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Optical rotation of quinolizidine alkaloids: an important variable in chemosystematic studies of *Fabaceae*

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Abstract: The anomalous distribution of (+)-lupanine and (–)-lupanine in *Podalyria* species provides circumstantial evidence that hydroxylation and subsequent esterification of lupanine is only possible when (–)-sparteine and (+)-lupanine are the precursors. The optical rotation of lupanine and/or sparteine isolated from different genera, in combination with literature data, provide evidence of a separate biosynthetic pathway which leads to lupanine-type esters rather than α-pyridones. The occurrence of this pathway can be predicted from the optical rotation of sparteine and/or lupanine, even in the absence of end products. Enantiomers-specificity is thus an important variable for establishing homology in comparative studies.

Quinolizidine alkaloids are important chemotaxonomic characters in the family *Fabaceae*, particularly at the generic and tribal levels (MEARS & MABRY 1971, SALATINO & GOTTLIEB 1980, KINGHORN & SMOLENSKI 1981, KINGHORN & BALANDRIN 1984). Most of the genera of this large family have now been studied and several new sources and new structures came to light recently. In the mainly African tribes *Podalyrieae, Liparieae* and *Crotalarieae*, we have recorded the widespread occurrence of hydroxylated lupanines and various esters of these compounds (VAN WYK & VERDOORN 1990; VERDOORN & VAN WYK 1990, 1991; VAN WYK & al. 1991; VEEN & al. 1991). In a detailed study of the genus *Podalyria* (VAN WYK & al. 1992), roughly half the species were found to have hydroxylated lupanines and their angelate and tigliate esters, while the other half accumulated large amounts of lupanine as the only major compound. In order to find out how this interesting dichotomy in *Podalyria* should be interpreted, the homology of sparteine and lupanine as chemotaxonomic characters was investigated. The study was then extended to examples from other genera to test the predictivity of optical rotation and to see if it can be universally applied to quinolizidine-bearing genera.

Material and methods

Pure samples of sparteine and/or lupanine were isolated from selected species of *Podalyria* and some other genera of African legumes. Voucher specimens of the material used are

given below (all in JRAU): *Podalyria argentea* SALISB. (VAN WYK 2761), *P. calyptrata* (RETZ.) WILLD. (VAN WYK 2676), *P. canescens* E. MEY. (VAN WYK 2682), *P. cordata* (THUNB.) R. BR. (VAN WYK 2771), *P. glauca* (THUNB.) DC. (VAN WYK 2945), *P. cuneifolia* VENT. (VAN WYK 2589), *P. sericea* (ANDR.) R. BR. ex AIT. fil. (VAN WYK 2461 c), *Aspalathus nivea* THUNB. (VAN WYK 2813), *Lebeckia cytisoides* THUNB. (VAN WYK 2439), *L. melilotoides* DAHLGR. (VAN WYK 2562 a), *Virgilia divaricata* ADAMSON (VAN WYK 2647).

The alkaloids were extracted by standard procedures (e.g., VAN WYK & al. 1992). Finely ground air-dried material was extracted with 0.1 N sulphuric acid for 20 min and filtered through celite. The filtrate was made basic with ammonia, extracted with dichloromethane and the organic layer concentrated under reduced pressure to yield the crude alkaloid mixtures. Individual alkaloids were isolated from selected samples by column chromatography and/or preparative thin layer chromatography (silica gel 60; 0.25, 0.5 or 2 mm layer thickness). Various eluent systems were used, of which cyclohexane : chloroform : diethylamine (5 : 4 : 1) [System I] and cyclohexane : diethylamine (9 : 1) [System II] were particularly useful in separating sparteine and lupanine from other alkaloids. Alkaloids were visualised with iodoplatinate spray reagent. The optical rotation of each purified sample of sparteine and lupanine was measured on a Jasco DIP-370 polarimeter. (–)-sparteine $[\alpha]_D^{20}$ – 14° ($CHCl_3$) [lit., – 17.1° (EtOH) (SOUTHON & BUCKINGHAM 1988)]. (+)-lupanine $[\alpha]_D^{20}$ + 60° ($CHCl_3$) [lit., + 61° (EtOH) (SOUTHON & BUCKINGHAM 1988)] (–)-lupanine $[\alpha]_d^{20}$ – 60° ($CHCl_3$) [lit., – 61° (EtOH) (SOUTHON & BUCKINGHAM 1988)].

Results

The results showed that sparteine and lupanine from the two groups within *Podalyria* are indeed non-homologous. In species which have hydroxylated lupanines and esters, sparteine is of the (–)-form and lupanine of the (+)-form. In those species accumulating large amounts of lupanine (and no hydroxylupanines or esters), the lupanine was invariably found to be of the (–)-form. Investigation of *Lebeckia* and *Aspalathus* (two genera of the tribe *Crotalarieae* not known to produce esters) showed that both agree with the ester-producing *Pearsonia* (VERDOORN & VAN WYK 1990, 1991) in having the (–)-sparteine – (+)-lupanine pathway. The same is true of *Virgilia divaricata*, from which we have isolated (–)-sparteine and (+)-lupanine. A summary of the results is given in Table 1.

