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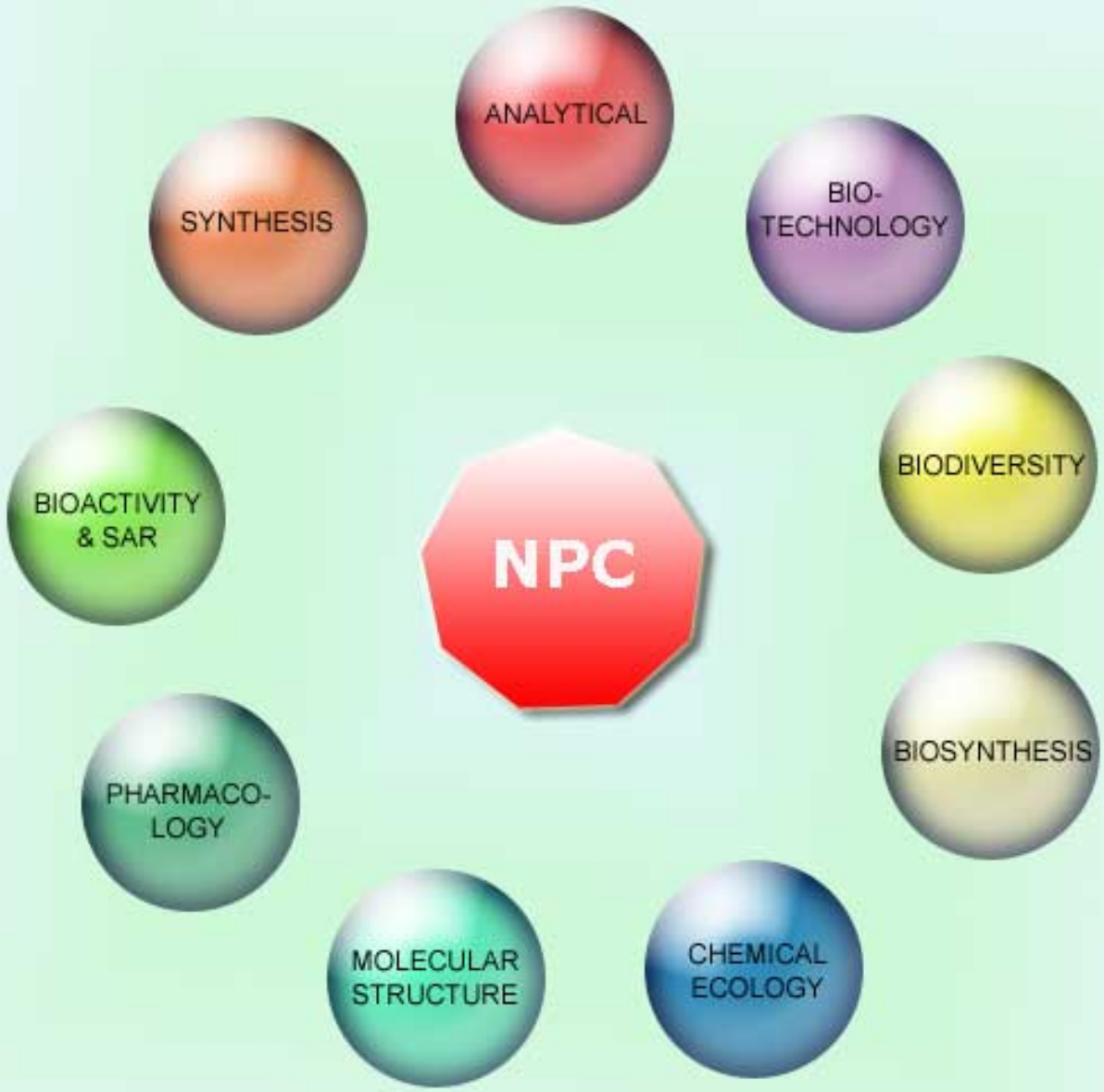

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Phytoecdysteroids in the Genus *Microsorum* (Polypodiaceae) of French Polynesia

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A chemical survey of the six species of *Microsorum* in French Polynesia has been performed to determine and quantify the phytoecdysteroids. The content and composition of these compounds in the fronds of each of the six species were established. The highest concentrations of ecdysteroids were found in *M. membranifolium* (1.6% w/w) and *M. scolopendria* (0.47%), used in Polynesian traditional medicine. Seven phytoecdysteroids were quantified in these species and the major components were ecdysone, 20-hydroxyecdysone, and 2-deoxy-20-hydroxyecdysone, besides the minor ones (inokosterone, makisterone A, makisterone C, and 2-deoxyecdysone). Both the fronds of *M. membranifolium* and the rhizomes of *M. scolopendria* grown in French Polynesia could be considered as uncommonly rich sources of ecdysteroids.

