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δ-Indomycinone: A New Member of Pluramycin Class of Antibiotics Isolated from Marine *Streptomyces* sp.†

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In a continuation of our investigation for bioactive secondary metabolites from marine microorganisms[1], we have isolated a new pluramycin type metabolite δ-indomycinone (**2**), along with the known antibiotic β-indomycinone (**1**)[2]. Both compounds were extracted from the culture broth of a *Streptomyces* strain (B 8300), obtained from the collection of marine actinomycetes at Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven, Germany. Pluramycin metabolites containing the 4*H*-anthra[1,2-*b*]pyran-4,7,12-trione nucleus to which amino sugars typically are attached at C-8 and C-10 positions, were most commonly isolated from terrestrial *Streptomyces* sp. The pluramycin analogues which lack any carbohydrate substitution are the pluramycinones such as indomycinones[3]. The pluramycins are a group of highly structurally evolved DNA reactive agents and found to have antimicrobial and anticancer activity[4]. We would now like to describe the taxonomy of the producing strain and the fermentation, isolation and structure determination of the new metabolite δ-indomycinone (**2**).

Taxonomy of Strain B 8300

The actinomycete strain B 8300 was isolated from sediment of the Laguna de Terminos at the Gulf of Mexico using cellulose medium[5] containing 50% seawater at an incubation temperature of 18°C. The pure culture was maintained on yeast extract-malt extract agar[6] (YMA). The strain forms extensive yellow aerial mycelium, with straight to flexuous (Rectiflexibiles) spore chains of about 20 μm length. Spores are cylindrical about 1.1 μm long and 0.4 to 0.5 μm in diameter with a smooth surface. The non fragmenting vegetative mycelium is reddish brown forming a reddish brown, diffusible pigment. The strain cannot grow at 4°C and 45°C on YMA. Poor growth occurs at 10°C and 37°C after 4 weeks. The temperature optimum is at about 30°C. Growth is obtained in the presence of 0.4 and 7% (w/v) sodium chloride but not in the presence of 10 or 13% sodium chloride on YMA. Further physiological characteristics are compiled in Table 1.

The cell wall peptidoglycan of the strain contains major diagnostic amounts of L-diaminopimelic acid (L-DAP) and glycine corresponding with wall chemotype I sensu LECHEVALIER and LECHEVALIER[8]. Whole-cell

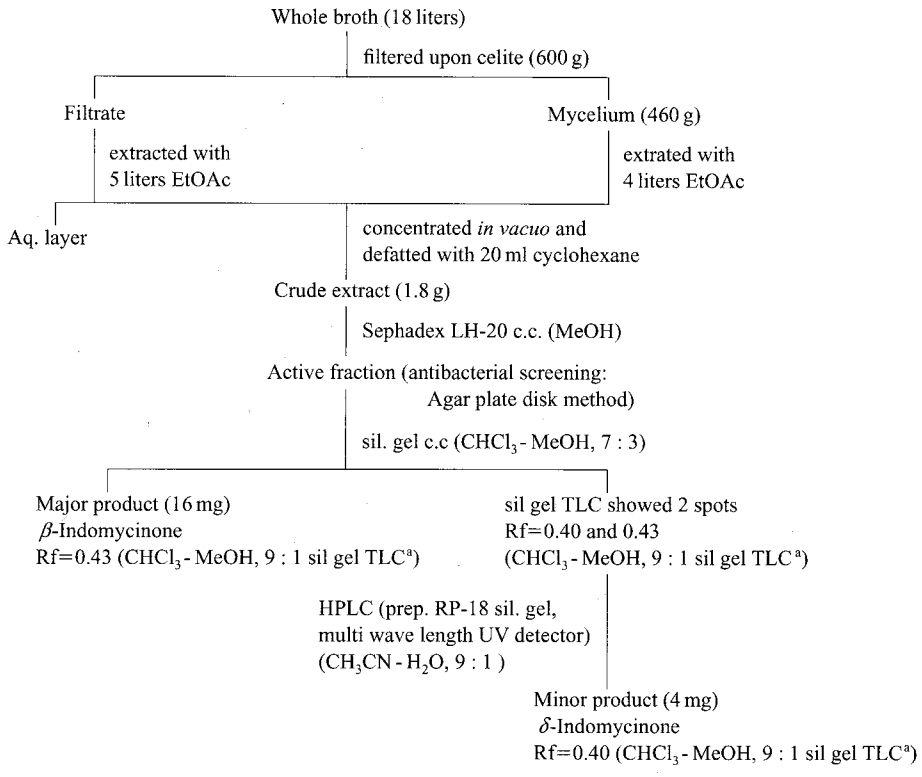
Table 1. Physiological characteristics of strain B 8300.

