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γ-INDOMYCINONE, A NEW PLURAMYCIN METABOLITE FROM A DEEP-SEA DERIVED ACTINOMYCETE

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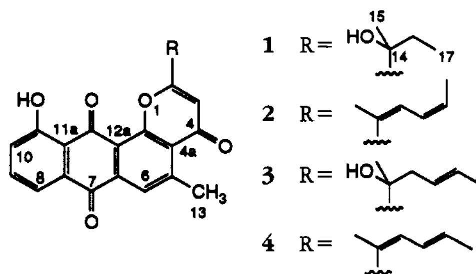
ABSTRACT.—A new member of the pluramycin class of antibiotics, γ-indomycinone [**1**], has been isolated along with the known compounds rubiflavinone C-1 [**2**] and β-indomycinone [**3**] from the culture broth of a *Streptomyces* sp. obtained from a deep-sea sediment core. Each compound is composed of an anthraquinone-γ-pyrone nucleus, but bears a different side-chain. Compounds **2** and **3** were identified by comparison of their spectral data with published data, while γ-indomycinone [**1**] was characterized using ^{1}H-nmr and mass spectrometry.

Marine bacteria are the subject of a growing number of natural products studies. Like their terrestrial counterparts, marine microorganisms are prolific producers of unique secondary metabolites (1,2). Interesting compounds have been isolated from bacteria cultured from a variety of marine habitats. Examples include the antimycin antibiotics urauchimycins A and B (3), isolated from the bacterium *Streptomyces* sp. found associated with an unidentified sponge; marinone and debromomarinone (4), isolated from an unidentified actinomycete obtained from estuarine sediments; and the macrolactins (5) and caprolactins (6), both isolated from unidentified Gram-positive bacteria obtained from deep-sea sediment samples.

We would now like to report the structure determination of a new pluramycin-type metabolite, γ-indomycinone [**1**], which was isolated along with the previously reported compounds rubiflavinone C-1 [**2**] (7) and β-indomycinone [**3**] (8), from the culture broth of an actinomycete identified as *Streptomyces* sp. obtained from a deep-ocean sediment sample. Pluramycin antibiotics (9), most commonly isolated from terrestrial *Streptomyces* sp., contain an anthraquinone-γ-pyrone nucleus, to which amino sugars are typically attached at C-8 and C-10.

The organism, isolate PC1/B2, was cultured for 3 days in 2-liter flasks each containing 1 liter of Difco marine broth supplemented with soluble starch. After adjusting the pH to 10, the entire culture broth was extracted with EtOAc to give 2.07 g of organic-soluble material. The crude extract, which was cytotoxic towards the KB cell line with an MIC value of ≤0.001 μg/ml, was then subjected to a solvent partition scheme. The resulting hexane-soluble fraction (800 mg) was chromatographed over Si gel (CH_2Cl_2-MeOH, 98:2), followed by extensive Si gel hplc, yielding rubiflavinone C-1 (**2**, 8



mg) and β-indomycinone (**3**, 6 mg). Similar chromatography of the CCl_4 fraction afforded γ-indomycinone (**1**, 0.6 mg).

The ^{1}H-nmr spectra for these compounds show a characteristic set of signals (see Table 1). For example, each contained three signals for protons on a 1,2,3-trisubstituted benzene ring [7.8 (dd, H-8), 7.7 (t, H-9), and 7.3 (dd) ppm], two olefinic or aromatic protons [8.0 (s, H-6) and ca. 6.5 (s, H-3) ppm], a hydrogen-bonded phenol proton [12.9 (s, C-11-OH) ppm], and a methyl group [3.0 (s) ppm]. Likewise, the ^{13}C-nmr spectra (Table 2) included signals for two quinone carbonyls (188 and 182 ppm) and a γ-pyrone (177 ppm), along with 14 additional sp^2 hybridized carbons, 9 of which were quaternary. The remaining signals were the result of the various side-chains attached at C-2.

Both rubiflavinone C-1 [**2**] and β-indomycinone [**3**] could be identified by comparison of their spectral data (ms, ir, and ^{1}H-nmr) with those reported in the literature (7,9). As previously reported by Nadig and Séquin (7), compound **2**, upon isolation, quickly began to isomerize to rubiflavinone C-2 [**4**], which contains a *trans* C-16,C-17 double bond, thus supporting the suggestion that compound **4** is an artifact. Because ^{13}C-nmr data for compounds **2** and **3** have not been previously reported, they are included in Table 2.

