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Synthesis of Brassinosteroids of Varying Acyl Side Chains and Evaluation of Their Brassinolide-like Activity

Shinya UESUSUKI[a], Bunta WATANABE[a], Shuji YAMAMOTO[a], Junko OTSUKI[a], Yoshiaki NAKAGAWA[a] & Hisashi MIYAGAWA[a]
[a] Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University
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Synthesis of Brassinosteroids of Varying Acyl Side Chains and Evaluation of Their Brassinolide-like Activity

Shinya UESUSUKI, Bunta WATANABE, Shuji YAMAMOTO, Junko OTSUKI, Yoshiaki NAKAGAWA,† and Hisashi MIYAGAWA

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

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Brassinosteroids containing various side chain moieties were synthesized and their activity was determined as the reciprocal logarithm of the ED_{50} (50% effective dose per plant in moles) in the rice lamina inclination assay using synergist indole-3-acetic acid (IAA). The introduction of a hydroxyl group in the α-position to the carbonyl group of the ester structure significantly enhanced the activity. 2α,3α-Dihydroxy-17β-[(2*R*,3*S*)-2-hydroxy-3-methylpentanoyl]oxy-B-homo-7-oxa-5α-androstan-6-one showed the highest activity, for which the pED_{50} was determined to be 10.5 under synergistic conditions with IAA. Under identical conditions, the pED_{50} values of brassinolide and castasterone were determined to be 13.6 and 12.3 respectively. With respect to the α-carbon of the acyl moiety, the *R*-form was 10 times more potent than the corresponding *S*-form. Substituting the terminal structure (Et) of the side chain to that of the most potent compound, brassinolide (*i*-Pr), did not increase the activity.

Key words: brassinolide; castasterone; brassinosteroids; plant hormone; rice lamina inclination assay

Brassinolide (**1**, BL in Fig. 1) is a known steroidal plant hormone and its structure, which characteristically has a 7-member B-ring moiety containing a 6-keto-7-oxa, was originally determined by Grove *et al.*[1] Later, castasterone (**2**, CS in Fig. 1) containing a 6-keto 6-member B-ring instead of a 7-member ring was also identified in chestnut insect gall.[2] In the last two decades, more than 50 BL analogs have been identified in plants and these brassinolide-like compounds are collectively called brassinosteroids (BRs).[3–5] Since they have excellent activity in evoking cell elongation and cell division, which are essential for plant growth and various forms of stress-resistance, they are expected to be used in the future as versatile plant growth regulators.[6–9]

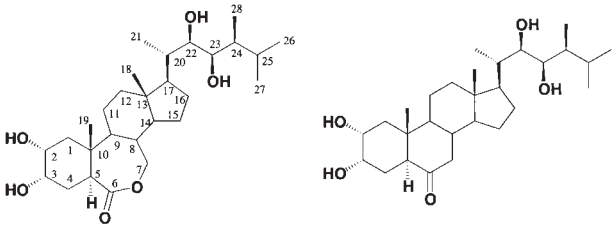


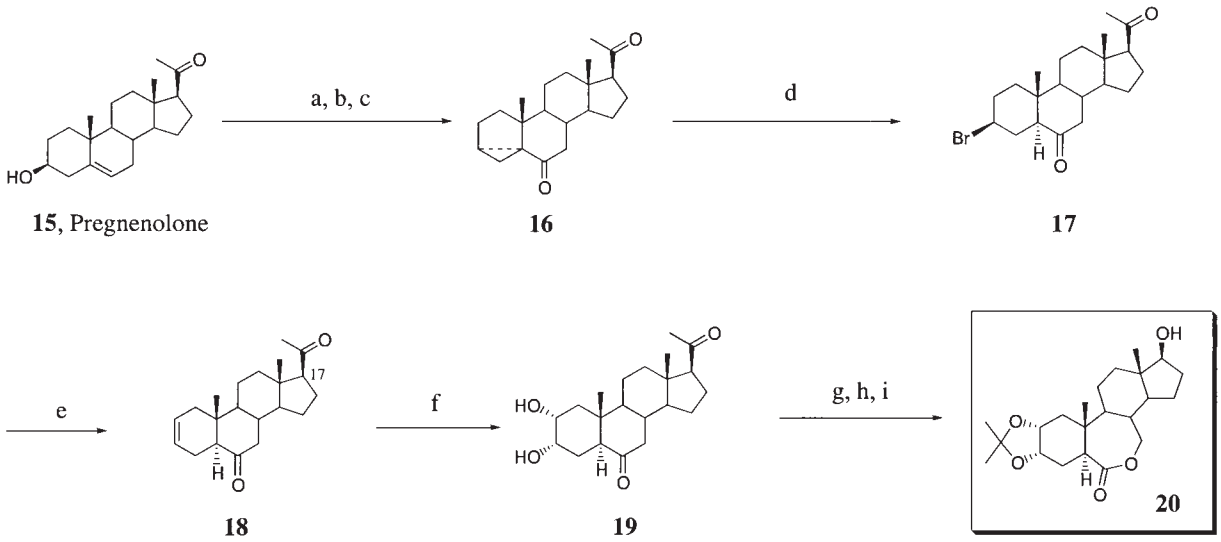
Fig. 1. Structures of Brassinolide (**1**) and Castasterone (**2**).

