



Penienone and Penihydrone, New Plant Growth Regulators Produced by the Fungus, *Penicillium* sp. No.13.

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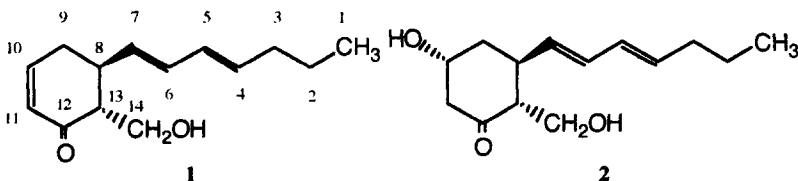
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Abstract: Penienone (1) and penihydrone (2) were isolated from the metabolite of the fungus *Penicillium* sp. No.13. as new plant growth regulators and their structures were established by NMR studies and their derivatives. Copyright © 1996 Elsevier Science Ltd

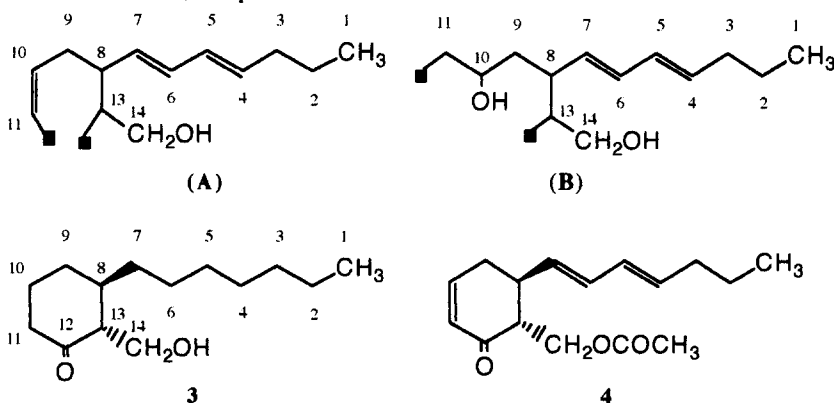
Recently, we have elucidated the structures of botryolactone¹ and cyclo-(L-tryptophyl-L-phenylalanyl)² as the growth regulators of lettuce seedlings. In our continuous screening search for plant growth regulators by the bioassay method using lettuce and rice seedlings, which might be potential use in developing new effective herbicides and as tools to analyze the growth of the organs in higher plants, we found the presence of the plant growth regulators in the culture filtrate of *Penicillium* sp. No.13. Here we describe the structural elucidation and biological activities of penienone (1) and penihydrone (2).



The fungus, *Penicillium* sp. No.13, was cultured stationarily in a potato extract medium containing sucrose (30 g/l) at 24 °C for 21 days. The culture filtrate was adjusted to pH 2.0 and then successively extracted twice with EtOAc. The combined solvents were concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel and then by preparative TLC. Purification by chromatography afforded penienone (1)³ as colorless plates in a yield of 16 mg/l and penihydrone (2)⁴ as colorless needles in a yield of 6 mg/l.

The molecular formula of 1 was established as C₁₄H₂₀O₂ by EIMS [(M⁺ - CH₂OH), *m/z* 189] and elementary analysis (Found: C, 76.00; H, 9.08. Calcd.: C, 76.33; H, 9.15 %). ¹H and ¹³C data of 1 are shown in the table. In the ¹H-NMR data of 1, one methyl signal at δ_H 0.91 (C-1), four methylene signals at δ_H

1.41 (C-2), 2.05 (C-3), 2.39 (C-9), and 3.72, 3.91 (C-14) two methine signals at δ_{H} 2.39 (C-13) and 2.67 (C-8), one D₂O exchangeable proton signal at δ_{H} 2.95 (OH) and six olefinic proton signals at δ_{H} 5.45 (C-7), 5.66 (C-4), 6.01 (C-5), 6.05 (C-11), 6.11 (C-6) and 6.97 (C-10) were observed. The IR absorption band at 1672 cm⁻¹ and one signal at δ_{C} 202.2 indicated the presence of an α,β -unsaturated carbonyl carbon. The two olefinic protons at δ_{H} 5.66 and 6.01 indicated the *E* geometry by their coupling constants ($J = 14.2$ Hz). Similarly, the two olefinic protons at δ_{H} 5.45 and 6.11 indicated the *E* geometry by their coupling constant ($J = 14.4$ Hz). On the other hand, the coupling constants ($J = 10.0$ Hz) of two olefinic protons at δ_{H} 6.05 and 6.97 showed the *Z* geometry. Homo-spin decoupling experiments in ¹H NMR revealed the presence of the partial structures of (A). From these results, together with the molecular formula and the data of paritantin isolated by Demetriadon et. al.,⁵ the planar structure of **1** was established.



