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SYNTHESIS AND EVALUATION OF PENICILLIN-V PRODRUG

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An ester prodrug of penicillin-V was prepared and its hydrolysis kinetics in phosphate buffer and in human serum was studied. The hydrolysis of the prodrug to yield penicillin-V was pH dependent. In 1 N hydrochloric acid at 37°C, the hydrolysis of the prodrug followed pseudo-first order kinetics and the half life of hydrolysis was 46 min. No hydrolysis was observed in human serum. The stability of the prodrug was investigated in phosphate buffer (pH 7.4) at different temperatures (37, 55 and 70°C) and the $t_{1/2}$ at 25°C was found to be 13.29 days. In vivo studies in rabbits showed that the AUC_{0-6} values for the penicillin-V and prodrug (III) were 53.63 and 69.97 µg/ml/h respectively.

Keywords : *Penicillin-V; Prodrug; Synthesis*

Introduction

Penicillin-V potassium salt [(3,3-Dimethyl) – 7 – oxo-6-[(phenoxyacetyl) amino]-2S-(2α, 5α, 6β)]-4-thia-1-aza-bicyclo[3,2,0]heptane-2-carboxylic acid mono potassium salt] is an orally used antibiotic for the treatment of gram positive infections(1,2). A mild taste and characteristic odor characterize it. Its bioavailability is about 60 percent(3). The penicillin administered by mouth was reported to cause oesophagitis or oesophageal ulceration(3).

The prodrug approach has received considerable attention for the improvement of the pharmaceutical and pharmacological properties of an existing drug via bio-reversible drivatisation(4-6). The pharmacokinetic behaviour of relatively hydrophobic drugs that are inefficiently absorbed from the intestinal tract could also be improved by the design of a prodrug with hydrophobic properties(6-8,13,14). The most common approach is to increase the hydrophobic nature of drugs containing carboxylic acid groups, including penicillins by converting them to bioreversible esters(6-14).

The present paper describes the preparation and evaluation of a prodrug for penicillin-V.

Materials and Methods

Melting points were determined on Gallenkamp melting point apparatus and were uncorrected. TLC was performed on silica gel plates (Sigma Chem. Co.) The IR spectra were determined on a Shimadzu Spectrophotometer IR-435. The ^{1}H-NMR spectra were recorded on 80 MHz Bruker-WP pulse spectrometer, and the chemical shifts were reported in units down field from the internal standard tetra-methylsilane. The MS were measured on 7070E VG analytical mass spectrometer. HPLC was carried out using a chromatographic system consisting of Merck Hitachi Lachrome pump-7100, Merck Hitachi Lachrome UV detector L-7400, a Rheodyne injector holding 50 µl loop and a Lichrosphere 100 RP-18 (250x4mm, 5µm) column. The signals from the detector were stored in a computer and processed using PE-Nelson 1022 software.

The phenoxymethyl penicillin (Penicillin-V) potassium salt was kindly provided by Al-Hikma Pharmaceutical Company(Jordan). All the solvents used in the HPLC analysis were of HPLC grade and the chemicals were of analytical grade. All other reagents were of

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reagent grade. Distilled and deionized water was used for HPLC.

Synthesis

Preparation of 2-hydroxyethyl ester of Penicillin-V (II)

To a stirred solution of penicillin-V potassium salt (**I**) (3.88 g, 10 mM) in 20 ml dimethylsulfoxide, bromoethanol (1.25 g, 10 mM) was added and the reaction mixture was stirred at room temperature for 2 days. Water was added to the reaction mixture and a viscous precipitate was formed. The precipitate was dissolved in 25 ml chloroform, washed twice with water (50 ml), and the chloroform layer was dried over anhydrous magnesium sulfate. The solvent was evaporated under vacuum and the crude residue was recrystallized from ethanol: water mixture to give a white, odorless and tasteless crystalline solid in 45% yield; mp 45°C; TLC with n-hexane: ethyl acetate: methanol (5:9.8:0.2 v/v/v), Rf=0.35; IR (cm^{-1}) 3500(OH), 3360(NH), 1780(COO), 1740(CONH), 1680 (CON<); ^{1}HNMR ($CDCl_3$) δ1.56 (s, 3H, CH_3), 1.65(s, 3H, CH_3) 1.89(s, br, 1H, OH), 3.91 (t, 2H,-CH_2-O), 4.35(t, 2H, CH_2OCO), 4.55(s, 1H, CHCOO), 4.69(s, 2H, OCH_2CON), 5.64(q, 1H, NCHCO), 5.82(d, 1H, NCHS), 6.87-7.47(m, 5H, Ar-H), 7.47 (s, br, 1H, NH); MS (m/e) 203(M^+-191), 191, 149, 81, 77, 69, 57, 55, 43, 41, 39, 32, 28.

