STUDIES ON MACROCYCLIC LACTONE ANTIBIOTICS. VII[1]

STRUCTURE OF A PHYTOTOXIN "RHIZOXIN" PRODUCED BY *RHIZOPUS CHINENSIS*

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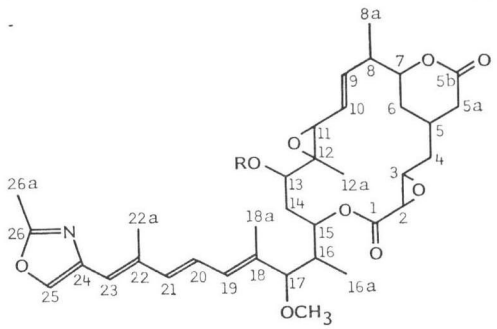
A new 16-membered macrolide designated as rhizoxin was isolated as a toxin produced by *Rhizopus chinensis*, the causal agent of rice seedling blight. The skeletal structure was determined by detailed NMR spectroscopic investigation of this compound and of its derivatives. Rhizoxin induced at a concentration of 10 ng/ml abnormal swelling of rice seedling roots, which is one of the characteristic symptoms of this disease. This compound also exhibited potent antifungal activity but little effect against bacteria.

Rice seedling blight occurring in nursery cases was found to be caused by fungi of *Rhizopus* sp.[2,3] Among them *Rhizopus chinensis* caused the most severe losses. The characteristic symptoms in an early stage of this disease are abnormal swelling of seedling roots[2,3]. Since no pathogen was detected in the tissues of the infected plant and an ether extract of the bed soil of infected rice plants caused the typical symptoms of this disease, it was suggested that a metabolite of the pathogenic fungi should be the principle of the disease[4,5].

Based on our knowledge mentioned above we attempted to isolate the toxin from the culture broth of *Rhizopus chinensis* Rh-2[4,5] (see Experimental part). The isolated toxin was designated as rhizoxin (**1**).

Fig. 1. Structures of rhizoxin (**1**) and of its acetate (**2**).



Rhizoxin (**1**) R=H
Acetate (**2**) R=Ac

Physico-chemical Properties of Rhizoxin

Rhizoxin (**1**), mp 131~135°C, $[\alpha]_D^{24}$ +201° (*c* 0.8, MeOH), showed its UV absorption maxima in methanol at 295 (ε 37,000), 308 (47,800) and 325 nm (35,000) indicating the presence of a linearly conjugated tetraene type of chromophore, and its IR bands (in $CHCl_3$) at 3400 and 1730 cm^{-1} attributed to

OH and O–C=O groups, respectively.

The ^{1}H NMR and ^{13}C NMR data are summarized in the Tables 1 and 4 (Experimental part), and 2, respectively. These NMR data suggested the presence of the following carbon units: $7\times$–CH_3, $4\times$ –CH_2–, $3\times$–$\underset{|}{C}H$–, $7\times$–$\underset{|}{C}H$–O, $1\times$–$\underset{|}{C}$–O, $7\times$ =CH–, $4\times$ =$\underset{|}{C}$, $2\times$–COO–.

The high resolution electron impact mass spectrum (HREIMS) of this compound exhibited the molecular ion peak (M^+) at m/z 625.3233. From its M^+ value taken together with its NMR and elemental analysis data (Experimental part) the molecular formula of rhizoxin (**1**) was determined as $C_{35}H_{47}O_9N$ (MW 625.3250).

NMR Spectroscopic Identification of the Partial Structures of Rhizoxin

The following units, **a** to **e**, as the partial structures of rhizoxin were elucidated by detailed analyses of ^{1}H and ^{13}C NMR of rhizoxin and of its derivatives **2** and **3**.

Table 1. ^{1}H NMR data of rhizoxin (**1**) in $CDCl_3$ (400 MHz).