To date a number of BRs including BL have been synthesized, and the structure-activity relationship (SAR) of BRs has been extensively analyzed.[10–21] The cumulative results of these studies indicate that the structure requirements for high activity are: (1) a 2α,3α-diol, (2) a *trans* A/B ring system, (3) a 6-keto or 7-oxa-6-keto moiety in the B-ring, (4) hydroxyl groups at C-22 and C-23, and (5) a methyl or ethyl substituent at C-24. The stereochemistry of the steroid ring system of BL (2α,3α-dihydroxy, *trans* configuration of A/B-ring fusion, configurations of 18 and 19-CH_3, C-17 configuration) is easily constructed from steroid compounds such as pregnenolone and stigmasterol using conventional methods.[4,22] But, the construction of the alkyl chain moiety with defined stereochemistry is not easy, because four asymmetric carbons exist consecutively in the side chain. Kohout and his coworkers combined 2α,3α,17β-trihydroxy-7-oxa-B-homo-5α-androstan-6-one containing the steroid moiety of BL with amino acids and found weak activity for some compounds using a bean hypocotyls elongation bioassay.[23] These compounds have an ester bond in the side chain moiety. Synthesis of the ester analogs is attractive, because the ester bond is easily constructed from alcohols and carboxylic acids to derive various analogs.

In this study, we synthesized a number of ester

† To whom correspondence should be addressed. Fax: +81-75-753-6123; E-mail: naka@kais.kyoto-u.ac.jp

Abbreviations: BR, brassinosteroids; BL, brassinolide; SAR, structure-activity relationship; RLI, rice lamina inclination; DMAP, dimethylaminopyridine; NMO, *N*-methylmorpholine *N*-oxide; PPTS, pyridinium *p*-toluenesulfonate; MsCl, methanesulfonyl chloride; *p*-TsOH, *p*-toluenesulfonic acid; TBDPS, *tert*-butyldiphenylsilyl; TBAF, tetra-*n*-butylammonium fluoride; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; BnBr, benzyl bromide; DEAD, diethyl azodicarboxylate; PDC, pyridinium dichromate; DCC, dicyclohexylcarbodiimide; IAA, indole-3-acetic acid



a) MsCl, Et_3N / toluene, b) $KHCO_3$ / acetone, H_2O, c) CrO_3, H_2SO_4 / acetone, d) HBr / AcOH,
e) LiBr, Li_2CO_3 / DMF, f) OsO_4, NMO / acetone, H_2O, g) $(CF_3CO)_2O$, H_2O_2 / $CHCl_3$,
h) K_2CO_3 / MeOH, H_2O, i) $(MeO)_2CMe_2$, PPTS / CH_2Cl_2

Scheme 1.

analogs and measured their BL-like activity in a rice lamina inclination (RLI) bioassay[24] using intact rice seedlings under the synergistic condition with indole-3-acetic acid (IAA). BRs were chemically prepared from the 17-hydroxy compound (**20**; key intermediate in Scheme 1), which compound was derived from pregnenolone (**15** in Scheme 1) and various carboxylic acids.

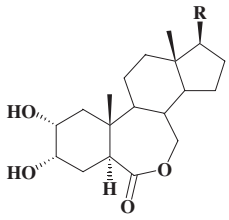
Materials and Methods

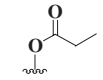
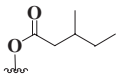
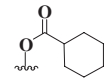
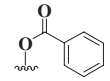
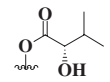
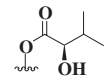
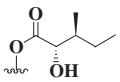
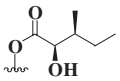
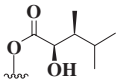
General. Reactions requiring anhydrous conditions were performed using oven-dried glassware and conducted under positive pressure using argon. Anhydrous solvents were either commercially available or prepared conventionally in the laboratory. Chemicals were purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wisconsin, U.S.A.), Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Nacalai Tesque Inc. (Kyoto, Japan), unless otherwise noted. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-300 or ARX-500 NMR spectrometer in deuteriochloroform ($CDCl_3$) with tetramethylsilane as the internal standard. Chemical shifts for carboxylic acids were not evident, because the signals are broad and small. IR spectra were recorded on a Shimadzu IR-420 spectrometer (Shimadzu, Kyoto, Japan). Optical rotations were measured on a JASCO DIP-1000 or P-1000 polarimeter. High-resolution mass spectra (HRMS) were measured on JMS600H (JEOL, Tokyo, Japan). Elemental analyses were performed at the Microanalytical Center at Kyoto University. Melting points were determined on a Yanako melting point apparatus (Yanagimoto Seisakusho Co., Ltd., Kyoto, Japan) and are uncorrected. The structures of the final compounds and their melting points and spectral data are shown in Table 1. HRMS data are shown for compounds **12–14** instead of their elemental analysis data. Spectral and elemental analysis data for intermediates are shown in the text.

*Construction of the key intermediate (**20**)* (Scheme 1).

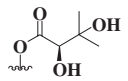
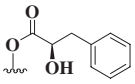
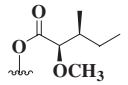
*3β-Bromo-5α-pregnan-6,20-dione (**17**).* Synthetic procedures are basically the same as those reported previously.[25] Acetic acid (150 ml) and 48% HBr (7.5 ml) were successively added to compound **16** (13.49 g, 42.9 mmol) and the mixture was dissolved following ultrasonification. After adding water (750 ml) to the mixture, the aqueous layer was extracted with toluene (150 ml × 4). Saturated aqueous $NaHCO_3$ (900 ml) was added to the combined organic layers and the mixture was stirred for 5 min. The aqueous layer was then extracted with toluene (300 ml × 2). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* to afford a colorless solid compound **17** (16.71 g, 99%), which was used without purification. NMR δ_H ($CDCl_3$): 0.63 (3H, s), 0.80 (3H, s), 1.23 (1H, qd, $J = 12.1$, 6.2 Hz), 1.26–1.39 (4H, m), 1.48 (1H, td, $J = 12.5$, 3.9 Hz), 1.61–1.74 (3H, m), 1.78–1.83 (2H, m), 1.90–2.04 (3H, m), 2.09 (1H, dt, $J = 12.2$, 3.2 Hz), 2.12 (3H, s), 2.14–2.19 (2H, m), 2.21–2.27 (2H, m), 2.34 (1H, dd, $J = 13.3$, 4.5 Hz), 2.54 (1H, t, $J = 9.1$ Hz), 3.93 (1H, tt, $J = 12.3$, 4.5 Hz). NMR δ_C ($CDCl_3$): 13.11, 13.37,

