Pergamon

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Tetrahedron Letters 44 (2003) 6017–6020

TETRAHEDRON LETTERS

Preparation of cruciferous phytoalexin related metabolites, (−)-dioxibrassinin and (−)-3-cyanomethyl-3-hydroxyoxindole, and determination of their absolute configurations by vibrational circular dichroism (VCD)

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Received 15 May 2003; revised 12 June 2003; accepted 13 June 2003

Abstract—Cruciferous phytoalexin related metabolites, (−)-dioxibrassinin (**1**) and (−)-3-cyanomethyl-3-hydroxyoxindole (**2**) were prepared from isatin as racemates and were resolved by chiral HPLC. Their absolute configurations were determined by the new chiroptical technique, vibrational circular dichroism (VCD), as well as by the conventional electronic circular dichroism (ECD). It is concluded that the absolute configurations of the naturally occurring (−)-**1** and (−)-**2** are both *S*.

Phytoalexins are defined as antifungal compounds produced by plants de novo after their exposure to physical, biological or chemical stress.[1] More than 20 indole or indole-related phytoalexins[2] have been isolated from the economically and dietary important plants (e.g. cabbage, turnip, Japanese radish, rapeseed, etc.) of the cruciferous family cultivated worldwide. In addition, the cruciferous family includes *Arabidopsis thaliana* which is an interesting model plant and the only example to date of a completely sequenced plant genome.[3] Therefore, cruciferous phytoalexins can be expected to be a model case for the study of a plant's chemical and biochemical defense mechanism. Recently, we reported the first stereochemical study regarding a representative cruciferous phytoalexin, (*S*)-(−)-spirobrassinin (**3**).[4] No other stereochemical studies have been reported, although several compounds among the cruciferous phytoalexins have asymmetric centers. Moreover, partially enantioenriched **3** and its derivative show an interesting enantiomeric enrichment phenomenon during nonchiral chromatographic separation.[5] This is an extremely novel effect involving natural products. Due to this curious stereochemical phenomenon and interests in biological activities of chiral cruciferous phytoalexins, as well as for a biosynthetic pathway study, we have continued our stereochemical studies of the cruciferous phytoalexins.[6] In this paper, we describe the determination of the absolute configurations of phytoalexin related metabolites, (−)-dioxibrassinin (**1**)[7] and (−)-3-cyanomethyl-3-hydroxyoxindole (**2**)[7,8] by means of the recently developed technique, vibrational circular dichroism (VCD),[9] as well as by conventional electronic circular dichroism (ECD). Also, we demonstrate the stereospecific chemical conversion from chiral dioxibrassinin to spirobrassinin. This is the first example of the application of VCD to an oxindole alkaloid, which is one of a large family of pharmacologically significant alkaloids.

Keywords: VCD; dioxindole; dioxibrassinin; phytoalexin; tertiary alcohol.
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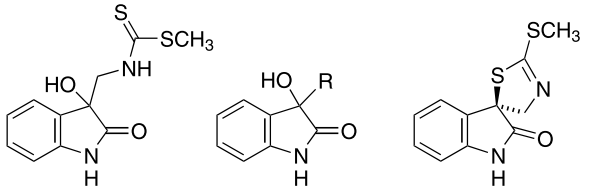
doi:10.1016/S0040-4039(03)01513-2



1 **2** R = CH_2CN, **4** R = CH_3 natural-(*S*)-(-)-**3**

(−)-Dioxibrassinin (**1**) and (−)-3-cyanomethyl-3-hydroxyoxindole (**2**) were first isolated from *Pseudomonas cichorii* inoculated cabbage (*Brassica oleracea* var. *capitata*) as minor phytoalexin metabolites.[7] Structural features readily suggest that intramolecular cyclization of **1** leads to **3**. Thus, (−)-**1** was believed to be a precursor of (*S*)-(−)-spirobrassinin (**3**). In fact, chemical transformation of racemic **1** to **3** was achieved by thionyl chloride in a moderate yield.[4b] However, it might be difficult to confirm the absolute configuration of **1** by utilizing this chemical transformation from a chiral **1** to the stereochemically known (*S*)-(−)-**3**, since there is no obvious stereochemical information regarding this cyclization mechanism. Another compound **2** was recently isolated again as a metabolite of indole-3-acetaldoxime from UV-irradiated turnip tissue during studies focusing on the biosynthetic relationship between indole glucosinolates and the indole phytoalexins.[8] These two compounds have one tertiary hydroxy group at the C3 chiral carbon on the oxindole nuclear framework. In general, determination of the absolute configuration of a tertiary hydroxy group is a difficult task, although there are several established methods to determine the absolute configuration of secondary alcohols such as Mosher's method.[10] In order to determine the absolute configurations of dioxindoles, as well as to establish a general method for the determination of absolute configuration of tertiary alcohols, we examined the VCD for these compounds. VCD has been developed recently as a chiral method of stereochemical analysis in which one measures the differential absorption of left versus right circularly polarized IR radiation by molecular vibrational transition.[9]