Preparation of Penicillin-V diester of ethylene glycol (III)

To a stirred solution of (**I**) in 20 ml dimethylsulfoxide, bromoethyltosylate (1.325g, 5mM) was added and the reaction mixture was stirred at room temperature for 20 hours. Water (50 ml) was added to the reaction mixture and a viscous precipitate was formed. It was dissolved in 25 ml chloroform and the chloroform layer was washed twice with water (50 ml). The chloroform layer was dried over anhydrous magnesium sulfate, the solvent was evaporated under vacuum and the crude residue was recrystallized from chloroform: tetrahydrofuran (THF) mixture to give a pure white and odorless crystalline solid in 53% yield; mp 105°C; TLC with n-hexane: ethyl acetate: methanol (5:9.8:0.2 v/v/v) Rf=0.62; IR (cm^{-1}) 3360(NH), 1775(COO), 1740(CONH), 1685(CON<); ^{1}HNMR ($CDCl_3$) δ1.53(s, 3H, CH_3), 1.63(s, 3H, CH_3), 4.43(s, 2H, CH_2OCO), 4H, COO-CH_2-CH_2-OCO) 4.5(s, 1H, CHCOO), 4.65(s, 2H, OCH_2CON), 5.63(q, 1H, NCHCO), 5.86(d, 1H, NCHS), 6.86-7.50(m, 5H, Ar-H), 7.83(d, 1H, NH); MS (m/e) 726 (M+), 535, 344, 327, 286, 250, 186, 168,114, 112,77.

Chromatographic conditions

The chromatographic analysis were performed by gradient elution. The mobile phase composition for the first 6 min was sodium dihydrogen phosphate buffer (0.05M, pH 3.25): THF: acetonitrile (13:6:1 v/v/v) which was suitable for the separation of penicillin-V(**I**) and the hydroxyethyl ester of penicilline-V (**II**). The composition was changed to the ratio 11:6:3 from 6 to 18 minutes. The flow rate of the mobile phase was 1.0 ml/min and the detector wavelength was set at 268 nm.

Calibration curve in aqueous phosphate buffers

Stock solution of penicillin-V potassium salt(**I**), penicillin-V esters (**II**, **III**) were prepared in water, THF and methanol respectively. For the calibration curve of compound (**I**), various dilution were made with water to obtain 10, 20, 40, 60, 80 and 100 µg/ml. The solutions were chromatographed and the respective areas were calculated. Similar calibration curve data for compounds **II** and **III** were obtained.

Calibration curve in human serum

50 µL of standard solutions of either compound **I** or compound **III** were added to spike human serum (final volume 1.0 ml) to provide calibration standards of 0.5, 0.75, 1.0, 2.0, 4.0, 8.0, 12.0, 16.0 and 24.0 µg/ml for *in vivo* rabbit studies. For the *in vitro* studies, the calibration curves were prepared using 2.0, 4.0, 8.0, 12.0, 16.0 and 24.0 µg/ml of compound **I** or **II** 200µl of these samples were precipitated using 600 µl of acetronitrile, vortexed and centrifuged at 4000 rpm for 5 mins and 50 µl of the supernatent was injected to the HPLC. Calibration data for compounds **I** and **III** were obtained. Separate aqueous samples (2 µg/ml each) of the compounds **I**, **II** and **III** were prepared and analyzed to check the separation of the drug peaks.

Kinetic Measurement

The hydrolysis of compound **III** was studied at 37±0.5°C in 1.0 N HCl, phosphate buffers (pH 4.6 and 7.4) and human serum. Compound **III** was dissolved in tetrahydrofuran to give a concentration of 2.0 mg/ml. 100 µl of compound **III** solution was added to 10 ml of the buffer or human serum and the sample was placed in a water-bath. The samples (200 µl) were withdrawn at appropriate time intervals. The buffer samples were analysed after suitable dilutions. The serum samples were precipitated using 600 µl of acetonitrile, centrifuged and the supernatants were analysed after suitable dilution. The first order rate constants for the hydrolysis of compound **III** were determined from the slope of the linear plot of log percent remaining versus time.

