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An easy and stereoselective rearrangement of an abietane diterpenoid into a bioactive microstegiol derivative

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ABSTRACT

Parvifloron D was isolated from *Plectranthus ecklonii* together with sugiol and mixtures of β-sitosterol and stigmasterol and ursolic and oleanolic acids. Treatment of parvifloron D [2α-(4-hydroxy)benzoyloxy-11-hydroxy-5,7,9(11),13-abietatetraen-12-one] with acid-washed molecular sieves gave the microstegiol derivative 2β-(4-hydroxy)benzoyloxy-11β-hydroxy-4(5 → 11),20(10 → 5)di*abeo*-5(10),6,8,13-abietatetraen-12-one in a moderate yield (26%). The new microstegiol derivative inhibited the growth of some *Staphylococcus* and *Enterococcus* species with significant MIC values ranging from 3.91 to 7.81 μg/ml.

1. Introduction

Abietanes and rearranged abietanes are a family of naturally occurring diterpenoids which have been isolated from a variety of terrestrial plant sources. These compounds exhibit an array of interesting biological activities which has generated significant interest from the synthetic community. Microstegiol (**1**, Fig. 1) (Ulubelen et al., 1992) is a rearranged abietane isolated from several Labiatae plants belonging to the *Salvia* (Li et al., 2000; Topcu and Ulubelen, 1996; Ulubelen et al., 1992, 1997) and *Ajuga* (Kökdil et al., 2002) genera and it has shown pharmacological properties as an antileukemic, cytotoxic (Topcu et al., 2003) and antibacterial agent (Kabouche et al., 2005; Ulubelen et al., 2000). Surprisingly, only one total synthesis of the hydrocarbon framework of microstegiol (**1**) and its naturally occurring 3-oxo derivative (Chyu et al., 2005) has been reported until now (Taj et al., 2009). In this communication, we wish to report a simple method for transforming parvifloron D (**2**) (Rüedi and Eugster, 1978; Van Zyl et al., 2008) into the 2β-(4-hydroxy) benzoyloxy derivative of microstegiol (**3**). This method could be an easy and stereoselective entry to other microstegiol derivatives starting from the parviflorons, that are natural diterpenoids widely distributed in several *Plectranthus* species (Abdel-Mogib et al., 2002; Gaspar-Marques et al., 2008; Narukawa et al., 2001; Rüedi and Eugster, 1978; Van Zyl et al., 2008). Compounds **2**, **3** and sugiol (**4**) were tested against a panel of Gram-negative and Gram-positive bacteria and *Candida albicans*, and their MIC (minimum inhibitory concentration) values determined.

2. Results and discussion

Repeated chromatographic processes on an acetone extract of *Plectranthus ecklonii* Benth. whole plant, allowed the isolation of parvifloron D (**2**) together with sugiol (**4**) and other constituents (Section 3.3).

Treatment of a dichloromethane solution of parvifloron D (**2**) with acid-washed molecular sieves for 24 h at room temperature gave a complex mixture of compounds. Column chromatography allowed the isolation of **3**, the main and most polar reaction product (Section 3.4). Compound **3** had a molecular formula $C_{27}H_{30}O_5$, as that of the starting material (**2**). The ^1H and ^{13}C NMR spectra of **3** (Table 1) were almost identical to those of microstegiol (**1**) (Ulubelen et al., 1992) and the observed differences were consistent with the presence in the former of a 4-hydoxybenzoyloxy group attached to the C-2 position instead of the C-2 methylene group of the latter. The HMBC correlations observed between the Me-20 protons and the C-5, C-6 and C-10 carbons, and between the Me-18 and Me-19 protons and the C-3, C-4 and C-11 carbons, as well as those between the 11-OH and the C-9, C-11 and C-12 carbons (Table 1), supported that **3** arises from the abietane skeleton of **2** by a 4(5 → 11),20(10 → 5)di*abeo*-rearrangement.

