A Bioactive *seco*-Rosane Diterpenoid from *Vellozia candida*

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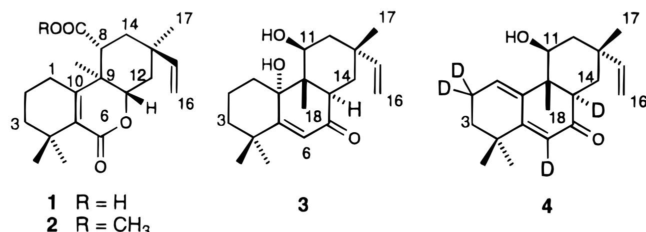
Bioassay-directed fractionation of the bioactive alcoholic extracts of *Vellozia candida* yielded a new 6,7-*seco*-rosane diterpenoid, candidalactone (**1**), which showed moderate toxicity toward DNA repair-deficient mutants of *Saccharomyces cerevisiae.* Another new but inactive rosane diterpenoid, candidenodiol (**3**), was also obtained.

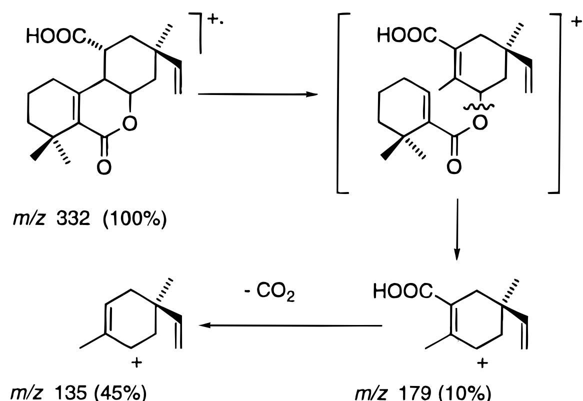
Vellozia candida Mikan (Velloziaceae)[1] inhabits the coastal mountains of Rio de Janeiro. Previous studies on this plant have afforded several rosane diterpenoids including epoxycorcovadin, epoxyvellozin,[2,3] and vellozi-olide,[4] a diterpenoid with a novel carbon skeleton. In our continuing search for potential anticancer agents utilizing a mechanism-based bioassay,[5,6] and continuing our phytochemical studies on Velloziaceae,[2−4] we have investigated alcoholic extracts of *V. candida.* In this paper, we report the isolation of a new bioactive 6,7-*seco*-rosane diterpenoid, candidalactone (**1**), and a new but inactive rosane diterpenoid, candidenodiol (**3**).



The dried and powdered whole plant of *V. candida* was sequentially extracted with hexane, EtOH, and MeOH. Of these, the latter two extracts showed moderate activity in our mechanism-based bioassay[5,7] and were subjected to bioassay-guided fractionation involving solvent−solvent partition, Sephadex LH-20 gel filtration, Si gel CC, and RP-HPLC, as appropriate, to afford the two new compounds **1** and **3**, of which **1** was found to be moderately bioactive.

