

Ecdysteroids from the medicinal fern *Microsorium scolopendria* (Burm. f.).

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Introduction

The fern *Phymatosorus scolopendria* (Syn. *Microsorium grossum*, *Phymatosorus grossus* - Tahitian name : Metua pua'a) belongs to the family Polypodiaceae. It is the most commonly used medicinal fern in the Society Islands, and probably in the entire Pacific. In Fiji, a decoction or pressed juice of the leaves are taken to treat weakness before and after childbirth, to treat influenza in children, to aid the healing of fractured bones, and as a remedy for pain in the lower chest, diarrhoea, stomach ache and strained muscles¹. In Samoa, the juice of the leaves is used in a poultice and an infusion of the scraped rhizomes and/or crushed leaves is taken as a potion for treating various kinds of inflammations and recalcitrant wounds². In Tonga, the ground leaves and stems are used to treat boils. In the Society Islands the juice of the leaves or rhizomes is used in popular remedies³ as a purgative and to treat pneumonia, bruises, sprains, gonorrhoea, leucorrhoea, stomach ache, sterility, dislocations and fractures. In New Caledonia, the young crushed fronds accelerate maturation of furuncles and are used in the treatment of bone fractures. The rhizome juice is used in nose and throat drops and to treat sinusitis⁴. The rhizome is eaten to soothe weeping eczema and is used to treat poisoning occasioned by eating fish (ciguatera)⁵. The fronds, crushed in coconut oil, are used as an anti-rheumatism liniment⁶. The medicinal uses of French Polynesian ferns have been recently reviewed⁷.



Experimental

Plant materials

The simple fronds were collected in Tahiti in the district of Pueu in July 2000 and December 2004, and were identified by Dr Jacques Florence. A voucher specimen (Vahirua Nr IVL-8) is deposited in the herbarium of the Laboratory of Natural Products, Institute Louis Malardé (Papeete Tahiti). A third sample of fronds (1.5 kg dry weight) was collected from a site located on the island of Tahiti in the district of Faaone in March 2005. A voucher specimen is deposited at the herbarium of the Museum of Tahiti and her Islands (Punaauia Tahiti).

Extraction and purification

Dried fronds (200 g) were milled to a fine powder and extracted with ethanol (2 x 1.5 L) with continuous stirring over 3 days each time. The extract was filtered and the filtrate evaporated to dryness. The residue was dissolved in methanol (200 mL), celite (50 g) was added and the whole was evaporated to dryness, suspended in chloroform (200 mL) and deposited at the top of a column containing Kieselgel Si60 (250 g; Merck, Darmstadt, Germany). The column was eluted with a step-gradient of methanol in chloroform: Ninety fractions (50 mL each) were collected and 10 mL of each were analyzed by TLC on silica F254 plates. Fractions 45 to 67 contained ecdysteroids; most of the ecdysone was present in fractions 54 to 57 and most of the 20-hydroxyecdysone in fractions 58 to 67. All of these fractions were then purified by semi-preparative HPLC and, whenever necessary, by using one or two additional analytical HPLC steps (see below).

HPLC methods

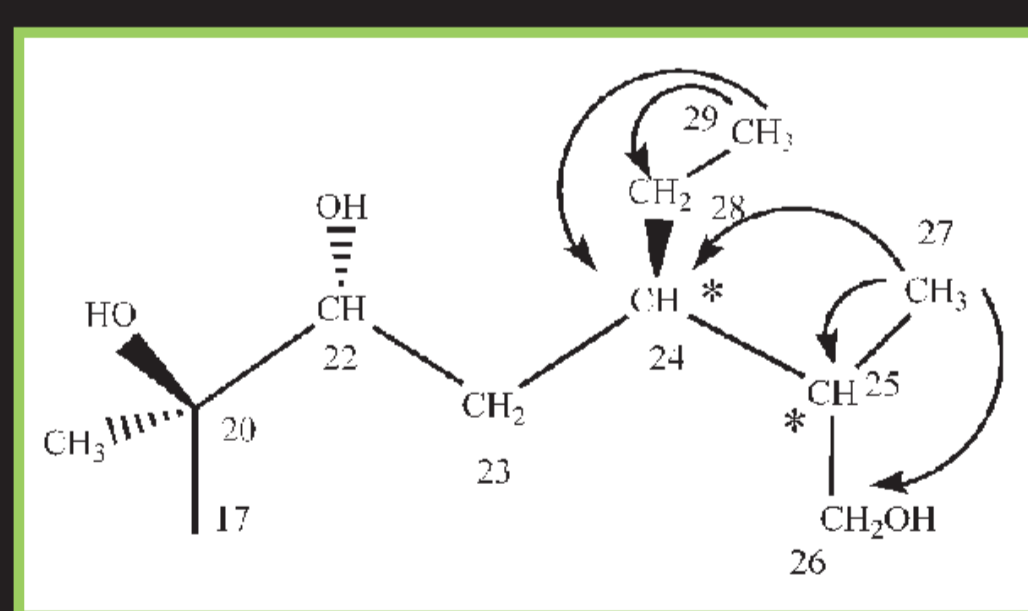
Semi-preparative NP-HPLC (system 1) employed a Zorbax®-SIL (DuPont, City? Country?) column (250 x 9.4 mm i. d.; particle size 5 mm) eluted with dichloromethane : propan-2-ol : water (125:40:3, v/v/v) with a flow-rate of 4 mL/min. Analytical NP-HPLC employed a Kromasil column (250 x 4.6 mm i.d.; - particle size 3.5 mm) eluted with dichloromethane : propan-2-ol water (125:30:1.5, v/v/v) (system 2) or cyclohexane : propan-2-ol ; water (100:30:1.5, v/v/v) (system 3) at flow rates of 1 mL/min. Analytical RP-HPLC used a Shandon Spherisorb -50DS2 column (250 x 4.6 mm i. d.; particle size 5 mm) eluted with either methanol : water (45:55) at a flow-rate of 0.7 mL/min (system 4) or a mixture of acetonitrile : propan-2-ol (5:2, v/v) and 0.1% trifluoroacetic acid in water (20:80, v/v) at a flow-rate of 1 mL/min.

