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Synthesis and Lateral Root-Inducing Activity of 3-Hydroxymethyl-2-Substituted-4-Butanolides

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A series of 3-hydroxymethyl-2-substituted-4-butanolides was synthesized and evaluated for their activity to induce lateral root in the lettuce seedlings. 2-Benzoyl-3-hydroxymethyl-4-butanolide stimulated the emergence of lateral root, while the 2-alkanoyl and 2-(α -hydroxybenzyl) analogs had no activity, indicating that the 2-benzoyl moiety was essential for lateral root-inducing activity. Among the compounds tested so far, 3-hydroxymethyl-2-(3-methoxybenzoyl)-4-butanolide showed the highest activity.

INTRODUCTION

In streptomycetes, some butanolides termed autoregulators have been isolated as primary signal molecules for triggering production of antibiotics and/or cytodifferentiation (Horinouchi and Beppu, 1992). The best studied compound among them is A-factor, [(3*R*)-3-hydroxymethyl-2-(6-methylheptanoyl)-4-butanolide], from *Streptomyces griseus*. Most of the autoregulators reported so far have a characteristic 2,3-disubstituted-4-butanolide skeleton as well as A-factor. These compounds are of particular interest because of their activity to regulate morphological differentiation such as aerial mycelium formation. As part of a program aimed at discovering new series of plant growth regulators, we have synthesized a number of 3-hydroxymethyl-2-substituted-4-butanolides, having a structural resemblance to A-factor, and evaluated their activity on the growth of lettuce seedlings. In the present paper we report that 3-hydroxymethyl-2-substituted benzoyl-4-butanolides induce lateral root formation of lettuce

A-factor

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seedlings and discuss the structure–activity relationships.

MATERIALS AND METHODS

Synthetic procedures

All melting points (mp) are uncorrected. The ^1H NMR spectra were determined with a JEOL JNM-FX100 spectrometer, using tetramethylsilane as an internal standard, and all samples were prepared in deuteriochloroform.

3-Formyl-4-butanolide (1)

Ozonized oxygen was bubbled through a solution of 3-vinyl-4-butanolide (5 g, 44.6 mmol) in anhydrous methanol (50 ml) at -78°C until the starting material had not been detected on TLC. To the solution was added dropwise dimethylsulfide (30 ml) and the mixture was stirred over night at room temperature. After removal of the solvent, the residue was chromatographed on silica gel by eluting with hexane–ethyl acetate (9:1). Concentration of the eluate under reduced pressure afforded **1** (4.37 g; 93%) as a colorless oil, b.p. $81\text{--}84^\circ\text{C}/2\text{ mmHg}$. ^1H NMR δ : 2.66 (1H, t, $J=8.2\text{ Hz}$), 2.87 (1H, t, $J=8.2\text{ Hz}$), 3.26–3.64 (1H, m), 4.23 (1H, t, $J=5.1\text{ Hz}$), 4.61 (1H, t, $J=5.1\text{ Hz}$), 9.76 (1H, d, $J=4.0\text{ Hz}$).

3-Hydroxymethyl-4-butanolide (2)

To a solution of **1** (4.73 g, 41.5 mmol) in ethanol (20 ml) was added sodium borohydride (490 mg, 51.9 mmol) at room temperature and the mixture was stirred for 2 h. To the mixture was added 2N HCl (25 ml) and the mixture was neutralized with 5% NaHCO_3 . After removal of the solvent, the residue was chromatographed on silica gel by eluting with hexane–ethyl acetate (1:2). Concentration of the eluate under reduced pressure afforded **2** (2.87 g; 54%) as a colorless oil, b.p. $150^\circ\text{C}/2\text{ mmHg}$. ^1H NMR δ : 2.08–2.85 (3H, m), 3.56 (2H, d, $J=6.0\text{ Hz}$), 4.12 (1H, t, $J=6.8\text{ Hz}$), 4.34 (1H, t, $J=6.8\text{ Hz}$).

3-(tert-Butyldimethylsilyloxymethyl)-4-butanolide (3)

To a solution of **2** (2.95 g, 25.4 mmol) in dry dimethylformamide (10 ml) was added imidazole (6.92 g, 100 mmol) and *tert*-butyldimethylsilyl chloride (TBDMS-Cl; 7.65 g, 50.8 mmol) at room temperature. After stirring for 3 days at room temperature, to the mixture was added water (20 ml) and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with brine and dried over sodium sulfate. After removal of the solvent, the residue was chromatographed on silica gel by eluting with hexane–ethyl acetate (4:1). Concentration of the eluate under reduced pressure afforded **3** (3.37 g; 62%) as a colorless oil. ^1H NMR δ : 0.92 (9H, s), 2.08–2.85 (3H, m), 3.56 (2H, d, $J=6.0\text{ Hz}$), 4.12 (1H, t, $J=6.8\text{ Hz}$), 4.34 (1H, t, $J=6.8\text{ Hz}$).

