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STRUCTURES AND SYNTHESIS OF THE GROWTH INHIBITORS BATATASINS IV AND V, AND THEIR PHYSIOLOGICAL ACTIVITIES

TOHRU HASHIMOTO and MISAO TAJIMA*

Institute for Physical and Chemical Research, Wakoshi, Saitama, 351, Japan

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Key Word Index—*Dioscorea batatas*; Dioscoreaceae; Chinese yam; bibenzyl; stilbene; batatasin; growth inhibitor.

Abstract—Two new compounds, batatasins IV and V were isolated from dormant bulbils of Chinese yam (*Dioscorea batatas*) and shown to be 2′,3-dihydroxy-5-methoxybibenzyl and 2′-hydroxy-3,4,5-trimethoxybibenzyl, respectively. An analogue, 3,4′-dihydroxy-5-methoxybibenzyl was synthesized. Inhibitory activities of these three compounds as well as batatasin I (6-hydroxy-2,4,7-trimethoxyphenanthrene) and batatasin III (3,3′-dihydroxy-5-methoxybibenzyl) in lettuce seed germination, lettuce hypocotyl elongation and wheat coleoptile section elongation tests are described.

INTRODUCTION

Previously batatasins I, II and III (B-I, B-II and B-III) were isolated from dormant Chinese yam† bulbils (*Dioscorea batatas* Decne.) [1] and the structures of B-I and B-III elucidated [2, 3]. The physiological role of these inhibitors in the dormancy of bulbils [4–7] and other physiological effects on auxin transport [8] and cell organell function [9] are being studied. We now report the isolation, structures and synthesis of batatasins IV and V. We have also synthesized an analogue of B-III and B-IV, 3,4′-dihydroxy-5-methoxybibenzyl, which does not occur in this plant.

RESULTS AND DISCUSSION

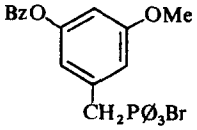
Batatasin IV

A characteristic MS fragmentation pattern with ions at *m/e* 244 (M^+), 137 (hydroxymethoxytropylium ion) and 107 (hydroxytropylium ion), and PMR signals at δ 3.76 (3H, *s*, MeO), 4.50 (2H, D_2O-exchangeable broad *s*, OH) and the signal in the aromatic region (δ 6.20–7.16) showed that the compound was a bibenzyl which possessed a methoxyl and a hydroxyl group on one ring and a hydroxyl group on the other. It showed intense UV absorption and the IR spectrum had peaks at 3300 (OH), 1616, 1589 and 1458 (aromatic) and 1193 cm^{-1} (≡C—O— of phenolic OH group). Treatment of B-IV with acetic anhydride and pyridine gave the diacetate (*m/e*: 328, M^+). Analysis of the PMR signals at δ 6.30 (3H, *m*), 6.85 (2H, *m*) and 7.05 (2H, *m*) in comparison with those of *o*-cresol and 3,3′-dihydroxy-5-methoxybibenzyl (B-III) located the OH groups and the MeO group as shown in structure **4**. The 4H singlet at δ 2.88 was assigned to ϕ—CH_2—CH_2—ϕ.

Wittig condensation of 3-benzyloxy-5-methoxytriphenylphosphoniumbromide (**1**) with 2-benzyloxybenzaldehyde (**2**) gave 2′,3-dibenzyloxy-5-methoxystilbene (**3**), which was then catalytically hydrogenated to give 2′,3-dihydroxy-5-methoxybibenzyl (**4**). B-IV was identical (IR, UV, PMR, MS and mp) with the synthesized bibenzyl.

*On leave from Nikken Chemicals Co., Ltd., Chuo-ku, Tokyo.

†In our previous papers the English name "yam" was used but "Chinese yam" is recommended.



