Neoclerodane and Labdane Diterpenoids from *Plectranthus ornatus*

Patrícia Rijo,[†] Cristina Gaspar-Marques,[†] M. Fátima Simões,[†] Aida Duarte,[†] María del Carmen Apreda-Rojas,[‡] Félix H. Cano,[‡] and Benjamín Rodríguez*[,§]

Faculdade de Farmácia da Universidade de Lisboa, Centro de Estudos de Ciências Farmacêuticas (CECF), Av. das Forças Armadas, P-1649-019 Lisboa, Portugal, Departamento de Cristalografía, Instituto de Química Física "Rocasolano", Consejo Superior de Investigaciones Científicas (CSIC), Serrano 119, E-28006 Madrid, Spain, and Instituto de Química Orgánica, Consejo Superior de Investigaciones Científicas (CSIC), Juan de la Cierva 3, E-28006 Madrid, Spain

Received April 29, 2002

Three new diterpenoids, a neoclerodane and two labdane derivatives, have been isolated from an acetone extract of *Plectranthus ornatus*. The structures of these compounds (plectrornatins A−C, **1**−**3**, respectively) were established mainly by spectroscopic means, particularly by 1D and 2D NMR studies and, in the case of the neoclerodane **1**, by an X-ray diffraction analysis. Compounds **1** and **3** showed moderate antimicrobial activity against five *Candida* species and selected Gram negative and Gram positive bacteria strains.

In continuation of our studies on biologically active diterpenoids from *Plectranthus* species,[1−4] we have now investigated *P. ornatus* Codd. (synonym *P. comosus* Hochst. ex Güerke, Labiatae), a species that has not hitherto been studied chemically or pharmacologically. We report here on the isolation and structure elucidation of three new diterpenoids, a neoclerodane[5] derivative (**1**) and two labdanes (**2** and **3**), found in the whole plant acetone extract, as well as the results of the assay of these substances as antimicrobial agents.

Results and Discussion

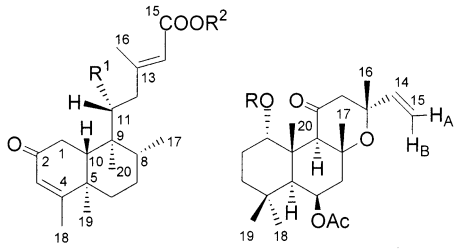
Repeated chromatographic processes on the acetone extract of *P. ornatus* whole plant allowed the isolation of the diterpenoids **1**−**3**. Combustion analysis and low-resolution mass spectrometry established a molecular formula $C_{23}H_{34}O_5$ for plectrornatin A (**1**). Its ^{1}H NMR spectrum showed signals for a secondary acetoxyl group placed between a fully substituted carbon and a methylene group (geminal proton of the acetate at δ 5.28 dd, J = 10.4 and 2.1 Hz; acetoxyl group at δ 1.98, 3H, s), a methyl ester group (δ 3.65, 3H, s), two olefinic protons (δ 5.73 quintuplet, J = 1.3 Hz, and δ 5.62 qd, J = 1.1 and 0.6 Hz), each one allylic coupled (COSY spectrum and double resonance experiments) with a different methyl group (δ 1.88, 3H, d, J = 1.3 Hz, and δ 2.12, 3H, d, J = 1.1 Hz, respectively), and finally two methyl singlets (δ 1.14 and 0.85, 3H each) and another methyl group attached to a methine carbon (δ 0.99, 3H, d, J = 6.2 Hz). The ^{13}C NMR spectrum of **1** (Table 1) was very similar to those reported[6,7] for 2-oxo-kolavenic acid (**4**) and its methyl ester derivative (**5**).[7,8] In fact, the C-1−C-8, C-10, C-15−C-19, and methyl ester carbon atom resonances of **1** (Table 1) were almost identical to those of **4**[6,7,9] and **5**,[7−9] whereas the observed differences in the chemical shifts of the C-9, C-11−C-14, and C-20 carbons [$\Delta\delta = \delta$(**1**) − δ(**5**): +4.7, +39.3, +7.4, −4.4, +3.2, and −5.6 ppm, respectively] are compatible only with the presence in **1** of an acetoxyl substituent (δ_C 170.3 s and 20.7 q) at the C-11 position (downfield shift of the C-11 α-carbon and in the C-9 and C-12 β-carbons; upfield shift for the C-13 and C-20 γ-carbons).[10]

