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Novel Sulfur-Containing Phytoalexins from the Chinese Cabbage *Brassica campestris* L. ssp. *pekinensis* (Cruciferae)[1]

Mitsuo TAKASUGI,* Kenji MONDE, Nobukatsu KATSUI, and Akira SHIRATA†,††
Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060
†National Institute of Agro-Environmental Sciences, Yatabe-machi, Tsukuba 305
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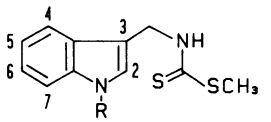
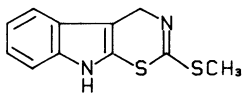
The first isolation and structure elucidation of four sulfur-containing indole phytoalexins from Chinese cabbage inoculated with *Pseudomonas cichorii* are described.

Accumulation of phytoalexins, antimicrobial compounds synthesized by plants after their exposure to micro-organisms,[2] has been described from over 20 plant families,[3] Because of their antimicrobial properties, an important role of phytoalexins in host defense mechanisms has been suggested.[4] We recently reported the isolation of two sesquiterpenoids,[5] a bibenzyl,[6] and two polyacetylenes[7] as phytoalexins from lettuce (*Lactuca sativa* var. *capitata*, Compositae), Chinese yam (*Dioscorea batatas*, Dioscoreaceae), and burdock (*Arctium lappa*, Compositae), respectively. The plant family Cruciferae includes many vegetables of economic importance such as cabbage, Chinese cabbage, radish, turnip, and so on.[8] However, no phytoalexins have been reported from this family. We reported, in a preliminary communication,[9] on the isolation and structures of three cruciferous phytoalexins, named methoxybrassinin (**1**), brassinin (**2**), and cyclobrassinin (**3**), from Chinese cabbage (*Brassica*



1 : R=OCH3
2 : R=H



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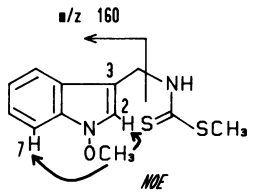
campestris L. ssp. *pekinensis*) inoculated with the bacterium *Pseudomonas cichorii*. In this paper we describe the details of the study and the isolation of an additional new phytoalexin, methoxybrassitin (**4**), together with a related compound **5**.

Results and Discussion

Sliced heads of Chinese cabbage were inoculated with the suspension of the bacterium *Pseudomonas cichorii* and incubated at 20 °C for 3 days. After being dried, the incubated tissue was extracted with acetone. Two-dimensional TLC bioassay[9] of the extracts revealed the presence of several antifungal compounds not detected in uninoculated control tissue. The acetone extracts from the inoculated tissue were submitted to sequential chromatography on silica gel and Sephadex LH-20 columns, giving five antifungal compounds **1**, **2**, **3**, **4**, and **5** in the yields (based on dry weight of the tissue) of 0.018%, 0.0022%, 0.0098%, 0.0011%, and 0.00047%, respectively.[10]

Methoxybrassinin (**1**) was obtained as a viscous oil. The molecular formula of **1** was determined to be $C_{12}H_{14}N_2OS_2$ by high-resolution mass spectrometry (HR-MS), together with ^1H and ^{13}C NMR spectral information. Its UV [λ_{max} (CH_3OH) 218 and 267 nm] spectrum suggested the presence of an *N*-methoxyindole nucleus[11] in **1**. The ^1H NMR spectrum ($CDCl_3$) showed signals for five aromatic protons, of which four [δ 7.60 (d, J=8 Hz, 4-H), 7.17 (ddd, J=8, 8, and 2 Hz, 5-H), 7.30 (ddd, J=8, 8, and 2 Hz, 6-H), and 7.45 (d, J=8 Hz, 7-H)] were assigned successively by spin-decoupling experiments and their locations were confirmed by nuclear Overhauser enhancement (NOE) difference experiments. Irradiation of N-OCH_3 protons at δ 4.10 resulted in enhancements of both the signals at δ 7.45 (7-H) and 7.34 (2-H), indicating that the position 2 of the indole nucleus is unsubstituted. Therefore, the position 3 of the nucleus should have a substituent. The ^1H and ^{13}C NMR spectra further indicated that the substituent contains one each of $-CH_2-$ [δ_H 5.02 (d, J=4 Hz) and δ_C 42.8], >NH [δ_H 7.0 (br s)], >C=S (δ_C 198.3), and $-SCH_3$ [δ_H 2.65 (s) and δ_C 18.2]. Its mass spectrum showed a base peak at *m/z* 160.0733 $(M-C_2H_4NS_2)^+$, indicating that the methylene in the substituent is attached to C-3 of the nucleus. Since ^1H NMR signals of the methylene changed from a doublet to a singlet on exchange with D_2O, it is further connected to the >NH leading to the whole structure **1**. The dithiocarbamate structure in **1** was further supported by the presence of a minor rotational isomer [δ 2.75 (s, $-SCH_3$), 4.75 (br d, J=4 Hz, $-CH_2-$), and 7.6 (br s, >NH)] due to hindered C-N rotation.[12] 1-Methoxyindole derivatives are rather rare as natural products[11] although the occurrence of indole alkaloids has been observed in many plants.[13]



