Communications to the Editor

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STUDIES ON MACROCYCLIC LACTONE ANTIBIOTICS. IX[1]
NOVEL MACROLIDES FROM THE FUNGUS *RHIZOPUS CHINENSIS*: PRECURSORS OF RHIZOXIN

Shigeo Iwasaki,* Michio Namikoshi, Hisayoshi Kobayashi, Jun Furukawa and Shigenobu Okuda

Institute of Applied Microbiology, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

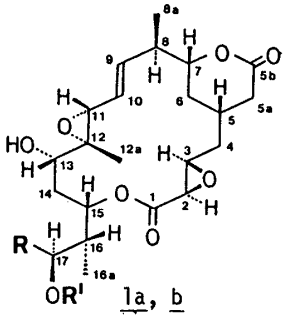
Five novel 16-membered macrolides (1b-3b), homologues of rhizoxin, were isolated and their structures were determined from their physicochemical and spectral properties.

KEYWORDS —— 16-membered macrolide; *Rhizopus chinensis*; phytotoxin; antifungal activity; anti-tumor activity

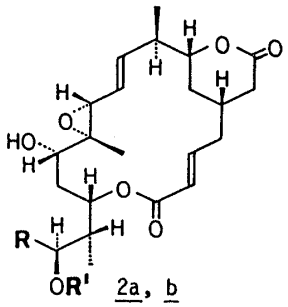
A novel 16-membered macrolide, rhizoxin (1a), was previously isolated as a toxin produced by *Rhizopus chinensis*,[2] the causal agent of rice seedling blight occured in nursery cases.[3] The potent antifungal and anti-tumor activities of this compound were established,[2,4] and its absolute structure has also been determined.[1]

This paper deals with the isolation and the structures of five additional compounds related to rhizoxin produced by *R. chinensis*.

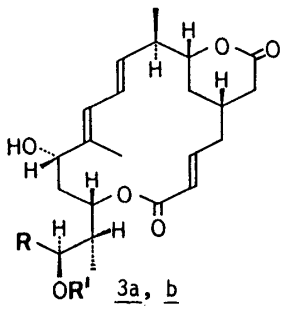
Figure 1



1a, b

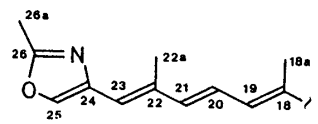


2a, b



3a, b

R =



1a: R' = CH_3
1b: R' = H

2a: R' = CH_3
2b: R' = H

3a: R' = CH_3
3b: R' = H

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R. chinensis Rh-2 strain was cultivated in a medium composed of 3% maltose, 1% polypepton, 0.25% KH_2PO_4, 0.75% K_2HPO_4, 0.25% $MgSO_4$ $7H_2O$, 0.20% $(NH_4)_2SO_4$ and 4% pharmamedia, at 28°C with shaking for 92 hours or in large fermentation tanks for 72 hours. The culture broth was first extracted with twice as much volume of acetone as broth. The filtrate after removal of cultured mycelia was then extracted with ethyl acetate. The extract was separated by successive silicagel and LH-20 column chromatographies to isolate rhizoxin (1a) and its related compounds 1b, 2a, 2b, 3a and 3b. The compounds were finally purified by HPLC using a Whatman M9 ODS-3 column eluted with mixtures of acetonitlile-water. These compounds could not be crystallized. Evaporation of the solvent from their acetone-hexane solution gave respective white powders. Their isolated yields per liter were calculated to be ca 20 mg, 0.01 mg, 2 mg, 0.04 mg, 0.1 mg and 0.1 mg for 1a, 1b, 2a, 2b, 3a and 3b, respectively.

