

ISOLATION AND STRUCTURE OF PYCNOPHORIN, A NOVEL DITERPENE α -PYRONE WITH
ANTIMICROBIAL ACTIVITY, PRODUCED BY PHYTOPATHOGENIC MACROPHOMA KUWATSUKAI

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Abstract: Pycnophorin, a new characteristic metabolite with antimicrobial activity, was isolated from the full-grown mycelia bearing pycnidia of M. kuwatsukai and determined as a novel diterpene α -pyrone shown as I.

The phytopathogenic fungus Macrophoma kuwatsukai, a causal agent of ring rot of apple, produces sesquiterpene-linked cyclohexenone epoxides, macrophorins A and D, having growth self-inhibiting activity¹). In the course of further search for characteristic metabolites produced by this fungus, we found a new metabolite in the full-grown mycelia bearing pycnidia that were induced by light²). The present communication deals with the isolation and structural determination of this novel diterpene α -pyrone (I) named pycnophorin.

M. kuwatsukai was grown on a potato-sucrose (commercial) medium under a fluorescent light for 30~40 days at ca. 25°C. The ethyl acetate-soluble portion of the mycelial extracts was chromatographed on silica gel with chloroform-isopropanol (100:1). A fraction giving a purple spot on a TLC plate by spraying with a vanillin-sulfuric acid reagent was purified by silica gel chromatography with chloroform-acetone (100:1) to afford pycnophorin as colorless crystals; mp 140.5~142°C, $[\alpha]_D^{18}$ -24° (MeOH, c=0.2). Its yield was about 4 times higher than that from the mycelia grown in the dark. Pycnophorin gave positive alcoholic ferric chloride (pale brown) and 2,4-dinitrophenylhydrazine tests, and showed antimicrobial activities against Helminthosporium maydis, H. setariae and M. kuwatsukai (MID 1, 20 and ca. 200 μ g/disc, respectively)¹), and Staphylococcus aureus and Bacillus subtilis (each MIC 6.2 μ g/ml).

Pycnophorin, C₂₇H₄₀O₄ (high EIMS: M⁺ obsd. 428.2915, calcd. 428.2926), possessed the following spectral properties: UV (MeOH) λ max 291 nm (ϵ 8300); IR (KBr) ν max 3200, 1702, 1662(br) and 1560(br) cm⁻¹; ¹H NMR (CDCl₃) δ 5.80(1H, OH), 5.086(1H, br t, $J=7.1$ Hz), 2.221(3H, br s), 1.953(3H, q, $J=0.7$ Hz), 1.688 and 1.580(each 3H, br s), 1.063 and 0.562(each 3H, s) (the NMR spectrum is shown in Fig. 1). On benzylation with benzoyl chloride/pyridine, it gave a mono-benzoate; IR (CHCl₃) ν c=O 1740 and 1702(br) cm⁻¹ (no hydroxyl absorption); UV (MeOH) λ max 300 (ϵ 7400) and 231 nm (ϵ 16000). These UV and IR spectra together with ¹H NMR signals of the methyl groups of pycnophorin (δ 2.221 and

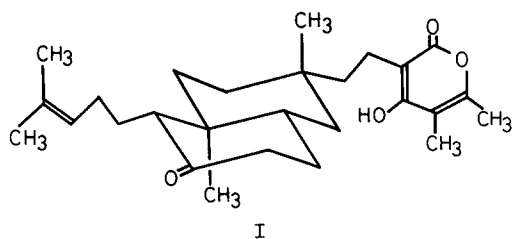


Fig. 1 ^1H and $^1\text{H}-^1\text{H}$ COSY NMR Spectra of Pycnophorin (CDCl_3 , 360 MHz)

