THE ALKALOID PATTERNS OF CELL SUSPENSION CULTURES AND DIFFERENTIATED PLANTS OF *BAPTISIA AUSTRALIS* AND THEIR BIOGENETIC IMPLICATIONS

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ABSTRACT.—Alkaloid extracts from *Baptisia australis* leaves, analyzed by capillary gas chromatography and gc/ms, were shown to contain sparteine (**1**), lupanine (**6**), cytisine (**3**), *N*-methylcytisine (**2**), anagyrine (**8**), 17-oxosparteine (**11**), 13-hydroxyanagyrine (**10**), 5,6-dehydrolupanine (**4**), tinctorine (**7**) (new for *Baptisia*), isotinctorine (**5**) (new alkaloid) and 13-acetoxyanagyrine (**9**) (new alkaloid).

The alkaloid content of cell suspension cultures was three orders lower than that of the differentiated plants; lupanine figured as the only alkaloid. A biogenetic pathway with lupanine biosynthesis as the central part, from which the α-pyridone alkaloids derive via 5,6-dehydrolupanine (**4**), is discussed.

Three lysine units build up the tetracyclic quinolizidine alkaloids such as sparteine (**1**) and lupanine (**6**) via their decarboxylation product, cadaverine (**12**) (1, 2).

Recently lysine decarboxylase and 17-oxosparteine synthase could be demonstrated as the principal enzymes of this sequence in cell suspension cultures and leaf chloroplasts of *Lupinus polyphyllus* (3–7). Isolated intact lupin chloroplasts produce lupanine upon feeding of cadaverine or 17-oxosparteine (**11**) but not of sparteine (**1**), whereas membrane-defective chloroplasts release sparteine (**1**) and 17-oxosparteine (**11**) instead of lupanine (**6**) (5). Cell suspension cultures of *Lupinus polyphyllus* and *Sarothamnus scoparius* accumulate only lupanine (**6**) irrespective of the alkaloid pattern of the differentiated plant (7, 8). Therefore, we assume lupanine (**6**) to be a central intermediate of quinolizidine alkaloid biosynthesis and not sparteine (**1**) as postulated by Schütte (1) and Nowacki and Waller (2). This view is further supported by $^{14}CO_2$-feeding experiments with *Lupinus* and *Thermopsis* species which revealed ^{14}C-lupanine (**6**) as the first labelled alkaloid without the occurence of ^{14}C-sparteine (**1**) (9, 10).

More than 9 quinolizidine alkaloids have been reported from *Baptisia* species (11, 12). Besides the common alkaloids such as sparteine (**1**) and lupanine (**6**), biogenetically more complex alkaloids like the α-pyridone alkaloids are present, which are thought to derive either from sparteine (**1**) or lupanine (**6**).

Cell suspension cultures of *Baptisia* should be suitable for the study of the biosynthetic sequence of lupin alkaloids, e.g., whether sparteine (**1**) or lupanine (**6**) figures as the precursor for the α-pyridone alkaloids such as anagyrine (**8**) and cytisine (**3**).

EXPERIMENTAL

PLANT MATERIAL.—Leaves of *Baptisia australis* (L.) R. Br. (Botanical Garden, Braunschweig), the same plant from which the cell cultures originated, were harvested in July 1977.

Genista tinctoria L. used for the isolation of reference alkaloids was collected from a site near Braunschweig. Callus cultures of *B. australis* were started in June 1977. The same medium and culture conditions as described for *L. polyphyllus* cell suspension cultures were employed (8).

After three months of callus culture, suspension cultures were established. They consisted of slow-growing green cell aggregates (about 6 mm mean diameter) and produced large amounts of phenolics. Suspension cultures were kept on rotary shakers at 120 rpm, 25°, 70% r.H. and continous illumination (Fluora- and Osram-L-lights). Cells were harvested after the twentieth passage and 18 months in culture.

ALKALOID EXTRACTION.—Cells or leaves were homogenized in 0.1 N HCl in a Waring blendor. After centrifugation, the supernatant was alkalinized with ammonia, and the alkaloids were extracted with methylene chloride. Following drying with anhydrous Na_2SO_4, the solvent was evaporated.

