Novel Bioactive Breviane Spiroditerpenoids from *Penicillium brevicompactum* Dierckx

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The structures of four new, naturally occurring bioactive spiroditerpenoids, (+)-breviones B, C, D, and E, potential allelopathic agents, have been determined from extracts of semisolid fermented *Penicillium brevicompactum* Dierckx. The structures display the novel breviane spiroditerpenoid skeleton. Structure elucidation was performed by chemical transformations and by homo- and heteronuclear 2D-NMR spectral data. On the basis of combined studies of the theoretical conformations and NOEDIFF data, their relative stereochemistry is proposed. A mixed biogenesis for this novel family of spiroditerpenoids is tendered. The levels of activity shown by breviones B, C, and E in the etiolated wheat coleoptiles bioassay, especially breviones E (100% inhibition) and C (80% inhibition) both at 10^{-4} M, suggest them as lead compounds for new agrochemicals.

Introduction

The discovery of new allelochemicals[1,2] from plants and microorganisms has attracted much interest over the last 20 years because of their ecological and utilitarian characteristics. They are bioactive natural products, produced within a specific territory, that profoundly influence their associated biosphere. Often, they offer new and diverse chemical templates which may be elaborated either by biotransformation, or limited synthesis, to produce environmentally compatible agrochemicals.[3] Among these are potential herbicides, antimicrobials, pesticides, and plant growth regulators. In addition, most organic natural products are intrinsically biodegradable. For example, it is becoming increasingly important to develop premixes of herbicides that employ different modes of action, to obtain both short- and long-term control of diverse weed populations.

The genus *Penicillium* is noted for producing a variety of bioactive metabolites possessing diverse biological properties including plant growth regulators.[4] In particular, *P. brevicompactum* produces polyketides, peptides, and a collection of diverse heterocyclic compounds with pyrrole, pyrroline, and oxazine structures[5] that exhibit several biological properties such as anti-juvenile-hormonal, insecticidal, and fungicidal activity and are potent agonists of D-*myo*-inositol-1,4,5-triphosphate receptors.[6]

In continuing our studies with microorganisms from New Zealand, we now report the discovery of four breviones from *P. brevicompactum* Dierckx.

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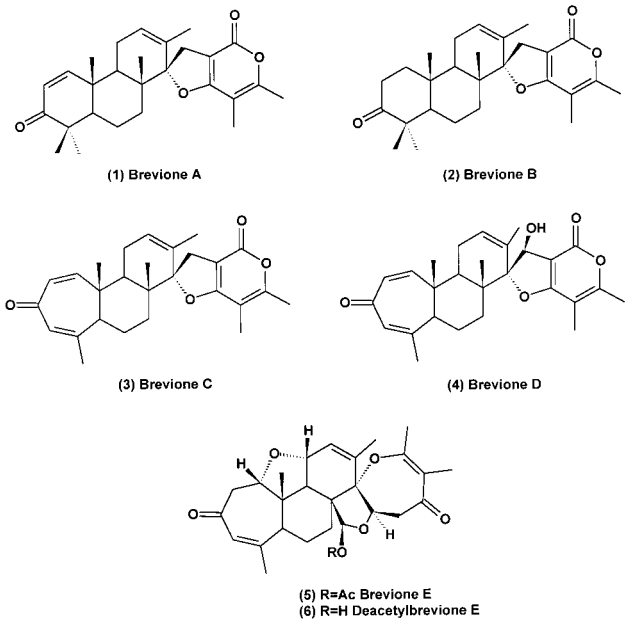

Figure 1. New isolated breviones from *Penicillium brevicompactum* Dierckx.

Results and Discussion

During the course of examining fungi for biologically active natural products, we accessed a strain of *P. brevicompactum* Dierckx from leaf litter collected in the Waipoua Forest, New Zealand. Upon subsequent semi-solid fermentation, compounds **1**−**5** were isolated from the ethyl acetate fraction using an etiolated wheat coleoptiles bioassay to detect bioactivity in the crude extract and to direct the fractionation.[7]

Recently, we reported the first of a new class of bioactive spiroditerpenoids, brevione A (**1**)[8] (Figure 1), a diterpenoid derivative with a polyketide moiety and a spiranic connection. Further studies of these fractions afforded four new related compounds, brevione B (**2**), C (**3**), D (**4**), and E (**5**).

Brevione B (**2**) gave an HREIMS with a molecular ion at *m*/*z* 425.2666, $[M + 1]^+$ corresponding to a molecular formula $C_{27}H_{36}O_4$. The IR showed absorption at 1709 cm^{-1} indicating the presence of carbonyl groups. The ^1H NMR spectrum of **2** (Table 1) strongly resembled that of brevione A (**1**).[8] Thus, seven singlets at δ 0.95, 1.05, 2 × 1.08, 1.64, 1.92, and 2.21 were assigned to the seven methyl groups of the breviane skeleton. The signal at δ 5.66 (brs, 1H) was assigned to the H-12 olefinic proton. The signals at δ 3.03 (1H, d, H-15a) and δ 2.89 (1H, d, H-15b) were typical of the methylene group of a spirane ring. The main difference in the ^1H NMR spectra between compound **2** and brevione A (**1**)[8] was the absence of signals corresponding to the C1−C2 double bond; these data clearly indicated that **2** is 1,2-dihydrobrevione A (Figure 1). A comparison between the ^{13}C NMR data of both compounds (Table 2) provided confirmation of the proposed structural assignments. The chemical shifts of C-1 and C-2 signals (δ 38.6 and δ 33.8), the downfield shift of C-3 resonance (δ 216.8, $\Delta\delta_{1,2}$ = 12.1), and the HMBC correlations confirmed the nature of the A ring of brevione B (**2**). Thus, compound **2** has been established as 3-oxo-14,3′-oxybrevia-12,2′,4′-triene 1′,5′-lactone.

