ABIETANE DITERPENOIDS FROM PLECTRANTHUS GRANDIDENTATUS

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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 grandidine C, grandidine D and 7-epigrandidone D, together with a mixture of fatty acid esters of 7α-acetyl-6β,12-dihydroxy-abieta-8,12-diene-11,14-dione. Some of these compounds showed moderate antibacterial activity.

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 grandidiones A–D and 7-epigrandidiones 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 grandidiones C and D and 7-epigrandidione 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-8,12-diene-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 1H 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 (δH 5.66 d, J = 1.8 Hz, δm-α-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-methylene) and δ 1.23 br s, 26 H (methylene) instead of the C-7α acetoxyl group of the former (δH 5.65 d, J = 2.1 Hz, δ 2.04 s, 3H, OAc).

Comparison of the UV spectra of 1 (λmax nm: 271 and 410) and 2 (λmax 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-myristyloxy (2.9%), -n-pentadecanoyloxy (2.9%), -myristoyloxy (2.9%), -n-β,12-dihydroxy-abieta-8,12-diene-11,14-dione.
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 grandiones. 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 ≥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 c 0.298). UVA (log ε): 273 (4.18), 409 (2.96), 274 (4.02), 519 (3.29), for a mean M_r 592. IR Vnm⁻¹: cm⁻¹: 3500, 3380 (OH), 1730 (ester), 1660, 1645, 1615 (quinone), 2930, 2850, 1460, 1380, 1250, 1160, 1150, 1100, 1050, 960, 900, 760. 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, 5 ml) and the reaction mixt. was left at room temp. for 24 hr. The reaction mixt. was then diluted with H₂O (50 ml), and treated with an excess of an ethereal soln of CH₂N₂ 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 (2.7%), 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-
Diterpenoids from *Plectranthus grandidentatus*

*coccus aureus* ATCC 25923, *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°C 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$_2$O 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°C.

**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°C 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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