DEACETYLATION OF BENZYL 6α-ACETOXYPENICILLANATE*

SAMIR M. BADR EL-DIN

National Research Centre, Dokki, Cairo, Egypt

and KENNETH R. HENERY-LOGAN

Department of Chemistry, University of Maryland, U. S. A.

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Benzyl 6α-acetoxypenicillanate was transformed into benzyl 6α-hydroxypenicillanate by enzymatic cleavage of the acetyl group. The preparation of several side chain oxygen analogues of the penicillins is described.

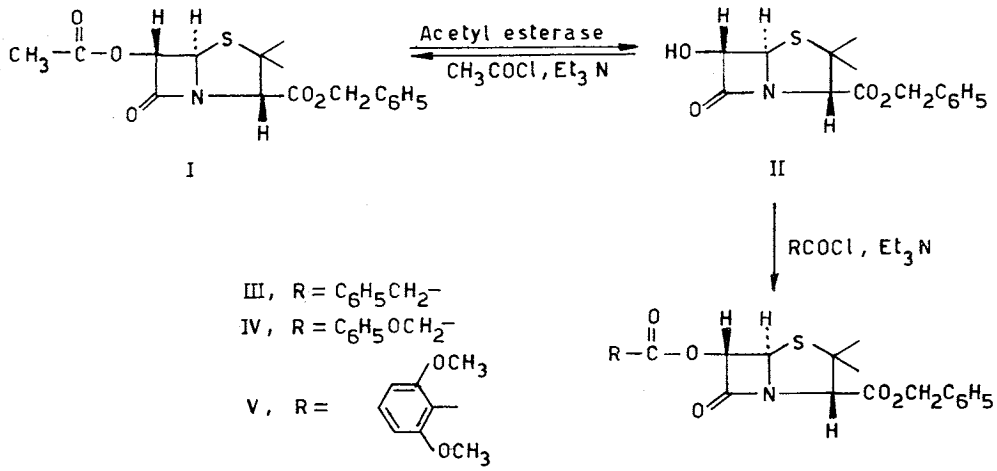
The synthesis of 6α-acetoxypenicillanic acid, a penicillin analogue containing an ester side chain at C-6, was first reported by BADR EL-DIN[1] and later by HAUSER and SIGG[2]. We wish to report the enzyme catalyzed deacetylation of benzyl 6α-acetoxypenicillanate (**I**) (an intermediate in the synthesis of 6α-acetoxypenicillanic acid[1]) to give an excellent yield of benzyl 6α-hydroxypenicillanate (**II**). The hydroxy compound **II** was acetylated to reform **I** and, in addition, was acylated to give several other penicillin analogues having an ester side chain at C-6.

The enzymatic deacetylation of **I** was investigated since acidic or basic hydrolysis would undoubtedly have resulted in concomitant opening of the β-lactam ring and probably other reactions. Deacetylation under acidic[3] and basic[4] conditions has been reported for the related, but more stable, cephalosporins, but the accompanying hydrolysis of the β-lactam ring disqualified these methods as ones of practical value. However, cephalosporins were transformed into the corresponding deacetoxycephalosporins in good yield with citrus acetyl esterase enzyme[4,5].

The diester **I** was hydrolyzed to the hydroxy ester **II** in high yield (96%) by the use of citrus acetyl esterase at pH 6.6. The benzyl ester remained intact since this enzyme is a specific esterase whose activity is greatest on esters of acetic acid[6]. The elemental analyses of the product isolated from the enzymatic reaction were consistent with the structure of **II**. However, the infrared spectrum (in Nujol or KBr) showed strong bands at 2.98 (hydroxy) and 5.7 μ (ester carbonyl) but lacked a band in the usual region of β-lactam carbonyls (5.62 μ). Compound **II** was conclusively identified by its reaction with acetyl chloride and triethylamine, which reformed compound **I** with the β-lactam band at 5.61 and the ester bands at 5.75 μ.

