Daleformis, a New Phytoalexin from the Roots of *Dalea filiciformis*: An Inhibitor of Endothelin Converting Enzyme

Ashok D. Patil, Alan J. Freyer,* Drake S. Eggleston, R. Curtis Haltiwanger, Brian Tomcowicz, Ann Breen, and Randall K. Johnson

Departments of Biomolecular Discovery and Analytical Sciences, SmithKline Beecham Pharmaceuticals, Research and Development, King of Prussia, Pennsylvania 19406-0939

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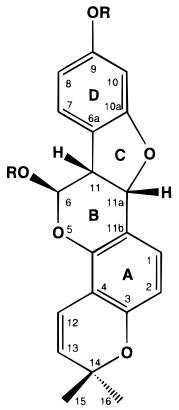
As part of a search for novel inhibitors of endothelin converting enzyme (ECE), the MeOH−CH_2Cl_2 extract of the roots of *Dalea filiciformis* was shown to be active. Bioassay-guided fractionation of the extract yielded a novel phytoalexin, daleformis (**1**), whose structure was determined by interpretation of spectral data and X-ray analysis. Daleformis (**1**) inhibited ECE with an IC_{50} of 9 μM.

Endothelin converting enzyme (ECE) is a membrane-bound neutral metalloprotease that catalyzes the conversion of a 38-residue inactive intermediate big-endothelin (B-ET) to a 21-residue potent vasoconstrictive peptide, endothelin-1 (ET-1). Overproduction of ET is associated with numerous disorders including hypertension and renal failure. An ECE inhibitor, by interfering with the ET biosynthesis pathway, should reduce production of endothelin and may have therapeutic utility for hypertension or renal failure.

As part of a search for biologically active natural products with potential utility in the treatment of hypertension, we initiated a high throughput screen to evaluate the ability of natural products extracts to inhibit ECE. The MeOH−CH_2Cl_2 extract of the roots of *Dalea filiciformis* Snader (Fabaceae) was found to be active in our ECE inhibitory screen and hence was selected for fractionation. A literature search revealed that there were no reports on the chemical examination of this plant. We now report the isolation and structure determination of a novel pterocarpinoid, daleformis (**1**), from the roots of *D. filiciformis*.



1 R = H
2 R = $COCH_3$

Extraction of dried, powdered roots with MeOH−CH_2Cl_2 afforded a residue that was passed through a column of polyamide to remove polyphenols and tannins. Bioassay-guided fractionation of this residue using RP-18 column chromatography followed by HPLC (Si gel) of the ECE active fraction yielded a colorless powder that crystallized from EtOAc−hexane to give daleformis (**1**) as white crystals.

Table 1. ^{1}H- and ^{13}C-NMR Assignments for **1** in CD_3OD

position	δ_C	δ_H
1	131.2	7.18 (1H, dd, 0.6, 8.4)
2	111.4	6.44 (1H, dd, 0.7, 8.4)
3	155.3	
4	111.5	
4a	150.1	
6	96.8	4.83 (1H, d, 8.2)
6a	119.0	
7	127.5	7.15 (1H, dd, 1.2, 8.1)
8	108.6	6.33 (1H, dd, 2.2, 8.1)
9	159.8	
10	98.4	6.24 (1H, d, 2.2)
10a	161.8	
11	47.1	3.26 (1H, ddd, 1.2, 7.6, 8.2)
11a	81.3	5.48 (1H, dd, 0.6, 7.6)
11b	114.1	
12	117.5	6.65 (1H, dd, 0.7, 10.0)
13	130.3	5.58 (1H, d, 10.0)
14	77.1	
15	27.9[a]	1.35 (3H, s))[a]
16	28.2[a]	1.36 (3H, s)[a]

[a] These assignments are interchangeable.

