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ABIETANE DITERPENOIDS FROM *PLECTRANTHUS GRANDIDENTATUS*

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Key Word Index—*Plectranthus grandidentatus*; Labiatae; diterpenes; abietanes; antibacterial activity.

Abstract—The acetone extract of the whole plant of *Plectranthus grandidentatus* provided the already known abietanes royleanone, 6,7-dehydroroyleanone, horminone, 6β-hydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone and the abietane dimers grandidone C, grandidone D and 7-epigrandidone D, together with a mixture of fatty acid esters of 7α-acyloxy-6β,12-dihydroxy-abieta-8,12-diene-11,14-dione. Some of these compounds showed moderate antibacterial activity. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

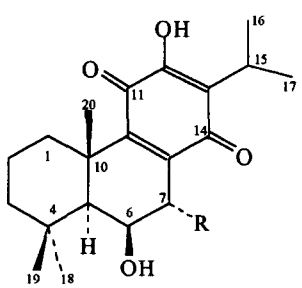
In continuation of our studies on biologically active diterpenoids from *Plectranthus* plants [1–3], we have now investigated *P. grandidentatus* (whole plant). From the leaf-glands of this species have been isolated [4] the abietanes 14-hydroxytaxodione and coleons U and V, and the abietane dimers grandidones A–D and 7-epigrandidones A, B and D. We report here on the isolation and identification of the abietane derivatives found in the whole plant acetone extract, as well as the results of the assays of some of these abietanes as antimicrobial compounds.

RESULTS AND DISCUSSION

Repeated chromatography of the acetone extract of *P. grandidentatus* (whole plant, see Experimental) allowed the isolation of the abietane dimers grandidones C and D and 7-epigrandidone D, previously found in the leaf-glands of this species [4], together with the already known diterpenes royleanone (12-hydroxy-abieta-8,12-diene-11,14-dione) [5, 6], 6,7-dehydroroyleanone (12-hydroxy-abieta-6,8,12-triene-11,14-dione) [5, 6], horminone (7α,12-dihydroxy-abieta-8,12-diene-11,14-dione) [5, 7], 6β-hydroxyroyleanone (6β,12-dihydroxy-abieta-8,12-diene-11,14-dione) [6, 8], 7α-acetoxy-6β-hydroxyroyleanone (**1**, 7α-acetoxy-6β,12-dihydroxy-abieta-8,12-diene-11,14-dione) [6, 8–10], all of them found in *Plectranthus* and *Coleus* species. In addition, we have also isolated another compound (**2**), whose structure was established.

The ^{1}H NMR spectrum of **2** was almost identical with that of **1** (Table 1), and the observed differences were consistent with the existence in the latter of a mixture of fatty acid esters at the C-7α position [$\delta_{H-7\beta}$ 5.66 *d*, J = 1.8 Hz, $\delta_{\omega-Me}$ 0.86, 3H, *t*, J = 6.9 Hz, δ 5.33 *m*, 0.3H (olefinic protons), δ 2.25 *m*, 2.2H allylic and α-methylenes) and δ 1.23 *br s*, 26 H (methylenes)] instead of the C-7α acetoxyl group of the former ($\delta_{H-7\beta}$ 5.65 *d*, J = 2.1 Hz, δ 2.04 *s*, 3H, OAc). Comparison of the UV spectra of **1** (λ_{max}^{MeOH} nm: 271 and 410) [6] and **2** (λ_{max}^{MeOH} nm: 273 and 409) further confirmed this deduction.

Hydrolysis of **2**, followed by methylation and GC-MS analysis of the crude of the reaction, allowed the identification of the fatty acid methyl esters and established that **2** was a mixture of 7α-palmityloxy (71.7%), -stearyloxy (12.8%), -oleyloxy (6.1%), -*n*-heptadecanoyloxy (3.9%), -*n*-pentadecanoyloxy (2.9%), -myristyloxy (2.9%), -6β,12-dihydroxy-abieta-8,12-diene-11,14-dione.



