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Preparation and Properties of 5-Phenylphenoxymethylpenicillin

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Cycloaddition of azidoacetyl chloride to benzyl D-5,5-dimethyl-5-phenyl-2-thiazoline-4-carboxylate (**1a**) gave 5-phenyl-6α-azidopenicillanate (**2a**). By catalytic reduction of **2a** and reaction with phenoxyacetyl chloride, 5-phenyl-6-epiphenoxymethylpenicillin benzyl ester (**4a**) was obtained. Oxidation of **4a** gave the sulfoxide **6**, which was isomerized in the presence of DBN. The sulfoxide **7** with the normal configuration could be isolated but deoxygenation of the sulfoxide was not successful. Isomerization of **4a** with DBN, either with or without silylation of the side chain, gave a mixture from which 5-phenylphenoxymethylpenicillin benzyl ester (**5**) was isolated. Compound **5** was debenzylated to 5-phenylphenoxymethylpenicillin potassium salt (**8**). The antibacterial activity of **8** was low, whereas the 6-epimer **9** was inactive. Contrary to published information, the 5-phenylpenam derivative **4c** could be prepared by the same method.

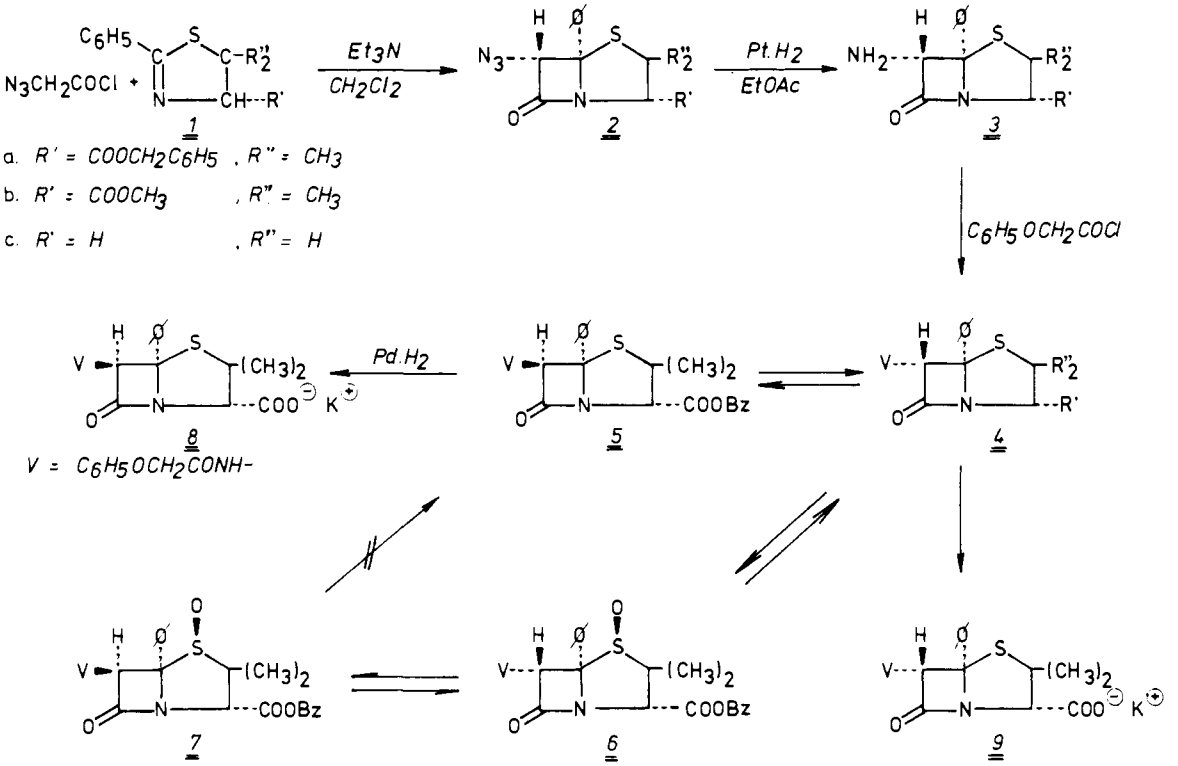
Recently several penicillins with additional or modified substituents in the penam part of the molecule have been synthetized. Substituents like alkyl,[1,2] halogen,[3] methoxy,[4–8] methylthio,[6–8] hydroxybenzyl,[9] hydroxymethyl,[10] formyl,[11] carboxy,[11] or hydroxy[12] groups have been introduced on C-6 of the molecule. Penicillin analogs with two hydrogen atoms[13] or one or two acetoxymethyl groups[14] on C-2 instead of two methyl groups have been prepared.

Penam derivatives with a *tert*-butyloxycarbonyl[15] or a methylthio group[16] on C-5 have been described. These compounds did not have a carboxy group on C-3 and an acylamino side chain, which are necessary for antibiotic activity. The 5-phenylpenicillanic esters with a phthalimido or a succinimido group on C-6, prepared some time ago by Sheehan and coworkers,[17] were inactive. It should be noted, however, that the configuration at C-5 and C-6 was not determined and that penicillin methyl esters with an imido side chain, having a normal configuration, present a very low degree of activity.

