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# Plant growth-regulating activities of batatasin III analogues

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Nine bibenzyls and 10 stilbenes were synthesized as analogues of batatasin III, a growth inhibitor isolated from dormant yam bulbils, and examined for their plant growth-regulating activities. The bioassays used were the elongation of dark-grown intact rice coleoptiles, auxin-induced elongation of excised oat coleoptiles, and germination of rape and barnyard grass seeds. In the elongation of intact rice coleoptiles, 3,3′-dihydroxy-5-methoxy- (batatasin III), 3,5-dimethoxy-3′-nitro-, 4′-bromo-3-nitro-, 3-amino-3′-chloro-, 3-amino-4′-chloro-bibenzyls and 3-benzyloxy-4′-bromo-5-methoxy-, 3-benzyloxy-3′,4′-dichloro-5-methoxy-stilbenes were inhibitory, and 4′-bromo-3-nitro-stilbene was promotive at a concentration of 100 mg/liter. The results obtained by the other bioassays were qualitatively consistent with these findings, although 3-amino-4′-chlorobibenzyl and 4′-bromo-3-nitrostilbene were not tested in all the bioassays.

In the seed germination, which was rather tolerant to the test analogues, batatasin III was inactive but 3-benzyloxy-4′-bromo-5-methoxy- and 3-benzyloxy-3′,4′-dichloro-5-methoxystilbenes were very active.

Thus, if substituted properly, bibenzyls and stilbenes are active without hydroxyl and methoxyl group(s) as the functional group.

Batatasin I, II and III isolated from yam bulbils have been shown to induce dormancy of stratified yam bulbils as well as retard the elongation of oat coleoptile segments (*1*), and batatasin III has been identified as 3,3′-dihydroxy-5-methoxy-bibenzyl (*2*). Lunularic acid, isolated as the growth inhibitor of liverworts, has been reported to possess a bibenzyl skeleton (*3, 4*). Thinking, therefore, that bibenzyl and stilbene compounds related to these natural growth inhibitors may have strong growth-regulating activities, we synthesized several substituted bibenzyls and stilbenes and examined their effects on seed germination and growth.

## Materials and methods

### *Synthesis*

Stilbenes and bibenzyls were synthesized as follows. Benzylbromide derivatives and triphenylphosphine were heated at about 100°C for 2 hr to form benzyl-triphenylphosphonium derivatives (Wittig salts). Condensation of the Wittig

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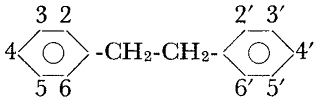
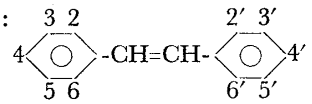
Table 1 *Synthesized batatasin III analogues*

