Site of Lupanine and Sparteine Biosynthesis in Intact Plants and *in vitro* Organ Cultures

Michael Wink

Genzentrum der Universität München, Pharmazeutische Biologie, Karlstraße 29, D-8000 München 2, Bundesrepublik Deutschland

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[^{14}C]Cadaverine was applied to leaves of *Lupinus polyphyllus, L. albus, L. angustifolius, L. perennis, L. mutabilis, L. pubescens*, and *L. hartwegii* and it was preferentially incorporated into lupanine. In *Lupinus arboreus* sparteine was the main labelled alkaloid, in *L. hispanicus* it was lupinine. A pulse chase experiment with *L. angustifolius* and *L. arboreus* showed that the incorporation of cadaverine into lupanine and sparteine was transient with a maximum between 8 and 20 h. Only leaflets and chlorophyllous petioles showed active alkaloid biosynthesis, whereas no incorporation of cadaverine into lupanine was observed in roots. Using *in vitro* organ cultures of *Lupinus polyphyllus, L. succulentus, L. subcarnosus, Cytisus scoparius* and *Laburnum anagyroides* the inactivity of roots was confirmed. Therefore, the green aerial parts are the major site of alkaloid biosynthesis in lupins and in other legumes.

Introduction

Considering the site of secondary metabolite formation in plants, two possibilities are given: 1. All the cells of plant are producers. 2. Secondary metabolite formation is restricted to a specific organ and/or to specialized cells.

Alkaloids are often found in the second class (for review [1, 2]), but whether a given alkaloid is synthesized in the root, the shoot, the leaves or the fruits virtually depends on the respective biogenetic type. *I.e.* there is no general rule and the site of biosynthesis has to be elucidated for every group of alkaloids or even every single alkaloid.

Quinolizidine alkaloids (QA) are characteristic metabolites of the Fabaceae and previous studies have indicated [1, 3], that only the aerial green parts of legume plants synthesize these alkaloids.

Recently it was shown, that pyrrolizidine alkaloids are formed in root cultures of *Senecio* [4]. Since pyrrolizidine alkaloids seem to be formally related to QA, we have reinvestigated the question concerning the site of biosynthesis of QA. We have studied the incorporation of [^{14}C]cadaverine, the sole precursor of the tetracyclic QA, into lupanine and sparteine in organs derived from intact plants and additionally *in vitro* cultures of roots, shoots and cotyledons.

Reprint requests to Priv.-Doz. Dr. M. Wink.

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Materials and Methods

Plants

Plants of *Lupinus polyphyllus, L. arboreus, L. subcarnosus, L. hartwegii, L. pubescens, L. perennis, L. albus, L. mutabilis, L. angustifolius*, and *L. succulentus* were grown in a green-house at 23 °C and under natural illumination or outside in an experimental garden.

In vitro organ cultures

Seeds of *L. polyphyllus, L. succulentus, L. subcarnosus, Cytisus scoparius, Laburnum anagyroides* were surface sterilized with ethanol and Na-hypochlorite and were germinated on sterile agar plates (15 g agar in 1000 ml tap water). When the seedlings were 3–6 cm long, they were dissected into roots, hypocotyls/epicotyls and cotyledons and were transferred to 100 ml Erlenmeyer flasks containing 20 ml RS medium [5]. Cultures were kept on rotary shakers, at 25 °C and 24 h light (neon light, 650 lux). The medium was renewed every 3 weeks. Organ cultures were used for the feeding experiments or GLC analysis, after 3–12 weeks *in vitro*. Root cultures consisted of slow-growing long root hairs with many lateral branches. They were heterotrophic and contained no chlorophyll. Shoot cultures derived from epi- and hypocotyls and developed small leaves (1 cm in diameter). In addition small roots were found in some cases. These shoots were green. Cotyledons could be

cultured for up to 5 weeks: They tripled their size and maintained their green appearance.

Alkaloid analysis

QA were extracted, isolated and analyzed by capillary gas-liquid chromatography according to standard techniques [6, 7].

