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Isolation of the Tetrapeptide Apicidins G, H and I from the Fungus *Fusarium semitectum*

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This study reports the isolation and characterization of three new tetrapeptides, apicidins G (**1**), H (**2**) and I (**3**), along with the known apicidin (**4**), apicidin A (**5**), apicidin C (**6**), diketopiperazine **7**, equisetin (**8**) and 7-hydroxy-2-(2-hydroxypropyl)-5-methylchromone (**9**). The structures of the new compounds were deduced by 2D NMR spectroscopic and MS data.

Keywords: Tetrapeptides, Apicidins, Fungus, *Fusarium*.

Fusarium semitectum (Syn. *Fusarium pallidoroseum*) is a fast growing fungus which was first described in 1875 from the petioles of banana leaves [1]. In common with other *Fusarium* species, *F. semitectum* has the ability to produce mycotoxins, such as nivalenol and (-)-zearalenone [2,3]. Other metabolites isolated include the antibiotic equisetin and α-pyrones, for example fusapyrone and deoxyfusapyrone [4,5]. In 1996, Singh *et al.* reported the isolation of the cyclic tetrapeptides apicidin and apicidin A from *Fusarium pallidoroseum* [6a]. Subsequent investigation of the same *F. pallidoroseum* sample by Singh *et al.* yielded apicidins B, C, D_1, D_2 and D_3 [6b-6c]. The unusual structural motif in apicidins is the presence of the amino acid 2-amino-8-oxo-decanoic acid (Aoda). Substitution of the Aoda residues has been reported for apicidins D_1-D_3 [6c]. All apicidins contain a (*D*)-pipecolic acid (Pip) unit, except for apicidin B, which has a (*D*)-proline (Pro) residue. Apicidin C has a (*L*)-valine residue instead of (*L*)-isoleucine (Ile). An *N*-methoxy-(*L*)-tryptophan is present in both apicidin and its congeners, except for apicidin A. This series of compounds has shown antiprotozoal activity by reversible blocking of histone deacetylase (HDAC) inhibitors [6d]. Apicidins are structurally related to trapoxin A, HC-toxin, WF-3161, Cyl-2 and chlamydocin [7-11]. The long chain amino acid with a terminal epoxy group in each of these cyclic tetrapeptides has been suggested to be responsible for their antiproliferative activity [12]. Jin *et al.* have identified the gene cluster responsible for apicidin biosynthesis in *F. semitectum*, and isolated apicidin E containing a 2-aminodecanoic acid unit [13]. Apicidin F, with *L*-phenylalanine (Phe) instead of Ile and *L*-2-aminooctanedioic acid instead of Aoda, has recently been identified from *F. fujikuroi* [14]. In this report, we describe the isolation and structure elucidation of three new tetrapeptides, apicidins G, H and I (**1-3**), together with six known compounds from *F. semitectum* (Figure 1). The stereochemistry of the new apicidins was proposed by comparison with the known apicidins and from biosynthetic considerations.

F. semitectum was isolated from a dead cicada skin collected from the Tawangmangu Botanic Garden in Central Java, Indonesia. The fungus was cultured in rice media, extracted with MeOH, then with EtOAc, to obtain a dark purple extract. This was chromatographed on silica gel and RP-HPLC to yield three new apicidins (**1-3**), together with the known (-)-apicidin (**4**), apicidins A (**5**) and C (**6**),

