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DRIMANES FROM THE EPICUTICULAR WAX OF THE FERN *NEPHROLEPIS BISERRATA*

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Key Word Index—*Nephrolepis biserrata*; Davalliaceae; Pteridophyta; leaf wax; drimane sesquiterpenes; acetylated hemiacetals.

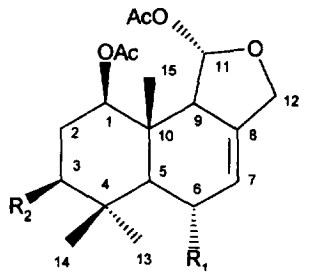
Abstract—From the thin epicuticular wax layer of the tropical fern *Nephrolepis biserrata*, three new drimane-type sesquiterpenes (1β,11α-diacetoxy-11,12-epoxydrim-7-ene, 1β,6α,11α-triacetoxy-11,12-epoxydrim-7-ene, 1β,3β,11α-triacetoxy-11,12-epoxydrim-7-ene) were isolated. All these new compounds contain a hemiacetal esterified with acetic acid.

INTRODUCTION

Diterpenes and triterpenes have been found before as constituents of the epicuticular material accumulated on fronds of ferns belonging to various taxa [1–3]. They form either farinose exudates, often together with flavonoids, e.g. in *Cheilanthes* and *Notholaena* species, or thin wax layers that cause the glaucous appearance of fern fronds, e.g. in *Lophosora quadripinnata* and *Polypodium aureum*. In the tropical fern *Nephrolepis biserrata* (Sw.) Schott, it is the rachis of its large fronds that bears a thin wax layer.

RESULTS AND DISCUSSION

The epicuticular wax recovered from the leaf rachis of *N. biserrata* contains three drimane-type sesquiterpenes (**1**–**3**) with an unusual acetylated hemiacetal. The pres-



	R_1	R_2
1	H	H
2	OAc	H
3	H	OAc

ence in all three of two acetates followed from typical singlets at about δ2.0 in the ^{1}H NMR spectra together with singlets of about 170 ppm and quartets (about 21 ppm) in the ^{13}C NMR spectra. Beside these six ester carbons, the ^{13}C NMR spectrum of **3** showed 3*q*, 3*t* (one oxygen-substituted at 69.2 ppm), 6*d* (three oxygen-substituted below 70 ppm, and an olefinic at 116.2 ppm) and 3*s* (one olefinic). From these data, the presence of two carbocyclic rings could be deduced. Bicyclic sesquiterpene skeletons with two singlets in the ^{13}C NMR upfield 60 ppm are quite rare; one possibility is the drimane-skeleton. Spin decoupling established the sequence C-1 to C-3 and C-5 to C-11. The olefinic proton H-7 coupled allylically to H-9, H-12 and H-12$_2$. The stereochemistry at C-6 could be deduced from the NOE of H-6 to two methyl groups (H-14 and H-15). The coupling pattern (*dd*, J = 11 Hz and J = 4 Hz) of the signal at δ4.67 allowed two positions for the third acetate: C-1 or C-3. NOE experiments (5% NOE from H-1 to H-9) and the coupling pattern of H-1 indicate that C-1 bears an equatorial acetate. The vicinal coupling constant of about 3 Hz (H-9 to H-11) is not sufficient for determination of the stereochemistry at C-11, because similar coupling constants were reported for acetates of drimeninol and isodrimininol [4, 5]. The stereochemistry at C-11 was established by a strong NOE-effect, H-11 to the methyl group H-15 (12%). The ^{1}H NMR-spectrum of **1** is similar to the ^{1}H NMR-spectrum of **2**, but obviously the acetate at C-6 is missing. There is one acetate at C-1 (5% NOE from axial H-1 to H-9) and another on the acetylated hemiacetal at C-11. **2** has acetates at C-1 and C-11 and an additional equatorial acetate at C-3. The coupling pattern and chemical shifts of H-1 and H-3 are very similar, but by NOE-experiments (effect from H-9 to H-1 and H-5, respectively, and from H-3 to H-5) they could be assigned unambiguously. All the ^{13}C NMR signals were assigned by HMQC. On EIMS none of the compounds **1**–**3** gave a molecular ion corresponding to its molecular formula deduced from the ^{13}C NMR spectra, but in each case a fragment corresponding to the loss of acetic acid present. The absolute stereochemistry of the new drimanes was not determined.

