Phytochemistry, Vol 22, No 5, pp 1275–1276, 1983
Printed in Great Britain

0031-9422/83/051275-02$03 00/0

SHORT REPORTS

A GROWTH INHIBITOR, 2-THIOXOTHIAZOLIDINE-4-CARBOXYLIC ACID FROM SAKURAJIMA RADISH SEEDLINGS

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(*Revised received* 27 *September* 1982)

Key Word Index—*Raphanus sativus*, Cruciferae; Sakurajima radish, 2-thioxothiazolidine-4-carboxylic acid; growth inhibitor.

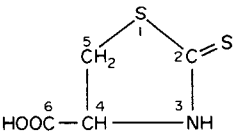
Abstract—A new growth inhibitor isolated from an acetone extract of light-exposed seedlings of Sakurajima radish, was characterized as 2-thioxothiazolidine-4-carboxylic acid by spectral data It inhibited the growth of hypocotyl sections of radish seedlings and intact hypocotyls of lettuce seedlings at concentrations higher than 3 mg/l.

INTRODUCTION

Light-induced inhibition of hypocotyl growth of Sakurajima radish (*Raphanus sativus* var. *hortensis* f. *gigantissimus* Makino) seedlings was reported to be caused by the increase in several endogenous growth inhibitors in relation to the exposure to light [1]. Of the growth inhibitors three neutral ones were isolated from light-exposed seedlings of Sakurajima radish and identified as 1-*β*,4-di-*O*-(4-hydroxy-3,5-dimethoxycinnamoyl) gentiobiose [2, 3], 1-*β*-*O*-(4-hydroxy-3,5-dimethoxycinnamoyl)-D-glucose [4] and 3-methoxy-4-methylthio-2-piperithione [5] by spectrometric analyses and chemical responses. In this paper we wish to report the chemical structure and biological activities of an acidic compound isolated from light-exposed seedlings of Sakurajima radish.

Table 1. ^1H and ^{13}C NMR chemical shifts (δ values from TMS) and multiplicities of **1** (in CD_3COCD_3) signals

Position	^1H	^{13}C
1	—	—
2	—	204.37 (*s*)
3	5.45 (*br s*)	—
4	4.97 (*ddd*, J = 8, 5 and 1 Hz)	67.05 (*d*)
5	3.85 (AB part of ABX, J = 12, 8 and 5 Hz)	38.34 (*t*)
6	5.45 (*br s*)	173.90 (*s*)



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RESULTS AND DISCUSSION

2-Thioxothiazolidine-4-carboxylic acid (**1**) was isolated as colourless needles from light-exposed seedlings of Sakurajima radish. Mass spectroscopy indicated that the molecular formula was $C_4H_5NO_2S_2$ (m/z 162.9772; calcd 162.9762). The IR spectrum revealed the presence of > NH (3340 cm^{-1}), carboxylic group (1736 cm^{-1}), S=C–N < (1480 cm^{-1}) and C=S (1208 cm^{-1}). The ^1H NMR spectrum (Table 1) in CD_3COCD_3 (60 MHz) showed signals for two protons of methylene (δ 3.85, AB

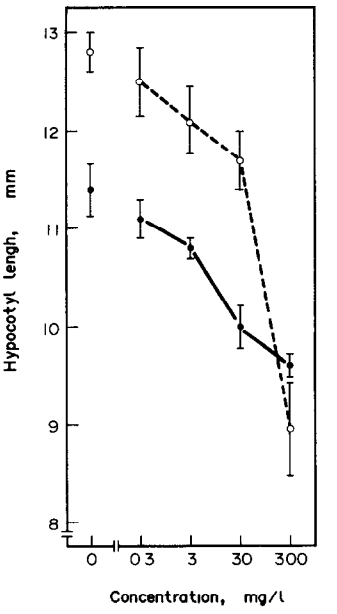

Fig 1 Effect of **1** on the growth of hypocotyl sections of etiolated Sakurajima radish seedlings (●) and intact hypocotyls of etiolated lettuce seedlings (○)

part of ABX, H-5), one methine proton (δ 4.97, X part of ABX, H-4) and proton(s) at δ 5.45 which disappeared on adding D_2O. The ^{13}C NMR spectrum (Table 1) allowed the assignments of one $\geq C=S$ (δ 204.37, C-2), one CH (δ 67.05, C-4), one CH_2 (δ 38.34, C-5) and one carboxylic carbon (δ 173.90, C-6). The mass spectrum showed a $[M+2]^+$ at m/z 165, a $[M]^+$ at m/z 163 and other prominent peaks at m/z 118 $[M-COOH]^+$ and 117 $[M-CH_2S]^+$.

