DORRIGOCINS: NOVEL ANTIFUNGAL ANTIBIOTICS THAT CHANGE THE MORPHOLOGY OF *ras*-TRANSFORMED NIH/3T3 CELLS TO THAT OF NORMAL CELLS

II. ISOLATION AND ELUCIDATION OF STRUCTURES

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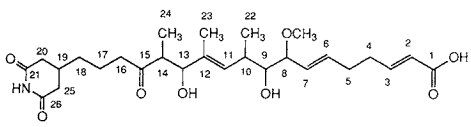
Two novel antifungal antibiotics, named dorrigocin A and B have been isolated from the fermentation broth and mycelium of *Streptomyces platensis* subsp. *rosaceus*. These closely related compounds were separated from one another by countercurrent chromatography on an Ito coil planet centrifuge. The structures of the dorrigocins were determined by NMR and IR spectroscopy and mass spectrometry. Each is a putative propionate-acetate derived straight chain fatty acid terminating in cycloheximide. The dorrigocins differ from one another only in their oxidation pattern.

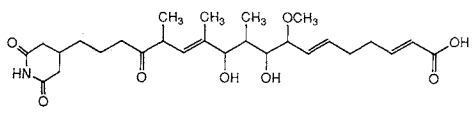
In the course of screening for antifungal antibiotics, two novel glutarimide compounds designated dorrigocin A (**1**) and B (**2**) were isolated from *Streptomyces platensis* subsp. *rosaceus*. These antibiotics are most closely related to the recently described glutarimide antibiotic BU-4146T (**3**)[1]. This paper will describe the isolation and separation of the dorrigocins and the elucidation of their structures. Two companion papers[2,3] describe the isolation, identification and fermentation of the producing culture and a biological evaluation of the dorrigocins.

Isolation of the Dorrigocins

Upon completion of fermentation, 30 liters of whole broth was treated with XAD-16 (3 liters) which was washed with water and eluted with methanol (12 liters). The methanol eluate was concentrated to dryness and triturated sequentially with 1 liter portions of; ethyl acetate, methanol, and water. The methanol soluble material was partitioned between hexane, ethyl acetate, methanol and water (0.2 liters of each). The lower layer from this partition was concentrated to dryness and chromatographed over a Sephadex LH-20 column developed with methanol. Active fractions from this column were combined and concentrated to a pale oil. This oil was subjected to countercurrent chromatography on an Ito multi-layered coil planet centrifuge in a solvent system of chloroform-methanol-water (1:1:1) with the lower layer stationary. Active fractions from this column were combined and concentrated to an oil. This oil was subjected to countercurrent chromatography on an Ito multi-layered coil planet centrifuge

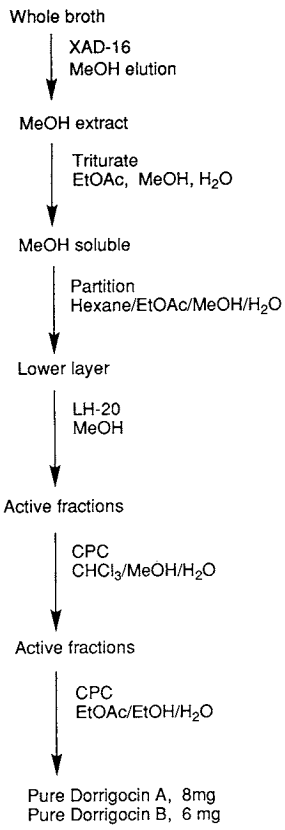


Dorrigocin A (**1**)



Dorrigocin B (**2**)

Fig. 1. Isolation of the dorrigocins.



in a solvent system of ethyl acetate - ethanol - water (3:1:2) with the lower phase stationary. Active fractions from this column were combined based upon their TLC behavior to yield pure dorrigocin A (8 mg) and dorrigocin B (6 mg).