Table 1. ^{13}C NMR Spectral Data for Compounds **1**−**3**[a]

carbon	**1**	**2**	**3**
C-1	36.0 (t)	71.3 (d)	75.1 (d)
C-2	199.0 (s)	25.5 (t)	21.7 (t)
C-3	125.6 (d)	36.4 (t)	36.9 (t)
C-4	171.9 (s)	33.8 (s)	33.7 (s)
C-5	40.1 (s)	47.8 (d)	49.1 (d)
C-6	35.5 (t)	70.1 (d)	69.5 (d)
C-7	27.5 (t)	46.3 (t)	46.2 (t)
C-8	36.2 (d)	76.2 (s)	75.7 (s)
C-9	43.4 (s)	58.9 (d)	58.2 (d)
C-10	46.0 (d)	41.8 (s)	40.5 (s)
C-11	74.5 (d)	208.3 (s)	206.2 (s)
C-12	41.7 (t)	49.8 (t)	49.1 (t)
C-13	155.9 (s)	74.7 (s)	74.6 (s)
C-14	118.4 (d)	146.2 (d)	146.7 (d)
C-15	166.4 (s)	112.2 (t)	112.4 (t)
C-16	18.8 (q)	31.3 (q)	31.7 (q)
C-17	17.6 (q)	29.4 (q)	29.5 (q)
C-18	19.0 (q)	32.8 (q)	32.9 (q)
C-19	18.7 (q)	22.8 (q)	22.9 (q)
C-20	12.2 (q)	18.0 (q)	17.4 (q)
COO*C*H$_3$	50.9 (q)		
1α-OAc			169.5 (s)
			21.4 (q)
6β-OAc		170.3 (s)	169.9 (s)
		21.8 (q)	21.8 (q)
11-OAc	170.3 (s)		
	20.7 (q)		

[a] All these assignments were in agreement with HSQC and HMBC spectra.

The ^{1}H and ^{13}C NMR data corresponding to the C-9 side chain of **1** clearly established that this diterpenoid pos-



1 R^1 = OAc, R^2 = Me **2** R = H

4 $R^1 = R^2$ = H **3** R = Ac

5 R^1 = H, R^2 = Me

* To whom correspondence should be addressed. Tel: +34 91 5622900. Fax: +34 91 5644853. E-mail: iqor107@iqog.csic.es.
[†] Faculdade de Farmácia da Universidade de Lisboa, CECF.
[‡] Departamento de Cristalografía, Instituto "Rocasolano".
[§] Instituto de Química Orgánica.

10.1021/np020203w CCC: $22.00
Published on Web 08/22/2002

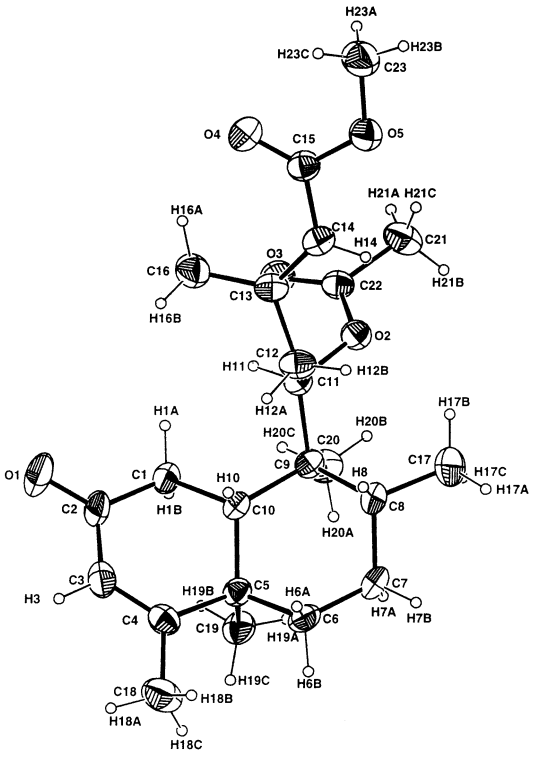


Figure 1. ORTEP[14] drawing of the molecular structure of plectrornatin A (**1**) with the numbering scheme. Displacement ellipsoids are drawn at the 30% probability level.