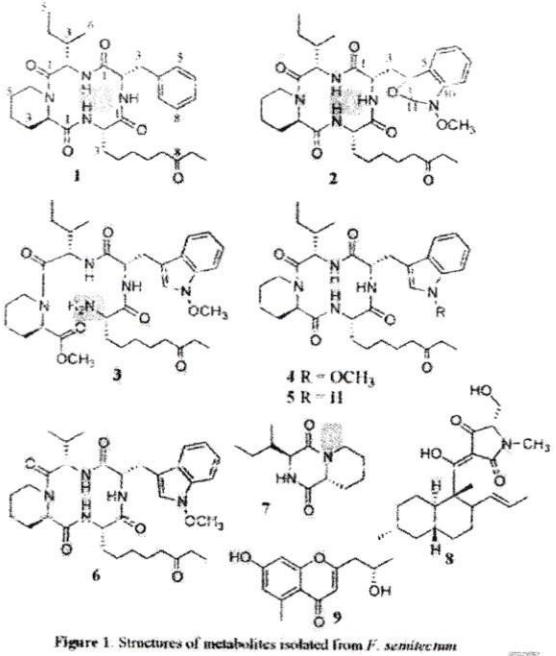

Figure 1. Structures of metabolites isolated from *F. semitectum*

(-)-cyclo-(*D*-pipecolinyl-*L*-isoleucine) (**7**) [15], (-)-equisetin (**8**) [6], and (+)-7-hydroxy-2-(2-hydroxypropyl)-5-methylchromone (**9**) [16].

Apicidin G (**1**) was isolated together with apicidin C (**6**) as a 1:1 mixture using RP-HPLC (MeOH/H_2O). The HRESIMS data of the fraction containing **1** suggested a nitrogenous compound from an adduct ion at *m/z* 577.3360 $[M+Na]^+$, corresponding to the molecular formula $C_{31}H_{46}N_4O_5$. The ^{1}H NMR spectrum of **1** (Table 1) showed signals for four α-protons (δ_H 5.06, 4.72, 4.16 and 3.80), three NH signals (δ_H 7.25, 6.46 and 6.43), five aromatic protons [δ_H 7.27 (2H), 7.19 (2H) and 7.22 (1H)] and three methyl groups

Table 1 NMR spectroscopic data for apicidins G, H and I.

Residue	Position	1 δ_C[a]	1 δ_H mult (J in Hz)[b]	Residue	Position	2 δ_C[a]	2 δ_H mult (J in Hz)[b]	3 δ_C[a]	3 δ_H mult (J in Hz)[b]
Pipecolic acid	1	171.6	-	Pipecolic acid	1	170.4	-	172.8	-
	2	50.8	5.06 br d (6.0)		2	51.0	3.49 br s	58.3	4.12 br d (10.0)
	3	24.1	a 2.01 m b 1.57 m		3	24.3	a 2.04 m b 1.59 m	26.7	a 2.13 br d (13.5) b 1.84 m
	4	19.2	a 2.14 m b 1.58 m		4	19.3	a 2.20 m b 1.62 m	21.8	a 1.89 m b 1.57 m
	5	25.4	a 1.80 m b 1.40 m		5	25.1	a 1.87 m b 1.41 m	22.1	1.80 m
	6	44.1	a 4.04 m b 3.04 br t (12.9)		6	44.0	a 3.83 br d (12.8) b 3.53 td (12.8, 2.0)	44.4	a 3.47 br d (12.0) b 2.92 td (12.0, 3.5)
Isoleucine	1	174.4	-		OMe			52.2	3.56 s
	2	54.4	4.72 t (10.3)	Isoleucine	1	171.2	-	172.7	-
	3	34.5	2.08 m		2	54.5	4.03 br t (9.5)	36.8	4.37 dd (8.0, 3.5)