In relation with our study of modified penicillins,[13] we wanted to examine the activity of 5-phenylphenoxymethylpenicillin having the same configuration as the natural product. For the preparation of this product we used the cycloaddition of azidoacetyl chloride to a thiazoline. This synthesis, which was discovered by Bose and coworkers,[18] yields a 5,6-*trans*-penicillin, which we have shown to have the 6-epi configuration.[19] The formation of methyl 5-phenyl-6-azidopenicillinate (**2b**), by reaction of azidoacetyl chloride with methyl 5,5-dimethyl-2-phenyl-2-thiazoline-4-carboxylate (**1b**) in the presence of triethylamine, has been described.[20] The configuration of **2b** has not been determined, but it was the same as that of the phthalimido- and succinimido-5-phenylpenicillanic acids prepared by Sheehan and coworkers.[17] As the preparation of **2b** and the transformation of **2b** into **4b** proceeded with satisfactory yields, we applied the same procedure to benzyl D-5,5-dimethyl-2-phenyl-2-thiazoline-4-carboxylate (**1a**). The benzyl ester of 5-phenyl-6-azidopenicillanic acid (**2a**) was obtained in fair yield (32%). Hydrogenation of **2a** in ethyl acetate in the presence of Adams catalyst gave benzyl 5-phenyl-6-aminopenicillanate (**3a**), which was transformed directly into benzyl 5-phenylphenoxymethylpenicillinate (**4a**) in 62% yield. We assume that **4a** had the 6-epi configuration in analogy with the course of the reaction with methyl 5,5-dimethyl-2-thiazoline-4-carboxylate.[18,19]

For the conversion of **4a** into a penicillin with normal configuration at C-6, we preferred the base-catalyzed isomerization of the sulfoxide derivative rather than the penicillin itself, because a more favorable ratio of normal to epi isomer was obtained with phenoxymethylpenicillin.[21] Only one product was obtained by oxidation of **4a** with *m*-chloroperbenzoic acid. The same product was formed upon oxidation of **4a** with ozone in acetone–water (1:1). Treatment of **6** with 1,5-diazabicyclo[4.3.0]non-3-ene (DBN) after silylating the amide side chain with *N,O*-bis(trimethylsilyl)acetamide (BSA) for 40 min at 0° gave a mixture of products with normal and epi configuration in the ratio 2:3. Treatment of **6** directly with DBN in the same conditions gave a

Scheme I



a. R' = $COOCH_2C_6H_5$, R'' = CH_3
b. R' = $COOCH_3$, R'' = CH_3
c. R' = H, R'' = H

1:2 ratio. When **6** or **7** were kept in the presence of DBN until equilibrium was reached (24 hr), a 1:1 ratio was obtained. The sulfoxides **6** and **7** were separated by column chromatography. We assume that both sulfoxides have the *S* configuration, because the oxidation of penicillins with a phenoxyacetamido side chain usually yields the (*S*)-sulfoxide. This assignment is supported by the rather large value of the difference of the chemical shifts of the two C-2 methyl groups. The value was smaller for different penicillin (*R*)-sulfoxides (Scheme I).[22]

The sulfoxide **7** could not be deoxygenated with an excess (8 mol) of phosphorus tribromide in dimethylformamide, even at 50°. The 6-epimer **6** could be transformed into **4a** in a 40% yield under these reaction conditions. It should be noted that the benzyl ester of phenoxymethylpenicillin sulfoxide was deoxygenated in 52% yield with phosphorus tribromide at 0°, whereas the 6-epimer was reduced in 98% yield under the same conditions. The lower yield observed for the isomer with the normal configuration can be explained by the existence of an hydrogen bond between the sulfoxide group and the amide side chain.[23] In compound **7** the sulfoxide group is apparently protected at the endo side by the hydrogen bond and at the exo side by the phenyl group. The hydrogen bond does not exist in the 6-epimer **6**, because of the greater distance between the groups involved, and thus reduction is possible.

As we were unable to transform **7** into **5**, we tried the epimerization of **4a** with DBN in dichloromethane for 20 min at 0° after silylation of the amide side chain with BSA. A 1:1 ratio of **4a** and **5** was obtained. This result was favorable for the preparation of **5**, which could be separated from **4a** by column chromatography. We also observed that **4a** could be epimerized wih DBN without protection of the amide side chain, in contrast with phenoxymethylpenicillin, where this derivation was necessary.[19] The treatment of **4a** with DBN yielded **5** in 60% yield. No **4a** was present in the reaction mixture, but several decomposition products, among them benzyl 5,5-dimethyl-2-phenyl-2-thiazoline-4-carboxylate (**1a**), were detected. Treatment of **4a** with lithium diisopropylamide, which has been used for the direct epimerization of phenoxymethylpenicillin methyl ester,[24] gave a 30% yield of a mixture containing **4a** and **5** in a 1:1 ratio.

