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ALKALOID DISTRIBUTION IN SEEDS OF *ORMOSIA*, *PERICOPSIS* AND *HAPLORMOSIA**

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Key Word Index—*Ormosia*; *Pericopsis*; *Haplormosia*; Leguminosae; chemotaxonomy; quinolizidine alkaloids; homopodopetaline.

Abstract—Alkaloid profiles were determined by capillary gas chromatography/mass spectrometry in seeds of 15 *Ormosia* species, of both South American and Asian origin, as well as in three *Pericopsis* species and *Haplormosia monophylla*. All samples contained alkaloids, and a total of 31 compounds were identified, comprising 23 lupine-type and seven *Ormosia*-type quinolizidine alkaloids, and the dipiperidine alkaloid, ammodendrine. Homopodopetaline, which has not previously been characterized as a natural product, was detected in extracts prepared from *O. coutinhoi*, *O. macrophylla* and *O. semicastrata* seeds. *Ormosia*-type quinolizidine alkaloids were restricted to the genus *Ormosia*, but were not observed in four members of this genus. The *Pericopsis* species accumulated predominantly α-pyridone quinolizidine bases, while two collections of *H. monophylla* contained mainly lupine-type quinolizidine alkaloids of the sparteine/lupanine class.

INTRODUCTION

The tribe Sophoreae is arranged taxonomically between the legume subfamily Caesalpinioideae and the remainder of the subfamily Papilionoideae. A constituent group of the Sophoreae, the *Ormosia* group, consists of the three genera, *Ormosia* (*ca* 100 species), *Pericopsis* (four species) and *Haplormosia* (one species). *Ormosia* species are found in the tropics of eastern South America and eastern Asia to northeastern Australia, but are absent in Africa [2]. Although *Ormosia* is generally regarded as constituting one genus, Yakovlev has recognized its segregation into six separate genera based on fruit structure and seed dispersal [3, 4].

Quinolizidine alkaloids have been reported to occur only in the 10 most primitive tribes of the Papilionoideae, and have a high chemosystematic significance [5–9]. The genus *Ormosia* is characterized by the occurrence of some of the most structurally complex quinolizidine alkaloids found in the Leguminosae [5–9], while only pyridone quinolizidine bases have been found to occur in *Pericopsis* [9–12]. There has been no prior report of alkaloids in *Haplormosia*.

In this study, we have investigated alkaloid profiles of seeds representing 15 *Ormosia* species, of both South American and Asian origin, as well as of three *Pericopsis* and one *Haplormosia* species. The objective of the investigation was to determine, in a preliminary manner, if the distribution of different structural types of quinolizidine alkaloids would support the proposed taxonomic subdivisions of the species examined. In view of the small quantities of alkaloids present, seed alkaloidal identifications were carried out by capillary GC/MS using a combination of two stationary phases.

RESULTS AND DISCUSSION

The dipiperidine alkaloid, ammodendrine, and 23 lupine-type and seven *Ormosia*-type quinolizidine alkaloids were identified in one or more of the *Ormosia*, *Pericopsis* and *Haplormosia* species studied in this investigation, by GC/MS comparison with authentic samples (Table 1). GC/MS has been widely applied towards the analysis of lupine alkaloids [9], and the data in Table 1 substantiate a previous observation [12] of the diverse nature of this class of quinolizidine alkaloids in the genus *Ormosia*. While relatively few of the species embraced in the present study have been examined before for lupine-type quinolizidine alkaloids, the various past identifications of α-pyridone bases in *O. emarginata* [13], *P. laxiflora* [11] and *P. mooniana* [12] were confirmed.

The *Ormosia*-type quinolidizine alkaloids are pentacyclic (C_{20}) and hexacyclic (C_{20} and C_{21}) compounds that are restricted in distribution to members of only a few genera in the Papilionoideae, including *Ormosia* [5–9, 14]. Such compounds are based on the same carbon skeleton and differ only in their degree of unsaturation and/or in their stereochemistry [9, 14]. While mass spectrometry is useful in assigning the molecular formulas of *Ormosia* alkaloids, even at low-resolution, this technique appears to be restricted in value in distinguishing between such

*Part 3 in the series 'Alkaloids of Papilionoideae'. For part 2, see ref. [1].

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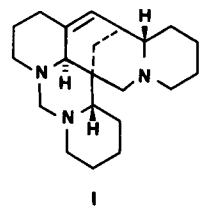
Table 1. Quinolizidine and dipiperidine alkaloids identified in *Ormosia*, *Pericopsis* and *Haplormosia* seeds in the present investigation