Keywords: *Microsorum*, Polypodiaceae, fern, French Polynesia, phytoecdysteroids, 20-hydroxyecdysone, 2-deoxyecdysone, ecdysone, 2-deoxy-20-hydroxyecdysone.

Ferns have always played an important part in the everyday life of the Polynesians as ornamental and medicinal plants. Nowadays, the use of plants has drastically decreased and only some species are still in use. Among them are *Microsorum scolopendria* and *M. membranifolium*, better known as "*metuapua'a"* in the Society Islands. Those ferns belong to the Polypodiaceae family and are employed in the preparation of many Polynesian traditional remedies [1-3]. The former genus name for these species was *Phymatosorus* (and sometimes *Polypodium*) [4-5].

The genus *Microsorum* comprises ca. 49 species. The taxonomy of this genus is complicated by the tendency of some species to hybridize [4]. Six species of *Microsorum* are found in French Polynesia: *M. scolopendria* (Burm.f.) E.B. Copeland, *M. membranifolium* (R. Brown) R.C. Ching, *M. maximum* (W.D. Brackenridge) E.B. Copeland, *M. punctatum* (C. Linnaeus) E.B. Copeland, *M. commutatum* (C.L. Blume) E.B. Copeland and *M. rubidum* (J. Smith ex G. Kunze) E.B. Copeland [4], but only the first two are used in traditional medicine. Some of these species show a very similar morphology, especially *M. scolopendria* and *M. rubidium*, which may induce confusion when collecting them for medicinal preparation purposes. Biologically active compounds belonging to the ecdysteroid family have been found in the genus *Polypodium* [6-8], and a phytochemical study of *M. scolopendria* revealed the presence of high amounts of ecdysteroids [9]. Ecdysteroids are known to have many pharmacological effects in mammals/humans, e.g. anabolic, hypoglycemic, hypocholesterolemic, tonic, hepatoprotective, antidepressant, and purgative effects [10-12], some of which are consistent with the properties of "*metuapua'a"*.

As part of an ongoing investigation on biologically active plants from French Polynesia, it was therefore decided to focus attention on the ecdysteroid content of the fronds belonging to the six species of the genus *Microsorum*.

Samples of fronds from the six species were extracted with methanol. After prepurification of the crude extract, HPLC analysis of all samples was performed in order to determine their ecdysteroid profile, and in the present study minor ecdysteroids were not considered. The concentration of ecdysteroids in each species was calculated by using ecdysone as an external standard. Seven ecdysteroids were identified: ecdysone, 20-hydroxyecdysone, inokosterone, makisterone A, makisterone C, 2-deoxy-20-hydroxyecdysone and 2-deoxyecdysone (supplementary data). The structures of these compounds are given in Table 1.

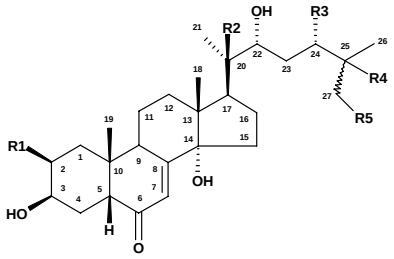

Table 1: Phytoecdysteroids present in the genus *Microsorum*.

	R_1	R_2	R_3	R_4	R_5
1: 20-Hydroxyecdysone	OH	OH	H	OH	H
2: Inokosterone	OH	OH	H	H	OH
3: Makisterone A	OH	OH	Me	OH	H
4: Ecdysone	OH	H	H	OH	H
5: 2-Deoxy-20-hydroxyecdysone	H	OH	H	OH	H
6: Makisterone C	OH	OH	Et	OH	H
7: 2-Deoxyecdysone	H	H	H	OH	H

The ecdysteroid content and composition of the six species of *Microsorum* of French Polynesia are compiled in Table 2. The major compounds are 20-hydroxyecdysone, ecdysone, 2-deoxy-20-hydroxyecdysone and 2-deoxyecdysone. From their ecdysteroid content, the six species can be classified as follows: two rich species (*M. membranifolium* and *M. scolopendria*), three poor ones (*M. x maximum*, *M. punctatum*, and *M. commutatum*), whereas *M. rubidium* contains at best only traces of ecdysteroids (data not shown).