sessed the C-13, C-14 olefinic double bond in an *E*-configuration, because the proton and carbon atom resonances of the C-13−C-16 structural part were almost identical to those of **4** and **5**[6−10] (see above) and very different from those reported for some structurally related diterpenoids with a 13*Z*-configuration.[11,12] On the other hand, NOE experiments supported the relative stereochemistry of the decalin part of **1**, as it is depicted in the formula, because irradiation at δ 0.85 (Me-20) caused NOE enhancement in the signals of the H-1α (+2.1%), H-7α (+2.1%), Me-17 (+1.6%), and Me-19 (+3.7%) protons, but not in the signal of the H-10β proton,[13] thus establishing that all these hydrogens (except for H-10β) are on the same side of the plane defined by the decalin (α-side in formula **1**) and that the A/B decalin ring junction is *trans*.

All the above data, together with 2D NMR experiments (COSY, HSQC, and HMBC), established a structure such as **1** for plectrornatin A, except for the stereochemistry at its C-11 stereogenic center. To define this configuration, an X-ray diffraction analysis of **1** was undertaken. Figure 1 shows the ORTEP[14] drawing of the result of the X-ray analysis, establishing that this diterpenoid possessed an *R*-configuration for its acetoxyl group at C-11. Neoclerodane derivatives, such as **1**, are frequent among the constituents of Labiatae plants,[15−17] but, to the best of our knowledge, this is the first report on a diterpene of this type from a *Plectranthus* species.

In the crystalline state, there are four formula units of **1** in the unit cell, bond lengths and angles are in good agreement with those found in analogous compounds,[8,18,19] and conformations of rings A and B are similar to those of **5**.[8] There are intermolecular hydrogen contacts through the symmetries $-x$, $y + 1/2$, $-z - 1/2$ and $-x$, $y - 1/2$, $-z - 1/2$, which build columns along the *b*-axis. The columns pack to form layers along the *a*-axis determined by contacts of the type C−H···C, C−H···O, and H···H, and these layers join to form the crystal. The distribution of the columns is closed distorted hexagonal.[20]

Another of the new diterpenoids isolated from *P. ornatus* was plectrornatin B (**2**, $C_{22}H_{34}O_5$). Its ^{1}H and ^{13}C NMR spectra showed characteristic signals for five methyl groups attached to fully substituted sp^3 carbon atoms, a vinyl group also on a quaternary carbon, a secondary hydroxyl group in an axial configuration and placed between a fully substituted sp^3 carbon and a methylene grouping (geminal proton at δ 4.42 dd, J = 3.4 and 2.5 Hz; δ_C 71.3 d), a (C)−CH−CHOAc−CH_2−(C) structural moiety in which the acetoxyl group must also be in an axial configuration (geminal proton of the acetate at δ 5.57 ddd, J = 3.4, 2.9, and 2.7 Hz, δ_C 70.1 d; acetoxyl group at δ_H 2.04, 3H, s, and δ_C 170.3 s and 21.8 q; methine group at δ_H 1.50 d, J = 2.7 Hz, δ_C 47.8 d; methylene grouping at δ_H 1.88 dd, J = 14.6 and 3.4 Hz, and δ 2.23 dd, J = 14.6 and 2.9 Hz, δ_C 46.3 t), and finally, another (C)−CH−CO−CH_2−(C) partial structure (ketone at δ_C 208.3 s; methine at δ_H 3.43 s and δ_C 58.9 d; methylene protons as an AB system at δ 2.63 and 2.70, both d, J_{gem} = 18.1 Hz).