Discussion

Available knowledge about quinolizidine alkaloid biosynthesis is summarised by ROBINS (1981, 1985), SCHÜTTE (1985), and WINK (1987). The biosynthesis of both enantiomers of sparteine has been studied by ROBINS and co-workers. They showed that α-pyridones such a methylcytisine and cytisine are formed from (+)-sparteine via tetracyclic intermediates, of which ring A degrades and ring D is converted into a pyridone (FRASER & al. 1988, SAITO & al. 1989). The stereochemistry of the enzymatic processes involved in the biosynthesis of hydroxylated lupanines is currently receiving attention (e.g., SAITO & al. 1993 a, b, 1994), and it is clear that these compounds are associated with (–)-sparteine and (+)-lupanine. The apparent absence of esterification with tiglic acid, angelic acid and pyrrolyl carboxylic acid in hydroxylated α-pyridones (such as baptifoline) suggests that the enzymes involved are enantiomer-specific and that only the (–)-sparteine (+)-lupanine

Table 1. Optical rotation of sparteine and/or lupanine in various species and genera of the *Fabaceae*

	α-pyridone pathway		ester pathway	
	(+)-sparteine	(−)-lupanine	(−)-sparteine	(+)-lupanine
Podalyria argentea		+		
Podalyria calyptrata		+		
Podalyria canescens		+		
Podalyria cordata		+		
Podalyria glauca		+		
Podalyria cuneifolia			+	+
Podalyria sericea			+	
Aspalathus nivea			+	+
Lebeckia cytisoides			+	
Lebeckia melilotoides			+	
Virgilia divaricata			+	+

series are normally esterified. Acetic acid esters of α-pyridones, however, such as 13-acetoxyanagyrine, have been reported from *Baptisia* and *Thermopsis* (Southon & Buckingham 1988).

To interpret the alkaloid pattern in a particular genus, it is therefore important to determine if the alkaloids involved are of the ester pathway or of the α-pyridone pathway. The mere presence or absence of sparteine and lupanine in two genera may not be homologous in the phylogenetic sense. The scheme in Fig. 1 shows the two non-homologous pathways, of which one or the other is usually present. In *Podalyria*, both are present, but in different species. Those species with high yields of lupanine almost completely lack any other compounds, while those with relatively low yields of lupanine have various hydroxylated lupanines and their esters. This pattern is summarised in Fig. 2. Predictably, even when high yields of sparteine were found in a species generally known to produce esters, the sparteine turned out to be of the (−)-form. In some species of *Podalyria*, the progression towards α-pyridones seems to be blocked after the lupanine step, resulting in an accumulation of high concentrations of (−)-lupanine (more than 4% dry weight in some species). Species with (−)-sparteine and (+)-lupanine on the other hand, have a wide range of hydroxylupanines and esters. Unfortunately, the relationships between the species of *Podalyria* are unknown, so that the full taxonomic implication of the two pathways in *Podalyria* is difficult to evaluate. Ester alkaloids are mainly found in the species with sericeous leaves [*P. cuneifolia* Vent., *P. sericea* (Ait.) R. Br. ex Ait. fil., *P. leipoldtii* L. Bol., *P. microphylla* E. Mey. and *P. pearsonii* Phill.], and these species probably form a monophyletic group. The reported presence of racemic sparteine in *Virgilia* needs confirmation but now seems

