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QUINOLIZIDINE ALKALOIDS IN *Genista acanthoclada* AND ITS HOLOPARASITE, *Cuscuta palaestina*

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Abstract—About 20 quinolizidine alkaloids were identified in *Genista acanthoclada* by capillary GLC and GLC-MS, such as sparteine, 11,12-dehydrosparteine, retamine, *N*-methylcytisine, cytisine, 17-oxosparteine, lupanine, α-isolupanine, 5,6-dehydrolupanine, 10-oxosparteine, *N*-carbomethoxycytisine, 17-oxoretamine, *N*-formylcytisine, *N*-acetylcytisine, and anagyrine. Its phloem-feeding holoparasite *Cuscuta palaestina* contained alkaloids too, such as sparteine, 11,12-dehydrosparteine, retamine, *N*-methylcytisine, cytisine, 17-oxosparteine, lupanine, *N*-carbomethoxycytisine, and anagyrine. Whereas sparteine, retamine, 17-oxosparteine, and cytisine are the main alkaloids of *G. acanthoclada*, lupanine, cytisine, *N*-methylcytisine, and anagyrine are abundant and enriched in *C. palaestina*. Since these alkaloids figure as antiherbivoral chemical defense compounds in *Genista*, it is assumed that the parasite can exploit the acquired allelochemicals for its own protection.

Key Words—*Genista acanthoclada*, holoparasite, *Cuscuta palaestina*, alkaloid sequestration, phloem transport, chemical defense.

INTRODUCTION

Hemi- and holoparasitic plants exploit their host plants in many ways: Both rely on water and inorganic ions that are transported in the vascular tissues. Whereas hemiparasitic plants partly use their own photosynthesis, the nonphotosynthetic

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holoparasites take all essential nutrients (i.e., amino acids and sugars) from their host plants (Steward and Press, 1990).

Plants have to defend themselves against herbivores and microorganisms. The production of chemical defense compounds or allelochemicals is a major means in this context (Harborne, 1988; Rosenthal and Janzen, 1979; Swain, 1977; Wink, 1988, 1992a–c).

Since parasitic and hemiparasitic plants are also endangered by herbivores and microorganisms, the question is whether and how they protect themselves. In general, the following alternatives exist: (1) no defense of their own, but relying on the defenses of the respective host plant; (2) mechanical protection by thorns, spines, or trichomes; (3) utilization of chemical defense by production of indigenous defense compounds and/or by sequestration of the allelochemicals from the host plant.

Genista acanthoclada DC. is a perennial scrub that inhabits arid and semi-arid coastal places of the eastern Mediterranean (Greece and the Aegean region) and often forms large associations. The aerial parts of *G. acanthoclada* consist of prickly spines, which certainly have an antiherbivore function. In addition, this species produces quinolizidine alkaloids, which are known herbivore deterrents and toxic allelochemicals (Wink 1985, 1987, 1988, 1992a,b). At the natural habitat in Crete, we could not observe any adverse effects by herbivory even though goats and sheep are abundant.

In eastern Crete we found many specimens of *G. acanthoclada* that were parasitized by *Cuscuta palaestina* Boiss., which formed thick mats on top of the broom. *Cuscuta* is known to be a predominantly phloem-feeding holoparasite.

In this study we analyzed whether *Cuscuta palaestina* is able to exploit the defense chemicals of its host plant, i.e., whether it takes up and stores them as acquired defense compounds.

METHODS AND MATERIALS

Plants. Aerial parts of *G. acanthoclada* DC. were collected in eastern Crete near Sitia in August 1991. *Cuscuta palaestina* Boiss. were manually picked from the broom host plants and stored separately.

Alkaloid Isolation and Analysis by Capillary GLC and GLC-MS. Plant material was homogenized in 0.5 M HCl and left standing overnight. Then the homogenate was made alkaline with ammonia (to pH 12) and poured onto a standard Chemelut column for solid–liquid extraction with CH_2Cl_2. The alkaloid-containing eluate was concentrated in vacuo and analyzed by high-resolution gas chromatography (HRGC) and GLC-MS. HRGC was performed with a gas chromatograph employing a capillary column (DB-5, 15 m × 0.258 mm,

0.1-μm film thickness; J&W Scientific, Folsom, California). GC conditions were: injector, 250°C; detector, 300°C; oven, 150°C; 1 min isothermal, 150–300°C with 15°C/min, at 300°C 10 min isothermal. GC-MS was performed with a Carlo-Erba 5160 GC and a Finnigan MAT 4515 quadropole mass spectrometer. EI-MS spectra were recorded at 40 eV and evaluated with the INCOS data system. Sparteine, lupanine, or cytisine were used as external standards for quantification.

