PERGAMON

Phytochemistry 52 (1999) 815–818

PHYTOCHEMISTRY

2,3,5-Tri-*epi*-brassinolide: preparation and biological activity in rice lamina inclination test

Hideharu Seto[a,*], Shozo Fujioka[a], Hiroyuki Koshino[a], Toshiro Suenaga[a], Shigeo Yoshida[a], Tsuyoshi Watanabe[b], Suguru Takatsuto[c]

[a]*The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, 351-0198, Japan*
[b]*Tama Biochemical Co. Ltd., 2-7-1 Nishishinjuku, Shinjuku-ku, Tokyo, 163-0704, Japan*
[c]*Department of Chemistry, Joetsu University of Education, Joetsu-shi, Niigata, 943-8512, Japan*

Received 4 December 1998; received in revised form 3 May 1999

Abstract

With a view to attaining more precise information on the biological activity of 2,3,5-tri-*epi*-brassinolide analogs, 2,3,5-tri-*epi*-brassinolide was prepared from 2,3-di-*epi*-brassinolide by direct epimerization at C-5. Biological activity of 2,3,5-tri-*epi*-brassinolide in the rice lamina inclination test was nil even at 1 μg/plant by the single application technique, while with co-application of indole-3-acetic acid, the activity was ca 1/1000th that of brassinolide, reconfirming that the A/B *trans*-fused ring junction of brassinosteroids is an essential structural factor for high biological activity.

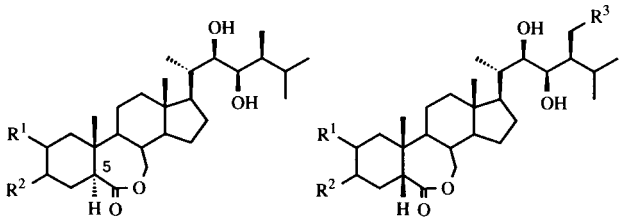
Keywords: 2,3,5-tri-*epi*-Brassinolide; Brassinosteroid; Structure–activity relationships; Rice lamina inclination test; Biological activity; Synthesis

1. Introduction

Brassinosteroids (BRs) have recently become an important subject in plant physiology due to their diverse phytohormonal activities and ubiquitous distribution in the plant kingdom (Fujioka & Sakurai, 1997). Regarding structure–activity relationships of BRs (Marquardt & Adam, 1991), we recently provided the first experimental proof that the A/B *trans*-fused ring junction of BRs is an essential structural factor for BR activity. The C-5 epimerization of brassinolide (**1**, BL), a biologically active natural BR, to 5-*epi*-BL (**3**) with an A/B *cis*-ring junction, resulted in nearly complete loss of the biological activity in the rice lamina inclination test (Seto et al., 1998). However, Brosa, Capdevila and Zamara (1996) and Brosa (1997) reported that a 5-*epi*-BL analog, 28-homo-2,3,5-tri-*epi*-BL (**5**), showed high activity comparable to that of **1** in the same test, from which they reached a conclusion conflicting with ours, namely that the activity of BRs mainly depends on the spatial position of the oxygen atoms at C-2, C-3, C-22, C-23 and C-6, rather than the A/B ring junction. However, we are sceptical about their activity evaluation in the absence of any dose dependence test; the tested dosage, 1 μg/plant, seems to be too high for such a comparative study. Thus, in order to attain more precise information on the biological activity of 2,3,5-tri-*epi*-BL analogs, we prepared 2,3,5-tri-*epi*-BL (**4**) and compared its activity to that of **1** in dose dependent tests. For direct comparison with **1**, **4** should be more suitable than **5**, because **1** and **4** have the same carbon framework.

* Corresponding author. Tel.: +81-48-462-1111 extn 5536; fax: +81-48-462-4674.
E-mail address: hseto@postman.riken.go.jp (H. Seto)

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00335-0



1 $R^1 = R^2 = \alpha$-OH
2 $R^1 = R^2 = \beta$-OH
3 $R^1 = R^2 = \alpha$-OH, R^3=H
4 $R^1 = R^2 = \beta$-OH, R^3=H
5 $R^1 = R^2 = \beta$-OH, R^3=Me

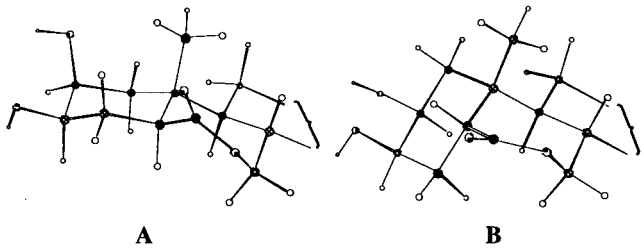


Fig. 1. Conformations of the A/B ring of 2,3-di-*epi*-brassinolide (**2**) and 2,3,5-tri-*epi*-brassinolide (**4**), **A** and **B**, respectively, in solution as deduced from ^{1}H NMR spectral data.

