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Candelalides A–C: Novel Diterpenoid Pyrones from Fermentations of *Sesquicillium candelabrum* as Blockers of the Voltage-Gated Potassium Channel Kv1.3

Sheo B. Singh,* Deborah L. Zink, Anne W. Dombrowski, Gabe Dezeny, Gerald F. Bills, John P. Felix, Robert S. Slaughter, and Michael A. Goetz

Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065-0900

sheo_singh@merck.com

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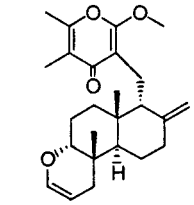
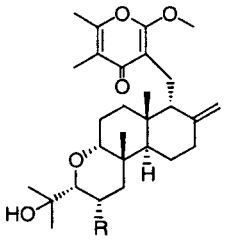
ABSTRACT



2: Candelalide A



3: R = OH, Candelalide B
4: R = H, Candelalide C

Blockers of the voltage-gated potassium channel Kv1.3 are potential immunosuppressants. Candelalides A–C are three novel diterpenoid pyrones that block this channel. The structure, stereochemistry, and activity against Kv1.3 are described.

The voltage-gated delayed rectifier potassium channel Kv1.3 is found in human T lymphocytes where it sets the resting potential.[1] Blockade of this channel results in depolarization of cells and limits the Ca^{2+} entry that normally occurs upon activation of the T-cell receptor. This action in turn results in a decrease in the lymphokine release and synthesis stimulated by calcium-dependent pathways, thus suppressing activation and proliferation of T cells. Therefore, Kv1.3 represents a potential novel target for immunosuppression. Blockers of Kv1.3 channel such as margatoxin (MgTX), a peptide, and correolide,[2] a *nor*-triterpenoid, that we reported suppress lymphokine production and cause membrane depolarization.[1]

More recently we reported nalanthalide (**1**), a diterpenoid pyrone isolated from *Nalanthamala* sp., that blocked this channel with an IC_{50} of 3.9 μM.[3] In this Letter we present

* To whom correspondence should be addressed. Fax (732) 594-6880.

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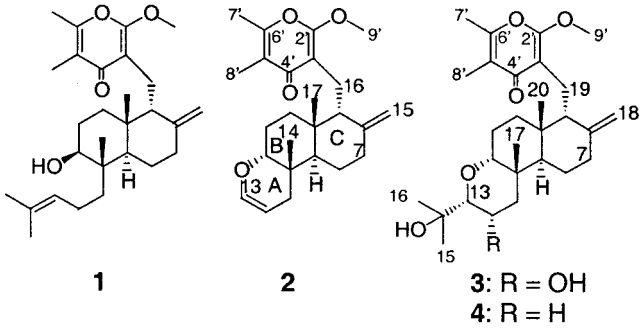


candelalides A (**2**), B (**3**), and C (**4**), three more complex, more potent, and novel diterpenoid pyrones isolated from fermentation broths of *Sesquicillium candelabrum*.[4] The isolation, structure elucidation including stereochemistry, and Kv1.3 channel blocking activities of these compounds are described.

Silica gel chromatography of methyl ethyl ketone extracts of the fermentation[5] broths of *S. candelabrum* followed by RP HPLC on a Dynamax C-18 column gave candelalide A (75 mg/L). Candelalides B (60 mg/L) and C (37 mg/L) were more efficiently purified from silica gel enriched fractions by RP HPLC on a Zorbax RX C-8 column using aqueous CH_3CN.[6]

Candelalide A (2). High-resolution EI mass spectral analysis of candelalide A showed a molecular ion at *m*/*z* 398.2451 which revealed a molecular formula of $C_{25}H_{34}O_4$ (calcd 398.2457). The formula was corroborated by the ^{13}C NMR spectra and suggested nine degrees of unsaturation. The UV spectrum of **2** showed an absorption band at λ_{max} 258 (log ϵ = 3.93) nm indicative of the methoxy pyrone structure. The IR spectrum exhibited an absorption band for a conjugated ketone (1669 cm^{-1}). The ^{13}C NMR spectrum of **2** showed signals for 25 carbons. The DEPT spectrum indicated the presence of five methyls (one methoxy), seven methylenes (one olefinic), and five methines (two aliphatic, one oxy, and two olefinic). By deduction this leaves eight quaternary carbons. Of these two were aliphatic, two shielded olefinic, three deshielded olefinic, and one carbonyl.

