Pterulinic Acid and Pterulone, Two Novel Inhibitors of NADH : Ubiquinone Oxidoreductase (Complex I) Produced by a *Pterula* Species

II. Physico-chemical Properties and Structure Elucidation†

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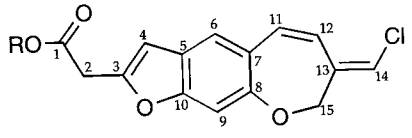
(Received for publication November 18, 1996)

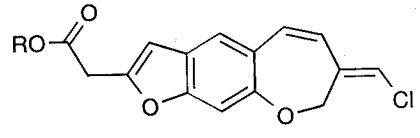
The structures of two novel fungal antibiotics, isolated from a *Pterula* species, that interfere with the NADH : ubiquinone oxidoreductase and inhibit the respiration of eucaryotes, were determined by spectroscopic techniques. Both compounds, pterulinic acid (**1a**) and pterulone (**2**), contain a 1-benzoxepin ring system and are chlorinated. Pterulinic acid (**1a**), which was obtained as a 1 : 5 inseparable mixture of the two isomers (*Z*)-**1a** and (*E*)-**1a**, in addition contains a furan. Their structures were determined by mass spectrometry and NMR spectroscopy, and 2D heteronuclear correlation experiments permitted the assignment of all NMR signals.

The fungal metabolites pterulinic acid (**1a**) and pterulone (**2**) were isolated from fermentations of a *Pterula* species in the course of a screening of basidiomycetes for the production of new antifungal compounds.[1] **1a** and **2** exhibit strong antifungal and weak cytotoxic activities. Both antibiotics are inhibitors of eucaryotic respiration, interfering with NADH:ubiquinone oxidoreductase.[1]

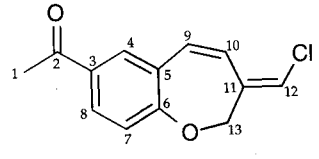
Pterulinic acid (**1a**) was obtained as a 1 : 5 mixture (calculated from the integrals in the ^1H NMR spectrum) of two isomers that could not be separated from each other. Although the ^1H and ^{13}C NMR data were very similar for the two components, indicating that they are geometric isomers, almost all of the NMR signals were resolved and the structures of the two could be determined independently. The relative intensities of the peaks in the EI mass spectrum was constant during the MS measurements, and there was no indication that the two components should not have the same composition. High resolution measurements of the mass of the molecular ion indicated that their elemental composition is $C_{15}H_{11}O_4Cl$, which corresponds to an unsaturation index of 10, and the signals for 10 pairs of protons and 15 pairs of carbons could be observed in the 1D NMR spectra recorded in $CDCl_3 : CD_3OD$ 95 : 5. The chemical shifts for the signals in the ^{13}C NMR spectrum suggested that the compounds contain one carbonyl function (δ 171.1) and 6 double bonds, and pterulinic acid (**1a**) should therefore contain 3 rings. The 1D NMR data are given in Tables 2 and 3, and pertinent data from HMBC and NOESY 2D NMR experiments are shown in Figs. 1 and 2. The major component was found to be the 13*E* isomer while the minor component is the 13*Z* isomer. The major differences between the NMR spectra of the two components can be seen in the vicinity of the chlorinated double bond, and the NOESY correlations observed



(*E*)-**1**



(*Z*)-**1**



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a: R=H; **b**: R=CH_3.

† Dedicated to Prof. Dr. H.-G. KUBALL on the occasion of his 65th birthday.

Table 1. Physico-chemical properties of pterulinic acid (**1a**) (a 1:5 mixture of the (13*Z*)-**1a** and (13*E*)-**1a**) and pterulone (**2**).

