

ALKALOIDS OF *SOPHORA* OF JUAN FERNANDEZ ISLANDS AND RELATED TAXA

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SUMMARY

Six species of *Sophora* were analyzed by GLC and GLC-MS, two from Juan Fernandez Islands (*S. masafuerana* and *S. fernandeziana*) two from continental Chile (*S. microphylla* and *S. macrocarpa*), one from Argentina (*S. linearifolia*) and one from New Zealand (*S. microphylla*). Eleven quinolizidine alkaloids were isolated (two of the matrine type, three of the sparteine type, two of the anagyryne type, and four of the cytisine type) as well as ammodendrine.

RESUMEN

Se estudiaron por GLC y GLC-MS seis especies de *Sophora*, dos de Juan Fernández (*S. masafuerana* y *S. fernandeziana*) dos de Chile continental (*S. microphylla* y *S. macrocarpa*), una de Argentina (*S. linearifolia*) y una de Nueva Zelanda (*S. microphylla*). Se aislaron once alcaloides quinolizidínicos (dos del tipo matrina, tres del tipo esparteína, dos del tipo anagirina y cuatro del tipo citisina) y además ammodendrina.

INTRODUCTION

The Juan Fernandez Islands, located 600 km off the coast of Chile at latitude 33°S, consist of two major islands, Más a Tierra (-Isla Robinson Crusoe) and Más Afuera (-Isla Alejandro Selkirk). These islands possess an unusual angiosperm flora of 147 native species of which 69% are endemic¹). As part of studies attempting to understand patterns and processes of evolution in endemic species of Juan Fernandez Islands, we report the alkaloid contents and alkaloid patterns of six related *Sophora* species. Two occur in the Islands, three occur on the South American continent, and one is found in New Zealand.

EXPERIMENTAL

Plant collection, *S. linearifolia* was collected in San Luis Dpto Capital, Los Puquios in November 1990 N° 447 and deposited in the herbarium of the Universidad Nacional de San Luis Argentina, *Sophora microphylla* of continental Chile was collected in Barrio Universitario Universidad de Concepción, *S. macrocarpa* was collected in Santa Juana Concepción 1990. *S. fernandeziana* (N° 11581) and *S. masafuerana* (N° 6296) were collected in Juan Fernandez Islands in 1990. The Chilean species are deposited in the Herbarium CONC, Concepción. *Sophora microphylla* of New Zealand is deposited in the Herbarium CHR N° 465466.

The plants were extracted with MeOH 75%, this extract was treated with CH₂Cl₂ then with CH₂Cl₂-HCl (pH 1) and CH₂Cl₂-K₂CO₃ (basic pH).

The separation and purification of the alkaloids was done by thin layer and column chromatography using silica and alumina (Table I).

TABLE I. Some alkaloids isolated from *Sophora macrocarpa*

Alkaloid	Rf a	Rf b	RT - GLC
sophoranol	0.95	0.34	6.011
α matrine	0.98	0.96	6.027
cytisine	0.28	0.04	3.750

Rf a: RF mobilphase $\text{CH}_2\text{Cl}_2:\text{MeOH}:\text{NH}_3 = 85:14:1$
 Stationary phase: silica gel (Merck) 60 F₂₅₄

Rf b: RF mobilphase: 2 x CHCl_3
 Stationary phase: alumina (Merck) 150 F₂₅₄

RT-GLC: RT (retention time: minutes)
 initial temperature 200°C
 final temperature 275°C
 increase of temperature 10°C/min
 initial time: 2 min; final time: 15 min
 column ULBON HR-52 $\varnothing = 0.53$ mm
 l = 15 m
 sample size 2 μ l
 chromatographer Shimadzu GC-9A

The identification was done based on GLC-MS (Table II)².