ALKALOID ANALYSIS.—Alkaloid mixtures were separated on glass capillary columns (0.25 mm x 25 m WCOT Cp-Sil 5) on a Perkin Elmer F 22 gas chromatograph. Alkaloids were separated and identified by gc/ms (AEI MS 30, Data System DS 50). For details see Wink *et al.* (8).

RESULTS AND DISCUSSION

Cell Suspension Cultures

Alkaloid accumulation of cell suspension cultures of *Baptisia australis* is quite low (1.2 μg total alkaloids per g cells, fresh weight) and amounts to only 0.1% of that found in the differentiated plant.

An alkaloid mixture from cell suspension cultures was separated by glass capillary gas chromatography (fig. 1). The only significant nitrogen-containing compound (gc peak 6) could be unequivocally identified by its retention time and gc/ms as lupanine (**6**) (table 1).

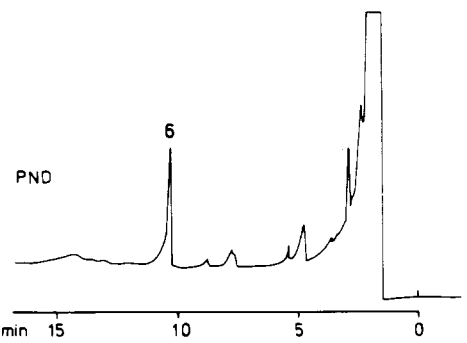

FIG. 1. Separation of an alkaloid mixture from cell suspension cultures of *Baptisia australis* by glass capillary gc. Temperature: 190°–270°, 4°/min. Phosphorus nitrogen detection (PND). **6**=lupanine.

Differentiated Plants

To evaluate the above given result, we have analyzed the alkaloid pattern of the mother plant. Nine nitrogen-containing compounds are present in the range of quinolizidine alkaloids in the chromatographic system (fig. 2) (8) applied. The identity of the gc peaks with the respective mass spectra (gc/ms) (table 1) was established by the use of mass chromatograms of the respective molecular ions and characteristic fragment ions.

Sparteine (gc peak **1**), *N*-methylcytisine (**2**), cytisine (**3**), lupanine (**6**), and anagyrine (**8**) displayed retention times identical to those of reference alkaloids which had been isolated from *Lupinus polyphyllus* (Sparteine (**1**), Lupanine (**6**)

TABLE 1. Identification of the alkaloids from *Baptisia australis* by gc/ms (compare figs. 1, 2, 3).

Gc-peak	M^+	Five characteristic ions[a]					Compound	References
1	234	137	98	193	234	110	sparteine (**1**)	[8, 15]
2	204	58	204	146	160	96	N-methylcytisine (**2**)	[14, 15]
3	190	146	147	190	134	160	cytisine (**3**)	[14, 15]
4	246	98	97	246	134	148	5,6-dehydrolupanine (**4**)	[16]
5	244	58	203	160	146	244	isotinctorine (**5**)	
6	248	136	149	248	98	110	lupanine (**6**)	[8, 15]
7	244	203	58	146	160	190	tinctorine (**7**)	[13]
8	244	98	244	146	136	160	anagyrine (**8**)	[14, 15]
9	302	96	302	243	146	160	13-acetoxyanagyrine (**9**)	

[a]Ions were ordered according to their decreasing abundance.

(8)), *Genista tinctoria* (N-Methylcytisin (**2**), anagyrine (**8**) (13)), and *Baptisia tinctoria* (Cytisine (**3**)) and could be easily identified by gc/ms (table 1) in comparison with corresponding reference alkaloids and reference spectra (8, 14, 15).

Additionally, 17-oxosparteine (**11**) and 13-hydroxyanagyrine (=baptifoline) (**10**) could be detected in the gc run. Both alkaloids, which display retention times of 9 and 18 min, respectively, were present in trace amounts only.

