Inhibition of Gibberellin Biosynthesis by Geraniol Derivatives and 17-Nor-16-azakauranes†

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Four analogues of the intermediates of gibberellin biosynthesis, 1-geranylimidazole (V) and three 17-nor-16-azakauranes (VIII), (IX) and (X) were found to inhibit gibberellin production of *Gibberella fujikuroi* by the procedure of bioassay which the author established. These compounds showed also growth retarding effects on rice seedlings.

Many researchers have recently focused their attention on the inhibitors of biologically active isoprenoid biosyntheses because these inhibitors are not only useful as a tool of the study on the biosynthetic pathways of isoprenoids, but also used as medicines or agricultural chemicals.^{1~3)}

The inhibitors of gibberellin biosynthesis have been used as plant growth retardants, and the typical plant growth retardants, AMO-1618 and phosphon D, were found to inhibit specifically the conversion of geranylgeranyl pyrophosphate (2) to copalyl pyrophosphate (3) by the kaurene synthetase purified from *Marah macrocarpus*.⁴⁾ These quaternary ammonium or phosphonium salts are active primarily on plants among the *Dicotyledonae*, but they are effective on a few species in *Monocotyledonae*.⁵⁾ These facts have stimulated the author to screen new inhibitors of gibberellin biosynthesis. The author now reports the biological activities, inhibition of gibberellin production and plant growth retarding effects of geraniol derivatives and azakauranes.

The biosynthetic pathway of gibberellins is as shown in Fig. 1⁶⁾ and many analogues of the intermediates of isoprenoid biosyntheses have been used as the biosyntheses inhibitors.¹⁾ Thus, the author prepared the analogues of geranylgeranyl pyrophosphate (2) and kaurene (4) as the candidate compounds of gibberellin biosynthesis inhibitors. The analogues of

FIG. 1. Biosynthetic Pathway of Gibberellins.

geranylgeranyl pyrophosphate were synthesized by the alkylation of the phenols or amines with geranyl bromide in the presence of sodium hydride in DMF. The geraniol derivatives thus prepared are compounds I to VII and the structures were confirmed on the basis of the IR, UV, NMR and mass spectra. The second series of the candidate compounds are 17-nor-16-azakauranes, VIII, IX and X, which were obtained by the reductive amination⁷⁾ of 17-nor-kauran-16-one derived from (–)-kaurene.

The gibberellin biosynthetic system in the mycelium of *Gibberella fujikuroi* was used for the bioassay of gibberellin biosynthesis inhibitors. The mycelium having the enzyme system for gibberellin biosynthesis was obtained by the cultivation of *G. fujikuroi* in the potato-glucose medium for 3 days. The wet mycelium which was washed with 0.5% NaCl was shown not to contain any gibberellins by the GC analysis of the methyl esters of its solvent extract. Inhibition of gibberellin production was measured by the GC analysis of

† Studies on the Inhibitors of Gibberellin Biosynthesis. Part I.

(I), O-geranylsesamol; (II), methyl *o*-geranyloxybenzoate; (III), R=H, N-geranyl, N-methylaniline; (IV), R=CO₂Me, methyl N-geranyl, N-methylantraanilate; (V), 1-geranylimidazole; (VI), 1-geranylbenzimidazole; (VII), methyl *p*-geranyloxybenzoate; (VIII), 17-nor-16-methylaminokaurane; (IX), 17-nor-16-dimethylaminokaurane; (X), 17-nor-16-dimethylaminokaurane methoiodide.

FIG. 2. GC of Methylated Acids Produced in Mycelium Suspensions.

the methyl esters of the acidic metabolites which were produced in the mycelium suspensions containing candidate compounds. The gas chromatogram of the methyl esters of the acidic metabolites produced in control suspension was as shown in Fig. 2 and the major peaks were assigned to GA₇, diacid, GA₁₃ and GA₃ using GC-MS analysis. The amount of GA₃ produced in the control was 270 μg.

When 100 ppm of compound V, 1-geranylimidazole, was added to the mycelium suspension, the production of gibberellins was completely inhibited as shown in Fig. 2. In the case of 50 or 25 ppm of V, the amounts of GA₃ decreased to 33 or 78 μg, respectively.

Table I represents the effects of geraniol

TABLE I. INHIBITION OF GIBBERELLIN PRODUCTION
Each value represents the percent inhibition.