RESULTS AND DISCUSSION

Quinolizidine Alkaloids in Genista acanthoclada. Alkaloid extracts of aerial parts of *Genista acanthoclada* were separated by capillary GLC and GC-MS (Figure 1), which allow the separation and identification of complex QA mixtures (reviewed in Wink, 1992c).

Since most of the alkaloids present had been analyzed before in our laboratories (Wink, 1992c; Wink et al., 1981, 1983, 1991; Wink and Witte, 1991; Wink and Römer, 1986), we could identify the following QA unambiguously according to their Kovats retention indices and mass spectra (Table 1). Thus sparteine, retamine, 17-oxosparteine, and cytisine can be considered as major and α-isosparteine, 11,12-dehydrosparteine, 17-oxoretamine, 5,6-dehydrolupanine, lupanine, α-isolupanine, *N*-methylcytisine, *N*-carbomethoxycytisine, *N*-formylcytisine, *N*-acetylcytisine, and anagyrine as minor components of the green parts of *G. acanthoclada*. A few minor alkaloids could not be identified with certainty, such as the dehydrosparteines (compounds 3, 4, and 6) and oxosparteines (10 and 15) (Tables 1 and 2) due to the scarcity of the material. Only cytisine, *N*-methylcytisine, anagyrine, retamine, and lupanine have been described for *G. acanthoclada* (Faugeras and Paris, 1971).

Quinolizidine Alkaloids in Cuscuta palaestina *Boiss.* About 1 g of dried plant material of *Cuscuta palaestina*, consisting of sprigs and flowers, which were definitely free of host-plant tissues, was extracted and analyzed by HRGC and GC-MS. As can be seen from Figure 1B, *Cuscuta palaestina* sequesters some of the alkaloids that are also present in the host plant. We assume that these alkaloids were not made by *Cuscuta palaestina* indigenously but were taken up from its host plant by phloem-feeding, since the overall pattern of alkaloids is rather similar. *Cuscuta reflexa* and *C. platyloba*, which were kept on *Lupinus albus* as a host plant were analyzed recently (Czygan et al., 1988; Bäumel et al., 1991). In this case an uptake of QA from the host plant was also observed. This is in agreement with earlier studies showing that QA are transported in the phloem (Wink, 1992a–c; Wink and Witte, 1991).

Major alkaloids of *C. palaestina* are cytisine-type alkaloids (such as cytisine, *N*-methylcytisine, and anagyrine), which show strong antiherbivore activ-