	4	24.7	a 1.59 m b 1.19 m		3	34.4	1.97 m	37.6	1.84 m
					4	24.7	a 1.71 m b 1.28 m	25.3	1.36 ddd (13.5, 7.2, 4.6)
	5	10.7	0.94 t (7.4)		5	10.6	0.91 t (7.5)	11.4	0.86 t (7.5)
	6	15.7	0.87 d (6.6)		6	15.6	0.90 d (6.5)	15.5	0.83 d (7.0)
	NH	-	7.25 d (10.0)		NH	-	7.12 m[d]	-	7.14 br d (7.5)
Phenylalanine	1	174.7	-	Tryptophan-*N*-OMe (epoxy)	1	171.3	-	172.2	-
	2	62.7	3.80 m		2	65.6	4.73 br d (9.9)	54.6	4.71 td (8.5, 6.5)
	3	35.3	a 3.72 dd (13.5, 11.2) b 3.25 dd (13.5, 5.8)		3	42.5	a 2.57 d (14.6) b 2.41 br d (14.6)	27.6	a 3.30 dd (15.0, 9.0) b 3.22 dd (15.0, 6.5)
	1'	127.2	-		1'	55.2	-	108.8	-
	5/9	129.1	7.19 m		5	129.6	-	123.9	-
	6/8	128.8	7.27 m		6	121.8	7.31 d (8.5)	119.3	7.67 d (8.0)
	7	127.3	7.22 m		7	124.3	7.09 td (7.8, 0.5)	122.5	7.19 td (8.0, 1.0)
	NH	-	6.43 d (6.8)		8	130.6	7.34 td (7.6, 0.5)	119.8	7.08 td (8.0, 1.0)
					9	114.9	7.04 d (7.9)	108.1	7.34 d (8.0)
					10	148.9	-	132.4	-
					11	97.9	5.99 s	122.4	7.31 s
					12	64.1	3.85 s	65.8	4.02 s
					NH	-	7.12 m[d]	-	8.29 br d (7.5)
Aoda	1	175.8	-	Aoda	1	173.1	-	172.4	-
	2	53.5	4.16 m		2	53.7	4.32 dd (9.5, 4.2)	55.3	4.18 br q (7.5)
	3	29.0	a 1.75 m b 1.52 m		3	32.9	a 2.03 m b 1.96 m	31.4	a 1.62 m b 1.55 m
	4	25.2	1.19 m		4	25.6	1.32 m	25.3	1.07 m
	5	28.6	1.24 m		5	29.2	1.31 m	28.4	1.10 m
	6	23.4	1.52 m		6	23.8	1.53 m	23.4	1.40 m
	7	42.1	2.34 t (7.4)		7	42.3	2.36 t (7.5)	42.1	2.29 t (7.0)
	8	211.8	-		8	211.8	-	212.1	-
	9	35.9	2.40 q (7.4)		9	36.0	2.38 q (7.4)	36.0	2.32 q (7.5)
	10	7.7	1.04 t (7.4)		10	7.9	1.01 t (7.3)	7.8	1.03 t (7.0)
	NH	-	6.46 d (10.4)		NH_2	-	6.19 br d (7.6)	-	8.35 br d (6.5)