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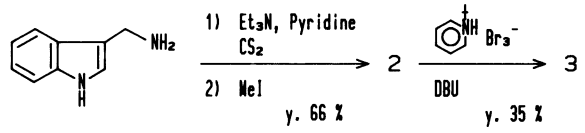
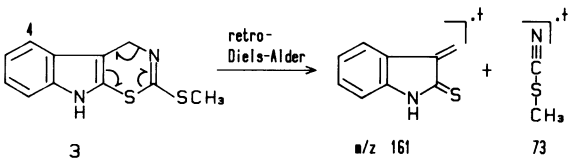
†† Present address: Sericultural Experiment Station, Yatabe-machi, Tsukuba 305.

Brassinin (**2**), mp 132—133 °C, has the molecular formula $C_{11}H_{12}N_2S_2$ by HR-MS. Its spectral data indicated **2** as a demethoxy derivative of **1**. The ^1H NMR spectrum was similar to that of **1** except that **2** showed a signal due to an indolic NH (δ 8.16) in place of the methoxyl group. The IR spectrum showed a sharp absorption (ν_{max} 3490 cm^{-1}) due to the indolic NH. The UV spectrum [λ_{max} (CH_3OH) 218 and 268 nm] was similar to that of **1**. The mass spectrum showed a base peak at *m/z* 130.0679 $(M-C_2H_4NS_2)^+$ and implied that the indole ring of **2** is connected to a methylene group of a side chain as in **1**. Similarly, NOE experiment confirmed the position of the side chain at C-3. Namely, irradiation of the indolic NH induced the enhancements of signals at δ 7.25 (2-H) and 7.42 (7-H). The side chain was shown to be the same as that of **1** based on ^1H and ^{13}C NMR spectra [δ_H 5.07 (d, J=4 Hz) and δ_C 43.2 (t), $-CH_2-$; δ_H 7.0 (br), >NH; δ_C 198.2, >C=S; δ_H 2.65 (s) and δ_C 18.2 (q), $-SCH_3$]. Therefore, the whole structure of brassinin should be represented by **2**. The structure of **2** was confirmed by the following synthesis. Treatment of 3-(aminomethyl)indole[14] with carbon disulfide in the presence of pyridine and triethylamine gave a dithiocarbamate salt, which was methylated with methyl iodide to give **2** in 66% yield. The both synthetic and natural specimens were identical in all respects.