Stability Studies

The hydrolysis of compound **III** was studied at different temperatures (37, 55 and 70°C) in phosphate buffer (pH 7.4). **III** was dissolved in THF to give a concentration of 2.0 mg/ml. Fifty µl of **III** solution was added to a 10 ml of the buffer. Samples (100 µl) were withdrawn at appropriate time intervals. The buffer samples were analysed after suitable dilutions. The first order rate constant for the hydrolysis of compound **III** was determined from the slope of the linear plot of log percent remaining versus time.

In-vivo studies

Compound **III** (72.5 mg) was suspended in 5 ml of 10% gum acacia. Penicillin-V potassium salt (78.8 mg) was dissolved in 5 ml of water. Either compounds (1 ml solution followed by 1 ml tap water) were given orally to Australian rabbits and the blood samples (400µl) were collected from the ear vein at appropriate intervals. The samples were centrifuged, 100µl of the serum was precipitated with 300µl of acetonitrile, centrifuged and the supernatants were analyzed. The concentrations of penicillin-V were plotted against time.

Results and Discussion

The prodrug of penicillin-V (**III**) was prepared by stirring a mixture of penicillin-V potassium salt and 2-bromotosylate in dimethylsulfoxide (DMSO) at room temperature. The hydroxyethyl ester of penicillin-V (**II**) was prepared by reacting the potassium salt of penicillin-V with 2-bromoethanol at room temperature in DMSO. The products were separated, recrystallized and characterized by various spectroscopic techniques. Compound **III** stored for more than a year at ambient temperature did not show any degradation products, as evident from the chromatographic analysis.

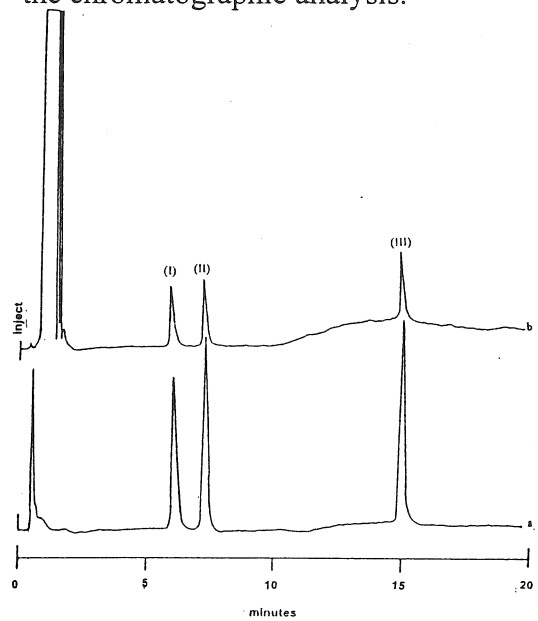
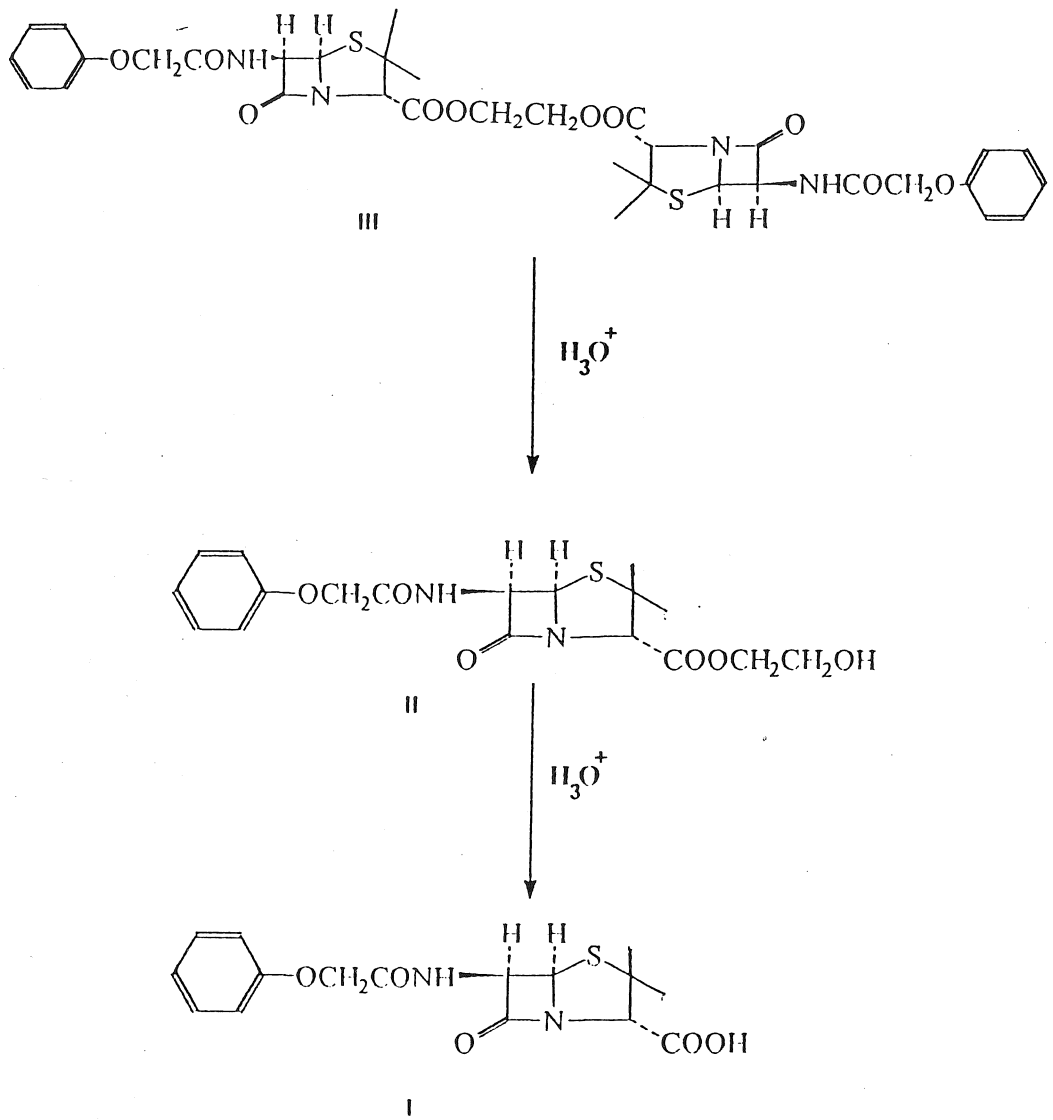


