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The phytoalexins from cauliflower, caulilexins A, B and C: Isolation, structure determination, syntheses and antifungal activity

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Abstract

Our continuous search for phytoalexins from crucifers led us to examine phytoalexin production in florets of cauliflower (*Brassica oleracea* var. *botrytis*) under abiotic (UV light) elicitation. Four known (isalexin, *S*-(−)-spirobrassinin, 1-methoxybrassitin, brassicanal C) and three new (caulilexins A–C) phytoalexins were isolated. The syntheses and antifungal activity of caulilexins A–C against the economically important pathogenic fungi *Leptosphaeria maculans*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, and the first synthesis of brassicanal C are reported.

Keywords: *Brassica oleracea*; Cruciferae; Brassicanal C synthesis; Brassitin; Cauliflower; Crucifer; *Leptosphaeria maculans*; 1-Methoxyindolyl-3-acetonitrile; *Phoma lingam*; Phytoalexin; Spirobrassinin

1. Introduction

Cauliflower (*Brassica oleracea* var. *botrytis*) is a crucifer vegetable cultivated and consumed worldwide. Despite the number of studies dealing with secondary metabolites of cauliflower (Llorach et al., 2003), including volatiles (Valette et al., 2006), glucosinolates (Tian et al., 2005; Menard et al., 1999) and oviposition stimulants (Hurter et al., 1999), no phytoalexins have been reported to date. Phytoalexins are secondary metabolites produced de novo by plants in response to various forms of stress, including microbial attack (Bailey and Mansfield, 1982). Most, if not all, of the phytoalexins produced by crucifers are derived from tryptophan and therefore contain an indole or oxindole moiety attached to a variety of functional groups (Pedras et al., 2000, 2003b). Due to the significance of phytoalexins in plant fitness, we have been investigating the phytoalexins of both cultivated and wild crucifers (Pedras et al., 2000, 2003b) and their interaction with economically important pathogens. Here we wish to report the isolation, structures, syntheses, and antifungal activity of caulilexins A (**1**), B (**2**), and C (**3**), three new phytoalexins produced by cauliflower, together with four known phytoalexins, isalexin (**4**) (Pedras et al., 2004b), *S*-(−)-spirobrassinin (**5**) (Takasugi et al., 1987; Suchy et al., 2001), 1-methoxybrassitin (**6**) (Takasugi et al., 1988) and brassicanal C (**7**) (Monde et al., 1991). In addition, the synthesis of brassicanal C (**7**) and its antifungal activity against three important crucifer pathogens is reported here for the first time.

2. Results and discussion

2.1. Isolation and structure elucidation

Preliminary experiments were carried out to establish the period for maximum production of induced metabolites in cauliflower florets. Analyses of extracts of cauliflower florets exposed to UV light (abiotic elicitation) using high performance liquid chromatography (HPLC) with photodiode array detection indicated the presence of seven elicited compounds (t_R = 3.8, 8.8, 9.5, 12.2, 16.6, 16.8 and 17.8 min) not detected in control tissues (no UV irradiation, Table 1). The

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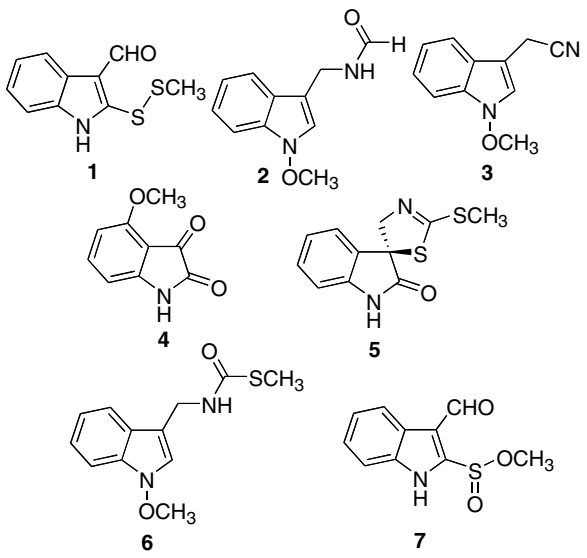


