and sugars from the extract. The flavonoid mixtures were run on 2D-TLC (three different concns for each extract) MN DC 6.6 polyamide; solvents: (1) toluene–MeOH–butanone–H_2O (70:35:10:4) (twice); and (2) H_2O–BuOH–Me_2CO–dioxan (15:3:2:1) (twice). The 2D chromatograms were photographed in black and white and on colour slides [3] and compared.

The substances (few mg of each) were obtained from prep. TLC plates run in the above solvent systems and C_6H_6–MeOH–butanone (4:3:3). Final purification was achieved over a small column of LH-20 Sephadex. Identification was supported by UV spectral data, acid hydrolysis to the aglycone (co-chromatography with authentic samples and UV data) and sugar (identified by GC or TMS ethers) and DCI/NH_3 MS (main constituents only).

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