Table 1. ^{1}H NMR data of compounds **1**–**3** ($CDCl_3$, 400 MHz, int. standard solvent peak = 7.26 ppm)

H	1			2			3		
1_{ax}	4.59	*dd*	(12;4)	4.67	*dd*	(11;4)	4.73	*dd*	(12;4)
2_{eq}	1.72	*dddd*	(13;4;4;4)	1.77	*dddd*	(13;4;4;4)	1.97	*ddd*	(12;4;4)
2_{ax}	1.61	*dddd*	(13;13;12;4)	1.65	*m*		1.72	*ddd*	(12;12;12;12)
3_{eq}	1.48	*ddd*	(13;4;4)	1.43	*		—		
3_{ax}	1.41	*		1.48	*		4.68	*dd*	(12;4)
5	1.39	*dd*	(12;5)	1.67	*d*	(10)	1.39	*dd*	(11;6)
6_1	2.03	*m*		5.55	*br d*	(10)	2.12	*br d*	(18)
6_2	2.19	*br d*	(18)	—			2.24	*br d*	(18)
7	5.59	*br s*		5.65	*br s*		5.60	*br s*	
9	2.58	*br s*		2.72	*br s*		2.55	*br s*	
11	6.30	*d*	(3)	6.37	*d*	(3)	6.28	*d*	(3)
12_1	4.20	*br d*	(12)	4.28	*br d*	(12)	4.21	*br d*	(11)
12_2	4.32	*br d*	(12)	4.39	*dddd*	(12;1;1;1)	4.32	*dddd*	(11;2;1;1)
13	0.89	*s*		0.92	*s*		0.87	*s*	
14	0.94	*s*		1.03	*s*		0.93	*s*	
15	0.94	*s*		1.00	*s*		0.96	*s*	
Ac-1	1.97	*s*		1.98	*s*		1.94	*s*	
Ac-2	2.03	*s*		2.03	*s*		2.01	*s*	
Ac-3	—	—		2.03	*s*		2.03	*s*	

*Overlapping.

Drimanes are widespread in nature. They are already known from a few families of higher plants (e.g. *Drimys sp.* [6, 7] and *Polygonum sp.* [8]), from liverworts (e.g. *Porella cordeana* [9]) and from fungi (*e.g. Phoma asparagi* [10] and the mushroom *Marasmius oreades* [11]). Acetic esters of a drimane-hemiacetal were isolated from the nudibranch *Dendrodoris grandiflora* [12]. The function of drimanes, especially of polygodial or protected forms of aldehydes from nudibranches [12] as defensive agents and antifeedants is well investigated, as is their synthetic chemistry [13]. To the best of our knowledge, this is the first report of drimane sesquiterpenes from ferns. It is also the first report of any sesquiterpenes forming a wax layer on fern fronds.

EXPERIMENTAL

Nephrolepis biserrata was propagated in a greenhouse of the Botanischer Garten der TH Darmstadt. Pinnae were removed from the fronds and the rachis were rinsed with $CHCl_3$ to dissolve the waxy material. The fractions obtained by CC of the concentrated crude soln (silica gel, hexane–EtOAc gradient) were examined by ^{1}H NMR spectroscopy. Sesquiterpene-containing fractions were further sepd by prep TLC (petrol–Et_2O 3:2) to obtain pure compounds that exhibit R_f 0.36 (**1**), 0.22 (**2**), and 0.20 (**3**), respectively.

1*β*,11*α*-*Diacetoxy*-11,12-*epoxydrim*-7-*ene* (**1**). MS *m/z* (rel. int.): 276 (100) $[M - HOAc]^+$, 261 (4) $[276 - CH_3]^+$, 217 (12) $[276 - OAc]^+$.

1*β*,6*α*,11*α*-*Triacetoxy*-11,12-*epoxy-drim*-7-*ene* (**2**). MS *m/z* (rel. int.): 334 (40) $[M - HOAc]^+$, 275 (5) $[334 - OAc]^+$.

Table 2. ^{13}C NMR data of compounds **1**–**3** ($CDCl_3$, 100.6 MHz, int. standard $CDCl_3$ 77.0 ppm)

C	1	2	3	
1	81.4	80.2	77.5	*d*
2	24.2	24.2	29.3	*t*
3	39.6	39.8	76.2	*t/d*
4	32.7	33.7	37.2*	*s*
5	48.9	53.1	46.7	*d*
6	23.3	70.3	22.5	*t/d*
7	117.6	116.2	117.0	*d*
8	135.1	140.6	134.9	*s*
9	57.9	57.3	57.7	*d*
10	37.4	42.1	37.4*	*s*
11	98.8	98.6	98.2	*d*
12	69.6	69.2	69.4	*t*
13	32.3	33.0	27.1	*q*
14	21.3	22.0	15.5	*q*
15	9.1	10.8	9.1	*q*
Ac	21.3	21.1	20.8	*q*
Ac	21.3	21.0	20.8	*q*
Ac	—	21.4	21.0	*q*
Ac	170.5	170.2	169.7	*s*
Ac	171.1	170.5	170.0	*s*
Ac	—	170.6	170.9	*s*

*Interchangable.

1*β*,3*β*,11*α*-*Triacetoxy*-11,12-*epoxydrim*-7-*ene* (**3**). MS *m/z* (rel. int.): 334 (20) $[M - HOAc]^+$, 275 (43) $[334 - OAc]^+$.

For ^{1}H NMR and ^{13}C NMR data see Tables 1 and 2.

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