incubation period required for maximum production of these elicited compounds was about four days. Of the elicited metabolites, three of them (**4**–**6**) appeared to be known compounds (UV spectra identical to those available in our HPLC-UV library) whose chemical structures were confirmed upon comparison of their spectroscopic data with those of authentic samples available in our laboratory. Thus, compound **4** ($t_R = 3.8$ min) was found to be isalexin, previously isolated from rutabaga tubers (Pedras et al., 2004b), compound **5** ($t_R = 12.2$ min) was identified as *S*-(−)-spirobrassinin ($[\alpha]_D^{24} - 109$, CH_2Cl_2, *c* 0.35) having absolute configuration *S* as confirmed by ^{1}H NMR chiral solvation (Pedras et al., 2004a), and compound **6** ($t_R = 17.8$ min) was found to be 1-methoxybrassitin (Takasugi et al., 1988).

Of the unknown elicited compounds, the compound with $t_R = 16.6$ min (**1**) showed in the ^{1}H NMR spectrum nine protons (four aromatic, one aldehydic, Me, and one (N)H) consistent with its molecular formula of $C_{10}H_9NOS_2$ (HREIMS). The ^{13}C NMR spectrum confirmed the presence of 10 carbons and suggested that the Me group was attached to a sulfur atom. Further structural analysis suggested the presence of a disulfide bridge and thus the chemical structure of compound **1** was assigned as 2-dithiomethylindole-3-carboxaldehyde. The structure of compound **1** was confirmed by synthesis, as described below. The molecular formula of the compound with $t_R = 8.8$ min (**2**) was deduced to be $C_{11}H_{12}N_2O_2$, by HREIMS. The ^{1}H NMR spectrum showed the expected 12 protons (five aromatic, a singlet at δ_H 8.14 indicating the presence of a formamide group, MeO, and methylene) and the ^{13}C NMR spectroscopic data corroborated the structural assignments based on ^{1}H NMR analysis. Thus the structure of compound **2** was assigned as 1-methoxyindol-3-ylmethylformamide and ultimately confirmed by synthesis. The compound with $t_R = 16.8$ min (**3**) had a molecular formula of $C_{11}H_{10}N_2O$ (HREIMS) and displayed in the ^{1}H NMR spectrum the expected 10 protons (five aromatic, three MeO, and methylene). Both the ^{13}C NMR (δ_c 119.6) and FTIR (ν_{max} 2249 cm^{-1}) spectra indicated the presence of a nitrile group. Therefore, the chemical structure of **3** was provisionally deduced to be 1-methoxyindol-3-ylacetonitrile. The proposed structure was confirmed by synthesis as described below. The compound with $t_R = 9.5$ min (**7**) displayed in the ^{1}H NMR spectrum nine protons (four aromatic, one aldehydic, OMe, and (N)H) consistent with its molecular formula of $C_{10}H_9NO_3$ (HREIMS) and ^{13}C NMR spectroscopic data. Comparison of the spectroscopic data with literature data indicated that the compound with $t_R = 9.5$ min was brassicanal C (**7**) (Monde et al., 1991), whose optical rotation could not be measured accurately due to the very small amount isolated. Eventually, this indication was confirmed by synthesis of brassicanal C (**7**), as described below.

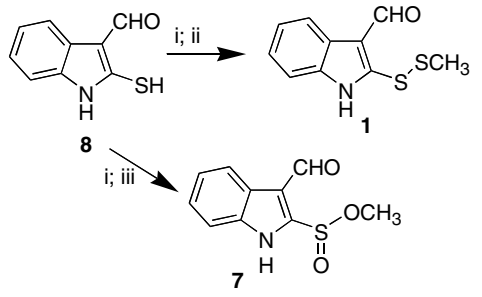
2.2. Syntheses of *1–3* and *7*

Synthesis of metabolites **1**–**3** and brassicanal C (**7**) was carried out in order to confirm their structures and to obtain sufficient amounts for testing for antifungal activity. Caulilexin A (**1**) was prepared from 2-sulfanylindole-3-carboxaldehyde (**8**) (Pedras and Okanga, 1999) by treatment first with iodine in pyridine followed by sodium thiomethoxide (Scheme 1). This one-pot process afforded caulilexin A in 33% yield from aldehyde **8**. Although brassicanal C (**7**) has been isolated previously, its synthesis has not been reported to date. Thus, a very simple preparation of **7**, similar to that of **1** but replacing sodium thiomethoxide