Tracer experiments

[^{14}C]Cadaverine was prepared from L-[^{14}C-U]-lysine with the aid of lysine decarboxylase according to [22]. Organs or organ cultures (1–4 g fresh weight) were incubated with 1–2 μCi [^{14}C]cadaverine (3 nmol/μCi) (in 5 ml tap water or RS medium) for 16 to 48 h. Alkaloids were extracted and chromatographed on silica thin-layer plates using cyclohexane and diethylamine as solvents (7:3). Alkaloids were detected with Dragendorff's reagent and identified by co-chromatography with authentic alkaloids and by capillary GLC. Radioactivity was monitored with a Berthold TLC scanner (II).

Results and Discussion

When [^{14}C]cadaverine is applied to leaves of *Lupinus polyphyllus, L. perennis, L. mutabilis, L. albus, L. angustifolius* and *L. pubescens* lupanine results as the major radioactive alkaloid, accompanied by minor alkaloids such as 13-hydroxylupanine, 13-tigloyl-oxylupanine (Fig. 1, Table I).

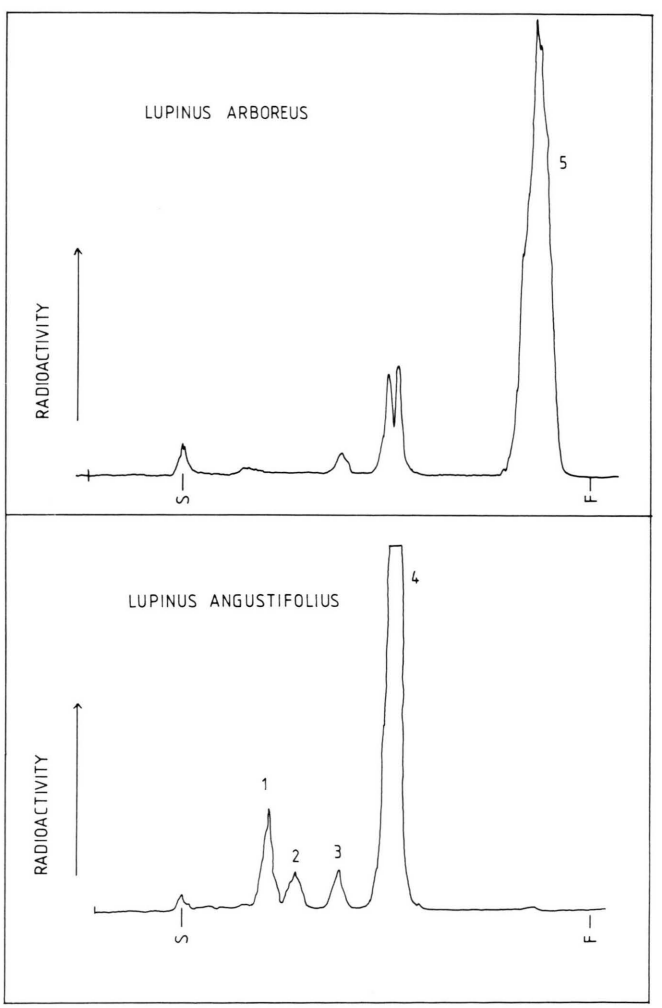

Fig. 1. Pattern of the incorporation of [^{14}C]cadaverine into quinolizidine alkaloids by leaves of *Lupinus angustifolius* and *L. arboreus*. Leaves were incubated with 2 μCi cadaverine (6 nmol). 1 = 13-hydroxylupanine, 2 = angustifoline, 3 = 13-tigloyl-oxylupanine, 4 = lupanine, 5 = sparteine, S = start, F = front.

Table I. Incorporation of [^{14}C]cadaverine into quinolizidine alkaloids. Complete leaves were incubated with [^{14}C]cadaverine (2 μCi) for 48 h. Alkaloids were extracted and separated by TLC. Radioactivity was detected by a TLC scanner and integrated online. Alkaloids: 1 = lupanine, 2 = 13-hydroxylupanine, 3 = multiflorine, 4 = angustifoline, 5 = esters of 13-hydroxylupanine, 6 = sparteine, 7 = lupinine, 8 = others, tr = traces.