[a]Chemical shifts (ppm) taken from 2D NMR spectra referenced to $CDCl_3$ (δ_C 77.16), data recorded at 500 MHz. [b]Chemical shifts (ppm) referenced to $CHCl_3$ (δ_H 7.26), data recorded at 500 MHz. [c]Assignments may be interchangeable. [d]Overlapping signals, assigned by 2D-TOCSY: NH for 2, NH2 for 3.

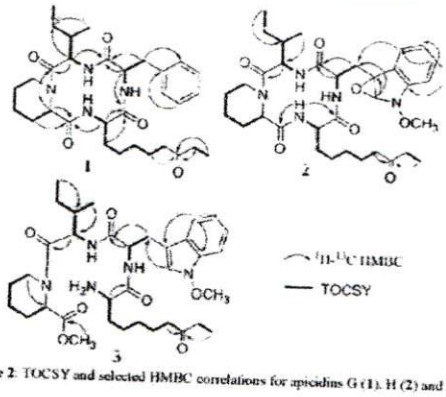

Figure 2. TOCSY and selected HMBC correlations for apicidins G (**1**), H (**2**) and I (**3**).

(δ_H 1.04, 0.94, 0.87). The ^{13}C NMR spectrum contained amide signals at δ_C 175.8, 174.7, 174.4 and 171.6 and a ketone signal at δ_C 211.8. The side chain signals of individual amino acids were determined by DQFCOSY and 2D-TOCSY data, and were consistent with the presence of Ile, Phe, Pip and Aoda residues. The signal at δ_H 5.06 (br d, 6.0 Hz) was assigned as the α-proton of Pip since it was linked to four methylene groups (including two downfield methylene protons at δ_H 4.04 and 3.04) by 2D-TOCSY (Figure 2). For Ile, the α-proton signal at δ_H 4.72 was a triplet with 10.0 Hz couplings to both NH (δ_H 7.25) and methine (δ_H 2.08) signals. The TOCSY data revealed cross peaks from this α-proton to the methyl groups at δ_H 0.94 (t, 7.4 Hz) and 0.87 (d, 6.6 Hz). The presence of Phe was established from an α-proton signal at δ_H 3.80, which showed couplings to methylene signals at δ_H 3.72 and 3.25 by TOCSY, and by HMBC correlations from the methylene protons to an aromatic carbon at δ_C 129.1. The amino acid 2-amino-8-oxo-decanoic acid (Aoda) was apparent from signals for five methylene groups [δ_H 2.34 (2H, t, 7.4), 1.75 (1H, m), 1.52 (3H, m), 1.24 (2H, m), 1.19 (2H, m)], an ethyl group [δ_H 1.04 (3H, t, 7.4 Hz), 2.40 (2H, q, 7.4 Hz)], and by HMBC correlations from the methyl group at δ_H 1.04 and the methylene protons at δ_H 1.52 and at δ_H 2.40 to the ketone signal at δ_C 211.8.

The amide carbons of Aoda, Phe, and Pip were assigned to the signals at δ_C 175.8, 174.7, and 171.6, respectively, by the $^3J_{CH}$ correlations from their β protons; the remaining amide signal at δ_C174.4 therefore belonged to Ile. $^2J_{CH}$ correlations from the respective α-protons fully supported these assignments. The sequence of the amino acid residues in apicidin G was determined from HMBC correlations, in particular from the α-proton of Pip to the carbonyl group (δ_C 174.4) of Ile, from the α-proton of Ile to the carbonyl group (δ_C 174.7) of Phe, and from the α-proton of the Aoda residue to the carbonyl group (δ_C 171.6) of Pip. The sequence was identical to that in apicidin (**4**) [6a] except for the Trp-*N*-OMe moiety, which was replaced by Phe in apicidin G (**1**).

The configuration of **1** was determined by analysis of proton NMR coupling constants compared with those in other apicidins [6]. The

α-proton of Phe was coupled to the adjacent NH proton with a *J* value of 6.8 Hz, and indicated a *syn*-relationship. In contrast, the 10.4 and 10.0 Hz coupling between the α-protons of the Aoda and Ile residues and the corresponding NH protons suggested an *anti*-relationship, as reported for apicidin (**4**). In the pipecolic acid unit the 6.0 Hz coupling of the α-proton matched the 5.5 Hz coupling for the corresponding signal in apicidin [6a]. In view of the co-isolation of (-)-apicidin (**4**), the absolute configurations of the shared amino acid constituents were inferred to be (*R*)-Pip, (*S*)-Ile, and (*S*)-Aoda. An (*S*)-Phe residue was inferred from the $J_{NH\,H\text{-}2}$ value [6a,6b], and by analogy with trapoxin-A [7]. Specific rotation measurements and verification of the amino acid configurations were not undertaken owing to the inseparable mixture of **1** and **6**.

