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Synthesis and Root Growth-promoting Activity of Capillarol and Its Derivatives

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The root growth-promoting factor, capillarol, and derivatives were synthesized from *p*-hydroxybenzaldehyde by photo-Fries rearrangement and their root growth-promoting activity for rice seedlings was studied.

Key words: synthesis; capillarol; photo-Fries rearrangement; root growth-promoting factor

Capillarol (**1**) was isolated in 1986 by Ueda *et al.* from *Artemisia capillaris* Thunb.[1] The structure of this compound was elucidated as methyl *p*-hydroxy-*m*-(3-methyl-2-butenoyl)cinnamate and it promoted rice root growth. We undertook the synthesis of **1** and its derivatives and studied their biological activity on Tan-ginbozu rice seedlings.

Materials and Methods

Experimental. IR spectra were recorded with Jasco IR-810 and Jasco FT/IR-5300 spectrometers. ^{1}H-NMR spectra were recorded with Hitachi R-600 (60 MHz) and Jeol JNM-EX400 (400 MHz) spectrometers in $CDCl_3$. ^{13}C-NMR spectra were recorded with a JNM-EX400 (125 MHz) spectrometer in $CDCl_3$. Chemical shifts for ^{1}H-NMR and ^{13}C-NMR data are expressed in ppm based on the signal of TMS at 0 ppm. Mass spectra were recorded with a Jeol JMS-AX505HA mass spectrometer (70 eV). UV spectra were recorded with a Hitachi U-2001 spectrometer, and UV irradiation was performed with an Eikohsha EHB-100 instrument, using an EV-H high-pressure mercury arc lamp. Column chromatography was performed on Merck Kieselgel 60 (Art Nr. 7734), and preparative silica gel TLC, on Merck Kieselgel F-254 (2 mm). Melting point data are uncorrected. GLC analysis was conducted with a Shimadzu GC-14A gas chromatograph, using a DB-5 capillary column (15 m × 0.25 mm, J&W Scientific).

Methyl p-(3-methyl-2-butenoyloxy)cinnamate (**3**). 3,3-Dimethyl-2-butenoyl choride (4.74 g, 40.0 mmol)) in benzene (10 ml) was added to methyl *p*-hydroxycinnamate **2** (5.00 g, 0.03 mol) and pyridine (4 g, excess) in benzene (20 ml) at room temperature, and the solution was stirred for 3 hours. The solution was poured into dil. hydrochloric acid and extracted with chloroform. The chloroform solution was successively washed with water, a 3% $NaHCO_3$ aq. solution and water, and dried over $MgSO_4$. Evaporation of the solvent gave a solid, which was recrystallized from hexane to give **3** as light yellow needles (5.98 g, 82%), mp 74–76°C; IR √max cm^{-1} (film): 1720, 1655, 1640, 1600, 1505, 1440, 1322, 1212, 1172, 1120, 1065; ^{1}H-NMR (60 MHz) δ: 2.05 (3H, s, CH_3), 2.25 (3H, s, CH_3), 3.81 (3H, s, CO_2CH_3), 5.90 (1H, brs, $Me_2C{=}CH$), 6.35 (1H, d, J=15.6 Hz, $CHCO_2Me$), 7.15 (2H, d, J=9.6 Hz, Ar-H), 7.55 (2H, d, J=9.6 Hz, Ar-H), 7.80 (1H, d, J=15.6 Hz, Ar-CH). *Anal.* Found: C, 68.99; H, 6.41%. Calcd. for $C_{15}H_{16}O_4$: C, 69.21; H, 6.20%.

p-(3-Methyl-2-butenoyloxy)benzaldehyde (**5**). *p*-Hydroxybenzaldehyde **4** (5.00 g, 0.04 mol) was treated with 3,3-dimethyl-2-butenoyl chloride (5.93 g, 0.05 mol) in the same manner as that for **3**. The crude product was recrystallized from ethanol to give **5** as light yellow needles (5.36 g, 64%), mp 75–77°C; IR √max cm^{-1} (nujol): 1735, 1695, 1645, 1600, 1590, 1505, 1350, 1300, 1260, 1220, 1180, 1160, 1130, 1120, 1070, 1010, 870, 845, 770, 760; ^{1}H-NMR (60 MHz) δ: 2.00 (3H, s, CH_3), 2.20 (3H, s, CH_3), 5.90 (1H, bs, C=CH), 7.25 (2H, d, J=8 Hz, Ar-H), 7.90 (2H, d, J=8 Hz, Ar-H), 10.00 (1H, s, CHO). *Anal.* Found: C, 70.82; H, 5.51%. Calcd. for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92%.