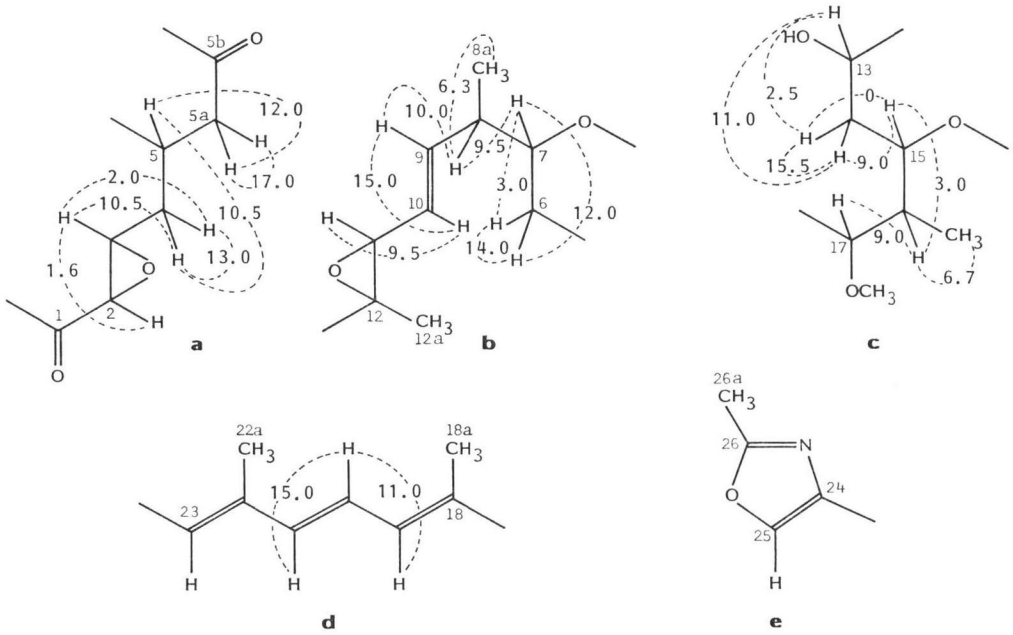
Proton	Chemical shift	Multiplicity and coupling constant (Hz)	Notes
H-2	2.96	d $J_{2,3}$=1.6	$^{2}J_{H,C(1)}$=6.0 Hz
H-3	3.27	ddd $J_{3,2}$=1.6, $J_{3,4}$=2.0 & 10.5	
H-4	2.33	ddd $J_{4,3}$=2.0, $J_{4,4}$=13.0, $J_{4,5}$=?[a]	
	0.79	ddd $J_{4,3}$=10.5, $J_{4,4}$=13.0, $J_{4,5}$=10.5	
H-5	2.05	m $J_{5,4}$=10.5, $J_{5,5a}$=12.0 & ?[a] $J_{5,6}$=2.0 & 12.0	
H-5a	2.72	dd $J_{5a,5}$=?[a], $J_{5a,5a}$=17.0	$^{2}J_{H,C(5a)}$=?[a]
	2.10	dd $J_{5a,5}$=12.0, $J_{5a,5a}$=17.0	
H-6	1.93	ddd $J_{6,5}$=2.0, $J_{6,6}$=14.0, $J_{6,7}$=3.0	
	0.93	ddd $J_{6,5}$=12.0, $J_{6,6}$=14.0, $J_{6,7}$=12.0	
H-7	3.87	ddd $J_{7,6}$=3.0 & 12.0, $J_{7,8}$=9.5	
H-8	2.30	m $J_{8,7}$=9.5, $J_{8,8a}$=6.3, $J_{8,9}$=10.0	
H-8a	1.20	d $J_{8a,8}$=6.3	
H-9	5.66	dd $J_{9,8}$=10.0, $J_{9,10}$=15.0	
H-10	5.38	dd $J_{10,9}$=15.0, $J_{10,11}$=9.5	NOE (12a→10)=+12%
H-11	3.20	d $J_{11,10}$=9.5	
H-12a	1.45	s	
H-13	3.20	dd $J_{13,14}$=2.5 & 11.0	$^{3}J_{H,C(12a)}$=3.0 Hz
H-14	2.05	ddd $J_{14,13}$=11.0, $J_{14,14}$=15.5, $J_{14,15}$=9.0	
	1.88	dd $J_{14,13}$=2.5, $J_{14,14}$=15.5, $J_{14,15}$=0	
H-15	4.63	dd $J_{15,14}$=0 & 9.0, $J_{15,16}$=3.0	$^{3}J_{H,C(1)}$=1.0 Hz
H-16	2.37	m $J_{16,15}$=3.0, $J_{16,16a}$=6.7, $J_{16,17}$=9.0	
H-16a	1.00	d $J_{16a,16}$=6.7	
H-17	3.23	d $J_{17,16}$=9.0	$^{3}J_{H,C(17-O)}$=3.0 Hz, $^{3}J_{H,C(18a)}$=4.0 Hz
17-OCH_3	3.15	s	
H-18a	1.82	s	
H-19	6.08	d $J_{19,20}$=11.0	$^{3}J_{H,C(18a)}$=8.0
H-20	6.60	dd $J_{20,19}$=11.0, $J_{20,21}$=15.0	NOE(18a→20)=+13%, (22a→20)=+13%
H-21	6.38	d $J_{21,20}$=15.0	$^{3}J_{H,C(22a)}$=3.0 Hz
H-22a	2.12	s	
H-23	6.27	s	$^{3}J_{H,C(22a)}$=9.0 Hz, $^{3}J_{H,C(25)}$=6.0 Hz
H-25	7.58	s	$^{2}J_{H,C(24)}$=15.0 Hz, $^{3}J_{H,C(26)}$=8.0 Hz
H-26a	2.45	s	$^{2}J_{H,C(26)}$=8.0 Hz

