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SKIN IRRITANT DITERPENE ORTHOESTERS OF THE DAPHNANE TYPE FROM *PEDDIEA AFRICANA* AND *P. VOLKENSII**

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Key Word Index—*Peddiea africana*; *P. volkensii*; Thymelaeaceae; irritants; diterpene esters; tumour promoters; antileukemic activity.

Abstract—From roots of *Peddiea volkensii* (Thymelaeaceae) the irritant factors V_1 and V_2 and from roots of *P. africana* the irritant factor A_1 were isolated. Their structures are the 9,13,14-*ortho*-(2,4,6-decatrienoates) of 5β-hydroxyresiniferonol-6α,7α-oxide (V_1) and of 5β,12β-dihydroxyresiniferonol-6α,7α-oxide (A_1) and the 12-*O*-acetate of the latter (V_2). Factors V_1 and V_2 do not exhibit tumour-promoting activity in the standard initiation–promotion protocol on mouse skin, although V_1 is a moderate irritant.

INTRODUCTION

Species of the Thymelaeaceae are known to contain, besides some tigliane type diterpene esters, mainly diterpene orthoesters of the daphnane and 1α-alkyldaphnane type [1–3]. Many of them exhibit irritant and tumour-promoting activities [4–7] as well as antileukemic activity [8, 9]. In the present investigation two *Peddiea* species, *P. volkensii* Gilg and *P. africana* Harv., were investigated for irritants and tumour promoters. Both species have tough barks with fibrous bast which is used as cordage. *Peddiea africana* is said to be used medicinally by Africans [10]. In a recent report, from the related *P. fischeri* new anticancer agents in the form of two coumarins and a benzoquinone have been isolated [11]. However, neither daphnane nor tigliane derivatives were found.

RESULTS AND DISCUSSION

From roots of *Peddiea volkensii* Peddiea factors V_1 and V_2 and from roots of *P. africana* the Peddiea factor A_1 were isolated (see Table 1). Their structures were identified as closely related diterpene orthoesters of the daphnane type. They all contain 2,4,6-decatrienoic acid as the 9,13,14-orthoester group as revealed by NMR, UV (absorption bands at *ca* 259, 269 and 279 nm, typical of a conjugated triene system) and mass spectra (base peak at *m/z* 149 corresponding to the acyl cation). The spectral data of factor V_1 are identical with those of the Excoecaria factor O_1 isolated from leaves of *Excoecaria oppositifolia* (Euphorbiaceae) [12]. Hence, factor V_1 represents the 9,13,14-*ortho*-(2,4,6-decatrienoate) (**1**) of 5β-hydroxyresiniferonol-6α,7α-oxide. The spectral data of factor V_2 are in accordance with those of Synaptolepis factor K_3 isolated from roots of *Synaptolepis kirkii* (Thymelaeaceae) [7]. Therefore, the structure of V_2 is the 12-acetate-9,13,14-*ortho*-(2,4,6-decatrienoate) (**2**) of 5β,12β-dihydroxyresiniferonol-6α,7α-oxide. The molecular weight of Peddiea factor A_1 is 16 mass units higher than that of V_1. In the NMR spectrum of A_1 the singlet of one proton at δ3.91 and the resonance of H-8 at δ3.73 (in contrast to that at δ2.98 in V_1) is indicative of a β-hydroxy group at C-12. Hence, factor A_1 is the 9,13,14-*ortho*-(2,4,6-decatrienoate) (**3**) of 5β,12β-dihydroxyresiniferonol-6α,7α-oxide.

The Peddiea factors exhibit weak (V_2, A_1) to moderate

*Part 4 in the series "On the Active Principles of the Thymelaeaceae; for part 3, see ref [6].

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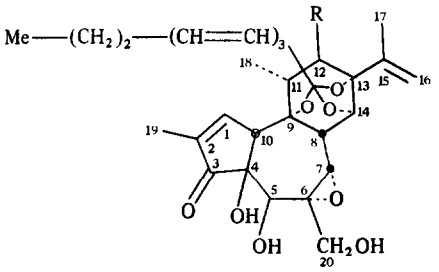
Table 1. Some characteristic data of irritant Peddiea factors isolated from roots of *Peddiea volkensii* (V_1, V_2) and *P. africana* (A_1)

Peddiea factor (structure)	Yield* (%)	R_f-value†	Molecular ion (*m/z*)	ID_{50}‡ (nmol/ear)
V_1 (**1**)	0.017	0.31	526	0.088
V_2 (**2**)	0.088	0.37	584	0.68
A_1 (**3**)	0.031	0.43	542	0.44

*By weight of the MeOH extracts.