Table 1. Structures of Newly Synthesized Compounds and Their Physical Properties



No.	R	mp/Optical rotation	Chemical shifts (ppm) for ^{1}H-NMR[a)]	Elemental analysis or HRMS
3		192 $[\alpha]_D^{29} + 25.3$ (c 0.54, $CHCl_3$)	0.82 (3H, s), 0.93 (3H, s), 1.14 (3H, t, $J = 7.6$ Hz), 2.33 (2H, q, $J = 7.6$ Hz), 3.13 (1H, dd, $J = 12.1$, 4.6 Hz), 3.71 (1H, ddd, $J = 12.0$, 4.7, 2.8 Hz), 4.02–4.13 (3H, m), 4.62 (1H, dd, $J = 9.1$, 8.0 Hz).	Found: C, 66.69%; H, 8.99% Calcd: C, 66.98%; H, 8.69%
4		175 $[\alpha]_D^{29} + 36.8$ (c 0.56, $CHCl_3$)	0.82 (3H, s), 0.89 (3H, t, $J = 7.4$ Hz), 0.93 (3H, s), 0.93 (3H, d, $J = 6.4$ Hz), 3.12 (1H, dd, $J = 12.0$, 4.5 Hz), 3.69–3.73 (1H, m), 4.02–4.09 (3H, m), 4.62 (1H, t, $J = 8.4$).	Found: C, 68.53%; H, 8.96% Calcd: C, 68.78%; H, 9.23%
5		200 $[\alpha]_D^{29} + 39.0$ (c 0.56, $CHCl_3$)	0.82 (3H, s), 0.93 (3H, s), 3.12 (1H, dd, $J = 12.1$, 4.6 Hz), 4.02–4.08 (3H, m), 4.61 (1H, t, $J = 7.9$ Hz).	Found: C, 69.35%; H, 9.17% Calcd: C, 69.61%; H, 8.99%
6		256 $[\alpha]_D^{29} + 81.1$ (c 0.38, $CHCl_3$)	0.94 (3H, s), 0.97 (3H, s), 3.14 (1H, dd, $J = 12.1$, 4.6 Hz), 4.03–4.12 (3H, m), 4.87 (1H, t, $J = 8.9$ Hz), 7.42–7.47 (2H, m), 7.54–7.57 (1H, m), 8.01–8.04 (2H, m).	Found: C, 70.46%; H, 7.66% Calcd: C, 70.56%; H, 7.74%
7		176 $[\alpha]_D^{27} + 34.8$ (c 0.94, $CHCl_3$)	0.84 (3H, s), 0.88 (3H, d, $J = 6.9$ Hz), 0.93 (3H, s), 1.04 (3H, d, $J = 6.9$ Hz), 2.73 (1H, d, $J = 6.0$ Hz), 3.12 (1H, dd, $J = 12.0$, 4.6 Hz), 3.69–3.74 (1H, m), 4.02–4.09 (4H, m), 4.74 (1H, dd, $J = 9.0$, 7.9 Hz).	Found: C, 65.45%; H, 8.58% Calcd: C, 65.73%; H, 8.73%
8		198 $[\alpha]_D^{31} + 47.8$ (c 1.04, $CHCl_3$)	0.84 (3H, s), 0.87 (3H, d, $J = 6.9$ Hz), 0.93 (3H, s), 1.02 (3H, d, $J = 6.9$ Hz), 2.74 (1H, d, $J = 6.1$ Hz), 3.12 (1H, dd, $J = 12.0$, 4.6 Hz), 3.68–3.76 (1H, m), 4.03–4.10 (4H, m), 4.67 (1H, dd, $J = 9.0$, 7.7 Hz).	Found: C, 65.73%; H, 8.43% Calcd: C, 65.73%; H, 8.73%
9		181 $[\alpha]_D^{31} + 39.0$ (c 0.95, $CHCl_3$)	0.84 (3H, s), 0.91 (3H, t, $J = 7.5$ Hz), 0.93 (3H, s), 1.00 (3H, d, $J = 6.9$ Hz), 3.13 (1H, dd, $J = 12.1$, 4.6 Hz), 3.69–3.75 (1H, m), 4.02–4.09 (4H, m), 4.72 (1H, dd, $J = 8.9$, 7.8 Hz).	Found: C, 66.17%; H, 8.71% Calcd: C, 66.35%; H, 8.91%
10		202 $[\alpha]_D^{31} + 45.1$ (c 1.05, $CHCl_3$)	0.82 (3H, d, $J = 6.8$ Hz), 0.84 (3H, s), 0.94 (3H, s), 0.96 (3H, t, $J = 7.4$ Hz), 3.13 (1H, dd, $J = 12.1$, 4.6 Hz), 3.70–3.76 (1H, m), 4.02–4.09 (1H, m), 4.18 (1H, d, 2.7 Hz), 4.72 (1H, dd, $J = 8.9$, 7.8 Hz).	Found: C, 66.05%; H, 8.97% Calcd: C, 66.35%; H, 8.91%
11		181 $[\alpha]_D^{26} + 43.3$ (c 1.08, $CHCl_3$)	0.80 (3H, d, $J = 6.8$ Hz), 0.83 (3H, s), 0.93 (3H, s), 0.95 (3H, d, $J = 7.1$ Hz), 1.00 (3H, d, $J = 6.6$ Hz), 2.72 (1H, d, $J = 5.7$ Hz), 3.13 (1H, dd, $J = 12.1$, 4.5 Hz), 3.70–3.73 (1H, m), 4.02–4.14 (3H, m), 4.32 (1H, dd, $J = 5.7$, 2.9 Hz), 4.67 (1H, dd, $J = 8.7$, 7.8 Hz).	Found: C, 66.74%; H, 9.03% Calcd: C, 66.93%; H, 9.07%

