NOTES

ISOLATION OF 7β-(5-HYDROXY-5-CARBOXYVARELAMIDO)-3-HYDROXYMETHYL-3-CEPHEM-4-CARBOXYLIC ACID FROM STREPTOMYCES SP.

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During the course of our screening work for new β-lactam antibiotics, we found that a Streptomyces strain numbered PA-41937 produces a substance active against Escherichia coli LS-1 (a mutant super sensitive to β-lactam antibiotics), sensitive to a cephalosporinase (from Enterobacter cloacae) and showing stronger acidic nature when compared with the known naturally occurring cepham compounds by paper electrophoresis. Since the acidic substance (tentatively called α-substance) was supposed to be a new cepham compound, it was isolated and the structure was elucidated to be 7β-(5-hydroxy-5-carboxyvarelamido)-3-hydroxymethyl-3-cephem-4-carboxylic acid (I). The strain probably belongs to Streptomyces griseus from preliminary taxonomical data and simultaneously produces four known cephem compounds, deacetylcephalosporin C¹, deacetyl-7-methoxycephalosporin C², deacetoxycephalosporin C³ and deacetoxy-7-methoxycephalosporin C⁴. The isolation and structure elucidation of I are presented in this report.

The strain PA-41937 was fermented by submerged culture using a 30-liter jar fermentor containing 20 liters of medium consisting of tomato paste 2.0%, dextrin 2.0%, dried yeast 1.0% and CoCl₂·6H₂O 0.0005% (pH 7.0 before sterilization) at 28°C for 72 hours under agitation of 300 r.p.m., aeration of 20 liters per minute and inner pressure of 0.5 kg/cm². The harvested broth was centrifuged by a Sharples centrifugal separator. The supernatant fluid (150 liters) obtained from 8 jar fermentors was adjusted to pH 7.0 and passed through a column (11 liters) of an Amberlite IRA-68 (Cl⁻) (Rohm and Haas Co., Ltd.). The column was washed with water and eluted with 5% NaCl. The active eluate fraction when assayed by pulp disk agar diffusion method on an E. coli LS-1 assay plate were then passed through a column (4 liters) of a Diaion HP-20 (Mitsubishi Kasei Kogyo Co., Ltd.) at pH 3.5. The column was eluted with water and then with 50% methanol. From the active eluate with water, a crude mixture of deacetylcephalosporin C and deacetyl-7-methoxycephalosporin C was isolated, and these compounds were identified by direct comparison with the authentic specimens by HPLC. The active eluate with 50% methanol was adjusted to pH 7.0 by NaOH, concentrated and freeze-dried to give a crude powder (42 g). The crude powder was subjected to chromatography on a column (5 × 40 cm) of QA-Sephadex A-25 (Pharmacia Fine Chemicals) with 0.2 M NaCl in 50 mM phosphate buffer, pH 7.0 (some 10 g portion could be charged for a run). Two active fractions appeared. From the former fraction, a crude mixture of deacetylcephalosporin C and deacetoxy-7-methoxycephalosporin C which gave respective identical peaks on HPLC with those of the authentic specimens was isolated. The latter fraction was adsorbed on an HP-20 column at pH 3.5 and eluted with 50% methanol. Adjustment of pH to 7.0, evaporation and freeze-drying gave a residue (1.2 g). The residue was purified by chromatography on a cellulose column (Microcrystalline Cellulose, Avicel) with 70% n-propanol. Evaporation and freeze-drying of the active eluate gave a powder (250 mg). Final purification was achieved by preparative HPLC on a column (10 × 250 mm) of Nucleosil 10 C¹₈ (Macherey-Nagel) with 10 mM phosphate buffer, pH 6.5. Peak fractions of the α-substance were collected. Adsorption and elution on an HP-20 AG column as above, adjustment of pH to 7.0 by NaOH, followed by freeze-drying gave the sodium salt of the α-substance (70 mg).

The sodium salt of the α-substance (I) is a colorless powder, easily soluble in water. It shows negative reaction to ninhydrin reagent. When
tested by paper electrophoresis with 50 mM phosphate buffer, pH 7.0, at 10 volt/cm for 2 hours, it moved to the anode with Rm (relative mobility to cephamycin C) 2.0. It shows a maximum at 260.5 nm in the UV spectrum (Fig. 1). In the IR spectrum (Fig. 2), absorptions at 1745 cm\(^{-1}\) (\(\beta\)-lactam), 1645 cm\(^{-1}\) (amide) and 1590 cm\(^{-1}\) (carboxylate) are shown. The substance was hydrolyzed with constant boiling hydrochloric acid or 5 N NaOH at 110°C for 20 hours, and the hydrolysates were analyzed by an automatic amino acid analyzer. A small amount of glycine was detected, but \(\alpha\)-aminoacidipic acid was not detected in any amount in the both hydrolysates.

The \(^{1}H\) and \(^{13}C\) NMR spectra were recorded with a Varian XL-100-12 A spectrometer in D\(_2\)O using tetramethylsilane as an external reference. When the \(^{1}H\) and \(^{13}C\) NMR data of I sodium salt were compared with those of deacetylcephalosporin C (II) sodium salt as a reference compound, close similarities were observed (Tables 1 and 2). Significant difference between NMR behaviors of I and II were shown as follows. (1) Compared with \(^{1}H\) signal of 16-CH of II, a corresponding signal of I shifted to lower field (\(\Delta\delta=-0.34\)). (2) Compared with \(^{13}C\) signals of 16-CH, 15-CH\(_2\) and 17-COO\(^{-}\) of II, re-
markable downfield shifts were found for the corresponding signals of I; $\delta \beta$ values were $+17.4$, $+3.5$ and $+6.6$, respectively. These signal shifts implied the replacement of an amino group on C-16 with a hydroxyl group. The CD spectrum of I sodium salt was substantially identical with that of II sodium salt; CD: $[\theta]_{355}^0, [\theta]_{315}^0 +230, [\theta]_{205}^0 +190, [\theta]_{257.5}^0 +26100, [\theta]_{239}^0 0, [\theta]_{225}^- -33800, [\theta]_{196}^- 0$ (c 0.0472, 10 mM phosphate buffer, pH 7.0).

I sodium salt was dissolved in methanol and methylated with diazomethane, and the product was subjected to measurement of FD/MS with a Hitachi RMU-8 GN spectrometer. A peak at m/z 403 (MH$^+$ of the dimethyl ester of I) was observed.

From the above data, we concluded the structure of I as shown in Scheme 1.

**References**