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RAPHANUSOL A, A NEW GROWTH INHIBITOR FROM SAKURAJIMA RADISH SEEDLINGS

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Key Word Index—*Raphanus sativus*; Cruciferae; Sakurajima radish; 1-β,4-di-*O*-(4-hydroxy-3,5-dimethoxycinnamoyl) gentiobiose; raphanusol A; growth inhibitor.

Abstract—A new growth inhibitor, raphanusol A, isolated from an acetone extract of light-exposed seedlings of Sakurajima radish, was characterized as 1-β,4-di-*O*-(4-hydroxy-3,5-dimethoxycinnamoyl) gentiobiose by chemical methods and spectral data.

INTRODUCTION

In a previous investigation [1], we reported that light-induced growth inhibition of Sakurajima radish (*Raphanus sativus* var. *hortensis* f. *gigantissimus* Makino) seedlings was caused by the increase in six endogenous growth inhibitors. Two of them (raphanusol A and B) have been isolated from light-exposed seedlings of Sakurajima radish, and one (raphanusol B) has been characterized as 1 - β - *O* - (4 - hydroxy - 3,5 - dimethoxycinnamoyl) - D - glucose [2, 3]. In this report we describe the characterization of raphanusol A.

RESULTS AND DISCUSSION

Raphanusol A (**1**) was crystallized from acetone–benzene as a colourless powder, mp 137–138°, $[\alpha]_D^{22}$ − 79.4° (MeOH; *c* 0.23) and $C_{34}H_{42}O_{19}$ (*m/z* 754). It gave a purple colour with Molisch's reagent, a violet colour with vanillin–sulphuric acid and a brown colour with ferric chloride solution. Its spectral data were as follows: IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2930 (Me), 1692 (conjugated ester), 1608, 1510, 1455 (arom. ring); ^1H NMR (60 MHz Me_2CO-d_6 + D_2O): δ 3.97 (3H × 4, *s*, –OMe), 5.56 (1H, *d*, *J* = 8 Hz, anomeric proton), 5.58 (1H, *dd*, *J* = 8 Hz, H-4 of D-glucose), 6.57 (1H, *d*, *J* = 16.6 Hz, *trans*-CH=CH–), 6.59 (1H, *d*, *J* = 16 Hz, *trans*-CH=CH–), 7.18 (4H, *s*), 7.85 (1H, *d*, *J* = 16 Hz, *trans*-CH=CH–) and 7.92 (1H, *d*, *J* = 16.6 Hz, *trans*-CH=CH–); UV λ_{max}^{EtOH} nm (log ε): 333 (3.44), 241 (3.37), 227 (sh) and 204 (3.44). Acetylation of raphanusol A with acetic anhydride–pyridine afforded an octa-acetate. The IR spectrum indicated no hydroxyl absorption and in the ^1H NMR spectrum, exhibited signals for eight acetoxyl methyl protons [δ 2.09 (3H × 6, *s*) and 2.31 (3H × 2, *s*)].

Acid hydrolysis of raphanusol A yielded D-glucose, but its aglycone could not be obtained. On methanolysis with methanol–hydrochloric acid, raphanusol A afforded mainly an aglycone and a monoglucoside. The aglycone, on hydrolysis with aq. 2M sodium hydroxide gave an acid, mp 202–204° whose IR spectrum was identical with that of authentic 4-hydroxy-3,5-dimethoxycinnamic acid. The methylate of the monoglucoside, on hydrolysis with aq. 2M sodium hydroxide gave 1,2,3,6-tetra-*O*-methyl-α-D-glucoside which was identical (IR, TLC) with an authentic sample of 3,4,5-trimethoxycinnamic acid. In addition, the ^1H NMR spectrum (δ 5.56, 1H, *d*, *J* = 8 Hz) and $[\alpha]_D^{22}$ value of raphanusol A showed that 4-hydroxy-3,5-dimethoxycinnamic acid was linked to C-1 of the monoglucoside in which 4-hydroxy-3,5-dimethoxycinnamic acid was linked to C-4 of D-glucose, with β-orientation. The permethylate of raphanusol A, on hydrolysis with methanol–hydrochloric acid soln, afforded 2,3-di-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-glucose which were identical (TLC, PC) with authentic samples. Raphanusol A, on methanolysis with sodium methoxide, yielded approximately equal amounts of the methyl ester of 4-hydroxy-3,5-dimethoxycinnamic acid and the biose whose acetate was identical (IR, TLC) with the authentic octa-acetate of gentiobiose. Furthermore, the presence of the terminal D-glucose was shown by peaks at *m/z* 331, 271, 211 and 169 in the mass spectrum of the acetate of raphanusol A. In raphanusol A, therefore, the D-glucose and 1 - β,4 - di - *O* - (4 - hydroxy - 3,5 - dimethoxycinnamoyl) - D - glucose are joined by a β1→6 link.