[a] Spin-spin coupling was observed but its constant could not be determined.

Table 2. ^{13}C NMR data of rhizoxin (**1**) in $CDCl_3$ (100 MHz).

Carbon	Chemical shift	Multiplicity & $^1J_{CH}$		Carbon	Chemical shift	Multiplicity & $^1J_{CH}$	
C-1	168.1	s		C-15	77.0	d	142
C-2	54.3	d	181	C-16	38.2	d	130
C-3	56.0	d	178	C-16a	9.6	q	127
C-4	36.1	t	125	C-17	89.4	d	138
C-5	38.2	d	125	17-OCH_3	56.2	q	140
C-5a	36.5	t	125	C-18	136.3	s	
C-5b	169.3	s		C-18a	11.5	q	127
C-6	34.4	t	125	C-19	129.4	d	150
C-7	82.5	d	145	C-20	123.8	d	148
C-8	45.4	d	125	C-21	123.8	d	150
C-8a	17.0	q	127	C-22	136.8	s	
C-9	139.7	d	155	C-22a	14.4	q	127
C-10	126.7	d	155	C-23	120.9	d	155
C-11	64.2	d	172	C-24	138.8	s	
C-12	65.2	s		C-25	136.1	d	206
C-12a	11.8	q	127	C-26	161.0	s	
C-13	77.5	d	141	C-26a	13.8	q	130
C-14	31.9	t	125				

Fig. 2. Partial structures of rhizoxin with their ^1H-^1H coupling constants (Hz).



Unit **a** (C-1 ~ C-5b)

In the ^1H NMR study of rhizoxin (**1**) the proton-proton coupling system of the unit **a** was determined by detailed decoupling experiments (see Table 1 and **a** in Fig. 2), and all of the protons on these carbons were assigned.

In ^{13}C NMR relatively large $^1J_{CH}$ values were observed for the carbon signals at 54.3 ($^1J_{C-2,H-2}$ =181 Hz) and 56.0 ppm ($^1J_{C-3,H-3}$ =178 Hz) which indicated an epoxide structure[4]. The ^1H-^1H coupling

between these two protons (J=1.6 Hz) indicated a *E*-orientation of the epoxide moiety[6,7]. The acyloxy carbonyl carbon signals at 168.1 ppm and 169.3 ppm exhibited long range couplings with H-2 (2.96 ppm) and H-5a (2.72 ppm) respectively, therefore, they were assigned to C-1 and C-5b. The partial structure C-1 through C-5b was thus established.

Unit **b** (C-6~C-12a)

Vicinal and geminal couplings of protons on C-6 to C-11 were likewise determined and all of the protons on these carbons were assigned (see Table 1 and **b** in Fig. 2).

