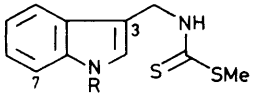
Isolation of Three Novel Sulphur-containing Phytoalexins from the Chinese Cabbage *Brassica campestris* L. ssp. *pekinensis* (Cruciferae)[1]

Mitsuo Takasugi,*[a] Nobukatsu Katsui,[a] and Akira Shirata[b]

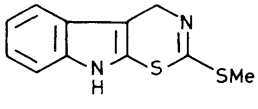
[a] *Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060, Japan*
[b] *National Institute of Agro-Environmental Sciences, Yatabe-machi, Tsukuba 305, Japan*

Inoculation of Chinese cabbage heads with the bacterium *Pseudomonas cichorii* induced the production of three major phytoalexins named methoxybrassinin (**1**), brassinin (**2**), and cyclobrassinin (**3**), whose structures have been elucidated on the basis of spectroscopic studies and synthesis.

Many important vegetables belong to the family Cruciferae. To our knowledge no phytoalexins, antimicrobial compounds synthesized by plants after their exposure to micro-organisms,[2] have been isolated from cruciferous plants. We report here the isolation and structure elucidation of the first cruciferous phytoalexins methoxybrassinin (**1**), brassinin (**2**), and cyclobrassinin (**3**), from Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*) heads inoculated with *Pseudomonas cichorii*. Ultraviolet irradiation or inoculation with *Erwinia carotovora* also induced the production of these compounds. They exhibited broad, moderate antifungal activity against 36 species, *e.g. Pyricularia oryzae*, with complete inhibition at 400 p.p.m., and moderate inhibition at 100 p.p.m.



(**1**) R = OMe
(**2**) R = H



(**3**)

Two-dimensional t.l.c. bioassay† of the acetone extracts from the inoculated tissue revealed the presence of several antifungal compounds, which were absent from uninoculated control tissue. The extracts (5.5 g from 486 g of the dried tissue) gave (**1**) (39 mg), (**2**) (8 mg), and (**3**) (20 mg) on repeated chromatography over silica gel and Sephadex LH-20.

† The developed silica gel t.l.c. sheet [i, Et_2O; ii, CH_2Cl_2–MeOH, 98 : 2] was sprayed with a conidial suspension of *Bipolaris leersiae* in a potato–glucose medium and incubated. Compounds (**1**), (**2**), and (**3**) showed spots at R_f 0.50, 0.44, and 0.52, and 0.61, 0.46, and 0.55 in solvents i and ii, respectively.

Methoxybrassinin (**1**), $C_{12}H_{14}N_2OS_2$, viscous oil, showed spectra data‡ consistent with a 3-substituted 1-methoxyindole:[3] ^{1}H n.m.r. (400 MHz; $CDCl_3$), assigned by spin decoupling and nuclear Overhauser enhancement (n.O.e.) difference, 5 × ArH: δ 7.17 (ddd, *J* 8, 8, and 2 Hz, 5-H), 7.30 (ddd, *J* 8, 8, and 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); OMe: δ 4.10 (s); ^{13}C n.m.r. ($CDCl_3$), δ 108.6, 119.0, 120.5, 122.7, and 123.1, 5 × –CH=; δ 106.4, 123.0, and 132.3, >C=; δ 66.0, OMe. Placement of the side chain at C-3 was supported by n.O.e. of 2- and 7-H on irradiation at δ 4.10 (1-OMe). The n.m.r. spectra further indicated that the side chain contained one $-CH_2-$ [δ_H 5.02 (d, *J* 4 Hz) and δ_C 42.8], one >NH [δ 7.0 (br. s)], one >C=S (δ_C 198.3), and one MeS [δ_H 2.65 (s) and δ_C 18.2]. The mass spectrum of (**1**) showed the base peak at *m/z* 160 ($C_{10}H_{10}NO$) indicating that the indole nucleus is attached to a methylene group. Since the methylene proton signal changed to a singlet on D_2O exchange, the methylene group is further connected to the >NH to give the whole structure (**1**).

Brassinin (**2**), $C_{11}H_{12}N_2S_2$, m.p. 132—133 °C, showed a similar ^{1}H n.m.r. spectrum to that of (**1**) except that an indolic NH signal replaced the methoxy signal. Cyclobrassinin (**3**), $C_{11}H_{10}N_2S_2$, m.p. 136—137 °C, showed n.m.r. spectra characteristic of a 2,3-disubstituted indole, suggesting the structure (**3**). The structures (**2**) and (**3**) were confirmed by synthesis.§

Chinese cabbage and other crucifers contain glucosinolates, which undergo enzymic hydrolysis on crushing of the tissues to give isothiocyanates.[4] Since (**1**), (**2**), and (**3**) were not detected in crushed Chinese cabbage tissue, they could not be simple products derived from glucosinolates. This is the first report of the isolation of sulphur-containing phytoalexins.

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‡ Additional data: (**1**), $\lambda_{max.}$ (MeOH) 218 (ε 37 300), 241 (trough, 11 500), 267 (15 800), 287 (sh, 9470), and 297 (sh, 5740) nm; *m/z* 266 (4%, M^+), 235 (54, M^+ – OMe), 218 (16, $M^+ - CH_4S$), 160 (100, $C_{10}H_{10}NO$), 145 (28), 129 (57), and 91 (23, $C_2H_3S_2$); i.r. ($CHCl_3$) $\nu_{max.}$ 3380, 1475, 1452, 1352, 1298, 958, and 922 cm^{-1}. The ^{1}H n.m.r. spectra of (**1**) and (**2**) indicated that they exist as a mixture of 2 isomers owing to hindered C–N rotation. For (**1**), minor ^{1}H n.m.r. signals were present at δ 4.75 (br. d, *J* 4Hz), 7.6 (br.), and 2.75 (s).

§ 3-Aminomethylindole[5] in a mixture of pyridine and triethylamine was treated with CS_2 at 0 °C for 1 h and then with MeI[6] at 3 °C overnight to give (**2**) in 66% yield. Bromination of (**2**) in CH_2Cl_2 with pyridinium hydrobromide perbromide at room temperature for 30 min followed by basification with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) under reflux for 10 min gave (**3**) in 35% yield.