Sample	Concentration (ppm)				
	500	200	100	50	25
I	14	—	—	—	—
II	20	—	—	—	—
III	44	17	27	21	—
IV	48	48	46	45	—
V	100	100	97	88	71
VI	100	100	79	46	33
VII	27	—	—	—	—
VIII	100	100	100	36	20
IX	100	100	78	39	23
X	100	100	82	54	53

derivatives and 17-nor-16-azakauranes on the gibberellin production of *G. fujikuroi*. 1-Geranylimidazole (V) showed about 100%

inhibition at over 100 ppm and 1-geranylbenzimidazole (VI) also showed 100% inhibition at over 200 ppm, but other geraniol derivatives showed 48% inhibition at the most even in a high concentration of 500 ppm.

17-Nor-16-azakauranes, VIII, IX and X showed about 80 to 100% inhibition at 100ppm. 1-Geranylimidazole (V) and the methoiodide (X) did not inhibit the mycelium growth of *G. fujikuroi* in liquid potato-glucose medium at the concentrations of below 100 ppm, whereas the compounds, VIII and IX, inhibited the mycelium growth at over 50 ppm. These facts suggest that V and X inhibit gibberellin biosynthesis without significant effects on the other metabolisms of *G. fujikuroi* at low concentrations of below 100 ppm, but VIII and IX are not specific inhibitors of gibberellin biosynthesis.

On the next, the plant growth retarding effects of the above derivatives except VII were examined using a standard rice seedling test described in EXPERIMENTAL. Table II shows

TABLE II. EFFECT OF GERANIOL DERIVATIVES AND 17-NOR-16-AZAKAURANES ON THE SECOND LEAF SHEATH AND ROOT GROWTH OF RICE SEEDLINGS (Normal cv., Kinmaze)

A denotes the relative growth of second leaf sheath (control: 100). B denotes the relative growth of root (control: 100).

Sample	Concentration (ppm)					
	300		100		30	
	A	B	A	B	A	B
I	90	110	89	93	93	92
II	85	99	85	105	91	100
III	78	120	84	126	87	117
IV	93	163	87	127	90	132
V	12	19	45	68	80	58
VI	70	12	89	51	87	59
VIII	0	0	34	0	74	6
IX	0	0	22	0	78	9
X	38	0	71	9	79	20

the effects of geraniol derivatives and 17-nor-16-azakauranes on the second leaf sheath and root growth of rice seedlings. Only the compounds having the inhibitory activity on gibberellin biosynthesis showed growth retarding effects on the second leaf sheath of rice seedlings. 1-Geranylimidazole (V) showed 88%

inhibition of the second leaf sheath at 300 ppm, but 1-geranylbenzimidazole (VI) only 30% inhibition at the same concentration.

On the other hand, 17-nor-16-azakauranes, VIII and IX, inhibited completely the shoot growth of rice seedlings at 300 ppm, and X showed 62% inhibition of the second leaf sheath growth at the same concentration. The decisive difference in the plant growth retarding activities of compound V and azakauranes have appeared in their effects on the root growth. Compound V showed 19% root elongation at 300 ppm, but azakauranes are considered to be very toxic to the root and only tiny roots appeared even in a low concentration of 30 ppm.

FIG. 3. Interaction between GA_3 and 1-Geranylimidazole Observed in the Second Leaf Sheath Growth of Rice Seedlings.

Then, the antagonistic effect of gibberellin on the inhibition of second leaf sheath growth induced by 1-geranylimidazole (V) was examined. The inhibitory effect of V was not completely nullified by a high concentration (1.5 ppm) of GA_3 as shown in Fig. 3. Downs and Cathey have reported that GA_3 nullified the inhibition of stem growth induced by AMO-1618 in red kidney bean plant.⁸⁾ These facts suggest that compound V might inhibit other growth process besides inhibition of gibberellin biosynthesis.

EXPERIMENTAL

IR spectra were obtained by JASCO IR-G Spectro-

photometer and UV spectra were measured in Hitachi EPS-3T Spectrophotometer. PMR spectra were recorded on a JEOL MH-100 Spectrometer with tetramethylsilane as internal standard.

MS and GC-MS were carried out using a JEOL D-100 Mass spectrometer fitted with a JEOL JGC-20K Gaschromatograph.