†On RP-18 plates in MeOH–H_2O (9:1).

‡Reference compound TPA (12-*O*-tetradecanoylphorbol-13-acetate): 0.016 nmol/ear.



1 R = H
2 R = OAc
3 R = OH

(V_1) irritant activity as compared to that of TPA (Table 1). Compounds with a functional group at C-12—a hydroxyl group in A_1 or an acetoxyl group in V_2—are less active than those lacking substitution, i.e. V_1 (Table 1). In the standard protocol for tumour-promoting activity it was shown [7] for Synaptolepis factor K_3, which is identical with Peddiea factor V_2, that it does not show tumour-promoting activity at a dose of 20 nmol/application over 24 weeks. Factor V_1, lacking a substituent at C-12, is also inactive as tumour promoter when assayed at 20 nmol/application over 24 weeks. On the other hand, simplexin, the corresponding (saturated) 9,13,14-*ortho*-decanoate of 5β-hydroxyresiniferonol-6α,7α-oxide is a strong tumour promoter, when assayed with 20 nmol/application [4, 6]. Hence it may be concluded that the presence of double bonds in the carboxylic acid moiety of the orthoester group diminishes or abolishes tumour promoting activity. Similar findings were reported previously for tigliane and ingenane type diterpene esters [2, 13, 14]. Generally, structures of this type are of interest as putative 'incomplete (or stage II) promoters' [13]. Since similar structures of the daphnane type with unsaturated acid moieties also exhibit potent anti-leukemic activity *in vivo* [8, 9] it may be of importance to know that their tumour promoting capacity seems to be low or nil.

EXPERIMENTAL

Plant material. Roots of *Peddiea volkensii* (5 kg) were collected by T. N. Gachathi, Nairobi University Herbarium, Nairobi, Kenya, in Kiriita Forest, Kenya, in 1982. Roots of *Peddiea africana* (1.5 kg) were obtained from L. G. Quillier, Botanist, Cape Town, South Africa.

Separation methods and spectra. See ref. [5].

Assay for irritant and tumour promoting activity. See ref. [15].

Extraction and separation procedures. Roots of *P. volkensii* (2.7 kg) and of *P. africana* (1.5 kg) were homogenized and extracted several times with MeOH using an ultra-turrax. The MeOH extracts (*P. volkensii*: 102 g; *P. africana*: 25.3 g) were partitioned between EtOAc and water, the aq. phase being extracted with EtOAc (×3), to yield the EtOAc extracts (*P. volkensii*: 22 g, IU > 1000 μg/ear; *P. africana*: 7.8 g, IU: 250 μg/ear).

The EtOAc extract of *P. volkensii* was filtered through a silica gel column, eluting with EtOAc–petrol mixtures of increasing polarity. The filtrate (8.9 g) was subjected to a Craig distribution in petrol–MeOH–H_2O (15:10:0.5) (z = 1020, V = 5 ml/3 ml, n = 1500 transfers) to yield two irritant sections, r = 45–90 (286 mg, IU: 5–10 μg/ear), and r = 91–114 (17 mg, IU: 1 μg/ear). Section r = 91–114 was separated by TLC in CH_2Cl_2–MeOH (19:1 × 2) to yield 10 mg of Peddiea factor V_1. Section r = 45–90 (40 mg) was separated in the same system to yield 12.5 mg of Peddiea factor V_2.

The EtOAc extract of *P. africana* was subjected directly to a Craig distribution (system as above, z = 30, V = 100 ml/100 ml, n = 60 transfers). Two sections r = 0–10 (6.76 g, IU: 500 μg/ear) and r = 11–29 (0.32 g, IU: 20 μg/ear) showed irritant activity. Column chromatography of the latter fraction did not afford TLC homogeneous irritants. Section r = 0–10 was filtered through a silica gel column with $CHCl_3$–EtOH (10:1) to yield 4.5 g of material which again was eluted on a silica gel column with CH_2Cl_2–Me_2CO (4:1). One of the fractions (130 mg) was further separated by TLC in CH_2Cl_2–Me_2CO (4:1) and subsequently in Et_2O–petrol–Me_2CO (1:1:1) to yield 7.8 mg of Peddiea factor A_1.