2. Results and discussion

The test compound **4** was prepared from 2,3-di-*epi*-BL (**2**) (Watanabe, Fujioka, Yokota & Takatsuto, 1998) by the direct C-5 epimerization previously reported (Seto et al., 1998). When **2** was refluxed in 1 M sodium methoxide solution in methanol (MeOH) for 6 h, C-5 epimerization occurred efficiently through the intermediary methyl esters, and the subsequent re-lactonization with Dowex-50W-2X resin (H^+ form) at pH 3–4 in MeOH–H_2O (4:1) at room temperature for 6 h gave 2,3,5-tri-*epi*-BL (**4**) (46%) along with the starting material **2** (52%).

The structure of **4** was verified by MS and NMR spectroscopy. NMR experiments on **4** and **2** were carried out at 600 MHz by PFG-DQFCOSY, PFG-HMQC and PFG-HMBC, by which all resonances were completely assigned (see Section 3). The conformation of the A/B-ring moiety of **4** deduced by the NMR study is worthy of comment. In the ^{1}H NMR spectrum, the resonances of 2-H at δ 3.68 and 5-H at δ 3.15 of **4** had large vicinal coupling constants, ($J_{2,1\beta} = 12.3$ and $J_{5,4\alpha} = 12.4$, respectively), indicating that both protons were located at axial positions. The 7α-H was δ 4.00 (*br dd*) with $J_{gem} = 12.2$ and $J_{7\alpha,8} = 10.2$, and the 7β-H signal was at δ 4.07 (*br d*) with $J_{gem} = 12.2$. These were consistent with conformer **B** (Fig. 1) which was one of the low energy conformers obtained from molecular dynamics calculations with energy minimization. A conformer **A** of **2** was also deduced by the NMR study, which was nearly the same as that of BL (**1**) previously reported (Seto et al., 1998). It is obvious that the A-ring of **4** has the chair conformation unlike that of **2**, illustrating the drastic change of A/B-ring conformation of **4** from that of **1**.

Biological activity of 2,3,5-tri-*epi*-BL (**4**) in the rice lamina inclination test (*Oryza sativa* cv. Tan-ginbozu) was examined and the activity was compared with BL (**1**), as shown in Fig. 2. Two assay methods were employed (Fujioka, Noguchi, Takatsuto & Yoshida, 1998): single application of BR, **1** or **4**, and co-application with indole-3-acetic acid (IAA) whose synergistic effect is known to significantly enhance the assay sensitivity (Takeno & Pharis, 1982). By a single application **4** exhibited no activity (Fig. 2A), while with co-application of IAA, considerable activity was observed at a dosage of 100 ng/plant, but which was ca 1/1000th that of **1** (Fig. 2B). These results were quite different from that of 28-homo-2,3,5-tri-*epi*-BL (**5**) reported by Brosa et al. (1996), who described **5** as having high activity comparable to that of **1** by a single application. They mentioned there that the cultivar Bahia employed had sensitivity as high as the cultivar Tanginbozu: the activity of **1** reached nearly the maximum at 10 ng/plant application to both cultivars. We therefore believe that a single dosage of 1 μg/plant as employed by them would be too high for such a comparative study describing the structure-activity relationships of BRs.

The conformational analysis described above suggested that distances between oxygen atoms at C-2, C-3, C-22, C-23 and C-6 of 2,3,5-tri-*epi*-BL (**4**) were more similar to those of BL (**1**), than to 5-*epi*-BL (**3**) (data not shown). However, **4** showed no significant effect in the rice lamina inclination test compared with **1** in our assay system, which was the same as the results of **3** previously reported (Seto et al., 1998). We therefore concluded that both the A/B *trans*-ring junction, i.e., an A/B ring conformation close to that of **1**, and the spatial position of the oxygen atoms should both be important for high biological activity, although Brosa et al. (1996) reported that the latter was much more contributive than the former.

3. Experimental

3.1. General

General experimental and molecular modelling details (Seto et al., 1998) and bioassay details (Fujioka et al., 1998) have been previously reported.

