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NOTE

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1-Hydroxy-1-norresistomycin, a New Cytotoxic Compound from a Marine Actinomycete, *Streptomyces chibaensis* AUBN$_1$/7[†]

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Abstract In our systematic screening programme for marine actinomycetes, a bioactive streptomycete was isolated from marine sediment samples of the Bay of Bengal, India. The isolate yielded a new cytotoxic compound. This was obtained by solvent extraction followed by chromatographic purification. The pure compound was identified from spectroscopic data as a quinone-related antibiotic, 1-hydroxy-1-norresistomycin (**1**). It showed a potent cytotoxic activity against cell lines *viz*. HMO2 (gastric adenocarcinoma) and HePG2 (hepatic carcinoma) *in vitro*. It also exhibited antibacterial activities against Gram-positive and Gram-negative bacteria.

Keywords *Streptomyces*, cytotoxic activity, antibacterial activity, quinone, 1-hydroxy-1-norresistomycin

Marine actinomycetes remain an important source in the search for novel bioactive compounds. So far terrestrial substrates have been predominantly exploited as sources of actinomycetes, where as the marine habitat has received much less attention. There are a few reports on bioactive compounds from marine actinomycetes in recent times [1～6]. The marine environment may be an important source of novel anti-cancer, anti-viral, antibacterial and antifungal antibiotics as well as industrially important enzymes.

Taxonomic characteristics of the isolate were determined by cultivation on various media as described by Shirling and Gottlieb [7], Waksman [8] and Arai [9]. Cell wall composition was analyzed by the method of Lechevalier and Lechevalier [10], using thin layer chromatography plates as described by Staneck and Roberts [11]. The taxonomic studies indicated that the isolate belongs to *Streptomyces chibaensis* and it was designated as *S. chibaensis* AUBN$_1$/7.

The cytotoxicities of **1** were assessed based on their effects on the growth of tumor cells *in vitro* according to the NCI guide lines [12]. The cell lines used were HMO2 (gastric adenocarcinoma) and HePG2 (hepatic carcinoma). Cells were grown in 96-well microtitre plates of RPMI 1640 tissue culture medium supplemented with 10% fetal calf serum at 37°C in a humidified atmosphere of 50% CO_2 in air. After 24 hours of incubation **1** (0.1～10.0 μg/ml) was added to the cells. After 48 hours incubation, the cells were fixed by addition of trichloracetic acid and cell protein was assayed with sulforhodamine B [13]. For each concentration tested, the GI_{50} (drug concentration causing 50% growth inhibition), TGI values (drug concentration causing 100% growth inhibition) and LC_{50} (minimum concentration which reduces the initial cell number to half) were determined (Table 2).

A full grown slant culture of the strain AUBN$_1$/7 on

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[†]The same compound (1-hydroxy-1-norresistomycin) which was isolated independently from different strains appears in this issue (pages 530–534).

starch casein agar (composition: soluble starch 1.0%, casein 0.03%, KNO_3 0.2%, NaCl 0.2%, K_2HPO_4 0.005%, $CaCO_3$ 0.002%, $FeSO_4 \cdot 7H_2O$ 0.001%, and agar 2.0%) was transferred into Erlenmeyer flasks (2×250 ml) containing 50 ml of seed medium (composition: soyabean meal 1.0%, cornsteep solids 1.0%, glucose 0.5% and $CaCO_3$ 0.5% with pH 7.0) and incubated for 2 days at 28°C on a rotary shaker (220 rpm).This culture was transferred as a 10% (v/v) inoculum into 200 ml aliquots of the production medium contained in 30×1 litre EM flasks. The medium composition was: soyabean meal 1.0%, corn steep solids 0.5%, soluble starch 1.0%, glucose 0.5% and $CaCO_3$ 0.7% with pH 7.0. The inoculated production flasks were incubated for 56 hours at 28°C on a rotary shaker (220 rpm).

The culture broth (6 litres) was centrifuged at 4000 rpm for 10 minutes, at 10°C and clear culture supernatant was separated. It was extracted twice with ethyl acetate (2×1.2 litres) and washed with 500 ml water at pH 7.0. The ethyl acetate layer was concentrated in vacuum at 35°C to give 5.0 g of the crude ethyl acetate extract. A polymer was obtained from the crude ethyl acetate extract, which did not possess any antimicrobial activity. The mycelium was extracted with acetone (1.0 litre) and then centrifuged (4000 rpm, 10 minutes, 10°C). The acetone extract was concentrated in vacuum and lyophilized to obtain 4.5 g of mycelium extract.