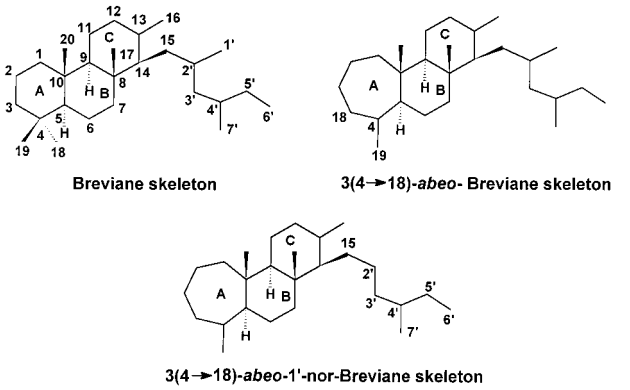

Figure 2. Breviane skeletons.

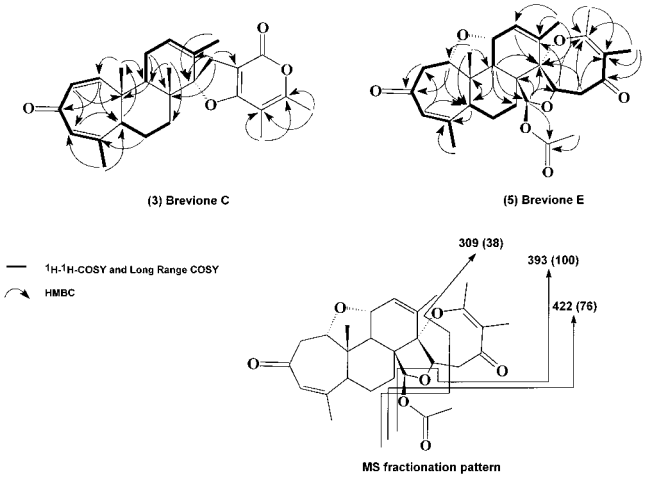

Figure 3. Selected 2D NMR correlations of brevione C (**3**) and E (**5**) and MS fragmentation of **5**.

Brevione C (**3**) HREIMS showed a molecular ion at *m*/*z* 420.2315 in accordance with the molecular formula $C_{27}H_{32}O_4$. The ^1H and ^{13}C NMR spectra had a typical breviane pattern. Indeed, the signals of the $\alpha,\beta,\gamma,\delta$-unsatured δ-lactone moiety connected to the spiranic ring and C ring are identical to brevione A (**1**) signals.[8] The main difference observed in **3** with respect to compound **1** was in the A ring. The ^1H NMR spectrum showed three signals at δ 6.48 (1H, d, H-1) δ 6.07 (1H, brs, H-18), and δ 5.86 (1H, dd, H-2) which were assigned to three olefinic protons of a cross-conjugated system. Studies of the ^1H−^1H COSY spectrum (Figure 3) allowed us to establish the correlations of the signals at δ 6.47 and δ 5.86 and the long-range COSY effects of the signal at δ 6.07 with δ 5.86, δ 2.94 (1H, dd, H-5) and δ 1.95 (1H, s, H-19) corresponding to a −CH═CH−CO−CH═C(CH_3)−CH−

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Table 1. ^{1}H NMR Data (399.95 MHz, *J* values in hertz) for Breviones A−E (1−5) and 6 ($CDCl_3$, signal of residual $CHCl_3$, centered at δ 7.25)

H	**1**[8]	**2**	**3**	**4**[a]	**5**	**6**
1α		1.52 m			-	-
	7.07 d *J* = 10.2		6.48 d *J* = 12.5	6.47 d *J* = 12.5		
1*β*		1.94 ddd *J* = 13.3, 7.2, 3.7			3.85 dd *J* = 10.8, 5.0	3.89 dd *J* = 11.0, 4.9
2α		2.40 ddd *J* = 15.8, 6.8, 3.7				
	5.85 d *J* = 10.2		5.86 dd *J* = 12.5, 2.2	5.85 dd *J* = 12.5, 2.2	2.85 m	2.87 m
2*β*		2.57 ddd *J* = 15.8, 11.1, 7.2				
5	1.58 m	1.34 m	2.94 dd *J* = 10.0, 7.0	3.01 brd *J* = 9.9	2.97 brd *J* = 10.4	2.97 brd *J* = 10.3
6α				1.63 m		
	1.58 m	1.55m	1.69 m		1.93 m	1.93 m
6*β*				1.73 dddd *J* = 13.4, 13.4, 13.4, 2.4		
7α	1.38 m	1.32 m	1.34 m	1.63 m	1.72 ddd *J* = 13.1, 13.1, 5.0	1.68 m
7*β*	1.61 m	1.54 m	1.52 ddd *J* = 13.0, 3.3, 3.3	2.61 ddd *J* = 12.9, 3.3, 3.3	1.64 m	1.59 m
9α	1.95 dd *J* = 12.0, 5.1	1.74 dd *J* = 9.6, 7.5	1.97 m	1.97 m	1.94 d *J* = 11.4	1.91 d *J* = 11.2
11α	2.27 ddd *J* = 17.7, 5.1, 5.1		2.30 brd *J* = 17.8		-	-
		2.04 m		2.26 m		
11*β*	2.14 brdd *J* = 17.7, 12.0		2.14 brdd *J* = 17.8, 12.0		4.45 ddd *J* = 11.4, 1.7, 1.7	4.58 ddd *J* = 11.2, 1.7, 1.7
12	5.70 brd *J* = 5.1	5.66 brs	5.73 brd *J* = 4.1	5.80 m	6.07 brdd *J* = 1.7, 1.4	6.06 brdd *J* = 1.7, 1.4
15a	3.05 d *J* = 15.8	3.03 d *J* = 15.7	3.03 d *J* = 15.8	5.42 d *J* = 3.2	-	-
15b	2.90 d *J* = 15.8	2.89 d *J* = 15.7	2.89 d *J* = 15.8		4.56 d *J* = 6.8	4.68 d *J* = 6.9
16	1.65 brs	1.64 brs	1.64 brs	1.51 brs	1.62 t *J* = 1.4	1.62 t *J* = 1.6
17	0.99 s	0.95 s	1.03 s	1.39 s	6.23 s	5.40 d *J* = 2.2
18	1.13 s	1.08 s	6.07 brs	6.06 brs	5.91 brs	5.94 brs
19	1.10 s	1.05 s	1.95 s	1.95 brs	1.90 brs	1.93 brs
20	1.18 s	1.08 s	1.09 s	1.11 s	0.90 s	1.13 s
2′	-	-	-	-	3.39 brd *J* = 20.1	3.38 brd *J* = 19.9
2′	-	-	-	-	3.19 ddq *J* = 20.1, 7.4, 1.9	3.21 ddq *J* = 19.9, 7.4, 1.9
6′	1.91 s	1.92 s	1.90 s	1.94 d *J* = 0.7	2.19 s	2.21 s
7′	2.21 s	2.21 s	2.21 s	2.24 d *J* = 0.7	1.94 brs	1.95 brs

