Note

Novel Acetamides Isolated from
Scolecotrichum graminis FuckelHiroyasu TABUCHI, Akitoshi TAJIMI*
and Akitami ICHIHARADepartment of Agricultural Chemistry, Faculty of
Agriculture, Hokkaido University,
Sapporo 060, Japan*Hokkaido National Agricultural Experiment Station,
Sapporo 061-01, Japan

Received June 6, 1991

In the course of our continuing research on phytotoxic compounds from *Scolecotrichum graminis* Fuckel, which causes a leaf streak disease of orchardgrass, we have reported the isolation of (+)-isocercosporin.¹⁾ Here are described the isolation and structural elucidation by the synthesis of novel acetamide compounds **1**, **2a** and **2b** from the same fungus, although these compounds have no significant activity. The isolation procedure for **1**, **2a** and **2b** is described in the experimental section.

HR-EI-MS measurement of **1**, $[\alpha]_D^{25} -69.8^\circ$ ($c=1.0$, EtOH), showed a molecular ion at m/z 143.0938 (calcd. for $C_7H_{13}NO_2$, 143.0946). The IR spectrum of **1** revealed absorption bands at 3300 (NH), 1720 (CO) and 1650 (HNCO) cm^{-1} . In the 1H -NMR spectrum of **1**, a broad singlet at δ 6.45 (1H) replaceable with D_2O was attributed to an amide proton. The signals of a triplet (3H) at δ 1.10 and two double-quartets (1H, 1H) at δ 2.48 and δ 2.60 showed the presence of an ethyl group, which was attached to a carbonyl carbon. A singlet (3H) at δ 2.01 was ascribed to acetyl protons. In spin decoupling experiment on the broad singlet (1H) at δ 6.45, a methine quintet (1H) at δ 4.62 collapsed to a quartet ($J=7.1$ Hz), which indicates the presence of a $-COCH(NHCO)CH_3$ moiety. Thus, the structure of compound **1** was elucidated to be 2-acetamido-3-pentanol.

The FD-MS of both **2a**, $[\alpha]_D^{22} -25.0^\circ$ ($c=1.0$, EtOH), and **2b**, $[\alpha]_D^{22} -188.0^\circ$ ($c=0.5$, EtOH), exhibited the $[MH]^+$ ion at m/z 146 and the $[2M+H]^+$ ion at m/z 291. The EI-HR-MS of **2a** showed 127.0981 $[M^+ - H_2O]$; calcd. for $C_7H_{13}NO$, 127.0996). The 1H -NMR spectra of both **2a** and **2b** were similar to that of **1**, except for two methine multiplets (1H, 1H) at δ 3.56 and δ 3.46, respectively. These results indicate that **2a** and **2b** were diastereomers of 2-acetamido-3-pentanol, which was also obtained by reducing **1** with sodium borohydride in a ratio of 2:1.

In order to determine the absolute configurations, **1**, **2a** and **2b** were synthesized *via* a Grignard reaction of 2-(*N*-phthaloylamino)propanal (**4**) from L-alanine (Scheme 1). Aldehyde **4**, which was obtained by *N*-phthaloylation

(phthalic anhydride, toluene, reflux, 3 hr), acid chloride formation (thionyl chloride, benzene, reflux, 12 hr) and Rosenmund reduction (H_2 , 10% Pd/C, toluene, 100–120°C, 2 hr) from L-alanine,^{2,3)} was reacted with ethylmagnesium iodide in tetrahydrofuran at $-78^\circ C$ for 2.5 hr to give a mixture of **5a** and **5b** in a ratio of 81:19. Resulting alcohol **5a**, which was separated from **5b** by silica gel column chromatography, was treated with dihydropyran catalyzed by *p*-toluenesulfonic acid in dichloromethane ($0^\circ C \rightarrow$ room temperature, 2 hr) to give **6a**. After removing the *N*-blocking group by hydrazine hydrate in ethanol (reflux, 1.5 hr),⁴⁾ the resulting crude amine was treated with acetic anhydride in methanol (room temperature, 2 hr) to afford acetamide **7a**. Removal of the THP group by *p*-toluenesulfonic acid in ethanol (room temperature, 2 hr) gave acetamide **2a**. By using the above-described procedures, **2b** was synthesized from **5b**. Furthermore, synthetic **2a** was converted to **1** by pyridinium dichromate.

