Note

Inhibiting Substance toward Tea Pollen Tube Growth from Marine Alga *Spatoglossum Pacificum*

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In the course of screening for substances which control the process of germination and tube elongation of the pollen from *Thea sinensis*, we found a new inhibitor, 6-acetoxylinoic acid (**1**), from the marine brown alga *Spatoglossum pacificum*.

One hundred and twenty-two species of algae were collected from various locations along the coast (Muran, Chiba, Kamakura, Izu and Okinawa) of Japan in 1986–1987. Methanol extracts of these were assayed for the germination and tube growth inhibition of tea pollen by Konishi's method.¹⁾ The nine species of *Callophyllis crispata*, *Cladophoropsis raucheriaeformis*, *Codium fragile*, *Coeloseira pacifica*, *Delisa fimbriata*, *Dictyota dichotoma*, *Rhodopeltis borealis*, *S. pacificum* and *Vidalia obtusiloba* strongly inhibited pollen germination and tube growth.

S. pacificum (11.45 kg) collected from the shore near Shirahama Shrine in Izu was freeze-dried and extracted with MeOH. The EtOAc-soluble fraction (31 g) from the MeOH extracts was fractionated twice in an SiO₂ column, and then by high-performance liquid chromatography (HPLC) in an ODS YMC AM324 column (100% MeOH, refractive index). Further purification of the active fraction by HPLC in a YMC PH A414 column, using 90% MeOH, gave a colorless and viscous inhibitor (**1**) in a yield of 7 mg. Compound **1** thoroughly inhibited pollen germination at a concentration of 1.0 µg/ml and reduced pollen tube

growth to 34% with a concentration of 0.1 µg/ml.

The ¹H-NMR (CD₃OD) spectrum showed signals of 34 protons assignable to a methyl at δ 0.95 (3H, t, *J* = 7.2 Hz), seven methylenes at 1.39 (8H, m) and 1.64 (6H, m), an acetyl at 2.07 (3H, s), two methylenes bonded to a double bond at 2.11 (4H, m), a methylene attached to a carboxyl at 2.32 (2H, t, *J* = 7.1 Hz), a methylene between double bonds at 2.81 (2H, m), a methine attached to an acetoxy group at 4.91 (1H, m), and two double bonds at 5.40 (4H, m). The presence of a free carboxyl group was confirmed by esterifying **1** with CH₂N₂ to give a monomethyl ester (**2**). Twenty carbons were observed in the ¹³C-NMR (CD₃OD) spectrum of **1** as follows: δ 14.4 (CH₃), 21.2 (CH₃), 23.6 (CH₂), 24.2 (CH₂), 26.0 (CH₂), 26.1 (CH₂), 26.5 (CH₂), 28.2 (CH₂), 30.4 (CH₂), 32.6 (CH₂), 34.9 (CH₂), 35.1 (CH₂), 35.3 (CH₂), 75.1 (CH), 128.8 (CH), 129.8 (CH × 2), 131.1 (CH), 172.8 (C) and 178.4 (C). The GC-FDMS of **1** gave an [MH]⁺ ion at *m/z* 339, indicating that MW of **1** was 338, and GC-EIMS gave fragment ions at *m/z* (rel. int.) 279 (5), 258 (0.1), 251 (1), 236 (1), 222 (1), 209 (1), 207 (1), 194 (1), 192 (1), 180 (1), 177 (3), 150 (47), 136 (4), 121 (8), 107 (12), 93 (30), 91 (15), 80 (67), 67 (47), 55 (33), 42 (100) and 40 (27). Considering the number of protons and carbons from the NMR data, the molecular formula of **1** was elucidated to be C₂₀H₃₄O₄. These data indicated that **1** was a monoacetoxy C₁₈ fatty acid with two double bonds jointed to the same methylene. The double bonds were shown to be *cis* since no trans unsaturation could be detected in the IR absorption (2920, 2850, 1711, 1694, 1657, 1383, 1088, 753 cm⁻¹) of **1**.

The position of an acetoxy group and double bonds was determined by GC-EIMS of derivatives of **1**. Hydrolysis of **1** with KOH and subsequent esterification with ethereal CH₂N₂ gave alcohol **3**. Oxidation of alcohol **3** with pyridinium dichromate PCC in CH₂Cl₂ resulted in the formation of ketone **4**. Alcohol **3** gave a fragment ion at *m/z* 145, which was yielded by cleavage of the C₆–C₇ bond, helping to locate the OH at C₆. Furthermore, in the case of **4**, cleavage at the α position of the keto function produced fragment ions at *m/z* 194, 165, 143 and 115. These fragmentation patterns showed that the position of the acetyl group was C₆.^{2,3)} In addition, **4** indicated fragment ions at *m/z* 137, 111 and 97, which were yielded by cleavage of the C₈–C₉, C₁₀–C₁₁ and C₁₁–C₁₂ bonds, respectively, confirming the presence of double bonds at C₉–C₁₀ and C₁₂–C₁₃. Thus **1** was characterized as 6-acetoxylinoic acid. Compound **1** was chiral, [α]_D²³ –1.04 (*c* = 0.5, CHCl₃), but the absolute configuration at C₆ remains to be solved.

C₈–C₁₁ fatty acids, their 2-hydroxy derivatives, and 3-hydroxydecanoic acid (myrmicacin) have shown inhibitory activity toward pollen germination.⁴⁾ These compounds completely inhibited the germination of *Camellia japonica* pollen at a concentration of 50 µg/ml, whereas **1** showed complete inhibition of *T. sinensis* pollen germination at the much lower concentration already described. Since linoleic acid has no activity, the acetoxy

- 1: R = H, R' = Ac
- 2: R = Me, R' = Ac
- 3: R = Me, R' = H

group at C₆ is necessary to exhibit the activity. There is a possibility that **1** could demonstrate activity after hydrolyzing the acetate. However, this has not yet been confirmed because 6-hydroxylinoleic acid could not be prepared due to the small quantity of **1**.

Recently, acetoxo and hydroxy fatty acids with biological activities have been found in marine plants, animals and basidiomycete fungus.⁵⁻⁷⁾ Such endogenous derivatives of fatty acids might have a common physiological role in various organisms.

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