Continued on next page

12		127 $[\alpha]_D^{27}$ + 38.0 (c 0.40, EtOH)	0.90 (3H, s), 0.91 (3H, s), 1.22 (3H, s), 1.25 (3H, s), 3.21 (1H, dd, J = 12.2, 4.4 Hz), 3.60 (1H, ddd, J = 12.1, 4.5, 2.7 Hz), 3.93 (1H, m), 3.94 (1H, s), 4.05 (1H, dd, J = 11.6 Hz), 4.21 (1H, dd, J = 12.5, 9.4 Hz), 4.70 (1H, dd, J = 9.0, 7.9 Hz).[b]	Calculated: 455.2645 ($C_{24}H_{39}O_6$) Found: 455.2635
13		204 $[\alpha]_D^{24}$ + 49.8 (c 0.50, $CHCl_3$)	0.76 (3H, s), 0.92 (3H, s), 2.80 (1H, d, J = 6.3 Hz), 2.98 (1H, dd, J = 14.0, 6.3 Hz), 3.10 (1H, dd, J = 14.0, 4.9 Hz), 3.11 (1H, dd, J = 12.0, 4.4 Hz), 3.70–3.73 (1H, m), 4.01–4.08 (3H, m), 4.46 (1H, dt, J = 5.1, 6.2 Hz), 4.62 (1H, dd, J = 8.8, 7.6 Hz), 7.20–7.29 (5H, m).	Calculated: 487.2695 ($C_{28}H_{39}O_7$) Found: 487.2708
14		77 $[\alpha]_D^{25}$ + 61.8 (c 1.09, $CHCl_3$)	0.84 (3H, s), 0.89–0.94 (9H, m), 3.13 (1H, dd, J = 12.1, 4.5 Hz), 3.37 (3H, s), 3.64 (1H, d, J = 4.4 Hz), 3.69–3.74 (1H, m), 4.02–4.14 (3H, m), 4.69 (1H, dd, J = 8.7, 8.0 Hz).	Calculated: 467.3008 ($C_{26}H_{43}O_7$) Found: 467.3010

a) Measured in $CDCl_3$ except for compound **12**.
b) Measured in CD_3OD.

21.28, 22.79, 24.12, 31.41, 32.31, 33.35, 37.59, 38.44, 39.16, 40.57, 44.33, 46.35, 50.30, 53.72, 56.74, 58.97, 63.35, 208.91, 209.00. IR ν_{max} (nujor) cm^{-1}: 1690, 1350, 1270, 1230, 1180, 1150, 710.

*5α-Pregn-2-en-6,20-dione (**18**).* Compound **17** (17.42 g, 44.1 mmol), $LiBr \cdot H_2O$ (23.22 g, 221 mmol), and Li_2CO_3 (24.61 g, 333 mmol) were suspended in anhydrous DMF and stirred for 2 h at 110 °C under an argon atmosphere. After cooling the mixture to room temperature, EtOAc (1 l) was added. The precipitate was removed by filtration and the filtrate was washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was evaporated *in vacuo* to yield a pale yellow solid compound **18** (14.17 g), which was used without purification. NMR δ_H ($CDCl_3$): 0.64 (3H, s), 0.72 (3H, s), 1.23–1.26 (1H, m), 1.33–1.38 (2H, m), 1.42–1.53 (2H, m), 1.63–1.80 (5H, m), 1.99–2.04 (4H, m), 2.09–2.13 (1H, m), 2.13 (3H, s), 2.15–2.20 (1H, m), 2.22–2.29 (1H, m), 2.36 (1H, dd, J = 9.4, 4.8 Hz), 2.38 (1H, dd, J = 13.2, 4.1 Hz), 2.55 (1H, t, J = 8.9 Hz), 5.56–5.58 (1H, m), 5.69 (1H, m). NMR δ_C ($CDCl_3$): 13.32, 13.53, 21.12, 21.74, 22.80, 24.15, 31.44, 37.52, 38.58, 39.39, 39.94, 44.26, 46.80, 53.32, 53.87, 56.85, 63.46, 124.38, 125.03, 209.02, 211.27. IR ν_{max} (nujor) cm^{-1}: 1690, 1650, 1350, 1230, 1190, 1160, 680.

