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STRUCTURE AND ANTIMICROBIAL ACTIVITY OF DITERPENES FROM THE ROOTS OF *PLECTRANTHUS HEREROENSIS*

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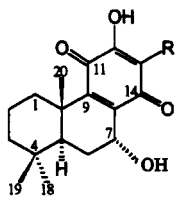
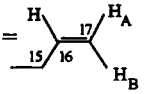
ABSTRACT.—Two abietane-type diterpenoids have been isolated from the roots of *Plectranthus hereroensis* (Labiatae), one being the already known horminone [**1**] and the other a new substance, 7α,12-dihydroxy-17(15→16)-*abeo*-abieta-8,12,16-triene-11,14-dione [**2**], whose structure was established by spectroscopic means. Compounds **1** and **2** showed antimicrobial activity against *Staphylococcus aureus, Vibrio cholerae, Candida albicans,* and *Pseudomonas aeruginosa.*

In our search for biologically active compounds from plants in the Labiatae, we have examined the roots of *Plectranthus hereroensis* Engl. In this communication we wish to report on the isolation, structure elucidation and antimicrobial activity of two abietane-type diterpenes found in this plant part.

Repeated chromatography of the Me_2CO extract of the roots of *Plectranthus hereroensis* (see Experimental) resulted in the isolation of two substances, one of which was identical with the previously known diterpene horminone [**1**], a compound found for the first time in *Horminium pyrenaicum* (1) [although prior to this it was known as semisynthetic substance (2)], and afterwards isolated from other Labiatae species (3), including *Plectranthus* spp. (4,5). The other substance isolated from *P. hereroensis* is a new natural compound whose structure [**2**] was established as follows.



1 R= 

2 R= 

Combustion analysis and low-resolution eims were consistent with the molecular formula $C_{20}H_{26}O_4$ for compound **2** and its uv spectrum [λ max (MeOH) 272 and 411 nm, log ε 4.00 and 2.83, respectively] was identical to that reported for horminone [**1**] (1,2,4), thus establishing that both compounds possess the same substituted chromophore. The ^{1}H-nmr spectrum of compound **2** (Table 1) was very similar to that of horminone [**1**] and the observed differences were in complete agreement with the existence in compound **2** of an allyl group at the C-13 position [δ 5.83, 1H, ddt, $J_{16,17B}$=17.1 Hz, $J_{16,17A}$=10.0 Hz, $J_{16,15A}$=$J_{16,15B}$=6.5 Hz (H-16); δ 5.11, 1H, ddt, $J_{17B,17A}$=1.6 Hz, $J_{17B,15A}$=$J_{17B,15B}$=1.6 Hz, (H_B-17); δ 5.03, 1H, ddt, $J_{17A,15A}$=$J_{17A,15B}$=1.4 Hz, (H_A-17); δ 3.18, 1H, ddt, $J_{15A,15B}$=13.0 Hz, (H_B-15); δ 3.16, 1H, ddt (H_A-15)] (4–10) instead of the C-13 isopropyl substituent of horminone [**1**: δ 3.16, 1H, septet, $J_{15,16(17)}$=7.1 Hz, (H-15) δ 1.22 and 1.21, 3H each, d, J=7.1 Hz (Me-16 and Me-17)] (4). Consequently, the new diterpenoid [**2**] is the 17(15→16)-*abeo*-16,17-didehydro derivative of horminone [**1**]. This conclusion was also supported by the allylic absorptions (3080, 1640,

TABLE 1. ^{1}H-Nmr Spectroscopic Data of Compounds **1** and **2**.[a]

Proton	**1**[b]	**2**	*J* (Hz)	**1**[b]	**2**
H-1β	2.70 dt	2.70 dt	1α,1β	13.4	13.0
H-2β	1.71 ddddd	1.74 ddddd	1β,2α	3.3	3.6
H-6α	1.96 ddd	1.97 ddd	1β,2β	3.3	3.6
H-7β	4.71 dd	4.73 dd	2β,1α	13.3	13.8
H-15	3.16 septet	—	2β,2α	13.3	13.8
H_A-15	—	3.16 ddt	2β,3α	13.3	13.8
H_B-15	—	3.18 ddt	2β,3β	3.5	3.6
H-16	—	5.83 ddt	6α,6β	12.9	12.8
H_A-17	—	5.03 ddt	6α,5α	1.1	1.4
H_B-17	—	5.11 ddt	6α,7β	1.4	1.5
Me-16[c]	1.22 d	—	7β,6β	4.6	4.5
Me-17[c]	1.21 d	—	15,16(17)	7.1	—
Me-18	0.98 s	0.98 s	15A,15B	—	13.0
Me-19	0.90 s	0.90 s	15,16	—	6.5
Me-20	1.21 d	1.22 br s	15,17A	—	1.4
OH-7[d]	3.01 s	2.99 s	15,17B	—	1.6
OH-12[d]	7.24 s	7.20 s	16,17A	—	10.0
			16,17B	—	17.1
			17A,17B	—	1.6
			20,1α	0.6	<0.4