Species	Alkaloid(s) identified	Number of unidentified alkaloids
Ormosia amazonica	5,6-Dehydrolupanine, 13-hydroxylupanine, lupanine, ormosanine, 17-oxo-lupanine, 17-oxosparteine, panamine, sparteine	13
O. balansae	β-Isosparteine, jamine, lupanine, 10-oxo-β-isosparteine, 17-oxolupanine, 17-oxosparteine, panamine, sparteine	5
O. cinerea	α-Isolupanine, α-isosparteine, β-isosparteine, lupanine, sparteine, tetrahydrorhombifoline	9
O. coutinhoi	Homo-6-epipodopetaline, homopodopetaline, lupanine, podopetaline	6
O. discolor	Anagyrine, 5,6-dehydrolupanine, α-isosparteine, lupanine, 17-oxolupanine, 17-oxosparteine, sparteine	3
O. emarginata	Ammodendrine, cytisine, *N*-formylcytisine, lupanine, *N*-methylcytisine, tetrahydrorhombifoline	3
O. fordiana	Ammodendrine, anagyrine, 5,6-dehydrolupanine, α-isolupanine, lupanine, 17-oxolupanine	2
O. henryi	Ammodendrine, cytisine, *N*-formylcytisine, *N*-methylcytisine	1
O. macrocalyx	Angustifoline, 5,6-dehydrolupanine, α-isoangustifoline,* β-isosparteine, lupanine, ormosanine, 17-oxolupanine, panamine, sparteine, 13α-tiglyoxylupanine	5
O. macrophylla	Homopodopetaline	3
O. nobilis	Angustifoline, homo-6-epipodopetaline, α-isoangustifoline,* α-isolupanine, lupanine, *N*-methylcytisine, sparteine, tetrahydrorhombifoline, 13α-tigloyloxylupanine	8
O. pachycarpa	Angustifoline, α-isoangustifoline,* lupanine, panamine, sparteine	2
O. panamensis	Anagyrine, baptifoline, *N*-formylcytisine, lupanine, *N*-methylcytisine, rhombifoline, thermopsine	5
O. semicastrata	Angustifoline, 6-epipodopetaline, homopodopetaline, α-isoangustifoline,* α-isolupanine, jamine, lupanine, ormosanine, 11-oxotetrahydrorhombifoline,* podopetaline, tetrahydrorhombifoline, 13α-tigloyloxylupanine	7
O. sumatrana	Ammodendrine, anagyrine, 5,6-dehydrolupanine, α-isolupanine, α-isosparteine, lupanine, ormosanine, panamine, sparteine	7
Pericopsis angolensis	Ammodendrine, anagyrine, cytisine, *N*-formylcytisine, *N*-methylcytisine	1
P. (Afrormosia) laxiflora	Cytisine, lupanine, *N*-methylcytisine, sparteine	0
P. mooniana	Cytisine, *N*-formylcytisine, *N*-methylcytisine	0
Haplormosia monophylla†	Anagyrine, 5,6-dehydrolupanine, 5,6-dehydro-α-isosparteine, α-isosparteine, lupanine, 10-oxo-β-isosparteine, sparteine	4
H. monophylla‡	Anagyrine, 5,6-dehydrolupanine, 5,6-dehydro-α-isosparteine, lupanine, sparteine	3

*Tentative identification.
†Collected in Liberia (Barker 1221).
‡Collected in Nigeria (Sankey *s.n.*).

stereoisomers on the basis of differential fragmentation patterns [9, 12, 14, 15]. However, the resolution attained on the capillary GC stationary phases used in this study was such that the available pairs of authentic *Ormosia* alkaloid stereoisomers were clearly separated, and, as a result, it was possible to identify seven *Ormosia* alkaloids among nine members of the genus (Table 1). The previous identifications of ormosanine in the seeds of *O. semicastrata* and *O. sumatrana* were confirmed, in addition to podopetaline in the former species, as established by McLean and co-workers [13, 15, 16].

To date, no C_{21} homo-derivative of an unsaturated pentacyclic $C_{20}H_{33}N_3$ *Ormosia* alkaloid has been identified as a constituent of any species in the genus. In this study, it was possible to detect homo-6-epipodopetaline and homopodopetaline (**1**) in several *Ormosia* species (Table 1). Homo-6-epipodopetaline has previously been found to occur in plant parts of *Acosmium panamense* (Benth.) Yakovlev and *A. subelegans* (Mohlenbrock) Yakovlev [14]. Homopodopetaline (**1**) is a new natural product, although it has been produced synthetically from podopetaline isolated from *Podopetalum ormondii* [16], which is now known as *O. ormondii*. The identification of **1** in extracts of *O. coutinhoi*, *O. macrophylla* and *O. semicastrata* was confirmed by GC/MS comparison with the



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aminal formed by the known reaction of podopetaline with formaldehyde [14, 16].