p-(3-Methyl-2-butenoyloxy)benzaldehyde ethyleneacetal (**6**). A mixture of benzaldehyde **5** (10.0 g, 0.05 mol), 2-ethyl-2-methyl-1,3-dioxolane (17.1 g) and a trace of *p*-toluenesulfonic acid in benzene (100 ml) was stirred overnight at room temperature. Sodium carbonate was added to the mixture, and the solution was washed with water and dried over $MgSO_4$. After the solvent had been removed, the residue was chromatographed over silica gel (200 g). Elution with hexane-ethyl acetate (15:1) gave a crude product, which was recrystallized from hexane-ethyl acetate to give pure **6** (5.21 g, 42%) as white needles, mp 77.0–78.5°C; IR √max cm^{-1} (film): 1730, 1650, 1610, 1595, 1515, 1310, 1225, 1200, 1170, 1125, 1075, 980, 960, 940, 875, 850, 835; ^{1}H-NMR (60 MHz) δ: 1.97 (3H, s, C=C-CH_3), 2.22 (3H, s, C=C-CH_3), 4.05 [4H, s, $(CH_2)_2$], 7.10 (2H, d, J=8 Hz, Ar-H), 7.50 (2H, d, J=8 Hz, Ar-H). *Anal.* Found: C, 68.01; H, 6.28%. Calcd. for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50%.

p-(1,3-Dioxolan-2-yl)-o-(3-methyl-2-butenoyl)phenol

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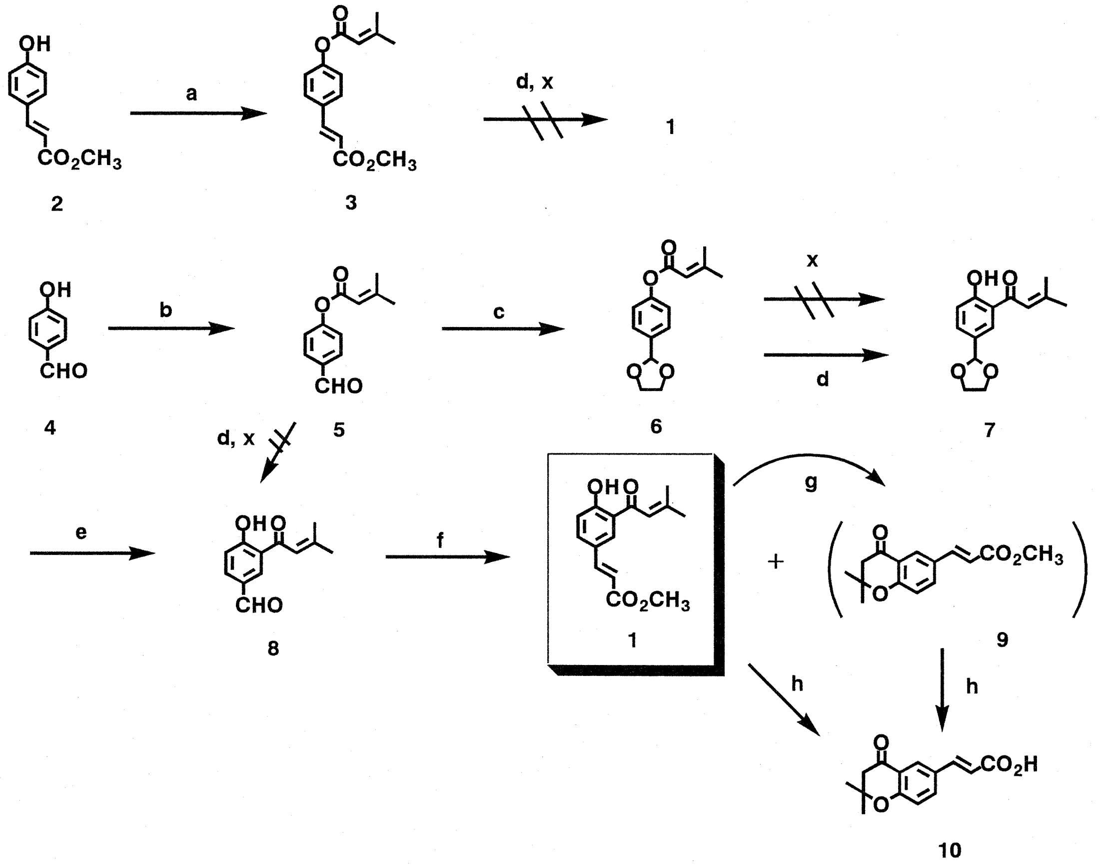

