DERIVATIVES OF OXETANOCIN: OXETANOCINS H, X AND G, AND 2-AMINOOXETANOCIN A

Sir:

Oxetanocin† is a novel nucleoside isolated from a culture filtrate of *Bacillus megaterium* NK84-0218[1]. It is the first natural product having an oxetanosyl-*N*-glycoside[2,3]. Recently, the potential usefulness of OXT-A† as an antiviral agent was disclosed by HOSHINO *et al.*[4]. Therefore, we have studied chemical and biological transformation of OXT-A to get its purine nucleoside analogues. In this communication, preparation of oxetanocins H, X and G, and 2-aminooxetanocin A, and their preliminary biological activities are described. The detailed biological activities will be published in separate papers.

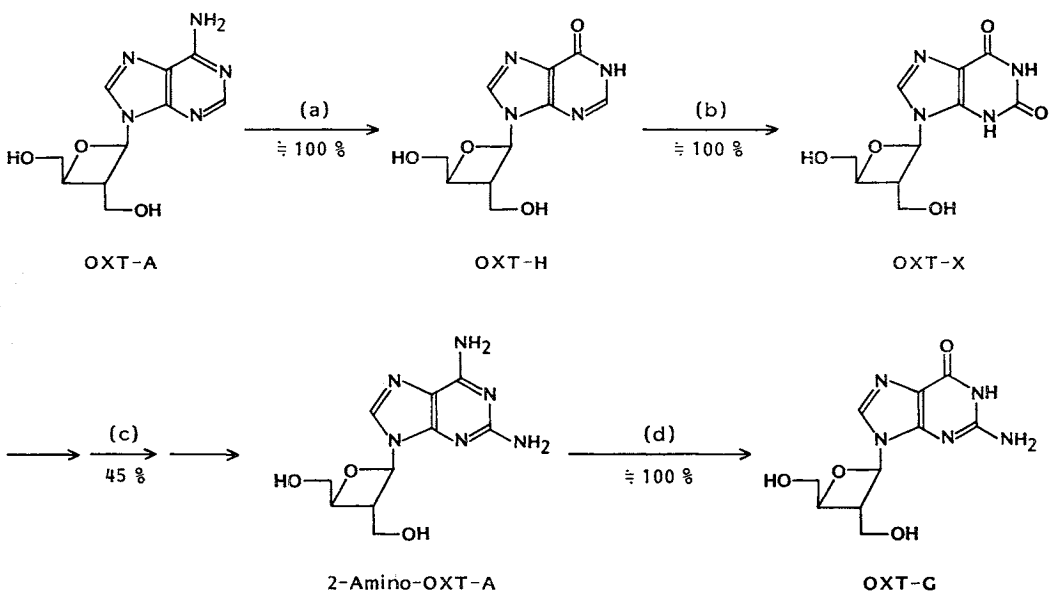
The scheme of chemical and biological transformation of OXT-A is shown in Fig. 1.

Oxetanocin H (OXT-H, the H comes from hypoxanthine) was readily derived from OXT-A by treatment with commercially available adenosine deaminase (Sigma, EC 3.5.4.4). MP 210°C; $[\alpha]_D^{25}$ −9.1° (*c* 1.0, H_2O); field desorption mass spectra (FD-MS) *m/z* 252 (M^+), calcd for $C_{10}H_{12}N_4O_4$: C 47.62, H 4.80, N 22.21, found: C 47.41, H 4.96, N 22.05; UV $\lambda_{max}^{H_2O}$ nm (log ε) 248.5 (4.10); $\lambda_{max}^{0.1N\ HCl}$ 249 (4.08); $\lambda_{max}^{0.1N\ NaOH}$ 254 (4.12).

For the microbial transformation, we tested 48 microorganisms including bacteria, actinomycetes, fungi and yeasts. The freshly harvested cells or mycelia were suspended in 1/20 M phosphate buffer (pH 7.0) containing OXT-A at 50 μg/ml and incubated for 18 hours at 37°C (for bacteria) or for 48 hours at 28°C (for other microorganisms). The transformation was analyzed by HPLC [column, Nucleosil 5C-18 (4.6×250 mm); mobile phase, 0.1 M citrate buffer (pH 4.0) - CH_3CN - MeOH (50:2:1); flow rate, 0.7 ml/minute; temp, 21°C; detection, UV at 259 nm]. The retention times for OXT-A and OXT-H were 17′30″ and 9′20″, respectively. Among the 48 microorganisms, 9 microorganisms including *Escherichia coli* 120551 converted OXT-A to OXT-H almost quantitatively.

In these studies we found that some microorganisms belonging to actinomycetes including *Nocardia interforma* coverted OXT-A to another nucleoside (retention time, 11′5″) *via* OXT-H.