The *Ormosia* species studied embraced all four sections of the genus, as enunciated by Rudd [17], as well as representatives in each of Yakovlev's genera [3, 4] *Ormosia*, *Fedorovia*, *Macroule*, *Placolobium*, *Ruddia* and *Trichocyamos*. About half these species were collected in South America and half in Asia. The study also included three of the four species of the closely related genus, *Pericopsis*, in addition to collections of *Haplormosia monophylla* from both Liberia and Nigeria, a species which constitutes a monotypic genus [2]. Reference to Table 1 shows that a number of the alkaloids that occurred in the seeds of the species investigated were unidentified. Most of these compounds were *Ormosia*-type quinolizidine stereoisomers for which no authentic standards were available for comparison purposes, although such compounds were of an assignable molecular formula. Therefore, the alkaloid constituents of the species studied have been divided into six classes, based on structural complexity and/or postulated biogenetic advancement, namely, (i) the dipiperidine alkaloid, ammodendrine; (ii) tetracyclic alkaloids of the sparteine/lupanine type; (iii) an ester of an alkaloid in (ii); (iv) tricyclic degradation products of the alkaloids in (ii); (v) *Ormosia* alkaloids based on the general structures A-H shown in Fig. 1, and (vi) α-pyridone quinolizidine bases. In all cases, unidentified alkaloids indicated in Table 1, with uncertain stereochemistry or position of oxygenated functionalities, could be included in one of these six categories. Alkaloids in each major class, expressed as a percentage of the total alkaloids in each *Ormosia*, *Pericopsis* and the *Haplormosia* species studied, are shown in Table 2. In this manner, it was felt that more definitive conclusions could be made concerning variations of alkaloid profiles in relation to taxonomic subdivisions of the species represented. It was also considered that the expression of total alkaloid percentages in groups (i)–(vi) would be more valuable than determinations of the % w/w yield of each alkaloid constituent in each seed investigated.

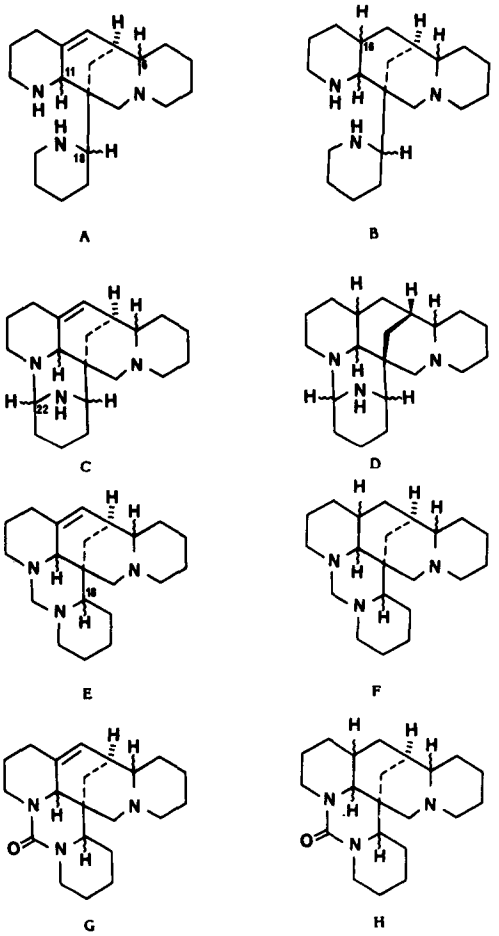

Fig. 1. General structural types of *Ormosia*-type quinolizidine alkaloids detected in this study. For elemental composition of types A-H, see text.

As may be seen from Table 2, *Ormosia*-type quinolizidine alkaloids were found in 11 of the 15 *Ormosia* species studied, that were indigenous to both South America and Asia. These compounds were present in species in the sections *Ormosia*, *Macrocarpae*, and *Unicolores*, but were absent in the two species in the section *Emarginatae*, namely, *O. emarginata* and *O. henryi*, which are also classified in Yakovlev's genus *Fedorovia*. *Ormosia* alkaloids were also not detected in *O. fordiana* and *O. panamensis* seeds, which are both in the section *Ormosia*. When *Ormosia* alkaloids were present, the most prevalent type was the hexacyclic $C_{20}H_{33}N_3$ variant (Fig. 1D), as represented by the compound, panamine. There was evidence for the accumulation of oxygenated *Ormosia* alkaloids in five species, with molecular formulas $C_{21}H_{31}N_3O$ and $C_{21}H_{33}N_3O$ (Fig. 1, G and H, respectively). Such compounds have not hitherto been observed as natural products, and exhibited mass spectral fragmentation patterns similar to those of homoxy-6-epipodopetaline ($C_{21}H_{31}N_3O$) and homoxyormosanine ($C_{21}H_{33}N_3O$), respectively, that were synthesized according to previous methodology [13, 14]. None of these oxygenated *Ormosia* alkaloids exhibited coincident column residence times to the two standards that were prepared, and thus they could not be provided with stereochemical assignments. No trace of *Ormosia*-type quinolizidine alkaloids was found in any species investigated in the genera *Pericopsis* and *Haplormosia* (Table 2).

As has been pointed out previously [9], among the quinolizidine alkaloid-bearing genera of the papilionates, there appears to be a mutual exclusivity in enzyme systems that elaborate *Ormosia*- and α-pyridone-base types, with the latter regarded as being more biogenetically advanced. In the present investigation, it was generally found that species that biosynthesized *Ormosia* alkaloids tended to produce no α-pyridones, and *vice versa*. However, traces of α-pyridones were found in seeds of *O. discolor* and *O. nobilis* that were also well represented by *Ormosia* alkaloids. In addition, data for *O. sumatrana* proved exceptional, in that anagyrine was found to constitute nearly 10% of the total seed alkaloids, and over 40% of the remainder were *Ormosia* alkaloids. The tetracyclic α-pyridone, anagyrine, however, may be regarded as being less biogenetically advanced than the tricyclic α-pyridones [18]. Given the tendency of *Ormosia* and α-pyridone alkaloids not to co-occur in a given *Ormosia* species, the present data showing that α-pyridones are the predominant quinolizidines in *O. panamensis* and *O. emarginata* contradict earlier reports on *Ormosia* alkaloids in these species published, respectively, by Lloyd and Horning [19] and Arthur and Loo [20]. We were able to obtain a