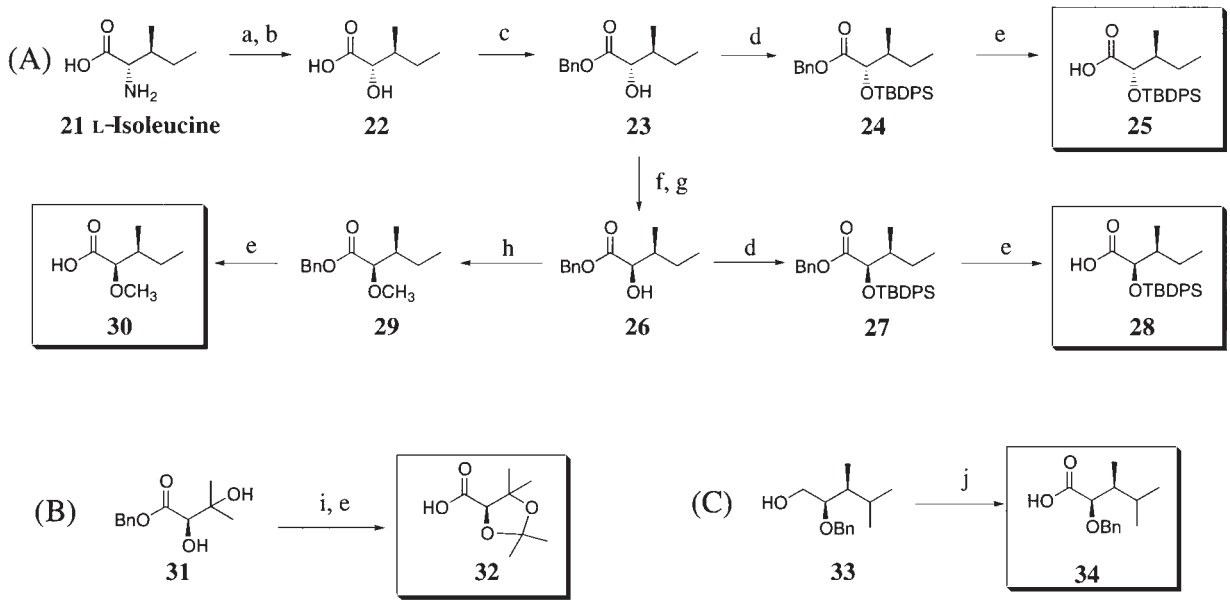
*17β-Hydroxy-2α,3α-isopropylidenedioxy-B-homo-7-oxa-5α-androstan-6-one (**20**).* Compound **19**, which was derived from compound **18** by osmium oxidation, was converted to the key intermediate **20** using conventional methods,[25] and is summarized in Scheme 1. Colorless crystal: m.p. 233 °C. $[\alpha]_D^{31}$ + 23.3° (*c* 0.98, $CHCl_3$). NMR δ_H ($CDCl_3$): 0.79 (3H, s), 0.90 (3H, s), 1.08–1.19 (3H, m), 1.32 (3H, s), 1.35–1.44 (3H, m), 1.46–1.54 (1H, m), 1.52 (3H, s), 1.59–1.65 (1H, m), 1.71–1.76 (1H, m), 1.78–1.86 (4H, m), 1.91 (1H, dq, J = 13.6, 3.3 Hz), 2.05–2.21 (1H, m), 2.34 (1H, J = dd, 16.0, 3.6 Hz), 3.29 (1H, dd, J = 10.2, 4.4 Hz), 3.67 (1H, td, J = 8.5, 5.6 Hz), 4.03–4.09 (2H, m), 4.35–4.40 (2H, m). NMR δ_C ($CDCl_3$): 11.20, 19.71, 22.57, 23.62, 23.82, 26.56, 27.70, 30.41, 33.53, 36.07, 36.51, 39.36, 40.27, 43.47, 46.68, 54.92, 70.60, 72.47, 73.08, 81.34, 107.60, 176.44. IR ν_{max} (nujor) cm^{-1}: 3500, 1710. Found: C, 69.53; H, 9.18. Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05.

Synthesis of hydroxycarboxylic acids (Scheme 2). α-Hydroxycarboxylic acids and their *O*-protected analogs were synthesized according to the conventional methods summarized in Scheme 2. Chiral isoleucine and valine were used as starting materials to prepare hydroxycarboxylic acids for compounds **7**–**10**, **14**.

*(2S,3S)-2-(tert-Butyldiphenylsilyl)oxy-3-methylpentanoic acid (**25**).* Colorless oil: $[\alpha]_D^{30}$ − 1.2° (*c* 1.20, $CHCl_3$). NMR δ_H ($CDCl_3$): 0.68 (3H, t, J = 7.4 Hz), 0.90 (3H, d, J = 6.9 Hz), 1.12 (9H, s), 1.13–1.30 (1H, m), 1.31–1.45 (1H, m), 1.53–1.66 (1H, m), 4.21 (1H, d, J = 3.8 Hz), 7.34–7.48 (6H, m), 7.60–7.69 (4H, m). NMR δ_C ($CDCl_3$): 11.61, 14.44, 19.36, 24.84, 26.96, 40.20, 76.26, 127.82, 127.85, 130.20, 132.16, 132.58, 135.79, 135.82, 174.02. Found: C, 71.23; H, 8.26. Calcd. for $C_{22}H_{30}O_3Si$: C, 71.31; H, 8.16.

*(2R,3S)-2-(tert-Butyldiphenylsilyl)oxy-3-methylpentanoic acid (**28**).* Colorless oil: $[\alpha]_D^{28}$ + 7.0° (*c* 1.22, $CHCl_3$). NMR δ_H ($CDCl_3$): 0.75 (3H, t, J = 7.4 Hz), 0.90 (3H, d, J = 6.9 Hz), 1.01–1.16 (1H, m), 1.16 (9H, s), 1.39–1.53 (1H, m), 1.56–1.69 (1H, m), 4.21 (1H, d, J = 3.2 Hz), 7.34–7.43 (6H, m), 7.61–7.66 (4H, m). NMR δ_C ($CDCl_3$): 11.85, 14.22, 19.50, 24.88, 27.01, 40.01, 76.38, 127.79, 130.14, 132.71, 135.34, 135.87, 135.89, 174.69. Found: C, 71.28; H, 8.21. Calcd for $C_{22}H_{30}O_3Si$: C, 71.31; H, 8.16.

