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SPIROSTAPHYLOTRICHINS U AND V FROM *CURVULARIA PALLESCENS*

WOLF-RAINER ABRAHAM,* HANS-PETER HANSSEN† and HANS-ADOLF ARFMANN

GBF—Gesellschaft für Biotechnologische Forschung mbH, Department of Microbiology, Mascheroder Weg 1, D-38124 Braunschweig, Germany; †IMB—Industrielle Mikrobiologie, Lurup 4, D-22927 Großhansdorf, Germany

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Key Word Index—*Curvularia pallescens*; Deuteromycotina; Hyphomycetes; phytotoxin; spirostaphylotrichins U and V; spiro-γ-lactams.

Abstract—The fungus *Curvularia pallescens* DSM 62482 produces spirostaphylotrichins A, C, D, R, Q and the new spirostaphylotrichins U and V in submerged culture under limited supply of nitrogen. The configuration of spirostaphylotrichin R could be determined as the 3,4-*cis*-diol. For spirostaphylotrichins U and V, no phytotoxic activity could be detected.

INTRODUCTION

Curvularia pallescens is a Deuteromycete (teleomorph = *Pseudocochliobolus pallescens*) causing black spot disease on food plants like maize (*Zea mays*), coriander (*Coriandrum sativum*), wheat (*Triticum vulgare*), beans (*Phaseolus* spp.) or garlic (*Allium sativum*) [1]. We used *Curvularia pallescens* DSM 62482 for biotransformations and detected some compounds which are secondary metabolites and were only formed if this strain grew under nitrogen-limited conditions. Here we report on their isolation and structure elucidation.

RESULTS AND DISCUSSION

The least polar product proved to be a mixture of two compounds. ^{1}H and ^{13}C NMR spectra of this mixture helped to identify these compounds as spirostaphylotrichins C (**2**) and D (**3**) [2]. The reason why we were unable to separate the mixture chromatographically could be seen in the ability of these epimers to interconvert. A base could form the alcoholate of **2** or **3** which opens the six-membered ring by a retro-aldol type reaction. This monocyclic intermediate can undergo an aldol reaction, again yielding both epimers **2** and **3**. The ratios of **2** and **3** in the mixtures obtained from the individual fermentations were 1:3 to 1:2, but never 1:1 indicating that racemization is an artifact of isolation. A third metabolite displayed very similar resonances in the NMR spectra (Table 1), but in the ^{13}C NMR spectrum a new resonance at $\delta_C = 64.9$ appeared, while C-3, C-5 and C-12 were deshielded and C-11 was shielded (Table 2). This pointed to a hydroxyl group at C-4. From the literature this compound was identified as spirostaphylotrichin A (**1**) [3]. Another spirostaphylotrichin could be separated from spirostaphylotrichin A only with difficulty. The ^{1}H NMR showed the resonance of a methyl group at $\delta_H = 1.70$ instead of the exomethylene group. The metabolite could be identified from its ^{13}C NMR data as spirostaphylotrichin Q (**4**), with its characteristically deshielded resonance of C-11.

Table 1. ^{1}H NMR data of spirostaphylotrichin R (**5**), U (**6**) and V (**7**) ($CDCl_3$, 400 MHz)

	5	**6**	**7**
4-H	4.10 *s*	3.82 *s*	3.81 *s*
6-H	4.75 *s*	4.75 *s*	4.87 *s*
8-H	5.94 *d*	5.98 *d*	5.96 *d*
9-H	7.10 *d*	7.04 *d*	7.07 *d*
11-H	1.68 *s*	1.59 *s*	1.46 *s*
12-H	6.18 *t*	6.36 *t*	6.17 *t*
13-H	2.20 *m*	2.26 *m*	2.29 *m*
13′-H		2.12 *m*	2.06 *m*
14-H	1.04 *t*	1.07 *t*	1.08 *t*
15-H	4.02 *s*	4.00 *s*	4.02 *s*
MeO	—	—	3.43 *s*

J (Hz): 8,9 = 10; 12,13 = 12,13′ = 7; 13,14 = 13′, 14 = 7.

The most polar fraction yielded three more spirostaphylotrichins. Two of them form a pair which we could not separate completely. The ^{13}C NMR data were in agreement with a 3,4-dihydroxy-spirostaphylotrichin and the minor compound of this mixture was identified as spirostaphylotrichin R. Unfortunately Sandmeier and Tamm [4], who first identified this spirostaphylotrichin, could not determine the configuration at C-3 of this compound. The isolation of the isomeric triol, named spirostaphylotrichin U, and characterization of the methoxy-derivative of one of these isomers, which is the third

*Author to whom correspondence should be addressed.