The hydroxy compound **II** was acylated to yield several side chain oxygen analogs of the penicillins. Reaction of **II** with phenylacetyl chloride, phenoxyacetyl chloride and 2, 6-dimethoxybenzoyl chloride afforded benzyl 6α-(phenylacetoxy) penicillanate (**III**), benzyl 6α-(phenoxyacetoxy)-penicillanate (**IV**) and benzyl 6α-(2, 6-dimethoxybentzoyloxy)-penicillanate (**V**), respectively. Compounds **III**, **IV**, and **V** each showed separate ester and β-lactam carbonyl bands in the infrared spectrum. Benzyl esters **III**, **IV**, and **V** are the side chain oxygen analogs of the benzyl esters of *epi*-penicillin G, *epi*-penicillin V and *epi*-methicillin, respectively.

* This work was carried out in the Department of Chemistry, University of Maryland, College Park, Maryland.



The shift of the β-lactam carbonyl band in the infrared spectrum of benzyl 6α-hydroxypenicillanate may be due to hydrogen bonds in the crystal lattice. There are previous examples of displacement of the β-lactam carbonyl to higher wavelengths in penicillin and cephalosporin derivatives. In one case, the crystalline sodium salt of deacetylcephalosporin C showed a strong band at 5.77 μ (in Nujol) but, unlike the spectrum of cephalosporin C, no band at 5.62 μ[4]. In another example, however, the infrared spectrum of an oil, benzyl 6-azopenicillanate, had only one band at 5.71 μ for both the β-lactam carbonyl and the benzyl ester carbonyl[7].

Experimental

All melting points were taken in a Mel-Temp melting point block and are uncorrected, all infrared spectra were obtained on a Beckman Model IR-5 spectrophotometer. Brinkmann silica gel G plates were used for the thin-layer chromatograms. Elemental analyses were carried out by Dr. FRANZ J. KASLER, University of Mayland.

Enzymatic Hydrolysis of Benzyl 6α-Acetoxypenicillanate (I)

Benzyl 6α-acetoxypenicillanate[1] was deacetylated by the use of a partially purified preparation of citrus acetyl esterase enzyme prepared according to the method of JANSEN *et al.*[5] with the modification of JEFFERY *et al.*[3]

Benzyl 6α-acetoxypenicillanate (104 mg, 0.3 m mole) was dissolved in isopropyl alcohol (2.5 ml) and water (10 ml). A solution of citrus acetyl esterase (5 ml) whose pH had been adjusted to 6.6 and warmed to 30°C was added to the solution. To the stirred reaction mixture (at 30°C), 0.02 N NaOH was added at such a rate that the pH of the solution remained in the region of 6.6. After 3 hours, the reaction was stopped and the solution was saturated with NaCl. The reaction mixture was then extracted with 80 ml of ethyl acetate. The ethyl acetate solution was treated with 10 ml of saturated sodium chloride solution, dried by filtering through filter paper and evaporated to give 89 mg of **II** (96% yield), m. p. 157~158°C. The product after recrystallization from acetone had m. p. 164~166°C (dec.), TLC showed one spot on TLC at R_f 0.55 (ethyl acetate-chloroform, 1 : 3, v/v), and its infrared spectrum (KBr) showed strong bands at 5.70 (benzyl ester carbonyl and β-lactam carbonyl) and 2.98 (hydroxyl) μ.

Analysis: Calcd. for $C_{15}H_{17}O_4NS$: C, 58.63; H, 5.58; N, 4.56; S, 10.41
Found: C, 58.65; H, 5.35; N, 4.75; S, 10.58

Acylation of Benzyl 6α-Hydroxypenicillanate (II)

Benzyl 6α-Acetoxypenicillanate (I)

To a stirred solution of **II** (153 mg, 0.5 m mole) and triethylamine (142 mg, 1.4 m mole) in acetone

(10 ml) was added a solution of acetyl chloride (110 mg, 1.4 m mole) in acetone (10 ml) over a period of 5 minutes. After 30 minutes at 5°C, the mixture was evaporated. The residue was dissolved in 30 ml of ethyl acetate, washed with two 5-ml portions of water, dried ($MgSO_4$) and concentrated to an oil (158 mg, 91 % yield). TLC showed one spot at R_f 0.55 (ethyl acetate-chloroform, 1 : 19, v/v). Crystallization from carbon tetrachloride-methyl cyclohexane afforded 110 mg of **I**, m. p. 50~51°C (dec.). The infrared spectrum (KBr) had strong bands at 5.61 and 5.75 μ and was identical to that of an authentic sample of **I**.

