in the first hour as indicated by TLC (the appearance of a single spot with the $R_f$ of VII). The dark brown reaction mixture was evaporated at 30–40 °C under reduced pressure to total dryness with a rotary evaporator. The dark brown residue was taken up in a small volume of water (10 ml) and repeatedly extracted with ether (20 ml × 10). The ether extract was washed with cold water (5 ml × 3) and dried over anhydrous sodium sulfate. A trace of pyridine (1 drop) was added and the ether solution evaporated at 30–40 °C under reduced pressure to afford colorless crystalline 18-OH-DOC (VII, mp 154–158 °C) in 94% yield (50 mg). The crude product was homogeneous as indicated by TLC: $R_f$ 0.23 ($SiO_2$ G), 0.31 ($SiO_2$ Eastman) in benzene–ethyl acetate (2:8). Recrystallization twice from acetone–hexane containing a trace of pyridine gave VII, mp 165–168 °C, $[\alpha]^{25}D$ +110.3° (*c* 0.21, $CHCl_3$) {lit.[19] $[\alpha]^{28}D$ +121° (aqueous 70% methanol)}. The infrared and mass spectra of VII were identical with those of an authentic sample of 18-OH-DOC obtained from Pappo.[8] The two samples showed identical mobility on TLC and paper chromatogram.

Pappo[8] pointed out that the melting point of 18-OH-DOC can vary depending on the solvent of crystallization.

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## References and Notes

(1) M. K. Birmingham and P. J. Ward, *J. Biol. Chem.*, **236**, 1661 (1961).
(2) F. G. Péron, *Endocrinology*, **69**, 39 (1961).
(3) J. M. Cortés, F. G. Peron, and R. I. Dorfman, *Endocrinology*, **73**, 713 (1963).
(4) G. J. Race and H. M. Wu, *Gen. Comp. Endocrinol.*, **4**, 199 (1964).
(5) J. C. Melby, T. E. Wilson, and S. L. Dale, *J. Clin. Invest.*, **49**, 64a (1970).
(6) J. C. Melby, S. L. Dale, and T. E. Wilson, *Circ. Res., Suppl. 11*, **28** and **29**, 143 (1971).
(7) J. T. Oliver, M. K. Birmingham, A. Bartova, M. P. Li, and T. H. Chan, *Science*, **182**, 1249 (1973).
(8) R. Pappo, *J. Am. Chem. Soc.*, **81**, 1000 (1959).
(9) R. Pappo, U.S. Patents 2 891 948 (1959) and 2 911 409 (1959).
(10) M. P. Li, C. P. Lantos, H. Traikov, M. V. Birmingham, and T. H. Chan, *J. Steroid Biochem.*, **1**, 259 (1970).
(11) M. Biollaz, J. Kalvoda, and J. Schmidlin, *Helv. Chim. Acta.*, **58**, 1475 (1975).
(12) D. N. Kirk and M. S. Rajagopalan, *J. Chem. Soc., Chem. Commun.*, 145 (1974).
(13) H. Wehrli, M. Cereghetti, K. Schaffner, and O. Jeger, *Helv. Chim. Acta*, **43**, 367 (1960).
(14) O. Jeger and K. Schaffner, U.S. Patent 3 131 180 (1964).
(15) C. Meystre, K. Heuster, J. Kalvoda, P. Wieland, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, **45**, 1317 (1962).
(16) H. Levy, D. I. Cargill, C. H. Cha, B. Hood, and J. Carlo, *Steroids*, **5**, 131 (1965).
(17) O. V. Dominguez, *Steroids, Suppl. II*, 29 (1965).
(18) P. Genard, M. Palem-Vliero, P. Coninx, M. Margonlies, F. Compernolle, and M. Vandewalle, *Steroids*, **12**, 763 (1968).
(19) M. P. Li, Ph.D. Dissertation, McGill University, 1973.
(20) J. Dillon and K. Nakanishi, *J. Am. Chem. Soc.*, **97**, 5417 (1975), and references cited therein.
(21) H. Wehrli, M. Cereghetti, K. Schaffner, J. Urech, and E. Vischer, *Helv. Chim. Acta*, **44**, 1927 (1961).
(22) R. Antonucci, S. Bernstein, R. Lenhard, K. J. Sax, and J. H. Williams, *J. Org. Chem.*, **17**, 1396 (1952).
(23) P. Buchschacher, M. Cereghetti, H. Wehrli, K. Schaffner, and O. Jeger, *Helv. Chim. Acta*, **42**, 2122 (1959).

# Determination of the Configuration of the Four D-Benzylpenicilloates

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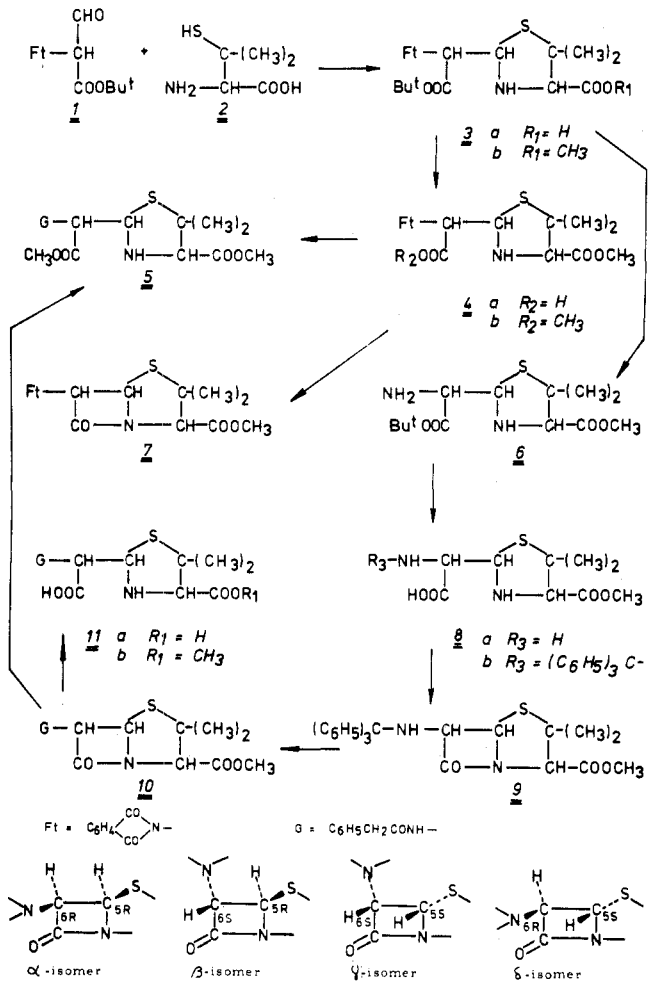
The configuration of the four dimethyl D-benzylpenicilloates was determined, and their stereochemical relationship to the dimethyl phthalimidopenicilloates, prepared according to Sheehan et al., was established, using a series of transformations and physicochemical techniques. The compounds designated as $\beta$ and $\gamma$ isomers in the Sheehan nomenclature were found to correspond to the $\gamma$ and $\delta$ isomers of "Chemistry of Penicillin", and to have the 5*R*,6*S* and the 5*S*,6*S* configuration, respectively. In the course of this study, 5-epi-6-epibenzylpenicillin methyl ester ($\gamma$-**10**) having the 5*S*,6*S* configuration was prepared for the first time. The isomer with the 5*S*,6*R* configuration, which was consequently denoted as $\delta$ isomer, was prepared from 5-epibenzylpenicillin methyl ester, and was identical with the $\beta$ isomer of "Chemistry of Penicillin". It was also established that sodium benzylpenicilloate $\alpha$ isomer, having the 5*R*,6*R* configuration of the natural penicillins, isomerized in aqueous solution to a mixture, containing mainly the $\delta$ isomer (5*S*,6*R*).

