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Alkaloids and Polyketides from *Penicillium citrinum*, an Endophyte Isolated from the Moroccan Plant *Ceratonia siliqua*

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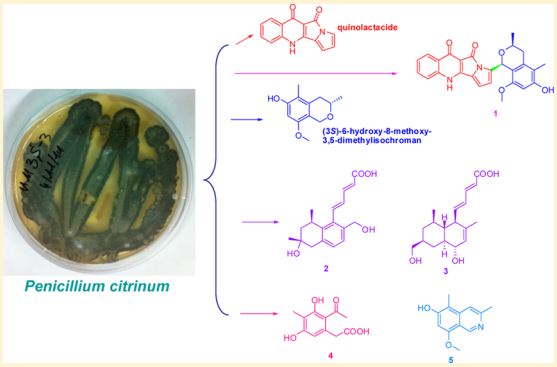
Supporting Information

ABSTRACT: The endophytic fungus *Penicillium citrinum* was isolated from a fresh stem of the Moroccan plant *Ceratonia siliqua*. Extracts of *P. citrinum* grown on rice and white bean media yielded five new compounds, namely, citriquinochroman (**1**), tanzawaic acids G and H (**2** and **3**), 6-methylcurvulinic acid (**4**), 8-methoxy-3,5-dimethylisoquinolin-6-ol (**5**), and one new natural product, 1,2,3,11b-tetrahydroquinolactacide (**6**), which had previously been described as a synthetic compound. In addition, 13 known compounds including seven alkaloids and six polyketides were isolated. The structures of the new compounds were unambiguously determined on the basis of one- and two-dimensional NMR spectroscopy as well as by high-resolution mass spectrometry. Citriquinochroman (**1**) features a new skeleton, consisting of quinolactacide and (3*S*)-6-hydroxy-8-methoxy-3,5-dimethylisochroman linked by a C−C bond. 1,2,3,11b-Tetrahydroquinolactacide (**6**) may be a biogenetic precursor of quinolactacide. Citriquinochroman (**1**) showed cytotoxicity against the murine lymphoma L5178Y cell line with an IC_{50} value of 6.1 μM, while the other compounds were inactive (IC_{50} >10 μM) in this assay.



Endophytes are microorganisms that live in the internal tissues of their host without causing any apparent disease symptoms.[1] Instead, endophytes affect their hosts in a positive way including growth enhancement and protection against pathogens and feeding damage.[2−4] Endophytes, especially endophytic fungi, are important sources of biologically active secondary metabolites, in that they have provided numerous secondary metabolites with novel structures, showing interesting biological and/or pharmaceutical activities.[5−8]

The genus *Penicillium* comprises more than 300 species,[9] which produce a variety of novel bioactive compounds. Well-known drug leads from this genus are penicillin antibiotics produced by *P. chrysogenum*[10] and the antifungal metabolite griseofulvin produced by *P. griseofulvum*[11] and *P. patulum*.[12] *P. citrinum* (syn. *P. steckii*) is known for the production of the mycotoxin metabolite citrinin.[13,14] Several other secondary metabolites have been described from *Penicillium* species, including isochroman toxins from *P. steckii*,[15] tanzawaic acids from *P. citrinum*,[16] and compactins from the same species.[17]

During our ongoing search for bioactive secondary metabolites from endophytic fungi,[18−21] *P. citrinum* was isolated from the inner stem tissues of the Moroccan plant *Ceratonia siliqua* L. (Fabaceae). Herein, we describe the isolation and structure elucidation of the new compounds, as well as their biological activities.

RESULTS AND DISCUSSION

The EtOAc extracts of both rice and white bean cultures of the endophytic *P. citrinum* were partitioned between *n*-hexane and 90% aqueous MeOH. The resulting MeOH phase was fractionated by vacuum liquid chromatography (VLC) on silica gel followed by size exclusion chromatography over Sephadex LH-20 and then semipreparative reversed-phase HPLC to yield five new compounds, citriquinochroman (**1**), tanzawaic acids G and H (**2** and **3**), 6-methylcurvulinic acid (**4**), 8-methoxy-3,5-dimethylisoquinolin-6-ol (**5**), and one new natural product, 1,2,3,11b-tetrahydroquinolactacide (**6**), which was previously known only as a synthetic compound. In addition, 13 known compounds, including 4-hydroxyquinolin-2(1*H*)-one,[22] quinolactacide,[23] penicinoline,[24] methylpenicinoline,[25] trichodermamide C,[26] citrinamide A,[27] indole acetic acid methyl ester,[28] tanzawaic acids B[16] and F,[14] arohynapene D,[29] (3*S*)-6-hydroxy-8-methoxy-3,5-dimethylisochroman,[30] (3*S*)-4,6-dihydro-8-me-

