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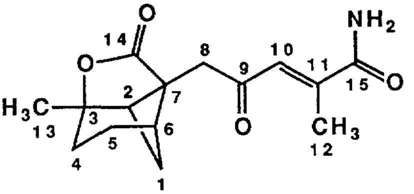
PINTHUNAMIDE, A NEW TRICYCLIC SESQUITERPENE AMIDE PRODUCED BY A FUNGUS, *Ampulliferina* sp

Yasuo Kimura*, Hiromitsu Nakajima and Takashi Hamasaki
Department of Agricultural Chemistry, Tottori University
Koyama, Tottori 680, Japan
Fumio Sugawara
RIKEN (The Institute of Physical and Chemical Research)
Wako, Saitama 351-01, Japan
Laszlo Parkanyi and Jon Clardy*
Baker Laboratory, Department of Chemistry, Cornell University
Ithaca, New York 14853-1301

Summary: *A new tricyclic sesquiterpene amide, pinthunamide, has been isolated from a fungus,* Ampulliferina *sp. and its structure was determined by single-crystal x-ray diffraction method together with assignments of ^1H- and ^{13}C-NMR data for pinthunamide.*

During the course of our screening program for new plant growth regulators among the fungal metabolites, it was found that a fungus, *Ampulliferina* sp. No. 27 produced a new plant growth substance which we have named pinthunamide (**1**). In this note, we report the isolation and structural elucidation of **1** by single-crystal x-ray diffraction method together with assignments of ^1H- and ^{13}C-NMR data.



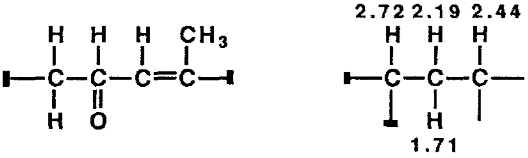
PINTHUNAMIDE (1)

The fungus was statically cultured in a malt extract medium at 24 °C for 31 days. The culture filtrate was treated with active charcoal at pH 2.0 (*dil.* HCl) and extracted with acetone. After evaporating the solvent, the residual brown solid was purified by column chromatography on silica gel (benzene-acetone) followed by Sephadex LH-20 (MeOH). The active principle thus obtained was recrystallized from ethyl acetate to afford pure needles in a yield of 5 mg per liter of the culture filtrate.

Pinthunamide (1) had the following physicochemical properties: colorless needles (from ethyl acetate); mp 190-193 °C; $[\alpha]_D^{20}$ +73.4° (*c*=1.0, EtOH); $C_{15}H_{19}NO_4$ (M^+ obsd. *m/z* 277.1317, calcd. 277.1315); *Anal.* found, C; 64.80, H; 6.90, N; 4.74 %, Calcd., C; 64.96, H; 6.91, N; 5.05 %. The IR spectrum of **1** (KBr) showed characteristic absorption bands at 3450, 3180 and 1680 (amide) 1760 (γ-lactone), 1675 and 1630 cm^{-1} (α, β-unsaturated ketone). The UV spectrum also supported the presence of α, β-unsaturated ketone group [UV λ_{max} (EtOH); 232 nm (ε 19,000)]. **1** was positive to potassium permanganate and NH_2OH-HCl-$FeCl_3$, but negative to $FeCl_3$, *p*-DABA, Ehrlich, Ninhydrin and Dragendorff reactions.

The ^{13}C-NMR spectrum[1] of **1** taken in $CDCl_3$ revealed the fifteen carbon signals indicating seventeen protons directly attached to carbons except for two protons of an amide function. In the ^{1}H-NMR spectrum of **1**, the deuterium exchangeable resonances in D_2O at δ_H 5.88 (br) and 6.18 (br) were assigned to primary amide protons. Proton spin decoupling experiments indicated the presence of an isolated methylene group [δ_H 2.92 (1H, d, *J*=18.4 Hz), 3.20 (1H, d, *J*=18.4 Hz)] and an allylic methyl group [δ_H 2.25 (3H, d, *J*=1.5 Hz)] coupled to a proton at δ_H 6.85 (1H, d, *J*=1.5 Hz).

