

Synthesis and Absolute Stereochemistry of a Cruciferous Phytoalexin, (–)-Spirobrassinin

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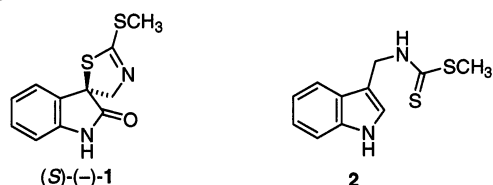
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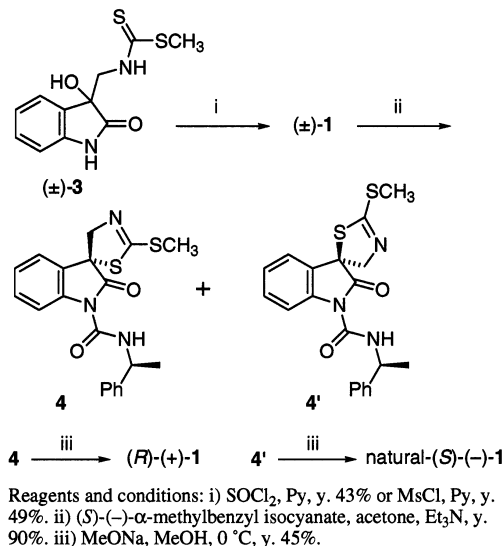
Synthetic (±)-spirobrassinin (**1**) was enantioresolved by a chiral auxiliary method giving (+)-**1** and natural (–)-**1**. The absolute configuration was unambiguously determined by X-ray crystallography of a (1'*S*,4'*R*)-camphanoyl derivative of (+)-**1**. Consequently, natural (–)-**1** has an *S* configuration. Their CD spectra supported this result.

Phytoalexins are defined as antifungal compounds produced by plants *de novo* after infection of microorganisms.¹ We reported the first cruciferous phytoalexins (e.g., brassinin (**2**)) from *Pseudomonas cichorii* inoculated Chinese cabbage (*Brassica campestris*).² Since cruciferous crops are cultivated worldwide and they are extremely valuable, various research groups have investigated the cruciferous phytoalexins and reported more than twenty phytoalexins³ as well as their interesting biological activities⁴ for the last decade. All these compounds are indole or indole-related compounds possessing one or two sulfur atoms, except for one case.⁵ In 1987, we isolated the first oxindole phytoalexin, spirobrassinin ((–)-**1**) from *P. cichorii* inoculated Japanese radish (*Rhaphanus sativus*).⁶ Although several compounds including **1** among cruciferous phytoalexin family have their asymmetric centers, no stereochemical study has been investigated.



Detailed chiral analysis of natural (–)-**1** revealed that it was not enantiomerically pure. Moreover, enantiomeric excesses of two natural spirobrassinin fractions separated by non-chiral chromatography were notably different (98% ee and 83% ee).⁷ This phenomenon called the enantiomeric enrichment is an extremely novel example about a natural product. The curious stereochemical phenomenon and interest in biological studies of chiral cruciferous phytoalexins strongly push us to confirm the absolute configuration of **1**. Herein, we describe about the absolute configuration of **1** by the X-ray crystallographic analysis of 1-[(1'*S*,4'*R*)-camphanoyl]-(*R*)-spirobrassinin (**6**). This is the first report concerning the absolute stereochemistry of the cruciferous phytoalexins.