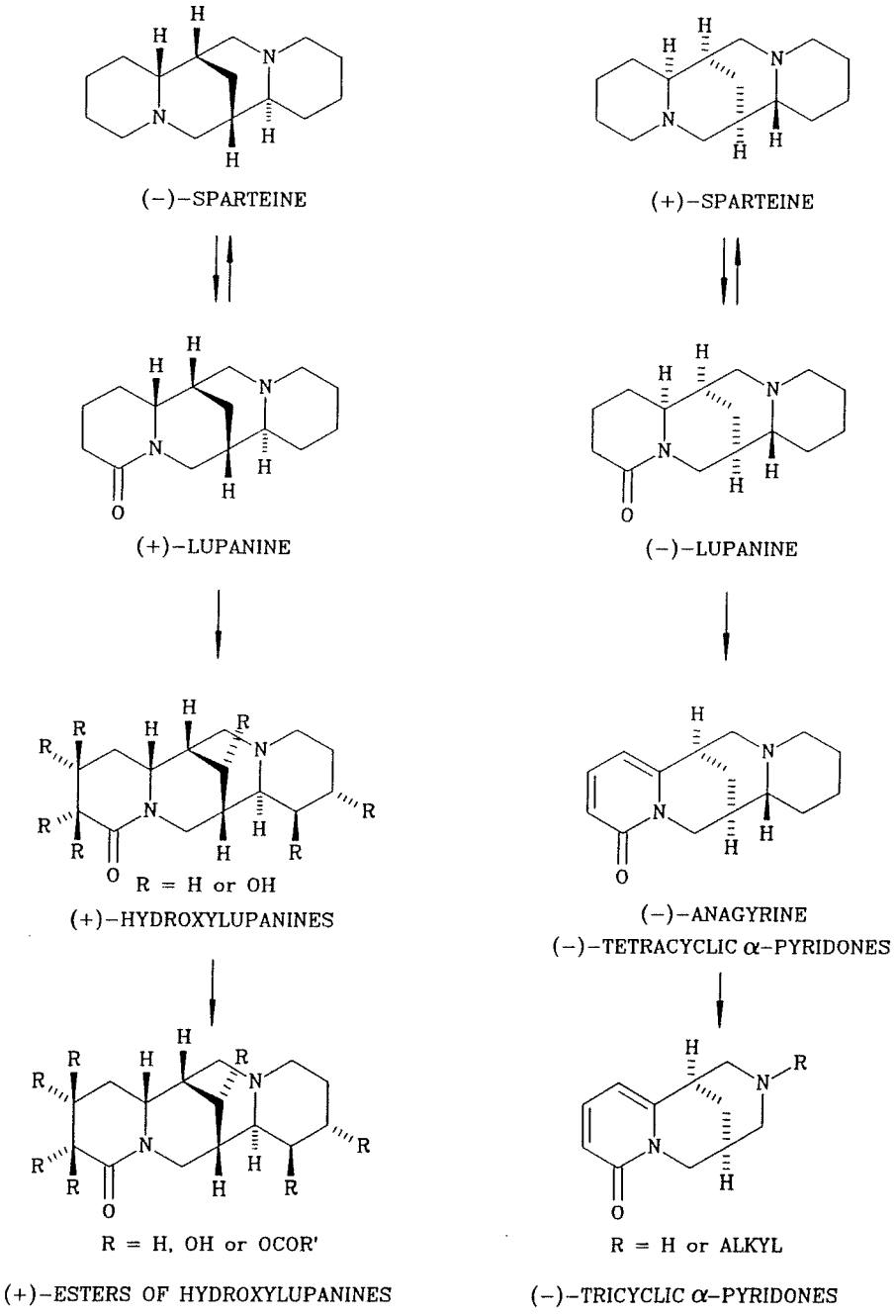

Fig. 1. Major tetracyclic quinolizidine alkaloids of the *Fabaceae*, showing the biogenetic relationships between different enantiomers in the ester pathway (left) and α-pyridone pathway (right)

unlikely. *Virgilia* has a characteristic combination of bi-, tri- and tetracyclic quinolizidines and several esters of alkaloids (VEEN & al. 1991), but the optical rotation of the major compounds was not reported in recent studies (GREINWALD & al. 1989, VEEN & al. 1991). According to our hypothesis, *Virgilia* should have the ester

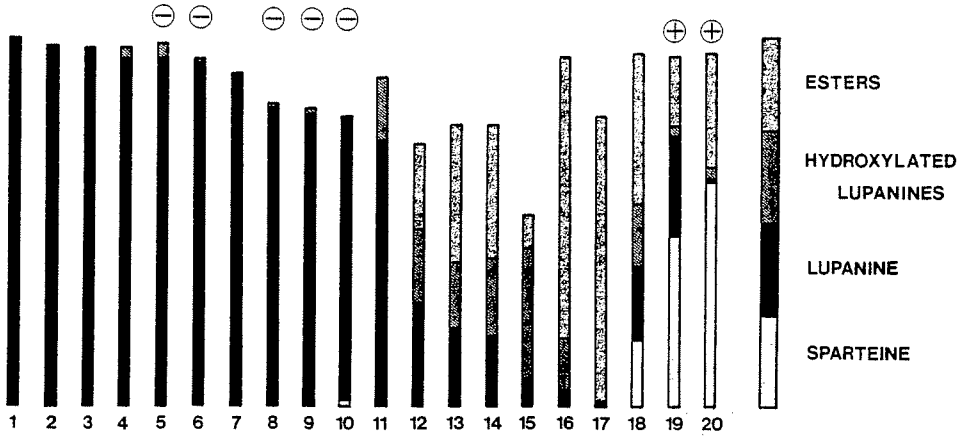

Fig. 2. Schematic summary of the dichotomous distribution of (–)-lupanine and (+)-lupanine in 20 species of the genus *Podalyria*. Species 1 to 10 accumulate large amounts of (–)-lupanine and virtually no other alkaloids. Species 12 to 20 accumulate (–)-sparteine, (+)-lupanine, together with various hydroxylupanines and their esters (data from Van Wyk & al. 1992)

pathway and our results indeed show that *V. divaricata* has the expected (–)-sparteine and (+)-lupanine. In view of the large number of chiral centres in sparteine and lupanine, it is difficult to imagine that a single plant would produce racemic mixtures. Even very small quantities of lupanine (optical rotation + 61°) contaminating a sample of sparteine (– 17.1°) could easily result in a reading of near zero, thus indicating racemic sparteine.