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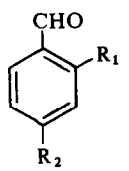
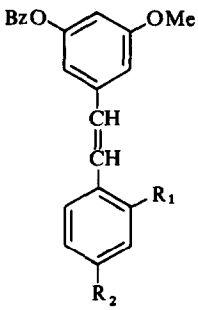


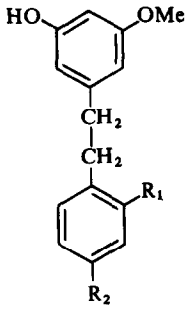
(2) R_1 = OBz, R_2 = H

(5) R_1 = H, R_2 = OBz



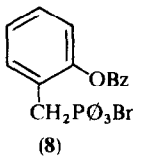
(3) R_1 = OBz, R_2 = H

(6) R_1 = H, R_2 = OBz



(4) R_1 = OH, R_2 = H

(7) R_1 = H, R_2 = OH



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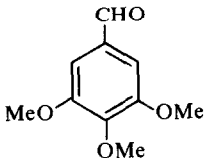

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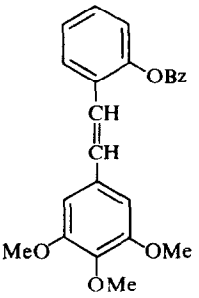

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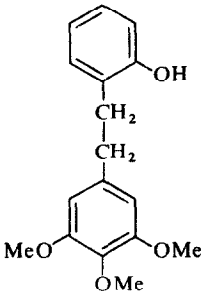

(11)

Batatasin V

The PMR signals at δ 2.88 (4H, *s*, ϕ—CH_2—CH_2—ϕ), 3.77 (6H, *s*, MeO) and 3.82 (3H, *s*, MeO), and the MS ions at *m/e* 288 (M^+), 181 (trimethoxytropylium ion) and 107 (hydroxytropylium ion) showed that B-V was a bibenzyl which consisted of trimethoxybenzyl and hydroxybenzyl. Acetylation of B-V gave the monoacetyl derivative, *m/e* 330 (M^+), 181 and 149 (acetoxytropylium ion). The presence of a sharp 2H singlet at δ 6.38 showed that the MeO groups were at positions 3,4,5 of the benzyl moiety. Analysis of multiplets at δ 6.80 and 7.03 located the OH group at position 2′ of the other benzyl moiety. Thus, the structure of B-V was deduced to be **11**, which was also supported by UV and IR spectra.

Condensation of 2′-benzyloxybenzyltriphenylphosphoniumbromide (**8**) with 3,4,5-trimethoxybenzaldehyde (**9**), followed by a catalytic hydrogenation, gave 2′-hydroxy-3,4,5-trimethoxybibenzyl (**11**) as an oil, with which natural B-V was identical (UV, IR, PMR and MS).

3,4′-*Dihydroxy-5-methoxybibenzyl*

The discovery of 2′,3-dihydroxy-5-methoxybibenzyl (B-IV) and 3,3′-dihydroxy-5-methoxybibenzyl (B-III) in nature stimulated us to synthesize their analogue having a OH group at position 4′. Condensation of a Wittig salt (**1**) with 4-benzyloxybenzaldehyde (**5**), followed by catalytic hydrogenation, gave the bibenzyl (**7**) as colourless needles. This compound is very stable in a crystalline state compared with B-III and B-IV. It showed the same R_f value as that of B-III in Si gel TLC (Table 1) but separation of the two compounds was achieved by GLC. This compound could not be detected in Chinese yam bulbils in spite of our thorough search.

Physiological activities

Batatasins I, III, IV and V, and 3,4′-dihydroxy-5-methoxybibenzyl inhibited elongation of wheat coleoptile sections, elongation of lettuce hypocotyl and lettuce seed germination (Figs. 1–3). When compared at concentrations higher than 10^{-4} M B-I was least effective but the other batatasins and the analogue were almost comparable in inhibitory activity. The observation of a slight but reproducible inhibitory activity at low concentrations was worthy of note. When applied alone to wheat coleoptile sections (Fig. 1) B-I showed some activity from 10^{-7} to 10^{-6} M, B-III from 10^{-6} to 10^{-4} M, B-IV from 10^{-8} to 10^{-6} M, and 3,4′dihydroxy-5-methoxybibenzyl at 10^{-6} M. B-V exerted no inhibition at such low concentrations. In lettuce hypocotyl elongation (Fig. 2) activity at low concentrations was not clear. In lettuce seed germination (Fig. 3) no inhibition at low concentration was discernible but when applied together with abscisic acid (ABA) B-III, B-IV, B-V and the 3,4′-dihydroxy-analogue showed a slight inhibition at 10^{-6} M. The dual inhibitory activity at low and high concentrations may be explained by assuming that batatasins at