Table 2. Percentage of major classes of alkaloids occurring in *Ormosia*, *Pericopsis* and *Haplormosia* seeds

Genus	Collection* location	Alkaloid class† I	II	III	IV	V_A	V_B	V_C	V_D	V_E	V_F	V_G	V_H	VI
Ormosia‡														
Section *Ormosia*														
Series *Amacrotropis*														
O. semicastrata	AS	0	0.8	1.4	12.4	62.5	22.0	0	0	0.4	0.3	0	0.2	0
Series *Amazonicae*														
O. amazonica	SA	0	43.5	0	0	0	14.6	0.6	38.6	0	1.2	0.8	0.7	0
O. fordiana	AS	3.6	93.5	0	0	0	0	0	0	0	0	0	0	2.9
O. sumatrana	AS	0.8	44.2	0	0	1.1	7.3	0	34.5	0.2	2.0	0	0	9.9
Series *Nobiles*														
O. discolor	SA	0	99.7	0	0	0	0	0	0	0	0.1	0	0	0.2
O. macrophylla	SA	0	0	0	0	0	4.5	26.9	65.0	2.1	1.5	0	0	0
O. nobilis	SA	0	2.7	1.6	1.5	0	t§	0	91.5	2.5	t	0	0	0.1
Series *Pachycarpae*														
O. pachycarpa	AS	0	68.9	0	15.8	0	6.1	0	2.7	0	6.5	0	0	0
Series *Panamenses*														
O. panamensis	SA	0	t	0	0	0	0	0	0	0	0	0	0	100.0
Section *Macrocarpae*														
O. balansae	AS	0	94.8	0	0	0	0	0	0.1	0	4.1	0	1.0	0
O. cinerea	SA	0	25.9	0	t	0	70.4	0	0	0	3.6	0	t	0
O. coutinhoi	SA	0	1.0	0	0	88.9	1.6	0	0	7.3	1.1	0.1	0	0
Section *Emarginatae*														
O. emarginata	AS	13.7	0.1	0	3.6	0	0	0	0	0	0	0	0	82.6
O. henryi	AS	3.4	6.0	0	0	0	0	0	0	0	0	0	0	90.6
Section *Unicolores*														
O. macrocalyx	SA	0	46.5	0	0.4	1.0	1.1	0	47.1	0	t	0	3.9	0
Pericopsis														
P. angolensis	AF	26.1	0.1	0	0	0	0	0	0	0	0	0	0	73.8
P. laxiflora	AF	0	1.5	0	0	0	0	0	0	0	0	0	0	98.5
P. mooniana	AF	0	0	0	0	0	0	0	0	0	0	0	0	100.0
Haplormosia														
H. monophylla	AF	0	88.7	0	0	0	0	0	0	0	0	0	0	11.3
H. monophylla	AF	0	86.8	0	0	0	0	0	0	0	0	0	0	13.2

*Key to continent in which collected: AF, Africa; AS, Asia; SA, South America or Mexico.

†Key to alkaloid classification: I, dipiperidine alkaloid, II, sparteine- lupanine-type; III, an ester of an alkaloid in group II; IV, postulated degradation products of alkaloids in group II; V, *Ormosia*-type, A, pentacyclic, $C_{20}H_{33}N_3$, B, pentacyclic, $C_{20}H_{35}N_3$,C, hexacyclic, $C_{20}H_{31}N_3$, D, hexacyclic, $C_{20}H_{33}N_3$, E, hexacyclic, $C_{21}H_{33}N_3$, F, hexacyclic, $C_{21}H_{35}N_3$, G, hexacyclic, $C_{21}H_{31}N_3O$, H, hexacyclic, $C_{21}H_{33}N_3O$; VI, α-pyridone type.

‡Classified according to Rudd [17].

§t = trace (< 0.05% w/w).

sample of the seeds used by Lloyd and Horning, and it is apparent that their seeds were originally misidentified as *O. panamensis* and are actually *O. macrocalyx* Ducke. The latter species has been represented in the present study, and it is significant that among its constituents (Table 1) are the *Ormosia* alkaloids, ormosanine and panamine, as originally identified by Lloyd and Horning [19]. Although we were unable to check on the identity of the material used by Arthur and Loo [20], this may also be a case of taxonomic misidentification, since in a later work on *O. emarginata* by McLean *et al.* [13], the α-pyridone base *N*-methylcytisine was the only quinolizidine alkaloid constituent detected, with *Ormosia* alkaloids being absent.