[a]At 300 MHz, $CDCl_3$ solution.
[b]Data obtained in this work; identical to those reported in Hensch *et al.* (4).
[c]Interchangeable assignments.
[d]Disappeared after addition of D_2O.

990, and 915 cm^{-1}) exhibited in the ir spectrum of compound **2**. Rearranged 17(15→16)-*abeo*-abietane derivatives have been isolated from several *Plectranthus* species (Labiatae) (5, 7–11) and also from *Staudtia kamerunensis* (Myristicaceae) (6).

The antimicrobial properties and the minimum inhibitory concentration (MIC) of the Me_2CO extract of *P. hereroensis* were determined. The extract, at the dose employed (3 mg/disk), exhibited marginal inhibition against *Staphylococcus aureus* (16 mm zone diameter), *Vibrio cholerae* (14 mm zone diameter), and *Streptococcus faecalis* (11 mm zone diameter), but was inactive against *Escherichia coli, Shigella dysenteriae, Salmonella typhimurium, Pseudomonas aeruginosa,* and *Candida albicans.* In dilution assay the extract inhibited the growth of *Vibrio cholerae* and *Staphylococcus aureus* at a 125 μg/ml dose MIC value while the growth of the others tested strains was unaffected (MIC value ≥500 μg/ml). *Staphylococcus aureus* was selected for detection and activity-guided fractionation of antimicrobial compounds by bioautography (see Experimental).

The MIC values of horminone [**1**] and compound **2** were determined against the same bacterial systems by the broth microdilution method. Horminone [**1**] showed higher antibacterial activity against *Staphylococcus aureus* and *Vibrio cholerae* (MIC values both 7.8 μg/ml) than compound **2**, which exhibited for the same strains MIC values of, respectively, 15.6 μg/ml and 31.2 μg/ml. The MIC values of both compounds were also determined against *Candida albicans* (**1**, 250 μg/ml; **2**, 125 μg/ml), *Pseudomonas aeruginosa* (**1** and **2**, 250 μg/ml), and *Escherichia coli, Shigella dysenteriae, Salmonella typhimurium, Streptococcus faecalis* (**1** and **2**, ≥500 μg/ml).

Compounds with structures closely related to those of **1** and **2** have shown significant biological activity as tumor inhibitors (12–14), antifeedants (15), and antibacterials (16).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Eims were determined at 70 eV on a VG 12-250 instrument. Uv and ir spectra were recorded on a Perkin-Elmer model Lambda 2 and on a Perkin-Elmer model 681 spectrophotometers, respectively. ^{1}H-Nmr spectra were measured on a Varian XL-300 instrument, operating at 300 MHz. Optical rotations were determined with a Perkin Elmer 241 MC polarimeter.

PLANT MATERIAL.—The plant material was produced and cultivated, from authentic seeds of *P. hereroensis*, in Lisbon during 1990–1991 (Lisbon Pharmacy Faculty Hortum). The material was collected in December 1991, the identification was confirmed by Dr. Belo Correia, and a voucher specimen is deposited in the Herbarium of Instituto Botânico, Universidade de Lisboa.

EXTRACTION AND ISOLATION.—Dried and powdered roots of *P. hereroensis* (220 g) were extracted with Me_2CO (3×1.5 liters) at room temperature for three days. The solvent was evaporated under reduced pressure at low temperature (40°) yielding a residue (9 g), which was subjected to cc (Si gel, Merck No. 7734, deactivated with 15% H_2O, w/v, 100 g).

Elution with petroleum ether (bp 65–68°) and petroleum ether/EtOAc mixtures gave impure horminone (**1**, 102 mg) from the fractions eluted with petroleum ether-EtOAc (9:1). From the fractions eluted with petroleum ether-EtOAc (7:3), was obtained impure **2** (27 mg). Final tlc purification yielded pure horminone (**1**, 32 mg) and pure **2** (11 mg) (Si gel plates, hexane-EtOAc 3:2 and 4:1, respectively, as solvent).