Candidalactone (**1**), $C_{20}H_{28}O_4$ (HREIMS), showed the presence of four methyl groups (δ 1.09 s, 1.17 s, 1.24 s, 1.32 s), a vinyl group [δ 5.71 dd (J = 17.9, 10.9 Hz), 5.17 d (J = 10.9 Hz)], all attached to quaternary carbons, two allylic protons (δ 2.21−2.26 m), a proton on an oxygenated carbon [δ 3.94 dd (J = 12.8, 3.7 Hz)], and a proton on a carbon bearing a CO_2H group [δ 2.51 dd (J = 12.2, 3.2 Hz)] in its ^{1}H-NMR spectrum. The presence of a carboxylic acid function and an $\alpha\beta$-unsaturated lactone moiety in **1** were inferred from its IR spectrum. The ^{13}C-NMR spectrum of candidalactone (**1**) analyzed with the help of its DEPT spectrum showed a lactone carbonyl (δ 163.4), a carboxylic acid carbonyl (δ 179.2), two tetrasubstituted sp[2] carbons (δ 131.7, 159.5), three tetrasubstituted sp[3] carbons (δ 33.3, 36.8, 41.9), and an oxygenated methine carbon (δ 77.4) in addition to the four methyl groups (δ 11.1, 27.1, 28.7, 31.0) and the vinyl group [δ 113.9 (CH_2) and 144.2 (CH)]. These data along with the knowledge of the presence of rosane diterpenoids in this plant species[2−4] suggested the presence of a modified rosane carbon skeleton in **1**. It was also apparent that the C_6−C_7 bond has undergone an oxidative cleavage and that one of the resulting CO_2H groups (at C-5) has formed a lactone with the OH group at C-11 usually present in *Vellozia* diterpenoids. The MS of **1** showed fragment ions at m/z 179 and 135, assignable to the fragments shown in Scheme 1, further suggesting that it has a 6,7-*seco*-rosane skeleton. The formation of the methyl ester **2** on treatment of **1** with CH_2N_2 confirmed the presence of a CO_2H group in candidalactone, and this was located at C-8 on the basis of biogenetic arguments. ^{1}H- and ^{13}C-NMR assignments of candidalactone (**1**) (see Table 1) were made by comparison with those data reported for rosane diterpenoids[3] and with the help of COSY-45 and HETCOR spectra, and these assignments confirmed the location of the carboxyl group at C-8.

Scheme 1. Proposed MS Fragmentation for Candidalactone (**1**)



The stereochemical assignments at C-8, C-9, C-11, and C-13 were made by the application of ^{1}H-NMR, ^{13}C-NMR, COSY-45, HETCOR, and NOESY spectroscopy. The ^{1}H-NMR signal for H-8 at δ 2.51, assigned on the basis of its chemical shift, appeared as a dd (J = 12.2,

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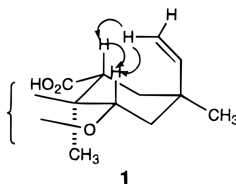
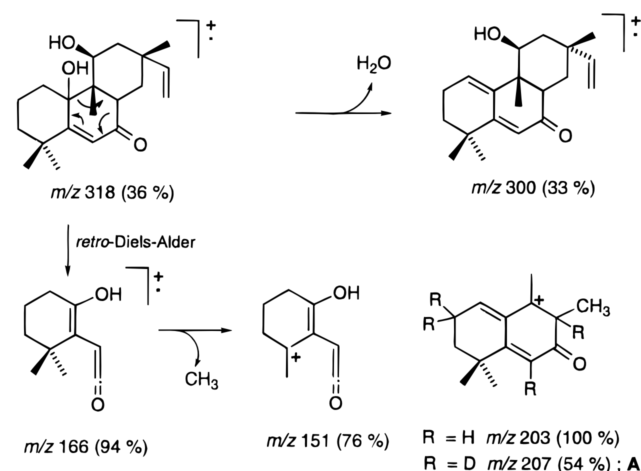


Figure 1. NOE interactions for compound **1**.

3.2 Hz), suggesting it to be axial. Consequently, the CO_2H function on C-8 must have an equatorial orientation. On the basis of similar arguments, the attachment of the lactone oxygen at C-11 was determined to be equatorial. An upfield shift of the ^{13}C-NMR signal of the C-20 methyl group [δ_C 11.1 as opposed to δ_C >12.0 for related rosane diterpenoids][3,8] suggested that it suffered a γ-gauche shielding effect from the C-8-CO_2H. In the NOESY spectrum of **1** cross peaks were observed between H-8 and H-11, H-8 and H-16a, and H-11 and H-16a (Figure 1), suggesting that these protons lie on the same side of the *seco*-rosane carbon skeleton. An examination of a computer-generated model revealed that these protons lie in close proximity to one another. From the foregoing evidence, candidalactone was identified as 6,7-*seco*-rosane-5(10),15-dien-7-oic acid 6,11-carbolactone (**1**). A compound that was shown to be identical with candidalactone also occurred in the hexane extract of *V. candida*, but no bioactivity data were obtained for this sample.[9]