	1a	**2**
Appearance	Yellowish crystals, mp 152~154°C	Yellowish crystals, mp 122~124°C
Molecular formula	$C_{15}H_{11}O_4Cl$	$C_{13}H_{11}O_2Cl$
HREI-MS (*m/z*)		
Observed	290.0347 M^+	234.0455 M^+
Calculated	290.0346 for $C_{15}H_{11}O_4Cl$	234.0448 for $C_{13}H_{11}O_2Cl$
EI-MS	292 (34%), 290 (M^+, 100%), 255 (68%), 247 (8%), 245 (24%), 227 (15%), 210 (35%), 181 (43%), 171 (34%), 152 (17%)	236 (33%), 234 (100%), 219 (60%), 199 (65%), 156 (22%), 145 (18%), 143 (17%), 130 (16%), 128 (27%)
UV (MeOH)		
λ_{max} nm (ε)	258 (39,800), 295 (25,000) 302 (infl. 23,500), 324 (infl. 8,700)	266 (44,200), 302 (14,400)
IR (KBr) cm^{-1}	3435, 2925, 1705, 1470, 1345, 1275, 1245, 1145, 1100, 960, 885 and 775	1680, 1600, 1495, 1355, 1330, 1280, 1255, 1125, 990, 830, 810, 780, 760, 660 and 575
TLC (Rf)	0.65[a], 0.67[b]	0.66[a], 0.60[b]

[a] Merck, Kieselgel 60 F_{254}: Toluene - acetone - AcOH (70:30:1).
[b] Merck, Kieselgel 60 F_{254}: Toluene - ethyl formate - formic acid (10:5:3).

Table 2. ^1H NMR data of the two pterulinic acid isomers (*E*)-**1a** and (*Z*)-**1a**, and pterulone (**2**).

Position	(*E*)-**1a**	(*Z*)-**1a**	**2**
1	—	—	2.57 (s)
2	3.72 (d; 0.9)	3.72 (d; 0.9)	—
4	6.51 (t; 0.9)	6.51 (t; 0.9)	7.91 (d; 2.2)
6	7.33 (s)	7.29 (s)	—
7	—	—	7.03 (d; 8.4)
8	—	—	7.78 (dd; 2.2, 8.4)
9	7.02 (s)	7.06 (s)	6.59 (dm; 11.8)
10	—	—	6.87 (dm; 11.8)
11	6.58 (dm; 12.0)	6.37 (dm; 12.0)	—
12	6.69 (dm; 12.0)	6.21 (dm; 12.0)	6.17 (m)
13	—	—	4.59 (dd; 0.6, 0.6)
14	6.03 (m)	6.22 (m)	
15	4.52 (m)	4.84 (m)	

The spectrum of the pterulinic acid mixture was recorded in $CDCl_3$: CD_3OD 95:5, and the spectrum of pterulone (**2**) in $CDCl_3$ at 500 MHz. The solvent signal of $CHCl_3$ (7.26 ppm) was used as reference.

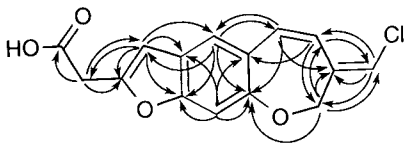
between 14-H and 15-H_2 in (*E*)-**1a** and between 12-H and 14-H in (*Z*)-**1a** determined the actual difference. 14-H gives HMBC correlations to C-12, C-13 and C-15, an oxygenated saturated carbon, and in addition weak COSY correlations between 14-H and 12-H as well as 15-H_2 were observed. In the coupled ^{13}C NMR spectrum it was noted that $^1J_{C,H}$ for C-14 is 195.8 Hz, a typical value for a R, R'C=CH, Cl group.[2] 15-H_2 give HMBC correlations to C-12, C-13, C-14 and also to C-8, which according to its chemical shift could be an oxygenated aromatic carbon. 11-H, which couples with 12-H with the coupling constant 12.0 Hz, gives HMBC correlations to C-6, C-8 as well as C-13, and the HMBC correlations from 6-H to C-8 and C-11, and from 12-H to C-7, establish the oxepin ring. The assignment of the chemical shifts for the benzene ring is facilitated by the HMBC correlations from 6-H to C-8 and C-10, and from 9-H to C-5, C-7, C-8 and C-10, and a correlation between 6-H and C-4 suggests that C-4 is attached to C-5. This is confirmed by the HMBC correlations between 4-H and C-3, C-5, C-6 and C-10, and also by the NOESY correlation between 4-H and 6-H, which, however, is weaker compared to the correlation between 6-H and 11-H. These data as well as the values of the chemical

Table 3. ^{13}C NMR data of the two pterulinic acid isomers (*E*)-**1a** and (*Z*)-**1a**, and pterulone (**2**).

