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THREE SULPHUR-CONTAINING STRESS METABOLITES FROM JAPANESE RADISH*

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Key Word Index—*Raphanus sativus*; Cruciferae; Japanese radish; stress metabolites; indole; dihydroindole; brassitin; *N*-methoxyspirobrassinol; *N*-methoxybrassinol methyl ether.

Abstract—Three new brassinin related stress metabolites, named brassitin, *N*-methoxyspirobrassinol methyl ether and *N*-methoxyspirobrassinol, were isolated from the Japanese radish 'Daikon' (*Raphanus sativus* var. *hortensis*) after inoculation with *Pseudomonas cichorii*. Their structures have been established on the basis of spectroscopic data and chemical reactions. *N*-Methoxyspirobrassinol has an unusual hemi-aminal structure, and occurs as a mixture of diastereomers. The occurrence of *N*-methoxyspirobrassinol methyl ether and *N*-methoxyspirobrassinol suggests the involvement of oxidized intermediates in the biosynthesis from brassinin to spirobrassinin

INTRODUCTION

Phytoalexins are defined as antimicrobial compounds which are produced by plants after their exposure to microorganisms [1–3]. Previously, we reported the first cruciferous phytoalexins, brassinin (**1**), methoxybrassinin (**2**) and cyclobrassinin (**3**) from Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) inoculated with the bacterium *Pseudomonas cichorii* [4, 5]. Several phytoalexins have been isolated so far from crucifers such as cabbage (*B. oleracea*) [6–8], turnip (*B. rapa* or *B. campestris*) [9, 10], rape (*B. napus* var. *oleifera*) [11], Indian mustard (*B. juncea*) [12, 13], false flax (*Camelina sativa*) [14] and arabidopsis (*Arabidopsis thaliana*) [15]. These compounds are structurally unique indole compounds possessing one or two sulphur atoms.

In a previous communication [16], we reported the first oxindole phytoalexin spirobrassinin (**4**) from inoculated Japanese radish 'Daikon' (*Raphanus sativus* var. *hortensis*), the most important cruciferous vegetable in Japan. Recently, spirobrassinin (**4**) was shown to be biosynthesized from brassinin (**1**), which originates from L-tryptophan [17, 18]. However, minor stress metabolites suggestive of the biosynthetic pathway of **4** could not be isolated from Japanese radish because of small amounts of available samples. Herein, we report the isolation and structure elucidation of three minor stress metabolites from *P. cichorii*-inoculated Japanese radish whose structures suggest a potential biosynthetic pathway to these cruciferous phytoalexins.

RESULTS

Sliced Japanese radish roots (*R. sativus* var. *hortensis*) were inoculated with *P. cichorii*. Following incubation for three days at 20°, the sliced tissue produced several antifungal compounds as evidenced by a 2D TLC bioassay [5]. The acetone extracts (172 g) from the inoculated slices (12 kg, dry wt) were separated by successive chromatography on Sephadex LH-20, silica gel, and HPLC to give the following compounds: brassitin (**5**) (6 mg), *N*-methoxyspirobrassinol methyl ether (**6**) (16 mg), *N*-methoxyspirobrassinol (**7a, b**) (6 mg); five known phytoalexins, brassinin (**1**) (120 mg) [5], methoxybrassinin (**2**) (726 mg) [5], spirobrassinin (**4**) (108 mg) [16], methoxybrassitin (**8**) (127 mg) [5], and brassicanal A (**9**) (24 mg) [19]; four related indole compounds, 3-indolecarbaldehyde (**10**) (173 mg), 1-methoxy-3-indolecarbaldehyde (**11**) (11 mg) [20], 3-indolacetonitrile (**12**) (12 mg), and 1-methoxy-3-indoleacetonitrile (**13**) (4 mg) [21].