Seven ecdysteroids were determined in the fronds of *M. membranifolium*, among which were four major ones: 20-hydroxyecdysone (2.13 $mg.g^{-1}$), ecdysone (4.91 $mg.g^{-1}$), 2-deoxy-20-hydroxyecdysone (7.07 $mg.g^{-1}$) and 2-deoxyecdysone (1.72 $mg.g^{-1}$). Six ecdysteroids were quantified in *M. scolopendria* with two major ones, 20-hydroxyecdysone (2.23 $mg.g^{-1}$) and ecdysone (1.45 $mg.g^{-1}$). The total content of the four other ecdysteroids was less than 0.5 $mg.g^{-1}$. Makisterone C was not detected.

M. maximum, probably the hybrid species from *M. scolopendria* and *M. punctatum* [5], is the third richest species in ecdysteroids. Its total ecdysteroid content is 0.91 $mg.g^{-1}$, i.e. much lower than that of the former species. The major component was 20-hydroxyecdysone (0.71 $mg.g^{-1}$). Four minor components were found, accounting for a total concentration of 0.20 $mg.g^{-1}$: makisterone A, inokosterone, 2-deoxy-20-hydroxyecdysone, and ecdysone.

M. punctatum and *M. commutatum* contain similar low concentrations of ecdysteroids (0.245 and 0.24 $mg.g^{-1}$, respectively). Three ecdysteroids were identified in *M. punctatum* (mainly 20-hydroxyecdysone) and only one (ecdysone) in *M. commutatum*.

Ecdysone was detected in the five ecdysteroid-containing species, and was a major component in three of them (*M. membranifolium*, *M. scolopendria*, and *M. commutatum*). 20-Hydroxyecdysone was detected in four species only, and it was the most abundant ecdysteroid in three of them. 2-Deoxy-20-hydroxyecdysone and 2-deoxyecdysone were only detected in *M. membranifolium*, where they occur in

Table 2: Phytoecdysteroid content ($mg.g^{-1}$ w/w) in fronds of five *Microsorum* species.

Species	***M. membranifolium***				***M. scolopendria***				***M. maximum***				***M. punctatum***				***M. commutatum***			
	mean	min	max	s.d.	mean	min	max	s.d.	mean	min	max	s.d.	mean	min	max	s.d.	mean	min	max	s.d.
1: 20-Hydroxyecdysone	2.13	1.9	2.35	0.15	2.23	1.13	3.33	0.70	0.71	0.61	0.82	0.07	0.19	0.01	0.24	0.07	n.d.	n.d.	n.d.	n.d.
2: Inokosterone	0.2	n.d.	0.6	0.21	0.36	0.29	0.43	0.05	0.04	0.02	0.08	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3: Makisterone A	0.12	n.d.	0.14	0.07	0.12	0.08	0.16	0.03	0.11	n.d.	0.03	0.04	0.03	n.d.	0.1	0.04	n.d.	n.d.	n.d.	n.d.
4: Ecdysone	4.91	4.77	5	0.08	1.45	1.37	1.52	0.05	0.02	n.d.	0.07	0.02	0.025	n.d.	0.1	0.04	0.24	0.18	0.34	0.06
5: 2-Deoxy-20-hydroxyecdysone	7.07	6.9	7.25	0.12	0.5	0.35	0.65	0.10	0.03	n.d.	0.03	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6: Makisterone C	0.48	0.35	0.6	0.08	n.d.	n.d.	n.d.	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7: 2-Deoxyecdysone	1.72	1.54	1.92	0.12	0.03	0.01	0.05	0.01		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total ecdysteroid content	16.63				4.69				0.91				0.245				0.24			

n.d.: not detected, s.d.: standard deviation.

large concentrations (7.07 and 1.72 mg.g^{-1}, respectively).Interestingly, the highest ecdysteroid contents are found in the two species used in traditional medicine.

This finding supports the hypothesis that the medicinal properties of "*metuapua'a*" are (at least in part) connected with the presence of ecdysteroids, although these data cannot be considered as proof of the hypothesis. A striking feature of the two *Microsorum* species is their high content of ecdysone, a rare phytoecdysteroid, which for that reason has not yet been specifically investigated for its pharmacological properties. A review of the different medicinal uses of French Polynesian ferns shows that the parts used are either the fronds or the rhizomes [13]. This prompted us to investigate ecdysteroid concentrations and patterns in the fronds and rhizomes of *M. scolopendria* and *M. membranifolium*. The data are compared in Table 3.

The concentration of ecdysteroids is more important in the rhizomes than in the fronds of *M. scolopendria* (11.17 and 4.69 mg.g^{-1}, respectively). The reverse is observed for *M. membranifolium*, which contains 16.63 mg.g^{-1} ecdysteroids in the fronds and 2.02 mg.g^{-1} in the rhizomes.