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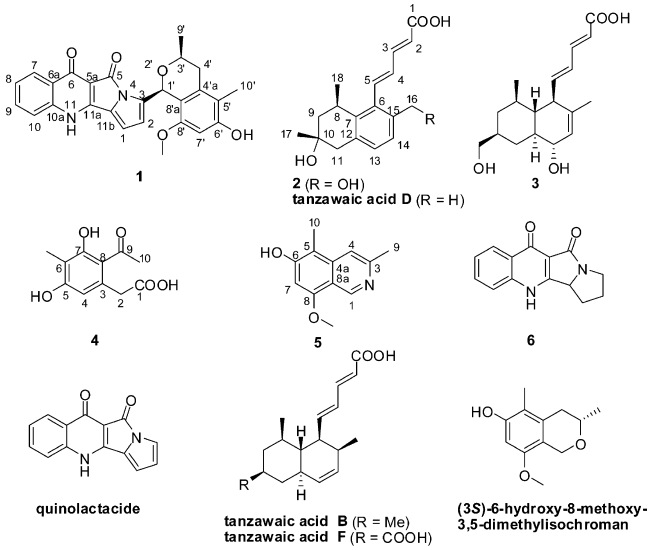
thoxy-3,5-dimethyl-6-oxo-3*H*-2-benzopyran,[31] and vanillic acid,[32] were obtained.



Compound **1** was isolated as a yellow, amorphous powder, and its molecular formula was determined as $C_{26}H_{22}N_2O_5$ on the basis of the pseudomolecular ion peak observed at *m/z* 443.1601 $[M + H]^+$ in the HRESIMS spectrum. Inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 1) revealed the presence of the two sets of signals corresponding to quinolactacide[23] and (3*S*)-6-hydroxy-8-methoxy-3,5-dimethylisochroman[30] units, respectively. The signals at δ_H 6.59 (1H, d, *J* = 3.7 Hz, H-1) and 5.84 d (1H, d, *J* = 3.7 Hz, H-2), showing vicinal couplings, and those at δ_H 8.23 (1H, dd, *J* = 7.7, 1.2 Hz, H-7), 7.41 (1H, ddd, *J* = 7.7, 7.2, 0.8 Hz, H-8), 7.72 (1H, ddd, *J* = 8.1, 7.2, 1.2 Hz, H-9), and 7.66 (1H, dd, *J* = 8.1, 0.8 Hz, H-10), representing typical signals for a 1,2-disubstituted benzene ring, were attributed to the quinolactacide unit.[23] The ^{13}C NMR spectrum also exhibited signals (Table 1) comparable to those of quinolactacide.[23] This assumption was supported by analysis of the HMBC spectrum, in which the cross-peaks between H-2, C-3 (δ_C 140.3), and C-11b (δ_C 123.9), between H-1, C-3, C-11b, and C-11a (δ_C 143.5), between H-7, C-6 (δ_C 177.0), C-9 (δ_C 134.0), and C-10a (δ_C 141.1), between H-8, C-10 (δ_C 119.4), and C-6a (δ_C 125.5), between H-9, C-7 (δ_C 126.2), and C-10a, and between H-10, C-8 (δ_C 125.5), and C-6a were discerned (Figure 1). The remaining resonances in the ^1H NMR spectrum include an aromatic proton at δ_H 6.39 (s, H-7′), a methyl group at δ_H 2.06 (s, H_3-10′), a methoxy group at δ_H 3.65 (s, 8′-OMe), two oxymethine protons at δ_H 5.96 (s, H-1′) and 3.90 (m, H-3′), an aliphatic methyl at δ_H 1.29 (d, *J* = 6.1 Hz, H_3-9′), and a methylene group at δ_H 2.70 (dd, *J* = 16.8, 3.5 Hz, H-4′$_{eq}$) and 2.37 (dd, *J* = 16.8, 11.1 Hz, H-4′$_{ax}$). These signals were indicative of the presence of a 6-hydroxy-8-methoxy-3,5-dimethylisochroman moiety,[30] as confirmed by the COSY correlations from H_3-9′ to H-3′ and from H-3′ to H_2-4′ and by the HMBC correlations from H_3-9′ to C-3′ (δ_C 65.7), C-4′ (δ_C 35.0); H_2-4′ to C-8′a (δ_C 115.1), C-4′a (δ_C 135.6), and C-5′ (δ_C 114.9); H_3-10′ to C-4′a, C-5′, and C-6′ (δ_C 156.2); H-7′ to C-5′, C-6′, C-8′ (δ_C 155.8), and C-8′a; H-1′ to C-3′, C-4′a, C-8′, and C-8′a; and 8′-OMe to C-8′ (Figure 1). The appearance of H-1′ as a singlet in this moiety and the absence of the H-3 signal in the quinolactacide unit indicated a linkage between C-1′ and C-3. The molecular formula suggested that the linkage has to be a C-1′/C-3 σ bond, as supported by the HMBC correlations from H-1′ to C-2 (δ_C 111.7) and C-3 (δ_C 140.3) and from H-2 to C-1′ (δ_C 69.3). This could explain the deshielded ^{13}C chemical shifts for both C-1′ (δ_C 69.3) and C-3 (δ_C 140.3) in **1** if compared to those in the individual units [$\delta_{C\text{-}1'}$ 64.6, in (3*S*)-6-hydroxy-8-methoxy-3,5-dimethylisochroman,[30] and $\delta_{C\text{-}3}$ 119.9, in quinolactacide[23]]. The relative configuration at C-1′ and C-3′ in **1** was established by a ROESY experiment. The large vicinal coupling constant ($^3J_{H\text{-}3',\ H\text{-}4'ax}$ = 11.1 Hz) between H-3′ and H-4′$_{ax}$ suggests their