Reduction of **1** with $NaBH_4$ in MeOH afforded a dihydro derivative **(2)**.[2] The IR spectrum of **2** showed the presence of -$CONH_2$ (3450, 3180 and 1675 cm^{-1}), γ-lactone (1760 cm^{-1}) and a double bond (1645 cm^{-1}). However, the absorption band at 1675 cm^{-1} due to a carbonyl group in **1** had disappeared. The UV maximum at 232 nm of **1** due to a α, β-unsaturated carbonyl group was also not observed. Upon irradiation of the methine signal at δ_H 4.77 (ddd, *J*=8.9, 8.9, 6.2 Hz) of **2** which appeared on the reduction of **1**, the signal showed couplings to a vinyl proton at δ_H 6.30 (1H, dd, *J*=1.5, 8.9 Hz) and methylene protons at δ_H 2.22 (1H, dd, *J*=8.9, 14.7 Hz) and 1.83 (1H, m). These results together with ^{1}H-NMR spin decoupling experiments of **1** showed the presence of two partial structures as shown in below.



The signal at δ_H 1.71 (1H, d, *J*=10.7 Hz) was only coupled to a proton at δ_H 2.19 (1H, ddd, *J*=10.7, 5,4, 5.4 Hz). The coupling constant (*J*=5.4 Hz) of the signal at δ_H 2.72 appearing as double doublets revealed w-type the long range coupling with the signal at δ_H 2.44 (m).[3] These results indicated that the dihedral angles between the signal at δ_H 2.72 and 1.71, and the signal at δ_H 2.44 and 1.71 were approximately 90°, respectively, and these protons must be in a rigid cyclic system.

In order to clarify the entire structure, a x-ray crystallographic analysis was carried out using a single crystal of **1** recrystallized from MeOH-H_2O. Crystals of **1** belonged to the triclinic, space group P1 with *a*=6.846(1), *b*=11.079(1), *c*=10.340(1)Å, α=77.47(1), β=90.04(1), γ=108.04(1)°, V=725.99(15)Å^3. Z=2. The structure was solved by direct methods, and block diagonal least-squares refinements with the 1914 observed ($|F_o| \geq 3\sigma(F_o)$) reflections, anisotropic nonhydrogen atoms, and isotopic hydrogens have converged to a final R factor of 0.048.[4]

The ^{1}H- and ^{13}C-NMR data for **1** and **2** were assigned from ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY spectrum and proton spin decoupling experiments as shown in Table.

Mass spectral analysis by EI for **1** gave two prominent peaks at *m/z* 259 (72.60 %) and 165 (19.0 %) together with a base peak at *m/z* 112 (100%). The fragment ions of *m/z* 259 and 165 were expected to have been derived from the molecular ion through the loss of H_2O and the

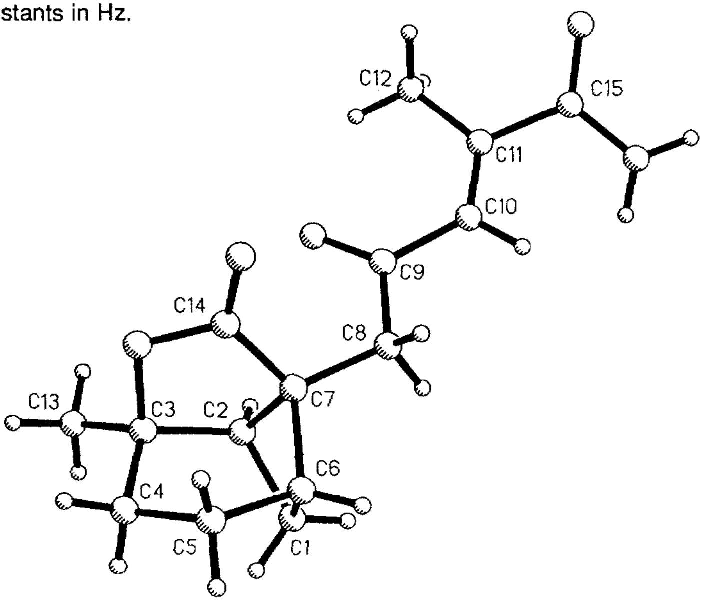
Table. ^{13}C- and ^{1}H-NMR spectral data for Pinthunamide **1** and **2**.[a]