Apicidin H (**2**) was obtained as a colorless oil by RP-HPLC using MeOH/H_2O. The HRESIMS data of **2** indicated a molecular formula of $C_{34}H_{49}N_5O_7$ from a sodiated adduct ion at *m/z* 662.3545, and established the presence of one additional oxygen in **2** with respect to apicidin (**4**). The ^{1}H NMR data suggested that **2** was structurally related to **4** from the characteristic signals corresponding to Pip [δ_H 3.49, 3.83 and 3.53], Ile [δ_H 4.65, 0.91 (3H), 0.90 (3H)] and Aoda residues [δ_H 4.32, 2.38 (2H), 2.36 (2H) and 1.01 (3H)]. The signal at δ_H 4.73 (d, 9.9) linked to a signal at δ_C 65.6 by HSQC was assigned as the α-proton of the remaining amino acid unit, and showed 2D-TOCSY correlations to a methylene group at δ_H 2.57 and 2.41, and to an NH proton (δ_H 7.12). A methine signal at δ_H 5.99 (H-11) was linked to a signal at δ_C 97.0 by HSQC. These chemical shifts, together with HMBC correlations from this methine proton to a methylene carbon (δ_C 42.5, C-3) and to a quaternary carbon (δ_C 84.2, C-4), were all consistent with an epoxy-derivatized Trp-*N*-OMe moiety. An amide signal at δ_C 171.3 was assigned to the epoxy-Trp-*N*-OMe moiety by $^3J_{CH}$ correlations from the β protons; HMBC correlations were seen from the NH proton (δ_H 6.19) of the Aoda residue to signals at δ_C 173.1 and 170.3, therefore the remaining amide signal at δ_C 171.2 could be assigned to Ile. This assignment is valid providing the sequence of amino acid constituents is uncharged from those in other apicidins. The signals at δ_C 173.1 and 170.3 were provisionally assigned to Aoda and Pip, respectively, with the δ_H 6.19/ δ_C 170.3 correlation representing an inter-unit correlation. Intra-unit correlations from the α-proton of epoxy-Trp-*N*-OMe to the carbonyl at δ_C 171.3 and from the α-proton of Ile to the carbonyl at δ_C 171.2 were observed.

The relative configuration of the epoxy ring was not determined. The specific rotation of **2** was -42, and when compared to literature values reported for apicidin ($[\alpha]_D$ -80.4) [6a], apicidin D_1 ($[\alpha]_D$ -72.6), apicidin D_2 ($[\alpha]_D$ -68.5), and apicidin D_3 ($[\alpha]_D$ -60.4) [6c], the three amino acid constituents shared between these various metabolites were suggested to be (*R*)-Pip, (*S*)-Ile, and (*S*)-Aoda. The structural similarity of apicidin H with other apicidin metabolites implies that the configuration of the epoxy-Trp-*N*-OMe residue should be *S*.

Apicidin I (**3**) was the final apicidin metabolite isolated from *F. semitectum*. The HRESIMS data of **3** exhibited an adduct ion at *m/z* 656.4037 $[M+H]^+$, corresponding to a molecular formula $C_{35}H_{53}N_5O_7$, which was 32 mass units larger than that of **4**. The ^{1}H and ^{13}C NMR spectra of **3** (Table 1) closely resembled those of **4**, revealing the presence of four α-protons (δ_H 4.71, 4.37, 4.18, and 4.12), five aromatic signals (δ_H 7.67, 7.34, 7.31, 7.19 and 7.08), two methyl triplets (δ_H 1.03, 0.86) and one methyl doublet (δ_H 0.83). The NMR spectra of **3** also indicated the presence of two amide protons (2 x NH, δ_H 8.29, 7.14), one amino group (NH_2, δ_H 8.35) and one additional methoxy group (δ_H 3.56, δ_C 52.2). Assignments of the amino acid residues were undertaken by HMBC, DQFCOSY, and 2D-TOCSY experiments, and by comparison with the data reported for apicidins [6a-6c]. TOCSY data revealed correlations from the NH_2 protons to the α-proton (δ_H 4.18) and to the five methylene groups of the Aoda residue. The methoxy group at δ_H 3.56 showed a HMBC correlation to the carbonyl group (δ_C 172.8) of the Pip unit. This information, together with the MS data, suggested a linear tetrapeptide as opposed to the cyclic tetrapeptide core of apicidin and its congeners. HMBC correlations were observed from the three α-protons at δ_H 4.37 (Ile), 4.71 (Trp-*N*-OMe) and 4.18 (Aoda) to the carbonyl groups at δ_C 172.7, 172.2 and 172.4, respectively. No inter-amino acid correlations were apparent in the HMBC spectrum, but the presence of Pip OMe and Aoda-NH_2 implies that apicidin I is an artefact from methanolysis of apicidin at the Pip residue. The same absolute configuration is therefore proposed. Apicidin I gave the same negative sign of specific rotation $[\alpha]_D$ -17.3 (*c* 0.16, MeOH) as previous apicidins.

