Communications to the Editor

OXETANOCIN, A NOVEL NUCLEOSIDE FROM BACTERIA

Sir:

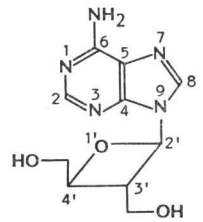
In the course of screening for new bioactive substances, a novel nucleoside named oxetanocin was isolated as crystals. The structure was determined to be 9-[(2*R*,3*R*,4*S*)-3,4-bis(hydroxymethyl)-2-oxetanyl]adenine (Fig. 1) by X-ray crystallographic analysis[1]. In this communication the production, isolation, and chemical and biological properties of oxetanocin are reported.

The oxetanocin-producing strain (our strain number: NK84-0218) was isolated from a soil sample collected in our premises and assigned to *Bacillus megaterium* NK84-0218 (unpublished). The strain was precultured in a 500-ml Erlenmeyer flask containing 100 ml of medium (soluble starch 2.0%, glucose 0.5%, soy bean meal (Prorich) 0.5%, peptone 0.5%, yeast extract 0.5%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCO_3$ 0.2%, pH 7.2 before sterilization) on a rotary shaking machine (200 rpm) at 27°C for 18 hours. The precultured broth (10 ml) was inoculated into a 5-liter Erlenmeyer flask containing 800 ml of the same medium described above and cultured under the same conditions. This cultured broth collected from 3 flasks (2.4 liters) was inoculated into a 200-liter stainless steel fermentor containing 120-liter of the following production medium; soluble starch 2.0%, soy bean meal (Prorich) 1.5%, KH_2PO_4 0.3%, Na_2HPO_4 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CoCl_2 \cdot 6H_2O$ 0.0002%, $FeSO_4 \cdot 7H_2O$ 0.0002%, antifoam (Pronal ST-1) 0.03%, pH 6.0 before sterilization. The fermentation was carried out at 37°C under aeration at 2/3 vol/vol/minute and agitation at 270 rpm for 43 hours.

The filtrate (290 liters) harvested from the 2 fermentors was charged on a Dowex 50WX8 (H^+, 19 liters) column and the adsorbed material was eluted by 0.5 N NH_4OH. The eluate having an antibacterial activity was passed through a carbon column (8 liters), and then eluted with aqueous acetone (stepwise increase of acetone content from 10% to 50%). After evaporation of the acetone from the active eluate under reduced pressure, it was passed through a Diaion HP-20 column (1.7 liters), and the adsorbed material was eluted with a stepwise elution of aqueous methanol (0~50%). The active eluate was dried under reduced pressure to give 14.3 g of crude material. The crude material of oxetanocin was further purified by chromatography on silica gel with a solvent composed of butanol and conc NH_4OH (10:0.2) followed by Diaion HP-20 column chromatography to give 3.50 g of pure oxetanocin. It was crystallized from water to give 2.95 g of colorless needles of oxetanocin, mp 197°C, $[\alpha]_D^{20}$ −44.3° (*c* 0.21, pyridine).

The molecular formula of oxetanocin was established as $C_{10}H_{13}O_3N_5$ (MW 251.24) by field desorption mass spectrometry and elemental analysis (M^+ *m/z* 251. Calcd: C 44.60, H 5.62,

Fig. 1. Structure of oxetanocin.



9-[(2*R*,3*R*,4*S*)-3,4-Bis(hydroxymethyl)-2-oxetanyl]adenine

Fig. 2. UV spectra of oxetanocin.

