83

Structure of Islandic Acid, a New Metabolite from *Penicillium islandicum* Sopp.

Yasuo Fujimoto,* Hiroshi Tsunoda, Jun Uzawa, and Takashi Tatsuno The Institute of Physical and Chemical Research, Wako-Shi, Saitama 351, Japan

The structure of islandic acid, a new anti-tumour metabolite isolated from *P. islandicum* Sopp., has been determined by the application of ${}^{13}C-{}^{1}H$ long range selective proton decoupling experiments on its methyl ester.

Penicillium islandicum Sopp. was isolated from imported yellowed rice by Tsunoda in 1948, and two metabolities, luteoskyrin and cyclochlorotine, which caused serious liver damage, have been isolated as major mycotoxins produced

by this fungus.¹ Modification of culture conditions much increased the yield of cyclochlorotine as well as production of a new metabolite. The new metabolite, which we have named islandic acid, showed cytotoxicity against Yoshida sarcoma cells in tissue culture and inhibited the transfection of *Bacillus* phage M_2 . We now describe the isolation and elucidation of the structure of islandic acid.

P. islandicum Sopp. was grown in yeast peptone-Czapek medium at 25 °C. After 2 weeks, the mycelium was resuspended in the medium, and cultivation was continued for a further 10 days. Islandic acid (1) $[C_{17}H_{18}O_8, \text{ m.p. }165-168 °C, \lambda_{max}(MeOH) 234, 260, and 335 nm (<math>\epsilon$ 32 000, 23 000, and 14 500)] was isolated from the culture filtrate by repeated chromatography on charcoal (acetone) and silica gel (chloroform-methanol, 4:1). Its i.r. spectrum [ν_{max} (KBr) 3300 (OH), 2800–2300 (CO₂H), 1750, 1700, and 1675 (CO₂) cm⁻¹] and its ready solubility in 5% NaCHO₃ showed that compound (1) is a carboxylic acid. Since (1) is not sufficiently soluble in CDCl₃ for its ¹³C n.m.r. spectrum to be recorded, it was converted (diazomethane) into the methyl ester (2) ($C_{18}H_{20}O_8$, m.p. 143–145 °C).

The ¹³C and ¹H n.m.r. data (100 MHz, CDCl₃) for (2) are shown in Figure 1. The chemical shifts of the hydrogenbearing carbon atoms were easily assigned by selective proton decoupling experiments. The assignments of the chemical shifts of the carbon atoms bearing no hydrogen and the arrangement of the partial structures derived from the ¹H and ¹³C n.m.r. data were determined by ¹³C-{¹H} long-range selective proton decoupling (l.s.p.d.) experiments.^{2,3}

Low power (0.4-0.5 G) irradiation of the $-\text{CO}_2\text{CH}_2$ protons (δ 5.18) transformed the carbon signals at δ 109.4 (t), 162.6 (m), and 166.0 p.p.m. (br. d) to two singlets (δ 109.4 and 162.6 p.p.m.) and a sharp doublet (δ 166.0 p.p.m.), suggesting that the hexadienoyl moiety was connected to the methylene protons resonating at δ 5.18 and that the other carboxy-carbon atom (δ 162.6 p.p.m.) was separated from the methylene protons by at the most three bonds.[†]

The substitution pattern between the hydroxymethyl and the α,β -unsaturated carboxylic ester moiety was confirmed by ¹³C-{¹H} and ¹³C-{¹H} {¹H} l.s.d.p. experiments. On selective irradiation of the β -hydrogen (δ 7.57) of the α,β -unsaturated carboxylic ester, the multiplet ¹³C signal at δ 154.2 p.p.m.



Figure 1. ¹H and ¹³C N.m.r. data for islandic acid methyl ester (2) (δ values for ¹H and ¹³C).

was decoupled and appeared as a quartet. This signal was more clearly decoupled, and appeared as a sharp doublet, and decoupling of the olefinic carbon signal at δ 118.4 p.p.m. also occurred on irradiation of the protons at δ 7.57 and 4.62 (CH₂OH).

Finally, the position of the methoxy-group was confirmed by selective irradiation of the protons at δ 4.62 and 5.18. In both these experiments, the ¹³C signal at δ 169.6 p.p.m. which was decoupled on irradiation of the methyl protons (δ 4.10) was decoupled to the same extent. Thus, the enolic carbon (δ 169.6 p.p.m.; =COMe) should be placed centrally between the hydroxymethyl and acyloxymethylene protons. These l.s.p.d. results are explained satisfactorily by the structure of islandic acid methyl ester shown in Figure 1. The biological activities of islandic acid and of its relatives will be published elsewhere.

We thank Drs T. Kihara and K. Isono (this institute) and Dr. K. Matsumoto and Prof. H. Hirokawa (Jōchi University) for the tests of cytotoxicity and transfection, respectively. This research was supported in part by a grant from the Ministry of Education, Science and Culture.

Received, 28th July 1981; Com. 916

References

- 1 M. Saito, E. Enomoto, and T. Tatsuno, Microbial Toxins, 1971, 6, 299.
- 2 S. Takenchi, J. Uzawa, H. Seto, and H. Yonehara, *Tetrahedron Lett.*, 1977, 2943.
- 3 J. Uzawa and M. Uramoto, Org. Magn. Reson., 1979, 12, 612.

[†] The presence of the (2Z,4E)-hexa-2,4-dienoyl moiety was confirmed by comparison of the ¹H-n.m.r. spectrum of (2) with that of methyl (2Z,4E)-hexa-2,4-dienoate which was prepared by photo-irradiation of methyl sorbate.