Pergamon

Tetrahedron: *Asymmetry* 13 (2002) 1875–1878

TETRAHEDRON: *ASYMMETRY*

Brz220 a novel brassinosteroid biosynthesis inhibitor: stereochemical structure–activity relationship

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Received 26 June 2002; accepted 29 July 2002

Abstract—The four stereoisomers of Brz220 (2*RS*,4*RS*-1-[4-propyl-2-(4-trifluoromethylphenyl)-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole), a novel brassinosteroid biosynthesis inhibitor, were separated by silica gel column chromatography and chiral high-performance liquid chromatography (HPLC). The absolute configuration of each stereoisomer was determined by a combination of asymmetric synthesis and NMR analysis. The effects of these stereoisomers on cress stem elongation clearly indicate that the (2*S*)-isomer has the greatest inhibitory activity.

1. Introduction

The brassinosteroids are a group of plant steroid hormones that regulate numerous aspects of plant growth and development, including stem elongation, leaf bending, tracheary element differentiation and photomorphogenesis.[1] The functions of endogenous brassinosteroids have been identified from the study of brassinosteroid-deficient mutants of *Arabidopsis*, pea, tomato and rice.[2] Brassinosteroid biosynthesis inhibitors, like these mutants, are useful for elucidating the functions of brassinosteroids, not only in other plant species, but also in tissues, and organs.[3–9] These inhibitors are useful tools that complement the analysis of mutants.[10,11] Recently we discovered Brz220, a novel potential brassinosteroid biosynthesis inhibitor, whose activity is comparable with that of known brassinosteroid biosynthesis inhibitors in its in vivo efficacy on cress.[12] Since it contains two stereogenic carbon atoms (C-2 and C-4, Fig. 1), there are four epimeric stereoisomers of Brz220.

Since the stereoisomers of azole compounds often have different biological activities,[13] we examined the relationship between the stereochemical structure and the biological activity of Brz220. Here, we report the synthesis of the (2*RS*,4*S*)-diastereomer **3**, the chromatographic separation of the stereoisomers, their absolute configurations and their biological activity.

2. Results and discussion

As shown in Fig. 1, racemic Brz220 was separated into two racemic diastereomers (racemate **1** and **2**) by silica gel column chromatography. Each of these racemic diastereomers was then separated into two enantiomers (**1a** and **1b**, and **2a** and **2b**, respectively) by chiral HPLC. In order to determine the absolute configuration of each stereoisomer, we used optically active (*S*)-1,2-pentanediol as the starting material for preparing **3** (Fig. 2, see Section 3). Chiral HPLC was used to separate the diastereomeric mixture of **3** into enantiomers **4** and **5**, which have known absolute configurations at C-4. Enantiomers **4** and **5** had identical ^{1}H NMR spectra to those of racemic **1** and **2**, respectively. Then, the specific rotations and retention times of **1a**, **1b**, **2a**, and **2b** in chiral HPLC were compared with those of **4** and **5** (Table 1). Compounds **1a** and **2b** were found to be identical to **4** and **5**, respectively, which have (4*S*)-configuration. In order to determine the configuration of enantiomers **1a** and **2b** at C-2, **2b** was subjected to intensive NMR analyses. The ^{1}H and ^{13}C NMR data for **2b** are shown in Table 2. The assignment of all ^{1}H and ^{13}C NMR signals was confirmed

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0957-4166/02/$ - see front matter
PII: S0957-4166(02)00475-5