Table 2. ^{13}C NMR data of spirostaphylotrichins **2**, **3** and **5**–**7** ($CDCl_3$, 75.5 MHz)

	2	3	5	6	7
C-1	164.1 *s*	165.0 *s*	167.3 *s*	166.4 *s*	166.1 *s*
C-3	137.3 *s*	138.2 *s*	86.6 *s*	90.5 *s*	94.8 *s*
C-4	n.d.	n.d.	68.7 *d*	73.1 *d*	68.2 *d*
C-5	53.8 *s*	53.8 *s*	56.7 *s*	56.7 *s*	57.4 *s*
C-6	73.6 *d*	73.2 *d*	73.4 *d*	73.4 *d*	73.8 *d*
C-7	194.3 *s*	194.6 *s*	197.0 *s*	195.4 *s*	197.2 *s*
C-8	121.6 *d*	121.5 *d*	120.8 *d*	120.0 *d*	121.4 *d*
C-9	149.8 *d*	150.4 *d*	153.0 *d*	154.1 *d*	153.0 *d*
C-10	128.8 *s*	127.4 *s*	128.7 *s*	127.4 *s*	128.9 *s*
C-11	91.8 *t*	91.9 *t*	23.7 *q*	18.7 *q*	17.0 *q*
C-12	147.2 *d*	147.6 *d*	150.6 *d*	152.7 *d*	149.3 *d*
C-13	24.6 *t*	22.7 *t*	23.4 *t*	23.7 *t*	23.7 *t*
C-14	12.7 *q*	13.1 *q*	13.4 *q*	13.1 *q*	13.4 *q*
C-15	63.0 *q*	63.0 *q*	64.6 *q*	64.7 *q*	64.3 *q*
MeO	—	—	—	—	51.0 *q*

n.d. = not detected.

compound of this complex and named spirostaphylotrichin V, gave us sufficient data to solve this problem. Spirostaphylotrichin R, U and V all have the 6*S*-hydroxy group as judged from the shift of 6-H in the ^{1}H NMR spectrum. To decide which of the triols is *cis*- and which one is *trans*- we compared their ^{13}C NMR resonances with those of (1*S*,2*S*)- and (1*R*,2*S*)-1,2-dihydroxy-3-menthene we produced in the biotransformation of α-terpinene with *Corynespora cassiicola* DSM 62474 [5]. In the *cis*-diol of these monoterpenes the adjacent methyl group is deshielded compared to the *trans*-diol (δ_C = 24.4 and δ_C = 20.1), while the resonance of the carbon bearing the secondary alcohol is shifted to higher field in the *cis*-alcohol compared to the *trans*-diol (δ_C = 71.8 and δ_C = 74.8) as C-1 is (δ_C = 70.1 and δ_C = 72.7). With this information in hand we could assign the stereochemistry of spirostaphylotrichin R, U and V. In spirostaphylotrichin R the C-11 methyl group resonated at δ_C = 23.7, C-3 at δ_C = 86.6 and C-4 at δ_C = 68.7, compared to δ_C = 18.7, δ_C = 90.5 and δ_C = 73.1 for spirostaphylotrichin U, respectively. This requires the *cis*-configuration **5** for spirostaphylotrichin R and the *trans*-configuration **6** for spirostaphylotrichin U. This assignment is further corroborated by the fact that in **5** an NOE is observed at 4-H after irradiation at the resonance of 11-H. The same arguments are applicable to the methoxy-ether, so its configuration was determined as **7**. Additionally a shielding of C-11 is observed here caused by the γ-effect of the methoxy group which is very near its predicted value of $\Delta\delta_C$ = − 7 ppm.

Spirostaphylotrichins U and V are new natural products while the configuration of spirostaphylotrichin R could be clarified. Spirostaphylotrichins A, C, D, Q and R are known from *Staphylotrichum coccosporum* [4]. Spirostaphylotrichins C and D were first isolated from *Drechslera tritici-repentis* (now *Pyrenophora tritici-repentis*) and named triticone A and B. Later on they were also found in *Curvularia lunata* [6]. Related compounds where the nitrogen is replaced by oxygen were reported from the fungus *Arthropsis truncata* [7]. Spirostaphylotrichin A possesses lipid-lowering activity [8] while