The mycelium extract (4.5 g) was chromatographed on a silica gel column (22×5 cm) and eluted with dichloromethane/methanol (95 : 5, 1 litre) to give ten fractions. The dried residues of all 10 fractions were dissolved, each in a specified volume of dichloromethane to give 1 mg/ml concentration and tested for their antimicrobial activities using *Bacillus subtilis* as test organism by disc-plate method. Among 10 fractions, fraction VI (49.8 mg) exhibited good activity and was purified by chromatography on Sephadex LH-20 (methanol) to obtain four fractions. The third fraction VIc was found to possess good antimicrobial activity while others have no or negligible activites. The active fraction VIc was further purified by chromatography on Sephadex LH-20 (acetone) resulting in two distinct fractions VIc.I (6 mg) and VIc.II (4.5 mg). Fraction VIc.I (**1**) had good antimicrobial activity (100 μg/ml) but negligible activity was observed with VIc.II. Compound **1** was found to be pure and was pale yellow solid.

The molecular formula of **1** was determined as $C_{21}H_{14}O_7$ on the basis of the EI-MS (70eV), which gave a ([M^+], 40) ion at *m/z* 378.1 and elemental analysis (Calculated C: 66.67%, H: 3.73% and O: 29.60%; Obtained C: 66.68%, H: 3.72% and O: 29.59%). UV λ_{max}^{MeOH} nm 200, 204, 288, 464, 516. IR ν_{max} (KBr) cm^{-1} 3422 (OH), 2924 (CH), 2854 (CH), 1639 (C=O), 1594 (C=C), 1145 (C–O), 1085 (C–O).

^{1}H-NMR ([D_6] DMSO, 300 MHz): δ=14.55 (br. s, 1 H, OH), 14.00 (br. s, 1 H, OH), 13.61 (br. s, 1 H, OH) , 7.48 (s, 1 H, 11-H), 7.08 (s, 1 H, 8-H), 6.39 (s, 1 H, 4-H) , 6.32 (br. s, 1 H, OH), 2.94 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3).

HMBC and HSQC Correlations: C-1: 12-H_3, 11-H; C-2: 12-H_3; C-2a: 4-H; C-4: 4-H; C-5: 4-H; C-5a: 4-H; C-6a: 8-H; C-8: 8-H, 14-H_3; C-9: 14- H_3; C-9a: 11-H, 14- H3, 8-H; C-11: 11-H; C-11a: 12-H_3; C-11b: 11-H; C-13: 12- H_3; C-14: 14-H_3, 8-H.

The ^{1}H NMR spectrum of **1** showed broad singlets at δ=14.55, 14.00 and 13.61 with intensities of 1 proton each. These indicate the presence of 3 chelated hydroxyl

Table 1 A comparison of ^{13}C-NMR data of 1-hydroxy-1-norresistomycin (**1**) and resistomycin (**2**)

Carbon	Compound (**2**)	Compound (**1**)
C-1	45.9	74.1
C-2	204.6	204.3
C-2a	102.5	102.5
C-3	170.2	169.9
C-4	100.1	100.3
C-5	169.7	169.7
C-5a	105.7	106.3
C-6	184.5	184.7
C-6a	106.4	107.0
C-7	167.9	168.2
C-8	119.2	119.4
C-9	151.5	152.5
C-9a	114.1	115.0
C-10	162.3	160.4
C-11	109.5	110.2
C-11a	152.2	152.3
C-11b	107.4	106.9
C-11c	139.5	139.2
C-11d	128.5	129.0
C-12 (ax)	28.4	33.3
C-13 (eq)	28.4	—
C-14	25.4	25.8

Table 2 Cytotoxic activities of 1-hydroxy-1-norresistomycin (**1**)

Compound	Cell line HMO2			Cell line HePG2		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	0.009	0.012	0.015	0.014	0.018	0.021

groups in the molecule. In the aromatic region three singlets at δ=7.48, 7.08 & 6.39 with relative intensity of one proton each were observed. This indicated the presence of three different protons. A broad singlet of one proton intensity appeared at δ=6.32, this indicates the presence of hydroxyl group attached to alicyclic or cyclic compound. The singlet of three proton intensity peak at δ=2.94 indicated the presence of a methyl group either on an aromatic ring or on heteroatom. Another peak at δ=1.48 with three proton intensity also indicates the presence of another methyl group attached to either a cyclic or an aliphatic moiety.