Table 1. ^1H and ^{13}C NMR Data of 1 (CD_3OD)

position	δ_C, type	δ_H, mult. (*J*)
1	114.1, CH	6.59, d (3.7)
2	111.7, CH	5.84, d (3.7)
3	140.3, C	
5	159.4, C	
5a	114.3, C	
6	177.0, C	
6a	125.5, C	
7	126.2, CH	8.23, dd (7.7, 1.2)
8	125.5, CH	7.41, ddd (7.7, 7.2, 0.8)
9	134.0, CH	7.72, ddd (8.1, 7.2, 1.2)
10	119.4, CH	7.66, dd (8.1, 0.8)
10a	141.1, C	
11a	143.5, C	
11b	123.9, C	
1′	69.3, CH	5.96, s
3′	65.7, CH	3.90, m
4′	35.0, CH_2	2.70, dd (16.8, 3.5)
		2.37, dd (16.8, 11.1)
4′a	135.6, C	
5′	114.9, C[a]	
6′	156.2, C	
7′	97.6, CH	6.39, s
8′	155.8, C	
8′a	115.1, C[a]	
9′	22.1, CH_3	1.29, d (6.1)
10′	10.3, CH_3	2.06, s
8′-OCH_3	55.7, CH_3	3.65, s

[a]Assignments within the same column may be interchanged.

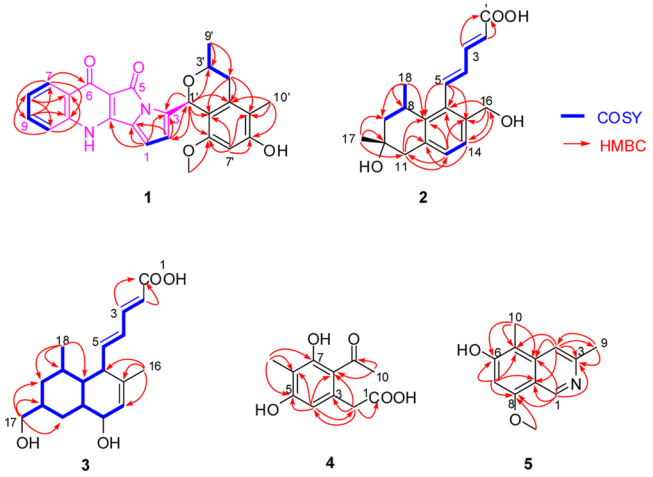


Figure 1. COSY and selected HMBC correlations of **1**–**5**.

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1,2-diaxial relationship. The ROESY correlations between H-3′ and H-1′ indicate a *cis*-configuration between them (Figure 2).

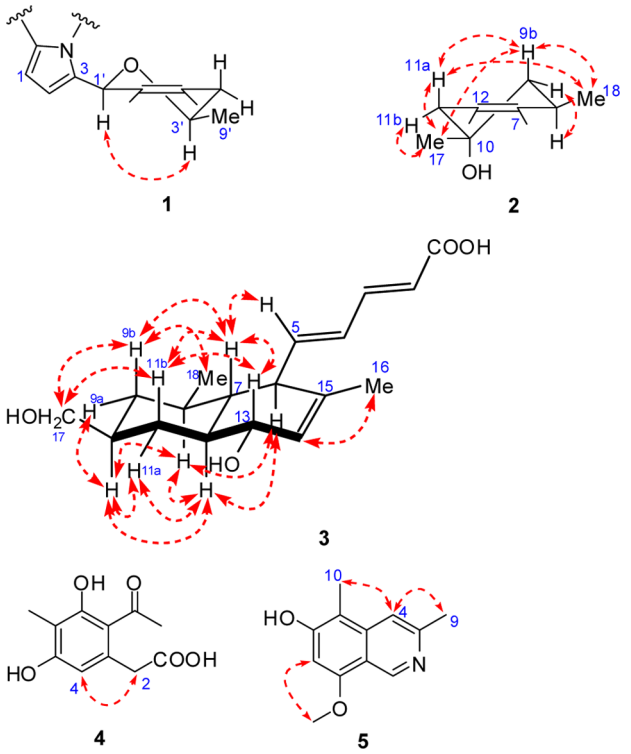

Figure 2. Selected ROESY correlations of **1**−**5**.

Since the co-isolated (3*S*)-6-hydroxy-8-methoxy-3,5-dimethylisochroman is a building unit of **1**, the stereocenter at C-3′ probably has the same *S* configuration in view of the same biogenetic pathway. Accordingly, the absolute configuration at C-1′ was assumed as *S*. Therefore, **1** was identified as a novel compound with a hitherto unknown quinolactacide-isochroman skeleton and was named citriquinochroman.