*(2R,3S)-2-Methoxy-3-methylpentanoic acid (**30**).* Colorless oil: $[\alpha]_D^{25}$ + 43.5° (*c* 1.15, $CHCl_3$). NMR δ_H ($CDCl_3$): 0.94 (3H, d, J = 6.9 Hz), 0.94 (3H, t, J = 7.4 Hz), 1.28–1.37 (1H, m), 1.49–1.56 (1H, m),



a) $NaNO_2$ / AcOH, H_2O, b) K_2CO_3 / MeOH, H_2O, c) BnBr, DBU / CH_3CN, d) TBDPS-Cl, imidazole / DMF
e) H_2, Pd-C / EtOH, f) DEAD, PPh_3, HCOOH / THF, g) NH_3 / MeOH, H_2O, h) CH_3I, NaH / THF,
i) $Me_2C(OMe)_2$, *p*-TsOH / CH_2Cl_2, j) PDC / DMF

Scheme 2.

1.87 (1H, m), 3.44 (3H, s), 3.73 (1H, d, $J = 3.7$ Hz). NMR δ_C ($CDCl_3$): 11.73, 14.05, 25.86, 38.05, 59.06, 83.42, 177.99. Found: C, 57.34; H, 9.58. Calcd for $C_7H_{14}O_3$: C, 57.51; H, 9.65.

*(2R)-2,3-isopropylidenedioxy-3-methylbutanoic acid (**32**).* Compound **31**, which was prepared according to the conventional method,[26] was used as the starting material. Colorless solid: $[\alpha]_D^{20} + 20.7^\circ$ (*c* 0.96, EtOH). NMR δ_H ($CDCl_3$): 1.24 (3H, s), 1.40 (3H, s), 1.51 (3H, s), 1.54 (3H, s), 4.39 (1H, s).

*(2R,3S)-2-Benzyloxy-3,4-dimethylpentanoic acid (**34**).* Compound **33**, which was derived from crotyl alcohol in our laboratory, was used as the starting material. Brown oil: $[\alpha]_D^{27} + 40.3^\circ$ (*c* 1.30, $CHCl_3$). NMR δ_H ($CDCl_3$): 0.87 (1H, d, $J = 6.1$ Hz), 0.92 (1H, d, $J = 6.2$ Hz), 0.97 (1H, d, $J = 6.2$ Hz), 1.72 (1H, m), 4.09 (1H, d, $J = 2.5$ Hz), 4.43 (1H, d, $J = 11.3$ Hz), 4.75 (1H, d, $J = 11.3$ Hz), 7.32–7.37 (5H, m). NMR δ_C ($CDCl_3$): 11.57, 19.76, 21.05, 29.81, 42.84, 73.02, 79.94, 128.00, 128.06, 128.43, 137.19, 177.91.

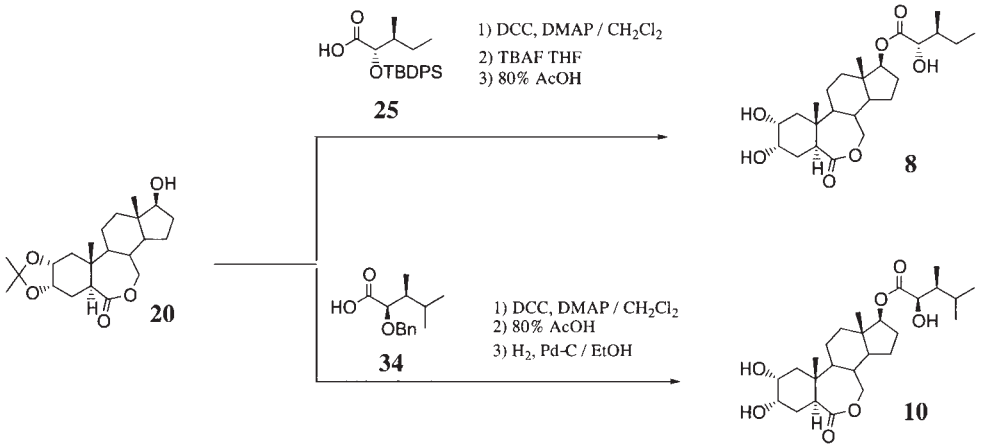
(R)-2-(tert-Butyldiphenylsilyl)oxy-3-phenylpropanoic acid. The corresponding hydroxycarboxylic acid for compound **13** was derived from D-phenylalanine. Colorless oil: $[\alpha]_D^{20} - 5.6^\circ$ (*c* 1.14, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.06 (9H, s), 2.87 (1H, dd, $J = 13.7$, 5.2 Hz), 2.95 (1H, dd, $J = 13.7$, 6.1 Hz), 4.47 (1H, t, $J = 5.5$ Hz), 7.11–7.58 (15H, m). Found: C, 74.08; H, 7.08. Calcd. for $C_{25}H_{28}O_3Si$: C, 74.22; H, 6.98.

(S)-2-(tert-Butyldiphenylsilyl)oxy-3-methylbutanoic acid. To derive the hydroxycarboxylic acid moiety of compound **7**, L-valine was used instead of L-isoleucine in Scheme 2. Colorless oil: $[\alpha]_D^{24} - 12.3^\circ$ (*c* 1.51, $CHCl_3$). NMR δ_H ($CDCl_3$): 0.88 (3H, d, $J = 6.9$ Hz), 0.90 (3H, d, $J = 6.9$ Hz), 1.13 (9H, s), 1.86–1.96 (1H, m), 4.13 (1H, d, $J = 3.6$ Hz), 7.26–7.45 (6H, m), 7.61–7.67 (4H, m). Found: C, 70.47; H, 7.74. Calcd. for $C_{21}H_{28}O_3Si$: C, 70.74; H, 7.92.