Scheme 1.

a) 3,3-dimethyl-2-butenoyl chloride, Pyr, benzene, 82%
b) 3,3-dimethyl-2-butenoyl chloride, Pyr, benzene, 64%
c) 2-ethyl-2-methyl-1,3-dioxolane, *p*-TsOH, benzene, 42%
d) *hν*, 300 W, K_2CO_3, hexane, 49%
e) AcOH aq., MeOH, 89%
f) $Ph_3P{=}CHCO_2CH_3$, CH_2Cl_2, 51% for **1** and 28% for **9**
g) CH_3SO_3H, CH_2Cl_2, 95%
h) 1M-NaOH (80% MeOH aq.), quant.
x) Lewis acid ($AlCl_3$, $TiCl_4$ or PPA)

(7).[2] A solution of the ester **6** (625 mg, 2.25 mmol) in hexane (500 ml) with anhydrous potassium carbonate (2.1 g) was irradiated for 16 hours with magnetic stirring, using a 300 W high-pressure mercury Arc (double jackets of quartz). After irradiation, the potassium carbonate was filtered off, and washed thoroughly with dichloromethane. The organic solutions were combined and concentrated *in vacuo*. The residue was submitted to chromatography, using benzene-ethyl acetate (10:1) as the eluent, to give crude **7** (306 mg, 49%); ^{1}H-NMR (60 MHz) δ: 2.05 (3H, s, CH_3), 2.22 (3H, s, CH_3), 4.06 [4H, s, $(CH_2)_2$], 5.75 (1H, s, O-CH), 6.80 (1H, s, =CH), 6.95 (1H, d, J=8 Hz, Ar-H), 7.55 (1H, d, J=8 Hz, Ar-H), 7.85 (1H, s, Ar-H).

p-Hydroxy-m-(3-methyl-2-butenoyl)benzaldehyde (**8**). Aqueous acetic acid (5%, 1 ml) was added to crude acetal **7** (99 mg, 0.40 mmol) in methanol (3 ml), and the mixture was stirred overnight at room temperature. The solution was extracted three times with dichloromethane, and the combined organic layer was successively washed with aqueous sodium hydrogen carbonate (5%) and water, and dried over $MgSO_4$. The organic layer was concentrated *in vacuo* to give a solid, which was recrystallized from hexane-ethyl acetate, yielding **8** as pure yellow needles (73 mg, 89%), mp 107–109°C; IR √max cm^{-1} (nujol): 1695, 1635, 1610, 1575, 1490, 1450, 1430, 1380, 1360, 1300, 1260, 1215, 1210, 1175, 850, 720; ^{1}H-NMR (60 MHz) δ: 2.10 (3H, d, J=1 HZ, CH_3), 2.25 (3H, d, J=1 Hz, CH_3), 6.85 (1H, bs, =CH), 7.06(2H, d, J=8 Hz, Ar-H), 7.95 (2H, dd, J=8,1 Hz, Ar-H), 8.30 (1H, d, J=1 Hz, Ar-H), 9.88 (1H, s, CHO). *Anal.* Found: C, 70.78; H, 6.23%. Calcd. for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92%.