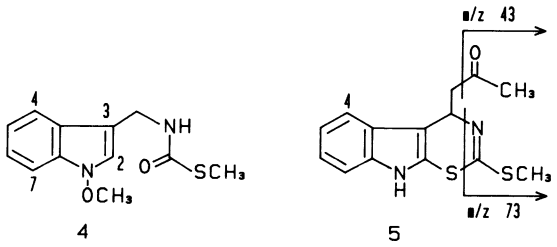


Cyclobrassinin (**3**), mp 136—137 °C, has the molecular formula $C_{11}H_{10}N_2S_2$ by HR-MS and showed a slightly different UV [λ_{max} (CH_3OH) 204, 227, 284, and 294 nm] spectrum from those of **1** and **2**. Its ^1H NMR spectrum showed signals for 2,3-disubstituted indole. In NOE difference experiment, irradiation of an indolic NH at δ 7.91 caused enhancement of the only one proton signal at δ 7.33 (7-H, indole numbering). Consecutive spin-decoupling experiments assigned the three remaining indole ring protons [δ 7.18 (6-H), 7.14 (5-H), and 7.49 (4-H)]. The spectrum further showed the presence of a methylene (δ 5.09, 2H, s) and a methylthio (δ 2.56, 3H, s) groups. The presence of nine sp^2 carbon atoms in the ^{13}C NMR spectrum, together with eight degrees of unsaturation implied a tricyclic structure of **3**. Its mass spectrum showed two peaks at *m/z* 161 $(M-C_2H_3NS)^+$ and 73 (C_2H_3NS), which suggested a



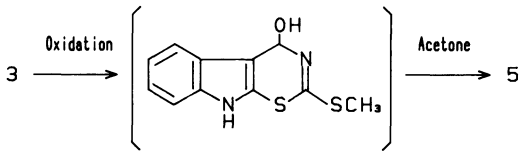
retro-Diels–Alder fission of the third ring in **3**. This fragmentation and the presence of a >C=N– group (δ_c 152.0; ν_{max} 1600 cm^{-1}) suggested that **3** contained a 4*H*-1,3- or a 4*H*-1,2-thiazine moiety. The former was preferred when we considered the low carbon chemical shift (δ 48.8) of the methylene and the co-occurrence of **3** with **2** in the inoculated Chinese cabbage tissue. In NOE difference experiment irradiation of the indole ring proton at δ 7.49 (4-H) resulted in the signal enhancement of the methylene protons and supported the proposed structure **3**. The structure was confirmed by transforming brassinin (**2**) to **3**. Bromination of **2** with pyridinium bromide perbromide, followed by dehydrobromination, gave **3** in 35% yield, which was identical with natural cyclobrassinin.

Methoxybrassitin (**4**). mp 94—96 °C, has a molecular formula of $C_{12}H_{14}N_2O_2S$. Comparison of its ^1H NMR spectral feature [δ 7.60 (4-H), 7.15 (5-H), 7.28 (6-H), 7.43 (7-H), 7.25 (2-H), and 4.07 (1-OCH_3)] with those of **1** suggested that **4** was also a 3-substituted *N*-methoxyindole. This view was supported by spin-decoupling and NOE difference experiments. Irradiation of the methoxyl protons and of the aromatic proton at δ 7.60 (4-H) caused signal enhancements of 2-H and 7-H protons, and methylene protons at δ 4.61 (d, J=5 Hz), respectively. The mass spectrum of **4** showed a base peak at *m/z* 160 $(M-C_2H_4NOS)^+$, indicating that the *N*-methoxyindole nucleus links a methylene group as in **1**. Since the methylene proton signals changed from a doublet to a singlet on exchange with D_2O, the methylene was further connected to a >NH. Namely, methoxybrassitin (**4**) differs from **1** in a substituent at the >NH. The substituent (C_2H_3OS) was assigned $-(C=O)SCH_3$ based on IR (1662 cm^{-1}, >C=O) and ^{13}C NMR [δ 167.6 (s), >C=O; 12.4 (q), $-SCH_3$] spectral data giving the structure **4**.



The fifth compound **5**, amorphous solid, has a molecular formula of $C_{14}H_{14}N_2OS_2$ and showed a very similar UV [λ_{max} (CH_3OH) 202, 227, 282, and 294 nm] spectrum to that of cyclobrassinin (**3**). The ^1H NMR spectrum [δ 7.59 (4-H), 7.14 (5-H), 7.17 (6-H), 7.31 (7-H), and 7.96 (br s, indole NH)] of **5** also showed characteristic of 2,3-disubstituted indole. The same ring system as in **3** was postulated when the IR (**5**: 1598 cm^{-1}; **3**: 1600 cm^{-1}) and ^{13}C NMR (**5**: δ_c 151.8; **3**: δ_c 152.0) absorptions of >C=N– groups were compared