Compound **2** was isolated as a white, amorphous powder, whose molecular formula was established as $C_{18}H_{22}O_4$ by HRESIMS. The ^{1}H NMR spectrum of **2** showed the presence of two vicinal aromatic protons at δ_H 7.03 (d, J = 7.8 Hz, H-13) and 7.24 (d, J = 7.8 Hz, H-14), four olefinic methine protons at δ_H 5.95 (d, J = 15.3 Hz, H-2), 7.49 (dd, J = 15.3, 11.0 Hz, H-3), 6.62 (dd, J = 16.0, 11.0 Hz, H-4), and 7.22 (brd, J = 16.0 Hz, H-5), one hydroxymethylene group at δ_H 4.58 and 4.52 (ABq, J = 12.4 Hz, H_2-16), and two methyl groups at δ_H 1.37 (s, H_3-17) and 1.17 (d, J = 7.0 Hz, H_3-18) (Table 2). The ^{13}C NMR spectrum of **2** showed a total of 18 carbons (Table 2), which include one carbonyl (δ_C 170.5), 10 sp^2 carbons (including six methines and four quaternary carbons), two oxygenated sp^3 carbons (including one methylene at δ_C 63.6 and one quaternary carbon at δ_C 70.1), three aliphatic sp^3 carbons (including two methylenes at δ_C 46.3 and 44.9 and one methine at δ_C 30.3), and two methyls (δ_C 30.9, 23.5), as supported by the DEPT-135 experiment. Detailed analysis of the 2D NMR spectrum (including COSY, HSQC, and HMBC) allowed us to establish its planar structure. A penta-2,4-dienoic acid side chain was located at C-6 of the benzene ring, as supported by the COSY correlations observed from H-2 (δ_H 5.95, d) to H-3 (δ_H 7.49 dd), H-3 to H-4 (δ_H 6.62 dd), and H-4 to H-5 (δ_H 7.22 brd) and by the HMBC correlations from H-2 and H-3 to C-1 (δ_C 170.5) and from H-5 to C-6 (δ_C 136.0), C-7 (δ_C 141.4), and C-15 (δ_C 138.2) (Figure 1). A CH_3(18)−CH(8)−CH_2(9) substructure was indicated by the COSY spectrum, in which the correlations from H_3-18 (δ_H 1.17, d) to H-8 (δ_H 3.36, m) and from H-8 to H_2-9 (δ_H 2.12 ddd and 1.60 dd) were observed. The HMBC correlations from H_3-17 (δ_H 1.37, s) to C-9 (δ_C 46.3), C-10 (δ_C 70.1), and C-11 (δ_C 44.9) further extend this substructure from C-9 to C-11 (Figure 1). Moreover, the C-8−C-11 moiety was fused to the benzene ring at C-7 and C-12, as suggested by the HMBC correlations from H_2-11 (δ_H 2.88 d and 2.71 dd) to C-7, C-12 (δ_C 136.2), and C-13 (δ_C 130.5), from H_3-18 to C-7, and from H-13 (δ_H 7.03, d) to C-7, C-11, and C-15. The remaining hydroxymethylene group was assigned at C-15, based on the observed HMBC correlations from H_2-16 (δ_H 4.58 d and 4.52 d) to C-6, C-14 (δ_C 127.4), and C-15, as well as from H-14 (δ_H 7.24, d) to C-6, C-12, and C-16 (δ_C 63.6). Therefore, the planar structure of **2** was established as shown in Figure 1. The relative configuration was deduced by analysis of the coupling constants and the ROESY spectrum. The large coupling constants of H-2/H-3 (15.3 Hz) and H-4/H-5 (16.0 Hz) indicated the 2*E*, 4*E* configurations of the double bonds in the side chain. The ROESY correlations between H_3-17/H-11a, H_3-17/H-9b, H-11a/H-9b, H_3-18/H-9b, and H_3-18/H-11a clearly indicated that H_3-18 and H_3-17 are in a *cis*-configuration (Figure 2). It is worth mentioning that a similar compound, tanzawaic acid D, was previously isolated

Table 2. ^{1}H and ^{13}C NMR Data of 2 and 3 (CD_3OD)

	2		3	
position	δ_C, type	δ_H, mult. (J)[a]	δ_C, type[b]	δ_H, mult. (J)[a]
1	170.5, C		170.5, C	
2	122.4, CH	5.95, d (15.3)	120.7, CH	5.81, d (15.3)
3	146.6, CH	7.49, dd (15.3, 11.0)	146.7, CH	7.25, dd (15.3, 11.0)
4	133.1, CH	6.62, dd (16.0, 11.0)	131.0, CH	6.28, dd (15.3, 11.0)
5	140.4, CH	7.22, brd (16.0)	150.7, CH	5.91, dd (15.3, 9.4)
6	136.0, C		51.3, CH	2.65, brt (8.4)
7	141.4, C		50.2, CH	1.02, ddd (11.0, 10.2, 7.7)
8	30.3, CH	3.36, m	40.7, CH	1.42, m
9	46.3, CH_2	2.12, ddd (14.1, 8.0, 2.5)	41.2, CH_2	1.73, ddd (13.2, 3.2, 2.4)
		1.60, dd (14.1, 6.5)		0.81, q (12.4)
10	70.1, C		40.7, CH	1.55, m
11	44.9, CH_2	2.88, d (15.8)	33.7, CH_2	2.32, ddd (12.8, 3.0, 2.4)
		2.71, dd (15.8, 2.4)		0.65, q (12.1)
12	136.2, C		47.3, CH	1.13, m
13	130.5, CH	7.03, d (7.8)	72.4, CH	3.70, brdd (9.2, 1.5)
14	127.4, CH	7.24, d (7.8)	130.9, CH	5.55, brs
15	138.2, C		135.2, C	
16	63.6, CH_2	4.58, d (12.4)	22.0, CH_3	1.59, s
		4.52, d (12.4)		
17	30.9, CH_3	1.37, s	68.7, CH_2	3.39, dd (10.7, 6.2)
				3.36, dd (10.7, 6.6)
18	23.5, CH_3	1.17, d (7.0)	23.1, CH_3	0.97, d (6.6)