The resulting synthetic **1**, **2a** and **2b** exhibited chromatographic and spectral characteristics (MS, IR and 1H -NMR) identical with natural **1**, **2a** and **2b**, respectively. Additionally, the observed optical rotation of synthetic **1**, $[\alpha]_D^{23} -60.0^\circ$ ($c=1.0$, EtOH), **2a**, $[\alpha]_D^{22} -25.0^\circ$ ($c=1.0$, EtOH), and **2b**, $[\alpha]_D^{22} -170.0^\circ$ ($c=0.4$, EtOH), were in good agreement with the values for natural **1**, **2a** and **2b**. These comparisons establish that the absolute configurations of **1**, **2a** and **2b** were all 2S. In order to establish the absolute configurations of the C-3 position of **2a** and **2b** according to Mosher's method,^{5,6)} **5a** was converted to the (*S*)-MTPA ester of **5a** (**11**) and the (*R*)-MTPA ester of **5a** (**12**). In a solution, the carbonyl proton, ester carbonyl and trifluoromethyl groups of the MTPA moiety are oriented on the same plane.⁵⁾ Due to the diamagnetic effect of the benzene ring, H_4 and H_5 of the (*R*)-MTPA ester of **5a** (**12**) showed 1H -NMR signals upfield relative to those of the (*S*)-MTPA ester of **5b** (**11**). On the other hand, H_1 and H_2 of **12** showed signals downfield of those of **11** (Scheme 1). These results indicate that the absolute configuration of the C-3 position of **5a** was *R*, and that those of **2a** and **2b** were *R* and *S*, respectively.

Although **1**, **2a** and **2b** showed no significant inhibitory activity toward the growth of lettuce seeds even at a high concentration of 500 ppm, these compounds are interesting from the standpoint of biosynthesis, which would involve transformation from L-alanine.

Experimental

The analytical instruments used were as follows: FD-MS, JEOL 01SG-2; EI-MS, JEOL DX-300; IR spectra, Hitachi Model 285; 1H -NMR spectra, Bruker AM-500 (500 MHz with $CDCl_3$ as the solvent); optical rotation, JASCO DIP-4.

Isolation and purification. Scolecotrichum graminis

Scheme 1.

* $\Delta\delta = \delta_S - \delta_R$. The numerical value denoting each proton is the chemical shift difference obtained by subtracting the chemical shift (δ_R) of a proton of the (*R*)-MTPA ester (**12**) from that (δ_S) of the (*S*)-MTPA ester (**11**) [$\Delta\delta$ values are given in ppm (CDCl_3 , 500 MHz)].

Fuckel supplied by Hokkaido National Agricultural Experiment Station, Sapporo, Japan, was seeded into 500-ml Erlenmeyer flasks containing 150 ml of a potato extract medium. After the fungus (143 flasks) had been

incubated at 25°C for 30 days in a surface culture, the culture filtrates (20 liters) were concentrated *in vacuo* to give a concentrate (1 liter), which was extracted with ethyl acetate. The extracts were dried over Na_2SO_4 and con-

centrated to dryness, and the residue was fractionated by silica gel column chromatography with CHCl_3 -MeOH (9:1) as the eluent into eight fractions (Fr. 1-8). Fr. 1 and Fr. 2, which showed a spot of $R_f=0.36$ and two spots of $R_f=0.26$ and 0.20 on silica gel TLC with ethyl acetate by the Rydon-Smith reagent, respectively, were subjected to silica gel preparative TLC with ethyl acetate to give **1** (14.5 mg), **2a** (11.0 mg) and **2b** (9.0 mg).

