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FLAVONOIDS AND PHENOLIC ACIDS IN *ADENOSTOMA*, A DOMINANT GENUS OF THE CALIFORNIAN CHAPARRAL

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Abstract—The flavonoid and phenolic acid pattern of *Adenostoma fasciculatum* and *A. sparsifolium*, two dominant, endemic species of the Californian chaparral, was analysed qualitatively and quantitatively. *Adenostoma sparsifolium* was found to secrete large amounts of lipophilic, unusually substituted flavonoids onto the leaf surface; *A. fasciculatum* produces five hydrophilic flavonol 3-*O*-glycosides of kaempferol, quercetin and isorhamnetin. The phenolic acid pattern differed quantitatively but not qualitatively between the species. The amounts of phenolic acids that could be detected within the leaves, leaf litter and soil beneath the shrubs seem too small to explain allelopathic effects as the main reason for the dominance of the two species.

INTRODUCTION

The genus *Adenostoma* consists of two species, *A. fasciculatum* Hook et Arn. and *A. sparsifolium* Torr., both of which are endemic to the arid foothill region of southern California (U.S.A.) and Baja California (Mexico) [1, 2]. Both species tend to form large, pure stands, particularly *A. fasciculatum* which is a dominant species of the Californian chaparral [2].

The Californian chaparral is defined as a special shrub community with a paucity of herbs beneath or between shrubs irrespective of the density of the canopy [2]. This vegetation pattern however is interrupted by fires, occurring at intervals averaging about 25 years which destroy all the above ground portions of the vegetation. In the first 2 or 3 growing seasons following a chaparral fire a luxuriant growth of many annual herb species can be observed. The shrub vegetation regenerates by growth of seedlings and resprouting from roots, and within 5–7 years the shrubs have attained their original dominance and the growth of herbs has largely ceased [3, 4].

A first report ascribed this phenomenon (also known as the fire cycle of the Californian chaparral) to the allelopathic potential of phenolic acids detected in the leaf leachates of *A. fasciculatum* [5, 6] even though other unidentified phytotoxins were also shown to be present. Further ecological studies however did not support the hypothesis of phenolic acids being the main allelopathic principle in the dominance of *Adenostoma* [7]. But since no detailed investigations of the phenolic acids from leaves of *Adenostoma* have been reported to date, we conducted a phytochemical analysis of both species of *Adenostoma*, with emphasis on the structural elucidation, quantification and localization of phenolic acids and flavonoids.

RESULTS AND DISCUSSION

The phenolic and flavonoid chemistry of both species of *Adenostoma* differed significantly. The leaves of *A. sparsifolium* are covered by a yellow, sticky resin comprising between 10 and 15% of the dry weight, and which is secreted by large (ϕ 50–70 μm), multicellular trichomes. The resin could be extracted with chloroform and although no phenolic acids could be detected, five lipophilic flavonoid aglycones were identified; three were new natural products and could be characterized by spectroscopic means as 5,6-dimethoxy-3,7-dihydroxyflavone, 5,6-dimethoxy-3,7,4′-trihydroxyflavone and 8-methoxy-3,5,7-trihydroxyflavone [8, 9]. Other flavonoids present in the resin were galangin and pinocembrin [8]. These flavonoids accounted for almost 60% of the total resin.

The production of high amounts of lipophilic flavonoids and their secretion in the epicuticular waxes on the leaf surface is a feature common to many plants from arid environments, e.g. *Larrea* (Zygophyllaceae) [10], *Hymenoclea salsola* (Asteraceae) [11], *Elytropappus rhinocerotis* (Asteraceae) [12], and *Notholaena* and *Pityrogramma* species from arid zones [13]. Although no definite conclusions can be drawn from the available data one can suggest that these lipophilic flavonoids most likely function in reflecting excess radiation [14, 15], in reducing water loss through the leaf surface [16, 17] and in protecting the plant against microbial infections [18].

The leaves of the other species, *A. fasciculatum*, are not resinous, but are covered with epicuticular waxes which comprise 5% of the dry weight. Neither flavonoids nor phenolics could be detected; however long chain, aliphatic compounds and pentacyclic triterpenes were the major constituents. Detailed studies on the composition of the epicuticular waxes and triterpenes will be published elsewhere.