A large coupling constant (15 Hz) between two olefinic proton signals due to H-9 (5.66 ppm) and H-10 (5.38 ppm) indicated an *E*-orientation of this olefinic linkage. The ^{13}C-signal at 64.2 ppm was assigned as C-11 by selective proton decoupling. A large $^1J_{C-11,H-11}$ value (172 Hz) suggested the existence of 1,2-epoxide between C-11 and C-12. Since a doublet at 3.20 ppm (H-11) exhibited only a vicinal coupling with C-10, C-12 was assigned as a tetrasubstituted carbon, and a ^{13}C singlet appearing at 65.2 ppm was assigned to this carbon. A NOE-enhancement (+12%) of the H-10 signal (5.38 ppm) upon irradiation of the H_β-12a singlet at 1.45 ppm suggested an *E*-orientation of the carbon chain about this epoxide moiety.

Unit **c** (C-13~C-17)

The H-13 signal appearing at 3.02 ppm in the spectrum of **1** shifted to 4.22 ppm in the spectrum of the acetate **2**. This fact indicated that the carbon bearing this proton is substituted by a hydroxyl group.

Although all the signals of the protons on C-13 to C-17 in compound **1** were finally assigned as shown in the Table 1 and **c** in Fig. 2, correlation of all the proton signals of this unit could initially be hardly achieved because of a very small coupling constant (smaller than 1 Hz) between the signals at 1.88 ppm (one of H_2-14) and 4.63 ppm (H-15), as well as overlapping of the H-14 signal with others. This problem was, however, solved by analysis of ^{1}H NMR spectrum of compound **3** obtained by an alkaline hydrolysis of **1** followed by methylation and acetylation (Fig. 3). In this spectrum the signals due to H_2-14 were separated from other signals, and the couplings of these protons with H-13 and H-15 could be well analyzed.

The position of the methoxyl group was determined from a long range coupling between the ^{1}H signal at 3.23 ppm due to H-17 and the ^{13}C signal at 56.2 ppm due to the methoxyl carbon ($^3J_{CH}$=3 Hz).

Unit **d** (C-18~C-23)

In the ^{1}H NMR spectrum of **1** vicinal couplings of the olefinic proton signals at 6.08 ppm (H-19), 6.60 ppm (H-20) and 6.38 ppm (H-21) were determined by decoupling experiments.

^{13}C-Methyl signals at 11.5 ppm and 14.4 ppm were assigned to C-18a and C-22a, respectively, from their respective long range couplings with H-19 ($^3J_{C-18a,H-19}$=8 Hz) and with H-23 ($^3J_{C-22a,H-23}$=9 Hz).

A large coupling constant between H-20 and H-21 (J=15.0 Hz) as well as a NOE-enhancement in the H-20 signal intensity (*ca.* +13%) upon irradiation of either H_β-18a or H_β-22a indicated that all of these three olefinic linkages are oriented in *E*-configurations.

Unit **e** (C-24~C-26a)

The remainder of rhizoxin (structure **e**, C_4H_4NO) requires three degrees of unsaturation. In the ^{13}C NMR spectrum of **1** a quartet at 13.8 ppm, two singlets at 161.0 ppm and 138.8 ppm and a doublet at 132.6 ppm were assigned to the carbons in the unit **e**. The doublet at 132.6 ppm exhibited a large $^1J_{CH}$ (206.0 Hz) with a ^{1}H signal at 7.58 ppm. These spectral data as well as the elemental composition left for this unit accounted for a 2,4-disubstituted oxazole structure. The values of ^{13}C chemical shifts

Fig. 3. Structure of seco-rhizoxin triacetate (3).

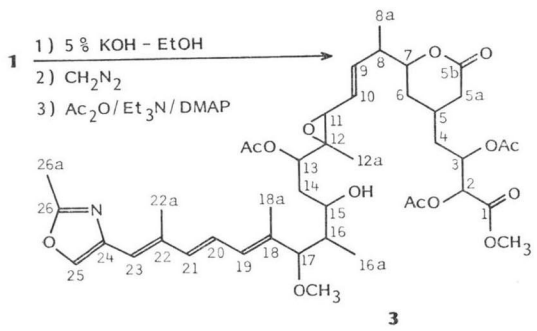


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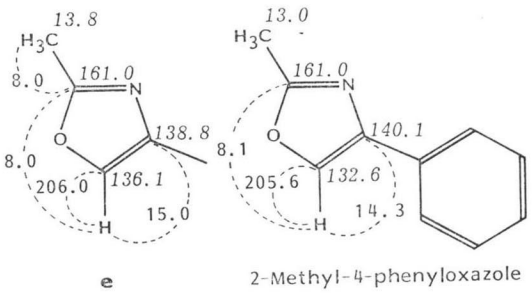
Fig. 4. ^{13}C Chemical shifts (ppm) and ^{13}C-^1H couplings (Hz) in oxazole rings of rhizoxin and 2-methyl-4-phenyloxazole.



and ^{13}C-^1H coupling constants shown in the partial structure **e** in Fig. 2 were, indeed, in good agreement with those in 2-methyl-4-phenyloxazole (Fig. 4)[8].