The first step in the Sheehan synthesis of penicillin[1] is the condensation of *tert*-butyl phthalimidomalonaldehydate (**1**) with D-penicillamine (**2**). When this reaction was performed in alcohol–water, containing sodium acetate, only two of the four possible[2] phthalimidopenicilloates,[3] called $\alpha$ and $\gamma$ isomers, were formed,[5] the $\gamma$ isomer being the major component. Using pyridine as solvent, the main product was the $\alpha$ isomer,[6] which has also been obtained by heating the $\gamma$ isomer in the same solvent.[1] It has been shown[1,5] that the $\alpha$ isomer has the same configuration as the natural penicillins, namely 5*R*,6*R*,[7] whereas the stereochemistry of the $\gamma$ isomer is unknown. When the *tert*-butyl group of **3b** was removed with acid at about 75 °C, another isomer of unknown configuration, designated as $\beta$ isomer, was formed. It could be cyclized to a 6-phthalimidopenicillanate (**7**) by treatment with thionyl chloride.[5,8]

During the wartime research on penicillin, four benzylpenicilloates (**5**), designated by the letters $\alpha$, $\beta$, $\gamma$, and $\delta$, were obtained.[4] Their configuration, except that of the $\alpha$ isomer, which corresponds to the natural penicillin, is unknown and their relation to the phthalimidopenicilloates (**3**) of Sheehan has not been investigated.

In order to determine the configuration of the $\gamma$ isomer of **3a**, we transformed this product into a penicillin using the second Sheehan synthesis,[9] which we had applied previously.[6] Hydrazinolysis of $\gamma$-**3b**, prepared by methylation of $\gamma$-**3a**, yielded $\gamma$-**6**, from which the *tert*-butyl group was removed with acid, to give $\gamma$-**8a**. This compound was transformed into $\gamma$-**8b**, by treatment with triphenylchloromethane, and cyclized with diisopropylcarbodiimide to methyl 6-tritylaminopenicillanate ($\gamma$-**9**). From the NMR spectrum a cis configuration of the hydrogen atoms on C-5 and C-6 could be deduced. As this compound was different from methyl (5*R*,6*R*)-tritylaminopenicillanate, prepared from natural 6-aminopenicillanic acid,[9] it can be concluded that the $\gamma$ isomers have a 5*S*,6*S* stereochemistry. Detritylation of $\gamma$-**9** and phenylacetylation



led to a benzylpenicillin methyl ester ($\gamma$-**10**), whose NMR spectrum confirmed the 5*S*,6*S* configuration. To our knowledge, this is the first time that a penicillin with 5-epi-6-epi configuration is reported.

On the other hand, $\gamma$-**3b** was transformed into dimethyl benzylpenicilloate (**5**), by cleavage of the *tert*-butyl ester and esterification to $\gamma$-**4b**, followed by removal of the phthaloyl group and phenylacetylation. This benzylpenicilloate ($\gamma$-**5**) was identical with the compound obtained by methanolysis of $\gamma$-**10**, which shows that no inversion of configuration occurred during the transformation of $\gamma$-**3b** to $\gamma$-**10**. Methanolysis was carried out in the presence of diazomethane, a method used for the first time in this work. This procedure was also applied to natural and 6-epibenzylpenicillin methyl ester, and gave the same yield as the more tedious triethylamine-catalyzed methanolysis. However, no alcoholysis of the $\beta$ lactam was observed when benzylpenicillin methyl ester was treated with ethanol and diazomethane. The physical constants of $\gamma$-**5** corresponded to those of the $\delta$ isomer of "Chemistry of Penicillin", which had been prepared by hydrolysis of benzylpenicillin in the presence of copper sulfate or by treatment of the $\alpha$-penicilloate with copper sulfate, followed by reaction of the copper salt with diazomethane.[10]

The $\beta$ isomer of **4a** could be obtained either from $\alpha$-**3b** or $\gamma$-**3b** by treatment with anhydrous hydrogen chloride at 75 °C. Using the procedure described for the DL analogue,[8] $\beta$-**4a** was cyclized to **7** with thionyl chloride. The methyl 6-phthalimidopenicillanate ($\beta$-**7**) thus obtained had the 6-epi configuration, and was identical with the product obtained by base-catalyzed epimerization of **7** having the natural configuration.[11,12] The products of the $\beta$ series consequently have the 5*R*,6*S* configuration.

In order to establish the correlation with the benzylpenicilloates described in "Chemistry of Penicillin", the methyl ester of 6-epibenzylpenicillin[13] was subjected to methanolysis. Dimethyl benzylpenicilloate (**5**), which was obtained in this way, had the same physical properties as the $\gamma$ isomer, which was prepared by condensation of methyl benzylpenaldate (methyl phenylacetamidomalonaldehydate) with penicillamine, followed by esterification of the thiazolidine carboxy group with diazomethane.[14]

A possible modification of the stereochemistry of C-5 or C-6, during the conversion of $\beta$-**4a** into **7**, was excluded by transformation of $\beta$-**4a** to $\beta$-**5**. Esterification of the carboxyl group of $\beta$-**4a** with diazomethane gave $\beta$-**4b**, which by removal of the phthaloyl group and phenylacetylation yielded $\beta$-**5**. The dimethyl benzylpenicilloate ($\beta$-**5**) thus obtained was identical with the compound obtained by methanolysis of the methyl ester of 6-epibenzylpenicillin, which confirms the previously assigned 5*R*,6*S* configuration to the $\beta$ series.

The fourth phthalimidopenicilloate, which we shall call the $\delta$ isomer, must have the 5*S*,6*R* configuration of the 5-epipenicillins. Methyl 6-phthalimido-5-epipenicillanate has been prepared recently[15] by opening of the thiazolidine of natural **7** with chlorine and ring closure of the methyl 2*S*-chloro-$\alpha$-(1-chlorothio-1-methylethyl)-4-oxo-3-phthalimido-1-azetidineacetate thus obtained with stannous chloride.

5-Epibenzylpenicillin methyl ester ($\delta$-**10**), which was prepared from the 5 epimer of **7**,[16] could not be transformed directly into **5** by reaction with methanol in the presence of triethylamine or diazomethane, a method which has been used for the preparation of other penicilloates, apparently because of a greater stability of the $\beta$-lactam ring. For this reason, $\delta$-**10** was hydrolyzed with sodium hydroxide, yielding a mixture of penicilloates (**11b**), from which $\delta$-**5** was isolated after reaction with diazomethane. This compound presented the physical properties of the $\beta$ isomer of "Chemistry of Penicillin", where it has been obtained by refluxing $\alpha$- or $\gamma$-**5** ("Chemistry of Penicillin" nomenclature) in toluene in the presence of some iodine.[17] Condensation of methyl benzylpenaldate with penicillamine methyl ester in boiling toluene also gave this $\beta$ isomer together with the $\gamma$ isomer (i.e., the $\delta$ and the $\beta$ isomer in the Sheehan nomenclature).[17]

The phthalimidopenicilloate of this series ($\delta$-**4b**) could not be prepared directly. It was detected by TLC in the reaction mixture obtained during the transformation of $\alpha$-**4b** or $\gamma$-**4b** into $\beta$-**4b** by treatment with *p*-toluenesulfonic acid in nitromethane solution. The NMR spectrum of the mixture provided the data of $\delta$-**4b**.