Species	Alkaloid pattern (total alkaloids = 100%)							
	1	2	3	4	5	6	7	8
L. polyphyllus	83.5	7.5	–	3.6	2.9	1.3	–	tr
L. mutabilis	72.4	6.9	–	–	14.6	3.2	–	tr
L. angustifolius	80.0	6.4	–	2.6	8.8	0	–	tr
L. albus a	59.3	3.4	29.3	–	4.2	1	–	tr
b	63.3	1	30.5	–	4.1	0.5	–	tr
c	tr	–	–	–	–	tr	–	–
L. arboreus	15.6	–	–	–	–	79.4	–	tr
L. hispanicus	–	–	–	–	–	–	80	20

a: Seeds from Crete, b: seeds from Syria, c: sweet variety "lucky".

Only in the case of *L. arboreus*, sparteine was the major labelled product (Fig. 1); in *L. hispanicus* it was lupinine. These labelling patterns correspond to the alkaloid patterns of the intact plants, in that lupanine figured as the major alkaloid in the first series, and sparteine in the second and lupinine in the third [6, 16]. The pattern of labelled QA is in agreement with the results from previous studies [1, 17–22].

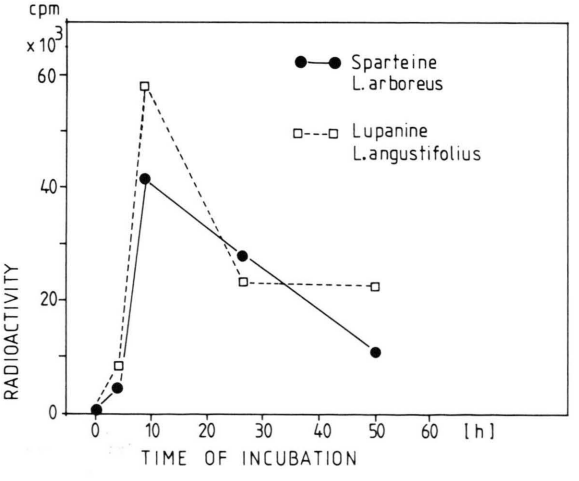

Fig. 2. Pulse chase experiment of lupanine and sparteine formation. Leaves of *Lupinus arboreus* and of *L. angustifolius* were incubated with 1 μCi cadaverine (3 nmol). After 8 h leaves were washed and incubated in tap water without [^{14}C]cadaverine. At the time intervals given, plant material was harvested and processed as described above.

The incorporation of [^{14}C]cadaverine into sparteine and lupanine is transient with a maximum between 8 and 20 h as indicated from a chase pulse experiment performed with leaves of *L. arboreus* and *L. angustifolius* (Fig. 2, [7, 8]).

In the next series of experiments we incubated leaflets, petioles and roots of *L. polyphyllus*, *L. arboreus*, *L. perennis* and *L. pubescens* with [^{14}C]cadaverine. Radioactive QA could be detected mainly in leaflets, to some degree in green petioles, but not in the roots (Table II, Fig. 3).

Organ cultures which were derived from seedlings of *Lupinus polyphyllus*, *Cytisus scoparius*, and *Laburnum anagyroides* contained QA as analyzed by GLC (Table III). Since QA are translocated from the cotyledons (which are extremely rich in alkaloids at the onset of germination) to hypocotyls and roots during germination [7], all organs contain alkaloids prior to *in vitro* cultivation. The occurrence of QA in *in vitro* cultured roots, shoots and cotyledons could be due either to *de novo* synthesis or to this translocation. Since the QA content of roots and cotyledons decreased with prolonged *in vitro* culture and was often hardly detectable (Table III), a *de novo* synthesis of QA in these plant parts was less likely. This could be confirmed by application of [^{14}C]cadaverine to *in vitro* cultured roots, shoots and cotyledons. As shown in Table II the label is only incorporated into

Table II. Site of quinolizidine alkaloid biosynthesis in plants and organ cultures. Plant parts were suspended in 5 ml water containing [^{14}C]cadaverine (2 μCi) and incubated at 25 °C and continuous illumination. After 20 h alkaloids were extracted and chromatographed on silica TLC plates. Radioactivity was recorded with a thin-layer scanner (s. Fig. 1, 2). ++ = Substantial incorporation in lupanine* or sparteine**; + = low incorporation; – = no radioactivity visible.