The ^1H- and ^{13}C-NMR spectral data (Table 1) of candidenodiol (**3**), $C_{20}H_{30}O_3$ (HREIMS), suggested it to be a rosane diterpenoid. Its UV and IR spectra were indicative of the presence of an enone moiety, which was confirmed from its ^1H- and ^{13}C-NMR spectra, which had signals at δ_H 6.20 (s), and δ_C 200.4, 166.4, and 123.7. The ^1H- and ^{13}C-NMR spectral data, in addition to the presence of signals due to four methyl groups and a vinyl group, all on quaternary carbons usually found in the rosane skeleton, indicated the presence of secondary [δ_H 4.36 dd (J = 11.8, 3.9 Hz); δ_C 67.8 (d)] and

Scheme 2. Proposed MS Fragmentation for Candidenodiol (**3**)



tertiary [δ_C 76.3 (s)] carbinol carbons. Location of the enone moiety and the two hydroxy groups, accounting for all three oxygen atoms present in the molecule, was achieved by analysis of COSY-45, HMBC, and mass spectra as described below.

The olefinic H of the enone moiety appeared as a sharp singlet, and no correlations were observed in the COSY-45 spectrum. The presence of significant fragments at m/z 203 and 166 in the MS of **3** (Scheme 2) suggested the possible location of the enone moiety in ring B; the latter fragment also indicated the presence of an OH group in ring A of **3**. The shift of the base peak from m/z 203 to 207 (fragment **A** in Scheme 2) in the MS of the tetradeuterated analog **4**[10] further supported the proposed MS fragmentation for **3**. The HMBC correlation observed between the enone olefinic H and the oxygenated quaternary carbon at δ_C 76.3 located the OH group at C-10. An HMBC correlation was also observed between this carbon and the protons of the Me group at δ_H 0.86; the latter signal correlated

Table 1. ^1H- and ^{13}C-NMR Spectral Data for Compounds **1** and **3** (δ in ppm, $CDCl_3$)

	1		**3**	
position	δH (m, J (Hz))[a]	δC[b,c]	δH (m, J (Hz))[a]	δC[b,c]
1[d]	2.21–2.26 (m)	26.1 (CH_2)	2.53 (bddd, 15.4, 11.2, 2.9)	34.8 (CH_2)
2[d]	1.66–1.74 (m)	18.4 (CH_2)	1.51–1.56 (m)	17.2 (CH_2)
3α	1.37 (dd, 13.9, 2.9)	39.6 (CH_2)	1.36–1.46 (m)[e]	39.2 (CH_2)
3β	1.47–1.53 (m)		1.51–1.62 (m)	
4		33.3 (C)		36.6 (C)
5		131.7 (C)		166.4 (C)
6		163.4 (C)	6.20 (s)	123.7 (CH)
7		179.2 (C)		200.4 (C)
8	2.51 (dd, 12.2, 3.2)	43.7 (CH)	2.88 (dd, 12.3, 3.6)	44.7 (CH)
9		41.9 (C)		47.8 (C)
10		159.5 (C)		76.3 (C)
11	3.94 (dd, 12.8, 3.7)	77.4 (CH)	4.36 (dd, 11.8, 3.9)	67.8 (CH)
12α	1.69 (t, 12.8)	37.8 (CH_2)	1.77 (ddd, 14.2, 3.9, 2.9)	43.4 (CH_2)
12β	1.86–1.92 (m)		1.33–1.46 (m)[e]	
13		36.8 (C)		36.7 (C)
14α	1.77 (t, 13.6)	37.1 (CH_2)	2.03 (ddd, 14.2, 3.6, 2.9)	31.5 (CH_2)
14β	1.86–1.92 (m)		1.33 (dd, 14.2, 12.3)	
15	5.71 (dd, 17.9, 10.9)	144.2 (CH)	5.72 (dd, 17.6, 11.0)	145.2 (CH)
16a	5.11 (d, 17.9)	113.9 (CH_2)	5.04 (dd, 17.6, 0.8)	113.0 (CH_2)
16b	5.17 (d, 10.9)		5.07 (dd, 11.0, 0.8)	
17	1.09 (s)	31.0 (CH_3)	1.04 (s)	31.0 (CH_3)
18	1.32 (s)	27.1 (CH_3)	1.30 (s)	31.1 (CH_3)[f]
19	1.24 (s)	28.7 (CH_3)	1.16 (s)	31.3 (CH_3)[f]
20	1.17 (s)	11.1 (CH_3)	0.86 (s)	9.6 (CH_3)