The most significant NMR values of the four isomers of the dimethyl penicilloates of the benzyl (**5**) and the phthalimido series (**4b**) are given in Table I. For the phthalimidopenicilloates (**4b**), the chemical shift of the C-3 proton appears at higher field in the compounds with 5*S* configuration than in the 5*R* isomers. This shielding had been observed previously in carboxythiazolidines[7] derived from D-penicillamine, when the C-3 proton was cis oriented relative to the C-5 proton, and it had led to the correct assignment of a 5*S* configuration to $\gamma$-**4b**.[18] This correlation between the chemical shift of the C-3 proton and the configuration at C-5 does not occur in the benzylpenicilloates (**5**). This difference can be explained by a difference of conformation, and/or by the greater flexibility of a phenylacetamido compared to a phthalimido group.

It has been observed[19,20] that the p*K* value of the protonated thiazolidine of benzylpenicilloate (**11a**) decreases from 5.3 to 4.7 when a neutral or alkaline solution of **11a** is kept for several hours. This change of p*K* was explained by the transformation of penicilloic acid to penamaldic acid.[19] This conversion was unlikely, because we observed that the solution even after storage for 50 h presented only the weak phenyl bands around 260 nm, and not the strong absorption at 280 nm of penamaldate. We also found[20] that there was a paral-

Table I. NMR Data[a] for Penicilloates

| | Nomenclature | | | | | |
|---|---|---|---|---|---|---|
| | Sheehan[b] | Chem. Pen.[c] | Configuration | H-3 | H-5 (J, Hz) | H-6 (J, Hz) |
| **4b** | α | | 5R,6R | 3.78 | 5.31 (10) | 4.93 (10) |
| | β | | 5R,6S | 3.87 | 5.86 (9.5) | 4.73 (9.5) |
| | γ | | 5S,6S | 3.59 | 5.34 (8) | 5.14 (8) |
| | δ[d] | | 5S,6R | 3.66 | 5.58 (7) | 5.15 (7) |
| **5** | α | α | 5R,6R | 3.31 | 5.09 (4) | 4.61 (4) |
| | β[d] | γ | 5R,6S | 3.43 | 5.10 (4.3) | 4.86 (4.3) |
| | γ[d] | δ | 5S,6S | 3.53 | 4.95 | 4.95 |
| | δ[d] | β | 5S,6R | 3.49 | 5.05 (2.5) | 5.15 (2.5) |

[a] In parts per million using $Me_4Si$ as internal reference. [b] Nomenclature used by Sheehan et al. [c] Nomenclature of "Chemistry of Penicillin". [d] Nomenclature used for the first time in this publication.

lelism between the change of p$K$ and the mutarotation of the solution of benzylpenicilloate. This change of rotation had already been observed.[21] When a solution of natural (α isomer) benzylpenicilloate was kept until a constant $[\alpha]_D$ was obtained (about 70 h) and lyophilized, and then treated with diazomethane, we found that the four isomers were present, but that the δ isomer (5S,6R) was the main component (about 70%).

## Experimental Section

Melting points were determined in open capillaries with a Büchi-Tottoli apparatus. Solvents were evaporated under reduced pressure below 30 °C. TLC was performed on silica gel F-254 plates (Merck) using the following mobile phases: I, $C_6H_6$–EtOAc–HCOOH, 20:10:0.25; II, $C_6H_6$–$Me_2CO$, 90:10; III, $C_6H_6$–$Me_2CO$, 80:20; IV, $n$BuOAc–$n$-BuOH–$H_2O$–MeOH–HOAc, 80:15:24:5:40. Spots were located by uv illumination and by exposure to iodine vapor. Column chromatography was performed on silica gel (Merck 0.06–0.2 mm). The optical rotation was measured at room temperature on a Thorn-NPL automatic polarimeter Type 243. Infrared spectra were recorded on a Perkin-Elmer 257 spectrometer. Mass spectra were recorded on an AEI MS-12 apparatus, and NMR spectra on a Hitachi Perkin-Elmer R 24 apparatus with tetramethylsilane for $CDCl_3$ solutions, and with the sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSSA) for $D_2O$ solutions, as internal standard.

**Methyl (3-Carbomethoxy-2,2-dimethyl-5-thiazolidino)-phthalimidoacetate α Isomer (5R,6R-4b).** *tert*-Butyl (3-carboxy-2,2-dimethyl-5-thiazolidino)phthalimidoacetate (α-**3a**) was prepared in 70% yield by condensing D-penicillamine (**2**) and *tert*-butyl phthalimidomalonaldehydate (**1**) in pyridine at 80 °C, as described by Hoogmartens et al.[6] This compound was dissolved in $CH_2Cl_2$ and esterified at 0 °C with diazomethane in ether. After evaporation of the solvent and recrystallization in absolute ethanol α-**3b** was obtained in 93% yield, mp 176–177 °C, TLC (system II) $R_f$ 0.73, $[\alpha]^{23}D$ −2.5° (c 2.0, dioxane), in agreement with previously reported values.[1]

Removal of the *tert*-butyl ester group of α-**3b** was performed by treatment of this compound with anhydrous hydrogen chloride in freshly distilled nitromethane at 0 °C over a period of 10 min, as described for the DL compound.[5] The resulting amino acid hydrochloride, which crystallized after addition of anhydrous ether to the cold concentrated nitromethane solution and after storage for 2 h at 0 °C (yield 70%, mp 108–110 °C dec), was suspended in anhydrous ether and treated with a slight excess of diazomethane in ether at 0 °C. Compound α-**4b** was obtained as an amorphous powder by evaporating the solvent under reduced pressure: TLC (system II) $R_f$ 0.67; $[\alpha]^{22}D$ −2° (c 1.0, MeOH); *m/e* 392; ir (KBr) 3340 (NH), 1780, 1715 (imide), 1740, 1215 (ester), 725 $cm^{-1}$ (phenyl); NMR ($CDCl_3$) δ 1.19 (s, $CH_3$), 1.64 (s, $CH_3$), 3.2 (br, NH), 3.71 and 3.73 (two s, $OCH_3$), 3.78 (s, H-3), 4.93 (d, $J$ = 10 Hz, H-6), 5.31 (d, $J$ = 10 Hz, H-5), 7.6–8.0 (m, $C_6H_4$). One of the two isomers, prepared[22] by condensation of methyl phthalimidomalonaldehydate and D-penicillamine, and esterification with diazomethane, had mp 138–139 °C, $[\alpha]^{25}D$ −5° (c 1, MeOH).

**Dimethyl Benzylpenicilloate α Isomer (5R,6R-5). A. From Sodium Benzylpenicillin.** Sodium benzylpenicillin (4.2 g, 11.8 mmol) was dissolved in 250 ml of methanol containing 2.0 ml (14.3 mmol) of triethylamine. After keeping for 45 h at room temperature, the solvent was distilled off under reduced pressure and the residue was taken up in 50 ml of water and 50 ml of ether. After cooling to 0 °C, the mixture was acidified (pH 3.6) with 1 N HCl, the ethereal phase was decanted, and the aqueous phase was further extracted with four volumes of ether. The combined organic layer was dried ($Na_2SO_4$) and evaporated. By crystallization from methanol–ether (1:9) 1.6 g (35%) of the C-7 methyl ester of benzylpenicilloate was obtained, mp 125.5–127 °C. This product was dissolved in 30 ml of dichloromethane and 10 ml of methanol, and treated with a slight excess of diazomethane in ether. The solvent was evaporated under reduced pressure, the residue was taken up in ether, and 2.36 g (12.4 mmol) of *p*-toluenesulfonic acid monohydrate was added. After storage for 2 h, the crystals were collected. They were suspended in ether, and the suspension was shaken with 5% $NaHCO_3$ solution and water. The ether layer was dried ($Na_2SO_4$) and evaporated, yielding 0.99 g (64% based on the monomethyl ester) of crystalline dimethyl benzylpenicilloate α isomer: mp 85–86 °C; TLC (system III) $R_f$ 0.52; $[\alpha]^{22}D$ +82° (c 0.5, MeOH); *m/e* 380 ($M^+$ very weak), 321 ($M - COOCH_3$)$^+$; ir (KBr), 3350 (NH), 3270, 1640, 1530 (amide), 1730, 1230 (ester), 725, 695 $cm^{-1}$ (phenyl); NMR ($CDCl_3$) δ 1.12 (s, $CH_3$), 1.43 (s, $CH_3$), 2.94 (br, NH), 3.31 (s, H-3), 3.62 (s, $CH_2$), 3.71 (s, two $OCH_3$), 4.61 (dd, $J$ = 4 and 9 Hz, H-6), 5.09 (d, $J$ = 4 Hz, H-5), 6.20 (d, $J$ = 9 Hz, NHCO), 7.34 (s, $C_6H_5$).