3-(tert-Butyldimethylsilyloxymethyl)-2-(α -hydroxybenzyl)-4-butanolide (4a)

To a solution of potassium bis(trimethylsilyl)amide (KHMDs; 0.5 M solution in toluene, 4.4 ml, 2.2 mmol) in dry THF (10 ml) was added dropwise a solution of **3** (0.4 g, 1.8 mmol) in dry THF (5 ml) at -70°C under an atmosphere of nitrogen. After stirring for 30 min at -70°C , to the mixture was added dropwise a solution of benzaldehyde (235 mg, 2.2 mmol) in dry THF (5 ml) at the same temperature. After stirring for 1 h at -70°C , the reaction mixture was quenched with 20 ml of aqueous ammonium chloride. The solution was allowed to warm up to room temperature and the product was extracted twice with ethyl acetate. The combined organic layers were washed with brine and dried over

sodium sulfate. After removal of the solvent, the residue was chromatographed on silica gel by eluting with hexane–ethyl acetate (7:1). Concentration of the eluate under reduced pressure afforded **4a** (440 mg; 71%) as a colorless oil. ^1H NMR δ : 0.96 (7.2H, s), 1.01 (1.8H, s), 2.80–3.48 (4H, m), 4.08–4.64 (2H, m), 5.04 (0.2H, d, $J=8.0$ Hz), 5.67 (0.8H, broad s), 7.40–7.60 (5H, m).

2-(α -Hydroxybenzyl)-3-hydroxymethyl-4-butanolide (5a)

To a solution of **4a** (180 mg, 0.5 mmol) in dry THF was added tetrabutylammonium fluoride (1 M solution in THF, 0.8 ml, 0.8 mmol) at 0 °C. After stirring for 3 h at 0 °C, the mixture was quenched by adding 5 ml of saturated aqueous ammonium chloride. The product was extracted three times with ethyl acetate and the combined organic layers were washed with brine and dried over sodium sulfate. After removal of the solvent, the residue was chromatographed on silica gel by eluting with benzene–ethyl acetate (3:2). Concentration of the eluate under reduced pressure afforded **5a** (70 mg; 59%) as a colorless crystal, mp 114–115 °C. ^1H NMR δ : 2.60–3.37 (4H, m), 3.86–4.48 (3H, m), 5.40 (1H, d, $J=4.0$ Hz), 7.26–7.46 (5H, m).

Compounds **5b–5d** were prepared from compound **3** in the same manner as compound **5a** with use of *n*-alkanals instead of benzaldehyde. Yields were calculated based on compound **3**.

3-Hydroxymethyl-2-(1-hydroxynonyl)-4-butanolide (5b)

Yield 38%; colorless oil; ^1H NMR δ : 0.92 (3H, t, $J=6.0$ Hz), 1.20–1.40 (12H, m), 1.44–1.64 (2H, m), 2.44–3.28 (4H, m), 3.76 (2H, d, $J=12.0$ Hz), 3.88–4.20 (1H, m), 4.00 (1H, t, $J=8.8$ Hz), 4.44 (1H, t, $J=8.8$ Hz).

2-(1-Hydroxyheptyl)-3-hydroxymethyl-4-butanolide (5c)

Yield 43%; colorless oil; ^1H NMR δ : 0.92 (3H, t, $J=6.0$ Hz), 1.26–1.40 (8H, m), 1.46–1.60 (4H, m), 2.58–3.00 (3H, m), 3.56 (2H, t, $J=2.8$ Hz), 4.00 (1H, t, $J=8.8$ Hz), 4.44 (1H, t, $J=8.8$ Hz).

3-Hydroxymethyl-2-(1-hydroxypentyl)-4-butanolide (5d)

Yield 47%; colorless oil; ^1H NMR δ : 0.95 (3H, t, $J=8.0$ Hz), 1.12–1.80 (6H, m), 2.42–3.08 (3H, m), 3.70 (2H, t, $J=6.6$ Hz), 3.86–4.56 (3H, m).