TABLE II. Identification of alkaloids by capillary GLC and mass spectrometry

Compound	M+	EI-MS				
ammodendrine	208	165(100)	136(60)	123(60)	208(55)	191(50)
anagyrene	244	98(100)	244(40)	146(20)	160(15)	136(15)
Baptifoline	260	114(100)	260(27)	160(13)	146(27)	96(31)
cytisine	190	146(100)	147(80)	190(65)	160(25)	134(25)
N-formylcytisine	218	146(100)	218(75)	147(45)	160(20)	134(15)
N-methylcytisine	204	58(100)	204(30)	146(10)	160(10)	
sparteine	234	237(100)	98(90)	234(44)	193(25)	84(10)
matrine	248	248(100)	205(80)	96(70)	150(60)	247(75)
lupanine	248	136(100)	149(60)	248(40)	150(34)	219(8)

EI-MS: 5 significant fragments and their relative abundance (%)

Since we had small amounts of plants from Juan Fernandez Islands, Argentina and New Zealand, two grams of the plant organs (stems, leaves, seeds) were extracted with MeOH 75%. These extracts are evaporated, then dissolved with distilled water and volume completed to 25 ml. Then, 20 ml of these solutions were eluted in an extrelut column, after 30 min the column is washed with 4 x 25 ml CH_2Cl_2 . The CH_2Cl_2 extract obtained was used for GLC-MS analysis to be able to determine the alkaloids present.

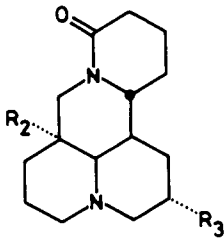
RESULTS AND DISCUSSION

The alkaloids of six *Sophora* species were studied by GLC and GLC-MS, two from Juan Fernandez Islands (*S. masafuerana* and *S. fernandeziana*) two from continental Chile (*S. microphylla* and *S. macrocarpa*)^{3,4,5,6,7}, one from Argentina (*S. linealifolia*) and one from New Zealand (*S. microphylla*). Alkaloids were identified according to their mass or NMR spectra.

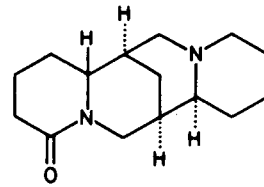
Leaves and stems of *S. masafuerana* afforded the following alkaloids, ammodendrine, lupanine, 5,6-dehydrolupanine, anagyrene, baptifoline, cytisine, N-methylcytisine. The stems afforded matrine in addition.

S. fernandeziana

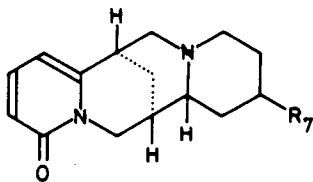
(Leaves): ammodendrine, matrine, lupanine, 5,6-dehydrolupanine, anagyrene, baptifoline, cytisine, N-methylcytisine and N-formylcytisine.



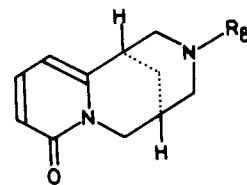
- 1 (+)-matrine: R₂ = H, R₃ = H,
 2 (+)-sophoranol: R₂ = OH, R₃ = H,



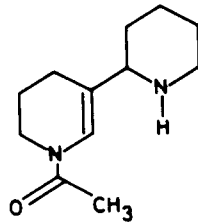
- 3 (+)-lupanine:



- 4 (-)-anagyrine: R₇ = H
 5 (-)-baptifoline: R₇ = OH



- 6 (-)-citisine: R₈ = H
 7 (-)-N-methylcitisine: R₈ = CH₃



8 ammodendrine

(Stems): matrine, lupanine, 5,6-dehydrolupanine, anagyrine, baptifoline, cytisine, N-methylcitisine and N-formylcitisine and ammodendrine.

S. macrocarpa

(Leaves): sparteine, matrine, dehydromatrine, sophoranol, lupanine, cytisine. The stems afforded spartein, matrine, sophoranol, lupanine, 5,6-dehydrolupanine, anagyrine, cytisine. Seeds afforded matrine, D-H matrine, sophoranol, lupanine, anagyrine, baptifoline, N-methylcitisine and cytisine.

***S. microphylla* from continental Chile**

(Leaves): matrine, D-H matrine, lupanine, 5,6-dehydrolupanine, cytisine, N-methylcitisine and ammodendrine.

(Stems): matrine, anagyrine, baptifoline, cytisine and N-methylcitisine, dehydromatrine.

(Seeds): matrine, lupanine, 5,6-dehydrolupanine, anagyrine, cytisine, N-methylcitisine, N-formylcitisine, iso N-methylcitisine and ammodendrine.