Table 1. Colour reactions, TLC Rf values and GLC retention times of batatasins

	B-I	B-II	B-III	B-IV	B-V	3,4′-diOH-5-MeO-bibenzyl
Colour reaction	Indigo	Pink	Orange	Pink	Pink	Pink
TLC R_f (a)	0.86	0.81	0.79	0.81	0.82	0.78
(b)	0.50	0.61	0.56	0.61	0.55	0.56
(c)	0.49	0.32	0.13	0.18	0.40	0.13
GLC retention time (min)	17.1	11.1	10.4	10.0	12.0	11.2

Sprayed with vanillin-H_2SO_4 reagent and heated at 75° for 15 min [10].
(a) Si gel plate, C_6H_6–*n*-BuOH–HCOOH (70:25:5).
(b) Si gel plate, Isopropylether–HOAc (95:5).
(c) Si gel plate, $CHCl_3$–HOAc (95:5).
GLC: 2 m × 3 mm column packed with 1.5% OV-17, 35 ml N_2/min, FID. The column temperature rose at a rate of 6°/min from 170°. Batatasins were tetramethylsilanized with 'Silyl-8' (Pierce Chemical Co.).

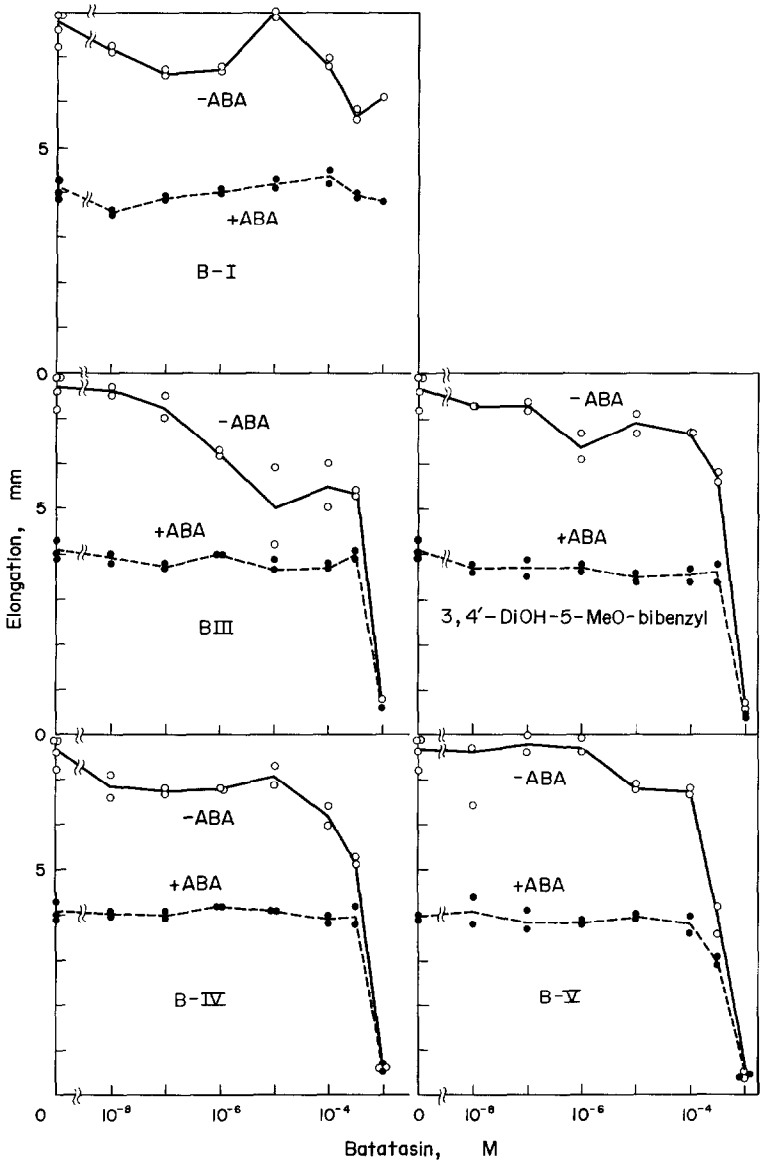

Fig. 1. Wheat coleoptile section test. Each point represents the average of 10 sections. Culture medium contained 2% sucrose and 6×10^{-7} M IAA, and when indicated, 3×10^{-6} M ABA.

medium concentration show latent promotive activity which may appear only when inhibition takes place.

Considering the R_f values of B-I and B-V and the low activity of B-I, the observed activity of the fractions which were referred to as B-I in our previous physiological work [4–7] appears now to be due mostly to B-V which is considered to have been included. Also, the B-III fraction is now thought to have contained B-IV which migrated closely on TLC (Table 1).

EXPERIMENTAL