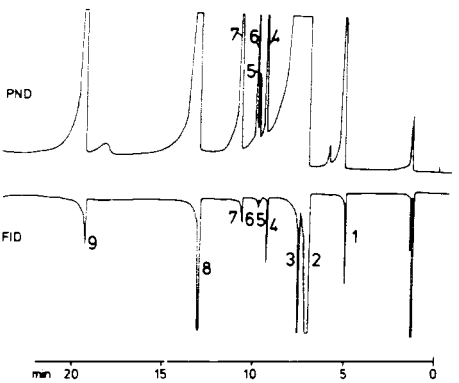

FIG. 2. Capillary gc of an alkaloid extract from *Baptisia australis* leaves. Conditions as in fig. 1. Identification of the gc peaks in fig. 3 and table 1.

Gc peak 4 results in a mass spectrum which is identical with that of 5,6-dehydrolupanine (**4**) isolated from *Thermopsis rhombifolia* (16), *Baptisia psammophila* (12) and *Baptisia tinctoria* (L. Witte unpublished results).

Gc peaks 5 and 7 both have a molecular ion at *m/z* 244 and intensive fragment ions at *m/z* 203 indicating a tricyclic alkaloid and *m/z* 58 which is characteristic for an *N*-methyl structure as in *N*-methylcytisine (**2**) (14, 15). Tinctorine (**7**) described from *Genista tinctoria* (13) displays the same retention time and mass spectrum as compound 7 (fig. 3C). We have reexamined tinctorine from *G. tinctoria* plants and could confirm the identity of **7** as tinctorine by gc/ms. This is the first report of this alkaloid in *Baptisia*.

The same fragmentation pattern as in tinctorine (**7**) was present in the mass spectrum of compound **5**, but the intensity of the fragment ions was changed,