The five known phytoalexins and two of the four related indole compounds, 3-indolecarbaldehyde (**10**) and 3-indoleacetonitrile (**12**), were identified by direct comparison with authentic samples. 1-Methoxy-3-indoleacetonitrile (**13**) was identified by comparison of its spectral data (^{1}H NMR, IR, UV, MS) with those reported by Nomoto and Tamura [21]. 1-Methoxy-3-indolecarbaldehyde (**11**) was synthesized by a Vilsmeier

*Part 20 in the series. 'Studies on Stress Metabolites'. For part 19, see ref. [18].

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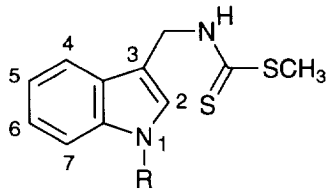

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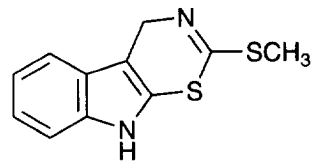

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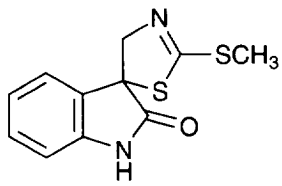

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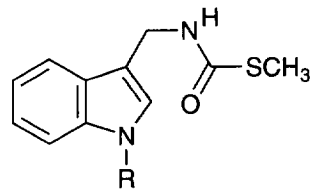

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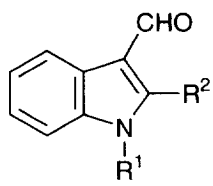
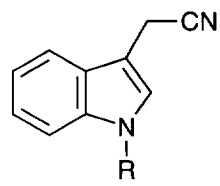
8 R=OCH_3



9 R^1=H, R^2=SCH_3
10 R^1=H, R^2=H
11 R^1=OCH_3, R^2=H



12 R=H
13 R=OCH_3

formylation of 1-methoxyindole according to a procedure reported by Somei *et al.* [22]. The spectroscopic data (^{1}H NMR, IR, UV, MS) of the synthetic compound were essentially identical to those of the natural one. Three new compounds **5**–**7** showed a clear antifungal activity against *Bipolaris leersiae* on a TLC sheet.

Brassitin (**5**), an amorphous solid, had a molecular formula of $C_{11}H_{12}N_2OS$. Comparison of its ^{1}H NMR (400 MHz, $CDCl_3$) spectrum, see Experimental, with those of brassinin (**1**) and methoxybrassitin (**8**) suggested it to be a 3-substituted indole. This view was supported by spin-decoupling and NOE difference experiments. Irradiation of the NH proton and of the aromatic proton at δ7.64 (4-H) caused signal enhancements of the H-2 and H-7 protons, and of the methylene protons at δ4.67 (*d*, *J* = 4.7 Hz), respectively. The mass spectrum of **5** showed a base peak at *m/z* 130 [M − C_2H_4NOS]$^+$, indicating that the indole nucleus was linked to 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 an amino group, i.e. **5** differs from **1** in a substituent at the amino group. This substituent (C_2H_3OS) was assigned as -(C = O)SMe based on the IR [$\nu_{max}^{CHCl_3}$ cm^{-1}: 1660 (C = O)] spectrum. The structure of **5** was further confirmed by synthesis from **1** by treatment with H_2O_2 in the presence of *p*-toluenesulphonic acid [23, 24].