Syntheses of geraniol derivatives

To a suspension of 1 mmol of NaH in 0.5 ml of dry DMF in an ice bath was added 1 mmol of a phenol or an amine in 1 ml of dry DMF with vigorous stirring. Geranyl bromide (1 mmol) in 0.5 ml of dry DMF was added dropwise over a period of 10 min at 0°C. The reaction mixture was stirred for 1 hr at 0°C. After continuous stirring for another 2~18 hr at room temperature, the reaction mixture was poured onto ice water and extracted with *n*-pentane or ethyl acetate. The product was isolated by preparative thin-layer chromatography.

Properties of geraniol derivatives

O-Geranyl*l*sesamol (I), oil. MS (*m/e*): 274 (M^+), 138, 119, 117. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 2760, 940 (methylenedioxy). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 296 (1370). PMR (CDCl_3) δ : 1.5 (3H, s), 1.65 (3H, s), 1.68 (3H, s), 4.40 (2H, d, $J=6$), 5.04 (1H, m), 5.40 (1H, t, $J=6$), 5.80 (2H, s).

Methyl *o*-geranyloxybenzoate (II), oil. MS (*m/e*): 288 (M^+), 152. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1730, 1715, 1600. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 295 (5000). PMR (CDCl_3) δ : 1.57 (3H, s), 1.64 (3H, s), 1.68 (3H, s), 3.80 (3H, s), 4.38 (2H, d, $J=6$), 5.00 (1H, m), 5.42 (1H, t, $J=6$).

N-Geranyl, *N*-methylaniline (III), oil. MS (*m/e*): 243 (M^+), 107. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1600, 690. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 250 (14450). PMR (CDCl_3) δ : 1.55 (3H, s), 1.63 (3H, s), 1.67 (3H, s), 2.82 (3H, s), 3.83 (2H, d, $J=6$), 5.00 (1H, m), 5.15 (1H, t, $J=6$).

Methyl *N*-geranyl, *N*-methylantranilate (IV), oil. MS (*m/e*): 301 (M^+), 286, 244, 232, 165. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1720, 1600. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 270 (5700), 338 (2260). PMR (CDCl_3) δ : 1.58 (3H, s), 1.62 (3H, s), 1.65 (3H, s), 2.72 (3H, s), 3.65 (2H, d, $J=6$), 3.80 (3H, s), 5.04 (1H, m), 5.24 (1H, t, $J=6$).

l-Geranylimidazole (V), oil. MS (*m/e*): 204 (M^+), 136. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1665. PMR (CDCl_3) δ : 1.60 (3H, s), 1.70 (3H, s), 1.75 (3H, s), 4.56 (2H, d, $J=6$), 5.10 (1H, m), 5.38 (1H, t, $J=6$), 6.90 (1H, s), 7.06 (1H, s), 7.48 (1H, s).

l-Geranylbenzimidazole (VI), oil. MS (*m/e*): 254 (M^+), 186, 118. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1665. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 250 (6600), 255 (6800), 266 (4300), 275 (4600), 281 (4600). PMR (CDCl_3) δ : 1.50 (3H, s), 1.60 (3H, s),

1.72 (3H, s), 4.68 (2H, d, $J=7$), 5.03 (1H, m), 5.36 (1H, t, $J=7$).

Methyl *p*-geranyloxybenzoate (VII), oil. MS (*m/e*): 288 (M^+), 152. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1720, 1710. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm(ϵ): 258 (19800). PMR (CDCl_3) δ : 1.60 (3H, s), 1.68 (3H, s), 1.75 (3H, s), 3.86 (3H, s), 4.60 (2H, d, $J=6$), 5.10 (1H, m), 5.48 (1H, t, $J=6$), 6.92 (2H, d, $J=9.2$), 8.00 (2H, d, $J=9.2$).