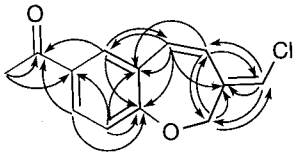
Carbon No.	(*E*)-**1a**	(*Z*)-**1a**	**2**
C-1	171.1 (s)	171.1 (s)	26.4 (q)
C-2	34.3 (t)	34.3 (t)	196.6 (s)
C-3	151.8 (s)	151.8 (s)	132.4 (s)
C-4	104.6 (d)	104.6 (d)	134.2 (d)
C-5	124.5 (s)	124.6 (s)	126.6 (s)
C-6	124.2 (d)	123.2 (d)	162.9 (s)
C-7	123.8 (s)	124.6 (s)	120.3 (d)
C-8	157.0 (s)	156.8 (s)	129.5 (d)
C-9	102.4 (d)	102.8 (d)	130.6 (d)
C-10	155.0 (s)	154.7 (s)	124.9 (d)
C-11	131.5 (d)	128.4 (d)	135.8 (s)
C-12	121.8 (d)	125.9 (d)	120.4 (d)
C-13	136.6 (s)	138.7 (s)	72.5 (t)
C-14	117.8 (d)	118.9 (d)	
C-15	73.2 (t)	69.1 (t)	

The spectrum of the pterulinic acid mixture was recorded in $CDCl_3$: CD_3OD 95 : 5, and the spectrum of pterulone (**2**) in $CDCl_3$ at 125 MHz. The solvent signal of $CDCl_3$ (77.0 ppm) was used as reference.

Fig. 1. Pertinent HMBC correlations observed with (13*E*)-pterulinic acid [(13*E*)-**1a**] and pterulone (**2**).



1a



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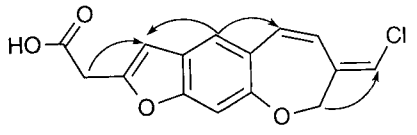
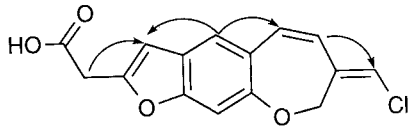
The corresponding correlations were observed also with the *Z*-isomer [(13*Z*)-**1a**].

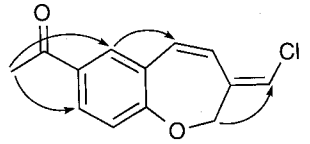
Fig. 2. Pertinent NOESY correlations observed with both pterulinic acid isomers (13*E*)-**1a** and (13*Z*)-**1a**, and with pterulone (**2**).



(*E*)-**1a**



(Z)-**1a**



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shifts of C-3 and C-4 are in agreement with the proposed furan, and the allylic ^1H-^1H coupling between 2-H_2 and 4-H as well as the NOESY correlation between them indicate that C-2 is bonded to C-3. This is confirmed by the HMBC correlations from 2-H_2 to C-3 and C-4, and also to C-1. The presence of a free carboxylic acid group in pterulinic acid (**1a**) was established by the transformation of **1a** to its methyl ester **1b**. The ^1H NMR spectrum of the methyl ester **1b** is very similar to that of pterulinic acid (**1a**), with the exception of the signal for the methoxy protons which appears at the expected value (3.75 ppm, see Experimental). The comparison of the EI-MS data of **1a** and **1b** show that while **1a** loses the chlorine and/or the carboxylic acid group (*m/z* 290, 255, 245 and 210), **1b** loses the chlorine and/or the carboxylic acid methyl ester group (*m/z* 304, 269, 245 and 210, elemental composition indicated by high resolution EI-MS).