Fig.1. High performance liquid chromatogram showing the hydrolysis of prodrug (**III**) to hydroxyethyl ester of penicillin-V (**II**) and penicillin-V (**I**) in acidic condition (a, 1N HCl) and in human plasma (b)

The hydrolysis profile of penicillin-V prodrug (**III**) and the hydroxyethyl ester of penicillin-V (**II**) were followed by specific and sensitive HPLC procedure. The retention time of penicillin-V (**I**), hyydroxyethyl ester of penicillin-V(**II**) and penicillin-V prodrug (**III**) were 6.0, 7.4 and 15.0 min respectively. No additional peaks were observed in the chromatogram throughout the study period at 37°C. As evident by the HPLC (Fig.1), the hydrolsis of prodrug (**III**) in

acidic condition was accompanied by the appearance of a peak with the same retention time as an authentic sample of the hydroxyethyl ester of penicillin-V (**II**), beside the peak corresponding to penicillin-V (**I**) which appeared during the degradation (Scheme 1). This is shown from the increase in the peak area of the intermediate (**II**) followed by the progressive disappearance of this peak. The hydroxyethyl ester of penicilline-V (**II**) was less stable than (**III**) under these conditions which also undergoes degradation to penicillin-V (**I**).

Fig. 2 shows the plot of the log percent remaining of penicillin prodrug (**III**) versus time in 1N HCl and in aqueous phosphate buffer of pH 4.6 and pH 7.4. The correlation coefficient (r) for these plots were always higher than that of the linear plot of percent remaining versus time and, therefore the degradation was assumed to follow a pseudo first order process. Table 1



Scheme 1

shows the first order rate constants and the corresponding half lives of the hydrolysis of prodrug (**III**) and hydroxyethylester of penicillin-V (**II**) in different medium. It is clear from table 1 (kinetic data-k, $t_{1/2}$) that the $t_{1/2}$ value of penicillin prodrug (**III**) is higher than the hydroxyethyl ester of penicillin-V (**II**).

The stability of the prodrug (**III**) was also studied at different temperatures in phosphate buffer (pH 7.4) (Fig.3). At 70°C it was hydrolysed to different compounds, as expected(2), other than penicillin and glycol (**II**). A representative chromatogram is shown in Fig. 4. The $t_{1/2}$ and $t_{10\%}$ for the penicillin prodrug at 25°C were 13.29 and 2.10 days.