Analysis: Calcd. for $C_{17}H_{19}O_5NS$: C, 58.42; H, 5.48; N, 4.01; S, 9.19
Found: C, 58.70; H, 5.30; N 3.97; S, 9.26

Benzyl 6α-(Phenylacetoxy) penicillanate (**III**)

To a stirred solution of **II** (307 mg, 1.0 m mole), triethylamine (152 mg, 1.5 m mole), and acetone (30 ml), cooled in an ice-bath, was added a solution of phenylacetyl chloride (232 mg, 1.5 m mole) in 10 ml of acetone over a period of 5 minutes. The reaction mixture was stirred at 0°C in the ice-bath for 30 minutes following the addition. A precipitate of triethylamine hydrochloride was removed by filtration and the filtrate evaporated to an oily residue. The residue was then dissolved in chloroform (30 ml), and water (10 ml) was added to the solution. The solution was titrated to pH 6.8 with 0.1 N NaOH. The chloroform layer was separated, dried over magnesium sulfate and evaporated to an oily product (259 mg). This product was chromatographed on a 20-g silica gel column using a solvent mixture of cyclohexane-chloroform (1 : 3, v/v). Compound **III** (164 mg) was isolated as an analytically pure oil. The infrared spectrum had strong bands at 5.62 and 5.75 μ.

Analysis: Calcd. for $C_{23}H_{23}NO_5S$: C, 64.93; H, 5.45; N, 3.29; S, 7.52
Found: C, 64.84; H, 5.25; N, 3.55; S, 7.37

Benzyl 6α-(Phenoxyacetoxy) penicillanate (**IV**)

To a solution of **II** (307 mg, 1.0 m mole) and triethylamine (112 mg, 1.1 m mole) in acetone (25 ml) cooled in an ice-bath was added while stirring phenoxyacetyl chloride (118 mg, 1.1 m mole) in 10 ml of acetone over a period of 5 minutes. The reaction mixture was stirred for 30 minutes, then the mixture was filtered to remove triethylamine hydrochloride and the filtrate evaporated to an oily residue. The residue was dissolved in chloroform and the resulting solution was washed with a 2 % sodium bicarbonate solution and with water. It was dried over magnesium sulfate and evaporated to an oil (313 mg, 71 % yield). The product became crystalline on storage at 5°C. Two recrystallizations from carbon tetrachloride-methylcyclohexane afforded analytically pure **IV** m. p. 75°C (dec.). The infrared spectrum (KBr) had strong bands at 5.62 and 5.73 μ.

Analysis: Calcd. for $C_{23}H_{23}O_6NS$: C, 62.58; H, 5.25; N, 3.17; S, 7.24
Found: C, 62.29; H, 5.19; N, 3.05; S, 7.25

Benzyl 6α-(2, 6-Dimethoxybenzoyloxy) penicillanate (**V**)

In a similar manner, acylation of **II** (307 mg, 1.0 m mole) with 2, 6-dimethoxybenzoyl chloride (253 mg, 1.26 m mole) in the presence of triethylamine (111 mg, 1.1 m mole) gave 313 mg (64 % yield) of crystalline product. Recrystallization from chloroform gave analytically pure **V** as a hemihydrate, m. p. 140~142°C. The infrared spectrum (KBr) had a broad medium intensity band with maximum at 2.98 (hydroxyl) and strong bands at 5.52 (β-lactam carbonyl) and 5.72 μ (ester carbonyls).

Analysis: Calcd. for $C_{24}H_{25}NO_7S \cdot 1/2H_2O$: C, 60.01; H, 5.45; N, 2.93; S, 6.66
Found: C, 60.18; H, 5.62; N, 2.70; S, 6.25

Acknowledgement

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