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STUDIES IN PENICILLIN CHEMISTRY

ISOLATION AND CHARACTERISATION OF METHYL [2s, 5R, 6R] 3, 3-DIMETHYL-6-HYDROXY-METHYL-8-OXO-4-THIA-1, 7-DIAZABICYCLO [3, 3, 0]-OCTANE (A) AND [1S, 4R, 5S] 4-HYDROXYMETHYL-7, 7-DIMETHYL-6-THIA-3, 8-DIAZABICYCLO [3, 2, 1] OCTANE-2-ONE (B)

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Abstract—Reduction of diphenylmethyl benzyloxypenicillinate with borane-tetrahydrofuran gives [2*R*, 2′*R*, 4′*S*] 2-benzyloxy-carbonylamino-2-[5′, 5′-dimethyl-4′-diphenylmethylcarboxy-2′-thiazolidine]-ethanol (**5**) as the main product. Treatment of this compound with methanolic potassium hydroxide gave the two title compounds A and B and some of the free acid corresponding to A. Full experimental details are given.

A compound with the β-lactam carbonyl replaced by a methylene group, such as 7-deoxypenicillin G (**1**), would be a transition-state analogue for enzymes that catalyse reactions in which the β-lactam is hydrolysed, or acylates a nucleophilic group. This idea is based on the familiar view that tetrahedral structures are intermediates in reactions of nucleophiles with CO groups. Hence 7-deoxy penicillins, or related compounds, would be expected to be powerful inhibitors of β-lactamases (and certain transpeptidases). Since we have been studying inhibitors of β-lactamases,[1] we decided to investigate the reaction that, according to Nataraj *et al.*,[2] would furnish a 7-deoxy penicillin. This utilized reduction with diborane. However, we have found, as have others,[3] that the 4-membered ring is opened during this reaction resulting in the formation of a 1, 3-amino-alcohol. During the course of this work, two novel compounds were isolated; their structures have been firmly established by spectroscopic and crystallographic[4] methods.

DISCUSSION

Penicillins contain at least three groups reduceable by borane, namely the CO groups of the amido side chain, the β-lactam CO and the carboxylic acid function. An attempt to reduce the β-lactam ring to an azetidine using borane–tetrahydrofuran (THF) might therefore be complicated by reduction of the other two CO groups. This applies in particular to the carboxylic acid group which is known to react very readily with borane.[5] A benzyloxycarbonyl side chain must also be considered less susceptible to a nucleophilic attack than the conventional acyl side chain. Nataraj *et al.*[2] prepared benzyloxypenicillin from 6-aminopenicillanic acid (6-APA)(**2**) and they then protected the acid as its benzyl ester. We preferred to protect the carboxylic acid first using diazo(diphenyl) methane and then to protect the 6-amino group.

The diphenylmethyl ester of 6-APA (**3**) has been prepared by Sheehan and Commons[6] as an oil in 31% yield by reaction of diazo(diphenyl)methane with 6-APA in a mixture of methylene chloride and methanol with a total reaction time of 44 hr. We prepared this compound in a more convenient way and isolated it as the *p*-toluenesulphonic acid salt in 85% yield. This involved reacting diazo-(diphenyl)methane with 6-APA in a 2:1 mixture of acetone/water in the presence of a molar equivalent of *p*-toluenesulphonic acid. The reaction time was only about 1 hr. The IR spectrum shows the characteristic β-lactam peak at 1797 cm^{-1} and the absorption due to the ester CO at 1745 cm^{-1}. In the NMR spectrum the H-5 and H-6 protons show up as doublets at δ5.13 and 5.57 respectively with a coupling constant of 4.28 Hz.

The benzyloxycarbonyl side chain was then introduced by the reaction of benzyl chloroformate with diphenylmethyl 6-aminopenicillanate in 60% acetone in the presence of sodium bicarbonate. The diphenylmethyl benzyloxypenicillinate (**4**) was isolated, after chromatography on a column of silica gel, in 77% yield. The IR spectrum shows strong CO peaks at 1792 and 1730 cm^{-1}. Purification of the protected penicillin was found to be unnecessary for the borane–THF reduction.