Six ecdysteroids were identified in the rhizomes of *M. scolopendria*, among which the two major components were 20-hydroxyecdysone (6.76 mg.g^{-1}) and ecdysone (3.41 mg.g^{-1}) at concentrations, respectively, 3 times and 2.35 times higher than those measured in the corresponding fronds.

Only five ecdysteroids were detected in the rhizomes of *M. membranifolium* and only three major compounds - ecdysone (0.87 mg.g^{-1}), 20-hydroxy-ecdysone (0.64 mg.g^{-1}) and 2-deoxy-20-hydroxy-ecdysone (0.40 mg.g^{-1}) - and two minor ones (inokosterone and makisterone C). Makisterone A and 2-deoxyecdysone were not detected. The total ecdysteroid content of rhizomes is eight times lower than in the fronds.

Based on the literature [1,3,14-16], the rhizomes of *M. scolopendria* constitute the most often used part in Polynesian traditional medicine, whereas the rhizomes of *M. membranifolium* are never used. The high level of ecdysteroids in the rhizomes of *M. scolopendria*, especially 20-hydroxyecdysone and ecdysone, accounts for its use.

Table 3: Phytoecdysteroids content (mg.g^{-1} dry wet) in fronds and rhizomes of *M. scolopendria* and *M. membranifolium*.

Species	***M. membranifolium***		***M. scolopendria***	
Concentration	Fronds	Rhizomes	Fronds	Rhizomes
1: 20-Hydroxyecdysone	2.13	0.64	2.23	6.76
2: Inokosterone	0.2	0.02	0.36	0.42
3: Makisterone A	0.12	n.d.	0.12	0.24
4: Ecdysone	4.91	0.87	1.45	3.41
5: 2-Deoxy-20-hydroxyecdysone	7.07	0.40	0.5	0.09
6: Makisterone C	0.48	0.09	n.d.	0.25
7: 2-Deoxyecdysone	1.72	n.d.	0.03	n.d.
Total ecdysteroid content	16.63	2.02	4.69	11.17

(n.d.: not detected).

In conclusion, among the six species of *Microsorum* grown in French Polynesia, the two species *M. scolopendria* and *M. membranifolium* are a rich source of ecdysteroids; especially the rhizomes of *M. scolopendria* and the fronds of *M. membranifolium*. The use of these latter plants in Polynesian traditional medicine can be fully justified by the numerous biological activities reported for ecdysteroids.

Previous studies of Formosan ferns reported the absence of ecdysteroids in some species including *M. membranifolium* [17]. This suggests the possibility to expect remarkable variations of ecdysteroid content under the influence of abiotic (or biotic) factors, and this will be investigated in forthcoming experiments.

Experimental

Plant material: Fronds of each species of *Microsorum* were collected from the archipelagoes of the Society Islands (Tahiti, Moorea, Raiatea) and Marquesas Islands (Nuku Hiva) in December 2004. Rhizomes of *M. membranifolium* and *M. scolopendria* were collected from the archipelago of the Society Islands (Tahiti) in February 2007. All samples (fronds and rhizomes of each species) were identified by the taxonomist Dr Jacques Florence (IRD). Six different samples from six locations were analyzed for each species to obtain a significant mean value of their ecdysteroid content. Voucher specimens are deposited in the Herbarium of French Polynesia at the 'Musée de Tahiti et ses îles' in Punaauia (PAP, Tahiti Island).

Extraction and HPLC methods: Dried fronds or rhizomes (100 mg) were homogenized using a blender and extracted with MeOH (5 mL) for 12 h. The extract was filtered and the filtrate evaporated to dryness. The obtained residue was dissolved in water and then chromatographied on a C_{18} Sep-Pak

cartridge using elution with MeOH/H_2O, 1/4, 13/9, 1/0 (10 mL each). The second fraction was evaporated to dryness, dissolved in H_2O/EtOH (3/1) and submitted to a low pressure chromatography on a polyamide column (16 x 0.5 cm, 3 mL), which was eluted with H_2O/EtOH, 3/1 (15 mL). Five fractions (3 mL each) were collected and 10 µL of each was analyzed by TLC on silica F254 plates. Fractions 2 and 3 contained ecdysteroids.

Quantification of ecdysteroids was achieved by reversed-phase HPLC using an UV DAD detector (Lichrospher C_{18} 5 µm, 250 x 4mm (Merck), flow rate 0.7 $mL.min^{-1}$, ACN/H_2O, 20/80 to 65/35 in 22 min). Ecdysteroids were detected by monitoring UV absorbance at 245 nm and characterized by their UV spectrum and retention time. The seven ecdysteroids were fully identified by MS and ^1H and ^{13}C NMR spectroscopy.

Supplementary data: ^1H and ^{13}C NMR data of the seven ecdysteroids are reported.

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