Other observations concerning compound identification include the occurrence of ammodendrine in four *Ormosia* species, all of Asian origin (Table 1). Thus far, this dipiperidine alkaloid has not so far been detected in any papilionate genus more taxonomically primitive than *Ormosia* [9], having been previously identified in a South American member of the genus [12]. Esters of sparteine/lupanine alkaloids were somewhat rare among the *Ormosia* species, and not found in any *Pericopsis* species or *H. monophylla* (Table 2). Analysis of *H. monophylla* seeds from two geographical locations revealed similar alkaloid profiles, with α-isosparteine being the most abundant alkaloid in both cases.

In conclusion, it may be pointed out that GC/MS is a very suitable approach for determining quinolizidine alkaloid profiles of papilionaceous species, in being sensitive, rapid and facile. Such methodology is particularly of value in chemosystematic studies, in that the absence of a particular class or classes of compounds may be established in a given species. Thus, since no *Ormosia*-type quinolizidine alkaloids were found in any of the *Pericopsis* species or *H. monophylla* in this preliminary study, support has been provided for retaining the taxonomic divisions between these genera and *Ormosia*. The prevalence of α-pyridone quinolidizine bases and the exclusion of *Ormosia* alkaloids in the specimens investigated, substantiates the proposed taxonomic subdivi-

sions [17] for *O. panamensis* (section *Ormosia*, series *Panamenses*) and *O. emarginata* and *O. henryi* (section *Emarginatae*). An extended GC/MS study of all the *Ormosia* species could therefore help provide a further understanding of the suitability of the proposals [3, 4, 17] for the subdivision of the genus, and help establish if the South American and Asian species differ phytochemically. Such studies might also provide chemical data which could help elucidate why the genus *Ormosia* is absent from Africa and whether the American or Asian representatives are the more primitive group of the genus.

EXPERIMENTAL

GC/MS. Finnigan GC/MS 4510, equipped with INCOS data system; Varian 1440 GC/Varian MAT 112S MS, modified with a Cook interface connected to a deactivated vitreous silica capillary tube direct line, and Varian 166 data system.

Plant material. Seeds of the following species (country of origin and voucher number in parenthesis) were obtained from the Herbarium, Royal Botanic Gardens, Kew [Krukoff Seed Collection (K) and the South China Institute of Botany (ISBC)]: *Ormosia amazonica* Ducke (Ecuador, Pennington 10787), *O. balansae* Drake (Guangzhou, China, Chen Pong-yu s.n., from ISBC), *O. cinerea* R. Ben. (Surinam, Wullschflagel 1493, K), *O. coutinhoi* Ducke (Surinam, Makauria and Supenaam 124a), *O. discolor* Spruce ex Benth. (Brazil, Krukoff 20822), *O. emarginata* (Hook. & Arn.) Benth. (Hong Kong, Dept. of Agric. s.n. 1975), *O. fordiana* Oliv. (China, Tsing Ying 1539), *O. henryi* Prain (Hainan, China, Cheng Pong-yu s.n., from ISBC), *O. macrocalyx* Ducke (Mexico, Souza 4211), *O. macrophylla* Benth. (Brazil, Zarucchi 1320), *O. nobilis* Tal. (Brazil, Murca Pires s.n.), *O. pachycarpa* Champ. ex Benth. (Hong Kong, Dept. of Agric, s.n. 1975), *O. panamensis* Benth. (Panama, Roy DB86), *O. semicastrata* Hance (Hong Kong, Krukoff 1974/26), *O. sumatrana* (Miq.) Prain (Thailand, Niyomdham 815), *Pericopsis angolensis* (Bak.) Van Meeuwen (Zimbabwe, Krukoff s.n.), *P.* (*Afrormosia*) *laxiflora* (Benth.) Van Meeuwen (Mali, Lafemere 80), *P. mooniana* Thw. (Borneo, Kostermans 6122), *Haplormosia monophylla* Harms. (Liberia, Barker 1221; Nigeria, Sankey s.n.).

Seed extractions and chromatographic methods. Each seed sample (*ca* 0.5 g) was ground and extracted with 75% EtOH (2 × 10 ml) at room temp. Seed EtOH extracts were evapd to dryness *in vacuo*, and moistened with 28% NH_4OH. On drying and acidification with N HCl, impurities were removed with CH_2Cl_2. The aqueous portion of each extract was made alkaline with 28% NH_4OH (pH 8.5), and alkaloids were extracted into CH_2Cl_2 and subjected to GC/MS analysis.

Using the Finnigan instrument, GC/MS was performed on a DB-5 column (J & W Scientific, Folsom, California) (30 m × 0.25 mm i.d. × 0.25 μm film thickness) with the column temp. held at 180° for 1 min, and then programmed 180–300° at 4° min. He head pressure, *ca* 0.70 kg/cm^2. Injector temp. 230°, interface separator temp. 270°, electron energy, 70 eV, emission current, 0.25 mA, scan-to-scan ratio, 1 sec, mass range scanned, 45–475 au. With the Varian instrument, GC/MS was conducted on a DB-1 column (J & W Scientific) (30 m × 0.32 mm i.d. × 0.25 μm film thickness). Other conditions were the same as those above, except that the programme was only continued to 270°, and the He head pressure was *ca* 0.28 kg/cm^2. In both cases, splitless injection with 1 μl of each diluted alkaloidal extract was used. Quantitation of each alkaloid as a percentage of the total alkaloids in a given extract was performed by internal normalization.