| Analogue no. | Structure | m. p. (°C) [a] | NMR (δ) [b] | IR ($cm^{-1}$) [c] |
|---|---|---|---|---|
| Bibenzyl compounds [d] | | | | |
| 1 | 3,3′-Dihydroxy-5-methoxy (batatasin III) | 93–94 | 2.80 (4H, s), 3.78 (3H, s) 6.3–7.2 (7H, m) | 3400, 1620 (KBr) |
| 2 | 4′-Bromo-3-hydroxy-5-methoxy | 102–103 | 2.85 (4H, s), 3.80 (3H, s) 6.3–7.8 (7H, m) | 3400, 1610 (KBr) |
| 3 | 3-Benzyloxy-3′-cyano-5-methoxy | oil | 2.86(4H, s), 4.80(2H, s) 3.80(3H,s), 6.2–7.6(12H,m) | 2220, 1600, 1150 (Neat) |
| 4 | 4′-Bromo-3,5-dimethoxy | oil | 2.79(4H,s), 3.68(6H,s) 6.3–7.2(7H, m) | 1600, 1460, 1200, 690 (Neat) |
| 5 | 3′-Chloro-3,5-dimethoxy | oil | 2.70(4H,s), 3.70(6H,s) 6.30(3H,s), 7.20(4H,m) | 1600, 1465, 1205, 1160, 1070, 845, 690 (Neat) |
| 6 | 3,5-Dimethoxy-3′-nitro | oil | 2.52(4H,s), 3.45(6H,s) 6.0–6.85(7H,m) | 3350, 1600, 1460, 1200 1150, 840, 690 (Neat) |
| 7 | 4′-Bromo-3-nitro | 173–174 | 2.80(4H,s), 7.1–7.3(8H,m) | 3400, 2600, 1600, 1580, 1510, 780, 690 (KBr) |
| 8 | 3-Amino-3′-chloro | 140–142 | 2.80(4H,s), 7.2(8H,s) 9.7(2H,s) | 3400, 2600, 1600, 1480, 780, 690 (KBr) |
| 9 | 3-Amino-4′-chloro | oil | | 3400, 2600, 1600, 810, 730, 670 (Neat) |
| Stilbene compounds [d] | | | | |
| 10 | 3-Benzyloxy-4′-bromo-5-methoxy | oil | 3.65(3H,s), 4.76(2H,s) 6.5–7.4(12H,m) | 1600, 1460, 1200, 1150 (Neat) |
| 11 | 3-Benzyloxy-3′-chloro-5-methoxy | oil | 3.5(3H,s), 4.80(2H) 6.3–7.1(12H,m) | 1600, 1450, 1150, 1050, 960, 740 (Neat) |
| 12 | 3-Benzyloxy-3′,4′-dichloro-5-methoxy | oil | 3.60(3H,s), 4.85(2H,s) 6.3–7.0(11H,m) | 1600, 1200, 1150, 1100, 1050, 890 (Neat) |
| 13 | 3-Benzyloxy-3′-cyano-5-methoxy | oil | 3.60(3H,s), 4.95(2H,s) 6.3–7.3(12H,m) | 2220, 1580, 1450, 1150, 960 (Neat) |
| 14 | 4′-Bromo-3,5-dimethoxy | 124–125 | 3.72–(6H,s), 6.30–7.52 (9H,m) | 1630, 1510, 1200, 960, 810 (KBr) |
| 15 | 3′-Chloro-3,5-dimethoxy | oil | 3.70(6H,s), 6.32–7.45 (9H,m) | 1600, 1460, 1205, 1070, 690 (Neat) |
| 16 | 3,5-Dimethoxy-3′-nitro | 114 | 3.80(6H,s), 6.40–8.30 (9H,m) | 1630, 1590, 1510, 1340, 1200, 1140, 1050, 950, 815, 720 (KBr) |
| 17 | 4′-Bromo-3-nitro | 114–115 | 6.50–8.45(8H,m) | 1630, 1515, 1350, 1065, 1000, 960, 800, 725 (KBr) |
| 18 | 3′-Chloro-3-nitro | 154–155 | 6.42–8.10(8H,m) | 1630, 1590, 1340, 1050, 960 (KBr) |
| 19 | 4′-Chloro-3-nitro | 135–136 | 6.35–7.65(8H,m) | 1630, 1580, 1510, 1340, 950, 815, 730, 620 (KBr) |

[a] Koffler block (uncorrected).
[b] In $CDCl_3$ with TMS as the internal standard; Varian EM-360 (60 MHz).
[c] $\nu_{max}$; Hitachi EPI-S 2.
[d] Bibenzyl:


Stilbene:


salts and benzaldehyde derivatives in dry ethanol in the presence of sodiumethoxide gave stilbenes. Stilbenes were converted to the corresponding bibenzyls by catalytic hydrogenation in ethanol using Pd-carbon. 4′-Bromo-3-hydroxy-5-methoxy- (No. 2), 3-amino-3′-chloro- (No. 8) and 3-amino-4′-chloro- (No. 9) bibenzyls were produced by catalytic hydrogenation of the corresponding 3-benzyloxy- (No. 10) and 3-nitro- (No. 18 and 19) stilbenes, respectively.

Products were purified by silica gel column chromatography or recrystallization.

The structures of the analogues shown in Table 1 were confirmed by NMR, MS and IR spectra as well as elemental analysis.

*Bioassay solution*

Test analogues were dissolved in a minimal amount of dioxane and detergent (OT-221, Nissan Chemical Co., Ltd.) and dispersed in distilled water or buffer solution.