Apicidin was screened against a panel of bacterial (Gram-positive and -negative) and fungal strains, but was without activity. There was insufficient quantity of the new apicidins for biological screening. In conclusion, three new tetrapeptides, apicidins G (**1**), H (**2**) and I (**3**) were isolated together with six known compounds from *Fusarium semitectum*. The results from our study have shown modification at the Trp-*N*-OMe unit with an epoxy group in apicidin H. The absolute configurations of the apicidin metabolites were proposed by comparison of *J* values, specific rotation values, and from the biosynthetic similarities between the various metabolites. Tetrapeptides such as apicidin and its congeners may be chemotaxonomic markers for this fungal species.

Experimental

General: NMR data of **1-3** were measured on a Bruker Avance 500 MHz pectrometer (5 mm inverse probe, gradient selection) in $CDCl_3$ at 298K. For HSQC and HMBC spectra, data were acquired using a $^1J_{C\text{-}H}$ of 135 Hz, while HMBC spectra were acquired using $^nJ_{C\text{-}H}$ of 8 Hz. TOCSY data (mixing time 60 msec) were determined in phase sensitive mode. Positive ion electrospray mass spectra were determined using either a Bruker Esquire HCT instrument (LRESIMS) or a MicroTof Q instrument (HRESIMS) with MeOH as solvent. Reverse phase HPLC was carried out on an Agilent 1100 series instrument fitted with either a Phenomenex Gemini C_{18} (250 x 10 mm i.d., 5 μ) column or a C_{18} analytical column with UV detection at 254 nm. Silica gel 60 G and silica TLC plates F_{254} were purchased from Merck. All solvents were either distilled or were of HPLC grade.

Fungal material: A white fungus isolated from a dead cicada skin was collected at the Tawangmangu Botanic Garden, Indonesia, and identified as *Fusarium semitectum* based on morphological comparison with *F. semitectum* [2,4]. A voucher specimen (SC-131208-1, AQIS IP09011654) is held in the School of Biology, UQ.

Culture conditions: The fungus was grown on Petri dishes containing PDA media at room temperature for 7 days. Rice media for fermentation was prepared by soaking 50 g of long grain rice in 50 mL of distilled water in 250 mL Erlenmeyer flasks. After 6 h, the rice media was autoclaved for 15 min. Thirty Erlenmeyer flasks containing rice media were inoculated with a small plug of agar containing the mycelia of *F. semitectum*, and the cultures were kept in the dark at room temperature for 21 days.

Extraction and isolation of metabolites: Mycelia and media were homogenized by stirring, then extracted with MeOH (3 x 700 mL) using ultrasonic vibration for 30 min. The extract was filtered, then concentrated *in vacuo* to an aqueous residue, which was partitioned

with EtOAc (3 x 300 mL) to give a dark purple oil (3.6 g). The extract was subjected to vacuum liquid chromatography using stepwise gradient elution (100% hexanes to 100% EtOAc) to obtain 11 fractions. Fraction 5 (225 mg) was subjected to RP-flash column chromatography using a stepwise elution of DCM/MeOH to give equisetin (**8**) (59.3 mg). Combined fractions 6 and 7 (570 mg) were chromatographed on silica using a stepwise elution of DCM/EtOAc/MeOH to give 11 fractions coded 6n7-1 to 6n7-11. Combined fractions 6n7-1 to 7-3 (17.4 mg) were purified by RP-HPLC (65-100% MeOH/H_2O) to yield (-)-apicidin (**4**) (3.7 mg) then (-)-equisetin (**8**) (1.8 mg). Fraction 6n7-5 (150 mg) was subjected to RP flash column chromatography with MeOH/H_2O to give apicidin (**4**) (16.2 mg), a mixture of apicidin (**4**) and equisetin (**8**) (41.1 mg) and fraction 6n7-5-1 (9.5 mg), which was further purified by RP-HPLC (70-100% MeOH/H_2O) to yield apicidin I (**3**) (2.4 mg). Fraction 6n7-6 (99 mg) was purified by RP-HPLC (80-100% MeOH/H_2O) and gave 7 fractions. (-)-Cyclo-(*D*-pipecolinyl-*L*-isoleucine) [15] (**7**) (0.9 mg) was in fraction 6n7-6-2. Fraction 6n7-6-5 contained a 1:1 mixture of apicidins G (**1**) and C (**6**) (5.4 mg). Apicidin (**4**) (36.4 mg) was in fraction 6n7-6-6. Fraction 6n7-6-4 (3.2 mg) was purified by RP-HPLC (75% MeOH/H_2O) affording apicidin H (**2**) (0.5 mg) and apicidin A (**5**) (1.3 mg). Combined fractions 6n7-7 and 7-8 (43.5 mg) were chromatographed using a RP Sep-pak™ (20-100% MeOH/H_2O) to give (+)-7-hydroxy-2-(2-hydroxypropyl)-5-methylchromone (**9**) [16] (2.2 mg), apicidin (**4**) (16.2 mg), and a mixture of apicidin (**4**) and equisetin (**8**) (5.5 mg).