[a] **4**: δ 2.71 d (*J* = 3.1, O*H* of C-15), **5**: δ 2.04 s (*CH*$_3$−CO), **6**: δ 2.35 d (*J* = 2.2, O*H* of C-17).

Table 2. ^{13}C NMR Data (100.577 MHz) for Breviones A−E (1−5) ($CDCl_3$, centered at 77.0 ppm)

C	**1**[8]	**2**	**3**	**4**	**5**[d]
1	157.7 d	38.6 t	154.1 d	154.5	85.2
2	125.5 d	33.8 t	128.2 d	130.2[c]	48.8 t
3	204.7 s	216.8 s	192.8	192.8	196.6
4	44.4 s	47.1 s	154.4	155.2	160.3
5	53.0 d	54.7 d	47.8	48.0	45.6
6	18.2 t	18.7 t	21.4	21.6	23.5
7	31.9 t	31.5 t	30.0	30.1	31.0
8	41.3 s	40.6 s	41.1[b]	42.7	49.7
9	42.1 d	46.7 d	41.3	41.8	53.9
10	38.9 s	36.2 s	41.4[b]	42.7	45.5
11	22.8 t	22.9 t	24.1	24.4	73.3 d
12	127.2 d	127.6 d	127.6	128.2	129.1
13	132.1 s	131.4 s	132.1	131.2	135.9
14	99.3 s	99.3 s	99.4[a]	100.2	97.2
15	28.5 t	28.4 t	28.4	75.1 d	84.7
16	18.2 q	18.2 q	18.1	19.1	18.3
17	16.3 q	15.7 q	15.8	16.1	100.9 d
18	27.7 q	26.2 q	130.8 d	130.7[c]	130.8
19	21.5 q	21.2 q	23.8	23.8	23.0
20	18.9 q	15.3 q	13.5	13.7	17.9
1′	170.9 s	170.8 s	171.0	171.1	-
2′	99.3 s	99.2 s	99.1[a]	100.2	39.3 t
3′	161.8 s	161.7 s	161.9	162.4	199.5
4′	102.7 s	102.6 s	102.8	103.3	106.9
5′	160.4 s	160.1 s	160.6	158.2	168.4
6′	9.5 q	9.4 q	9.6	9.4	29.1
7′	17.1 q	16.9 q	17.2	17.4	12.7

[a,b,c] These values may be interchanged within the same column.
[d] **5**: δ 169.2 s (CH$_3$−*CO*), δ 21.4 q (*CH*$_3$−CO).

moiety. The shielded signal of C-3 in the ^{13}C NMR spectrum (δ 192.8) suggested its placement between two double bonds, and the HMBC experiment (Figure 3) corroborates that this seven-carbon moiety consisted of the carbons of the A ring which had undergone a bond migration from C3−C4 to C3−C18 with respect to the breviane skeleton.

The relative stereochemistry was assigned on the basis of a NOESY experiment (mixing time = 600 ms) (Figure 4) where all chiral centers of compound **3** retained the relative configuration of compound **1**. On the basis of the observed NOE effects, the relative stereochemistry of H-5 and H-20 were established. Clear effects between H-11*β*, H-17, and H-20 ("*β*" side) and between H-5, H-7α, and H-9 ("α" side) allow for the assignment of an α and *β* orientation for H-5 and H-20, respectively.

Brevione C (**3**) is the first naturally occurring 3(4→18)-*abeo*-breviane, and its systematic name can be established as: 3-oxo-14,3′-oxy-3(4→18)-*abeo*-brevia-1,4(18),-12,2′,4′-pentaene 1′,5′-lactone, (Figure 2). Biogenetically a correlation between **3** and brevione A (**1**), as indicated (Figure 6), can be proposed.