TABLE III. Alkaloid profile of *Sophora* species from Juan Fernandez Islands as compared to those from the South American continent. * = plants from New Zealand. O = major, + minor alkaloid.

		Ammodendrine	Sparteine	Matrine	D-H matrine	Lupanine	5,6-dehydro-lupanine	Cytisine	N-Methyl-cytisine	Anagyrene	Baptifoline	N-formyl-cytisine	Sophoranol
<i>S. masatauerana</i>	Leaves	+				O	O	O	O	O	O	O	O
	Stems	+		+		O	O	O	O	O	O	O	+
<i>S. fernandeziana</i>	Leaves	+		O		O	O	O	O	+	+	+	+
	Stems			O		O	O	O	+	+	+	+	+
<i>S. macrocarpa</i>	Leaves		O	O	+	+	+	O	O	+	+	+	O
	Stems		O	O	+	+	+	O	O	+	+	+	O
	Seeds			O	+	O	O	O	O	+	+	+	O
<i>S. microphylla</i> Chile	Leaves	+		O	+	+	+	+	+	+	+	+	
	Stems			O	+	+	+	O	O	+	+	+	
	Seeds	+		O	+	+	+	O	O	+	+	+	
<i>S. microphylla</i> NZ*	Leaves	O		O	+	O	+	+	+	+	+	+	
	Stems	+		O	+	+	+	O	O	+	+	+	
	Seeds	+		O	O	O	+	O	O	+	+	+	
<i>S. linearifolia</i>	Leaves		O	O	+	O	O	+	+	+	+	+	+
	Stems			O	+	+	+	O	O	+	+	+	+

***S. microphylla* from New Zealand**

(Leaves): matrine, several dehydromatrines, lupanine, 5,6-dehydrolupanine, cytisine and ammodendrine.

(Stems): matrine, dehydromatrine (M⁺, 246), 5,6-dehydrolupanine, lupanine, anagryne, N-methylcytisine, cytisine and ammodendrine.

(Seeds): afforded matrine, three dehydromatrines, 5,6-dehydrolupanine, lupanine, anagryne, baptifoline, cytisine and N-methylcytisine and ammodendrine.

***S. linearifolia* from Argentina**

(Leaves): matrine, dehydromatrine, sophoranol, lupanine, sparteine, cytisine and N-methylcytisine.

(Stems): matrine, dehydromatrine, lupanine, anagryne, baptifoline and cytisine.

Besides matrine, we found several dehydromatrines (M⁺, 246); the exact structures could not be determined because of scarcity of material.

In Table III alkaloid profiles are shown in an overview in which major and minor alkaloids are differentiated. The matrine-type alkaloids are less abundant in plants from the Juan Fernandez Islands, as compared with plants from continental South America.

An abundant alkaloid from the Juan Fernandez plants is 5,6-dehydrolupanine, which only occurs as minor alkaloid in the other taxa. On the other hand, sparteine is only known from continental taxa.

Our data show that the *Sophora* species from Juan Fernandez Islands differ in their alkaloid profiles from the other taxa. This is especially true for *S. masafuerana*. We conclude that these *Sophora* species must have been isolated from the continental ones for some time, thus allowing the evolution of different alkaloid patterns. A similar phenomenon can be seen for *S. microphylla* (Table III). Plants from New Zealand already differ in their alkaloid pattern, especially in the abundance of lupanine.

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REFERENCES

1. Skottsberg, C. Derivation of the flora and fauna of the Juan Fernandez Islands. Nat. Hist. Juan Fernandez and Easter Islands, 1, 193-438 (1956).
2. Wink, M. In "Methods in Plant Biochemistry" (P. Waterman, ed.) Vol. 8, p. 187-239, Academic Press, London, New York (1992).
3. R. Negrete, B. Cassels, G. Eckhardt. *Phytochemistry*, **22**, 2069 (1983).
4. R. Negrete, N. Backhouse, B. Cassels. *J. Nat. Prod.*, **45**, 652 (1982).
5. R. Negrete, N. Backhouse, B. Cassels. *Bol. Soc. Chil. Quím.*, **27**, 263 (1982).
6. M. Silva, M.V. Medina, P.G. Sammes. *Phytochemistry*, **7**, 661 (1968).
7. A. Reyes, M. Miranda, R. Martinez. *Rev. Lat. Quím.*, **19**, 32 (1988).