[a]The methylene proton assigned in the upper row was referred to as proton "a", while the other one as proton "b". [b]Data extracted from HSQC and HMBC spectra.

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from *P. citrinum*,[16a] the only difference being that compound **2** has a hydroxymethylene group at C-16 instead of a methyl group as in tanzawaic acid D. The similar NMR data, coupling constants, and ROESY correlations for both compounds, especially their similar specific optical rotation values [**2**: $[\alpha]_D^{23}$ +74.0 (*c* 0.1, MeOH); tanzawaic acid D: $[\alpha]_D$ +118 (*c* 0.57, MeOH)[16a]], indicated that both share the same absolute configuration. Therefore, compound **2** was determined as 16-hydroxytanzawaic acid D and named tanzawaic acid G.

The molecular formula of **3** was established as $C_{18}H_{26}O_4$ by HRESIMS. Its NMR data (Table 2) were similar to those of tanzawaic acid B,[16] indicating that **3** was a tanzawaic acid derivative. A penta-2,4-dienoic acid side chain was recognized by the diagnostic olefinic protons signals at δ_H 7.25 (dd, H-3), 6.28 (dd, H-4), 5.91 (dd, H-5), and 5.81 (d, H-2) in the ^{1}H NMR spectrum of **3**, like those of tanzawaic acid B.[16] A comparison of the NMR data further indicates that one of the methyl groups in tanzawaic acid B was replaced by a hydroxymethylene function (δ_H 3.39 dd and 3.36 dd, H_2-17; δ_C 68.7, C-17) in **3**. This hydroxymethylene group was positioned at C-10, as it showed a COSY correlation to H-10 (δ_H 1.55, m) and HMBC correlations to C-9 (δ_C 41.2), C-10 (δ_C 40.7), and C-11 (δ_C 33.7). A further detailed analysis of the COSY spectrum allowed us to establish a long spin system within the molecule starting from CH-2 and sequentially extending until CH-14 (Figure 1). In addition, the COSY correlations from H_3-18 (δ_H 0.97, d) to H-8 (δ_H 1.42, m) and from H-7 (δ_H 1.02, ddd) to H-12 (δ_H 1.13, m) were observed. Moreover, a methyl group (δ_H 1.59, s, H_3-16) was suggested to be attached to C-15, since it showed HMBC correlations to C-6 (δ_C 51.3), C-14 (δ_C 130.9), and C-15 (δ_C 135.2). Thus, the planar structure of **3** was elucidated as shown in Figure 1, which has hydroxyl substituents at C-13 and C-17 and a C14/15 double bond. The configurations of the double bonds were deduced as 2*E*, 4*E*, 14*Z* on the basis of the coupling constants ($^3J_{H\text{-}2,\ H\text{-}3}$ = $^3J_{H\text{-}4,\ H\text{-}5}$ = 15.3 Hz) and of the ROESY correlation between H-14 (δ_H 5.55, brs) and H_3-16. Similarly, the relative configuration of the other stereocenters was determined from the ROESY spectrum together with proton−proton coupling constants. The axial orientations of H-7, H-8, H-9b (δ_H 0.81, q), H-10, H-11b (δ_H 0.65, q), H-12, and H-13 (δ_H 3.70, brdd) were indicated by the large vicinal coupling constants ($^3J_{H\text{-}7,H\text{-}6}$ = 7.7 Hz, $^3J_{H\text{-}7,H\text{-}8}$ = 11.0 Hz, $^3J_{H\text{-}7,H\text{-}12}$ = 10.2 Hz, $^3J_{H\text{-}9b,H\text{-}8}$ = $^3J_{H\text{-}9b,H\text{-}10}$ = 12.4 Hz, $^3J_{H\text{-}11b,H\text{-}10}$ = $^3J_{H\text{-}11b,H\text{-}12}$ = 12.1 Hz, and $^3J_{H\text{-}13,H\text{-}12}$ = 9.2 Hz). The ROESY spectrum further suggested that H-5, H-7, H-9b, 11b, H-13, and H_3-18 were cofaced based on the observed correlations of H-9b/H-7, H-7/H-11b, H-11b/H-13, H-7/H-13, H-5/H-7, H_3-18/H-9b, H_2-17/H-9b, and H_2-17/H-11b, while the correlations of H-6 (δ_H 2.65, brt)/H-8, H-6/H-12, H-8/H-12, H-12/H-11a (δ_H 2.32, ddd), H-12/H-10, H-10/H-11a, and H-10/H-9a (δ_H 1.73 ddd) indicated that they were directed to the other face (Figure 2). Thus, the relative configurations at C-6, C-7, C-8, C-10, and C-12 were established to be the same as those in tanzawaic acid B.[16] On consideration of the same biogenetic pathway, compound **3** should have the same absolute configurations as those present in tanzawaic acid B.[33] The name tanzawaic acid H was given to this new derivative.

