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AN ANTIMICROBIAL ABIETANE FROM THE ROOT OF *PLECTRANTHUS HERERoensis*

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Key Word Index—*Plectranthus hereroensis*; Labiatae; abietane diterpene; antimicrobial activity; antibacterial activity; antiviral activity.

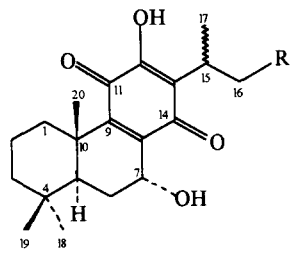
Abstract—A new abietane diterpene, 16-acetoxy-7α,12-dihydroxy-8,12-abietadiene-11,14-dione, has been isolated from the acetone extract of the root of *Plectranthus hereroensis* and its structure established by spectroscopic means. This compound showed antibacterial activity against *Staphylococcus aureus* and *Vibrio cholerae*, and antiviral activity against *Herpes simplex* type II.

INTRODUCTION

In a previous communication [1], we reported on the isolation of the abietane diterpenes horminone (**1**) [2, 3] and 7α,12-dihydroxy-17(15→16)-*abeo*-abieta-8,12,16-triene-11,14-dione from the acetone extract of the root of *Plectranthus hereroensis*.

These compounds showed antimicrobial activity against *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Candida albicans*. This biological behaviour prompted us to investigate the more polar fractions of the extract of the plant.

In addition, the absence of substances effective against *Herpes* infection led us to assay the activity of **2** against the two more important *Herpes* viruses.



1 R=H

2 R=OAc

RESULTS AND DISCUSSION

From the chromatographic fractions obtained after elution of 7α,12-dihydroxy-17(15→16)-*abeo*-abieta-8,12,16-triene-11,14-dione [1], we have isolated a new substance whose structure, 16-acetoxy-7α,12-dihydroxy-8,12-abietadiene-11,14-dione (**2**), was established as follows.

Low-resolution mass spectrometry and combustion analysis indicated the molecular formula $C_{22}H_{30}O_6$ for **2**. Its UV spectrum showed absorptions at 272.5 and 407.5 nm (log ε 4.18 and 2.98, respectively), identical to those reported [3] for horminone (**1**), thus establishing the presence of the same chromophore in both compounds.

The ^{1}H NMR spectrum of **2** unambiguously supported the structure of this substance, because it was identical to that of horminone (**1**) [2–4] in the signals corresponding to the H-1β, H-7β and C-18, C-19 and C-20 methyl group protons (see Experimental), whereas **2** showed signals which must be attributed to a 2-acetoxy-isopropyl group [δ_H3.36 *sextet* (H-15), 1.21 *d* (3H, Me-17), and 4.27 and 4.25 both *dd*(2H-16); $J_{16A,16B}$=13.0 Hz, $J_{15,16}=J_{15,17}$ =7.3 Hz, and 2.00 *s* (3H, OAc)] [5–7] instead of the isopropyl group at C-13 of horminone (**1**).

Abietane derivatives like **2**, with oxygenated functions at C-16, have previously been found in Labiatae plants [5–7] and particularly in those belonging to the *Plectranthus* genus [7–11].

From a biogenetic point of view, it is of interest to note that, in general, the abietane diterpenoids isolated from the aerial parts of *Plectranthus* species [7–11] are more oxygenated than those found in the roots of these plants (ref. [1] and this work).

An antimicrobial activity-guided fractionation by bioautography led to the isolation of the bioactive abietane **2**.

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The antimicrobial activity of **2** was evaluated against Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Salmonella typhimurium, Vibrio cholerae*), Gram-positive bacteria (*Staphylococcus aureus, Streptococcus faecalis*) and *Candida albicans*. The MICs of **2** against *Staphylococcus aureus* and *Vibrio cholerae* were 31.2 $\mu g\,ml^{-1}$ and 15.6 $\mu g\,ml^{-1}$, respectively. This substance was inactive against all the other micro-organisms tested.

Compound **2** (with a 2-acetoxy-isopropyl at C-13) was less active than 7α,12-dihydroxy-17(15→16)-*abeo*-abieta-8,12,16-triene-11,14-dione (with an allyl at C-13) [1], and these two were less active than **1** (with an isopropyl at C-13) [1]. These results are in agreement with those reported in ref. [12]. The authors described the antimicrobial activity of several abietane derivatives and found that the presence of an acetoxyl at C-16 decreased the activity [12].

The antiviral activity of **2** was tested against two types of viruses (HSV_1 and HSV_2). It was concluded that only HSV_2 plaque formation is inhibited by 60% with 0.2 $\mu g\,ml^{-1}$ (the maximum concentration not cytotoxic) of the compound. Recently, antiviral activity of abietane derivatives was reported [13].

EXPERIMENTAL

The plant material was produced and cultivated from authentic seeds of *Plectranthus hereroensis*, in Lisbon during 1990–1991 (Lisbon Pharmacy Faculty HORTUM). The material was collected in December 1991, and a voucher specimen was deposited in the Herbarium of 'Instituto Botânico, Universidade de Lisboa'.