Racemic dioxibassinin (**3**), which was a minor phytoalexin related metabolite isolated from cabbage, was synthesized from isatin by the previously reported method.⁸ Cyclization of (±)-**3** was achieved with thionyl chloride⁹ or methanesulfonyl chloride leading to (±)-**1**. The racemic **1** reacted with (S)-(–)-α-



Scheme 1. Synthesis of (+)-**1** and (–)-**1**.

methylbenzyl isocyanate giving two urea derivatives **4** and **4'**.¹⁰ As expected, the diastereomeric mixture was separated by simple SiO₂ column chromatography. Following deprotection of **4** and **4'** by sodium methoxide gave (+)-**1** (91% ee) and (–)-**1** (92% ee), respectively (Scheme 1).^{11,12} Their CD spectra were complete mirror images, and (–)-**1** showed an exciton type split at 221 (Δε +25.9) and 204 (Δε –21.0) nm with a moderate A value (+46.9). UV data of 3,3-dimethyloxindole (λ_{max} 205 nm, ε 30000)¹³ and CH₃(CH₂)₄CH₂–N=C(SCH₃)₂ (**5**) (λ_{max} 219 nm, ε 8590)¹³ suggested that two chromophores in **1** (oxindole and N=C(S–)SCH₃ moiety) would induce the split Cotton effects in its CD spectrum.¹⁴ In the case of natural (–)-(**1**), two axes of their transition dipole moments should be clockwise to give positive chirality leading to the *S* configuration of (–)-(**1**) (Figure 1).

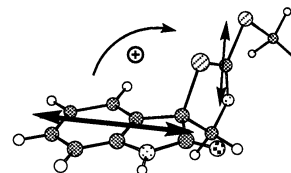
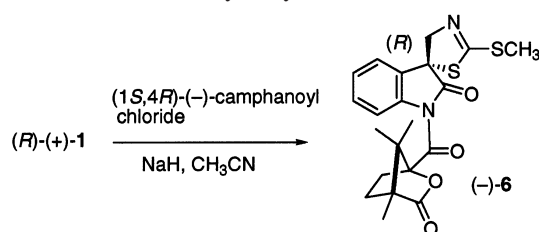


Figure 1. A structure of natural (S)-(**1**). Two arrows on the molecule indicate axes of transition dipole moments.

To confirm this prediction, X-ray crystallographic study has been performed. Unfortunately, numerous attempts to crystallize (+)-**1**, (–)-**1**, **4**, and **4'** for the X-ray study were unsuccessful. Therefore, (+)-**1** and (–)-**1** were examined to derive to crystalline compounds. Camphanoyl group was chosen as a crystalline

inducing part as well as its advantage having its known absolute configuration as an internal standard. Compounds (+)-**1** and (–)-**1** reacted with (1*S*,4*R*)-(–)-camphanoyl chloride to give two crystalline compounds **6** and **6'**, respectively (Scheme 2). Careful recrystallization of **6'** from CH₂Cl₂–hexane afforded colorless prisms suitable for the X-ray analysis.



Scheme 2. Synthesis of a camphanoyl derivative from (R)-(+)-**1**.

Figure 2 shows an ORTEP drawing of **6** determined by the single-crystal X-ray diffraction analysis.¹⁶ The absolute configuration of **6**, derived from (+)-**1**, was doubly confirmed as *R* by the internal reference method and by the Bijvoet method using the heavy atom (sulfur) effect. Consequently, the absolute configuration of natural (–)-**1** was unambiguously concluded as *S*, which is consistent with the result predicted by CD data.

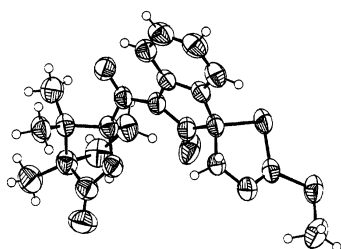


Figure 2. An ORTEP drawing of (–)-**6** with 50% probability ellipsoids.

In summary, we have succeeded in the determination of the absolute configuration of natural (–)-spirobrassinin. This is the first report concerning the absolute stereochemistry of cruciferous phytoalexin family. Further investigation on chiral properties of **1** and its analog is in progress and the results will be reported in detail elsewhere.

