Drimanes and Other Terpenoids from the Fern *Thelypteris hispidula* (Decne.) REED

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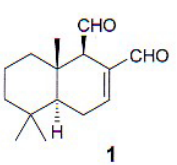
The drimane-type sesquiterpenoids (−)-polygodial (**1**), (−)-isopolygodial (**4**), drimenin (**5**), and isodrimenin (**6**) were isolated from a pungent Argentine collection of the fern *Thelypteris hispidula*, along with other terpenoids. As the mentioned drimanes have been previously found in liverworts, the present results strongly support the theory that Pteridophytes and Bryophytes share the same line of evolution. Our present investigations indicate that two chemotypes of *T. hispidula* are widespread in the northwest of Argentina. Plants belonging to the pungent chemotype contain drimanes, while nonpungent plants lack drimanes.

Introduction. – The genus *Thelypteris* SCHMIDEL is spread worldwide. *T. hispidula* grows abundantly in shaded humid forests of the northwest of Argentina, and no reports have been made on its chemistry so far.

Polygodial (**1**), a pungent drimane-type sesquiterpenoid, has been reported from some species of the families Hepaticae, Polygonaceae, Winteraceae, and Canellaceae [1–6]. It was not until 2001 that **1** was isolated for the first time from a Pteridophyte of the family Hymenophyllaceae [7]. This finding was of great importance, as it brought additional chemical evidence for the evolutionary relationship between Bryophytes and Pteridophytes. The role of pungent components in Bryophyte and Pteridophyte evolution has been also pointed out [7]. Polygodial (**1**) is fungitoxic against *Cladosporium sphaerospermum* [8]. In addition, it inhibits aphid feeding and colonization, as well as transmission of some plant viruses [9]. It also displays antifeedant activity against a number of Lepidopteran species, including the army-worms *Spodoptera littoralis* and *S. exempta* [10][11].

Continuing with our chemical investigations on Bryophytes and Pteridophytes [12–16], we now report on the constituents of the pungent chemotype of the fern *T. hispidula*. It yielded small amounts of 21-epifern-9(11)-ene (**2**), (−)-3-hydroxycalamenene (**3**), as well as the drimanes (−)-polygodial (**1**), (−)-isopolygodial (**4**), drimenin (**5**), and isodrimenin (**6**). We have also tested for the presence of drimanes in ether extracts, from pungent and nonpungent plants over an eight-month period by means of GC/MS to find that two chemotypes of this species are widespread in Tucumán province, Argentina.

Results and Discussion. – The air-dried fronds of pungent *T. hispidula* were ground and then extracted with Et_2O. The extract was subjected to column chromatography



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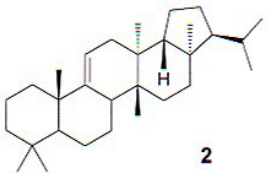

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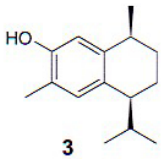

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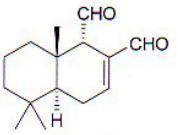

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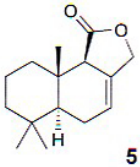

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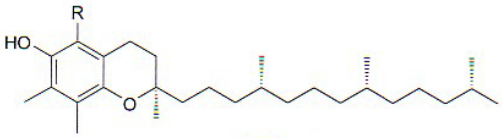
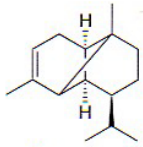


7 R = Me
8 R = H



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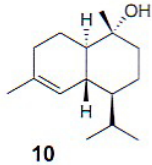

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(SiO_2; hexane/AcOEt) to give five fractions (*Table*). *Fr. I* yielded 21-epifern-9(11)-ene (**2**) as a colorless solid. The identification of this compound was accomplished by comparison of its spectroscopic features, optical rotation, and melting point with published data [17]. GC/MS Analysis of *Fr. II* showed the presence of α-tocopherol (**7**), γ-tocopherol (**8**), 3-hydroxycalamenene (**3**), α-copaene (**9**), and δ-cadinol (**10**). Reverse-phase HPLC (C_{18}) of this fraction gave (−)-3-hydroxycalamenene (**3**). This

Table. *Isolated Constituents of* Thelypteris hispidula

Fraction	Fraction weight	Constituents	Amount	Content[a])
I	7.8 mg	21-Epifern-9(11)-ene (**2**)	7.8 mg	0.78%
II	52 mg	(−)-3-Hydroxycalamenene (**3**)	2.3 mg	0.23%
		α-Tocopherol (**7**)	1.0 mg	0.10%
		γ-Tocopherol (**8**)	1.0 mg	0.10%
		α-Copaene (**9**)	Not isolated	–
		δ-Cadinol (**10**)	Not isolated	–
III	80 mg	(−)-Polygodial (**1**)	6.4 mg	0.64%
		(−)-Isopolygodial (**4**)	3.2 mg	0.32%
		Drimenin (**5**)	1.0 mg	0.10%
		Isodrimenin (**6**)	1.0 mg	0.10%
		Fitol	13.0 mg	1.30%
IV	213 mg	Sitosterol	Not isolated	–
		Campesterol	Not isolated	–
V	430 mg	Chlorophylls	Not separated	–

[a]) In weight-percent relative to the crude extract.

compound had been previously isolated from species of the families Ulmaceae [18], Tiliaceae [19], and Hepaticae [2], but this is the first report of the isolation from a Pteridophyte. Its antifungal effect against *Cladosporium cucumerinum* has been reported [19].

