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Spirobrassinin, a Novel Sulfur-containing Phytoalexin from the Daikon Rhaphanus sativus L. var. hortensis (Cruciferae)[1)]

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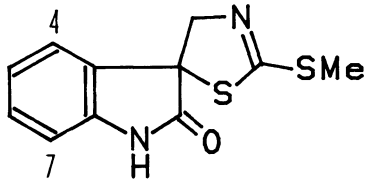
The isolation and structure elucidation of spirobrassinin, the first phytoalexin of oxindole structure from the daikon Raphanus sativus L. var. hortensis, is described.

The plant family Cruciferae contains many important vegetables. Recently, we reported the first isolation of three sulfur-containing phytoalexins, methoxybrassinin (**2**), brassinin (**3**), and cyclobrassinin (**4**) from the Chinese cabbage Brassica campestris L. ssp. pekinensis inoculated with the bacterium Pseudomonas cichorii.[2)] In a continuation of our search for newly induced antifungal compounds of the Cruciferae, we have investigated the phytoalexin formation of daikon (Raphanus sativus L. var. hortensis Ochse), the most important cruciferous vegetable in Japan.

Inoculation of sliced daikon roots with P. cichorii induced the production of several antifungal compounds as evidenced by two dimensional TLC bioassay.[2)] The acetone extracts from the inoculated slices (300 g, dry wt) gave **2** (57 mg), **3** (2 mg), and **5** (9 mg)[3)] after repeated chromatography over silica gel and Sephadex LH-20. From the more polar active fractions, a new compound named spirobrassinin (**1**), 18 mg, was isolated. The compound **1** suppressed completely the fungal growth of Pyricularia oryzae at the concentration of 400 ppm.

Spirobrassinin (**1**), $C_{11}H_{10}N_2OS_2$ (m/z, 250.0231, M^+), mp 158-159 °C and $[\alpha]_D$ - 69.5° (c 1.14, $CHCl_3$), exhibited the following spectra: MS m/z (%) 250 (62, M^+), 203 (59, M^+ - SMe), 177 (100, M^+ - C_2H_3NS), and 145 (34, M^+ - $C_2H_3NS_2$); UV (MeOH) 215 (ε 23500), 250 (8200), and 300 nm (1900); IR ($CHCl_3$) 3220 (>NH), 1718 (>C=O), and 1618 cm^{-1} (>C=N). The ^1H NMR spectrum (400 MHz, $CDCl_3$) of **1** indicated the presence of one methylthio (δ 2.62, 3H, s), one methylene (δ 4.51 and 4.68, each 1H, d, J = 15.1 Hz), and one >NH (δ 8.61, 1H, br s, D_2O exchangeable) groups and four aromatic protons on an ortho-disubstituted benzene ring. Irradiation of the >NH proton (δ 8.61) caused NOE enhancement of an aromatic proton signal at δ 6.92 (1H, d, J = 7.8 Hz, 7-H). Consecutive decoupling experiments assigned three other aromatic protons (δ 7.26, 1H , ddd, J = 7.8 , 7.8, and 1.5 Hz, 6-H; δ 7.09, 1H, ddd, J = 6.8, 7.8, and 1.0 Hz, 5-H; δ 7.36, 1H, d, J = 6.8 Hz, 4-H). The ^{13}C NMR spectrum ($CDCl_3$) of **1** revealed the presence of one sp^3 quaternary (δ 64.7, s), one >C=N- (δ 164.1, s), and one

carbonyl (δ 178.4, s) carbon atoms and also of one methylthio group (δ 15.7, q), one methylene (δ 75.1, t), and one ortho-disubstituted benzene ring (δ 110.4, 123.7, 124.5 and 129.7, each d; δ 131.3 and 139.5, each s). These spectral data and eight degrees of unsaturation derived from the molecular formula indicated **1** to be a tricyclic compound with a spiro ring system. The low-field methylene chemical shifts (δ 4.51 and 4.68) and the two fragments in the mass spectrum at m/z 177 and 145 indicated the presence of a $-CH_2N=C(S-)SMe$ moiety in **1**, leading to either an oxindole (**1**) or an indoxyl structure (**6**) for spirobrassinin. The two possibilities were discriminated by NOE experiment. Irradiation of the aromatic proton at δ 7.36 (4-H) induced enhancement of one of the methylene proton signals at δ 4.51, preferring the oxindole structure (**1**). Comparison of the UV spectral data of 3,3-dimethyloxindole (λ_{max} 247 and 276 nm)[4] with those of 2,2-dimethylindoxyl (λ_{max} 237, 257, and 398 nm)[5] also supported the former ring system. Hence, the structure of spirobrassinin is represented by formula **1**. Although many alkaloids with spirooxindole systems have been reported,[6] this is the first phytoalexin having such a ring system.



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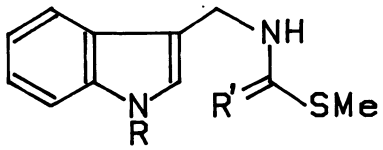
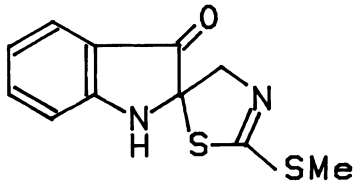


2: R=OMe, R'=S
3: R=H, R'=S
5: R=OMe, R'=O



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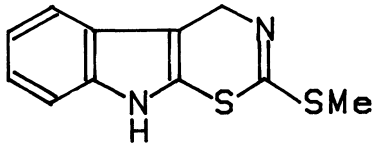

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References

1) Part 6 in the series "Studies on Stress Metabolites." For part 5, M. Takasugi, S. Kawashima, N. Katsui, and A. Shirata, Phytochemistry, in press.
2) M. Takasugi, N. Katsui, and A. Shirata, J. Chem. Soc., Chem. Commun., 1986, 1077.
3) The compound **5** has been isolated from P. cichorii-inoculated Chinese cabbage and will be reported in a full paper together with **2**, **3**, and **4**.
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