The reduction of **4** was carried out essentially according to Nataraj *et al.*[2] with a commercial borane–THF solution. During the work-up it was decided to partition the products between ethyl acetate and a sodium bicarbonate solution instead of an ammonium chloride solution in case mild acidic conditions were sufficient to cause epimerisation around C′-2 in the product. The open ring hydroxy compound (**5**) was isolated as the main product from this reaction (Scheme 1). This is contrary to the results reported by Nataraj *et al.*,[2] but Sammes and Smith[3] have independently from us found **5** and not the azetidine to be the main product from this reaction. In the IR spectrum, the β-lactam CO peak is now absent. The NMR spectrum shows the signal due to the penicillin H-6 (H-2 in the product) as a multiplet at δ3.65. The penicillin H-5 signal (H-2′ in the product) is also shifted upfield and appears as a doublet at δ4.75 with a coupling constant of 7.1 Hz. The signal due to the exchangeable OH and thiazolidine protons is very broad and appears in the region δ3.0–3.5.

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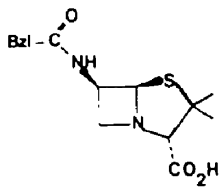
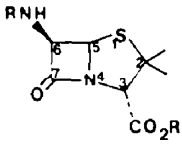


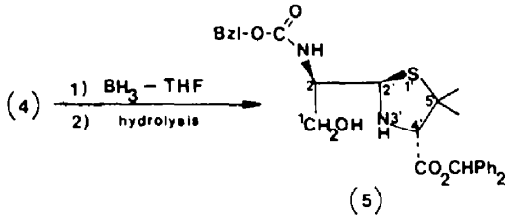
(1)



(2) R = R' = H

(3) R = H ; R' = $CHPh_2$

(4) R = Bzl-O-C(=O)- ; R' = $CHPh_2$



Scheme 1.

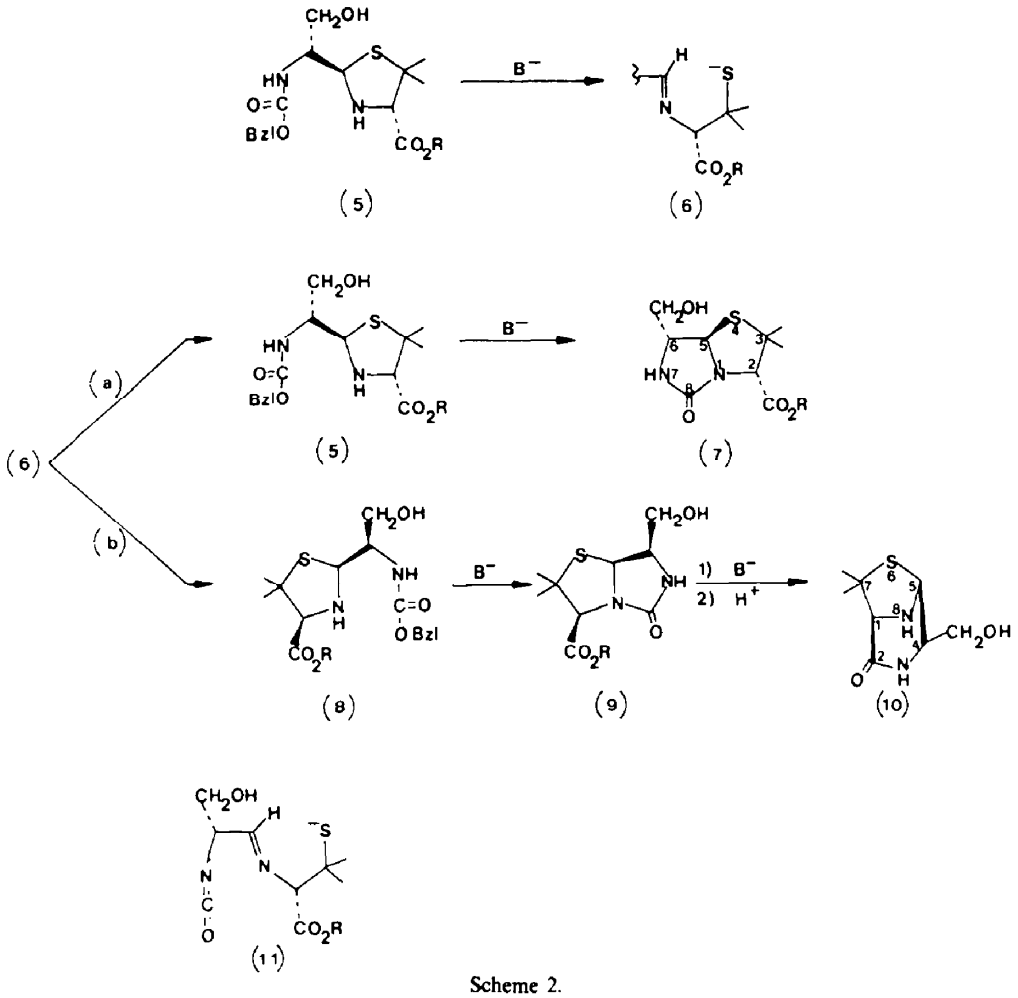
Before all the data that showed **5** and not the azetidine to be the product from the reduction were available, it was decided to deprotect the carboxylic acid function by treatment with a molar equivalent of potassium hydroxide in methanol. After leaving the reaction at room temperature overnight a TLC examination on silica gel (chloroform/methanol 9:1) showed two new major products, compound A, R_f 0.36, and compound B, R_f 0.18. In addition a considerable amount of polar material did not move from the origin in this solvent system. Both compounds, A and B, were isolated pure after chromatography on a column of silica gel. The slow moving material was also isolated after elution with chloroform/methanol 1:1 and after methylation was shown to be largely the free acid corresponding to A.