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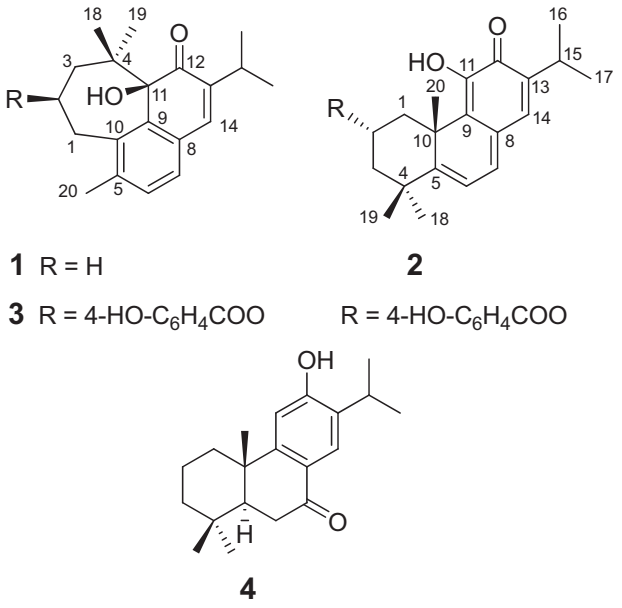

Fig. 1. The structures of the diterpenoids.

The absolute configuration of **3** and the stereochemistry of its seven-membered ring substituents were established as follows. Obviously, the C-2 asymmetric centre must have the same absolute stereochemistry in **2** (Rüedi and Eugster, 1978) and **3**, and the H-2α proton of **3** is axially oriented because it showed two *trans* diaxial ($J_{2\alpha,1\beta}$ = 11.8 Hz and $J_{2\alpha,3\beta}$ = 12.3 Hz) and two *cis* axial-equatorial ($J_{2\alpha,1\alpha}$ = 2.1 Hz and $J_{2\alpha,3\alpha}$ = 3.7 Hz) vicinal couplings (Table 1). Moreover, NOE experiments were in agreement with a *cis* spatial relationship between the 2β-4-hydroxybenzoate and the hydroxyl group at C-11 because irradiation at δ 2.73 (H-3β axial proton) caused NOE enhancement in the signals of the H-1β, H-3α, 11-OH and Me-18 protons (δ 4.04, 1.65, 4.63 and 0.85, respectively), whereas the signals of the H-1α, H-3α and Me-19 (δ 3.11, 1.65 and 0.88, respectively) were enhanced when the H-2α proton (δ 4.85) was irradiated. Furthermore, irradiation of the C-11 hydroxyl proton (δ 4.63) produced NOE enhancement only in the signals of the H-1β and H-3β protons.

The formation of a compound such as **3** starting from parvifloron D (**2**) under acid catalysis should be rationalized as it is outlined in Fig. 2. Compound **2** rearranges, via a C-10 carbonium ion intermediate (**A**) (Karanatsios et al., 1966; Matsumoto et al., 1995), to the 4,5-*seco*-20(10 → 5)*abeo*-abietane **B**, a class of rearranged abietanes that have been found in several Labiatae plants (Sexmero-Cuadrado et al., 1992; Li et al., 2000; Ulubelen et al., 1997). 12-*O*-deprotonation of **B** followed by a stereoselective attack of the 9,11-aromatic bond from the *Si*-face on the 3,4-olefin produces compound **3**. The total stereoselectivity for the conversion of **2** into **3** could be attributed to the presence in the former of a chiral centre at C-2 (Acuña et al., 2009).

The formation of the C-4–C-11 bond in **3** promoted by a 12-*O*-deprotonation of the intermediate **B** may be in competition with an alternative reaction in which a nucleophilic attack of the 11-OH on the 3,4-olefin may produce an 8-membered cyclic ether, like in the acid-catalyzed rearrangement of the quinone methide diterpene fuerstion (Karanatsios et al., 1966). In the case of the reaction of parvifloron D (**2**) with acid-washed molecular sieves the formation of cyclic ether derivatives could not be excluded because the characterization of all the reaction products was unsuccessful (Section 3.4).