1 R = OAc

2 R = Fatty acid esters

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Table 1. ^{1}H NMR spectral data of compounds **1*** and **2** [300 MHz, $CDCl_3$, δ values relative to residual $CHCl_3$ ($\delta = 7.25$), J values in Hz]

H	1	2	H	1	2
1β	2.63 *dt*	2.63 *dt*	OH (C-12)	7.18 s	7.18 s
6α	4.31 *t*	4.30 *t*	OAc	2.04 *s*	—
7β	5.65 *d*	5.66 *d*	Fatty esters ω-Me	—	0.86 *t* (3H)
15	3.16 *sept*	3.15 *sept*	Methylenes	—	1.23 *br s* (26H)
Me-16[a]	1.22 *d*	1.22 *d*	Allylic and α methylenes	—	2.25 *m* (2.2*H*)
Me-17[a]	1.19 *d*	1.18 *d*	Olefinic protons	—	5.33 *m* (0.3H)
Me-18	0.93 *s*	0.93 *s*			
Me-19	1.21 *s*	1.22 *s*			
Me-20	1.60 *s*	1.60 *s*			

J **1**: 1α,1β = 12.6, 1β,2$\alpha \approx$ 1β,2β = 3.5, 5α,6α = 6α,7β = 2.1,15,16(17) = 7.1.
2: 1α,1β = 12.2, 1β,2$\alpha \approx$ 1β,2β = 3.0, 5α,6α = 6α,7β = 1.8,15,16(17) = 7.0; fatty esters: Me, CH_2 = 6.9.

*These values have been specially obtained by us for this work and are in agreement with the previously reported values [6, 8–10].

[a]Interchangeable methyl groups.

The biautographic agar overlay assay with *Staphylococcus aureus* [11, 12], was used to detect and activity-guide the fractionation of antimicrobial compounds, and has revealed activity for all the royleanones isolated, but not for the grandidones. The activity showed in bioautography for the royleanones was confirmed by the determination of the minimum inhibitory concentration (MIC) against *S. aureus*. Compound **1**, with a MIC value of 31.2 μg ml^{-1} and horminone (MIC value previously described) [1] showed higher antibacterial activity than royleanone, 6,7-dehydroroyleanone, and 6β-hydroxyroyleanone (MIC values $\geq$125 μg ml^{-1}). The MIC determination against the other standard bacterial strains and a yeast (see Experimental) showed that all these assayed royleanones present MIC values of 15.6 μg ml^{-1} against *Vibrio cholerae* and less activity against the rest of the strains (MIC $\geq$125 μg ml^{-1}) (see Experimental). The activities of **1** and horminone against *S. aureus* and the activities against *V. cholerae* were found to be bactericidal, considering that the MIC and the MBC (minimum bactericidal concentration) values are the same.

EXPERIMENTAL

General. Seeds of *P. grandidentatus* Gürke were provided by the National Botanic Garden, Kirstebosh, Claremont, South Africa. The plants were cultivated in the Faculty of Pharmacy HORTUM, Lisbon University, through vegetative propagation of the first specimen obtained from seed. The material was collected in July–October 1994, and voucher specimens are deposited in the Herbarium of the Department of the Organic Chemistry, Faculty of Pharmacy, University of Lisbon, Portugal.

Extraction and isolation of the diterpenoids. Dried and powdered *P. grandidentatus* (whole plant, 2.56 kg) were extracted with Me_2CO (5 × 6 l) at room temp. for 5 days. The solvent was evapd under red. pres. and low temp. (40°) yielding a residue (65.5 g), which was subjected to CC (silica gel Merck N°. 9385, 500 g). From the frs eluted with hexane–EtOAc (4:1) (12 g), the following compounds were obtained in order of increasing chromatographic polarity: royleanone (10 mg) [5, 6], 6,7-dehydroroyleanone (11 mg) [5, 6], horminone (18 mg), compound **2** (72 mg) [5, 7], 6β-hydroxyroyleanone (6 mg) [6, 8], 7α-acetoxy-6β-hydroxyroyleanone (**1**, 210 mg) [6, 8–10], grandidone D (4 mg) [4] grandidone C (26 mg) [4] and 7-epi-grandidone D (26 mg) [4].