Compound **4** was isolated as white, amorphous powder. The HRESIMS spectrum exhibited a pseudomolecular ion peak at *m*/*z* 225.0757 $[M + H]^+$, indicating a molecular formula of $C_{11}H_{12}O_5$. The NMR data (Table 3) were similar to those of curvulinic acid, previously isolated from *P. canescens*,[34] the only difference being that one of the aromatic protons in curvulinic acid was replaced by a methyl group [δ_H 2.04 (s), δ_C 8.0 (CH_3)] in **4**, as suggested by the molecular weight of **4** being 14 amu higher than that of curvulinic acid. This methyl group was attached to C-6, as it showed correlations to C-5 (δ_C 160.8), C-6 (δ_C 111.8), and C-7 (δ_C 161.3) in the HMBC spectrum. Thus, compound **4** was deduced as 6-methylcurvulinic acid. This structure was confirmed by further analysis of the HMBC correlations as shown in Figure 1, as well as by the ROESY correlations as shown in Figure 2.

Table 3. ^{1}H and ^{13}C NMR Data of 4 and 5 (CD_3OD)

	4		5	
position	δ_C, type[a]	δ_H, mult. (*J*)	δ_C, type	δ_H, mult. (*J*)
1	175.1, C		141.0, CH	9.25, s
2	41.4, CH_2	3.76, s		
3	134.9, C		143.2, C	
4	112.3, CH	6.29, s	119.4, CH	7.94, s
4a			142.2, C	
5	160.8, C		109.5, C	
6	111.8, C		166.0, C	
7	161.3, C		101.1, CH	6.84, s
8	118.5, C		159.7, C	
8a			115.3, C	
9	206.2, C		19.0, CH_3	2.74, s
10	32.0, CH_3	2.57, s	9.5, CH_3	2.39, s
6-Me	8.0, CH_3	2.04, s		
8-OCH_3			57.0, CH_3	4.07, s

[a]Data extracted from HSQC and HMBC spectra.

A nitrogen-containing compound, **5**, was isolated as a yellow, amorphous powder. It has a molecular formula of $C_{12}H_{13}NO_2$, as indicated by HRESIMS measurement. The ^{1}H NMR spectrum of **5** exhibited two methyl singlets (δ_H 2.39 and 2.74), one methoxy singlet (δ_H 4.07), and three singlets in the aromatic region (δ_H 6.84, 7.94, and 9.25) (Table 3). The ^{13}C NMR and DEPT-135 spectra revealed the presence of nine sp^2 carbons (δ_C 166.0, 159.7, 143.2, 142.2, 141.0, 119.4, 115.3, 109.5, and 101.1), one methoxy group (δ_C 57.0), and two other methyl carbons (δ_C 9.5 and 19.0) (Table 3). The significantly downfield shifted olefinic methine signal at δ_H 9.25 (H-1) and its carbon at δ_C 141.0 were reminiscent of an isoquinoline skeleton.[35] This was confirmed by HMBC and ROESY experiments. The HMBC correlations from H_3-9 (δ_H 2.74) to C-3 (δ_C 143.2) and C-4 (δ_C 119.4), from H-1 to C-3, C-4a (δ_C 142.2), C-8 (δ_C 159.7), and C-8a (δ_C 115.3), and from H-4 (δ_H 7.94) to C-3 and C-9 (δ_C 19.0) indicated a methyl group being substituted at C-3 (Figure 1). Moreover, the correlations from H_3-10 (δ_H 2.39) to C-4a, C-5 (δ_C 109.5), and C-6 (δ_C 166.0), together with the correlations from H-4 to C-4a, C-5, and C-8a allowed the assignment of a methyl group to C-5. In addition, the correlations from H-7 (δ_H 6.84) to C-5, C-6, C-8, and C-8a, as well as 8-OMe (δ_H 4.07) to C-8 suggested the methoxy group being located at C-8 and adjacent to the aromatic proton H-7. The cross-peaks in the ROESY spectrum between H-4 and both H_3-9 and H_3-10 (Figure 2) confirmed the positions of Me-9 and Me-10, while the ROESY correlation between H-7 and 8-OMe was in agreement with their *ortho* positions. Therefore, **5** was determined as a new isoquinoline alkaloid and named 8-methoxy-3,5-dimethylisoquinolin-6-ol.

Compound **6** was identified as a new natural product by comparison of its spectroscopic data to those of 1,2,3,11b-tetrahydroquinolactacide,[36] which was an intermediate in the

synthesis of quinolactacide. However, only proton NMR data had been reported in the literature.[36] In the present study, the full set of NMR data is presented for this new natural product for the first time by using the 1D and 2D NMR techniques. This compound is probably a biogenetic precursor of quinolactacide, as they co-occurred in the title fungus.