It is of interest to indicate that, to the best of our knowledge, this is the first report on the transformation of an abietane diterpene into a microstegiol derivative and this rearrangement constitutes

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compound **3**.[a]

Position	δ_C	δ_H	HMBC (H → C)
1	33.60 *t*	3.11 *dt* (12.9,2.1), eq[b] H-1α[c]	C-2, C-3, C-5, C-9, C-10
		4.04 *dd* (12.9, 11.8), ax[b] H-1β	C-2, C-3, C-5, C-9, C-10
2	70.22 *d*	4.85 *dddd* (12.3, 11.8, 3.7, 2.1), ax H-2α	C-7′
3	47.61 *t*	1.65 *ddd* (12.8, 3.7, 2.1), eq H-3α[c]	C-1, C-2, C-4, C-11, C-18, C-19
		2.73 *dd* (12.8, 12.3), ax H-3β	C-1, C-2, C-4, C-11, C-18, C-19
4	41.12 *s*	–	
5	136.12 *s*	–	
6	130.67 *d*	7.13 *br d* (7.9)	C-5, C-7, C-8, C-10
7	127.81 *d*	6.97 *d* (7.9)	C-5, C-6, C-8, C-9, C-14
8	128.90 *s*	–	
9	139.54 *s*	–	
10	139.43 *s*	–	
11	83.51 *s*	[d]	
12	205.74 *s*	–	
13	141.25 *s*	–	
14	141.05 *d*	7.00 *t* (1.2)[e]	C-7, C-8, C-9, C-12, C-13, C-15
15	27.14 *d*	3.03 *septuplet of d* (6.8, 1.2)	C-12, C-13, C-14, C-16, C-17
16[f]	20.93 *q*	1.22 *d* (6.8)	C-13, C-15, C-17
17[f]	22.08 *q*	1.16 *d* (6.8)	C-13, C-15, C-16
18	28.96 *q*	0.85 *s*, eq Me-18	C-3, C-4, C-11, C-19
19	21.87 *q*	0.88 *s*, ax Me-19	C-3, C-4, C-11, C-18
20	21.38 *q*	2.42 *s*	C-5, C-6, C-10
1′	123.20 *s*	–	
2′,6′	131.92 *d*	7.98 *d* (8.8)	C-1′, C-3′, C-4′, C-7′
3′,5′	115.15 *d*	6.86 *d* (8.8)	C-1′, C-2′, C-4′
4′	159.85 *s*	[g]	
7′	165.75 *s*	–	

[a] In $CDCl_3$ solution, at 500 MHz (^{1}H) and 125 MHz (^{13}C); chemical shifts are expressed in ppm and *J* values (Hz) are given in parentheses.
[b] ax and eq designate axial and equatorial hydrogen, respectively, and for positions 18 and 19 axial or equatorial methyl substituent.
[c] The H-1α and H-3α equatorial protons showed a *W*-type coupling (*J* = 2.1 Hz).
[d] Hydroxyl proton at δ 4.63 *s*, that showed HMBC correlations with the C-9, C-11 and C-12 carbons.
[e] The H-14 proton is long-range coupled with both H-6 and H-15 protons (*J* = 1.2 Hz).
[f] For these positions, the numbering system is interchangeable.
[g] Hydroxyl proton at δ 5.74 *br*.

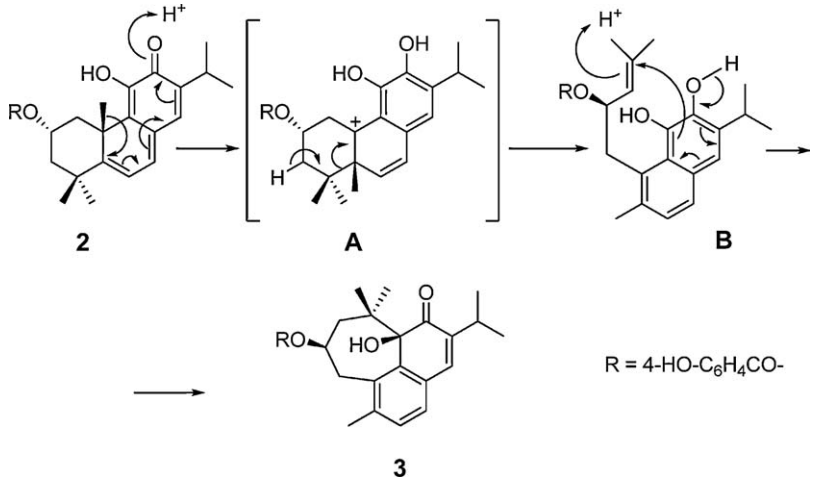

Fig. 2. Formation of compound **3** from parvifloron D (**2**).

the experimental evidence of the biogenesis of the microstegiol skeleton.