Methyl p-hydroxy-m-(3-methyl-2-butenoyl)cinnamate (Capillarol), (**1**) and *Methyl 6-(2,2-Dimethyl-4-chromanone)acrylate* (**9**). To aldehyde **8** (100 mg, 0.490 mmol) in absolute dichloromethane (2 ml) under a nitrogen gas atmosphere at 5°C, methyl (triphenylphosphoranylidene)acetate (180 mg, 0.540 mmol) in dichloromethane (2 ml) was added dropwise, and the solution was stirred for 2 hours. After the solution had been stirred at 25°C for an additional 15 hours, a saturated aq. sodium chloride solution was introduced. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was separated by preparative TLC [hexane-$CHCl_3$-AcOEt (10:10:1)] as the eluent to give capillarol (**1**) and **9**. Analytical samples were obtained by recrystallization from hexane-ether to give pure **1** (65 mg, 51%) and **9** (35 mg, 28%), respectively. The column oven temperature for GLC was programmed at 170°C for 2 min, before elevation to 300°C at 30°/min.

Data for **1**: Mp 115.5–116.0°C; IR √max cm^{-1} (film): 1702, 1635, 1610, 1570, 1500, 1435, 1360, 1320, 1300, 1275, 1255, 1220, 1200, 1175, 1040, 1020, 860; UV λmax (in EtOH) nm(e): 355 sh(300), 300 sh(20900), 280 (30235); MS m/z (rel. int.): 260 [M^+](90), 245 (100), 213 (100), 205 (73), 173 (70), 145 (40); ^{1}H-NMR (400 MHz) δ: 2.09 (3H, d , J=1 Hz, CH_3), 2.24 (3H, d, J=1 Hz, CH_3), 3.81 (3H, s, CO_2CH_3), 6.34 (1H, d, J=16 Hz, Ar-CH=C), 6.80 (1H, m, $W_{1/2}$=4 Hz, CH=C), 7.00 (1H, d, J=9 Hz, Ar-H), 7.65 (1H, d, J=16 Hz, C=$CHCO_2$), 7.65 (1H, dd, J=2, 9 Hz, Ar-H), 7.90 (1H, d, J=2 Hz, Ar-H), 13.08 (1H, s, hydrogen-bonded OH); ^{13}C-NMR (100 MHz) δ: 21.6, 28.4, 51.7, 115.9, 119.40, 119.43, 120.5, 125.2, 130.7, 134.3, 143.8, 159.7, 165.1, 167.5, 195.8. *Anal.* Found: C, 68.90; H, 6.51%. Calcd. for $C_{15}H_{16}O_4$: C, 69.21; H, 6.20%.

Data for **9**: Mp 134–135; IR √max cm^{-1} (film): 3080, 3050, 2960, 2930, 1705, 1690, 1630, 1603, 1570, 1490, 1460, 1430, 1370, 1308, 1290, 1260, 1190, 1175, 1100, 990, 930; UV λmax (in EtOH) nm(e): 292 (23,000), 249 (19,600); MS m/z (rel. int.): 260 [M^+](80), 245 (100), 229 (20), 213 (30), 205 (75), 185 (5), 173 (45), 145 (25); ^{1}H-NMR (400 MHz) δ: 1.48 (6H, s, $CH_3 \times 2$), 2.75 (2H, s, CH_2), 3.80 (3H, s, CO_2CH_3), 6.37 (1H, d, J=16 Hz, Ar-CH=C), 6.97 (1H, d, J=8 Hz, Ar-H), 7.64 (1H, d, J=16 Hz, C=CH-CO_2CH_3), 7.64 (1H, dd, J=2, 8 Hz, Ar-H), 8.02 (1H, d, J=2 Hz, Ar-H); ^{13}C-NMR (100 MHz) δ: 26.65, 48.74, 51.69, 79.96, 116.93, 119.20, 120.17, 126.74, 127.32, 135.04, 143.45, 161.32, 167.39, 191.84. *Anal.* Found: C, 68.95; H, 6.45%. Calcd. for $C_{15}H_{16}O_4$: C, 69.21; H, 6.20%.