Table 1
Production of phytoalexins[a] in cauliflower florets (*Brassica oleraceae* var. *botrytis*) upon elicitation with UV light (250 nm)

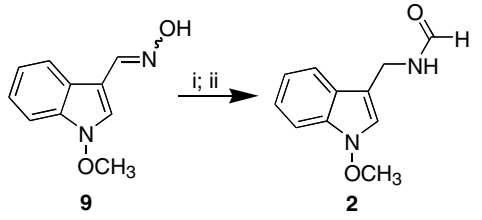
Phytoalexin	48 h μmol/100 g[b]	72 h μmol/100 g[b]	96 h μmol/100 g[b]	120 h μmol/100 g[b]
Caulilexin A (**1**)	0.059 ± 0.004	0.078 ± 0.019	0.17 ± 0.01	0.13 ± 0.08
Caulilexin B (**2**)	0.084 ± 0.018	0.18 ± 0.02	0.23 ± 0.05	0.064 ± 0.009
Caulilexin C (**3**)	0.012 ± 0.001	0.055 ± 0.013	0.039 ± 0.008	0.020 ± 0.001
Isalexin (**4**)	0.062 ± 0.014	0.23 ± 0.03	0.49 ± 0.05	0.46 ± 0.06
S-(−)-spirobrassinin (**5**)	0.051 ± 0.007	2.44 ± 0.65	3.97 ± 0.18	1.11 ± 0.25
1-Methoxybrassitin (**6**)	0.17 ± 0.00	0.075 ± 0.010	0.054 ± 0.006	0.030 ± 0.00
Brassicanal C (**7**)	0.22 ± 0.05	0.49 ± 0.09	0.43 ± 0.09	0.30 ± 0.09

[a] Results are presented as means ± standard deviation.
[b] Fresh floret tissue.

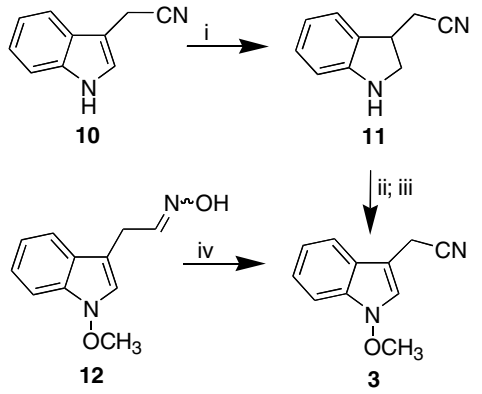


Scheme 1. Synthesis of caulilexin A (**1**) and brassicanal C (**7**); reagents and conditions: (i) I_2, pyridine; (ii) CH_3SNa, 33% from **8**; (iii) MeOH, 22% from **8**.

with methanol, was developed (22% yield, Scheme 1). To prepare caulilexin B (**2**), 1-methoxyindole-3-carboxaldehyde oxime (**9**) was reduced to the corresponding 1-methoxyindol-3-ylmethyl amine (Pedras et al., 2003b), which was then formylated in refluxing ethyl formate (85% yield, Scheme 2). Caulilexin C (**3**) was prepared by Ac_2O-mediated dehydration of 1-methoxyindol-3-yl acetaldoxime (**12**), which was obtained as previously reported (Pedras et al., 2003b) (42% yield, Scheme 3). An alternate route to **3** started with $NaBH_3CN$-AcOH-mediated reduction (Gribble, 1998) of commercially available indol-3-ylacetonitrile (**10**, Scheme 3) which yielded the indoline intermediate **11**. Subsequently, H_2O_2–Na_2WO_4-mediated oxidation of **11** (Somei and Kawasaki, 1989), followed by treatment with dimethyl sulfate afforded caulilexin C (**3**, Scheme 3) in 10% yield, based on recovered indolyl-3-acetonitrile (**11**). Although the yields of caulilexin C (**3**) obtained by both methods are rather low, previous syntheses appear to be less efficient (Acheson et al., 1984; Somei et al., 1985).