Hydrogenolysis of **5** in the presence of Pd/C gave 5-phenylphenoxymethylpenicillin (**8**) in 45% yield. Compound **4a** was transformed into 6-epimer **9** in the same way.

The assignment of the configuration is based on the chemical shifts of the C-6 hydrogen. The chemical shifts of H-6 in penicillins **4a** and **5** are 5.69 and 5.44; for the penicillin sulfoxides **6** and **7** the δ values are 5.95 and 5.70. In the penicillins with normal configuration (**5** and **7**), where C-5 phenyl and C-6 hydrogen are in cis position, the proton resonates at higher field. The same observation was with several phenyl-substituted β-lactams.[25] Another argument for the configuration is the observation that in compound **5** the methylene protons of the side chain appear as a singlet (δ 4.55) whereas in **4a** they show an AB pattern (δ 4.18, J = 15 Hz). This is an indication that in **4a** the phenyl group at C-5 and the side chain at C-6 are at the same side.

When this work was almost finished, a publication[26] appeared in which it was stated that the reduction of the related compound **2c** and the reaction of **3c** with phenoxyacetyl chloride did not give **4c**, but gave a substance having a phenoxy group at C-6 instead of a phenoxyacetamido group. When we repeated the reduction of **2c** using our usual reaction conditions (4 hr at room temperature), we obtained the expected product **4c** in good yield. The unusual reaction was explained by the scission of the β-lactam ring of **2c** or **3c** with the formation of the thiazoline **1c**, which would react with phenoxyacetyl chloride.[26] A cleavage of the β-lactam ring seems possible, considering the formation of the phenylthiazoline **1a** by treatment of **4a** with DBN. It should be noted that these authors[26] kept compound **2c** for 70 hr in ethyl acetate solution during the hydrogenation, whereas the duration of this operation performed in our laboratory was only 4 hr.

The potassium salt of 5-phenyl-6-epiphenoxymethylpenicillin (**9**) had no antibiotic activity when tested at 500 μg/

ml against *Staphylococcus aureus* ATCC 6538P, whereas the normal isomer **8** had a MIC of 37 μg/ml vs. a MIC of 0.025 μg/ml for phenoxymethylpenicillin. The presence of the bulky phenyl group on C-5 has a very unfavorable effect on the activity.

Experimental Section

Melting points were determined with a Büchi-Tottoli apparatus and are corrected. Microanalyses were performed by A. Bernhardt (Elbach über Engelskirchen, West Germany). For the TLC we used Merck precoated silica gel F-254 plates. Spots were located by uv illumination and exposure to iodine vapor. Column chromatography was performed over silica gel (Merck, 0.05–0.2 mm). Ir spectra were run on a Perkin-Elmer 257 spectrometer using KBr disks unless otherwise stated. Mass spectra were recorded on a A.E.I. MS-12 mass spectrometer, 8 kV accelerating potential, 100 μA trap current, and 70 eV ionization energy. The direct heating inlet system was used at temperatures varying from 80 to 150° dependent on the volatility of the examined product. NMR spectra were taken on a Hitachi Perkin-Elmer R-24 instrument in $CDCl_3$ (unless otherwise noted) with tetramethylsilane (TMS) as internal standard. Peak positions were expressed in δ values.

Benzyl D-5,5-Dimethyl-2-phenyl-2-thiazoline-4-carboxylate (1a). A mixture of 7.31 g (0.039 mol) of ethyl benzimidate hydrochloride,[27] 7.31 g (0.039 mol) of D-penicillamine hydrochloride, and 9.5 ml (0.078 mol) of dry triethylamine in 100 ml of dry methanol was stirred for 48 hr at room temperature. The methanol was removed under reduced pressure, and the residue was suspended in 250 ml of dichloromethane and treated with 7 ml of concentrated hydrochloric acid. After two washings with water, the organic layer was dried (Na_2SO_4) and evaporated to a white solid. Recrystallization from boiling ether gave 6.4 g (70%) of 5,5-dimethyl-2-phenyl-2-thiazoline-4-carboxylic acid: mp 123–125°; $[\alpha]^{25}D$ +49° (*c* 1, acetone); NMR (DMSO-d_6) δ 1.45 and 1.74 (3 H, s, *gem*-dimethyl), 4.86 (1 H, s, tertiary H-4), 7.4–7.86 (br, m, aromatic).

A solution of 12.71 g (0.055 mol) of D-5,5-dimethyl-2-phenyl-2-thiazoline-4-carboxylic acid, 7.77 ml (0.06 mol) of dry triethylamine, and 7.587 ml (0.06 mol) of freshly distilled benzyl bromide in 150 ml of dimethylformamide was stirred for 4 hr at room temperature. The mixture was poured into ice–water and the suspension was extracted with EtOAc (5 × 250 ml). The combined organic layer was washed ($NaHCO_3$–H_2O), dried (Na_2SO_4), and evaporated to a yellow oil (13.9 g, 80%): uv max (cyclohexane) 243 nm (ε 17,620); $[\alpha]^{25}D$ +3° (*c* 1, acetone); mass spectrum *m/e* 325; NMR ($CDCl_3$) δ 1.45 (3 H, s, CCH_3), 1.72 (3 H, s, CCH_3), 4.90 (1 H, s, H-4), 5.25 (2 H, s, $CH_2C_6H_5$), and 7.3–8 (m, aromatic).