If optical rotation is not specified, the reported presence or absence of sparteine and lupanine in a particular genus is ambiguous. In terms of chemosystematics, this means a loss of important information. For phylogenetic analyses, it is possible to interpret each of the biogenetic steps along the two pathways as irreversible evolutionary changes (even if the enzymes are unknown at this stage), thus resulting in almost ideal data for cladistic analysis. This is the only intellectually satisfying way to interpret any particular alkaloid profile. Research on the enzymatic processes is making steady progress, and this will add important supportive evidence for the stereospecificity of some of the compounds. Saito & al. (1993 a), for example, have shown that esterification with angelic or tiglic acid is also not random but specific. In *Pearsonia* species, hydroxylupanines are esterified exclusively with angelic acid (Verdoorn & Van Wyk 1990), in *Cytisus scoparius* exclusively with tiglic acid (Saito & al. 1993 a) and in *Lupinus* species with both angelic and tiglic acids (Mühlbauer & al. 1988). In *Cadia* (Van Eijk & Radema 1976, 1977), *Calpurnia* (Van Eijk & Radema 1977, Radema & al. 1979, Asres & al. 1986), *Virgilia* (Veen & al. 1991), *Priestleya* (Van Wyk & al. 1991) and the yellow-flowered species of *Podalyria* (Van Wyk & al. 1992), the major alkaloids are esterified with pyrrolyl carboxylic acid. The types of acids are therefore further phylogenetic characters which can be added to the scheme in Fig. 1 as additional apomorphies. Another exciting aspect of this approach is the possibility to distinguish between homologous and non-homologous absences. The absence of α-pyridones in *Podalyria* for example, is not due to a lack of the precursors [(+)-

sparteine and (–)-lupanine] but to the apparent absence of the appropriate enzyme(s) between (–)-lupanine and (+)-anagyrine (see Fig. 1).

A literature study of the known distribution of the ester- and the α-pyridone pathways within the genera of the *Fabaceae* (SOUTHON & BUCKINGHAM 1988), as judged by the reported co-occurrence of (+)- and (–)-sparteine and (–)- and (+)-lupanine, revealed that any particular genus is likely to have only one or the other. Some large genera have both, but the presence of both pathways in the same species remains to be demonstrated convincingly. Thus in the genera *Genista* and *Lupinus*, alkaloid esters and α-pyridones are not known to co-occur within the same species. The relation between (–)-sparteine and hydroxylated lupanines on the one hand, and (+)-sparteine and α-pyridones on the other hand, seems universal. Not a single reliable literature report (where optical rotation has actually been measured) could be found where this association does not hold. Most genera for which data are available, can therefore readily be classified into one of the two groups.

Conclusion

We propose that the two enantiomer pairs of sparteine and lupanine are the precursors of two separate and independent biosynthetic pathways, the one leading to α-pyridone alkaloids and the other to esters of tetracyclic quinolizidines via hydroxylupanine intermediates. In *Podalyria* species with high yields of lupanine and no esters, alkaloid biosynthesis follows the α-pyridone pathway, but the progression towards α-pyridones seems to be blocked after the lupanine step, resulting in an accumulation of (–)-lupanine. In those species with hydroxylupanines and esters, alkaloid biosynthesis follows the ester pathway. Hydroxylation and subsequent esterification of lupanine only seem possible when (–)-sparteine and (+)-lupanine are the precursors. Species from the genera *Aspalathus, Lebeckia, Pearsonia,* and *Virgilia* all show the expected combination of (–)-sparteine and (+)-lupanine. Literature data further support our hypothesis that the biosynthetic dichotomy applies to all quinolizidine-bearing genera. The reported occurrence of racemic mixtures of sparteine (and lupanine) in some species needs confirmation.

Chemotaxonomic comparisons of alkaloid profiles can be simplified by measuring the optical rotation of sparteine and lupanine. The recognition of a separate ester pathway provides a sound basis for comparing genera and species in a rigorous way. The relevant biosynthetic pathway can thus be predicted, even when the end products (α-pyridones or esters) are not present. This generalisation greatly simplifies the complexity and represents an important step towards a more complete understanding of the taxonomic and evolutionary significance of quinolizidine alkaloids in the subfamily *Papilionoideae*.

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