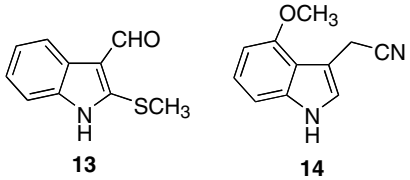
Scheme 2. Synthesis of caulilexin B (**2**); (i) $NaBH_3CN$, $TiCl_3$, $AcONH_4$, $MeOH/H_2O$; (ii) HCOOEt, reflux, 85% yield from **9**.



Scheme 3. Synthesis of caulilexin C (**3**); (i) $NaBH_3CN$, AcOH; (ii) H_2O_2, $Na_2WO_4 \cdot 2H_2O$; (iii) K_2CO_3, $(CH_3)_2SO_4$, $MeOH/H_2O$; 10% yield from **10**; (iv) Ac_2O, reflux, 42% yield from **12**.

2.3. Antifungal activity

The antifungal activity of metabolites **1**–**3**, **7**, **13** and **14** against the phytopathogenic fungi *Leptosphaeria maculans* (Desm.) Ces. et de Not. [asexual stage *Phoma lingam* (Tode ex Fr.) Desm.], *Sclerotinia sclerotiorum* (Lib.) de Bary and *Rhizoctonia solani* Kuhn, was determined using a mycelial radial growth antifungal assay. The phytoalexins brassicanals A (**13**) and C (**7**) and arvelexin (**14**) were used in the bioassays as standards due to their structural resemblance to caulilexins A (**1**) and C (**3**). The results of these antifungal bioassays are shown in Table 2. Caulilexin A (**1**) appeared to exhibit the strongest antifungal activity among the various metabolites, causing complete growth inhibition of *R. solani* (at 5.0×10^{-4} M) and *S. sclerotiorum* (at 1.0×10^{-4} M), that is substantially higher activity than brassicanal A (**13**) but showed lower activity against *L. maculans* (slightly lower than that of brassicanal A (**13**)). Caulilexin C (**3**) caused complete growth inhibition (5.0×10^{-4} M) of *R. solani* and had a smaller effect on *L. maculans* (77% inhibition), appearing to be slightly more antifungal than arvelexin (**14**), whereas caulilexin B (**2**) exhibited the lowest antifungal activity against the tested fungi. In the TLC bioassay against *C. cucumerinum*, caulilexin A (**1**) caused complete growth inhibition at 1.0×10^{-8} M, whereas all other tested compounds caused inhibition of mycelial growth at 100 times higher concentrations (1.0×10^{-6} M).



3. Conclusions

Three metabolites were isolated from stressed cauliflower florets (**1**–**3**), their structures were elucidated, the syntheses were carried out and the antifungal activity was established. Compounds **1**–**3** display antifungal activity, are elicited metabolites produced by cauliflower under abiotic elicitation (UV light) and are not detectable in control plants. Thus, metabolites **1**–**3** satisfy the conditions to be new

Table 2
Antifungal activity[a] of caulilexins A-C (**1**–**3**), brassicanals A (**13**) and C (**7**), and arvelexin (**14**) against *Leptosphaeria maculans*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*