[a] Measured at 400 MHz. [b] Measured at 100.57 MHz. [c] Type of carbon (in parentheses) determined by a DEPT experiment. [d] Signal due to one of the two protons at this position: the remaining proton could not be assigned. [e] Overlapping signals. [f] Interchangeable.

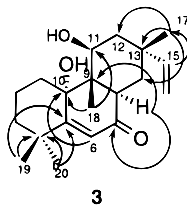


Figure 2. HMBC connectivitied (^{1}H → ^{13}C) of compound **3**.

with the carbinol carbon at δ_C 67.8, thus locating the secondary OH function at C-11. The proton signal (dd) at δ 2.88 showed correlations to the quaternary carbon at δ_C 47.8. Thus, the ^{1}H-NMR signal at δ_H 2.88 was assigned to H-8 and the ^{13}C-NMR signal at δ 47.8 to C-9. These and other significant HMBC correlations for **3** are shown in Figure 2. The coupling pattern of the ^{1}H-NMR signal at δ_H 4.36 (dd, J = 11.8, 3.9 Hz), which showed a HETCOR correlation with δ_C 67.8 (see above), indicated that this proton should be axially oriented. The chemical shift of the Me-20 (δ_C 9.6) suggested that it is subjected to a γ-gauche effect from the OH group at C-11 and, consequently must be in an axial orientation. The coupling pattern observed for H-8 at δ_H 2.88 (dd, J = 12.3 and 3.6 Hz) suggested it to be axially oriented. Thus, the B,C ring junction of **3** must be *trans*.

The absolute stereochemistry of candidenodiol (**3**) was derived from an analysis of its CD spectrum in MeOH. As for other αβ-unsaturated 6-membered ring ketones,[11] the CD spectrum of **3** showed two bands of lowest energy; n → π^* at 332 nm and $\pi \rightarrow \pi^*$ at 262 nm with positive and negative Cotton effects, respectively. Application of helicity rules[11] indicated the absolute configuration of candidenodiol to be as shown in structure **3** leading to its identification as 10(*S*),11(*R*)-dihydroxy-5(6),15-rosanedien-7-one.

Candidalactone (**1**) exhibited moderate but selective activity in our mechanism-based yeast bioassay for DNA-damaging agents[5-7] with the following IC_{12} values: RS 322YK (*rad* 52Y), 87 μg/mL; RS 188N (RAD+) >200 μg/mL. These results indicated that candidalactone represents the major bioactive compound in the crude extract of *V. candida*.