Characteristics	B 8300	Characteristics	B 8300
Production of		Carbon source utilization[a]	
Catalase	+	Adonitol	+
Nitrate reductase	−	L-Arabinose	+
		D-Fructose	+
Hydrolysis of		D-Glucose	+
Starch	+	*i*-Inositol	−
Gelatin	+	D-Mannitol	+
Casein	+	D-Melibiose	−
Chitin	+	D-Melizitose	−
Esculin	+	D-Raffinose	−
		L-Rhamnose	+
Melanin formation on		Sucrose	−
Peptone yeast extract iron agar[a]	−	D-Xylose	+
Tyrosine agar[a]	−	Xylitol	−

[a] After SHIRLING and GOTTLIEB[7].

† Art No. 10 on secondary metabolites of marine bacteria. Art. No. 9: see ref. 1.

Fig. 1. Isolation procedure of indomycinones.



[a]Silica gel Polygram SIL G/UV_{254} 4×8 cm (Macherey-Nagel & Co., Düren, Germany).

hydrolysates lack diagnostic sugars. The fatty acid profile shows larger amounts of saturated *iso*-(14:0, 15:0, 16:0, 17:0), *anteiso*-(15:0, 17:0) and straight chain (16:0) fatty acids. The saturated straight chain 14:0, 15:0, 17:0 fatty acids as well as the unsaturated straight chain 16:1 and 17:1 and the *iso*-16:1, *iso*-17:1 and *anteiso*-17:1 fatty acids represent only minor components; 10-methyl branched fatty acids are not present. The strain B 8300 can be assigned to the genus *Streptomyces* due to its morphological features, especially by the spore chain morphology and its biochemical properties (cell wall type I, no diagnostic sugars present). Strain B8300 is deposited in the culture collection of marine actinomycetes at the Alfred-Wagner-Institute for Polar and Marine Research, Bremerhaven, Germany.

Fermentation and Extraction

The slant culture of *Streptomyces* sp. B 8300 grown at 27°C for 3 days was inoculated into 2 litres of culture medium composed of malt extract 20 g, yeast extract 8 g, glucose 8 g, in 1 litre normal water and 1 litre synthetic sea water, adjusted to pH 7.8, distributed into 10 one litre Erlenmeyer flasks, and incubated for 72 hours at 28°C. Fermentation was carried out in a 20-litre jar fermentor containing 18 litres of culture medium described above. The medium was sterilized for 1 hour at 121°C, prior to sterilisation the pH was adjusted to 7.8. Incubation was carried out at 28°C for 72 hours with automatic addition of 2 N HCl or 2 N NaOH to maintain the pH value between pH 6~7, and polypropylene glycol to control foaming. Sterile air was supplied (5 litres/minute) and agitation was at 120 rpm. Active principles were extracted with ethyl acetate from the filtered broth. Purification of the active substances were carried out according to the procedure shown in Fig. 1.

Physico-chemical Properties

The physico-chemical properties of β- and δ-indomycinone (**1** and **2**) are summarized in Table 2. The ^1H NMR and ^{13}C NMR data of **2** are summarized in Table 3.

β-Indomycinone (**1**) was obtained as a yellow solid, its HR-EIMS showed *m/z* 404.1283, calcd. for $C_{24}H_{20}O_6$. HPLC diode array screening revealed that its UV absorption λ_{max} 239, 267 and 416 nm and retention time 12.16 (minutes; ODS analytical column, CH_3CN:H_2O; 9:1)

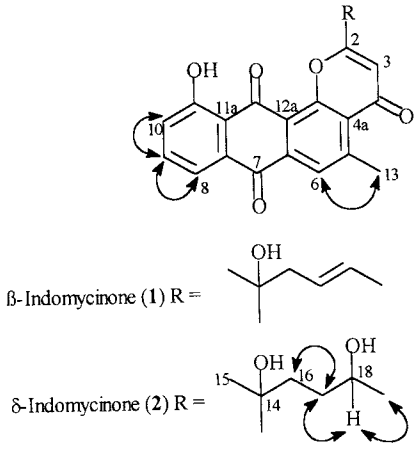


Table 2. Physico-chemical properties of β- and δ-indomycinone (**1** and **2**).