2-Benzoyl-3-(tert-butyldimethylsilyloxymethyl)-4-butanolide (6a)

To a solution of oxalyl chloride (1.24 ml, 1.4 mmol) in dry dichloromethane (6 ml) was added dropwise a solution of dimethylsulfoxide (1.26 ml, 1.8 mmol) at –70 °C in a nitrogen atmosphere. After stirring 15 min. at –70 °C, to the mixture was added dropwise a solution of **4a** (240 mg, 0.7 mmol) in dry dichloromethane at the same temperature. After stirring for 30 min. at –75 °C, to the mixture was added triethylamine (0.5 ml, 3.5 mmol) and stirred for 1 h at the same temperature. The reaction mixture was quenched by adding saturated aqueous ammonium chloride and allowed to warm up to room temperature. The product was extracted three times with ethyl acetate and the combined organic layers were washed with brine and dried over sodium sulfate. After removal of the solvent, the residue was chromatographed on silica gel by eluting with hexane–ethyl acetate (7:1). Concentration of the eluate under reduced pressure afforded **6a** (196 mg; 82%) as a colorless oil. ^1H NMR δ : 1.04 (9H, s), 3.00–3.40 (1H, m), 3.62 (2H, t, $J=4.1$ Hz), 4.00–4.64 (3H, m), 7.10–7.64 (3H, m), 7.86–8.20 (2H, m).

Desilylation of **6a** was carried out in the same manner as described for the preparation of compound **5a**.

2-Benzoyl-3-hydroxymethyl-4-butanolide (7a)

Yield 42%; colorless oil; [¹H]NMR δ: 1.80–1.92 (1H, broad s), 3.08–3.46 (1H, m), 3.72 (1H, d, *J*=6.0 Hz), 4.00–4.72 (3H, m), 7.12–7.72 (3H, m), 7.84–8.16 (2H, m).

By the same procedure, Compounds **7b–7n** were prepared from the corresponding 3-(*tert*-butyldimethylsilyloxymethyl)-2-substituted-4-butanolide **4b–4n**. Yields were calculated based on compound **3**.

3-Hydroxymethyl-2-*n*-nonanoyl-4-butanolide (7b)

Yield 35%; colorless oil; [¹H]NMR δ: 0.82 (3H, t, *J*=8.0 Hz), 1.19–1.21 (10H, m), 1.58–1.78 (2H, m), 2.40–3.04 (3H, m), 3.48–3.68 (1H, m), 3.80 (2H, t, *J*=2.4 Hz), 4.22 (1H, t, *J*=8.8 Hz), 4.50 (1H, t, *J*=8.8 Hz).

2-*n*-Heptanoyl-3-hydroxymethyl-4-butanolide (7c)

Yield 7%; colorless oil; [¹H]NMR δ: 0.88 (3H, t, *J*=8.0 Hz), 1.14–1.34 (8H, m), 2.12–2.40 (1H, m), 2.40–3.00 (2H, m), 3.60–3.80 (2H, m), 4.04–4.56 (3H, m).

3-Hydroxymethyl-2-*n*-pentanoyl-4-butanolide (7d)

Yield 23%; colorless oil; [¹H]NMR δ: 0.94 (3H, t, *J*=8.0 Hz), 1.32–1.72 (4H, m), 1.72–2.08 (2H, m), 2.60–3.00 (1H, m), 3.08–3.57 (1H, m), 3.70 (2H, t, *J*=4.8 Hz), 3.80–3.98 (1H, m), 4.00–4.60 (3H, m).

3-Hydroxymethyl-2-(2-methoxybenzoyl)-4-butanolide (7e)

Yield 36%; colorless oil; [¹H]NMR δ: 3.10 (1H, broad s), 3.70 (2H, t, *J*=4.4 Hz), 3.84 (3H, s), 4.05 (1H, t, *J*=8.8 Hz), 4.17 (1H, d, *J*=9.0 Hz), 4.46 (1H, t, *J*=8.8 Hz), 6.84–7.80 (4H, m).

3-Hydroxymethyl-2-(3-methoxybenzoyl)-4-butanolide (7f)

Yield 10%; colorless oil; [¹H]NMR δ: 2.22 (1H, broad s), 3.12–3.50 (1H, m), 3.64–3.96 (2H, m), 3.86 (3H, s), 4.05–4.68 (4H, m), 6.76–7.78 (4H, m).

3-Hydroxymethyl-2-(4-methoxybenzoyl)-4-butanolide (7g)

Yield 53%; colorless oil; [¹H]NMR δ: 3.05–3.42 (1H, m), 3.74 (2H, d, *J*=5.0 Hz), 3.88 (3H, s), 4.22 (1H, t, *J*=6.0 Hz), 4.32 (1H, d, *J*=6.0 Hz), 6.94 (2H, d, *J*=10 Hz), 7.02 (2H, d, *J*=10 Hz).