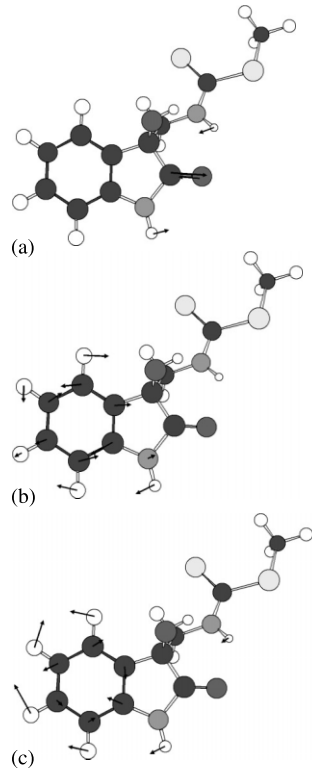


Figure 1. Vibrational modes of **1**. (a) 1725 cm^{-1}, (b) 1624 cm^{-1}, (c) 1473 cm^{-1}.

Racemic **1** and **2** were synthesized from isatin by the previously reported methods, respectively.[7] Enantiomeric resolutions were performed by chiral HPLC on a CHIRALPAK® AD after screening several chiral stationary phase. Practical scale separations (up to 3 mg for one injection) were carried out for racemic compounds. The first-eluted enantiomers were identified as the natural ones by comparison of their optical rotations, while the second ones were unnatural ones.[11] To simplify the theoretical calculations of their VCD spectra, a model compound, 3-hydroxy-3-methyloxindole (**4**) was also prepared and separated enantiomerically by the same method.[11]

IR and VCD spectra were measured at 4 cm^{-1} resolution using a BioTools Chiral*IR* spectrometer. All spectra were obtained in DMSO-d_6 using CaF_2 cell of 6 μm pathlength at the concentration of approximately 2 M. The VCD spectra of the enantiomers were complete mirror images in every case. All compounds show the same characteristic three bands near 1730, 1620, and 1470 cm^{-1} (Fig. 1) with several complex bands in the fingerprint region. Two characteristic bands of three were readily assigned as the contribution of the C=O stretch (1730 cm^{-1}) and an aromatic ring stretch (1620 cm^{-1}) (Fig. 2). To determine their absolute configurations, the measured VCD spectrum of each compound was compared with theoretical predictions. For the latter purpose, ab initio quantum calculations of the (*S*)-enantiomers **1**, **2** and **4** have been carried out. First, conformational analyses were carried out using molecular mechanics with an MM2+ force field from the CONFLEX program.[12] Several lower conformations were selected so that the cumulative sum of the Boltzmann weighted populations of the conformers considered to be covered over 95%. Geometry optimizations and harmonic frequency analysis were performed by ab initio molecular orbital calculations to obtain the accurate energetics, IR and VCD spectra.[13] The spectra of conformers whose energies are within 1 kcal/mol from the lowest conformer were superposed with Boltzmann weights.[13] Several calculated conformers of each molecule differed in the orientation of the OH group and the side chain group. Signs of the three characteristic bands showed reasonable agreement between the observed and calculated VCD spectra with slight difference of their wavenumber frequencies (Fig. 2).[15] These comparisons allow one to conclude that the absolute configurations of the naturally occurring (−)-**1** and (−)-**2** are both *S*.

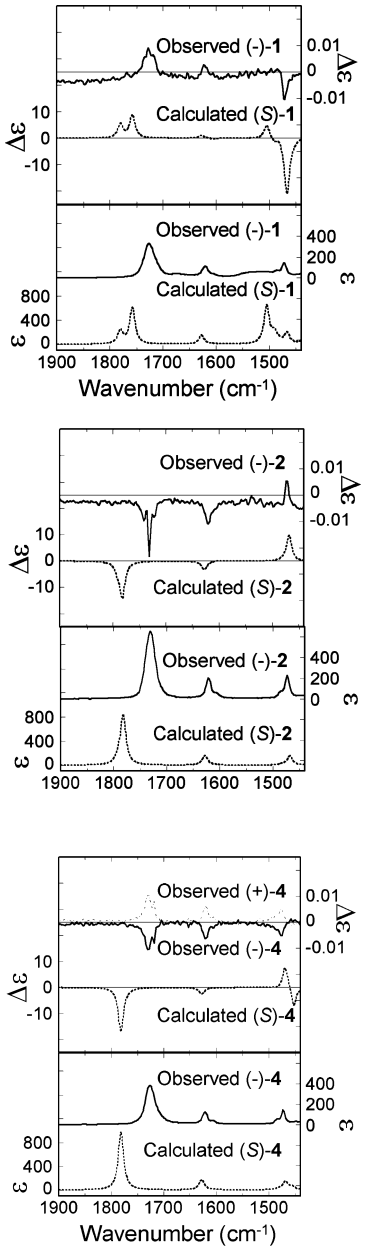
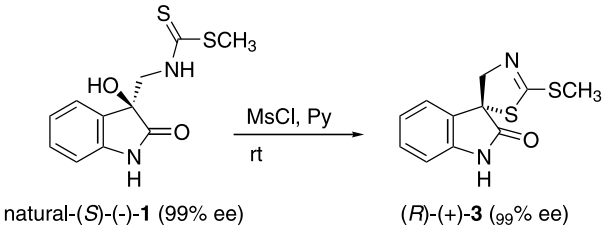


Figure 2. Comparison of observed IR and VCD spectra of (a) (−)-**1**, (b) (−)-**2**, (c) (+)-**4** and (−)-**4** with Boltzmann-population-weighted sum of spectra of (*S*)-**1**, (*S*)-**2** and (*S*)-**4**.