Benzyl 5-Phenyl-6α-azidopenicillinate (2a). Et_3N (0.027 mol in 250 ml of CH_2Cl_2) was added to a refluxing solution of 9 g (0.027 mol) of **1a** and 3.21 g (0.027 mol) of azidoacetyl chloride[28] in 450 ml of CH_2Cl_2 under high dilution conditions[17] over a period of 20 hr. The solvent was removed in vacuo and the residue was extracted with anhydrous ether. The oily residue (11.8 g) obtained by the removal of the solvent was chromatographed over 150 g of silica gel using benzene as eluting solvent to yield 3.526 g (32%) of a white solid, which was crystallized from ether–*n*-pentane as white needles: mp 95.5–96.5°; $[\alpha]^{25}D$ +9° (*c* 0.5, acetone); mass spectrum *m/e* 380 ($M - N_2$)$^+$; ir 2109 (N_3), 1790 (β-lactam), 1748 cm^{-1} (ester); NMR ($CDCl_3$) δ 1.14 (3 H, s, CCH_3), 1.68 (3 H, s, CCH_3), 4.65 (1 H, s, H-3), 4.91 (1 H, s, H-6), 5.16 (2 H, s, $CH_2C_6H_5$), 7.2–7.6 (m, aromatic). Elution with benzene–chloroform (50:50) yielded 5.31 g (59%) of starting material **1a**. Anal. ($C_{21}H_{20}O_3N_4S$) C, H, N.

5-Phenyl-6-epiphenoxymethylpenicillin Benzyl Ester (4a). A solution of 2 g (4.9 mmol) of **2a** in 100 ml of EtOAc was shaken with 2 g of PtO_2 (Adams catalyst) at room temperature for 4 hr in the presence of H_2 at atmospheric pressure. The solution was then filtered and evaporated under reduced pressure to yield a yellow, viscous oil, **3a**: ir (CH_2Cl_2) 3400 (br, NH_2), 1775 cm^{-1} (β-lactam). This material was dissolved in 200 ml of CH_2Cl_2 and 0.73 ml (5.3 mmol) of Et_3N, and a solution of 0.883 g (5.3 mmol) of phenoxyacetyl chloride in 45 ml of CH_2Cl_2 was added with stirring and cooling to −10° over a period of 1 hr. The mixture was stirred for an additional 2 hr at room temperature. The solution was then successively washed with dilute HOAc, with a 5% solution of $NaHCO_3$, and with water. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to a white solid to yield 1.57 g (62%): mp 122–124° (CH_2Cl_2–*n*-heptane); $[\alpha]^{25}D$ +12° (*c* 0.5, acetone); ir 3265 (NH), 1780 (β-lactam), 1728 (ester) and 1668 cm^{-1} (amide); mass spectrum *m/e* 516; NMR ($CDCl_3$) δ 1.16 (3 H, s, CCH_3), 1.70 (3 H, s, CCH_3), 4.66 (1 H, s, H-3), 4.18 (AB pattern, *J* = 15 Hz, 2 H, OCH_2CO), 5.17 (2 H, s, $CH_2C_6H_5$), 5.69 (1 H, d, *J* = 9 Hz, H-6), 6.5–7.64 (m, aromatic). Anal. ($C_{29}H_{28}O_5N_2S$) C, H, N.

5-Phenyl-6-epiphenoxymethylpenicillin Sulfoxide Benzyl Ester (6). (a) Oxidation with *m*-Chloroperbenzoic Acid. A suspension of 130 mg (85%, 0.76 mmol) of *m*-chloroperbenzoic acid in dry chloroform was added to a solution of 400 mg (0.76 mmol) of **4a** in 10 ml of dry chloroform at 0°. The reaction mixture was stirred for another 30 min at 0°. The solution was then diluted with 40 ml of chloroform and extracted with 50 ml of a solution of 5% $NaHCO_3$ in water, dried (Na_2SO_4), and evaporated until crystallization occurred, yielding 295 mg (72%): mp 82–83° ($CHCl_3$–*n*-pentane); $[\alpha]^{25}D$ −0.2° (*c* 1, acetone), −0.6° (*c* 1, chloroform); ir 3320 (NH), 1785 (β-lactam), 1750 (ester), 1690 (amide), 1055 cm^{-1} (sulfoxide); mass spectrum *m/e* 532; NMR ($CDCl_3$) δ 0.76 (3 H, s, CCH_3), 1.65 (3 H, s, CCH_3), 4.21 (AB pattern, *J* = 15 Hz, 2 H, OCH_2CO), 4.76 (1 H, s, H-3), 5.25 (2 H, s, $CH_2C_6H_5$), 5.95 (1 H, d, *J* = 9 Hz, H-6), 6.49–7.40 (m, aromatic). Anal. ($C_{29}H_{28}O_6N_2S$) C, H, N.