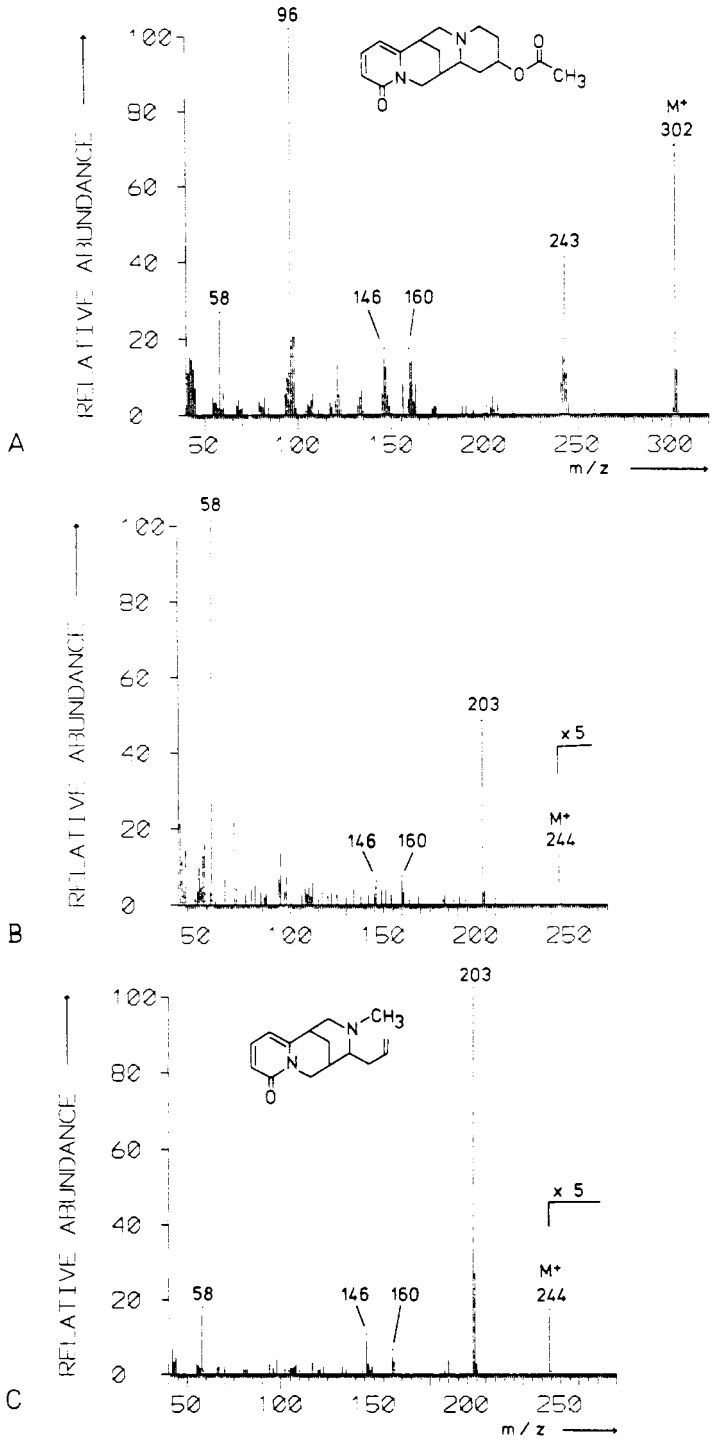

FIG. 3. Mass spectra of quinolizidine alkaloids: A, 13-acetoxyanagyrine; B, isotinctorine; and C, tinctorine.