N-Methoxyspirobrassinol methyl ether (**6**) had a molecular formula of $C_{13}H_{16}N_2O_2S_2$ (*m/z* 296.0629, [M]$^+$) and showed no carbonyl group absorption in the IR spectrum ($\nu_{max}^{CHCl_3}$ cm^{-1}: 1575, 1462 and 1210). The ^{1}H NMR spectrum showed the presence of one methylthio (δ2.57, 3H, *s*), two methoxyl (δ3.70, 3H, *s*; 3.95, 3H, *s*), one methylene (δ3.90, 1H, *d*, *J* = 15 Hz and 4.96, 1H, *d*, *J* = 15 Hz), one methine (δ4.94, 1H, *s*), and four aromatic (δ6.94, 7.02, 7.25 and 7.33) protons. The ^{13}C NMR spectrum ($CDCl_3$) [δ15.0 (*q*, -SMe), 59.7 (*q*-OMe), 63.9 (*q*, -OMe), 69.9 (*t*, -CH_2-), 109.0 (*d*, > CH-), 112.9 (*d*, = CH-), 123.8 (*d*, = CH-), 123.9 (*d*, = CH-), and 129.6 (*d*, = CH-)] supported the presence of the above groups. The spectrum also indicated the presence of one sp^3 quaternary [δ_c 68.8 (*s*)] and one sp^2 quaternary [δ_c 163.4 (*s*)] carbon atoms. ^{1}H–^{1}H decoupling experiments assigned successive aromatic protons [δ7.33 (*br d*, *J* = 7.4 Hz, H-4), 7.02 (*ddd*, *J* = 7.4, 6.4, and 1.0 Hz, H-5), 7.25 (*ddd*, *J* = 7.8, 6.4, and 1.0 Hz, H-6), 6.94 (1H, *br d*, *J* = 7.8 Hz, H-7)]. Irradiation of the methoxyl protons at δ3.95 resulted in NOE enhancement of the H-7 proton and also of the methine proton at δ4.94, thereby suggesting the presence of a *N*-methoxy-2,3-dihydroindole ring

system. The UV spectrum, [λ_{max}^{EtOH} nm: 210 (ε 26400), 234 sh (14400) and 291 (2880)] is also suggestive of this ring system [25]. Taken together, these spectral data and its seven degrees of unsaturation suggested that **6** was a tricyclic compound with a spiro ring at C-3 position as in spirobrassinin (**4**). However, the large difference in chemical shifts of the methylene protons (δ3.90 and 4.96) indicated that these two methylene protons were in a different environment. Therefore, the remaining methoxyl group and methine proton at δ4.94 must be located at the C2 position of the *N*-methoxy-2,3-dihydroindole ring. Detailed differential NOE experiments revealed the full structure of *N*-methoxyspirobrassinol methyl ether as shown in formula **6** except for the absolute configuration.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of the amorphous solid **7** indicated that is a mixture of two closely related compounds. After careful HPLC separation, the mixture **7** was isolated as two compounds, **7a** and **7b**. However after several hours, **7a** was converted into the same mixture of **7a** and **b**. Similarly, **7b** gave rise to the mixture of **7a** and **b** after several hours, indicating that both are in equilibrium. The ratio of **7a** to **b** before the HPLC separation was estimated as 2.5 : 1 based on the peak areas in the HPLC chromatogram. Therefore, all spectra were measured as a mixture.

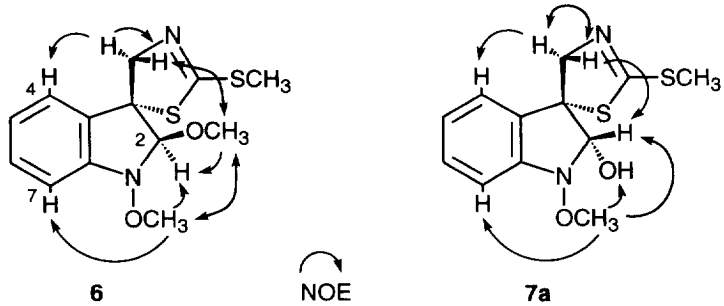
The amorphous solid **7** has a molecular formula of $C_{12}H_{14}N_2O_2S_2$ (*m/z* 282.0500, $[M]^+$). The ^{1}H NMR spectrum clearly showed two sets of signals: **a** and **b** indicate the signals of the major and minor components, respectively. The ^{1}H NMR spectrum of the major component **7a** showed that it has one methylthio (δ2.57, *s*), one methoxyl (δ3.97, *s*), one methylene (δ4.41, *d*, *J* = 16 Hz and 4.65, *d*, *J* = 16 Hz), one methine (δ4.80, *d*, *J* = 12 Hz), one D_2O exchangeable (δ3.36, *d*, *J* = 12 Hz), and four aromatic [δ6.94 (*dd*, *J* = 8 and 1 Hz), 7.04 (*ddd*, *J* = 8, 8 and 1 Hz), 7.29 (*m*) and 7.29 (*m*)] protons. The ^{1}H NMR spectrum of the minor component **7b** was very similar to that of the major one. Therefore, these two compounds must be diastereomers. The IR spectrum indicated the presence of a hydroxyl group. Observation of a dehydration fragment (*m/z* = 264.0390) in the HR-EI-mass spectrum indicated that **7a** and **b** must each have one hydroxyl group.