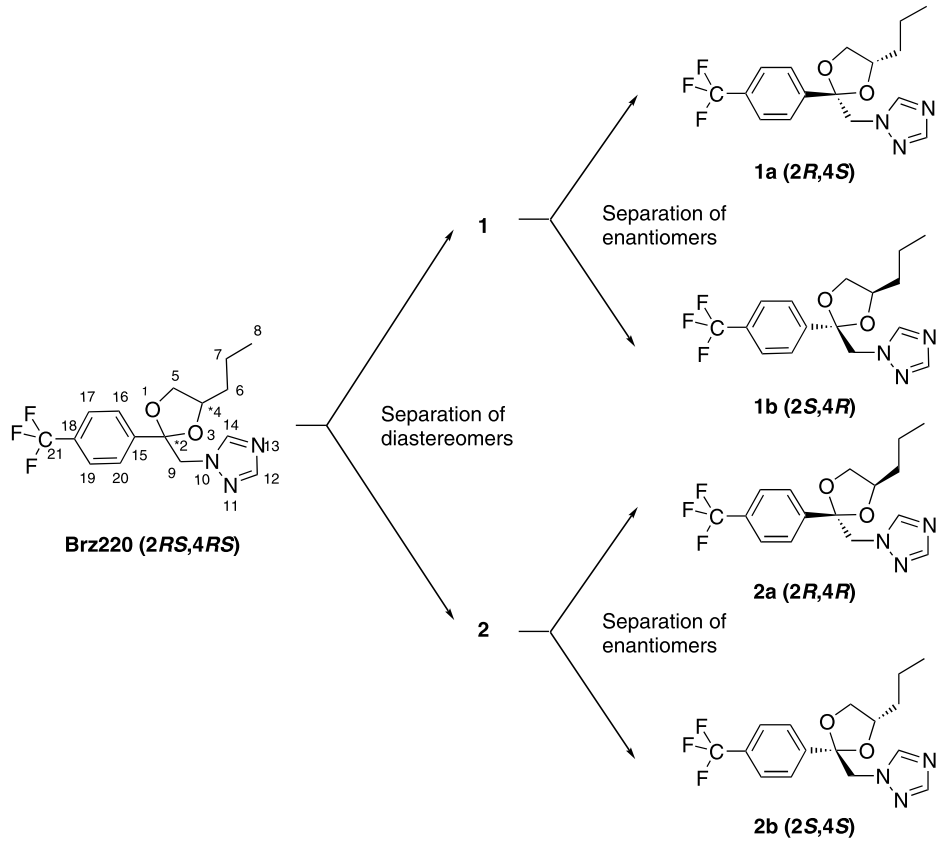

Figure 1. Preparation of the four stereoisomers of Brz220.

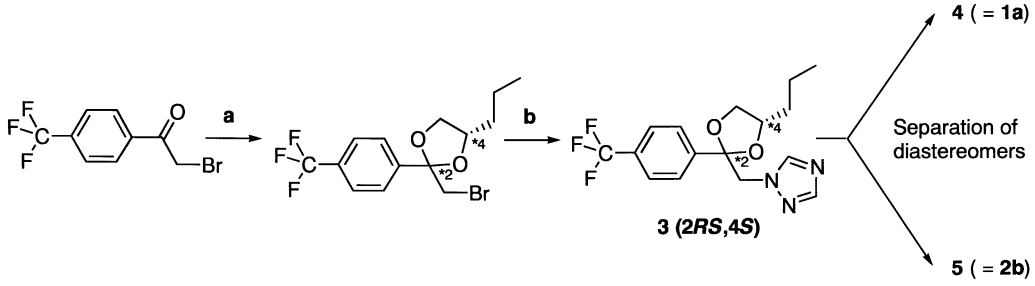

Figure 2. Synthetic scheme for **3** and separation of stereoisomers **4** and **5**. (a) (*S*)-1,3-pentanediol, *p*-toluenesulfonic acid, toluene. (b) 1,2,4-triazole, KOH, DMSO.

Table 1. Specific rotations and retention times of **1a**, **1b**, **2a**, **2b**, **4** and **5** in chiral-HPLC

Compound	$[\alpha]_D^{27}$[a]	Retention time (min)
1a	+7.5 (*c* 0.29)	5.6
1b	−8.9 (*c* 0.32)	10.4
2a	+2.3 (*c* 0.30)	6.4
2b	−3.1 (*c* 0.32)	11.2
4	+7.1 (*c* 0.30)	5.6
5	−3.4 (*c* 0.29)	11.2

[a] Measured in methanol solution.

from DQFCOSY, HMQC, HMBC NMR data and NMR data of a Brz220 analogue.[14] The HMBC data indicated that there was a chemical shift of C-2 that was correlated with H-5β, not H-5α, indicating that the dioxolane ring is not planar, but is in a torsional strain conformation. In the NOE difference spectrum, H-6 and H-16 and H-20 showed a clear NOE correlation (Table 2). Other NOE correlations were observed between H-4 and H-9. These results demonstrate that the *n*-propyl group and the triazole moiety are on opposite sides of the dioxolane ring, i.e. **2b** has (2*S*,4*S*)-configuration. By contrast, its diastereomer **1a** has (2*R*,4*S*)-configuration because these substituents are on the same side of the dioxolane ring. Based on these analyses, the absolute configurations of C-2 and C-4 positions for **1b** and **2a** were determined to be those shown in Fig. 1.

The inhibition of brassinosteroid biosynthesis by the four stereoisomers of Brz220 was examined using the cress (*Lepidium sativum* L.) stem elongation test, since cress is very sensitive to brassinosteroid deficiency.[3] The

Table 2. NMR data ($CDCl_3$) for 2*S*,4*S*-1-[4-propyl-2-(4-trifluoromethylphenyl)-1,3,-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole [**2b** (=**5**)]

