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

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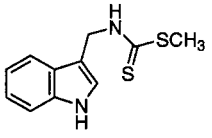
(Received May 16, 2000; CL-000471)

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 phytoalexins[3] as well as their interesting biological activities[4] 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.

(*S*)-(–)-**1**



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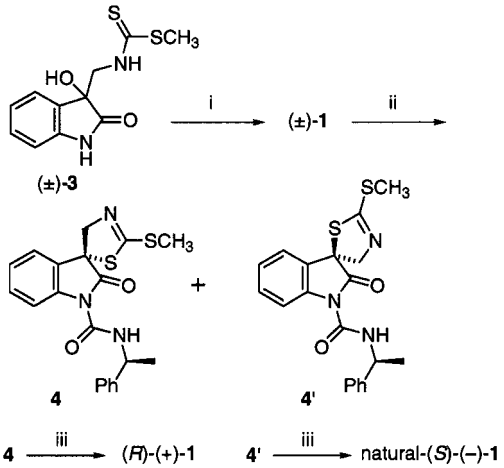
Detailed chiral analysis of natural (–)-**1** revealed that it was not enantiomerically pure. Moreover, enantiomeric excesses of two natural spirobrassinin fractions separated by non-chiral chromatography were notably different (98% ee and 83% ee).[7] This phenomenon called the enantiomeric enrichment 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 dioxibrassinin (**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 chloride[9] or methanesulfonyl chloride leading to (±)-**1**. The racemic **1** reacted with (*S*)-(–)-α-



Reagents and conditions: i) $SOCl_2$, Py, y. 43% or MsCl, Py, y. 49%. ii) (*S*)-(–)-α-methylbenzyl isocyanate, acetone, Et_3N, y. 90%. iii) MeONa, MeOH, 0 °C, y. 45%.

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 SiO_2 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 $CH_3(CH_2)_4CH_2$–N=C$(SCH_3)_2$ (**5**) (λ_{max} 219 nm, ε 8590)[13] suggested that two chromophores in **1** (oxindole and N=C(S–)SCH_3 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).

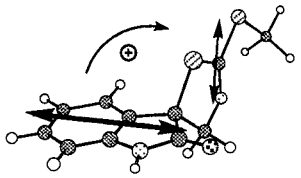
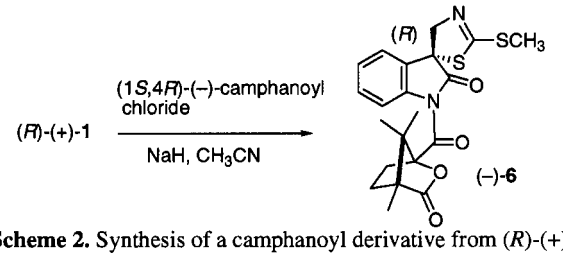


Figure 1. A structure of natural (*S*)-(–)-**1**. Two arrows on the molecule indicate axes of transition dipole moments.

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**[15] from CH_2Cl_2–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.

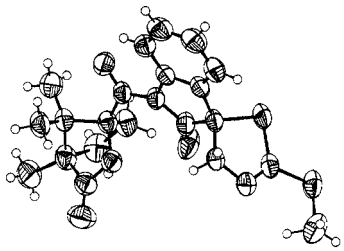


Figure 2. An ORTEP drawing of (–)-**6** with 50% probability ellipsoids.

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.

P. K. thanks the Grant Agency for Science of the Slovak Republic (No. 1/6080/99) for financial support.

References and Notes

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10 Selected data for **4**: amorphous solid: $[\alpha]_D^{20}$ +92.4° (*c* 0.17, CH_2Cl_2); EI-MS (%) *m/z* 397 (M^+, 10), 250 (100), 203 (20), 177 (30), 149 (8), 105 (12); IR ($CHCl_3$): 3320, 1736, 1720 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) 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); ^{13}C NMR (75 MHz, $CDCl_3$) 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° (*c* 0.17, CH_2Cl_2); EI-MS (%) *m/z* 397 (M^+, 10), 250 (100), 203 (20), 177 (30), 149 (8), 105 (12); IR ($CHCl_3$): 1738, 1726, 1722 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) 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); ^{13}C NMR (75 MHz, $CDCl_3$) 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.

11 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.

12 (+)-**1**: colorless needles: mp 143–145 °C; $[\alpha]_D^{20}$ +142.7° (*c* 0.25, CH_2Cl_2); 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° (*c* 0.25, CH_2Cl_2); 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.

13 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, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5 (C=N), 53.0 (CH_2), 31.6 (CH_2), 30.6 (CH_2), 27.1 (CH_2), 22.6 (CH_2), 14.5 (CH_3), 14.4 (CH_3), 14.0 (CH_3).

14 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.

15 Selected data for **6**: colorless prisms: mp 177–179 °C; $[\alpha]_D^{20}$ –16.9° (*c* 0.20, CH_2Cl_2); 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 ($CHCl_3$) 1787, 1772, 1718 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 (CH_2), 65.7 (C), 57.3 (C), 54.5 (C), 30.4 (CH_2), 29.5 (CH_2), 17.6 (CH_3), 16.6 (CH_3), 15.6 (CH_3), 9.7 (CH_3).

16 Crystal data for **6**: $C_{21}H_{22}N_2O_4S_2$, *M* = 430.54, colorless prisms (0.42 × 0.28 × 0.20 mm), orthorhombic, space group $P2_12_12_1$ (#19), *a* = 16.076(3), *b* = 17.194(4), *c* = 7.554(1) Å, *V* = 2088(7) $Å^3$, *Z* = 4, D_c = 1.369 g cm^{-3}, D_m = 1.359 g cm^{-3} by flotation using a CCl_4–hexane solution, radiation Cu K_α (1.54178 Å), unique data $F_o > 3\sigma(F_o)$, 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.