Results and Discussion

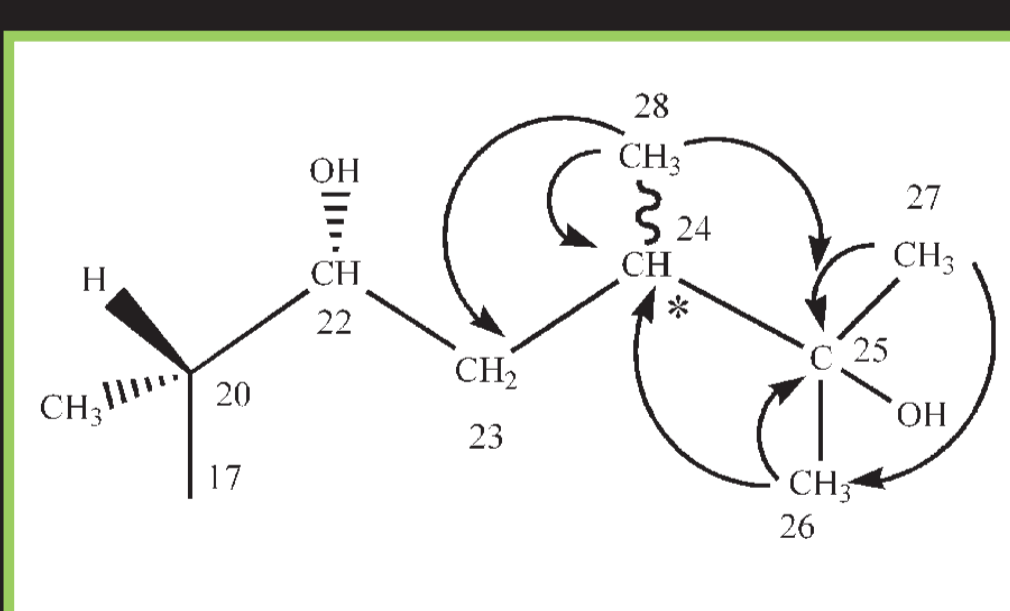
HPLC analysis of a crude sample (5 mg dry weight) was performed using HPLC system 3 and showed the presence of ecdysone and 20-hydroxyecdysone (Fig. 1). The crude extract of fronds was purified by CC over silica and the different ecdysteroid-containing fractions were purified by semi-preparative NP-HPLC. Different procedures led to the isolation of 11 different ecdysteroids. Ecdysone, 20-hydroxyecdysone, inokosterone (two epimers, mainly [= 95%] the least polar (25R) epimer on RP-HPLC: Yingyongnarongkul et al., 2005), makisterone A, makisterone C and poststerone are common ecdysteroids and they were identified by comparison with reference molecules available from previous work and by comparison with published MS and NMR data [Lafont et al., 2002].

The whole set of isolated compounds presented 1H- and 13C-NMR spectra with no significant differences of chemical shifts for the core steroid in comparison with 20-hydroxyecdysone. Thus, all these products bear only modifications to their side-chains.