Apicidin G 1
Colorless film
^{1}H NMR and ^{13}C NMR ($CDCl_3$): Table 1.
HRESIMS: *m/z* [M+Na]$^+$ calcd for $C_{31}H_{46}N_4NaO_5$: 577.3360; found: 577.3360.

Apicidin H 2
Colorless film
$[\alpha]^{24}_D$: -42 (*c* 0.03, MeOH)
^{1}H NMR and ^{13}C NMR ($CDCl_3$): Table 1.
HRESIMS: *m/z* [M+Na]$^+$ calcd for $C_{34}H_{39}N_5NaO_7$: 662.3524; found: 662.3545.

Apicidin I 3
Colorless film
$[\alpha]^{24}_D$: -17 (*c* 0.16, MeOH)
^{1}H NMR and ^{13}C NMR ($CDCl_3$): Table 1.
HRESIMS: *m/z* [M+H]$^+$ calcd for $C_{35}H_{54}N_5O_7$: 656.4018; found: 656.4037.

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References

[1] (a) Desjardins AE. (**2006**) *Fusarium Mycotoxins: Chemistry, Genetics, and Biology*. The American Phytopathology Society: Minnesota. (b) Geriach W, Nirenberg H. (**1982**) *The Genus Fusarium. A Pictorial Atlas*. Biologische Bundesanstalt für Land-und Firstwirtschaft, Berlin.

[2] Zaccardelli M, Balmas V, Altomare C, Corazza L, Scotti C. (**2006**) Characterization of Italian isolates of *Fusarium semitectum* from alfalfa (*Medicago sativa* L.) by AFLP analysis, morphology, pathogenicity and toxin production. *Journal of Phytopathology*, *154*, 454–460.

[3] Marasas WFO, Nelson PE, Toussoun TA. (**1984**) *Toxigenic Fusarium Species: Identity and Mycotoxicology*. The Pennsylvania State University Press, London.

[4] Wheeler MH, Stipanovic RD, Puckhaber LS. (**1999**) Phytotoxicity of equisetin and *epi*-equisetin isolated from *Fusarium equiseti* and *F. pallidoroseum*. *Mycological Research*, *103*, 967–973.

[5] Evidente A, Conti L, Altomare C, Bottalico A, Sindona G, Segre AL, Logrieco A. (**1994**) Fusapyrone and deoxyfusapyrone, two antifungal α-pyrones from *Fusarium semitectum*. *Natural Toxins*, *2*, 1–13.