Structure Elucidation of the Dorrigocins

Structure Elucidation of Dorrigocin A

A fast atom bombardment positive ion mass spectrum of dorrigocin A had a highest mass peak at *m/z* 530 with a substantial peak at *m/z* 508 suggesting a molecular weight of 507. (The 530 and 508 peaks representing sodium and hydrogen adducts, respectively.) A high resolution positive ion mass spectrum of dorrigocin A matched the sodium adduct peak at *m/z* 530.2733 indicating a molecular formula of $C_{27}H_{41}NO_8Na$ (calculated *m/z* 530.2730) and hence $C_{27}H_{41}NO_8$ for dorrigocin A. A ^{13}C NMR spectrum of dorrigocin A contained only 25 carbon signals (see Table 1). An infrared spectrum containing bands at 3202 and 1698 cm^{-1} together with the carbon signal at δ 175.5 suggested an imide functionality. The presence of a cycloheximide, common in antifungal antibiotics, would account for the two degenerate carbon signals implied by the apparent disparity between mass spectral and nuclear magnetic resonance data. A cycloheximide ring was defined by an imide carbon signal at δ 175.5 (C-21 and C-26) which was coupled long range in an HMBC experiment[4] to the protons (see Table 2) of a four proton pair of methylene signals at δ 2.62 and δ 2.31 (C-20 and C-22) which were in turn coupled in a COSY experiment to a single methine proton signal at δ 2.14 (C-19). This system could be extended *via* a COSY experiment to show the attachment of an additional three methylenes with proton signals at δ 1.38 (C-18), δ 1.61 (C-17) and δ 2.60 (C-16). This δ 2.60 methylene proton signal was long range coupled to a ketone carbonyl signal at δ 216.5 (C-15) which was also coupled into an isolated spin system with a methine proton signal at δ 2.77 (C-14) coupled to a methyl signal at δ 0.87 (C-24) and an hydroxymethine at δ 4.00 (C-13). A trisubstituted olefin is next attached with a proton signal at δ 5.28 (C-11) and carbon signals at δ 135.8 (C-12) and δ 133.5 (C-11), the latter of which shows long range coupling to δ 4.00 (C-13), δ 1.62 (C-23) and δ 0.97 (C-22). A nuclear Overhauser effect (NOE) was observed in a ROESY[5] experiment between the olefinic proton at δ 5.28 (C-11) and the hydroxymethine proton signal at δ 4.00 (C-13) indicating that the trisubstituted olefin was of *trans* stereochemistry. This same olefinic proton signal (δ 5.28) is coupled to a methine proton signal at δ 2.71 (C-10) which is further coupled to the methyl proton signal at δ 0.97 (C-22) as well as a methine at δ 3.21 (C-9) which further couples to a methine at δ 3.54. This methine signal at δ 3.54 (C-8) correlates to a carbon signal at δ 84.4 in the HMQC[6] experiment. This shows long range coupling to a methoxyl group with proton signal at δ 3.22 which must therefore be

Table 1. ^{13}C NMR data for dorrigocin A and dorrigocin B (in CD_3OD).

Carbon number	Dorrigocin A	Dorrigocin B	Carbon number	Dorrigocin A	Dorrigocin B
1	170.5 (Q)	170.8 (Q)	14	50.1 (CH)	47.2 (CH)
2	123.6 (CH)	123.9 (CH)	15	216.5 (Q)	213.7 (Q)
3	149.5 (CH)	149.2 (CH)	16	43.6 (CH_2)	41.4 (CH_2)
4	32.7 (CH_2)	32.6 (CH_2)	17	21.1 (CH_2)	21.6 (CH_2)
5	31.9 (CH_2)	31.9 (CH_2)	18	35.2 (CH_2)	35.3 (CH_2)
6	135.1 (CH)	136.4 (CH)	19	31.5 (CH)	31.5 (CH)
7	130.2 (CH)	129.0 (CH)	20, 25	38.5 (CH_2)	38.6 (CH_2)
8	84.4 (CH)	86.1 (CH)	21, 26	175.5 (Q)	175.5 (Q)
9	78.9 (CH)	75.8 (CH)	22	16.4 (CH_3)	8.4 (CH_3)
10	35.8 (CH)	38.3 (CH)	23	10.8 (CH_3)	12.3 (CH_3)
11	133.5 (CH)	80.6 (CH)	24	14.5 (CH_3)	16.4 (CH_3)
12	135.8 (Q)	140.2 (Q)	8-OCH_3	56.4 (CH_3)	56.2 (CH_3)
13	81.8 (CH)	127.8 (CH)			

Table 2. ^1H NMR data for dorrigocin A and dorrigocin B (in CD_3OD).