The ^{13}C-NMR spectrum exhibited two signals at δ=204.3 & 184.7 and these peaks indicated the presence of two carbonyl groups. In addition to this, 17 signals in the aromatic region and two signals in the aliphatic region were observed. The HMBC and HSQC data of **1** presented in Fig. 2 were crucial in determining the structure and are in full agreement with the structure. The important evidence of the structure comes from the HMBC correlation noticed for C-1 carbon with the protons of the methyl group 12-H_3 (1.48) and the proton at C-11 (7.48). The above spectral data suggest the structure for **1** as shown in Figs.1 and 2. A comparison of ^{13}C-NMR data of the pure **1** with that of the reported compound resistomycin [14, 15] was made (Table 1). The ^{13}C-NMR data of the pure **1** is almost similar to resistomycin (**2**) except at C-1 because of the presence of hydroxyl group.

The spectral information of the **1** was searched in different databases like Chapman and Hall, SciFinder, Beilstein and Antibase and no hints were observed in any of the databases. This indicated that 1-hydroxy-1-norresistomycin (**1**), {1,3,5,7,10-pentahydroxy-1,9-dimethyl-2*H*-benzo[cd] pyren-2,6(1*H*)-dion} is not reported so far (Figs. 1 and 2) and it is the only resistomycin analogue reported to date. The minimum inhibitory concentrations (MIC) of the **1** against different test organisms were determined by the broth dilution method (Table 3).

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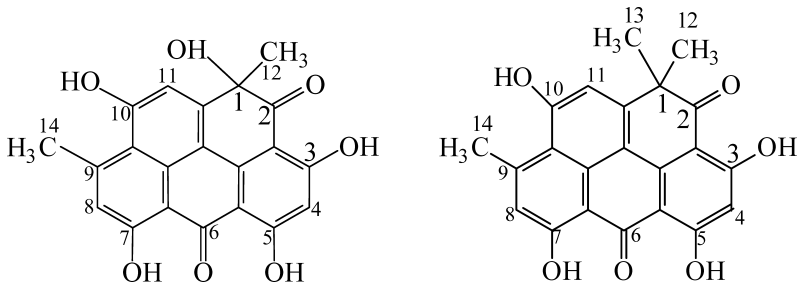

Fig. 1 1-Hydroxy-1-norresistomycin (**1**) and resistomycin (**2**).

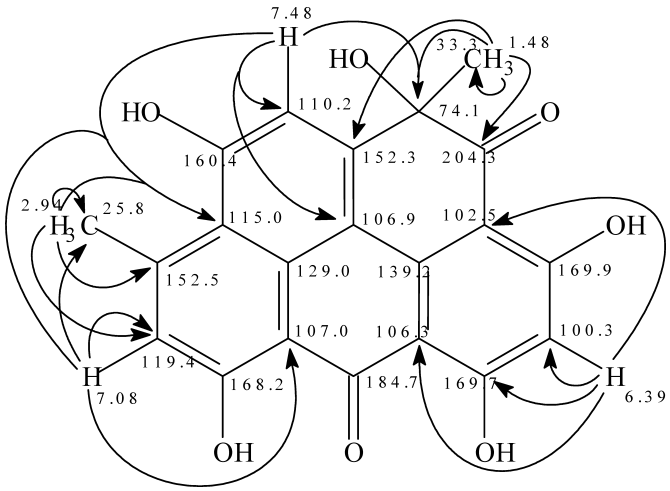

Fig. 2 CH-Correlations with HMBC and HSQC of **1**.

Table 3 Antimicrobial activities of 1-hydroxy-1-norresistomycin (**1**)

Test organism	MIC (μg/ml)
Bacillus subtilis ATCC 6633	16
Bacillus pumilus ATCC 19164	16
Staphylococcus aureus ATCC 29213	64
Escherichia coli ATCC 26	128
Pseudomonas aeruginosa ATCC 27853	>128
Proteus vulgaris ATCC 6897	>128

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