Skeletal Structure of Rhizoxin

The connection mode of the structural units identified above was established as **e-d-c-b-a** by further investigation of NMR and mass spectra of rhizoxin.

Connections between **e** and **d**, **d** and **c**, and **b** and **a** units through the respective single bonds, C-24 ~ C-23, C-18 ~ C-17 and C-13 ~ C-12, were determined by respective ^{13}C-^1H long range couplings between C-25 and H-23 ($^3J_{CH}$=6 Hz), C-18 and H-17 ($^2J_{CH}$=4 Hz), and C-12a and H-13 ($^3J_{CH}$=3 Hz).

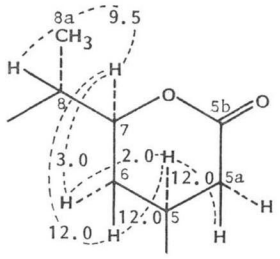
The partial structure C-17 through C-26a was supported on the one hand by HREIMS exhibiting the base ion peak at m/z 232.1330 which corresponds to $C_{14}H_{18}O_2N$ (232.1336) formed by scission of C-17 ~ C-16 bond, and on the other hand by its tetraene type of UV absorption.

Assignment of H-4 to H-6 of **1** in the proton NMR measured in $CDCl_3$ was difficult, because part of these proton signals overlapped with others. Its spectrum measured in benzene-d_6 (Table 4 in the Experimental part) or in a mixture of benzene-d_6 - $CDCl_3$ (95: 5), however, could separate these signals. A spectrum in the latter solvent system exhibited separate signals for H-4 at 0.13 and 1.62 ppm, H-5 at 1.15 ppm, H-5a at 1.46 and 2.18 and for H-6 at 0.07 and 1.20 ppm. Irradiation of H-5 decoupled the signals at 0.13 (H-4), 1.46 (H-5a) and 0.07 (H-6). The partial structure including H-4 through H-6 was hence established and all of units **a** to **e** were connected spectroscopically.

The 16-membered lactone structure was determined by observation of a long range coupling between C-1 and H-15 (Table 1), and the remaining ester linkage was then assigned as a 6-membered lactone involving C-5b carbonyl and C-7 oxy-function.

The relative stereochemistry of three chiral centers at C-5, C-7 and C-8 were elucidated as in Fig. 5, since one of the H_2-6 signals appearing at 0.93 ppm showed large coupling constants with each of H-5 and H-7 ($J_{6,5}$=$J_{6,7}$=12.0 Hz) and one of the H_2-5a signals at 2.10 ppm also showed $J_{5,5a}$ as 12.0 Hz, demonstrating their 1,2-diaxial interaction, and since a NOE enhancement of the H-7 signal of about 6% was observed by irradiation of H_3-8a.

Fig. 5. Relative stereochemistry of the system C-5 to C-8.



The structure of **1** elucidated as above was confirmed also by combining the structures assigned to its degradation products. The degradation experiments will, however, be discussed elsewhere together with the foregoing study on the stereo-structure of rhizoxin.

Bioactivity

Rhizoxin (**1**) induced, at less than 10 ng/ml concentration, abnormal swelling of rice seedling roots, forming malformed shapes that is one of the characteristic symptoms of the rice seedling blight[4,5].

The potent activity of this compound was established against a variety of phytopathogenic fungi by assaying the mycelium growth inhibitory activity (Table 3). MIC against 10 tested fungi were less than 1 μg/ml except for *Rhizopus chinensis* (rhizoxin producing fungus) and *Fusarium oxysporum*. Growth of some of the tested fungi was inhibited even at 0.1 μg/ml but little activity was observed against bacteria[9].

Table 3. Antifungal activity[a] of rhizoxin.