Batatasin IV. Dormant Chinese yam bulbils (100 kg ft. wt) were extracted with 80% aq. Me_2CO. After the Me_2CO was removed *in vacuo* the aq. residue was extracted with EtOAc at pH 7.0. Chromatography of the extract on a Si gel column eluted with EtOAc–C_6H_6 (1:100 → 1:10) followed by PLC (Si gel GF_{254}, $CHCl_3$–HOAc, 95:5) gave a brown gum, which was decolourized by charcoal in EtOAc and crystallized from $CHCl_3$–CCl_4 to give needles (45 mg), mp 99.5–100.5°. (Found: C, 73.0; H, 6.3. $C_{15}H_{16}O_3$ requires: C, 73.7; H, 6.6%). UV λ_{max}^{EtOH} nm (log ε): 283 sh (3.66), 276 (3.699) and 226 sh (4.209), λ_{min}^{EtOH} 247 (2.681). λ_{max} (EtOH + 0.5 mM KOH): 295 sh (3.929), 283 (3.998), 277 sh (3.978), 243 sh (3.959) and 214 (4.017), λ_{min} 265 (3.895). IR ν_{max}^{KBr} cm^{-1}: 3300, 1616, 1589, 1458, 1193, 1155, 1059 and 758. MS (probe) 75 eV *m/e* (rel. int.): 244 (M^+; 87), 138 (68), 137 (52), 108 (21) and 107 (100). PMR (100 MHz, TMS, $CDCl_3$ + $(CD_3)_2CO$): δ 2.88 (4H, *s*), 3.76 (3H, *s*), 4.50 (2H broad *s*, D_2O-exchangeable), 6.30 (3H, *m*, W = 16 Hz), 6.85 (2H, *m*, W = 18 Hz) and 7.05 (2H, *m*, W = 19 Hz). Acetylation with Ac_2O-py gave the diacetate as an oil, MS *m/e* (rel. int.): 328 (M^+; 100), 286 (77), 269 (66), 244 (51), 138 (72), 137 (73) and 107 (62). The colour reaction of the acetate with vanillin-H_2SO_4 reagent [10] was pink.

Batatasin V. The first part of the isolation procedure was common to that of B-IV. The 4–6% EtOAc eluate from the column described of B-IV were combined and purified by repeated PLC (Si gel GF_{254}, $CHCl_3$–HOAc, 95:5) and a Sephadex LH-20 column (30% aq. MeOH) to give a colourless