Experimental Section

General Experimental Procedures. UV spectra were taken in MeOH on a Beckman DU-50 spectrophotometer. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter in $CHCl_3$ solutions. NMR spectra (δ ppm, J in Hz) were obtained on a Varian Unity 400 spectrometer (400 MHz) for compounds **1** and **3**, and on a Bruker WM-400 (400 MHz) for compound **2** using TMS as internal standard. EIMS were obtained using a VG Quattro instrument at 70 eV. Column chromatography (CC) employed Si gel 60 (230–400 mesh) and Sephadex LH-20 (25–100 μM). TLC and PTLC were performed by using precoated Si gel 60 F_{254} and Si gel GF 1000 μM plates, respectively, and detection was accomplished by UV_{254} irradiation and by spraying with ceric sulfate/H_2SO_4 followed by heating. HPLC separation of compound **3** was carried out on a Whatman Partisil 5 μm column with a Waters 990 Series photodiode array detector at 248 nm.

Plant Material. *V. candida* was collected in the Corcovado mountain, Rio de Janeiro, RJ, Brazil in February 1977. A voucher specimen is deposited at the Departamento de Botânica of the Universidade de São Paulo, São Paulo, SP, Brazil.

Biological Assays. The bioassays using DNA repair deficient mutants of *Saccharomyces cerevisiae* were performed as previously described.[6] The IC_{12} values refer to the concentration in μg/mL required to produce a zone of inhibition of 12 mm diameter around a 100 μL well during a 48 h incubation period at 32 °C.

Extraction and Isolation. The dried and powdered whole plant of *V. candida* (2.3 kg) was sequentially extracted with hexane, EtOH, and MeOH. Evaporation yielded hexane (79.3 g), EtOH (12.5 g), and MeOH (6.0 g) extracts. A portion of the EtOH extract (3.1 g) was partitioned between *n*-BuOH and water. The bioactive *n*-BuOH fraction was then fractionated between hexane and 80% aqueous MeOH. Water was then added to the bioactive aqueous MeOH fraction to give 60% aqueous MeOH, and this fraction was extracted thoroughly with $CHCl_3$ to yield 1.2 g of the bioactive $CHCl_3$ fraction. The $CHCl_3$ fraction was subjected to gel permeation chromatography on Sephadex LH-20, eluting initially with hexane–CH_2Cl_2 (1:4), followed by CH_2Cl_2–acetone (2:3), CH_2Cl_2–acetone (1:4), and finally with MeOH. A portion of the bioactive fraction (198 mg) from the hexane–CH_2Cl_2 (1:4) fraction (497.6 mg) of the above Sephadex column was submitted to Si gel CC using a gradient solvent system from 2% *i*-PrOH in $CHCl_3$ to MeOH yielding 60 fractions. The bioactive fractions (nos. 25–33; 51.3 mg) were combined and rechromatographed on Si gel CC using 5% *i*-PrOH in $CHCl_3$, yielding 42 fractions. Fractions 21–36 were found to be bioactive. On the basis of their TLC behavior, two combined fractions (nos. 21–29; 8.7 mg and 30–36; 27.9 mg) were obtained, and these were separately submitted to Si gel CC. Compound **1** (2.5 mg) was isolated from 6.0 mg of fractions 21–29 on separation by Si gel CC using hexane–*i*-PrOH (7:1) followed by hexane–*i*-PrOH (4:1) and then crystallization using hexane–EtOAc (5:1).

A portion of the MeOH extract of *V. candida* (2.89 g) was partitioned as for the EtOH extract (see above). The bioactive $CHCl_3$ fraction (1.32 g) was submitted to Sephadex LH-20 gel permeation chromatography eluting initially with hexane, followed by 5%, 30%, 50%, and 80% CH_2Cl_2 in hexane, 10% and 80% acetone in CH_2Cl_2, and finally with MeOH. The bioactive 50% CH_2Cl_2 in hexane fraction (429.6 mg) was chromatographed on a Si gel CC using hexane–*i*-PrOH (9:1) to MeOH, yielding 49 fractions. A portion of the bioactive fractions (nos. 13–36; 42.5 mg) was submitted to PTLC separation on Si gel using 6% *i*-PrOH in $CHCl_3$ containing 1% of AcOH as the eluant, yielding 6.3 mg of a solid that by successive crystallization from hexane–EtOAc (5:1) yielded 1.1 mg of compound **1**. A portion (24.3 mg) of the combined inactive fraction (54.9 mg) was submitted to Si gel HPLC using 2% *i*-PrOH in $CHCl_3$ as the mobile phase, yielding compound **3** (8.4 mg).