After crystallisation the structures of both A and B were determined by X-ray crystallography.[4] Compound A, methyl [2*S*, 5*R*, 6*R*] 3, 3-dimethyl-6-hydroxy-methyl-8-oxo-4-thia-1, 7-diazabicyclo [3, 3, 0] octane-2-carboxylate, has the fused immidazolidone–thiazolidine structure (**7**; R = Me). This bicyclic structure has been reported earlier[7] and the 2, 5-dicarboxylic acid is produced from the reaction of carbon dioxide with 6-APA.[7d-f] The high positive rotation of its dimethyl ester $[\alpha]_D^{23} + 218$ (*c* 1.4, EtOH)[7d] agrees well with that found for our compound $[\alpha]_D^{20} + 250$ (c 1.0, MeOH). The spectroscopic data of **7** were consistent with the structure.

Compound B, [1*S*, 4*R*, 5*S*] 4-hydroxymethyl-7, 7-dimethyl-6-thia-3, 8-diazabicyclo [3, 2, 1] octan-2-one (**10**) was isolated in 35% yield. Not enough work has been done to state without reservations how **10** is formed. The formation of this bicyclic system was first proposed for a crystalline material isolated when a D-penicilloate diester was treated with two equivalents of sodium hydroxide in aqueous alcohol.[8] Since then, this system has been shown to be formed from penilloic acids in acetic anhydride.[9-11] The formation of **7** and **10** is best discussed together. The stereochemistry of **7** is 2*S*, 5*R*, 6*R*, that is, the same around all three asymmetric C atoms as in the starting material. In **10**, however, the stereochemistry is 1*S*, 4*R*, 5*S* (note that the systematic numbering is different from that used by convention in penicillins). Here the stereochemistry around C-1 and C-4 (C-3 and C-6 in penicillins) is retained but C-5 has epimerized. Epimerisation around position 5 is known to occur readily[12] and this is borne out by the formation of **10**. It is therefore noteworthy that no sign of the C-5 epimer of **7** could be found at the end of our reaction.