Compound	Concentration (M)	*Leptosphaeria maculans*[b]	*Sclerotinia sclerotiorum*[c]	*Rhizoctonia solani*[d]
Caulilexin A (**1**)	5.0×10^{-4}	55 ± 7	100 ± 0	100 ± 0
	2.5×10^{-4}	39 ± 3	100 ± 0	83 ± 9
	1.0×10^{-4}	21 ± 7	100 ± 0	50 ± 8
	5.0×10^{-5}	10 ± 6	90 ± 7	13 ± 2
	1.0×10^{-5}	n.d.	71 ± 12	n.d.
Caulilexin B (**2**)	5.0×10^{-4}	31 ± 8	31 ± 1	18 ± 7
	2.5×10^{-4}	17 ± 6	20 ± 2	n.i.
	1.0×10^{-4}	n.i.	n.i.	n.i.
Caulilexin C (**3**)	5.0×10^{-4}	77 ± 2	30 ± 8	100 ± 0
	2.5×10^{-4}	45 ± 5	n.i.	80 ± 7
	1.0×10^{-4}	30 ± 10	n.i.	48 ± 11
Brassicanal C (**7**)	5.0×10^{-4}	70 ± 5	33 ± 9	53 ± 4
	2.5×10^{-4}	62 ± 7	11 ± 4	33 ± 7
	1.0×10^{-4}	30 ± 5	n.i.	n.i.
	5.0×10^{-5}	13 ± 4	n.i.	n.i.
Brassicanal A (**13**)	5.0×10^{-4}	70 ± 5	33 ± 9	53 ± 4
	2.5×10^{-4}	62 ± 7	12 ± 5	33 ± 7
	1.0×10^{-4}	30 ± 5	8 ± 2	n.i.
	5.0×10^{-5}	13 ± 4	n.i.	n.i.
Arvelexin (**14**)	5.0×10^{-4}	59 ± 3	37 ± 3	70 ± 3
	2.5×10^{-4}	36 ± 3	17 ± 3	17 ± 7
	1.0×10^{-4}	15 ± 2	n.i.	n.i.

[a] % Inhibition $= 100 - [(\text{growth on treated/growth on control})\times100]\pm$ SD; results are the means of at least three separate experiments; n.i., no inhibition; n.d., not determined.
[b] Results after 4 days of incubation.
[c] Results after 2 days of incubation.
[d] Results after 3 days of incubation.

phytoalexins, for which we propose the names caulilexins A (**1**), B (**2**) and C (**3**). Caulilexins **1**–**3** accumulate in florets of cauliflower to levels comparable to those of other phytoalexins (Pedras et al., 2003a). Caulilexin A (**1**) is the first example of an indolyldisulfide phytoalexin and is the most active of all phytoalexins against *S. sclerotiorum*. Caulilexin B (**2**) represents yet another example of a non-sulfur containing indolyl phytoalexin. Caulilexin C (**3**) is the third indol-3-ylacetonitrile found to be a phytoalexin; previously, indolyl-3-acetonitrile was found to be a phytoalexin of *B. juncea* and arvelexin (1-methoxyindol-3-ylacetonitrile) a phytoalexin of stinkweed (*Thlaspi arvense*) (Pedras et al., 2003a). It is worthy to note that nitriles seem to be rather active against *L. maculans* and *R. solani* but less active against *S. sclerotiorum*. Nitrile **3** was previously isolated from Chinese cabbage (Nomoto and Tamura, 1970) and was also obtained from incubations of an indole glucosinolate with myrosinase (Hanley et al., 1990; Agerbirk et al., 1998). However, this is the first time that compound **3** is reported to be an induced metabolite and to display antifungal activity. In agreement with previous results (Pedras et al., 2004b, 2003a), the current bioassay results using three different plant pathogens suggest that crucifer phytoalexins display selective antifungal activities.

Crucifer vegetables are known to be biologically active against carcinogenesis due to their ability to induce phase II conjugating enzymes, and have additional positive effects in mammalian systems (Seow et al., 2005; Hsu et al., 2005; Eberhardt et al., 2005). Therefore, not surprisingly, some indolyl phytoalexins (brassinin, spirobrassinin, brassilexin, camalexin, 1-methoxyspirobrassinin) have been found to possess significant activity against various cancer cells (Mezencev et al., 2003). While it is likely that the effect of phytoalexins on human health is not readily noticeable (due to the very small amounts produced), structures like caulilexin A (**1**) might be good leads for the design of compounds with potential antiproliferative and chemopreventive activities in mammals.

4. Experimental

4.1. General

All chemicals were purchased from Sigma–Aldrich Canada Ltd., Oakville, ON. Analytical HPLC analysis was carried out with a high performance liquid chromatograph equipped with quaternary pump, automatic injector, and photodiode array detector (wavelength range 190–600 nm), degasser, and a Hypersil ODS column (5 μm particle size silica, 4.6 i.d. ×200 mm), equipped with an in-line filter. Mobile phase: H_2O–CH_3CN, 75:25 to 100% CH_3CN, in 35 min, linear gradient, and 1.0 ml/min flow rate. Samples were dissolved either in CH_3CN or in MeOH.