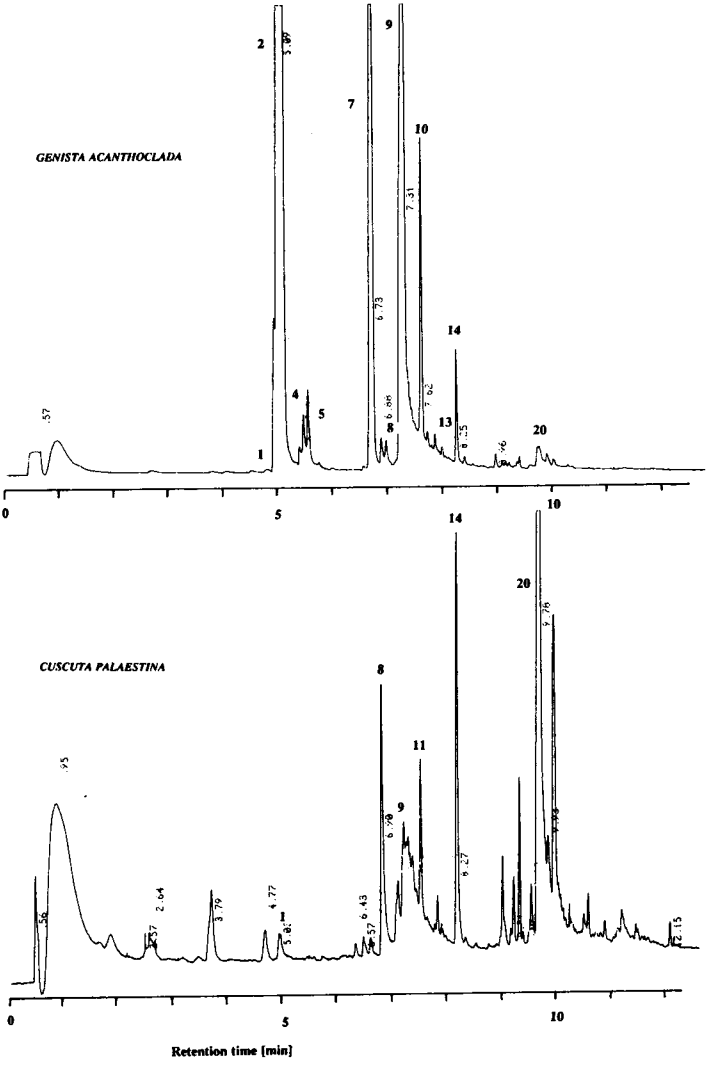

FIG. 1. Separation of alkaloid extracts from *Genista acanthoclada* and its holoparasite *Cuscuta palaestina* by capillary GLC (nitrogen-specific detection) Numbering is according to Table 1.

ities, e.g., cytisine activates acetylcholine receptor-coupled ion channels of neurons (Wink, 1992a–c). The cytisine level found in *Cuscuta* should be high enough to provide significant antiherbivore protection.

Considering the survival strategies mentioned in the introduction, it is likely

TABLE 1. IDENTIFICATION OF QUINOLIZIDINE ALKALOIDS FROM *Genista acanthoclada* AND *Cuscuta palaestina* BY GC-MS

Alkaloid	RI	M+	5 abundant ions (abundance %)
1. α-Isosparteine	1710	234	234(25) 193(15) 137(50) 98(100) 84(20)
2. Sparteine	1785	234	234(20) 193(30) 137(100) 98(100) 84(20)
3. Dehydrosparteine	1810	232	232(50) 191(15) 148(30) 134(100) 98(95)
4. Dehydrosparteine	1825	232	232(65) 189(30) 148(30) 134(90) 98(100)
5. 11,12-Dehydrosparteine	1840	232	232(35) 191(5) 148(20) 134(100) 97(85)
6. Dehydrosparteine	1855	232	232(45) 191(10) 148(20) 134(40) 98(100)
7. Retamine	1940	250	250(5) 232(20) 207(25) 134(35) 98(100)
8. *N*-Methylcytisine	1952	204	204(15) 146(5) 58(100)
9. Cytisine	1990	190	190(65) 160(25) 147(80) 146(100) 134(25)
10. Oxosparteine	2015	248	248(25) 229(10) 150(35) 135(25) 97(100)
11. 17-Oxosparteine	2070	248	248(40) 220(20) 136(40) 110(75) 97(100)
12. α-Isolupanine	2105	248	248(20) 149(30) 136(70) 98(30) 57(100)
13. 5,6-Dehydrolupanine	2128	246	246(12) 134(12) 98(100) 97(35) 84(10)
14. Lupanine	2165	248	248(30) 149(50) 136(100) 110(20) 98(25)
15. 10-Oxosparteine	2180	248	248(45) 220(15) 136(95) 110(60) 97(100)
16. *N*-Carbomethoxycytisine	2240	248	248(25) 160(15) 146(100) 102(90) 58(50)
17. 17-Oxoretamine	2250	264	264(25) 247(80) 136(25) 98(100) 97(75)
18. *N*-Formylcytisine	2310	218	218(50) 190(5) 160(20) 146(100) 134(15)
19. *N*-Acetylcytisine	2320	232	232(15) 218(30) 190(5) 160(20) 146(100)
20. Anagyrine	2382	244	244(20) 136(20) 122(20) 98(100)

TABLE 2. ALKALOID PROFILES OF *Genista acanthoclada* AND ITS PARASITE *Cuscuta palaestina*