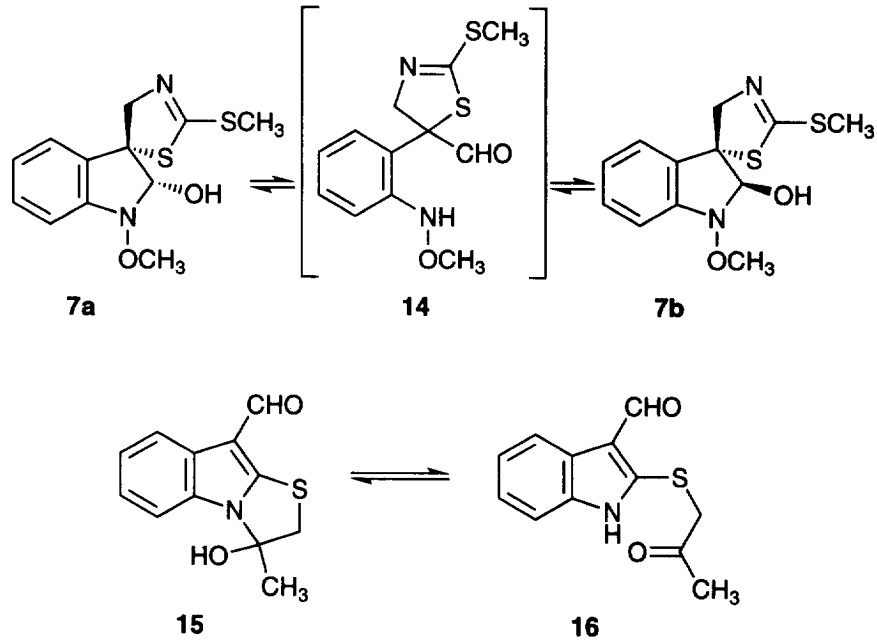
^{1}H–^{1}H decoupling experiments assigned the four successive aromatic ring protons of **7a** [δ6.94 (*d*, *J* = 8 Hz, H-7), 7.04 (*dd*, *J* = 8 and 8 Hz, H-5), and 7.29 (*m*, 4, H-6)]. Irradiation of the methoxyl protons at δ3.97 resulted in enhancement of the aromatic proton signals at δ6.94 (H-7) suggesting the presence of a similar *N*-methoxyindoline ring as in **6**. A similar UV spectrum [λ_{max}^{EtOH} nm: 211 (ε 27300), 246 (15500) and 292 (3630)] to that of **6** also supported the presence of the same ring system in **7a** and **b** [25]. The base peak at *m/z* 161.0318 $[M - OMe - OH - N = C\text{-}SMe]^+$ in the HR-EI-mass spectrum and an IR absorption at 1575 cm^{-1} (C = N) supported the moiety − N = C-SMe. Low field chemical shifts (δ4.41 and 4.65) of the methylene group in **7a** indicated that it is attached to the nitrogen atom of -N = C-SMe. The compounds **7a** and **b** had a partial structure [CH_2-N = C-SMe] as in **6**. These considerations and the detailed difference NOE experiments (Fig. 2) revealed the full structure of the major compound as **7a**. The relative stereochemistry of the minor one is, therefore, represented by the formula **7b**.