3.2. Preparation of 2,3,5-tri-epi-brassinolide (4)

A mixture of 2,3-di-*epi*-brassinolide (**2**) (5.2 mg) and

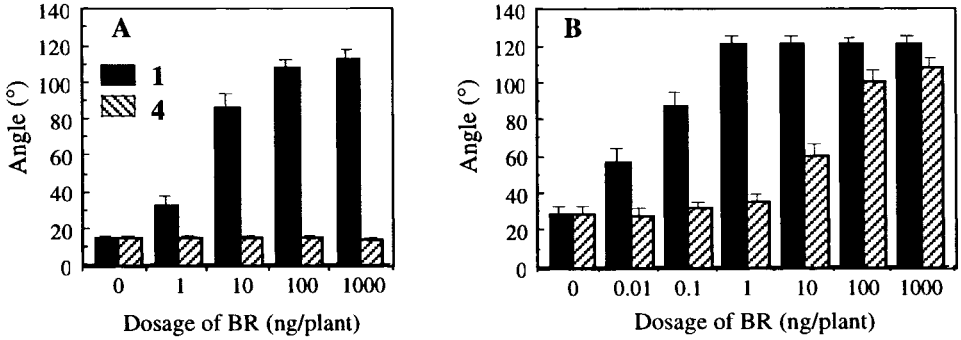


Fig. 2. Rice lamina inclination test (*Oryza sativa* cv. Tan-ginbozu) of brassinolide (**1**) and 2,3,5-tri-*epi*-brassinolide (**4**): (A) application of BR alone; (B) co-application with IAA (5 μg/plant). Each data point represents the mean of 30 replicates ± SE.

1 M NaOMe solution of MeOH (0.5 ml) was stirred at refluxing temperature for 6 h, after which MeOH (1 ml) and H_2O (0.5 ml) at 0°C were added. The mixture was acidified with Dowex-50W-2X resin (H^+ form) to pH 3–4 and stirred at RT for 6 h, then filtered through a glass filter. The filtrate was evaporated and the residue was subjected to column chromatography. Elution with $CHCl_3$–EtOH (20:1) gave the starting material **2** (2.7 mg, 52%), and elution with $CHCl_3$–EtOH (10:1) gave 2,3,5-tri-*epi*-brassinolide (**4**) (2.4 mg, 46%).

3.3. 2,3-di-epi-brassinolide (*2*)

(Watanabe et al., 1998) δ_H (600 MHz; $CDCl_3$–CD_3OD, 10:1) 0.72 (3H, *s*, 18-H_3), 0.84 (3H, *d, J* 6.8, 28-H_3), 0.89 (3H, *d, J* 6.8, 21-H_3), 0.94 and 0.96 (each 3H, each *d, J* 6.8 Hz, 26-H_3 and 27-H_3), 1.07 (3H, *s*, 19-H_3), 1.07 (1H, *m*, 9-H), 1.17 (1H, *m*, 14-H), 1.18 (1H, *m*, 24-H), 1.22 (1H, *m*, 12α-H), 1.28 and 1.69 (each 1H, each *m*, 15-H_2), 1.28 and 1.98 (each 1H, each *m*, 16-H_2), 1.32 (1H, *dd, J* 15.1 and 3.4, Hz 1α-H), 1.45 and 1.73 (each 1H, each *m*, 11-H_2), 1.47 (1H, *m*, 20-H), 1.57 (1H, *m*, 17-H), 1.62 (1H, *m*, 25-H), 1.62 (1H, *ddd, J* 12.2, 3.9 and 2.9 Hz, 4α-H), 1.75 (1H, *m*, 8-H), 1.98 (1H, *m*, 12β-H), 2.27 (1H, *dd, J* 15.1 and 3.4 Hz, 1β-H), 2.34 (1H, *ddd, J* 12.2, 12.2 and 11.7 Hz, 4β-H), 2.88 (1H, *dd, J* 12.2 and 3.9 Hz, 5-H), 3.51 (1H, *dd, J* 8.8 and 1.5 Hz, 22-H), 3.59 (1H, *ddd, J* 11.7, 2.9 Hz and 2.9, 3-H), 3.69 (1H, *dd, J* 8.8 and 1.5 Hz, 23-H), 3.94 (1H, *ddd, J* 3.4, 3.4 and 2.9 Hz, 2-H), 4.03 (1H, *dd, J* 12.7 and 9.3 Hz, 7α-H), 4.11 (1H, *dd, J* 12.7 and 1.5 Hz, 7β-H); δ_C (150 MHz; $CDCl_3$–CD_3OD, 10:1) 9.94 (C-28), 11.57 (C-18), 11.63 (C-21), 17.35 (C-19), 20.54 and 20.67 (C-26 and C-27), 22.50 (C-11), 24.55 (C-15), 27.37 (C-16), 27.43 (C-4), 30.51 (C-25), 35.77 (C-10), 36.70 (C-20), 38.79 (C-8), 39.58 (C-12), 40.03 (C-24), 42.29 (C-13), 44.05 (C-1), 46.96 (C-5), 51.12 (C-14), 52.13 (C-17), 59.06 (C-9), 68.57 (C-2), 69.82 (C-3), 70.65 (C-7), 73.07 (C-23), 74.21 (C-22), 176.43 (C-6).