All these data pointed toward a structural hypothesis such as **2** for this diterpenoid, since its NMR spectral data were very similar to those reported[21−23] for forskolin and other related 8,13-epoxy-14-labdenes isolated from *Coleus* species. A careful inspection of the COSY, TOCSY, HSQC, and HMBC NMR spectra of **2** confirmed the proposed structure for this substance. In particular, the HMBC spectrum of **2** showed connectivities between the 11-ketone carbon and the C-9 methine and both C-12 methylene protons, whereas the 14-olefinic carbon was correlated with the C-12 and C-15 methylene and Me-16 protons. Furthermore, the C-1 hydroxylic carbon showed cross-peaks with the C-2, C-3, C-5, C-9, and Me-20 protons, and the carbonyl carbon of the acetate (δ 170.3 s) correlated with the H-6α proton (δ 5.57), which, in turn, was connected with the C-4, C-5, C-7, C-8, and C-10 carbons. The relative stereochemistry of **2** was in agreement with NOE experiments, because irradiation at δ 3.43 (H-9α axial proton) caused NOE enhancement in the signals of the H-5α (+3.5%), H-7α (+3.7%), and H-12α (+1.2%) axial protons, as well as in H-14 and H_B-15 protons (+1.7 and +1.1%, respectively) of the vinyl group, thus establishing that all these hydrogens are on the same side of the plane of the molecule (α-side). Moreover, a strong NOE enhancement (+3.5%) was observed in the signal of the Me-20 protons when the H-1β proton (δ 4.42) was irradiated, and on irradiation at δ 5.57 (H-6α equatorial proton) the signals of the H-5α, H-7α, and Me-19 axial protons and those of the H-7β and Me-18 equatorial substituents were enhanced (+3.9, +3.7, +2.3, +2.4, and +4.7%, respectively).

The last diterpenoid found in *P. ornatus*, plectrornatin C (**3**, $C_{24}H_{36}O_6$), was the 1α-*O*-acetyl derivative of **2**, as was evidenced by its ^{1}H and ^{13}C NMR data [downfield shift of the H-1β proton of **3** (δ 5.53, t, J = 2.8 Hz) with respect to that of **2** ($\delta_{H-1\beta}$ 4.42 dd, J = 3.4 and 2.5 Hz); an additional acetoxyl group in **3** (δ_H 1.95, 3H, s; δ_C 169.5 s and 21.4 q)]. This was rigorously confirmed by the fact that acetic anhydride−pyridine treatment of **2** yielded a compound identical in all (TLC, mp, $[\alpha]_D$, and ^{1}H NMR and mass spectra) respects to natural **3**.

The absolute stereochemistry of **2** and **3** was not ascertained. However, on biogenetic grounds, we suppose that they belong to the *normal*-labdane series, like forskolin and related substances,[21−23] all of them isolated from *Coleus* species, a genus of the Labiatae family botanically very close to the genus *Plectranthus*. The negative specific rotation values showed by **2** and **3** (see Experimental

Section) and other structurally related diterpenoids[21,22] seem to support this assumption.

Compounds **1** and **3** were tested as antimicrobial agents against yeast and Gram negative and Gram positive bacteria strains, showing moderate antifungicidal activity, MIC values 62.5 μg/mL, against *Candida albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. guilliermondii*. The antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* revealed MIC values ≥ 125 μg/mL. In autobiography, plectrornatin B (**2**) showed activity against *S. aureus* (data not measured). It was not possible to perform other assays of antimicrobial activity with **2**, due to the scarcity of the sample available.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. IR spectra were obtained on a Perkin-Elmer Spectrum One spectrophotometer. The UV spectrum was recorded on a Perkin-Elmer Lambda 2 UV/vis spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Varian INOVA 400 apparatus at 400 and 100 MHz, respectively, and chemical shifts are reported with respect to residual $CHCl_3$ (δ 7.25) for protons and to the solvent signals (δ_{CDCl_3} 77.00) for carbons. ^{13}C NMR assignments were determined by gHSQC and gHMBC spectra. Mass spectra were registered in the positive EI mode on a Hewlett-Packard 5973 instrument (70 eV). Elemental analyses were made with a Carlo Erba EA 1108 apparatus. Merck Si gel no. 7734 (70−230 mesh) was used for column chromatography. Merck 5554 Kieselgel 60 F254 sheets were used for TLC analysis.

Plant Material. *Plectranthus ornatus* Codd. was cultivated in the Faculty of Pharmacy Hortum, Lisbon University, from seeds provided by the Herbarium of the Botanical Garden of Lisbon, Portugal. Whole plants of this species were collected in May 1998, and voucher specimens were deposited in the Herbarium of the Botanical Center of the "Instituto de Investigação Científica Tropical", Lisbon (ref. C. Marques, S/N° LISC).