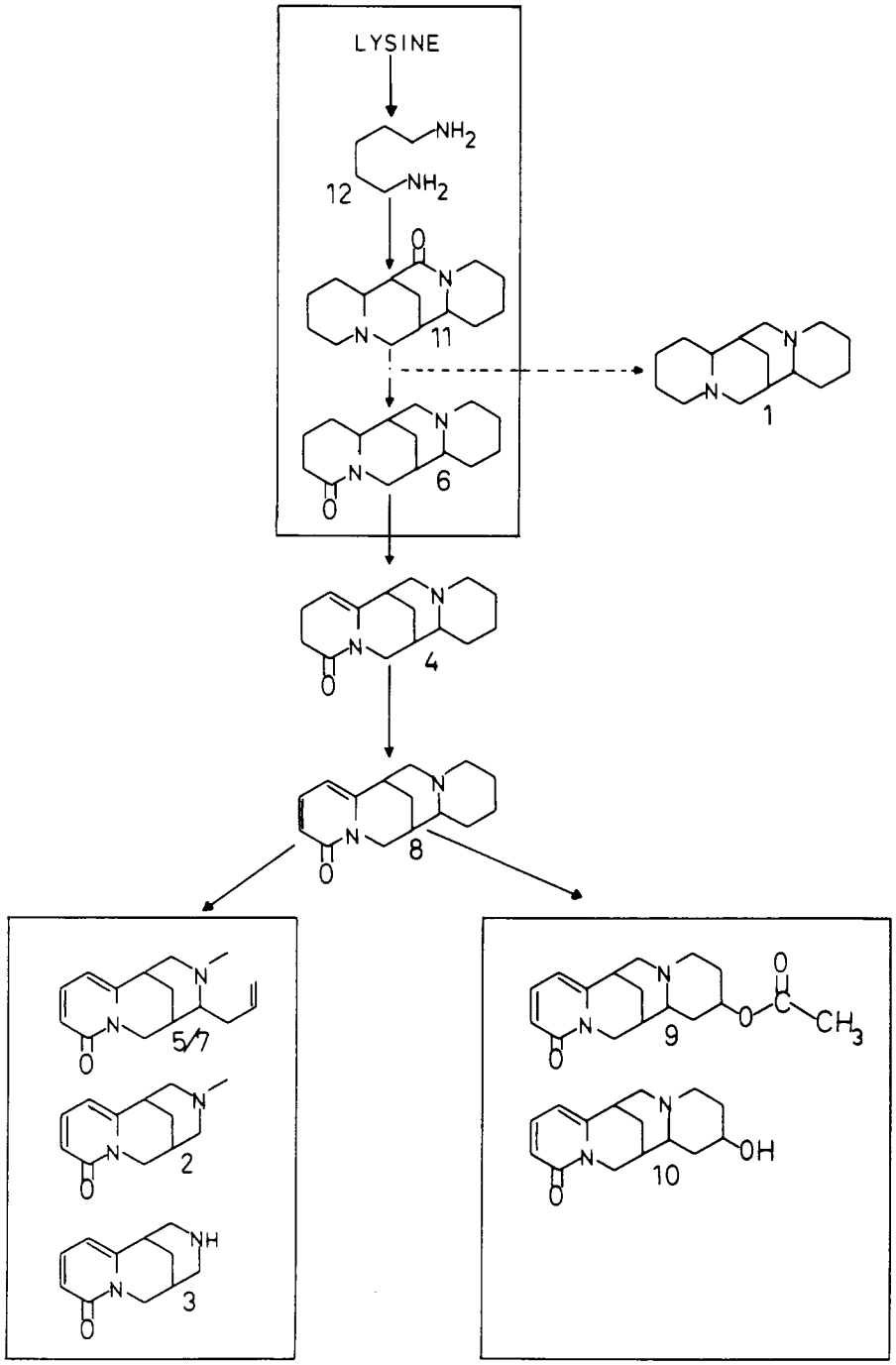

FIG. 4. Biogenetic pathway of quinolizidine alkaloids in *Baptisia*. (**1**) Sparteine, (**2**) N-methylcytisine, (**3**) cytisine, (**4**) 5,6-dehydrolupanine, (**5**) isotinctorine, (**6**) lupanine, (**7**) tinctorine, (**8**) anagyrine, (**9**) 13-acetoxyanagyrine, (**10**) 13-hydroxyanagyrine (=baptifoline), (**11**) 17-oxosparteine, (**12**) cadaverine.

e.g., *m/z* 58 became the base peak (fig. 3B). We have found the same compound (identical gc-peaks) to be more abundant in *G. tinctoria* by gc/ms. We assume that compound **5** is a stereoisomer of tinctorine (**7**). In analogy to the isomers of sparteine (**1**) and lupanine (**6**), such as α-isosparteine and α-isolupanine, we propose to call this alkaloid isotinctorine; this assumption has to be confirmed in further studies.

A molecular ion at *m/z* 302 was found for gc peak 9 (fig. 3A); upon hydrolysis with methanolic KOH an alkaloid with a molecular ion at *m/z* 260 was obtained. The respective mass spectrum was identical with that of 13-hydroxyanagyrine (=baptifoline) (**10**) (17). The loss of 42 mass units after hydrolysis indicates acetic acid as the acid component of this ester alkaloid. After catalytic reduction of the ester alkaloid with palladium charcoal, we obtained a mass spectrum with an intensive fragment at *m/z* 246 and a molecular ion at *m/z* 306. Analogous esters (with *m/z* 246 as base peak) of 13-hydroxylupanine are known to occur in *Lupinus* and *Cytisus* species (1, 2, 8). Therefore, we consider this alkaloid to be a 13-acetoxyanagyrine, the first ester alkaloid found in the genus *Baptisia*.

Biogenetic Implication of the Alkaloid Composition of Plant and Cell Culture

Alkaloid composition of *Baptisia* plants is subjected to a strong intraspecific variation (18). This feature and the more advanced analytical methods employed in this study may explain the differences in the alkaloid pattern of *B. australis* in relation to literature data (11, 18).

The main alkaloid produced by cell suspension cultures is lupanine (**6**). Other alkaloids which are thought to derive from lupanine, such as anagyrine (**8**), which are more abundant in the plant are missing. Assuming that only those products which are less advanced in biogenetic sequence will accumulate in cell cultures of quinolizidinal plants (**7**), it might be concluded that lupanine is the main alkaloid in this sequence and that the other alkaloids such as sparteine (**1**) and the α-pyridone alkaloids are derived from it. This assumption is supported by *in vivo* experiments (19) in which ^{14}C-lupanine was fed to *B. leucopheya* plants. Radioactive sparteine (**1**), anagyrine (**8**), cytisine (**3**), *N*-methylcytisine (**2**) and 13-hydroxyanagyrine (**10**) were isolated as subsequent conversion products.

From these and the other data mentioned in the introduction (3–10) we consider a lupanine biosynthesis without sparteine as an intermediate to be the main pathway of quinolizidine alkaloid biosynthesis (fig. 4). As in lupins (3–5, 7), 17-oxosparteine (**11**) is probably the first alkaloid intermediate because the presence of 17-oxosparteine synthase in *Baptisia* is indicated by recent experiments (unpubl. results). The 5,6-dehydrolupanine (4) has been reported so far only from species which contain both lupanine (**6**) and α-pyridone alkaloids (12, 16, 20). It is plausible to place this compound as the intermediate between lupanine (**6**) and anagyrine (**8**).

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