The structures of compounds 1b-3c were determined by comparing their physicochemical properties (Table 1) and their ^{1}H-NMR data, listed in Table 2 in which the multiplicities and chemical shift values of all the proton signals are given. Proton assignments for each compound were made by analysis of their spin-spin couplings and also by comparisons with the ^{1}H-NMR data of rhizoxin (1a) previously reported in detail.[1,2]

The UV-absorption spectra of compounds 1b-3b showed the presence of the same chromophore as in the rhizoxin molecule. The NMR signals of H-18a to H-26a of these compounds (Table 2) also proved the identity of the partial structure of C-18 through C-26a.

The molecular formulas of these compounds determined by high resolution electron impact mass spectroscopy (HREIMS) indicate that the b-series compounds are composed of one CH_2 unit less than the corresponding a-series compounds. The ^{1}H-NMR spectra of compounds 1b, 2b and 3b exhibited no signal due to the OCH_3 group which appeared in the spectra of 1a, 2a and 3a at δ 3.15, 3.15 and 3.17 ppm respectively, and the H-17 signals appearing at δ 3.23-3.26 ppm for the a-series compounds shifted to δ 3.89-3.91 ppm for the b-series compounds. This shows that the 17-OCH_3 group present in 1a, 2a and 3a is replaced by an OH group in 1b, 2b and 3b. Since the ^{1}H-NMR spectra of each pair of compounds showed all the other proton signals at the comparable positions, it was concluded that the only structural difference in the pair of compounds is the substituent at C-17.

On the other hand, comparison of the molecular formulas of 1a, 2a and 3a (and also of 1b, 2b and 3b) show that their atomic composition differs only in the numbers of oxygen atoms and that 2a and 3a are composed of one atom and two atoms respectively less than 1a.

In the spectrum of 1a, H-2 and H-3 signals appeared at δ 2.96 and 3.27 ppm as the protons on the carbon bearing an epoxy group, while those signals for 2a and 3a appeared at δ 5.61 and 6.82 ppm and δ 5.61 and 6.77 ppm in the respective spectra. These signals of 2a and 3a were assignable to the olefinic protons of disubstituted $\alpha\beta$-unsaturated ester moieties, and an E-orientation of the olefinic linkages were indicated by large coupling constants between H-2 and H-3 (15.8 Hz for 2a, and 15.2 Hz for 3a). This partial structure change at C-2,3 also caused lower field shifts of the H-4 signals (see Table 2). On comparison of the ^{1}H-NMR spectra of 1a and 3a the H-11 and H-12a signals shown at δ 3.20 and 1.45 ppm in the former shifted to δ 5.81 and 1.79 ppm respectively in the latter. This suggested the presence of a

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Table 1. Physico-Chemical Characteristics of 1a - 3b

Compd.	Mol. Formula	HREIMS (calcd. val.)	$[\alpha]_D^{24}$(MeOH)	UV(MeOH) λ_{max} nm(ε)
1a	$C_{35}H_{47}O_9N$	M^+ 625.3233 (625.3250)	+155.5°(c=0.80)	295(42300) 308(54000) 325(39000)
1b	$C_{34}H_{45}O_9N$	M^+-1 610.3009 (610.3012)	+116.3°(c=0.11)	297(32300) 309(41000) 323(30100)
2a	$C_{35}H_{47}O_8N$	M^+ 609.3300 (609.3299)	+136.8°(c=0.71)	297(46000) 308(64400) 323(46300)
2b	$C_{34}H_{45}O_8N$	M^+ 595.3135 (595.3142)	+99.3°(c=0.61)	297(39100) 308(50300) 323(36600)
3a	$C_{35}H_{47}O_7N$	M^+ 593.3382 (593.3414)	+287.1°(c=0.48)	216(25900) 235(24700) 238(24500) 297(38700) 309(49900) 323(36500)
3b	$C_{34}H_{45}O_7N$	M^+ 597.3165 (597.3137)	+246.0°(c=0.66)	215(28400) 232(26200) 238(25700) 297(42500) 308(55100) 323(40300)

Table 2. Chemical Shifts of ^{1}H-Signals of 1a - 3b (in $CDCl_3$, in δ Value)