The mass spectrum of γ-indomycinone [**1**] supported a molecular formula of $C_{22}H_{18}O_6$, 26 mass units (C_2H_2) fewer than that of **3**. Losses of M^+-18 and M^+-29 suggested the presence of a hydroxyl group and an easily lost ethyl group. In addition to the signals associated with the basic structure, the ^{1}H-nmr spectrum (Table 1) contained signals assigned to a diastereotopic pair of methylene protons [2.10 (dq) and 1.98 (dq) ppm] and two methyl groups, one a singlet (1.67 ppm) and one a triplet (0.97 ppm), while lacking the olefinic side-chain signals present in the ^{1}H-nmr spectrum of β-indomycinone [**3**]. Decoupling of the triplet methyl caused each of the methylene signals to collapse to a doublet. These data are consistent with a 1-hydroxy-1-methylpropyl side-chain as in **1**.

Until now, terrestrial *Streptomyces* spp. have been the sole source of pluramycin antibiotics, a quickly growing class of compounds which exhibit a variety of interesting biological activities (10–12). Even though strain PC1/B2 was isolated from a deep-sea sediment, it grew well on most complex and synthetic media, but only moderately on a medium containing glycerol and asparagine as carbon and

TABLE 1. ^{1}H-Nmr Data for Compounds **1–3**.

Proton	Compound		
	1	**2**	**3**
3	6.52 (s)	6.38 (s)	6.56 (s)
6	8.00 (s)	7.99 (s)	8.07 (s)
8	7.83 (dd, 7.5, 1.2)	7.78 (dd, 7.6, 1.3)	7.82 (dd, 7.6, 1.3)
9	7.69 (t, 7.9)	7.68 (t, 8.0)	7.68 (t, 8.0)
10	7.37 (dd, 8.3, 1.2)	7.33 (dd, 8.4, 1.3)	7.36 (dd, 8.5, 1.3)
13	3.03 (s)	2.96 (s)	3.02 (s)
15	1.67 (s)	2.06 (s)	1.70 (s)
16A	2.10 (dq, 14.2, 7.5)	8.37 (d, 11.7)	2.91 (dd, 14.4, 8.5)
16B	1.98 (dq, 14.2, 7.5)		2.78 (dd, 14.4, 7.2)
17	0.97 (t, 7.5)	6.53 (ddq, 11.7, 10.7, 1.7)	5.38 (m)
18	—	6.08 (dq, 10.7, 7.6)	5.70 (dq, 11.1, 6.8)
19	—	2.16 (dd, 7.6, 1.7)	1.64 (d, 6.8)
OH-11 ...	12.83 (s)	12.90 (s)	12.85 (s)

TABLE 2. ^{13}C-Nmr Data for Compounds **2** and **3**.

Carbon	Compound	
	2	**3**
2	164.4	171.8
3	109.7	109.6
4	179.6	179.2
4a	126.8	126.3
5	150.1	150.0
6	125.6	125.7
6a	136.8	136.0
7	182.2	181.8
7a	132.8	132.3
8	119.5	119.5
9	136.6	136.5
10	125.4	125.4
11	163.0	162.7
11a	117.3	116.8
12	188.0	187.4
12a	119.4	119.6
12b	156.6	156.1
13	24.2	24.2
14	123.5	73.7
15	12.4	26.1
16	131.0	38.2
17	125.6	123.1
18	135.4	129.6
19	14.2	13.0

nitrogen sources. The color of the substrate mycelium ranged from medium yellow on yeast-malt extract to medium to light yellow-brown on inorganic salts starch medium with no soluble pigments or melanin produced. The organism produced an extensively branching substrate mycelium as well as an abundant yellow-white aerial mycelium, which was transformed into sheathed chains of arthrospores. The chains of arthrospores ranged in length from 20 to 30 spores per chain and were classified as *Rectiflexibilis*. Scanning-electron microscopy revealed cylindrical spores with a smooth surface. Sporangia, zoospores, and sclerotia were not observed.

An analysis of the whole-cell hydrolysate of strain PC1/B2 revealed the presence of only the LL-isomer of diaminopimelic acid. A type 2c fatty acid pattern (13) consisting mainly of saturated iso and anteiso fatty acids was noted. The phospholipid pattern was determined to be type II based on the presence of phosphatidylethanolamine and diphosphatidylglycerol and the absence of phosphatidylcholine or other unidentified phospholipids containing glucosamine (13). Based on the taxonomic properties above, the strain PC1/B2 is considered to be a member of the genus *Streptomyces* (14), and it was designated as *Streptomyces* sp. PC1/B2. Further studies for species identification are in progress and will be reported elsewhere.