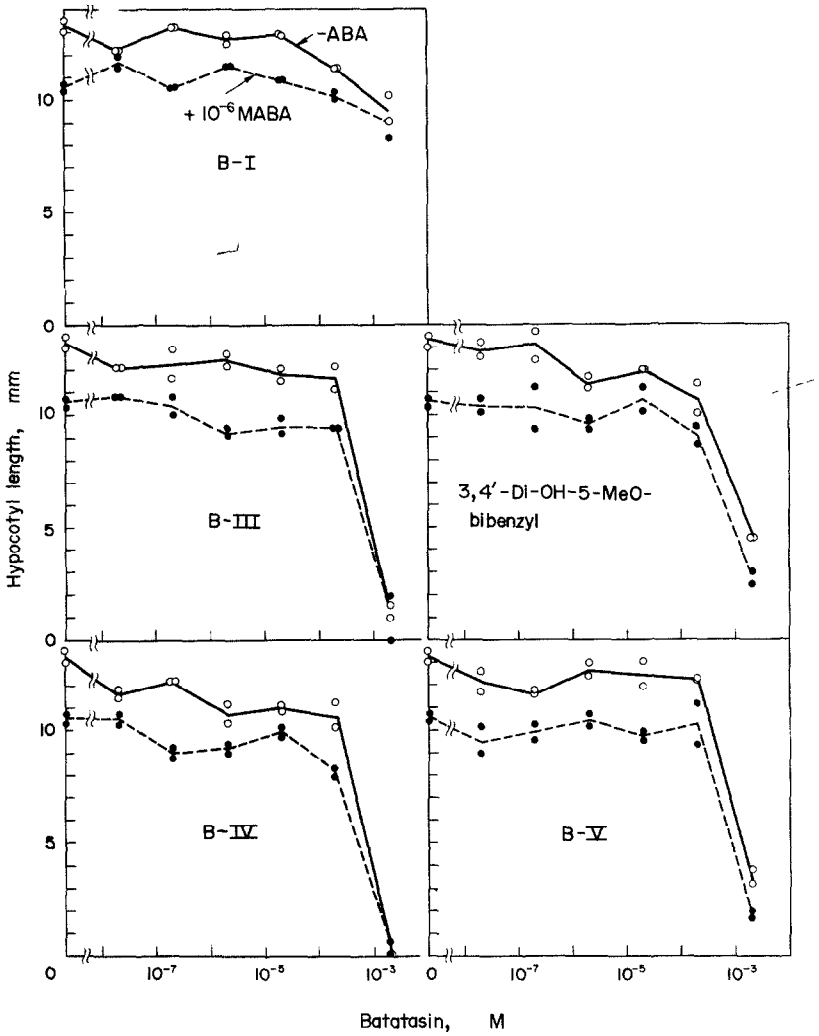

Fig. 2. Lettuce hypocotyl elongation test. Each point represents the average from 10 seedlings. The concentration of ABA was 10^{-6} M.

oil (15 mg), which was shown to be pure by GLC and LC. UV λ_{max}^{EtOH} nm (log ε): 281 sh (3.439), 275 (3.511) and 215 (4.322), λ_{min} 242 (2.881). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1592, 1508, 1455 (the above three, aromatic), 1238, 1125 and 752. MS *m/e* (rel. int.): 288 (M^+; 100), 182 [43], 181 [60], 149 [14] and 107 [19]. PMR ($CDCl_3$): δ 2.88 (4H, *s*), 3.77 (6H, *s*), 3.82 (3H, *s*), 6.38 (2H, *s*), 6.80 (2H, *m*, *W* = 24 Hz) and 7.03 (2H, *m*, *W* = 22 Hz). B-V acetate (Ac_2O – Py), an oil, MS *m/e* (rel. int.): 330 (M^+; 85), 181 (100), 149 (21), and 107 (11).