Extraction and Isolation. Dried and powdered *P. ornatus* Codd. whole plants (3.4 kg) were extracted with Me_2CO (10 L × 3) at room temperature for 2 weeks. After filtration and evaporation of the solvent under reduced pressure at low temperature (40 °C) a residue (184 g) remained. This residue was subjected to column chromatography (Si gel, 1100 g) eluting with a solvent gradient from 100% petroleum ether (bp 50−70 °C) to 100% EtOAc. The fractions eluted with EtOAc−petroleum ether (3:1) yielded a residue (9.1 g), which was rechromatographed [Si gel column, EtOAc−petroleum ether (2:1) as eluent], giving, in order of increasing chromatographic polarity, impure **3**, **2**, and **1**. Final purification on PTLC plates [Si gel, eluted with EtOAc−petroleum ether (1:1)] and, in the case of **1** and **3**, crystallization yielded pure **1** (73 mg, 0.0021% on dry plant material), **2** (5 mg, 0.00015%), and **3** (14 mg, 0.00041%).

Plectrornatin A [Methyl 11*R*-acetoxy-2-oxoneocleroda-3,13*E*-dien-15-oate (11*R*-Acetoxy-2-oxokolavenic acid methyl ester)] (1): colorless needles (EtOAc−*n*-hexane), mp 170−171 °C; $[\alpha]^{22}_D$ −60.9° (*c* 0.651, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 221 (4.27), 239 (sh) (4.13) nm; IR (KBr) ν_{max} 1725, 1245 (OAc), 1710 (α,β-unsaturated COOMe), 1665 (α,β-unsaturated ketone), 2990, 2950, 2880, 1440, 1390, 1280, 1215, 1115, 1045, 1025, 970, 950, 920, 860 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.73 (1H, quint., J = 1.3 Hz, H-3), 5.62 (1H, qd, J = 1.1, 0.6 Hz, H-14), 5.28 (1H, dd, J = 10.4, 2.1 Hz, H-11), 3.65 (3H, s, COO*Me*), 2.61 (1H, ddd, J = 17.7, 3.5, 1.3 Hz, H-1β), 2.49 (1H, dd, J = 17.7, 13.7 Hz, H-1α), 2.34 (1H, ddd, J = 13.3, 2.1, 0.6 Hz, H_B-12), 2.28 (1H, dd, J = 13.3, 10.4 Hz, H_A-12), 2.12 (3H, d, J = 1.1 Hz, Me-16), 1.98 (3H, s, OAc-11), 1.97 (1H, dd, J = 13.7, 3.5 Hz, H-10β), 1.88 (3H, d, J = 1.3 Hz, Me-18), 1.82 (1H, dt, J = 13.1, 2.9 Hz, H-6α), 1.52 (1H, ddq, J = 11.9, 6.2, 4.5 Hz, H-8β), 1.50 (1H, dddd, J = 13.4, 12.2, 11.9, 2.9 Hz, H-7α), 1.48 (1H, dddd, J = 13.4, 4.5, 3.6, 2.9 Hz, H-7β), 1.34 (1H, ddd, J = 13.1, 12.2, 3.6 Hz, H-6β), 1.14 (3H, s, Me-19), 0.99 (3H, d, J = 6.2 Hz, Me-17), 0.85 (3H, s, Me-20); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m/z* (rel int) 390 $[M]^+$ (1), 375 (1), 358 (1), 347 (1), 330 (3), 317 (8), 315 (2), 299 (4), 283 (2), 235 (28), 205 (100), 191 (36), 189 (13), 185 (37), 143 (20), 135 (41), 125 (41), 121 (48), 111 (27), 109 (47), 107 (23), 95 (29), 83 (35), 81 (23), 79 (21), 55 (25), 43 (53); *anal.* C 70.67%, H 8.63%, calcd for $C_{23}H_{34}O_5$, C 70.74%, H 8.78%.