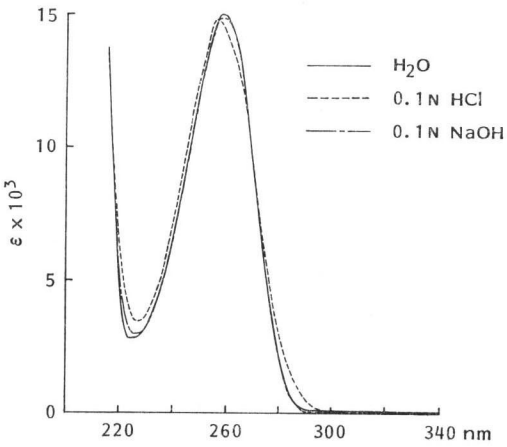
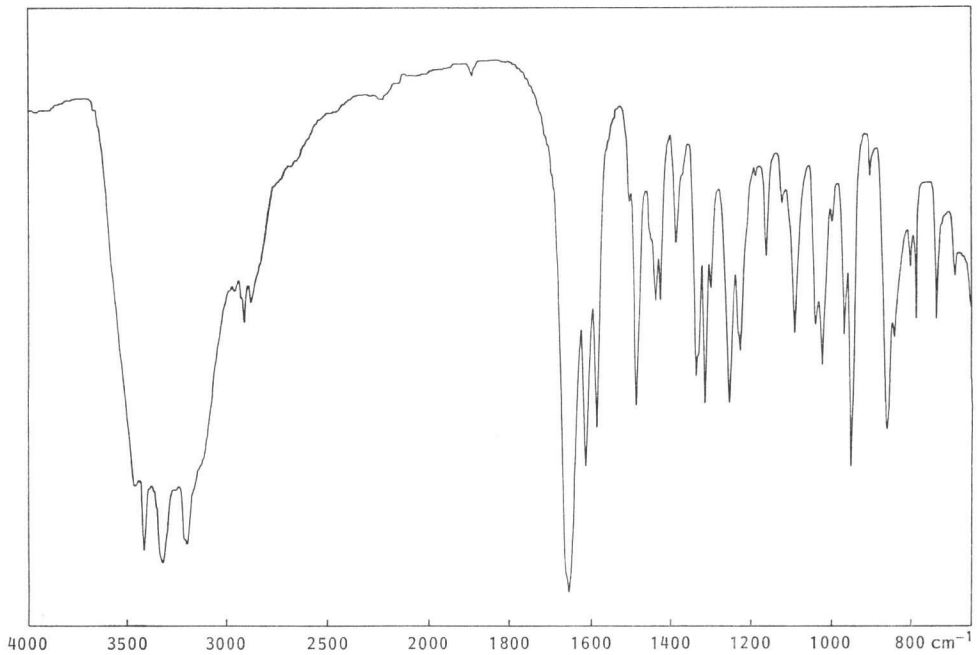


Fig. 3. IR spectrum of oxetanocin (KBr).



N 26.01. Found: C 44.75, H 5.58, N 25.98.). The UV spectra are shown in Fig. 2: $\lambda_{max}^{H_2O}$ (log ε) 259 nm (4.18), $\lambda_{max}^{0.1N\ HCl}$ 257 nm (4.17), $\lambda_{max}^{0.1N\ NaOH}$ 259 nm (4.18). These spectra suggested the presence of an adenine chromophore. The IR spectrum is shown in Fig. 3. The ^1H NMR spectrum measured in DMSO-d_6 at 400 MHz (internal TMS reference) suggested the presence of a branched-chain sugar. After the structure determination by X-ray crystallographic analysis, the definite assignment was made as follows: δ 8.65 (1H, s, 8-H), 8.18 (1H, s, 2-H), 7.37 (2H, s, 6-NH_2), 6.42 (1H, d, J=5.5 Hz, 2′-H), 5.40 (1H, s, 4′-CH_2OH), 5.04 (1H, s, 3′-CH_2OH), 4.55 (1H, m, 4′-H), 3.66~3.78 (5H, m, 3′-H, 3′-CH_2 and 4′-CH_2). The ^{13}C NMR chemical shifts measured at 100.4 MHz (internal TMS reference) were assigned in comparison with those of adenosine[2]: δ 156.0 (s, 6-C), 152.6 (d, 2-C), 149.0 (s, 4-C), 139.8 (d, 8-C), 118.7 (s, 5-C), 80.5 (d, 2′-C), 77.8 (d, 4′-C), 62.7 (t, 4′-CH_2), 58.4 (t, 3′-CH_2), 45.1 (d, 3′-C).

Oxetanocin showed activity against herpes simplex virus-II (DNA virus) at 5.8 μg/well (50% inhibition of cytopathic effect), while the cytotoxicity against Vero cells was 132.6 μg/well (50% inhibition of cell growth). However, oxetanocin did not show activity against vesicular stomatitis virus (RNA virus) at 100 μg/well. Oxetanocin inhibited the growth of HeLa cells *in vitro* (IC_{50} 47 μg/ml). It also showed strong antibacterial activity against the following bacteria on peptone agar: *Staphylococcus aureus* 209P (MIC<0.1 μg/ml), *Bacillus subtilis* PCI 219 (<0.1), *Bacillus polymyxa* IAM 1210 (<0.1), *B. cereus* IAM 1072 (<0.1), *Bacillus megaterium* ATCC 14945 (1.56). Other bacteria, fungi and yeast so far tested were not inhibited at 100 μg/ml. Adenine and adenosine were antagonistic against oxetanocin in terms of the antibacterial activity, while guanosine and inosine showed the weak antagonistic effect. Intravenous injection of 4 mg of oxetanocin to mice (*ca.* 200 mg/kg) did not show any sign of toxicity.

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References

1) NAKAMURA, H.; S. HASEGAWA, N. SHIMADA, A. FUJII, T. TAKITA & Y. IITAKA: The X-ray structure determination of oxetanocin. J. Antibiotics 39: 1626~1629, 1986
2) JOHNSON, L. F. & W. C. JANKOWSKI: Carbon-13 NMR Spectra. A Collection of Assigned, Coded, and Indexed Spectra. Spectrum No. 376, John Wiley & Sons, New York, 1972