Reference alkaloids. Authentic samples of the following alkaloids, either in the form of free bases or salts, were available to us, as described previously [1, 12, 21–24]: ammodendrine, angustifoline, anagyrine, baptifoline, cytisine, 5,6-dehydrolupanine, 11,12-dehydrosparteine (5,6-dehydro-α-isosparteine), 13-*epi*-hydroxylupanine (jamaidine), 6-epipodopetaline (sweetinine), *N*-formylcytisine, homo-6-epipodopetaline, 13-hydroxylupanine, α-isolupanine, α-isosparteine, β-isosparteine, lupanine, *N*-methylcytisine, ormosanine, ormosinine, 10-oxo-β-isosparteine, 17-oxolupanine, 17-oxosparteine, panamine, podopetaline, sparteine, templetine, tetrahydrorhombifoline and thermopsine. Jamine (homo-ormosanine), as well as a mixture of 13α-angeloyloxylupanine and 13α-tigloyloxylupanine, and a *Sophora secundiflora* (Ort). Lag. ex DC. extract containing rhombifoline [25], were kindly supplied by other workers in this area. Homopodopetaline and homotempletine were synthesized from podopetaline and templetine, respectively, by reaction with formaldehyde [14, 16, 26]. 13β-Tigloyloxylupanine was prepared by the general method of ref. [27], by reaction of tigloyl chloride with 13-epihydroxylupanine. Homoxy-6-epipodopetaline was prepared from (±)-6-epipodopetaline (2 mg) by dissolution in benzene (3 ml), addition of triethylamine (0.3 ml), cooling, and passage of phosgene gas for 2 min; after standing overnight and flushing with N_2, the homoxy derivative was obtained as a white powder [14, 28]. The homoxy derivative of ormosanine was prepared in a similar way.

Identification of alkaloids. The following compounds (arranged in the classification used in Table 2) were identified by direct comparison (RR_t to lupanine on DB-5 and DB-1 columns, respectively; MS) to authentic alkaloids: (i) dipiperidine alkaloid, ammodendrine, RR_t: 0.57, 0.47; MS: *m/z* 208 $[M]^+$ [29]; (ii) sparteine-/lupanine-type quinolizidine alkaloids, 5,6-dehydrolupanine, RR_t: 0.95, 0.94; MS: *m/z* 246 $[M]^+$ [30]; 11,12-dehydrosparteine, RR_t: 0.46, 0.40; MS: *m/z* 232 $[M]^+$ [12]; 13-hydroxylupanine, RR_t: 1.41, 1.69; MS: *m/z* 264 $[M]^+$ [30]; α-isolupanine, RR_t: 0.91, 0.90; MS: *m/z* 248 $[M]^+$ [30]; α-isosparteine, RR_t: 0.35, 0.34; MS: *m/z* 234 $[M]^+$ [9, 14]; β-isosparteine, RR_t: 0.51, 0.49; MS: *m/z* 234 $[M]^+$ [31]; lupanine, RR_t: 1.00, 1.00; MS: *m/z* 248 $[M]^+$ [32]; 10-oxo-β-isosparteine, RR_t: 1.03, 1.12; MS: *m/z* 248 $[M]^+$ [24]; 17-oxolupanine, RR_t: 1.32, 1.45; MS: *m/z* 262 $[M]^+$ [32]; 17-oxosparteine, RR_t: 0.86, 0.88; MS: *m/z* 248 $[M]^+$ [32]; sparteine, RR_t: 0.44, 0.38; MS: *m/z* 234 $[M]^+$ [30]; (iii) ester of alkaloid in (ii), 13α-tigloyloxylupanine, RR_t: 2.05, 2.47; MS: *m/z* 346 $[M]^+$ [27]; (iv) tricyclic degradation products of alkaloids in (ii), angustifoline, RR_t: 0.88, 0.85; MS: *m/z* 234 $[M]^+$ missing, 193 [30]; tetrahydrorhombifoline, RR_t: 0.83, 0.80; MS: *m/z* 248 $[M]^+$ missing, 207 [22]; (v) *Ormosia* quinolizidine alkaloids, $C_{20}H_{33}N_3$ (Fig. 1A): 6-epipodopetaline, RR_t: 1.37, 1.41; MS: *m/z* 315 $[M]^+$ [23]; podopetaline, RR_t: 1.43, 1.73; MS: *m/z* 315 $[M]^+$ [9, 14]; $C_{20}H_{35}N_3$ (Fig. 1B): ormosanine, RR_t: 1.43, 1.60; MS: *m/z* 317 $[M]^+$ [12]; $C_{20}H_{33}N_3$ (Fig. 1D): panamine, RR_t: 1.56, 1.81; MS: *m/z* 315 $[M]^+$ [12]; $C_{21}H_{33}N_3$ (Fig. 1E): homo-6-epipodopetaline, RR_t: 1.55, 1.67; MS: *m/z* 327 $[M]^+$ [23]; homopodopetaline, RR_t: 1.61, 1.75; MS: *m/z* 327 $[M]^+$ (100), 312 (8), 284 (8), 244 (22), 243 (36), 229 (53), 98 (8), 84 (3), 55 (5), 41 (12); $C_{21}H_{35}N_3$ (Fig. 1F): jamine, RR_t: 1.67, 1.90; MS: *m/z* 329 $[M]^+$ [9, 14]; (vi) α-pyridone quinolizidine bases, anagyrine, RR_t: 1.42, 1.55; MS: *m/z* 244 $[M]^+$ [30]; baptifoline, RR_t: 1.82, 1.90; MS: *m/z* 260 $[M]^+$ [1]; cytisine, RR_t: 0.78, 0.71; MS: *m/z* 190 $[M]^+$ [33]; *N*-formylcytisine RR_t: 1.33, 1.24; MS: *m/z* 218 $[M]^+$ [34]; *N*-methylcytisine, RR_t: 0.78, 0.66; MS: *m/z* 204 $[M]^+$ [33]; rhombifoline, RR_t: 1.00, 0.99; MS: *m/z* 244 $[M]^+$ missing, 203 [30]; thermopsine, RR_t: 1.25, 1.38; MS: *m/z* 244 $[M]^+$ [30].