Compound **1** had a molecular ion at *m/z* 338 in its ESIMS (with two exchangeables), and its composition of $C_{20}H_{18}O_5$ was deduced by HRDCIMS and supported by the ^{13}C-GASPE NMR spectrum. The ^{1}H-NMR spectrum (summarized in Table 1) showed the presence of nine multiplets between δ 7.18 and 4.83, an aliphatic ddd at δ 3.26, and two methyl singlets at δ 1.36 and 1.35. Homodecoupling experiments revealed the presence of an ABX, two AB, and an ABC spin system, while the ^{13}C-GASPE NMR spectrum confirmed the existence of eight quaternary, ten methines, and two methyl carbons. The aromatic ring D carbon assignments were based on their heteronuclear correlations with the ABX protons in which the chemical shifts of C-9 (δ 159.8) and C-10a (δ 161.8) indicated that they were oxygenated. The H-7 doublet of doublets shared a long-range 1.2 Hz coupling with benzylic H-11 and correlated with the C-11 (δ 47.1) methine. The H-11 proton at δ 3.26 in ring B shared vicinal couplings with the H-6 (δ 4.83) and H-11a (δ 5.48) methine protons. The chemical shifts of C-11a (δ 81.3) and C-6 (δ 96.8) were typical of an oxygenated and a doubly oxygenated carbon, respec-

* Phone: (610) 270-6315. FAX: (610) 270-4022. E-mail: Alan_J_Freyer@sbphrd.com.

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tively. The HMBC spectrum readily identified C-4a at δ 150.1 and C-11b at δ 114.1 and established that C-6 bore a hydroxyl group and was also connected to a pyran oxygen as evidenced by its downfield shift.

Because one of the two exchangeable hydrogens in **1** is the C-6 alcohol, only one of the oxygenated ring D carbons (C-9 and C-10a) could be phenolic. This was supported by the acetylation reaction product obtained from **1**. The ^{1}H-NMR spectrum of diacetate **2** contained two singlets (3H each) at δ 2.13 (alcoholic) and δ 2.39 (phenolic). Because the position of C-9 was too remote to be part of a ring system, it had to bear the phenol substituent, while the oxygenated C-10a was incorporated into furan ring C, which also included the oxygenated C-11a. The H-2 proton at δ 6.44 (J = 0.7, 8.4 Hz) in ring A was coupled to H-1 and showed a long-range 0.7 Hz coupling with aromatic H-12 in the adjacent ring. The HMBC correlations about ring A identified C-3 at δ 155.3 and C-4 at δ 111.5, but there were no correlations that might suggest that the pyran ring was attached to C-3. The assignments of the remaining 2,2-dimethylpyran ring carbons were based on HMBC correlations between H-12 (δ 6.65) and C-3 (δ 155.3), C-4 (δ 111.5), C-4a (δ 150.1), and C-14 (δ 77.1) as well as between the CH_3-15 and -16 protons and the two methyl carbons, the oxygenated quaternary C-14 and C-13 (δ 130.3).

Thus, **1** had a C-6 hydroxyl group that differed from phytoalexin 1, which has a C-11 hydroxyl substituent.[1] Considering the relative stereochemistry of the three consecutive methine protons in ring B of daleformis (**1**) H-6 shared an 8.2 Hz coupling constant with H-11, and H-11 shared a 7.6 Hz coupling with H-11a. These values suggested that H-11 shared either a 0° (coperiplanar) or a 180° (transperiplanar) dihedral angle with H-6 and H-11a. NOE saturation of H-11 produced an intense increase in the H-11a signal and a much smaller enhancement of the H-6 signal. Therefore, H-11 and H-11a were coperiplanar, and H-11 and H-6 were transperiplanar.

Treatment of daleformis (**1**) with Ac_2O and pyridine yielded its diacetate (**2**), which crystallized from MeOH. The crystal and molecular structure of compound **2** was firmly established by a single-crystal X-ray diffraction study. The structure was confirmed to be that of a novel benzofuran−benzopyran framework. The benzofuran−benzopyran ring fusion is cis leading to a roughly perpendicular bend in the molecular conformation. The molecular structure of daleformis diacetate **(2)** is shown in Figure 1, and the final atomic coordinates for the nonhydrogen atoms are given in Table 2.