The absolute configurations at C-8 and C-13 in **1** were determined by CD spectrum, NOE and homo-spin decoupling experiments in ¹H-NMR of a hexahydro-derivative (**3**)⁶ prepared by catalytic hydrogenation of **1** over PtO₂ in EtOH. The absence of NOE effects and the coupling constant of 11.5 Hz between each methine proton at C-8 and C-13 in the derivative showed those protons were in the position of *trans*-diaxial.⁷ Furthermore, the CD spectrum showed a positive Cotton effect at λ_{max} . 290 nm [θ] +3620. These results indicated the absolute configurations at C-8 and C-13 in the derivative were both *R* configurations. From the results of the absolute configurations of the derivative, the absolute configurations at C-8 and C-13 in **1** were determined to be *S* and *R*, respectively.

The molecular formula of **2** was established as C₁₄H₂₂O₃ by EIMS [(M⁺), m/z 238] and elementary analysis (Found: C, 70.31; H, 9.09. Calcd.: C, 70.55; H, 9.31 %). ¹H- and ¹³C-NMR data of **2** are shown in the table. In the ¹H-NMR data of **2**, one methyl signal at δ_{H} 0.85 (C-1), five methylene signals at δ_{H} 1.36 (C-2), 1.88 (C-9), 2.00 (C-3), 2.34, 2.63 (C-11) and 3.59 (C-14), three methine signals at δ_{H} 2.30 (C-13), 2.82 (C-8) and 4.36 (C-10), two D₂O exchangeable proton signals at δ_{H} 2.98 and 3.98 (OH) and four olefinic proton signals at δ_{H} 5.49 (C-7), 5.59 (C-4), 6.02 (C-5, C-6) were observed. Two double bonds at C-4 and C-5, and C-6 and C-7 were determined to be *E* by the coupling constants of $J = 14.2$ Hz (C-4H) and 13.9 Hz (C-7H). The ¹³C-NMR data showed one carbonyl carbon (δ_{C} 212.1). Homo-spin decoupling experiments in ¹H-NMR revealed the presence of the partial structure of (B). From these results, together with the molecular

formula the planar structure of **2** was established.

The absolute configurations at C-8, C-10 and C-13 in **2** were determined by NOE and homo-spin decoupling experiments in $^1\text{H-NMR}$ of **2** and the physicochemical properties of its acetyl derivative (**4**)⁸ derived by treatment with $\text{Ac}_2\text{O/pyridine}$. The absence of NOE effects and the coupling constant of 11.3 Hz between each methine proton at C-8 and C-13 of **2** showed those protons were in the position of *trans* - diaxial. Since the NOE effects and approximately the same couplings ($J = 3.4$ and 3.9 Hz) were observed between the methine proton at C-10 and two geminal protons at C-11, the methine proton at C-10 was in the equivalent position to two protons at C-11.⁹ Furthermore, the physicochemical properties including the sign and magnitude of the specific rotation of the acetyl derivative corresponded to those of acetyl derivative (**4**) of **1**, in which the absolute configurations had been determined as described above. From these results, the absolute configurations at C-8, C-10 and C-13 in **2** were determined to be *S*, *R* and *R*, respectively.

Table 13C (67.80 MHz) and 1H (270.05 MHz) NMR Data for 1 and 2

Carbon Number	1*			2*		
	δ_{C}	δ_{H}		δ_{C}	δ_{H}	
1	13.7 q	0.91	(3H, t, 7.3)	14.4 q	0.85	(3H, t, 7.3)
2	22.3 t	1.41	(2H, tq, 7.3, 7.3)	23.6 t	1.36	(2H, tq, 7.3, 7.3)
3	34.6 t	2.05	(2H, dt, 7.3, 6.8)	35.8 t	2.00	(2H, m)
4	135.0 d	5.66	(1H, dt, 14.2, 6.8)	135.2 d	5.59	(1H, dt, 14.2, 6.8)
5	129.4 d	6.01	(1H, dd, 14.2, 10.3)	131.7 d	6.02	(1H, m)
6	132.7 d	6.11	(1H, dd, 14.4, 10.3)	132.4 d	6.02	(1H, m)
7	131.3 d	5.45	(1H, dd, 14.4, 9.0)	134.4 d	5.49	(1H, dd, 13.9, 9.3)
8	40.9 d	2.67	(1H, m)	50.1 d	2.82	(1H, m)
9	33.0 t	2.39	(2H, m)	39.7 t	1.88	(2H, dd, 9.6, 3.6)
10	149.9 d	6.97	(1H, ddd, 10.0, 5.6, 2.7)	69.1 d	4.36	(1H, br. s)
11	129.5 d	6.05	(1H, d, 10.0)	39.9 t	2.34	(1H, dd, 14.2, 3.9)
					2.63	(1H, dd, 14.2, 3.4)
12	202.2 s			212.1 s		
13	52.8 d	2.39	(1H, m)	57.5 d	2.30	(1H, ddd, 11.3, 5.9, 3.9)
14	60.8 t	3.72	(1H, m)	60.7 t	3.59	(2H, m)
		3.91	(1H, m)			
10 OH					3.98	(1H, br. s)
14 OH		2.95	(1H, br.s)		2.98	(1H, br. t)