Facile interconversion between **7a** and **b** is explained by the intervention of a hemi-aminal **14** as shown in Scheme 1. Such an equilibrium was previously observed in the case of brassicanal B (**15**) [19], which was isolated as a minor phytoalexin from inoculated Chinese cabbage (Scheme 1). The equilibrium between **15** and **16** is shifted toward the hemiaminal **15**. To our knowledge, **7** and **15** are novel natural compounds having hemiaminal partial structures which cause this equilibrium.

Since the structures of brassitin (**5**) and methoxybrassitin (**8**) are very similar to those of brassinin (**1**) and methoxybrassinin (**2**), respectively, except for their side chains, the thiocarbamate moieties of these **5** and **8** could be derived from the respective dithiocarbamates **1** and **2** by way of an oxidation process. Chemical transformation of **1** to **5** by H_2O_2 treatment supported this hypothesis. Moreover, Murai *et al.* have reported that when sliced potato tubers were exposed to microorganisms, the amount of H_2O_2 in the tissue increased markedly as compared with that of the control tissue [26]. These results suggested that **5** would be synthesized from **1** owing to the increasing H_2O_2 concentrations occurring in the inoculated tissue.

Our previous feeding experiments [17, 18] revealed that both cyclobrassinin (**3**) and spirobrassinin (**4**) are biosynthesized from **1**. However, labelled **3** was not incorporated to **4**. These results suggested that **4** is directly derived from **1**, and not by way of **3**. The structures of **7** imply that the corresponding hemiaminal **19** might be the direct precursor for oxindole ring in **4**





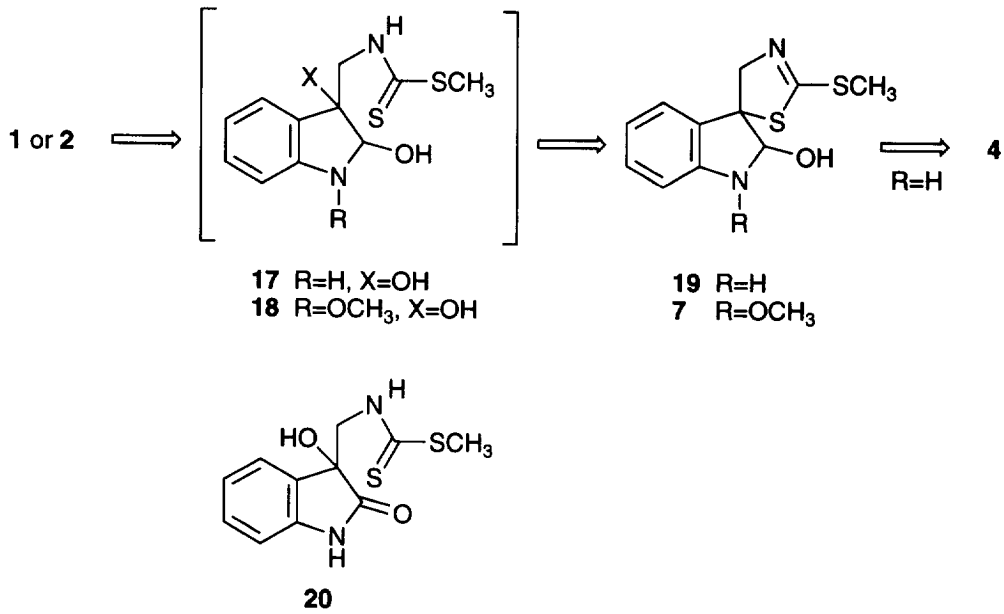
Scheme 1. Equilibria of hemi-aminal compounds.

(Scheme 2). The presence of a bulky methoxyl group at N-1 of **7** would prevent further oxidation of the secondary hydroxyl group at C-2 and give **7** and **6** as minor components.