Scheme 1.

Many natural products having the 3-hydroxyoxindole moiety have been found.[16] However, no reliable method for the determination of their absolute stereochemistry has been published, except for one case. Recently, Aimi et al. reported a useful empirical CD correlation,[17] although no theoretical calculation has yet been carried out to test this correlation. To compare our results with the prediction by their method, the ECD of three enantiomers **1**, **2** and **4** were measured. All three enantiomers show the two or three Cotton effects at the long-wavelength region (300–260 nm) and the shorter wavelength region (260–220 nm),[11] which is consistent with the CD correlation and our conclusions.

The chemical transformation from dioxibrassinin to spirobrassinin was mentioned earlier. This result suggested that the spirobrassinin was biologically synthesized directly from dioxibrassinin. However, labeled racemic dioxibrassinin would not incorporate into spirobrassinin, while labeled brassinin and cyclobrassinin were incorporated into spirobrassinin in relatively good yield using UV irradiated turnip tissue.[18] To confirm these results in the light of stereochemical detail, the chemical transformation to spirobrassinin was performed. A naturally occurring (*S*)-(−)-**1** (99% ee) was transformed by methanesulfonyl chloride into spirobrassinin, whose absolute configuration was checked by ECD and found to be the unnatural type *R* while maintaining its ee as 99% (Scheme 1).[19] Obviously, intramolecular S_N2 reaction with inversion occurred. Again, this supports the previous incorporation studies of dioxibrassinin at least from the viewpoint of its chemical reactivity.

In conclusion, we have determined the absolute stereochemistry of naturally occurring (−)-dioxibrassinin (**1**) and (−)-3-cyanomethyl-3-hydroxyoxindole (**2**) as *S* by comparing theoretical calculations and observed spectra of the VCD, with particular emphasis on the three characteristic VCD bands of the 3-hydroxyoxindole moiety. The chirality of the three VCD bands was obviously transferred from the single chiral carbon of the tertiary alcohol to neighboring carbonyl and aromatic functions by coupling between the vibrational modes of these groups. While VCD no doubt is present in vibrational modes primarily associated with the single chiral carbon atom, in this case such modes are not conveniently located in isolation in the VCD spectrum as are the modes of neighboring carbonyl and aromatic groups. This concept of vibrational chirality transfer from a certain chiral functional group to other readily detectable functional groups might be very useful in the establishment of a general method for determination by VCD of absolute stereochemistry of the tertiary alcohols, whose stereochemistry can not otherwise be determined easily. To our knowledge, this is the first case of the determination of absolute configuration of chiral tertiary alcohols by VCD.

Acknowledgements

This research was supported in part by a Grant-in-Aid for scientific research from Ministry of Education, Culture, Sports, Science and Technology (Grants 14658181) and by NEDO for 'Research and Development on Glycocluster Controlling Biomolecules'.

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11. Naturally occurring **(*S*)-(−)-dioxibrassinin (2)**: $[\alpha]_D$ −7.3° (MeOH; *c* 0.2); UV (MeOH) λ_{max} (ε) 208 (27,300), 252 (12,500), 284 (sh, 6,000) nm; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 211 (+31.0), 247 (−19.6), 268 (+2.3), 295 (+2.4) nm. **(*R*)-(+)-Dioxibrassinin (2)**: $[\alpha]_D$ +6.0° (MeOH; *c* 0.2). Naturally occurring **(*S*)-(−)-3-cyanomethyl-3-hydroxyoxindole (3)**: $[\alpha]_D$ −34.8° (MeOH; *c* 0.2); UV (MeOH) λ_{max} (ε) 208 (30100), 251 (6600), 291 (1800) nm; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 209 (–40.7), 238 (+26.3), 264 (−10.6), 288 (−1.4) nm. **(*R*)-(+)-3-Cyanomethyl-3-hydroxyoxindole (3)**: $[\alpha]_D$ +25.7°(MeOH; *c* 0.2). **(*S*)-(−)-3-Hydroxy-3-methyloxindole (3)**: $[\alpha]_D$ −50° (MeOH; *c* 1.0); 99% ee; UV (MeOH) λ_{max} (ε) 207 (28200), 252 (6400), 286 (1600) nm; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 208 (−40.7), 237 (+25.4), 262 (−10.0) nm. **(*R*)-(+)-3-Hydroxy-3-methyloxindole (3)**: $[\alpha]_D$ +50° (MeOH; *c* 1.0).
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