Methylation of **1** with diazomethane gave a monomethyl ester which showed ester absorption (1750 cm^{-1}) in its IR spectrum and in its mass spectrum a $[M]^+$ at m/z 177, a $[M-COOMe]^+$ at m/z 118 and a $[COOMe]^+$ at m/z 59.

These spectral data show that **1** has a 2-thioxothiazolidine carboxylic acid structure, the position of the carboxylic acid being determined by the fact that the signal of the methine proton (*ddd*, J = 8, 5 and 1 Hz, H-4) was changed to a sharp double doublet (J = 8 and 5 Hz) by adding D_2O, showing that the methine proton coupled with the exchangeable proton of the thioamide group. Thus, the structure of **1** is shown to be 2-thioxothiazolidine-4-carboxylic acid.

The results of bioassays of **1** are shown in Fig. 1. Compound **1** inhibited the growth of hypocotyl sections of etiolated Sakurajima radish seedlings and intact hypocotyls of etiolated lettuce seedlings at concentrations greater than 3 mg/l. These results suggest that **1** is a new growth inhibitor which may be involved in the light inhibition of hypocotyl growth of Sakurajima radish seedlings.

EXPERIMENTAL

Extraction and isolation. 2-day-old etiolated seedlings of Sakurajima radish were irradiated with white fluorescent light (2 W/m^2) for 1 day at 25°. 10 kg seedlings were harvested, washed with H_2O and frozen at −20°. The frozen seedlings were homogenized in 30 l cold 80% Me_2CO in a homogenizer. The extract was filtered through Whatman filter paper No. 2 and the filtrate 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 extract was discarded. The aq. residue was then adjusted to pH 2.8 with H_3PO_4 and extracted with EtOAc. The EtOAc extract was evaporated to dryness after drying over Na_2SO_4. The EtOAc fraction (5.8 g, brown oil) was chromatographed on Si gel with a C_6H_6–EtOAc solvent system containing increasing amounts of EtOAc. Biological activity was determined using the Sakurajima radish hypocotyl test [1]. An active part (60% EtOAc in C_6H_6, 400 mg) was purified by TLC ($CHCl_3$–HOAc, 19:1, isopropyl ether–HOAc 19:1). Brown needles (65.9 mg) were yielded by concentrating the active eluate from TLC plates. Repeated recrystallization from $CHCl_3$ yielded colourless needles (50 mg).

2-Thioxothiazolidine-4-carboxylic acid (**1**). Mp 169–171°, $[\alpha]_D^{28}$ −66.7° (MeOH, *c* 0.27). (Found: *m/z* 162.9772, calcd for $C_4H_5NO_2S_2$ 162.9762.) IR ν_{max}^{Nujol} cm^{-1}: 3340, 1736, 1480, 1208 and 1050. ^1H and ^{13}C NMR: see Table 1. EIMS (70 eV) *m/z* (rel. int.): 165 (10), 163 $[M]^+$ (100), 118 $[M-COOH]^+$ (52), 117 $[M-CH_2S]^+$ (12) and 99 (20).

Methylation of **1** *with* CH_2N_2. Compound **1** (2 mg) in MeOH and Et_2O, was methylated with CH_2N_2–Et_2O at room temp., the solvent evaporated and the residue chromatographed on a column of Si to give a monomethyl ester. Amorphous. IR ν_{max}^{Nujol} cm^{-1}: 3200 (NH), 1750 (ester), 1480 (S=C–N), 1210 (C=S) and 1050. EIMS *m/z* (rel. int.): 177 $[M]^+$ (39), 118 $[M-COOMe]^+$ (44), 99 (6), 59 $[COOMe]^+$ (40) and 41 (100).

Bioassays of **1**. 10 5-mm sections, excised 3–8 mm below the hook of 2-day-old etiolated seedlings of Sakurajima radish, and 10 uniform 2-day-old etiolated lettuce seedlings each were cultured in a 4.5 cm Petri dish in the dark for 1 day at 25° on 1.5 ml of medium containing **1**.

Acknowledgement—We thank Miss I. Fukidome for valuable technical assistance.

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