*Rice coleoptile growth assay*

Twenty seeds of rice (*Oryza sativa* L., c.v. Nihonbare) were germinated and grown in 10 ml of test solution in a 6-cm petri dish for 4 days at 30°C in the dark and the lengths of the coleoptiles were measured to the nearest 1 mm.

*Oat coleoptile segment straight growth assay*

Dehusked seeds of oat (*Avena sativa* L., c.v. Victory I) were sown on the surface of distilled water at 27°C and after 24 hr, germinated seeds were transferred onto 0.5% agar. After growing for 18 hr in the dark at 27°C, the seedlings were illuminated with red light (a 15-watt white fluorescent lamp covered with 3 sheets of red cellophane) for 8 hr and returned to the dark. After a further 18 hr, 6.5-mm segments were excised 3 mm below the coleoptilar tips and 12 segments were incubated in 10 ml of test solution in a 6-cm petri dish at 27°C. After 18-hr incubation, the lengths of the segments were measured to the nearest 0.1 mm.

*Seed germination assay*

Twenty seeds each of rape (*Brassica campestris* L.) and barnyard grass (*Panicum Crus-galli* L.) were sown on 2 sheets of filter paper in a 9-cm petri dish containing 5 ml of test solution and placed in the laboratory room. Four and 8 days later, the numbers of germinated seeds were recorded.

## Results and discussion

In the elongation of intact rice coleoptiles, analogues No. 6, 7, 8, 9, 10, and 12 were inhibitory in addition to No. 1 (batatasin III), and No. 17 was promotive (Table 2). In the auxin-induced elongation of excised oat coleoptiles, all analogues tested were inhibitory; No. 1, 6, 10, and 12 were particulary very active (Table 3). In the seed germination of rape, analogues No. 7, 10, and 12 were active. In the germination of barnyard grass seeds, No. 6 in addition to the above three analogues showed an inhibitory activity comparable to that of No. 7. No. 17 was promotive (Table 4). The active compounds were generally common for the four bioassays,

Table 2 *Effects of batatasin III analogues on elongation of intact rice coleoptiles*

| Analogue no.[a] | Elongation (mm)[b] | % Inhibition |
|---|---|---|
| Control | | |
| Bibenzyls | 21.8±2.20 | — |
| 1 | 13.5±2.20[c] | 38 |
| 2 | 26.5±2.04 | −22 |
| 3 | 18.4±1.90 | 16 |
| 4 | 18.4±2.45 | 16 |
| 5 | 21.4±1.94 | 2 |
| 6 | 11.5±1.84[d] | 47 |
| 7 | 14.8±1.28[d] | 32 |
| 8 | 12.6±1.55[d] | 42 |
| 9 | 10.8±1.49[d] | 50 |
| Stilbenes | | |
| 10 | 13.3±1.76[d] | 39 |
| 11 | 20.0±2.48 | 8 |
| 12 | 12.5±1.12[d] | 43 |
| 13 | 22.4±1.33 | −3 |
| 14 | 22.1±2.42 | −1 |
| 15 | 20.8±1.78 | 5 |
| 16 | 21.3±2.44 | 2 |
| 17 | 30.5±2.09[d] | −40 |
| 18 | 26.5±1.83 | −22 |
| 19 | 22.1±2.85 | −1 |

[a] Test solutions contained 100 mg/liter of each test analogue, 0.2% dioxane and 0.2% detergent.
[b] Mean ± SEM (n=20).
[c] Significantly different from the control at $P<0.05$.
[d] Significantly different from the control at $P<0.01$.

Table 3 *Effects on auxin-induced elongation of oat coleoptile segments*

| Analogue no.[a] | Elongation (mm)[b] | % Inhibition |
|---|---|---|
| Control | 3.69±0.14 | — |
| 1 | 1.03±0.08 | 72 |
| 4 | 2.58±0.09 | 30 |
| 5 | 2.87±0.08 | 22 |
| 6 | 2.17±0.05 | 41 |
| 7 | 2.63±0.08 | 29 |
| 9 | 2.38±0.10 | 35 |
| 10 | 1.32±0.08 | 64 |
| 12 | 1.20±0.07 | 67 |

[a] Test solutions contained 100 mg/liter of each test analogue, 0.2% dioxane, 0.2% detergent, 0.35 mg/liter of indole-3-acetic acid, 2% sucrose and 0.01 M citrate-phosphate buffer (pH 5.0).
[b] Initial length, 6.5 mm. Mean ± SEM (n=12). All data were significantly different from the control at $P<0.01$.