with those of **3**. The NMR spectra of **5** further showed the presence of a methylthio group (δ_H 2.51 and δ_C 15.1) attached probably to the above >C=N- and also of a moiety CH_3COCH_2- [δ_H 2.21 (3H, s) and δ_C 30.9, $-CH_3$; δ_C 206.5, >C=O; δ_H 2.87 (dd, J=8 and 15 Hz) and 2.99 (dd, J=6 and 15 Hz), and δ_C 49.0, $-CH_2-$] attached to a methine group [δ_H 6.00 (dd, J=6 and 8 Hz) and δ_C 56.0]. In NOE difference experiments, irradiation of the proton at δ 7.59 (4-H) resulted in enhancements of signals due to both the methine and the methylene protons. Therefore, the structure **5** was proposed. Two diagnostic mass fragments at m/z 43 (C_2H_3O) and 73 (C_2H_3NS) were reasonably explainable by the structure **5**. The compound **5** may be an artefact derived from **3**, since it showed no optical activity and acetone was used as a solvent for extraction. The artefact-forming process, however, requires an oxidation step. Long time stirring of **3** with acetone in the presence of air showed no indication of the formation of **5**. Therefore, **5** might be a condensation product of



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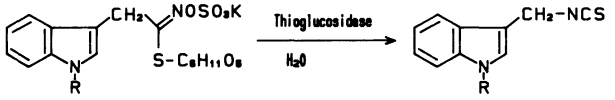
acetone with a hydroxy derivative **6** produced in the inoculated tissue, although such compound has not been isolated to date.

Cruciferous plants, including Chinese cabbage, contain thioglucosides called glucosinolates,[15] which undergo enzymic hydrolysis on crushing of the plant tissues to give isothiocyanates. Since Chinese cabbage contains neoglucobrassicin and glucobrassicin (**7** and **8**, respectively)[15] the formation of methoxybrassinin (**1**) and brassinin (**2**) might be explained by reactions of the respective isothiocyanates (**9** and **10**) derived from the glucosinolates (**7** and **8**) with methanethiol



7: R=OCH₃
8: R=H

9: R=OCH₃
10: R=H

possibly generated in the course of cutting the tissue. This possibility was ruled out, however, from the following experiments: i) crushed uninoculated Chinese cabbage tissue gave none of these compounds when they were incubated for 0, 1, 2, 3, and 4 days at 20 °C; ii) none of these compounds **1** to **4** were detected when the excised Chinese cabbage tissue without the bacterium inoculation was incubated under the same conditions as those of the inoculated tissue (20 °C, 3 days); iii) production of these compounds was also induced by ultraviolet irradiation. In this case, tissue is not mechanically injured.

It is interesting to note that Nomoto and Tamura reported the isolation of 1-methoxyindoleacetonitrile from clubroots of Chinese cabbage.[11] They supposed it to be a metabolite of the roots infected with *Plasmodiophora brassicae*. We have also observed the presence of this compound in *P. cichorii*-inoculated daikon (*Raphanus sativus* L. var. *hortensis*).[16]

These four compounds **1**, **2**, **3**, and **4** exhibited broad, moderate antifungal activity against over 31 plant pathogenic fungi, e. g. *Pyricularia orizae*, with complete inhibition at 400 ppm, and moderate inhibition at 100 ppm.

Although many phytoalexins (over 200 compounds) have been isolated to date,[3] this is the first report of sulfur-containing phytoalexins.

Experimental

All the melting points were uncorrected. IR spectra were recorded with a JASCO A-120 spectrometer, UV spectra with Shimadzu UV 240 spectrometer, ^1H and ^{13}C NMR spectra with JEOL JNX-GX400 or JEOL JNM-FX200 spectrometer in deuteriochloroform solution containing tetramethylsilane as an internal standard, low and high resolution mass spectra with a JEOL JMS-D300 spectrometer, with direct inlet system operating at 70 eV. HPLC separation was performed with a Hitachi 635S equipped with a JASCO UVIDEC-100V detector, using an analytical or a preparative μ-Porasil column (Waters Associates).