3-Hydroxymethyl-2-(3,4-methylenedioxybenzoyl)-4-butanolide (7h)

Yield 5%; colorless oil; [¹H]NMR δ: 3.16–3.48 (1H, m), 3.76 (2H, t, *J*=6.1 Hz), 4.24 (1H, t, *J*=8.1 Hz), 4.55 (1H, d, *J*=5.0 Hz), 4.56 (1H, t, *J*=8.1 Hz), 6.07 (2H, s), 6.27–7.80 (3H, m).

2-(2-Chlorobenzoyl)-3-hydroxymethyl-4-butanolide (7i)

Yield 12%; colorless oil; [¹H]NMR δ: 3.18–3.38 (2H, m), 3.80 (2H, d, *J*=8.0 Hz), 4.00–4.28 (1H, m), 4.44 (1H, t, *J*=10.1 Hz), 4.46 (1H, d, *J*=8.0 Hz), 4.48 (1H, t, *J*=10.1 Hz), 7.16–7.80 (4H, m).

2-(3-Chlorobenzoyl)-3-hydroxymethyl-4-butanolide (7j)

Yield 47%; colorless oil; [¹H]NMR δ: 2.40 (1H, broad s), 3.12–3.52 (1H, m), 3.72 (2H, d, *J*=6.0 Hz), 4.21 (1H, t, *J*=8.8 Hz), 4.52 (1H, t, *J*=8.8 Hz), 4.56 (1H, d, *J*=10.0 Hz), 7.20–8.08 (4H, m).

2-(4-Chlorobenzoyl)-3-hydroxymethyl-4-butanolide (7k)

Yield 7%; colorless oil; [¹H]NMR δ: 1.60 (1H, broad s), 3.24–3.56 (1H, m), 3.80 (2H, d, *J*=8.0 Hz), 4.28 (1H, t, *J*=6.8 Hz), 4.52 (1H, t, *J*=6.8 Hz), 4.58 (1H, d, *J*=8.0 Hz), 7.44 (2H, d, *J*=8.4 Hz), 8.04 (2H, d, *J*=8.4 Hz).

3-Hydroxymethyl-2-(3-phenylpropanoyl)-4-butanolide (7l)

Yield 12%; colorless oil; ^1H NMR δ : 2.56–3.10 (4H, m), 3.16–3.40 (1H, m), 3.64 (2H, t, $J=4.6$ Hz), 4.12 (1H, d, $J=6.8$ Hz), 4.26 (1H, d, $J=4.0$ Hz), 4.42 (1H, t, $J=6.8$ Hz), 7.04–7.40 (5H, m).

2-Cinnamoyl-3-hydroxymethyl-4-butanolide (7m)

Yield 9%; colorless oil; ^1H NMR δ : 3.28–3.56 (1H, m), 3.74 (2H, t, $J=6.1$ Hz), 4.00 (1H, d, $J=8.0$ Hz), 4.24 (1H, t, $J=6.1$ Hz), 4.32 (1H, t, $J=6.1$ Hz), 6.44 (1H, d, $J=12.0$ Hz), 6.64 (1H, d, $J=12.0$ Hz), 7.04–7.86 (5H, m).

3-Hydroxymethyl-2-(2-naphthoyl)-4-butanolide (7n)

Yield 21%; mp 147–148 °C; ^1H NMR δ : 3.18–3.62 (1H, m), 3.80 (2H, d, $J=6.0$ Hz), 4.12–4.68 (3H, m), 4.76 (1H, d, $J=6.0$ Hz), 7.40–8.20 (7H, m).

Biological evaluations

Lettuce (*Lactuca sativa* L. cv. Sacramento) seeds were used in seedling test. Acetone solution (1 ml) containing the proper amount of a test compound was poured on a filter paper in a petri dish of 9 cm in diameter. After evaporation of the solvent, 20 seeds were placed on a filter paper and water (5 ml) was added. Plants were maintained at 25 ± 1 °C in light (photosynthetically available radiation; $50 \mu\text{mol m}^{-2} \text{s}^{-1}$, photoperiod; 12 h, relative humidity; $60 \pm 5\%$). After 5 days of incubation, the length of the primary roots was measured and the emergence of the visible lateral roots was inspected. A primary root was considered responsive when it contained at least one lateral root. In controls the emergence rate of lateral roots was less than 2%. The growth rates were calculated as percentages of the averaged lengths of primary roots of treated plants to those of controls (deionized water). All experiments were triplicated.