It is known that fermentation of fungi on different culturing media can provide large numbers of fungal metabolites to be used for biological and chemical evaluation.[37] The present study also indicated that the secondary metabolites belonging to different structure classes could be produced by culturing *P. citrinum* on different media. For example, the rice culture of the title fungus mainly produced isochromans (arohynapene D and (3*S*)-6-hydroxy-8-methoxy-3,5-dimethylisochroman), quinolactacide derivatives (**6**, quinolactacide, and methylpenicinoline), and their mixed quinolactacide-isochroman product (**1**), while upon changing to a white bean medium the fungus mainly produced tanzawaic acid derivatives (**2**, **3**, and tanzawaic acid F) and other alkaloids (**5**, trichodermamide C, citrinamide A, and indole acetic acid methyl ester).

All isolated compounds were examined for their effects on the growth of the L5178Y mouse lymphoma cell line using the MTT assay. Compound **1** showed cytotoxicity (IC_{50} 6.1 μM) comparable with the positive control kahalalide F (IC_{50} 4.3 μM). The remaining compounds exhibited no activity (IC_{50} > 10 μM). Moreover, all compounds were evaluated for their antimicrobial activities against *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, and *Escherichia coli* ATCC 25922; however, none exhibited any significant activity at a concentration of 64 μg/mL.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer-241 MC polarimeter. ^{1}H (600 MHz), ^{13}C (150 MHz), and 2D NMR spectra were recorded on a Bruker AVANCE DMX 600 NMR spectrometer. The chemical shifts (δ) are in ppm referring to the deuterium solvent peaks at δ_H 3.31 (CD_3OD) and 2.50 (DMSO-d_6) and δ_C 49.0 (CD_3OD) and 39.5 (DMSO-d_6) for ^{1}H and ^{13}C, respectively. Mass spectra were measured by an LC-MS HP1100 Agilent Finnigan LCQ Deca XP Thermoquest, and high-resolution mass spectroscopy (HRESIMS) spectra were recorded with a FTHRMS-Orbitrap (Thermo Finnigan) mass spectrometer. HPLC analysis was performed with a Dionex P580 system coupled to a photodiode array detector (UVD340S). The detection was set at 235, 254, 280, and 340 nm. The analytical column (125 × 4 mm, L × i.d.) was prefilled with Europhere 10 C_{18} (Knauer, Germany). HPLC separation was performed on a semipreparative HPLC system of Lachrom-Merck Hitachi (pump L7100; UV detector L7400; column Europhere 100 C_{18}, 300 × 8 mm, Knauer, Germany) with a flow rate of 5.0 mL/min. Column chromatography was performed using Merck MN silica gel 60 M (0.04−0.063 mm) or Sephadex LH-20 as stationary phase. For TLC analyses and preparative TLC precoated silica gel 60 F_{254} plates (Merck) were used followed by detection under UV 254 and 366 nm or after spraying with anisaldehyde reagent. Solvents were distilled before use, and spectral grade solvents were used for spectroscopic measurements.

Fungal Material. The fungus (strain no. MM 3S-3) was isolated under aseptic conditions from a healthy stem of *C. siliqua* L. (Fabaceae) collected in Morocco in 2011 according to the procedure described by Kjer et al.[37] The identification was performed according to a molecular biological protocol by DNA amplification and sequencing of the ITS region (GenBank accession no. JQ901861) as described previously.[37] The fungal strain was cultivated on solid rice or solid white bean medium, which was prepared by autoclaving 100 g of rice or beans and 100 mL of water in a 1 L Erlenmeyer flask. For each medium, fermentation was performed in five flasks for 30 days at room temperature under static conditions.

Extraction and Isolation. After fermentation, the fungal culture in each flask was extracted with ethyl acetate (2 × 400 mL). The obtained crude extract from rice medium (4.0 g) or bean medium (6.0 g) was partitioned between *n*-hexane and 90% MeOH in water. Each methanolic extract was subjected to VLC on silica gel 60 eluting with a gradient of *n*-hexane−ethyl acetate and of dichloromethane (DCM)−MeOH to give several subfractions. Further purification was achieved by size exclusion chromatography over Sephadex LH-20 using MeOH or DCM−MeOH (1:1) as mobile phase and/or by semipreparative HPLC (Merck Hitachi L7100) using MeOH−H_2O as mobile phase to yield **1** (2.0 mg), **4** (1.4 mg), **6** (3.5 mg), 4-hydroxyquinolin-2(1*H*)-one (1.8 mg), quinolactacide (7.5 mg), methylpenicinoline (5.0 mg), tanzawaic acid B (3.9 mg), arohynapene D (1.3 mg), and (3*S*)-6-hydroxy-8-methoxy-3,5-dimethylisochroman (14.5 mg) from rice cultures, and **2** (2.5 mg), **3** (1.1 mg), **5** (12.8 mg), penicinoline (5.5 mg), trichodermamide C (1.8 mg), citrinamide A (6.5 mg), indole acetic acid methyl ester (1.6 mg), tanzawaic acid F (1.0 mg), (3*S*)-4,6-dihydro-8-methoxy-3,5-dimethyl-6-oxo-3*H*-2-benzopyran (8.8 mg), and vanillic acid (1.1 mg) from bean cultures.