Fr. III was repeatedly purified by regular and reverse-phase HPLC, leading to the isolation of (−)-polygodial (**1**), (−)-isopolygodial (**4**), drimenin (**5**), and isodrimenin (**6**). Identification of **1** was accomplished by comparison of its optical rotation, and ^{1}H-NMR and EI-MS spectra with those of an authentic sample. Compound **4** was identified by its optical rotation, and EI-MS spectrum, and the structures of compounds **5** and **6** were elucidated by EI-MS and IR spectroscopy. *Fr. IV* and *V* contained mixtures of sterols and chlorophylls, respectively, and were not further investigated.

During plant collection, we tasted small amounts of fresh material to find pungent and nonpungent individuals in the same region. GC/MS Profiles of Et_2O extracts from pungent and nonpungent plants differed only in drimane-type sesquiterpenoids, which were detected solely in the *pungent* extracts.

We also carried out a greenhouse experiment, in which we kept five pungent and five nonpungent plants in flowerpots during a period of eight months. The plants were kept in soil from where they had been collected. GC/MS Analyses were performed every 15 d. We found that pungent plants remained pungent, and that nonpungent individuals did not produce any drimane during the whole experiment. Therefore, we conclude that there are two different chemotypes for this species. In both chemotypes, we detected the following common plant constituents: neophytadienes, phytol, α- and γ-tocopherol, sitosterol, and campesterol.

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Experimental Part

General. For thin layer chromatography (TLC), pre-coated SiO_2 plates (*Kieselgel 60* F_{254}; *Merck*) were employed. Spots on the plates were detected with the *Godin* reagent [20]. Silica gel (70–230 mesh) was used for column chromatography (CC). HPLC Separations were carried out on a C_{18} column (*Luna*; 5 μm, 10 × 250 mm) and on a *Develosil 60-5* column (*ChemcoPak*; 5 μm, 10 × 250 mm). Detection was accomplished by the use of UV and refractive-index detectors. GC/MS analyses were carried out on a *Hewlett-Packard 6890* gas chromatograph coupled to a *HP-5973* mass-selective detector. The separations were performed on a *HP-5 MS* capillary column coated with phenyl methyl siloxane (30 m × 0.25 mm i.d., 0.25 μm film thickness), using He as carrier gas. Optical rotations were measured with a *HORIBA SEPA-300* high-sensitive polarimeter at 25°. ^{1}H-NMR spectra were recorded on a *Bruker AM-500* spectrometer in $CDCl_3$, with Me_4Si as internal standard.

Extraction and Isolation. Pungent *Thelypteris hispidula* was collected in July 2003 at San Javier, Tucumán, Argentina (GPS data: 1207 o.s.l.; 26°45.865′ South; 65°21.980′ West). A voucher specimen (LIL 606796) was deposited at the Herbarium of the *Fundación Miguel Lillo*, Tucumán, Argentina. Dried fronds (44 g) were cut into pieces and extracted with Et_2O at r.t. for 6 d. The extract was filtered, evaporated to dryness (1.0 g), and purified by CC (SiO_2; hexane/AcOEt gradient) to afford five fractions. *Fr. I* (7.8 mg), eluted with pure hexane, contained **2** as a colorless solid. The presence of compounds **3** and **7**–**10** in *Fr. II* (52 mg) was determined by GC/MS. Processing of this fraction by RP-HPLC (C_{18}; MeOH) yielded small amounts of α- and γ-tocopherol, together with **3** (2.3 mg), obtained as a yellow oil. *Fr. III* (80 mg) was first chromatographed by normal-phase HPLC (SiO_2; hexane/AcOEt 85 : 15) to give three distinct fractions *A*–*C*, with retention times of t_R 33, 41, and 48 min (flow rate 1.5 ml/min). Further purification of *Fr. A* by RP-HPLC (C_{18}; MeOH/H_2O 80 : 20; 1.5 ml/min)

yielded **4** (3.2 mg; t_R 23 min). Processing of *Fr. B* by RP-HPLC (MeOH/H_2O 95:5; 1.0 ml/min) gave **6** (1 mg; t_R 29 min) and **5** (1 mg; t_R 31 min). RP-HPLC (MeOH/H_2O 85:15, 1.5 ml/min) of *Fr. C*, which contained a hot-tasting substance, afforded **1** (6.4 mg; t_R 19 min). *Fr. IV* and *V* contained unresolved mixtures.

Greenhouse Experiment. Five hot-tasting and five nonpungent plants were placed in flowerpots in a greenhouse, with daily watering. Plants had been collected in March 2004 at the location described in the previous paragraph. Vouchers of nonpungent and punget *T. hispidula* were deposited at the Herbarium of the *Fundación Miguel Lillo* (LIL 607161 and LIL 607192, resp.). Every 15 d, samples of each plant were extracted on a mortar with Et_2O, and the extracts were filtered through cotton, and evaporated to dryness, before being analyzed by GC/MS.

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