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- Selected data for **4**: amorphous solid: $[\alpha]_D^{20} +92.4^\circ$ (*c* 0.17, CH₂Cl₂); EI-MS (%) *m/z* 397 (*M*⁺, 10), 250 (100), 203 (20), 177 (30), 149 (8), 105 (12); IR (CHCl₃): 3320, 1736, 1720 cm^{−1}; ¹H NMR (80 MHz, CDCl₃) δ 8.86 (1H, d, *J* = 7 Hz), 8.24 (1H, m), 7.57 (8H, m), 5.15 (1H, quintet, *J* = 7 Hz), 4.72 (1H, d, *J* = 15 Hz), 4.47 (1H, d, *J* = 15 Hz), 2.65 (3H, s), 1.69 (3H, d, *J* = 7 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 179.1, 163.9, 150.6, 142.9, 139.3, 130.2, 128.8, 128.3, 127.5, 126.0, 125.6, 123.7, 116.6, 75.5, 65.5, 50.1, 22.7, 15.7. **4'**: amorphous solid: $[\alpha]_D^{20} +26.8^\circ$ (*c* 0.17, CH₂Cl₂); EI-MS (%) *m/z* 397 (*M*⁺, 10), 250 (100), 203 (20), 177 (30), 149 (8), 105 (12); IR (CHCl₃): 1738, 1726, 1722 cm^{−1}; ¹H NMR (80 MHz, CDCl₃) δ 8.86 (1H, d, *J* = 7 Hz), 8.24 (1H, m), 7.37 (8H, m), 5.15 (1H, quintet, *J* = 7 Hz), 4.78 (1H, d, *J* = 15 Hz), 4.52 (1H, d, *J* = 15 Hz), 2.65 (3H, s), 1.62 (3H, d, *J* = 7 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 179.1, 164.0, 150.6, 142.8, 139.2, 130.1, 128.8, 128.3, 127.5, 126.0, 125.6, 123.7, 116.6, 75.6, 65.5, 50.1, 22.7, 15.7.
- The enantiomeric excesses were determined by HPLC analysis using a Sumichiral OA-4700 chiral column (*i*-PrOH–dichloroethane–hexane 2:8:90) monitoring by photodiode array and HPLC–CD detectors.
- (+)-**1**: colorless needles: mp 143–145 °C; $[\alpha]_D^{20} +142.7^\circ$ (*c* 0.25, CH₂Cl₂); UV (EtOH) λ_{max} (ε) 218.0 (28800), 260.0 (sh, 6480), 295.6 (1550) nm; CD (EtOH) λ_{ext} (Δε) 204.4 (21.0), 221.0 (−25.9), 240.0 (2.6), 248.8 (−1.0), 263.6 (5.9), 308.2 (5.1) nm. (–)-**1**: colorless needles: mp 142–144 °C; $[\alpha]_D^{20} -143.6^\circ$ (*c* 0.25, CH₂Cl₂); UV (EtOH) λ_{max} (ε) 218.0 (28800), 260.0 (sh, 6480), 295.6 (1550) nm; CD (EtOH) λ_{ext} (Δε) 204.4 (−21.0), 221.0 (25.9), 240.0 (−2.6), 248.8 (1.0), 263.6 (−5.9), 308.2 (−5.1) nm.
- 3,3-Dimethyloxindole was prepared by a published method, see: D. Döpp and H. Weiler, *Chem. Ber.*, **112**, 3950 (1979). Compound **5** was synthesized from *n*-hexylamine by two steps. Detailed experimental conditions and its data will be published elsewhere. **5**: ¹H NMR (400 MHz, CDCl₃) δ 3.39 (2H, t, *J* = 7.1 Hz), 2.54 (3H, s), 2.36 (3H, s), 1.65 (2H, q, *J* = 7.1 Hz), 1.40 (2H, m), 1.32 (4H, m), 0.89 (3H, t, *J* = 7.0 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 156.5 (C=N), 53.0 (CH₂), 31.6 (CH₂), 30.6 (CH₂), 27.1 (CH₂), 22.6 (CH₂), 14.5 (CH₃), 14.4 (CH₃), 14.0 (CH₃).