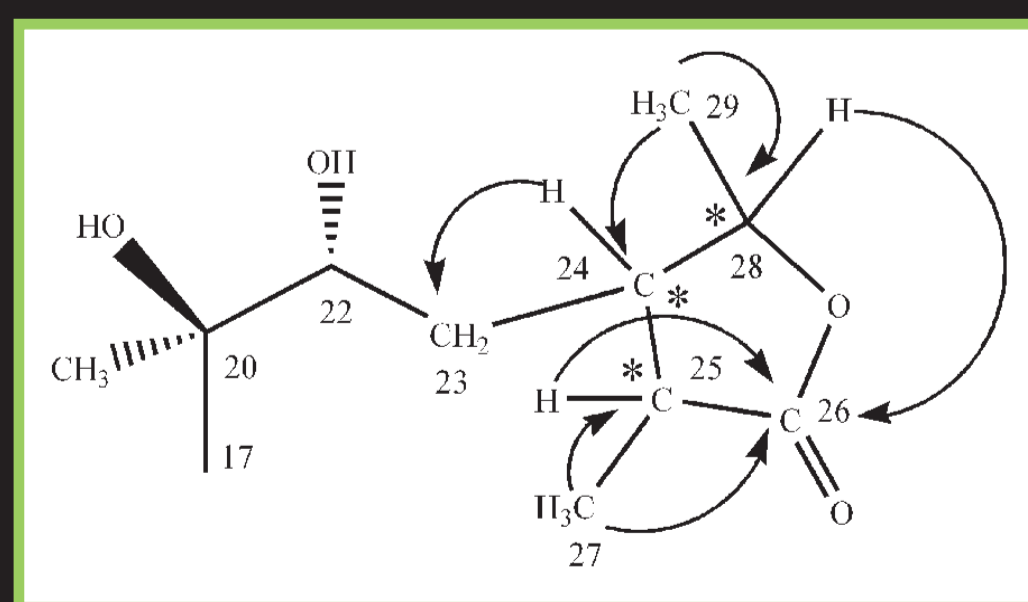
Key 1H-13C-HMBC correlations of side-chain of amarasterone A epimers



..... the side-chain of 20-deoxymakisterone A



... and the side chain of 24,28-diepi-cyasterone



Key 1H-NOESY correlations of the side chain of 24,28-diepi-cyasterone

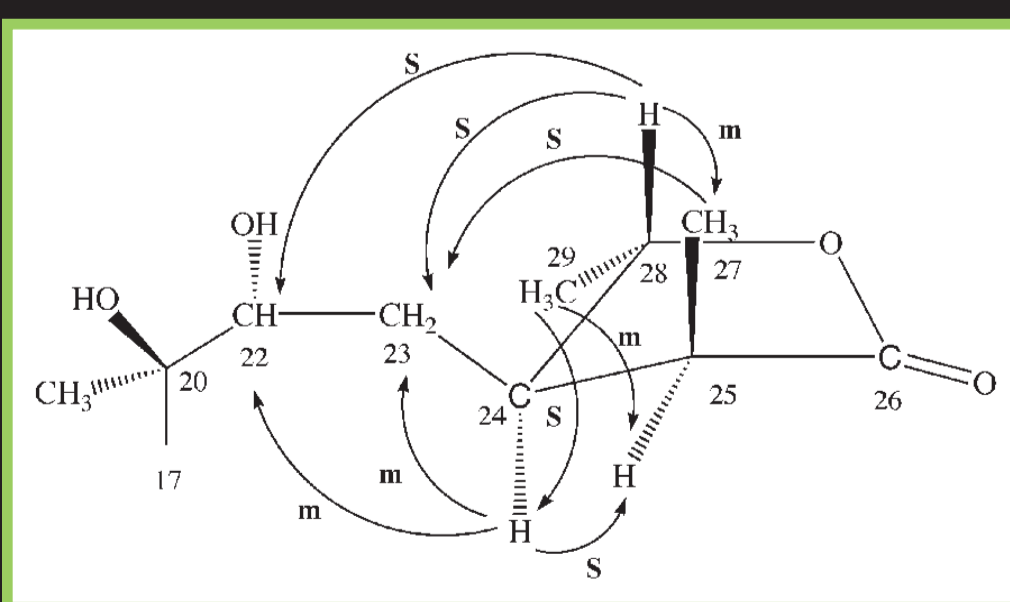


Table 1. HPLC (retention time, min) of the different ecdysteroids isolated.

| Compound | Retention time (min) | | | |
|-------------------------------|----------------------|----------|----------|----------|
| | System 2 | System 3 | System 4 | System 5 |
| Ecdysone | 19.0 | 25.4 | 21.6 | 22.8 |
| 20-Hydroxyecdysone | 27.8 | 33.0 | 10.9 | 8.8 |
| Amarasterone A (epimer 1) | 15.6 | 20.8 | 24.9 | 33.5 |
| Amarasterone A (epimer 2) | 12.0 | 17.8 | 31.0 | 36.8 |
| 25-Deoxyecdysone 22-glucoside | 16.7 | 20.3 | 29.8 | 42.7 |
| Inokosterone (epimer 2) | 24.9 | 31.5 | 11.5 | 11.0 |
| 24,28-Diepi-cyasterone | 10.2 | 34.8 | 11.5 | 13.8 |
| Makisterone A | 19.0 | 27.3 | 16.0 | 14.9 |
| Makisterone C | 11.1 | 16.6 | 35.9 | 44.7 |
| 20-Deoxymakisterone A | 12.4 | 17.8 | 29.1 | 43.1 |
| Poststerone | 9.6 | 19.3 | 12.3 | 13.3 |

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Acknowledgements

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