4-Pentadecylpyridine: A Metabolite from *Taphrina pruni*

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Contrary to normal fruit, plums infected with *Taphrina pruni* show various disease symptoms. A comparative study of chemical constituents has proved, by means of GC and GC/MS, that only infected plums contain 4-penta-decylpyridine. Its structure was confirmed by synthesis: a phosphinenickel complex catalyzed the reaction between 4-bromopyridine and penta-decylmagnesium bromide.

Extraction and chromatography of the cultivated fungus have demonstrated that the compound is a fungal metabolite.

Introduction

In early summer 1994 plum trees *(Prunus domestica)* in the vicinity of Bayreuth had been affected by the fungus *Taphrina pruni.* Just after fruit formation, the diseased plums grew abnormally fast. They had a curved shape and their skin was yellowish green. Different from non-infected plums, they did not develop a stone and partly became three times greater in size. At the end of July the affected fruit dried up and fell off the tree.

These discrepancies stimulated us to investigate if there were also differences in chemical constituents. In this context, we report on the identification of a fungal metabolite.

Results and Discussion

Infected as well as non-infected fruits of one tree were harvested at the end of June and equally processed by extraction with solvents of increasing polarity. After chromatography and derivatisation the fractions were analysed by GC and GC/MS. GC-runs of ethylacetate-extracts revealed a compound in the extracts of infected plums which was absent in not infected ones. The low amount of the compound only allowed its identification by MS: the mass spectrum showed a strong molecular ion at mass 289 and thus indicated the presence of a compound containing nitrogen. Peaks at intervals of 14 mass units pointed to a straight aliphatic chain consisting of at least 13 carbons. A strong key fragment was observed at mass 106. Such ions are typical for pyridines substituted in position 3 or 4 (Medina and Spiteller, 1979; Walther and Spiteller, 1993; Biemann, 1962; John and Lamberton, 1967). They are formed by a double hydrogen shift and represent a fragment with a C2 residue (Fig.1):

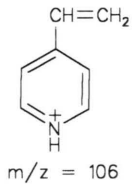

Fig. 1. Key fragment for monoalkylsubstituted pyridines.

Comparison of spectra with 2, 3 and 4-alkylsubstituted pyridines suggested that the compound was 4-pentadecylpyridine (Hardy and Mushrush, 1988).

In order to prove the proposed structure, 4-pentadecylpyridine was synthesized analogously to Kumada *et al.* (1978). A phosphine nickel complex catalyzed the reaction between 4-bromopyridine and pentadecylmagnesium bromide (Fig.2).

Mass spectrum and retention index (RI = 2260) of the natural and the synthetic product were identical.

To ensure whether 4-pentadecylpyridine was a fungal metabolite or *de novo* synthesized from the plum, *Taphrina pruni* was cultivated in petri dishes. For analogous extraction and chromatogra-

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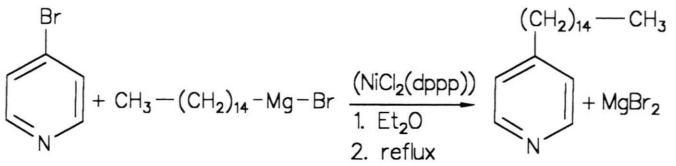

Fig. 2. Synthesis of 4-pentadecylpyridine.

phy of a methanolic extract of the fungus 4-tridecylpyridine was employed as internal standard. This proved that 4-pentadecylpyridine is in fact a compound of fungal metabolism.

4-Pentadecylpyridines with a long aliphatic chain in position 4 have not been found in nature so far. Nevertheless, C-12 to C-14 side chains bound to pyridines in position 3 have been detected in egg tar (K. Tsuji, 1976). Pyridines having short alkyl chains are common aroma constituents (Thomas *et al.*, 1992; Nakamura *et al.*, 1989).

A fungal metabolite of some *Fusarium* species is the pathotoxin fusaric acid (5-*n*-butylpicolinic acid, Fig.3). Fusaric acid was the first wilting toxin that was purified and identified. It was shown that its carboxyl group inhibits fungal spore germination and polyphenoloxidase activity whereas the butyl side chain affects water permeability. This influence on water permeability increases with the length of the alkyl substituent (Kern, 1972; Becker, 1972).