*: **1** in CDCl_3 and **2** in CD_3COCD_3

The s, d, t and q in parentheses of ^{13}C NMR spectrum showed multiplicities determined by INEPT experiments.

Also, the s, d, t, q, m and the numbers in parentheses of ^1H NMR spectrum showed multiplicities and coupling constants, respectively.

Penienone (**1**) completely inhibited the hypocotyl elongation and the root growth of lettuce seedlings¹⁰ at a concentration of 300 mg/l. Penihydrone (**2**) inhibited the hypocotyl elongation by 41 %, but accelerated the root growth by 280 % at the same concentration. **1** completely inhibited the total length, the second leaf sheath length and the primary root growth of rice seedlings¹¹ at a concentration of 300 mg/l. **2** showed the weak inhibitory activities against the total length and the second leaf sheath length at a concentration of 300 mg/l. However, **2** inhibited the primary root growth by 5 % at the same concentration.

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3. The physicochemical properties of this compound are as follows. Mp 61-63 °C. $[\alpha]_{\text{D}}^{20} -45^{\circ}$ (c 1.0, EtOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 230 (26,400). CD (c 0.3, EtOH)[θ] (nm): + 2,200 (325). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3914, 1672.
4. The physicochemical properties of this compound are as follows. Mp 85-87 °C. $[\alpha]_{\text{D}}^{20} +5^{\circ}$ (c 1.0, MeOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 233 (19,000). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3402, 1702.
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6. The physicochemical properties of this compound are as follows. Colorless oil. $[\alpha]_{\text{D}}^{20} -23.8^{\circ}$ (c 0.21, MeOH). CD (c 0.11, EtOH)[θ] (nm): +3,620 (290), -640 (225). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3412, 1704. EIMS m/z : 226 (M^+), 208, 137, 127, 110, 97, 83, 67. $^1\text{H-NMR}$ (TMS, CDCl_3): 0.88 (3H, t, $J=7.3$ Hz), 1.27 (12H, s), 1.45 (1H, m), 1.62 (1H, m), 1.70 (1H, m), 1.97 (1H, m), 2.09 (1H, m), 2.25 (1H, ddd, $J=11.5, 6.5, 2.8$ Hz), 2.32 (1H, m), 2.38 (1H, m), 2.70 (1H, m), 3.71 (1H, m), 3.85 (1H, m).
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8. The physicochemical properties of acetyl derivatives of penienone and penihydrone are as follows. Colorless oil. $[\alpha]_{\text{D}}^{20} -32.3^{\circ}$ (c 0.31, MeOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 230 (22,500). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 2964, 1743, 1680. EIMS m/z : 262 (M^+), 202, 189, 173, 159, 145, 133, 117, 107, 91, 77, 68. $^1\text{H-NMR}$ (TMS, CDCl_3): 0.91 (3H, t, $J=7.3$ Hz), 1.41 (2H, tq, $J=7.3, 7.3$ Hz), 2.01 (3H, s), 2.03 (2H, dt, $J=7.3, 7.3$ Hz), 2.43 (3H, m), 2.82 (1H, m), 4.20 (1H, dd, $J=11.1, 3.9$ Hz), 4.58 (1H, dd, $J=11.1, 3.2$ Hz), 5.46 (1H, dd, $J=14.7, 8.8$ Hz), 5.65 (1H, dt, $J=14.7, 7.3$ Hz), 5.96 (1H, m), 6.05 (1H, m), 6.08 (1H, m), 6.95 (1H, ddd, $J=10.3, 5.5, 2.4$ Hz).
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