(2*S*)-2-acetamido-3-pentanone (**1**). $[\alpha]_D^{25} -69.8^\circ$ ($c=1.0$, EtOH). EI-MS m/z (%): 143 $[\text{M}]^+$ (0.12), 69 (100). EI-HR-MS m/z : 143.0938 (M^+ ; calcd. for $\text{C}_7\text{H}_{13}\text{NO}_2$, 143.0946). IR ν_{max} (film) cm^{-1} : 3300, 1720, 1650. $^1\text{H-NMR}$: δ 6.45 (1H, br. s, NH), 4.62 (1H, quintet, $J=7.1$ Hz, 2-H), 2.60 (1H, dq, $J=18.0$, 7.3 Hz, 4-H), 2.48 (1H, dq, $J=18.0$, 7.3 Hz, 4-H), 2.01 (3H, s, $\text{CH}_3\text{CO-}$), 1.35 (3H, d, $J=7.1$ Hz, 1-H), 1.10 (3H, t, $J=7.2$ Hz, 5-H).

(2*S*,3*R*)-2-acetamido-3-pentanol (**2a**). $[\alpha]_D^{22} -25.0^\circ$ ($c=1.0$, EtOH). EI-MS m/z (%): 127 $[\text{M}-\text{H}_2\text{O}]^+$ (2.85), 69 (100). EI-HR-MS m/z : 127.0981 ($\text{M}^+ - \text{H}_2\text{O}$; calcd. for $\text{C}_7\text{H}_{13}\text{NO}$, 127.0996). IR ν_{max} (film) cm^{-1} : 3350, 1640. $^1\text{H-NMR}$: δ 5.68 (1H, br. s, NH), 4.01 (1H, m, 2-H), 3.56 (1H, m, 3-H), 1.99 (3H, s, $\text{CH}_3\text{CO-}$), 1.40-1.60 (2H, m, 4-H), 1.25 (1H, br. s, 3-OH), 1.10 (3H, d, $J=6.9$ Hz, 1-H), 0.99 (3H, t, $J=7.4$ Hz, 5-H).

(2*S*,3*S*)-2-acetamido-3-pentanol (**2b**). $[\alpha]_D^{22} -188.0^\circ$ ($c=0.5$, EtOH). FD-MS m/z (%): 291 $[2\text{M}+\text{H}]^+$ (16.40), 146 $[\text{MH}]^+$ (100). IR ν_{max} (film) cm^{-1} : 3300, 1630. $^1\text{H-NMR}$: δ 5.68 (1H, br. s, NH), 4.01 (1H, m, 2-H), 3.46 (1H, m, 3-H), 2.00 (3H, s, $\text{CH}_3\text{CO-}$), 1.40-1.60 (2H, m, 4-H), 1.25 (1H, br. s, 3-OH), 1.20 (3H, d, $J=6.8$ Hz, 1-H), 0.96 (3H, t, $J=7.5$ Hz, 5-H).

Acknowledgments. We are grateful to Mr. Kenji Watanabe and Mrs Eri Fukushima (both of Hokkaido Univ.) for measuring mass spectra.

References

- 1) H. Tabuchi, A. Tajimi and A. Ichihara, *Agric. Biol. Chem.*, **55**, 2675 (1991).
- 2) H. Newman, *J. Am. Chem. Soc.*, **95**, 4098 (1973).
- 3) Y. Moriyama, Y. Saitoh, Y. Igarashi, T. Sugimoto, T. Takahashi and H. Khuong, *Koen Yoshishu-Tennen Yuki Kagobutsu Toronkai*, 22nd, 532 (1979).
- 4) H. Newman, *J. Org. Chem.*, **39**, 100 (1974).
- 5) J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
- 6) I. Ohtani, T. Kusumi, M. O. Ishitsuka and H. Kakisawa, *Tetrahedron Lett.*, **30**, 3147 (1989).