Candidalactone (1): colorless crystals [hexane–AcOEt (5:1)]; mp 176–177 °C; UV λ_{max} ((MeOH) (log ϵ) 231 (3.85) nm; $[\alpha]^{24}_D$ +69.4 (c = 2.5, $CHCl_3$); IR ν_{max} (KBr) 3500–3000 br, 1715, 1700, 1631, 1404, 1276, 1236, 1078, 997, 898 cm^{-1}; HREIMS found [M$^+$] 332.1970, $C_{20}H_{28}O_4$ requires 332.1988; ^{1}H- and ^{13}C-NMR spectra, see Table 1; EIMS m/z (rel int) 332 [M$^+$]

(100), 317 (37), 179 (10), 135 (45), 121 (21), 107 (28), 43 (41), 41 (42).

Candidalactone Methyl Ester (2). Candidalactone (70.0 mg), isolated from the hexane extract,[8] was dissolved in Et_2O (2 mL), and a saturated ethereal solution of CH_2N_2 was added. After the excess CH_2N_2 was destroyed with AcOH, the solvent was evaporated, and the solid obtained was crystallized from hexane–AcOEt (9:1), yielding **2** (71.6 mg): $[\alpha]^{24}_{D}$ +86.8 (c = 0.95, $CHCl_3$); IR ν_{max} (KBr) 2950, 1720, 1704, 1630, 1429, 1355, 1264, 1211, 1164, 1067, 1018, 929 cm^{-1}; ^1H-NMR ($CDCl_3$, 400 MHz) 1.05 (H_3-17, s), 1.15 (H_3-20, s), 1.22 (H_3-19, s), 1.31 (H_3-18, s), 2.48 (H-8, dd, J = 11.0, 5.0 Hz), 3.68 (3H-$OCOCH_3$, s), 3.92 (H-11, dd, J = 13.0, 4.0 Hz), 5.08 (H-16a, d, J = 18.0 Hz), 5.13 (H-16b, d, J = 11.0 Hz), 5.69 (H-15, dd, J = 18.0, 11.0 Hz); ^{13}C-NMR ($CDCl_3$, 100.57 MHz) 11.20 (C-20), 18.40 (C-2), 25.60 (C-1), 27.00 (C-18), 28.70 (C-19), 30.90 (C-17), 33.20 (C-4), 36.70 (C-12), 37.00 (C-13), 37.60 (C-12), 39.60 (C-3), 41.90 (C-9), 43.60 (C-8), 51.90 (OCO*C*H_3), 77.20 (C-11), 113.50 (C-16), 131.40 (C-5), 144.20 (C-15), 159.60 (C-10), 163.20 (C-6), 175.10 (C-7); EIMS m/z (rel int) 346 $[M^+]$ (100), 331 (20), 135 (37), 121 (13), 107 (18), 43 (15), 41 (28).

Candidenodiol (3): oil; UV λ_{max} ((MeOH) (log ϵ) 240 (3.4) and 308 (2.9) nm; $[\alpha]^{24}_{D}$ +58.4 (c = 4.5, $CHCl_3$); IR ν_{max} (film) 3450, 1660 cm^{-1}; HREIMS $[M^+]$ 318.2195, $C_{20}H_{30}O_3$ requires 318.2194; ^1H- and ^{13}C-NMR data, see Table 1; EIMS m/z (rel int) 318 $[M^+]$ (36), 300 (33), 285 (45), 249 (24), 229 (26), 203 (100), 193 (76), 179 (26), 166 (94), 151 (76), 139 (83), 135 (38), 123 (19), 109 (28).

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