The ^1H NMR analysis (Table 1) of candelalide A showed complementary proton signals. Analysis of 2D-COSY and TOCSY spectra of **2** revealed four $^1H-^1H$ spin systems leading to four partial fragments assigned to C1−C3, C5−C7, C9−C19, and C11−C13. Exocyclic methylene protons (H_2-18) showed correlations to H_2-7 due to long-range allylic couplings. An HMQC experiment was used to establish one-bond carbon−proton connectivity, and an HMBC experiment was used to connect the partial fragments to the remainder of the molecule. The pertinent HMBC correlations of **2** are shown in Figure 1A. The two- and three-bond HMBC

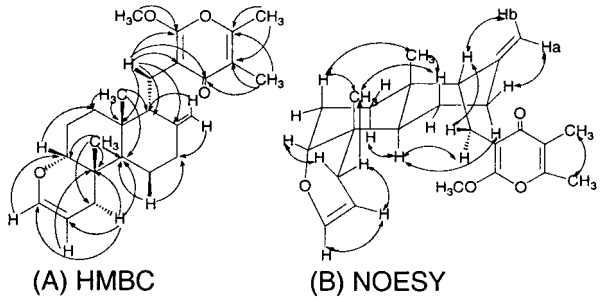


Figure 1. Selected HMBC ($^nJ_{CH}$ = 7 Hz) and NOESY (mix time = 300 ms) correlations of candelalide A (**2**).

correlations from the methyl and methylene groups H_3-7′, H_3-8′, H_2-19, and H_3-9′ helped to establish the methoxy pyrone unit. The methyl group H_3-17 displayed HMBC correlations to C-1, C-5, C-9, and C-10; the olefinic methylene protons H_2-18 showed three-bond HMBC correlations to C-7 and C-9; the methyl group H_3-14 gave correlations to C-3, C-4, C-5, and C-11. These HMBC correlations established the connectivity of the respective COSY-derived fragments to each other and to the remaining quaternary carbons. Finally, the HMBC correlations of H-3 to C-13 and H-13 to C-3 led to formation of a dihydropyran ring.

The mass spectral analysis[7] of **2** showed a characteristic fragment ion for methoxy pyrone at *m*/*z* 167 (Figure 2). It

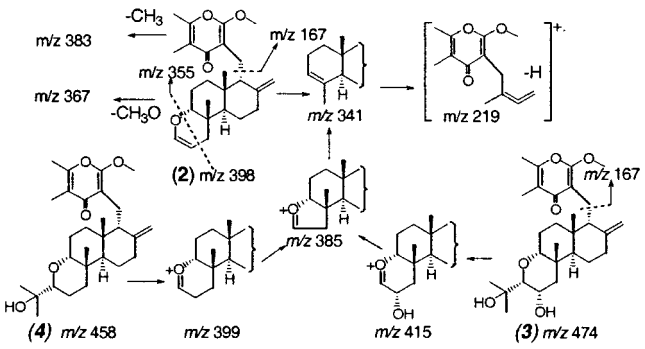


Figure 2. EIMS fragmentation of **2**−**4**.

also showed ions at *m*/*z* 355 and *m*/*z* 341 due to cleavages of the dihydropyran ring with loss of either two or three carbons with oxygen, respectively.

The relative stereochemistry of candelalide A was elucidated by measurement of scalar coupling and NOESY

(4) *Sesquicillium candelabrum* (Bonorden) Gams was cultured from a sporodochium of *Bactrodesmium* sp. collected on decayed wood, Scott's Run, Fairfax Co., VA. The strain (MF6374) is maintained in the Merck Microbial Resources Culture collection, Rahway, NJ.

(5) For growth conditions ,see: Dombrowski, A.; Jenkins, R.; Raghoobar, S.; Bills, G.; Polishook, J.; Pelaez, F.; Burgess, B.; Zhao, A.; Huang, L.; Zhang, Y.; Goetz, M. *J. Antibiot.* **1999**, *52*, 1077. Solid-state vermiculite based (220 mL liquid on 675 CC vermiculite) AD2 production medium was used.

(6) **2** $[\alpha]^{22}_D$ −23.1° (*c* 0.26, CH_3OH), **3** $[\alpha]^{22}_D$ −50.9° (*c* 0.49, CH_3OH), **4** $[\alpha]^{22}_D$ −67° (*c* 1.03, CH_3OH), each obtained as an amorphous powder after lyophilization.

(7) All mass spectral fragments were assigned by high-resolution EIMS measurements. The observed and calculated mass values were within ±0.0005 amu.

