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Fusoxysporone - a New Type of Diterpene from *Fusarium oxysporum*

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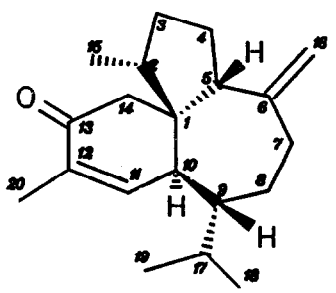
Abstract: A novel diterpene, fusoxysporone **1**, was isolated from liquid cultures of the fungus *Fusarium oxysporum* IMB FO 1/82. The skeleton of **1** - related to the viscidane type - is described for the first time and named fusoxysporane. Additionally, fusaric acid and dehydrofusaric acid were identified in extracts of the culture medium.

Introduction

Fusarium oxysporum Schlecht.: Fr. (Deuteromycotina) is an economically important soilborne plant pathogen. The taxonomic disposition of individual *Fusarium* strains is somewhat problematic, as members of this genus may vary widely in morphological and nonmorphological characteristics[1]. For *Fusarium oxysporum* over 120 formae speciales and races have been described[2]. From *Fusarium* species a number of secondary metabolites have been isolated including terpenes, polyketides and compounds derived from amino acid metabolism[3,4]. Some of these metabolic products like sesquiterpenes derived from the trichothecane skeleton or the gibberellins exhibit biological activity.

Results and Discussion

Fusarium oxysporum IMB FO 1/82 was cultivated for 7 weeks in a liquid biomalt medium. Main compound of the distillates is an UV-absorbing substance which displayed a molecular formula of $C_{20}H_{30}O$ in the mass spectra. ^{13}C NMR showed four carbons belonging to two double bonds since they resonated between δ_C 122.0 and δ_C 149.1. Another carbon at δ_C 200.5 is part of an α,β-unsaturated ketone forming the chromophore of the UV-absorption. Taking these informations together a tricyclic skeleton is required to give the molecular formula. This tricyclus must be a spiro compound because all methyl groups adjacent to sp^3-carbons displayed doublets in the ^1H NMR spectra and a singlet is found in the ^{13}C NMR at δ_C 48.4. ^1H NMR showed two protons at δ_H 4.93 and δ_H 4.74 belonging to an exomethylene group. These protons had couplings to two protons displaying fourfold doublets at δ_H 2.49 and δ_H 2.09 and to a broad triplet at δ_H 2.83. The analysis of



Fusoxysporone **1**

the COSY-45 $^1H\{^1H\}$ NMR revealed a 4,5,5-trisubstituted 2-methyl-cyclohexen-2-one-1. To this ring a seven-membered ring containing the exo-methylene group and an isopropyl moiety is attached and the spiro-centre is completed by a five-membered ring bearing a methyl group. 2D ^1H-detected one-bond ^{13}C-^1H correlation (HMQC)[5] allowed the assignment of all carbons. The carbon framework of a tricyclo[8.4.0.0^{1,4}]tetradecene-11 deduced from the COSY-45 was confirmed by the 2D ^1H-detected long-range ^{13}C-^1H shift correlation (HMBC)[6], via $^2J_{CH}$ and $^3J_{CH}$ (Table 1). This led to the constitution of 2,12-dimethyl-9-isopropyl-6-methylene-tricyclo[8.4.0.0^{1,4}]tetradecen-11-one-13 for this metabolite. This constitution presents a diterpenic backbone not observed so far in nature.