"Chemistry of Penicillin" [23] gives mp 87–89 °C, $[\alpha]^{25}D$ +81.5° (c 1, MeOH).

**B. From Benzylpenicillin Methyl Ester.** Benzylpenicillin methyl ester[13,24] (2 g, 5.7 mmol) was dissolved in 5 ml of absolute methanol and 5 ml of dry ether, and 10 ml of ether containing 6 mmol of diazomethane was added. After standing overnight, the solution was evaporated under reduced pressure, and the resulting oil was dried in vacuo over $P_2O_5$. After addition of dry ether, 1.540 g (71%) of crystals was obtained, with the same physical constants as the product described under A.

***tert*-Butyl (3-Carboxy-2,2-dimethyl-5-thiazolidino)phthalimidoacetate γ Isomer (γ-3a).** This compound was prepared by condensing equimolecular amounts of *tert*-butyl phthalimidomalonaldehydate and D-penicillamine hydrochloride in ethanol–water containing sodium acetate, as described by Sheehan et al.[1] After storage for 16 h at room temperature a first crop of crystals was obtained, followed by a second one, obtained by partial evaporation of the filtrate. The purity of γ-**3a**, after two recrystallizations from MeOH, was checked by TLC in the system I ($R_f$ 0.23), which allowed the distinction from α-**3a** ($R_f$ 0.34). A certain amount (15%) of α-**3a** was isolated by heating the residue, obtained by evaporation of the filtrates, in pyridine at 80 °C. Total yield of γ-**3a** 42%; mp 155–157 °C; $[\alpha]^{22}D$ −19° (c 1.0, dioxane), +6° (c 1.0, HOAc); NMR [$CDCl_3$–$(CD_3)_2SO$ (2:1)] δ 1.22 (s, $CH_3$), 1.41 (s, *t*-Bu), 1.60 (s, $CH_3$), 3.43 (s, H-3), 4.87 (d, $J$ = 9 Hz, H-6), 5.17 (d, $J$ = 9 Hz, H-5), 7.75 (m, $C_6H_4$). Sheehan et al.[1] reported a melting point of 145–146 °C and $[\alpha]^{23}D$ +22° (c 1, HOAc), and Hoogmartens et al.[6] give $[\alpha]^{25}D$ −13° (c 1, dioxane). The more negative rotation reported here is probably the correct one, since in those reports the γ isomer was purified by recrystallization from the more polar solvent system methanol–water. The observed difference in optical rotation may be easily explained by assuming a contamination of γ-**3a** with a small amount of β isomer, which in analogy with the values of the isomers of **4b**, should have a high positive $[\alpha]_D$ and a $R_f$ value similar to that of the γ isomer.

***tert*-Butyl (3-Carbomethoxy-2,2-dimethyl-5-thiazolidino)-phthalimidoacetate γ Isomer (γ-3b).** Compound γ-**3a** (8.48 g, 20.2 mmol) was dissolved in 350 ml of dry dichloromethane, and treated at 0 °C with an ethereal solution of diazomethane until a yellow color persisted. By evaporation of the solvent and crystallization in absolute ethanol, 6.54 g (74.5%) of γ-**3b** was obtained: mp 126–128 °C; TLC

(system II) $R_f$ 0.64; $[\alpha]^{22}D$ −18.5° (c 1.0, dioxane); NMR ($CDCl_3$) δ 1.17 (s, $CH_3$), 1.40 (s, t-Bu), 1.58 (s, $CH_3$), 3.30 (br, NH), 3.51 (s, H-3), 3.67 (s, $OCH_3$), 4.92 (d, J = 9 Hz, H-6), 5.22 (d, J = 9 Hz, H-5), 7.6–7.8 (m, $C_6H_4$). Upon addition of $D_2O$, the signal at 3.30 ppm disappeared and a sharpening of the signals at 3.51 and 5.22 ppm was observed. Melting point and [α]D are in agreement with those previously reported[1] for the same compound, prepared in 43% yield by esterification in dioxane.

***tert*-Butyl (3-Carbomethoxy-2,2-dimethyl-5-thiazolidino)-aminoacetate Hydrochloride γ Isomer (γ-6).** A solution of 4.34 g (10 mmol) of γ-**3b** in 80 ml of pure dioxane and 0.6 ml (12 mmol) of hydrazine hydrate was stored for 21 h at room temperature under a nitrogen atmosphere, as described for the DL compound.[5] The reaction mixture was freeze dried, and the residue was dried in vacuo over $P_2O_5$. The white powder was stirred for 2 h in 64 ml of 0.2 N HCl, and after cooling for 15 min, the precipitate of phthalhydrazide was filtered off; the filtrate was lyophilized and dried over $P_2O_5$ and KOH. Crystallization from MeOH–$Et_2O$ yielded 2.62 g (77%) of γ-**6** in two fractions: mp 152–154 °C dec; TLC (system IV) $R_f$ 0.55; ir (KBr) 3250 (NH), 2890 ($NH_3^+$), 1740, 1720, 1215, 1155 $cm^{-1}$ (ester).

**Methyl 6-Tritylaminopenicillanate γ Isomer (5*S*,6*S*-9).** A solution of 3.7 g (10.9 mmol) of γ-**6** in 60 ml of anhydrous nitromethane was cooled to 0 °C, and a stream of dry HCl was bubbled through the stirred solution for 30 min. The reaction mixture was stored for 4 h (storage for a longer time gave a lower yield) at 0 °C, and the major part of the HCl was removed under reduced pressure. Anhydrous $Et_2O$ (120 ml) was added gradually, and the precipitate was filtered off, washed with $Et_2O$, and dried for 3 h in vacuo over $P_2O_5$ and KOH. Recrystallized triphenylchloromethane (7.45 g, 25.5 mmol) was added to the dried precipitate (**8a** HCl), followed by a solution of 150 ml of anhydrous $CH_2Cl_2$ and 7.72 g (60 mmol) of *N*-ethyldiisopropylamine. The reaction mixture was immediately cooled in dry ice–butanol, stirred for 0.5 h, and stored for 20 h at −13 °C. Until this stage of the procedure contact with humidity was avoided as much as possible. The reaction mixture was poured into 150 ml of ice–water, and immediately adjusted to pH 6 with dilute $H_3PO_4$. The organic layer was decanted, washed twice with water, dried ($Na_2SO_4$), and evaporated at room temperature. The oily residue of **8b** was dissolved in 70 ml of $CH_3NO_2$, containing 2.81 g (22.4 mmol) of diisopropylcarbodiimide (DICI), stored overnight at room temperature, and evaporated. Rapid chromatography of the residue over silica gel (100 g) using benzene as eluent, followed by a second chromatographic purification of the collected fractions containing the desired product, yielded 1.2 g (2.55 mmol, 22%) of 5*S*,6*S*-**9** as an amorphous product: TLC (in benzene) $R_f$ 0.1; $[\alpha]^{20}D$ −96° (c 1.0, $CHCl_3$), −96° (c 1.0, n-BuOAc); *m/e* 472; ir (KBr) 3290 (NH), 1780 (β-lactam), 1740, 1210 (ester), 745, 705 $cm^{-1}$ (phenyl); NMR ($CDCl_3$) δ 1.50 (s, two $CH_3$), 3.30 (br, NH), 3.60 (s, H-3), 3.78 (s, $OCH_3$), 4.04 (d, J = 4.5 Hz, H-5), 4.38 (br, H-6, upon addition of $D_2O$, d, J = 4.5 Hz), 7.35 (s, $C_6H_5$).