To explain these observations we propose the sequence of intramolecular reactions shown in Scheme 2. Here the first step is epimerisation around the thiazolidine 2′ carbon. Following this **7** is formed from the 2′*R* epimer (**5**) by a nucleophilic attack of the thiazolidine N on the urethane CO group. In this compound the immidazolidone ring and the ester group are on opposite sides of the thiazolidine ring (reaction path a). A similar reaction by the 2′-*S* epimer (**8**) would give **9**. This compound was not isolated from the reaction. Here the immidazolidone ring and the ester group are on the same side of the thiazolidine ring and the OH group or the immidazolidone N are sterically well placed for a nucleophilic attack on the ester. Whether **10** is formed via a cyclic ester or directly by a nucleophilic attack by the immidazolidone N cannot be stated, but the absence of **9** at the end of the reaction suggests that it is an intermediate in the formation of **10**.

If **7** could be induced to epimerize around C-5 it should also go on to form **10**. To test this **7** (26 mg) was treated with two equivalents of potassium hydroxide in methanol. Most of the ester is hydrolysed before epimerisation, which is a prerequisite for the intramolecular attack on the ester, takes place. In spite of that a small amount (1 mg) of **10** was isolated and identified by a TLC comparison with an authentic sample and by its proton NMR spectrum. The epimerisation that precedes the formation of **10** probably happens via intermediate **11**.



Scheme 2.

In the above discussion no mention has been made of the fact that the starting material (5) was a diphenylmethyl ester but 7; (R = Me) was isolated as the methyl ester. In the methanolic medium of the reaction this must happen by a base catalysed transesterification.

EXPERIMENTAL

M. ps are uncorrected. IR spectra were recorded with a Perkin Elmer 297 spectrometer. The ^{1}H and ^{13}C NMR spectra were obtained with a Bruker WH300 instrument at 300 and 75.47 MHz respectively. Chemical shifts are relative to TMS. Mass spectra were obtained with a VG Micromass ZAB1F instrument. TLC was carried out on Merck 5735 plastic silica gel sheets, and the column chromatography was carried out on Merck 0.05–0.2 mm, 7734, silica gel.

Diphenylmethyl 6-aminopenicillanate (2) *p-toluene-sulphonic acid salt*. 6-Aminopenicillanic acid (6.5g, 30 mmol) and toluene sulphonic acid (5.7 g, 30 mmol) were dissolved in acetone (600 ml) and water (300 ml). Diazo-(diphenyl) methane (14 g, 72 mmol) was added in three portions to the stirred soln over a period of about 1 hr. After the purple colour had disappeared most of the acetone was evaporated and more water (300 ml) and ether (300 ml) were added followed by the gradual addition of $NaHCO_3$ (7.2 g, 90 mmol) in water (300 ml). The organic layer was separated and toluenesulphonic acid (5.7 g, 30 mmol) in acetone (150 ml) added slowly while stirring. The colourless ppt (14 g, 85%) had m.p. 155–156° (dec). $[\alpha]_D^{20}$ + 131.7, (c 1.06, MeOH). ^{1}H-NMR (DMSO-d_6): δ1.28, 1.64, 3H singlets, ($-CH_3$)$_2$; 2.29, 3H s, Ph-CH_3; 4.73, 1H s, H-3; 5.13, 5.57, 1H doublets, J 4.28Hz, H-5 and H-6; 6.93, 1H s, Ph_2CH; 7.1–7.5, 14H m, aromatic; 8.9, broad, $^+NH_3$. ν_{max} (nujol), carbonyl peaks: 1797 s; 1745 cm^{-1} s. (Found: C61.07, H5.65, N5.31. $C_{28}H_{30}N_2O_6S_2$ requires: C60.63, H5.45, N5.05%).