Table 4 *Effects on seed germination*

| Analogue no.[a] | Rape | | Barnyard grass | |
|---|---|---|---|---|
| | Incubation period (days) | | | |
| | 4 | 8 | 4 | 8 |
| Control | 90[b] | 90 | 50 | 55 |
| 1 | 90 | 100 | 55 | 55 |
| 6 | 90 | 95 | 25 | 35 |
| 7 | 45 | 65 | 25 | 35 |
| 9 | 70 | 80 | 25 | 30 |
| 10 | 0 | 0 | 0 | 20 |
| 11 | 90 | 90 | 45 | 50 |
| 12 | 5 | 30 | 15 | 35 |
| 13 | 90 | 100 | 55 | 65 |
| 14 | 90 | 90 | 40 | 60 |
| 17 | 90 | 90 | 50 | 70 |
| 19 | 90 | 90 | 55 | 55 |

[a] Test solutions contained 1000 mg/liter of each test analogue, 1.6% dioxane and 0.7% detergent.
[b] Figures show percentages of germinated seeds with 20 seeds sown.

although the activities of No. 7 and 9 were weak in excised oat coleoptiles compared with those in intact rice coleoptiles.

Germination of rape and barnyard grass seeds was very tolerant to the test analogues (Table 4). Batatasin III was inactive, but 4′-bromo- (No. 10) and 3′,4′-dichloro- (No. 12) 3-benzyloxy-5-methoxystilbenes were very active.

As the results with analogues No. 6, 7, 8, and 9 in Table 2 and No. 6, 7 and 9 in Tables 3 and 4 show, the presence of the hydroxyl or methoxyl group or groups in the bibenzyl skeleton was not required for the activity.

Stilbenes were also active if the benzene rings were properly substituted. 3-Benzyloxy-5-methoxystilbenes substituted with bromine at position 4′ (No. 10) or chlorine at positions 3′ and 4′ (No. 12) were active in all the bioassays tested, whereas one with chlorine at position 3′ (No. 11) was inactive. Interestingly, 4′-bromo-3-nitrostilbene (No. 17) showed considerable promotive activity (Tables 2 and 4), but this was not observed with bibenzyls.

To examine the effect of the compounds on gibberellin-mediated growth, the rice leaf sheath test was performed with rice c.v. Nihonbare by the microdrop method of Murakami (*5*). Gibberellin $A_3$ (17 ng) and each test analogue (2.5 $\mu$g) in 50% aqueous acetone were applied to each seedling. None of the tested analogues (No. 6, 7, 9, 10, 12, 16, 17, and 19) could suppress the gibberellin-induced elongation of the second leaf sheaths (data not shown). This result suggests that the inhibitory activities of the analogues are not due to interference with gibberellin-mediated processes, although whether or not sufficient amounts of the test analogues reached the active site in the tissues is not clear. The data with excised oat coleoptiles support the suggestion that one possible attack point of these analogues is a process(es) involved in the auxin action, but its elucidation must await further investigation.

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**References**


(1) Hashimoto, T., K. Hasegawa and A. Kawarada: Batatasins: new dormancy-inducing substances of yam bulbils. *Planta* 108: 369–374 (1972).

(2) Hashimoto, T., K. Hasegawa, H. Yamaguchi, M. Saito and S. Ishimoto: Structure and synthesis of batatasins, dormancy-inducing substances of yam bulbils. *Phytochem.* 13: 2849–2852 (1974).

(3) Valio, I. F. M., R. S. Burdon and W. W. Schwabe: New natural growth inhibitor in the liverwort *Lunularia cruciata* (L.) Dum. *Nature* 223: 1176–1178 (1969).

(4) Pryce, R. J.: Lunularic acid, a common endogenous growth inhibitor of liverworts. *Planta* 97: 354–357 (1971).

(5) Murakami, Y.: A new rice seedling test for gibberellins, 'microdrop method', and its use for testing extracts of rice and morning glory. *Bot. Mag. Tokyo* 81: 33–43 (1968).