Proton on carbon number	Dorrigocin A	Dorrigocin B
2	5.81 (d, 1H, J=15.5 Hz)	5.83 (d, 1H, J=15.5 Hz)
3	6.92 (dt, 1H, J=15.5, 6.7 Hz)	6.90 (dt, 1H, J=15.5, 6.9 Hz)
4	2.36 (m, 2H)	2.32 (m, 2H)
5	2.30 (m, 2H)	2.28 (m, 2H)
6	5.71 (dt, 1H, J=15.5, 6.5 Hz)	5.76 (dt, 1H, J=15.5, 6.5 Hz)
7	5.49 (br dd, 1H, J=15.5, 8.6 Hz)	5.23 (ddt, 1H, J=15.5, 8.6, 1.2 Hz)
8	3.54 (dd, 1H, J=8.6, 4.1 Hz)	3.49 (dd, 1H, J=8.6, 2.4 Hz)
9	3.21 (dd, 1H, J=6.9, 4.1 Hz)	3.34 (dd, 1H, J=8.2, 2.4 Hz)
10	2.71 (dq, 1H, J=9.9, 6.9 Hz)	1.74 (m, 1H)
11	5.28 (d, 1H, J=9.9 Hz)	3.96 (d, 1H, J=8.2 Hz)
13	4.00 (d, 1H, J=9.8 Hz)	5.28 (d, 1H, J=10.2 Hz)
14	2.77 (dq, 1H, J=9.8, 6.9 Hz)	3.51 (d, 1H, J=10.2 Hz)
16	2.60 (m, 2H)	2.49 (m, 2H)
17	1.61 (m, 2H)	1.57 (m, 2H)
18	1.38 (m, 2H)	1.35 (m, 2H)
19	2.14 (m, 1H)	2.10 (m, 1H)
20, 25	2.62 (m, 1H), 2.31 (m, 1H)	2.63 (m, 1H), 2.30 (m, 1H)
22	0.97 (d, 3H, J=6.7 Hz)	0.91 (d, 3H, J=7.0 Hz)
23	1.62 (br s, 3H)	1.64 (br s, 3H)
24	0.82 (d, 3H, J=7.2 Hz)	1.10 (d, 3H, J=6.5 Hz)
8-OCH_3	3.22 (s, 3H)	3.24 (s, 3H)

attached at C-8. The C-8 ether methine proton signal was also coupled to an olefinic proton signal at δ 5.49 (C-7) which was further coupled to an olefinic proton signal at δ 5.71 (C-6). The coupling constant of 15.5 Hz between the two olefinic protons as well as the lack of an NOE between the two indicated a *trans* stereochemistry for this olefin. The olefinic proton signal at δ 5.71 (C-6) was coupled to a methylene proton signal at δ 2.30 (C-5) which was further coupled to another methylene proton signal at δ 2.36 (C-4). The δ 2.36 (C-4) methylene proton signal was coupled to an olefinic methine at δ 6.92 (C-3) which was further coupled only to another olefinic proton signal at δ 5.81 (C-2). The coupling constant of 15.5 Hz between the two olefinic protons indicated a *trans* stereochemistry for this olefin. Each of the two olefinic proton signals at δ 5.81 (C-2) and δ 6.92 (C-3) showed long range coupling to a carbonyl carbon signal at δ 170.5 (C-1) which must define an α,β-unsaturated acid or ester system. That this must be a carboxylic

acid system is evidenced by the molecular formula which allows for only CO_2H remaining with one unit of unsaturation.