Methyl 6-tritylaminopenicillanate, prepared by reaction of triphenylchloromethane with natural 6-aminopenicillanic acid followed by esterification with diazomethane,[9] had mp 163–165 °C; $[\alpha]^{31}D$ +100° (c 1, n-BuOAc); NMR ($CDCl_3$) δ 1.22 (s, $CH_3$), 1.48 (s, $CH_3$), 3.26 (br, NH), 4.33 (s, H-3), 4.42 (m, J = 4 Hz, H-5 and H-6), 7.32 (m, $C_6H_5$).

**Benzylpenicillin Methyl Ester γ Isomer (5*S*,6*S*-10).** A solution of p-toluenesulfonic acid monohydrate (190 mg, 1 mmol) in 20 ml of anhydrous $Me_2CO$ was added to a solution of γ-**9** (472 mg, 1 mmol) in $Me_2CO$ (20 ml). The reaction mixture was stirred at room temperature for 30 min and evaporated to dryness. The resulting oil was washed twice with $Et_2O$, and dried in vacuo over $P_2O_5$ for 2 h. This tosylate salt (420 mg) was dissolved in 30 ml of a solution of dry $CH_2Cl_2$ and 101 mg (1 mmol) of freshly distilled triethylamine. At 0 °C, solutions of triethylamine (111 mg, 1.1 mmol) and phenylacetyl chloride (166 mg, 1.1 mmol) in $CH_2Cl_2$ (20 ml for each) were added gradually to the cooled and vigorously stirred solution. After stirring for 2 h, the reaction mixture was washed successively with 0.01 N HCl (2 × 10 ml), 5% $NaHCO_3$ (2 × 20 ml), and water. The organic layer was dried ($Na_2SO_4$), evaporated, and purified by chromatography over silica gel (15 g) using benzene–acetone (99:1) as eluent. The fractions containing the desired product were collected and evaporated, yielding 160 mg (46%) of γ-**10** as an oil: TLC (system III) $R_f$ 0.60; $[\alpha]^{20}D$ −236° (c 0.7, acetone); *m/e* 348; ir (KBr) 3200, 1660, 1515 (amide), 1780 (β-lactam), 1740, 1210 $cm^{-1}$ (ester); NMR ($CDCl_3$) δ 1.30 (s, $CH_3$), 1.58 (s, $CH_3$), 3.61 (s, $CH_2$), 3.74 (s, $OCH_3$), 3.78 (s, H-3), 5.21 (d, J = 4 Hz, H-5), 5.51 (dd, J = 4 and 9 Hz, H-6), 6.40 (d, J = 9 Hz, NHCO), 7.35 (s, $C_6H_5$).

**Methyl (3-Carbomethoxy-2,2-dimethyl-5-thiazolidino)-phthalimidoacetate γ Isomer (γ-4b).** The *tert*-butyl group of γ-**3b** was removed by treatment with anhydrous hydrogen chloride at 0 °C for a period of 10 min, as described for the DL compound.[5] The amino acid hydrochloride (γ-**4a**) crystallized directly in 93% yield from the cold nitromethane solution (mp 120–122 °C dec). This compound was suspended in ether and treated with a slight excess of diazomethane in ether at 0 °C. By concentration of the solution, γ-**4b** was obtained in 84% yield: mp 145–147 °C; TLC (system II) $R_f$ 0.51; $[\alpha]^{22}D$ −28.5° (c 1.0, MeOH); *m/e* 392; ir (KBr) 3300 (NH), 1780, 1720 (imide), 1740, 1200 (ester), 710 $cm^{-1}$ (phenyl); NMR ($CDCl_3$) δ 1.21 (s, $CH_3$), 1.65 (s, $CH_3$), 3.6 (br, NH), 3.75 and 3.80 (two s, $OCH_3$), 3.59 (s, H-3), 5.14 (d, J = 8 Hz, H-6), 5.34 (d, J = 8 Hz, H-5), 7.6–8.0 (m, $C_6H_4$). One of the two isomers, prepared[22] by condensation of methyl phthalimidomalonaldehydate with D-penicillamine, and esterification with diazomethane, had mp 146–147 °C; $[\alpha]^{27}D$ −28° (c 1, MeOH).

**Dimethyl Benzylpenicilloate γ Isomer (5*S*,6*S*-5). A. From γ-4b.** An amount of 1.6 g (4.08 mmol) of γ-**4b** was dissolved in 40 ml of dioxane, and 0.240 g (4.8 mmol) of hydrazine hydrate was added. After keeping for 20 h at room temperature, the solvent and excess hydrazine were removed by lyophilization. The residue, after drying in vacuo over $P_2O_5$ for 2 h, was stirred for 2 h at room temperature in 25 ml of 0.2 N HCl. After cooling to 0 °C for 15 min, phthalhydrazide was removed by filtration and the filtrate was lyophilized. The residue (0.85 g, 2.8 mmol) was dissolved in 60 ml of $CH_2Cl_2$ at 0 °C, and 14.3 ml of a solution of 1.4 ml (10 mmol) of $Et_3N$ in 49 ml of $CH_2Cl_2$ was added, followed by a simultaneous and dropwise addition of 15.8 ml (3.1 mmol) of the same $Et_3N$ solution and of 4.1 g (3.1 mmol) of phenylacetyl chloride in 15 ml of $CH_2Cl_2$. The mixture was stirred for 2 h at 0 °C, and then extracted successively with 20 ml of 0.1 N HCl, 50 ml of 5% $NaHCO_3$ solution, and water. After drying ($Na_2SO_4$), the organic solvent was evaporated, and the residue was purified on a column of 10 g of silica gel using benzene–acetone (98:2) as eluent. The fractions containing the desired component were evaporated and crystallized from ether: 185 mg (12% overall yield); mp 106–108 °C; TLC (system III) $R_f$ 0.50; $[\alpha]^{22}D$ −41° (c 0.3, MeOH); *m/e* 380 ($M^+$ very weak), 321 $(M - COOCH_3)^+$; ir (KBr) 3280 (NH), 3240, 1640, 1555 (amide), 1740, 1210 (ester), 725, 695 $cm^{-1}$ (phenyl); NMR ($CDCl_3$) δ 1.11 (s, $CH_3$), 1.59 (s, $CH_3$), 3.2 (br, NH), 3.53 (s, H-3), 3.61 (s, $CH_2$), 3.74 and 3.75 (two s, $OCH_3$), 4.95 (m, H-5 and H-6; upon irradiation of NHCO, a singlet appeared), 6.50 (m, NHCO, coupled with H-6 and virtually coupled with H-5), 7.28 (s, $C_6H_5$). For dimethyl benzylpenicilloate δ isomer of "Chemistry of Penicillin" a mp 116–117 °C and $[\alpha]^{23}D$ −40° (c 1, MeOH) is given.[10]

**B. From Benzylpenicillin Methyl Ester γ Isomer.** A quantity of 175 mg (0.5 mmol) of γ-**10** was dissolved in 0.5 ml of absolute methanol and 1.5 ml of ether containing 0.6 mmol of diazomethane, and kept overnight. After evaporation of the solvent, the oily residue was chromatographed over silica gel (5 g) using benzene–acetone (99:1) as eluent. The first fraction (35 mg) contained starting material, the second one (50 mg) consisted of a mixture of α-**5** and γ-**5** in a ratio of 2:1 (as determined by NMR), and the third one (50 mg) contained almost pure γ-**5** (5*S*,6*S*-**5**), which was identical with the product prepared by method A.

