Verticillium rexianum (SACC.) SACC., a New Producer of Monacolin K

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Z. Naturforsch. **53c,** 289–290 (1998); received February 16, 1998

Monacolin K, *Verticillium rexianum, Nectriopsis exigua*, Mycophilic Fungi, Pectate Lyase

From the broth of submerged cultures of *Verticillium rexianum* (teleomorph: *Nectriopsis exigua)*, a mycophilic fungus growing on myxomycetes, monacolin K was isolated as a weak inhibitor of pectate lyase. Monacolin K is the first secondary metabolite described from *V. rexianum*.

Introduction

Mycophilic fungi are widespread in nature and their host range comprises higher fungi as well as lower fungi (Hawksworth, 1981). Nectrotrophic fungi e.g. fungi that kill their hosts, have been found to be a good source for antimicrobial metabolites (Schneider *et al.*, 1997, Wagner *et al.*, 1995, Zapf *et al.*, 1995).

Erwinia carotovora is the causal agent of soft rot of potatoes and other important crops in different climatic regions. The production of the extracellular enzyme pectate lyase (PL) enables the pathogen to macerate host tissue and therefore, this enzyme is discussed as a main virulence factor (Hugouvieux-Cotte-Pattat *et al.*, 1996). In order to detect new natural compounds with plant protectant activity, a screening of fungi for the production of inhibitors of PL was carried out. Among 1064 extracts tested, only two were active. One of them was an extract obtained from cultures of *Verticillium rexianum,* UR 573. *V. rexianum* (teleomorph: *Nectriopsis exigua*) is one of the most common mycoparasites on myxomycetes (Helfer, 1991). As no secondary metabolites were known from this fungal species, the inhibitor was isolated and its structure elucidated. In this note we report *V. rexianum* as a new producer of monacolin K (Endo, 1979), and the effect of the compound on pectate lyase.

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Materials and Methods

Producing organism

Verticillium rexianum, UR 573 was isolated from *Lycogala epidendrum* collected in the Bavarian forest, Germany (Helfer, 1991).

Screening for pectate lyase inhibitors

A primary screening for inhibitors of the PL (E. C. 4.2.2.2.) from *E. carotovora* was carried out using the thiobarbituric acid assay (Easton and Rossall, 1985). The reaction mixture consisted of 200 µl substrate solution containing polygalacturonic acid (0.3%, w/v), pectin N (average molecular weight 30.000, Roth, Karlsruhe), Tris-HCl[tris(hydroxymethyl)aminomethane] 0.6 M, $CaCl_2$ 1.2 mM, pH 8.7 and 5 µl PL (0.85 U/ml; Serva Biochemicals, Heidelberg). After 40 minutes of incubation at 40 °C, the reaction was stopped by adding 10 µl NaOH (0.5 N) and 10 µl $ZnSO_4$ (0.9%). Excess substrate and enzyme were precipitated by centrifugation at 780 g. To 100 µl of the supernatant 30 µl HCl (1 N) and 100 µl thiobarbituric acid (0.5%, w/v) were added and incubated at 70 °C for 15 h. Absorbance of the supernatant was measured at 550 nm.

Fermentation of V. rexianum and isolation of the active compound

Fermentations were carried out in a 100 liter fermentor (Deutsche Metrohm, Filderstadt) with aeration (0.15 vvm) and agitation (150 rpm) in YMG medium consisting of glucose 0.4%, yeast extract 0.4%, malt extract 1%, pH 5.5. The compound was isolated from the culture fluid (80 liters) by adsorption onto HP 21 resin (Mitsubishi) and elution with methanol (5 liters). The crude extract (4.8 g) obtained by concentration was applied in portions of 2.4 g onto a column (40 mm x 140 mm) with silica gel (Merck 60, 0.063 ~ 0.2 mm)

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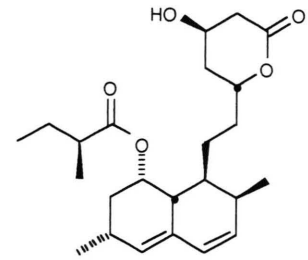
and eluted with 500 ml cyclohexane – ethyl acetate (2:3). Further purification was achieved by preparative HPLC on Merck LiChroSpher RP-18 (7 μm, 250 x 25 mm) and elution with acetonitrile. Followed by preparative HPLC on Merck LiChro-Gel PS I (10 μm, 250 x 25 mm) and elution with 2-propanol (5 ml/minute). Bioactivity-guided isolation yielded 1 mg monacolin K per liter of culture filtrate.



Monacolin K

Results and Discussion

The structure of the isolated compound was determined by mass spectrometry and NMR spectroscopy, and the comparison of the spectral data with those published for monacolin K (or mevinolin) (Endo, 1979; Alberts *et al.*, 1980) revealed its identity.

Monacolin K is a weak inhibitor of PL as shown in Table I, and its mode is competitive.

Table I. Inhibition of pectate lyase (PL) by monacolin K (247 μM) at different substrate concentrations.

Polygalacturonic acid [μM]	Inhibition of PL (%)
50	50
100	33
167	0

Monacolin K and its analogues are known from a large number of ascomycetes and their anamorphs, like *Aspergillus terreus, Emericella unguis, Monascus ruber, Paecilomyces viridis, Penicillium brevicompactum,* and *P. citrinum* (Endo *et al.*, 1997). *Verticillium rexianum* is the first mycophilic species found to produce monacolin K. Whether monacolin, which also exhibits antifungal activity, plays a role in the natural habitat of *V. rexianum* remains to be investigated.

Acknowledgements

We thank H. Besl for providing the strain *V. rexianum* UR 573. Financial support from the BMBF, BASF AG and the Swedish Science Research Council is gratefully acknowledged.

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