3.4. 2,3,5-tri-epi-brassinolide (*4*)

Mp 227–228°C (colorless prisms from Et_2O); δ_H (600 MHz; $CDCl_3$–CD_3OD, 10:1) 0.72 (3H, *br s*, 18-H_3), 0.84 (3H, *d, J* 6.8 Hz, 28-H_3), 0.90 (3H, *br d, J* 6.4 Hz, 21-H_3), 0.95 and 0.97 (each 3H, each *d, J* 6.8 Hz, 26-H_3 and 27-H_3), 1.08 (3H, *br s*, 19-H_3), 1.18 (1H, *m*, 14-H), 1.19 (1H, *m*, 24-H), 1.23 and 1.66 (each 1H, each *m*, 15-H_2), 1.31 and 1.98 (each 1H, each *m*, 16-H_2), 1.35 and 2.02 (each 1H, each *m*, 12-H_2), 1.36 (1H, *m, J* 14.4 Hz from DQFCOSY, 1β-H), 1.49 (1H, *m*, 20-H), 1.52 and 1.66 (each 1H, each *m*, 11-H_2), 1.58 (1H, *m*, 17-H), 1.64 (1H, *m*, 25-H), 1.66 (1H, *m*, 9-H), 1.88 (1H, *m, J* 14.4 Hz from DQFCOSY, 1α-H), 1.92 (1H, *m*, 8-H), 2.04 (1H, *m*, 1β-H), 2.30 (1H, *br dd, J* 12.4 Hz and 12.4, 4α-H), 3.15 (1H, *br d, J* 12.4 Hz, 5-H), 3.51 (1H, *dd, J* 8.3 and 1.0 Hz, 22-H), 3.68 (1H, *br m, J* 12.3 Hz from DQFCOSY, 2-H), 3.69 (1H, *dd, J* 8.3 and 1.0 Hz, 23-H), 4.00 (1H, *br dd, J* 12.2 and 10.2 Hz, 7α-H), 4.06 (1H, *br m*, 3-H), 4.07 (1H, *br d, J* 12.2, 7β-H); δ_C (150 MHz; $CDCl_3$–CD_3OD, 10:1) 9.99 (C-28), 11.48 (C-18), 11.73 (C-21), 20.59 and 20.72 (C-26 and C-27), 22.30 (C-11), 22.57 (C-19), 24.65 (C-15), 27.42 (C-16), 29.37 (C-4), 30.56 (C-25), 36.75 (C-20), 37.62 (C-10), 38.53 (C-8), 39.33 (C-12), 39.53 (C-1), 40.06 (C-24), 42.28 (C-13), 43.30 (C-9), 50.89 (C-14), 51.12 (C-5), 52.09 (C-17), 66.38 (C-2), 67.04 (C-3), 71.46 (C-7), 73.10 (C-23), 74.25 (C-22), 177.25 (C-6); HR-FAB-MS *m/z* ($[M+1]^+$: positive ion, glycerol): Found, 481.3524. Calc. for $C_{28}H_{49}O_6$, 481.3529.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (C) to H. Seto (No. 09672183)

from the Ministry of Education, Science, Sports and Culture of Japan.

References

Brosa, G. (1997). In E. J. Parish, & W. D. Nes, *Biochemistry and function of sterols* (p. 201). New York: CRC Press Inc.

Brosa, G., Capdevila, J. M., & Zamara, I. (1996). *Tetrahedron*, *52*, 2435.

Fujioka, S., & Sakurai, A. (1997). *Nat. Prod. Rep.*, *14*, 1.

Fujioka, S., Noguchi, T., Takatsuto, S., & Yoshida, Y. (1998). *Phytochemistry*, *49*, 1841.

Marquardt, V., & Adam, G. (1991). Ebing, W. (Ed.), *Chemistry of plant protection* (7, p. 103). Springer–Verlag New York.

Seto, H., Fujioka, S., Koshino, H., Suenaga, T., Yoshida, S., Watanabe, T., & Takasuto, S. (1998). *J. Chem. Soc., Perkin Trans. 1*, p. 3355.

Takeno, K., & Pharis, R. P. (1982). *Plant and Cell Physiol.*, *23*, 1275.

Watanabe, T., Fujioka, S., Yokota, T., & Takatsuto, S. (1998). *J. Chem. Res. (S)*. p. 744.