Method of Bioassay. For two-dimensional TLC bioassay, developed silica-gel sheet (i, Et_2O; ii, CH_2Cl_2-CH_3OH, 98:2) was sprayed with a dense conidial suspension of *Bipolaris leersiae* in a potato-glucose medium, and incubated in a moist box at 25 °C for 2 days. Fungitoxic spots appeared white against a dark gray background. Antifungal activity of each chromatographic fraction was monitored by one-dimensional TLC bioassay using the solvent system i or ii.

Induction and Isolation of the Phytoalexins. Chinese cabbage heads (*Brassica campestris* L. ssp. *pekinensis*) were cut into small pieces and kept in a moist chamber at 20 °C for 1 day, and then inoculated with *Pseudomonas cichorii* (ca. 10^8 cells ml^{-1}). After being incubated at 20 °C for 3 days they were air-dried at 60 °C. The dried tissue (1418 g) was extracted twice with acetone at room temperature. The extracts were evaporated under reduced pressure below 35 °C to give an acetone extract (20.0 g), which was triturated with ethyl acetate to remove inactive insoluble material (2.27 g). The filtered ethyl acetate solution was passed through a short silica-gel column and the column was eluted with ethyl acetate. The eluate (14.15 g) from the column was separated into three fractions by chromatography on silica gel: F-1-1 (0.97 g), inactive fraction eluted with CH_2Cl_2-CH_3OH (99.5:0.5); F-1-2 (4.55 g), less polar active fraction eluted with the above eluent; F-1-3 (8.87 g), more polar active fraction eluted with CH_2Cl_2-CH_3OH (99.5:0.5, and 7:3). Further fractionation of F-1-3 will be reported in future.

Fraction F-1-2 was separated into five fraction (F-2-1—F-2-5) by chromatography on Sephadex LH-20 with CH_3OH-EtOAc (1:1). Fraction F-2-3 (525 mg) was further separated

into 6 fractions (F-3-1—F-3-6) by chromatography on silica gel with hexane-diethyl ether (4:1). Fraction F-3-2 (265 mg) was further purified by chromatography on silica gel with CH_2Cl_2–CH_3OH (99.5:0.5) to give methoxybrassinin **1**, 250 mg). Fraction F-3-4 (15.8 mg) was purified by three sequential preparative HPLC on a μ-Porasil column using CH_2Cl_2–CH_3OH (99.5:0.5), benzene–EtOAc (20:1), and hexane–diethyl ether (4:1) to give a compound **5** (6.7 mg). Fraction F-3-5 (40.2 mg) was purified by a silica-gel column with benzene–EtOAc (10:1), followed by preparative HPLC on a μ-Porasil column using CH_2Cl_2–hexane–CH_3OH (800:200:1), yielding methoxybrassitin (**4**, 15.9 mg). Fraction F-2-4 (226 mg) was fractionated by chromatography on silica gel with CH_2Cl_2 to give cyclobrassinin (**3**, 139.1 mg) and brassinin (**2**, 30.8 mg).

Characterization of the Compounds 1—5. Methoxybrassinin (1): $C_{12}H_{14}N_2OS_2$ (*m/z* 266.0550, M^+); viscous oil; IR ($CHCl_3$) 3380, 1475, 1452, 1352, 1298, 1088, 958, and 922 cm^{-1}; UV (CH_3OH) 218 (ε 37300), 241 (trough, 11500), 267 (15800), 287 (sh, 9470), and 297 (sh, 5740) nm; ^1H NMR δ=2.65 (s, $-SCH_3$), 4.10 (s, $-OCH_3$), 5.02 (d, *J*=4 Hz, $-CH_2-$), 7.0 (br, >NH), 7.17 (ddd, *J*=8, 8, and 2 Hz, 5-H), 7.30 (ddd, *J*=8, 8, 2 Hz, 6-H), 7.34 (s, 2-H), 7.45 (d, *J*=8 Hz, 7-H), and 7.60 (d, *J*=8 Hz, 4-H). The spectrum showed minor signals (1/4 intensity of the major signals) due to a rotamer at δ= 2.75 (s, $-SCH_3$), 4.75 (br d, *J*=4 Hz), and 7.6 (br, >NH); ^{13}C NMR δ=18.2 (q, $-SCH_3$), 42.8 (t, $-CH_2-$), 66.0 (q, $-OCH_3$), 106.4 (s), 108.6 (d), 119.0 (d), 120.5 (d), 122.7 (d), 123.0 (s), 123.1 (d), 132.3 (s), and 198.3 (s, >C=S); MS *m/z* (%) 266 (4, M^+), 235 [54, $(M-OCH_3)^+$], 218 [16, $(M-SCH_3-H)^+$], 160 (100, $C_{10}H_{10}NO$), 145 (28), 129 (57), and 91 (23, $C_2H_3S_2$).

