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# Brassicanals A and B, Novel Sulfur-Containing Phytoalexins from the Chinese Cabbage *Brassica campestris* L. ssp. *pekinensis*1)

Kenji MONDE, Nobukatsu KATSUI, Akira SHIRATA,† and Mitsuo TAKASUGI*
Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060
†National Institute of Sericultural and Entomological Science, Tsukuba 305

The isolation and structure elucidation of two sulfur-containing indole phytoalexins, brassicanals A and B, are described. Unusual $^1$H NMR spectra of brassicanal B are also discussed.

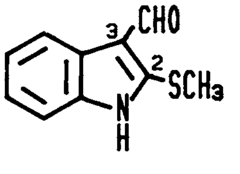
In previous papers2) we reported the first isolation of cruciferous phytoalexins, brassinin (3) and related compounds, from Chinese cabbage inoculated with *Pseudomonas cichorii*. These compounds are structurally characterized by the presence of an indole nucleus with the appendage containing two sulfur atoms. We wish to report here the isolation and structural elucidation of two new phytoalexins from Chinese cabbage.

The more polar part of the acetone extract obtained from the inoculated Chinese cabbage (1.9 kg, dry weight) was repeatedly chromatographed on silica gel, Sephadex LH-20, and HPLC columns to give brassicanals A (1) and B (2) (7 and 55 mg, respectively), spirobrassinin3) (24 mg), and 3-indolecarbaldehyde (6 mg). The compounds 1 and 2 completely inhibited the conidial germination of *Bipolaris leersiae* at concentrations of 300 and 400 ppm, respectively.

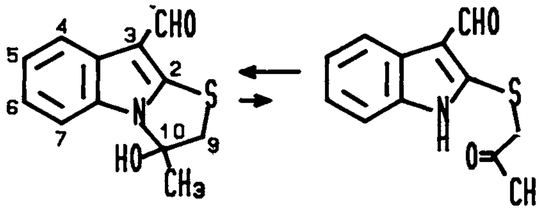
The $^1$H NMR spectrum ($CD_3COCD_3$) of brassicanal A (1), $C_{10}H_9NOS$, mp 210-213 °C, showed signals for formyl ($\delta$ 10.19, s), methylthio ($\delta$ 2.72, s), and >NH ($\delta$ 11.3, br s) groups, together with an ortho-disubstituted benzene ring.4) Its UV spectrum was similar to that of 3-indolecarbaldehyde, suggesting it to be 3-formyl-2-(methylthio)indole. Vilsmeier formylation of 2-(methylthio)indole5) gave the desired compound, which was identical with natural brassicanal A(1).

The UV spectrum6) of brassicanal B (2), $C_{12}H_{11}NO_2S$, mp 169-170 °C, $[\alpha]_D$ 0°, was similar to that of 1. The $^{13}$C NMR spectrum ($CDCl_3$) of 2 showed signals for formyl ($\delta$ 182.7), methyl ($\delta$ 24.8), methylene ($\delta$ 48.7), and quaternary carbon ($\delta$ 92.0) atoms. The presence of a hydroxyl group in 2 was deduced from strong IR ($CHCl_3$) absorption at 3300 $cm^{-1}$. Irradiation of the methyl protons ($\delta$ 2.07, s) in the $^1$H NMR ($CDCl_3$) of 2 resulted in the NOE enhancement of both signals for the aromatic ($\delta$ 7.56, m, 7-H) and one of the methylene protons ($\delta$ 3.87, d, $J$ = 11.7 Hz). On the other hand, irradiation of the formyl proton at $\delta$ 9.65 led to the NOE enhancement of the *very broadened* 4-H signal at $\delta$ 7.82.6) These findings suggested brassicanal B to be present as a hemiaminal structure depicted as 2, structure of which was confirmed by the synthesis7) of 2. In the $^1$H NMR spectrum of 2 in $CD_3OD$, the gradual disappearance of methylene proton signals was observed, suggesting the hemiaminal (2) and a keto form (2') are present in an equilibrium mixture in CD$_3$OD.

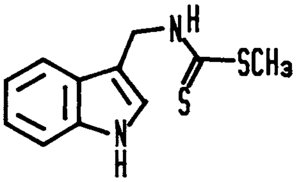
Fig. 1 shows the $^1$H NMR spectra of 2 at 25 °C and -50 °C in CD$_3$OD. A singlet (δ 9.85,CHO) and a broad signal (δ 7.95,4-H) splitted into seemingly two singlets (δ 9.99, 9.66) and two signals (δ 8.00, 7.85), respectively, at -50 °C. These results indicate that two rotamers about C3-CHO exist in CD$_3$OD. However, in CDCl$_3$ the dynamic behavior of 2 is not so simple, e.g., singlet signal (CHO) at 25 °C changed into two singlets and shifted to higher field at -50 °C (Fig.2). This phenomenon may be attributed to the cooperativity of hindered rotation about C3-CHO and molecular association of 2. The spectrum also showed marked dependence on sample concentration in CDCl$_3$.