**Methyl 6-Phthalimidopenicillanate β Isomer (5*R*,6*S*-7).** A stream of anhydrous HCl was passed for 7 min through a solution of 3.12 g (7.2 mmol) of α-**3b** in 60 ml of redistilled nitromethane, kept at 71–76 °C in an oil bath. The solution was kept for 16 h in the refrigerator. The crystals of β-**4a** hydrochloride were filtered off, washed with $CH_2Cl_2$, and dried over $P_2O_5$ and KOH in vacuo, yielding 1.50 g (50%), mp 149–150 °C dec. This product was suspended in 22.5 ml of purified thionyl chloride and 75 ml of dry $CH_2Cl_2$. The mixture, which became clear after 1.5 h, was refluxed for 4 h with stirring. After evaporation of the solvent, the residue was dissolved in 30 ml of $CH_2Cl_2$ and washed with 5% $NaHCO_3$ solution, 3 N HCl, and water. After drying over $Na_2SO_4$, the solvent was evaporated. The residue was dissolved in benzene and chromatographed over 8 g of silica gel using benzene and benzene–acetone (50:1) as eluent. The eluate was monitored by TLC in the system II. The fractions, containing the component with the highest $R_f$ value, were collected and evaporated. Treatment of the residue with ligroin yielded 130 mg (10%) of crystals. Recrystallization in acetone–ether–ligroin gave 85 mg of β-**7**: mp 178.5–180 °C; $[\alpha]^{20}D$ +203° (c 0.5, $CHCl_3$), +216° (c 0.5, dioxane); *m/e* 360; ir (KBr) 1785, 1728 (imide), 1770 (β-lactam), 1750, 1215 $cm^{-1}$ (ester); NMR ($CDCl_3$) δ 1.49 (s, $CH_3$), 1.67 (s, $CH_3$), 3.81 (s, $OCH_3$), 4.64 (s, H-3), 5.40 (d, J = 2 Hz, H-6), 5.59 (d, J = 2 Hz, H-5), 7.6–8.1 (m, $C_6H_4$).

The same physical constants are described for methyl 6-epiphthalimidopenicillanate, obtained by base-catalyzed epimerization of methyl 6-phthalimidopenicillanate.[11,12]

**Methyl (3-Carbomethoxy-2,2-dimethyl-5-thiazolidino)-phthalimidoacetate β Isomer (β-4b).** An amount of 2.37 g (5.7 mmol) of the hydrochloride salt of β-**4a**, which has also been used as

starting product for preparing β-7, was suspended in 80 ml of dry dichloromethane, and treated at 0 °C with an ethereal solution of diazomethane until a yellow color persisted. After evaporation of the solvent, the oily residue was purified by chromatography over silica gel (50 g), using benzene–acetone (97:3) as eluent, and crystallized from a minimum amount of absolute MeOH, yielding 0.53 g (23%) of β-**4b**: mp 119–122 °C; TLC (system II) $R_f$ 0.53; $[\alpha]^{22}D$ +121° (*c* 1.0, MeOH); *m/e* 392; ir (KBr) 3340 (NH), 1780, 1715 (imide), 1740, 1215 (ester), 725 $cm^{-1}$ (phenyl); NMR ($CDCl_3$) δ 1.23 (s, $CH_3$), 1.56 (s, $CH_3$), 3.7 (br, NH), 3.75 and 3.80 (two s, $OCH_3$), 3.87 (s, H-3), 4.72 (d, *J* = 9.5 Hz, H-6), 5.86 (d, *J* = 9.5 Hz, H-5), 7.6–8.0 (m, $C_6H_4$).

**Dimethyl Benzylpenicilloate β Isomer (5*R*,6*S*-5). A. From 6-Epibenzylpenicillin Methyl Ester.** 6-Epibenzylpenicillin methyl ester[13] (1.8 g, 5.3 mmol) was dissolved in 100 ml of absolute MeOH, and 0.76 ml (5.3 mmol) of freshly distilled $NEt_3$ was added. The optical rotation (0.5-dm tube) of the solution changed from 155° to a constant value of 79° after 65 h. The solvent was evaporated under reduced pressure. The residue was taken up in 50 ml of $CHCl_3$ and 50 ml of water, and acidified with 11 ml of 0.5 N HCl. The organic layer was decanted, washed with water, and dried with $Na_2SO_4$. After evaporation of the solvent, the residue was taken up in ether, and 1.4 g (70%) of β-**5** was obtained: mp 108–109 °C; TLC (system III) $R_f$ 0.46; $[\alpha]^{22}D$ +120° (*c* 1.0, MeOH); *m/e* 380 ($M^+$, very weak), 321 ($M - COOCH_3)^+$; ir (KBr) 3360 (NH), 3310, 1645, 1530 (amide), 1730, 1215 (ester), 730, 695 $cm^{-1}$ (phenyl); NMR ($CDCl_3$) δ 1.12 (s, $CH_3$), 1.30 (s, $CH_3$), 3.20 (br, NH), 3.43 (s, H-3), 3.61 (s, $CH_2$), 3.66 and 3.70 (two s, $OCH_3$), 4.86 (dd, *J* = 4.3 and 9 Hz, H-6), 5.10 (d, *J* = 4.3 Hz, H-5), 6.25 (d, *J* = 9 Hz, NHCO), 7.30 (s, $C_6H_5$).

By methanolysis of 6-epibenzylpenicillin methyl ester in MeOH–$Et_2O$ in the presence of a slight excess of diazomethane, as described for α-**5**, compound β-**5** was obtained in 72% yield. This method is more straightforward than the base-catalyzed methanolysis.

For dimethyl benzylpenicilloate γ isomer of "Chemistry of Penicillin" mp 110–110.5 °C and $[\alpha]^{22}D$ +122° (*c* 1, MeOH) are reported.[14]

**B. From β-4b.** Removal of the phthaloyl blocking group from β-**4b** (0.53 g, 1.3 mmol) and N-phenylacetylation of the resulting methyl (3-carbomethoxy-2,2-dimethyl-5-thiazolidino)aminoacetate hydrochloride were performed as described for the γ isomer (γ-**4b**), yielding, after column chromatographic purification and crystallization from ether, 0.20 g (40%) of β-**5**, which was identical with the product (5*R*,6*S*-**5**) prepared by method A.