Synthesis of 2',3-*dihydroxy*-5-*methoxybibenzyl*. A mixture of 3-benzyloxy-5-methoxybenzyltriphenylphosphoniumbromide (**1**) (2.46 g) and 2-benzyloxybenzaldehyde (**2**) (1.06 g) was refluxed in dry EtOH in the presence of NaOEt for 3.5 hr. The reaction mixture was concentrated, dissolved in Et_2O, washed with H_2O, and evapd to dryness, to give a brown oil (3.26 g). It was chromatographed on a Si gel column eluted with C_6H_6 to give 2',3-dibenzyloxy-5-methoxystilbene (**3**), an oil (1.69 g, yield 75%). IR ν_{max}^{neat} cm^{-1}: 3030, 1605, 1590, 1452, 1385, 1114, 975 and 704. MS *m/e*: 422 (M^+, base peak) and 331. It showed a single spot on TLC but separated into two peaks on GLC (OV-17, 2 m × 3 mm column at 280°). PMR ($CDCl_3$) showed that this was a 1:1 mixture of *cis*- and *trans*-isomers. Hydrogenation of the stilbene (**3**) in EtOH with Pd–C (5%) at room temp for 1.5 hr gave 2',3-dihydroxy-5-methoxybibenzyl (**4**) as needles (0.74 g, yield 76%), mp 99.0–101.0° (from $CHCl_3$–CCl_4). UV λ_{max}^{EtOH} nm (log ε): 280 sh (3.609), 275 (3.639) and 224 sh (4.175). IR ν_{max}^{KBr} cm^{-1}: 3300, 1616, 1589, 1492, 1458, 1367 w, 1272 w, 1191, 1152, 1058, 974 and 757. PMR ($CDCl_3$ + $(CD_3)_2CO$): δ 2.85 (4H, *s*), 3.72 (3H, *s*), 6.32 (3H, *m*, *W* = 13 Hz), 6.83 (2H, *m*, *W* = 24 Hz) and 7.06 (2H, *m*, *W* = 22 Hz). MS (probe) *m/e*: 244, 138, 137 and 107.

Synthesis of 3,4'-*dihydroxy*-5-*methoxybibenzyl*. A mixture of salt **1** (3.93 g) and 4-benzyloxybenzaldehyde (**5**) (1.70 g) was refluxed in the same way as for stilbene **3** for 2 hr to give stilbene **6** A Si gel column chromatography (*n*-hexane–C_6H_6, 1:1 → 1:2) separated the *cis*-isomer, an oil (0.92 g) and the *trans*-isomer, colourless needles (3.58 g), mp 94.5–96.5°. The oil; UV λ_{max}^{EtOH} nm (log ε): 286 (4.117) and 236 sh (4.296), λ_{min} 254 (3.824). IR ν_{max}^{neat} cm^{-1}: 1588, 1451, 1430, 1246, 1156, 1046, 1031, 868 and 836. PMR ($CDCl_3$): δ 3.62 (3H, *s*, MeO—), 4.88 (2H, *s*, 4'—O—C<u>H</u>$_2$—φ), 5.01 (2H, *s*, 3—O—C<u>H</u>$_2$—φ), 6.46 (5H, *m*, *W* = 27 Hz), 6.83 (2H, *d*, *J* = 8 Hz, 3',5'—H), 7.21 (2H, *d*, *J* = 8 Hz, 2',6'—H) and 7.32 (10H, *m*, *W* = 40 Hz). MS *m/e*: 422 (M^+; base peak), 331 and 227. The crystals UV λ_{max}^{EtOH} nm (log ε): 338 sh (4.254), 320 (4.491), 307 (4.490) and 239 sh (4.223), λ_{min} 312 (4.487) and 256 (3.569). IR ν_{max}^{nujol} cm^{-1} 1596, 1447, 1275, 1236, 1158 and 964 (*trans*—CH=CH—). PMR ($CDCl_3$): δ 3.78 (3H, *s*, MeO—), 5.06 (4H, *s*, 3,4'-O—C<u>H</u>$_2$—φ), 6.46 (1H, *t*, *J* = 2.2, 4-H), 6.67 (1H, *t*, *J* = 2.0, 2-H or 6-H), 6.74 (1H, *t*, *J* = 2.0, 2-H or 6-H), 6.97 (4H, *q*, *J* = 6.0 and 2.0, 2', 3', 5', 6'-H), 7.28 (1H, *d*, *J* = 13, φ—C<u>H</u>=CH—φ), 7.37 (10H, *s*) and 7.52 (1H, *d*, *J* = 13,