Protons	1a	1b	2a	2b	3a	3b
H-2 (d)	2.96	2.98	5.61	5.72	5.61	5.63
H-3 (ddd)	3.27	3.29	6.82	6.85	6.77	6.79
H-4 (ddd)	2.33	2.35	2.56	2.58	2.53	2.55
(ddd)	0.79	0.82	1.80	1.79	1.74	1.75
H-5 (m)	2.05	2.08	1.80	1.83	1.75	1.80
H-5a (dd)	2.72	2.74	2.78	2.79	2.76	2.77
(dd)	2.10	2.07	2.10	2.10	2.09	2.10
H-6 (ddd)	1.93	1.99	1.98	1.98	1.98	1.97
(ddd)	0.93	0.95	0.72	0.76	0.68	0.70
H-7 (ddd)	3.87	3.89	3.79	3.78	3.67	3.70
H-8 (m)	2.30	2.32	2.32	2.13	2.27	2.29
H-8a (d)	1.20	1.22	1.19	1.20	1.19	1.20
H-9 (dd)	5.66	5.63	5.55	5.57	5.15	5.18
H-10 (dd)	5.38	5.37	5.34	5.36	6.23	6.24
H-11 (d)	3.20	3.23	3.24	3.27	5.81	5.79
H-12a (s)	1.45	1.43	1.42	1.44	1.79	1.81
H-13 (dd)	3.02	3.07	3.07	3.15	3.90	3.99
H-14 (ddd)	2.05	2.08	2.10	2.13	2.14	2.16
(dd)	1.88	1.95	1.81	1.94	1.70	1.83
H-15 (dd)	4.63	4.66	4.61	4.69	4.58	4.69
H-16 (m)	2.37	2.34	2.40	2.34	2.27	2.12
H-16a (d)	1.00	1.02	1.00	1.00	1.00	0.97
H-17 (d)	3.23	3.89	3.25	3.91	3.26	3.89
17-OCH_3 (s)	3.15	---	3.15	---	3.17	---
H-18a (s)	1.82	1.89	1.84	1.92	1.89	1.88
H-19 (d)	6.08	6.11	6.09	6.16	6.12	6.21
H-20 (dd)	6.60	6.53	6.59	6.55	6.63	6.58
H-21 (d)	6.38	6.37	6.37	6.39	6.41	6.40
H-22a (s)	2.12	2.12	2.34	2.13	2.15	2.13
H-23 (s)	6.27	6.25	6.25	6.25	6.26	6.25
H-25 (s)	7.58	7.54	7.54	7.53	7.55	7.54
H-26a (s)	2.45	2.46	2.46	2.46	2.46	2.46

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double bond at C-11, 12 in 3a instead of an epoxy group in 1a. Evidence for a conjugated diene system in 3a (C-9 through C-12) was also provided by its UV spectrum (see Table 1). An E-orientation of the C-11, 12 double bond was determined by an NOE-enhancement of the H-10 signal observed on irradiation of H_3-12a.

On the basis of the evidence described above, it was concluded that the only structural difference between 1a and 2a is at the C-2,3 position and between 1a and 3a at the C-2,3 and C-11, 12 positions. The structures of 2a (accordingly of 2b) and of 3a (accordingly of 3b) were thus established as shown in Figure 1.

Assignments of the relative stereochemistry of compounds 1b - 3b were based on comparisons of the coupling modes of their proton signals with those of 1a, although the details of the NMR data of compounds 1b - 3b could not be presented in this communication. The absolute structures of these compounds were also tentatively proposed as shown in Figure 1 in consideration of their biogenetic relationship to rhizoxin, since these compounds can be regarded as the biogenetic precursors of rhizoxin (1a).

The potent activities of these compounds against rice seedling roots, fungi and tumour cells were also established by assaying their growth inhibitory activity. Such biological data will be reported elsewhere and will provide very useful information about the structure-activity relationship of the rhizoxinoid compounds.

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