Although only marginally cytotoxic to the human colon cancer cell line HCT-116, a mixture of rubiflavinones C-1 and C-2 (**2** and **4**) has demonstrated differential cytotoxicity against the CHO cell line UV20, which is deficient in its ability to perform DNA excision repair (15), versus the DNA repair-proficient cell line BR1 (16). The UV20 cell line is susceptible to agents which form bulky adducts with DNA. Other agents which are active in this assay are mitomycin C and cisplatin. This result is consistent with earlier reports that the rubiflavin antibiotic complex, which contains the indomycinones and rubiflavinones (8,9), was shown to bind to DNA, inhibiting DNA synthesis (17).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All ^{1}H- and ^{13}C-nmr data were recorded in $CDCl_3$ on a GE Omega-500 instrument at 500 and 125 MHz operating frequencies, respectively. Chemical shifts are referenced to solvent peaks: δ_H 7.26 (residual $CHCl_3$) and δ_C 77.0. Ir spectra were obtained using a Perkin-Elmer 1600 Ft-ir spectrometer. Mass spectral data were obtained on a VG-70SE mass spectrometer operating in the ei mode. Uv spectra were obtained on a Milton Roy Spectronic 3000 diode array spectrophotometer.

CULTURE CONDITIONS.—The bacterium, designated PC1/B2, was obtained from a deep ocean sediment sample (# PC001 in University of Hawaii Corelab) which was drilled at 11°5.1′S latitude 172°51.2′W longitude at a depth of 4680 m on March 16, 1982. A sample of sediment was diluted 1000:1 with sterile artificial sea water and plated onto marine agar 2216 (Difco) petri plates. The isolated culture was grown in 38 two-liter flasks each containing one

liter of marine broth 2216 (Difco) supplemented with 10% soluble starch for 3 days at 23–25° on an orbital shaker at 250 rpm.

TAXONOMIC STUDIES.—Cultural and physiological studies were carried out according to Shirling and Gottlieb (18) and Gordon *et al.* (19). Characteristics were assessed after 14 to 28 days incubation at 28°. The color and hue indicated are those of the National Bureau of Standard Centroid Color Charts, Publication 440, Washington, DC. Spore chain morphology was determined using a scanning electron microscope (JEOL, Model 6300V).

Whole-cell sugars were determined using standard gc-ms methods (20). The isomers of diaminopimelic acid (DAP) present in the cell wall were determined using gc-ms on a Chirasil-Val column after derivatization as their *N*-heptafluorobutyryl *n*-butyl esters (21). Phospholipids were extracted and analyzed using two-dimensional tlc (22). The preparation and analysis of whole-cell fatty acid methyl esters was carried out using the Microbial Identification System (23).

EXTRACTION AND ISOLATION.—The crude hexane fraction (800 mg) obtained from a modified Kupchan solvent partition scheme (24) was chromatographed over Si gel with eluents of increasing polarity from 100% CH_2Cl_2 through 40% CH_3OH in CH_2Cl_2. The fractions were assayed for cytotoxicity, and the most active fraction was extensively chromatographed using normal-phase hplc over Si gel (CH_2Cl_2/MeOH/aqueous NH_3, varied ratios), yielding the previously reported pluramycin-type metabolites rubiflavinone C-1 [**2**, 8 mg] and β-indomycinone [**3**, 6 mg]. Similar chromatography of the CCl_4 fraction afforded a new member of the pluramycin family, γ-indomycinone [**1**, 0.6 mg].

γ-Indomycinone [**1**].—Uv (EtOH) λ max 420 (sh), 270 (sh), 236, 208 nm; ir (film) ν max 3417, 2922, 2852, 1738, 1715, 1644, 1455, 1378, 1263, 1188 cm^{-1}; ^1H-nmr data, see Table 1; eims *m/z* 378 (40), 362 (15), 360 (23), 350 (21), 349 (100), 281 (61); hreims *m/z* 378.1113 (calcd for $C_{22}H_{18}O_6$, 378.1103).

Rubiflavinone C-1 [**2**].—Uv (EtOH) λ max 240, 229, 402 nm; ir (film) ν max 2924, 2360, 1642, 1453, 1268 cm^{-1}; ^1H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; hreims *m/z* 386.1146 (calcd for $C_{24}H_{18}O_5$, 386.1154).

β-Indomycinone [**3**].—Uv (EtOH) λ max 240, 267 (sh), 410 (sh) nm; ir (film) ν max 3354, 2920, 1641, 1584, 1452, 1380, 1314 cm^{-1}; ^1H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; eims *m/z* 404 (0.4), 386 (3), 350 (23), 349 (100), 334 (24), 281 (27); hreims *m/z* 404.1254 (calcd for $C_{24}H_{20}O_6$, 404.1260), 386.1157 (calcd for $C_{24}H_{18}O_5$, 386.1154).

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