Brassinin (2): $C_{11}H_{12}N_2S_2$ (*m/z* 236.0418, M^+); mp 132—133 °C (CH_2Cl_2–hexane); IR ($CHCl_3$) 3490, 3380, 1480, 1455, 1092, and 922 cm^{-1}; UV (CH_3OH) 218 (ε 38300), 236 (trough, 8740), 268 (16100), and 287 (9070) nm; ^1H NMR δ=2.65 (s, $-SCH_3$), 5.07 (d, *J*=4 Hz, $-CH_2-$), 7.0 (br, >NH), 7.18 (ddd, *J*=8, 8, and 1 Hz, 5-H), 7.25 (ddd, *J*=8, 8, and 1 Hz, 6-H overlapped with 2-H), 7.42 (d, *J*=8 Hz, 7-H), 7.64 (br d, *J*=8 Hz, 4-H), 8.16 (br s, 1-H), and minor signals (1/4 intensity of the major ones) due to a rotamer at δ=2.75 (s, $-SCH_3$), 4.80 (d, *J*=4 Hz, $-CH_2-$), and 7.6 (br, >NH); ^{13}C NMR δ=18.2 (q, $-SCH_3$), 43.2 (t, $-CH_2-$), 110.9 (s), 111.5 (d), 118.7 (d), 120.4 (d), 122.8 (d), 124.0 (d), 126.5 (s), 136.3 (s), and 198.2 (s, >C=S); MS *m/z* (%) 236 (46, M^+), 188 [3, $(M-SCH_3-H)^+$], 162 (16, C_9H_8NS), and 130 (100, C_9H_8N).

Synthesis fo Brassinin (2): To a mixture of 3-(aminomethyl)indole[14] (730 mg), pyridine (2.0 ml), and triethylamine (0.70 ml) was added carbon disulfide (330 µl) at 0 °C and the mixture was kept at the temperature for 1 h. The reaction mixture was then treated with methyl iodide (312 µl), kept at 3 °C for 24 h, and poured into 1.5 M H_2SO_4 (1 M=1 mol dm^{-3}) (50 ml).[17] The crude product extracted from the reaction mixture with diethyl ether was crystallized from CH_2Cl_2–hexane to yield **2** (782 mg), mp 132—133 °C, in 66% yield.

Cyclobrassinin (3): $C_{11}H_{10}N_2S_2$ (*m/z* 234.0264, M^+); mp 136—137 °C (CH_2Cl_2–hexane); IR ($CHCl_3$) 3450, 1600, 1450, 1430, 1338, 980, and 910; UV (CH_3OH) 204 (ε 24700), 227 (34000), 259 (trough, 6330), 284 (7830), and 294 (8170) nm; ^1H NMR δ=2.56 (3H, s, $-SCH_3$), 5.09 (2H, s, $-CH_2-$), 7.14 (1H, ddd, *J*=7, 7, and 2 Hz, 5-H), 7.18 (1H, ddd, *J*=7, 7, and 2 Hz, 6-H), 7.33 (1H, dd, *J*=7 and 2 Hz, 7-H), 7.49 (1H, dd, *J*=7 and 2 Hz, 4-H), and 7.91 (1H, br, 1-H); ^{13}C NMR δ=15.2 (q, $-SCH_3$), 48.8 (t, $-CH_2-$), 103.9 (s), 110.7 (d), 117.2 (d), 120.3 (d), 122.0 (d), 122.4 (s), 125.2 (s), 136.7 (s), and 152.0 (s, >C=N); MS *m/z* (%) 234 (10, M^+), 161 (100, C_9H_7NS), 117 (47, C_8H_7N), and 73 (6, C_2H_3NS).