The previously known diterpenoids were identified by their ^{1}H NMR and MS and, in some cases, by comparison (TLC) with authentic samples.

7-Fatty acid esters of 6β,7α-dihydroxyroyleanone (**2**). Yellowish thick oil; $[\alpha]_D^{21}$ + 11.7°, $[\alpha]_{578}$ +8.0°, $[\alpha]_{546}$ −17.1°, $[\alpha]_{436}$ −36.6°, $[\alpha]_{365}$ −63.8° ($CHCl_3$; *c* 0.298). UV λ_{max}^{MeOH} nm (log ε): 273 (4.18), 409 (2.96), $\lambda_{max}^{MeOH+NaOMe}$ nm: 223 (4.47), 274 (4.02), 519 (3.29), for a mean M_r 592. IR ν_{max}^{NaCl} cm^{-1}: 3500, 3380 (OH), 1730 (ester), 1660, 1645, 1615 (quinone), 2930, 2850, 1460, 1380, 1250, 1160, 1150, 1100, 1050, 960, 900, 760. ^{1}H NMR: Table 1.

Hydrolysis of **2** *and identification of fatty acid methyl esters.* To a soln of **2** (20 mg) in EtOH (5 ml) was added a soln of KOH in EtOH (10%, w/v. 5ml) and the reaction mixt. was left at room temp. for 24 hr. The reaction mixt. was then diluted with H_2O (50 ml), acidified (pH $\approx$ 3) with aq. 1.5 M H_2SO_4 and extracted with $CHCl_3$ (4 × 20 ml). The organic extract was dissolved in Et_2O (5 ml) and treated with an excess of an ethereal soln of CH_2N_2 for 2 hr at room temp. The solvent was evapd and the crude residue subjected to GC-MS analysis under standard conditions, by using a Hewlett Packard 5890 gas chromatograph coupled to a HP 5971A mass detector. Myristic (2.6%), *n*-pentadecanoic (2.9%), palmitic (71.7%), *n*-heptadecanoic (3.9%), stearic (12.8%) and oleic (6.1%) acids methyl esters were identified.

Microorganisms. Gram negative bacteria: *Escherichia coli* ATCC 25922, *Shigella dysenteriae* ATCC 13313, *Salmonella typhimurium* ATCC 43971, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio cholerae* ATCC 11623; Gram positive bacteria: *Staphylo-*

coccus aureus ATCC 2592^{-1}3, *Streptococcus faecalis* ATCC 10541; yeast: *Candida albicans* CIP 3153 A.

Bioautography. TLC plates with 0.15 mg of each sample (silica gel, hexane–EtOAc) were covered with a suspension of the indicator strain *S. aureus* with a final concentration of 10^6 cfu ml^{-1} and incubated at 37° for 24 hr [11, 12].

Minimum inhibitory concentration (MIC). The MIC value for bacteria and yeast was determined using the three-fold serial broth microdilution assay [13] over the concentration range 500 μg ml^{-1} to 7.8 μg ml^{-1}. The test compounds were added to sterile Mueller–Hinton broth medium for bacteria and YMA broth Medium for the yeast as a soln in MeOH–H_2O and inoculated with a microorganism concentration of *ca* 10^5 cfu ml^{-1}. Solvent blanks were included. The MIC value was taken as the lowest concentration of compound which inhibited the growth of the test organisms after 24 hr incubation at 37°.

Minimum bactericidal concentration (MBC). The bactericidal effects of the tested compounds were examined after the determination of MIC. A replica plating from each clear tube was done into a Mueller Hinton agar. After 24 hr at 37° the MBC was determined as the lowest concentration of the test sample in which no recovery of microorganisms was obtained [13].

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