RESULTS AND DISCUSSION

Synthesis

The general synthetic pathway for the preparation of 3-hydroxymethyl-2-substituted-4-butanolides is shown in Fig. 1. 3-Formyl-4-butanolide (**1**) was prepared by ozonolysis of 3-vinyl-4-butanolide (Kondo and Mori, 1974) and subsequent treatment with dimethyl sulfide. Reduction of compound **1** with sodium borohydride followed by silylation with *tert*-butyldimethylsilyl chloride provided compound **3**. The aldol condensation of compound **3** with a variety of aldehydes using potassium bis(trimethylsilyl)amide (KHMDs) as a base afforded the corresponding aldol adducts **4a–n**, each of which was an inseparable mixture of stereoisomers. Disilylation of compounds **4a–d** with tetrabutylammonium fluoride afforded diols **5a–d**. Oxidation of compounds **4a–n** with oxalyl chloride and dimethyl sulfoxide in the presence of triethylamine followed by desilylation gave compounds **7a–n**.

Biological activity

Table 1 shows the effects of 3-hydroxymethyl-2-substituted-4-butanolides on root growth of lettuce seedlings. Compounds with a hydroxy group at the 2-position of 4-butanolides (**5a–d**) did not induce lateral roots at all, while in the lettuce seedlings treated with 2-benzoyl-4-butanolide **7a**, the emergence of lateral roots was observed (Fig. 2). The 2-alkanoyl analogs (**7b–d**), which promoted significantly primary root growth, showed much less or no lateral root-inducing activity, suggesting that a benzoyl

Fig. 1. Synthesis of 3-hydroxymethyl-2-substituted-4-butanolides
 Reagents and conditions: (a) O_3 , MeOH, -78°C ; (b) Me_2S ; (c) $NaBH_4$, EtOH, room temperature; (d) TBDMs-Cl, imidazole, DMF, room temperature; (e) KHMDS, RCHO, THF, -78°C ; (f) $(n-Bu)_4NF$, MeCN, 0°C ; (g) $(COCl)_2$, DMSO, CH_2Cl_2 , -78°C .

Fig. 2. Representative samples of lettuce seedlings after treatment for 5 days with water (right) and 100 ppm of compound **7a** (left)

group at the 2-position of 4-butanolide was important for resulting in lateral root emergence in the lettuce seedlings. The introduction of a methoxy group at the *meta* or *para* position on the benzene ring (**7f** and **7g**) increased lateral root-inducing activity in comparison with that of the unsubstituted compound **7a**, while the 2-methoxybenzoyl analog **7e** had almost the same activity as that of **7a**. The 3,4-methylenedioxybenzoyl analog **7h** exhibited activity comparable to that of the most effective compound **7f**. The 3-chlorobenzoyl analog **7j** showed some lateral root-inducing activity but not as high as 3-methoxybenzoyl analog **7f**, while the 2- and 4-chlorobenzoyl analogs had no activity. Replacement of the benzoyl group with a 3-phenylpropanoyl or a cinnamoyl group gave analogs with much lower activity (**7l** and **7m**). The 2-naphthoyl analog **7n** showed higher activity than the benzoyl compound **7a**.

The dose-effect curves of compounds **7f** and **7g** on lateral root emergence of the lettuce seedlings are shown in Fig. 3. Induction of lateral root by compounds **7f** and **7g**

Table 1. Effects of 3-hydroxymethyl-2-substituted-4-butanolides on root growth of lettuce seedlings

No.	R	Emergence rate of lateral root at 100 ppm (%)	Growth of primary root at 100 ppm (% of control)
5a		0	110
5b		0	24
5c		0	140
5d		0	102
7a		22	71
7b		0	190
7c		0	218
7d		2	184

(Table 1 continued)

7e		20	128
7f		79	85
7g		50	138
7h		78	78
7i		0	45
7j		42	94
7k		0	131
7l		7	115
7m		9	181
7n		39	105

was slightly observed at a concentration of 1 ppm. The emergence rate of lateral root correlated well with the treated concentration.

The growth of the root is a complex phenomenon under the influence of environmental factors and mainly controlled by hormones (Wightman *et al.*, 1980). The formation of lateral root is interesting from a developmental point of view because it involves the initiation and emergence of several new meristematic areas in the primary root. It is known that auxins and cytokinins have an important role in the regulation of lateral root formation, however, the precise mechanism of the hormonal control of the lateral root initiation and development is unclear (MacIsaac *et al.*, 1989).

Although the mode of action of 3-hydroxymethyl-2-substituted benzoyl-4-butanolides described in this article has not been studied yet, these 4-butanolides might be a valuable prove for elucidating the formation of lateral root and represent reasonable leads for the development of new plant growth regulators. Further studies on the biological activities and the structure-activity relationships of this series of compounds are in progress.

Fig. 3. Effect of compounds **7f** and **7g** on lateral root emergence of the lettuce seedlings

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