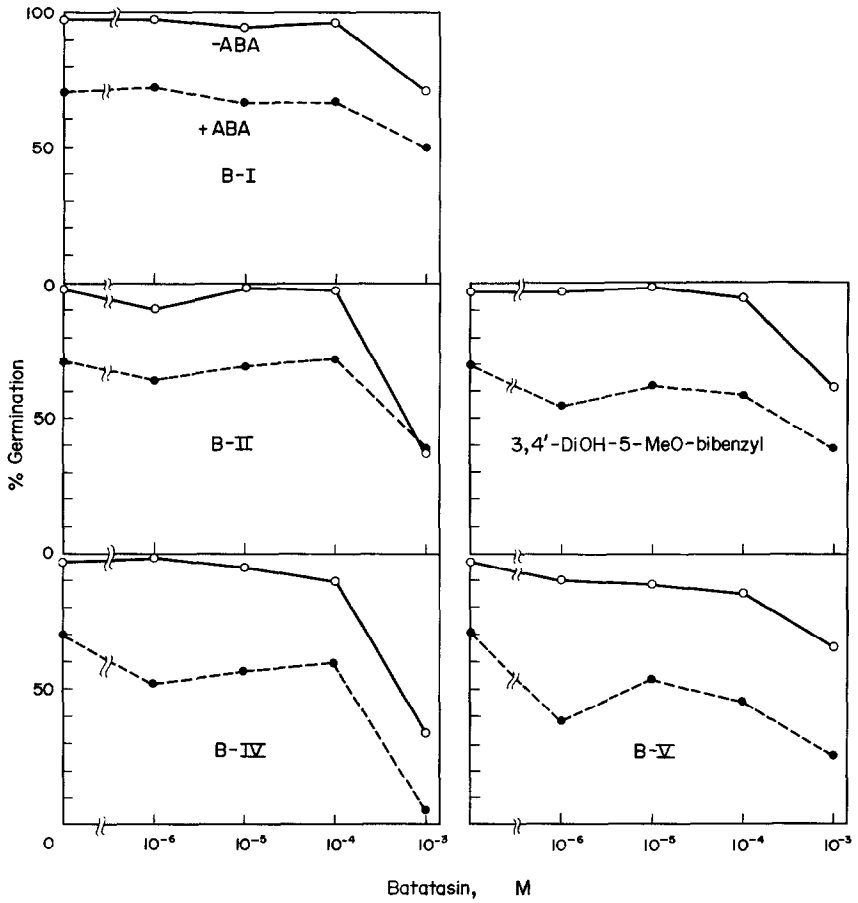

Fig. 3. Lettuce seed germination test. Each point represents the average from triplicate dishes each having 30 seeds. The concentration of ABA was 10^{-6} M.

ϕ—CH=CH—ϕ). MS *m/e*: 422 (M^+; base peak), 331 and 227. Catalytic hydrogenation of both isomers in the same way as for compound **4** gave 3,4'-dihydroxy-5-methoxybibenzyl (**7**) as colourless needles (yield 84%), mp 112.5–113.5° (from hot C_6H_6). UV λ_{max}^{EtOH} nm (log ε): 280 (3.555), 277 sh (3.544) and 225 (4.297), λ_{min} 248 (2.484) and 219 (4.267). IR ν_{max}^{KBr} cm^{-1}: 3310, 1621, 1592, 1512, 1342, 1296, 1195, 1155, 1145, 1060, 987, 832, 819 and 687. PMR ($CDCl_3$ + $(CD_3)_2CO$): δ 2.79 (4H, *s*, ϕ—CH$_2$—CH$_2$—ϕ), 3.73 (3H, *s*, MeO—), 5.70 (2H, broad *s*, —OH), 6.29 (3H, *s*), 6.77 (2H, *d*, J = 8 Hz, 3',5'—H) and 7.04 (2H, *d*, J = 8 Hz, 2',6'—H). MS *m/e* (rel. int.): 244 [M^+; 29), 138 (19), 137 (10) and 107 (100).