(R)-2-(tert-Butyldiphenylsilyl)oxy-3-methylbutanoic acid. To derive the hydroxycarboxylic acid moiety of compound **8**, D-valine was used instead of L-isoleucine in Scheme 2. Colorless oil: $[\alpha]_D^{23} + 13.8^\circ$ (*c* 0.93, $CHCl_3$). NMR δ_H ($CDCl_3$): 0.87 (3H, d, $J = 6.9$ Hz), 0.89 (3H, d, $J = 6.9$ Hz), 1.11 (9H, s), 1.31–1.93 (1H, m), 4.15 (1H, d, $J = 3.6$ Hz), 7.36–7.59 (6H, m), 7.64–7.67 (4H, m). Found: C, 71.02; H, 7.76. Calcd for $C_{21}H_{28}O_3Si$: C, 70.74; H, 7.92.

Synthesis of BL analogs (Scheme 3). Condensation of a key intermediate with the corresponding carboxylic acid was performed using dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) in CH_2Cl_2, and protective groups were removed as summarized in Scheme 3.

Bioassay. BL activity was measured in a dwarf rice lamina inclination assay developed by Fujioka *et al.*[24] Briefly, the seeds of dwarf rice *Oryza sativa* cv. Tanginbozu were soaked in aqueous 0.2% Benlate-T solution for 2 d at 30 °C under light. Germinated seeds were planted on 20 ml of 1% agar medium (*ca.* 1 cm depth) in a beaker (50 ml volume), then incubated 3 d under the conditions mentioned above to obtain the seedlings (*ca.* 3 cm). A 0.5 μl ethanol solution of indole-3-acetic acid (IAA, 50 mM) was applied to the top portion of the



Scheme 3.

lamina by micro-syringe, then various doses of test compounds were applied in ethanol solution (0.5 μl) with a microsyringe to the same part for the IAA treatment. After maintaining the plants for 2 d under identical growth conditions, the external angle between the lamina and its leaf sheath was measured using a circular protractor. Seven seedlings were planted in each beaker, and three sets were used for each dose, yielding 21 observations. Both a negative (solvent) and a positive control (1 nmol of BL) were used in each experiment.

Results and Discussion

Synthetic study

Pregnenolone **15** was converted to its methanesulfonate, then solvolyzed with $KHCO_3$ in aqueous acetone. Subsequent Jones oxidation yielded 3α,5-cyclo ketone **16** in 77% yield from **15**. Treatment of **16** with HBr in AcOH gave **17** quantitatively. This ring-opening reaction proceeded instantaneously, and the prolonged reaction time caused epimerization with respect to C-17. Although the treatment of **16** with *p*-toluenesulfonic acid (*p*-TsOH) in sulfolane[27] directly afforded **18**, this was unfavorable in that the products contained 30% of the undesired epimer of **18** with respect to C-17. The low yield of **18** (28% from **15**) previously reported[25] is probably due to the epimerization at C-17. This epimerization could not be avoided, even though multiple reaction conditions were attempted (*p*-TsOH and NaBr in DMF).[28]

Dehydrobromination of **17** was performed with LiBr/Li_2CO_3 in DMF[29] to give compound **18** in high yield as well as its regioisomer. Without Li_2CO_3, C-17 epimerization occurred. Crude **18** was then submitted to further dihydroxylation using OsO_4/*N*-methylmorpholine *N*-oxide (NMO) without purification. The regioisomer was inert under these conditions and was easily separated from **19** by column chromatography. After recrystallization, the 2α,3α-diol analog **19** was obtained in 51% yield from **16**. Compound **19** was subjected to Baeyer-Villiger oxidation followed by basic hydrolysis and protection of 2α,3α-diol to afford 7-oxalactone **20** in 43% yield from **19**. The total yield of **20** from starting material **15** was 17% in 10 steps.

Hydroxycarboxylic acid derivatives were derived from α-amino acid according to the method reported by Irie *et al.*,[30] as summarized in Scheme 2. L-Isoleucine **21** was treated with $NaNO_2$ in AcOH followed by hydrolysis with K_2CO_3 to give **22**. Selective benzylation of **22** afforded the benzyl ester **23**. After protecting the hydroxyl group of compound **23** by *tert*-butyldiphenylsilyl (TBDPS), the benzyl group was removed to afford compound **25**. (2*R*)-2-Hydroxycarboxylic acid derivative **28** was derived from compound **23** using the Mitsunobu reaction.[31] 2-Methoxycarboxylic acid **30** was converted from **26** using conventional methods. The synthesis of 2-benzyloxycarboxylic acid **34** was performed by treatment of alcohol **33** with pyridinium dichromate (PDC) in DMF (Scheme 2).[32]

BL analogs having various ester substructures at the C-17 position of the steroid skeleton were synthesized by condensing the key intermediate **20** with the corresponding carboxylic acids (Scheme 3) using DCC and DMAP. Deprotection of the hydroxyl groups was performed using hydrogen and palladium-carbon. Yields in the condensation and deprotection steps were between 45–88%. Although compounds **4** and **6** were already synthesized and reported to be active in the bean hypocotyls elongation assay, the evaluated activity was qualitative.[23]