Resolution of lupine-type quinolizidine alkaloid isomers. Four pairs of isomers, epimeric at C-11 or C-6, were separable by analysis of their RR_ts to lupanine on both stationary phases used, namely, anagyrine and thermopsine, α-isolupanine and lupanine, α-isosparteine and sparteine, and 10-oxo-β-isosparteine and 17-

oxosparteine. The epimeric pairs, 13-epihydroxylupanine (RR_t 1.47, 1.62, on DB-5 and DB-1, respectively) and 13-hydroxylupanine, and 13α-tigloyloxylupanine and 13β-tigloyloxylupanine [RR_t 2.43, 3.12; MS, m/z 346 $[M]^+$ (2), 281 (27), 246 (98), 207 (51), 148 (22), 134 (43), 55 (100)] were also separable with the chromatographic systems employed. In addition, 13α-tigloyloxylupanine was resolvable from its geometrical isomer, 13α-angeloyloxylupanine (RR_t 1.99, 2.37).

Ormosia-*type quinolizidine alkaloid identification.* In contrast to previous GC/MS work using a packed column [12], the available stereoisomers of pentacyclic and hexacyclic *Ormosia*-type quinolizidine alkaloids were separable on both the stationary phases employed in the present work. Hence, compounds based on the following carbon skeletons were resolved in this study (RR_t on DB-5 and DB-1, respectively): $C_{20}H_{33}N_3$, 6-epipodopetaline and podopetaline; $C_{20}H_{35}N_3$, ormosanine and templetine (RR_t 1.52; 1.75; MS, m/z 317 $[M]^+$ [12]); $C_{21}H_{33}N_3$, homo-6-epipodopetaline and homopodopetaline; $C_{21}H_{35}N_3$, jamine (homo-ormosanine) and homotempletine [RR_t 1.63, 1.85; MS, m/z 329 $[M]^+$ (33), 328 (100), 281 (8), 246 (7), 245 (2), 231 (2), 207 (6), 163 (2), 98 (1), 84 (1), 44 (21), 41 (27)]. The hexacyclic *Ormosia* alkaloid, panamine ($C_{21}H_{31}N_3$) was resolved from the dimeric *Ormosia*-alkaloid, ormosinine ($C_{40}H_{66}N_6$), a compound which shows closely comparable EIMS data [9, 35].

The two homoxy-*Ormosia* alkaloids prepared by synthesis exhibited the following data (RR_t on DB-5 and DB-1, respectively): homoxy-6-epipodopetaline ($C_{21}H_{31}N_3O$): RR_t 2.20, 2.72; MS, m/z 341 $[M]^+$ (12), 298 (1), 257 (3), 243 (8), 160 (2), 146 (3), 98 (100), 67 (7), 55 (11) homoxyormosanine ($C_{21}H_{33}N_3O$): RR_t 2.26, 2.80; MS, m/z 343 $[M]^+$ (100), 314 (5), 300 (27), 286 (4), 258 (13), 245 (10), 172 (3), 98 (64), 69 (8), 55 (14).

Tentative alkaloid identifications. One compound was tentatively identified as α-isoangustifoline in several extracts, and exhibited RR_t (DB-5, DB-1) of 0.88 and 0.80, and MS data identical to angustifoline, with which it co-eluted in all cases. This compound has previously been described as a constituent of *L. polyphyllus* leaflets, as a minor alkaloid [27]. Its shorter column residence time than the parent compound, angustifoline (with an 11α-H substituent), is consistent with this isolate being epimeric at C-11 [36]. A second compound that may be classified as a degradation product of the sparteine- lupanine-type quinolizidine alkaloids, 11-oxotetrahydrorhombifoline, was tentatively identified by comparison with literature mass spectral data, as described previously [12, 37]. This compound was first isolated from the bark of *O. countinhoi* [37], but was not present in the seeds of this species in the present study.