Analysis of the methanolic extracts from both species of *Adenostoma* revealed the presence of phenolic acids bound as glycosidic esters. The phenolic acid pattern was analysed after alkaline hydrolysis and found to differ quantitatively but not qualitatively, with protocatechuic, *p*-hydroxybenzoic, benzoic, *p*-coumaric, ferulic, and cinnamic acids identified in both species. The total phenolic

Table 1. Analysis of phenolic acids in leaf, leaf litter and soil samples of *A. fasciculatum* and *A. sparsifolium*

	A. fasciculatum			*A. sparsifolium*		
	leaf	litter	soil	leaf	litter	soil
Total amount (mg/g dry wt)	2.2	0.2	0.007	16.9	0.48	0.062
% distribution						
Protocatechuic	t	27	t	t	8	t
p-Hydroxybenzoic	77	64	71	t	4	16
Benzoic	9	9	t	8	5	4
p-Coumaric	14	t	29	92	50	16
Ferulic	t	t	t	t	20	32
Cinnamic	t	t	t	t	13	32

content comprises 0.2% of the dry weight for *A. fasciculatum* and 1.7% of the dry weight for *A. sparsifolium*, respectively. The main compound in *A. fasciculatum* is *p*-hydroxybenzoic acid and in *A. sparsifolium* *p*-coumaric acid. In *A. fasciculatum*, five flavonol glycosides were identified as quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoglucoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rhamnoglucoside and isorhamnetin 3-*O*-rhamnoglucoside. The total amount of flavonoid is 0.07% dry weight.

The methanolic extract of *A. sparsifolium* contains no measurable amounts of flavonol or flavone glycosides, but probably proanthocyanidins due to the fact that the extract turns red upon heating with HCl [M. Proksch, unpublished]. Previous investigations have suggested that the allelopathic properties of the phenolic acids could be responsible for the dominance of *Adenostoma* in semi-arid and arid habitats [5, 6]. Leaves, leaf litter and soil were therefore collected beneath the *Adenostoma* shrubs and analysed. No flavonoids could be detected, but the same phenolic acids present in the leaves were also found in the leaf litter and soil samples though in very small concentrations (Table 1). Soil samples yielded 7 μg/g dry weight of phenolic acids from *A. fasciculatum* and 62 μg/g dry weight for *A. sparsifolium*, respectively. Soil samples from corn fields or stands of *Celtis laevigata* for which allelopathic phenomena have been demonstrated, contained 400 μg/g and 1000 μg/g respectively [19, 20].

Thus in the case of *Adenostoma* the amounts of phenolic acids extractable from litter and soil seem too little to explain allelopathic effects. Though a seasonal [21] or populational fluctuation in phenolic acid content may occur, it seems unlikely that phenolic acids play the major role in the dominance of the genus *Adenostoma* in the Californian chaparral. These findings are in accordance with a recent report [21] in which unidentified toxins of microbial origin are held responsible for the inhibition of seed germination in *Adenostoma* stands.

EXPERIMENTAL

Leaves, leaf litter and soil were collected in spring 1982 in Oak Grove, California (U.S.A.). In order to obtain information on the localization of the compounds to be analysed, the dried leaf material was extracted by dipping into $CHCl_3$ for approximately 1 min prior to grinding and extraction with 80% MeOH. Thus two fractions were obtained, one containing the lipophilic compounds of the leaf surface ($CHCl_3$ extract), the other containing the hydrophilic compounds localized within the leaf (MeOH extract). The isolation, identification and quantification of the lipophilic flavonoids in the $CHCl_3$ extract of *A. sparsifolium* were performed as described previously [8, 9, 22]. The glycosidic flavonoids in the MeOH extract of *A. fasciculatum* were isolated by prep. TLC on cellulose and identified according to standard procedures [23]. The phenolic acids were analysed after alkaline hydrolysis of the MeOH extract as described in [24].

Leaf litter and soil samples were extracted with 80% MeOH; these MeOH extracts were then hydrolysed with NaOH and analysed as described in ref. [24].

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