(b) Oxidation with Ozone. Ozone was bubbled through a cooled solution of 0.516 g (1 mmol) of **4a** in 50 ml of acetone–water (3:2) for 0.5 hr with a flow rate of oxygen of 150 ml/min. During the reaction the temperature was kept at 0–2°. The solution was concentrated and the suspension was freeze-dried and afforded 0.520 g (98%) of amorphous material. From the spectral data it was concluded that the product was identical with the compound described under (a).

5-Phenylphenoxymethylpenicillin Sulfoxide Benzyl Ester (7). (a) Isomerization with BSA and DBN. A solution of 1 g (1.8 mmol) of **6** in 20 ml of anhydrous CH_2Cl_2 was treated with 1.5 ml (5 mmol) of *N,O*-bis(trimethylsilyl)acetamide (BSA) and stirred for 15 min. After cooling the solution to 0°, 0.25 ml (1.8 mmol) of DBN was added at once and stirring was continued for 40 min at 0°. The reaction mixture was first diluted with 130 ml of CH_2Cl_2 and then washed with a solution of 0.5 ml of HOAc in 150 ml of water and further washed twice with water (100 ml), dried (Na_2SO_4), and evaporated to a yellow oil. The NMR spectrum showed a ratio of 2:3 for normal product and the 6-epimer. A solution in benzene was chromatographed over 25 g of silica gel using benzene–acetone (99:1 v/v) as eluting solvent for **7**. The starting product (503 mg, 50%) was eluted with benzene–acetone (97:3 v/v). **7** was taken up in ether and 310 mg (31%) of crystalline **7** was obtained: mp 179–181° dec; $[\alpha]^{25}D$ +63° (*c* 0.2, acetone); ir 3375 (NH), 1803 (β-lactam), 1750 (ester), 1050 cm^{-1} (sulfoxide); mass spectrum *m/e* 532; NMR ($CDCl_3$) δ 0.68 (3 H, s, CCH_3), 1.68 (3 H, s, CCH_3), 4.57 (2 H, s, OCH_2CO), 4.91 (1 H, s, H-3), 5.24 (2 H, s, $CH_2C_6H_5$), 5.70 (1 H, d, *J* = 11 Hz, H-6), 6.94–7.69 (m, aromatic), 8.59 (1 H, d, *J* = 11 Hz, NH). Anal. ($C_{29}H_{28}O_6N_2S$) C, H, N.

(b) Isomerization with DBN. A solution of 0.1 g (0.18 mmol) of **6** in 2 ml of anhydrous CH_2Cl_2 containing 0.025 ml (0.18 mmol) of DBN was kept at 0° for 50 min. The reaction was stopped with 0.2 ml of HOAc, 1 *N*. After dilution with 5 ml of CH_2Cl_2, the organic layer was washed with water, dried (Na_2SO_4), and evaporated to a yellow oil, which consisted of **6** and **7** in the ratio 2:1 (NMR). When **6** was kept with the same reagents for 24 hr at 25°, a ratio of 1:1 was obtained. The same ratio was found when **7** was treated with DBN in the same way.

Deoxygenation Experiments with 5-Phenylphenoxymethylpenicillin Sulfoxide Benzyl Ester. (a) With PBr_3. PBr_3 (0.22 ml, 2.24 mmol) was added to a solution of 150 mg (0.28 mmol) of 5-phenylphenoxymethylpenicillin sulfoxide benzyl ester (**7**) in 20 ml of anhydrous DMF, cooled to 0°. The reaction mixture was stirred for 45 min at 0° and poured into an ice-cold solution of 0.2 g of $NaHCO_3$ in 30 ml of water. The suspension was extracted twice with 30 ml of EtOAc. Evaporation of the reaction mixture gave 135 mg (90%) of the starting material. Reduction of 5-phenylphenoxymethylpenicillin sulfoxide benzyl ester (53.2 mg, 0.1 mmol) under more severe reaction conditions (0.9 ml of PBr_3 at 25° and at 50° for 50 min) was not successful. Degradation products (spots at the start) on TLC were found in the reaction performed at 50°. Reduction of the 6-epimer **6** with PBr_3 at 50° gave the 5-phenyl-6-epiphenoxymethylpenicillin ester (**4a**) in a 40% yield.

(b) With $SnCl_2$. 5-Phenylphenoxymethylpenicillin sulfoxide benzyl ester (**7**, 26.6 mg, 0.05 mmol) was dissolved in acetonitrile (5 ml) and dimethylformamide (2 ml) and stirred at 0°; 24.8 mg (0.055 mmol) of stannous chloride and 39.2 mg of acetyl chloride were added. This mixture was stirred at room temperature for 1 hr. The acetonitrile was removed in vacuo; the residue was poured

into water and extracted with 25 ml of ethyl acetate. The organic layer was washed with 0.5 *N* hydrochloric acid solution (20 ml), 5% sodium bicarbonate solution (25 ml), and twice with water (50 ml), dried (Na_2SO_4), and evaporated to give 23 mg of starting product.

