Pergamon 0031-9422(95)00646-X

Phytochemistry, Vol. 41, No. 2, pp. 571–573, 1996

0031-9422/96 $15.00 + 0.00

A REARRANGED ABIETANE DITERPENOID FROM *PLECTRANTHUS HERERoensis*

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(*Received* 6 *June* 1995)

Key Word Index—*Plectranthus hereroensis*; Labiatae; rearranged abietane diterpene; 17(15 → 16); 18(4 → 3)-bisabeo derivative; antibacterial activity.

Abstract—A new abietane diterpenoid has been isolated from the aerial parts of *Plectranthus hereroensis*, together with two known diterpenes. The structure of the new substance, 3β-acetoxy-6β,7α,12-trihydroxy-17(15 → 16);18(4 → 3)-bisabeo-abieta-4(19),8,12,16-tetraene-11,14-dione, was established by spectroscopic means and by comparison with closely related compounds. This diterpene showed moderate antibacterial activity.

INTRODUCTION

In previous communications [1–3], we reported on the isolation and structure elucidation of some abietane diterpenoids, such as horminone (**1**) [1] and **2** [2], and other constituents [3] from the acetone extract of the root of *Plectranthus hereroensis*. Because horminone (**1**) and compound **2** showed antimicrobial activity [1, 2], we decided to investigate the diterpene constituents of the aerial parts of this plant.

RESULTS AND DISCUSSION

Repeated chromatography of the acetone extract of the aerial parts of *P. hereroensis* (see Experimental) allowed the isolation of the previously known abietanes **1** [1] and **2** [2], together with a new substance whose structure (**3**) was established as follows.

Combustion analysis and low-resolution mass spectrometry indicated the molecular formula $C_{22}H_{26}O_7$ for compound **3**. Its UV spectrum showed absorptions at 272.5 and 421 nm (log ε 4.02 and 2.81, respectively), identical to those reported [1, 2] for **1** and **2**, thus establishing that the new diterpenoid (**3**) possessed a hydroxy-*p*-benzoquinone structural moiety. Inspection of the ^{1}H and ^{13}C NMR spectra of **3** (Table 1) revealed striking similarities with other rearranged abietane derivatives previously isolated from *Plectranthus* [4, 5], *Coleus* [6, 7], and *Solenostemon* [8] species.

The ^{1}H NMR spectrum of **3** (Table 1) showed signals for an allyl group at C-13, a chelated phenolic proton at C-12, two secondary hydroxyl groups at the C-6β and C-7α positions (geminal protons at δ4.37 *t*, J = 2.1 Hz and δ4.67 *dd*, J = 3.5 and 2.1 Hz (transformed into a *d*, J = 2.1 Hz, after addition of D_2O), H-6α and H-7β, respectively) and a C-20 methyl group almost identical to those of lanugone D (**4**; δ3.21, 2H, *m*, $J_{15,16}$ = 6.5 Hz (2H-15); δ5.07, 1H, *m*, $J_{17A,16(cis)}$ = 10.1 Hz (H_A-17); δ5.15, 1H, *m*, $J_{17B,16(trans)}$ = 17.1 Hz (H_B-17); δ5.86, 1H, *m*, (H-16); δ4.45, 1H, *m*, $W_{1/2}$ = 5 Hz (H-6α); δ4.64, 1H, *m*, $W_{1/2}$ = 2 Hz (H-7β); δ1.58, 3H, *s*, (Me-20)] [4]. Moreover, the chemical shifts of the C-7 to C-17 and C-20 carbon atoms of compounds **3** and **4** (Table 1 and [4], respectively) were identical, thus establishing that both compounds possessed the same partial structure in their B and C rings.

The remaining signals of the ^{1}H and ^{13}C NMR spectra of **3** (Table 1) revealed the existence of a 4(19)-exocyclic methylene, a C-5 methine group, a geminal methyl-acetoxyl grouping at C-3, and two contiguous methylene groups (C-1 and C-2) identical to those found in ring A of spirocoleone 16 (**5**) [5] and other related diterpenoids [8, 9] (e.g. **5**: $\delta_{H\text{-}1\beta}$ 2.96 *br d*, J_{gem} = 13.5 Hz; δ_{OAc} 2.08 *s*; $\delta_{Me\text{-}18}$ 1.61 *s*; $\delta_{H\text{-}5\alpha}$ 2.40 *br s*, $W_{1/2}$ = 5.5 Hz; $\delta_{H\text{-}19}$ 5.19 and 5.30, both *br s*, $W_{1/2}$ = 3.5 and 3.0 Hz respectively [5]).