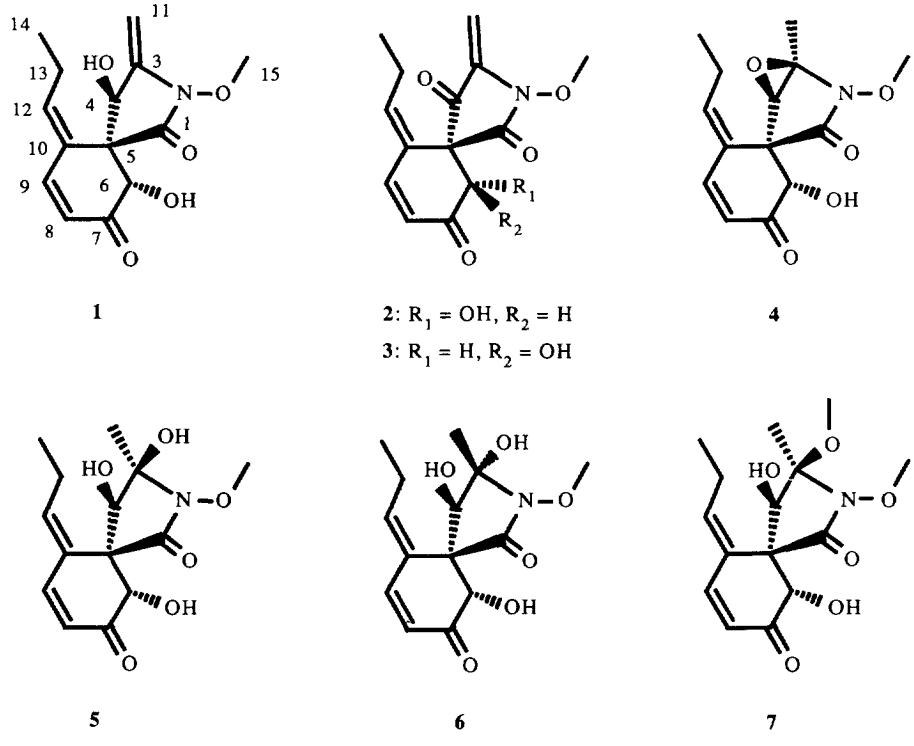

Fig. 1. Spirostaphylotrichins from *Curvularia pallescens* DSM 62482.

spirostaphylotrichins C and D are phytotoxins. The latter inhibit photosynthetic electron transport in chloroplasts [9]. For spirostaphylotrichins U and V we could not find any phytotoxic activity. *Curvularia pallescens* DSM 62482 also produces some metabolites which do not belong to the spirostaphylotrichin group. They are currently under further study.

EXPERIMENTAL

Curvularia pallescens DSM 62482 was grown in 10 × 1 l flasks filled with 200 ml of the medium containing glucose (10 g), NH_4Cl (0.1 g), K_2HPO_4 (0.5 g), KCl (1 g), $MgSO_4 \times 7H_2O$ (0.2 g), $CaCl_2 \times 2H_2O$ (0.1 g) in 1 l deionized water. After 10 days the culture broth was filtered and extracted × 3 with EtOAc. After drying with Na_2SO_4 the solvent was evapd and the crude extract was sepd on a Si-60 column with a *n*-hexane/EtOAc gradient (changing from 19:1 to 0:1). When necessary the collected frs were purified further by prep. TLC on silica Si-60.

The ^1H and ^{13}C NMR spectra were obtained at 400 and 75.5 MHz, respectively. $CDCl_3$ was the solvent and TMS the int. standard. Mass spectra were recorded with 70 eV. IR spectra (DRIFT) were measured on KBr, UV spectra in MeOH.

From 2 l of culture broth **1** (6 mg), **2** and **3** (15 mg), **4** (24 mg), **5** (3 mg), **6** (6 mg) and **7** (5 mg) were obtained.

Spirostaphylotrichin U **6**. Not completely sepd from **5**. R_f 0.56 (EtOAc). UV: λ_{max} 290 nm. IR: 3385, 1685 cm^{-1}. MS *m/z*: 297.1210 (297.1212 calcd for $C_{14}H_{19}NO_6$) (4%), 279 (35), 224 (100), 177 (47), 149 (52).

Spirostaphylotrichin V **7**. Glassy, yellowish crystals. R_f 0.55 (EtOAc). UV: 289 nm. IR: 3383, 1687 cm^{-1}. MS (*m/z*): 311.1355 (311.1369 calcd for $C_{15}H_{21}NO_6$) (2%), 279 (100), 224 (45), 207 (59), 149 (94).

$$[\alpha]^{27} = \frac{\begin{matrix}589\text{ nm} & 578\text{ nm} & 546\text{ nm}\end{matrix}}{\begin{matrix}-147.6^\circ & -151.2^\circ & -155.0^\circ\end{matrix}} \quad (c = 0.50)$$

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