Organism	Concentration of rhizoxin (μg/ml)		
	10	1	0.1
Alternaria kikuchiana	+	+	−
Colletotrichum lindemuthianum	+	+	+
Fusarium nivale	+	+	+
Fusarium oxysporum f. *lycopersici*	−	−	−
Helminthosporium oryzae	+	+	+
Pyricularia oryzae	+	+	−
Pythium aphanidermatum	+	+	+
Rhizoctonia solani	+	+	−
Rhizopus chinensis	−	−	−
Sclerotinia trifoliorum	+	+	+

[a] Mycelium growth inhibitory activity: +; inhibition, −; no inhibition.

Experimental

General

Melting points were taken using a Yamato MP-1 apparatus and are uncorrected. UV spectra were measured on a Shimadzu apparatus (model UV-300), the maxima are given in nm (extinction ε). IR spectra were measured on a Japan Spectroscopic Co. apparatus (model A-102) and are recorded in cm^{-1}. ^1H and ^{13}C NMR spectra were measured on a Jeol apparatus JNM FX-400 (^1H; 400.5 MHz, ^{13}C; 100.7 MHz) machine, chemical shifts are given in ppm (in δ) relative to TMS (0 ppm) as an internal standard, and coupling constants are recorded in Hz (J). Mass spectra were measured on a Shimadzu LKB-9000.

Thin-layer chromatography was carried out on Merck DC-Fertigplatten (Kieselgel 60F-254), and high performance liquid chromatography was performed on a Shimadzu LC-3A apparatus.

Isolation of Rhizoxin (1)

Rhizopus chinensis Rh-2 strain was cultivated in a medium composed of glucose 1%, Polypepton 1%, $MgSO_4 \cdot 7H_2O$ 0.05%, KH_2PO_4 0.05% and $CaCl_2 \cdot 2H_2O$ 0.01% at 32°C by standing for 2 days. The ether extract of the culture broth was separated by successive silica gel and LH-20 column chromatographies. Colorless powder of the almost pure toxin was obtained in *ca.* 1 mg/liter culture. This was further purified for spectroscopic analysis by high performance liquid chromatography (HPLC) using a Whatman M9 ODS-3 column eluted with a mixture of MeOH - H_2O (80: 20). The compound could not be recrystallized but addition of hexane to the acetone solution of the compound followed by evaporation of the solvent gave a pale yellow powder which melted at 131~135°C. *Anal* Calcd for $C_{35}H_{47}O_9N$: C 67.18, H 7.57, N 2.24, O 23.01. Found: C 66.94, H 7.78, N 2.04; $[\alpha]_D^{24}$ +201° (*c* 0.8, MeOH); UV (MeOH) λ_{max} nm (ε) 295 (37,000), 308 (47,800), 325 (35,000); IR ($CHCl_3$) ν_{max} cm^{-1} 3400 (OH), 2960, 2925, 1730 (–OCO–), 1580, 1460, 1380, 1190, 1110, 1085, 1045, 965; ^1H NMR see Table 1 (in $CDCl_3$) and Table 4 (in benzene-d_6); ^{13}C NMR see Table 2; HRIMS *m/z* 625.3233 (M^+); EIMS 625 (M^+, 1), 610 (0.5), 607 (0.4), 593 (0.5), 578 (0.1), 550 (0.1), 532 (0.1), 515 (0.2), 315 (3), 284 (1), 268 (1), 232 (100), 200 (29), 190 (12), 158 (22), 131 (22), 109 (21), 91 (22), 81 (24), 55 (27), 43 (32), 41 (32).

Table 4. ^{1}H NMR data of rhizoxin (**1**) in benzene-d_6 (400 MHz).