Position	δ_C	δ_H (mult, *J* in Hz)	Correlations	
			HMBC	NOE
2	107			
4	78.6	β 3.76 (m)		H-5β, H-6
5	70.4	α 3.33 (t, 8.3)	C-4, C-6	
		β 4.00 (dd, 5.7, 8.3)	C-2	
6	35.0	1.26 (m)		
		1.49 (m)		H-4β, H-5α, H-16
7	18.9	1.24 (m)		
		1.35 (m)		
8	13.9	0.89 (t, 7.0)	C-6, C-7	
9	56.8	4.47 (d, 14.6)	C-2	H-4β, H-11, H-16
		4.48 (d, 14.6)	C-11	H-4β, H-11, H-16
11	144.5	8.21 (s)	C-13	H-4β, H-9
13	151.3	7.95 (s)	C-11	
15	131.0			
16	126.2	7.66 (s)	C-2, C-18, C-21	H-5α, H-6, H-9
17	125.5	7.66 (s)	C-2, C-18, C-21	H-5α, H-6, H-9
18	131.1			
19	125.5	7.66 (s)	C-2, C-18, C-21	H-5α, H-6, H-9
20	126.2	7.66 (s)	C-2, C-18, C-21	H-5α, H-6, H-9
21	123.9			

inhibitory activities listed in Table 3 are expressed as I_{50} values. Of the four stereoisomers of Brz220, the (2*S*,4*R*)-isomer **1b** was the most active; the (2*S*,4*S*)-isomer **2b** was slightly less active than **1b**, and the (2*R*,4*S*)-**2a** and (2*R*,4*R*)-**1a** showed only weak activity. These results indicate that the *S* configuration at C-2 produces the greatest inhibitory activity, and this is enhanced by the *R* configuration at C-4.

Table 3. I_{50} values of the four stereoisomers of Brz220 in cress stem elongation

Compound	I_{50}[a] (μM)
1a	0.94
1b	0.01
2a	1.04
2b	0.12

[a] The concentration that produces 50% inhibition of cress hypocothyl elongation compared to the control (non-treated) condition.

In conclusion, we have clarified the stereochemical structure–activity relationships of Brz220. In inhibiting brassinosteroid biosynthesis, the (*S*)-configuration of Brz220 at C-2 predicts whether a stereoisomer can bind to its receptor site on a cytochrome P450 in brassinosteroid biosynthesis pathway, as occurs with brassinazole, triazole-type brassinosteroid biosynthesis inhibitor.[15] Further study to reveal the site of action of Brz220, both in vivo and in vitro is now in progress.

3. Experimental

3.1. General

Chemicals were purchased from KANEKA Co. Ltd. (Osaka) and Tokyo Kasei Co. Ltd. (Tokyo). Flash column chromatography was performed on silica gel (FL-60D, Fuji Silica Chemical Ltd.). Optical rotations were measured on a JASCO P1010 polarimeter. ^1H, ^{13}C NMR, DQFCOSY, HMQC, HMBC and difference NOESY spectra were recorded on a JOEL alpha 400 spectrometer with a field gradient unit. ^1H NMR chemicals shifts were recorded using tetramethylsilane (TMS) as an internal standard and ^{13}C NMR chemical shifts are reported in ppm relative to TMS. The melting points (mp) were determined with a Yanagimoto melting point apparatus and are uncorrected.

3.2. Synthesis

A mixture of the diastereomers of (2*RS*,4*S*)-1-[4-propyl-2-(4-trifluoromethylphenyl)-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole, **3** was prepared from 2-bromo-1-(4-trifluoromethylphenyl)ethanone and (*S*)-1,2-pentanediol (99% ee /GLC) according to the procedure reported previously.[12]

3.3. Diastereomer separation of Brz220 and 3

Two racemates **1** and **2** were obtained by separating racemic Brz220 with silica gel chromatography, using ethyl acetate-*n*-hexane (85:15) as the eluent. Compounds **4** and **5** were obtained by separating **3** by HPLC with a chiral stationary phase column (Daicel Chem. Ltd., CHIRALPAK AS, 4.6 mm×250 mm), using *n*-hexane–2-propanol (9:1) as the eluent, at a flow rate of 1 mL/min, using a detection wavelength at 254 nm.

Compounds 1 and 4: Colorless crystals; mp 46–47°C; ^1H NMR ($CDCl_3$) δ 0.92 (3H, t, $J=7.0$ Hz), 1.26–1.45

(4H, m), 3.24 (1H, t, $J=6.6$ Hz), 3.91 (2H, m), 4.50 (2H, s), 7.66 (4H, s), 7.94 (1H, s) 8.23 (1H, s).

Compounds 2 and 5: Colorless crystals; mp 55–56°C; ^{1}H NMR data for **2** were identical to those for **5** (Table 2).

3.4. Resolution of the racemates, 1 and 2

Racemates **1** and **2** were separated into optically active compounds **1a** and **1b**, and **2a** and **2b**, respectively, in a manner similar to that used to separate **4** and **5**. The enantiomeric excess (ee) of each isomer was greater than 99% as determined from peak areas.

3.5. Bioassay

Cress seeds (*L. sativum L.*) were purchased locally. The seeds were sterilized in 1% NaOCl for 20 min and washed with sterile distilled water five times. The seeds were sown on a 0.8% agar-solidified medium containing half-strength Murashige and Shook salts and 1.5% sucrose (w/v) in Agripots (Kirin Brewery Co., Ltd, Tokyo, Japan) with or without test chemicals. The test chemicals were dissolved and diluted with DMSO. Cress seeds were grown under a 16 h light (240 μE m^{-2} s^{-1}) and 8 h dark photoperiod in a growth chamber at 25°C for 8 days.

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