These data indicated that the structure of raphanusol A is 1 - β,4 - di - *O* - (4 - hydroxy - 3,5 - dimethoxycinnamoyl) gentiobiose (**1**).

EXPERIMENTAL

Extraction and isolation. Seedlings of Sakurajima radish grown in the dark for 2 days at 25° were irradiated with white fluorescent light (2 W/m²) for 1 day at 25°. 10 kg

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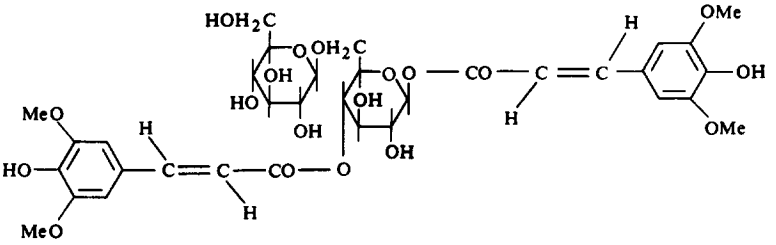

1 Raphanusol A

seedlings were extracted with 80% Me_2CO. The Me_2CO soln was concd *in vacuo* to give an aq. residue. The residue was adjusted to pH 7.5 with KPi buffer and extracted with EtOAc. The EtOAc fraction (35.4 g, brown oil) was chromatographed on Si gel with a C_6H_6–EtOAc solvent system containing increasing amounts of EtOAc, then with Me_2CO, and finally with MeOH. The MeOH eluate was purified by TLC ($CHCl_3$–HOAc, 19:1, $CHCl_3$–MeOH, 4:1). Biological activity was determined using the Sakurajima radish hypocotyl test [1]. An active eluate from the TLC plate was further purified by HPLC (Waters Associates system 500, 25 kg/cm^2, H_2O–MeOH, 1:1, Prep Pak-500/C_{18}) to yield a yellowish oil which on crystallization (Me_2CO–C_6H_6) gave a colourless powder (37 mg).

Raphanusol A (1). Mp 137–138°, $[\alpha]_D^{22}$ −79.4° (MeOH; *c* 0.23). (Found: C, 52.46; H, 5.59. $C_{34}H_{42}O_{19}\cdot H_2O$ requires: C, 52.84; H, 5.70%). UV λ_{max}^{EtOH} nm (log ϵ): see Results and Discussion; IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 1692, 1630, 1608, 1510, 1455, 1428, 1370, 1335, 1280, 1250, 1220, 1170, 1152, 1110, 1050, 992, 930 and 830; ^{1}H NMR: see Results and Discussion; FD/MS *m/z* 777 $[M+23(Na)]^+$.

Acetylation. Treatment of raphanusol A with Ac_2O–pyridine overnight at room temp. gave the octa-acetate of raphanusol A. IR: no OH absorption; ^{1}H NMR (60 MHz): δ 2.09 (3H×6, *s*), 2.31 (3H×2, *s*) and 3.88 (3H×4, *s*); MS: *m/z*: no $[M]^+$, 537, 494, 331, 271, 211, 169, 109 and 43 (base peak).