Isolation of 16-acetoxy-7α,12-dihydroxy-8,12-abietadiene-11,14-dione (**2**). The Me_2CO extract of the roots of *P. hereroensis*, obtained as previously described [1], was subjected to CC (silica gel Merck no. 7734, deactivated with 15% H_2O, w/v, 100 g). Elution with petrol–EtOAc (9:1) and (7:3) yielded horminone (**1**) and 7α,12-dihydroxy-17(15→16)-*abeo*-abieta-8,12,16-triene-11,14-dione, respectively [1]. Further elution with petrol–EtOAc (3:2) yielded impure **2** (36 mg), which was purified by prep. TLC (silica gel plates, petrol–EtOAc, 3:2 as eluent) giving 30 mg of pure yellow amorphous solid, mp 45–55°; $[\alpha]_D^{16}$ −105.5° ($CHCl_3$; *c* 0.055). IR ν_{max}^{KBr} cm^{-1}: 3530 (OH), 3360–2700 br (phenolic OH), 1745, 1255, 1235 (OAc), 1660, 1630, 1600 (*p*-benzoquinone), 2940, 2870, 1460, 1400, 1375, 1150, 1060, 1035, 960, 950, 900, 770; UV λ_{max}^{EtOH} nm (log ε): 272.5 (4.18), 407.5 (2.98); ^1H NMR (200 MHz, $CDCl_3$): δ 7.30 *br s* (1H, OH-12),* 4.73 *m* (1H, $W_{1/2}$ = 7 Hz, H-7β), 4.27 and 4.25 both *dd* (1H each, J_{gem} = 13.0 Hz, J_{vic} = 7.3 Hz, 2H-16), 3.36 *sextet* (1H, *J* = 7.3 Hz, H-15), 2.95 *br s* (1H, OH-7α),* 2.68 *dt* (1H, J_{gem} = 13.1 Hz, $J_{1\beta,2\alpha} = J_{1\beta,2\alpha}$ = 2.9 Hz, equatorial H-1β), 2.00 *s* (3H,OAc), 1.21 *s* (3H, Me-20), 1.21 *d* (3H, *J* = 7.3 Hz, Me-17), 0.97 and 0.90 both *s* (3H each, Me-18 and Me-19) (*Exchangeable with D_2O); EIMS (70 eV, direct inlet) *m/z* (rel. int.): 390 $[M]^+$ (0.1), 375 $[M-Me]^+$ (0.02), 372 $[M-H_2O]^+$ (0.04), 330 $[M-HOAc]^+$ (0.7), 315 $[M-HOAc-Me]^+$ (0.4), 312 $[M-HOAc-H_2O]^+$ (0.4), 297 $[M-HOAc-Me-H_2O]^+$ (0.5), 193 (4), 145 (2), 133 (3), 129 (3), 128 (4), 123 (11), 105 (6), 95 (8) 91 (13), 83 (10), 79 (10), 77 (12), 69 (28), 55 (34), 53 (11), 43 (100), 41 (28). Found: C, 67.29; H, 7.89. $C_{22}H_{30}O_6$ requires: C, 67.67; H, 7.74%.).

Micro-organisms. Escherichia coli ATCC 25922, *Shigella dysenteriae* ATCC 14314, *Salmonella typhimurium* ATCC 43971, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio cholerae* ATCC 11623, *Staphylococcus aureus* ATCC 25923, *Streptococcus faecalis* ATCC 10541 and *Candida albicans* CIP 3153 A.

Quantitative antibacterial and antifungal evaluation. The MIC values for bacteria and yeast were determined using the 3-fold serial broth microdilution assay [14] over the concn range 500 $\mu g\,ml^{-1}$–7.8 $\mu g\,ml^{-1}$. The test compound was added to sterile Mueller–Hinton broth medium for bacteria and YMA broth medium for the yeast as a soln in MeOH–H_2O (2:1). Solvent blanks were included. The MIC value was taken as the lowest concn of compound which inhibited the growth of the test organisms after incubation for 24 hr at 37°.

Bioautography. A bioautographic agar overlay assay for detection and activity-guided fractionation of antimicrobial compounds using *St. aureus* as indicator strain was developed [15, 16].

Maximum cytotoxicity concentration. Vero cell monolayers were incubated with different compound concns for 72 hr (37°). After this time, the dye uptake assay was performed [17] to determine the per cent of living cells related to the control (cells with DMSO).

Antiviral assays. Vero cell monolayers were infected with *Herpes simplex* type I (HSV_1) and type II (HSH_2) at a multiplicity of injection of 0.1. After adsorption for 2 hr at 37°, the cells were covered with DME medium with 2% NCS and 50 $\mu g\,ml^{-1}$ of gentamicine containing 2% Sephadex G-50, and incubated for 3 days at 37°. After this time the number of viral plaques was counted [18].

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