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REFERENCES

1. Balandrin, M. F., Robbins, E. F. and Kinghorn, A. D. (1982) *Biochem. Syst. Ecol.* **11**, 307.
2. Polhill, R. M. (1981) in *Advances in Legume Systematics* (Polhill, R. M. and Raven, P. H., eds) p. 191. Royal Botanic Gardens, Kew, Surrey.
3. Yakovlev, G. P. (1971) *Bot. Zhurn.* **56**, 652.
4. Yakovlev, G. P. (1973) *Noveosti Sist. Vyssh. Rast.* **10**, 193.
5. Mears, J. A. and Mabry, T. J. (1971) in *Chemotaxonomy of the Leguminosae* (Harborne, J. B., Boulter, D. and Turner, B. L., eds) p. 73. Academic Press, London.
6. Salatino, A. and Gottlieb, O. R. (1980) *Biochem. Syst. Ecol.* **8**, 133.
7. Kinghorn, A. D. and Smolenski, S. J. (1981) in *Advances in Legume Systematics* (Polhill, R. M. and Raven, P. H., eds) p. 585. Royal Botanic Gardens, Kew, Surrey.
8. Gottlieb, O. R. (1982) *Micromolecular Evolution, Systematics and Ecology. An Essay into a Novel Botanical Discipline* pp. 104–119. Springer, Berlin.
9. Kinghorn, A. D. and Balandrin, M. F. (1984) in *Alkaloids: Chemical and Biological Perspectives* Vol. 2 (Pelletier, S. W., ed.) p. 105. Wiley–Interscience, New York.
10. Fitzgerald, M. A., Gunning, P. J. M. and Donnelly, D. M. X. (1976) *J. Chem. Soc., Perkin Trans. I.* 186.
11. Adesogan, E. K. (1976) *Phytochemistry* **15**, 2025.
12. Kinghorn, A. D., Balandrin, M. F. and Lin, L.-J. (1982) *Phytochemistry* **21**, 2269.
13. McLean, S., Lau, P. K., Cheng, S. K. and Murray, D. G. (1971) *Can. J. Chem.* **49**, 1976.
14. Balandrin, M. F. (1982) Ph.D. Dissertation, University of Illinois at the Medical Center, 177 pp.
15. McLean, S. and Misra, R. (1974) *Can. J. Chem.* **52**, 1907.
16. McLean, S., Misra, R., Kumar, V., and Lamberton, J. A. (1981) *Can. J. Chem.* **59**, 34.
17. Rudd, V. (1965) *Contrib. U.S. Natl. Herb.* **32**, 279.
18. Asres, K., Phillipson, J. D. and Mascagni, P. (1986) *Phytochemistry* **25**, 1449.
19. Lloyd, H. A. and Horning, E. C. (1958) *J. Am. Chem. Soc.* **80**, 1506.
20. Arthur, H. R. and Loo, S. N. (1967) *Aust. J. Chem.* **20**, 809.
21. Kinghorn, A. D., Selim, M. A. and Smolenski, S. J. (1980) *Phytochemistry* **19**, 1705.
22. Balandrin, M. F. and Kinghorn, A. D. (1981) *J. Nat. Prod.* **44**, 495.
23. Balandrin, M. F. and Kinghorn, A. D. (1981) *J. Nat. Prod.* **44**, 619.
24. Kim, I.-C., Balandrin, M. F. and Kinghorn, A. D. (1982) *J. Agric. Food Chem.* **30**, 796.
25. Keller, W. J. and Hatfield, G. M. (1979) *Phytochemistry* **18**, 2068.
26. Fitzgerald, T. J., LaPidus, J. B. and Beal, J. L. (1964) *Lloydia* **27**, 107.
27. Wink, M., Scheibel, H. M., Witte, L. and Hartmann, T. (1982) *Planta Med.* **44**, 15.
28. McLean, S., Roy, M. L., Liu, H.-J. and Chu, D. T. (1972) *Can. J. Chem.* **50**, 1639.
29. Fitch, W. L. and Djerassi, C. (1974) *J. Am. Chem. Soc.* **96**, 4917.
30. Cho, Y. D. and Martin, R. O. (1971) *Arch. Mass Spectral Data* **2**, 328.
31. Keller, W. J. and Zelenski, S. G. (1978) *J. Pharm. Sci.* **67**, 430.
32. Schumann, D., Neuner-Jehle, N. and Spiteller, G. (1968) *Monatsh. Chem.* **99**, 390.
33. Neuner-Jehle, N., Nesvadba, H. and Spiteller, G. (1964) *Monatsh. Chem.* **95**, 687.
34. Ohmiya, S., Otomasu, H., Murakoshi, I. and Haginawa, J. (1974) *Phytochemistry* **13**, 643.
35. Bhacca, N. S., Balandrin, M. F., Kinghorn, A. D., Frenkiel, T. A., Freeman, R. and Morris, G. A. (1983) *J. Am. Chem. Soc.* **105**, 2538.
36. Kinghorn, A. D. and Smolenski, S. J. (1980) *Planta Med.* **38**, 280.
37. McLean, S., Harrison, A. G. and Murray, D. G. (1967) *Can. J. Chem.* **45**, 751.