Specific rotations, $[\alpha]_D$ were determined at ambient temperature on a polarimeter using a 1 ml, 10 cm path length cell; the units are 10^{-1} deg cm^2 g^{-1} and the concentrations (c) are reported in g/100 ml. NMR spectra were recorded on 500 MHz spectrometers. For ^{1}H NMR (500 MHz) the chemical shifts (δ) are reported in parts per million (ppm) relative to TMS. The δ values were referenced to CD_3CN (CD_2HCN at 1.94 ppm). Other conditions are as previously reported (Pedras and Okanga, 1999).

Plant material: cauliflower florets were purchased from local markets.

4.2. Fungal cultures and antifungal bioassays

Leptosphaeria maculans isolate BJ-125, *R. solani* isolate AG 2-1 and *S. sclerotiorum clone #33* were obtained from AAFC, Saskatoon, Canada. Cultures were handled as described previously: *L. maculans* (Pedras and Okanga, 1999); *R. solani* (Pedras and Liu, 2004); *S. sclerotiorum* (Pedras and Ahiahonu, 2002). Radial growth antifungal bioassays were carried out as described previously: *L. maculans* (Pedras et al., 2003a); *R. solani* (Pedras and Liu, 2004); *S. sclerotiorum* (Pedras and Ahiahonu, 2002). *Cladosporium cucumerinum* TLC bioassay was performed as described previously (Pedras and Sorensen, 1998).

4.3. Isolation and identification of phytoalexins from UV elicited cauliflower florets

Cauliflowers were cut vertically in 10–15 mm thick slices or pieces and were incubated for 24 h in a closed chamber, 100% humidity. The slices were irradiated with a UV light (250 nm) for 15 min on each side. Control tissues were not UV elicited but otherwise were treated similarly. Control and elicited tissues were incubated further at 20 °C in darkness; one of the control slices and one of the elicited slices were collected at 24 h intervals for five days. Each slice was ground in a blender and the resulting material was stirred in EtOAc (150 ml) for 12 h. The macerates were filtered, the filtrates were dried and concentrated. The residues were dissolved in CH_3CN and were analysed by HPLC.

To isolate phytoalexins, cauliflower florets (12 kg) were treated as described above. After four days of incubation, slices were ground in a blender and the resulting material was stirred in EtOAc (18 l) for 24 h. The macerates were filtered, the filtrates were dried and were concentrated. The residue (4.05 g) was fractionated by FCC (CH_2Cl_2–hexane and CH_2Cl_2–MeOH gradient elution). Fractions containing elicited metabolites (HPLC analysis) were combined and were further fractionated using FCC, preparative TLC and reversed phase chromatography. Elicited metabolites were obtained as follows: caulilexin A (**1**, 1 mg), preparative TLC (CH_2Cl_2–Et_2O, 95:5); caulilexin B (**2**, 1 mg), FCC (hexane–Et_2O, 1:4, reversed phase chromatography, CH_3CN–H_2O, 3:7); caulilexin C (**3**, 1 mg), FCC (silica gel, hexane–EtOAc, 9:1; silica gel reversed phase CH_3CN–H_2O, 1:3); isalexin (**4**, 1 mg), FCC (silica gel, CH_2Cl_2–MeOH, 9:1: silica gel reversed phase, CH_3CN–H_2O, 9:1); *S*-(−)-spirobrassinin (**5**, 4 mg), FCC (silica gel, hexane–Et_2O, 1:1; silica gel reversed phase, CH_3CN–H_2O, 1:1); 1-methoxybrassitin (**6**, 2 mg), FCC (silica gel, hexane–Et_2O, 3:2; silica gel reversed phase, CH_3CN–H_2O, 1:1); brassicanal C (**7**, 1 mg), FCC (silica gel, CH_2Cl_2–MeOH, 9:1; silica gel reversed phase, CH_3CN–H_2O, 4:1).