In vitro hydrolysis studies of the penicillin-V prodrug **III** in serum indicated that there was no hydrolysis of this compound to hydroxyethyl ester of penicillin-V or penicillin-V at 37°C. But a decrease in the concentration of penicillin-prodrug (**III**) with respect to time was observed, which indicated that

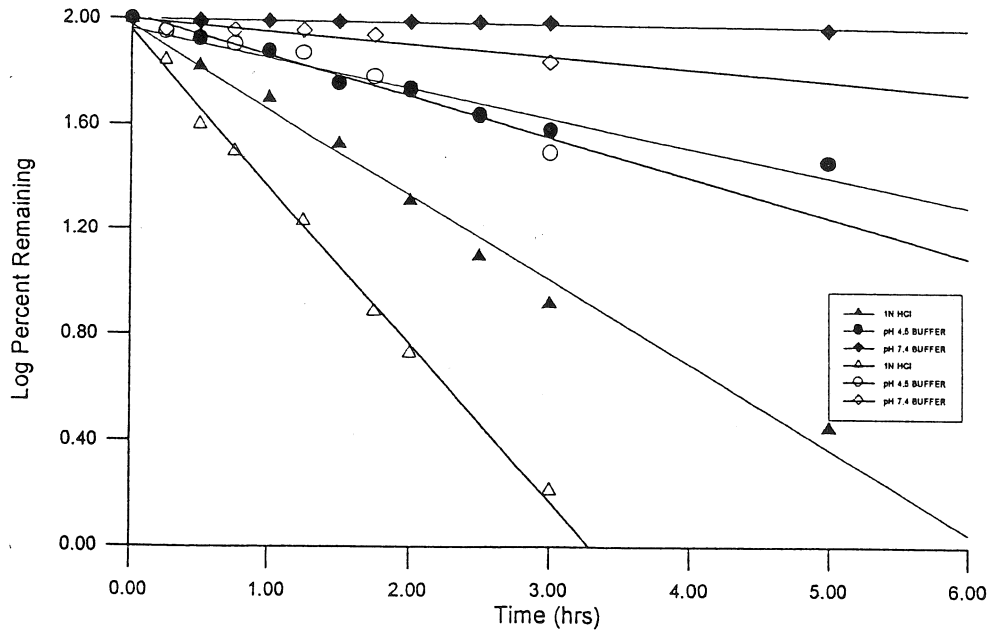


Fig.2. pH-hydrolysis profile of penicillin-prodrug (**III**) (——) and penicillin glycol (II)(- - -)

Table 1. Kinetic data for synthesized compound **II** and **III**

Compounds	Condition	K (hr^{-1})	$T_{1/2}$ (hr)
Penicillin prodrug (**III**)	1.0 N HCl	0.9078	0.763
	pH 4.6 Buffer	0.2374	2.918
	pH 7.4 Buffer	0.0188	26.813
Penicillin glycol (**II**)	1.0 N HCl	1.3956	0.496
	pH 4.6 Buffer	0.3535	1.960
	pH 7.4 Buffer	0.1075	6.446

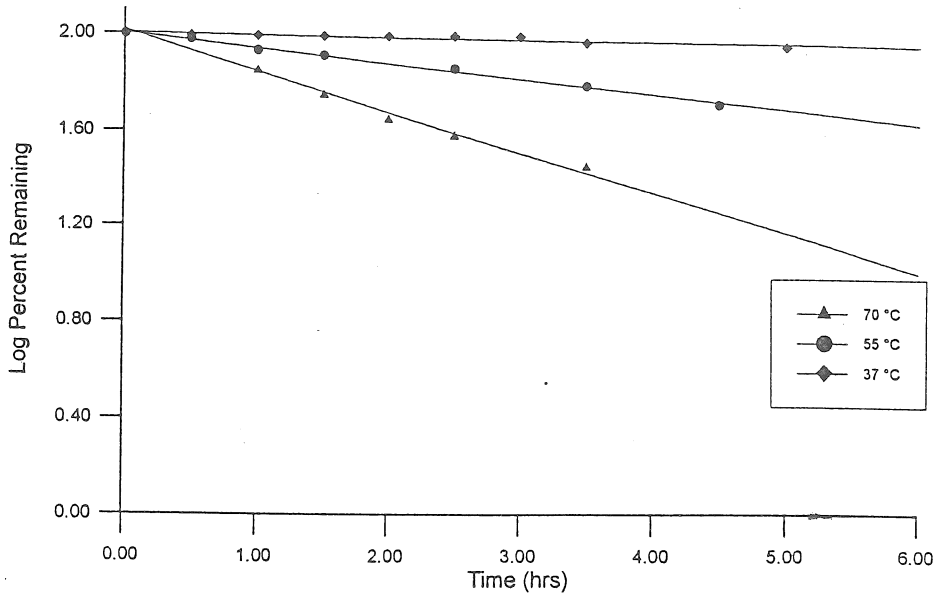


Fig.3. Stability of penicillin prodrug (**III**) at different temperatures in phosphate buffer (pH 7.4)