Compound **9** *from capillarol* (**1**). A mixture of compound **1** (10.0 mg, 0.040 mmol) and a drop of methanesulfonic acid in dichloromethane (5 ml) was heated for 0.5 hr at 40°C and then allowed to stand at room temperature for 1 hr. The addition of powdered sodium hydrogen carbonate to the mixture and subsequent filtration afforded a solid, which was recrystallized from hexane to give pure **9** (9.5 mg) as colorless crystals in a 95% yield.

6-(2,2-Dimethyl-4-chromanone)acrylic acid (**10**). Ester (capillarol) **1** (26 mg, 0.10 mmol) was dissolved in 2 ml of 1 M NaOH (80% MeOH), and the solution was left overnight at room temperature. Acidification and subsequent dichloromethane extraction afforded carboxylic acid **10** (24 mg) in a quantitave yield. The crude solid was recrystallized from hexane to give a pure crystal, mp 230–233°C; IR √max cm^{-1}: 3200–2800, 1690, 1685, 1620, 1610, 1565, 1490, 1460, 1430, 1310, 1280, 1260, 1210, 1190, 1140; UV λmax (in EtOH) nm(e): 210(4301), 249(9890), 277(7384), 341(1123); MS m/z (rel. int.): 246[M^+](60), 231(100), 213(30), 191(65), 145(15); ^{1}H-NMR (60 MHz) δ: 1.51(6H, s, $CH_3 \times 2$), 2.72(2H, s, CH_2), 6.35(1H, d, J=16 Hz, Ar-CH=C), 6.95(1H, d,J=8 Hz, Ar-H), 7.65(1H, d, J=16 Hz, C=CH-CO_2), 7.65(1H, dd, J=2, 8 Hz, Ar-H), 8.05(1H, d, J=2 Hz, Ar-H). *Anal.* Found: C, 68.44; H, 5.41%. Calcd. for $C_{14}H_{14}O_4$, C, 68.28; H, 5.73%.

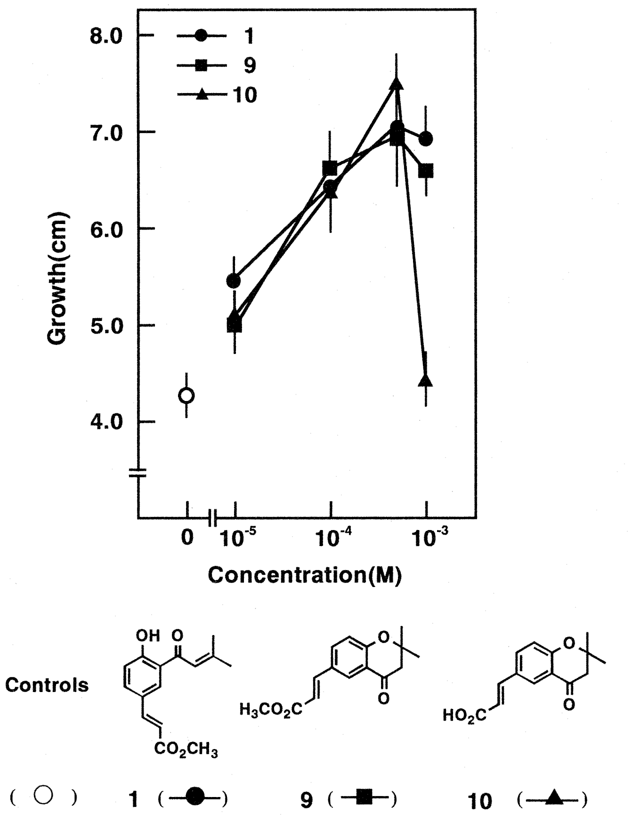

Fig. 1. Effects of Capillarol and Related Compounds on the Seminal Root Growth of Tan-ginbozu Rice Seedlings.

Chromanone ester **9** was treated in a similar manner as that for capillarol **1** to give acid **10** in a quantitative yield.