Daleformis (**1**) had an IC_{50} of 9 μM for inhibition of ECE, while the diacetate **(2)** was totally inactive. Compound **1** was inactive in a functional assay and therefore will not be considered as an ECE lead.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. All homonuclear and heteronuclear 1D and 2D NMR data were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$. ESIMS were obtained in the negative mode on a Perkin-Elmer Sciex API-III triple quadruple mass spectrometer. The HRDCIMS were acquired on a VG-70SE using CH_4 and NH_3 gases.

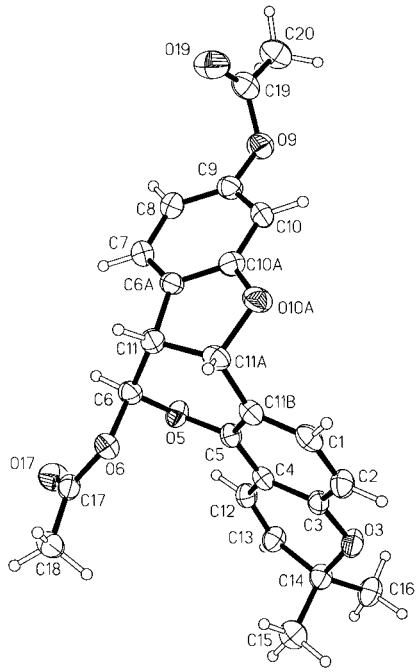

Figure 1. View of **2** showing the numbering scheme employed. Anisotropic displacement ellipsoids for nonhydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius.

Table 2. Atomic Coordinates [$\times 10^4$] and Equivalent Isotropic Displacement Parameters [Å$^2 \times 10^3$] for X1372A1

	x/a	y/b	z/c	U(eq)[a]
O3	−4565(2)	12566(5)	3048(1)	35(1)
O5	354(2)	11972(5)	2964(1)	30(1)
O6	1151(2)	8252(4)	3437(1)	29(1)
O9	2160(3)	15927(5)	106(1)	39(1)
O10A	104(3)	8849(5)	1196(1)	37(1)
O17	1914(3)	10755(5)	4307(1)	40(1)
O19	3977(3)	14117(6)	−395(1)	52(1)
C1	−2437(4)	8408(7)	2067(2)	33(1)
C2	−3582(4)	9522(7)	2341(2)	33(1)
C3	−3401(3)	11436(7)	2807(2)	27(1)
C4	−2074(4)	12290(7)	3012(2)	26(1)
C5	−945(3)	11071(7)	2736(2)	24(1)
C6A	1966(4)	10929(7)	1716(2)	28(1)
C6	1425(4)	10185(7)	2946(2)	29(1)
C7	2989(4)	12704(7)	1738(2)	34(1)
C8	3088(4)	14321(8)	1184(2)	36(1)
C9	2157(4)	14105(8)	623(2)	33(1)
C10	1129(4)	12312(7)	582(2)	31(1)
C10A	1062(4)	10736(7)	1140(2)	30(1)
C11	1553(4)	9025(8)	2243(2)	30(1)
C11A	152(4)	8054(7)	1923(2)	31(1)
C11B	−1087(4)	9159(7)	2259(2)	29(1)
C12	−1958(4)	14445(7)	3460(2)	28(1)
C13	−3064(4)	15293(7)	3766(2)	30(1)
C14	−4434(4)	13968(7)	3698(2)	30(1)
C15	−4590(4)	12165(8)	4295(2)	38(1)
C16	−5644(4)	15761(8)	3647(2)	39(1)
C17	1405(3)	8812(7)	4117(2)	29(1)
C18	983(4)	6771(8)	4579(2)	36(1)
C19	3182(4)	15806(8)	−365(2)	37(1)
C20	3121(5)	18091(8)	−804(2)	46(1)

[a] U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

Analytical and preparative TLC were carried out on precoated Si gel G (Kieselgel G_{254}) and reversed-phase (Whatman KC18F) plates. A Rainin HPXL solvent delivery system equipped with a refractive index detector, model 156, was used for HPLC separations employing a Lichrosorb Si 60 column. Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter. Re-

agent grade chemicals (Fisher and Baker) were used throughout.