Brevione D (**4**) was isolated as a minor compound with molecular formula $C_{27}H_{32}O_5$, obtained from HRMS. The ^{1}H NMR spectrum (Table 1) showed three double bond signals at δ 6.47 (1H, d, H-1), δ 6.06, (1H, brs, H-18) and δ 5.85 (1H, dd, H-2) identical to those of the A ring of brevione C (**3**). Comparison of the ^{13}C NMR spectra (Table 2) of **3** and **4** confirmed that **4** had a 3(4→18)-*abeo*-breviane skeleton. Considering that in the ^{1}H NMR spectrum a doublet appeared at δ 5.42 (1H, H-15) coupled with the hydroxyl proton signal (D_2O interchangeable)

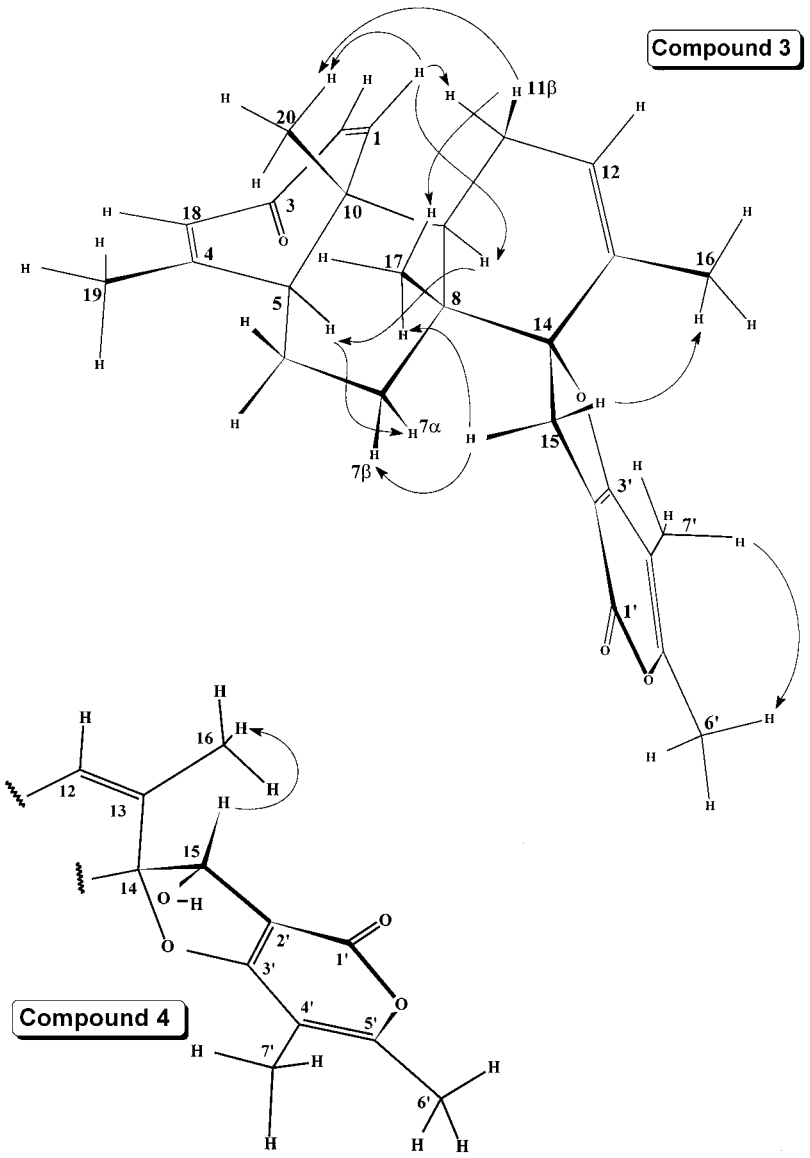

Figure 4. Selected NOE effects of compound **3** and of spiro fragment of compound **4**.

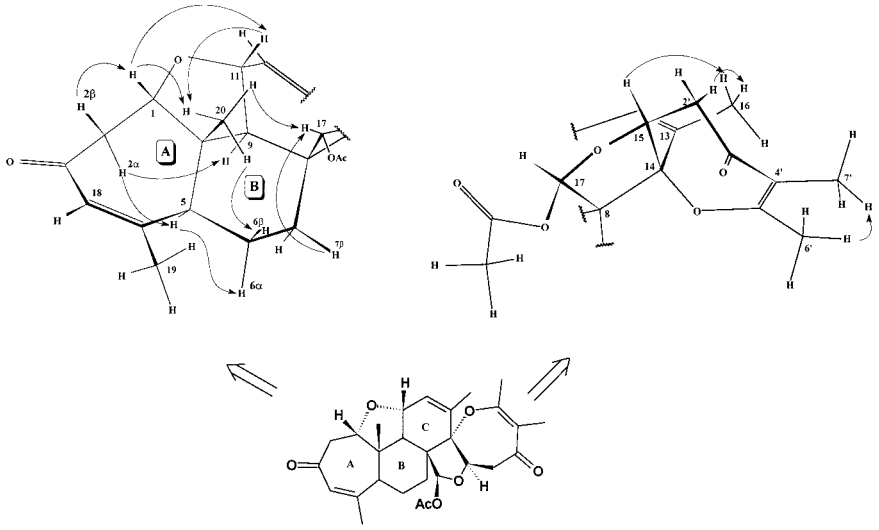

Figure 5. Selected NOE effects of two fragments protons of **5**.

at δ 2.71 (1H, d, O*H*) and the typical signal for methylene C-15 of brevione C (**3**) had disappeared, we propose that compound **4** is 15-hydroxybrevione C (Figure 1). The presence of the signal δ 75.1 d in ^{13}C NMR and a hydroxyl