Structure Elucidation of Dorrigocin B

A fast atom bombardment positive ion mass spectrum of dorrigocin B had the same parent peak as that of dorrigocin A and a high resolution spectrum gave the same molecular formula match for the sodium adduct at $C_{27}H_{41}NO_8Na$ indicating a molecular formula for dorrigocin B of $C_{27}H_{41}NO_8$. Evaluation of the 2-D homonuclear and heteronuclear spectral data of dorrigocin B identified systems identical to dorrigocin A for the C-1 through C-9 and for C-15 through the cycloheximide functionality (see Tables 1 and 2). In dorrigocin B, however, the ketone carbonyl signal at δ 213.7 (C-15) shows long range coupling to a methine proton signal at δ 3.51 (C-14), to a methyl proton signal at δ 1.10 (C-24) and to an olefinic proton signal at δ 5.28 (C-13). This δ 5.28 proton signal correlates to a carbon signal at δ 127.8 (C-13) which shows long range coupling to proton signals for a methyl at δ 1.64 (C-23) and to the hydroxymethine at δ 3.96 (C-11). An NOE observed between the olefinic proton signal at δ 5.28 (C-13) and the hydroxymethine proton signal at δ 3.96 (C-11) indicates a *trans* stereochemistry for this (C-12 ~ C-13) olefin. The δ 3.96 proton signal is coupled to a methine signal at δ 1.74 (C-10) which is further coupled to a methyl signal at δ 0.91 (C-22) and to a methine at δ 3.49 (C-8). This methine signal at δ 3.49 (C-8) correlates to a carbon signal at δ 86.1 which shows long range coupling to a methoxyl group with proton signal at δ 3.24 which must therefore be attached at C-8 as in dorrigocin A. The C-8 proton signal can be followed by COSY peaks through the same C-8 through C-1 chain as for dorrigocin A. Thus dorrigocin A and B differ from one another only as a 1,3-hydroxy shift through the allylic hydroxyl functionality of their carbons 11 through 13.

Experimental

General Procedures

Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter in a 10 cm cell. Rf values were acquired on Merck Kieselgel 60 F_{254} TLC plates visualized with ceric sulfate spray reagent[7]. Fast atom bombardment mass spectra were measured on a Kratos MS-50 mass spectrometer. Ultraviolet spectra were recorded on a Perkin-Elmer Lambda 3B UV-visible spectrophotometer. Infrared spectra were recorded on a Nicolet model 60SX FT-IR. Nuclear magnetic resonance spectra were acquired on a General Electric GN500 spectrometer.

Characterization of the Dorrigocins

Dorrigocin A, $C_{27}H_{41}NO_8$, clear oil, $[\alpha]_D$ +91° (*c* 0.13, MeOH), had an Rf of 0.71 in MeOH, an Rf of 0.34 in acetone and an Rf of 0.24 in EtOAc-MeOH (4:1). An ultraviolet spectrum acquired in methanol or acidic methanol has λ_{max} 204 nm (ε 9,300). An ultraviolet spectrum acquired in basic methanol has λ_{max} 209 nm (ε 11,000). An infrared spectrum acquired as a thin film contains bands at ν_{max} 3446, 3203, 3085, 2966, 2930, 2876, 1708, 1698, 1657, 1451, 1375, 1263, 1197, 1149, 1112, 1073, 1048, 982, 871 and 753 cm^{-1}. ^1H and ^{13}C NMR data appear as Tables 1 and 2.

Dorrigocin B, $C_{27}H_{41}NO_8$, a clear oil, $[\alpha]_D$ +16° (*c* 0.33, MeOH), had an Rf of 0.73 in MeOH, an Rf of 0.38 in acetone and an Rf of 0.46 in EtOAc-MeOH (4:1). An ultraviolet spectrum acquired in methanol or acidic methanol has λ_{max} 206 nm (ε 12,000). An ultraviolet spectrum acquired in basic methanol has λ_{max} 208 nm (ε 14,000). An infrared spectrum acquired as a thin film contains bands at ν_{max} 3452, 3207, 3093, 2971, 2930, 2827, 1710, 1700, 1657, 1450, 1381, 1262, 1149, 1114, 1087, 982, 878 and 752 cm^{-1}. ^1H and ^{13}C NMR data are appear in Tables 1 and 2.

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