Horminone [**1**].—Mp 175–177° (from *n*-hexane); $[\alpha]^{20}D$ −128.3° (*c*=0.169, $CHCl_3$). ^{1}H nmr, see Table 1. Identical with the previously described compound (mp 178°, $[\alpha]D$ −130°)(1,4,14). Comparison (mmp, hplc, tlc) with an authentic sample (3) confirmed this identity.

7α,12-Dihydroxy-17(15→16)-abeo-*abieta-8,12,16-triene-11,14-dione* [**2**].—Mp 55–60° (red amorphous solid);$[\alpha]^{19}D$ −75.7° (*c*=0.111, $CHCl_3$). Uv λ max (MeOH) (log ε) 272 (4.00), 411 (2.83) nm; ir ν max (NaCl) 3530 (OH), 3380–2600 (phenolic), 3080, 1640, 990, 915 (allyl group), 1655, 1630, 1600 (*p*-benzoquinone), 2920, 2860, 1460, 1370, 1250, 1220, 1150, 1060, 1020, 970 cm^{-1}; ^{1}H nmr, see Table 1; eims (70 eV, direct inlet) *m/z* 330 $[M]^+$ (90), 315 (30), 312 (48), 297 (79), 269 (23), 259 (34), 243 (32), 216 (34), 193 (93), 123 (38), 109 (30), 91 (38), 81 (33), 69 (32), 55 (72), 43 (61), 41 (100); *anal.* found C, 72.58; H, 8.09, $C_{20}H_{26}O_4$ requires C, 72.70; H, 7.93%.

MICROORGANISMS.—*Escherichia coli* ATCC 25922, *Shigella dysenteriae* ATCC 13313, *Salmonella typhimurium* ATCC 43971, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio cholerae* ATCC 11623, *Staphylococcus aureus* ATCC 25923, *Streptococcus faecalis* ATCC 10541, *Candida albicans* CIP 3153A.

QUALITATIVE ANTIMICROBIAL EVALUATION.—The disk-diffusion method (17) was used to measure the antimicrobial activity of the plant extract. The different organisms were seeded over previously sterilized Mueller-Hinton agar for bacteria and Casitone agar for the yeast. The zones of growth inhibition were observed around dried disks (Whatman 6 mm diameter) containing 3 mg of plant extract. The plates were incubated at 37° for 24 h. Solvent blanks were included.

QUANTITATIVE ANTIMICROBIAL EVALUATION.—The MIC values of the extract and compounds **1** and **2** for bacteria and yeast were determined using the twofold serial broth microdilution assay (18) at concentrations ranging from 500 μg/ml to 7.8 μg/ml. Each test compound was added to sterile Mueller-Hinton broth for bacteria and YMA broth for the yeast as a solution in Me_2CO or MeOH. Solvent blanks were included. The MIC value was taken as the lowest concentration of the compounds which inhibited the growth of the test organisms after 24 h of incubation at 37°.

BIOAUTOGRAPHY.—A simple bioautographic agar overlay assay for detection and activity-guided fractionation of antimicrobial compounds by tlc has been developed (19). The suspension of the indicator strain, *S. aureus* was prepared at a final concentration of ca. 10^8 cells/ml (20). Mueller-Hinton agar was used as the solid medium for the overlays. The molten medium was maintained in a H_2O bath at 45° and 2,3,5-triphenyltetrazolium chloride (Sigma) was added to 0.1% (w/v) (20). The microbial suspension was then mixed with the overlay medium and applied immediately to the tlc plate. After the medium had solidified the overlayed tlc plate was placed in a box and incubated overnight at 37°. Where microbial growth had been inhibited pale spots could be seen against a deep pink-red background. In the bioautogram of the crude extract 0.15 mg were sufficient to observe four main inhibition zones at R_f 0.54, 0.43, 0.36, 0.08 (Si gel, hexane-EtOAc, 3:2). A bioautogram of pure horminone [**1**] and of the diterpene **2** revealed clear inhibition zones [**1**, 0.15 mg, R_f 0.63; **2**, 0.15 mg, R_f 0.46 (hexane-EtOAc, 3:2)] that correspond to the inhibition zones of the crude extract of R_f 0.54, and R_f 0.43.

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