Bioassay. The aqueous culture method for rice seedlings (*Oryza sativa* L. cv Tan-ginbozu) was used for a root growth-promoting bioassay in the same manner as that in the literature.[1] The test compound was dissolved in a small amount of solvent and put into a sterilized test tube of 2.8 cm diameter. After evaporating the solvent to dryness *in vacuo*, 1 ml of distilled water was added to the test tube. Rice seedlings (10) just after germination were transferred to the test tube, which was then covered with polyethylene film. Each test tube was kept under continuous white fluorescent light (FLR 40S D/M, Matsushita Electric Industrial Co., Japan) of 8.6 Wm^{-2} at 30°C for 5 days. At the end of the incubation period, the root length of the seedling was measured.

Results and Discussion

The key point in the synthesis of capillarol (**1**) was the introduction of a butenoyl group at the ortho position to the hydroxyl group of the desired molecule. A convenient method was Fries rearrangement of a phenolic ester such as **3** (Scheme 1), which proved an easy way to get capillarol directly. However, under the usual Fries rearrangement, using a Lewis acid ($AlCl_3$, $TiCl_4$ or PPA) as the catalyst, it is difficult for the acyl group possessing a conjugated double bond such as a butenoyl group to migrate.[3] In addition the α, β-unsaturated ester at the benzylic position like a cinnamate is unstable to a Lewis acid at high temperature. Rearrangement was initially tried on compound **3**, which was derived from methyl *p*-hydroxycinnamate **2** and 3,3-dimethyl-2-butenoyl chloride in an 82% yield; however, some multiple-polymeryzed or indistinct degradation products were observed with any catalyst used. So we chose *p*-hydroxybenzaldehyde **4** as our starting material, whose formyl group is easy to convert to an unsaturated ester group.

3,3-Dimethyl-2-butenoyl chloride was added to aldehyde **4** to give phenolic ester **5** as slightly yellow needles (mp 75–77°C, 64%), and protection of the formyl group of **5** as ethylene acetal with 2-ethyl-2-methyl-1,3-dioxolane afforded **6** (mp 77.0–78.5°C, 42%). Although rearrangement to **8** or **7** of these compounds (**5** or acetal **6**) were tried with several Lewis acids, no migration products were obtained. So another photo-Fries rearrangement, which is predominantly an intramolecular free-radical process with UV light in the absence of any catalyst to protect the unstable functional group from the Lewis acid,[2] was investigated.

After irradiating the phenolic ester group of **3**, **5** and **6** in hexane with a high-pressure mercury lamp in the presence of potassium carbonate, only compound **6** gave rearranged product **7** in a 49% yield, while the other compounds gave multiple indistinct products. Acetal **7** was hydrolyzed by aq. acetic acid to give aldehyde **8** as a yellow crystal (mp 107–109, 89%).

Finally, condensation of aldehyde **8** with methyl (triphenyl-phosphoranylidene)acetate in dichloromethane at 0°C gave capillarol (**1**) as yellow needles (mp 115.5–116.0°C, 51%), as a single *E*-isomer, no *Z*-isomer being apparent by a ^{1}H-NMR (400 MHz) analysis. In addition a GLC analysis of **1** also showed a single peak. In this step, chromanone **9** (mp 134–135°C, 28%) was produced together with **1** by an intramolecular Michael addition. Treatment of **1** with methanesulfonic acid also yielded **9** in a 95% yield, and carboxylic acid **10** could be prepared from **1** or **9** by NaOH-MeOH in a quantitative yield. The ^{1}H-NMR, UV and MS spectral data for synthetic **1** and **9** were identical with those of the natural products,[1] and the ^{13}C-NMR and IR spectral data for synthetic **1** and **9** showed reasonable structural features.

Fig. 1 shows the effect of **1**, **9** and **10** on the root elongation of the dwarf rice cultivated, Tan-ginbozu. All of these compounds promoted the elongation of the roots. Compound **1** was more active than **9** and **10** at concentrations between 10^{-5} M and 5×10^{-5} M. The biological activities of **1** and **9** leveled off at concentrations higher than 10^{-4} M. In contrast, the biological activity of **10** increased linearly up to 5×10^{-4} M. The subsequent disappearance of the biological activity of **10** at 10^{-3} M seems to indicate that this concentration was an overdose and thereby nullified the growth effect.

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