Plectrornatin B (6β-Acetoxy-8α,13*R-epoxy-11-oxo-14-labden-1α-ol) (2):** colorless thick oil; $[\alpha]^{22}_D$ −66.3° (*c* 0.205, $CHCl_3$); IR (NaCl) ν_{max} 3455 (OH), 3080, 940 (vinyl group), 1738, 1240 (OAc), 1714 (ketone), 2925, 2854, 1455, 1390, 1210, 1070, 1040, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.94 (1H, dd, J = 17.4, 10.7 Hz, H-14), 5.57 (1H, ddd, J = 3.4, 2.9, 2.7 Hz, H-6α), 5.15 (1H, dd, J = 17.4, 0.9 Hz, H_B-15), 5.04 (1H, dd, J = 10.7, 0.9 Hz, H_A-15), 4.42 (1H, dd, J = 3.4, 2.5 Hz, H-1β), 3.43 (1H, s, H-9α), 2.70 (1H, d, J = 18.1 Hz, H-12β), 2.63 (1H, d, J = 18.1 Hz, H-12α), 2.23 (1H, dd, J = 14.6, 2.9 Hz, H-7β), 2.09 (1H, dddd, J = 14.6, 13.5, 3.9, 2.5 Hz, H-2β), 2.04 (3H, s, OAc-6β), 1.88 (1H, dd, J = 14.6, 3.4 Hz, H-7α), 1.64 (1H, ddd, J = 14.6, 13.5, 3.6 Hz, H-3α), 1.50 (1H, d, J = 2.7 Hz, H-5α), 1.47 (1H, m, H-2α), 1.47 (3H, s, Me-17), 1.37 (3H, s, Me-20), 1.28 (3H, s, Me-16), 1.09 (1H, ddd, J = 14.6, 3.9, 2.5 Hz, H-3β), 0.97 (3H, s, Me-19), 0.95 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m/z* (rel int) 378 $[M]^+$ (1), 363 (5), 351 (1), 345 (2), 318 (1), 303 (3), 285 (8), 279 (11), 267 (15), 189 (14), 167 (34), 149 (100), 109 (26), 95 (26), 81 (29), 71 (31), 69 (29), 57 (36), 55 (34), 43 (77); $C_{22}H_{34}O_5$ M_r 378.

Plectrornatin C (1α,6β-Diacetoxy-8α,13*R-epoxy-14-labden-11-one) (3):** colorless needles (EtOAc−*n*-pentane), mp 234−235 °C; $[\alpha]^{20}_D$ −81.5° (*c* 0.114, $CHCl_3$); IR (KBr) ν_{max} 3090, 1642, 948 (vinyl group), 1732, 1238 (OAc), 2953, 2861, 1453, 1394, 1364, 1210, 1144, 1036, 993, 913 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.92 (1H, dd, J = 17.4, 10.7 Hz, H-14), 5.57 (1H, ddd, J = 3.8, 2.9, 2.6 Hz, H-6α), 5.53 (1H, t, J = 2.8 Hz, H-1β), 5.19 (1H, dd, J = 17.4, 1.1 Hz, H_B-15), 5.04 (1H, dd, J = 10.7, 1.1 Hz, H_A-15), 3.23 (1H, s, H-9α), 2.67 (1H, d, J = 18.6 Hz, H-12β), 2.60 (1H, d, J = 18.6 Hz, H-12α), 2.26 (1H, dd, J = 14.6, 2.9 Hz, H-7β), 2.05 (3H, s, OAc-6β), 2.00 (1H, dddd, J = 15.5, 13.6, 3.6, 2.8 Hz, H-2β), 1.95 (3H, s, OAc-1α), 1.90 (1H, dd, J = 14.6, 3.8 Hz, H-7α), 1.71 (1H, dddd, J = 15.5, 3.4, 2.8, 2.7 Hz, H-2α), 1.47 (1H, d, J = 2.6 Hz, H-5α), 1.46 (3H, s, Me-17), 1.45 (1H, ddd, J = 13.8, 13.6, 3.4 Hz, H-3α), 1.42 (3H, s, Me-20), 1.26 (3H, s, Me-16), 1.11 (1H, ddd, J = 13.8, 3.6, 2.7 Hz, H-3β), 0.99 (3H, s, Me-19), 0.97 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m/z* (rel int) 420 $[M]^+$ (0.5), 405 (0.3), 377 (1), 360 (2), 300 (2), 285 (5), 247 (8), 215 (11), 190 (8), 173 (11), 163 (7), 147 (7), 119 (10), 109 (11), 107 (11), 95 (13), 91 (11), 81 (13), 69 (14), 67 (11), 55 (20), 43 (100), 41 (13); *anal.* C 68.39%, H 8.58%, calcd for $C_{24}H_{36}O_6$, C 68.54%, H 8.63%.