Diphenylmethyl benzyloxypenicillinate (3). Diphenylmethyl 6-aminopenicillanate toluenesulphonic acid salt (1.11 g, 2.00 mmol) was dissolved in acetone (30 ml) and water (20 ml) containing $NaHCO_3$ (400 mg, 4.6 mmol). The mixture was cooled on ice and benzyl chloroformate (284 μl, 2 mmol) was added. The mixture was allowed to warm to room temp and left for 90 min. Ether (50 ml) was added and after shaking, the two layers were separated. The aqueous layer was extracted with more ether (50 ml) and the combined ether extracts washed with $NaHCO_3$ aq (400 mg in 20 ml) and with sat brine (20 ml). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The syrupy residue contained two components, R_f 0.21 (minor) and R_f 0.44 (major). The main component, diphenylmethyl benzyloxypenicillinate, was purified on a column of silica gel at 4° with ether/light petroleum (b.p. 60–80°), 3:2 (v/v) as an eluent and isolated as a foam, yield 794 mg, 77%. $[\alpha]_D^{20}$ + 186.4 (c 1.0, MeOH) ^{1}H-NMR ($CDCl_3$): δ1.28, 1.62, 3H singlets, ($-CH_3$)$_2$; 4.55, 1H s, H-3; 5.14, 2H s, $PhCH_2$; 5.6, 3H m, H-5, H-6 and NH; 6.95, 1H s, Ph_2CH; 7.35 15H m, aromatic. After D_2O shake signal centred on δ5.6 goes to a 2H doublet FD Mass spectrum gave M$^+$ 516, ν_{max} ($CHCl_3$) main peaks: 3430 w; 3030 w; 2980 w; 1792 s; 1730 s; 1510 m; 1458 cm^{-1} w. (Found: C67.26, H5.50, N5.45. $C_{29}H_{28}N_2O_5S$ requires: C67.41, H5.46, N5.42%).

[2R, 2'R, 4'S] 2-*Benzyloxycarbonylamino*-2- [5', 5'-*dimethyl*-4'-*diphenylmethylcarboxy*-2'-*thiazolidine*]-*ethanol* (**4**). Compound **3** (4 g; prepared as described, dried over P_2O_5 but not purified on silica gel) was dissolved in dry THF (80 ml) and cooled on an ice/salt bath. One molar borane/THF soln (22 ml) was added taking the usual precautions for the exclusion of air and moisture. The mixture was allowed to reach room temp overnight (20 hr). A soln of $NaHCO_3$ (80 ml, 0.5 M) was added and the mixture stirred at room temp for 1 hr. EtOAc (100 ml) was added; the aqueous layer was extracted with more EtOAc (50 ml) and the combined organic extracts washed with sat brine (40 ml), dried ($MgSO_4$) and evaporated to dryness. The main component (R_f 0.38, EtOAc-light petroleum (b.p. 60–80°), 2:3 (v/v) was purified on a column of silica gel (250 g) and identified as the title compound (1.6 g). $[\alpha]_D^{20}$ + 84.7 (c 1.0, MeOH) ^{1}H-NMR ($CDCl_3$): δ0.99 and 1.57, 3H singlets (s), $(-CH_3)_2$; 3.0–3.5, 2H, broad signal, OH and 3'-NH; 3.65, 1H m, H-2; 3.30, 2H m, 2 × H-1; 3.84, 1H s, H-4'; 4.75, 1H d, $J_{2H'\text{-}H2}$ 7.1Hz, H-2'; 5.11, 2H s, Ph-CH_2-; 5.56, 1H broad, urethane NH; 6.96, 1H s, Ph_2CH; 7.3–7.4, 15H m, aromatic. After D_2O shake signals at δ3.0–3.5 and 5.56 disappear. FD Mass spectrum gave M^+ 521. ν_{max} ($CHCl_3$), main peaks: 1720–1730 s; 1960 w; 2860 w; 2930 w; 3350 m; 3440 cm^{-1} m. (Found, C66.95, H6.44, N5.10. Calc. $C_{29}H_{32}N_2O_5S$ requires: C66.90, H6.20, N5.38%).