Table 1: NMR data of fusoxysporone **1**, fusaric acid **2** and dehydrofusaric acid **3** ($CDCl_3$)

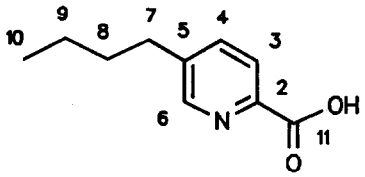
	1			**2**		**3**	
C		crosspeaks with H ($^1J_{CH}$)					
		H	H'	C	H	C	H
C-1	48.4 s	-	-	-	-	-	-
C-2	50.8 d	1.85 m	-	144.8 s	-	145.0 s	-
C-3	28.7 t	1.79 m	1.39 m	124.7 d	8.19 d	124.7 d	8.19 d
C-4	25.1 t	1.82 m	1.67 m	138.1 d	7.80 d br	138.2 d	7.80 d br
C-5	51.7 d	2.83 dd br	-	143.0 s	-	141.9 s	-
C-6	149.1 s	-	-	148.1 d	8.65 s br	148.3 d	8.65 s br
C-7	41.0 t	2.49 dddd	2.09 m	34.4 t	2.74 t	32.3 t	2.85 t
C-8	27.4 d	1.58 m	1.17 dddd	32.7 t	1.66 tt	32.8 t	2.44 dt
C-9	49.5 d	1.64 m	-	22.1 t	1.37 tq	136.3 d	5.82 m
C-10	45.1 d	2.61 m	-	13.6 q	0.94 t	116.2 t	5.02 m
C-11	145.8 d	6.56 dq	-	165.3 s	-	165.3 s	-
C-12	136.3 s	-	-				
C-13	200.5 s	-	-				
C-14	38.5 t	2.31 d	2.08 d				
C-15	15.4 q	0.79 d	-				
C-16	122.0 t	4.93 m	4.74 ddd				
C-17	30.8 d	1.59 m	-				
C-18	21.6 q	0.98 d	-				
C-19	21.4 q	1.00 d	-				
C-20	16.2 q	1.80 dd	-				

J(Hz): **1**: 2,15=6.5; 3',15>0; 4,5=4'5=9; 5,16'=1.7; 7,7'=15; 7,8=5.5; 7,8'=2.5; 7,16=0.7; 7',8'=12.5; 7',16'=1.7; 8,8'= 14.2; 8',9=8; 10,11=4; 10,20= 2.5; 11,20= 1.5; 14,14'=17.7; 16,16'= 1.7; 17,18=6.0; 17,19=6.5. **2**: 3,4=5;7,8=8,9=9,10=7. **3**: 3,4=5;7,8=8,9=7.

The relative configuration could be deduced from the close similarity to the sesquiterpene acorenone and the assignments of the ^{13}C resonances of the diastereomers acorenone-A, acorenone-B, 4-epi-acorenone-A, and 4-epi-acorenone-B by Wolf and coworkers[7]. Only the acorenone-A showed a

resonance of the methylene adjacent to the keto function at δ_C 39.0 while this carbon of the other three compounds resonated between δ_C 45.6 and δ_C 49.6. The reason for this upfield shift are the γ-gauche-effects of the methyl- and the isopropyl group which are syn to this methylene. The observation that C-14 of the compound from *Fusarium oxysporum* displayed a resonance at δ_C 38.5 in ^{13}C NMR which is very close to the corresponding one of acarenone-A requires the (1RS,2SR,5SR)-configuration of the diterpene. Since C-9 of the seven-membered ring which is in γ-position to C-14 shielded this carbon only of $\Delta\delta$ 0.5 ppm, requiring a dihedral angle close to 180°, the configuration at C-10 is SR. This results in an axial position of 10-H which is in agreement with the large homoallylic coupling between 10-H and 20-H and the absence of a W-coupling between 10-H and 14-H. The $^3J_{CH}$ couplings observed in the HMBC spectra corroborate these assignments. $^3J_{CH}$ couplings between δ_C 38.5 (C-14) and δ_H 2.83 (5-H), between δ_C 122.0 (C-16) and the same proton, and between δ_C 50.8 (C-2) and δ_H 2.31 (14-H_{ax}) confirm the relative configuration at the cyclopentane part of the molecule. A $^3J_{CH}$ coupling between δ_C 45.1 (C-10) and δ_H 1.17 (8-H_{ax}) corroborates the configuration at C-10. The stereochemistry at C-9 was deduced from the fact that a $^3J_{CH}$ coupling between δ_C 30.8 (C-17) and δ_H 1.58 (8-H_{eq}) was seen. Also in the DNOE spectra an enhancement at δ_H 1.64 (9-H) was observed after irradiation at δ_H 2.83 (5-H), although the DNOE spectra were of low quality due to the fact that the region between δ_H 1.85 and δ_H 1.58 was crowded by the resonances of seven protons with coupling patterns of higher order.