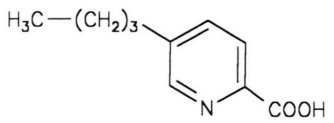

Fig. 3. Fusaric acid.

Experimental

4-bromopyridine was obtained from Fluka Chemie AG (Neu-Ulm).

Analytical GC was carried out using a WCOT glass capillary OV 101 column (30 m x 0.3 mm), temperature program from 80° to 280° at 3°min^{-1}. The temperature of injector and detector (FID) were kept at 270° and 290°, respectively. Carrier gas was H_2 and the split ratio 1: 30. Retention indices were calculated according to Kovats (Kovats, 1958) with *n*-alkanes (C_{10}-C_{36}) as reference compounds.

GC-MS measurements were performed on a fused silica gel column DB1 (30 m x 0,3 mm) with the same temperature program used for analytical GC. The column was connected to a double-focussing spectrometer and EIMS were recorded at 70 eV.

NMR measurements were performed with a Bruker AM 500 NMR spectrometer.

Taphrina pruni was grown on malt extract/agar 2%.

Culture conditions: A suspension of 11.6g malt extract, 8.7g agar and 580ml H_2O was adjusted to pH 7 with diluted KOH and boiled for one hour. The hot solution was applied to plastic petri dishes.

Dichloro(1,3-bis(diphenylphosphino)propane)-nickel(II) was synthesized according to Kumada *et al.* (1978).

4-Pentadecylpyridine: A 1l three-necked flask was charged with 0.25g of dichloro(1,3-bis(diphenylphosphino)propane)nickel(II), 31.4 g (0.2 mol) 4-bromopyridine and 150 ml of dry ether. The mixture was cooled in an ice bath and 125.6 g (0.4 mol) pentadecylmagnesiumbromide in 200 ml of dry ether were added with stirring. Then the mixture was allowed to warm up to room temperature. Within 30 minutes an exothermic reaction started. The solution was stirred at room temperature for 2 more hours and then refluxed for another 6 h. Then the mixture was cooled in an ice bath and hydrolyzed with 250ml 2 N hydrochloric acid. The aqueous layer was extracted twice with ether, washed with water and dried over anhydrous calcium chloride. The crude product was purified by destillation (150°C, ca.10^{-2} torr), yield: 61% (35.2g; 0,12 mol).

^{1}H-NMR: (Me_2CO-d_6) as solvent: δ 0,87 (3H, t, *J*=7 Hz, H-20); δ 1,2–1,4 (24H, br m); δ 1,62 (2H, m, H-2′); δ 2,61 (2H, m, H-1′); δ 7,18 (2H, dd, 3*J*= 4 Hz, 4*J*=1 Hz, H-2 and H-4); δ 8,41 (2H, dd, 3*J*= 5 Hz, 4*J*=1 Hz, H-1 and H-5).

MS: EIMS (probe) 70eV, *m/z* (rel. int.): 289 [M+] (9), 288 (7), 107 (29), 106 (100), 93 (36), 57 (6), 43 (22).

Isolation of 4-pentadecylpyridine: At the end of June 200 g of infected and 200 g of non-infected plums of *Prunus domestica* were harvested near Bayreuth from one tree. The fruits were frozen in liquid nitrogen, homogenized and extracted four times with 500ml MeOH at 20°C. After evaporation of the solvent in vacuum the residue was redissolved in H_2O and extracted three times with 300 ml solvent: first with hexane (HEX) then with ethylacetate (EA) and finally with butanol (BuOH). The EA-extract (3.05g) was chromatographed on silica gel (50g) and eluted with solvents of increasing polarity (CH (cyclohexane), CH/EA 9:1, CH/EA 4:1, CH/EA 2:1, CH/EA 1:1, CH/EA 1:2, CH/EA 1:4). The CH residue was further fractionated by preparative TLC (CH/EE 2 : 1, R_f= 0.59–0.64, layer thickness 0.75mm, UV 254nm).

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