From all the above data, it was evident that the new diterpene possessed the structure depicted in **3**, except for

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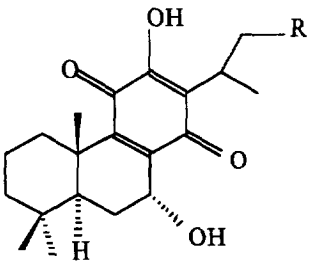
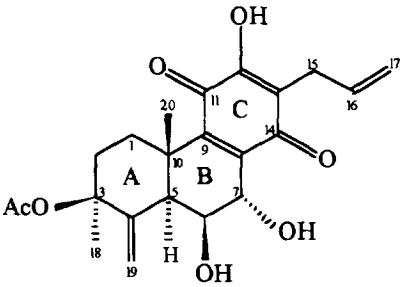


R

1 H

2 OAc



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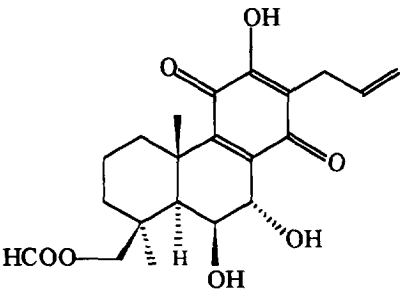

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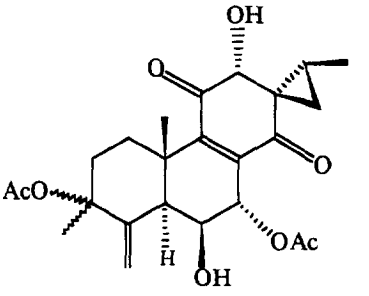

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Table 1. ^{1}H and ^{13}C NMR spectral data of compound **3***

C	δ_H	δ_C	C	δ_H	δ_C	$J_{H,H}$	Hz
1(α)	1.45 *td*	34.9 *t*	13	–	117.9 *s*	1α,1β	13.5
(β)	2.56 *ddd*		14	–	188.4 *s*	1α,2α	4.3
2(α)	2.37 *ddd*	32.4 *t*	15	3.19 *dq*	26.9 *t*	1α,2β	13.5
(β)	2.29 *td*		16	5.83 *qt*	133.6 *d*	1β,2α	2.9
3	–	83.2 *s*	17(A)	5.04 *dq*†	116.5 *t*	1β,2β	4.7
4	–	145.8 *s*	(B)	5.13 *dq*‡		2α,2β	13.5
5(α)	2.52 *br s*§	44.7 *d*	18	1.61 *s*	25.7 *q*	5α,6α	2.1
6(α)	4.37 *t*	70.2 *d*	19(A)	5.17 *br d*	107.8 *t*	6α,7β	2.1
(OH)	1.53 *s*¶		(B)	5.32 *br d*		7α,OH	3.5
7(β)	4.67 *dd***	67.8 *d*	20	1.41 *s*	20.6 *q*	15,16	6.5
(OH)	2.85 *d*¶		OAc	2.06 *s*	169.6 *s*	15,17A	1.4
8	–	140.9 *s*			22.5 *q*	15,17B	1.6
9	–	147.9 *s*				16,17A	10.0
10	–	38.8 *s*				16,17B	17.0
11	–	183.1 *s*				17A,17B	1.5
12(OH)	7.16 *s*¶	151.7 *s*				19A,19B	<0.5
						19A,5α	1.7
						19B,5α	1.2

*500 MHz (^{1}H) and 125.7 MHz (^{13}C) in $CDCl_3$ solution. Chemical shifts are relative to the solvent (residual $CHCl_3$ for ^{1}H, δ7.25, and δ_{CDCl_3} 77.00 for ^{13}C). ^{1}H spectral parameters were obtained by first-order approximation. All these assignments were in agreement with H-COSY and HMQC spectra.

†*cis* Hydrogen with respect to H-16.

‡*trans* Hydrogen with respect to H-16.

§$W_{1/2}$ = 5 Hz.

¶Disappeared after addition of D_2O.

**Collapsed into a *d* (J = 2.1 Hz) after addition of D_2O.

the sterochemistry at the C-3 asymmetric centre. This structural feature was established by NOE experiments. Thus, irradiation at δ1.61 (Me-18) caused NOE enhancement in the signals of the H-1α (δ1.45, 2% enhancement), H-2α (δ2.37, 1%) and H-5α (δ2.52, 4% protons, whereas irradiation at δ2.52 (H-5α) produced NOE enhancement in the signals of the Me-18 group (δ1.61, 2%) and the H-1α (δ1.45 4%) and H-6α (δ4.37, 5%) protons, but not in the signal of the Me-20 group (δ1.41). These results firmly established that in **3** the methyl-18 group at C-3 is α and axial and, consequently, that the C-3 acetoxyl subsituent has an equatorial β-configuration. Furthermore, in **3** the junction of rings A and B in *trans* (no NOE between H-5α and methyl-20, see above) and these rings possess a chair conformation ($^{10}C_3$ and $^{8}C_5$, respectively). The coupling values between the C-1 and C-2 methylene and the C-5, C-6 and C-7 methine protons (Table 1) further supported this point.