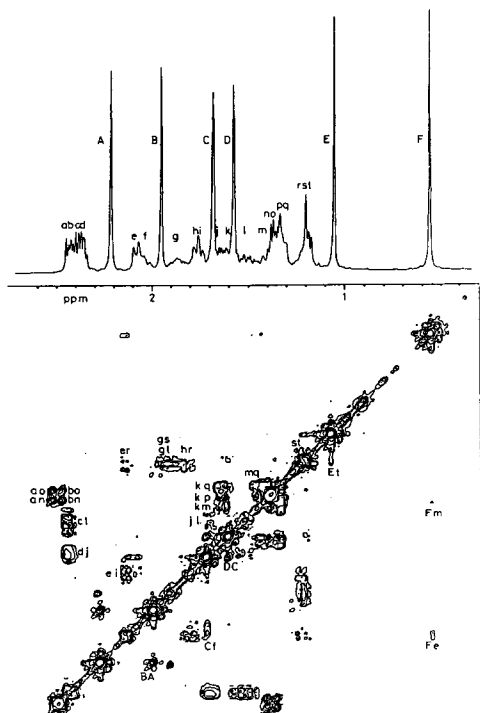


Fig. 2 EIMS Fragment Peaks of Pycnophorin (m/z , %)

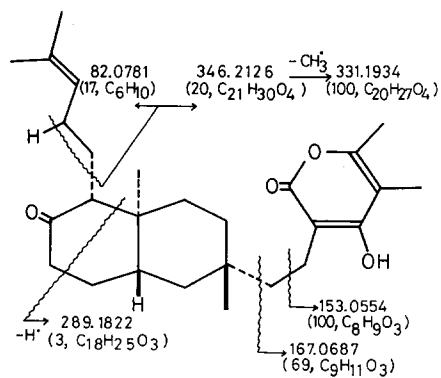
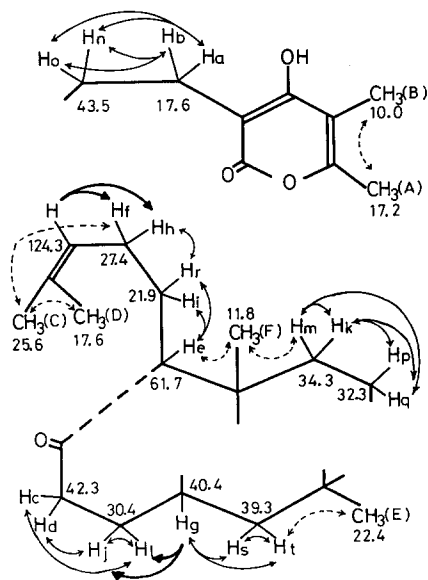


Fig. 3 Partial Structures of Pycnophorin



$^1\text{H}-^1\text{H}$ (\longleftrightarrow and \dashrightarrow) and $^1\text{H}-^{13}\text{C}$ (δ , ppm) COSY NMR data, and decoupling experiments (\longrightarrow)

1.953: a strong NOE was observed between them) indicated the presence of a 3-substituted 4-hydroxy-5,6-dimethyl- α -pyrone moiety $C_7H_7O_3$ ³⁾. ^{13}C NMR signals of the α -pyrone ring were observed at δ 163.6(C-2), 103.5(C-3), 165.0(C-4), 106.3(C-5) and 155.3(C-6). In addition, the presence of a two-carbon side chain on this UV chromophore was revealed by high-resolution mass spectrometry (Fig. 2). These observations indicated that pycnophorin includes a bicyclic keto-diterpene residue $C_{20}H_{33}O$ in the molecule.

The 1H NMR and MS spectra of pycnophorin (δ 5.086(1H, br t), 1.688 and 1.580(each 3H, br s); m/z 359(M^+ -69) and 69) indicated the presence of an isopentenyl group. 1H - 1H COSY NMR spectrum of pycnophorin is shown in Fig. 1. Two partial structures of the diterpene residue were deduced in combination with 1H - ^{13}C COSY NMR data and additional decoupling experiments (summarized in Fig. 3). The symmetrical five-line multiplet of protons a and b (Fig. 1, δ 2.416, X'_2 of A'_2 X'_2 in the 400 MHz NMR spectrum) that was shifted downfield by 0.6 ppm at a higher concentration in deuteriochloroform was assigned to the above-mentioned methylene group having the α -pyrone ring. The multiplet of remaining protons c and d at the lower field could be therefore assigned to the methylene group adjacent to the keto group (IR $\nu_{C=O}$ 1702 cm^{-1} ; ^{13}C NMR δ 212.3) in the terpene moiety (Fig. 3). These assignments were supported by 1H NMR data (note 6 described below) of the sodium borohydride reduction product of pycnophorin. Furthermore, the proximity of this keto group and the isopentenyl side chain was confirmed by the rearrangement peaks at m/z 346 and 82 (Fig. 2) in the MS spectrum and by the chemical shift (δ 2.086, br d, $J=8.6$ Hz) of the methine proton e.