The use of acid-washed molecular sieves for obtaining 4(5 → 11),20(10 → 5) di*abeo*-rearranged derivatives from suitable abietane diterpenoids looks particularly attractive due to the noteworthy simplification of the experimental procedure, the avoidance of more acidic promoters (Karanatsios et al., 1966; Matsumoto et al., 1995; Sexmero-Cuadrado et al., 1992; Simões et al., 1986) and the mildness of the reaction conditions.

Compounds **2**–**4** were tested for their antibacterial activity against *Staphylococcus* and *Enterococcus* species, including methicillin- and vancomycin-resistant strains. Most of the strains tested in this work were not used in the antibacterial activity studies on some abietanes reported previously (Kabouche et al., 2005; Ulubelen et al., 2000).

Parvifloron D (**2**), previously reported as an antibacterial metabolite from *P. ecklonii* (Nyila et al., 2009), inhibited *E. faecalis* ATCC 51299 (low-VRE: vancomycin resistant *Enterococcus*) and *E. faecalis* FFHB with MIC values of 7.81 and 3.90 μg/ml, respectively, and showed lower MIC values (15.62 μg/ml) against both *S. aureus* ATCC 43866 and CIP 106760.

Compound **3** was tested against Gram-negative and Gram-positive bacteria, and *Candida albicans*. The results obtained revealed very interesting MIC values against *S. aureus* ATCC 6538 and *S. aureus* ATCC 43866 (3.90 μg/ml), and against *S. aureus* CIP 106760 (MRSA: methicillin resistant *S. aureus*), *S. epidermis* ATCC 12228, *E. faecalis* ATCC 51299 (low-MRSA) and *E. hirae* ATCC 10541 (7.81 μg/ml). Compound **3** also showed MIC values of 31.25 μg/ml against *S. aureus* ATCC 700699 and *S. aureus* FFHB 29593 (MRSA) and showed higher MIC values (62.50 μg/ml) towards *S. aureus* ATCC 25923, *M. smegmatis* and *C. albicans*. Though microstegiol (**1**) has been reported (Kabouche et al., 2005; Ulubelen et al., 2000) as a weak microbial growth inhibitory compound, its derivative **3** revealed a higher activity against six of the Gram-positive strains tested, those that gave MIC values ranges from 3.91 to 7.81 μg/ml.

The lower MIC value (62.5 μg/ml) exhibited by sugiol (**4**) was against *E. faecalis* FFHB 427483, which is in agreement with a very low antibacterial activity reported previously for this compound (Politi et al., 2003; Ulubelen et al., 2000).

3. Experimental

3.1. General experimental procedures

Melting-points, uncorrected: Kofler block. Optical rotations: in $CHCl_3$ solution (Perkin-Elmer 241 MC polarimeter). IR: in KBr (Perkin-Elmer Spectrum One spectrophotometer). UV: in MeOH solution (Perkin-Elmer Lambda 2 UV/vis spectrophotometer). ^1H and ^{13}C NMR spectra (at 500 and 125 MHz, respectively): in $CDCl_3$ solution at room temperature (Varian SYSTEM 500 MHz spectrometer). ^1H and ^{13}C NMR chemical shifts are reported with respect to the residual $CHCl_3$ signal (δ 7.26) and to the solvent signals (δ 77.00), respectively. EIMS: positive mode (Hewlett–Packard Model 5973 instrument, 70 eV). HRESIMS: Agilent 6520 Accurate-Mass QTOF LC/MS apparatus. Merck silica gel (70–230 mesh and 230–400 mesh, for gravity flow and flash chromatography, respectively) was used for column chromatography. Merck 5554 Kieselgel 60 F_{254} sheets were used for thin-layer chromatographic (TLC) analysis.