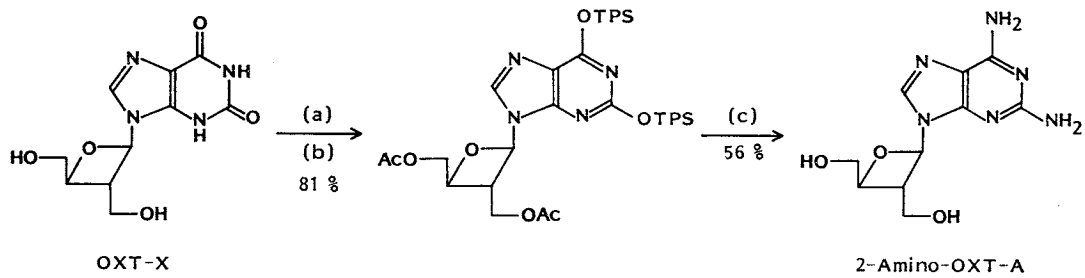
Fig. 1. Chemical and biological transformation of oxetanocin A.



(a) Adenosine deaminase or *Escherichia coli* 120551. (b) *Nocardia interforma* (from OXT-A against OXT-H). (c) Three step chemical reaction (see Fig. 2). (d) Adenosine deaminase.

† Hereafter, oxetanocin[1] is named as oxetanocin A (abbreviation: OXT-A). The A comes from adenine chromophore.

Fig. 2. Chemical transformation from OXT-X to 2-amino-OXT-A.



TPS: 2,4,6-Triisopropylbenzensulfonyl.

(a) Ac_2O - Et_3N (4-dimethylaminopyridine), acetonitrile, room temp, 4 hours. (b) 2,4,6-Triisopropylbenzensulfonyl chloride - Et_3N (4-dimethylaminopyridine), CH_2Cl_2, room temp, 4 hours. (c) Liq. NH_3, EtOH, 110°C, 3 days.

This new nucleoside was found to be oxetanocin X (OXT-X, the X comes from xanthine). MP 205°C (dec); $[\alpha]_D^{25}$ −22.0° (*c* 0.64, H_2O); FD-MS *m/z* 269 $(M+H)^+$, calcd for $C_{10}H_{12}N_4O_5 \cdot H_2O$: C 41.96, H 4.93, N 19.57, found: C 41.84, H 4.95, N 19.43; UV $\lambda_{max}^{H_2O}$ nm (log ε) 250.5 (4.01), 276.5 (3.95). The conversion from OXT-A to OXT-X by *N. interforma* was almost quantitative.

We were successful in the chemical transformation of OXT-A to 2-amino-OXT-A, which is readily transformed to OXT-G (G from guanine), *via* the 2,6-dichloropurine derivative[5], but the overall yield was only 7.2%. Therefore, we tried microbial transformation from OXT-X to OXT-G, but this attempt was not successful. The effective transformation was achieved by three step chemical reaction followed by enzymic deamination. The scheme of the chemical transformation is shown in Fig. 2. Treatment of OXT-X with acetic anhydride and triethylamine in the presence of a catalytic amount of 4-dimethylaminopyridine gave the di-*O*-acetate in 90% yield. It was treated with 2,4,6-triisopropylbenzensulfonyl chloride and triethylamine in the presence of 4-dimethylaminopyridine to yield the di-*O*-sulfonyl derivative in 90% yield. The ammonolysis of the product at 110°C for 3 days in a pressure vessel gave 2-amino-OXT-A in 56% yield. MP 119°C (dec); $[\alpha]_D^{25}$ −16.4° (*c* 0.5, H_2O); FD-MS *m/z* 266 (M^+), calcd for $C_{10}H_{14}N_6O_3 \cdot H_2O$: C 42.25, H 5.67, N 29.57, found: C 42.46, H 5.85, N 29.44; UV $\lambda_{max}^{H_2O}$ nm (log ε) 256 (3.96), 278 (3.95).

Treatment of 2-amino-OXT-A with adenosine deaminase yielded OXT-G in almost quantitative yield. MP 112°C (dec); $[\alpha]_D^{25}$ −19.8° (*c* 0.75, 0.1 N NaOH); FD-MS *m/z* 268 $(M+H)^+$, calcd for $C_{10}H_{13}N_5O_4 \cdot H_2O$: C 42.10, H 5.30, N 24.55, found: C 42.04, H 5.18, N 24.48; UV $\lambda_{max}^{PH\,6.0,\,H_2O}$ nm (log ε) 253.5 (4.09).

Table 1. Antiviral activities of oxetanocins against HSV-II.

	50% inhibition of CPE[a] (μg/well)	50% growth inhibition of Vero cells (μg/well)
OXT-A	5.56	132.6
OXT-H	87.5	>400
OXT-X	107.5	240
OXT-G	9.71	330
2-Amino-OXT-A	17.68	165.8
Ara A[b] (control)	4.36	22.3

[a] Cytopathic effect.

[b] Ara A: 9-β-D-Arabinofuranosyladenine.

All of the new derivatives presented in this paper did not show antibacterial activities except for 2-amino-OXT-A: *Staphylococcus aureus* 209 P (MIC: 3.13 μg/ml in 0.5% peptone agar). *Bacillus cereus* IAM 1072 (3.13 μg/ml).

Antiviral activities against herpes simplex virus type-II (HSV-II) are shown in Table 1. Antiviral activities against human immunodeficiency virus *etc.* will be presented in separate papers.

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