Synthesis of Cyclobrassinin (3): To a solution of **2** (237 mg) in CH_2Cl_2 (24 ml) was added pyridinium bromide perbromide (336 mg) in small portions at room temperature and the mixture was kept at the temperature for 20 min and then basified with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and refluxed for 10 min. The reaction mixture was evaporated and the residue was applied on a silica-gel column and eluted with CH_2Cl_2 to give crystalline material (107 mg), which on recrystallization from CH_2Cl_2–hexane gave **3** (81 mg) in 35% yield, indistinguishable from natural cyclobrassinin.

Methoxybrassitin (4): $C_{12}H_{14}N_2O_2S$ (*m/z* 250.0772, M^+); mp 94—96 °C (Et_2O–EtOH); IR ($CHCl_3$) 3430, 1662, 1482, 1455, and 1182 cm^{-1}; UV (CH_3OH) 219 (ε 32100), 247 (trough, 2360), 273 (5200), 287 (5200), 295 (sh, 4250) nm; ^1H NMR δ=2.38 (3H, s, $-SCH_3$), 4.07 (3H, s, $-OCH_3$), 4.61 (2H, d, *J*=5 Hz, $-CH_2-$), 5.51 (1H, br s, >NH), 7.15 (1H, ddd, *J*=8, 8, and 1 Hz, 5-H), 7.25 (1H, s, 2-H), 7.28 (1H, ddd, *J*=8, 8, and 1 Hz, 6-H), 7.43 (1H, br d, *J*=8 Hz, 7-H), and 7.60 (1H, br d, *J*=8 Hz, 4-H); ^{13}C NMR δ=12.4 (q, $-SCH_3$), 36.8 (t, $-CH_2-$), 65.9 (q, $-OCH_3$), 108.0 (s), 108.5 (d), 119.1 (d), 120.3 (d), 122.1 (d), 122.8 (s), 123.0 (d), 132.5 (s), and 167.6 (s, >C=O); MS *m/z* (%) 250 (42, M^+), 219 (33), 202 [43, $(M-SCH_3-H)^+$], 176 (48), 171 [33, $(M-SCH_3-OCH_3-H)^+$], 160 [100, $(M-NHCOSCH_3)^+$], 145 (36), 129 (57), 102 (31), 75 (54), and 47 (66).

Compound 5: $C_{14}H_{14}N_2OS_2$ (*m/z* 290.0550, M^+); IR ($CHCl_3$) 3460, 1705, 1598, 1445, 1423, 1355, 1338, 1260, 1158, 965, and 918 cm^{-1}; UV (CH_3OH) 202 (ε 18900), 227 (28000), 259 (trough, 5740), 282 (6490), and 294 (6690) nm; ^1H NMR δ=2.21 (3H, s, $-COCH_3$), 2.51 (3H, s, $-SCH_3$), 2.87 (1H, dd, *J*=15 and 8 Hz), 2.99 (1H, dd, *J*=15 and 6 Hz), 6.00 (1H, dd, *J*=8 and 6 Hz, >CH–), 7.14 (1H, ddd, *J*=7, 7, and 2 Hz, 5-H), 7.17 (1H, ddd, *J*=7, 7, and 2 Hz, 6-H), 7.31 (1H, dd, *J*=7 and 2 Hz, 7-H), 7.59 (1H, dd, *J*=7 and 2 Hz, 4-H), and 7.96 (1H, br, >NH); ^{13}C NMR δ=15.1 (q, $-SCH_3$), 30.9 (q, $CO\underline{C}H_3$), 49.0 (t, $-CH_2-$), 56.0 (d, >CH–), 106.3 (s), 110.7 (d), 117.8 (d), 120.6 (d), 122.2 (s), 122.3 (d), 124.8 (s), 136.6 (s), 151.8 (s, >C=N-), and 206.5 (s, >C=O); MS *m/z* (%) 290 (21, M^+), 275 (14), 217 (31), 199 (31), 184 (74), 175 (100, $C_{10}H_9NS$), and 73 (32, C_2H_3NS), and 43 (62).

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