Acid methanolysis. Raphanusol A (15 mg) was dissolved in MeOH, and refluxed with 2M HCl for 2 hr, diluted with H_2O and extracted with Et_2O. The Et_2O soln was evaporated to dryness; the residue (8 mg) was shown to be a complicated mixture (TLC, Si gel). The H_2O layer was neutralized with basic ion exchange resin (Amberlite 400), and evaporated to dryness. The presence of D-glucose in the residue (3 mg) was shown by PC (*n*-BuOH–HOAc–H_2O, 4:1:2, R_f 0.33).

Mild methanolysis. Raphanusol A (15 mg) was dissolved in MeOH containing a drop of conc HCl, and heated for 1 hr at 50°. After dilution with H_2O, the soln was extracted with Et_2O. The Et_2O soln was washed with H_2O, dried, and evaporated to dryness. The residue (MS *m/z*: 238 $[M]^+$, ^{1}H NMR: δ 3.64 (3H, *s*) and 3.86 (3H×2, *s*)) was refluxed in aq. 2M NaOH for 1 hr, diluted with H_2O and extracted with Et_2O. The Et_2O extract was evaporated to give an acid, mp 202–204° whose IR spectrum (3500–2400, 1660, 1621, 1597 and 1515 cm^{-1}) was identical with that of authentic 4-hydroxy-3,5-dimethoxycinnamic acid [4]. The aq. layer was extracted with EtOAc. The residue from the aq. layer was too small an amount to be analysed. The EtOAc extract was evaporated to give a monoglucoside: MS *m/z* 400 $[M]^+$; ^{1}H NMR: δ 3.34 (3H, *s*, –OMe), 3.99 (3H×2, *s*, –OMe), 6.35 (1H, *d*, *J* = 16.4 Hz, *trans*-CH=CH–), 6.96 (2H, *s*) and 7.63 (1H, *d*, *J* = 16.4 Hz, *trans*-CH=CH–). The monoglucoside was methylated by the Purdie method, and the permethylate was refluxed in aq. 2M NaOH for 1 hr to give 1,2,3,6-tetra-*O*-methyl-α-D-glucoside which was identical with an authentic sample [IR ν_{max}^{nujol} cm^{-1}: 3550, 1305, 1190, 1108, 1050, 995, 942, 908 and Si gel TLC (2.5% MeOH–$CHCl_3$, R_f (0.52)], and 3,4,5-trimethoxycinnamic acid whose IR spectrum (3500–2500, 1716, 1687, 1640, 1590, 1500, 1330, 1261 and 1115 cm^{-1}) was identical with that of the authentic compound [4]. 10 mg of raphanusol A was dissolved in MeOH and NaOMe, and heated for 25 min at 50° and below. The mixture was diluted with H_2O and extracted with Et_2O. The Et_2O extract was evaporated to dryness. The residue (4 mg, *m/z* 238 $[M]^+$) was identical with the methyl ester of 4-hydroxy-3,5-dimethoxycinnamic acid. Treatment of the aq. layer (7 mg) with Ac_2O–pyridine overnight at room temp. gave the octa-acetate of gentiobiose which was identical with authentic sample [IR ν_{max}^{nujol} cm^{-1}: 1752, 1430, 1363, 1220, 1040, 982, 930, 908) and Si gel TLC (3% MeOH–$CHCl_3$, R_f 0.30 (cf. octa-acetyl maltose, R_f 0.41)].

Permethylation. Raphanusol A (6 mg) treated by the Purdie method, was hydrolysed with MeOH containing a drop of conc HCl followed by aq. 2M NaOH. The aq. phase was neutralized with an acidic ion exchange resin (Amberlite IRC-120), and evaporated to dryness. Si gel TLC (5% MeOH–$CHCl_3$) and PC (*n*-BuOH–HOAc–H_2O, 4:1:2) of the residue showed the presence of 2,3-di-*O*-methyl-D-glucose (TLC, R_f 0.21, PC, R_f 0.76; authentic sample: TLC, R_f 0.20, PC, R_f 0.76) and 2,3,4,6-tetra-*O*-methyl-D-glucose (TLC, R_f 0.68, PC, R_f 0.90; authentic sample: TLC, R_f 0.68, PC, R_f 0.90).

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