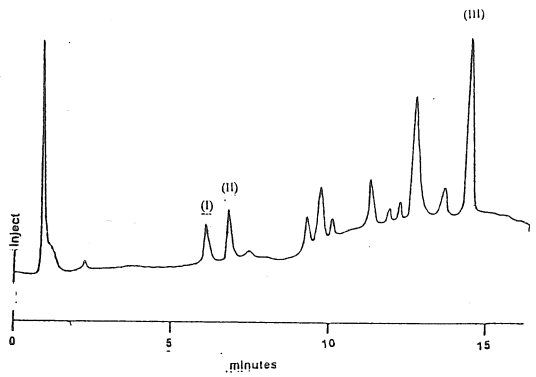


Fig.4. High performance liquid chromatogram showing the hydrolysis of prodrug (**III**) to hydroxyethyl ester of penicillin-V (**II**), penicillin-V (**I**) and unidentified components at 70°C in phosphate buffer (pH 7.4)

penicillin-V prodrug (**III**) might be bound to the serum proteins.

From the *in vivo* studies in rabbits (Fig.5) the AUC_{0-6} for penicillin-V from prodrug (**III**) and penicillin-V potassium salt were 69.97±3.75 and 53.63±2.95 μg/ml/hr respectively (n=4). This indicated that the amount of penicillin-V in the blood from the administration of prodrug **III** was higher than that of penicillin-V potassium salt. The t_{max} and C_{max} for the penicillin prodrug and penicillin-V potassium salt showed no significant difference. These values were 1.50±0.21 hr, 23.94±2.51 μg/ml and 1.50±0.27 hr, 19.75±1.97 μg/ml respectively. As it is evident from the *in vitro* and *in vivo* studies, most of the penicillin prodrug (**III**) was hydrolysed to penicillin-V (**I**) in acidic conditions *via* the hydroxyethyl ester of penicillin V. Prodrug **III** was absorbed in the unhydrolysed form as well (Fig. 1b).

In conclusion, the prodrug of penicillin-V was prepared, which can enable improvement of its bioavailability.

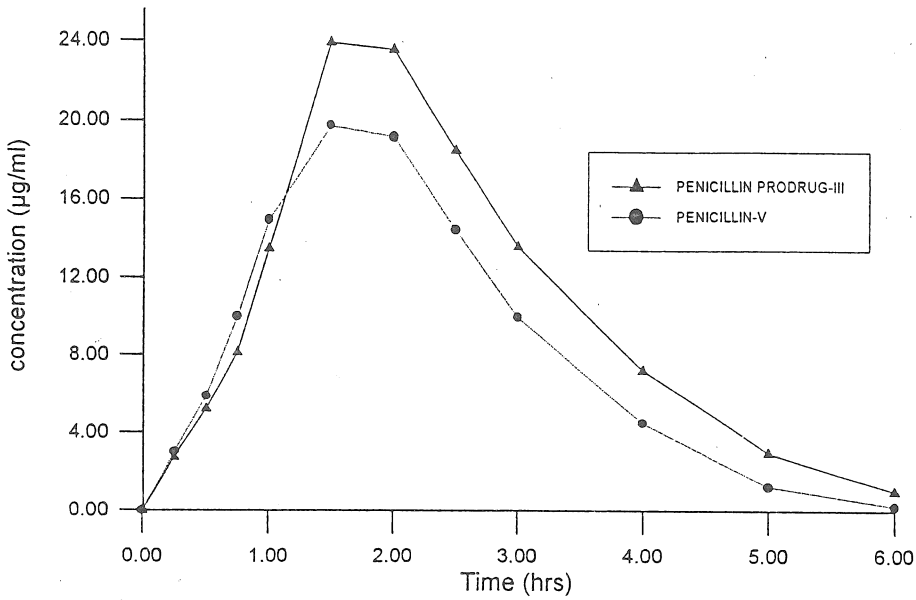


Fig.5. Serum concentration versus time plot for penicillin prodrug (**III**) and penicillin-V

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