X-ray Crystallographic Analysis of Plectrornatin A (1). Crystals of **1** suitable for X-ray diffraction analysis were obtained by recrystallization from EtOAc−*n*-hexane. A colorless prism of **1** (0.48 × 0.37 × 0.09 mm) was selected for the data collection. Crystal data: $C_{23}H_{34}O_5$; M_r = 390.50 g·mol^{-1}; orthorhombic a = 17.8476(19) Å, b = 12.8447(11) Å, c = 9.7760(8) Å, V = 2241(4) Å^3, space group $P2_12_12_1$, Z = 4, D_{cal} = 1.157 Mg·m^{-3}. Data collection: Seifert XRD 3000S diffractometer, 293.(2) K; 3993 independent reflection intensities were collected between 5° and 67° in θ, in the $\omega/2\theta$ scan mode, with Cu Kα monochromated radiation (λ 1.54180 Å). No decay was observed in two reference reflections measured every 150 min, and 3557 reflections were considered as observed at the $2\sigma(I)$ level.

The structure was solved by direct methods (SHELX-97)[24] and difference Fourier techniques; no absorption correction was applied (μ = 0.644 mm^{-1}). The structure was refined using full matrix least-squares on F^2. All non-H atoms were refined with anisotropic thermal parameters. Since **1** crystallized in a polar space group, polar axis restraints were applied.[24] The H atoms were assigned geometrically and treated using

appropriate riding models. The refinement converged to R final indices [$I > 2\sigma(I)$] $R_1 = 0.042$, $wR_2 = 0.122$ and R indices (all data) $R_1 = 0.043$, $wR_2 = 0.128$. All calculations were done with the program SHELX-97.[24] All the geometric calculations were performed with the program PARST,[25] and scattering factors, anomalous dispersion, and absorption coefficients were taken from *International Tables for X-ray Crystallography*.[26,27]

Acetic Anhydride−Pyridine Treatment of Plectrornatin B (2) To Give Plectrornatin C (3). Treatment of **2** (3 mg) with acetic anhydride−pyridine (1:1, 2 mL) at room temperature for 24 h yielded, after usual workup, a substance (2.3 mg) identical in all (TLC, mp, $[\alpha]_D$, ^{1}H NMR and mass spectra) respects with **3**.

Biological Assays. Antimicrobial activities of **1** and **3** were tested against *Pseudomonas aeruginosa* ATCC 27853, *Escherischia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Candida albicans* CIP 3153A, and four clinical yeasts *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. guilliermondii*, obtained from the Microbiology Laboratory, Faculty of Pharmacy, Lisbon. The compounds were dissolved in DMSO and graded concentration with a Mueller-Hinton broth ranging from 250 to 3.9 μg/mL. The minimum inhibitory concentration (MIC) was performed by a broth microdilution method according to NCCLS[28] and was defined as the minimum concentration of the compound that showed no microorganism growth compared with the control without compound.

Acknowledgment. The authors thank Dr. E. S. Martins, "Centro de Botânica do Instituto de Investigação Científica Tropical", Lisbon, Portugal, for the identification of the plant material. This work was supported by funds from the Spanish "Dirección General de Investigación Científica y Técnica" (DGICYT, grant no. BQU 2000-0868) and from Portuguese FCT (I&D no. 8/94), POCTI (QCA III), and FEDER projects. One of us (C.G.-M.) thanks the Praxis Program for a fellowship (Praxis XXI/BD/18046/98).