Taking all these informations together the main metabolite of *Fusarium oxysporum* is (1RS,2SR,5SR,9SR,10RS)-2,12-dimethyl-9-isopropyl-6-methylene-tricyclo[8.4.0.0^{1,4}]tetradecen-11-one-13 which is named fusoxysporone. The diterpene skeleton which is found here for the first time is called fusoxysporane. A related skeleton lacking the bond between C-9 and C-10 is viscidane but has a different configuration at C-5[8]. It was reported from *Eremophila* [9].



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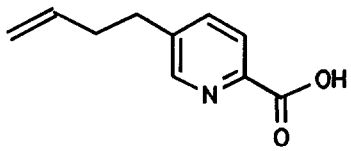

3

Only diterpenes of the kaurane and gibberellane type are known from the genus *Fusarium*. To our knowledge only the latter was reported from *Fusarium oxysporum*[10,11]. The *Fusarium oxysporum* strain studied here forms also some sesquiterpenes whose structure elucidation is in progress.

From the diethyl ether extracts, we could additionally isolate a mixture of fusaric acid **2** and dehydrofusaric acid **3**. The compounds were identified by their NMR data (Table 1). These metabolites have formerly been described from other *F. oxysporum* strains[12,13]. Their biosynthesis and possible role in the host-pathogen relationship have been studied intensely[3].

Experimental:

Fusarium oxysporum IMB FO 1/82 was cultivated in a biomalt (2%)-liquid medium in 1000 ml Fernbach flasks. After 7 weeks, the cultures were harvested and the volatiles were obtained by circulation steam

distillation. The culture broth was additionally extracted with diethyl ether. The crude extracts (441mg) were chromatographed on a Lobar Si-60 column (Merck) using a gradient from pure n-hexane to pure ethyl acetate. When necessary the collected fractions were further purified by prep. TLC. From 3 l of medium 39.5 mg of fusoxysporone and 155.9 mg of a mixture (4:5) of fusaric acid and dehydrofusaric acid were isolated.

GLC analyses of the volatiles were performed using a glass capillary SE-54 (25m x 0.32mm i. d.), FID and a computing integrator. Operating conditions: linear temperature programme 80 - 220°C, 2°C/min.

NMR: All spectra were recorded at 300°K on a Bruker AM 600 NMR spectrometer locked to the deuterium resonance of the solvent. The value of the delay to optimise one-bond correlations in the HMQC spetrum and supress them in the HMBC spectrum was 3.45 ms and the evolution delay for long-range couplings in the latter was set to 70 ms. All spectra were recorded using the standard Bruker software package and data manipulation of the 2D spectra were performed on a Bruker Aspect X32 Data station. All chemical shifts are given in ppm relative to TMS and couplings in Hz[14]. MS analyses were carried out on a Varian MAT 112S mass spectrometer (80eV).

Fusoxysporone (1): Colorless viscous liquid, R_f 0.39 (n-hexane/EtOAc 19:1), IR ($CHCl_3$): 1665 cm^{-1}. UV (CH_3OH): 241 nm. HR-MS (m/z): 286.2289 (M^+, 286.2297 calc. for $C_{20}H_{30}O$). GLC-MS: 286 ($[M]^+$, 42% rel. intensity), 271 ($[M\text{-}CH_3]^+$, 5), 258 ($[M\text{-}CO]^+$, 6), 243 ($[M\text{-}C_3H_7]^+$, 45), 175 (34), 161 (52), 148 (65), 136 (100), 135 (88), 121 (27), 109 (51).

$$[\alpha]^{25°} = \frac{\begin{array}{cccc}589nm & 578nm & 546nm & 436nm\end{array}}{\begin{array}{cccc}+146.6° & +153.4° & +174.6° & +292.6°\end{array}} \quad (CHCl_3,\ c=0.50)$$

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