HYDROXYNYBOMYCIN: ISOLATION, STRUCTURE AND BIOACTIVITY

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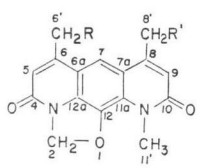
Nybomycin (**1**)[1] and deoxynybomycin (**2**)[2] comprise a thus-far unique structural class of antibiotics that contain a linearly fused pyridoquinolone ring system and an angularly fused oxazoline unit. Recently, we isolated a third member of this unusual class, hydroxynybomycin (**3**), which occurs as a minor metabolite of the nybomycin-producing organism, *Streptomyces* sp. D-57.[3] Its isolation, structure elucidation and bioactivity are reported herein.

Hydroxynybomycin was isolated as its di-*n*-butyrate (**4**). Crude nybomycin (30 g, *ca.* 70% pure),[3] was converted to nybomycin *n*-butyrate (**5**) using the method previously described.[4] Several crystallizations from tetrahydrofuran gave pure **5** (mp 203~204°C). Concentration of the resulting mother liquors afforded a mixture of **4** and **5** which was separated by column chromatography [silica gel; chloroform - ethanol (98: 2)] and preparative TLC. Recrystallization of the minor component (0.38 g) from ethanol, containing a trace of chloroform, produced light yellow needles of hydroxynybomycin di-*n*-butyrate (**4**): mp 193~194°C; IR (KBr) 1740 (s), 1665 (s), 1635 (s), 1600 (s) and 1170 cm^{-1} (s); ^1H NMR ($CDCl_3$) δ 0.96 (6H, t, J=7 Hz), 1.70 (4H, sextet, J=7 Hz), 2.40 (4H, t, J=7 Hz), 3.90 (3H, s), 5.28 (2H, d, J=1 Hz), 5.31 (2H, d, J=1 Hz), 6.36 (2H, s), 6.62 (1H, t, J=1Hz), 6.67 (1H, t, J=1 Hz), 7.38 (1H, s); MS *m/e* (relative intensity, %) 454 (63, M^+), 384 (26), 296 (25), 71 (41), 43 (100).



1: R=H, R'=OH
2: R=R'=H
3: R=R'=OH
4: R=R'=$OCOCH_2CH_2CH_3$
5: R=H, R'=$OCOCH_2CH_2CH_3$

Anal. Calcd. for $C_{24}H_{26}N_2O_7$: C, 63.43; H, 5.76; N, 6.16. Found: C, 63.32; H, 5.81; N, 6.01.

The structure of the di-*n*-butyrate was readily deduced by the comparison of its ^1H NMR spectrum to that of nybomycin *n*-butyrate.[4] The two spectra were quite similar except for replacement of the allylic methyl doublet at δ 2.47 (J=1.2 Hz) for C-6′ of **5** by a new methylene doublet at δ 5.33 (J=1.3 Hz) having almost the same chemical shift as the methylene doublet (δ 5.35, J=1.3 Hz) for C-8′ of **5** and **4**. In addition, the relative integrated peak intensities for the butyrate protons were twice those of **5**, indicating a dibutyrate ester. These data, combined with the observed downfield shift (0.2 ppm) of the olefinic proton at C-5, established hydroxynybomycin di-*n*-butyrate as **4**. Structure **4** is also supported by the microanalytical, IR, and MS data provided above.

The parent antibiotic, hydroxynybomycin (**3**), was obtained from **4** by hydrolysis in concentrated hydrochloric acid at 25°C. Dilution of the mixture with water gave the crude antibiotic, which was recrystallized from hot dimethylformamide to provide pale yellow crystals of **3**: mp>360°C, IR (KBr) 3600~3200 (s), 1680~1600 cm^{-1} (s); ^1H NMR (CF_3CO_2H) δ 4.44 (3H, s), 5.41 (2H, s), 5.48 (2H, s), 6.75 (2H, s), 7.38 (1H, s), 7.64 (1 H, s), 8.14 (1 H, s); MS *m/e* (relative intensity, %) 314 (19, M^+), 57 (20), 55 (19), 43 (30), 41 (18), 28 (100).

Anal. Calcd. for $C_{16}H_{14}N_2O_5$: mol wt, 314.0903. Found: mol wt, 314.0909 (HRMS)

The ^1H NMR spectrum of **3** in trifluoroacetic acid was almost identical to that of nybomycin itself[5] except for the appearance of a broad, two-proton singlet at δ 5.42 in place of the allylic methyl resonance at δ 2.87. High resolution mass spectral analysis confirmed the molecular formula as $C_{16}H_{14}N_2O_5$, as noted above. Pure **3** had an Rf value identical to that of the slower moving, minor component of the crude antibiotic mixture in three separate chromatographic solvent systems. ($CHCl_3$ - EtOH, 90:10, Rf 0.27; C_6H_6 - *i*-PrOH, 85:15, Rf 0.10; EtOAc-HOAc, 70:30, Rf 0.07).

Antibacterial test data were obtained for compounds **1** through **5**. Agar-diffusion data show that hydroxynybomycin's antibacterial

Table 1. Antibacterial assays

Test organism	Agar diffusion, zone diameter, mm[a,b] (Broth dilution, MIC, μg/ml)[c]				
	1	**2**	**3**	**4**	**5**
Bacillus subtilis	13	18	17	0	27
Graphium fructicolum	tr	tr	0	0	13
Klebsiella pneumoniae	18 (<1.6)	16 <(1.6)	19 (25)	0 (>100)	25 (12.5)
Lactobacillus casei	9	7	8	10	15
Mycobacterium avium	14	16	9	tr	25
Sarcina lutea	10	9	10	0	21
Staphylococcus aureus	0 (100)	0 (3.1)	0 (>100)	0 (>100)	16 (3.1)
Streptococcus pyogenes	0	9h	9	9h	19

[a] tr=trace; h=hazy zone.

[b] Total of 20 μg of compound per 6.35 mm disc (0.02 ml of a 1 mg/ml solution or suspension). Compounds, **1**, **2**, **3** and **4** were dissolved in dimethylformamide, **5** in dimethyl sulfoxide.

[c] In 2-fold dilution test. Compounds **1**, **2**, **3** and **4** were tested as partial suspensions.

spectrum is similar to those of nybomycin and deoxynybomycin, although it is less potent (Table 1). In contrast to the improved *in vitro* activity of nybomycin *n*-butyrate over nybomycin, the di-*n*-butyrate of hydroxynybomycin is essentially inactive.

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