Biological Assays. Biological assays were performed using a previously described procedure.[2]

Plant Material. *Dalea filiciformis* Snader (Fabaceae) was collected near Puebla, Mexico, for phytochemical investigators by the USDA under a cooperative agreement with the National Cancer Institute in January 1963. A voucher sample (B-613594) is preserved at the National Herbarium, Washington, DC.

Extraction and Isolation. Exhaustive extraction of part (25 g) of the total dried roots (0.85 kg) of *D. filiciformis* with MeOH−CH_2Cl_2 (1:1, 3 × 150 mL) afforded a brown residue (3.1 g) that was subjected to a polyamide column and eluted with MeOH. The eluent obtained from the polyamide column was evaporated under reduced pressure and temperature to yield the ECE active yellow residue (2.49 g), which was applied to a column of RP-18 Si gel. Elution with H_2O−MeOH (20:80), then with increasing percentages of MeOH in H_2O and finally with neat MeOH afforded several fractions that were collected and monitored by TLC. Like fractions were combined to produce 11 (A−K) individual fractions. The Si gel preparative TLC of the ECE inhibitory fraction D (0.163 g) using MeOH−CH_2Cl_2 (5:95) followed by Si gel HPLC (EtOAc−hexane 35:65; flow rate 3.5; RI detection) yielded four compounds of which only daleformis, (**1**) (32 mg) was shown to inhibit ECE.

Daleformis (**1**). white powder; $[\alpha]_D$ −8.6° (*c* 1.04, MeOH); UV (MeOH) λ_{max} 238 and 289 nm; IR (KBr) ν_{max} 3243, 3000−3100, 1642, 1625, 1609, 1585, 1497, 1375, 1076, and 732 cm^{-1}; ^1H- and ^{13}C-NMR data, see Table 1; LRESIMS *m/z* 338 (M^+); HRDCIMS 338.1143 (M^+, calcd for $C_{20}H_{18}O_5$, 338.1154).

Acetylation of Daleformis (1). Daleformis (10 mg) was dissolved in Ac_2O−pyridine (1:1, 1 mL), and the reaction mixture was left at room temperature for 12 h. H_2O was then added, and the mixture was extracted with $CHCl_3$. Diacetate **2** was purified by Si gel preparative TLC with an EtOAc−hexane mixture (10:90) as a colorless powder that crystallized from MeOH as white crystals (11 mg): $[\alpha]_D$ −11.4 (*c* 0.31, MeOH); UV (MeOH) λ_{max} 235 and 285 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29 (1H, d, J = 8.1 Hz), 7.21 (1H, d, J = 8.2 Hz), 6.65 (1H, dd, J = 1.2, 8.1 Hz), 6.59 (1H, d, J = 10.0 Hz), 6.55 (2H, m), 6.21(1H, d, J = 8.0 Hz), 5.70 (1H, d, J = 8.1 Hz), 5.55 (1H, d, J = 10.0 Hz), 3.79 (1H, ddd, J = 1.2, 7.4, 8.1 Hz), 2.39 (3H, s), 2.13 (3H, s), 1.41 (3H, s), 1.39 (3H, s); LRESIMS *m/z* 422 (M^+); HRDCIMS 422.1358 (M^+, calcd for $C_{24}H_{22}O_7$, 422.1365).