	β-Indomycinone (**1**)	δ-Indomycinone (**2**)
Appearence	Yellow solid	Yellow solid
Molecular formula	$C_{24}H_{20}O_6$	$C_{24}H_{22}O_7$
M.P.	178°C	181°C
EI-MS	404 (M^+)	422 (M^+)
HREI-MS (*m/z*)		
Found	404.1283	422.1362
Calcd.	404.1250	422.1311
UV λ_{max} nm (log ε)	239, 267 and 416	220, 260 and 416
IR (KBr) ν cm^{-1}	3300 (br), 2930, and 1640	3350 (br), 2930, 1640

Table 3. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR data (125 MHz, $CDCl_3$) of δ-indomycinone (**2**).

C-Atom	^{13}C NMR	^1H NMR	C-Atom	^{13}C NMR	^1H NMR
2	172.77 s		11	161.90 s	
3	113.03 d	6.30 (s, 1H)	12a	119.02 s	
4	181.10 s		12b	158.22 s	
4a	127.78 s		13	24.29 q	3.01 (s, 3H)
5	150.58 s		14	73.50 s	
6	126.35 d	8.09 (s, 1H)	15	26.44 q	1.30 (s, 3H)
6a	137.69 s		16	30.97 t	[a] 1.90 (m, 2H)
7	182.75 s		17	30.52 t	[a] 1.95 (m, 2H)
7a	132.45 s		18	69.45 d	3.22 (m, 1H)
8	120.10 d	7.82 (dd, 7.5, 1.0, 1H)	19	14.54 q	1.57 (d, 8.1, 3H)
9	137.69 d	7.70 (t, 8.0, 1H)	—		12.77 (s, OH)
10	125.99 d	7.39 (dd, 8.1, 1.0, 1H)	—		12.58 (s, OH)
11a	116.00 s		—		4.80 (s br, OH)
12	189.00 s		—		3.80 (s br, OH)

Coupling constants in [Hz], δ in ppm, TMS as internal standard.
[a] Assignment may be interchanged.

was highly comparable with the known pluramycin class antibiotic β-indomycinone. Hence the structure of compound **1** was determined as β-indomycinone (**1**) and identification was confirmed further by comparison with an authentic sample[2,9].

δ-Indomycinone (**2**) was isolated as yellow solid. The UV and IR spectra of **2** showed close similarity with compound **1** (see Table 2), HR-EIMS showed *m/z* 422.2362, calcd. for $C_{24}H_{22}O_7$; 404 (M^+-18), 389 ($M^+-H_2O-CH_3$). Many of the ^1H NMR and ^{13}C NMR spectral signals were observed at the same chemical shifts as those for compound **1** (see Table 3). The side chain double bond observed in compound **1** however, was not present in compound **2**. On the other hand, signals in the ^1H NMR spectrum of compound **2** indicate a tertiary methyl (δ 1.30), a secondary methyl (δ 1.57, 3H, d, J=8.1), a methine proton (δ 3.22, m, 1H) and two methylene protons at δ 1.90 ~ 1.95 (m, 4H). The ^{13}C NMR spectrum of compound **2** also showed signals at δ 14.54 (q), 26.44 (q), 30.57 (t), 30.98 (t), and 69.45 (d) which were not exhibited in β-indomycinone (**1**). These data unequivocally showed that compound **2** has a similar basic structure as β-indomycinone (**1**), but a different side chain. Correlation of the proton at δ 3.22 (m, 1H) with the signals at δ 1.57 (d, 8.1, 3H) and 1.90 (m, 2H), 1.95 (m, 2H) were established by analysis of the H,H-DQFCOSY spectrum. The above spectral data are consistent with the structure of 2-(2,4-dihydroxyhexane)-4*H*-anthra[1,2-*b*]pyran-4,7,12-trione (**2**). This new antibiotic was named as δ-indomycinone, with comparison of γ-indomycinone[10].

Biological Activity

Antimicrobial activity was determined by serial broth dilution method using nutrient broth (Difco). β-Indomycinone (**1**) and δ-indomycinone (**2**) showed MIC at

100 μg/ml and 113 μg/ml against *B. subtilis*. Paper disc method against *E. coli*, *S. aureus*, *C. albicans*, *Streptomyces* sp. Tü 824 showed antibacterial activity at 100 μg/ml. β-Indomycinone (**1**) and δ-indomycinone (**2**) showed antioxidative activity in an antioxidative test carried out by DPPH method[11].

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