Treatment of [2R, 2'R, 4'S] 2-*benzyloxy-carbonylamino*-2-[5', 5'-*dimethyl*-4'-*diphenylmethylcarboxy*-2'-*thiazolidine*]-*ethanol* (**4**) *with methanolic potassium hydroxide*. The title compound (1.04 g, 1.99 mmol) was dissolved in MeOH (8 ml) and methanolic KOH (2.2 ml, 1.0 M) was added. The soln was left at room temp overnight (22 hr). More KOH (1 ml, 1.0 M) was added and the reaction left for a further 7 hr. Methanolic HCl (3.2 ml, 1.0 M) was added and KCl separated by filtration and the filtrate evaporated. The crude residue was placed on a column of silica gel and eluted with $CHCl_3$/MeOH 9:1 and then 4:1. Early fractions containing benzyl- and benzhydryl-alcohol were discarded. Two compounds were isolated, compound A R_f 0.36 ($CHCl_3$/MeOH 9:1) and compound B R_f 0.18. Compound A, methyl [2*S*, 5*R*, 6*R*] 3, 3-dimethyl-6-hydroxymethyl-8-oxo-4-thia-1, 7-diazabicyclo [3, 3, 0] octane-2-carboxylate (**7**, R = Me) (150 mg, 30%) was crystallized from water, m.p. 135–136°, $[\alpha]_D^{20}$ + 250.3 (c 1.0, MeOH). ^{1}H-NMR ($CDCl_3$): δ1.46, 1.57, 3H singlets (s), $(-CH_3)_2$; 3.51, 1H broad, OH; 3.70, 2H m, $-CH_2OH$; 3.76, 3H s, OCH_3; 3.86, 1H m, H-6; 4.62, 1H s, H-2; 5.37, 1H d, $J_{H5\text{-}H6}$ 1.33Hz, H-5; 6.41, 1H broad, NH. After D_2O shake signals at δ3.51 and 6.41 disappear. ^{13}C-NMR ($CDCl_3$): δ26.42, 33.01 quartets (q) $(-CH_3)_2$; 52.09 q, $-OCH_3$; 58.05 s, C-3; 58.44 d, C-2; 64.55 t, CH_2OH; 68.02, 70.78 doublets, C-6 and C-5; 162.65, 169.86 singlets, CO carbons. FD Mass spectrum gave $M^+ + 1$ 261. ν_{max} ($CHCl_3$) main peaks; 1670 cm^{-1} to 1760 (s); 3460 shoulder to 3200 and down to 2850. (Found: C45.99, H6.15, N10.81. $C_{10}H_{16}N_2O_4S$ requires: C46.15, H6.20, N10.77%).

Compound B, [1*S*, 4*R*, 5*S*] 4-hydroxymethyl-7, 7-dimethyl-6-thia-3, 8-diazabicyclo [3, 2, 1] octan-2-one (**10**) (140 mg, 35%) was crystallized from MeOH/acetone, m.p. 172–173°. $[\alpha]_D^{20}$-8.2 (c 1.0, MeOH). ^{1}H-NMR (D_2O): δ1.30, 1.35, 3H singlets (s), $(-CH_3)_2$; 3.49, 3H m, CH_2OH and H-1; 3.72, 1H m, H-4; 4.99, 1H d, $J_{H4\text{-}H5}$ 3.31Hz, H-4. Double resonance decoupling by irradiation at δ4.99 reduces multiplet at 3.72 to an apparent triplet, $J_{H5\text{-}CH_2OH}$ 6.8Hz. ^{13}C-NMR (D_2O): δ25.02, 31.64 quartets, $(-CH_3)_2$; 58.29 d, C-1; 59.82 s, C-7; 61.80 t, CH_2OH; 65.54, 70.78 doublets, C-4 and C-5; 170.98 s, carbonyl carbon. FD Mass spectrum gave M^+ 202. ν_{max} (nujol) main peaks: 1670 cm^{-1} (s) shoulder at 1620; 2300–2500 (w); 3200 (w) – 3500 (s). Elemental analysis: C/N ratio found 3.46. $C_8H_{14}N_2O_2S$ requires: 3.43. Further elution of the column with $CHCl_3$/MeOH 1:1 gave a third compound, the free acid corresponding to A, which was identified after methylation by comparison with compound A. Additional yield 60 mg, 12%.

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