I. Intact plants	Plant part		
	Leaflets	Petioles Stems	Roots
*L. polyphyllus**	++	+	–
*L. arboreus***	+	++	–
*L. perennis**	++	–	–
*L. pubescens**	++	–	–
II. Organ cultures	**Cotyledons**	**Hypocotyls Epicotyls Leaflets**	**Roots**
*L. polyphyllus**	–	++	–
*L. succulentus**	–	++	–
*L. subcarnosus**	–	++	–

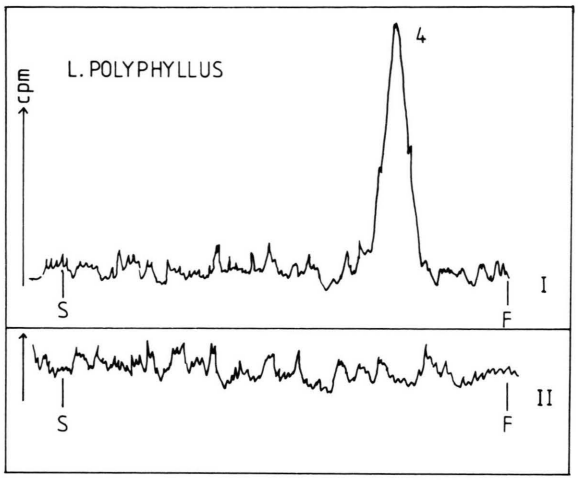

Fig. 3. Site of quinolizidine alkaloid biosynthesis: Incorporation of [^{14}C]cadaverine into lupanine. Leaflets (I) and roots (II) of *Lupinus polyphyllus* (weight: 2–3 g) were incubated with 1 μCi [^{14}C]cadaverine (3 nmol) for 20 h. Alkaloids were extracted and separated by TLC. I = leaflets, II roots, S = start, F = front, 4 = lupanine.

QA by cultured shoots, but not by roots or cotyledons.

Our feeding experiments with intact plants and *in vitro* organ cultures thus confirm the view that the major sites of QA biosynthesis are the green aerial parts (except the cotyledons) of a lupin, especially the leaflets and the mesophyll-containing petioles. We have previously shown, that QA biosynthesis can be localized in chloroplasts [9, 10]. After synthesis QA are translocated *via* the phloem all over the plant [11], where they are accumulated by epidermal tissue in particular [12–14]. Consequences of the complex alkaloid physiology for the production of QA by cell cultures have been discussed in [2, 15].

Although QA could be considered homologous to pyrrolizidine alkaloids, from a chemical point of view, the physiology of both types of alkaloids differs significantly [4], a fact which stresses the importance to study each group of alkaloids individually.

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Table III. Alkaloid concentrations and alkaloid patterns of organ cultures. Seedlings were dissected into cotyledons, hypo-/epicotyls and roots and cultured for 4–8 weeks in RS medium. Alkaloids were extracted and analyzed by capillary GLC.

Species		Alkaloid content μg/g f.w.	Alkaloid pattern (total alkaloid = 100%) 1	2	3	4	5	6	7	8	9
L. polyphyllus	C	0									
	S	76	68	19	13						
	R	4	48	16	21	18					
Cytisus scoparius	C	0									
	S	64	38			43	19				
	R	0									
	R	77	43			38	19				
Laburnum anagyroides	C/S	21	1					14	69	10	6
	R	0									

Alkaloids: 1 = lupanine, 2 = 4-hydroxylupanine, 3 = 13-hydroxylupanine, 4 = tetrahydrorhombifoline, 5 = dehydrolupanine, 6 = cytisine, 7 = N-methylcytisine, 8 = anagyrine, 9 = 5,6-dehydrolupanine; C = cotyledons, S = shoots, R = roots.

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