X-ray Crystal Determination of Daleformis Diacetate (2). Data collection and structure refinement. A suitable crystal grown by slow evaporation from MeOH was flash cooled in a steam of N_2 gas to 223(2) K. Lattice parameters were determined from the setting angles of 25 reflections well distributed in reciprocal space measured on an Enraf Nonius CAD-4 diffractometer. A full sphere of intensity data to $2\theta \leq 125°$ were collected on the diffractometer using graphite monochromated CuKα radiation and an $\omega - 2\theta$ variable speed scan technique.[3] Three orientation controls were monitored to assess any crystal movement during the experiment. The intensities of three reflections measured at the beginning, end, and every 3 h of exposure time showed a variation of 7.6%. Data were corrected for this variation and for Lorentz and polarization effects. No corrections were made for absorption. The 3732 reflections measured were averaged, R_{merge} = 0.031, to 3178 measurements of which 1383 were Friedel mates. The structure was solved by direct methods using the SHELXS program[4] and refined using the SHELXL-93 program.[5] Positions for non-hydrogen atoms were eventually refined with anisotropic displacement parameters. The positional and isotropic displacement parameters for the hydrogens attached to heteroatoms were refined. Other hydrogen atoms were included in idealized positions riding on the atom to which they are attached with isotropic displacement factors assigned as a constant (1.2) times U(eq) of the attached atom. The full-matrix least-squares refinement (on F^2) of 284 parameters converged (Δ/σ_{max} = 0.00) to values of the conventional crystallographic residuals R = 0.045 for 2445 observed data [$I > 2\sigma(I)$] and R = 0.074 (wR_2 = 0.114) for all 3178 data. The function minimized was $\Sigma w(F_o^2 - F_c^2)^2$. Weights ($w$) were eventually assigned to the data as $w = 1/[\sigma^2(F_o^2) + (0.0491P)^2 + 0.3235P]$ where $P = [MAX(F_o^2,0) + 2F_c^2]/3$. A final difference Fourier map showed residual density between +0.19 and −0.18 e $Å^{-3}$. Values of the neutral atom scattering factors and real and imaginary dispersion corrections were taken from the *International Tables for X-ray Crystallography*.[6]

The absolute configuration was assigned on the basis of an R-factor ratio test and the least-squares absolute structure parameter.[7,8] The ratio of weighted R values, 0.1142/0.1140 = 1.0018 permits assignment with a confidence level greater than 99%. Consistent with this, the absolute structure refined to a value of 0.1(3) for the reported absolute configuration and 0.9(3) for the inverted model.[9]

Crystal Data. $C_{24}H_{22}O_7$, M = 422.42, clear colorless needles, 0.44 × 0.06 × 0.04 mm, space group $P2_1$, Z = 2, a = 9.639(2) Å, b = 5.386(2) Å, c = 19.363(4) Å, β = 92.28(2)°, V = 1004.4(5) $Å^3$, D(calcd) = 1.397 Mg m^{-3}, F(000) = 444, μ(Cu Kα) = 0.856 mm^{-1}.

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References and Notes

(1) Lyne, R.; Mulheirn, L.; Leworthy, D. *J. Chem. Soc. Chem. Comm.* **1976**, *19*, 498.
(2) Patil, A. D.; Freyer, A. J.; Breen, A.; Carte, B.; Johnson, R. K. *J. Nat. Prod.* **1996**, *59*, 606−608.
(3) *CAD-4 Operations Manual*; Enraf-Nonius: Delft, The Netherlands, 1977.
(4) Sheldrick, G. M. *Acta Cryst.* **1990,** *A46*, 467−473.
(5) Sheldrick, G. M. SHELX93. *Program for the Refinement of Crystal Structures*, University of Gottingen: Gottingen, Germany, 1993.
(6) *International Tables for Crystallography*, Wilson, A. J. C., Ed.; Kluwer Academic: Dordrecht, 1992; Vol. C.
(7) Flack, H. D. *Acta Cryst.* **1983**, *A39*, 876−881.
(8) Flack, H. D.; Schwarzenbach, D. *Acta Cryst.* **1988**, *A44*, 499−506.
(9) The author has deposited atomic coordinates for **2** with the Cambridge Crystallographic Data Centre. The coordinates can be obained, upon request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, U.K.

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