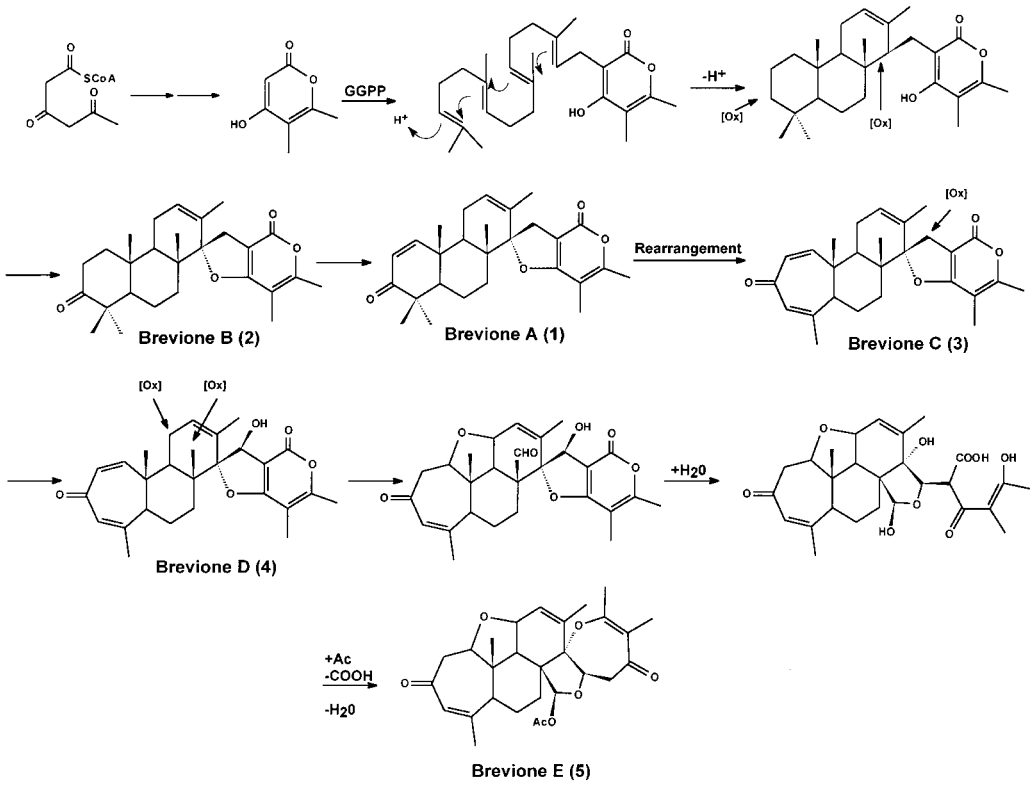

Figure 6. A postulated biogenetic origin of breviones A−D (**1**−**5**).

signal at 3320 cm^{-1} IR confirmed the structure of brevione D (**4**).

The relative stereochemistry of the chiral center at C-15 was established as *S** because of the important deshielded effect of the H-7β signal (δ 2.61 ddd, $\Delta\delta_{4,3}$ = 1.09) and was confirmed with the observed NOE effect between H-15 and H-16 (Figure 4). The systematic name of **4** was determined as 15(*S**)-15-hydroxy-3-oxo-14,3′-oxy-3(4→18)-*abeo*-brevia-1,4(18),12,2′,4′-pentaene 1′,5′-lactone (Figure 2).

Brevione **E** (**5**) was isolated as a colorless oil. The ^{1}H NMR (Table 1) showed that **5** had a structure, a priori, unrelated with compounds **1**−**4**. This spectrum had diterpene moiety signals, but not the cyclic $\alpha,\beta,\gamma,\delta$-unsatured δ-lactone moiety signals. The ^{13}C NMR, APT, and ^{1}H−^{13}C HETCOR spectra revealed a total number of 34 protons attached to carbons (8CH, 4CH_2, and 6CH_3) and 10 additional quaternary carbons. The HRMS showed a molecular ion at 482.2309 corresponding to a molecular formula $C_{28}H_{34}O_7$. The 12 unsaturation degrees required by the molecular formula were assigned, after a detailed study of HMQC and HMBC experiments, to three carbonyls (^{13}C NMR: δ 199.5, 196.6, 169.2), three double bonds [^{13}C NMR: δ 135.9 and 129.1; δ 160.3 and 130.8 (conjugated with a carbonyl group); and δ 168.4 and 106.9 (enol double bond)], structurally leaving six rings. The IR spectrum showed no hydroxyl absorptions, but contained an ester carbonyl (1750 cm^{-1}) and two α,β-unsatured carbonyl bands at 1677 and 1644 cm^{-1}. The ^{1}H−^{1}H COSY (Figure 3) showed correlation between δ 3.85 (1H, dd, H-1β) and δ 2.85 (2H, m, H-2). The H-2 signal showed a long-range correlation with δ 5.91 (1H, brs, H-18), and this olefinic signal had allylic couplings with δ 1.90 (3H, brs, H-19) and δ 2.97 (1H, brd, H-5). These correlations suggested the arrangement −O−CH−CH_2−CO−CH=C(CH_3)− as in the A ring of **3** (4→18)-*abeo*-breviane skeleton the C-1 being oxygenated; the HMBC data confirmed this sequence. The signal at δ 2.97 indicated the connection between the A and B rings. So, the ^{1}H−^{1}H COSY (Figure 3) showed correlations between H-5, H-6 (δ 1.93, 2H, m), H-7α (δ 1.72, 1H, ddd), and H-7β (δ 1.64, 1H, m). The long-range HMBC correlation observed between the proton signal at δ 0.90 (3H, s, H-20) and the carbon signals at δ 85.2 (C-1), δ 53.9 (C-9), and δ 45.6 (C-5) implied the presence of the angular methyl (H-20) between the A and B rings.

The signal at δ 6.07 (1H, brdd, H-12) gave ^{1}H−^{1}H COSY coupling with δ 4.45 (1H, ddd, H-11β) and allylic coupling with δ 1.62 (3H, t, H-16). The H-11β signal showed a correlation with δ 1.94 (1H, d, H-9α). This COSY sequence suggested a −CH−CHOR−CH=C(CH_3)− substructure; ^{13}C NMR data and HMBC correlation of these carbons determined that this moiety was C9/C11/C12/C13/C16 (Figure 3), being a C-11 oxygenated carbon. The absence of hydroxyl bands in the IR of **5** suggested the presence of an ether bridge between C-1 and C-11.