Preparation of 17-nor-16-methylaminokaurane (VIII)

A solution of 54.8 mg (0.2 mmol) of 16-nor-kauran-16-one, 67.6 mg (1.0 mmol) of methylamine hydrochloride and 25 mg (0.8 mmol) of NaBH_3CN in 2.4 ml of absolute MeOH was stirred for 72 hr at room temperature. The methanol was removed under reduced pressure, and the residue was dissolved in 8 ml of 0.5 N HCl and washed with two 10 ml portions of ether. The aqueous layer was brought to pH 10 with 6 N KOH, saturated with NaCl, and extracted with three 20 ml portions of ethyl acetate. The extracts were dried over Na_2SO_4 and evaporated to give 49 mg of VIII as a colorless powder in 82% yield. mp 47.5~49°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 2770. PMR (CDCl_3) δ : 0.80 (3H, s), 0.84 (3H, s), 1.02 (3H, s), 2.40 (3H, s). MS (*m/e*): 289 (M^+), 274 ($M^+ - \text{CH}_3$), 258 ($M^+ - \text{CH}_3 - \text{CH}_4$), 233 ($M^+ - 2 \times \text{CH}_3 - \text{CH}_4$).

Preparation of 17-nor-16-dimethylaminokaurane (IX)

IX was prepared by the same procedure used for the preparation of VIII in 89% yield as a colorless powder. mp 103~106°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 2810, 2770, 2720 ($-\text{N}(\text{CH}_3)_2$). PMR (CDCl_3) δ : 0.80 (3H, s), 0.85 (3H, s), 1.04 (3H, s), 2.20 (6H, s). MS (*m/e*): 303 (M^+), 288 ($M^+ - \text{CH}_3$).

Preparation of 17-nor-16-dimethylaminokaurane methiodide (X)

A solution of 50 mg of IX and 0.2 ml of methyl iodide in 2 ml of methanol was heated under reflux for 2 hr. After evaporation of methanol the residue was crystallized from methanol to give 51 mg of X as colorless prisms in 57% yield. mp 293~298°C (decomp.). PMR (CDCl_3) δ : 0.80 (3H, s), 0.85 (3H, s), 1.00 (3H, s), 3.49 (9H, s). MS (*m/e*): no M^+ , 303 ($M^+ - \text{CH}_3\text{I}$), 288 ($M^+ - \text{CH}_3\text{I} - \text{CH}_3$).

Bioassay of microbial gibberellin biosynthesis

Surface mycelium of *G. fujikuroi* grown for 3 days on potato-sucrose agar slant which is a kind gift from Prof. N. Takahashi was inoculated into 100 ml of potato-glucose medium in 500 ml Sakaguchi flask. After 3 days, vegetative cell cultures were transferred (2 ml per 100 ml fresh medium) and shaken at 30°C for 3 days. The mycelium was filtered through filter paper and washed with 40 ml of 0.5% NaCl solution 5 times.

The washed mycelium (0.3 g) was soaked in 20 ml of acetone for 2 days and repeated the same treatment twice. The acetone was evaporated and the residual solution was made alkaline and extracted with 5 ml of EtOAc, and then acidified to pH 2 and extracted with 5 ml of EtOAc twice. No gibberellins were detected in the methyl esters of the acidic extract by GC.

A sample was added to the bottom of a test tube as EtOAc solution and the solvent was evaporated. The sample was then suspended in 0.5 ml of 8% Tween 20. To the test tube 0.3 g of the wet mycelium and 4.5 ml of 0% ICI medium¹⁰⁾ were added and shaken at 30°C for 2 days. The mycelium was then filtered and the filtrate was made alkaline to pH 10 and washed with 5 ml of EtOAc to remove the neutral metabolites, and then acidified to pH 2 and extracted with 5 ml of EtOAc twice.

The acidic extract was concentrated and methylated with diazomethane. The methyl esters of the acidic metabolites were analyzed by GC: 3% OV-1 on Gas-chrom Q (80~100 mesh), 1 m, carrier; 0.8 kg/cm² of N₂, column temp. 215°C.

The acidic metabolites produced in the control suspension were subjected to GC-MS analysis and the major peaks were identified as the methyl esters of GA₇, diacid, GA₁₃ and GA₃ by comparison with the mass spectra of authentic compounds.⁹⁾

Rice seedling test

Ten rice seeds (*Oryza sativa* L. normal cv., Kinmaze) just after germination were transferred into a sample

tube (7×3.2 cm), to which 1 ml of distilled water containing a finite amount of a test sample was added. The sample tube was covered with a polyethylene cap and kept at 30°C in 12 hr light condition.

After 6 days, the lengths of second leaf sheath and root were measured.

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