Proton	Chemical shift	Multiplicity and coupling constant (Hz)	Proton	Chemical shift	Multiplicity and coupling constant (Hz)
H-2	2.64	d $J_{2,3}$=1.6	H-10	5.03	dd $J_{10,9}$=15.5, $J_{10,11}$=9.5
H-3	3.10	ddd $J_{3,2}$=1.6, $J_{3,4}$=2.0 & 11.0	H-11	2.96	d $J_{11,10}$=9.5
			H-12a	1.44	s
H-4	1.52	ddd $J_{4,3}$=2.0, $J_{4,4}$=13.0, $J_{4,5}$=3.0	H-13	3.22	dd $J_{13,14}$=3.5 & 10.5
			H-14	2.15	ddd $J_{14,13}$=10.5, $J_{14,14}$=?[a], $J_{14,15}$=8.0
	0.07	ddd $J_{4,3}$=11.0, $J_{4,4}$=13.0, $J_{4,5}$=11.0	H-15	4.87	dd $J_{15,14}$=0 & 8.0, $J_{15,16}$=6.5
H-5	1.05	m $J_{5,4}$=11.0, $J_{5,5a}$=5.0 & 12.5, $J_{5,6}$=3.0 & 12.5	H-16	2.72	m $J_{16,15}$=3.0, $J_{16,16a}$=6.5, $J_{16,17}$=9.5
H-5a	2.14	dd $J_{5a,5}$=5.0, $J_{5a,5a}$=18.0			
	1.42	dd $J_{5a,5}$=12.5, $J_{5a,5a}$=18.0	H-16a	1.11	d $J_{16a,16}$=6.5
H-6	1.20	ddd $J_{6,5}$=3.0, $J_{6,6}$=13.0, $J_{6,7}$=2.5	H-17	3.18	d $J_{17,16}$=9.5
			17-OCH$_3$	3.01	s
	−0.07	ddd $J_{6,5}$=12.5, $J_{6,6}$=13.0, $J_{6,7}$=12.5	H-18a	2.02	s
			H-19	6.13	d $J_{19,20}$=11.0
H-7	3.14	ddd $J_{7,6}$=2.5 & 12.5, $J_{7,8}$=10.0	H-20	6.73	dd $J_{20,19}$=11.0, $J_{20,21}$=15.0
			H-21	6.52	d $J_{21,20}$=15.0
H-8	1.80	m $J_{8,7}$=10.0, $J_{8,8a}$=7.0, $J_{8,9}$=10.0	H-22a	2.25	s
			H-23	6.40	s
H-8a	1.00	d $J_{8a,8}$=7.0	H-25	7.08	s
H-9	4.51	dd $J_{9,8}$=10.0, $J_{9,10}$=15.5	H-26a	1.98	s

[a] Spin-spin coupling was observed but its constant could not be determined.

Table 5. ^{1}H NMR data of rhizoxin acetate (**2**) (400 MHz).

Proton	Chemical shift		Proton	Chemical shift	
	in $CDCl_3$	in benzene-d_6		in $CDCl_3$	in benzene-d_6
H-2	2.96	2.64	13-OAc	—	1.79
H-3	3.30	3.12	H-14	2.06	—
H-4	—	1.52		—	—
	—	0.07	H-15	4.68	5.02
H-5	—	—	H-16	—	1.76
H-5a	2.74	—	H-16a	0.96	0.96
	—	—	H-17	3.26	3.38
H-6	—	1.21	17-OCH$_3$	3.18	3.05
	—	−0.07	H-18a	1.81	2.07
H-7	3.90	3.12	H-19	6.26	6.75
H-8	—	—	H-20	6.58	6.85
H-8a	1.20	1.08	H-21	6.50	7.08
H-9	5.65	4.44	H-22a	2.14	2.38
H-10	5.33	4.98	H-23	6.30	6.97
H-11	3.18	3.01	H-25	7.54	7.00
H-12a	1.46	1.50	H-26a	2.46	1.96
H-13	4.23	4.68			

Rhizoxin Acetate (**2**)

Rhizoxin was acetylated in benzene using 1.5 equivalents of acetic anhydride and pyridine with a catalytic amount of 4-dimethylaminopyridine. The solution was washed with dil HCl and water. After drying the solution over sodium sulfate the solvent was evaporated. The crude product was purified by silica gel column chromatography to give the acetate **2** in an almost quantitative yield. Addition of hexane in its acetone solution followed by evaporation of the solvent gave a white powder.

Table 6. ^{1}H NMR data of compound **3** in $CDCl_3$ (400 MHz).