Breviane E (**5**) presented a modified methyl group at C-8. The singlet at δ 6.23 (1H, H-17) corresponding to δ 100.9 (C-17) presented long-range HMBC correlations with δ 97.2 (C-14), δ 49.7 (C-8), and δ 31.0 (C-7) which is in agreement with a modified H-17. The chemical shift of C-17 indicated the presence of an acetal function.

The HMBC correlations between δ 2.04 (3H, s, *CH_3*−CO) and δ 169.2 (CH_3−*CO*) determined the presence of an acetyl group in **5**. Further correlations between H-17 and CH_3CO− suggested the existence of an acetyl ester at C-17. The hydrolysis of **5** with 0.05M H_2SO_4 (80 °C for 1 h) gave deacetylbrevione E (**6**). Its ^{1}H NMR (Table 1) showed an important shielding of the H-17 signal (δ 5.40, 1H, d, $\Delta\delta_{5,6}$ = 0.83) and its coupling with a hydroxyl proton signal at δ 2.35 (1H, d, D_2O exchanged). These data confirmed the position of an acetyloxy group at C-17

and determined that the resulting hemiacetal remained relatively stable, as expected with a cyclic stabilization.

The last subunit was established with $^1H-^1H$ COSY correlations (Figure 3) between methylene protons δ 3.39 (1H, brd, H-2′), δ 3.19 (1H, ddq, H-2′); methyne proton δ 4.56 (1H, d, H-15); and methyl protons δ 1.94 (3H, brs, H-7′). The H-7′ was in a dimethylenolate system and was confirmed with chemical shifts of C-4′ (δ 106.9) and C-5′ (δ 168.4), and the long-range HMBC correlations of H-6′ (δ 2.21, 3H, s) and H-7′ with C-3′ (δ 199.5), C-4′ (δ 106.9), and C-5′(δ 168.4). The long-range COSY correlation between H-2′and H-7′ suggested the presence of a $-CHOR-CH_2-CO-C(CH_3)=C(CH_3)-O-$ subunit (Figure 3), which was confirmed by HMBC correlations.

The existence of a seven-member spiranic ring attached at C-14 and a pentacyclic acetyl hemiacetal between C-15 and C-17 allows for a total of six rings for compound **5**, and the stability of an hemiacetal of **6**. The long-range HMBC connections (Figure 3) of H-17 with C-15 (δ 84.7); H-15 with C-14 (δ 97.2); C-13 (δ 135.9) and C-5′; and H-2′ with C-14, and the long-range COSY correlation between H-17 and H-15, confirmed the nature of these two rings.

A detailed study of the MS fragmentation pattern of **5** showed, as more abundant peaks, the fragments corresponding to the acetyl group and the D and E rings (Figure 3).

The complete structure of brevione E (**5**) was further supported by NOESY experiments. The relative stereochemistry of **5** was deduced from the combined analyses of NOESY data and $^1H-^1H$ coupling constants (Figure 5). The A, B, and C rings had a disposition similar to compounds with the 3(4→18)-*abeo*-breviane skeleton. The relative α orientation of the C−O bonds in the tetrahydrofuranic ring was established by the observed NOE effects between H-1β, H-11β, and H-20. The C-17 had a relative β orientation, according to the observed NOE effects of H-17 and protons of the acetyl group placed in the "β" side of **5** (H-17 with H-20 and H-11 with CH_3−CO) and the deshielding observed effects for H-11β, H-15, and H-20 signals of the deacetyl compound **6**. The relative stereochemistry of C-15 was assigned as *S** as in **4**, based on the observed NOE effects between H-15 and H-16. The relative stereochemistry of the final new chiral center at C-17 was assigned as *R**, based on the coplanarity of H-17 and H-15 according to a ^4J coupling observed in $^1H-^1H$ COSY by a "W" path. The coplanarity of both protons were in agreement with the lack of NOE effects between each.

Brevione E (**5**) is the first naturally occurring 3(4→18)-*abeo*-1′-nor-breviane (Figure 2), and its systematic name can be established as (15*S**,17*R**)-17-acetoxy-3,3′-dioxo-1,11:14,5′:15,17-trioxy-3(4→18)-*abeo*-1′-nor-brevia-4(18),-12,4′-triene. This compound presents an atypical cyclic arrangement that may be biogenetically related to brevione D (**4**) as indicated (Figure 6).

Possible Mixed Biogenetic Origin of the Breviones A−E. The mixed biogenetic origin of mycophenolic acid has been reported for *P. brevicompactum*.[9] Recent experiments have demonstrated that its biogenesis proceeds through combined biogenesis from acetate and mevalonate pathways. Three units of acetyl-SCoA may be the precursor of the α,β,γ,δ-unsatured δ-lactone moiety. The methylation may possibly occur prior to the ring closure, as in mycophenolic acid biogenesis[9] (Figure 6).

An addition of geranylgeranyl, as the side-chain, may be similarly proposed as in mycophenolic acid, followed by cyclization, as proposed for the spongian diterpenes,[10] to obtain the breviane skeleton. An oxidation of C-3 and C-14 and cyclization to produce the spirocycle could lead brevione B (**2**). A dehydrogenation of **2** could yield brevione A (**1**). The rearrangement of the A-ring of **1** following to a dehydrogenation could yield brevione C (**3**) (Figure 3). The oxidation of C-15 of brevione C (**3**) may generate brevione D (**4**) (Figure 6).