References and Notes

(1) Batista, O.; Duarte, A.; Nascimento, J.; Simões, M. F.; de la Torre, M. C.; Rodríguez, B. *J. Nat. Prod.* **1994**, *57*, 858−861.
(2) Batista, O.; Simões, M. F.; Duarte, A.; Valdeira, M. L.; de la Torre, M. C.; Rodríguez, B. *Phytochemistry* **1995**, *38*, 167−169.
(3) Batista, O.; Simões, M. F.; Nascimento, J.; Riberio, S.; Duarte, A.; Rodríguez, B.; de la Torre, M. C. *Phytochemistry* **1996**, *41*, 571−573.
(4) Teixeira, A. P.; Batista, O.; Simões, M. F.; Nascimento, J.; Duarte, A.; de la Torre, M. C.; Rodríguez, B. *Phytochemistry* **1997**, *44*, 325−327.
(5) Although the hydrocarbon skeleton of clerodanes such as **1** is biogenetically derived from an *ent*-labdane and although they should be named *ent*-clerodanes, we prefer to use the term *neoclerodane* proposed by Rogers et al. (Rogers, D.; Unal, G. G.; Williams, D. J.; Ley, S. V.; Sim, G. A.; Joshi, B. S.; Ravindranath, K. R. *J. Chem. Soc., Chem. Commun.* **1979**, 97−99) for defining the absolute configuration, because it is the nomenclature used in the majority of the articles published on this subject since 1979.
(6) Aquino, R.; Ciavatta; M. L.; De Tommasi, N.; Gàcs-Baitz, E. *Phytochemistry* **1992**, *31*, 1823−1825.
(7) Lopes, L. M. X.; Bolzani, V. Da S.; Trevisan, L. M. V. *Phytochemistry* **1987**, *26*, 2781−2784.
(8) Avila, D.; Medina, J. D.; Deeming, A. J. *J. Nat. Prod.* **1992**, *55*, 845−850.
(9) In the article of E. Gàcs-Baitz and co-workers,[6] the assignment of the ^{13}C NMR spectrum of **4** is correct. However, other data published[7,8] on the ^{13}C NMR assignments of **4** and **5** contain several ambiguities and mistakes. In particular, the assignments of the C-4 and C-15 carbons of **5** are interchanged in ref 8.
(10) It is of interest to indicate that the C-11 acetoxyl substituent of **1** causes small downfield shifts on the C-1, C-5, C-7, and C-17 δ-carbons with respect to **4** ($\Delta\delta$ +1.2, +0.2, +0.7, and +1.9 ppm, respectively), whereas the C-14 olefinic carbon of **1** is strongly deshielded ($\Delta\delta$ +3.2 ppm).
(11) Fang, N.; Yu, S.; Mabry, T. J.; Abboud, K. A.; Simonsen, S. H. *Phytochemistry* **1988**, *27*, 3187−3196.
(12) Tamayo-Castillo, G.; Jakupovic, J.; Bohlmann, F.; Castro, V.; King, R. M. *Phytochemistry* **1989**, *28*, 139−141.
(13) Irradiation at δ 0.85 (Me-20) in **1** under NOE experimental conditions also caused an enhancement (+1.6%) in the signal of the H-11 proton.
(14) Hall, S. R.; Flack, H. D.; Stewart, J. M. *Xtal System*; University of Western Australia, 1999.
(15) Merritt, A. T.; Ley, S. V. *Nat. Prod. Rep.* **1992**, *9*, 243−287.
(16) Rodríguez-Hahn, L.; Esquivel, B.; Cárdenas, J. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, Ch., Eds.; Springer-Verlag: Vienna, 1994; Vol. 63, pp 107−196.
(17) Hanson, J. R. *Nat. Prod. Rep.* **1997**, *14*, 245−258; **1998**, *15*, 93−106; **1999**, *16*, 209−219; **2000**, *17*, 165−174; **2001**, *18*, 88−94.
(18) Bomm, M. D.; Zukerman-Schpector, J.; Lopes, L. M. X. *Phytochemistry* **1999**, *50*, 455−461.
(19) *Cambridge Structural Database System*; Cambridge Crystallographic Data Centre: Cambridge, U.K., 1992.
(20) Martínez-Alcázar, M. P.; Cano, F. H.; Martínez-Ruiz, P. *J. Mol. Struct.* **2001**, *562*, 79−88.
(21) Gabetta, B.; Zini, G.; Danieli, B. *Phytochemistry* **1989**, *28*, 859−862.
(22) Roy, R.; Mishra, A.; Varma, N.; Tandon, J. S.; Saux, M.; Carpy, A. *Phytochemistry* **1993**, *34*, 1577−1580.
(23) Dolmazon, R.; Albrand, M.; Pollet, P.; Mahmout, Y. *Bull. Soc. Chim. Fr.* **1993**, *130*, 501−508.
(24) Sheldrick, G. M. *SHELX97 (SHELXS and SHELXL). Manual of Programs for the Refinement of Crystal Structures*; University of Göttingen: Göttingen, Germany, 1997.
(25) Nardelli, M. *Comput. Chem.* **1983**, *7*, 95−98.
(26) *International Tables for X-ray Crystallography*; Kluwer Academic Publications: Dordrecht, Germany, 1992; Vol. C.
(27) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained (CCDC 183944), free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(28) Wayne, P. A. In National Committee for Clinical Laboratory Standards; NCCLS: USA, 2000; 5th ed.; approved standard M7-A5.

NP020203W