3.2. Plant material

P. ecklonii Benth. was grown in the Hortum of the Faculty of Pharmacy (Lisbon University, Portugal) from seeds provided by the Herbarium of the National Botanic Garden of Kirstenbosch, South Africa. Whole plants were collected in July 1998 and voucher specimens, identified by Enrico S. Martins, are deposited in the Herbarium of the Instituto de Investigação Cientifica Tropical, Lisbon (ref. C. Marques S/No. LISC).

3.3. Extraction and isolation

Dried and powdered *P. ecklonii* (whole plant, 7.0 kg) was extracted with Me_2CO (6 × 7 l, room temperature, 15 days). Filtration and evaporation of the solvent under *vacuum* at low temperature (40 °C) yielded a residue (284.4 g, 4.06% of dry plant material), which was subjected to column chromatography over silica gel (Merck, no. 7734, 3 kg), using *n*-hexane–EtOAc mixtures of increasing polarity (1:0–0:1) and EtOAc–MeOH (1:0–0:1) as eluents. Eight crude fractions were obtained, accordingly to differences in composition, as monitored by TLC. Successive chromatographic processes (over silica gel and using *n*-hexane–EtOAc mixtures as eluents) on the initial fractions eluted with *n*-hexane–EtOAc (7:3) to EtOAc (100%) yielded, in order of chromatographic polarity, the following compounds: sugiol (Kupchan et al., 1969; Rodríguez, 2003) (**4**, 18.9 mg, 0.00027%, on dry plant material), a mixture of β-sitosterol and stigmasterol (1.61 g, 0.023%), a mixture of ursolic and oleanolic acids (105 mg, 0.0015%) and parvifloron D (**2**, 19.2 g, 0.27%) (Rüedi and Eugster, 1978; Gaspar-Marques et al., 2008).

All the isolated compounds were identified by their physical (mp, $[\alpha]_D$) and spectroscopic (IR, ^1H and ^{13}C NMR and mass spectra) data.

3.4. Preparation of compound 3 from parvifloron D (2)

To a solution of **2** (351 mg) in CH_2Cl_2 (30 ml) was added acid-washed 4 Å molecular sieves AW-300 (12.5 g, purchased from Aldrich, 1.6 mm pellets) and the mixture was left at room temperature until disappearance of the initial red colour of the solution (24 h). Then, after filtration and washing of the molecular sieves with CH_2Cl_2 (3 × 15 ml), the organic solution was evaporated to dryness yielding 180 mg of a mixture of several compounds (TLC). Flash column chromatography [silica gel, *n*-hexane–EtOAc (2:1) as eluent] gave 91.2 mg of pure **3** (26% yield, most polar compound).

3.5. Compound characterization

2β-(4-Hydroxy)benzoyloxy-11β-hydroxy-4(5→11),20(10→5) diabeo-5(10),6,8,13-abietatetraen-12-one (**3**): Fine yellowish needles from EtOAc–*n*-hexane, mp 192–195 °C; $[\alpha]_D^{20}$ +271.2 (*c* 0.229, $CHCl_3$); UV (MeOH), λ_{max} nm (log ε): 336 (3.89); IR (KBr), ν_{max} 3402, 2965, 2929, 2868, 1706, 1679, 1655, 1609, 1592, 1513, 1466, 1372, 1312, 1275, 1165, 1097, 1027, 950, 852, 773, 698 cm^{-1}; EIMS 70 eV, *m*/*z* (rel. int.) 434 $[M]^+$ (1), 296 (76), 240 (11), 227 (100), 121 (27), 93 (5); HRESIMS: *m*/*z* 435.2170 $[M + H]^+$ (calc. for $C_{27}H_{31}O_5$: 435.2166). For ^1H and ^{13}C NMR spectra (see Table 1).

3.6. Biological evaluation

The strains tested in this work were selected from strain collections of the Microbiology Laboratory, Faculty of Pharmacy (University of Lisbon). The analyses were performed as described previously (Gaspar-Marques et al., 2006). The antimicrobial activity of the solvent (DMSO) was evaluated and ampicillin, methicillin and vancomycin were used as control antibiotics. Vancomycin showed MIC values in the range 1.95–7.81 μg/ml against *Staphylococcus* strains and 0.97–62.50 μg/ml for *Enterococcus* strains.

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