4.4. Caulilexin A (*1*)

A solution of 2-sulfanylindole-3-carboxaldehyde (**8**, 53 mg, 0.30 mmol) in pyridine (1 ml) was added to a solution of iodine (76 mg, 0.30 mmol) in pyridine (0.5 ml), cooled to 0 °C. The mixture was stirred for 10 min at 0 °C, CH_3SNa (42 mg, 0.60 mmol) was added and the cooling bath was removed. After stirring for 2 h at r.t., the mixture was diluted with 1.5 M H_2SO_4 (15 ml) and was extracted with EtOAc. The combined organic extract was dried, concentrated and the residue subjected to FCC hexane–EtOAc (4:1) to afford caulilexin A (**1**) (22 mg, 33%) as a slightly yellow solid, m.p. 114–116 °C. HPLC t_R = 16.2 min; ^{1}H NMR (500 MHz, CD_3CN): δ 10.42 (*br s*, D_2O exchangeable, 1H), 10.20 (*s*, 1H), 8.09 (*d*, $J = 8$ Hz, 1H), 7.56 (*d*, $J = 8$ Hz, 1H), 7.33 (*dd*, $J = 8$, 8 Hz, 1H), 7.28 (*dd*, $J = 8$, 8 Hz, 1H), 2.60 (s, 3H). ^{13}C NMR (125 MHz, CD_3CN): δ 185.5 (*s*), 143.8 (*s*), 138.1 (*s*), 128.0 (*s*), 126.7 (*s*), 125.4 (*d*), 124.1 (*d*), 121.0 (*d*), 113.1 (*d*), 24.1 (*q*). HREIMS m/z [M^+] measured: 223.0121 (223.0126 calc. for $C_{10}H_9NOS_2$); EIMS m/z (% relative abundance): 223 [M^+] (58), 176 (100), 121 (19), 77 (16). FTIR ν_{max}: 3213, 2920, 1632, 1578, 1433, 1373, 1242, 846, 745 cm^{-1}. UV (MeOH) λ_{max} (log ε): 213 (4.4), 252 (4.2), 307 (4.0) nm.

4.5. Caulilexin B (*2*)

Crude 1-methoxyindolyl-3-methylamine (34 mg, 0.20 mmol) obtained from 1-methoxyindole-3-carboxaldehyde oxime (**9**) (Pedras et al., 2003b) was dissolved in HCOOEt (2 ml) and the mixture was refluxed for 16 h with stirring. The mixture was concentrated and the residue was subjected to FCC (CH_2Cl_2–MeOH, 95:5) to afford caulilexin B (23 mg, 85% based on oxime **11**), m.p. 71–73 °C, CH_2Cl_2–MeOH. HPLC: $t_R = 8.9$ min. ^{1}H NMR (500 MHz, CD_3CN): δ 8.14 (*s*, 1H), 7.65 (*d*, $J = 8$ Hz, 1H), 7.46 (*d*, $J = 8$ Hz, 1H), 7.38 (*s*, 1H), 7.27 (*dd*, $J = 8$, 8 Hz, 1H), 7.13 (*dd*, $J = 8$, 8 Hz, 1H), 6.76 (*br s*, D_2O exchangeable, 1H), 4.52 (*d*, $J = 6$ Hz, 1H), 4.07 (*s*, 3H); ^{13}C NMR (125 MHz, CD_3CN): δ 162.2 (*s*), 133.8 (*s*), 124.3 (*s*), 124.0 (*d*), 123.8 (*d*), 121.2 (*d*), 120.6 (*d*), 110.6 (*s*), 109.7 (*d*), 67.0 (*q*), 33.7 (*t*); HREIMS m/z [M^+] measured: 204.0898 (204.0899 calc. for $C_{11}H_{12}N_2O_2$); EIMS m/z (% relative abundance) 204 [M^+] (92), 173 (64), 145 (39), 118 (100), 91 (21); FTIR ν_{max}: 3282, 2935, 1661, 1523, 1452, 1382, 1226, 1101, 1010, 740 cm^{-1}; UV $(MeOH)_{max}$ (log ε): 221 (4.4), 290 (3.7) nm.