5-Phenylphenoxymethylpenicillin Benzyl Ester (5). (a) Isomerization with BSA and DBN. A solution of 1 g (1.9 mmol) of **4a** in 25 ml of anhydrous CH_2Cl_2 was treated with 1.5 ml (5 mmol) of BSA and stirred at room temperature for 15 min; 0.5 ml (3.8 mmol) of DBN was added and the solution was stirred for another 20 min at room temperature. After dilution with 150 ml of CH_2Cl_2 the solution was poured into a mixture of 200 ml of water and 0.5 ml of HOAc. The organic layer was washed twice with 150 ml of water, dried (Na_2SO_4), and evaporated to a brown oil. The NMR spectrum showed a ratio of 1:1 for normal product and the 6-epimer. Chromatography over 50 g of silica gel with benzene–EtOAc (99:1 v/v) as eluent afforded **5** (400 mg, 40%). Starting material (350 mg, 35%) was eluted with benzene–EtOAc (98:2 v/v). **5** was crystallized from dichloromethane–*n*-heptane: mp 158–159° $[\alpha]^{25}D$ −14° (*c* 0.5, acetone); ir 3350 (NH), 1780 (β-lactam), 1718 (ester), 1697 (amide I), 750 cm^{-1} (double peak, phenyl); mass spectrum *m/e* 516; NMR ($CDCl_3$) δ 1.08 (3 H, s, CCH_3), 1.67 (3 H, s, CCH_3), 4.55 (2 H, s, $CH_2OC_6H_5$), 4.60 (1 H, s, H-3), 5.16 (2 H, s, $CH_2C_6H_5$), 5.44 (1 H, d, *J* = 9 Hz, H-6), 7.0–8.0 (m, aromatic and NH). Anal. ($C_{29}H_{28}O_5N_2S$) C, H, N.

(b) Isomerization with DBN. A solution of 0.516 g (1 mmol) of **4a** in 15 ml of anhydrous CH_2Cl_2 was treated with 0.125 ml (1 mmol) of DBN at 25° for 24 hr. The main reaction product on TLC (benzene–acetone 95:5) was **5**; several other products, among them **1a**, were also found. Chromatography over 20 g of silica gel and elution with benzene–EtOAc afforded 0.301 g of **5**. No **4a** was found. On the other hand, treatment of the natural compound **5** with DBN leaves the starting product unchanged.

(c) Isomerization after Lithium Diisopropylamide. A solution of 100 mg (0.2 mmol) of **4a** in 4 ml of anhydrous tetrahydrofuran was treated at −80° with 0.54 ml (0.5 mmol) of lithium diisopropylamide[24] during 1 min. This treatment was followed by the addition of 8 μl (0.2 mmol) of methanol, and 0.1 ml of formic acid afforded a 30% yield of a 1:1 ratio of **4a** and **5** (NMR). Chromatography over 3 g of silica gel and elution with benzene–EtOAc (99:1 v/v) and with benzene–EtOAc (98:2 v/v) yielded, respectively, 14 mg of **4a** and 15 mg of **5a**.

5-Phenyl-6-epiphenoxymethylpenicillin Potassium Salt (9). A solution of 450 mg (0.67 mmol) of **4a** in 35 ml of EtOAc was hydrogenated over 450 mg of 10% Pd/C for 6 hr at room temperature and at a pressure of 1.6 kg/cm^2. The catalyst was filtered off and washed with a total volume of 250 ml of EtOAc. The combined filtrates were concentrated to 40 ml. The potassium salt was extracted at pH 7 (0.25 *N* KOH) in 50 ml of water. Freeze-drying of the aqueous layer yielded 140 mg (45%) of **9** as a hygroscopic and amorphous powder. The organic layer was dried (Na_2SO_4) and filtered and addition of *n*-heptane gave 180 mg of **9** (40%). Crystalline material was obtained by precipitation with ether from a concentrated solution of **9** in EtOAc: mp 132–136° dec; $[\alpha]^{25}D$ +3° (*c* 0.1, water); ir 3410 (NH), 1755 (β-lactam), 1668, 1535 (amide I and II), 1597 (COO^-), 750 and 688 cm^{-1} (phenyl). Anal. ($C_{22}H_{21}O_5N_2SK$) C, H, N.