Compounds	**1**		**2**	
carbon number	δ_C	δ_H	δ_C	δ_H
1	23.1 t[b]	1.71 (d, 10.7)[c] 2.19 (ddd, 10.7, 5.4, 5.4)	24.8 t	1.75 (d, 10.7) 2.32 (ddd, 10.7, 5.4, 5.4)
2	47.6 d	2.72 (dd, 5.4, 5.4)	49.1 d	2.66 (dd, 5.4, 5.4)
3	88.9 s		90.9 s	
4	30.1 t	1.93 (2H, s)	31.6 t	1.83, 1.9-2.1 (4H, m)
5	23.0 t	1.93 (2H, s)	24.2 t	
6	40.7 d	2.44 (m)	38.0 d	2.46 (m)
7	53.9 s		56.4 s	
8	44.2 t	2.92 (d, 18.4) 3.20 (d, 18.4)	44.2 t	1.82 (m) 2.22 (d, 14.7, 8.9)
9	198.6 s		67.0 d	4.77 (ddd, 8.9, 8.9, 6.2)
10	128.3 d	6.85 (d, 1.5)	133.0 d	6.30 (dd, 8.9, 1.5)
11	145.2 s		140.4 s	
12	15.1 q	2.25 (d, 1.5)	14.0 q	1.85 (d, 1.5)
13	25.1 q	1.54 (s)	25.9 q	1.43 (s)
14	178.2 s		181.8 s	
15	170.5 s	5.88 (br) 6.18 (br)	174.8 s	

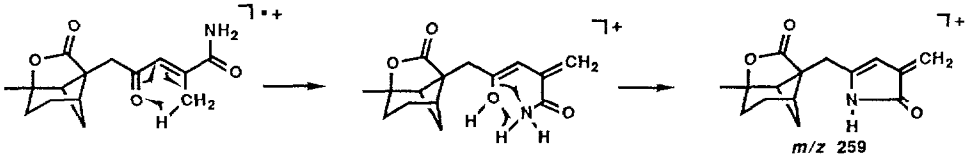
[a] **1** in $CDCl_3$ and **2** in CD_3OD.

[b] s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad.

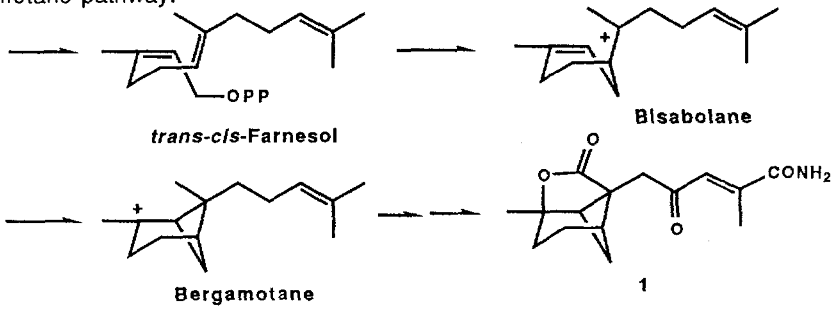
[c] Coupling constants in Hz.



cleavage between C-8 and C-9 carbons, respectively.



Pinthunamide (1) has a unique structure combining four-, five- and six-membered rings and appears to be biosynthesized through the mevalonate--*trans-cis*-farnesol--bisabolane--bergamotane pathway.[5]



The biological activity was examined on lettuce seedlings according to the method of Frankland and Wareing with some modifications.[6] Pinthunamide (1) accelerated the root growth by 150 % at a dose of 300 mg/liter over control seedlings. However, **1** at the concentrations tested scarcely affected hypocotyl elongation.

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REFERENCES AND FOOTNOTES

1) ^{13}C- and ^1H-NMR spectra were obtained on a JEOL GX-270 spectrometer operating at 67.8 MHz and 270 MHz, respectively. Chemical shifts are expressed in ppm using TMS as internal standard.

2) **2**: mp 164-165 °C. IR ν_{max} (KBr) cm^{-1}: 3450, 3180, 1760, 1675, 1645, 1600, 1200, 1080. MS *m/z*: 279 (M^+), 262, 244, 215, 165, 114.

3) L. M. Jackman and S. Sternhell, "*Application of Nuclear Magnetic Resonance Spectroscopy in Organic chemistry.*" Pergamon press, Oxford, **1969**, pp. 334-341.

4) Crystallographic parameters have been deposited with the Cambgidge Crysttalographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K. Please give a complete literature citation when ordering. The other references will have to be renumbered.

5) T. K. Devon and A. I. Scott, "*Handbook of Naturally Occurring Compounds.*" Vol. II. Terpenes, Academic Press, New York and London, **1972**, p57.

6) B. Frankland and P. F. Wareing, *Nature*, **185**, 255 (1960).

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