4.6. Caulilexin C (3)

4.6.1. Method A

A solution of 1-methoxyindolyl-3-acetaldoxime (**12**, 95 mg, 0.46 mmol) (Pedras et al., 2003b) in Ac_2O (1 ml) was stirred under reflux for 1 h. The reaction mixture was concentrated and the residue was subjected to FCC (CH_2Cl_2–MeOH, 98:2) to afford caulilexin C (**3**, 36 mg, 42%) as a semi-solid.

4.6.2. Method B

To a stirred solution of indol-3-ylacetonitrile (**10**, 100 mg, 0.64 mmol) in AcOH (2 ml), $NaBH_3CN$ (120 mg, 1.8 mmol) was added and the reaction mixture was stirred at r.t. After 3 h, the reaction mixture was diluted with H_2O (40 ml) and was extracted with CH_2Cl_2. The combined organic extract was dried, was concentrated and the residue was subjected to FCC (CH_2Cl_2) to afford indoline **11** (40 mg, 40% yield) and recovered indolyl-3-acetonitrile (**10**, 60 mg, 60%). Indoline **11** (40 mg, 0.25 mmol) was dissolved in MeOH (2 ml) and a solution of $Na_2WO_4 \cdot 2H_2O$ (22 mg, 0.07 mmol) in H_2O (160 μl) was added with stirring. The stirred mixture was cooled to −18 °C (ice + NaCl) and an aqueous solution of H_2O_2 (300 μl, 3 mmol) was added dropwise over a 15 min period. After stirring for further 30 min at −18 °C, K_2CO_3 (300 mg, 2.2 mmol) and $(CH_3)_2SO_4$ (80 μl, 0.85 mmol) were added and the mixture was stirred for 5 h at r.t. The mixture was diluted with H_2O (5 ml) and was extracted with CH_2Cl_2, with the combined organic extract dried and concentrated. The residue was subjected to FCC (EtOAc–hexane, 3:7) to afford 7 mg (10% yield from indolyl-3-acetonitrile (**10**)) of caulilexin C (**3**).

HPLC: $t_R = 16.8$ min. ^{1}H NMR (500 MHz, CD_3CN): δ 7.65 (*d*, $J = 8$ Hz, 1H), 7.50 (*d*, $J = 8$ Hz, 1H), 7.46 (*s*, 1H), 7.32 (*dd*, $J = 8$, 8 Hz, 1H), 7.18 (*dd*, $J = 8$, 8 Hz, 1H), 4.24 (*s*, 3H); 3.91 (*s*, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 133.4 (*s*), 124.3 (*d*), 123.6 (*s*), 123.4 (*d*), 121.3 (*d*), 119.7 (*d*), 119.6 (*s*), 109.6 (*d*), 102.2 (*s*), 67.0 (*q*), 14.5 (*t*); HREIMS *m/z* [M^+] measured: 186.0795 (186.0793 calc. for $C_{11}H_{10}N_2O$); EIMS *m/z* (% relative abundance) 186 [M^+] (98), 171 (37), 155 (100), 128 (58), 101 (23); FTIR ν_{max}: 2937, 2249, 1454, 1359, 1240, 1097, 1029, 739 cm^{-1}; UV $(MeOH)_{max}$ ($\log\varepsilon$): 219 (4.4), 271 (3.8) nm.

4.7. Brassicanal C (7)

A solution of 2-sulfanylindole-3-carboxaldehyde (**8**, 50 mg, 0.28 mmol) in MeOH (1.5 ml) was added to a solution of iodine (140 mg, 0.55 mmol) in MeOH (1.5 ml) and pyridine (200 μl) and the mixture was stirred for 4 h at r.t. The reaction mixture was concentrated and the residue was subjected to FCC (hexane–EtOAc, 4:1) to afford brassicanal C (14 mg, 22%) as a slightly yellow solid, m.p. 148–149 °C, hexane–EtOAc. HPLC: $t_R = 9.5$ min. Except for the optical rotation, the spectroscopic data of the synthetic sample were identical to those previously reported (Monde et al., 1991).

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