Synthesis of 2'-hydroxy-3,4,5-trimethoxybibenzyl. 2-Benzyloxybenzyltriphenylphosphoniumbromide (**8**) (5.38 g) and 3,4,5-trimethoxybenzaldehyde (**9**) (1.96 g) were dissolved in dry EtOH and refluxed for 1.5 hr to give *cis*- and *trans*-2'-benzyloxy-3,4,5-trimethoxystilbenes (**10**), respectively, as an oil (1.17 g) and colourless needles (2.14 g), mp 102–104° (from C_6H_6–*n*-hexane). The oil; UV λ_{max}^{EtOH} nm (log ε): 299 (3.9445), 238 sh (4.124) and 207.5 (4.405). λ_{min} 259 (3.806). IR ν_{max}^{nujol} cm^{-1}: 2950, 1582, 1448, 1330, 1242, 1128, 1012, 758 and 702. PMR ($CDCl_3$): δ 3.55 (6H, *s*, MeO—), 3.78 (3H, *s*, MeO—), 5.40 (2H, *s*, —O—CH$_2$—ϕ), 6.42 (2H, *s*, ϕ—CH=CH—ϕ), 6.53 (1H, *s*, 2—H or 6—H), 6.62 (1H, *s*, 6—H or 2—H) and 6.69–7.26 (9H, *m*). The needles; UV λ_{max}^{EtOH} nm (log ε): 322 (4.137), 306 (4.126) and 209 (4.396), λ_{min} 312 (4.1255) and 259 (3.792). IR ν_{max}^{nujol} 2958 w, 1578, 1257, 1130, 962 w (*trans* —HC=CH—), and 752, PMR ($CDCl_3$): δ 3.84 (9H, *s*, MeO—), 5.12 (2H, *s*, —O—CH$_2$—ϕ), 6.70 (2H, *s*, 2,6—H) and 6.87–7.64 (11H, *m*). MS *m/e*: 376 [M^+], 285 (base peak) and 257. Catalytic hydrogenation of stilbenes **10** (3.31 g) performed in the same way as for compound **4** gave 2'-hydroxy-3,4,5-trimethoxybibenzyl (**11**) as an oil (1.99 g) after prolonged shaking. UV λ_{max}^{EtOH} nm: 281 sh, 274 and 216. IR ν_{max}^{KBr} cm^{-1}: 3400, 1598, 1513, 1460, 1426, 1243 and 1130. PMR ($CDCl_3$): 2.88 (4H, *s*), 3.76 (6H, *s*), 3.82 (3H, *s*), 4.52 (1H, broad *s*), 6.37 (2H, *s*), 6.79 (2H, *m*, W = 25 Hz) and 7.03 (2H, *m*, W = 22 Hz). MS *m/e*: 288 (M^+), 182, 181 (base peak), 151 (weak), 149 and 107.

Wheat coleoptile section test. Wheat seeds (*Triticum aestivum* cv Norin No. 61) were germinated and grown on wet paper tissues in a tray under red light at 23° for the first 48 hr, and then in the dark for 22 hr. From 25 to 30 mm coleoptiles 5-mm sections were excised 4 mm below the tips and cultured on media for 18 hr in the dark at the same temp. Batatasins were dissolved in EtOH, a required amount of the soln was added to a layer of filter paper in a 3cm petri dish and dried. To the dishes so prepared 1 ml of a soln containing 1% MeOH, 0.05% Tween 20, 2% sucrose and the required concentrations of IAA and (*R, S*)-ABA were added.

Lettuce hypocotyl elongation test. Lettuce (*Lactuca sativa* cv Great Lakes No. 54) seeds were germinated in the dark at 23° for 42 hr, and the seedlings were transferred and grown in petri dishes under the same conditions for 42 hr. The length of hypocotyls was measured. Culture petri dishes were prepared as described for the wheat coleoptile section test except that sucrose and IAA were omitted and the amount of medium added was 0.5 ml/dish.

Lettuce seed germination test. In petri dishes prepared as described for the lettuce hypocotyl elongation test, 30 seeds of lettuce were sown in the dark at 23° and after 42 hr the % germination was determined.

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