Alkaloid	Alkaloid composition (total alkaloids = 100%)		
		C. palaestina	
	G. acanthoclada	Sample 1	Sample 2
1. α-Isosparteine	tr		
2. Sparteine	46.1	tr	tr
3. Dehydrosparteine	0.2		tr
4. Dehydrosparteine	0.9		tr
5. 11,12-Dehydrosparteine	1.2		tr
6. Dehydrosparteine	tr		
7. Retamine	16.6		tr
8. *N*-Methylcytisine	0.4	7.5	5.9
9. Cytisine	25.3	33.1	12.1
10. Oxosparteine	tr		
11. 17-Oxosparteine	4.9		4.9
12. α-Isolupanine	tr		
13. 5,6-Dehydrolupanine	tr		
14. Lupanine	1.2	25.4	7.2
15. 10-Oxosparteine	tr		
16. *N*-Carbomethoxycytisine	tr	tr	
17. 17-Oxoretamine	tr		
18. *N*-Formylcytisine	tr		
19. *N*-Acetylcytisine	tr		
20. Anagyrine	0.7	24.9	56.1
Alkaloid content (μg/g dry weight)	3560	ND	680

that *Cuscuta* exploits the allelochemicals of its host plant as acquired defense chemicals. The exploitation of host plant-produced allelochemicals seems to be a general phenomenon in parasitic plants, examples are: uptake of QA by *Cuscuta reflexa* and *C. platyloba* from *Lupinus*, *Cytisus*, or *Spartium* host plants, respectively (Czygan et al., 1988; Bäumel et al., 1991); by *Orobanche rapum-genistae* from *Cytisus scoparius* (Wink et al., 1981); by *Pedicularis semibarbata* from *Lupinus fulcratus* (Stermitz et al., 1989); by *Viscum cruciatum* growing on *Lygos sphaerocarpa* (Cordero et al., 1989); of PA, QA, and iridoid glycosides by *Castilleja* species from *Lupinus*, *Senecio*, or *Penstemon* host plants (Stermitz et al., 1986; Stermitz and Harris, 1987).

A remarkable difference can be seen in the abundance of individual alkaloids in *G. acanthoclada* and *C. palaestina* (Table 2) in that sparteine, 11,12-dehydrosparteine, and retamine are missing in *Cuscuta* or are only present in traces, whereas *N*-methylcytisine, anagyrine, and lupanine are clearly enriched

(Table 2). Since sparteine and retamine are abundant in the same organ of the host plant parasitized by *C. palaestina* and since their phloem transport has been demonstrated in other instances (Wink and Witte, 1991), we assume that QA uptake and storage by *C. palaestina* is, in part, a specific process. In *Viscum cruciatum* from *Lygos sphaerocarpa* lupanine, anagyrine, cytisine, and *N*-methylcytisine were detected as well as retamine, whereas the host plant contained mainly retamine and sparteine (Cordero et al., 1989), showing a somewhat similar qualitative discrimination as found in our *C. palaestina* plants. QA do not pass biomembranes, such as the tonoplast, by simple diffusion but by carrier-mediated transport (Mende and Wink, 1987; Wink and Mende, 1987). In larvae of the pyralid moth, *Uresiphita reversalis*, which sequesters QA from its host plant, we observed a substantial chemical discrimination in that alkaloids of the 10-oxosparteine-type were not resorbed, but eliminated with the feces, whereas the more toxic cytisine and derivatives were stored. Since we can explain this discrimination in terms of a specific QA carrier in midgut epithelia (Wink et al., 1991), we would expect, in analogy, the presence of a selective alkaloid transporter (at least for *N*-methylcytisine and anagyrine) in the haustoria or other biomembranes of *C. palaestina*, which has not been described in biochemical terms so far. In addition, we cannot rule out that sparteine was taken up and metabolized by *Cuscuta* into lupanine and 17-oxosparteine. When comparing the QA profiles from *C. platyloba* and *L. albus*, it was found that 13-hydroxylupanine was enriched in *C. platyloba* whereas 13-*trans*-cinnamoyloxylupanine had decreased. Bäumel et al. (1991) explain this difference by hydrolysis of the ester alkaloids in *Cuscuta*. Thus, biotransformation could be a second factor besides selective transport to influence the alkaloid profiles of the parasite.

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