The spiro ring systems in **4**, **6** and **7** could be formed by intramolecular nucleophilic attack at C-3 by the respective dithiocarbamate side chains in **1** and **2**. Plausible intermediates **17** and **18** have respective leaving groups at C-3. The structures of **17** and **18** are supported by the presence of dioxibrassinin **20** in *P. cichorii*-inoculated cabbage [7] (Scheme 2). Such diol intermediates were proposed by Scott in biosynthetic studies of indole alkaloids [27, 18]. However, the diol intermediates have not been isolated to date because of their instability. Further methylation or methanolysis of **7** would lead to **6**.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra in $CDCl_3$ containing tetramethylsilane as an int. standard, low and high



Scheme 2. A plausible biosynthetic pathway of the cruciferous phytoalexins.

resolution ms with a Jeol JMS-D300 spectrometer, with direct inlet system operating at 70 eV. HPLC separation was performed with a Jasco 800S equipped with a Jasco UVIDEC-100V detector, using an analytical or a prep. μ-Porasil column, or Radial-Pak cartridge CN column (Waters Associates).

Method of bioassay. For 2D TLC bioassay, developed silica gel sheets (i, Et_2O; ii, CH_2Cl_2–MeOH, 49:1) were sprayed with a dense conidial suspension of *Bipolaris leersiae* in a potato-glucose medium, and incubated in a moist box at 25° for 2 days. Fungitoxic spots appeared white against a dark gray background. The antifungal activity of each chromatographic fraction was monitored by 1D TLC bioassay using solvent systems i or ii.

Induction and isolation of phytoalexins. Japanese radish roots were sliced and kept in a moist chamber at 20° for 1 day, and then inoculated with *Pseudomonas cichorii* (*ca* 10^8 cells ml^{-1}). After being incubated at 20° for 3 days, they were air-dried at 60°. The dried tissue (12 kg) was extracted twice with Me_2CO at room temp. The extracts were evapd under red. pres. below 35° to give an Me_2CO extract (172 g), which was triturated with EtOAc to remove inactive insoluble material (135 g). The filtered ethyl acetate solution was passed through a short silica gel column and the column was eluted with ethyl acetate to yield active eluate (31.4 g).

The eluate was separated into 5 fractions (F-1-1–F-1-5) by chromatography on Sephadex LH-20 with MeOH. Fr. F-1-2 (2.239 g) was further sepd into 3 fractions (F-2-1–F-2-3) by chromatography on silica gel with CH_2Cl_2–MeOH (49:1). Fraction F-2-1 (417 mg) was further purified by chromatography on silica gel with hexane–Et_2O (1:1) to give 5 frs (F-3-1–F-3-5). F-3-1 (107 mg) was sepd into 4 fractions (F-4-1–F-4-4) by chromatography on silica gel with CH_2Cl_2–MeOH (999:1). F-4-1 (34 mg) was further purified by prep. TLC on silica gel with CH_2Cl_2–MeOH (49:1), followed by chromatography on silica gel with hexane–Et_2O (10:1), yielding 1-methoxy-3-indoleacetonitrile (**13**) (4 mg). F-4-3 (36 mg) was purified by prep. TLC on silica gel developed $\times 3$ with hexane–Et_2O (1:1) to give *N*-methoxyspirobrassinol methyl ether (**6**) (16 mg). F-3-2 (165 mg) was further purified by a silica gel column with CH_2Cl_2–MeOH (199:1), yielding methoxybrassitin **8** (66 mg). Fraction F-2-2 (297 mg) was further sepd into 5 fractions (F-5-1–F-5-5) by chromatography on silica gel with hexane–Et_2O (1:1). F-5-2 (60 mg) was recrystallized from MeOH to give spirobrassinin (**4**) (40 mg). Fraction F-5-1 (43 mg) was purified by two sequential silica gel chromatography using C_6H_6–EtOAc (9:1) and CH_2Cl_2–MeOH (199:1), followed by prep. HPLC on a Radial-Pak CN column using *i*-PrOH–hexane–CH_2Cl_2 (1:100:100) to give *N*-methoxyspirobrassinol (**7a**, 3.6 mg; **7b**, 2.1 mg).