Peddiea factor V_1 (**1**). MS m/z: 526 $[M]^+$, 495, 477, 149 (base peak). UV λ_{max}^{MeOH} nm (ε): 194 (12 660), 248 sh (22 300), 258 (33 000), 268 (42 200), 278 (32 000). ^{1}H NMR: δ 7.65 (1H, *m*, H-1), 5.05 (1H, *s*, H-16a), 4.93 (1H, *s*, H-16b), 4.46 (1H, *d*, J = 2.5 Hz, H-14), 4.28 (1H, *d*, J = 0.5 Hz, H-5), 3.85 ± 0.04 (2H, AB, J_{AB} = 12 Hz, H_2-20), *ca* 3.80 (1H, *m*, H-10 superimposed to H_2-20), 3.49 (1H, *d*, J = 0.5 Hz, H-7), 2.98 (1H, *d*, J = 2.5 Hz, H-8), 1.85 (6H, *m*, H_3-17 and H_3-19, superimposed); acid moiety: 6.77 (1H, *dd*, J_1 = 15 Hz, J_2 = 10 Hz, 1 olefinic H), 5.6–6.4 (5H, *m*, 5 olefinic H), 2.48 (2H, *m*, CH_2–C=C), 0.93 (3H, *t*, terminal Me). Spectral data are identical with those obtained for Excoecaria factor O_1 (12).

Peddiea factor V_2 (**2**). MS m/z: 584 $[M]^+$, 149 (base peak). UV λ_{max}^{MeOH} nm (ε): 196 (17 300), 248 sh (25 200), 260 (37 150), 269 (45 200), 279 (36 000). ^{1}H NMR: δ 7.58 (1H, *m*, H-1) 5.0 (3H, *m*, H_2-16, and *s*, H-12, superimposed), 4.77 (1H, *d*, J = 2.5 Hz, H-14), 4.26 (1H, *br s*, H-5), 3.86 (3H, AB, H_2-20, and *m*, H-10, superimposed), 3.53 (2H, *d*, J = 2.5 Hz, H-8, and *s*, 7-H, superimposed), 1.83 (6H, *m*, H_3-17 and H_3-19, superimposed); acid moieties: 6.72 (1H, *dd*, J_1 = 15 Hz, J_2 = 10 Hz, 1 olefinic H), 5.6–6.4 (5H, *m*, 5 olefinic H), 0.9 (3H, *t*, term. Me); 2.02 (3H, *s*, acetate). Spectral data are identical with those obtained for Synaptolepis factor K_3 [7].

Peddiea factor A_1 (**3**). MS m/z: 542 $[M]^+$, 524, 511, 506, 149 (base peak); UV λ_{max}^{MeOH} nm (ε): 193 (16 600), 247 sh (26 200), 259 (39 150), 268 (44 000), 279 (37 500). ^{1}H NMR: δ 7.55 (1H, *m*, H-1), 5.10 (2H, *br s*, H_2-16), 4.70 (1H, *d*, J = 2.5 Hz, H-14), 4.22 (1H, *s*, H-5), 3.91 (1H, *s*, H-12), 3.82 ± 0.07 (3H, AB, J_{AB} = 12 Hz, H_2-20, and *m*, H-10, superimposed), 3.73 (1H, *d*, J = 2.5 Hz, H-8), 3.53 (1H, *s*, H-7), 1.87 (3H, *br s*, H_3-17), 1.80 (3H, *m*, H_3-19); acid moiety: 6.66 (1H, *dd*, J_1 = 15 Hz, J_2 = 10 Hz, 1 olefinic H), 5.5–6.4 (5H, *m*, 5 olefinic H), 2.48 (2H, *m*, CH_2–C=C), 0.90 (3H, *t*, term. Me).

Biological activities. For irritant activity of the Peddiea factors see Table 1. Factor V_1 was assayed for tumour promoting activity at a dose of p = 20 nmol/application. Within 24 weeks none of the 15 animals of the group showed any tumour. Due to lack of compound, factor A_1 was not assayed.

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