5-Phenylphenoxymethylpenicillin Potassium Salt (8). A solution of 350 mg (0.67 mmol) of **5** in 25 ml of EtOAc was hydrogenated over 350 mg of 10% Pd/C for 6 hr at room temperature and at a pressure of 1.6 kg/cm^2. The catalyst was filtered off and washed with EtOAc (5 × 40 ml). The combined filtrates were concentrated to 30 ml, and 40 ml of water was added. The cooled and stirred mixture was adjusted to pH 7 with 0.25 *N* KOH. Freeze-drying of the aqueous layer yielded the potassium salt of **8** as a hygroscopic and amorphous powder (140 mg, 45%). The powder was dissolved in EtOAc and crystals of **7b** were obtained by adding ether: mp 116–119° dec; $[\alpha]^{25}D$ −10° (*c* 0.2, water); ir 3400 (NH), 1767 (β-lactam), 1680, 1513 (amide I and II), 1600, 1444 (COO^-), 750 and 690 cm^{-1} (double peaks, two phenyl groups). Anal. ($C_{22}H_{21}O_5N_2SK$) C, H, N.

Methyl 5-Phenyl-6α-azidopenicillanate (2b). The condensation of 2.49 g of methyl D-5,5-dimethyl-2-phenyl-2-thiazoline-4-carboxylate[17] (**1b**) with 1.19 g of azidoacetyl chloride, using the procedure described for the synthesis of **2a**, gave 1.49 g (34%) of **2b**: mp 97–99° (ether–n-hexane); $[\alpha]^{25}D$ +14° (*c* 0.2, acetone); mass spectrum *m/e* 304 $(M - N_2)^+$; ir 2109 (N_3), 1791 (β-lactam), 1746 cm^{-1} (ester); NMR ($CDCl_3$) δ 1.24 (3 H, s, CCH_3), 1.72 (3 H, s, CCH_3), 3.74 (3 H, s, $COOCH_3$), 4.60 (1 H, s, H-3), 4.85 (1 H, s, H-6), 7.2–7.82 (m, aromatic). Starting material (47%) was eluted with benzene–chloroform (25:75).

5-Phenyl-6-epiphenoxymethylpenicillin Methyl Ester (4b). Reduction of **2b**, using Adams catalyst under conditions outlined for **4a**, afforded **3b** as a viscous oil: ir (CH_2Cl_2) 3400 (br, NH_2), 1773 (β-lactam), 1745 cm^{-1} (ester). This product was used for the next operation without further purification. Treatment with phenoxyacetyl chloride afforded **4b**: mp 129–130° (Et_2O–*n*-hexane); $[\alpha]^{25}D$ +15° (*c* 1, acetone); ir 3260 (NH), 1780 (β-lactam), 1735 (ester) and 1670 cm^{-1} (amide); mass spectrum *m/e* 440; NMR ($CDCl_3$) δ 1.17 (3 H, s, CCH_3), 1.70 (3 H, s, CCH_3), 4.67 (1 H, s, H-3), 5.66 (d, *J* = 9 Hz, 1 H, H-6), 6.5–7.6 (m, aromatic). A mp of 127–129° was given for the same product,[19] but the configuration at C-3 was not stated.

6-Azido-7-oxo-5-phenyl-4-thia-1-azabicyclo[3.2.0]heptane (2c). A solution of 2.02 g (0.02 mol) of triethylamine in 150 ml of CH_2Cl_2 was added, under high dilution conditions, to a refluxing solution of 3.32 g (0.02 mol) of 2-phenyl-2-thiazoline[29] (**1c**) and 2.38 g (0.02 mol) of azidoacetyl chloride in 400 ml of CH_2Cl_2 over a period of 15 hr. The solvent was removed in vacuo, the residue was extracted with anhydrous ether, and the solvent was evaporated to a viscous residue. Chromatography over 50 g of silica gel, using benzene as eluting solvent, afforded 2.99 g (62%) of a white solid, which was crystallized from ether–n-hexane as white needles: mp 65–67°; mass spectrum *m/e* 218 $(M - N_2)^+$; ir 2107 (N_3), 1780 cm^{-1} (β-lactam); NMR ($CDCl_3$) δ 3.27, 4.32 (4 H, m, CH_2 groups), 4.9 (1 H, s, H-6), 7.4 (5 H, s, phenyl).