Fraction F-1-3 (2.256 g) was further sepd into 8 frs (F-6-1–F-6-8) by chromatography on silica gel with CH_2Cl_2–MeOH (49:1). F-6-2 was identified as methoxybrassinin (**2**) (726 mg). Fraction F-6-3 (66 mg) was further purified by chromatography on silica gel with C_6H_6, followed by prep. HPLC on a μ-Porasil column using hexane–Et_2O (2:1) to give 3-indoleacetonitrile (**12**) (12 mg). Fraction F-6-4 (88 mg) was purified by chromatography on silica gel with hexane–Et_2O (4:1), yielding methoxybrassitin (**8**) (61 mg) and 1-methoxy-3-indolecarbaldehyde (**11**) (11 mg). Fraction F-6-5 was further purified by chromatography on silica gel with hexane–Et_2O (1:1) to give brassinin (**1**) (120 mg). Fraction F-6-6 (71 mg) was further sepd into 7 fractions (F-7-1–F-7-7) by chromatography on silica gel with C_6H_6–EtOAc (4:1). F-7-3 (34 mg) was further purified by prep. HPLC on a μ-Porasil column using hexane–Et_2O (1:1), yielding brassitin (**5**) (6 mg). F-7-4 was recrystallized from MeOH to give brassicanal A (**9**) (24 mg). Fraction F-6-7 (638 mg) was sepd into 10 fractions (F-8-1–F-8-10) by silica gel chromatography using hexane–Et_2O. F-8-7 was identified as 3-indolecarbaldehyde (**10**) (173 mg). F-8-6 was further purified by two sequential CC on silica gel using hexane–CH_2Cl_2–*i*-PrOH (10:10:1) and CH_2Cl_2–MeOH (99:1), yielding spirobrassinin (**4**) (68 mg).

Brassitin (**5**). Amorphous, $C_{11}H_{12}N_2OS$ (*m/z* 220.0675 $[M]^+$). EI-MS *m/z* (rel. int.): 220 (33), 205 (7), 172 (8), 145 (7), 144 (8), 130 (100), 129 (56), 118 (9), 102 (30). UV λ_{max}^{EtOH} nm: 218 (ε 36 800), 271 (5 910), 277 (5 910), 287 (4 830), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3440, 3340, 1660, 1490, 1185. ^1H NMR (400 MHz, $CDCl_3$): δ2.39 (3H, *s*, SMe), 4.67 (2H, *d*, J = 4.7 Hz, CH_2), 5.50 (1H, *br s*, D_2O exch., CONH), 7.16 (1H, *dt*, J = 7.3 and 1.0 Hz, H-5), 7.17 (1H, *d*, J = 2.4 Hz, H-2), 7.24 (1H, *td*, J = 8.3 and 1.0 Hz, H-6), 7.38 (1H, *br d*, J = 8.3 Hz, H-7), 7.64 (1H, *br d*, J = 7.3 Hz, H-4), 8.11 (1H, *br s*, D_2O exch., NH).

Synthesis of brassitin (**5**). To a solution of brassinin (**1**) (59 mg, 0.25 mmol) in MeOH (4 ml) was added slowly 30% aq H_2O_2 (0.20 ml, 1.76 mmol) at 0°C and the mixture was kept at 0°C for few min. To the mixture was added solid *p*-toluenesulphonic acid (53 mg, 0.27 mmol) at the same temperature. The reaction mixture turned red and was kept at 0°C for 45 min. The reaction mixture was then treated with triphenylphosphine (466 mg, 1.78 mmol, Nacalai tesque) at 0°, kept at the same temperature for an additional 5 min, and warmed to room temp. After 1.5 h, the mixture was filtered through a Celite pad, and the filtrate was evapd *in vacuo*. The residue was dissolved in EtOAc (30 ml) and the organic phase was washed with aq $NaHCO_3$ (2×30 ml), and then concd *in vacuo* to give the crude product (548 mg). This was purified by CC on silica gel (CH_2Cl_2) and by prep. TLC on silica gel to give brassitin (5 mg) in 8% yield.