- a) N. Harada and K. Nakanishi, "Circular Dichroic Spectroscopy – Exciton Coupling in Organic Stereochemistry," University Science Books, Mill Valley (1983). b) K. Nakanishi and N. Berova, in "Circular Dichroism – Principles and Applications," ed. by K. Nakanishi, N. Berova, and R. W. Woody, VCH Publishers, New York (1994), p. 361.
- Selected data for **6**: colorless prisms: mp 177–179 °C; $[\alpha]_D^{20} -16.9^\circ$ (*c* 0.20, CH₂Cl₂); EI-MS (%) *m/z* 430 (*M*⁺, 58), 402 (10), 383 (12), 357 (100), 329 (19), 249 (38); UV (EtOH) λ_{max} (ε) 205.6 (30700), 237.2 (sh, 14900), 269.4 (sh, 5290), 297.0 (sh, 1340) nm; CD (EtOH) λ_{ext} (Δε) 203.4 (6.2), 213.6 (−15.7), 226.4 (2.1), 240.2 (−6.2), 258.2 (4.3), 274.8 (5.7), 306.2 (−1.3); IR (CHCl₃) 1787, 1772, 1718 cm^{−1}; ¹H NMR (400 MHz, CDCl₃) δ 7.52 (1H, d, *J* = 8.1 Hz), 7.39 (1H, dd, *J* = 7.4 and 1.3 Hz), 7.27 (1H, ddd, *J* = 8.1, 7.6, and 1.3 Hz), 7.17 (1H, ddd, *J* = 7.6, 7.4, and 1.0 Hz), 4.82 (1H, d, *J* = 15.4 Hz), 4.40 (1H, d, *J* = 15.4 Hz), 2.92 (1H, ddd, *J* = 13.5, 9.4, and 4.4 Hz), 2.55 (3H, s), 2.27 (1H, ddd, *J* = 13.5, 10.8, and 4.4 Hz), 1.89 (1H, ddd, *J* = 12.8, 10.8, and 4.4 Hz), 1.72 (1H, ddd, *J* = 12.8, 9.4, and 4.4 Hz), 1.22 (3H, s), 1.04 (3H, s), 0.90 (3H, s); ¹³C NMR (100 MHz, CDCl₃) δ 177.4 (C), 175.1 (C), 171.1 (C), 163.9 (C), 138.2 (C), 129.9 (CH), 129.4 (C), 125.9 (CH), 124.6 (CH), 113.7 (CH), 92.9 (C), 76.0 (CH₂), 65.7 (C), 57.3 (C), 54.5 (C), 30.4 (CH₂), 29.5 (CH₂), 17.6 (CH₃), 16.6 (CH₃), 15.6 (CH₃), 9.7 (CH₃).
- Crystal data for **6**: C₂₁H₂₂N₂O₄S₂, *M* = 430.54, colorless prisms (0.42 × 0.28 × 0.20 mm), orthorhombic, space group *P*2₁2₁1 (#19), *a* = 16.076(3), *b* = 17.194(4), *c* = 7.554(1) Å, *V* = 2088(7) Å³, *Z* = 4, *D*_c = 1.369 g cm^{−3}, *D*_m = 1.359 g cm^{−3} by flotation using a CCl₄–hexane solution, radiation Cu K_α (1.54178 Å), unique data *F*_o > 3σ(*F*_o), 2040. The skeletal structure was solved by the direct method and successive Fourier syntheses. All hydrogen atoms were found by the difference Fourier syntheses. Absorption correction and full matrix least-squares refinement of positional and thermal parameters, including anomalous scattering factors of sulfur, oxygen, nitrogen, and carbon atoms, led to the final convergence with *R* = 0.0330 and *R*_w = 0.0472, while *R* = 0.0450 and *R*_w = 0.0648 for the mirror image structure. The absolute configuration was also determined by measurement of the Bijvoet pairs.