6-Phenoxyacetamido-7-oxo-5-phenyl-4-thia-1-azabicyclo-[3.2.0]heptane (4c). A solution of 1.2 g (4.8 mmol) of **2c** was dissolved in 100 ml of EtOAc and was hydrogenated in the presence of 1.2 g of Adams catalyst for 4 hr at atmospheric pressure. The catalyst was filtered off and washed five times with 30 ml of EtOAc. The combined filtrates were evaporated, leaving a residual oil (**3c**): ir (CH_2Cl_2) 3480 (NH_2), 1778 cm^{-1} (β-lactam); NMR ($CDCl_3$) δ 1.58 (s, br, NH_2), 3.21 (3 H, m, CH_2CH), 4.25 (1 H, m, CH), 4.56 (1 H, s, H-6), 7.4 (5 H, s, aromatic) (singlet at 1.58 disappeared when D_2O was added). The product was dissolved, without further purification, in 120 ml of anhydrous CH_2Cl_2 and 0.84 ml (6 mmol) of Et_3N, and a solution of 1.02 g of phenoxyacetyl chloride (6 mmol) in 50 ml of CH_2Cl_2 was added with stirring and cooling to −5° over a period of 1 hr. The solution was stirred 2 hr at 25°, washed with five portions of water, dried, and evaporated to a yellow gum. This gum was chromatographed over 60 g of silica gel using benzene. Some starting material (**2c**) was eluted with 600 ml of benzene and with 500 ml of benzene–acetone (99:1) 1.21 g (71%) of **4c**: mp 117.5–118° ($CHCl_3$–*n*-hexane); r 3260 (NH), 1782 (β-lactam), 1670 cm^{-1} (amide); mass spectrum *m/e* 354; NMR ($CDCl_3$) δ 3.22 (3 H, m, CH_2CH), 4.17 (2 H, d, *J* = 15 Hz, $OCH_2C_6H_5$), 4.23 (1 H, m, CH), 5.62 (1 H, d, *J* = 9 Hz, H-6), 6.43–7.37 (11 H, m, phenyl and NH) (doublet at 5.62 becomes a singlet when D_2O was added). Anal. ($C_{19}H_{18}O_3N_2S$) C, H, N.

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Stereospecific Synthesis of the 6β-Hydroxy Metabolites of Naltrexone and Naloxone

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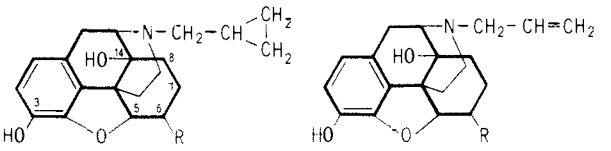
The narcotic antagonists naltrexone (1a) and naloxone (**2a**) were stereospecifically reduced to the corresponding 6β-hydroxy epimers **1b** and **2b**, respectively, with formamidinesulfinic acid in an aqueous alkaline medium. The reaction products were obtained with no detectable quantity of the 6α epimers **1c** and **2c**. The products **1b** and **2b** were formed in yields of 88.5 and 40%, respectively, and characterized by spectral methods. Compared to **1a** and 2a, the stereospecific reduction products **1b** and **2b** and their 6α epimers **1c** and **2c** are all significantly less potent as narcotic antagonists in mice. Only **1c** and **2c** also possess antinociceptive activity.

The ability of narcotic antagonists such as naltrexone (**1a**) and naloxone (2a) to block the euphorigenic and dependence producing effects of narcotics forms the pharmacologic basis for the use of these drugs in the treatment of opiate dependence. Compared to naloxone, naltrexone has been found to be more potent and to have a longer duration of antagonist action in laboratory rodents[1,2] and man.[3] In addition, naltrexone is an effective antagonist in man at oral doses of 30–50 mg/day, while an equieffective oral dose of naloxone would be much larger (up to 3000 mg/day).[3,4]

In man the major metabolite of naloxone is the 3-glucuronide,[5] whereas the 6-keto reduction product, a 6β-hydroxy derivative (**1b**), is the major metabolite of naltrexone.[6,7] Comparative studies of the biotransformation of both 1a and **2a** have revealed species variation in the stereochemistry of the alcohol resulting from reduction of the 6-keto group.[8] Our interest in both the role of biotransformation in the relatively long duration of action of **1a**, and observed differences in biotransformation of **1a** and **2a**, necessitated a quantity of the appropriate 6β epimeric alcohol metabolites **1b** and **2b** for use as analytical standards and for pharmacologic characterization.

Chemical methods are readily available for the synthesis of 6α-hydroxy epimers **1c** and **2c**. These compounds are known as N-substituted 14-hydroxydihydronormorphines and are accessible through hydride reduction of the N-substituted 14-hydroxydihydronormorphinones.[9] No claim was found in the literature of a stereospecific chemical reduction of 6-keto compounds (having the morphine nucleus) to yield the 6β-hydroxy epimers. Although attempts have been made,[6,10] no successful chemical synthesis of **1b** and **2b** has been reported. The purpose of this report is to describe a method for the synthesis of **1b** and **2b** by a stereospecific reduction of the respective 6-keto compounds **1a** and **2a**.



1a, R = =O
b, R = ⁻OH, H
c, R = ⁻H, HO

2a, R = =O
b, R = ⁻OH, H
c, R = ⁻H, HO

Initial attempts by us to synthesize **1b** included a reduction procedure involving lithium tri-*sec*-butylborohydride[11] and **1a**. However, this reagent[12] yielded solely the 6α-hydroxy epimer **1c**. This course was therefore abandoned in view of the state of knowledge concerning hydride reductions of compounds of this class.[6,10,13] Our objective of a successful synthesis of **1b** and **2b** was, however, achieved in a procedure using the reaction of formamidinesulfinic acid,[14,15] in alkaline solution, with naltrexone (**1a**) and naloxone (**2a**). This procedure was a modification of that of Nakagawa and Minami in the reduction of various ketones.[16] The reduction of 1a yielded the 6β-hydroxy derivative **1b** in a yield of 88.5%, with no indication of the

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