N-Methoxyspirobrassinol methyl ether (**6**). Gum, $C_{13}H_{16}N_2S_2O_2$ (*m/z* 296.0629 $[M]^+$). $[\alpha]_D^{18}$ $-1.9°$ ($CHCl_3$; *c* 1.57). EI-MS *m/z* (rel. int.): 296 (10), 267 (10), 266 (17), 265 (100), 264 (27), 234 (29), 233 (62), 218 (18), 192 (47), 191 (35), 161 (86), 160 (47), 117 (40), 87 (39). UV λ_{max}^{EtOH} nm: 210 (ε 26 400), 234 (sh, 144 00), 291 (2 880). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1575, 1462, 1210. ^1H NMR (400 MHz, $CDCl_3$): δ2.57 (3H, *s*, SMe), 3.70 (3H, *s*, OMe-2), 3.90 (1H, *d*, J = 15 Hz, aromatic side methylene H), 3.95 (3H, *s*, N1-OMe), 4.94 (1H, *s*, H-2), 4.96 (1H, *d*, J = 15 Hz, methoxy side methylene H), 6.94 (1H, *br d*, J = 7.8 Hz, H-7), 7.02 (1H, *ddd*, J = 7.4. 6.4 and 1.0 Hz, H-5), 7.25 (1H, *ddd*, J = 7.8, 6.4 and 1.0 Hz, H-6), 7.33 (1H, *br d*, J = 7.4 Hz, H-4). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ15.0 (*q*), 59.7 (*q*),

63.9 (*q*), 68.8 (*s*), 69.9 (*t*), 109.0 (*d*), 112.9 (*d*), 123.8 (*d*), 123.9 (*d*), 128.0 (*s*), 129.6 (*d*), 148.1 (*s*), 163.4 (*s*).

N-Methoxyspirobrassinol (**7a**, **b**). Gum, $C_{12}H_{14}N_2S_2O_2$ (*m/z* 282.0500 $[M]^+$). $[\alpha]_D^{20}$ 0° ($CHCl_3$; *c* 0.52). EI-MS *m/z* (rel. int.): 282 (2), 264 (3), 251 (43), 250 (67), 234 (22), 177 (26), 161 (100), 160 (20), 150 (24), 149 (49), 148 (43), 117 (86), 116 (21), 102 (20), 90 (23), 89 (31), 87 (66), 77 (26), 76 (20), 74 (20), 72 (54), 51 (20), 45 (30), 44 (33), 40 (51), 39 (21). UV λ_{max}^{EtOH} nm: 211 (ε 27 300), 246 (15 500), 292 (3 630). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490, 1575, 1463, 1221, 1209, 1146. ^1H NMR (400 MHz, $CDCl_3$): major peaks: δ2.57 (2.4H, *s*, SMe), 3.36 (0.8H, *d*, *J* = 12 Hz, D_2O exchangeable, OH-2), 3.97 (2.4H, *s*, N1-OMe), 4.41 (0.8H, *d*, *J* = 16 Hz, methine side methylene H), 4.65 (0.8H, *d*, *J* = 16 Hz, aromatic side methylene H), 4.80 (0.8H, *d*, *J* = 12 Hz, H-2). 6.94 (0.8H, *dd*, *J* = 8 and 1 Hz, H-7), 7.04 (0.8H, *ddd*, *J* = 8, 8 and 1 Hz, H-5), 7.29 (1.6H, *m*, H-4,6), minor peaks; 2.57 (0.6H, *s*, SMe), 3.06 (0.2H, *d*, *J* = 5 Hz, OH-2), 3.95 (0.6 H, *s*, N1-OMe), 4.12 (0.2H, *d*, *J* = 16 Hz, methylene H), 4.93 (0.2H, *d*, *J* = 16 Hz, methylene H), 5.30 (0.2H, *d*, *J* = 5 Hz, H-2). 6.95 (0.2H, *br d*, *J* = 8 Hz, H-7), 7.03 (0.2H, *ddd*, *J* = 8, 8, and 1 Hz, H-5), 7.29 (0.4H, *m*, H-4, H-6).

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