Brevione D (**4**) as precursor of brevione E (**5**) may be proposed if we consider the fact that the relative stereochemistry *S** for C-15 is the same in compounds **4** and **5**. An oxidation of C-1, C-11, and C-17 could be the genesis of the tetrahydrofuranic cyclization between the A and B ring and an aldehyde group at C-8. The opening of the spiranic and lactone rings might allow for the formation of the hemiacetal ring and final acetylation. Decarboxylation and formation of the seven-membered spirocycle may yield brevione E (**5**).

Bioassay Data and Discussion. The most active metabolite tested against etiolated wheat coleoptiles was brevione E, with significantly inhibited growth 100% at 10^{-4} M ($P < 0.01$), relative to controls. Brevione C induced an 80% inhibition at 10^{-4} M, while brevione A gave 40% inhibition only at 10^{-3}M, and brevione B gave 38% inhibition at 10^{-3} M (all significant inhibition at $P < 0.01$). Unfortunately, not enough of brevione D or deacetylbrevione E was available for bioassay. All those metabolites exhibiting biological activity possessed certain structural features associated with compounds that have been shown to be bioactive. These include lactones and/or α,β-unsaturated ketones. On the basis of these results, we also suggest that an expansion of the A ring, in compounds C and E, may account for an increase in bioactivity.

The levels of activity shown by breviones B, C, and E in the coleoptiles bioassay, especially brevione **E** (100% inhibition) and C (80% inhibition) at 10^{-4} M, suggest that they may be lead compounds for new agrochemicals.

Experimental Section

General. Mass spectra were obtained using a HRMS spectrometer at 70 eV. Optical rotations were determined using a polarimeter set on the sodium D line. ^1H NMR and ^{13}C NMR spectra were made at 399.952 and 100.577 MHz, respectively, using $CDCl_3$. The resonance of residual chloroform at δ_H 7.25 and δ_C 77.00 was used as the internal reference for ^1H and ^{13}C spectra, respectively. Column chromatography was performed on silica gel (70−230 mesh), and TLC analyses were carried out using aluminum precoated silica gel plates. High-performance liquid chromatography separations on Li-Chrosorb silica 60 were in normal mode phase using a differential refractometer (RI) and a UV detector. All solvents were spectral grade or distilled from glass prior to use.

Fungal Material. A strain of *P. brevicompactum* Dierckx was collected in New Zealand. A voucher specimen is deposited at Mercer University with the code NZ-481.

Description of Fungi Characterization. Penicilli were irregular on all standard media (CYA, MEA & G25N) but

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slightly more regular of Difco oatmeal agar, which was the only medium to promote intense sporulation, yielding a lawn of deep, uniform color (glaucous gray-green). Penicilli on all media were terverticillate, or more frequently with one or more rami terverticillate and the other(s) quaterverticillate or with one or more rami biverticillate and the other(s) terverticillate. Regular biverticillate penicilli were rare in comparison, but were always readily located with some searching. Most penicilli had two (sometimes three, rarely four) rami and frequently had metulae and phialides, and/or metulae and rami at the same branching level. Most frequently, one or more phialides or metuale displayed indeterminate growth, i.e., proliferating as a hyphae which might bear another penicillus at a distance. Penicilli on G25N exhibited up to five or more levels of branching, but were also highly irregular.

It is often the tendency of penicilli toward irregularity and (especially on G25N) proliferation to higher branching orders (pentaveticillate or hexaverticillate on some rami) that makes NZ-481 difficult to assign with certainty. The stipes are also somewhat shorter than described for *P. brevicompactum*. Pitt [11] comments on a *P. monstrosum* Sopp which is not described in standard references on *Penicillium*, but which has irregular, inflated penicilli and is placed into synonymy with *P. brevicompactum*.

Semisolid Fermentation. The fungus was cultured on potato dextrose agar slants at 26 °C for 10 days and then maintained at 5 °C.

The fungus was transferred to Fernbach flasks (2.8 L), each containing 100 g of shredded wheat, 200 mL of Difco mycological broth (pH 4.8), 2% yeast extract, and 20% sucrose[12] for secondary metabolite production. Inoculated flasks were incubated at 24 °C for 19 days.

Extraction and Isolation. Following incubation, 300 mL of acetone was added to each semisolid fermentation flask. The mycelia and substrate were macerated with a Super Dispax homogenizer, and the suspension was strained through cheesecloth to remove the pulp. The liquid was then filtered through Whatman No. 1 filter paper on a Büchner funnel. The clarified filtrate was reduced under vacuum to yield an aqueous phase. This was extracted twice with ethyl acetate; each volume of solvent was equal to twice that of the aqueous portion. Combined ethyl acetate extracts were dried over anhydrous sodium sulfate and reduced to a small volume under vacuum and chromatographed over a silica gel column (365 g silica gel 60, 10 × 13 cm). The column was stepwise eluted with C_6H_6 (1 L), diethyl ether (1 L), EtOAc (1 L), Me_2CO (1 L), and CH_3CN (1 L). Each fraction was bioassayed in the etiolated wheat coleoptile bioassay.[13,14] The EtOAc fraction inhibited the growth of etiolated wheat coleoptiles 100% ($P < 0.01$) relative to controls.

The EtOAc fraction (7.2 g) was partitioned by column chromatography on silica gel using hexane:acetone (4:1) to afford six fractions. Fractions C and D were studied. The major product from fractions D and E was mycophenolic acid.[15]

Fraction C (1.6 g) was chromatographed using silica gel and eluted with hexane:acetone (9:1), yielding nine fractions. Fraction F (270 mg) was chromatographed using silica gel and eluted with hexane:acetone (4:1). Product **2** (brevione B) was obtained and further purified using silica gel and eluting with chloroform, yielding 50 mg of **2**.