Bioassay

The synthesized compounds were subjected to the rice lamina inclination bioassay.[24] The dose-response curves for BL **1** and compounds **9** and **10** are shown in Fig. 2. In each curve, the inclination angles caused by BL treatment and control were set as 100% and 0% respectively. From these dose-response curves, a 50% effective dose (ED_{50}; mol) was evaluated by probit transformation[33] and the reciprocal logarithmic value of ED_{50}, pED_{50}, was used as an index of BL-like activity. The pED_{50} values of the newly synthesized compounds

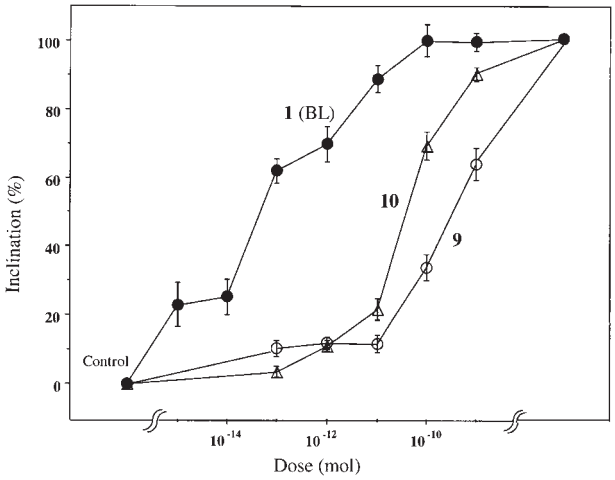


Fig. 2. Dose-response Relationships for BL (1,●), Compound **9** (○) and **10** (△).

Table 2. Brassinolide-like Activity of Brassinosteroids in the Rice Lamina Inclination Assay

Compound	pED_{50} (mol)[a]	Compound	pED_{50} (mol)
1[b]	13.6 ± 0.4 (n = 4)[c]	**8**	9.6
2[d]	12.3 ± 0.1 (n = 3)[c]	**9**	9.3
3	<8.0 (13%)	**10**	10.5
4	<8.0 (41%)	**11**	10.1
5	<8.0 (48%)	**12**	8.3
6	<8.0 (44%)	**13**	<8.7 (16%)
7	8.6	**14**	9.1

a) Percent values in parentheses are the inclination % at the corresponding dose. 100% for BL treatment and 0% for control.
b) Brassinolide.
c) Mean ± standard deviation for repetition (n).
d) Castasterone.

are listed in Table 2. A activity in terms of pED_{50} was not obtained for compounds **3**–**6**, but three compounds (**4**–**6**) gave a significant response (>40%) at the highest doses.

Structure-activity relationship study

Since most of the BRs contain both 22- and 23-hydroxyl groups in the side chain moiety, we assumed that the carbonyl oxygen of these ester compounds plays the role of the 22-hydroxyl oxygen. Based on this hypothesis we introduced a hydroxyl group at the alpha position relative to the carbonyl of the ester moiety to mimic the 23-hydroxyl group of BL. The activity was enhanced dramatically by introduction of the hydroxyl group next to the carbonyl group of compound **4** (vs **9** or **10**). As shown in Table 2, the *R*-form was 10 times more potent than the corresponding *S*-isomer (**7** vs **8**, and **9** vs **10**), which is consistent with the fact that the *R* configuration at C-23 is favored for BR activity.[34] Thus, we concluded that the α-OH group of ester compounds corresponds to the 23-OH group of BL.

In a further study, we found that conversion of the α-hydroxyl group of the side chain of **10** to a methoxy group (**14**) caused a 25-fold reduction in activity. Previously Luo and his coworkers measured the BL-activity of BL and its *O*-methylated analogs in the presence and absence of IAA. They found that under assay conditions similar to those in our studies, the conversion of 22-OH of BL to 22-OCH_3 resulted in a 10-fold decrease in activity. Methylation of the 23-OH, which is presumed to be the α-OH group of our ester compounds (**7**–**13**), was detrimental to activity.[35] This observation is not consistent with the fact that the activity was maintained by the methylation of the α-OH group of the ester side chain. These contradictory results may be due to different physicochemical properties such as hydrophobicity and steric effect differences between compounds containing an ester versus an alkyl moiety. In fact, inactive 23-OMe BL becomes active upon methylation of the 22-OH group corresponding to a similar increase in the molecular hydrophobicity.[35] Considering these results, we concluded that the oxygen of the 22-OH group works as a hydrogen bond acceptor similar to carbonyl oxygen.

Introduction of the hydroxyl group at the terminal of the ester side chain decreased activity by 20 times (**8** *vs* **12**). Pharis and his coworkers synthesized the 25- and 26-hydroxy derivatives of BL and found that these hydroxylated compounds showed lower activity than BL.[20] Generally, if compounds are hydroxylated they are easily removed from the target tissues, even though the potent BL has four OH groups. The hydroxylation at C2 of typhasterol enhanced activity by 10 times (**2** *vs* typhasterol).[6] Examination of the activity of more hydroxylated compounds will be interesting from the viewpoint of BL metabolism in the regulation of hormonal activity.

When the side chain of compounds **7** and **8** was elongated, activity increased 10 times (**7** *vs* **9**, and **8** *vs* **10**). But further addition of a methyl group at the γ position of compound **10**, which makes the terminal alkyl structure similar to that of BL, was not favorable to activity (**10** *vs* **11**). Modification of the terminal *i*-Pr moiety (**8**) to benzyl (**13**), caused a subsequent loss of activity. As explained above, the hydrophobic and steric effects of the side chain moiety must correlate with the activity. In order to delineate the essential physicochemical property, a three-dimensional structure-activity relationship study is in progress for the expanded set of compounds.

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