**Dimethyl Benzylpenicilloate δ Isomer (5*S*,6*R*-5). A. From 5-Epibenzylpenicillin Methyl Ester.** A sample of 1.050 g (3 mmol) of 5-epibenzylpenicillin methyl ester[16] was dissolved in 54 ml of MeOH and 21 ml of water. To this solution was added dropwise 29 ml of 0.1 N NaOH over a period of 60 min. Methanol was distilled off in vacuo, and the residue was extracted with two 25-ml portions of ethyl acetate. The organic layer, from which 40% of starting material could be recovered, was extracted with water. The combined water layers were covered with EtOAc, cooled in ice–water, and acidified with $H_3PO_4$ (10%) to pH 3. The organic phase was decanted, and the water layer was extracted with two volumes of EtOAc. The combined organic layer was dried ($Na_2SO_4$) and evaporated. The residual oil was dissolved in 15 ml of $Et_2O$ and 15 ml of $CH_2Cl_2$, cooled in ice–water, and treated with a slight excess of ethereal diazomethane. After chromatography of the mixture over silica gel, using benzene–acetone (97:3) as eluent, three fractions were collected. The first fraction, evaporated to dryness and dissolved in ether, yielded, after standing for several days, 60 mg of crystalline δ-**5**: mp 110–111 °C; TLC (system III) $R_f$ 0.53; $[\alpha]^{22}D$ +1° (*c* 0.5, MeOH); *m/e* 380 ($M^+$ very weak), 321 ($M - COOCH_3)^+$; ir (KBr) 3330, 1655, 1520 (amide), 1735, 1210 (ester), 725, 695 $cm^{-1}$ (phenyl); NMR ($CDCl_3$) δ 0.83 (s, $CH_3$), 1.54 (s, $CH_3$), 3.49 (s, H-3), 3.68 and 3.70 (two s, $OCH_3$), 3.63 (s, $CH_2$), 5.15 (dd, *J* = 2.5 and 9.5 Hz, H-6), 5.05 (d, *J* = 2.5 Hz, H-5), 6.32 (d, *J* = 9.5 Hz, CONH), 7.30 (s, $C_6H_5$). From the third fraction, 80 mg of a crystalline compound was obtained, which was shown by TLC, NMR, and optical rotation to be the enantiomer of 5*R*,6*S*-**5**, and the second fraction consisted of a mixture of these two isomers together with some traces of other isomers of dimethyl benzylpenicilloate. The total yield from the methanolysis of methyl 5-epibenzylpenicillin, as determined by NMR, amounted to about 20% of 5*S*,6*R*-**5** and 20% of the enantiomer of 5*R*,6*S*-**5**, taking into account the 40% of recovered starting material. The formation of the enantiomer of 5*R*,6*S*-**5** during this transformation may be explained by assuming a partial epimerization of 5-epipenicillin methyl ester at position 3 before the hydrolysis took place.[16] By carrying out the reaction in other solvents such as dioxane–water, or by maintaining the pH during hydrolysis at a constant value of 9, 10, or 10.5, lower yields were obtained. Treatment of the 5-epipenicillin ester with diazomethane in methanol–ether for 16 h at room temperature gave only starting material. Dimethyl benzylpenicilloate β isomer of "Chemistry of Penicillin"[17] has mp 113–114 °C and $[\alpha]^{26}D$ +24.5° (*c* 0.5, MeOH). It should be noted that this isomer was obtained by refluxing in toluene the α or γ isomer ("Chemistry of Penicillin" nomenclature), which have a high $[\alpha]D$, and that the product probably was not pure.

**B. From Natural Benzylpenicilloic Acid (5*R*,6*R*-11a).** The $[\alpha]D$ of the monosodium salt of benzylpenicilloic acid[19–21] fell from 128° to 100° (after 5 h), 60° (after 20 h), 36° (after 40 and 65 h). Monosodium benzylpenicilloic acid (3.35 g) was dissolved in 150 ml of water, and the pH was adjusted to 7 with 2 N NaOH. After standing for 70 h, the solution was cooled in ice–water, and acidified in the presence of 100 ml of EtOAc with $H_3PO_4$ (20%) to pH 2.6. The EtOAc was decanted, and the water was extracted twice with EtOAc. The combined organic layer was dried ($Na_2SO_4$) and evaporated. The oily residue was dissolved in $Et_2O$–$CH_2Cl_2$ and esterified with diazomethane at 0 °C. The mixture, containing about 70% of the δ isomer, 15% of the α isomer, and 10% of N-methylated penicilloate (based on NMR), was separated by column chromatography over silica gel (50 g), using benzene–acetone (97:3) as eluent. Thus δ-**5** (1.6 g, 42%) was obtained. It has the same NMR spectrum as the product prepared by method A.

**Isomerization of γ-4b (5*S*,6*S*-4b) in Acidic Solution.** Anhydrous *p*-toluenesulfonic acid[25] (5.1 g, 30 mmol) was added to a solution of 3.9 g (10 mmol) of γ-**4b** in 120 ml of freshly distilled nitromethane. After the mixture was stirred at room temperature for 4 h, the solvent was evaporated, and the oily residue was taken up in $CH_2Cl_2$ and successively washed with $NaHCO_3$ (5%) and water. The organic layer was dried ($Na_2SO_4$) and evaporated to an oil, which was taken up in anhydrous ether. After standing for several days, 0.39 g (10%) of colorless crystals were filtered off. The physical constants of this product (melting point, TLC, and NMR) were found to be identical with those of the starting isomer γ-**4b**. Analysis of the filtrate by TLC revealed the presence of at least three different compounds ($R_f$ 0.67, 0.52, and 0.48, system II), which were identified as isomeric dimethyl phthalimidopenicilloates, since the mass spectrum of the mixture was identical with that of pure α or γ isomer. From the NMR spectrum, it was deduced that the mixture, in addition to a small amount (5%) of α-**4b** ($R_f$ 0.67), consisted mainly of β- and γ-**4b** ($R_f$ 0.53 and 0.51, respectively) and of δ-**4b** ($R_f$ 0.48): NMR ($CDCl_3$) δ 1.26 (s, $CH_3$), 1.55 (s, $CH_3$), 3.5 (br, NH), 3.75–3.80 (two s, $OCH_3$), 3.66 (s, H-3), 5.15 (d, *J* = 7 Hz, H-6), 5.58 (d, *J* = 7 Hz, H-5), 7.6–8.0 (m, $C_6H_4$). Chromatography of the filtrate over silica gel (120 g), using benzene–acetone (98:2) as eluent, yielded 1.8 g (50%) of a mixture of γ-**4b** and β-**4b** in the ratio of 2:3 (by NMR).

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**Registry No.**—α-**3a**, 1056-69-5; γ-**3a**, 59168-65-9; α-**3b**, 59054-13-6; γ-**3b**, 59054-14-7; β-**4a** HCl, 59121-40-3; α-**4b**, 59054-15-8; β-**4b**, 59054-16-9; γ-**4b**, 59054-17-0; δ-**4b**, 59054-18-1; α-**5**, 57628-09-8; β-**5**, 59054-19-2; γ-**5**, 59054-20-5; δ-**5**, 59054-21-6; γ-**6** HCl, 59054-22-7; β-**7**, 19788-66-0; γ-**8a** HCl, 59054-23-8; γ-**8b**, 59054-24-9; γ-**9**, 59054-25-0; α-**10**, 653-89-4; β-**10**, 21794-95-6; γ-**10**, 59054-26-1; δ-**10**, 59034-27-4; α-**11a**, 493-39-0; sodium benzylpencillin, 69-57-8; benzylpenicilloic acid C-7 methyl ester, 59054-27-2.