Fraction D (2.29 g) was chromatographed using silica gel eluting with hexane:EtOAc (3:7). Five fractions were obtained. Fraction C (315 mg) was chromatographed using silica gel and eluted with chloroform:acetone (4:1); four fractions were obtained. Fractions DA and DB were evaluated.

Fraction DA was chromatographed using silica gel and eluted with chloroform:acetone (9:1) and following preparative TLC developed with hexane:acetone (4:1); product **3** (brevione C, 5 mg) was obtained.

Fraction DB was chromatographed using silica gel and eluted with chloroform:acetone (9:1). Two compounds were obtained, which were purified by semipreparative TLC eluting with chloroform:acetone (9:1). Compound **5** (brevione E, 20 mg) and **4** (brevione D, more polar) were obtained. Compound **4** was purified using HPLC with a Si 60 5 μm column and eluted with hexane:acetone (4:1), using 1 mL min^{-1} flow rate and a RI detector, yielding 2 mg of **4**.

Brevione B (2): colorless oil; $[\alpha]^{25}_D = +65.9°$ ($CHCl_3$, $c = 0.24$); IR $\nu_{max}^{(neat,\ KBr)}$ cm^{-1}: 2948; 1709 (carbonyl groups); 1446; 754; ^{1}H NMR and ^{13}C NMR are listed in Tables 1 and 2; EIMS, *m/z* (rel int): 424 $[M]^+$ (100), 409 $[M - CH_3]^+$ (31); 382 $[M - 42]^+$ (12); 352 $[M - 72]^+$ (26); 271 (15); 218 (22); HREIMS *m/z* calcd for $C_{27}H_{36}O_4$ 424.2609 found 425.2666 $[M + 1]^+$.

Brevione C (3): colorless oil; $[\alpha]^{25}_D = +18.2°$ ($CHCl_3$, $c = 0.22$); IR $\nu_{max}^{(neat,\ KBr)}$ cm^{-1}: 2925; 1722 (ester carbonyl); 1656 (conjugated carbonyl); 1440; 753; ^{1}H NMR and ^{13}C NMR are listed in Tables 1 and 2; EIMS, *m/z* (rel int): 420 $[M]^+$(66), 405 $[M - CH_3]^+$ (10), 378 $[M - 42]^+$ (4), 348 $[M - 72]^+$ (7), 267 (16), 217 (6); HREIMS calcd for $C_{27}H_{32}O_4$ 420.2301, found 420.2315.

Brevione D (4): colorless oil; $[\alpha]^{25}_D = +26.6°$ ($CHCl_3$, $c = 0.1$); IR $\nu_{max}^{(neat,\ KBr)}$ cm^{-1}: 3320 (hydroxyl group), 2930, 1718 (ester carbonyl), 1662 (conjugated carbonyl), 1442, 753; ^{1}H NMR and ^{13}C NMR are listed in Tables 1 and 2; EIMS, *m/z* (rel int): 436 $[M]^+$(22), 418 $[M - H_2O]^+$ (4), 285 (22); HREIMS calcd for $C_{27}H_{32}O_5$ 436.2250, found 436.2259.

Brevione E (5): colorless oil; $[\alpha]^{25}_D = +34.5°$ ($CHCl_3$, $c = 0.7$); IR $\nu_{max}^{(neat,\ KBr)}$ cm^{-1}: 2938, 1750 (acetyl carbonyl), 1677 (conjugated carbonyl), 1644 (conjugated carbonyl), 1225 (acetyl ether), 754; ^{1}H NMR and ^{13}C NMR are listed in Tables 1 and 2; EIMS, *m/z* (rel int): 482 $[M]^+$(19), 422 $[M - CH_3COOH]^+$ (75), 393 (100), 309 (38), 295 (24), 281 (16) (Figure 3); HREIMS calcd for $C_{28}H_{34}O_7$ 482.2305, found 482.2309.

Preparation of Deacetylbrevione E (6). Product **5** (2 mg) was dissolved in 0.5 mL of methanol and 2 mL of 0.05 M sulfuric acid and stirred continuously at 80 °C for 1 h. After the usual workup, the product was purified using HPLC with a Si 60 5 μm column and eluted with hexane:acetone (13:7), using 1 mL min^{-1} flow rate and a RI detector, yielding 1 mg of hydrolyzed product. ^{1}H NMR is listed in Table 1.

Bioassay. Wheat seeds (*Triticum aestivum* L. cv. Wakeland) were sown on fine moist vermiculite in trays and grown in the dark at 22 °C for 4 days.[16] The etiolated seedlings were removed from the trays, and the roots and caryopsis were removed from the shoots. The latter were placed in a Van der Weij guillotine, and the apical 2 mm were cut off and discarded. The next 4 mm of the coleoptiles were removed and used for bioassay. All manipulations were done under a green safelight.[17] Crude extracts, fractions, or pure compounds to be assayed for biological activity were added to text tubes (approximately 20 μL/tube) and evaporated under nitrogen to dryness. The assay was duplicated. Two milliliters of phosphate−citrate buffer containing 2% sucrose[17] at pH 5.6 was added to each test tube. Following the placement of 10 coleoptiles in each test tube, the tubes were rotated at 0.25 rpm in a roller tube apparatus for 24 h at 22 °C in the dark.

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The coleoptiles were measured by projecting their images (×3) from a photographic enlarger.[18] Data were statistically analyzed.[19]

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Supporting Information Available: Copies of ^{1}H NMR for breviones B−E, ^{13}C NMR for breviones B, C, and E, COSY and NOESY for breviones C and E, and HMQC and HMBC for brevione E. This material is available free of charge via the Internet at http://pubs.acs.org.

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