Proton	Chemical shift	Multiplicity and coupling constant (Hz)	Proton	Chemical shift	Multiplicity and coupling constant (Hz)
1-OCH_3	3.80	s	H-11	3.68	d $J_{11,10}$=4.0
H-2	5.30	d $J_{2,3}$=3.5	H-12a	1.10	s
H-3	5.34	ddd $J_{3,2}$=3.5, $J_{3,4}$=3.5 & 10.0	H-13	4.90	dd $J_{13,14}$=5.0 & 11.5
H-4	1.87	ddd $J_{4,3}$=10.0, $J_{4,4}$=14.0, $J_{4,5}$=?[a]	H-14	1.95	ddd $J_{14,13}$=5.0, $J_{14,14}$=13.0, $J_{14,15}$=2.5
	1.43	ddd $J_{4,3}$=3.5, $J_{4,4}$=14.0, $J_{4,5}$=?[a]		1.45	ddd $J_{14,13}$=11.5, $J_{14,14}$=13.0, $J_{14,15}$=11.5
H-5	1.90	m $J_{5,4}$=?[a], $J_{5,5a}$=?[a], $J_{5,6}$=12.0 & ?[a]	H-15	3.46	ddd $J_{15,14}$=2.5 & 11.5, $J_{15,16}$=7.0
H-5a	2.76	dd $J_{5a,5}$=?[a], $J_{5a,5a}$=16.0	H-16	1.87	m $J_{16,15}$=7.0, $J_{16,16a}$=7.0 $J_{16,17}$=4.0
	2.03	dd $J_{5a,5}$=?[a], $J_{5a,5a}$=16.0			
H-6	1.90	ddd $J_{6,5}$=?[a], $J_{6,6}$=13.0, $J_{6,7}$=3.0	H-16a	1.12	d $J_{16a,16}$=7.0
			H-17	3.68	d $J_{17,16}$=4.0
	1.27	ddd $J_{6,5}$=12.0, $J_{6,6}$=13.0, $J_{6,7}$=12.5	17-OCH_3	3.22	s
			H-18a	1.76	s
H-7	4.15	ddd $J_{7,6}$=3.0 & 12.0, $J_{7,8}$=6.5	H-19	6.15	d $J_{19,20}$=11.0
			H-20	6.61	dd $J_{20,19}$=11.0, $J_{20,21}$=15.0
H-8	2.52	m $J_{8,7}$=6.5, $J_{8,8a}$=7.0, $J_{8,9}$=7.0	H-21	6.42	d $J_{21,20}$=15.0
			H-22a	2.15	s
H-8a	0.93	d $J_{8a,8}$=7.0	H-23	6.28	s
H-9	5.75	dd $J_{9,8}$=7.0, $J_{9,10}$=16.0	H-25	7.58	s
H-10	5.68	dd $J_{10,9}$=16.0, $J_{10,11}$=4.0	H-26a	2.47	s

[a] Coupling constant could not be determined.

UV (MeOH) λ_{max} nm (ε) 296 (38,300), 308 (48,900), 323 (36,200); IR ($CHCl_3$) ν_{max} cm^{-1} 2970, 2940, 1735, 1580, 1445, 1370, 1310, 1245, 1180, 1107, 1083, 980, 970; ^{1}H NMR see Table 5, EIMS 667 (M^+, 9), 652 (3), 636 (1), 232 (100), 200 (32), 190 (10), 158 (25), 131 (18), 109 (22), 95 (23), 91 (23), 81 (19), 55 (40), 43 (61), 41 (49).

Alkali Treatment of Rhizoxin

Rhizoxin was dissolved in 5% ethanolic NaOH. The solution was heated to reflux for one hour. After neutralization of the solution with dil HCl the solvent was evaporated. The residue was dissolved in ether and the solution was washed with water and dried over sodium sulfate. The crude product was methylated with diazomethane and acetylated in benzene with 1.5 equivalent of acetic anhydride and pyridine with a catalytic amount of 4-dimethylaminopyridine. A silica gel column chromatography of the crude products mixture gave compound **3** as a major product in *ca.* 43% yield. UV (MeOH) λ_{max} (ε) 297 (45,600), 308 (55,600), 323 (43,200); IR ($CHCl_3$) ν_{max} cm^{-1} 3500, 2960, 1750, 1580, 1445, 1380, 1240~1200, 1115, 1090, 1030, 970; ^{1}H NMR see Table 6; EIMS 801 (M^+, 6), 786 (2), 770 (1), 741 (3), 726 (1), 710 (1), 700 (1), 681 (1), 639 (1), 232 (100), 200 (22), 190 (6), 158 (11), 148 (19), 131 (8), 60 (14), 55 (7), 43 (68).

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