Table 1. NMR Assignments of Candelalides A−C (**2**−**4**) in $CDCl_3$[a]

	2		**3**		**4**	
position	δC	δH (*J* in Hz)	δC	δH (*J* in Hz)	δC	δH (*J* in Hz)
1	28.76	ax: 2.36, dd, 14.0, 4.4 eq: 1.04, td, 13.2, 3.2	28.29	ax: 2.32, m eq: 0.86, td, 13.2, 3.6	29.17	ax: 2.36, m eq: 1.00, m
2	23.94	ax: 1.95, ddd, 15.0, 3.6, 2.4 eq: 1.75, ddd, 14.0, 6.8, 3.6	24.50	ax: 1.89, ddd, 14.0, 3.6, 2.0 eq: 1.67, ddd, 14.0, 6.8, 3.6	24.57	ax: 1.94, m eq: 1.66, m
3	78.97	3.71, dd, 4.0, 2.0	82.89	3.25, dd, 3.6, 2.4	81.69	3.23, t, 2.8
4	33.71		34.72		34.75	
5	34.51	1.98, dd, 12.8, 3.6	35.02	3.00, dd, 9.6, 3.2	33.72	2.18, dt, 10, 3.2
6	22.92	ax: 1.36, dq, 13.2, 4.8 eq: 1.57, m	22.77	ax: 1.28, m eq: 1.71, m	22.38	ax: 1.31, m eq: 1.52, m
7	31.40	ax: 2.42, m eq: 2.12, m	31.58	ax: 2.35, m eq: 2.14, m	31.34	ax: 2.41, m eq: 2.12, m
8	149.23		151.26		149.38	
9	56.66	1.86, dd, 12.6, 3.6	57.43	1.84, dd, 7.2, 5.6	56.72	1.86, dd, 11.2, 3.2
10	37.46		37.62		37.48	
11	32.59	ax: 1.59, m eq: 2.10, dd, 17.6, 6.0	41.69	ax: 0.93, dd, 14.8, 3.6 eq: 2.27, dd, 15.2, 2.8	36.05	ax: 1.02, dt, 4.0, 14.4 eq: 1.97, td, 3.2, 13.6
12	97.65	4.53, dt, 6.0, 2.0	67.22	4.05, ddd, 4.4, 3.2, 2.0	22.03	ax: 1.34, m eq: 1.62, m
13	142.66	6.37, dd, 6.4, 2.4	81.81	3.04, d, 2.5	83.35	3.15, dd, 11.6, 2.4
14	23.48	0.90, s	73.79		72.05	
15	108.93	Ha: 4.49, dd, 2.8, 2.0 Hb: 4.17, dd, 3.0, 2.0	23.50*	1.37, s	25.75*	1.17, s
16	19.84	Hβ: 2.70, dd, 12.8, 3.6 Hα: 2.44, dd, 12.8, 11.6	23.11*	1.32, s	23.80*	1.19, s
17	23.56	0.99, s	23.11**	0.75, s	22.93**	0.80, s
18			107.68	Ha: 4.52, dd, 2.8, 2.0 Hb: 4.30, dd, 2.8, 2.0	108.88	Ha: 4.49, dd, 2.8, 2.4 Hb: 4.19, dd, 2.8, 2.4
19			21.31	Hβ: 2.98, dd, 12.8, 4.4 Hα: 2.12, dd, 13.6, 7.6	19.81	Hβ: 2.76, dd, 12.8, 3.2 Hα: 2.46, dd, 12.8, 11.2
20			23.99**	0.92, s	23.28**	0.95, s
2′	162.84		163.12		162.88	
3′	103.62		104.52		103.66	
4′	180.36		179.90		180.32	
5′	118.59		118.66		118.61	
6′	154.76		154.75		154.80	
7′	16.99	2.23, s	16.97	2.23, q, 0.8	16.97	2.23, q, 0.8
8′	10.09	1.89, s	10.09	1.89, q, 0.8	10.06	1.89, q, 0.8
9′	55.38	3.83, s	55.25	3.85, s	55.32	3.84, s

[a] ax = axial H; eq = equatorial H; *, ** may be interchanged.

correlations. H-3 (δ 3.71) appeared as a doublet of doublets and showed two small couplings (J = 4.0, 2.0 Hz). The magnitude of these couplings is indicative of the equatorial orientation of H-3, thus placing the oxygen axial with respect to B ring. The methyl groups H_3-14 and H_3-17 showed NOESY correlations to the common axial protons H-2 and H-6, and H-5 showed correlations to the axial H-1 and Hα-16 (Figure 1B). These correlations suggest that the bicyclic ring B/C is trans fused and both of the methyl groups are axially oriented on the β-face. This will lead to the axial orientation of H-5 and C-16 placing the methoxy pyrone at the α-face. On the basis of these collective data, structure **2** is assigned to candelalide A.