To some extent, the spectroscopic data for pterulone (**2**) are similar to those of pterulinic acid (**1a**). However, the mass spectrum reveals that it is smaller, and data from high resolution measurements suggest that its elemental composition is $C_{13}H_{11}O_2Cl$ and that the unsaturation index is 8. The benzene ring is 1,3,4-trisubstituted, as shown by the ^1H-^1H coupling pattern of the remaining aromatic protons, and the presence and the position of an acetyl group is indicated by the HMBC correlations from 1-H_3 to C-2 as well as C-3, and the NOESY correlations between 1-H_3 and 4-H as well as 8-H. The HMBC correlations observed for the aromatic protons facilitate the assignment of the remaining aromatic carbons (C-5 and C-6), and indicate that the carbon skeleton of pterulone (**2**) continues from C-5 with C-9. The ^1H-^1H coupling constant between 9-H and 10-H is similar to that for the corresponding protons in pterulinic acid (**1a**), and the presence of an oxepin ring is supported by the HMBC correlations from 10-H to

C-11, C-12 and C-13, and from 13-H_2 to C-6, C-10, C-11 and C-12. The NOESY correlation between 12-H and 13-H_2 show that the C-11/C-12 double bond is *E*, corresponding to the major isomer of pterulinic acid (**1a**).

Only a few 1-benzoxepin natural products have previously been reported, and pterulinic acid (**1a**) and pterulone (**2**) are as far as we know the first two produced by a fungus. The heartwood of *Ptaeroxylon obliquum* has yielded several chromones containing an oxepin ring,[3] and so have the plants *Cneorum pulverulentum*[4] and *Cnidium monnieri*.[5] However, no bioactivities of these compounds have been reported.

Experimental

General

The isolation of pterulinic acid (**1a**) and pterulone (**2**) is reported in the preceding paper.[1] Trimethylsilyldiazomethane used for methylation of pterulinic acid was purchased from Aldrich, as a 2 M solution in hexane. UV spectra were obtained with a Perkin Elmer λ 16, and IR spectra with a Bruker IFS 48. The melting points, which are uncorrected, were determined using a Reichert microscope. Mass spectra were recorded with a Jeol JMS-SX102 spectrometer, and NMR spectra were recorded at room temperature with a Bruker ARX 500 spectrometer with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were performed with gradient enhancements using sine shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH}=145$ Hz and $^2J_{CH}=10$ Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001).

Methyl Pterulinate (**1b**)

Methyl pterulinate (**1b**) was obtained as a yellowish oil, as a 1:5 mixture between (13*Z*)-**1b** and (13*E*)-**1b**, by the methylation of pterulinic acid (**1a**) with TMS-diazomethane. Pterulinic acid (2 mg) was dissolved in methanol (1 ml) and 5 drops of TMS-diazomethane in hexane (2 M) was added. The solution was kept at room temperature for 15 minutes whereafter the volatiles were evaporated by a stream of nitrogen gas. UV (MeOH) λ_{max} (ε): 256 (29,400), 294 (18,000), 302 (infl., 15,800) and 324 (infl., 6,300). IR (KBr): 2930, 1745, 1470, 1250, 1205, 1145, 1100, 845 and 775 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz), δ (chemical shifts for the minor isomer (*Z*)-**1b** are given in parenthesis), multiplicity, *J* (Hz): 7.39 (7.33), s, 6-H; 7.09 (7.12), s, 9-H; 6.75 (6.42), dm, $J_{11\sim12}=12.0$, 12-H; 6.63 (6.26), dm, $J_{11\sim12}=12.0$, 11-H; 6.57 (6.55), m, 4-H; 6.07 (6.27), m, 14-H; 4.58 (4.89), s, 15-H_2; 3.80 (3.80), s, 2-H_2; 3.75 (3.75), s, 1-OCH_3. MS (EI, 70 eV), *m/z*: 306 ($M^+ +2$, 35%), 304.0521 (M^+, 100%, $C_{16}H_{13}O_4Cl$ requires 304.0502), 269.0807 ($M^+ -Cl$, 62%, $C_{16}H_{13}O_4$ requires 269.0814), 247 (13%), 245.0359 ($M^+ -CO_2CH_3$, 35%, $C_{14}H_{10}O_2Cl$ requires 245.0369), 210.0677 ($M^+ -Cl-CO_2CH_3$, 38%, $C_{14}H_{10}O_2$ requires 210.0681), 181 (37%), 171 (26%), 152 (12%), 94 (15%), 73 (28%).

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft and the Swedish Natural Science Research Council. M. ENGLER was a fellow of the Graduiertenkolleg "Biotechnologie".

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