**Brassicanal A (1)**



**Brassicanal B (2)** **2'**



**Brassinin (3)**

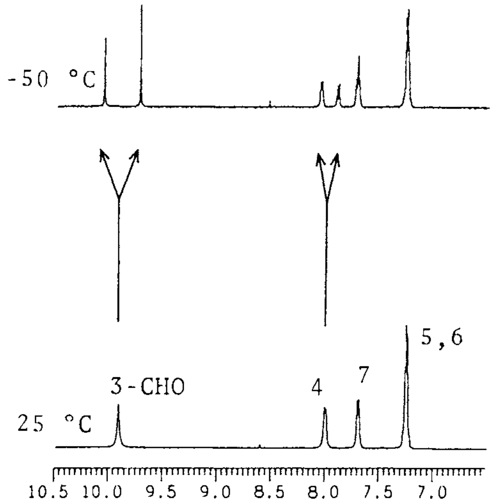


Fig. 1. $^1$H NMR of 2 in CD$_3$OD.

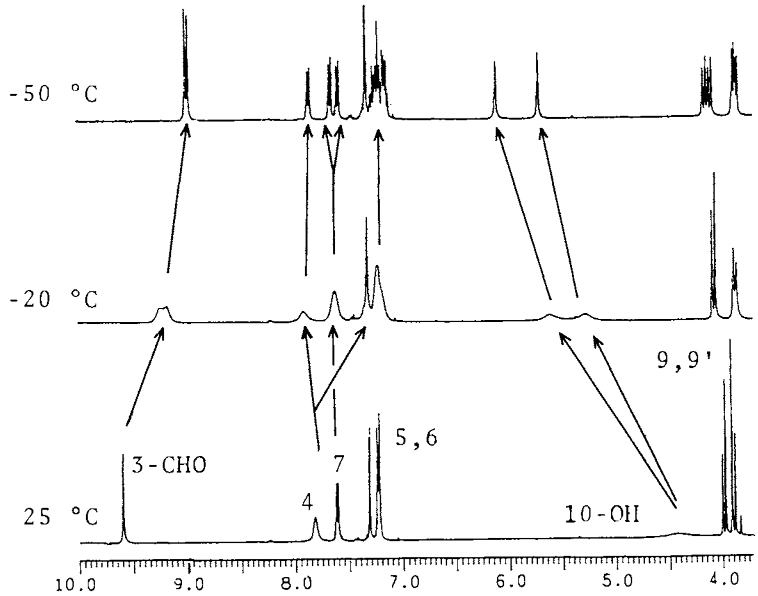


Fig. 2. $^1$H NMR of 2 in CDCl$_3$.

References

1) Part 8 in the series "Studies on Stress Metabolites." For previous papers, see ref. 2.

2) M. Takasugi, K. Monde, N. Katsui, and A. Shirata, Bull. Chem. Soc. Jpn., 61, 285 (1988) and references cited therein.

3) M. Takasugi, K. Monde, N. Katsui, and A. Shirata, Chem. Lett., 1987, 1631.

4) Additional data of 1: UV (MeOH) 210 (ε 26700), 255 (8100), 276 (9550), and 311 nm (9740); $^1$H-NMR δ 7.43 (1H, m) and 7.21 (2H, m ).

5) T. Hino, K. Tsuneoka, M. Nakagawa, and S. Akabori, Chem. Pharm. Bull., 17, 550 (1969).

6) Additional data of 2: UV (MeOH) 207 (ε 25800), 220 (25400), 252 (24100), 277 (14100),and 315 nm (11700); $^1$H-NMR δ 7.19 (2H,m), 4.12 (1H, br s, D$_2$O exchangeable),and 3.92 (1H, d, J = 11.7 Hz); $^{13}$C-NMR δ 110.0 (s), 110.4 (d), 119.5 ( a broad signal), 122.8 (d),123.0 (d),124.4 (s), 130.8 (s), and 133.1 (s); MS m/z 233 (M$^+$; 10),215 (M$^+$-H$_2$O) 100), 214 (69), and 200 (33).

7) Treatment of 2-indolinethione with bromoacetone followed by Vilsmeier formylation gave 2 in 33% yield.

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