*Citriquinochroman (**1**):* yellow, amorphous powder; $[\alpha]_D^{23}$ −14.1 (*c* 0. 1, MeOH); UV λ_{max} (PDA) 202.5, 289.4, and 336.2 nm; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data in CD_3OD, see Table 1; ESIMS *m*/*z* 443.2 $[M + H]^+$ and 441.4 $[M - H]^-$; HRESIMS *m*/*z* 443.1601 $[M + H]^+$ (calcd for $C_{26}H_{23}N_2O_5$, 443.1601).

*Tanzawaic acid G (**2**):* white, amorphous powder; $[\alpha]_D^{23}$ +74.0 (*c* 0.1, MeOH); UV λ_{max} (PDA) 203.8, 248.1, and 293.6 nm; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data in CD_3OD, see Table 2; ESIMS *m*/*z* 320.0 $[M + NH_4]^+$, 605.0 $[2M + H]^+$, 622.0 $[2M + NH_4]^+$, 627.0 $[2M + Na]^+$, 301.4 $[M - H]^-$, and 603.1 $[2M - H]^-$; HRESIMS *m*/*z* 325.1410 $[M + Na]^+$ (calcd for $C_{18}H_{22}O_4Na$, 325.1410).

*Tanzawaic acid H (**3**):* white, amorphous powder; $[\alpha]_D^{20}$ +28.0 (*c* 0.1, MeOH); UV λ_{max} (PDA) 269.5 nm; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data in CD_3OD, see Table 2; ESIMS *m*/*z* 305.3 $[M - H]^-$, 610.8 $[2M - H]^-$; HRESIMS *m*/*z* 329.1723 $[M + Na]^+$ (calcd for $C_{18}H_{26}O_4Na$, 329.1723).

*6-Methylcurvulinic acid (**4**):* white, amorphous powder; UV λ_{max} (PDA) 220.8 and 284.7 nm; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data in CD_3OD, see Table 3; ESIMS *m*/*z* 224.9 $[M + H]^+$ and 222.9 $[M - H]^-$; HRESIMS *m*/*z* 225.0757 $[M + H]^+$ (calcd for $C_{11}H_{13}O_5$, 225.0757), 247.0576 $[M + Na]^+$ (calcd for $C_{11}H_{12}O_5Na$, 247.0577).

*8-Methoxy-3,5-dimethylisoquinolin-6-ol (**5**):* yellow, amorphous powder; UV λ_{max} (PDA) 211.8, 260.6, 372.9 nm; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data in CD_3OD, see Table 3; ESIMS *m*/*z* 204.2 $[M + H]^+$ and 202.2 $[M - H]^-$; HRESIMS *m*/*z* 204.1017 $[M + H]^+$ (calcd for $C_{12}H_{14}NO_2$, 204.1019).

*1,2,3,11b-Tetrahydroquinolactacide (**6**):* yellow, amorphous powder; $[\alpha]_D^{28}$ −75.5 (*c* 0.2, DMSO); UV λ_{max} (PDA) 214.6, 251.1, and 311.2 nm; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.74 (1H, s, NH), 8.17 (1H, dd, *J* = 7.8, 1.0 Hz, H-7), 7.72 (1H, ddd, *J* = 8.1, 7.2, 1.0 Hz, H-9), 7.58 (1H, brd, *J* = 8.1 Hz, H-10), 7.40 (1H, dd, *J* = 7.8, 7.2 Hz, H-8), 4.68 (1H, dd, *J* = 9.5, 6.9 Hz, H-11b), 3.47 (1H, dt, *J* = 10.8, 8.1 Hz, H-3a), 3.13 (1H, m, H-3b), 2.29 (1H, m, H-1a), 2.16 (2H, m, H_2-2), 1.48 (1H, m, H-1b); ^{13}C NMR (150 MHz, DMSO-d_6) δ 172.2 (C-6), 171.1 (C-5), 163.2 (C-11a), 139.6 (C-10a), 132.4 (C-9), 127.4 (C-6a), 125.6 (C-7), 124.1 (C-8), 118.7 (C-10), 108.5 (C-5a), 60.8 (C-11b), 42.7 (C-3), 28.4 (C-1), 27.8 (C-2); ESIMS *m*/*z* 241.2 $[M + H]^+$, and 239.4 $[M - H]^-$; HRESIMS *m*/*z* 241.0971 $[M + H]^+$ (calcd for $C_{14}H_{13}N_2O_2$, 241.0972), 263.0790 $[M + Na]^+$ (calcd for $C_{14}H_{12}N_2O_2Na$, 263.0791).

Cytotoxicity Assay. Cytotoxicity was tested against L5178Y mouse lymphoma cells using the microculture tetrazolium (MTT) assay and compared to that of untreated controls, as described previously.[38] As negative controls, media with 0.1% EGMME−DMSO were included in the experiments. The cytotoxic depsipeptide kahalalide F, isolated from *Elysia grandifolia*, was used as a positive control.[38]

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Antibacterial Assay. All isolated compounds were tested for their antimicrobial activities against *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, and *Escherichia coli* ATCC 25922, following the broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). For preparation of the inoculum the direct colony suspension method was used.[39]

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, 2D NMR, and MS spectra for compounds **1**–**6** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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