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MONOENE- AND DIENE-TYPE VALEPOTRIATES FROM *PHYLLACTIS PULVINATA*

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Key Word Index—*Phyllactis pulvinata*; Valerianaceae; valepotriates; isolation; structure elucidation; ^{13}C NMR; FDMS.

Abstract—Four new valepotriates from *Phyllactis pulvinata* have been isolated, two are of the monoene type and two the diene type. In addition unknown valepotriates, presumably belonging to the IVHD-type, were detected in minor amounts. In relation to known valepotriates the new substances can be described as 1β-aceacevaltrate, 1-dehydroacevaltrate, 11α-acedidrovaltrate and 11α-acehomodidrovaltrate. The valepotriate content of the leaves was more than four times greater than that of rhizomes, mostly due to the monoene derivatives.

INTRODUCTION

In a continuation of our investigation on the distribution of valepotriates [1–4] within the Valerianacean family, *Phyllactis pulvinata* Rauh et Willer from the Peruvian Andes was analysed for its valepotriate content. Schild and Seitz [5] had previously detected the known diene type valepotriates valtrate and acevaltrate, the monoene didrovaltrate and IVHD in this species. Additionally unknown valepotriates of monoene, diene and hydrine types were assumed to be present from their chromogenic behaviour with benzidine HCl on TLC. Today more powerful separation techniques [6] are available. Furthermore it has been shown [7] that the main structural problems within the classes of valepotriates can be fully resolved by ^{13}C NMR. In this paper the isolation and structure elucidation of four new valepotriates from the leaves of *P. pulvinata* is described. In addition their distribution together with other known valepotriates in leaves and rhizomes is reported.

RESULTS AND DISCUSSION

Air dried leaves and roots of *P. pulvinata* were extracted in dichloromethane [8] and analysed by TLC and HPLC. Preparative TLC followed by repeated analytical scale HPLC was performed until at least 15 mg of each compound was obtained. Structural investigations were performed by ^{13}C chemical shift correlation with respect to the ^{13}C NMR data of known valepotriates [6, 7, 9]. Since EIMS yielded no, or only very low abundancies of, molecular ions from this class of compounds, FDMS was used for M_r determinations.

A comparison of the ^{13}C shifts of **1** and **2** (Fig. 1) with those reported for acevaltrate [7] confirms the diene-type structure and locates the acyloxy residue and the (β-OAc)-i-Val O moiety at the primary allylic centre C-11 and the secondary allylic centre at C-7 respectively. The acyloxy residue is characterized by two carbonyl chemical shifts, namely δ170.4 ± 0.05 and 169.55 ± 0.05. For structural assignments it is not necessary to give an unambiguous assignment of the two carbonyl resonances within the (β-OAc)-i-Val O moiety. The same holds for the (β-OAc)-i-Val O residue of **1** located at C-1 [9]; thus **1** can be described as 1β-aceacevaltrate. Compound **2** contained an unsaturated acyloxy chain in position 1 [9]. It could be derived by elimination of water from an α- or β-hydroxylated isovaleroyloxy chain present in other valepotriates [4, 6]. Compounds **3** and **4** are monoene type valepotriates. With reference to [7] **3** is an 11α-acedidrovaltrate and **4** its related 1-homoderivative, i.e. an 11α-acehomodidrovaltrate.

If we assume that a β-OAc substituent within an O-i-Val moiety would deshield the parent carbonyl resonances [7] of C(7)-O-i-Val (172.5 ppm) and C(1)-O-i-Val (170.3 ppm) by the same increment it is reasonable to interchange the reported [7] chemical shift assignments for the carbonyl resonance within the C(7)-O-(β-OAc)-i-Val moiety. With our given assignments (Table 1) one may conclude that a β-OAc substituent within a O-i-Val moiety deshields the parent isovaleroyloxy carbonyl resonance by an increment of 2.9 ± 0.1 ppm.

FDMS supported these results. The molecular ions, representing the most abundant signals in the upper mass range ($M^{+\cdot}$ 538, 478, 482 and 496 for compounds **1**–**4**, respectively). The substituents located at position 1 (as proved by ^{13}C NMR) gave rise to the respective acyl ions. These were the most abundant of the fragmentation ions [m/z 143 (30%), 83 (100), 85 (20) and 99 (12) for **1**–**4** respectively].