Candelalide B (3). High-resolution EIMS analysis of candelalide B (**3**) gave a molecular ion at *m*/*z* 474.2975, suggesting a molecular formula of $C_{28}H_{42}O_6$ which was supported by ^{13}C NMR spectrum. This formula contains an extra $C_3H_8O_2$ moiety and one less degree of unsaturation compared to candelalide A. The UV and IR spectra of the two compounds were identical except for the presence of a hydroxy absorption band at 3359 cm^{-1} in **3**. The ^1H and ^{13}C NMR spectra (Table 1) of candelalide A and B were similar except for the absence of the signals for the C12−C13 olefin and the presence of five additional signals attributed to two oxymethines δC 67.22 (δH 4.05, ddd, J = 4.4, 3.2, 2.0 Hz), δC 81.81 (δH 3.04, d, J = 2.5 Hz), an oxygen-bearing quaternary carbon (δ 73.79), and two methyl groups δC 23.50 (δH 1.37, s) and δC 23.11 (δH 1.32, s). These signals were assigned to C-12, C-13, C-14, C-15, and C-16, respectively, by COSY and HMBC (Figure 3A) experiments. Accordingly, the dihydropyran ring was established form HMBC correlations of H-3 to C-14 and H-14 to C-3.

The proposed structure of candelalide B was fully supported by mass spectral fragmentation as shown in Figure

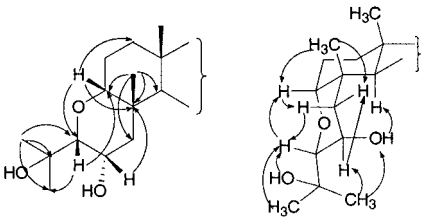
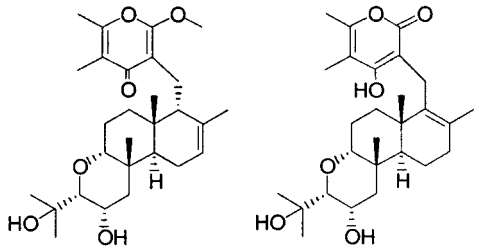


Figure 3. Selected HMBC ($^nJ_{CH}$ = 7 Hz) and NOESY correlations (mix time = 300 ms) of candelalide A (**3**).

2. Like candelalide A the stereochemistry of candelalide B (**3**) was deduced by application of scalar coupling and NOESY correlations (Figure 3B). The stereochemistry of the new stereocenters was established as follows. The H-12 showed three couplings with magnitudes all less than 5 Hz indicating its equatorial orientation in a chair conformation of the pyran ring. H-13 showed a doublet with 2.5 Hz coupling and displayed 1,3-diaxial NOESY correlations to H-11 and H-3 (axial with respect to pyran ring) thus placing it at the axial position at C-13.

Candelalide C (4). A molecular formula of $C_{28}H_{42}O_5$ (*m*/*z* 458, M^+)[7] was determined by high-resolution EIMS analysis. It differs from **3** by having one less oxygen. The ^1H and ^{13}C NMR spectra (Table 1) of **3** and **4** were similar except C-12 oxymethine (δ 67.22) was replaced by a methylene group (δ 22.03) in candelalide C. This assignment was substantiated by analysis of the COSY spectrum and mass spectral fragmentation (Figure 2).

Treatment of candelalide B with (+)-camphor sulfonic acid in dichloroethane produced a 1:1 mixture of compounds **5** (λ_{max} 258 nm) and **6** (λ_{max} 290 nm). Selective enol ether hydrolysis of only compound **6** is intriguing. Reactions with a 10-fold excess of (*R*) and (*S*) MTPA chloride led to the exclusive mono-esterification of the *tert*-hydroxy group at C-14, indicating a significant steric compression around the axial C-12 hydroxy group.



The stereochemistry at C-3 of candelalides A−C is similar to that of colletotrichins[8] but opposite to that found in nalanthalide, viridoxins,[9] and sesquicillin.[10] Candelalides B and C are formed by cyclization of the isoprene unit to a pyran ring; elimination of 1 mol each of acetone and water gives candelalide A.

Candelalides A−C (**2**−**4**) blocked the $^{86}Rb^+$ efflux in CHO−Kv1.3 cells[2b] with IC_{50} values of 3.7, 1.2, and 2.5 μM, respectively. Candelalide B is 3-fold more potent than nalanthalide (IC_{50} 3.9 μM) and candelalide A and 2-fold more potent than candelalide C indicating the importance of C-12 hydroxy group. Immunosuppressant activity of subglutinols,[11] diterpenoid hydroxy pyrones, have been reported with no mention of mechanism of action.

In summary, we describe here the isolation and structure elucidation of three novel diterpenoid pyrones that are novel blockers of the voltage-gated potassium Kv1.3 channel and are potential immunosuppressants.

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