## References and Notes

(1) J. C. Sheehan and K. R. Henery-Logan, *J. Am. Chem. Soc.*, **79**, 1262 (1957); **81**, 3089 (1959).
(2) In this paper only penicilloates, derived from D-penicillamine (*S*-penicillamine), will be considered.
(3) Penicilloates with a phthalimido side chain will be called phthalimidopenicilloates, whereas the compounds with a phenylacetamido side chain will be designated as benzylpenicilloates, according to the nomenclature of "Chemistry of Penicillin".[4]
(4) H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., "The Chemistry of Penicillin", Princeton University Press, Princeton, N.J., 1949, p 535.
(5) J. C. Sheehan and P. A. Cruickshank, *J. Am. Chem. Soc.*, **78**, 3677 (1956).
(6) J. Hoogmartens, P. J. Claes, and H. Vanderhaeghe, *J. Med. Chem.*, **17**, 389 (1974).
(7) To avoid confusion the penam numbering will also be used for the penicilloates, and for carboxythiazolidines, derived from D-penicillamine.
(8) J. C. Sheehan and P. A. Cruickshank, *J. Am. Chem. Soc.*, **78**, 3680 (1956).
(9) J. C. Sheehan and K. R. Henery-Logan, *J. Am. Chem. Soc.*, **84**, 2983 (1962).
(10) Reference 4, pp 576–577 and 619.
(11) S. Wolfe and W. S. Lee, *Chem. Commun.*, 242 (1968).
(12) R. D. G. Cooper, P. V. De Marco, and D. O. Spry, *J. Am. Chem. Soc.*, **91**,

1528 (1969).
(13) A. Vlietinck, E. Roets, P. J. Claes, G. Janssen, and H. Vanderhaeghe, *J. Chem. Soc., Perkin Trans. 1*, 937 (1973).
(14) Reference 4, pp 584 and 616–617.
(15) S. Kukolja, *J. Am. Chem. Soc.*, **93**, 6267, 6269 (1971).
(16) R. Busson and H. Vanderhaeghe, *J. Org. Chem.*, following paper in this issue.
(17) Reference 4, p 615.
(18) I. McMillan and R. J. Stoodley, *Chem. Commun.*, 11 (1968).
(19) H. D. C. Rapson and A. E. Bird, *J. Pharm. Pharmacol.*, **15**, 222T (1963).
(20) C. Deherdt, Ph.D. Thesis, Universiteit Leuven, 1965.
(21) Reference 4, p 573.
(22) J. C. Sheehan and D. A. Johnson, *J. Am. Chem. Soc.*, **76**, 158 (1954).
(23) Reference 4, p 612.
(24) H. T. Huang, T. D. Seto, and G. M. Shull, *Appl. Microbiol.*, **11**, 1 (1963).
(25) *p*-Toluenesulfonic acid monohydrate was heated in vacuo at 105 °C for 2 h, and upon cooling in an inert atmosphere, the anhydrous form of *p*-toluenesulfonic acid spontaneously crystallized out.

# Preparation and Isomerization of 5-Epibenzylpenicillins

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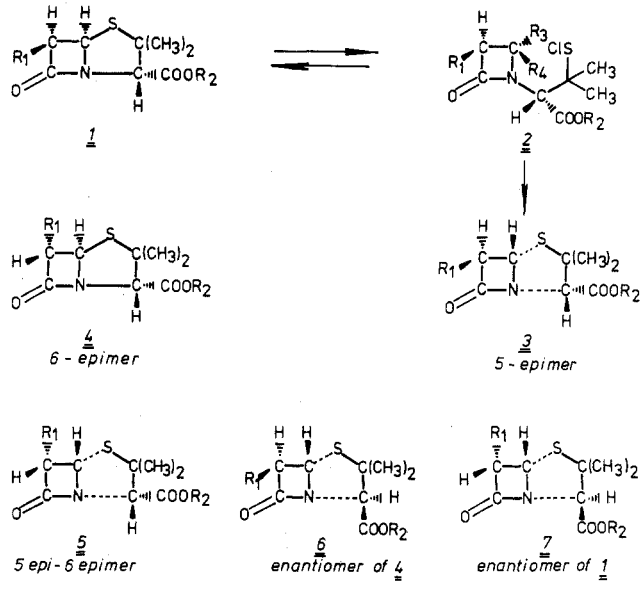
5-Epibenzylpenicillin methyl and benzyl ester were obtained by replacement of the phthalimido side chain of the corresponding 6-phthalimido-5-epipenicillanates, which were prepared from the natural isomer by the method of Kukolja. Base-catalyzed isomerization of silylated 5-epibenzylpenicillin methyl ester in the presence of triethylamine and DBN was investigated. With triethylamine, no epimerization was observed, whereas a mixture of 5 epimer and of the enantiomers of the 6 epimer and natural isomer was obtained when DBN was used as catalyst. These observations, which indicate an epimerization at position 3, are compared with the results of isomerizations of penicillanates with a different configuration and with other side chains. The general mechanism of epimerization of penicillanates is discussed. The antibiotic activity of the sodium salt of 5-epibenzylpenicillin, prepared by hydrogenolysis of the benzyl ester, is less than 0.1% of that observed for natural benzylpenicillin.

In the course of the study of the configuration of the four D-benzylpenicilloates,[1] 5-epibenzylpenicillin was needed. At that moment, only methyl and benzhydryl 6-phthalimido-5-epipenicillanate had been described.[2] Compound **3bx** was prepared by transformation of **1bx** into **2bxv** and **2bxw** by chlorinolysis of the $S_1$–$C_5$ bond, and by recyclization of **2bxw** with $SnCl_2$. This method has been applied for the preparation of several 5-epipenicillins having a cyclic or an acyclic imido side chain.[3]

The replacement of the phthalimido side chain by a phenylacetamido group seemed to be impossible in the penicillin series, because treatment with hydrazine causes cleavage of the β-lactam ring.[4] For this reason the procedure of Kukolja[2] was applied to the methyl ester of benzylpenicillin (**1ax**). Treatment of **1ax** with an equivalent amount of chlorine yielded a mixture of 25% of **2axv** and 75% of **2axw** (as determined by NMR). Attempts to cyclize this mixture with $SnCl_2$ gave several compounds of unknown structure but no 5 epimer could be detected. It should be noted that chlorinolysis of the $S_1$–$C_5$ bond in benzylpenicillin in the presence of an excess of chlorine has led to an olefinic azetidinone, which has been cyclized to a thiazabicycloheptenone.[5]

This negative result prompted an attempt to remove the phthaloyl group with hydrazine in dimethylformamide, a method which has been used successfully in the cephalosporin series.[6] Application of this procedure to **3bx** gave methyl 6-amino-5-epipenicillanate (**3cx**) in 75% yield. The success of this reaction is probably due to the greater stability of the β-lactam in the 5 epimer, because treatment of **3bx** with hydrazine in dioxane also gave **3cx**, albeit in lower yield, whereas reaction of **1bx** with hydrazine in dimethylformamide still caused cleavage of the β-lactam ring. By reaction of **3cx** with phenylacetyl chloride, 5-epibenzylpenicillin methyl ester (**3ax**) was obtained.

In order to prepare a salt of 5-epibenzylpenicillin, we applied the same scheme to the benzyl ester. Benzyl 6-phthalimidopenicillanate (**1by**), obtained by esterification of 6-phthalimidopenicillanic acid[7] with benzyl bromide in dimethylformamide, was treated with chlorine as described for the methyl ester.[2] The resulting 2-chloro-α-(1-chlorothio-1-methylethyl)-4-oxo-3-phthalimido-1-azetidineacetate was obtained in good yield but it could not be crystallized although it contained practically only the trans isomer (**2byw**). Treatment of this material with $SnCl_2$ afforded **3by** in 85% total yield. Since the presence of water greatly affects the stereoselectivity of the reductive cyclization,[2] it is necessary to perform this step with anhydrous $SnCl_2$ under strictly anhydrous conditions, in order to obtain a high yield of **3by**, because **1by** cannot be separated from the desired isomer **3by** by column chromatography. The presence of appreciable




a. $R_1 = C_6H_5CH_2CONH$ -
b. $R_1 = C_6H_4(CO)_2N$ -
c. $R_1 = Cl^{\ominus}H_3N^{\oplus}$ -
d. $R_1 = C_6H_5OCH_2CONH$ -

x. $R_2 = CH_3$ -
y. $R_2 = C_6H_5CH_2$ -
z. $R_2 = Na$

v. $R_3 = H$, $R_4 = Cl$
w. $R_3 = Cl$, $R_4 = H$