In addition to these newly described diene and monoene compounds, two valepotriates were identified in the leaves. Also four valepotriates were detected in the

Table 1. Valepotriate[*] content (%/dry wt) in leaves and rhizomes of *P. pulvinata*

	Diene-type									Monoene-type					
	V[†]	Iso V	Homo V	Ace V	DIA V	11α-Aceiso V	1β-Aceace V	1-Dehydroace V	Total	Didro V	11α-Ace-didro V	11α-Ace-homodidro V	IVHD-subtype	Total	Total valepotriates
Leaf	0.450	0.031	0.032	0.643	0.025	0.041	0.159	0.167	1.548	0.724	5.984	0.101	0.124[‡]	6.933	8.481
Rhizome	0.967	0.045	0.006	0.167	0.082	Tr[‖]	—	Tr	1.267	0.482	0.034	Tr	0.117[§]	0.633	1.900

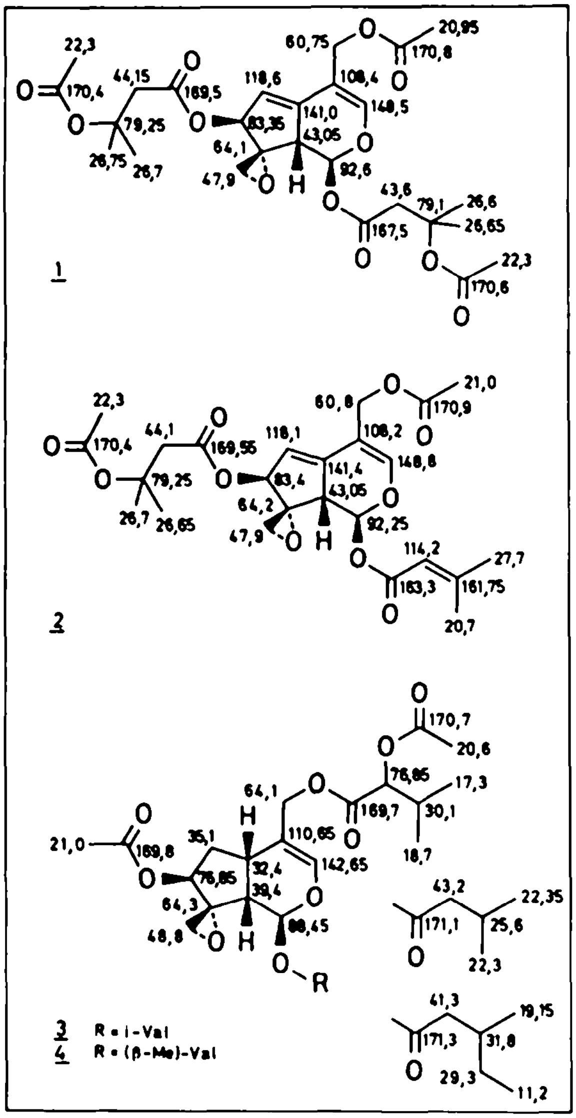
[*] For a structural overview see refs [6, 7].

[†] V, Valtrate.

[‡] Two compounds.

[§] Six compounds.

[‖] Tr, detectable by TLC and HPLC but less than 0.001 %.



1

2

3 R = i-Val

4 R = (β-Me)-Val

Fig. 1. The structures of new monoene- and diene-type valepotriates and their ^{13}C NMR chemical shifts ($CDCl_3$, TMS as int. standard). The previously reported [7] chemical shift assignments for the carbonyl resonances within the 7-O-(β-OAc) i-Val moiety have been interchanged.

rhizomes. Based on their chromogenic reaction with DNPH reagent on TLC [10] they belong to the IVHD-type valepotriates. Their quantity, however, was too small (0.01–0.080 % calculated as IVHD) for isolation from the limited amount of plant material available.

Regarding the quantitative distribution in leaves and rhizomes (Table 1) it is interesting to note that the leaves had a much greater valepotriate content than the rhizomes. The quantity of monoene-type valepotriates in leaves exceeded the maximum of 5 % reported for the rhizomes of *Valeriana pinnatifida* R. et P. [5].

Valepotriates have so far only been reported for the family Valerianaceae. Within the family the species vary in their type and content of valepotriates. Up to now there is insufficient published data to be able to discuss species interrelationships on the basis of chemical evidence.

EXPERIMENTAL

Material and methods. P. pulvinata was collected by B.T. in the Peruvian Andes. A herbarium specimen is deposited in the Museo de Historia National del Peru, Lima. The material was air-dried and extracted according to ref. [8].

Isolation procedures are described in ref. [6]. ^{13}C NMR: see refs [7, 11]. FDMS: VG Analytical Micromass 7035; source potential + 4 kV, cathode potential – 7 kV, emitter heating current 0–18 mA. HPLC: Column: Lichrospher 100 CH-18/2 (5 μm) Merck (250 mm × 4 mm i.d.). Operating conditions: 10 min linear gradient elution starting with 75 % pump B to 95 %. Pump A $MeOH-H_2O$ (1:3); pump B $MeOH-H_2O$ (9:1); flow rate 1 ml/min. Detection for diene valepotriates: 254 nm, for monoene valepotriates 208 nm; internal standard: *n*-pentylbenzene (Merck). The relative retention indices to acevaltrate for the newly isolated compounds were as follows: **1**, 0.72; **2**, 0.85; **3**, 0.88; **4**, 1.4.

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