[6] (a) Singh SB, Zink DL, Polishook JD, Dombrowski AW, Darkin-Rattray SJ, Schmatz DM, Goetz MA. (**1996**) Apicidins: novel cyclic tetrapeptides as coccidiostats and antimalarial agents from *Fusarium pallidoroseum*. *Tetrahedron Letters*, *37*, 8077–8080. (b) Singh SB, Zink DL, Liesch JM, Dombrowski AW, Darkin-Rattray SJ, Schmatz DM, Goetz MA. (**2001**) Structure, histone deacetylase, and antiprotozoal activities of apicidins B and C, congeners of apicidin with proline and valine substitutions. *Organic Letters*, *3*, 2815–2818; (c) Singh SB, Zink DL, Liesch JM, Mosley RT, Dombrowski AW, Bills GF, Darkin-Rattray SJ, Schmatz DM, Goetz MA. (**2002**) Structure and chemistry of apicidins, a class of novel cyclic tetrapeptides without a terminal α-keto epoxide as inhibitors of histone deacetylase with potent antiprotozoal activities. *Journal of Organic Chemistry*, *67*, 815–825; (d) Darkin-Rattray SJ, Gurnett AM, Myers RW, Dulski PM, Crumley TM, Allocco JJ, Cannova C, Meinke PT, Colletti SL, Bednarek MA, Singh SB, Goetz MA, Dombrowski AW, Polishook JD, Schmatz DM. (**1996**) Apicidin: a novel antiprotozoal agent that inhibits parasite histone deacetylase. *Proceedings of the National Academy of Sciences*, *93*, 13143–13147.

[7] Itazaki H, Nagashima K, Sugita K, Yoshida H, Kawamura Y, Yasuda Y, Matsumoto K, Ishii K, Uotani N, Nakai H, Terui A, Yoshimatsu S, Ikenishi Y, Nakagawa Y. (**1990**) Isolation and structural elucidation of new cyclotetrapeptides, trapoxins A and B, having detransformation activities as antitumour agents. *Journal of Antibiotics*, *43*, 1524–1532.

[8] Liesch JM, Sweeley CC, Staffeld GD, Anderson MS, Weber DJ, Scheffer RP. (**1982**) Structure of HC-toxin, a cyclic tetrapeptide from *Helminthosporium carbonum*. *Tetrahedron*, *38*, 45–48.

[9] Umehara K, Nakahara K, Kiyoto S, Iwami M, Okamoto M, Tanaka H, Kohsaka M, Aoki H, Imanaka H. (**1983**) Studies on WF-3161, a new antitumor antibiotic. *Journal of Antibiotics*, *36*, 478–483.

[10] Hirota A, Suzuki A, Aizawa K, Tamura S. (**1973**) Structure of cyl-2, a novel cyclotetrapeptide from *Cylindrocladium scoparium*. *Agricultural and Biological Chemistry*, *37*, 955–956.

[11] Closse A, Huguenin R. (**1974**) Isolation and structure elucidation of chladomycin. *Helvetica Chimica Acta*, *57*, 533–545.

[12] Shute RE, Dunlap B, Rich DH. (**1987**) Analogues of the cytostatic and antimitogenic agents chlamydocin and HC-toxin: synthesis and biological activity of chloromethyl ketone and diazomethyl ketone functionalized cyclic tetrapeptides. *Journal of Medicinal Chemistry*, *30*, 71–78.

[13] Jin JM, Lee S, Lee J, Baek SR, Kim JC, Yun SH, Park SY, Kang S, Lee YW. (**2010**) Functional characterization and manipulation of the apicidin biosynthetic pathway in *Fusarium semitectum*. *Molecular Microbiology*, *76*, 456–466.

[14] von Bargen KW, Niehaus EM, Bergander K, Brun R, Tudzynski B, Humpf HU. (**2013**) Structure elucidation and antimalarial activity of apicidin F: an apicidin-like compound produced by *Fusarium fujikuroi*. *Journal of Natural Products*, *76*, 2136–2140.

[15] Mitova M, Tutino ML, Infusini G, Marino G, De Rosa S. (**2005**) Exocellular peptides from Antarctic psychrophile *Pseudoalteromonas haloplanktis*. *Marine Biotechnology*, *7*, 523–531.

[16] Kashiwada Y, Nonaka GI, Nishioka I. (**1984**) Studies on rhubarb (*Rhei rhizoma*) V. Isolation and characterization of chromone and chromanone derivatives. *Chemical and Pharmaceutical Bulletin*, *32*, 3493–3500.

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