The stereochemistry of the C-3 carbon of spirocoleone 16 (**5**) has not been ascertained [5], although it is reasonable to assume that it possesses the same C-3 configuration as **3**, because the methyl-18 and C-19 protons resonate at the same field in both compounds (Table 1; for **5** see above and [5]). However, an opposite 3β-methyl, 3α-acetoxyl arrangement has been established in the structurally related 7-deoxy-12-*O*-deacetyl-3-acetoxycoleone *N* by using the pyridine-induced shifts method [8].

From a biogenetic point of view, it is of interest to note that compound **3** could be derived from **5** by hydrolysis of the C-7 acetate and an oxidative cyclopropane-opening reaction [10].

Bioautography revealed that compound **3** has moderate antibacterial activity against *Staphylococcus aureus* (see Experimental).

EXPERIMENTAL

Mp: uncorr. The plant material was produced and cultivated, from authentic seeds of *P. hereroensis* Engl., in Lisbon during 1990–91 (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, Lisbon, Portugal.

Extraction and isolation of the diterpenoids. Dried and powdered *P. hereroensis* aerial parts (1370 g) were extracted with Me_2CO (3 × 4 l) at room temp. 3 days. The solvent was evapd under reduced pressure and low temp. (40°) yielding a residue (127 g), which was subjected to CC (silica gel Merck N° 9385, 400 g). Elution with petrol and petrol–EtOAc mixtures gave horminone (**1**, 8 mg) from the fractions eluted with petrol–EtOAc (9:1) and 16-acetoxy-7α,12-dihydroxy-abieta-8,12-diene-11,14-dione (**2**; 100 mg) [2] from the fractions eluted with petrol–EtOAc (3:2). Further elution with petrol–EtOAc (1:1) yielded impure **3** (38 mg), which was purified by CC (silica gel, petrol–EtOAc 1:1) and prep. TLC (silica gel, CH_2Cl_2–MeOH, 9:1) giving 13 mg of **3**.

The previously known diterpenoids were identified by their ^{1}H NMR and MS and by comparison (TLC) with authentic samples [1, 2].

3β-*Acetoxy*-6β,7α,12-*trihydroxy*-17(15 → 16;18(4 → 3)-*bisabeo-abieta*-4(19),8,12,16-*tetraene*-11,14-*dione* (**3**). Mp 194–195° decomp. (EtOAc, *n*-hexane, yellow needles), $[\alpha]_D^{20}$ + 66.7°, $[\alpha]_{578}^{20}$ + 71.4°, $[\alpha]_{546}^{20}$ + 57.1°, $[\alpha]_{436}^{20}$ + 285.7°, $[\alpha]_{365}^{20}$ + 3657.1° ($CHCl_3$; *c* 0.021). IR ν_{max}^{KBr} cm^{-1}: 3580–3000 *br* (OH), 1720, 1265 (OAc), 1670, 990, 910 (allyl), 1655, 1640, 1610 (*p*-benzoquinone), 880 (exocyclic methylene), 2980, 2950, 2880, 1380, 1370, 1340, 1210, 1120, 1030, 805; UV λ_{max}^{MeOH} nm (log ε): 272.5 (4.02), 421 (2.81); ^{1}H and ^{13}C NMR: Table 1; EI-MS (70 eV, direct inlet) *m/z* (rel. int.): 402 $[M]^+$ (0.2), 355 (1), 342 $[M - AcOH]^+$ (1), 324 $[M - AcOH - H_2O]^+$ (5), 309 (4), 296 (7), 295 (6), 281 (6), 141 (5), 115 (9), 109 (8), 105 (6), 95 (5), 91 (11), 77 (11), 69 (7), 65 (8), 55 (14), 53 (14), 43 (100), 41 (24). (Found: C, 65.38; H, 6.42. $C_{22}H_{26}O_7$ requires: C, 65.66; H, 6.51%.)

Bioautography. A bioautographic agar overlay assay for detection and activity-guided fractionation of antimicrobial compounds using *S. aureus* as indicator strain was developed [11, 12].

Acknowledgements—This work was financially supported by the Portuguese Junta Nacional de Investigação Cientifica e Tecnológica (research project N° PBIC/C/BIO/1100/92), Centro de Estudos de Ciências Farmacêuticas, and the Spanish Dirección General de Investigación Cientifica y Técnica (grant N° PB93-0154).

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