Long-range couplings between the tertiary methyl group F and the protons e and m, and E and t (Fig. 3), and apparent NOE's between the tertiary methyl group F and the protons i and t, and E and g, indicated that the diterpene moiety consists of a trans-decaline ring and, consequently, that the methyl groups E and F, and the protons e, g, m and t are axially positioned. Irradiation of the methylene protons s and t⁴⁾ collapsed the isolated one-proton multiplet of the proton g (δ 1.874) to a double doublet ($J=3.5$, ca. 12 Hz), suggesting a trans-diaxial relationship between the protons g and l (1: δ 1.521, dq, $J=4.2$, 11.7 Hz in the 400 MHz NMR spectrum). Stereostructure of this decaline skeleton was well interpreted by estimates of the ^{13}C NMR chemical shifts (remaining quaternary carbons; δ 42.0(angular) and 33.1(ring))⁵⁾. Sodium borohydride reduction of pycnophorin gave an alcohol with an axial hydroxyl group, mp 212~213°C⁶⁾, which yielded two alkyl naphthalenes by Zn-dust distillation⁷⁾; one (UV (EtOH) λ_{max} 319, 304, 283(sh) and 273 nm) of which was identified as 2-methylnaphthalene by direct comparison with an authentic sample (UV, HPLC and GC-MS). Therefore the stereostructure of pycnophorin, excluding its absolute configuration, was elucidated as shown in I.

Treatment of pycnophorin with ethereal diazomethane yielded a 2-methoxy- γ -pyrone derivative⁸⁾; UV (MeOH) λ_{max} 255 nm (ϵ 8300); IR ($CHCl_3$) $\nu_{C=O}$ 1700 and 1667 cm^{-1} . This showed a negative Cotton effect Δ -3.2 at 294 nm in the CD spectrum, indicating the absolute stereochemistry of pycnophorin to be that

shown in structure I. Pycnophorin is an unusual meroterpene which has a new diterpene skeleton as well as a trisubstituted 4-hydroxy- α -pyrone ring⁹⁾. Its biosynthesis in the diterpene moiety is of interest, since it may include a methyl migration similar to that in rosenonolactone biosynthesis¹⁰⁾ and one of the two alternative pathways, i.e., through oxidation of a bicyclic intermediate or oxidative cleavage of a tricyclic one.

Acknowledgements. The authors thank Dr. Y. Suzuki, the Institute of Physical and Chemical Research, for the 400 MHz NMR measurements and Dr. K. Okada, Yamagata University, for CD measurement. They also thank Dr. K. Tsuji for his advice in manuscript preparation.

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- 6 MS m/z 430(M^+); UV (MeOH) λ_{max} 291 nm (ϵ 7400); IR (KBr) ν_{max} 3200, 1663 and 1560 cm^{-1} .
¹H NMR (CDCl₃) δ 4.05(1H, br d, $J=2.6$ Hz), 2.40(Ha and Hb, X₂' of A₂' X₂'), ca. 1.8, 1.6 and 1.05(Hc, Hd and He), 2.20, 1.91, 1.69 and 1.61(each 3H, br s), 0.97 and 0.90(each 3H, s).
The configuration of the generated hydroxyl group was assigned as α -axial on the basis of ¹H NMR data.
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- 8 MS m/z 442(M^+); ¹H NMR (CDCl₃) δ 3.93(3H, s), 2.25, 1.93, 1.70 and 1.60(each 3H, br s), 1.03 and 0.55(3H, s). The minor product, a 4-methoxy- α -pyrone derivative, was also obtained; MS m/z 442(M^+); UV (MeOH) λ_{max} 300 nm; IR (CHCl₃) $\nu_{C=O}$ 1699(br) cm^{-1} ; ¹H NMR (CDCl₃) δ 3.75(3H, s), 2.19, 1.90, 1.67 and 1.57(each 3H, br s), 1.02 and 0.54(3H, s).
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(Received in Japan 18 February 1986)